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SUSTAINABLE BIOFUELS PRODUCTION (BIOETHANOL-BIODIESEL-BIOHYDROGEN PROCESSES) from BIOMASS FEEDSTOCKS -LIGNOCELLULOSIC WOOD, MICROALGAES, OLEAGINEOUS MICROORGANISMES & WCO AND FOCUS ON VALUE ADDED BY-PRODUCTS CHEMICALS & BIOACTIVE COMPOUNDS RECOVERY A MULTIDISCIPLINARY APPROACH

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PREFACE

This Thesis work will be based on utilising bioenergies from biomass to biofuels through involvement of various modern techniques and recent innovative approaches are put together permit to help the society and strategy of biofuels. I have in mind to express this strategy through contributing new dispositif for the evolution of environmental impacts.

Thereby,I focus here the bioenergy that may compete over the increase of price of fossil fuels in the next decades as depletion .

There are many options referred on my thesis work ultimtely reflects on improving biofuels strategy. One among the option is the recombinant engineering the yeasts and fungal strain that helps and play a remarkable role on biofuel yields other than improving downstream processing.

In regards to Up-stream processing,many methods are proposed in view of easier production and recovery of main substrates molecules for fermentation and recovering chemical byproducts and bioactive compounds.

Improved Bioenergy operative methods leads to improve th environment through CO2 sequesteration and reducing proportionately GHGE, climate change etc. In this presentation of work,Biohydrogen by Biophotolysis method including dark fermentation are well discussed in comparison to PEM process;Biomass availability from various resources are mainly focussed to obtain mainly biothanol and biodiesel other than bioactive compounds separation.

Microalgaes strategy will be clearly mentioned in regards to GHGE,.

Other than microalgaes,waste cooking oil are showing a clean start-Up procedure on transforming biodiesel that meets ASTM and EN and other international stanadards.

. From MouttouCoumarassamy roland

SUMMARY

INTRODUCTION

The aim of this study is to acquire the bioenergy by convenient method of processing includes conventional &other innovative technological approach taken into consideration in view of viabilise the production process more economical, more competitaive towards sustainability.

The perspective of the study is focussed on issue of biofuels producing as bioethanol, biodiesel etc.. the alternative to traditional fossil fuels in which biomasses are considered as the only ideal substitut , widespread,abundant,inexpensive and sustainable resources.

The idea behind the biomass usage to produce bioenergy is the increase in demand to reduce green house gas emission(GHGE), improve soil quality and water quality and to provide economic developments and other socio-economic benefits.

GHGE are the gases present in the atmosphere that absorb and emit thermal radiation process often referred as green house effect having the constituents such as water vapour, CO2,CH4,NO, O3 etc.. According to climate change protocol, the environmental impacts caused by the sectors such as power plant ,industrial processes, transportation sectors contributes major GHGE as a result of fossil fuels specifically coal, natural &gas petroleum that releases carbon and stored in non-renewable energy form as CO2.Hence bioenergy comes into the reality to having the potential to neutralise Carbon through balancing the amount of carbon releases in the form of bioenergy stored in plants,tissues, other materials etc...

During the recent decades, The fossil fuels are in depletion stage and facing surplus utilisation strategy in future through certain drawbacks such as higher energy prices, their role in global warming and their non-renewable nature as energy source whereas biofuels makes more attraction at present generation and stands to improves the above strategy through possible CO2 sequesteration.

Early1897, ethanol was used as transportation fuel in internal combustion engine of the car invented by Nicolas Otto. Later there was not much interest in ethanol due to usage of fossil fuels . As a result of oil crisis and climatic change, most of the developed countries decided to decrease CO2 emissions and hence alternative fuels like Bioethanol came on potential way in order to have the positive impacts on society. Ethanol can be blended with gasoline upto 30% without any necessary changes in the engine of the vehicule as it improves the fuel combustion and reduces the emission of CO2 **.**

In 2008,GMR DC,SNL conducted a joint biofuel system analysis study in USA in view of assessing feasibility ,impacts ,limitation& other enabling factors of large scale production of biofuels .The report of finding was 90 billions gallons per year of biomass derived ethanol distribution,15 millions gallons ethanol per year from corn based grain and balance from cellulosic ethanol. The production of 45 billions gallons per year of cellulosic ethanol requires 480 millions of biomass of which 215 millions tons from perennial energy crops (equilvalent to 48 millions planted cropland).

In Brazil, ethanol is regularly produced from sugarcane, wheat,corn,barley,rye,and other cereals. Ethanol used in vehicules equipped with ethanol compatible materials and (timing)with on board electronic engine managements are running on pure ethanol. In sweden, Ethanol is being reinforced by swedish National board of Technical & other industrial Developments sectors since 30 years as an alternative fuel and proving financial support for the exploration with the University team. Since 2004, swedish pilot plants were started and established functioning within one of the atleast renewable source fuel are used in practice distributing more than 1000 m3 per year at the filling

station. Moreover nearly 1500 stations offering E-85 as fuel in Sweden.

In USA,ethanol is mainly produced from corn and in Europe, (France, Germany, Spain) the main producers transforms mainly from Cereals, and also from waste and good potatoes.

In India, The Ministry of Petroleum issued a gazette notification making mandatory for OMC in 2013.through involvement of important concern(BP,HP,IOC) additising and blending 5%ethanol with petrol. to overcome the energy crisis and to consider potentially Carbon neutrality for sustainablity.

The most commonly used blends are E85 & E10. This shows that bioethanol is possible as a fuel engine that helps to enhance the ignition or engine performance.

and usually produced from feedstock such as corn, sugarcane etc. which affect not only food chain but also increase in production cost. Thereby, This research study will focus on several aspects towards the utilisation of biomasses of various origins. categorised as Lignocellulosic wood (LCW), Waste cooking oil(WCO),

Microalgaes, Livestock manure for Biomethane, Biochar oil etc.. depends on aiming the final target products recovery. The relevant methods are proposed to coupling with recent modern technologies that are finely described in order to maximize the yield from the biomass serving greater extent.

This project is aiming for discussion on various research methods and technologies and contribution towards the improvement of environments that will be discussed in multidisciplinary criteria .This project is well known for various biofuels production and major products recovery upon integrating Biorefinery concept obtainable from cheapeast bioresource materials. This project is to find high comparative study over existing methods and biofuels resources and in view of this aspect ,the recombinant metabolic engineering approach is being practiced with well known industrial species such as **S.Cerevisiae, Yarrow Lipolytica** etc.. towards the bioethanol and biodiesel production as second and third generation biofuels.

This technological project shows certain challenges over in association with algaes -WCO feedstocks and other fuel program studies meets ASTM properties specifications and thereby focusses on new strategy principles and increasing the demand as alternative biofuels.

This project may also focuss on enhancing the efficiency of algal cultivation and and mode of harvesting through different technologies more adoptable for the bioprocessing industries have been given due consideration for the future perspective offering the opportunity which is many times higer than that of plants for CO2 accumulations and throughCO2 sequesteration .

Though it has disadvantages such as geographically available biomass that makes expensive distribution & transportation hence,raw materials needs to be processed in semi-produce form while in on-site of production of locations like forests, cultivation land etc..

The current investigations indicates the applicability of lab findings of bioethanol evolution through different methodologies and other innovational approaches and biodiesel become a challenge in scaling-Up of the design of bioreactor and mode of recovering algal-oil for ultimate processing through transesterfication.

The spent algal biomass can be considered on three axes or pillars subsequently that may be
Upgraded towards the Improvement of environmental conditions.

- Food and Feed &Neutraceutical applications
- BioFuel production
- Environmental Improvements VIA Upgrading Biodiesel purifications routes of algal- Biomass for

renewable diesel etc..
This project covers the state-of-the-art processes involved in bioethanol production, including pretreatments, hydrolysis, fermentation, bioethanol recovery, integrated product recoveries, LCA, techno-economic analysis, &process simulation.
The bioethanol produced globally in 2018 was 110 billions liters and is expected to increase to 140 Billions liters in 2022 with compound annual growth rate(CAGR) of 7.6% due to anticipated economic feasibility of the process.

1.1 STRATEGY & BIOMASSES AVAILABILITY

The bioenergy is the part of global carbon cycle in which atmospheric CO2is taken up by plants and converting into tissues(referred as Sequesteration -C).Then the plant biomass is released back Carbon into the atmosphere while burning directly during the course of fuel conversion and vice versa.
Considering the availability of resources present in our planet system among the various biomass materials,Lignocellulosic wood materials considered as a potential bioenergy feedstock , an alternative & renewable source for bioethanol production and clean energy.
LCBW is being considered due to relatively low cost,of acquisition,availability,& sustainability of supply.This biomass has the capacity to increase the current production rate of bioethanol & being speculated to produce approximately 442 billions liters per year.globally.
Bioethanol is the most promising biobased fuels possessing modified physicochemical properties applicable for internal combustion engines since it has similar properties to gasoline in terms of high octane number.,high flame speed,low stochiometric air-fuel ratio and low heating value etc..
Bio-energy is considered to be renewable form of energy and derivatives of organic materials of the living plants and wastes.The various forms bioenergy include power,heat,solid,liquid and gaseous biofuels which is increasing in demand in response to concerning energy security,energy independence,and environmental &other chemical impacts associated with the use of other non-renewable energy resources.
Biomass availability is to meet the renewable energy goal through influence of land availability,competing land uses,yield potential,yield gaps,producer profitability,enhancing rural livilihoods resulting provision of the supply chain to meet the challenges today.
Fuels from Biomasses are distributed geographically and it can be conveniently storable and used as fuel in liquid,solid or Gas forms.It can be burnt without any significant toxic emissions.
The major advantages of utilisation of biomasses is the modifying climate face change that reduces proportionately Green House Gas emission(GHGE) responsible for Impact of global warming.
Biomass comes from a variety of sources that include;
Agricultural wastes,forest residues,municipal solid wastes(MSW),wood wastes,Wastes from waste paper and industrial processing wastes,,energy crops,cellulosic agricultural wastes include crop waste such as wheat straw,corn stover(leaves,stalks and cobs),rice straw, and sugarcane-bagasses ,wood chips from forest wastes etc..other than waste cooking oil(WCO),microalgaes,Oleagineous microrganismes etc..
Algal bioethanol is gaining attraction possibly due to high carbohydrates presence and lack of lignin **(Refer Fig-.A&B...)**
Biomasses are always available in abundency that can be produced as a renewable energy source.The unit for biomass is calculated as (g/m2) and also in Kg/m2,Lb /ft2 etc..The biomass is an important source of energy considerable after coal,oil & natural gas.Biomass helps climate change by

reducing green house gas (GHGE)that gives impact to the global warming and also helps clean our environment.It is considered historically as carbon-neutral renewable energy source signifying carbon emitted and carbon removed from the atmosphere that are essentially balanced.

***1.2* SCOPE OF THE PROJECT**

The scope of this study is to acquire the bioenergy in simpler method of processing comprises recent developments including conventional &other innovative technological approaches taken into consideration in view of viabilise the production process more economical ,more competitaive towards sustainability

> The IDEA of the Thesis project is focussing on various number of research-methods,developing a method of producing bioethanol through Lignocellulosic wood materials (LCWB)& other industry wastes that does not compete with the food chain in regards to sustainability ,cost,energy & efficient over global remedies.

> The project proposes here focussing on second and third generation biofuels that replaces fossil fuels towards the sustainability using undepleted biomasses realisable through variable modern technologies in combination with conventional methods other than the recombinant metabolic engineering approach with well known industrial species such as S.Cerevisiae,Yarrow Lipolytica etc. This shows the promising strategy for the high yield of bioethanol & biodiesel.

CO2 sequesteration and waste water remediation are well discussed for proper utilisation of biomass in view of improving the yield of biodiesel etc..;

According to climate change protocol, bioenergy comes into the reality to having the potential to neutralise Carbon through balancing the amount of carbon releases in the form of bioenergy stored in plants,tissues,other materials etc...

The idea behind the sustainablity is to produce bioenergy through increase in demand to reduce green house gas emission(GHGE),improve soil quality and water quality and to provide economic developments and other socio-economic benefits.

Therefore,food chain can not be affected and these biomasses can be considered as an important source of energy after the Coal,Oil and natural gas etc.;-

This project is aiming for discussion on various research methods and technologies and contribution towards the improvement of environments that will be discussed in multidisciplinary criteria .This project is well known for various biofuels production and major products recovery upon integrating Biorefinery concept obtainable from cheapeast bioresource materials.

Thereby , the classification of biofuels production can divide into four categories as generation biofuels.

1st generation biofuels;

-1-G Ethanol Technology referred to food crop feedstocks produced mainly and directly from plant Corn,Sugarcane,rice crops containing starch,and sugar materials.

2nd Generation biofuels;-

-2-G -Ethanol Technology referred to Product from non food integral part feedstocks of the plants such as Lignocellulosic Wood materials containing lignin, Cellulose and hemicellulose etc. and industrial wastes, Energy crops,Pulp &Pulp processing wastes and other crop residues, Municipal solid wastes(MSW)etc...

This is how we are not compete with esential food crops chains by separating them from1st

generations biofuels.
These biofuels are known as advanced generation fuels that can supply in large proposition towards global demand sustainably,affordably and with greater environmental benefits.
For effective degradation of cellulose,acid and enzyme hydrolysis processes needed to be given a greater attention so as to produce various chemicals and fuels sucessfully and inexpensive manner.
Ethanol can be produced from every sort of carbohydrates materials such as starch,sugars and lignocellulosic raw materials.Higher propotions of sugar presence enhances the easy fermentability index in sugar beets,sugarcane etc

3rd Generation biofuels:-

3-G-Ethanol Technology referred to Micro-algal biomasses by utilising the oil through Tranesterfication process and reported to be 19%influences higher biodiesel extraction.and this also includes WCO,fast growing trees,perennial grasses possible as feedstocks,expected to be meeting higher demand.and increase steadily with public incentive policies.

4th Generation biofuels:-

-This is referred to most sustainable energy production:
CO2 is captured and stored.These can be produced where CO2 and H2O found in sufficient concentrations**.**

ANOTHER -FOURTH GENERATION BIOETHANOL;-

4G-Ethanol is obtained from the modification of E.Coli gene alterations through application of metabolic engineering or sysytem biology strategies.

BIOMASSES FOR BIOFUELS PRODUCTION:-

1;3 HYPOTHESIS (Biofuels production sustainability)

The author describes here suggesting on three major issues towards sustainable biofuel production (BIOETHANOL)and to meet phenomenal characterstics of following criterias &schema;-

These are known as ;
Three **Ps**(**People-Plant-Profit**)**
or
Three **Es**(**Environment-Economy-Equity**)**

*This can be referred **as** **R-T-Q** through following schema of concept;

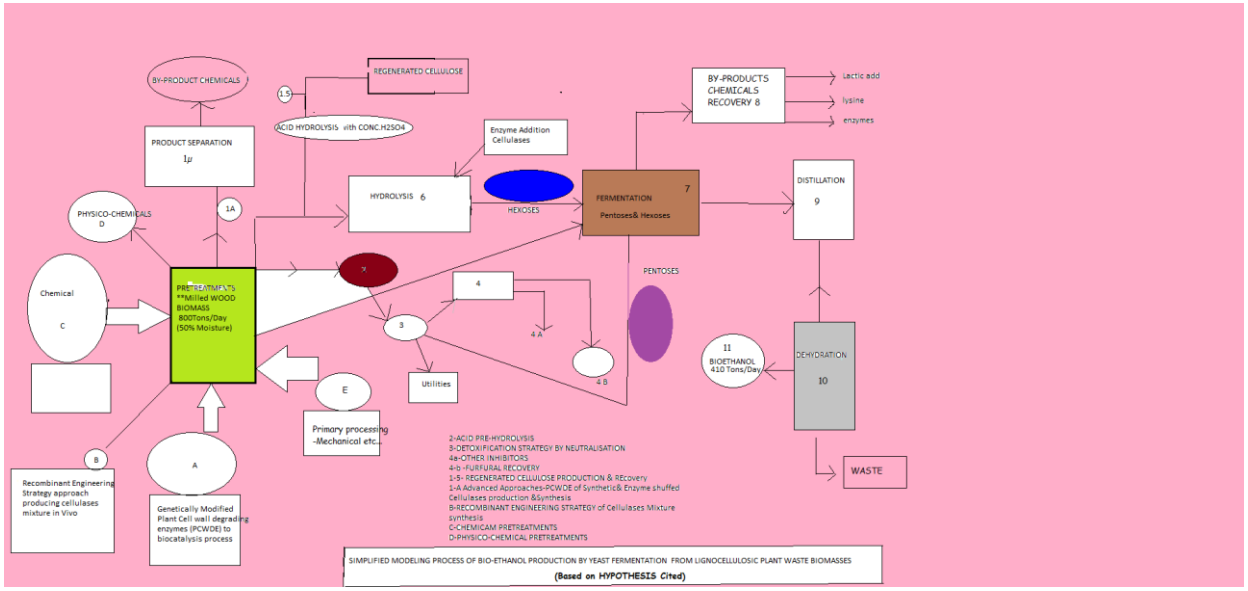
R >>>>>>T >>>>> **BIOFUELS +Value added by-products chemicals**

where **R** denotes Reneweable biomass may be multifaceted origines ,from various agricultural resources and highly variable in regards to pretreatment processing and other evoultive methods etc. applicable for future biofuel transformation and it is need to standaridise them and utiliseable as substrates in conjunction with other substrates for the aim of convenient processing.

T- stands for recent Technological methods referring to most modern techniques involved in combination with other conventional processing to produce higher yields of biofuels and to recover most valuable products upon integration of biorefinery concept allowing to equilibriate the whole **CONSOLIDATED BIOPROCESSING** for a minimal period of time (in the case of direct microbial treatment takes between 35-60 days)towards the sustainablity and stability.

Q-Quality Assurance (QA)applicable towards the whole process integration stages involves highly oriented technologies adoptable for consolidated bioprocessing in which QC analysis can be practiced from raw materials,to the stages of intermediate products and upto the final target product obtaineable during upstream and downstream processing of biomasses towards qualitative and quanitative productivity of biofuels for sustainability.

The results obtained by above equational concept influencing specific BIOETHANOL strategy **Refer(FIGURE -A**) would be more sustainable in accordance with the type of feedstock utilisation,modern technology practiced and other higher technique involvement in combination with conventional methods,recombinant strategy of strains,other upstream processing such as cocktail enzymes,(Laccases etc..) successive Down stream processing
methods(fermentation,separation and purification by Ion exchange resin,Polysulfone Membrane Ultrafiltation etc;;) leads to produce very successful biofuel of quality meeting the international standards & specification (ASTM,EN etc..)..



Route- 1 -5 signifies Pretreatments by acid hydrolysis (partial bifurcation possible towards Enzyme process)& subsequent recovery of Regenerated -Cellulose through High Conc.Acidic method.
Route-2 (B)means Microbial Prereatment processing followed by Consolidated processing (CBP)with microbial strains &enzymes Cocktail-MIX & Yeast fermentation ,purification&Distillation.
Route-3 (A)proposes Advanced Plant cell wall Degrading Enzymes (PCWDE)Techniques (Recombinant Enzymes& Synthesis) Pretreatments followed by actual Hydrolytic Enzyme Processing & Fermentation.& purification & Distillation .
Route-4 (C&D) focusses on Normal Pretreatments methods by Physico-chemical processing Impregnation by H2SO4 & steam explosion)followed by Enzymes Hydrolysis ,evoporation to distill the glucose level(saccharification)& Yeast fermentation &purification of ethanol.

BIOMASS TO LIQUID BIOFUELS

2;0 - BIOETHANOL PRODUCTION

Ethanol can be produced from various kinds of substrates.This will vary from countries to countries due to their farming conditions.In USA,Corn is the dominant substrate whereas in Brazil,sugarcane is regularly used as principle raw materials to produce ethanol followed by wheat,corn,barley,rye,and other cereals.In Europe,ethanol produced from cereals thereby second generation bio-fuels comes into practice from integral part of plants such as hemicelluloses,celluloses etc.

So,methodlogy are much developed to process them mainly from agricultural wastes,forest residues,municipal solid wastes(MSW)Wastes from waste paper and industrial processing wastes,,energy crops,cellulosic agricultural wastes include crop waste such as wheat straw,corn stover(leaves,stalks and cobs),rice straw, and sugarcane-bagasses ,wood chips from forest wastes. etc..considered as biomass feedstocks.

Ethanol production involves two major processing such as Dry and wet milling;

PROPERTIES AND USES OF BIOETHANOL;-

Bioethanol is the colourless and flammable liquid and considered either most used liquid biofuel as a fuel or as a gasoline enhancer.and facilitates better fuel combustion and It is easily bidegradable. and consider as a vehicule fuel produces Green house gas emission(GHSE)compared to petrol or octane..Ethanol has some fine properies over conventional fossil fuels such as high heat of Vapourisation,low flame temperature,greater gas volume changes and high specific energy**.(Refer Tab-1.).**

Ethanol has higher 35% oxygen content than any other biofuels. that allows better combustion of hydrocarbons with reduction in CO emissions and other dreadful hydrocarbons.Bioethanol is completely miscible with H2O in all proportions while gasoline and water are immiscibleThis may cause the blended gasoline containing H2O results in corrosion related problems on the mechanical components in engine especially made of copper,brass or aluminium.Ethanol has greater octane booster properties but low cetane number thereby reduces the use of toxic additives like benzene. and influences on thermal efficiency and compression ratios of engine respectively in compared to gasoline alone..This permits additional O2 to burn relatively more completely during combustion process leads to finally and possibly CO and HC emissions becomes lower.than gasoline.

Bioethanol is a safer alternative to MTBE.a toxic compound,the most common additive used in petrol to provide cleaner combustion .In order to use ethanol as a effective engine fuel,It is important to understand its physico-chemical properties and its blends proportions towards the engine performance.This can be determined according to a ASTM methods and guidelines.

Ethanol ,having the lower boiling point helps in obtaining better combustion efficiency and lower energy density admits lower vapour pressure than gasoline. This reflects directly influences on fuel consumption owing to light weight designed products**.**

2;1 PELLETS FROM HERBACEOUS -GRASS;-

Bioenergy is the renewable energy derived from living biological materials .Bioenergy is increasing in response to concerns about energy,security,energy independence & environments and climatic impacts associated with the use of non-renewable energy resources.

WOOD PELLETS PROCESSING;-

Bioenergy are made from shavings,sawdusts;chips,slabs or any wood matter after pulverisation or broken-down with other mechanical means to small grain sized particles.The process includes drying mechanism to reduce excess moisture content from 50%to around 10% .then the materials is then heated ,pressed and moulded using the natural based lignin cells as a binding agents.Then the pellets can be used & converted into gases,liquid or biofuels.

The short rotation crops (SRC)such as Hybrid Poplar and willow yield pulpwood products other than *energy harvestable could be chipped on site. These chips are combined often with other feedstocks in view of densification during the course of wood chips pelletisation.SRC provides long term yield potential and environmental benefits such as wild life habitats,soil erosion prevention and water quality improvements etc*..

Perennial-Grass, Crops based biomass production & utilisation;-

In the case of cellulosic crops that include herbaceous and woody perennials and leaves,stems,stalks etc.. can be burnt directly to generate electricity or can be convertable into liquid biofuels,energy gas & chemicals through conventional technologies.

PERENNIAL CROPS PROCESSINGS;-

Grass are used to produce a type of herbaceous biomass, that is used for direct-energy . Different energy grasses such as Switch grass,(giant Panicum),Miscanthus (x-Giganteus)Reed Canarygrass (Phalaris Arundinacea),Indianagrass(sorghastrum Nutans) used for biomass conversion to solid fuels in the form of pellets, briquet ,cubes and can be converted into liquid fuels like ethanol,methanol & other advanced biofuels(alkanes). These can be burnt directly either alone or co-fired with another sources.

These grasses are often harvested only once or twice per year.Most of the highest yielding grasses are tropical or sub-tropical origins referred as warm season possessing C4 pathways for photosynthesis generates higher grown rate in contrary to cool season that have C3 photosynthetic pathways better suited for biomass production. Normally grass is harvested in a dry form 10-12%moisture and burnt on whole bales,powdered or burned without any aditional processing of densification..The later involves drying,grinding,pressing etc.into a more dense form provides uniform sets physical size(56mm *7mm).This improves handling,storage,& transportation as well as better control over the combustion process.

If the end product is ethanol then the complex carbohydrates must be broken down into simpler sugars using biocatalysts in presence of heat or other chemicals such as lime,ash contents etc.The presence of two components (lignin & ash)may be resolved by utilising new generation enzymes.The economic considerations for grass biomass are indicated as it shows that the minimum input costs to be considered assuring optimum yield for a viable production system of a typical different yield scenarios.(;;;)

In the case of switchgrass, CelA (modified microbial strain) achieved 60% conversion of xylan showing its potential for industrial processes using mild or no pretreatments.

There is unusual, usual and highest scenarios status obtained during the course of 10 years of switchgrass harvesting with a difference cost involvement in scenarios 5 for adding 50 lbs.of fertilizers. This shows clearly having the overall lower cost benefit per unit.

PRODUCTIONAL COSTS of Perennial Crops Pellets;-

The available meadow can be used through harvesting followed by pelletisation to produce 2 tons of grass pellets per acre which is equivalent to 20 tons per 10 acres..The heating value is 7900 BTU/lb for the grass, generate the heat equivalent to 2000 gallons of fuel oils that is equivalent to 270 MMBTU.
The cost incurred on harvest the grass using a mobile harvesting equipment as follows:
Cost of harvesting/ 10 acres = USD 800
Cost of pelletising = USD 2800
In order to process grass pellets through heating,the provisions are needed to install such as conversion of space heating equipments,boilers or furnaces and able to burning pellets at the location.In order to facilitate the installation,the cost associated with mobile pelletizing equipments(in USA) would be in the order of USD 14000 (USD 4000 to harvest the grass and USD 10000 required for convert equipments to burn pellets).

BIOETHANOL PRODUCTION FROM LIGNOCELLULOSIC WOOD BIOMAS S(LCB)
BIOETHANOL FROM STARCH AND LIGNOCELLULOSIC MATERIALS;-
Lignocellulosic materials are considered as a primary renewable resource material for BIOFUELS PRODUCTION that involves five main steps of processing.
LCB is abundant ,renewable source of carbohydrates,convertable into liquid fuel.The major advantages is not competing with human food chain and improves CO2 balance in the atmosphere.
Hence we found out the possible ways to improve upon giving added value to the waste materials but considering the cost of production ,the technical feasibility of the conversion process are validated as principal biomass substrates applicable towards the nature of the region .
Various number of researches have been conducted from a simple conversion processes of biomasses such as sugarcane,corn,banana peels,rice straw etc..through fermentation or utilising LCB in multistage conversion methods for transforming into bioethanol.

2;2 Chemical composition of LIGNOCELLULOSIC WOODS MATERIALS:-
The chemical composition of LCB are categorised into three major elementary compounds such as Cellulose (30-50% dry wt),Hemicellulose(20-40% dry wt) and Lignin (10-20%dry wt).The molecular structure is represented in **Figure (1**).

CELLULOSE;
Cellulose is a hexose sugars,linear long polymers of glucose monomers(D-glucose comprise of Cellobiose,a dimer of glucose units linked each other to Beta-1-4 glycosidic bonds enclosed into the microfibril bundles of woody biomass as the building blocks of elements and this is considered to be having higher degree of polymerisation among the other polymers.The number of glucose units in one polymer strand can be 10000 or higher.The structural elucidation is based on beta configuration at the anomeric carbons giving rise to stretched chain conformation binding through hydrogen bonds into flat sheets.These linear conformation permits the arrangement of numerous cellulose strands into crystalline fibers exerting to structural matrix alignment.The high molecular weight of cellulose ,low flexibility of its polymer chain,inter& intra molecular hydrogen bonding, hydrophobic forces are exerting top & bottom surfaces of the molecules enabling Vanderwaals forces interaction develops between the above stretched chain conformation. Hence this phenomenon makes a limiting factor for insolubilization in water & other most organic solvents.This may be essential points that can be

approchable for the range of biochemicals products from this plateform..

Extensively High pressure &High temperature reaction profile are needed for hydrolysis of cellulose into reducing sugars susceptible to decompose under variable harsh conditions.This can be discussed later in separate chapter.

Cellulose rich plant material producing 50-60% glucose compounds and 40-50% of Xylose sugars that can not be used for fermentation into ethanol by yeasts. The studies (2004) shows that engineering the yeast to change the metabolism helps in increase the efficiency to convert Xylose to ethanol. This will be discusssed in details in another chapter.(Recombinant yeast studies)

The schematic representation **(Fig1;2,,)** gives the concept on biorefinery plateforms from LCW as the precursor for the production of Miscellaneous Chemicals and biofuels. This can be realised through biological processes leading to wide range of substances such as Bioethanol, Organic
acids(lactic acid, acetic acid & Levulinic acid (an intermediate compounds for ,Glycerol, Sorbitol, Mannitol, Fructose etc.), enzymes,biopolymers (DHA)etc.. through metabolic pathway of microorganismes. Many toxic compounds can be derived upon chemical pretreatments methods and enzymatic hydrolytic process recommendable for conversion of HMF ,as an important intermediate plateform for the production of DMF(Dimethyl furan),furfurals ,sugars & other phenolic compounds.

HEMICELLULOSES:

Hemicelluloses serve as major constitutional portion in woody biomass of the plant matter.This is a short,highly branched Hetero-polymer of pentose sugars(D-Xylose&L-Arabinose)and hexoses(D-Glucose&D-Mannose&D-Galactose) and sugar acids of which Xylan is most abundant in nature represents 70-90% associated to cellulose present in the form of Xyloglucans or Xylans as the large source of carbons.Hemicelluloses are the branched polymers,amorphous in nature susceptible to hydrolyse very easily than cellulose.In other words,Hemicelluloses are heterogeneous polysaccaharides tends to degrade by acid into the various conversion products such as monomeric compounds such as glucose,galactose,Xylose&Arabinose etc..and present on the outer surface of cellulose fibers and acts as a barrier.that hinders the accessibility cellulase enzymes over cellulose molecules..Hemicelluloses are present as the presiding resource of softwood,Hardwood & other various plants.Beta glycosidases is supplemented from another source to cellulases like Trchoderma and Pencillium Sp;, found to be good in their capacity to hydrolyse pretreated softwoods etc..;

Hydrolysis of hemicelluloses can be done by enzymes like glycoside hydrolases,carbohydrate esterases,endo-hemicellulases,Polysaccharides lyases,which include endo 1-4 beta xylanases,beta xylosidases,beta mannase,beta mannosidases alpha-L-Arabinofuranosidases etc. **Thereby, hemicellulases like xylanases, xylosidases are included in enzyme cocktails.**

Xylose can be biologically converted by yeasts that involves pentose sugars fermentation to produce Single Cell Protein(SCP).and variety of fuels & solvents.using the yeast strains such as Pichia Sitipis,candida Sheratal etc.. The various compounds as described in the schema**(Fig1.3...)** on which microorganismes utilises Xylose substrates via NADPH as a reductase activity enzyme to produce Xylitol and various polymers(PHKA),polylactates and a series of organic acids(succinic acid,propionic acid,acetic acid,lactic,&butyric),solvents(butanol& acetone) and other fuel additives(DMF,butanol& 3 butanediol) etc.

LIGNIN;-

Precursor alcohols structure in lignins:

Lignin provides the mecanical strength to plants & trees.Lignin is an organic compounds originated or derived from glucose through development of different precursors alcohols such as coniferyl alcohol,Synapyl alcohol and p-Coumaryl alcohol etc..joined together through various functional groups like Methoxyl,carbonyl,Hydroxyl showing the high polarity property to the lignin molecules resulting set of linkages create a high thick matrix &acts as a building blocks of lignin compounds.These components was degraded by various oxidoreductases like lignin peroxidases,Manganese peroxidases ,Laccases, etc.; The action of these enzymes activity not only accessible to cellulose& hemicellulose substrates but also generate oxidative species that may attack inhibitors produced during pretreatment process and make more effective and convenient by these enzymes.

Its structure is complexe,hydrophobic,cross-linked with above building blocks elements of aromatic polymers of phenolpropane

Hence it is considered to be a obstacle to the fermentation of LCw and these high rigid structure are unaffected by chemical & biological degradation which in turn reflects on bioethanol productional quality upon further attacks mostly possible through Fungi & some Actinomycetes.

Refer Figure 2008,2009,2015)

Extractives of LCW materials are the sources of Terepenoids,steroids(soluble in non polar organic solvents.fats(saturated&unsaturated),waxes,metal compounds (Mg,Ca,Na& P) etc &other Phenolic constituents.(**Refer Fig-1:4**)

2:3 PRETREATMENT METHODS OF BIOETHANOL PROCESSING OF LIGNOCELLULOSIC BIOMASSES ;-

Bioethanol can be produced from every sort of carbohydrates materials .

Various number of researches have been conducted from a simple conversion processes of biomasses such as sugarcane, corn ,banana peels, rice straw etc. through fermentation or utilising LCB in multistage conversion methods for transforming into bioethanol

The conversion process is based on type of lignocellulosic materials used for bioethanol production comprises different stages as mentioned below.

a) Pretreatments of feedstocks,& Overliming

b) Hydrolysis of Cellulose &HemiCelluloses(saccharification)

C) Fermentation

d) Separation by Distillation & Purification of ethanol to meet Fuels specifications

e) Waste treatments.

PRETREATMENT METHODS;-

Pretreatments are the process of breaking down complex cellulose structure into simpler sugars units accounting the productional cost approximately 0.3US$/gallons of ethanol.

In bioprocessing, Pretreatments has played a major role during final stage of production in regards to quality &quantity of the process. There are several methods available to be used for pretreatments that can be classified into 4 categories as Physical,Chemical, Physico-chemicals & Biological processing**.(Refer Fig-1.1)**

The most advanced techniques may be either acid-base fractionation or Ionic Liquid-based

fractionation(ILF).Chemical pretreatments are the most efficient and predominant one.

1;2 PRETREATMENT TECHNOLOGIES;-

The processing technologies shows an influential effect on overall process of bioethanol from LCW and make easier accessible for hydrolytic conversion of bioethanol. **Table 2;0** summarises the advantages &disadvantages of different pretreatment processes technologies ;

It is the process of reducing the particle sizes of biomass feddstocks enabling to increase the surface or volume ratio and easier to make accessible for subsequent processing.Then the saccharification process comes into appearence for producing fermentable sugars from cellulosic materials via enzymatic degradation,acidic&Ionic hydrolysis.

1;2;0 PHYSICAL PRETREATMENTS PROCESSING;-

The aim of the process is to produce fermentable sugars such as Hexoses, & Pentoses from lignocellulosic materials leaving behind the Lignin structure used for production of electricity & direct conversion of biofuels.

This involves breaking down of the size of LCWB and crystallinity by Physical processing such as Ball milling, colloid milling, hammer milling, grinding, Irradiation & High pressure steam, Extrusion, expansion and Pyrolysis etc. principley employed as pretreatments methods in view of hydrolysis & to produce fermentable sugars such as Pentoses (Xyloses etc.) & hexoses (Mannases etc..) from LCB leaving Lignin as byproduct. This increases in surface area, & pore size of the biomass enabling to have the access in enzymatc activity.

The problem associated with the production of second generation of biofuels is addressed possibly to extract the compounds from the woody or fibrous biomass where useful sugars are locked in cellulose, hemicellulose & lignin molecules. Hence the research studies have now focussed their attraction towards the cellulose hydrolysis & Depolymerisation of the polymers into the bioethanol production.

During the recent days, as a result of modern biotechnology advancements, the pretreatments methods of hemicellulose of softwood and the hardwood (Xylose compounds) are being practiced either by enzymatic or by physical & chemical pretreatments processing methods. This results in increase of sugar yield greater than90% (therotical yield) and signifies that cellulose is more susceptible to enzymatic action when its crystalline structure is disrupted otherwise enzymes tends to bind on the surface of lignin resulting cellulose chain not in hydrolysable stage.

1;2;1 MECHANICAL TREATMENTS:-

This is an important stage of pretreatment process for improving the bioconversion efficiency through particle densification for enzymatic accessibility, and the overall transformation of LCB materials leads into biofuels without generation of toxic side streams.

The process involves breakdown of LCW materials generates new surface area, improve flow properties, increases the bulk density and porosity proceed through a combination of mechanical processess of chipping, grinding or milling to reduce the cellulose crystallinity. The size of the material is usually between 10mm to 30mm after chipping and 0.2mm to 2mm after milling or grinding. The energy requirement are dependant on the final particle size that does not require any additional chemicals.so any forms of inhibitors are not generated. This can be done through attrition milling,ball milling or compression milling to destruct lignin compounds giving better access for

enzymes to attack cellulose &hemicellulose during enzymatic hydrolysis process.

1;2;2 *MICROBIAL TREATMENT OR NO PRETREATMENTS.;*
- (BIOLOGICAL PRETREATMENTS)

These pretreatments methods are considered to be cheap alternative,efficient and eco-friendly manner.There are several micro-organismes naturally exploitable and capable in assimilating the inhibitory compounds.This include yeast(S.Cerevisiae,fungi & bacterias).Certain number of microorganisms are able to release cellulases &hemicellulases enzymes degrading only lignin molecules results LC substrates hydrolysed into fermentable sugars under mild conditions during a short time.

The commonly used microorganismes are filamentous fungi that are ubiquitous,isolatable from soil,living plants or lignocellulosic waste materials.

Wood degrading microrganismes such as bacteria &Brown rot fungi,white rot,soft rot fungi are employable in biological pretreatments among which fungi play a major role on distinct degradation characterstics on LCB.So it is essential to indicate that brown rot fungi mainly attack cellulose material while White &soft rot fungi attack both lignin &cellulose molecules.The advantages & disadvantages are briefly summarized **in Table- 2 .**

Pretreatments is one ofthe important steps makes viablising the process commercially for production of fuels and chemicals until biomass developed that lacks typical recalcitrance.The elimination of pretreatments step during initial processing of biomass is highly beneficial owing to incurring of cost involvements.

It involves minimal energies input with incubation of microorganismes that produces extracellular enzymes modifying biomass to a greater extent through coupling with biological or thermochemical processing.

A poplar species ,Caldicellulosiruptor source of biomass degrading enzymes considered for this role.The aim is to create biopulping with indigeneous microorganismes preferably fungal species as described earlier to accelerate and control the processing.White rot fungi is one among the delignification species usually consuming time about to process the biomass varying from 28-60 days determining the production process more economically viable.(refer Chen et al2010,Zong et al 2011)

1;2;3 PHYSICO-CHEMICAL PRETREATMENTS:-
STEAM EXPLOSION PROCESS(AUTO-HYDROLYSIS)

This method is most commonly used for hardwood materials,&agricultural residues where it is less effective method for softwood due to the presence of lower content of acetyl group elements.

In this method,LCB is exposed at a temperature of higher pressure saturated steam about 160-260 °C and a corresponding pressure of 5atm-50 atm.are generated for few minutes. followed by release of pressure gradually make swelling LCB matrix which in turn causes individual fibers of cell wall structure to separate from matrix disrupted.Acid can be added as a catalyst during steam explosion but not essential.If not added,these can be termed as Auto Hydrolysis(.Refer Tab-2)

Though it has some disadvantages the processing softwood materials,the increase of SO_2 or addition of H_2SO_4 has been proposed or recommended as one of most effective pretreatments methods**.**

1;2;4 AMMONIA FIBER EXPLOSION METHOD(AFEX);-

In this liquid ammonia process,NH3 is added to the biomass under moderate pressure(100psi to 400psi) treated at a temperature of 70°C-200°C before releasing pressure rapidly.This leads to disrupt the lignin bonds and influencing decrease in cellulose crystallinity.The important parameters of the process are optimized to be the temperature of the reaction,residence time,ammonia loading and water boardings etc...(**refer Tab-2**)

Ammonia fiber explosion pretreatments exerts the increase in digestibility of LCB and enhance the yield during hydrolytic enzymatic process but it does not show any inhibition on subsequent processes stating that phenolic fragments of lignin remain on the surface of cellulose while explosion process. The research study have been conducted on Switch grass(Panicum Virgatum)using NH3 fiber explosion method controlling temperature profile at 100°C at a ratio of 1;1 yielding 0,2 g ethanol/gm of dry biomass with enzyme cocktails and higher sugar yield obtainable as 520 gm sugar/kg biomass as compared with the standard method showing the yields of 410 gm sugar/kg biomass indicating a good yield strategy possible with the enzyme process followed by ammonia treatments.

1;3;0 CHEMICAL TREATMENTS ;

Advanced pretreatments methods for lignocellulose:

These methods are targeted at reducing cost of ethanol production by fractionating the cellulose in such a way generate value added co-products under a mild conditions like 50°C in 1 atmospheric pressure using cellulose as solvents that enhances cellulose accessibility & separation of cellulose,hemicellulose & lignin finally to produce value added co-products.This is called as Cellulose solvent based lignocellulose fractionation(CSLF).The operation helps to reduce quantities of enzymes required for subsequent enzyme process and could be used for varieties of feedstocks. This include **1)Acid 2) ILS**

Acid -Mediated Fractionation;-

The cellulose reagents such as phosphoric acid and organic solvents like acetone or ethanol are used in mild conditions of 1 atm. at 50°C to separate the biomass molecules based on solubility properties of principle three compounds in above solvents ,water respectively.Lignin separates from other two molecules fraction helping to reduce substrate recalcitrance & unwanted sugar degradation,cost ,inhibitors etc..This shows that this method will be suitable for treating varieties of feedstocks like bamboo,corn stover,sugarcane,switchgrass,elephant grass etc...

***1;3;1 ACID PRETREATMENTS*;-**

The process is more popular one and highly efficient in biodegradation of complex materials where dilute H2SO4 is used commonly to separate cell wall components enhances hydrolytic degradation phenomenal of Hemicellulose & cellulolignin compounds .

The process can be performed at a temperature range between 120°C-180°°C with a residence time of 15-60 minutes whereas it is advisable to note that applying low temperature profile is recommended making cell wall matrix to loosen through degradation of hemicellulose and the process is not cost effective & does not affect the lignin molecules but the hydrolytic cellulose microfibrils leads to produce high yields of monomeric sugars,essential for fermentation. (**Refer Tab-2)**

Other acidic substances such as Hcl,acetic acid&oxalic acid(C2H2O4) have shown the promising

results.(Ref ;2011,2012,2013).Eulaliopsis Binate has been treated with 0,5%dilute acid H_2SO_4 at a concentration of solid liquor ratio 1;5,at a controlled temperature 160°C shows the recovery of 21,02 % total sugars with a low inhibitor production levels.

1;3;2 ALKALI PRETREATMENTS;-

The pretreatments method applied with alkali is the simpler operational process yielding high conversion of monomers within a short period of time.Then the advantages of the process is the optimal utilisation of lower temperature & pressure causing less sugars degradation and elimination of inhibitors.

Among the alkali reagents used,KOH,NaOH,Hydrazine(N_2H_2),anhydrous ammonia,$Ca(OH)_2$are typically recommended for better yield of recovery of sugars.

NaOH is one of the most pretreatments methods applicable for bioethanol production that can enhance swelling phenomenon having a high accessibility and decrease in crystallinity and lower polymerization degree.Presence of higher Lignin content in softwood comprises of mannoses are compartively effective than that of lower lignin level in hardwood,Herbaceous crops and agricultural residues.that can be hydrolyzed either by Chemical or enzymatic methods.

CHEMICAL HYDROLYSIS :-

During chemical hydrolysis method,dilute acid hydrolysis (0,5-1 % H_2SO_4) are carried out at higher temperature profiles resultingthe higher glucose yield obtaineableat 220°C and mannose compound obtained at temperature below 200°C.During two stages,separation of mannose is possible followed by glucose at higher temperature hence choosing two-stage process is more effective than one-stage process.During concentrated acid hydrolysis process,H_2SO_4 or HCl is used to recover high sugar content and high yield ethanol but drawbacks fall on extremely corrosive in nature therefore the process needs expensive alloys or non-metallic construction that leads to high production maintenance cost**.**

1;3;3 ORGANOSOLV PRETREATMENTS;-

This process removes the carbohydrates and improves cellulose and hemicelluloses processing directly linked with significant cost of purchasing solvents and interrelates with processing cost of extensive removal of solvents from the biomass while controlling organic emissions.These can be commercialised on a large scale for biofuels production.

The rice straw is pretreated with alkali (6%NaOH)than CaOH&KOH at 25°C for 24 hours.and found that the above concentration (equivalent to Gm/Gm dry rice straw)responded to a steady result achieving 85% increase of sugar yield through enzymatic hydrolysis process and also efficiently increase in cellulose accessibility followed by NH_4OH soaked in $Ca(OH)_2$.

1;3;4 OZONALYSIS PRETREATMENTS:-

O_3 is a powerful oxidant,highly reactive towards incorporating conjugated double bonds and elements having higher electron density of functional group.This is particularly true with lignin content having C=C bonds susceptible to oxidation.O_3 is used to degrade the lignin & hemicellulosic molecules of LCB material like wheat straw,pineapple,peanut,cotton straw,bagasse,poplar straw dust etc..The mechanism of Ozonalysis,is to clear the carbon-carbon bonds occuring at higher temperature or in catalytic beds leads to less pollution into the environment.Employing Ozonization

with diluted H2SO4 treated sugarcane bagasse showed the result in increase of delignification and various monomers sugar production.To study the bagasse in a fixed bed reactor it has shown 46%glucose yield at 80%(w/w) moisture content than that of 6% more monomers than at 40%(wt/wt)moisture content.The later favours the inhibitory compound formations as a result of low water content***.(Tab-2)***

1;3;5 IONIC LIQUIDS(ILS);-

Ionic liquids are organic salts composed of organic cations and anions based either from organic or inorganic sources.Four groups of ionic liquids based cations are generally used such as Quarternary ammonium,N-Alkylpyridinium,N-alkyl-iso-Quinolinium,and 1-alkyl-3methylimidazolium compounds etc..This has possessing unique properties like low vapour pressure and high thermal & chemical stability besides characterising as powerful solvent for cellulose.

The pretreatment methods should be focussed on the basis of selecting appropriate cations&anions ionic liquids (ILS) having the properties such as hydrophobicity,solvent power,polarity etc.. suggestable as environmentally friendly and as green solvents.that helps in tuning and that could be adjusted to achieve desired results .In addition,it is possible to conduct the process based on the cost effectiveness,other physical properties,toxicity,corrosivity;biodegradability,water tolerance limit etc.influencing overall efficiency of ILS methods**.(Refer Tab-2)**

In order to treat LCB among the usage of ILS,the [EMIM][AC] and [BMIM]C are mostly used as effective solvents(Refer2012) otherwise certain number of ILS can cause cellulose dissolution & other structural alignment& modification during direct hydrolytic process.Inproper choosing ionic liquids ,results in ILS accumulation in residual biomass itself which could interfere with hydrolytic process & subsequent downstream fermentation stages.Then the remedy is to recover it from antisolvents after regeneration process through flash distillation that can be reusable.

3;0 EFFECTS OF INHIBITORS ON ETHANOL PRODUCTION;-

Inhibitory compounds on fermentation with S.Cerevisiae;-

The effect of inhibitors mainly depends on type of microorganisms,medium concentration used,type of fermentation and number of inhibitors etc***.;(Refer page...)***

The advantages of Bio-Processing method is to launch the pretreatments prior to enzymatic hydrolysis for final production of bioethanol yield rate in terms of quantity and quality.

DETOXIFICATION STRATEGY METHODS ;

(FOR INHIBITORS FORMATION)

This is an important aspect of processing the biomass that depends on method practiced to reduce the inhibitor concentration and convert them into non-toxic compounds.The concentration of inhibitors depends on the type of raw materials and operational condition of hydrolysis.During the pretreatments and hydrolytic processing methods ,compounds formed alongwith the sugars reported to be many of the toxic compounds such as acetic acid,Furfural,HMF,and phenolic compounds etc. which are derivatives of cellulose ,hemicellulose and Lignin. affecting the fermentation process.

< To overcome these effects,the encapsulation of yeast can be proposed to stabilise the productivity of alcohol and provides higher biomass in continuous fermentation.

<In addition to that,Detoxification can be improved through cell concentration

increase(Immobilisation)or by genetic modfications of cells ,changing fermentation factors such as pH in order to reduce the effect of carboxylic acid inhibition while in batch cultivation mode.where population grown in static set & fixed condition (temperature,pressure and aerations).

<There are different ways of minimising the inhibitors in hemicellulosic hydrolysates such as developing microorganisms in presence of inhibitors effects that can withstand and converting toxic compounds into products prior to fermentation but these effect will not interfere with the metabolism.

Inhibitor formation as a function of Severity Pretreatments ;-

The inhibitor formation and hydrolysis of cellulose are proportional functionality of pretreatments methods severity.which is often influenced by reaction ,temperature,retention time &acid concentration.etc..(refer page1987).Overend et al developed an equations involving reaction time & temperature through combined severity factor (CSF) relationship can be expressed by an equations as

$$CSF = t \exp[T-Tref/14.75]$$

Where t= residence time

Tref = reference temperature sets at 100°C(usually)

T = Temperature in °C

1**)FURAN ALDEHYDES;-**

1)One among the inhibitors are Furfural and HMF formed through hexoses and Pentose sugars that will affect the growth. in high concentration.Furfural can affect enzymes ADH,PDH and ALDH (ADH-Alcohol Dehydrogenase,PDH-Pyruvate dehydrogenase ,ALDH-Aldehyde Dehydrogenase etc.)damages the cell membrane.Furfural is toxic in batch cultivations and its presence converts them into furfural alcohol through the formation of acetic acid leads to high amount of acetate and finally exhibits lower ethanol yield.

ADH helps in conversion and lower concentration of furfural has a positive effect on growth of the cell whereas its high concentration leads to stop the growth of fermentation.

In Batch cultivations,Furfural is toxic in nature and its presence converts them into furfural alcohol through the formation of acetic acid leads to high amount of acetate and finally exhibits lower ethanol yield and it is important to note that presence of furfural exceeding 1Gm/Liter will decrease CO2 evolution and viability.During the Fermentation of sugars,the higher concentration of furfural (4 Gm/liter)will inhibit the growth by 80% and ethanol production by 97% oberved with S.Cerevisiae.

2) CARBOXYLIC ACIDS;-

After the furfural,the next inhibiting factor for the biomass formation and ethanol production is the carboxylic acids.Weak acids like acetic acid will cause ATP depletion,toxic anion accumulation and the inhibition of aromatic amino acids **uptake.Acetic acid is normally derived from acetyl group of hemicellulose.Under low** pH conditions,acetic acid will be in its dissociated form and can diffuse through the plasma membrane.The increase of pH 7.4 can lead to dissociation in cytoplasm(release of protons enhances the decrease in internal pH exerting cellular activity inhibition).

3)FORMIC ACIDS;-

It is the inhibitorier than Levulinic acid due to its low molecular size.Then the acetic acid inhibition level depends upon medium conditions such as pH & Concentration of Oxygen. etc..Finally,ethanol

production can be accelerated by presence of 10Gm/liter acetic acid in medium free of other inhibitory compounds.

A biorefinery point of view,the above inhibitors are the compounds transformable while developing microorganismes prior to fermentation and converts toxic components into products which will not interefer with metabolisms**.(FIGURE ...°)**

4;0 HYDROLYSIS PROCESS;-

The pretreatments methods have been reported through research make the substrates more conducive for hydrolysis process and considered to be crucial step before saccharification.This can be classified into two categories*;*

-1)Acid Hydrolysis

2) Enzymatic-Hydrolysis;-

4;1 ACIDIC HYDROLYSIS:-

Hydrolysis can be performed either by dilute acid or concentrated often catalysed by H2SO4 or HCl.Dilute acid hydrolysis is the most commonly used method in industries with dilute H2SO4 as catalyst hydrolysable at 120°C-220°C to produce large oligomers having less glucose units ,as the aim is to remove hemicellulose selectively.The lower acid hydrolysis can be carried out at higher temperature and generate large number of inhibitors than concentrated acid hydrolysis in which later process is controllable at lower temperature resulting 90% sugar recovery at a short period of time..However,

the optimum reactional conditions are essential by several interrtelated parameters such as time,acid concentration,type of biomass etc.. for the better yield of bioethanol productivity.

In other words,it is the two stage process where dilute acid for hemicellulose followed by concentrated acid used for hydrolysis of cellulose molecules.

The influence of inhibitors exerting the hydrolytic behaviour of biomass substrate are clearly discussed herein**.(Refer Tab-2)**

The disadvantages of the method is the high cost of production due to difficulty in acid recovery,disposal,concentratiion control & recycling other than degradation of sugar monomers due to acidic environments.

One among the,research studies carried out on employing high pressure two stage acid hydrolysis(1% H2SO4 in first stage followed by 0.5%H2SO4) to obtain high conversion of 189 gm Xylose /Kg and 219 Grams Glucose /Kg and formation of Furfural &HMF observed while using with rice straw.

In another conditions,the sugar beet pulp hydrolysis is identified through hydrolysis with 1.1 gm.H2SO4 per gram sugar beet pulp controllable at 80°C for 90 minutes showing the results as 86.3% and 7.8% of cellulose & Hemicellulose hydrolysis respectively

4;2 ENZYMATIC HYDROLYSIS PROCESS;

Enzyme catalysed hydrolysis uses enzymes to hydrolyse complex polysaccharides into sugar monomers under mild operating conditions of temperature 45-50°C and pH 4.8-5.0. This method is efficient that influence on higher sugar recovery without inhibitors formation.This is mostly influenced by factors such as pH,enzyme loading,time,temperature and substrate concentration.

Hydrolysis is carried out by three different cellulases enzymes such as 1,4-beta

endoglucanases,exoglucanases,Beta-glucosidases,Cellobiohydrolases where polysaccharides are broken down into shorter sugar chains(endoglucanases)followed by cellobiose moities(by exoglucanases)subsequently degrade cellobioses and oligosaccharides to glucose(beta-glucosidases).
The hydrolysis of hemicellulose is susceptible for degradation easier than cellulose owing to the nature of amorphous properties. The hemicellulose contains 10-15% and 10-35% of xylan in soft & hard woods respectively which has main & outer chains and this can be degraded the main chain using endo 1,4 beta Xylanase(EC3.2.1.8) into a short chain xylan oligosaccharide then further degraded to a pyranose form of xylan as Xyropyranose by Beta Xylosidase(EC 3.2.1.37).On the contrary,the outer chain of Xylan can be degraded by enzymes namely accessory Xylanolytic enzymes like Feruloyl esterase (EC.3.1.1.73) alpha L-arabinofuranosidase (EC.3.2.1.55) Alpha-Glucuronidase .
Cellulases &Xylanases are the important bioactives catalysts employable to hydrolyse cellulose & Hemicelluloses compounds where the correct proportions of enzymes cocktails are needed to produce cost effective Ethanol.
Various factors affect the biological hydrolysis process namely substrate concentration,cellulase activity,reaction time(temperature/pH/other parameters)and influence on strong inhibitory compounds presence .The rate of hydrolysis also dependant on several structural parameters of substrate include molecular structure,crystallinity,surface area fiber materials,degree extent of fiber swelling,degree of polymerization and association of lignin with other compounds in LCB.
The cost of enzymes also impacts on the overall cost of the production and above potential enzymes used in contemporary times secreting by microorganismes such *asClostridium,Cellulomonas,Erwinia,Thermonospora,Bacteriodes,Bacillus,Ruminoccus,Acetovibrio,Streptomyces and other fungi likeTrichoderma,Penicillium,Fusarium,Phanerochaete,Humicola & Schizophillium* Sp..Among the species,TrichodermaSpecies are most commonly used for hydrolysis than other microbial enzymes which is lacking on stability,catalytic efficiency,substrate & product inhibition.In such cases,recombinantDNA engineering approach are applied to improve upon strategy of enzymes usage and make them more robust & economic feasible.
The efficiency of cellulose hydrolysis can also be improved by the addition of Polyethylene Glycol(PEG)or Tween 20 resulting to increased saccharification & reduction in the adsorption of cellulose on lignin.
Various number of research studies have been focussed on optimization of enzyme process reaction condition in regards to usage of substrate and presence of inhibitors. etc ..are.susceptible to enhance the final recovery of ethanol production.The improvement of hydrolytic characterstics phenomenon are indicated as follows:

1)Presence of glycerol&Sorbitol in higher quantities stimulates the negative effects on above process containing commercial enzymatic cocktails mix.

2)There is a influence on bioethanol production using pulp& banana peels waste as substrate pretreated with dilute acid followed by enzyme process.

3) Combined pretreatments methods of NH3 &CO2 on Miscanthus Sinensis Grass followed by enzymatic hydrolysis with 20 FPU per gram cellulose at 50°C for 72 Hours possible to attain saccharification efficiency 93.6% with 31.2Gm/L glucose.

4)Triticale straw as substrate undergoes steam explosion pretreatment at 200°C for 10 minutes showing highest cellulose saccharification(92%).

The most comparative results of enzyme process yielding with reducing sugars &total reducing sugars are shown in **Table-3** the hydrolysis yield can be calculated using the equation as follows;-

Product of Glucose(Gm/L)
Hydrolysis yield(%) = ----------* 100
1.111 Glucan in sample(Gm/L)
where the conversion factor is 1.111 applied to hydrolysis of Glucan to Glucose.

COMBINATIONS OF NOVEL ENZYMES SYSTEM FOR IMPROVED COST EFFECTIVE BIOFUEL PRODUCTION ;-

The hydrolysis is the most critical step as complete saccharification and liquefaction of plant polymers essential for economic production of bioethanol .To maximize the hydrolytic efficiency, we need to supplement deficient enzyme cocktails with accessory enzymes like beta glycosidase,xylanase,beta xylosidase and esterases etc..

Hydrolytic processing of forest residues etc,,requires pretreatments with dilute acids to make cellulose accessible to cellulase and other enzymes enabling to proceed for conversion into glucose and other 5 or 6 C sugars in contrary to expensiveness in competing with corn based ethanol.Hence NREL & partners (USA) developed employing cocktails of cellulase enzymes based on fungi and bacterial sources(endoglucanases,Exoglucanases and beta -glucosidases.)working in very different ways efficiently at releasing plant sugars ..

ENZYMES ADVANTAGES & POTENTIAL IMPACTS ;-

Cellulase Caldicellulosiruptor Bescii Cel A, a high active, stable hydrolytic enzymes with multiple functions domain over other fungal and bacterial-cellulases in biofuels conversion owing to the properties such as specific activity,stability at elevated temperature and novel digestion mechanisms.Hence,NREL isolated a high active cellulases -CelA with novel digestion mechanism through X-ray study on primary protein components of CelA comparing with binary mixture containing Trichoderma Reesei Cel7A exoglucanase and A.Cellulolyticus Cel5 A endoglucanases on several substrates through TEM(transmission Electron Microscopy).This shows that celA retained high activity at all temp. tested converting 60% glucan at 85°C compared to28% glucan conversion for endo/exo cellulase mixture (Cel7A/CelA) at the optimal temp.of 50°C.This can be explained as difference in activity to 7 fold increase at the molecular level for CelA.

1;;3 ROLE OF* LACCASES ENZYMES *IN BIOFUELS DELIGNIFYING STRATEGY;-

Laccases are the one of enzymes being investigated not only for potential use as pretreatments agents in biofuel strategy mainly as Delignifying enzyme but also act as a biotechnological tool for removal of inhibitors (mainly phenolic) of subsequent enzymatic process for optimum results and adoption of biorefinery concept.This decides upon removing lignin compounds to release or exposing sugars to the hydrolytic enzymes which in part dependent on cost effective and benign pretreatment of biomass..

In addition to that,various oxidoreductases (lignin peroxidases,Managanese peroxidases,Laccases etc.;are involved on lignin decomposition but also generates various oxidative species that may attack inhibitors produced during pretreatments thereby process becomes viable and more effective.

IMPROVING THE SACCHARIFICATION;

1;: ***ROLE OF BETA- GLUCOSIDASE ENZYMES IN BIOFUELS;-***

The overall efficiency of biomass is determined by involvement of cellulase in correct proportions to get optimum ratio in commercial cocktail cellulases enzymes containing beta glycosidase that enhances the saccharification yields by tracking the trapped sugars from complex polymers to produce final glucose oligomers units for ultimate biofuels ..Inappropriate ratio of these enzymes (endo/exo/beta glycosidase) will lead to accumulation of cellobiose through inhibition activity of cellulases.

Pencillium Decumbens 114-2,filamentous fungi considered to be the best source of beta glycosidase works at optimum conditions such as;

Temp;65-70°C, pH; 4.5-5.5

and showing higher activity causes higher hydrolysis of biomass and found to be good blend and similar to that cellulases from T.reesei.

Novel bifunctional glycoside hydrolases enzyme having the properties both beta glycosidase and Xylosidases in Pencillium Piceum strain capable to act on Xylotriose to produce xylobiose and D-xylose and shows that xylooligomers are the most powerful inhibitors of saccharification process than cellobiose and glucose *resulting better results in cocktail activities.*

Other Applications:-

Beta glucosidases possess broad substrate specificity having a huge applications across various industries.and.degrade the intermediate Gluco-oligosaccharides that can cause both hydrolysis and reverse hydrolysis(transglycosylation)results not only influencing the properties suitable for biofuel conversion but also produces a glycon moiety(as antitumour agent) and lower viscosity Gelan..These enzymes has played a vital role on synthesis of surfactants (o-alkyl-glycosides)by reverse hydrolysis.

Activators& Other Conditions influencing the enzymes;

For effective cellobiose hydrolysis, Thermostable glucosidases isolated from the two strains -A.Cremonium Thermophilum(AtBG3) and Thermoasces Auranticus(TaBG3) are susceptible to hydrolysis a greater extent of cellobioses as compared to the commercial enzymes.This shows that beta glycosidases having the two factors such as strength of glucose tolerance and inhibition and affinity towards cellobiose clevage playing a role significatif in biomass yields.Changing carbon source substrates like Lactose can influence on glucose tolerance ability of enzymes.Clavispores and other strains Candida Sp. are resistant to above inhibitors.

Addition of $MnCl_2$ ions may influence on increase in activity by Phaffa Rhodozyme cultivateable in a culture media**.**

*Enzyme **IMMOBILISATION** for enhancing activity;-(research study)*

Immobilization is the technique for the enhancement of its activity and stability of enzyme facilitates efficient recovery and reuse the enzymes.Their properties differs or vary from physicochemical characterstics ,increase in thermo stability and different pH optima.

It is reported to be an increase in Km and decrease Vmax value and reusable for infinite times to make the process viable in biofuel conversion.The techniques can be carried out on various inorganic compound and organic polymers like chelated magnetic metal ion nanoparticles(NP)magnetic chitosan,Alginates,polyacrylamides gel(PAG) agarose and silica

.These techniques offers additional adavantages of higher surface area to volume ratio facilitates higher enzyme loading and biocatalytic efficiency. for industrial applications.

Immobilization of beta glycosidase on magnetic Fe3O4 NP combined with Agarose showed enhanced activity and prolonged usability more than 90%even after 15 sucessive cycles .10% saccharification efficiency increase is possible with above criteria than normal.

XYLANASES ENZYMES;-.

XYLANASES ACTIVITY TOWARDS BIOFUEL PRODUCTION &YIELD;-

Number of agricultural wastes like wheat bran used as substrate to enhance the productivity of Xylanases enzymes in SSF and SBF in industrial scale...Xylanases (Endo-1,4 ,beta -D-Xylan,Xylan Hydrolases,EC-3.2.18) catalyses the hydrolysis of xylan ,the major constituents of hemicellulose present in plants and microbes) in random clevage to produce Xylose,Xylobiose and Xylo-oligosaccharides..The well known applications are studied in details in various sectors such as paper bleaching,biofuelling,fruit juice clarifications, bread panification etc...

Xylanases are essential in biomass hydrolysis as they initiate through mediating progressive cell wall penetration by other celluolytic enzymes.Xylan biorefinery is not limited to fuel market whereas biohydrolysis of xylan such as pentoses oligomers and monomers are used as intermediate in the production of bio-chemicals.

5;0 FERMENTATION PROCESS:-

The process of digesting fermentable sugars into ethyl alcohol & other by-products under anaerobic conditions are referred as Fermentation as the conversion of biomass carried out through microorganisms by yeasts,fungi and bacteria etc...

The most commonly used yeast species Saccharomyces Cerevisiae converts the hydrolysates of cellulose & hemicellulose like glucose,mannose or fructose compounds transformed into ethyl alcohol and CO2 at a temperature20-35°C with pH 4.0-5.0 having initial sugar concentration of 8% for a period of 96 hours and the agitational speed of 150-200 Rpm. showing the yield of 150gm/L EtOH **Refer** (**TABLE 2**) **.**

C6H12O6 + Yeast >>>> 2C2H5OH + 2CO2

The corn wet milling employs continuous fermentation and dry milling shows batch process whereas the advantages of batch operation are the higher product ethanol titer value and reduces risk of contamination.This shows cell cycling is not feasible resulting in additional cost involvement in fresh yeast seed culture in every batch.

Yeast Cell Propagations

In continuous fermentation ,bacterial contamination arises that can be performed in a successive five stage operations.CSTR (continuous stirreed tank reactor)becomes larger operates in combined approached form with Plug Flow reactor (PFR) then The first stage often uses increase in yeast cell densities showing performance such as higher ethanol volumetric productivity at high ethanol titers.

This can be produced on off-site and if produces on on-site, it increases capital , equipments ,reactor volume costs and time etc..Therefore cell recovery offers a promising solution including fermentation at high cell densities with significantly ethanol volumetric productivity .This avoids or decreases the need of introducing fresh yesat seed culture in every batch.

High cell viabilities must be maintained through the end of fermentation employing cell recycling in the process so that non-viable cells does not accumulate and able to achieve the ethanol titer

value of 18%v/v and even higher achievable for the range of process configurations and feedstocks. but cell viabilities shown dropping precipitously at titer value 12% v/v ethanolwhich needs necessitating operation of ethanol concentration***.***

<u>Cells recovery;-</u>

This includes 1- Centrifugation,2-Floccullation-sedimentation,
3-Immobilization 4-membrane separation
The first 2 strategies are widespread adoptation in industries.Performing fermentation at high cell densities and recovering by centrifugation namely Melle-Boinot process are realisable where 95% yeast cells harvested.that makes up the difference in fermetation so that no additional yeast not required.

The recycled cells are diluted by 50% with water ,acidified to pH 1.8-2.5 to inactivate bacterial contamination..

The fed -Batch version process,30% of reactor volume is initially loaded with concentrated yeast cream to which high concentrated hydrolysate (cellulotic derivatives of sugar solution)added periodically that can be optimised for ethanol productivity over the course of fermentation.This results in influencing the short fermentation times of 6-10 Hours and corresponding high ethanol productivities achievable at high cell densities (though maximum titer value is limited to12 %v/v due to substantial decrease in cell viability)

<u>Contamination by other strains over fermentation;-</u>

Sometimes,long term cell recycling leads into risk of contamination by indigeneous wild type yeast strains typically outcompete and replace starter strains in processes.The robust yeast strains can be replaced through isolation and now employable as the seed culture.

The bacterial contamination is the substantial problem tends to adopt mostly at mesophilic temperatures that might be controlled through the use of antibiotics and allow to make unfavourable for its growth on low pH.If not controlled,bacteria compete with the yeast by consuming the available C source and tends to produce compounds such as acetic ,lactic acid that affects directly ethanol yield & yeast growth.Bacteria often have shorter doubling times than yeast and tend to dominate the process especially in extended fermentation &continous processes.

FERMENTATION TECHNOLOGIES ;-

The technologies used for fermentation of monomeric units of sugar into ethanol by the following fermentation processes that are commonly exploitable as follows ;

A) Separate hydrolysis &Fermentation(SHF)
B)Simultaneous saccharifications and Fermentations(SSF)
C)Simultaneous saccharification and Co-Fermentation(SSCF)

This can be carried out either by batch-or continuous -cultivation or fed-batch in presence of inhibitors which affect the fermentation influencable by detoxification strategy**.**

DETOXIFICATION STRATEGY;-

BATCH CULTIVATIONS;-

This is the simplest process as it is flexible for a range of products,easy to control & has multi vessel.This involves adding the substrates,microorganismes,culture medium and nutrients at the

beginning of operations in a closed system at a predetermined time under favourable conditions and possible to withdraw the products only at the end of process.
The disadvantages are the low yield,long fermentation time,and high labour cost making batch process unattractive for commercial production.The presence of high sugar concentration exerts the inhibition of cell growth & ethanol productivities***.***
Microorganisms grow in static or set cultivation medium during the fixed condition such as temperature,pressure and Aeration.In batch cultivation,detoxification may be improved by increasing cell concentration.For Example, Cell Immobilization or genetic modification of the cells to increase the inhibitor tolerance and changing the fermentation parameters such as pH to reduce the inhibitory effects of Carboxylic acids.
During batch cultivations & fermentation,the yeast may be inoculated at 1* 107g/L viable cells/ml equivalent to (0.1-0.5g/L dry cellweight(DCW) ,using preconditioned active dry yeast that doubles more than 5 folding during exponential phase growth. The air sparging will help in maintaining redox potential & high cell viability and N is added as Urea to generate the growth.

CONTINUOUS CULTIVATIONS;-
This process involves adding substrates,culture medium, and nutrients into a fermentor containing active microorganismes and possible to withdraw the products such as ethanol,cells, residual sugar etc..continuously makes it more beneficial.The advantages of the process are high productivity,smaller volume and low investments,operational cost etc..The disadvantages include product contamination,potential decline in yeast capability to support ethanol concentration due to long cultivation&low in detoxification of inhibitory compounds..In continuous cultivations of LCB hydrolysates,the encapsulation of yeast responds to detoxification strategy & provides higher biomass in comparison to freely suspended cells in bioreactor**.(Refer-Table-5.0**)

Fed Batch fermentation Process;-
This is the combination of Batch & continuous fermentation process involves charging the substrate into the fermentors without removing the medium.Comparing other two processes,this shows higher productivities,more dissolved O2 ,shorter fermentation time and lower toxic effect of the medium .The inconveniency is the productivity is limited by cell concentration & fedd rate.
Sacchromyces Cerevisiae is the most commonly advanced and recommendable species other than pentose and hexoses fermenting yeast strains used in bioethanol production under different conditions of fermentation but it is proportionally related to the strains such as bacterias like Zymomonas &Escherchia Coli and fungal Aspergillus fiber species.
The most recent studies on ethanol production from different LCWB materials using enzymatic hydrolysis and fermentation methods by S.Cerevisiae are reported in **(Table.3).** Ethanol yield can be calculated as a percentage of theortical yield using equation -3;

$$\text{Ethanol yield}(\%) = \frac{\text{Ethanol produced (gram/L)}}{1.11(\text{initial Wt.of mass in fermn. medium(Gm/L)} * 0.51 * \text{Glucan in sample(Gm/L)})} \quad \ldots(3)$$

The other processes of fermentation are compartively discussed.

5;1 SIMULTANEOUS SACCHARIFICATION & FERMENTATION(SSF)

The microorganisms ,S.Cerevisiae is able to conduct the simultaneous saccharification and fermentation processes at the same time breaks down the cellulose molecules into simpler sugars to produce Ethanol**.(Refer Fig -1;5**)

In SSF,the fungal cellulases are mostly active at 50°C to 55°C while the microbes ferment effectively at 35°C .This fermentation process has been preferred step for the production of chemicals and fuels ,as the operation of hydrolysis process and fermentation are conducted in the same reactor therby reduction of cost is possible and the chance of contamination is minimal due to the presence of ethanol and reduce proportionately the feedback inhibition of cellulase enzymes activites.

The disadvantages of SSF is the variation of optimum temperature required for both enzymes & microorganismes that might reduce hydrolytic ability and fermentation efficiency. trespectively.

The important study was realised using mixture of S.Cerevisiae and Pichia Sitipitis after 79 hours fermentation at 30°C yielding ethanol of 74% of theortical value using SSF process. Mandarine peel waste is used for producing bioethanol obtaining 6.8Gm ethanol per 100Gm biomass(6.8%) using SSF method in presence of S.Cerevisiae(ECT 1329) ,used as a strain.The advantages and disadvantages of SSF process are reported in**(Tab-3)**

5;2:SIMULTANEOUS SACCHARIFICATION &CO-FERMENTATION(SSCF);-

(Refer Fig-1;6)SSCF presents a process model of illustration of SSCF. SSCF fermentation involves application of employing the mixed microbes to ferment hexoses and pentoses (Xylose, Arabinose, Galactoses, Glucose, fructose etc...Normally, SSCF and separate hydrolysis Co-fermentation(SHCF)has been suggested for the conversion of both pentoses and hexoses to produce higher rate of bioethanol production.The basic phenomenon is the use of mixed microbes tends to grow by respective ability in the hexose fermenting medium than pentoses microbes results in higher rate of ethanol obtainable from hexoses.

This involves hydrolysis & saccharification processes proceeding in the single unit with the co-fermentation of pentose sugars like Xylose which cannot be assimilated by normal yeast species as it suffers from glucose and ethanol inhibition whereas in SSCF,recombinant engineered yeast come into reality and there is reduction in glucose inhibition during hydrolysis and increases xylose to glucose concentration ratio as most of the organismes consume xylose.

Like SSF,SSCF has then advantages of lower cost,higher ethanol yield and shorter processing time.

Figure 8.
Saccharification coupled with co-fermentation (SCCF) [51].

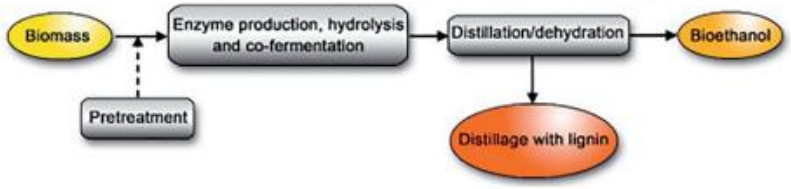


Figure 9.
Consolidated bioprocessing [51].

(Refer Tab-2).SSCF gives a comparative statements on bio-ethanol derived 4 lignocellulosic feedstocks with SSCF Process.The mass flow of cellulose in each stock is 35556Kg/h. This explains herbaceous feedstocks showing lower ethanol yield due to high moisture content not suitable for pretreatment reactor but additional water is required during washing stages.Whereas sugarcane bagasse shows pêrspective strategy for tropical sugar producing countries.The simulation shows the use of paper waste (newspaper,waste paper of chemical pulp etc.) can be the potential feedstock for bioethanol taking into consideration for its higher cellulose content but it is necessary to evaluate the usage of raw material such as municipal solid waste etc..

5;3 SEPARATE HYDROLYSIS FERMENTATION(SHF);

The main characterstics of the process is the approach of hydrolytic enzyme process separately with LCB materials as the operating conditions(pH,temperature etc..) are well optimised.However,glucose & cellulobiose accumulation will have the inhibiting effect on cellulase enzymes.SHF and SSF are complementary to one another as referred **in (TABLE-2.)...**

. This combined process can be used for economic assessment and process optimization of the production process of ethanol.

Fig-1;7presents a model illustrating the process of SHF**.**

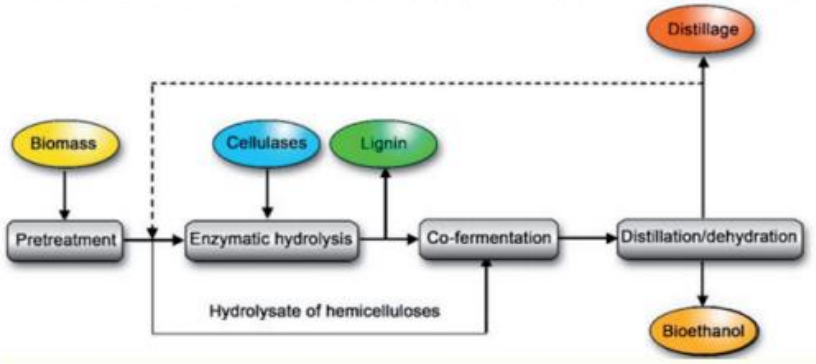


Figure 7.
Separate hydrolysis and co-fermentation (SHCF) [51].

SHF process are used to obtain a maximum of 29.4 gm of ethanol from 100 gm of mandarine peel waste as biocatalyst using popping pretreatment and enzymatic hydrolysis supported by S.Cerevisiae KCTC 7906 strain.

Table-3 shows bioethanol production is from agricultural residues by SHF and SSF methods using S.Cerevisiae as biocatalalyst for fermentation.The Table-3 also shows different biomass substrates like unripe banana peels,Matooke peels,energy grass, sugarcane bagasse,A.Salmiana,G.verru cosa,Empty palm fruit bunch fibers,rice straw,corn stover,switch grass ,pinewood ,agave tequilana bagasse,corn stalks,orange peels,sunflower stalk etc..are commonly usable for high yield ethanol production and these are referred to different LCB materials processable in different locations of the regions worldwide.The classification of bioethanol fuel from LCB are currently being developed to meet sustainabilty and fuel quality standards as possibly requirement for road,Aviation & electricity.

INTEGRATED PROCESSES;-(IP)

This involves combining one or more processes for the purpose of optimization in order to obtain increase of yield & minimum production cost .The example of IP is the membrane reactor where both the hydrolytic reaction & separation of fermentation products occur simultaneously upon integrating SHF &SSF processes .This shows that offering opportunities for the temperature of the cellulases (45-55°C)& S.Cerevisiae (<32°C)are to be controlled separately for the efficient operation of the process.This allows above organismes adoptable for fermenting both hexoses & pentoses in a single step process giving rise to a method known as Separate hydrolysis & Co-Fermentation (SHCF).

The disadvantages of the enzyme process is the inhibition of cellulases caused by high concentration of glucose produced and the challenges can be solved by increasing concentration of enzyme or by using SSF.Thus SSF allows the glucose transforming directly into ethanol in the same reactor.This approaches has been further developed to a technology namely as CBP.

5;4CONSOLIDATED BIO-PROCESSING(CBP);-

This processing involves the enzyme production,hydrolysis and fermentation to be carried out in single unit mostly by microorganismes such as Clostridium Thermocellulm as it has the capacity of synthesizing cellulases to produce ethanol after degradation of lignocellulolytic materials.

This process has advantages such as less energy intensive,cheaper enzyme costs,low cost investments ,less possibility of contamination etc..

Figure 8.
Saccharification coupled with co-fermentation (SCCF) [51].

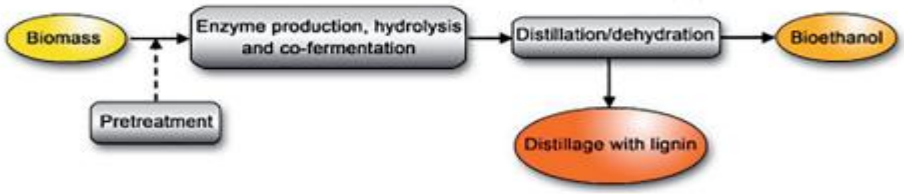


Figure 9.
Consolidated bioprocessing [51].

MICROALGAL SPECIES RELATED TO BIOETHANOL YIELDS;-

Refer Table-5.1 show microalgal species producing bioethanol due to the presence of higher level carbohydrates as 3 rd generation biofuels and shows the advantages as it lacks from lignin contents which does not require any pretreatments***.***

6;0 BIOETHANOL SEPARATION& PURIFICATION:-

After the fermentation,the products obtainable such as metabolites and ethanol needs to be separated followed by purification by distillation.Though Ethanol is purified through distillation through effective liquid-liquid separation.which involves certain disadvantages like cost and limitation over separating volatile organic molecules.Hence,other improved techniques are appeared industrially in alternative to distillation.

After fermentation,byproducts from yeast metabolism needs to be separated essentially cellulose & hemicelluloses derivatives(esters,organic acids and higher alcohol)and lignin derived cyclic and hetero-cyclic compounds.
The typical Implementation of bioethanol process from Lignocellulosic feedstocks;-(USA origin) is schematically represented**.**
After the pretreatments,The non-converted biomass removed by a filter module(FIL-1),then cooled by coller equipments(EX-2)till reaches 34°C thus permits enter into the fermentation reactor(FERMENTA)where stream of Urea is added having the mass ratio of feed (442.5:1).
The output stream from the fermentation reactor is inserted to FILT-2 where the solid comportements are removed.Next,the liquid stream is fed into a battery of three distillation coulmn to separate the ethanol.(**Refer Fig- 1.8)**.
To stimulate each column,the module -RadFac is necessary.The 1st column (STRIPP-C)designed with 33 stages without condenser and the feedstream is located in stage1 and the side stream rich in ethanol has a flow of 38000 Kg/H and extracted in stage 4 with a operating pressure 1 atm. having column pressure drop of 0.68atm..The recovery of ethanol is found to be 99wt%.Onthe other hand,,ETOH-rich stream is fed at stage 22 of second column called as **RECTIFIC-C.**
Bioethanol dehydration shows receiving the diluted stream in the range of 5-12%wt ethanol after fermentation needs to be concentrated from binary azetrophe EtOH-H2O (95.63%wt composition) to over 99-99.8%Wt ethanol through following steps. The operation is constrained by the azeotrophic nature of EtOH-water solution that can be carried out based on the principles of distillation(leveraging the difference in boiling point of the components of the solution)and problem is overcome by using a separating agent that alters relative volatility of key components.
This column has 30 stages and a partial vapor-liquid condenser with a reflux ratio3.3 and the column pressure is 1 atm. reaching 95wt% of recovery .In the 3 rd column called RECOVERY ,a glycerol stream is added to overcome the Azeotrophic point being designed with 15 stages,reflux ratio of 0.3 and total condenser allows 99wt%ethanol recovery..The rich ethanol stream from the second column inserted at stage 12 while the glycerol column at stage 2.The ETOH:Glycerol mass ratio is 1:1 having a operating pressure(1 atm.) of column .
Distillation is an important industrial efficient purification techniques applicable to separate ethanol from water where high columm rejects the water at the bottom and low boiling volatile components are concentrated with ethanol in vapour phase. It involves repetitative condensation and vapourisation
.The simplified flow sheet (**FIG-1.9)**explains fuel ethanol produced from LCB by SSCF Process involves Azetropic Distillation using benzene as entrainer.

OTHER METHODS OF PURIFICATION;-
Other techniques used in recovery of ethanol include Diffusion distillation,extractive distillation,Vaccuum distillation,Membrane distillation,Chemical dehydration. etc.
The most conventional techniques include liquid-liquid extraction,Azeotrophic distillation &Extractive distillation.The former one is the predominantly used for large scale operations.
2) **Adsorption** is another separation technique utilises large surface area of adsorbent such as Activated alumina,Activated carbon etc.where compounds are adsorbed due to physico-chemical characterstics nature related similar polarity and tends to be adsorbed more.Ethanol is polar

compound containing other particles as impurities influences non-polar surface and pore distribution more favourable for separation.

3)**Ozonation** is the process of oxidation permits to decompose the compounds using its strong oxidation potential O3 can remove impurities whereas it not allows the oxidation under atmospheric condition .

4) **GAS STRIPPING** is the separation technique utilising volatilities differences among compounds and the efficiency is based on Henry'S law. H = P vap / C sat.

Where H= Henry constant

P vap= partial pressure of pure compound(atm)

C sat = saturation conc.of pure compound in liquid phase (mol or mg/L).

Henry's law varies based on vapor and liquid phase variation.Hence ,the compounds having low boiling points like methanol,acetaldehyde can be stripped more easily considered as impurities in ethanol.

LIFE CYCLE ASSESSMENTS (LCA);-

The assessment is made to measure the environmental impacts of bioethanol production using different feedstocks.LCA tools helps in identifying the potential impacts during a process design and for decision making in order to improve the process prior to scaling-Up.

LCA methodlogy consists of four main stages includes goal ,scope,life cycle inventory analysis (LCIA),Impact Assessments, and interpretation of results. whereas LCIA conducted by using methodologies like CML2002,ecoindicator99,ReCiPe,LIME,LUCAS,TRACL that all depends on impact categories ,selection of indicators etc....The (**TABLE- 5.2)** indicates that bioethanol has the capacity to reduce GHGE and global warming potential substantially hence facilitates the protection of ozone layer.

COMPUTER -PROCESS SIMULATIONS;-

As described in Simulation flow chart, (**Refer Fig 1;8)**this pictorial representation describes chemical,physical &biological & other technical processes and unit operations in a simulation software. which helps in design of environmentally -friendly and safer processes,reduction of capital & operating cost,to provide functionality & flexibility needed for modelling efficient biofuels processes through optimal process design,regulatory compliance& operational analysis of biofuels process.After evaluation of three processes ,the simulation results shows highest yield 23.6% for SSCF and lowest yield of 18.5% for SHF. The results concluded that enzymatic technologies could be used *for microalgal production of bioethanol.*

6;1 ANALYTICAL METHODOLOGY OF BIOETHANOL

The following methodologies can be practiced for determining purity of bioethanol content.The other metabolites (cellolbiose,glucose,acetic & lactic acid etc..)produced during the course of enzyme or microbial processing of ethanol production can be estimated by one among the methods including HPLC as cited as below;

QUANTITATIVE ETHANOL DETERMINATION;-

1- Direct injected GC method;-

A sample solution (0.5 ml) dispensed into 1 ml caped sample then 5ml of 1% internal

standard solution (eq.to 50mg) was added.After mixing,0.1 µL of sample solution injected directly into a GC with syringe.Ethanol content is calculated according to following equation;

Ethanol(mg/ml) = As/Ais *(Wis/RRF)*1/V

where V=sample volume(ml)

2-DICHROMATE OXIDATION METHOD;-

A sample solution (1-5ml) was steam distilled to obtain alcoholic eluate(>50ml) & then oxidised with acidified dichromate.The excessive potassium dichromate was the titrated with ferric oxide.the ethanol content in the sample should be calculated by noting volume difference of potassium dichromate consumption between sample & control solution.

3-Distillation Hydrometric method;-

Alcoholic volatile compounds in samples were separated by distillation and the gravity of the distillate was measured by hydrometer.and the ethanol content is then converted.

GLUCOSE ANALYSIS;-

Primarily,YSI-27 to be standardized as per the instruction in the manual and membrane is to be checked.The biomass hydrolysate is weighed and diluted 3-4 times of weight and allowed to mix for 2 minutes and filtered.Then the first filtrate is to be injected into the previous standardised instrument.The results are recorded by setting the clear button.Then the second filtrate is injected to confirm the standards as described earlier.The next five or six specimens is to launched gives the dilution factor(divided by2)that is expressed as milligrams/ml(usually 90-95/2= 92.5units of glucose).

Materials&Methods;-

YSI#27 analyser manual,Blank membrane,Glucose-Dextrose membrane,Blender and standards purchasable from company.

Scale,filter paper,funnel,distilled water etc...

The specimens range allow to be in middle.500 or 200mg/100ml can be diluted to appropriate amount.

((Parts of std./parts of std plus## parts of water equals to dilution factor))

(dilution factor times std value equals to new volume value)

The equation below is used for the determination of glucose presence. in the sample;

{ V1A V10=V 2A V20]

ESTIMATION OF ETHANOL BY HPLC;-

It is the analytical techniques practiceable which utilises liquid as a mobile phase instead of gas of GC.Here ,the samples are not heated at the injection port.Thus non-volatile compounds or heat sensitive compounds can be analysed with HPLC.

EXPERIMENTAL DESIGN OF BREEZE HPLC SYSTEM;-

To improve the productivity of fermentation process,certain stress factors including relative concentration of inhibitors formation such as glucose,ethanol,acetic acid,lactic acid etc.affecting the activity of yeast which can be carefully regulatable the above process through monitoring the above elements.

To optimise fermentation,Water Breeze HPLC system can easily provide information within 20-30

minutes operatable even through plant operators.

MATERIALS;-

Dextrins,maltotriose,maltose,GMH,L+lactic acid,glycerol,acetic acid,96%H2SO4,C2H5OH were used .Dextrins is a mix of 92% polysaccharides,2.7% maltotriose,1.7% maltose,2.6%glucose and 0.9% unidentifiable carbohydtrates and these data can be used to calculate the standards concentraions.

Preparation of standard solution;-

The stock solution prepared by weighing each components into a 25 ml flask and then diluted to 5%,10%,30%,50% and 70% with deionised water and filtered using 0.45µm,25mm dia.syringe filter.

Mobile phase;-

Two step dilution of dil.H2SO4(0.5mM)to be prepared .Firstly,1.6 ltr of deionized water added into 5.5 ml of 96%H2SO4 in a 2 ltr flask and further diluted to make 50mM H2SO4 solution.then 10 ml of 50mM transfered to 1 liter flask and made into 0.5mM. H2SO4 as mobile phase.

HPLC Breeze system is equipped with an IC Pak Ion Exclusion Column used for analysis of 8 major components within 10 minutes.This requires lesser than 50%time performance than current HPLC analysis. of the recommended

A possibility to test the accelerated methodlogy of HPLC is the examination of small changes effect in the mobile phase concentration on peak retention times.Hence,experiments are run with mobile phases ranges from 0.452 mM.H2SO4 to 0.515 mM.H2SO4 of the recommended 0.50 mM .H2SO4 mobile phase concentration.Atleast 20 injections can be done to calculate the results for each mobile phase concentration.

The Figure1 shows the fast separation optimisable by changing combination of column dimension,column temperature and concentration of mobile phase and flow rate.This relates to the peaks identification through chromatograms of individual components under the same conditions.Then calibration curves are generated automatically in Breeze water from the series of chromatograms of standard mixtures at different concentrations.The relationship between peak area and the concentration was linear over entire concentration range examinable.

ESTIMATION BY OLFACTOMETRY;-

Olfactometry is a sensory analysis usually coupled with GC.Typically,GC column is connected with a separator where anaytes are separted in two ways,Olfactometry and a detector such as FID,PID and MS.

Olfactometry is a simple open end column system and a panellist records the odour character and intensity of analytes corresponds with a peak in chromatogram.Olfactometry provides flavour data rather than stoichiometry chemical data that helps in flavour development in alcoholic beverages etc..

Analysis of BIO-Ethanol using Hearty -Cut System;-

ASTM-D4806 is the quality standards for bioethanol prescribing GC quantitation for the concentration of ethanol and methanol(specified by ASTM D-5501)However ,the later uses a 100-150m column resulting a long analysis times.

<u>Heart-Cut System equipped with FID;-</u>

Having a column 150m this analysis takes over 40 minutes.This system separates EtOH,MeOH

from other hydrocarbon in 1st column.(RtX-1) and conducts more precise separation in 2nd column.(Rtx-Wax).The analysis time for 2nd column is significantly shorter than D(5501) method.quantifies accurate alcohol concentration in bioethanol.

ANALYSIS OF BIOETHANOL BY GAS CHROMATOGRAPHY;- (AGILENT TECHNOLOGY)

GC has the advantages over the resoultion of analysis whereas IR is convenient for routine quality assurance and classification of ethanol.

Gas Chromatography is an analytical techniques more reliable for volatile and semi-volatile compounds based on impurities presence and nature of the different origin of bioethanol.

The sample is vapourised at an injection port by heat ,then sent to column packed with vapourised absorbent or adsorbent.Each component is separated inside the column based on the physico -chemical characterstics properties of the sample and then the detector measures the concentration of compounds in end of the column.Based on Target compounds separation ,detectors and coating must be choosen for the column(since it has many coatings) analytes and MS identifies them and accelerates the bioethanol analysis.

METHOD-1;-

The GC method by Agilent Technologies developed for detecting presence of impurities in bioethanol.this application describes a method for analysis of N2,O2,CO2 ,ethanol from the head space of biofuel reactor.

The fermentation process can be monitored by analysing head space of fermentation vessel through Agilent Technologies series 7890 GC equipped with six port gas sample valve,a split/splitless inlet ,a four port switching valve,a Pora Plot Q column and a Mol Sieve Column. opertable with combination of hardware and allows to obtain separation components O2,CO2,N2 in a simpler way and ethanol on a single injection at above ambient temperatures.

There are three ways in which sample can be introduced with above configuration :

1)directly from the process through connection of gas sample valve 2)Syringe injection through gas sample valve :3)Syringe injection directly into GC inlet. This configuration allows for simultaneous separation of all components while eliminating possible contamination of MolSieve column.

METHOD-2:

GC method is the most appropriate and rapid method for determination of ethanol contents.

Materials&Methods;-

LC grade (>99% purity),ethanol,acetonitrile,1-propanol, acetone,Isopropanol;1-butanol,tertiary Butanol were used from ALPS(Taiwan)

Analysis of GC condition;

This study was carried out by GC 2000(thermoquest,Milan)equipped with computer integrator software(Chrom-card Version 1.06 for GC)and an FID detector.The flow rates of H2 and air were set at 30°C & 300 ml/min respectively.The temperature of FID detector and injection port was set at 285°C and 255°C respectively.N2 at a flow rate of 2ml/min was used as carrier gas .with the CP WAX 58CB separation column (30mm*0.5mm)

Chromopak,Netherland.Oven temperature was set initially at 45°C for 2 minutes and then increased

rapidly to final temperature of 245°C in 6 minutes at the rate of 45°C /min .Ethanol & acetonitrile were eluted to 80°C and 100°C respectively.The sample components will be eluted very rapidly in 7-8 minutes to complete the sample analysis.

The ethanol sample may be added with minute quantity of 1propanol ,acetonitrile,acetone,1-Butanol& t-Butanol etc..Then the ethanol content can be measured as above conditions.The results has shown that retention time of 6 std.solution were 4.43,4.37,4.32,4.06,5.96 & 5.72 minutes respectively.Meanwhile,the GC peaks of acetonitrile & ethanol were closer to each other other than other components.

The results was the retention time of ethanol std.solution said to be 2.73 minutes and the resolution of megapore capillary column(Rs=5.8) was better than the packed GLC method .Rs=1.4-1.9) as described in AOACmethods.

7;1 RESEARCH STUDY 1;-

HIGH YIELD REGENERATED CELLULOSE CONVERSION WITH CONCENTRATED H2SO4;-

a Comparative study with normal LCW-BIOMASS;- (research study)

****The study may be beneficial giving an identity to the combined pretreatments methods exclusively while using acidic process in order to determine high yield cellulose conversion and morphology of biomass structure. .

The appreciable Depolymerization (DP) was observed when H2SO4 concentration reached 65% causes swelling& dissolving the cellulose materials compared to 45-65% where,in a state high crystalline nanoparticle having **Cl** crystalline polymorph obtainable. The water dilution permits the acidic solution of cellulose makes regenerated and precipitation in the form of low molecules amorphised flocs having crystalline Polymorph of **Cll**-type. **(refer Fig 2.1 &2.2)** Increased temperature of acidic treatments promotes hydrolysis and dissolution of cellulose.

MATERIALS &METHODS:-

A homogeneous suspension of microcrystalline cellulose (2.05g,5%H2O by mass)prepared in presence of solution LiCl2(10gm) in conc.HCl(150ml) and total mass added to extraction.

CH2Cl2 (500ml) is added repeatedly for continuous extraction heavier than H2O containing NaSO4 which is then heated at 65°C having a provision of agitation,for more than 12 hours.Then LiCl (5gm) in conc.HCl (75ml)added to extraction chamber for further extraction.This is emptified for every 6 hour and replaced with CH2Cl2.Then combined solvents are recovered through distillation and the residual oil chromatographed with (Si gel,CH2Cl2;EtOH 2;1)and graded to CH2Cl2;MeOH 95;5) to give

- 5(CH2Cl Furfural (1.233 g,71%),2(2-Hydroxy acetyl)Furan(0.116gm,8%),
-5(HMF) - (0.082g,5%) , Levulinic acid (0.011 Gm, 1%)

In the case of Sucrose and Glucose use as substrates ,the former yields compartively higher as 76%,6%,4% and 5% and humic materials etc ..whereas the glucose yields 71%,7%;8% and 3%respectively the compounds obtainable as indicated above.

In general,the yield is comparatively higher than other crude raw materials.

Then major yield 5CH2 Cl2Furfural (1.24 g) dissolved in ethanol(60ml)and allowed to stir at ambiant RTfor 8 hours and excess EtOH recovered through distillation and residue is chromatographed to get yellow pale liquid.(Si-gel,CH2Cl;EtOH 2;1) to yield(1.26 g ,95%).

Viewing on above approaches,these can be carried out with PdCl2 results in obtain the colourless

liquid having the yield of (0.78g,87%).

RESULTS &DISCUSSIONS;-

Cellulose Hydrolysis;-

The cellulose sample is hydrolysed with mixture of Celluloytic enzymes(cellulasesNS-50013 & Beta glucosidases NS 50010-NovoZym,Denmark) in presence of 10ml of 50mM/L acetate buffer (pH4.8) to obtain total volume 20mLof liquid phase and conc.comes to 50 G/L.then incubated at 50°C and shaken 180Rpm for 48 hours.Then it is centrifuged at 4000G for 10 minutes.to separate cellulose residue from liquid phase.then the sediments is washed and dried at 60°C and further overnight dried till gets constant weights.

The conversion degree of cellulose (CD)at the hydrolysiscan be calculated as follows:-

CD = 100 { 1- (W/Wo)]

Analysis5;

The degree of Depolymerisation can be measured by viscosity method using diluted solution of Cellulose.

Analysis 6;-

Cellulose structural characterstics can be measured by X-Ray Diffraction studies(XRD) using RigaKu-Ultima Plus Diffractometer. Diffractogram is recorded (Theta=2)angle ranges from 5-80°.and then these was separated and selected X-ray pattern can be corrected and normalised(Cu K alpharadiation, Lamda=0.15418nm).Then diffraction from crystalline and non-crystalline separation realisable through Computerised method.The crystallinity degree can be calculated as per the following equations;-

$$X, \% = 100 \frac{Ie\ d}{Io\ d}$$

Then the contents of Cll -crystalline polymorph can be calculated using XRD calibration method of inner standards:

Cll, % = **200** (**I 12** I **I 15** + **I 16**).

According to XRD investigation, the higher acid concentration 64-65% permits hydrolysis lead to form,regenerated cellulose having Cll polymorph only and low crystallinity (X=25-30%) and low DP(40-50%)These results permit to find following optimal conditions for the production of AMORPHISED CELLULOSE in commercial scale as raw materials for biofuel production.

Analysis7:

Shape-Size of NP by Scanning Electron Microscope(SEM);-

The particular investigation of size and shapeof NP can be done by SEM Hitachi S-4700.

The diluted dispersion of cellulose NP subjects to ultrasonic treatments for 5 minutes. The results shows that DP 60-70% was observed at 65%Wt contained about 1-2% sulphonic group. This leads about 66-68% and DP of 60-70%.**(Refer Fig;2;5)**

CONCLUSIONS:

The conclusions of whole study shows that the cellulose sample dissolved completely and regenerated cellulose is precipitated having **Cll** polymorph with decrease in degree of crystallinity (25-30%) and low **DP**(40-50%) as indicated earlier.Increasing furtherH2SO4 to exceeding 65% lead to

decrease in yield of regenerated cellulose again due to the presence of acidic environment.This gives higher DP and forming watersoluble polymers.

Combination of optimal acidic treatments with high power of disentegration permits obtaining NCP (150-200 * 10-20 nm)with improved recovery (about 70%)is possible.(**refer Tab-5.)**

7;2;RECOMBINANT GENETIC ENGINEERING OF S.CERVISIAE FOR IMPROVING BIOETHANOL PRODUCTION FROM DIRECT STARCH FERMENTATION;-RESEARCH STUDY 2

The process methodology is more adoptable for **wet or dry corn** milling ethanol production in order to improve the bioethanol yields**(1G-Technology**). Bioengineering the yeast strain is the most promising solution through recombinant DNA technology offering a valuable choice for CBP of biomass fermentation to increase the ethanol yield from several sources of carbon such as starch ,lignocellulosic feedstocks etc.. with effective cost reduction possible..

This determines starch necessarily to be hydrolyzed by acid pretreatments and saccharified with alpha 1-4& 1-6 debranching hydrolases using alpha-amylases and /or glucoamylases& alpha glucosidases before fermentation. .The yeast is not able to ferment starch naturally but genetic modulation will help in improve upon cost of the fuel production through specific gene expression gaining new properties & improve the metabolic pathways.

Genetic engineering tools offer a solution more adoptive for the saccharification process before fermentation such as the expressing genes of Rhizopus Oryzae capable of break down both Alpha 1-4&1-6 glycosidic bonds efficiently&successfully transfered to S.Cerevisiae yielded 80% starch utilization during 100 hour fermentation period.

S.Cerevisiae var.Diastatistics was not efficient to degrade alpha 1-6 glycosidic bond of of amylopectin units whereas starch domain A.Nigergenes has been fused with STA-1shows remarkable hydrolysis of insoluble starch

Glucoamylase expressing phenomenon in S.Cerevisiae:-

Gluamylases(1-4 Alpha-D-glucan Glucohydrolases) is capable to hydrolyse starch from non reducing ends to release beta D-Glucose units and saccharification of polymers.

A High ethanol production (**0.71g/hour/liter**)was observed during 300 hour completion of repeated fermentation process through recombinant **S.cereviae** strains **(YF207/pGA11**) which expresses R.Oryzae Glucoamylases. encoded by Gla and GlaB on cell surface possessing divergent kinetic properties & activities.

Starch fermentation ability is influenced resulting highest ethanol production of 15Gm/liter in 24 Hour) signified by the exhibiting activities of 9*10^9U/cell.It is important to note that the increase in glucoamylase activity from Awamori through optimising codon comparing to normal(791nKAT & 591 n KAT) and able to transform the recombinant gene into industrial S.Cerevisiae.

Alpha Amylases expression in S.Cerevisiae;-

The basic concept is the expression high amount of amylases by S.Cerevisiae under aerobic condition for effective starch fermentation and to provide long term durability,the co -expression of both enzymes on the cell wall of yeast are required. as the efficient factor for ethanol production.

S.Cerevisiae expressing recombinant alpha amylase genes(**LKA1 & LKA2**)obtained from Lipomyces Konanenkoae have shown a proven result in direct conversion of starch but ethanol productivity is found to be low(17.2Gm/L in 200 hour)owing to its inadequecy.

S.Cerevisiae for the stable starch materials;-

The purpose of gene integration is to provide longterm stabilty & activities and could be increased 20 fold by means of delta integration sequence with respect to conventional transformation. The delta integration of Ty retrotransposon or rDNA sequence of yeast strain are generally used elements for chromosomal integration of recombinant gene shown that 90% initial starch content was fermented by this yeast coexpressing both enzymes transformed via delta -integration.

The **Novel strategy** comes into practice to obtain high level of biomass and ethanol production through cell fusion techniques used to make haploid,diploid,& tetraploid of S.Cerevisiae targeting two separate DNA sites for efficient cloning of two or more genes through combination of rDNA and delta-integration resulting strains grow faster ,proliferated and fermenting starch more efficiently than parent strains and producing ethanol **0.55,0.72,,0.93** Gm/L/hour respectively..

Cell wall anchoring enzymes through expression of S.Cerevisiae:-

Co-expression of enzymes have been standardized through anchoring upon secretion into the fermentation medium for direct utilization of starch molecules.Though it has advantages & disadvantages ,its secretion is not favourable to the environment due to loss of stability in early stage of fermentation.

Addition of Calcium ion will improve the stability of alpha amylase during repeated 10 cycles of raw starch fermentation.This shows addition of reagents not required for starch utilising enzymes while use of cell surface engineered yeast.

In another study,co-expressing S.Cerevisiae on cell surface shows the result possible with continuous 23 cycles of ethanol fermentation for direct starch use without loss of enzymatic activities.This produce more ethanol(60 gm/L in 100hour fermn.)than higher starch degradation rate observed with only cell coexpressed with Glucoamylases strains(50gm/liter in 120 Hour .)

The table describes the genetic engineering of S.Cerevisiae for high efficiency ethanol production;-(**TABLE-----6.0)**

Co-expression of alpha-amylase and Glucoamylases;-

Co-expressing both enzymes through additional genetic manipulations have emerged a later strategy for enhancing bioethanol production synergistically from corn & wheat.The several studies have been reported on constructing S.Cerevisiae expressing both enzymes through genes of **A.Awamori(GA1**),Debaryomyces Occidentalis(**GAM1**) and alpha amylases **(AMY**) encoding on plasmids for direct conversion of starch into ethanol.The yeast containing all three genes exhibits highest Glucoamylases activities(1020 U/l) comparedto only the presence of GAM1 and GAI1(790 U/l) and (560 U/l) respectively indicates synergistic activity.

Victor et al(2013) reported that ethanol production capacities have been tested with alpha amylases & Gluco-amylases from Aspergillus Tubingenis T8.4 expressing lab strain S.cerevisiae Y294 and semi-industrial strain S.Cerevisiae Mnu-alpha1;Y294 and Mnu-alpha1 that produces 9.03 and 6.67G per liter resply. from a substrate load of 200 Gm.per/ liter of raw corn starch during 10 days fermentation in the absence of total heat treatments .

A study was based on utilisation of bacterial Pullulanase co-expressing alpha amylases& glucoamylases resulted in complete utilisation 99% of starch matter.

Conclusions;-

Hence starch& other substrates,as a potential feedstocks maintains their availability,accessibility and relatively low cost in comparison to sucrose & glucose based feedstocks.Genetic engineering of popular industrial strains needs attention in order to increase the stability and activities of enzymes,lower the cost of process & higher yield of ethanol and rate of fermentation etc .considered to be significant.

7;3 RESEARCH STUDY 3

RICE HUSKS-DELIGNIFICATION FOR BIOETHANOL PRODUCTION :-

The research was carried out by RVCE scientists in Bangalore,India towards the production of ethanol from rice husk.The objective of this study is to find out alternative source of biofuel as prime source of bioenergy. INDIA produces larger amount of rice husks from agricultural wastes as byproducts-raw material.Achieving to produce high yield of sugar,the pretreatments methods such as chlorite and alkali can be proposed and subsequently followed by hydrolytic enzymes and fermentation with fungal strains to determine effect of sugar for ethanol conversion.

Thereby,the alkaline hydrolysis process is judged as a critical method to pretreat the plant biomass involving presence of lignin contents towards the saponification of intermolecules ester bonds cross-linking Xylan hemicelluloses and other components..This pretreated materiel are used with cellulase enzymes effectively for depolymerisation and subsequent fermentation process.

The conversion of LCB involves pretreatments followed by enzymatic hydrolysis into simpler sugars and yeast fermentation.The presence of lignin in cell walls shows negative impacts during processing methods.The chemical treatments of rice husks involves usage of NaOH for effective removal of lignin due to their strong alkalinity.The concentration of NaOH and NaCl (1-5%) used have shown the best results at 5 % for both solutions thereby enhances the susceptibility to enzymatic hydrolysis process at 30°C.The above tretaments causes profound deacetylation and milder delignification of rice husks and there is no apparent loss of cellulose.In addition to above,fungal species -**Trichoderma Reesei** was used to study for higher conversion yield of sugar consequently higher ethanol obtained were 250 mg per gram of biomass after 6 days of fermentation with S.Cerevisiae**.(Refer Fig;4..3& 4.4).**

Pretreatments of Rice Husks;-

The chlorite oxidation and wet oxidation are used as promising oxidative delignifying strategy and the results shown as follows with wheat straw used as substrates and the conversion of 85% yield is possible with cellulose substrates into glucose.

Substrate ; 20gm/liter), Temperature;70°C ,

Time:5-10 minutes and Yield;85 %

The Na chlorite treatments have yielded 90% delignification in woody materials.The bioconversion process comprises of 3 major steps;Pretreatments,hydrolysis and fermentation.

Trichoderma reesei,fungal species produces commercial cellulases having wide cellulolytic activities capable to breakdown substrate into monomeric units.The action of cellulases involves concerted action of 1)endogluconases(endo 1,4-beta glucanases)hydrolyse internal bonds preferably in cellulose amorphous regions releasing new terminal ends,which randomly affect the internal beta 1,4 linkages 2)Cellobiohydrolases (exo-1,4 beta glucanases)act on existing or endoglucanases generated chain ends 3)Beta 3-glucosidases,which hydrolyses cellobiose to glucose.

MATERIALS & METHODS;-

The raw material is the source from rice husk and powdered in mill used as C-source.

*-Microorganisms-**Trichoderma Reesei(MTCC-4876)** obtained from MTCC,Chandigarh.These fungi produces various cellulolytic enzymes that converts carbohydtrate polymers into fermentable sugars followed by inoculation by S.Cerevisiae to produce ethanol.*

INOCULAM PREPARATION;-

*Fungal culture inoculated on PDA medium in petri dishes and the spores (7 days old slant) are transfered and dispersed in sterile distilled water containing 0.1% Tween and vortexed.Then the spore content is measured with Haemocytometer adjusted to 2*106 Spores/ml by optical density**.***

RESULTS AND DISCUSSIONS;-

Pretreatments;- Compositional analysis of lignocellulosic substrates:

A)Cellulose(3-50%)Hemicellulose(20-35%)& Lignin((10-25%)

B) Pretreatments strategies;-(Refer FIGURE-1 &2)

The effect of NaOH and Na chlorite treatments on rice husks was also studied for varied concentration and pretreatments time.The maximum potential results were obtained with 5%NaOH and 5% Na Chlorite as shown in (Figure..)

C)SACCHARIFICATION;-

-Delignified substrate have shown the effects upon Treating with cellulase enzyme as follows;The substrate were treated with FPase dosage for saccharification.The optimum sugar release(740.35+- mg/G.dry solids) was with rice husk.

MICROBIAL SACCHARIFICATION OF DELIGNIFIED SUBSTRATES:-

(Refer Fig.3). indicates the study of one factor at a time of approach of microbial saccharification using Fungi-T.Reesei of delignified substrate.It reveals that the optimum sugar release(127.1+- 4.21 mg/Gm) was obtained at the seventh day of saccharification.

FERMENTATION OF DETOXIFIED ACID HYDROLYSATES;-

*Ethanol production increased with increase in incubation time till 7 days of fermentation time.However the maximum ethanol obtained was (3.20+-0.36 Gm/L) with and yield of ethanol (0.27 Gm/ g of total sugars)(**Refer FIGURE-4..1&4.2).***

CONCLUSIONS;-

The conclusion of the study reported to be the bioethanol production process is possible by direct fermentation with enzymes that shows higher results than microorganisms but was not cost effective but this can be replaced by chemical and other pretreatment (alkali & sodium chlorite)processes.and considered to be more adoptive one.Detoxification strategy developed to eliminate fermentation inhibitors from the hydrolysates.Therefore saccharification was taken forward with fermentation efficiency to obtain more bioalcohol.

(SMALL SCALE APPROACH);-

PROTOCOL FOR BIOMASS PRETREATMENTS

10 gm biomass in dry basis is pretreated with 100ml H2SO4 overight to ensure penetration of liquid and separated through centrifugation and placed 3 equal quantities in small reactors require Fluidized sandbath to reach temperature 140°-200°C safely. and the reactor pretreated in boiling water for 2 minutes.to accelerate the heating in sandbath.

Then the reactor are plunged into ice water for 2-3 minutes with agitation initial and quickly quench the thermal treatments after reaching desired incubation in primary sand bath.Then the contents are transferred with wood rod from dried reactor into the 50ml disposable centrifuge. In the case of woody biomass,these may be ejected the solids as it swells.

Then pretreated biomass is used after pH adjustments for the evaluation of solids in the absence of free sugars present in the solid substrates so washing removes solubilised sugars and other materials that might impact fermentation.The initial wash involves 10ml H2O per gram requiring 25ml of water for one reactor in the tube for centrifugation(1000g/min)and then liquid is withdrawn for analysis and subsequently biomass is washed with a total of 100ml/g.biomass in a centrifuge bottle. (if filtration is needed requires 3 layers).

Finally ,after pretreatments a portion can be removed for compositional analysis before and after washing.It is essential to combine four reactors set,mix, and remove three 0.5 gm wet samples approximately into the individual drying for compositional analysis at 45°C This yields 0.1 Gm dry basis (db) each generates db data for carbohydrates and Lignin analysis.

BIOMASS ANALYSIS OF PRETREATED SUBSTRATES;-

During processing ,the moisture determination is essential otherwise the material flow can not be followed.After centrifugation,the packed biomass shows typically 15%db or 20 %dry matter where no free liquid is visible.It is important to note that 80-85% liquid portion contains soluble sugars constituents bulk free liquids and these materials should be included in compositional evaluations that determines through extensive washing with water or buffer to remove soluble substituents.

For material balance analysis,it may be initiated to weigh both liquids and solids of various fractions so tracing the process and material flow is possible for further analysis.

Lignin is analysed by determining acid insoluble lignin by Muffle Furnace degradation and insoluble Lignin by absorbence with UV-Spectrometers.The carbohydrates methods can be scaled down from 300mg to 100 mg dry biomass if it runs in triplicate.This is especially useful when fermentation is scaled to 10 Grams on db as starting materials ,necessarily common for analysis through out the process.

Quantitative saccharification analysis is the requirements of dilute acid pretreatments before and after washing the solids to determine the degree of HemiCellulose and Cellulose material release(FIGURE-24;1)

The result shows that significant decrease in Xylose and apparent increase in the level of Cellulose clearly explains on the basis of high degree susceptibility of hemicellulose,Xylose linkages compared to Cellulse and Glucose linkages.

The enzyme dose of cellulases are based on cellulose contents basis and then HMF&Furfural levels signifies the severity pretreatments generating these compounds which can be analysed by HPLC. with P column.(after Neutralisation)to confirm severity of process.Ideal pretreatments generates low levels of various acid degradion products.

5;1 (Small Scale Approach)

PROTOCOL FOR BIOMASS FERMENTATION ;-

Hydrolysis of polymeric cellulose and hemicellulose moleciules to fermenting reducing sugars essentially done by fermentation.in three ways:-

SSF/SSCF/SHF/CBP

a) ***RESULTS-ANALYSIS OF BIOMASS FERMENTATION***

Characterisation of fermentation results uses similar approaches with HPLC primarily equipped with Bio-rad Aminex HPX-87 Column with an acidic mobile phase(5mM.H2SO4) The analysis can generate quantitaive data based on standards for ethanol,butanol,Butyric acid,Lactic acid,Acetic acid and other Cyanic acids such as Formic acid as well as Xylose a& Glucose whereas the other biomass sugars do not separate enough with this column so separate analysis may be recommended using HPX-87 column with distilled water.

It is finally recommended to complete the analysis of fermentation broth with above separation methods.(Aminex HPX-87H& 87P) to extract as much as data required regarding yield and substrate usage during fermentation.

A typical analysis of fermentation broth is shown

The presence of free fermentable sugars indicates enzymes in activation but the fermentation of these available sugars is inhibited by ***C.Thermocellulum*** *signifying pair-enzyme production which leads to subsequent liberation of sugars.In addition,COMT transgenic liberates more free sugars that supports earlier data in regards to hydrolytic processing enzymes of COMT switchgrass compared*

to,wild types.It is hypothesized that the inhibitor substances generated by action of enzymes portfolio in Caldicellulosiruptor different from C.Thermocellulam and GC-MS analysis studies the composition of fermentation broth from later species detects the previous unknown intermediaters in Lignin pathway (Iso-Sinapyl alcohol)

for Switchgrass not present in sufficient level to explain Inhibition.This shows that blocking of an intermediates step in Lignin production and results in new side reaction thrpough back-Up of pathway substrates owing to complex kinetics of bioconversion process enhances weight loss therein.obtainable through determination of venting the bottles after incubation at 58°C in viewof warming the bottle and also to remove the pressure which in turn improving the yield at the rate of 36%obtainable for transgenic switchgrass comparing the yeast based fermentation magnitude realisable to eliminate thr impact prevention at 18hour .

CASE STUDY OF SSF PROCESS;-

Fermentation can be done with 1gm biomass on dry basis in a container having volume - 100ml serum Vials or serum bottles. This can be tracked routinely by weight loss by venting CO2 liberation .It should have the provision to puncture by a needle.The weight is recorded again to obtain exact biomass weight in each of the bottle in triplicate alongwith no biomass control in triplicate containing all components except biomass.This allows in detecting fermentable substrates or residual sugars in the inoculam.

2) For yeast SSF,the stock buffer is 0.1M sodium citrate (pH4.8) is to be diluted with water to the final volume to reach 50mM concentration of buffer.E.Coli or Zymomonas can be approached foe SSF using a different pH.A final volume of 20mL yield a 5% biomass loading using 1 gm.sealed loosly and autoclaved for 30 minutes.and care should be taken not to clinging to the sides of container to avoid improper fermentation.After cooling,10% yeast extract is added to initiate fermentation at the rate of 0.5% .If water is lost during autoclaving ,0.5mL overnight culture of microorganismes and additional water is added.The industrial mixture enzymes having 15 filter paper units per gram Cellulases may be added at a consistent dose levels .Other protocols explains adding enzymes possibly based on protein weight due to impact level in fermentation.

3)Hemicellulases ,Beta -glucosidases,pectinases can be added at one quaters of volume cellulases.This can be modified by addition of other components.As far as yeast fermentation is

concerned streptomycin at a rate of 62.5Microgram /mL(50Microliterrof stock 25mg/mL into 20mL.) can be included to minimize the mesophilic anaerobes for the precutionery measures otherwise this can be skipped

Then the OD at 60nm are measured and this can be noited that yeast culture grown on overnight in YPD broth can reach 10 OD -600nm units.

It is advisable to freeze the inoculam portions after cell removal to determine residual sugars and product concentration (inoculam).

4)Fermentation container can be sealed and time zero (To) is recorded the weight before incubation at the desired temperature and then the container are kept upright to have the full access of solids to enzymes and microorganismes to minimize the biomass coatingalongthe sides of as it poses not falling into bulk liquid subjected for fermentation.but shaking at 100-125Rpm is required.

5)The weight loss is tracked routinely by piercing with 25 gauge pressure needle and permit the CO2 to escape .This can be done through venting 20seconds or longer initially but not more than this limit as the internal gas contract and drawing in air.After venting,it is weighed and recorded six or less times to minimize differential cooling.It is obvious to note that the fermentation is most active during first 24-48 hours but it is recommended to continue venting and weighing until fermentation weight loss profile exceeds the continual equilibrium level.So,Venting can be done between 18 hour and 24 hours after that it provides excellent data on the progress of fermentation showing the fermentation profile for switchgrass during SSF bioconversion.

6)Upon completion of fermentation,the contents is poured into tared centrifuge 50mL disposable tube.then it is centrifuged at 1000Rpm for 20 minutes to separate the solids from the broth and poured off the liquid into the tared centrifuge tube.Then all the tubes are weighed in proportions tocheck the progress of the process.

SSf conversion approaches describes earlier showing that trangenic COMT lines produces more ethanol than wild type lines with the requirement of four fold decrease of cellulase enzymes.The fermentation process was clear with no indication of yeast inhibition. in compared with CBP where yielding the same results are possible with C.thermocellulum but no addition of cellulase enzymes is required .

5;2 CASE STUDY FOR SHF PROCESS;-

1)The biomass,buffer and water are prepared in container and then autoclaved ,cooled then followed by addition of desired enzyme cocktails on the basis of cellulose content and total biomass.It is incubated at desired temperature at 80 Rpm to avoid foaming provided by better mixing the biomass non-sticky on the side of vessel.

2) This can be incubated for upto 5 days with NovoZym enzymes and expection of hydrolysis time is to be completed in 4-5 days. It is noted that examination of bottles are swirled gently off the sides any clinging biomass.The viscosity drops significantly within 24 hours.After the hydrolysis,it can be removed and the containers are necessarily cooled..1ml of mixed sample are removed and frozen for analysis of free sugars contents for optimal hydrolysis time determination.This can be done by setting-Up 10 identical hydrolysis bottles and removing a pair of them sequentially and daiy after exclusion of liquids..

3)If hydrolysis is complete,the former approach can be practiced to make rapid.If substrate is more difficult to hydrolyse then initiate the fermentation with the solid providing additional time for enzyme to continue to act while fermentative microorganismes converts the free sugars to products(It is known from the results that switchgrass is susceptible to hydrolysis and fermentation of liquid

whereas a woodysubstrates-Populus may be fermented with the solids present requiring additional enzymes hydrolysis.

The analysis is referred to weighing the fermentation container having a ventable top,serum vials.For yeast conversion,yeast extract is added at a rate of 0.5V/V and 0.5mL of overnight yeast culture added alongwith Streptomycin and water so as to reach preferred volume(depends on other organismes demand).Hence it is repeated as SSF necessarily track the progress of fermentation .Incubation needs optimum temperature for fermentative microorganisms provides shaking anysolids with microorganisms presence followed by venting done by 6-12 times since fermentation is appeared to be rapid.This should be continuously monitored until fermentation is complete.Then the contents are mixed and poured into theappropriate centrifuge containers.Then the subsequent step should be repeated as described earlier.in Paragraph6.in SSF

5;3 CASE STUDY FOR CONSOLIDATED BIOPROCESSING(CBP)-FERMENTATION

CBP requires anaerobic fermentative microorganismes such as Clostridium Thermocelllum,Calci-Cellulosiruptor and Thermo anaerobium bacterium species that are able to produce Cellulases,Hemicellulases, and accessory enzymes utilising polymeric carbohydrates other than simple sugars generating during the course of fermentation.These are essential for microorganismes growth and metabolism while biomass breakdown products.into acetic acid,ethanol and small amounts of Lactic acid.

The preparation of CBP fermentation container is the use of sealable rubber Vials or bottles to improve airtight seal for strict anaerobes .A near stationary phase inoculam should be prepared in which water is added to final volume nearly two times strength to facilitate preparation of bottles on small scale.This is followed by the vials sealed after blowing air using either Vacuum or fill the station with O2 free N2 for 10 minutes. then inoculated for 20 minutes and repeat the recharging the vials with O2 free N2 gas for 20 minutes to draw off O2.Then it is cooled and inject the preplanned volume of medium and inoculam.The existing anaerobic conditions requiring avoid the back pressure and adsorbent pad may be used to protect spillage of inoculam..It is advisable to use reducing agent such as Cystein to remove or reduce the residual O2 presence.Use of Resazurin is helpful to note 02 presence.

The completed vials are incubated in shaker controllable in RT after measuring weight loss as with SSF & SHFto check the progress of fermentation followed by venting.The primary difference is performed the venting by needles itself to eliminate O2 in an anaerobic condition. especially with Thermophiles. as itn provides cooling.phenomenon.Therefore it is necessary heat the vials at the start and then venting in anaerobic condition to equalise the pressure that occurs as a result of heating at the beginning to avoid apparent weight loss resulting from heating.

Then the fermentation is completed and the fermentation samples is drawn for analysis through sezparation of solids, from liquids as described in SSF.(Step 6).

8;0 <u>MISCELLANEOUS VALUE ADDED BYPRODUCTS CHEMICALS;-</u>(From Wood &Other waste Resources;-)

BIOCHAR-BIOOIL-BIOGAS;-

Here,we refer the pyrolysis process ,as the method applied for converting a solid lignocellulosic wood materials into carbonaceous char,condensable oil and gases and heat production..

SLOW PYROLYSIS,A PATH TO CHAR;-

Slow pyrolysis is the method to make char the wood materials through slow heating that maximise the production of solid carbonaceous materials and production of water from dehydrated reactions with the having larger particles more than >2mm sizes and slow heating rates <10°C/min and a temperature difference between 400-600°C are the common operational parameters resulting yields of liquids(30-50%) and a char yields of 25-35% possible to obtain with the residual vapours (not escaping within reactors)remain for 5-30 minutes.

USES of bio-char:-

Slow pyrolysis typically yields of oil (30-50wt%)referred to Two phase oil typically from combustion or gasification.It is possible to extract some valuable chemicals from aquaeous phase of slow pyrolysis oil such as Acetone/ketones (~5% on dry weight basis),Methanol (162%),formic/acetic acid (5-8%).

Bio-char is used as a soil amendment and nutrient adsorber for farmers that can improve properties of soil or effluents from anaerobic digestion..

FAST PYROLYSIS, A PATH TO OIL;-

Fast pyrolysis process is the method to heat the smaller particles lesser than 2mm and fast heating rates (>100°C) to temperature between 400-650°C resulting yield of liquids 60-75wt% and char yields of 15-25% and non condensable gas yields of 10-15wt% observed.occured in a designed reactor to remove and condense the vapours lesser than 2 seconds.Compounds obtainable from pyrolysis of lignocellulosic materials can be upgraded in a variety ways to produce fuels and 3000 chemical compounds.

Crude oil containing 46-48%O or 22 moles% O2 in the form of alcohol,ether, carbonyl acid etc.. are necessarily removed for <0.5%

and simultaneous stablisation require to upgrade the oil fuel quality of total liquid 40 wt%..To achieve this process and properties, the catalytic reaction with H2 gas of 2 step hydro-treatments is necessary with 2 different catalysts and having low temperature.(~170°C) followed by (~400°C) leads to O2 removal as water and conversion of carbonyl groups into alcohols.

USES of bio-oil;-

The oil typically contains 10-30% water depends on the moisture contents and appear as dark brown liquid (red to dark green),very viscous smells like campfire crossed with barbecue sauce (see properties of bio-oil)FIG - 26.4.The basic mass composition of the bio-oil on dry basis is 44-47%C, 6-7%H2,46-48% 02 and 0-0.2%N.

Bio-oil is advantageous owing to its efficiencies of Rankin cycles for producing electricity through causes problems in gas turbines,diesel engines due to nature variation in ash content and low cetane.

By this method,gasoline,diesel and jet fuels equivalent can be produced at lab scale & pilot plant levels.

FUELS AND BY-PRODUCTS CHEMICALS FROM LIGNOCELLULOSIC WOOD

Microwave Pyrolysis -Catalytic process-(MAP)

The usage of catalysts mentioned in this process may influence on selectivity of desired

products(fuels/Chemicals)through improved properties such as low heating value,high viscosity,thermal and chemical stability etc..

Microwave assisted Pyrolysis (MAP) induces heating within the body of substances through direct energy conversion enhances improvements of the selectivity of desired products by using two types catalysts such as metal oxides and Zeolithes (CaO ,NiO, K2CO3, MgO, CuSO4, AlCl3, MgCl2, HZSM-5).

This has possessing undesirable properties (low heating value,high viscosity and thermal& chemical stability of pyrolysed product energy efficiency and reduces the capital cost.

The results have shown that the addition of additives such as activated carbon,SiC,char etc..had influenced a positive effect on heating rate,volatile yield,and improvement in bio-conversion rate and a influence on selectivity of product such as Phenols,Hydrocarbons,Furfurals,Guaicols,furan contained in liquid product,H2,CO included in SynGas etc..

PRODUCTS FROM HEMICELLULOSES,CELLULOSES,LIGNINS in BIOREFINERY CONCEPTS;-

Woody biomass is comprised of at least four components; Extractives ,Hemicellulose, Lignins, Celluloses. A preference should be given to a biorefinery process characterstics such as Non-pretreatment

,and detoxification absence ,are the criteria essential for separation of major components. The extractives and hemicelluloses are least resistant to chemical and thermal degradation whereas cellulose is most resistant to chemical,biological and thermal one.

During pretreatments and hydrolysis process,many toxic compounds are regenerated as an important industrial products such as Hydroxy Methyl Furfural(HMF) and then Levulinic acid and Formic acids from Hexoses (Rhamnose Glucose, Mannose, Galactose) etc.. and Furfural derived from Pentoses (Xylose, Arabinose) which in turn gives Formic acid and acetyl groups of hemicelluloses gives Acetic acid.. whereas Lignin compounds after Delignification gives rise to phenolic compounds. These are the inhibitory compounds formed during dilute -acid hydrolysis process methods but affects fermentation stage.

Water based biorefinery process such as Hot water extraction is the first process to extract value while improving the quality of remaining solid materials .Extractives from wood biomass are the hemicelluloses are largely removed in extraction liquor. Whereas lignin and Cellulose largely remain in residual woody structure. Xylo-Oligomers ,aromatics and acetic acid in the hardwood extract are the major components influencing greater potential for developments.

Dilute acid hydrolysis of concentrated wood extracts renders wood extract with monomeric sugars whereas high concentration produces Xylose monomers in abundence comparatively during short period of high temperature reaction time .

Hence,acid hydrolysis provides a perfect opportunity for the removal or separation of aromatic materials from wood hydrolysates.After solid removal from hot water wood extract ,hydrolysates can be purified by Nano membrane Filtration (NMF)to yield a fermentable sugar stream.The main biofuels like bioethanol can be produced from such stream without a detoxification stage.

HIGH VALUE CHEMICAL INTERMEDIATES(LEVULINIC ACID) and BIOFUELS THROUGH ZEOLITE SYNTHESIS;-

Zeolithes are nano structured materials comprising mesoporous and Nanoporous elements-chemically known as Aluminium Silicates (Al2SiO3) ,heart of the catalytic process for the synthesis of Levulinic acid ,as intermediate compound obtainable while converting substrates like Glucose

,Fructose etc..and it is considered as a well known precursors for various plateform chemicals and biofuels.
The research study involved on synthesis of array of zeolites through convenient methods and certain technics like Heterogeneous catalyst synthesis,HPLC,XRD,GC/MS and other kinetic studies including catalyst design permits to strengthen the project and makes industrial feasible in energy sector and to meet the challenges of raw materials in another energy application as a promising startegy.

Needs Investigation for Technofeasibilty;-
MAP of biomass process are technoeconomically assessed one and viable,profitable hence the lot of technical barrier needs to overcome or investigated before scaling-Up such as reactor design,development of cost effective catalyst,Uniform-Heating,Optimum reaction condition,kinetic studies,and reaction mecanisms.These parameters determines the global focus on viability of the process.

Metabolic Engineering of Cornybacterium Gluatamicum for fermentative production of chemicals in Biorefinery;-
Various technologies that utilises microbial host strains from renewable biomass have been developed for sustainable production of plateform chemicals and fuels through Cornybacterium Glutamicum.
C.Glutamicum is non-pathogenic industrial promising species for industrial production of L-glutamate and Lysine production and biobased chemicals separation possible through its flexible metabolism that allows the broad spectrum utilisation of C-sources and production of various amino acids(AA).
For such production,systems,classical breedings,synthetic biology and metabolic engineering approaches have been used to improve its application ranging from traditional AA to modern biorefinery system production of Value added plateform chemicals .This can be performed by metabolic pathways in combination with recombinant genetic engineering approach for bio based production of major C2-C6 plateform chemicals.

LACTIC ACID PRODUCTION FROM AGRICULTURAL WASTES;-
Agricultural feedstocks residues are evaluated for lactic acid production by SSF process using Lactobacillus Delbrueckii and lactobacillus Planatarum without any additional nutrients.
L.planatrum shows the lactic acid produced from alfalfa fiber and soya fiber was 46 and 44 grams/100 g.respectively. whereas **L.Delbrukeii** shows the lactic acid production in soya fiber was 44 grams/100 g.that of Alfalfa was 32 g./100G.fiber.

Lactic acid from Wood;-
L.Delbruckeii NRRL-B-445 was used to convert both glucose and cellobiose into lactic acid through SSF process where wood subjected to delignification treatments and swelling process and then hydrolysed and fermented in a single stage to produce lactic acid.

USEFUL CHEMICALS FROM LIGNOCELLULOSIC Wood& BIO-OILS;-
3000 &Many more compounds including high valuable chemicals can be produced through extraction techniques according to Garcia (2014) & Czernik(2004).These are ;

-Calcium salts & Phenolates useful for SOx capturing in coal combustion and allowed to react with carboxylic acid(~1.2-2.1mol/Kg organics)and phenols (~1.8-2.1 mol/kg organics) with lime.
-Terepenoids &Phenols can replace Creasotes as wood preservatives.
-Heaviest fraction of pyrolysis oil can be used as tar for roofing or roads as well as glues and sealants.
-Fertilizers can be produced by reacting with carbonyl (1.8-6.2 mol/Kg organis) with NH3 or by spraying biochars with pyrolysis oil.
-Aldehydes & ketones (phenolics) naturally present in aqueaous phase,are useful for meat browning.
-Road deicers can be produced by reaching the aqueoues phase of bio-oil with calcium salts.
-Resins and plastics produceable from oligomeric lignin and sugars.
-Methanol and acetic acid and acetone can be recovered from slow pyrolysis oil.
The products of catalytic process are aromatics ,as a useful fuel additives or intermediates for the production of chemicals(Brigewater2000)

RESULTS AND DISCUSSIONS

The biofuel staretgy are discussed herein will give a overall concept upon using different biomass feedstocks for bioethanol by variable processing technology in a sustainable way .In other words,it is a tactic approach on processing the feedstock upon utilisation of available biomasses for efficient biofuel conversion.

Bioethanol having the superior characterstic properties such as high cetane number,low heating value,high flamme speed etc.. are possibly obtainable in accordance with the application of selective processing methods. Normally,this can be performed through CBP methods or by Acid-Enzyme hydrolytic conversion process that can meet the challenges todays of biofuel staretgy to recover maximum number of value added by-products chemicals other than principle bioethanol produces on biorefinery concept.

Efffective Pretreatments;

The classification of pretreatments methods, hydrolysis,Fermentation methods have the significant effects on physicochemical properties of bioethanol influencing the internal combustion engines otherwise it may have the change in combustion behaviour due to the different operating conditions..

The CBP strategy(otherwise called as Direct microbial Conversion(DMC)) finds a solution over successful anaerobic fermentation for the conversion of glucans by 60% than 28% obtainable over fungal & bacterial cellulases mixture (Cel17A/Cel15A)especially applicable with Cellulase cardio cellulosiruptor Bescii species or other anaerobes such as Clostridium thermocellulum,thermo anaerobes species etc..considered to be a significant one owing to nature of producing cellulases &hemicellulases in Vivo where temperature is acceptable for growth of organismes and permit the biomass degradation during the course of acetic acid,ethanol,lactic acid production resulting gives a successful fermentation in oxygen free environments. CBP strategy shows 60% conversion of Xylan by CelA in native switchgrass showing its potential as industrial process possible using mild or no pretreatments.This shows difference in activity translates to a seven fold increase in activity for CelA at the molecular level.

Among the different processing methods as discussed earlier,Ozonalysis is an efficient pretreatment methods recommended for energy grasses obtainable a delignification startegy by 51% while pretreating by alkali(1%) or ozonalysis followed by enzyme processing capable to produce

467.9mg/Gm and 431.9mg/g respectively.
Upon treating switchgrass with ionic liquids (IL)at 100°C for 3 hours followed by hydrolysis by cellulases (0.3%w/w/g) using S.Cerevisiae strain BY4741 at 30°C for 20 hours having agitational speed 200rpm influences higher bioethanol yield of 85.7gm and reported to be one among the promising strategy of IL pretreatments methods

Enzymes:

Among the pretreatments by enzymes,Xylanases are considered to be an important Xylan degrading enzymes where Xylooligomers formation can act as powerful inhibitors of saccharification process than Cellobiose & Glucose.Inappropriate ratio of Beta Glucosidases will lead to accumulation of cellobioses that inhbits the activity of cellulases as it catalyses the rate limiting step in breakdown of cellulose molecules.Hence strategy is developed to maximise the saccharification through inclusion of endo1,4 beta Xylanases ,beta Mannosidases,beta Mannases, Pectinases, Beta Glucosidases, L-Arabinofuranosidases etc..in appropriate levels necessarily required.in a cocktail mix.
Laccases are one among the potential pretreatments agents in removal of lignin compounds in biofuel and act as a biotechnological tool for removing phenolic inhibitors to arrive an optimal results and adptation of biorefinery concept..Various Oxido-reductases (lignin peroxidases, Manganese peroxidases) generates various oxidative species on lignin that helps in attacking inhibitors formation thereby the process becomes viable and more effective
Though endogeneous level of beta glycosidase activity is not sufficient for higher saccharification , commercial celluclast from trichoderma reesei is supplementable with thermoacidophilic beta glycosidases from Tolypocaladium specis syzx4 showing in saccharification yield achieveable upto 88.4%.under optimal hydrolysis conditons

Enzyme Activity Loss;-

The enzyme activity loss for less sugar yield can be differed with other factors such as composition of lignin,pretreatments methods and type of biomass etc..The presence of high Guaiacyl content in comparaison to Syringyl content in Lignin confers to increase capacity on entrap the enzyme leads to activity loss.

Inhibitors formation &Detoxification strategy Methods:-

In addition to that,the beta glcosidases presence play a role on the basis of selectivity towards higher concentration of glucose & cellobiose and supporting level of higher ethanol concentration.(occured in CBP) since enzyme saccharification & fermentation conducts in separate reactors and these are insensible to the inhibitors like HMF,Furaldehyde etc..& influencing add-up costs.
To overcome this effects,the encapsulation of yeast can be viable solution to stabilise the productivity of alcohol compared to freely suspended cells.There are certain number of methods available to minimise the inhibitors formation such as developing microorganismes in static or set cultivation medium where fixed condition (temp/pressure/aeration) to be regulated.
The other alternative solution is to increase the cell concentration (Immobilisation)or by Genetic engineering or modification of cells in which change in pH reduces the carboxylic acid fermentation of specific compounds like Xylooligomers,acetic acid,glucose etc. that exerts a negative effects on bioethanol yield.In other words,engineered yeast is resistant to fermentative inhibitors by overexpression of enzymes conferring to improved resistance to phenolics,aliphatic acid etc..

Ion exchange,Biocatalyst & liquid-liquid extraction etc.; are recommended as the better option for detoxification of hydrolysates. Overliming (addn. of CaOH) has emerged as one of the efficient methods through precipitation nature of toxic elements to reach good overall ethanol yield(OEY).
In the case of SSF process,high yield,high productivity,high product titer other than process water recirculation are the important aspects to choose the design where enzyme inhibition can be avoided by sugar or by Fed-batch or by Continuous means rather than batch process.

Yeast fermentation strategy;-
This project proposes an improved fermentation strategy of all chemically treated susbstrates via microbial saccharification where cellulose conversion into glucose can be possibly done in presence of lower lignin contents.Neutralisation is the better detoxification strategy employable where enzyme saccharification is optimised with 20 UFP/gm db.in pH5.5 after 36 hours at 30°C.
The hexose sugars present in a mixture of pentose sugars may influence on fermentation that helps in increasing the productivity at 30°C in contrary to ethanol production affected adversely by lowering or elevationg pH.The supplementation with soyabean meal at a rate of 1.2% enhances the ethanol productivity by reducing the fermentation time.Addition of non-ionic surfactants (Tween 80)showed significant increase in saccharification.
To check the progress of fermentation,HPLC analysis equipped with Bio-Rad Aminex 87-H gives the characterstics of fermentation broth based on standards.
Cassava starch used as a substrate where Zymomonas mobilis act as a ethanologenic organismes at pH 5.0 with a inoculam size 6%(V/V) having a agitational speed 25rpm at 30°C achieved a ethanol concentration 13.3 g/L at a substrate level 1g/L equivalent to 0.51 g.EtOH/gm of sugar as compared with 0.57gm EtOH/gm of hydrolysates of Xylan & glucan polysaccharides.This shows that the above feedstock is economically feasible with the species.
Pitchia Sitipis showed more efficient cell growth & bioethanol yield as compared with Kleuyveromyces Marxians while using G.Verrucosa as biomass feedstocks..
S.Cerevisiae shows a maximum bioethanol yield of 0.45g/Gm glucose than Pichia Sitipis & Zymomonas sp. and concluded to be enzymatic hydrolysis ,a promising step produces a higher yield than acid hydrolysis fermented by S.cerevisiae and explained as metabolic engineering construction is needed to degrade the lignocellulosic biomasses.
In SSF,fungal cellulases shows a promising activity at 50-55°C while the microbes ferment effectively below 35°C and act as a preferred step for the production of biofuels and chemicals due to nature of two operations occur in same reactors in contrary to CBP. The release of sugars is not controllable as all cellulases are added & consumed spontaneously by microorganismes resulting low sugar concentration reducing enzymes inhibition but SSF is often effective while coupling with dilute acid process.
In SSCF,employing the mixed microbes involved in fermentation of hexose & pentoses sugars are limited by the respective ability usually grow faster resulting higher rate of conversion from hexoses.It is reported to be the reduction in glucose inhibition owing to the nature of two different or one recombinant microorganismes activity performance whereas SHF process suffers inhibition while xylose assimilation occurs in glucose & ethanol concentration.

Regenerated Cellulose.-
As per the route proposed by this project study(Route-Ia) ,regenerated cellulose can be produced

through tailor made approach upon treating lignocellulosic feedstocks through acidic hydrolysis process with dilute H2SO4 and part of hydrolysates transformable into bioethanol via saccharolytic enzyme processing.
According to XRD-investigational study,regenerated cellulose may be recommended as precursor in acidic solution and precipitatable amorphised flocs having Cll type polymorph characterstics with 64-65% H2SO4 having low crystallinity (X=25-30%) and low DP (40-50%).This results shows to find a optimal conditions necessary for the production of amorphised cellulose in commercial scale as raw materials for biofuels.

Recombinant strategy of microorganismes.
The part of this project also focussed on yeast cell engineering (S.Cerevisiae)or other species like E.Coli,Zymomonas Mobilis, etc..may influence on bioethanol production through synthetic pathways but also essential for other factors such as reducing toxic product inhibition,tolerance towards the osmotic condition(concentration of ethanol) and to widen the range of substrates such as high solids loading at the beginning, high temperature profiles in simultaneous saccharification stage.This shows broad substrate specificity possible on various biomass materials towards the improved biofuels production in addition to the formation of organic acid ,enzymes,lycopene,vitamins,HMF,furfurals etc..after purification.
An alternative search for a novel pathways ,there is a need to use independant culture techniques such as metagenomics,bioengineering novel strain etc. realisable through genetic tools(MAGE/CRISPR/Cas,ZFN,TALEN) helps in improving GMO with desired industrial characterstics.

Upgrading Bio oils strategies& Liquid fuel production;-
There are many pathways to reach liquid biofuels and to improve the strategies for upgrading,dilution can be done to decrease viscosity and to improve ageing properties of oils with the inclusion of solvents possible through either biodiesel,methanol,ether or other alcohols.
The products of catalytic process are aromatics ,as a useful fuel additives or intermediates for the production of chemicals(Brigewater2000).
The sugars produced from cellulose in biomass (predominently Levuloglucosan) can be collected by fractional condensation and fermented into ethanol or lipids.The sugar yields can be improved through washing biomass with mild acids before pyrolysis influences negative effects of bio oil aldehydes and ketones over the microbes.

9;0 CONCLUSIONS

< SHF is more efficient than SSF since ability to carry out each step under optimal conditions of saccharifications at 45°C for better performance during fermentation stage.The yeast can be recycled after fermentation of the hydrolysate during SHF process as compared to inconducive nature of SSF process.The disadvantages he activity of cellulases.Both the processes are complementary to one another as combination can be used for economic assessment & optimisation of production process. .

< Different methods of detoxification are proposed to carry-out successful saccharification stage in order to remove maximum number of inhibitors formation before fermentation..CBP strategy shows 60% conversion of Xylan by CelA in native switchgrass showing its potential as industrial process possible using mild or no pretreatments. showing difference in activity to a seven

fold increase in activity for CelA at the molecular level.

< The quantity of bioethanol(1G) produced globally is increasing 110 billions liters in 2018 that could be 140 billions in 2022 with USA & Brazil ,the highest producers in the world .Due to high feedstock cost involvement,now it has been decided to produce 2G,3G,4G bioethanol etc..2G constitutes < 3% of total production and shows higher GHG potential compared to 1G.

< In order to increase the yield of bioethanol and minimize the cost of processing,integrated apporoaches may be done through coupling the processes(SHCF,SSF,SHF,CBP).Based on LCA assessments,the environmental impacts depends on the feedstocks availability and technology used for converting the bioethanol.

Future Prospectives:-

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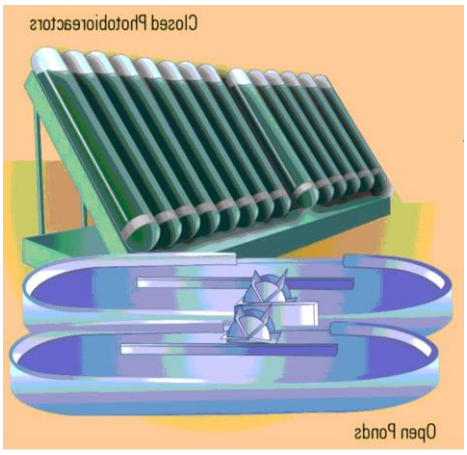


BIODIESEL FROM MICRO-ALGAES

Further suggestion needs to focus on enzyme stability and kinetic properties through certain approaches like changing AA sequences,creation of sulfide bridge within molecules and changing 3D-Configurations helping to increase the potential applications of Xylanases under extreme condition.

10;0 BIODIESEL METHODS & PRODUCTION PROCESSES

Biodiesel produced from Microalgaes considered as Third generation biofuels.BIODIESEL is alternative ,renewable,domestic energy resource and very similar to Petroleum Diesel but they are not identical whereas biodiesel is non-Toxic and possessing high lubricity in nature,that offers several advantages to the environments since it is biodegradable and exhibit low CO2 emissions - (GHGE), low SO2 etc,,It is safe for use in all conventional diesel engines -offer same performance like petroleum diesel.

Properties of Biodiesel:-

Biodiesel considered to be transportation fuel having the chemical properties like increase the volatility,thermal stability, and to reduce the viscosity. thereby additization is required for the fuel

performance to avoid FAME degradation .It is non inflammable,non toxic,reduces tail-pipe emissions, visible smoke and noxious fumes,& odours.Since it has low or no S content and it is often used as an additive to ultra-low Sulphur diesel(ULSD) fuel.It has been shown high lubricity than any other fuel.It has high cetane number and produces less particulates-CO and hydrocarbon emissions.It improves the environment quality with a pleasent fruit odour.It can be produced easily from a variety of raw material of various resources including recycled waste oil .

It can be produced easily from a variety of raw material of various resources such as Waste cooking oil,Microalgaes,oleagineous microorganismes etc.;including recycled waste oil .

The method of extraction & production techniques are the important determinants for the successful of ecofuel synthesis.

10;1 BIODIESEL PRODUCTION FROM MICRO ALGAE

PRODUCTION OF BIODIESEL from ALGAL CULTURES;-

Producing biofuels from algal cells consists of three basic steps involving cells harvesting storable in large tanks by harnessing their photosynthetic substrates.

The second step is the extraction of lipid content exists in the form of polar and non-polar lipids.Currently fuel production is focussed on the use of non-polar lipids constituting a small fraction of total lipids produced by algaes.

The third step involves lipids used for further processing depends on target of biofuels aimed .This can be done by basic three methods such as Biochemical conversion,Thermochemical conversion and Chemical conversion.

Here we will be discussing briefly about Various microalgaes cultivation for the production of biofuels.and co-products obtainable by biorefinery concept.

ALGAL- BIOMASS CULTIVATION & FOR ADVANCED FOR BIODIESEL PRODUCTION:-

There are different mode of cultivation of algae such as 1)Heterotrophic,2)Photoautotrophic, 3)Mixotrophic, growths considerable for harvesting that are based on requirement of light for energy and carbon sources.

PHOTOAUTOTROPHIC ALGAE

The photoautotropic cultivation mode utilises light energy requirements and inorganic C as carbon source and large variation of lipid contents can be manipulated through N limitation.

There are about 40000 types of algae species identified and classified in multiple major grouping:-

1)Cyanobacteria(Cyanophyceae)
2)Green Algae (Chlorophyceae)
3)Diatoms(BacilloarioPhyceae)
4)Yellow Green Algae(Xanthophyceae)
5)Golden Algae(Chrysophyceae)
6)RedAlgae(Rhodophyceae)
7)BrownAlgae(Phaeophyceae)
8)Dinoflagellates(Dinophyceae)
9)PicoPlankton(Prasinophyceae) etc..

All these categories algaes vary in lipid content as shown in the (Figure.-

To grow Algae biomass for biofuel production in large scale,the algal species should contain higher lipid content(minimum of 35%)that vary from strains to strain.Contrarily,The output of oil recovery from algal biomass accumulates as high as 58700 L of oil per hectare .

HETEROTROPHIC & MIXTOTROPHIC ALGAE

Heterotrophic algae can not use sunlight but works under dark conditions and uses inorganic carbon from CO2 unlike Photoautotrophic categories but sugar is required as a source of organic carbon.

Some strains of algae like Mixotrophic can take the adavantages of both Phototrophic and Heterotrophic nutrition modes and utilises C from CO2 as well as Sugars as organic nutrients source where cultures are cultivated in waste water with CO2 injection. These heterotrophic algae can produce higher amounts of biomass by utilising unlimited sunlight exposure,

In addition to this,the mode of major limitation is associated with the availability of cheap source of organic carbon and economical in investment and other operation costs,Currently SOLOZYME, the leading organisation involved on biofuel production and By-products transformation remarkably significant .

OLEAGINEOUS MICROORGANISMES FOR BIODIESEL PRODUCTION:-

Oleagineous micro-organismes are the promising sources for the production of renewable biofuel owing to their efficient photosynthetic capabilities that are capable to channel the majorities of their energies into cell division enhances biomass productivity.

CULTIVATION & HARVESTING TECHNIQUES OF MICROALGAES;

The research describes the use of algal biomass as a sustainable feedstock for biodiesel production.The perspective of this recent option (efficient Biomass) are based on three major process steps as follows:

a)Algal strain selection(different types)through Characterisation of Algae for its applications.

b) Algal cultivation -algal growth system-photobioreactor,Open raceways &Fermenters)

c)Biomass harvesting and Dewatering

d) Algal oil cultivation& conversioninto Biofuels and Valuable production (mechanical, chemical-transesterfication, enzymatic,supercritical etc..)

e) Separation & Purification.

MICROALGAL STRAIN SELECTION FOR BIOFUEL ;

Upon consumption of raw material, these are often usage of residuary waters comprising poor of organic carbon but rich in other nutrients.Microalgal based processes are unique because they associate with CO2 fixation while biodiesel production.Hence the strain selection and screening are very important steps to make the process economically viable,In addition to that, the rôle of genetic engineering and isolation of new species will play in near future.

Strain selection is directly linked with the mode of cultivation.Certain desirable qualities for strain selection include tolerance & high CO2 concentration,high biomass productivity,tolerance to temperature variation and importantly CO2 fixation rates are taken into consideration.

10;2 GROWTH SYSTEMS FOR BIODIESEL PRODUCTION

There are different modes of microalgal Cultivation technologies may be proposed or adopted for the commercial plants such as

**Extensive Ponds(lagoon)

1)OPEN PONDS/Raceway Ponds and Circular Ponds2)Flat -Plate Photobioreactor

3)Inclined Tubular Photobioreactor4)Horizontal/Continuous Photobioreactor

5)Fermenters(Algae grown on organic substrate in dark condition)

OPEN SYSTEM;

These are popoularly known as Raceways ,economical for mass cultivation of Algae.The nutritient rich algae growth is mixed through paddlewheel and can be scaled-up on many acres of lands.

FERMENTERS:

Heterotrophicalgae is used to ferment in large vertical tanks installed to greater heights of 12 stories(Winchester Kentucky by Alltech Inc.)(REF FIGURE 6.3).

PHOTOBIOREACTORS

There are closed systems providing a controlled environments for growing Photosynthetic algae under sterile conditions.These are designed to have adequate light exposure for Photosynthesis through artificial or natural lights.This reactor provides algae growth parameters(pH,Temperature,Mixing etc..)that can be controlled to maximize or increase the algal biomass under sterile conditions.Common designs of Photobioreactor are described as in the Process **)....**

-Flat Plate..(a)

-Tubular(a)

-Hanging bags(c)

-Bubble column(abc)

- Closed column(d)

PHOTOBIOREACTOR DESIGN;-

Once the algal strain selection is made ,Cultivation could be done in open as well as Closed system on well designed photobioreactors.

Open system of different categories(circular ponds,raceways and modified raceways,unstirred ponds etc..) offers easiest way of operation and more economical than closed system.These open system shows low potentiality such as poor light utilisation efficiency,huge evoporative losses and biological contamination.In addition to that,open system are not viable as gas mixing due to low depth of pond for the high rate of CO2 Sequesteration, highly suitable on employing into the closed type Photobioreactor.

The advantages and other conveniencies of type photobioreactor are based on setting various design parameters include type of reactor,height to diameter ratios,mode of delivery,minimization in temperature variations, optimum intensity of light,maximum utilisation of light,proper mixing,usage of optimum light,-dark cycles,minimization od CO2 losses etc.

The multiple design of photobioreactors shows typical opportunities and challenges upon comparing with Open Systems.These include tubular horizontal and vertical type reactors,stirred tank types and flat plate reactors etc,,

Tubular Photobioreactors are easy to operate on working with biomass productivities having

higher illumination surface areas.This system involves incorporating and sparging the gas to provide the overall mixing and high gas transfer efficiency.and possessing very large illumination surface area and suitable for outdoor cultures.

Concerning **Vertical tank reactors**, it may be observed that the algal sequesteration of CO2 could be increased through implementation of multiple photobioreactor in order to scale-Up the process.

In the case of **membrane photobioreactor**,the gas(CO2)exchange efficiency improved through the introduction of membrane module that leads to minimization losses whereas in membrane carbonation photobioreactor,lesser the bubble,lesser the gas transfer that allows delivering the precise control of Co2 then leads to minimize or reduces the losses of Co2 to atmosphere.

In **Airlift bioreactor**,the light intensity can be increased through better design where light utilisation is comparatively higher compared to bubble column.Even in optimum concentration, The removal of excess biomass continuously is to be considered in order to provide maximum spread area to volume ratios,this factor not to be ignored.

CONFIGURATION OF THE REACTORS;-

As described earlier ,certain technologies are proposed in the form of artificiel open pond or shallow Raceway in which the suspension is mixed through Paddle-wheel(cheapest way to construct &OPERATE).The inconveniency associated with the production relates to lower productivities and low mass yield,limited number of species grown in ponds,water losses through evoporation,vulnerability to contamination by other algae,bacteria,lower efficiency to Co2 use,salinity etc..

The use of inoculum ratio to pond capacity make growth the microalgae strain in extreme condition**.(usually few days**)so as to minimize evoporation losses &contamination,Addition of NaHCo3 tends to raise the pH to minimize Chrolella invasion of Sprirulina culture and N source to decrease the amoeba grazers.

-The two major types of Photobioreactor(PBR) are tubular &Plate types helping to reduce evoporation but immune to contamination.The temperature,pH,salinity,better controllable but surface area to volume ratio(S/V) facilitate higher volumetric productivities&cell concentration.PBR designed for proposing the biodiesel production and achieving higher photosynthetic efficiencies&productivities.The major issues are considered to be;Constructing suitable materials,efficient mixing/cooling,Co2 supply and O2removal,the high cost involves on reduced stability,light duration via external surfaces or internal light conducting structures and the use of genetically modified strains.

The industrial feasibility of PBR is substantially reduced than open Ponds ;In this case,the average total cost of lipid production are 12,73$ /gallon for open tank comparing31,61/gallon for PBR.To improve the industrial effectiveness of algal cultivation,the optimal way is the combining PBR and raceway ponds for biomass production through coupling where higher culture grown as inoculam at a larger capacity but parallel risk of contamination decreases.

FACTORS AFFECTING ALGAL PRODUCTIVITY;-

Lipids and Carbohydrates are accumulated upto 65%of dry weight but with lower biomass recoverable that enhances productivities under abnormal conditions of temperature,salinity,light intensity,nutrients presence.Some of the factors which are not influencing algal productivities are N2depletion,temperature-variation,configuration of the reactor,Osmotic pressure,pH shift,Co2 supplement and irradiance etc..

BASIC CULTURE CONDITION AND NUTRITIONAL REQUIREMENTS;

The major nutrients and bioactive contents are influenced by irradiation& temperature variation mainly based on microalgae strains.These in turn alters the physical properties of membrane allowing disfunctioning regularly in photosynthesis respiration process and membrane transport which in turn affect the biochemical composition and quantity of cellular lipid&fatty acids.

The efficiency of light energy supply becomes one of the limiting factors for outdoor&indoor cultivation. Other than solar irradiation,the fluorescent tubes are normally used to emit either blue or red light spectrum that are essentiel for photosynthesis.

The key factors for uniform sufficient irradiation reflects on operation depth affecting light penetration,& its availability and mixing stimulates the light distribution& uniformity.Temperature changes affects light-unsaturation in membrane lipids.The optimal operation temperature are 16-28 °C.Temperature below 16°C may result in reducing cell growth and falls on photosynthetic deficiency while reaches optimal.

CULTURE MEDIA FOR MASS-MICROALGAE PRODUCTION;-

The output of oil recovery from algal biomass estimated to be as high as 58700 L of oil per hectare

Microalgae production needs to be done on very large scale to make it profitable based on low cost media differes from,culture media laboratory.The success of cultivation depends on culture media developments and evolution of large scale processes and implication of nutrient recycling in biorefineries**.**

10;3 DOWNSTREAM PROCESSING OF MICROALGAE:

Harvesting &Dewatering ;

The simplest & low energy effective downstream processing methods can be enhanced through cultivation of producing mass algae.Harvesting &Dewatering in dilute suspension at concentration lesser than 1g/L (ponds) and 3-5g/L(PBR) are recommended.Dewatering about 20-30% water content is necessary to reduce volume&Weight and to minimize the transporation& downstream cost and to extend shelf -life of algal concentrate.

To get the desired product quality,different methods -Physical,chemical& biological are proposed on which gravity sedimentation& Coagulation are low energy process allowing algae to naturally settle at the bottom.Flotation lifts algae to the surface and some algae float naturally through induced by micro-air bubble.

Dissolved air flotation and with chitosan or hydrophobic adsorbents thicken the materials to 10% dry wt.content (100g/L).Centrifugation produces g-forces between 5000-10000 to separate algal particles out but not recommended as high gravitation forces damage the cell structure of algae and requirement of large capital investments and other operating costs.

For effective dewatering of microalgae, biofloculation was done using biopolymers such as cationic starch or polyAlpha glutamic acid(μPGA) produced by Bacillus Licheniformis that could greatly reduce the cost as it requires little or no energy consumption and easy to operate and as effective as Chemical flocculation,The μPGA has been used to concentrate fresh water Desmodesmus Sp,51 with efficiency increases from 43,8 to 98,2 % as the initial culture pH changes from 7,2 to 3,0. With optimum dosage of 2,5mL /L, flask mixing rate of 150 rpm for 1 min and slow mixing rate of 80 rpm for 2min carrying out the efficiency of 99% have been reported suggesting that high performance for optimal recovery & applicability possible in commercial scale harvesting of microalgae.

Electrochemical Harvesting(ECH)can be used for product recovery safe and cost effective for implementation at large scale.ECH of chlorella Sorekiniam & S.Obliqus investigated to overcome the metallic comtamination then the addition of electrolyte -NaCl increases the recovery efficiency (RE)of of chrolella Spfrom 65.99 to 94.52%with energy consumption of 1.6KWh/kg-1 and no deteriorating effect observed on lipid extraction FA composition whereas S.Obliqus shows higher recovery efficiency of 83% at 1.5A,initial pH9.0 and 6gL-1 NaCl with power consumption of 3.8KWh Kg-1. RE with ECH is comparable to Centrifugation,Filtration,Chemical flocculation but with lower power consumption.The influence of electrolyte will enhance 22%lipid extraction showing ECH, a possible Process to launch microalgae biomass.for biodiesel production.

Harvesting &reactivation techniques based on magnetic nanoparticle(MNPs) is a novel apporoach developed for rapid separation of algal cells applicable to more on Cyanobacteria Microcystis Aeruginosa separation with a efficiency of 99.6%.

10;4 **LIPID STRATEGIES of MICROALGAE OVER BIODIESEL PRODUCTION (Research study)**

Presence or absence of light availablity and nutrients should influence lipid composition ,fatty acids and membrane lipid synthesis(mainly chloroplast).Comparing the strain-C.Vulgaris,the biomass and lipid productivities have shown at cellular lipid content of 38% in autotrophic growth than heterotrophic and mixrotraphic conditions.

Heterotrophic cells of chlorella Zofingiensis fed with 30g/Lof glucose increased the oleic acid from 17.9 to 35.2% TFA as compared to photoautotrophic cells and oils from above species appears to more suitable for biodiesel conversion.Chlorella shows higher total lipid content(0.661 G/liter) when cultured at 0.1 g/liter(lower concn.of urea) but with maximum lipid productivity of 0.124g/L/day.

The main fatty acids present in lipids of chlorella sp.are normally short chain fatty acids(C14-C18) which had been cultivated in industrial scale bioreactors produces 2.4 w/w lipids(calculated as sum of FAME in dry mass).These lipids contain higher amount of neutral lipids,sphingolipids,glycolipids than phospholipids.

The highest lipid accumulation have been achieved with N.Oculta,T.Suecica,L.Galbasa and P.Lutheri as 37.3,23.6,28.3,and 37.2gm resply. with slight reduced cell growth of 0.64,0.49,0.54 and 0.38 g/L culturing under deficiency conditions of 10-65 g/L KNO3,3-7.5g/L NaHPO4 and 2.5 g/L FeCl 3.

The reactor conditions,nutritional manipulations and culture conditions are all effective factors to improve the productivities of microalgae cultivations at optimum photoperiod and light intensity in regards to growth kinetics of above four species.The cultivation of these species at optimum pH,salinity,photoperiod,light intensity and macro nutrients are discussed through Table.....The high cell density and biomass in 5L PBR and 300 L open tank are shown in(**Refe**r **TABLE..6;0)**

This shows the major components in all four microalgal species are tetradecanoic acid(C14;0),Pentadecanoic acid(C15;0),palmitic acid(C16;0),Heptanoic acid(C17;0),Stearic acid(C18;0),Oleic Acid(C18;1)Linoleic acid(C18;2)Linolenic acid(C18;3),Eicosanoic acid(C20;0),Eicodienoic acid(C20;2),Eicosatrienoic acid-ETE(C20;3),Eicosapentaenoic acid(EPA)(C20;5),Eicosatetraenoic acid(ETA)(C20;4), and docosahexaenoic acid (DHA)(C22;6).The synthesised FA in algae are commonly in medium length ranging from 16 to 18 carbons specifically C16;0,C16;1,C18;0,C18;1,C18;2 in green algae and C16;0,C16;1 in brown algae.The total SFA 44.3-63.8%and 30.4-55.03%; monounsaturated fatty acids(MUFA) 6.1-7.0 and 4.2-13.1% and PUFA8.3-22.3% and 1.02-15.2% resply. are obtained in 5L PBR and 300 L tank.

For P.Lutheri in PBR,palmitic acid(34.4%) remains high while both EPA (8.4%) and DHA(6.9%)slightly increased with total SFA(47.9) and MUFA(30.9%) remains comparable with PUFA(18.9%) elevated under optimal condition of light and illumination.

The lipid classes of P.Lutheri cultivated in semi continuous mode with neutral lipids and glycolipids as the major constituents accounted for 57% and 24% of TFA residues resply. with emphasis on EPA(C20:5n-3)and DHA (C22;6n-3).

EPA and octadecatetraenoic acid distributed as lipid fraction in Tetraselmis sp. contrary to absence in Chlorella sp,.EPA and DHA found in higher amount in Amphidimium similar to high presence EPA in other species.These lipids containing omega-3 long chain PUFA finds application in food and aquaculture industries.

Saturated fatty esters(SFE) possess high octane number and superior stability whereas PUF esters has improved low temperature properties.Modifying fatty esters such as enhanced proportions of oleic acid (C18;1)ester can provide above properties together therefore it promotes quality of biodiesel conversion owing to the presence of high oleic acid.Over 65% FA are saturated and MUFA(C16;0,C18;0 and C18;1) are well suitable for biodiesel conforms the EN of FAME with four or more double bonds 1 mol%.

For integrated and optimal bioprocesses,the microalgal residues after lipid extraction and cellulosic materials can be co-digested in anaerobic digester for biogas production and also waste water treatments to balance -C/N ratio in optimum range of 20;1--25;1.

10;5LIPID-EXTRACTION METHODS OF MICROALGAES:-

Once the algal biomass is dehydrated then it is subjected to proceed for lipid extraction process which is relatively difficult process due to the presence of thick wall preventing the release of interlipids.Hence the use of mechanical methods are proposed for other terrestial bearing oil crops unlike algal biomass.There are many pathways available for Downstream processing of microalgae in recovering the oil. To improve algal lipid extraction,the methods like autoclaving,Supercritical CO2 and Ultrasonification are needed for optimization during scale-up which will be discussed later.

MECHANICAL METHODS:-

A simple oil press can be used to press the dry-biomass in extracting the oil.These oil presses used sucessfully for the extraction of oil from seed crops(sunflower,Canola,Olives) but yet not for microorganismes..This is operatable mechanically crushing the biomasses in an oil pressSo algae require drying prior to pressing it and this option is less cost effective.

ENZYMATIC CONVERSION:-

Natural or enzymatic enzymes can be utilized for hydrolysis of cell wall and then water is used as a solventfor fractionation of oil.More expensive enzymes are required but appeared to be less affected by water commercially unpracticed.

CATALYTIC CRACKING:-

The objective of the process is to break down the longer chain molecules into smaller chain compounds which can be further refined the gasoline or other fuels.

SUPERCRITICAL FLUID METHODS:-

SCCO2 -Supercritical CO2 possessing the dual properties of both liquid and gas could be used as a solvent for the oil extraction from algae.SCCO2 is liquefied under pressure and heated to the point where it possess dual properties.SFE is the mass transfer process at the optimum pressure and temperature operating condition where supercritical CO2 is used for fractionation of biodiesel.A biodiesel separation yield of 99.94% obtainable at 40°C under a pressure of 30MPa and a flow

rate of 7mL/min CO2 with a retention time of 90 minutes.

OTHER SOLVENT EXTRACTION PROCESS:-

Extraction using chemical solvents are the most selective method practiced in laboratory scale research owing to its selectivity and solubility nature.Solvents such as n-Hexane,Methanol,Ethanol, and mixed polar/non-polar solvents(MeOH/CHCl4,Hexane/Propanol) are effective on algal strains.

To select proper chemical solvent, the following several issues may be considered for extraction towards commercial scale.

**Solvent toxicity and safety

**additional energy input are considerable for solvent recovery

** additional cost incurred on waste water treatments

**requirement of solvent in large quantity for effective lipid production.

CHEMICAL METHODS;-

Prior to oil extraction,the algae cells require pretreatment in view of cell disruption subjected to undergo Ultrasonification to release oil.Then transesterfication is a well known process of converting algae oil to biodiesel in presence of alkali as catalyst(KOH,NaOH) in which the triglycerides are reacted with methanol or ethanol to produce biodiesel at particular temperature and time.The final product is 90% biodiesel as FAME with 10% glycerol as byproduct and various impuities obtainable as soaps,FFA,methanol etc...

10;5 PRODUCT EXTRACTION AND FRACTIONATION

The cell wall is highly rigid composed of complex polysaccaharides and GlycoProteins with high mecanical and chemical resistance.Breaking cell wall of the algae may require energy to release the intracellular lipids and facilitating the access of solvent into the lipid mixture.Then the lipid move to the solvent phase from which it is separated . A cell disruption technique done with the use of H2O2 and FeSO4 under optimal condition of time permits to accelerate the lipids extraction -twice comparing undisrupted cells.

There are four cells disruption pretreatments methods available such as *Ultrasonification*(US),*Microwave*(MW),*autoclave*(AC) and *Electroflotation by alternative current* (EFAC)reported to be total lipid yield of 5.3-33(MW),7.1-24.8(MW)2.3-15.4(AC)and 3-13.3%(US) resply..Comparing all four methods,EFAC can give the best results and good option for simultaneous microalgae harvesting and cell disruption.The integral harvesting &lipid extraction may reduce the cost of downstream processing.

Two TiO2 photocatalysts(anatase/rutile bicrystalline) and (anatase/brooklin)used for flocculation through injection of aminoclay-conjugated TiO2 into chlorella Sp.KR1 feedstock produces 85%harvesting efficiency followed by UVirradiated at 365nm for 3 hour causing cell disruption by85%.

Bioseparation process include utilising SFE(SuperFluidExtraction) whereas the prior operation consists of fermentation,Extraction,Enzyme pretreatment ,physical fractionation or size reduction.in which SFE operation carried out and then chromatographically separated.

The yield of high value arachidonic acid (ARA) from wet fungal-Mortierella Sp.biomass extraction with Dimethyl ether(DME)with a pressure4MPa.at 40-60°C.are obtainable which are substantially higher than usingCO2 at 30MPa.This shows the extraction of polar&nonpolar lipids could be liquified completely with DME.

A complete fractionation of of valuable compounds can be acieved in a single processing plateform by modifying the extractive condition and solvents thereby SCCO2 is reported to be

obtaining oil rich in μ-linoleic acid (ALA)with a low solvent to dry algae ratio 6:3..The maximum yield is obtained at 60 °C operatable at pressure 30MPa with 0.4Kg/h of CO2 and 5% Cosolvent ethanol which is more selective method than soxhlet extraction.

Direct synthesis or insitu supercritical tranesterification method have the potential to disrupt the cells for extract the lipids in single step with NannoChloropsis Gaditana species.Wet80% moisture and dry cells have been used for direct synthesis of biodiesel through direct tranesterfication with no added catalyst possible using supercritical methanol.The main process parameters are optimised with an initial methanol to dry ratio(10:1vol/wt)and reaction time for synergistic effect(50min)and a temperature at255-265°C to get maximum biodiesel yield of 0.46-0.48 g/g lipids from wet & dry resply.

The study with *Bortryococcus Braunii* shows the usage of compressed CO2 dissolved in expanded methanol increases the selectivity of lipids in biodiesel production as the characterstics of solvents expands in volume and decreases in polarity.This solid phase extraction is reported to be extraction of 21mg biodiesel desirable lipids per ml. of organic solvent as compared to 3mg/ml neat MeOH or CHCl4/MeOH mixture.

CHEMICAL CONVERSION;-

.One of the most popular method is the Ultrasonification Technology applcable for easier biodiesel conversion through transesterfication process from WCO.To handle in efficient manner,lipid strategy are throughly studied as mentioned earlier and the following approach.is obviously needed for effective processing of WCO and algal biomass.

10;6 PRETREATMENTS OF HIGH FFA FEEDSTOCKS;-

(for Waste Cooking Oil(WCO/Microalgaes etc..)

Many of the feedstocks contain large amount of FFA leads to the formation of soaps with alkali catalyst that inhibit the reaction of separation process between ester & glycerol.Soaps may allow emulsification causing less separation and produces water that can hydrolyse the triglycerides contributing more soap formation.At this stage,the catalyst concentration is no longer available to accelerate the reaction.

If FFA level exceeds **>1%** then it is essential to add extra alkali catalyst to neutralise FFA through soap formation leaving behind to act rest of the catalyst.

The amount of additional catalyst can be calculated by following formula as indicated below:-

(1 mol. of catalyst to neutralise 1 mol.of FFA)

NaOH..... .....[%FFA](0.114)+1%

KOH ....[%FFA](0.197/0.86)+1%

Sodium Methoxide.....[%FFA](0.190)+0.5%

FFA levels as high as **5-6%,**the above dosage may be recommended and depend on the basis of type of emulsifier presence and especially true with non-availability of water. If FFA level is between 2-3% then the trace amount of water may be considered.

If Feedstocks containing higher amounts of FFA between **5-30%,**then the addition of extra catalyst is not recommended owing to the gel formation of soap.Additionally,it tends to separate glycerol layer from ester and finally leads FFA into waste product subsequently low yield of biodiesel possible.

There are **Few Techniques** available to convert FFA into biodiesel:-

1**)** **Enzymatic method** (already discussed in previous chapter).

2) Glycerolysis;-

Adding Glycerol to feedstock and heated to 200°C in presence of ZnCl2 as a catalyst allowed reacting with FFA to form mono and diglycerides.This describes FFA level decrease in a batch process using animal fats.The drawback of above reaction is high temperature process and reaction is relatively slow whereas the advantages of the process does not require methanol during pretreatments thereby water is formed and removed through evaporation from the reaction mixture.

FFA+ Glycerol>>>>>>>monoglycerides +H2O

3) **ACID CATALYSIS**;-

This technique involves H2SO4 used to catalyse esterfication FFA into alcohol ester which is relatively fast and completed in 1 hour at 60°C. however,transesterfication of triglycerides is very low taking several days normally to complete.

FFA+Methanol>>>>>>>>>>>>>Methyl ester +H2O

Contrarily,the reaction heated to 130°C could accelerate reaction within 30-45 minutes but. having the problem of H2O production which in turn inhibits the reaction at the final stage.

4)**ACID CATALYSIS COUPLED WITH ALKALI TREATMENTS**;-

Acid used for FFA conversion into methyl ester in order to accelerate catalyst and make relatively fast as pretreatments methods and poses water accumulation.When FFA reduces lower than 0.5%,an alkali catalyst is added to convert triglycerides into methyl esters alongwith transformation of FFA feedstocks is considered to be during pretreatments stage that leads to not limiting the reaction of molar ratio of alcohol to FFA as high as 40:1 recommended.

The disadvantages is that more energy required to recover excess methanol.Another approach is to let acid catalysed esterfication to proceed for inhibit water formation and allowed to boil off alcohol and water.If FFA is still too high ,addition of methanol can be added ,if necessary and then acid catalyst can be used to continue the reaction and to be repeated for multiple steps for less usage of methanol and allowed to settle for few hours .This leads to methanol-water mixture rising to the top and can be removed .This can be repeated to continue the reaction(Patent-EarlHammond,ISU) with the addition of methanol and acid.Alternatively,using fluids such as glycerol and ethylene glycol may be recommended to wash the water from the mixture.

10;7 BIODIESEL PRODUCTION PROCESS

BATCH PROCESSING;-

Batch processing is considered to be simplest method producing alcohol esters where alcohol to glycerides ratio reported to be 4;1 to 20;1.The normal ratio is 6;1 opertable at 65°C and usage of NaOH ranges from 0.3-I.5%by weight of oil.At the beginning,more mixing is required to mix properly oil,catalyst and alcohol followed by less mixing at the end of the reaction permits inhibiting the glycerol to separate from ester-oil phase and reported to be 85-94% in single step

In some cases uses a two step reaction where glycerol removal is possible between the stages to enhance the final reaction upto 98%.Higher temperature and higher alcohol-oil ratio can accelerate the reaction towards completion within 20 minutes to 60 min.

This shows a process flow diagram of typical BATCH system where oil is charged followed by a catalyst,methanol and agitated during reaction time then stopped for settling esters-glycerol phase followed by alcohol and salts removal by gentle washing with acidified hot water then dried.The finished biodiesel is transferred to storage and glycerol stream may be neutralised before refining.

Yellow grease ,animal fats for examples,having higher FFAsusceptible to modify with acid

esterfication vessel and storage for acid catalyst as indicated in system process flow.The feedstock is dried below 0.4% ,filtered,charged into tank where methanol mixture and H2SO4 added & agitated.The same temperature are used otherwise may be pressurized or co-solvent is added to avoid the glycerol formation.If it is two stage process the stirring is done until methanol phase separation removal cleared.Then stirring phenomenon decides upon requirement of methanol and H2S04 addition.Once the equilibrium is reached during methyl ester conversion The multiphase methanol-water-acid mixture separation can be done by settling or Centrifuge.The remaining mixture converted into soap by neutralisation with excess base catalyst as described in Batch stage process.

CONTINUOUS BIODIESEL-PROCESS SYSTEMS;-

As described ,the plug flow reaction system shows a popular variation batch process as the use of continous stirred tank reactor (CSTR)in series.CSTR permits to allow a longer residence time in CSTR-1to achieve the reaction time to a greater extent .When the glycerol is decanted then the reaction in CSTR-2 is rapid with 98% more completion process.The specificity and design of CSTR is based on through mixing input system ensures largely constant medium composition and increases the dispersion of glycerol in the ester phase as a result of extending phase separation.Mixing carried out either from pump to initiate esterfication and ends up tranesterfication through pipe reactor with a provision of mixing in the reactor.This results in moving the reaction mixture in continuous plug with axial directed mixing ,called as Plug flow reactor(PFR)directs the functioning chain series of small CSTR together.

In conclusion,this continuous system require short residence times as low as 6-10 min for completion of reaction.PFR enhances decantation of glycerol in middle of the stages.The whole system operates at elevated temperature and pressure to increase the reaction rate.

NOTE on FREE FATTY ACID SYSTEMS;-

The maximum amount of FFA for a base catalysed system ia about 2% weight of oil but preferably 1%is required.Caustic stripping is normally carried out toi stripp of Na soaps through Centrifuge or water extraction in which some triglycerides are remov soaps and these can be sent to transesterfication unit for further treatment after drying.Here,acid esterfication play a role to avoid FFA and waste water removal.

The requirement of high alcohol to FFA ratio is 20;1 and 40;1 as summarized depends on direct esterfication requiring large amount of acid catalyst.The esterfication of FFA with methanol produces byproduct water

supposed to be removed but afetr drying then the resulting mixture of ester and triglycerides can be used directly in conventional base -catalysed system.The water can be removed by vapourisation ,settling or centrifugation as methanol-water mixture.

The countercurrent continuous flow system will wash out the water alongwith the existing stream of acidic methanol.Upon using H3PO3 ,as the initial catalyst in acid catalysed system ensuring neutralisation with excess of KOH and repeated the process upon completion.Then insoluble potassium phosphate is recovered ,washed and dried ,used as a fertilizer as described .

An alternative procedure to process high FFA feedstock to hydrolyse into pure(Product) FFA and glycerine through counter-current system reactor using H2SO4 or Sulfonic acids streams where typically the output is obtainable the above pure Product.The pure FFA are then acid esterfied in another counter-current reactor to transfer into methyl esters then It is neutralised,dried resulting

90% yield.possible with acid-resistant process equipments.

As described **,** another alternative approach can be performed where high FFA feedstocks uses base catalysts forming soaps could be rezcovered then oil dried using conventional base catalyst system.This strategy can not lead a economical status since soap stock is discarded and effective price of feedstock increases due to oil presence and may be converted into esters using acid catalysed reaction.The main problem with this strategy is the presence of large amount of water to be removed before biodiesel meets specifications.

FIXED BED REACTOR SYSTEM:-

This system uses insoluble base,$CaCO_3$ as a catalyst where water is removed and output is obtainable with clear ester separation from glycerol and avoids the problem of using high FFA in variation through fixed bed catalysed reactor system.

NON-CATALYSED -BIOX PROCESS SYSTEM;-

BIOX process system are designed to overcome slow reaction owing to extreme low solubilty nature of alcohol in TG phase.This process uses a co-solvent-THF(TetraHydroFuran)to solubilize methanol catalyses the fast reaction in the order of 5-10 minutesleaving no residues either in ester or glycerol phase.This system is opertable low temp.at30°C since the boiling point of THF is closer to methanol and these two solvents may be recovered in single step.

In another approach,MTBE used as aco solvent and leads to obtain clear ester-glycerol separation with free ofcatalyst and H20.But it requires large volumal equipments duye to the usage of additional volume of cosolvent for the same quantity of same solvents.

This shows Biox cosolvent process system are subjected to air-toxic (EPA) and hazardous nature with slow down the time required leak proof equipment for recyclage and recovery of methanol/cosolvent completelyremovable from glycerol and biodiesel.

NON CATALYSED -SUPERCRITICAL PROCESS SYSTEM;-

This sysytem dipicts , a conception of configuration for a supercritical esterfication process .A fluid or gas is subjected to temperature and of pressure where number of unusable properties exhibiting in excess of its critical-point such as distinct liquid and vapour phase confined tofluid phase. A non catalytic process of undergoing ester production is the use of high (42:1) alcohol to oil ratio under supercritical conditional parameters (350°C-400°C and < 80 atm or 1200psi)enhances the completion of reaction within 4 minutes .Solvents having OH group such as water,primary alcohol behave on the properties of super acids.The greater energy consumption leads to higher capital costs and operating costs. contibuting alongwith feedstock estimated to be 7% product cost.

10;8 POST-REACTION PROCESSING:-

The objective of the step is to recover ester phase from the reaction mixture include ester/ glycerol separation,ester washing,ester drying and other ester treatments and additization.The FAAE and glycerol are sparingly soluble based on density difference between the phases and presence of methanol in one or more phases affects the solubility of ester in glycerol and vice versa.

Hence ester washing step uses to neutralise the residual catalyst and subsequently remove any soaps formed during esterfication and also to remove residual free glycerol and methanol.To

meet the specifications ASTM,ester drying is required on the amount of water present in final product.In addition,other treatments may be used to reduce colour bodies in the fuel,removing S or P or glycerides etc..

Additization is the addition of material enhances specific functionality of one or more fuel properties.The examples are cloud point/pour point additives,antioxidants or any stabiity-enhancing agents ...

Glycerol phase having the density of 1.05G/cc or more that depends the amount of methanol,water & catalyst present in glycerol whereas the acid alcohol ester have the density of 0.88g/cc .The a bove criteria resolves two phase separation through simpler gravitational techniques.

It is recommended that slow mixing is necessary at the beginning to slow down the time required for phase separation contrary to several hours.If intensive mixing is done for the entire reaction,the glycerol can be dispersed in very fine droplet to coalesce into distinct glycerol phase on this phenomenon.

This can be explained as neutral pH will coalesce quickly the glycerol phase thereby,the requirements of total catalyst will be minimized. It is important to deal the final mixture having significant quantities of mono,di,triglycerides that lead to the formation of emulation layer at the glycerol inetrface.This signifies a net loss of product unless it is recovered and separated otherwise the entire process should return in order to meet the specifications.

In addition to that,the esterfication is run with excess of alcohol to ensure attaining the complete higher reaction then the residual alcohol acts as a dispersant for the ester into glycerol phase and for the glycerol into the ester phase requiring additional biodiesel processing to conform the standards.

PROCESS EQUIPMENTS FOR ESTERS/GLYCEROL SEPARATIONS;-

There are three types of equipments used for Ester/Glycerol separations.They are :

a)Decanter System b)**Centrifuge System** c**) HydroCyclone System**

DECANTER Separation system works on the basis of variation of densities and residence times.For a small batch system,the separation will be last about 1-8 Hours.More the flow rate of product mixture then the size of the unit will be bigger in terms of rater tall or narrow to allow physical separation between ester and glycerol phase and works on ratio of L/D between 5-10.It again depends upon the temperature factor affecting solubility of alcohol in both phases and viscosity of two liquids.Higher temperature causes residual alcohol to flash and restricting flow of ester phase.whereas low temperature increases viscosities of two phases and tends to slow down the Coalescence.The presence of emulsion is indicative of mono and diglycerides forming between the phases and these must be recovered in continuous operation system having the provision of not filling the decanter.

CENTRIFUGAL SYSTEM;-

Centrifuges are mostly operatable for phase separation in continuous plants.The higher speed system creates an arificial, high gravity field exerting centrifugal effects that separates two phase system of ester-glycerol.These can be operated also at smaller capacity where use of batch centrifuge in a continuous process system require a surge tank to match batch-cycle time.

HYDROCYCLONE PROCESSING;-

It acts on density based separation and operates on Bernoulli's principles of implying pressure acclerated in an incompressive flowing system.The basis is similar to centrifuge with the heavier

material pass towards the wall and downwards and the lighter material forced towards center and upwards...The liquid mixture enters into the hydrocyclone at a moderate prssure (125psi) then the system pressure decreases and velocity increases as the liquid passes for wider to the narrower part of the inverted cone. .

The rapid reduction of pressure in device enhances flashing of volatile liquid such as alcohol,disrupting or inhibiting the separation process.Hence excess methanol should be removed from the system before introducing into the HydroCyclone.These are at the experimental stage in Biodiesel applications.

ESTER WASHING;-

The objective is to remove the soaps formed during transesetrfication as described earlier.The water is used acts as a medium for addition of acid to neutralize the remaining catalyst and to remove the product salts.The residual should be removed before wash stepsthrough other processes with wash water.Generally use of warm water at 120-140°C prevents precipitation of esters and delaying the formation of emulsion with gentle washing results rapid and complete phase separation.

Softener water is slightly acidic in nature eliminates Ca &Mg contamination and tends to neutralize base catalyst .Similarly removal of iron-Fe and Cu eliminates a source of catalyst that decreases the fuel stability.The resulting phase separation is typically clean between ester and water.However the equilibrium solubility of water in ester is higher than B100 therefore it remains even after washing steps.

To solve above problem,Vaccuum Driers and Falling Film Evoporaters are mostly used to remove water content where system is operatable under low pressure allowing water to evoporate at lower temperature in the former case whereas in the later case, the product is in direct contact with high heating surface and rate of evoporation is higher at reduced pressure results more water removal and care should be taken to avoid darkeneing of fuels while in contact with heating surface.This shows the indication of polymerisation of PUME(poly unsaturated methyl ester) as darkens.

Molecular sieves or silica and removal Gels can also be used for the esters containing large amounts of water owing to the passive nature but the disadvantages is that the units must be regenerted periodically.

OTHER ESTERS TREATMENTS;-

Magnesol considered to be adsorbent and tends to adsorb hydrophilic materials such as glycerol,mono,Diglycerides.To conform ASTM norms,an activated carbon bed is used to remove excessive colour in biodiesel.Additionally,Vaccuum distillation has added benefits of deodourisation and removal of other contaminants than elimination of S compounds. Then the filtration play a essential rôle while biodiesel leaving the plant with 5micro -grams ensuring no contaminants than feedstock of 100micro grams .Then it has been suggested that fuels to be cooled before filtration as it tends to crystallize of saturated esters results this will lower the cloud point.

ADDITIZATION OF FUELS ;-

For the reason of improving the performance against Lubricity,detergency,Oxidative stability,Corrosion resistance,conductivity and many other properties,additives are added to treat above characterstics in biodiesel where the technology is less advanced and it needs to be improved as it contains large number of double bond molecules susceptible to less oxidation stability than petroleum diesel.

TREATMENTS AND RECOVERY OF SIDE STREAM;-

The Non-esters side streams necessarily to be treated as a part of overall biodiesel process.

1) Excess alcohol(methanol) to be recycled within system
2) glycerol as a byproduct
3) Waste water stream from the process

Methanol is to recycled since required in excess amounts for transesterfication and saves the input costs and eliminates its emission to the environment owing to its nature of inflammable and toxic properties,

Glycerol is partially refined and recovered as a co-product and estimated to be 10 % by weight of input reactants.Waste water related to operating cost of the plant again depends on water consumption of its treatments.

METHANOL MANAGEMENTS ;-

Methanol is more soluble in esters but not finally miscible and comparatively lower soluble in fats and oils(approx,10wt/wt % at 65°C in tallow).Methanol is fully miscible with H2O and withGlycerol.This leads the solvent-methanol prefering two-phase system.Moreover,the low solubility phenomenon in fats and oils enhances limited solubility phase of overall transesterfication reactions.

In addition,methanol having relatively low boiling point ,64.7°C signifies fairly volatile and recovery- recycling methanol is necessary and these can be removed from oils ester by flash evoporation.

When the two phases are glycerol and esters remains then the methanol tends to distribute between the two phases at the ratio of 90:10wt/wt%.and signifiying the distribution approx.60:40wt%.between the two phases.If methanol allowed to stay in phase separation that acts a stabiliser and delaying the rate of gravity separation.Hence this solvent is to removed .
before separation and these could be recovered using distillation either conventional or Vaccuum or partial single flash recovery process.An alternative method is the falling Film Evoprative distillation could be adoptive.

GLYCEROL REFINING;-

The recovered glycerol after the reaction consists of residual alcohol,catalyst residue,carry-over fats and oils and some esters.In addition to above,the glycerol from certain feedstocks may also contain phosphatides,S compounds,proteins,Aldehydes and ketones and other insoluble matters(Dirt,minerals,bones or fibers).Hence, refining steps could be performed through following methods;-

a)Chemical refining;-

There are several factors to be considered in chemical refining.The base or acid catalyst tends to concentrate in glycerol phase and needs to neutralise it to form salt precipitation.The soaps are formed during reaction parallel to this phenomenon ,must be removed by coagulation and precipitation with AlSO4 or Ferric Chloride and needs to complete the process by Centrifugal separation.Here the control of low pH leads to glycerol dehydration and higher pH tends to polymerise the glycerol molecules.These can be bleached through Activted Clay or earth.

PHYSICAL REFINING;-

The first stage of refining process is the removal of fatty particles,insoluble matter or precipitated solids through Fitration and or Centrifugation.This removal may require pH adjustments and the water is removed through evoporation & whole processing sets at 150-200°F then glycerol is

obtainable in less viscous form and stable.

The final purification step can be completed using Vaccuum distillation followed by steam Injection and activated carbon bleaching.This is the well establish technology but it has disadvantages such as intensive energy requirements, high capital cost etc.;Then Vaccuum Distillation is best suited of the operation > 25 Tons/day.

In the case of ION exchange resin separation process,the minerals,catalysts and other impurities may be removed through use of Cations,Anions and Mixed Bed Systems.The glycerol is first diluted with softened water at a conc.15-35%before subjected to pass through resin bed system followed by H2O removal using Vaccuum distillation or Flash drying to get a concentrated partial refined Glycerol.

This system is best suited for smaller capacity plants and operations whereas the disadvantages are reported to be the fouling of resins causes by FA.

WASTE WATER MANAGEMENT DURING GLYCEROL-REFINING:-

Approximate 1 gallon of water is required for one gallon of ester wash.All the process water must be softened to eliminate Ca,Mg and Na salts and also Fe and Cu metals.The ester wash water shows fairly high biochemical oxygen demand(BOD) from the residual fats:oils,esters and glycerol.

As a result of regeneration of Ion exchange resin process,large quantities of low salt waters are produced during glycerol refining.In addition,the use of water softener,Ion exchange resin and cooling water system produces moderate dissolved salts necessarily to conform the norms before dispose towards the local municipal sewage plants.

The methanol is to be recycled and recovered at the maximum level internally before present in waste water disposal that leads to cost savings and easier process access permission from the pollution control board.

11;0 CO2 SEQUESTERATION

METHODS FOR HARVESTING ALGAL BIOMASS AFTER Co2 SEQUESTERATION:-

The whole harvesting process of algae biomass can be done for bioenergy generation for economical criteria base on scale-up process,size and density of culture .This could be performed through various harvesting techniques like Centrifugation,Flocculation,Gravity sedimentation,Filtration ,Elctrophoresis etc..The high harvesting performance can result through Centrifugation process but it is cost and energy intensive.

Flocculation is the process of heavy particle aggregation that makes settling easy.This can be done through NaOH or through Chemical flocculants such as ferric chloride(FeCl3) or Aluminium Sulphate(Al(So4)3.

Gravity sedimentation is the process of sedimenting the algae particle highly dependent on size& density of the biomass and can be increased using flocculants.

Filtration involves allowing the algal biomass to pass through screen of particular pore size whereas the problem associated with the filtration is not permitting the higher concentration of culture presence..

Electrophoresis process is the process where application of electric current result in inducing the electrostatic field forces charged the algal cultures for moving out of the solution.

RECENT DEVELOPMENTS OF CO2 SEQUESTERATION IN LARGE SCALE:

In China ,ENN group developed a technology on fixing CO2 from coal production resulting biofuel conversion possible through sequesteration..The equipments are installed for microalgae cultivation in a pilot plant scale where CO2 absorption is done followed by oil extraction and

biodiesel production.This equipped system can absorb 110 tonnes of CO2 capable of producing 20 tonnes of biodiesel and 5 tonnes of protein per year.Based on this principles,ENN group subsequently demonstrated a project in Dallate(Mongolia) in 2010 utilising microalgae by absorbing CO2 emitted from flue gas of coal derived methanol and coal derived dimethylether(DME) production equipments.This in turn will produce biodiesel and feed finally.

Sweden has set up a pilot plant in Eastern Germany utilising microalgae to absorb green house gas emission from coal fired power plant then pumped into a plastic tank containing broth where algae is cultivated.

Highlighting the algal process ,the conclusion is that Algal is efficient in nutrient removal and has more lipid content whereas macoralgae is rich in carbohydrate.Algae strain screening are considered to be most important for its optimization of technologies through combined processing.).

11;0 WASTE WATER REMEDIATION;-

The aim of the study is to develop technologies adpotable for large scale production using oil rich algal biomass from waste water***;-***

DUAL PURPOSE MICROALGAE & BACTERIA BASED SYSTEM FOR PRODUCTION OF BIODIESEL AND CHEMICAL PRODUCTS WHILE WASTE WATER TREATMENTS:(research study1)

The research studies was conducted towards biorefinery design upon integrating municipal waste water treatment together with use of sea water supplemented with anaerobically piggery waste for cultivation of Arthrospora (spirulina),recovery of oleagineous microalgae and producing biogas,biodiesel,biohydrogen,and other high value added products.

According to Life cycle Analysis(LCA)studies,these type of system could help to improve the competitiveness of biofuels production since it is not competing with fresh water resource in agriculture and add-up value into the wastewater treatment itself..Isolation of Cyanobacteria or Consortia & other species related to population dynamics in mixed culture. are studied that depends on various factors such as biomass,lipid productivity of individual strain,waste water characterstics ,resource of strain habitats, and climatic conditions in treatment plants etc..

Several alternative technologies were also focussed to harvest the biomass aiming at a low cost such as cell immobilization,biofilm formation,flocculation,bio-flocculation etc..offering a new strategies and cost effective opportunities and competative one.This contributes overall enhancement of the economic viability of whole integrated system of biofuels***.***

WASTE WATER FOR BIOMASS FEEDSTOCK PRODUCTION THROUGH CULTURES OF MICROALGAE-CHLAMYDOMONAS REINHARDTII.:-(Research Study2)

The aim of the study is to develop technologies adpotable for large scale production using oil rich algal biomass from waste water.The strain -***Chalmydomonas Reinhardtii*** grown in artificial media together with the presence of waste water in three different stages of treatment process namely-influent,effluent,centrate etc..containing different levels of nutrients.The specific growth of algae was monitored over a period of 10 days in several cultural substrates and the biomass evaulation is done in proportion to removal of N& P during influence of CO2 & pH .This shows influence of growth possible in presence of optimum nutrients level but denotes inhibition of algal growth as contributed by the higher level of nutrients in the beginning stage.

The studies shown that optimal range pH7.5 with air injection and moderate CO2 promotes algal growth whereas high degree of CO2 inhibits algal growth through shift of pH. resulting bio-oil yield of 2.Gm/liter/day in bio coil signifies the above strain obtainable with dry biomass yield of 25.25%(w/w) . FeCl3 was found to be effective flocculants helps the algae to settle

for easy harvesting & separation from the culture media carried out on photobioreactor resulting as 55.8 mg/L nitrogen and 17.4mg/L Phosporous effectively removal from centrate.

BIODIESEL PRODUCTION FROM INDIGENEOUS MICROALGAE GROWN IN WASTE WATER¿-

The main aim of the research study is to reduce the total N2 content 55.4 to 83.9% and the coliform removal was as high as 99.8%. Sustainable biofuel is possible from microalgae grown in waste water namely Desmodesmus sp.,Oscillatoria and Artrospira species that has shown higher biomass conc. of 0.58g/L and the other two native mixed culture reached 0.45g/L resply.This shows highest lipid content and FAME yield .

Upon Treating through Ozone,this could be used as combinative method for harvesting and reducing FAME unsaturation since it exhibits greater oxidative stability due to its higher degree of saturation.

MICROALGAE CULTURE FROM ANAEROBIC DIGESTED WASTEWATER THROUGH MPBR;-(Research Study 3)

The study was focussed on microalgae cultivation in Novel membrane photobioreactor(MPBR) fed with anaerobic digested waste water (ADW) using several microalgae species such as Scenedesmus species -S.dimorphus,S.Quadricauda,Sorokiniana,and Chrolella Vulgaris ESP-6 evaluated for efficient removal ammonia and Phosporus during 9 days incubation period.

The study shows MPBR utilizes waste water schemes without pretreating algaes for successful production of biofuels and protein feed.

AQUACULTURE WASTE WATER AS GROWTH MEDIUM FOR BIOFUELS AND BIOMASS:-

Platymonas Subcordiformis was used as growth medium using aqua-culture waste water for biomass and biofuels coinversion utilisable in various stages containing different levels of nutrients.The above species removes nitrogen and P with an average efficiency of 87-95% and 98-99% resply. and the algal density increased by 8.9 times than initial level.

LIPID PRODUCTION FROM NANNOCHLOROPSIS Species;-

This species represents as a genus marine microalgae having photosynthetic efficiency,capable to convert CO2 and store the lipids mainly in the form of Triacylglycerol(TCG) and omega-2 long chain PUFA and EPA.

MICROALGAE BASED TECHNOLOGY GREEN PROCESS FOR BIOFUELS PRDUCTION AND WASTE WATER REMEDIATION;-

The highlighting featuring of microalgal production is the limited investment requirement for biofuels production & waste water remediation.

2) Algae is efficient in nutrient removal 3)Microalgae expressing more lipid content 4) Future perspectives of algae based technologies (bio oil efficiency is improved by 41% through combination. of chemical & biological process optimization).

Algal mediated waste water treatment and CO2 sequesteration could provide a status against clean water,air and energy and effectively stimulates treating complex contaminants such as heavy metals,polyclinic aromatic compounds etc..

These algae based technologies can be considered as green process and selling of algal bio oil becomes acceptable (2$/L)than 1§ per L in next decades contributing 75% of markets.

Waste water are considered to be one of best options for sustainablity,zero emissions production of biofuels as sourcing nutrients for biomass production than potable water or sea water remarkbly adds up the cost of biomass.

12;O UPGRADING ROUTES FOR BIODIESEL PURIFICATIONS:-*.,*

Deoxygenation process appears to be promising as produces low C diesel in comparison with Cracking and esterfication.Cracking is not more attractive due to higher yield of light C(C1-C4) and short alkanes (C3-C15)with lower densities.For this reason,silicates,Alumina,Zeolithes and fluid cracking catalysts are typically used for this reaction.Hence,the fuel properties of renewable diesel having good cetane number,obtanaible by catalytic deoxygenation.So designing new catalytic system and to optimize the process condn.could maximise the yield of unsaturated hydrocarbons and alcohols.This pathway can be considered for produce of high value chemicals.On the other hand,deoygenation process,can not be focussed for the biodiesel production owing to the nature of extra steps involvement,increase in capital and operational costs etc..

BIODIESEL PURIFICATION;-

Tranesterfiction of triglycerides can be carried out to produce biodiesel as FAAE(fatty acid alkyl esters) then the purification step comes into reality to separate glycerols as byproduct from two phase of biodiesel via gravitational settling or centrifugation. towards the removal of vegetable oil,alcohol,catalyst,soap and FFA etc( )There are different categories of purification methods such as Equilibrium-based ,affinity based,membrane based,solid-liquid and reaction based reaction etc.;Hence proper combination of purification methods are required usually to obtain robust biodiesel purification technology.

1;1 EQUILIBRIUM-BASED SEPARATION PROCESSES;-

Absorption,distillation,SFE and liquid liquid extraction(LLE)are some of the examples of procesess.Absorptionis commonly utilized for separating particles and impurties from gaseous mixtures*.*

1;2 ***DISTILLATION****;-*

Conventional distilation is the mostly common method used for separation of volatile compoundsfrom heavier substances. in a liquid mixture.There are different techniques include conventional distillation (normal/Vacuum and steam distillation),Azetrophic(*distillation,extractives & molecular distillation(MD)) etc..In the case of MD,these may be carried out under high vacuum, and the molecules freepath is longer than evporation and distance of condenser surface , therefore these evoporated molecules without deflect on collison with foreign gas molecules results in higher separation yield of biodiesel from waste cooking oil and possible to achieve 98% separation at a evporater temperature of 120°C..*

1;2 LIQUID -LIQUID EXTRACTION;-

SCCO2, a solvent extraction process ,most commonly used to purify biodiesel encompassing all techniques developed for wet washing.The use of deionized water is being practiced to remove soaps,catalyst,alcohol and other contaminant of biodiesel enhances the purification step as volume and temperature of water considered as a key factor..Consequently a superior purification can be expected using higher water volume at elevated temperature with a ratio of 1;2(H2O/biodiesel) for 20 minutes decreased glycerol content 0.09331-0.09%.

More purification is possible with acidified water followed by water wash.The most common acids are used prior to washing are phosphoric acids,H2SO4,HCl etc.;reported to be hydolysis of soaps into FFA and subsequent decrease in emulsification tendency.This phenomenon can be performed via Vacuum-flask-evporation,Hot-air-bubbling,other-water adsorbents ,convective heat drying anhydrous salts etc..

In view of water consumption,several studies are reported to be 3-10 Liters of H2O required

for every 1 Liter of biobiesel resulting an equivalent volume of water needs to be treated.Then a novel method is proposed to reduce the water consumption by approaching MicroFilration (MF)accompanied with sand filtration carbon(AC) and showed 15%lower water consumption through dilution-rate with make-up water to purify biodieselcontaining 1000ppm in the final product .

1;3 SUPER FLUID EXTRACTION (SFE);

SFE is the mass transfer process at the optimum pressure includes shorter purification time,no water consumption, and no waster water production ,temperature operating condition at which supercritical CO2 is used for fractionation of biodiesel.A biodiesel separation yield of 99.94% obtainable at 40°C under a pressure of 30MPa and a flow rate of 7mL/min CO2 with a retention time of 90 minutes.

2;1 AFFINITY BASED SEPARATION PROCESS;-

Ion exchange &Adsorption are the most specific example of separation processess also known as DRY WASHING methods.

An appropriate adsorbent is used selectively to adsorb on its surface from the liquid phase.These methods offers several advantages over wet washing including shorter purification time,no water consumption, and no waster water production ,smaller unit sizes etc.. resulting biodiesel having acceptable water content limit less than 500 ppm conforms ASTM standards***.***

ADSORPTION;-

This is the process of adsorbing to a solid surface by ions,atoms or molecules(adsorbents)from a substance (mostly liquid or gas).During adsorption,the component penetrates or dissolves in the bulk of adsorbent where surface adhesion occurs.Adsorbents are natural or synthetic materials of amporphous or microcrystalline structure owing to the basic or acidic adsorption where polar substances such as glycerol and methanol can be adsorbed and filtered out of biodiesel.

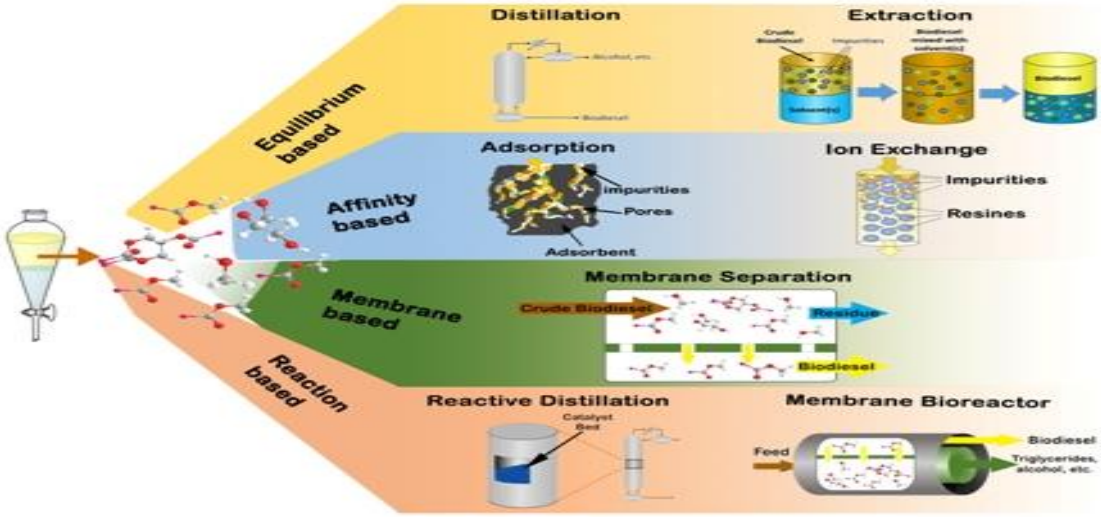
The adsorption process for purification can be classified and based on 1)Silica-Adsorbents (Magnesol and Trisyl),biobased adsorbents-(LC substrates) and activated compounds include activated fiber,AC and activated clay etc...

SILICA BASED ADSORBENTS;-

Silica gel,zeolithes and molecular sieves are the industrial adsorbents used for purification.Silica gel owns a hydrophilic surface due to the presence of hydroxyl group making it proper adsorbent for water,alcohol,and other polar molecules and shows promising potential for purification at room temperature for removal of glycerolfrom WCO.This can be achieved by using crushed silica -gel and sieved to 10-40 mesh with 0.1-0.2 % glycerol at a space velocity of 3-11cm/min and reported to be 0.13 gm of glycerol per gram of 1-1.5mm particle size silica adsorbent in a fuel capacity bed.

Magnesol is commonly available silica based adsorbents ,commonly based on inorganic matrix MgCl2.and anhydrous NaSO4 offers a great potential for purification owing to selective adsorption of hydrophilic impurities of crude biodiesel.

Hence biodiesel needs to be thoroughly mixed with 1% Magnesol powder and 2% silical gel for certain duration before undergoing filtration to remove adsorbents in the final stage.This shows a promising results in terms of removal of soap,methanol,and H2O contents having the values of 1670ppm, 2.13% and 1300mg/Kg resply. and decreased the values to 60,85ppm,0.19%and 500ppm resply.



BIOMASS BASED ADSORBENTS;-

Corn starch,rice strarch are having polyhedral structure whereas potato and cassava starch having ellipsoidal and semispherical structure resply.The dry washing can be performed using admixing 1-10% adsorbents for 10 min at RT with 150 rpm.followed by filtration using filter paper and shows the results as decrease in acidity index and a removal of 0.13% free glycerol and decrease in turbidity while using 5% potato starch and 1-2% cassava and &1% RICE starch.

Rice husk ash(RHA) showing higher performance in purification owing to the presence of higher silica content and the presence of meso and macropores in its structures considered to be acceptable than using acidified water (1% H3PO4 and 1% Magnesol.)

ACTIVATED COMPOUNDS;-

Activated fiber and activated alumina are the most commonly used adsorbents owing to its physico-chemical properties such as large porous volume & high surface area produceable from carbonaceous organics(charcoal,wood,petroleum cokes,coconut shell etc.

AC purification leads to better biodiesel yield (91.50%-93.75%) with respect to water washed product (86-89%) on both the feedstocks(SCO& SFFO)-Spent fish frying oil.

ION EXCHANGE RESIN (IEC)METHODS FOR DRY-PURIFICATIONS;-

This is the process of exchanging the ions between the solution and a solid exchanger phase due to the affinity by electroststic force and the functional group on the surface.This is based on functionality & density of charge structure to strongly acidic cation(**SAC**),weakily acidic cation**(WAC),**Strongly basic anions(**SBA)** and weakly basic anios resins**.(WBA)**

Among the resins,SAC are commonly used for dry-washing purifications methods.In order to choose proper IEC,the structural properties (degree of cross linking,porosity;particle size)exchange capacity,stability,strength & density of charge are to be considered .

PD206 & BD 10 dry cationic resins may be used for purifications of biodiesel from WCO and rapeseed oil where soap and glycerol removal performance is achieved in contrary to methanol.The **LEWATIT GF202** comes into reality having a great potential for CH3OH removal alongwith the capacity in decrease of soaps and glycerol content in biodiesel. The recyclage of this resin shows the conveniency by using 15 cm bed of Lewatit GF202 in a column of 30 cm length with 5cm diameter circulates on a constant flow rate of 236 cm3/hour.This shows a positive impact in decrease of acidity and viscosity comparing water washing methods although the final product is not clear with

Na & K content.

The other industrially available resins such as **T45BD & T45BDMP(Thermax)** alongwith **BD10(Dow Chemical**)shown the better results in terms of filtration,Adsorption and soaps removal by glycerol affinity etc.;during resin process. In view of removal of Na soaps,the resins showed better performance compzred withnK soaps in proptions to decrease in particle size of resins.

It can be concluded that IER is particularly more effctive in removal of metal compounds in the case of heterogenous -transesterfication catalysis process where metals are leached out of a solid catalyst.

SOLID LIQUID SEPARATION PROCESS IN BIODIESELPURIFICATIONS;-

This process is limited to filtration mechanism mostly after heterogeneous transesterfication or by a dry-washing methods where soap removal is possible in presence of methanol acting as Co-solvent and tends to precipitate upon this solvent.This is particularly effective method in the case of high conc.of Na soap than K soap which tends to solidify or gel formation occur at RT.

12;1 MEMBRANE BASED-SEPARATION TECHNOLOGIES-IN BIODIESEL PURIFICATIONS;(for Two phase Separation)

This is the method to facilitate the two phase separation possibly realisable through the technologies mentioned as follows based on characterstics of feedstocks as it avoids potential emulsions;-

1) Micro-Filtration(0.1Micrometer)

2)Ultra-Filtration(1-20Nanometer)

3)Nano-Filtration(pore size of 1nm.)

PHASE BEHAVIOUR OF BIODIESEL IN MEMBRANE SEPARATIONS;-

The aim is to selectively permeate FAME the biodiesel through phase-separation behaviour of other components among the impurities(unreacted triglycerides,methanol/ethanol,glycerols and soaps).The existance of droplets suspension of glycerols is dependant on temperature & composition of unreacted oils (di &tri) can also form the droplets in membrane reactors comprising bi-model distributionwhose charcterstics is based on origin of oil

.In such case,WCO forming larger droplets due to size exclusions than unprocessed one allowing easier separation of unreacted oil.This can be explained through saponification products forms glycerol-alkali bonds forming reverse-micelle structure and poses problem in separation of the above elements.In addition to that,alcohol and saponification can prevent glycerol droplets from reaching necessary size for effective separation due to the role as surfactants which tends to decrease the surface tension in the droplets exerting increase in overall glycerol permeability.

The biodiesel phase behaviour is addtionally dependant on temperature in a ternary system(Methanol-Oil-FAME) which can be tested at three temp.(20°C,40°C,60°C)facilitating membrane separation.

Here,the **two-phase region** is noticeably narrower at higher temperature implying Temp.and Conc. dependance whereas **single phase** signifies total lack separation beyond the two phase region but functionally varying from crude retentate in terms of composition.During the initial feed of Oil;FAME;MeOH of 26;54;20 wt% ,the permeate had no triglycerides at 20°C and further increase of temp. at 40°C and 60°C with a oil produce of 26%.possible.

Based on above work,the unreacted oil and methanol are insoluble together with the increase of FAME accelerate the two phase system into single phase region.

Further studies are carried out on Octanol-water coefficients to calculate volume fractions of

methanol and biodiesel required to produce hetrogeneous two-phase flow.Then the semi-empirical model are evaluating the phase inversion study occur with volume fractions of methanol lower than 0.31 implies methanol-rich phase that inhibits the separations and leads to complete lack of permeate flux.

ORGANIC & POLYMERIC MEMBRANE USES:-

This includes Polysulfone(PS),Polyamides(PA),Polycarbonates(PC),regenerated Cellulose (RC)and PolyVinylidenfluoride (PVDF),Polyacrylonitrile (PALN)etc..commonly employed in biodiesel purification.

A comparative study carried out between the Polysulfone (PS)and (PALN)membranes in obtaining high purity biodiesel in a range of 97.5%to 99% as in the former achievable with a ester losses of 8.1wt% and 10.3wt% resply.It is recommended that PS is effective always for the high purity without additiional steps.This has been confirmed through successful implementation of UF Poly(ether-sulfone)membrane and MF cellulose ester membrane for the glycerol separation in the final product with a nominal molecular weight cut off (NMWCO) of 10KDa of molecules with special reference to poly-ether sulfone membrane used in UFwhere 0.02wt%,glycerol could be reached in the permeate conforming the international (EN) standards.

Since glycerol is difficult to separate from final biodiesel Hence organic membrane such as modified hydrophilic PALN have been employed at 100KDa NMWCO that was able to separate glycerol more easily with high water contents from3% in pure FAME to 63% in 0.2wt% water after 180 minutes of time on stream. For a P-Sulphone ether membrane at 10kDa NMWCO,small addition of water (upto 2wt% by mass)drastically removes glycerol droplets from 0.02% to 0.009% in the permeate..

<u>MEMBRANE BIOREACTORS FOR BIODIESEL SEPARATIONS;-</u>

Polysulfone(PS) is the most attractive among the organic membrane utilizable due to temp.resistance &inert charcterstics to organic membrane.4ery ammonium was grafted to chloromethylated membrane acts as a catalyst in anion-exchange membrane fuel cells.The above concept can be applied in biodiesel refining process.

The study was performed with n-Hexane due to lower toxicities and conversion is possible with the increased proportions of co-solvent from 65.7% at 30 wt% to 95.8 % at 60 wt%n-hexane.The conversion was still better (87.% versus 95.3%)with no water in the mixture in comparison to-ve effect showed by 5wt% water during refining.

In addition to above phenomenon,the presence of FFA content at a level of 2.5wt% showing 91.3%yield than absence of FFA level recoverable at 95.3% on biodiesel refining.

<u>Membrane Bioreactor</u>;-

The study was focussed on Inorganic-membrane reactor for subsequent purification and conversion is improved through a base catalyst (1wt%NaOH)achievable at 96% level at the flow rate of 6.1mL/min than using acid catalyst (2wt%H_2SO_4) operatable at 65°C results 64% conversion.This is particularly effective in membrane technology taken into account due to the factors such as amount of catalyst,appropriate residence time for complete conversion and methanol-oil ratio factor for maximizing conversion.This is proportional to unreacted oil inside the reactor. KOH,as a catalyst,fed at a rate of 157.04mg/cm3 at 70°C enhances the yield conversion upto 93.5% achievable than 91.5% with 250mg/cm3 regulated at the same temperature.

The study was conducted Using TiO_2-UF membrane
on the residence times with a pore size of 30nm for the variety feedstocks oil conversion as low as 35

min loading a catalyst of 0.5 and 1.4wt%at 65°C.

TiO2 ,as a membrane having NMWCO of 300KDa used to unhinder the FAME production between the range of 0.0355 to 0.042 Kg/min not related to recycling amount.and the transmembrane pressure and glycerol concentration shows recycling build-Up at 100% and 75% resply.It has been observed that methanol-oil rich phase avoids the fouling mecanism through agglomeration of glycerol& ,methanol recycling at a ratio of 10;1 are utilized in soyabean,Palm,Canola oil. at a temperature of 65°C and 0.5wt%NaOH acts as a catalyst.

This reaction system was able to outperform the batch reactor at similar conditions with H2O washing methanol conforming finally ASTM standards (<0.24wt%)for glycerol presence.

13;0 RENEWABLE DIESEL;-

.The Renewable diesel have been proposed through catalytic conventional catalyst from biomasses to produce hydrocarbons in the range of diesel fuel as an alternative to petroleum diesel & biodiesel in view of minimising adverse properties of biodiesel such as moisture absorption,corrosiveness etc..

Deoxygenation Pathways;-

Catalytic deoxygenation of biobased oils will ensure in obtaining NO3 free diesel.Hence,monometallic catalysts supported commercial silicates(SiO2),Alumina(Al2O3),Zeolithes,Carbon(typically carbon supported noble metals) and fluid catalytic cracking catalysts are typically used for this type of reaction under inert atmosphere (N,He,Ar**).**

As an alternative ,Deoxygenation can be done using hydrotreating catalyst in presence of hydrogen helps in removing unwanted heteroatoms such as S,N etc..for ultimate improvement of quality of products realisable through Hydrodeoxygenation**(HDO)** and decarboxylation(**DCO**)processes**.**

Choice of catalysts& supports;-

There are three groups of upgrading catalysts available such *as* **Ni &Co** *promoted* **Mo&W sulfides** conventionally supported on silicates and or alumina materials.

The second category falls on Carbon supported noble metals **(*Pd & Pt).***

The ***third group*** comprises transition metal **Carbides,Nitrides&Phosphides** determinable for being active in HDO reaction and selective towards long chain paraffins& olefins.

The 1st group catalyst are developed in refineries mainly for removing S atoms (as its presence may worsen properties)while regulating the hydrotreating catalysts at elevated temperature & pressure but it shows drawbacks as it needs co-feeding of S source(like H2S)continuously in view of maintaining catalytic activity resulting biofuels free of S in nature.

In the case of 2nd group catalyst**,Pd & Pt** used as supported mono & bimetallic catalysts where co-feeding of S source is not much required due to the nature of insensitiveness to water but it is able to activate water at low /moderate temperature.

This catalysts have shown excellent performance for deoxygenation of various model compounds of bio-oils.Oleic acids are studied over four alumina supported metallic catalysts showing catalytic activities in the order of Co>Pd>Pt>Ni whereas Co/Al2O3 deoxygenates both through DCO & HDO pathways hence it needs specifc attention required to industrialise the catalysts for producing renewable diesel.

Transition metal *carbides* exhibits higher catalytic activity,better selectivity towards HDO rather than DCO as compared to Pd& Pt catalysts.Mo2C/ CNF found to be excellent catalyst for *paraffin*

production due to its high hydrogenation ability and W2C/CNF reported to be appropriate one for *olefin* process.

Transition metal *nitrides* is known for adsorption & activate Hydrogen than carbides hence used for desulphurisation& hydrodenitrogenation which acts as bifunctional catalysts(both in acidic & metallic sites).For a reason,MoN is selective towards HDO producing n-Octadecane exclusively from oleic acid whereas hydrotreating canola oil over Mo2N/Al2O3 for 450 hours time on streams exhibits a high stability of catalyst and higher O2 removal of 90% and a biodiesel yield of 38-48%possible

Carbon materials have been used extensively as suppports for biodiesel upgrading industrially due to its unique properties such as high surface area,ability to disperse highly the catalyst in active phase ,inertness or functionality etc..and works on the basis of source of substrate material and type of techniques used**.**

Mesoporous Aluminium silicates could be an excellent choice for the supports used in HDO catalyst process where it can act and tune the strength of acid sites by changing the structure of Silica-Alumina as well as quantity of aluminium incorporated into the silica frame-work.(Si/Al ratio) by improving dispersion phenomenon.

For a reason,Mesoporous silicates (MCM-41,SBA-15,SBA-16) have been recommended as effective biodiesel upgrading catalyst and these structure are known for dispersing active phase of large molecules present in vegetable oil.For example,stearic acid deoxygenation can be done on Pd/SBA-15, and Pd/C catalysts showing higher turn over frequency (in Pd/SBA-15) indicates the effectiveness of this support.These can be tuned the active sites via Ion Exchange resin adsorption or by grafting techniques.

Effect of other operating parameters(while HDO):

This involves mainly sulfidation, H2 pressure,temperature products & contact time etc..in deciding upon the conversion rate and yield of desired products .Moreover,the chain length & degree of unsaturation of feedstocks are known to be affecting the product yield and extent of side reactions.

Applying *H2 pressure* favours heavily HDO over DCO facilitating Hydrogenolysis &Hydrogenation reactions through improving activity & stability of catalysts.

The effect of *temperature* translates into faster kinetics and final products obtainable at a higher rate but it is not preferable for the better results(higher yield etc..).For a instance, higher temperature(>400°C) give rise to undesired cracking low (C4-C14) & heavy hydrocarbons (C19-C30) products and subjected to reduce biodiesel yields in the final product streams.The isomerisation products can be increased possibly upon improving cold flow properties of diesel at the expense of cetane number.

Contact time is an important factor in determining kinetics of the reaction that provide yields data for optimisation and to design scale-Up process .

In Continuous system,activity & selectivity over reaction time is indicative of catalyst deactivation,stability & surface structure changes.Hence ,longer reaction time will lead to higher conversion of products of saturated compounds under H2 atmosphere exhibiting lower yield due to excessive cracking & DCO reactions whereas space velocity can regulate the contact time between feed & catalyst determining the feed conversion & selectivity.

CONCLUSIONS;-

Renewable diesel have been investigated & proposed the biomass as a raw

material(feedstock) to replace petroleum diesel hence it needs to be upgraded the biodiesel due to suffering nature of the properties as described earlier.

In an effort to obtain an alternative method, an initial hydro-Deoxygenation step followed by hydro-Isomerisation process ensures high cetane number,excellent cold flow properties and environmental friendliness in renewable diesel possible as compared with petroleum diesel & biodiesel.

The commercial production of renewable biodiesel exceeds the petroleum diesel.NEXBTL was introduced this products as the first one in 2005 by Finnish Co. followed by NesteOil,Petrolas/H-Bio,British Petroleum,Conoco Philips/Tyson & Dynamic fuels, Syntroleum/tyson etc..

JET FUELS FROM ALGAES;-

The method for use of algae feed stock to make aviation fuels are discussed as certain challenges associated with algal and cell biojet fuel programs.This shows a product meet ASTM fuel property specification. Concepts are illustrated on biojet fuel approaches include use of extracted materials(lipids&Carbohydrates) while other utilizes the whole algae(liquefaction & gasification)..

***A* COMPARATIVE STUDY OF OIL YIELD FROM MICRO-ALGAE WITH MARINE MACRO-ALGAE ;**

Macroalgae are the multicellular,macroscopic algae growing largely in marine environment.These are red,green,or brown algae((eg.***Sushi-Porphyria)*)**and these are explored for bioenergy option via anaerobic digestion(biogas) and fermentation(ethanol) etc..The approximate yield of oil recovery from these algae is about 70%range.

The research study shows that utilisation of SC-CO2 & Hexane for extracting lipids from marine algae species-***Chlorococcum*** is possible for ultimate biodiesel production. This has shown the poor yield of oil at a range of wt.7.1% to dry marine algal biomass having the fatty acid profile such as C18:1(~ 63wt%),C16:0(~19wt%),C18:2(4wt%),C16:1(4wt%),C18:0(3wt%).

SC-CO2 extraction methods enhances the lipids yields whereas Hexane extraction permit the inclusion of isopropanol as a Cosolvent under continuous operation of soxhlet for the increase in lipid extraction.

Marine microalgaes & marine microorganismes are well developed for its efficient metabolisms owing to their environmental adaptation and these are exploited to produce neutraceuticals value fatty acids having higher content of PUFA and DHA &EPA than fresh water species.

14;0 (WASTE COOKING OIL (WCO) AS FEEDSTOCKS)>

TRANSESTERFICATION USING HIELSCHER ULTRASONIFICATION TECHNOLOGY FOR BIODIESEL PRODUCTION :-(PATENT PROCESS)

Hielscher Ultrasonification process shows promising strategies on improving transesterfication kinetics significantly provides necessary activational energies Therefore lower excess methanol and less catalyst are required for industrial biodiesel processing .In normal production,slow kinetics and poor mass transfer not enhancing the plant capacity neither qualitywise nor quantitywise

Biodiesel is produced commonly in batch reactors using heat &mechanical mixing as energy input;

14;1 ULTRASONIFICATION PROCESSING& SEPARATION OF BIODIESEL:- ***Processing Parameters.-***

-CATALYST KOH : 0.2-0.4 Kg
-Methanol catalyst (premix Tank) : 8.2 L
-Substrate(Raw Material) (Vegetable oil) : 66L
-Recirculation Time : 20 minutes
-Heating Temperature :45-65°C
-Pressure : 1-3 Gauge Bar (15-45psi)
-Time taken for separation of GLYCERINE : 30-60 minutes

Primarily,dissolving the catalyst into methanol is required in premix tank and then the catalyst premix is mixed with heated animal fat& vegetable oil(forms methyl ester) or ethanol(forms ethyl ester) in presence of sodium or potassium methoxide or hydroxide.Then the mixture is heated(temperature :45-65°C) and The pump feeds the mixture into flow cell then the pressure is adjusted to 1-3 gauge bar through back-pressure valve.Then the recirculation of above mixture is done through Ultrasonic biodiesel reactor for about 20 minutes which undergoes Ultrasonification sonicated in line for 5-15 seconds.During this time,oil is converted into biodiesel.In the meantime,the pump and ultrasonification are switched off.

Then,Glycerine drops outthat can be separated through centrifuges.and the converted biodiesel is washed with water.

The Sonification process is performed normally at an elevated pressure 1-3 gauge bar using a feed pump and adjustable back pressure valve next to flow cell.

Industrial biodiesel processing does not require much Ultrasonific energy .On the bench top scale ,the ultrasonification of 1 KW amount of energy required for scaling-up process.

Cost Of Ultrasonification :

Hielscher Ultrasonic devices help to reduce the utility cost and makes viable and green.The resulting cost will vary betweeen 0.1 centimes to 1.0 per liter(0.4ct-1.9 cts/gallon

(BATCH PROCESSING) <u>SMALL -SCALE SET UP:</u>-

In the case of conventional esterfication process that works under Batch system ,it tends to be slow and phase separation of glycerine is time consuming often taking 5 hours or more.

The picture shows processing the oil compound in a small scale having a capacity of 60-70 liters (16-19 gallons).The schema shows as follows:

-one 500 or 1000 W Ultrasonic device(20KHz) with booster,sonotrode,and flow cell.
-Power meter for reading power and energy and power.
-80 Liter processing tank(eg.HDPE)
-Heating element (1-2 KW)
-10L catalyst premixer with stirrer
-Pump (mono or geared Centrifuge) for 10-20 L/min at 1-3 gauge Bar pressure.
-back-pressure valve for adjusting pressure in a flow cell.
- Pressure gauge for measuring feed pressure.

14;2 (CONTINUOUS PROCESSING)

<u>BIODIESEL CONVERSION USING VEGETABLE OILS BY ULTRASONIFICATION</u>:-

Manufacturing biodiesel from vegetable oils(Eg.Soya,canola,Jatropha,sunflower seed,or algae) or animal fats considered as a very good raw material where bio-based transesterfication of fatty acids takes place with methanol or ethanol to give corresponding methyl esters or ethyl esters.Glycerin is the major byproducts of this reaction.basically,triglycerides are the esters composed of three chains of fatty acids bound by glycerine molecules.During the transformation of

triglycerides,heavier alcohol and glycerine are combined with fatty acids that leads to the formation of corresponding esters with a catalyst.

The glycerine-the heavier phase will immerse to the bottom whereas biodiesel(FAME) ,lighter phase floats on top which can be separated by decanters or through centrifuge.

In the case of conventional esterfication process that works under Batch system ,it tends to be slow and phase separation of glycerine is time consuming often taking 5 hours or more.

14;3 BIODIESEL PRODUCTION USING SIMULTANEOUS SC-CO2 PROCESS (MICRO-ALGAES) Research Study 4

The research work shows a promising sustainable & alternative source to non-renewable petrol diesel in which oil is extracted from micro-algae through transesterfication process in one step. Simultaneous addition of Immobilized enzyme-LIPASE as a catalyst and supercritical CO2(SC-CO2) used as a solvent and reaction medium at a temperature of 35°C and reaction time of 6 hours with a Methanol;oil (M.O) molar ratio (8:1) reporting the results found to be 19.3% biodiesel yield contributing promise strategy on biodiesel production .

In this work,the use of lipase enzyme reduces pretreatments or soap formation in comparison to conventional processing that involves complexive solvent separation from the products which adds up the downstream processing cost.

.Among the organic solvents,n-hexane is by far the most commonly used solvent and found to be 94.8 % yield obtainable using ***Mucor MiChei Lipase*** during 5 hours comparing with other conventional organic solvent that require additional separation unit.

Therefore SC-CO2 are suggested to proceed with enhancment of oil extraction from different resources and simultaneous reaction processes for easier product separation via simple depressurization

In order to minimize the overall cost associated with microalgae converted biodiesel process,it is necessarily to avoid all complexity for which SERP(Simultaneous extraction rection process) has been proposed for testing ***Chrolella and Tetraselmis*** **suecia** species using H2SO4, as a catalyst with a optimum conversion rate of 91% achievable after 8 hours of reaction time at 60°C.Though use of acid catalyst is not recommended for fuel production due to its corrosivness, it is advantageous on using the enzyme over chemical catalysts to study SERP process for industrial feasiblity.

MATERIALS AND EXPERIMENTAL METHODS ;-

A dried biomass of **Scenedesmus sp**. is prepared by Algal oil Ltd.Phillipines ,cultivated in organic fertilizer (NPK-(14-14-14) and sun dried .,Methanol purity,(>99%) from Fischer chemicals,USA),Enzymes(activity-11900 PLU/g)from Novozymes,Denmark),n-Hexane(96% purity)from Daejung co,Korea and standard solution of FAME containing Myristic acid(C14;0),10% Palmitc acid(C16;0),6%Stearic acid(C18;0),35%Oleic acid(C18;1),36% *Linoleic acid(C18:2),2%Arachidonic acid(C20:0),Behenic acid(C22;0) obtained from Sigma-Aldrich,USAthen Ultrapure-02 from Air Products, Abu-Dhabi (UAE).

Primarily,the harvested microalgae lyophilised in freeze drier at -80°C with a pressure maintained at 0.01mBar for 6 hours.This freeze dried cells are ground for a duration of 15 seconds to have the particle size 150-355 Micro-meter.The total oil content found to be **5.8 +- 0.16%**.using Folch method with a Choloroform;methanol solvent mixture ratio((2;1 v/v).

The experimental setup include C02 cylinder,High pressure syringe pump,with a capacity 500 bar(Model 260D,USA),Pump controller (ISCO,USA),High pressure stainless steel reaction

cell(VOL;10ml)and temperature controlled incubator with max.temp.of 150°C (ISCO,USA).The temperature in a incubator and pressure in chamber are measured and controlled.The precision of measurement is reported to be +-0.1°C as shown in the Figure...))

A sample of lyophilised cells (1gm) was taken alongwith 2.7w/w enzyme and prespecified amount of methanol in reaction cell covered by two 5 /8 filters.Glassworks placed at the top and bottom of samples to revent particle carry-over.Then the cells are heated up to desired temperature then SC-CO2 are passed from CO2 cylinder into high pressure syringe pump to reach desired pressure then the reaction cells are filled with SC-CO2.The reaction starts at this point and the product is dissolved in solvent after a specified reaction time which is eluted by depressurizing the cell.Then the reaction products is diluted to 10ml of n-hexane and analysis of FAME is done through gas chromatograph(GC) under a pressure 400 Bar at different temperature (35,45 & 50°C) with different methanol;oil ratio(M:O)(8.1;12;1,16;1).

Then it is found to be pressure operatable at 400 bar considered as a rate determining step in this work due to fact that extraction yield of same strain of microalgae increases with increase of SC-CO2.This is propotional to increase in density of algae influencing positive effect on solubility and high pressure will not have enough -Ve effect on enzymes.

Then the FAME is calculated as determined using Folch method expressed as follows;

$$\text{FAME yield} = \frac{\text{m FAME}}{\text{m. oil content}} * 100\%$$

*where **m FAME & m.oil** content are the weights of FAME produced and the oil in biomass used resply.*

RESULTS & DISCUSSIONS;-

The comparative study shows that the FAME production yield is 19.3% after 6 hours and the oil extracted from same microalgae at the same time but a lower pressure of 200 bar having the similar enzyme loading for the same molar ratio of (M:O)reports a much better yield of 62% obtained at 4 hour period.The study shows that the two processes takes place simultaneously as lipid extraction is the rate determining step due to difference of pressure((400 bar in place of 200bar))in SCCO2 process decreases due to redn. in SC-CO2 density thereby lipid extraction effciency reduced .

It shows that higher M:O ratio of 12:1 operated in 4&6 hour time at the same temperature and pressure,it leads to decrease of yield with the increase of M:0 ratio at both tested the time because of inhibition of methanol.

The main challenges of the study is now focussed on low extraction rates affects proportionally the biodiesel production due to presence of low lipid in biomass .It is recommended to test other microalgae biomass having a higher lipid content for mass efficient extraction.

14;4 RESEARCH STUDY-5

PRODUCTION OF BIODIESEL FROM WASTE COOKING OIL (WCO)USING IMMOBILISED LIPASE ENCAPSULATED IN K-CARAGEENAN:-

This is a novel technique for processing biodiesel using Lipase immobilization by encapsulation and its physical properties ,stability characterstics are well studied on this study.The normal enzymatic processing poses many of the problems linked with Chemical processing.However,it requires only moderate operating condition to yield the higher quality product with higher level of conversion and constateable in most favourable life cycle assessment of enzymatic biodiesel

production for environmental consequences.

The associated chemical processing problems of treating waste water are lessened and no issue of soap formation that means used as feed stock the waste oil with high FFA .In addition to that,byproduct glycerol does not require any purification and saleable at higher price.The remedy is to reduce the processing cost as enzyme can not be recyclable and its removal is difficult due to its imperfect solubilization nature..

The major drawbacks of the process is the limitation of mass transfer,enzyme leakage,and lack of availability of versatile commercial immobilized enzyme and involves presence of toxic chemicals in certain time.

In order to minimize the drawbacks,an attempt is made with immobilized enzyme used in degradable polymers(K-Carrageenan) as a carrier for lipase immobilization.

14;5. **(Research Study-6)**

BIODIESEL PRODUCTION FROM WASTE COOKING OIL(WCO) THROUGH IMMOBILIZATION BY T.L LIPASES

The research study was conducted with cheaper waste cooking oil as a potential substitutes and a secondary raw materials to the production of biodiesel.The amount of of cooking oil produced every year is very immense over 15 millions tonnes ,if converted,can satisfy to a larger extent the world demand of biodiesel.This allows for 21%crude oil and 96% in fossil energy savings as it shows challenging startegies over higher yield of biodiesel.

Generally,conventional chemical process poses problems in glycerol recovery and removal of inorganic salts, supporting high temperature & undesirable side reactions and further pretreatments requirements.Therefore ***enzymatic catalysed process*** comes into reality as it shows main advantages such as

-No need of pretreatments in removal of FFA,water etc..

-Higher efficiency, and lower energy consumption and conversion of free FFA etc.;

- -Product purity-easier separation of Glycerol etc..

The enzyme-catalysed reaction can be influenced by base catalysts which require higher dosage,(in the case of Immobilization),difficulties to reproduce in lab scale(design & reactor scale-Up). Hence immobilization permits to increase the usage & time and kinetics of the reaction. Thereby immobilized Thermomyces lanuginsus (TL) Lipase was used on Hydrotalcite in this study able to catalyse transestrification ofWCO linked on interacting with citric acid and residual oleic acid exposed its polar tailor to the medium modified on Fe204 /Au nanoparticles (NP) consisting of magnitite N¨P supporting Au-NP through physical adsorption includes effects of interfacial actiavtion.

This ensures almost homogeneous catalysis of waste oil which is considered as a key role of support and further elucidated the role of Au conduction through NP size -Cu effect to facilitate the transfer of electron and appropriate enzyme orientation and thus increases enzyme loading activities.

EXPERIMENTAL MATERIALS& METHODS;-

All chemicals were of analytical grade from Aldrich Chemicals Co. WCO obtained from olive oil'Sigma-Aldrich (01514) after a simulated cooking(temp.180°C for 5 hours)

1:2 Physicochemical characaterstics of WCO;-

Physicochemical characteristics of WCOwere obtained prior to biodieselproduction that include saponification,water content,Iodine value and acid value.Then the experiments were run in

triplicate and mean value are given.**Refer Table 6;3 f**or phsico-chemical properties.

1;3 SYNTHESIS OF Fe304/Au NP s ligand exchange to obtain hydrophilic NPs LIPASE IMMOBILIZATION;-

Fe3O4/Au @OA Nano particles were prepared by two different samples increasing the amount of Au NPs precursor(HAu Cl4)Nano from 42 mg to 62 mg.

Modified NP were mixed with 10 mL of buffer solution (phosphate buffer 0.1M to give at pH =3.0)with 2 mg of TL (Solution; 1,00000U/g) and shaken at 200 RPM ,T=4°C for 180 minutes to obtain TL immobilised enzyme lipase named NP @TL where pH 3.0 is choosen as isoelectric point for immobilization have a stable enzyme.

SYNTHESIS;-

The Vessel reactor having a capacity of 25ml volume continuously stirred with 200 RPM for methyl ester production at two different temperatures 45 and 55°C .These different experiments were performed with 1 gm of WCO in ratio presence of different conc.of free or immobilised enzymes(5%,10% &20%) oil/Methanol ratio 1;6 etc..Furthermore,the three experiments were done with different oil/Methanol were washed with hot water at 60°C and finally dried with anhydrous NaSO4 to obtain pure biodiesel.Then the oil conversion to methyl ester formation(biodiesel)determined through simple equation

$$\text{Yield} = \frac{\text{m Ester}}{\text{M oil}} * 100\%$$

The analysis of FAME produced was carried by a GC-MS (ThermoFischer) equipped with a Trace Gold Capillary Column (0.25mm * 60m)

***GC-MS Configuration*; -**

Initial temperature;120 °C for 4 m

- rate 1= 6.5 °C/min to, 170°C

-rate 2 =2..75°C/min to 250°C for 9 min.

Injecter & detecter temperature were set 250°C and 230°C resply.Helium used as carrier gas.Methnol BF3 method was used for WCO derivatization to obtain composition and time of retention FAME compared with known concentration of FAME mixture and biodiesel conversion.The retention of time of biodiesel produced shows the similar results .Then EN 14214 was used for methyl ester content evaluation in produced biodiesel and the measurements were performed in triplicate.

RESULTS AND DISCUSSIONS;-

As shown **in Figure 6;10,** the experiments was conducted using different molar ratios of oil/methanol on biodiesel productions with Immobilized Lipase operatable at 45°C for 24 Hours time reaction.It shows that the biodiesel yield are favourable at an oil/Methanol molar ratio of 1;3 results equal to 81.8% then it increases further to a level of 84.5% for an oil/methanol ratio of 1;6..

Biodiesel obtained from WCO through immobilized Lipase after 24 Hour synthesis presents a linolenic methyl ester content of 0.54+- 0.03 in agreement with EN 14214 and it is equivalent to modified method as 97.8+- 0.21%.Then Iodine value is calculated as per above normal results equal to 66.75(g.Iodine/100G) and it appears to be high enzyme activity though it is lower than that different oils due to the presence of FFA,water and degraded product contents.The conversion of biodiesel can be obtained higher than 90% for longer times because of progressive enrichment in shorter fraction along the cooking.The results of characterstization are reported in

TABLE...5 ,the feasibility of WCO as fuel.

The olive oil containing 13 Fatty acids(FA) before &after cooking simulation were detected by GC-MS charcaterization .This shows light different activity of enzyme as the length of chain to be converted increases.

The difference of biodiesel synthesis was evaluated by GC-MS analyses derivatized olive oil,derivatizaed WCO and biodiesel.The spectra were reported in Figure-....S1,S2,S3 resply.....

The evidence of the results are indicated in **TABLE6;4...**in comparison with between GC-MS of derivatized WCO and biodiesel showing the ability of nanocatalysts converts FA into methyl esters**. In conclusion**,the difference exists on the yield of calculated biodiesel yield 100% and after 24 Hours may be due to presence of unconverted acids significantly explains more pronceable as longer chain to be converted.

Research Study-

LIPID EXTRACTION FROM SPIRULINA,SPECIES AND SCHIZOCHYTRIUM SPECIES USING SUPERCRITICAL CARBON-DI-OXIDE WITH METHANOL (co-solvent)()Research Study-

SPRIULINA sp, and SCHIZOCHLYTICUM sp,was studied in pilot plant using SCCO2 with 200 gm of biomass for 6 hour where methanol is added as a solvent at a volume ratio of 4 %.The result is shown that adding methanol in SCCO2 increased lipid extraction yield significantly (on both species) under a operating conditional pressure of 4000 psi then lipid extraction yields increased by 80 and 72 respectively.These have been also observed in comparison with soxhlet extraction having methylene chloride/Methanol ratio of (2.1%v/v),the methanol SCCO2 demonstrated high effectiveness of lipid extraction yield after loading 5 fold biomass. This shows a good potential for Scaling -Up and kinetic studies shows Methanol-SCCO2extraction influences on lipid extraction yield shows through the graphs**.Refer (Fig-11**

15;0 ***APPLICATION OF METABOLIC ENGINEERING TECHNOLOGIES TO IMPROVE LIPID PRODUCTION IN OLEAGINEOUS SPECIES;-***

METABOLIC ENGINEERING APPROACHES OF MICROALGAE & OLEAGINEOUS MICROORGANISMES FOR BIOFUEL PRODUCTION :-

(to enhance Lipid production strategies)

Microalgaes are single cell photosynthetic microorganismes capable of capturing sunlight and converting CO2 and water into organic matter-Triacylglycerol(TCG).

This is possible to manipulate the metabolic pathway of algae such as Cyanobacteria,a blue green algae are thought to be better candidate for undergoing study on genetic engineering in order to produce improved recovery of biodiesel.These blue green algae do not accumulate storage lipids but they are carbohydrate producing secondary metabolites,Some strains can be doubled lesser than 10 hours and some other strains can fix atmospheric N2 &produce H2,Moreover many of the strains are genetically modified showing higher productivities of biodiesel as attractive organism..

Oleagineous microorganismes such as fungi,yeasts,microalgaes etc considered to be the perfect candidates as they fulfill the lipid production status used for energy or neutraceutical purposes.The approach on biochemical & metabolic mechanismes related to biosynthesis and accumulation of fatty acid synthesis are the premitive step considered for enhancing the lipid prodution through designing modeling tools.This could predict the difference in behaviour of every change & help in designing the most suitable modification.(**Refer FIG- 7:0)**

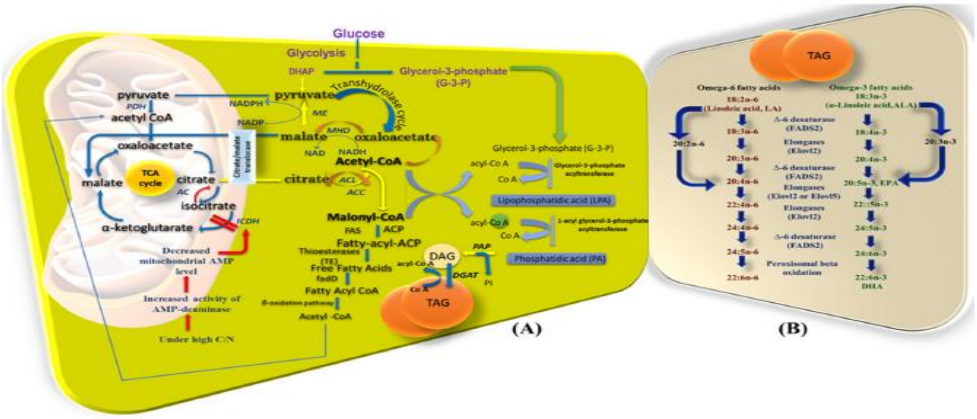


Figure 3. (**A**) *De-novo* fatty acid synthesis in oleaginous microorganisms (adapted from [13,16,18,221,243,244]), and enzymes involved in lipid accumulation. AC, aconitase; ACC, acetyl-CoA carboxylase; ACL, ATP-citrate lyase; ACP, acyl carrier protein; FAS, fatty acid synthetase; ICDH, iso-citrate dehydrogenase; MD, malate dehydrogenase (cytoplasmic); PD, pyruvate dehydrogenase; PAP, phosphatidic acid phosphohydrolase; DGAT; diacylglycerol acyltransferase; FAS: fatty acid synthase. (**B**) Biosynthesis pathway of omega-3 and -6 fatty acids (EPA and DHA) from parent fatty acids (LA and ALA) through a series of desaturation and elongation reactions [17,161,245,246].

The overexpression of genes or enzymes of biosynthetic pathways,suppression,blocking or knock-out of genes of competitaive pathways, regulation of pass pathways, multigenes approaches etc..could find a suitable solution for synthesis,storage & profile of lipids as per the adoptivity of microorganismes into the environments.This results in change in production rates both for biofuels energy and neutraceutical purposes

Research studies are conducted upon pathways related to the synthesis ,storage & profile of lipids as per the adaptivity of microorganismes to the environments results in change in production rates.The major areas in which manipulation can be done through overexpression of genes or enzymes of biosynthetic pathways ,suppression,blocking or knock-out of genes of competitative pathways,regulation of by-pass pathways,multi-genes approaches etc..

The basic mechanism over synthetic pathways for enhancing fatty acid profiles is summarised in **Figure-7;2** in bacteria model where acetyl-CoA constitues the central molecules and it leads to malonylCo-A followed by the production fatty acyl carrier protein (fatty ACP or moiety)to get the final transformation into free fatty acids (FFA)by thioesterases..These FFA can be evolved to PUFA with the help of specific desaturases and elongases enzymes.

The above pathways can be modified through one of indicated methods such as overexpression of key enzymes of genes that encode ACC &FAS among the first choices.In some cases,the co-expression of more genes isnecessary for the sucessful increase of lipid synthesis whereas the later step of pathways could limit the results as acyl-ACP inhibits the over-expression of ACC in E.Coli cells similar to TAG synthesis improvement through overexpression in Kennedy pathways(like DGA& KG).

The second technique is the regulation of pass pathways involves genes repairing regulates the molecules appear inexistance in basic lipid biosynthetic pathways. On the other hand, suppression or knock-out of genes related to lipid Beta-oxidation ,degradation & their synthesis inhibition constitutes improved accumulation of lipids determinable through the aspects of inactivation or dysfunctioning enzymes.

Hence,the multi-genes approach shows overexpression of more than one genes of keypoints

of lipid metabolisms otherwise it can be done in combination with knock-out of others that influences the lipid metabolism by about 20 times.These are specifically overexpressed with three genes and knock-out one normally.

By knocking out the acetyl Co-A synthetase,they stopped the degradation of FA while overexpression ACC produces more malonyl-CoA followed by overexpression of two thioesterases (an endogeneous& exogeneous)in final stage.This increases in short chain FA profiles through decrease in inhibition of acyl-ACP.

Similarly,the enhancement of biodiesel production can be achieved through increase of lipid profiles in a study realised with the overexpression of the two key genes ACC1 & DGAT1 in oleagineous yeast(yarrow Lipolytica) leads to produce by 2 times & 4 times respectively whereas the overexpression in combined form results in 5 times greater lipid accumulation than control indicates their synergistic effect.

CONCLUSIONS;-

This shows the enhancement of fatty acid production possible for their use in nutritional or energistic purposes. In conclusion,the combination of De -Novo and Ex Novo pathways and use of metabolic engineering could lead to even greater accumulation of lipids.

Whenever the suppression or activation of genes are required in genes modification,the methods such as mutagenesis,homologus recombination,the use of micro RNA (miRNA) and short interfering RNA (siRNA) can be practiced based on type of microorganismes,the strains,their genetic profiles and the desired results etc.. .

Enhance LIPID production from LignoCellulosic Hydrolysates through Yeast

Yarrowia Lipolytica is the common biotechnological plateform for the production of Lipids ,as a preferred feedstock for the Biofuels and Chemicals.To reduce the cost of microbial lipid production,the in-expensive Carbon sources such as LignoCellulosis Biomass(LCB hydrolysates can be extremely used but it contains often toxic substances like Xyloses considered as precursors which cannot be assimilated or used by Yeasts.and this species are successfully engineered by Overexpressing of the native genes. of species

***COMPARISON OF PRODUCTIVITY STUDIES of DELECTED CROPS*: (OIL YIELD expressed in *L/Hectare*)**

CORN 172
SOYABEAN 446
CANOLA 1190
RAPESEED 1190
JATROPH 1892
OIL PALM 5950
MICRO-ALGAE(30% oil)..... 58700
MACROALGAE(70% oil)..... 136000

The information given here will help us to realise third generation biofuel production.Green growth stores vitality as lipids that can be changed over into different energies.(ethanol,Hydrogen,diesel,Gas,CH4,Alkane compounds in heterotrophic maturation forms and even stream fuels etc..)

.....)

***16;0* OLEAGINOUS MICRO-ORGANISMES AND THEIR ROLE IN BIO-DIESEL & OMEGA-3 FATTY ACIDS PRODUCTION.**

Micro-organismes are known to be natural oil producers and accumulate more than 20% w/w of lipids in their cellular components on dry weight basis often referred as Oleagineous Micro-organismes.They are capable of
synthesising vast majority of fatty acids from short chain hydrocarbonated chain(C6) to long hydrocarbonated chain (C36) such as saturated (SFA),monosaturated(MUFA) or polyunsaturated(PUFA**refer Tab-7.0)** Microalgae can use both inorganic and inorganic carbon sources through different modes of cultivation such as *Autotraphic,Mixtotrophic,Heterotrophic &Photoautotrophioc* etc....

Therefore microbial oils from single cell microorganismes considered to be efficient feedstock due to similarities with vegetable oils. and enhances higher productivity in comparing with other resources and easier to upgrade in regards to Upstream&downstream processing, easier genetic manipulation possible towards the specificity of the product in addition to criteria of environmental controlled growth.

As described earlier several species of unicellular organisms can produce more than 20%w/w of lipids in contrary to other marine species of macroalgae which can yield 70%w/w depends on cultivation condition under C/N ratio having 4-28 unbranched carbon chain length translated as saturated or unsaturated fatty acids either in MUFA or PUFA..

* The actual mechanism involve as TAG synthesis takes place mainly in sub-Cellular compartments-Chloroplasts & endoplasmic reticulum as a result of enzymatic reactions. .This may be explained as accumulation of lipids through fatty acid synthesis in Chloroplast then the assembly of glycerolipids in endoplasmic reticulum leads to TAG accumulations into oil bodies via NOVO Pathways starts in chloroplast by CO2 in fixation into sugars and further metabolise to form Acetyl CoA,precursor of fatty acid synthesis.

In the past decades,heterotrophic cultivation of algaes showed many advantages over Photoautotrophic cultivation such as Cost-effectiveness and easier Cultivations &maintenance etc. that can be used in any fermenters utilizable for yeasts & Bacteria without illuminations.Various inexpensive agricultural raw materials are used as alternatives to Glucose as C source such as rice,sugarcane bagasse,wheat straws,Corn stover,waste molasses,Soy Whey,industrial waste water,birch,spruce and beech etc...that supports heterotrophic cultivations.

Table-7.1 shows resolving problem in replacement with SCO in comparison to Fish oil towards the Neutraceuticals and biofuels production.

YEAST&FILAMENTOUS FUNGI AS OLEGINEOUS MICRO-ORGANISMES FOR BIOFUEL PRODUCTION ;-

Oleaginous yeast are the well studied microorganismes which include species such as Candida, Rhodosporidium,Yarrowia,Cryptococcus,Rhodotorula,Lipomyces and Trichosporon etc.. can accumulate lipids upto 80%wt/wt of their dry cell weight.These strains are reported to be .

The oleaginous filamentous fungi are the promising microbes for biofuel production having unique characterstics over FA profiles with Alpha -linoleic acid(GLA) but synthesised not in higher amounts..Fungi can be cultivated on inexpensive feedstocks such as Molasses,MSG,waste water,sewage

OLEGINEOUS BACTERIA FOR BIOFUELS:-

Bacteria are also the good source of TAG in biofuels compared with microalgae and yeasts.Some important strains are Rhodococcus sp.,Gordonia Sp.,Acinetobacter Sp.,& Arthrobacter Sp.,capable to grow in versatile substrates.It is important to note that Rhodococcus Sp.,was studied extensively for its potential activity to degrade lignin and assimilate lignin monomeric compounds into the lipid

accumulation pathway containing a lipid content of 26.8%cultivating on aromatics obtained from Organosolv pretreatment of loblolly pine, alongwith lignocellulosic effluents containing various sugars.

OLEGINEOUS MICROORGANISMES FOR NEUTRACEUTICALS PRODUCTION:-

Traustochytrids,microalgaes&filamentous Fungi are rich in PUFA considered for neutraceuticals.A list of olegineous microorganismes involved in EPA &DHA production are presented **in Tab 7.1.**

OLEAGINEOUS TRAUSTOCHYTRIDS:-(for neutraceuticals)

Traustochytrids are heterotrophic fungus like clade of Stramenpiles ,often referred as Algae.These are a good source of DHA and improved technologies have been developed for commercial production. requiring higher temperature between 25-30°C for optimal growth whereas lower temperature of 15°C growth enhances DHA production at the reduced growth level.The species have a wide pH tolerance ranging between 5--8 with a optimal salinity level reflects on strains to strains growth variation capable of growing in several cultivation conditions.

Among the strains of Traustochytrids,Schizochytrium Spp., are able to produce approximately 35-40%w/w of total FA as DHA in larger scale production whereas the marine species Auroantiochytrium Sp.,T66(ATCC PRC.276) in heterotrophic cultivations using forest biomass hydrolysate (30g/L glucose)in flask implying dry cell weight & total lipids of 10.38g/L and 4.98g/L respectively shows the recovery of 25.98%DHA constitution compared to bioreactor cultivation which shows as 11.24g/L and total lipids of 5.90g/L and DHA content of 35.76%of total lipids.

This shows that there is a great potential in valorising sustainable resources for DHA production.

OLEAGINEOUS MICROALGAE & DIATOMS:

Marine microalgaes & marine microorganismes are well developed for its efficient metabolisms owing to their environmental adaptation and these are exploited to produce neutraceuticals value fatty acids having higher content of PUFA and DHA &EPA than fresh water species.The marine oleaginous diatoms-Fistulifera Solaris cultivated in photoautotrophic conditions reported to be producing EPA with a optimum level of 135.7mg/L/day whereas the heterotrophic growth marine diatom-Nitzschia Laevis upon supplementation with glucose results in EPA production of 174.6g/L/day .

OLEAGINOUS YEASTS &FUNGI FOR NEUTRACEUTICALS:-

The first microbial strain,Mucor Circinenelloids,a filamentous fungus was used for commercial production of Gamma Linolenic acid rich oil.The other species Morteriella Alpina 1S-4 ,a oleaginous fungus is a good source of amino acid production capable to produce EPA &AA through n-3 and n-6 PUFA biosynthetic pathways respectively.

Yarrow Lipolytica are reported to be well studied strain for genetic manipulations and unique ability to grow on hydrophobic substrates.produces EPA yield of 161.04mg/g/day.The cultivation of yeast can enhance the productivity using low cost substrates such as waste glycerol or sugar from lignocellulosic biomasses.make viable & feasible..This organism is considered as a model microorganism to understand mechanismes behind the uptake of hydrophobic substrates.

16.1 OLEAGINEOUS MICROORGANISMES;-

PRETREATMENT METHODS FOR LIPID RECOVERY :-

To improve lipid extraction efficiency, a preteatment step is often necessary to disrupt the cellular integrity of microorganismes that can extract directly from wet biomasses. .Currently, various pretreatment methods have been employed in laboratory scale such as high pressure homogenisation,Bead beating,Microwave,Ultrasonification,osmotic shocks,and autoclaving but

none of them are effective in operation for large scale processes.

The **oil expeller presses** are the simplest mechanical methods and has been tested for algal biomasses but not yet for microorganismes.This is operatable mechanically to crush biomasses in an oil press whereas bead beating works with an agitated beads break the cells by shaking the vessel that already filled up .This is applicable for all types oleaginaous organismes susceptible to extract the lipids.This method can increase the extraction efficiency of lipids containing higher pigments contents contributing overall cost of downstream processing.but the disadvantages is the need of dry-processing of low moisture samples &propotionate increase in cost involvements.

Another **Mechanical methods** is the Micro-wave preteatment of biomasses for dewatering the microalgae and the major advantages of the processes are the lower energy input together with rapid treatment,high yield and purity of the product possible and negligence of using hazardous substances and low cost process.

Osmotic shock is the another method where hypo & hyper osmotic conditions are created by varying salt concentration leads to play an important rôle in lipid removal which is based on high concentration of salt equilibriated with water or fluids moving intracellularly causing the cells to swell or burst.The osmotic shock is applied alongwith a mixture of polar & non-polar solvents from wet Chlamydomonas Reinhardtii cells resulting an increase of lipid content by two times compared to other processes where NaCl induced osmotic This method has its own limitation in commercial application owing to the nature of damaging the cell wall properties of specific species.Oxidative agents such as free nitrous acids(FNA) can treat microalgal cells towards the increase of lipid extraction by 2.4 fold.

Electroporation is another efficient techniques involves increase in lipid recovery though does not affect the composition and quality of lipid.

Ultrasound assisted extraction is another physical method offers several benefits as simple,ecofriendly and time jconditions.The major disadvantages of the method is the prolonged use of Ultrasonification giving the deterimental free radicals in extracted lipids.

Apart from above,the **biological methods** have been tested to facilitate lipid extraction from organismes to which a recombinant Glucomannanase(p1MAN5C),was used for degradation of cell wall of microwave pretreated cells of Rhodosporidium Toruloides Y4 resulted Algaenan, a resistant insoluble non-hydrolysable biopolymers of cell walls disrupts the cell algae..

Finally,a method is to **lyse the cell** wall of Gram Negative microorganismes through the use of antibiotics -Beta lactum for not only to restrict the growth but it is also applied to interfere with peptiglycan synthesis rendering the cell unable to maintain osmotic pressure with subsequent release of intracellular materials after disruption.

Yarrow Lipolytica & S.Cerevisiae have been engineered to excrete higher amount of extracellular FFA similar to E.Coli .This model system has been brought into concept as a Plug&Play secretion system useable in other microorganismes including yeasts etc..

16;2 TRANSESTERIFICATION PROCESS;- (*for OLEAGINOUS MICROORGANISMES)*

The downstream processing of SCO production consists of four steps involves oil extraction,transesterfication,Purifications and final extraction.In the case of Direct or In -situ transesterfication,the multiple steps are eliminated as with most of the cases where lipid extraction &Transesterfication are achieved in one step.

The process can be characterized as homogeneous or heterogeneous depends on the catalyst finally occupying the same phase or not with the reaction mixture .

In *homogeneous catalysis*, use of alkali affects the downstream processing through above phenomenon.Contrarily,inorganic acid can be recommended and ideal but lower yield possible but increase in cost for commercial exploitation. In the case of ***Heterogeneous catalysis***,the catalyst appear in different phase than that of reaction mixture and reusable while separation from the above then it appears to be selective,.

Finally,to state that two widely tranesterfication methods such as Supercritical &Microwave guided techniques are applicable to special characterstics related to production of highly purified products and reduced energy cost with first method whereas then second one appears to be highly efficient on yield on time. possessing advantages such as chemicals ,energy and cost reduction and limited to single step extraction-transesterfication with reduced downstream processing cost. .

PROCESS FOR REUSE OF METHANOL

An alternative non catalytic conversion process is for biodiesel translating on triglycerides transesterfication under supercritical methanol process above (293°°C,8.1 M.Pa) in absence of catalyst working under high pressure where methanol can be recycled and reused. Efforts are used to reuse the catalyst making it as GREEN..

***PURIFICATION OF BIODIESEL*:-**

The final product quality is the major concern of biodiesel where immense purification is required to filter the crude-multiphase components containing soaps,enzymes,metal ions,water,acid or base solvents and non desirable lipids needs to be separated.

For example,if the substrate is considered to be the microalgal biomasses then it is necessary to separate the lipids with gravitational or centrifugation techniques where fatty acids can be better clarified for its purification by more than single step methods Different methods have been reported for this step such as use of solvents in combination with Vaccuum,NaSO4 and other filtration for removal of byproducts..Some of the most usual crucial techniques are DRY Washing,Wet washing and membrane separation.

WET WASHING;-

This is considered to be well known ,traditional & conventional method for purifications and suitable for removing excess contaminants& chemicals present as from the previous step.The major disadvantages is the demand of high amount of water and need for drying final product through absolute H2O removal and further requirement of extra water are needed to treat the waste water before

DRYING WASHING

This technique is replacing above method as there is no need for water,no product loss and provides added advantages upon selecting proper adsorbents.This includes use of efficient compounds such as silica,starch,Cellulolytic derivatives &Ion Exchange resins etc...

Other than above two methods,certain Novel methods are gaining attraction among the use of membrane Technology which has the special characterstics composing of support & coating materials and whole process based on rejection coefficients.The two membranes currently practiced are **PVDF**(PolyVinyldiene fluoride,PolyDimethyl siloxane**(PDS)** together with ceramic materials suitable for organic materials.

In conclusion,use of combinative two stage process are suggested starts with wet & continual washing helps to produce high quality biodiesel that conforms the norms ASTM and EN .So purification process has to be balanced among the environment,operation and purchase costs and efficiency.

16;3 CONCENTRATION & PRODUCTION OF OMEGA FATTY ACIDS:-

SCO can be used as a food supplements for the food & neutraceutical industries as a renewable energy source.The first application are Omega-3 & 6 lipids and the second applications is the production of FAAE as biodiesel.

For enrichment of (omega)w-3 and w-6 lipids with desired compounds, the most commonly used methods are Winterization,Molecular distillation & Urea Complexation.

The by-products such as Monoacyl,Diacyl glycerols and FFA can be removed through urea complexation coupled with molecular Distillation process involves increase in cost of the process.

Winterization are recommended as an alternative & selective method ,increases PUFA content to a greater extent through treatment of oil with organic solvent under low temperature (0,-20°C,-80°C) for some cycles leading to crystallization of some compounds .The usage of solvent is to separate PUFA from other compounds. Saturated FA based on solubility and melting points that creates oil fragments and enhances doubling of omega -3 content of lipids.In coupling with urea complexation process,the saturated & less unsaturatedFA are separated from PUFA through reduction of FFA resulting in upto 95 % DHA purity possible with the crystalline urea.Then urea tends to create the crytstals with FFA and then PUFA are finally concentrated & made into relatively clear at- the final mixture.This is based on the ratio between urea presence &FFA content during the time of crystallization process that tends to increase the DHApurity from 30 % to 60 %.In general,these methods are used alone or in combination to enhance the quantity & quality of omega-3 &omega-6 lipids through the basis of available condition & presence of microorganismes and desired compounds to be separated in order to achieve the optrimal results of the process & product.

In conclusion,microbial oil can be plausible alternatives resource for food & fuel applications;In order to reduce the high cost involved on feedstock associated with the growth media for the cultivation of microorganismes,these can be profitably utilized with the renewable carbon energy resource probably through lignocellulosic materials etc...

16;4 MICROALGAL BIO-REFINERY:-

Algaes are biofactories for the production of number of high value compounds-Micro algae lipids contains essential FA such as EPA and DHA and other high value acids (omega-3,gamma linolenic acid etc.. The neutral lipids notably triglycerides(TAG) are well suitable for biodiesel production as FAME.

-Carbohydrates accumulated as reserved material or become the main component of cell wall (cellulose,pectin,and sulphated polysaccharides etc..)The species such as chlorella,dunalella,Chalmydomoas have reported to be 16-60% on Dry basis with 75% algal complex hydrolysable into hexoses or 80% therortical ethanol yield possible

-The secondary metabolites include high value specific pigments and vitamins.

-Algal proteins have high nutritive qualities compared to other referential proteins having higher proportions of amino acids balance.

- Among the myriad compounds produced by algae,polysaccharides,mycosporin like Amino acids(**MAA**),halogenated compounds,polyhydroxyalkanoates**(PHA**) are reported to be effective.Polysaccharides shows a specific role as antihumor,antiviral, and immunostimulant activities that can be used as emulsion stabiliser,as bioflocculant,as thickener,for modify the water regards to rheological characterstics and elimination of heavy metals during polluted water treatments etc..some MAA can act as antioxidants and provides protection against photo-oxidative

stress by ROS.

Halogenated compounds produced naturally by marine red and brown algae comes on various metabolism stage include Indole,Terepenes,Acetogenins,phenols,FA and volatile hydrocarbons.The derivatives of sesquiterepenees,polyhalogenated terepenes and halogenated FA play an vital role on pharmacological activities including antibacterial etc...

Polyhydroxyalkanotes(PHA) is microbial,procaryotic carbon -energy sources material whereas PHB (poly-beta hydroxy butyrates)is natural polyester capable in showing biodegradability but these two can be synthesized by cyanobacteria,Spirulina Sp., Nostoc Sp;, and Synechocystis sp..It stimulates various biological activities include antioxidatives,*antimicrobial,antifungal,antiviral,etc..

ANTIOXIDANTS;-

Environmental conditions such as high intensity light,high temperature,salts stress may be effective to boost some antioxidant production in Microalgae .This involves chlorophyll-alpha and pigments involved in photosynthesis boost the photoprotective agents like secondary carotenoids(astaxanthin,beta carotene,Zeoxanthin)

The above 2 antioxidants involve single step cultivation and two stage cell growth with the species-Haemotococcus pluvialis for Astaxanthin production.the first stage producing green biomass under optimal growth condition referred to green stage and the second stage exposing the culture to adverse environmental condition to induce astaxanthin yield(11,5 mg/L/day) can be attained at the lab scale under continual illumination

ALGAES AS HIGH VALUE BIOACIVE COMPOUNDS ;-

. Among potential neutraceuticals from microalgae,these includePUFA, phenolic compounds,antioxidants,pigments,carotenoids, Pigments),,Vitamins(Betacarotene), polysaccharides,lipidcomponents(Phospholipids,Glycolipids,sterols,etc..), dietary fibers,Hydrocolloids,Proteinaceous compounds include Peptides &Amino acids etc..Halogenated derivatives and phenolic compounds etc..

FATTY ACIDS &GLYCEROLS :-

Mono or PUFA are the principle constituents for the production of nucleic acid,proteins,biomembranes.PUFA,a key rôle player in cellular and tissue metabolism include regulation of membrane fludity,electron and O2 transport,thermal adaptation .

The stress environmental condition affect the FA production and alter the biomembrane composition and functioningthrough lipid peroxidation by reactive oxygen species(ROS).Thereby,lipid ,FFA content affect the biodiesel conversion.Glycerol accumulation acts as osmoticum(regulate osmotic pressure between cell and environment) that produces high value bioactive compounds for food and other applications.

PIGMENTS ;-(Chlorophyll,carotenoids,Phycobilins)

Algal species are known for pigments.Chlorella zofingiensis,C.Vulgaris,Dunaliella salina,Haematococcus Pluvialis are well important strains known commercially in large scale cultures..In addition to that,Chlorococcum sp.,Scenedesmus Sp.,Chlorella ;,Chalmydomonas Sp.,etc are the potential producer of Astaxanthin & Lutein.

Pigments are colurful compounds absorbing and reflect certain wavelength of visible light.These are referred to Chlorophyll,Phycobillins,Carotenoids acts as a light energy absorber.&Betacarotene as vitamins.

Chlorophyll (Cl)are divided into 4 categories such as -a,b,c,d etc..present in higher plants show greenish colour through photosynthetic way containing lipid soluble ,a substituted Porphyrin ring

with centrally bound Magnesium atom and further esterified to diterpene-alcohol to form chlorophyll.).

Carotenoids,during Photosynthesis play a role in light harvesting,photoprotection,superoxideO2),scavengin,excess energy dissipation and structural stabilisation.Carotenoids form pigment-protein complexes with peptides mainly located in chloroplasts or plastids.The selected algal acetylenic carotenoids are;1)Astaxanthin,2)Fucoxanthin,3)Beta carotene,4)Lutein etc..

Astaxanthin,a 2nd carotenoid element ,found everywhere esp.in marine environment having color of pinkish red Hue in shrimp,crayfish etc.Lutein, primary carotenoid involves in maintaining structure & functioning photosystems.Beta-carotene, a secondary carotenoid Astaxanthin.

16;5 BIOLOGICAL PROPERTIES OF MICRO-ALGAES;-

1:0 as Anti-oxidative & anti-protozoas:

Pigments having the antioxidative properties are high value compounds typically functioning as food preservatives or additives or as health promoting supplements..

Chlorophylls and its derivatives-Pheophorbide possess antioxidant properties.Phycobilliproteins can be utilised as natural colorants,or pharmacological properties such as fluorescent agents with antioxidants,anticancer,antiinflammatory,neuroprotectives,Hepatoprotectives etc.;

Several species such as dunaliella tertiolecta,nannoc hlorlopsis Oculat,Spirulina Platensis,tetraselnis Suecica and Eylena Gracillis produce viamins,Vitamin C,E & B12 reported to be promote neuroprotective activity as well as radical scavenging ability.The products undergo clinical showing specific therapeutical targets comprising ion channels,metabolic enzymes,microtubules,DNA etc..Table-2 shows antioxidant properties of seven species of cyanobacteria and 3 microalgaes species:-

***1;2* As an Antimicrobial and Antifungal activity;-**

Several marine algal species shows potent antimicrobial activities include P.Tricornutum cell lysates against gram +ve and -ve bacterias even at lower micro moles concentration attributing EPA,GLA(gamma-linoleic acid),ARA ,DHA a health benefits of PUFA.Marine Protists such as Thraustochytrium ,Schizochytrium,Crypthecodinium etc..are rich source of DHA. .

1;3; As an Anti Viral activity;-

Microalgae are the potential sources of many antiviral compounds..Sulphated polysaccharides have shown anti-viral activity against two enveloped Rhabdoviruses such as VHSV(Viral haemorrahagic septicemia virus of salmond fish & african swine fever virus(ASFV)The red species of microalgae containing sulphated polysaccharides mainly xylose,glucose,galactose etc.; exhibits the features of antiviral properties.

Due to their antiviral spectrum properties against HSV and HIV-1 viruses,the sulphate exopolysaccharides from marine microalgaes are expected to interfere with stage-I ,one of enveloped Viruses.In the case of HIV,they may inhibit choicefully Reverse Transcriptase preventing the creation of new viral particle after invasion(injection).Thereby,the inhibitory effect is possible due to the interaction with positive charge on cell surface of virus particle prevents penetration into host cells..

1:4; as an Anti-tumoral activity;-

.Fucoxanthin(fucoxanthinol) from crysophytic,pheophytic algal species acts as accessory pigments show potent antioxidant properties,antiinflammatory,,anticancer etc.. .

RESULTS AND DISCUSSIONS;-

Biodiesel is the alternative to petroleum diesel and offers the several advantages to the environments since it exhibits lower CO2 emissions(GHGE) meaning that global climate change,carbon neutrality etc..

In order to utilise the fuel property efficiently right from the storage,distribution etc.; it has to be upgraded like moisture content,low SO2 contents,cold flow properties,viscosity reduction thereby additization is required to improve the fuel performance having the higher cetane number and may not contribute the net accumulations of GHGE.

Waste cooking oil(WCO),Microalgaes,oleagineous microorganismes etc..are considered to be the potential feedstocks for biodiesel production through transesterfication reaction.Heterotrophic microalgaes can produce higher amount of biomasses by unlimited sunlight exposure though it works under dark conditions.

The output of oil recovery is estimated to be accumulated as high as 58700 liters per hectare of microalgaes cultivation .

Among the bioreactor design,photobioreactor(PBR) in combined status with open Raceway pond are strongly recommended for the cultivation of microalgaes,varying from species to species for the reason of convenient way of operations and to avoid huge evoporation losses,reducing the biological contamination,proper mixing mode minimization of CO2 losses etc..

Additionally, It may be observed that algal sequesteration of CO2 could be increased through implementation of multiple PBR to scale-Up the process.varying from species to species.The reactor configuration with special reference to Open pond where paddle wheel system proposed will have the industrial feasibility of PBR having the lipid production cost estimated 31.6$/gallons compared to 12.73$/Gallons for open pond.This shows higher biomass production possible at a large capacity in parallel to decrease of the risk of contamination.

The optimal growth temperature requiring from 16-28°C helps in photosynthetic efficiency and sufficient irradiation are essentially needed for boosting-up the major nutrients.and bioactive contents of microalgaes .This can be done normally by blue or red light spectrum fluorescent tubes for photosynthesis.

Harvesting& dewatering microalgaes are possible with a concentration of 1-3gm/L biolymers on fresh water Desmodesmus Sp51 having the increase of efficiency appears from 43.8 to 98.2% with a initail pH 7.2 to 3.0 whereas optimal dosage 2.5ml/L with a mixing rate of 150rpm for 1minute and slow mixing of 80 rpm for 2 minute exhibits the yield of 99%realisable in commercial scale harvesting.

The highest recovery efficiency (RE)83% was obtainable with S.Obliquus at 1.5A and initial pH 9.0 and 6 g/L NaCl with a power consumption of 3.84 KWh/Kg.whereas Chlorella Sorekiniam shows increase of RE from 66 to 94.52% having consumption of 1.6Kwh/kg and observed no fatty acid deterioration.The recovery efficiency with ECH is highly compareable to Centrifugation,Filtration,Chemical flocculation etc...Lipid extraction enhances by 22% electrolytes without any adverse effects which makes ECH ,a possible step in commercial microalgaes biomass recovery & biofuels production

The highest lipid accumulation have been achieved with **N.Oculta,T.Suecica,L.Galbasa and P.Lutheri** as 37.3,23.6,28.3,and 37.2 resply. with slight reduced cell growth of 0.64,0.49,0.54 and 0.38 g/L culturing under deficiency conditions of 10-65 g/L KNO3,3-7.5g/L NaHPO4 and 2.5 g/L FeCl 3**..**

EPA and DHA found in higher amount in Amphidimium similar to high presence EPA in other species like Tetraselmis Sp..These lipids containing omega-3 long chain PUFA finds application in food and aquaculture industries.

Saturated fatty esters(SFE) possess high octane number and superior stability whereas Poly Unsaturated fatty(PUF) esters have improved low temperature properties.Modifying fatty esters such as enhanced proportions of oleic acid (C18;1)ester can provide above properties together therefore it promotes quality of biodiesel conversion owing to the presence of high oleic acid.Over 65% FA are saturated and MUFA(C16;0,C18;0 and C18;1) are well suitable for biodiesel conforms the EN standards. of FAME (four or more double bonds 1 mol%).

For integrated and optimal bioprocesses,the microalgal residues after lipid extraction and cellulosic materials can be co-digested in anaerobic digester for biogas production and also waste water treatments to balance C/N ratio in optimum range of 20;1--25;1.

To improve algal lipid extraction,the methods like autoclaving,Supercritical CO2 and Ultrasonification are needed for optimization.

Hexane is the commonly used solvent than methanol,ethanol and a mixture of polar & non-polar solvents(MeOH/CHCl4), (Hexane/propanol)are effective on algal species.

Scenedesmus Sp.,NannoChloropsis Sp..,ChloroCoccus etc..,to enhance oil extraction in one step with simultaneous addn.of immobilised lipase catalyst coupled with Super critical CO2 (SERP) process as a extractive solvent principle conducted at 35°C shows the promising sustainable strategy explained as 19.3% recovery possible at a molar ratio 8;1(M;O) within 6 hours than easier separation of other compounds . Though the yield is low,the successful production of biodiesel is achievable to simplify the system and make it more economical.

Immobilised thermomyces Lanuginsus (TL) Lipases used in Hydrotalcite at a rate of 20% able to catalyse transesterfication of pretreated WCO linked on Citric & residual oleic acid and further exposed on tailor medium modified on Fe_2O_3/Au nanoparticles (NP) consisting magnitite shows a very high yield of biodiesel(upto90%)during 24 hours reaction time.

This shows a fast kinetic & higher activity in formation of methyl esters (34.6% for 3 hours and 70.1%after 6 hours)as NP exhibits a combinative properties (favourable enzyme orientation on the support & support surface functionality etc..)Additionally the immobilised lipase activity exists above 74% after 3 cycles of use with a biodiesel yield 97.8(+-)0.21 of ester contents and a linolenic methyl esters contents of 0.53(+-) conforms EN14214 standards.

A biodiesel separation produces the yield of 99.94% with SCCO2 at 40°C under a pressure of 30MPa and a flow rate of 7mL/min CO2 possibly with a retention time of 90 minutes investigated Comparing all four methods,EFAC can give the best results and good option for simultaneous microalgae harvesting and cell downstream processing.

Direct synthesis or insitu supercritical transesterfication method can be suggested as potential method to above processing of disruption of cells & vfextracting lipids in single step with NannoChloropsis Gaditana sp.(having 80% moisture&dry cells) in view of synthesising biodiesel with no added catalyst using supercritical methanol optimised at 255-265°C for 50 minutes at a CH_3OH to dry cell ratio(10;1) releasing biofuel yield of 0.46-0.48g/Gm lipids of higher yield quality product & higher level of conversion from wet & dry respectively.

WCO;

This can be used as potential feedstock and secondary raw material to the biodiesel if converted can satisfy to a larger extent the world demand of biodiesel.

Ultrasonfication serves as a better option to yield higher quality product & higher level of conversion. irrespective of argumentable enzymatic processing. This method of transesterfication require lower ethanol and less catalyst and consume 1KW energy for scale-Up having the variable cost between 0.1 cts. to 1 /L/gallon.

Batch processing are the simplest method of producing alcohol esters at 65°C as FAME within 20-60 minutes whereas continuous Plug Flow Reactor(PFR) require short residence times as low as 6-10 minutes operatable at elevated pressure & temperature.

POME considered to be a attractive natural source for biodiesel due to the presence of lipid concentration as high as 4-8g/L and shows high cetane number meets the demand for cleaner & greener energy.

Post processing the biodiesel,a complicated step involving separation of ester phase from the reaction mixture having the difference of densities arises between methanol,soaps,FFA, moisture or more phases..The centrifugal systems can help this in continual operations.

Separation through Water wash;-

For a reason,acidified water followed by water wash reported to be more beneficial for hydrolysis of soaps into FFA& tends to decrease the emulsification tendency.Then second step needs to be dehydrated the process in order to decrease water contents via Vacuum flask evporation,hot -air bubbling,convective heat drying,anhydrous salts,other water adsorbents etc...

A novel method is highly practiceable to reduce the normal process water content usage (3-10 liters of H_2O required for 1 liter of biodiesel) but it can be performed through Microfiltartion followed by sand filtration,Activated carbon etc..showing 15% lower water consumption through dilution rate with make-Up water to purification which is significantly having 1000 ppm in the final product.

To solve this,Vacuum drier,& falling film evoporater are mostly used to remove water contents operatable under low pressure. Magnesol is used commonly with inorganic matrix-$MgCl_2$ & $NaSO_4$ to adsorb hydrophilic materials (mono&Diglycerides,Glycerols etc..).An activated carbon bed is used to remove excessive colours in biodiesel than removal of S compounds ,odours by Vacuum distillation.

Upgrading biodiesel&renewable diesel;-

In view of upgrading the biodiesel purification,the deoxygenation pathways appears to be promising route for the Renewable diesel transformation.using Silica,Alumina,Zeolithes and fluid cracking catalysts.

This will enhance the cetane numbers by catalytic deoxygenation but this pathways can not be used for biodiesel due to the involvements of nature of extra steps, increase in capital& opeartional costs etc..Hence,proper combined status process are essentially required to obtain a robust biodiesel purification.

Recycling Glycerol

In future,non-ester side streams to be treated in parallel to overall biodiesel process such as recyclage the glycerol,excess methanol to be recycled estimated to be 10% by weight of input reactants.within the system and waste water stream.

Since methanol delays the rate of gravity separation distributed approximately 60;40wt% between the two phases of glycerol esters and this can be suggested through recovery of solvent either by conventional or vacuum distillation or partial single phase recovery process.

During the final stage,glycerol refining can be overperformed by chemical methods through the

formation of salts by neutralisation such as addition of FeCl2 or Al2SO4 and then complete the process by centrifugal separation followed by bleaching by activated clay are recommended.

Ion Exchange resin play an important role on removal of minerals,catalysts and other impurities through cation,anions & mixed bed system at the final polishing stage but it needs the regeneration of other beds simultaneously.In the case of heterogeneous system,Ion exchange methods are considered for removal of metal catalysts .PD 206 &BD10 dry cationic bed are recommended for purification of biodiesel from WCO & rapeseed oil alongwith soap & glycerol removal in contrary to methanol whereas LEWATIT GF 202 have the potential to remove methanol but the capacity to remove soaps & glycerol are not to the similar extent with the former case.

Waste management;-

Methanol is to be recycled and able to recover at a maximum level before disposal into waste water stream .This make the process easier access permission from the pollution control board.

. Membrane separation finds more phenomenal characterstics solution upon implying the membranes such as PolySulfone(PS) & PolyAcrylonitrile(PALN) etc..through successful implementation of UltraFiltration (ethersulfone)& MicroFiltration Cellulose ester membrane systems for the glycerol separation having 0.02wt% to 0.009% limit attainable in the permeate by adding 2wt% by mass thus finally conforms EN & ASTM standards.

PS is the most attractive organic membrane applied in biodiesel refining and a study was performed with n-Hexane, as alower toxicity co-solvent increase the conversion from 65.7% at 30 wt% to95.8% at 60 wt%n-hexane..

Unicellular Organism,as a source of lipids;-

Unicellular Oleagineous microorganismes ,a potential source accumulate more than 20% w/w lipids in contrary to other marine macro algaes species(70% lipids w/w)in their cellular components on dry weight basis capable to synthesize vast majority of fatty acids from short chain(C6) to long chain hydrocarbons(C36) depends on cultivational condition under C/N ratio translates as saturated or unsaturated FA.

Genetic Manipulation for improving oil contents;-.

The overexpression of genes or enzymes of biosynthetic pathways,suppression,blocking or knock-out of genes of competitaive pathways, regulation of pass pathways, multigenes approaches etc..could find a suitable solution for synthesis,storage & profile of lipids as per the adoptivity of microorganismes into the environments.This results in change in production rates both for biofuels energy and neutraceutical purposes.

The above strategy are realisable through the expression of two key genes ACC1 & DGAT1 in oleagineous yeast (yarrow Lipolytica)by 2 times & 4 times respectively whereas the overexpression in combined form results in 5 times greater lipid accumulation than control indicates their synergistic effect.

Cyanobacteria,a blue green algaes consider as a model organismes known for genetic recombination to find a change in metabolic pathways in which lipids not in accumulation in contrary to carbohydrates production as secondary metabolites.

Yarrow Lipolytica , an indiustrial yeast is well studied strain for genetic manipulations and unique ability to grow on hydrophobic substrates.produces EPA yield of 161.04mg/g/day. The cultivation of yeast can enhance the productivity using low cost substrates such as waste glycerol or sugar from lignocellulosic biomasses thereby the process makes viable & feasible..This organism can be considered as a model microorganisme to understand the mechanismes behind the uptake of

hydrophobic substrates.

Bioactive compounds, as Neutraceuticals;-

Fistulifera Solaris ,a Marine microalgaes &microorganism are well developed for its efficient metabolisms owing to their environmental adaptation and these are exploited to produce neutraceuticals value fatty acids having higher content of PUFA and DHA &EPA than fresh water species especially cultivated in photoautotrophic conditions reported to be producing EPA with a optimum level of 135.7mg/L/day whereas the heterotrophic growth marine diatom-Nitzschia Laevis upon supplementation with glucose results in EPA production of 174.6g/L/day ..

Traustochytrids(fungus like clade of stramenpiles),a good source of DHA has been recommended for commercial production through improved technology requiring 25-30°C temperature for optimal growth and reduced temperature 15°C enhances DHA production at the reduced growth level.

(17.0) CONCLUSIONS;-

Biodiesel is the alternative to petroleum diesel and offers the several advantages to the environments since it exhibits lower CO2 emissions(GHGE) meaning that global climate change,carbon neutrality etc..

The exploration of a sustainable resource of PUFA is needed through vegetable oils that can be considered as a alternative source of linoleic(C18:4,n-6AA)..The growth of SC microorganismes can be done in large cultivations conditions with the Glycerol as carbon sources that could be considered & used as a cheapest materials estimated to be 80% cost of productions of biodiesel and neutraceuticals

In order to utilise the fuel property efficiently right from the storage,distribution etc.; it has to be upgraded like moisture content,low SO2 contents,cold flow properties,viscosity reduction thereby additzation is required to improve the fuel performance having the higher cetane number and may not contribute the net accumulations of GHGE.

Additionally, It may be observed that algal sequesteration of CO2 could be increased through implementation of multiple PBR to scale-Up the process.varying from species to species.The reactor configuration with special reference to Open pond where paddle wheel system proposed will have the industrial feasibility of PBR having the lipid production cost estimated 31.6$/gallons compared to 12.73$/Gallons for open pond.This shows higher biomass production possible at a large capacity in parallel to decrease of the risk of contamination

Designing Photobiorectors (for algaes) that certainly provide a status and maximise the productivity that reflect on acquiring clean water,Clean air,clean energy and effectively stimulates treating the complex contaminents-heavy metals and polycyclic aromatic compounds.etc..while replicating in real condition .

CO2 Sequesteration & waste remediation;-

Waste water are considered to be one of best options for sustainablity,zero emissions production of biofuels as sourcing nutrients for biomass production than potable water or sea water remarkbly adds up the cost of biomass.

The waste management point of view,Methanol is to be recycled and able to recover at a maximum level before disposal into waste water stream .This make the process easier access permission from the pollution control board.

Microalgae production needs to be done on very large scale to make it profitable based on low cost media differes from,culture media laboratory.

AND AIM TO DEVELOP PROCEDURE TO INCLUDE LONG CHAIN ALCOHOL FOR LOWERING FREEZING POINT OF BIODIESEL; (applicable for cold countries) (study realised by Worcester PolyTech Institute)

OBJECTIVE OF STUDY ;-

- The aim of the study is to develop a procedure for producing long chain biodiesel that enhances on easier separative methods. and then would decrease CP so that it can facilitate the lower freezing point of biodiesel blend with optimal alcohol length as determined as Iso-butanol in view of withstand colder climates .

--The second aim of the study is to use different alcohol to produce biodiesel having the complexity to increase wax formation through Crystallization results becoming harder and subsequently lower freezing point.(**Refer)**

-The next goal of the study is to predict how biodiesel will freeze.Then the experimental study was correlated with theortical CP predictions equations in compared with control samples from JetA fuel and Diesel 2D.

Processing Biodiesel with different Alcohol;-

Higher the temperature to process the reactants,faster the reaction obtainable in proportion to not exceeding boiling point of alcohol.Then additional time is required for the catalyst to dissolve in alcohol approx.5-20 minutes based on solubility of base in alcohol.

During refining biodiesel,CH3OH influences two-phase separation between unrefined esters and glycerol layer that settles to the bottom .Hot water is used to purify the esters further through evoporation however ethyl or long chain alcohol give rise to a single phase separation where unrefined esters are purified through hot water to boil out alcohol and condense later and phase is more attainable through excess alcohol removal.In the case of longer chain alcohol,there may be another approach to separate unrefined biodiesel through density separation using a preferable centrifuge.(**Refer Fig-8.1,2,3,4,5,6,7,8N etc..)**

Following equations can be used for calculating the cold flow properties of biodiesel which may be estimated on the basis of total saturated FA alkyl esters(Sats) concentration.

Equation 1:-

CP = 1.44 * [Sats]-24.8

-used to calculate CP on the basis of saturation point with +- 2 level of accuracy.

-CP modeling based on also n-alkane content.

Equation-2

$$\text{Lamda i i} = \frac{-2\,(\text{delta h sblm2} - RT)}{Z}$$

CFPP =Cold flow Plugging Point

where requiring minimum temperature to filter 20 ml through 45 nm wire mesh under 0.02 atm.Vacuum during 60 seconds.

CFPP = 0.438[Sats]-8.93

Equations-3

$$\ln(x) = \text{Delta H}\left[\frac{1}{Rg}\right] - \left[\frac{1}{Tf\ Mp}\right]$$

where X=solute mole fraction

Rg = Gas constant

Equation-4

CP= 18.134 Nc -- 0.79 UFAME

where Nc = weighted average number of C atoms

UFAME = Composition of FAME in biodiesel

EXPERIMENTAL STUDY AND METHODLOGY OF BIODIESEL TO FIND OUT THE LOWEST FREEZING POINT.-

Methodology;-

CP is determined for biodiesel based on chain length through a prediction method(1)(2)(3)(4).

Method-1

DSC scan was used to analyse the melting onset temperature(MP),maximum peak temperature(PH) and enthalpy of heating(Delta Hp) in which this parameters used to solve the Hildebrand equations-X-for ideal solution for predicting crystallization onset temperature of solute in solution within 5°C.

Method-2.

This is used for analysing n-alkane of methyl ester in biodiesel fuel blends and would be ideal if n-alkane values are available for modeling CP.The equation -X- summarizes the solid -liquid equilibrium functions using the composition in both

Here UNIQUAC solid phases non-ideality used as a predictive model method .The calculations -X-X predicts the fluid behaviour at low temperature.

Method-3

n-alkane of methyl ester predicted with a set of equations suggested by ASTM test procedure.but equation X-X interprets in difficulty and translates which method would best to run.

Method-4

Cold flow properties of biodiesel may be determined based on total presence of Total saturated fatty acid alkyl esters (Sats) concentration as expressed by the equation(A).

Equation X- used for calculate CP on the basis of +- 2°C level of saturation point accuracy.The whole method shows increase in CP with increase of C content.

Prediction method for PP:-

This is based on previous study results comparable with experimental data.Multiple sources used to provide most accurate set of PP. Referring-FigX- theortical PP indicate an increase in temperature correlating to decrease number of C chains presence.

RESULTS &DISCUSSIONS;-

The samples was analysed for CP & PP.The results can be seen under the respective ester carbon length in fuels(FIGURE-12.0&12.1&12.2&12;3)

(CP) Cloud Point;-

The cloud point is the temperature at which waxes first to start crystallise.In other words,it is the temperature at which oils gets solidify.It is the indication of lowest temperature at which fuel can be used before wax crystals blocks the fuels filters.Therefore it predicts suggesting lower operating temperature for engine operation.

CP are tested according to ASTM methods applicable to biodiesel as follows;-

3ml purified biodiesel is measured out in a glass tube then it is placed in a large container corked with a thermometer.and another thermometer into ice batch are inserted.Then the bath temperature is recorded at thge start of the experiment .For every -1°C increase in temperature the change is recorded for clouding.In thecase of Iso-butyl and Iso-pentyl esters ,the step is modified with dry ice bath allowing for colder freezing temperature.

(PP)POUR POINTS;-

I t is the point at which fuel flows at the lowest temperture .Beyond this,it becomes a waxy gel. ASTM standards for untreated #2 oil is 17°F.Additive or kerosene are added to heating oil during winter ensuring the flow.

The experimental design is same as that of Cloud point and repeatable as indicated above;-

3ml of purified biodiesel is measured in glass tube with corked thermometer & placed in large container and the other one into the ice bath are inserted.and recorded at the start.

For every -1°C increase of temperature,the measure is recorded for solidification when the fluid is no longer able to be poured.In the case of iso-butyl & isopentyl esters ,the step-3 are to be modified to a dry ice bath allowing for colder freezing temperature.

In order to drive the reaction typically NaOH or KOH is used since base catalyst is less complicated than acid alternative and then to optimise the experimental study ,the cost,separation,feasibility and product yield were considered.KOH is easier to use in industry for recycled oils but not only creates the product yield but is highly recommended for better two phase separation and more expensive.

Methyl-Esters;-

Methanol based biodiesel results in 2 phase separation betweenn glycerol and methyl esters layerin which glycerol removal is possible and the remaining esters subjected to wash yielding high purity level esters based on reaction kinetic.The results are indicated through 3ml sampling for CP & PP.Tab-0000

Trouble shooting (TS)of Ethyl esters;-

The limiting factor is the separation between layers and one of the three attempts was to drive the separation of glycerol through addition of excess 10 ml glycerol and to be shaken to the motif of separating by density.Another attempt was to separate by using freezing point where glycerol freeze and leave behind liquid biodiesel.The problem is that freezing didnot drive glycerol sink to a bottom layer but the whole solution becomes frozen.The last attempt was realised to reach optimal separation through addition of NaCl in order to have layer separation through ***solubility .The optimal*** separation of glycerol are done through centrifuge at 300Rpm for 15 minutes.The results of CP & PP are indicated through 3ml sampling .

Trouble shooting (TS) Butyl esters;-

The by-products of transesterfication is the more formation of water due to saponification.

TS of Iso-Butyl Esters;-

Isobutanol is not successful in phase separation so centrifugation is necessary at 2500 Rpm to be done for 15 minutes.Then the top layer was extracted out and 3ml were sampled for CP and Pp as below:

TS of IsoPentyl esters:-

Isopentyl was the appropriate biodiesel based alcohol tested for longest 5-carbon to the chain of the alkyl ester.Then the top layer of the solution was extracted and the the sampling was repeated as above.compared with Diesel 2D and JetA as control

CONTROLS;-

JetA & Diesel 2D was used as a control to validate CP and freezing point test methods.3ml were sampled for the above analysis shown below.

CP of various fuels;-

The results are determined through a graph against CP temperature in decreasing order of C length..Isobutanol based biodiesel had the lowest CP of -8.5°C while methanol based fuel had the highest CP of -3°C.

PP of various fuels;-

Jet A was not able to freeze in bath conditions.Then Diesel 2D used as control with a recorded PP of -30°C matching actual PPfuel exactly.hence we may predict that isobutanol has the lowest PP and methanol had the highet PP.

In both the cases,increase in PP is possible with the increase in carbon content.

CONCLUSIONS;-

The objectif of the experiment is to determine optimal method to achieve lowest freezing point in biodiesel fuel through investigation of CP & PP of methanol,ethanol,Isobutanol and Iso-pentanol.The addition of complex alcohol in transesterfication process was expected to be increase in carbon chain to the esters results the lower freezing temperature.

RECOMMENDATION AND CONCLUSION OF EXPERIMENTATION SUGGESTING FOR OPTIMAL ALCOHOL

The remedy is to improve tha quality of the product through evoporation but excess alcohol during experimentation as well as to wash all final product to make sure of quality esters as pure as possible.

Hence recommendation is to use the optimal alcohol length as ***isobutanol*** *based biodiesel as a blend for colder climates conditions.These combined biodiesel fuel shows having the lower cloud point biodiesel would yield desirable freezing point that would be more competitative with petrol fuels.*

Isobutanol determines to be the optimal performance fuel in lower climates. having lower pour points and cloud points than methanol.

17:1ANALYTICAL METHODOLOGY OF *BIODIESEL*

The quality control of biodiesel is greatly significant on the basis of commercialisation and market acceptance.The assessemnt of biodiesel is to be done through determination of various chemical parameters such as Acid value,Saponification Value,Iodine value,Calorific value,cetane index,flash point, ash content,refractive index,viscosity ,specific gravity,,fatty acid composition of individual essential oils etc.and this can be determined also through Gas Chromatoghraphy methods,Spectroscopic methods,Nuclear magnetic Resonance Spectroscopy etc..Near-Infra-red spectroscopy,HPLC helping in to characterise and assessing the quality of biodiesel.

QUALITATIVE ESTIMATION OF BIODIESEL AND OTHER & IMPURITIES PRESENCE;-

FATTY ACIDS TITRATION METHODS;-

It means to determine Neutralisation Number(NN).Two methods are developed in determining strong acids and free fatty acids(FFA).One of the method is Potentiometery Method:-and other one is two acid base indicators(neutral red,Phenolpthalein) .

First method is more reliable even with use of two indicators.NN derived from titration method are 10- 20%relatively greater than activity sample

Apart from this,wet chemical methods plays a role in determining fatty acid profiles in which iodine and saponification values are analysed.

SAPONIFICATION VALUE;-

It is the process of breaking down or degrading neutral fat or oil into glycerine & fatty acids by treating with hot caustic or alkali. It is the value or saponification number related to the average molecular weight of fatty compounds.Longer the chain fatty molecules have low saponification number and shorter chain fatty acids have higher saponification numbers.

IODINE VALUE;-

It is the expression of degree of unsaturation of fat and it is measured the value by the amount of Iodine required to react upon absorption by 100 gms of given oil under prescribed condition.It is the measure of unsaturation of fats and oils.Higher the value indicates higher the unsaturation determines by measuring number of double in fatty compounds.

Iodine absorption occurs at double bonds giving higher IV number that indicates higher quantity of double bonds leads greater potential to polymerise hence lesser stability.

BAILEY &WALKER METHOD FOR FAT &OIL ANALYSIS;-

Materials& Methods;-

Balance,Mortar&Pestle,Cylinder,Petroleum ether,Electric Oven,Condenser Vials,paper Thimbles,Electric hot plate with water circulation system etc..

Procedures;-

100 gms of oil biomass sample to be weiged and then Paper thimbles to be oven dried followed by biomass to be placed in the preweighed thimble.Then 40 ml of petroleum ether is added into the vials containing above materials in the thimble.It is to assured that enough ether is filled just below the top of the thimble(1/4th) and water circulation is to be assured before start of the equipments through the condenser.Then the electric hot plate is tuned on high level allow the ether to boil & recirciulate by setting it to low temperature refluxing for 1 hour .After 1 hour ,the thimbles are removed and dry it for 1/2 hour in air Oven regulated at 100°C.Then the thimbles are weighed after extraction of oil to calculate % oil extraction.The following equation is used for calculating oil content in the sample.

$$\%OIL = \frac{\text{grams of oil extracted}}{\text{Initial weight of sample}} * 100$$

TESTING FFA CONTENT;-

During and after transesterfication ,the product has much impurities like FFA ,Glycerol etc. in which .FFA can be estimated by following simpler methods;-

Materials &methods;-

Automatic Burette having glass reservoir bottle,two hole rubber stopper,rubber bulb,pinch clamp,rubber tubing connecting tip to burette,Erlenmeyer flask (wide mouth 250 ml)dropping bottle for indicator solution,NaOH(0.1N),Isopropyl alcohol,Phenolpthalein indicator(1gm/50cc H20).

Procedure;-

Graduate is filled with isopropyl alcohol & emptified into erlenmeyer flask and 3-4 drops of above indicator is then added followed by adding 0.1N NaOH drop by drop until isopropyl alcohol first changes into pink colour from white.Then 32.5ml oil is filled in graduate (200°F)to be tested for FFA contents. and oil is emptified completely into pink colored isopropyl alcoholin erlenmeyer flask.Then flask is to be shken vigourously to mix the oil & alcohol till color change into color of oil.

0.1N NaOH is filled to the top of the burette mark and to be assured before each test is made. The oil sample is titrated in erlenmeyer flask with NaOH through shaking flask until color changes

into pink.This is repeated until color does not appear.Please note that oil does not require extra heating.However,if analysis is made on cold oil sample,then alcohol & oil mixture should be warmed to 150°F before titration for best results.Then the amount of 0.1N NaOH consumed in titration is to be determined. 1.0ml NaOH is equivalent to 0.1 FFA

For, 0.5 ml = 0.05%FFA

5.2ml = 0.52%FFA

QUICK DETERMINATION OF FAT CONTENT BY REFRACTOMETER;-

Bausch & Lomb"Abbe56"refractometer or equivalent can serve us to determine the oil content of biomass directly readable as mentined in chart (Table24.1)

The procedure is above 50 gms biomass to be weighed and equivalent amount of n-heptane is added and make blended at high speed for 2 minutes followed by decanting and filtering it in funnel and cover it with watch glass to minimise the evoporation.If first part of filtrate is cloudy,then discard it and collect few ml.of clear filtrate and place 3-4 drops of them into the refractometer prism and read the refractive index**.**

ANILINE POINT/ CETANE NUMBER;-

It is the relative measure of the interval between the beginning of injection and auto ignition of fuels.Higher the cetane number of fuel, the shorter the delay interval and the greater its combustibility.Fuels having low cetane numbers will result in difficult starting ,noise and exhaust smoke.Diesel engines can be operated better with fuels having cetane number(higher50).

DENSITY;-

Density is the weight per unit volume.Oils are denser contains more energy whereas Petrol and diesel gives comparable energy by weight whereas diesel is denser and hence gives more energy per liter.

ASH MEASUREMENTS

Samples can be analyzed to determine total ash minerals content in a Microfurnace controlled at 550°C kept in overnight till gets constant weight.

MOISTURE DETERMINATION;-

Moisture can be determined either by Toluene distillation method or by Vaccuum Oven method or by Infra-red method.

Infra-red Method;-

Infra-red method provides the product sample to be placed with an attached heating elements.This shows continual indication of weight decrease in moisture loss throughout the drying cycle until constant moisture loss is present.

Equipments used;-

Infra-red heating elements equipments,drying dishes,Top loading balance etc..

Procedure;-

Accurately 5 grams are weighed directly into a dish on the balance.Lamp is tuned on and left it until no further change in weight occurs.Then the weight is recorded & Moisture content is calculated.

ESTIMATION OF MONO-DI-TRIACYL GLYCEROLS ,METHYL ESTERS AND GLYCEROLS THROUGH ***GEL PERMEATION CHROMATOGRAPHY(GPC)***:-

This method is meant for analysing above products as variables that affects transesterfication of rapessed oil and using a refractive index detector and TetrahydroFuran as mobile phase. It is similar to HPLC but reproducibility is better through means of standard deviation expression at

different rates of conversion.

HPLC METHOD:-

The basis of the HPLC method is a small column packed with adsorbent on which sample is loaded and elutable with a solvent under high pressure using pump system.The component are screened by detector system after comes out of the column and the data is recorded in forms of peaks and percentages.

Reaction mixtures obtained from Lipase catalysed transesterfication process is analysed by HPLC using evoporative light scattering detector(ELSD).This method helps to quantify esters,FFA, and various forms of acylglycerols.

The composition of reaction mixture can be determined by modified HPLC method of Holcapek et al(1999)using Hitchi7000, equipped with a degasser ,a binary pump and autosampler with chromatography column-Zorbax eclipse XDB-C18 capillary column(4.6 mm-250nm-5µm) and UV-VIS detector.

Solvent A methanol and solvent B(Isopropanol/n-hexane,5:4 by volume) were used as a mobile phase.The samples of reaction mixture at different time intervals were centrifuged at 1.677 XgRCF for 10 minutes;a known of upper layer was dissolved into the mixture of Isopropanol/n-hexane.5:4v/v and injected using an autosamples.All the samples and solvents were filtered using 0.4µm millipore filter.The flow rate of a binary solvent mixture(methanol,solventA and isopropanol/n-hexane,5:4 by volume,solvent B)was 1 ml/min with a linear gradient from 100%A to 40%.At 60%B in 30 minutes.Column temperature was maintained at a constant value of 40°C.The components were detected at 205nm.The fatty acids were identified by comparison of retention time of oil components with those of standards.The relative HPLC areas and the components mass were caliberated using known standard composition.The percentage conversion was taken as the conversion of triglycerides to methylesters,monoglycerides and diglycerides

STUDY TO FINDOUT TAG PROFILE WITH HPLC;-

The study with TAG of jatropa oil profile can be done with HPLC equipped with ELSD-800 detector.and then separated using column inertsil ODS3(250*4.6mm) having the mobile phase containing mixture of acetonitrile and dicholoromethane(60;40).set at a flow rate of 0.8mL per min. with a pressure of 2.3 bars shows TAG peaks identifiable based on retention time with commercial TAG standards. Then the purification of glycerol can be determined using HPLC Shimadzu LC10 with a refractive index detector. packed with a column Shim Pack scr-10N(7.9mm*30mm) having the mobile phase with H2O with a flow rate of 0.5mL per minute at 50°C**.**

GAS CHROMATOGRAPHY ;-

This is the prescribed method for measuring free and total glycerols as per standards ASTM methods D6584.

The principles of this method is to treat with N,O-bis(trimethylsily)trifluroacetamides(BSTFA) to give corresponding trimethylsilyl(TMS)derivatives since it improves chromatographic properties of hydroxylated molecules and in case of coupling to mass spectrometer. That explains facilitating mass spectra interpretation.The sample is injected into a microsyringe where steam of inert gas carries it into the detector of the analyser.The detector give rise to electric signals when components passing through it which is then amplified before fed to recorder.This traces the progress of analysis in the form of series of peaks.The accuracy can be further influenceable by factors such as base line drift ,overlapping signal etc..in order to compensate above in biodiesel.

The first Gas Chromatograph method has been developed to determines simultaneously the amount of glycerol(in derivatized form),mono et diacylglycerols,triacylglycerols and methylesters in biodiesel sample.The derivatised glycerol is firstmaterial to be eluted followed by methyl esters,and derivatized above three acyl glycerols.

Trimethylsilylation of glycerol,mono and di-acylglycerides allows to determine and then followed by Gas Chromatography using 10 m capillary coated with 0.1mm film DB-5 permit to analyse all analytes in single run.

Biodiesel sample can be analysed in employing Flame-ionisation detector(FID) .The determination of composition of oil (C16;0,C16;1,C18;0,C18;2,C18;3) is done using fused Silica capillary column 60m*0.32mm(ID) at the split ratio 1;5 and the oven temperature controllable at 150°C for 1minute then heated to 30°C per minute at 240°C.Helium used as carrier gas with a flow rate 1mL per minute and an auxillary gas for FID.1ml of each diluted sample with Dichloromethane is injected.

DETERMINATION FFA through Agilent GC:-

The composition of seed oil is determined using Agilent Gas Chromatography 6890 equipped with Ioniosation detector and a capillary column(30mm*0.25mm*0.25mm).About 1ml oil converted into methyl ester using 1mlNaOMe(1M) in 1ml Hexane before subjected into GC.The detector temperature programmed at 240°C with a flow rate of 0.8ml/min.The injector temperature set at 240°C .Hydrogen used as a carrier gas and then peaks are identifieable by retention times by comparing authentic standards analysed under same conditions.

ANALYSIS of FFA ,FAEE.. through HP Model 6890Chromatograph;-

Jatropha oil is analysed using gas Gas chromatgraphic Analyser of oil ethyl esters made into EE using 2%H2SO4 as catalyst in presence of excess dry CH2OH .Then the chromatographhic analysis carried out using Hewlett Packard Model 6890 Chromatograph having a capillary column of 30m length and 530micro.m inner diameter packed with Apioezon.The temperature od detector,injection,Colum etc.. set as 280°C,300°C and 100°C to 240°C at 15°C per minute.respectively.

1;2:1VISCOSITY DETERMINATION ANALYSIS;

It is referred to thickness of the fuel that resists to flow.Gasoline has low viscosity flows easily than higher viscosity greases.

Viscosity applied to determine the conversion of vegetable oil into methyl esters resulting from transesterfication is referred to the value of 1. kinematic viscosity has been included in biodiesel standards(1.9-6.0 mm2/sec(ASTM) & 3.5-5mm2/sec in EN 14214).

Viscosity determined at two temperatures (20 &37.8°C)are in good agreement with gas chromatography analysis for verification purposes.The difference in viscosity between oil and esters can serve to monitor the progress of reactions.

In order to obtain the dynamic viscosity over the temperature ranges upto 300°C ,a modified saybolt viscometer is designed to measure the efflux times for a quantity of 60ml methyl or ethyl esters in a sample. and this viscometer can be calibrated using a standard oil and can be used to determine kinematic viscosity(<0.056mm/sec with 2% repetability).

Decresaing temperature ,viscosity increases and often reported to be 5°F during winter in compared to normal basement storage

shows 60°F. Whereas the cold bioheat causes poor atomisation,delayed ignition,noisy flames,pulsation and possible sooting etc,,

The new patented technology SVM 4001determines the viscosity index easier and faster than ever.The new double cell design instrument enables simuntaneously measurement of kinematic viscosity in the sample at 40°C and 100°C.

This fast measurement automatically calculates viscosity index fully compliant with with ASTM-d2270 and permits the results displaying on the screen within few minutes.No externalPC or software is required to perform the Calculations.This instrument can be employed in areas where speed is essential and this viscosity can guide as where the lines are clear of old batches than other new product batches ,ready for distribution.

1:2:2 VISCOMETERS FOR BIOFUELS;-

Anto Paar SVM3001 is a high precise viscometer with an integrated density measuring cell.A single measuring cycle on a small volume yields kinematic viscosity,density,dynamic viscosity,viscosity index and more whereas one combined measuring cells covers entire measuring range of viscosity,density and temperature,a filled in one.According to the standards,a minimum sample of only 2mL is sufficient for multiparameters results that enables to measure at the broad range of temperature between-60°C to +135°C from jet fuels,heavy fuels and crude oils.This innovative double cell design allows simultaneously measurements at 40°C and100°C from a single syringe.

According to D2270 as well as freely selective API calculations,the viscosity index results are automaticully extrapolated on the touchscreen.

1:2:3ANALYSIS-ULTRA LOW-SULPHUR IN BIOFUELS:

Biodiesel has no sulfur content in it .The S content of heating oil ranges from 0.5% to 0.05% when S burns ,it reacts with O to form SO2 and SO3.The SO3 reacts with water vapour during conclusion to create H2SO4 aerosol.The acid adheres to exchanges inside chimney,it creates scaly to red crust that makes 50% deposit and downgrade the effeciency by 1-4% during the year .So blending with ultralow S fuel is necessary to eliminate the scale.

Tagaku designed a Micro-Z ULS for detecting low level Sulphur compounds analysis.X-ray Fluorescence(WDDXRF) instrument measures both S peak and the background density and ideal solution for S analysis with lower limit of detection (LLD) of 0.3ppm sulphur.The ability of measurements and changes in structure fuels delivers a better net peak intensity mesurements resulting superior caliberation and enhancing real precisions.

RIGAKU MICRO-Z ULS FOR ULTRA LOW SULPHUR IN FUELS;-

The above instrument is a dispersive X-RAY Fluorescence (WDXRF) instrument that measures both S peak and background intensity.The ability to measure and correct for changes in intensity delivers a better net peak intensity measurements resulting in super caliberation and enhanced real world precision.

This instrument is ideal solution for S analysis of biofuels etc..with a lower limit of detecting (LLD)of 0.3PPM sulphur.

1:2:4 UNCOOLED METHANE GAS DETECTION BY INFRARED CAMERA:-

FLIR-GF77 Gas find IR is engineered specifically to detect methane in order to improve gas inspections and reduce the chance of false readings.It allows to find potentially dangerous invisible methane leaks in renewable energy production facilities and other industrial plants.

This provides the gas detection capability at half of the price of colled gas inspection thermal cameras in view of reducing emissions and ensures safety work environments**.**

1:2:5 CLOUD POINT &POUR POINT:-

The methodlogy has been already discussed in previous Biodiesel chapter.(refer Lower Freezing point literature).

It is the temperature at which wax crystals begins to form in the fuel greater than 10-20°F pour point .The crystal formation can clog filters restricting the fuel flow .Both pour point & cloud point will affect the winter performance if no proper treatments occurs.

MPC -6 is designed by Tanaka,japan simplifying the test for cloudpoint of Biofuels.Preheating and cooling sequence are run automatically and cloud point is obtainable in a single run.The new SPE will test the pour point with different pressures in order to optimize the parameters.The final data is recoverable through data storage.

WATER & SEDIMENTS;-

Accumulation of water during storage causes the formation of sludge and ice.Sludge is referred to presence of largely oil and water. that permit not to mix but presence of organic sediments acts as a binder to stabilize tha above and lead to form the milky substances susceptible to unburn ASTM limit for water is 0.1%.

COLOR;-

Presence of murky appearence may indicate a fuel quality problem irrespective of the problem darkness of color.

IGNITION POINT;-

The ignition or fire point is the lowest temperature at which rapid combustion of a fuel takes place in air.It is the temperature at which all the fuel has been heated and vapourize sufficiently to continue to burn at least 5 seconds.

FLASH POINT;-

The minimum temperature at which the fuel will ignite (flash)upon applying an ignition source.It varies inversely with fuels volatility.Presence of very small amount of alcohol will lead to significant drop in the flash point.This is

sufficiently greater at 150°F .It is the maximum temperature at which it can be stored and handled safely without serious hazard .In other words,minimum flash point temperature are required for safetyness and acts as an index for biofuel storage indicating purification strategy of biofuels.

Grabner instruments propose a new miniflash FP unit anayser that detects lowest flash points combustion analysis through aliquot 1-2ml requirements requirements.It has the advantges of reading the results automatically and flexible to use for detailed analysis with cockpit premium software having the temperature range between -25°C to +120°C.

18;0 BIO-HYDROGEN PRODUCTION

H2 is not an energy source but it is the energy carrier.It is the secondary form of energy,manufacturable like electricity.It is not primary energy existing freely in nature whereas it is alternate energy vector and linked to a sustainable energy future.H2 can be produced from different technologies and also from wide variety of primary energy source .Biomass has the potential to accelerate the realisation of H2 as a major fuel of the future

PROPERTIES OF HYDROGEN AND ITS USES;-

Hydrogen stores three times more energy (in terms of Volume)than gasoline and seven times more than coal.H2 needs to be stored in super insulated vessel due to its low boiling point and low energy density ,it is still easier than storing electric energy .Besides special properties of H2 leads to its occurance in elemental forms,usually bound in compounds and rarely

in a pure molecular form of H2.,Therefore ,to obtain H2,it is often necessary to break down the compounds that contain H2..Therefore ,the selection of H2 production process and H2 containing substrates are based on cost of anlayses of processes,the abundance of substrates and number of expected moles of H2 obtained from a mole of substrates..

HYDROGEN PRODUCTION ROUTES;-

At present,half of all current status production of H2 are based on Thermocatalytic and gasfication process using heavy oil as a starting material.Hence,Biomass gasification offers earliest and most economical route for the production of renewable source.Recently H2 is produced for an industrial applications from petrochemical Cracking (crude oilor natural gas cracking) ,coal based processes or water Electrolysis..

Petrochemical and Coal based processes are related to steam reforming of hydrocarbons.(mainly CH4).H2 is produced mainly from natural gas in a two stage process called steam methane reforming(SMR). This process is limited but can not be used for hydrocarbons heavier than Naptha..

MISCELLANEOUS -OTHER POSSIBLE HYDROGEN ROUTES;-

There are several other processesss to obtain H2 such as

-Water splitting,Decomposition of biomass or NH3.

-Water splitting into H2 and O2 during electrolysis.,

-Applying heat from other chemical reactions (Thermochemical Splitting),using biological processes,or

-solar energy(Biophotolysis),

-Microbial- biocatalytic electrolysis process(anode working -potential produced by microbial cells,

-In electrical discharges(Plasmolysis),

-Applying magnetic Induction(Magnetolysis)or Irradiation by radioactive materials(Radiolysis.....)

-Hydrogen can be obtained by fermentation of biomass in several thermochemical (Gasification ,Pyrolysis) and biological (Biophotolysis,Fermentation,and biological gas shift) processes.

-Biomass Pyrolysis involves heating of substrate under less O2 (anaerobic ocndition).

- Biomass gasification is a H2 production and generation by decomposition of biomass under limited presence of an oxidiser(air,steam ,CO2 etc.;)

Among many hydrogen production methods,high purity of Hydrogen can be obtained by Electrohydrolysis of water.In terms of sustainability and environmental impacts,PEM water elecrolysis was considereblv more efficient promising method for high pure H2 production from renewable energy sources and emits only oxygen as byproduct without any gas emissions.

BIOMASS GASIFICATION PROCESS ;-

Biomass Gasification is a mature technology method, as a viable pathway process determines its potential uses a controlled system involves a steam,heat,and O2 converting biomass to H2 & other product.

*Gasfication involves conversion of organic material biomass containing 15% moisture preferable for producing clean fuel or syngas by reacting them at high temperature(800°C-1000°C)in the absence of oxygen or steam.*The conversion product is syngas containing H2(6-55%) and CO(8-53%) with CH4 as a co-product (2-26%)

The entire process can be briefly *explained through following equations:*

C6H12O6(biomass))+(thermal energy+ steam) >>> >>>>>>>>>>>

H2+CO2+CO+ H20+CH4 + Hydrocarbons+ash

Biomass conversion technologies can be divided into two ;

1)Direct production routes

2)Conversion of stable intermediates

Both the classes have thermochemical and biological routes considerable for minimising the transportation costs and shipping may be directly possible to the central and large scale H2 production .

Hydrocarbons+Ash

The proposed catalyst such as Dolomite ,alkali catalyst and noble metals. are used with a Temperature & pressure of the process varying from raw materials namely sewage sludge (180-250°C and 1.5MPa),& for a wood material ranges from 950°C-1500°C&for a lignocellulosic biomass having a (Copper-Zinc catalyst,T= 700°C-800°C) and for rapeseed having optimum temperature 750°C.The CH4 produced in the process can be later used for synthesis of H2 in CH4 forming processes.

According to Moreno et Dufour ,the process is suitable for wood residues such as eucalyptus ,almond etc..which require high energy temperature around 1000°C producing 0.26m3 Hydrogen from 1.32 Kg Pinewood and 1.01 Kg Vine. In the same way, 0.31m3 hydrogen generation is possible from 1.44 Kg of almond .

The CH4 obtained through this process can be further used for H2 production by gasification or SMR(Stream methane reforming).This process depends on requirement of catalysts (gasifying agents),biomass properties and temperature.Comparing Dark fermentation,Biomass gasification produces more polluants and require more energy to generate 1m3 Hydrogen..

BIOMASS PYROLYSIS PROCESS-

It is the thermal decomposition reaction with biomass based on catalysts(gasifying agents)heating of organic wastes to high temperatures 400°C-600°C under a pressure of 0.1-0.5MPa .in the absence of oxygen.

The product of the process are H2,CO,CH4,biochar, and oils.

Biomass +>>>>>>CO +CO2 +CH4 + Hydrocarbons +Ash

Hemicelluloses are optimally degraded between 250°C-350°C

Celluloses ..... **325°C--400°C**

Lignin ..... .**300°C-500°C**

These can be fragmented and dried.The obtained products of biomass can be used further generation of H2. that requires less heat than steam reforming methods but more contrary than dark fermentation.

a) &b)Water gas Shift reaction:-(as described below)

The two stage process reactions ,pyrolysis carried out in absence of O2 and produces other hydrocarbon compounds in the gas mixture. An extra precaution isto be taken to reform Hydrocarbon with a catalyst to yield a clean Syngas ,mixture of H,CO, & CO2.During the second stage of process,a shift reaction step is carried out in order to convert CO reacting with water to form CO2 and more hydrogen obtainable via a water -shift reaction.Adsorbers or special membranes can separate H2 from the gas stream. and purified.

STEAM METHANE REFORMING(SMR) AND PYROLYSIS;-

SMR is the process requiring high temperature and pressure and hydrogen generation happens during reactional steps (1)(2) producing with methane/CO and H2O participation under pressure varying from P=1.5-3MP.

CH4 +H2O>>>>>>>H2 + CO

Delta-H=206.1KJ/mol, T+700-900°C.....(1)

CO + H2O >>>>> >CO2 +H2, + heat (small amount)... (

delta-H =-41.1KJ/mol ,T= 90-230°C .....(2)

18;1 <u>FOCUS ON BIOHYDROGEN PRODUCTION METHODS & SUBSTRATES</u>

HYDROGEN PRODUCTION BY CONVENIENT SUBSTRATES-

The substrates suitable for H2 production can be based on according to their complexity,kind of materials,method required for their pretreatments,energy demand for H2 production or production costs.

Traditional methods use substrates related to fossil fuels ie. Primeval biomass.The alternative source of H2 is contemporary from 1 to 3 usually 2Hydrogen atom per molecule(H2O,NH3,H2S,HCl)

-Suitable raw materials for dark fermentation include wastes containing a high fraction of carbohydrates such as Lignocelluloses,sugar -containing and starch crops ,chitin,starches,Hemicelluloses ,starches in water cellulose,glucose,sucrose,organic municipal wastes,wastes from dairy products,manures,compost and waste water from food industries etc..

Complex substrates are not the preferred feedstocks for H2 producing biocatalysts but it is necessarily transform them by pretreatment methodlogies and make the complex into simpler one .The pretreatments of biocatalysts can provide a fundamental basis for the development of H2 production system owing to the physiological difference phenomenon between H2 producing acidogenic microbes and H2 consuming methanogenic bacterias.This in turn facilitates choosing the parent inoculam by pretreatments for selective enrichment of acidogenic bacteria for H2 production.**(REFER TABLE 8;0)**

The type of process can be classified as direct (one stage H2 production)or indirect hydrogen sources.(Two stage process)include dark & photo fermentation. **The primary biological routes integrated with Various secondary process for effective H2 production is schematically represented.(refer FIG-9.2)**

Table-7shows resources of complex raw materials for the potential H2 production;The traditional complex raw materials like coal,oil etc can be replaced by animal manure via dark fermentation,gasfication etc..

*it can be discerned that the ratio of O2 to Carbon (O;C)in short chained organic compound(C<6)(being hydrogen source especially for **dark fermentation**) is*

1. Exceptions are butyric,propionic,lactic,maleic acid glutamic acids raw materials for photofermentation and alcohols for plasmolysis.

2-The larger organic compounds such as sucrose,maltose,and lactose, etc..ratio of O;C comes to 0.92 .In the case of starch and cellulose ,the ratio O;C in molecule is 0.83.

Table-7 shows resources of complex raw materials for the potential H2 production;The traditional complex raw materials like coal,oil etc can be replaced by animal manure via dark

fermentation,gasfication etc..

Sequential Cultivation technologies;-

Sequential dark and photofermentation processes gives overall H2 yields higher than in separate processes although lower production rate is possible due to drawbacks with photofermentation.

These can be carried out in different vessels needs optimization of each separate stage and require transfer of materials and space requirements much higher than in single stage processes.and both microbial systems must be controlled with operational parameters.

A three stage process (PHOTOSYNTHESI/DARK/PHOTOFERMENTATION)has been considered testing for high yielding H2(7.1-8.3 mol H2/Hexoses).Microalgaes such as **Dunaliella** and **Chlamydomonas** produced a phototrophic accumulation of polysaccharides subsequent fermentation with classical two stage processes.The disadvantages(bottleneck) of the pretreatment process of algae is the prior to transfer to heterotrophic reactor.(frozen/cocentrated cell)

HYDROGEN PRODUCTION BY BIOLOGICAL METHODS;-

18;2 ***BIOPHOTOLYSIS METHODS***;-

Biophotolysis is a process of water decomposition by photoautotrophic organismes such as green microalgaes like Scenedesmus Obliquus,Chlamydomonas Reinhardtii,Chlorella, & Cyanobacteria (Anabaena Variabilis ,Nostoc punctiforme& Synechocystis etc... ability to split water into hydrogen in presence of sunlight having the wave length between 380-750 nm of visible light.The ability of water splitting using photolysis is below 1.5 and it can be increased to the range from 3-10% after O2 removal in which organism absorb .The photo hetertrophic organism produces two enzymes. Hydrogenase- a and Mo-Nitrogenases etc..and Nitrogenase(Cyanobacteria) .

Biophotohydrolysis include direct,indirect, and two stage indirect processes**;**

Hydrogenases are classified by metal composition of actIve part of enzyme; Fe-S-Hydrogenase,Ni-Fe-Hydrogenase and Fe-S-Hydrogenase (in cyanobacteria).Fe-hydrogenase is very acive enzyme allowing the production simultaneously 02 and H2 in the ratio 1;2. .

H2 generation is sensitive to the presence of Sulphur..Besides cyanobacteria,lower amounts of N allows higher production H2.

In the case of direct biophotolysis,there are no intermediates,photosynthesis absorb light,ejected electron are transported linearily from water with potential 0.82V to ferrodoxin with potential(0.44V)as per the equation.below;-

2H+ +2FD(Re)- >>>>>>>>>>>>>>>>H2 + 2FD(Ox).

In the case of indirect biophotolysis, H2 production generation processes do not occur simultaneously irrespective of of O2 presence.In the above reaction,both the enzymes(nitrogenases and Mo-Fe Hydrogenases) takes part where photosynthesis converts light energy into chemical energy as carbohydtrate molecues ,reused and produce H2. using green algaes

PHOTOFERMENTATION;-

It is a special process occuring in presence of Visible light (radiation of 45%) emitted by sun.The photoheterotrophic bacteria used for the process called as Purple Non Sulphur(PNS)bacteria like Rhodospillum rubrum or Rhodobacter decomposes organic acids such as lactate,acetate& butyrates to produce Hydrogen & CO2 in anaerobic and anoxic conditions by capturing solar energy . This organism use a source of light energy and organic carbon to produce H2 as a byproduct of ATP generation with oxidation of CO which inhibits hydrogenase enzymes.It is found to be the

species like Cyanobacteria generates H2 using both hydrogeneases and nitrogenases.

Other inhibitors of hydrogenases like EDTA,O2 etc.. and other optimum conditions of this enzyme depends also on species and set the parameters are as follows;

Temp;(T):55°C for R.Rubrum

T= 70°C for R.Sul dophilus

pH= 6.5- 7.5

- Optimum carbon source for Photofermentation based on bacterial species. Most of the bacteria prefers Lactate(Rhodo8604,Rhodo capsulatus,R.Spareoides),Butyrate(R.Spareoides RV),Pyruvate(R.CapsulatusZ1),Malate(R.SpearoidesO.U.001) and acetate(R.Monas).

The process can be improved by mixing photo heterotrophic with another bacteria group like Acrogenic Lactobacteria Delbrueckii or with anaerobic fermentative bacteria like Clostridium butyricum,C.Pasteuranium and Enterobacter Aerogenes.

.In the case of Hybrid process, higher the pH 7.5 shows lower efficiency than dark fermentation. The attempts are made with another hybrid system such as dark ,photofermentation or combination of these two processes can lead to generation of 12 moles of hydrogen per mole of hexoses.

18;3 BIO-CATALYST ELECTROLYSIS;-

(MEC-MICROBIAL CATALYSIS PROCESS)

MEC process is a technology to produce hydrogen by combining bacterial metabolism from the microbial decomposition of organic compound by applying electric current which transfer the electron to the anode in anaerobic condition and protons are released in the solution.

MEC process is introduced by two independant organisation namely Penn State University & Waginengen University ,Netherlands, in 2005. This is also called as Microbial electrolysis Cell (MEC) uses micro-organisms to activate reaction on electrodes which is normally built from several Polycarbonate plates.Bacteria like ***Geobacter,Shewanella or Pseudomonas*** are the electroactive organismes are allowed to grow on surface of anode and these microorganism decomposes complex organic matter into carbon dioxide(CO2),protons, and electrons that needs a 1.2 V-voltage to decompose the water The hydrogen production rate is about 0.2- 3 m3 of H2 per 1m3 of water per day.The energy produced by bacteria is too low for water splitting and needs to be reinforced by an external energy source to generate hydrogen.According to Logan et al, potential 0.3 V produced by bacteria should be increased to 1.23V for water splitting at neutral pH which can be solved by MERC(microbial Reverse -Electro Dialysis)designing methods.

Anode(Oxidation); $CH_3COOH + 2H_2O >>>>> 2CO_2 + 8e + 8H^+$

Cathode(Reduction): $8H^+ + 8e^- >>>>>>>>> 4H_2$ .....(1).

H2 production can be achieved by organic matter by MEC including renewable biomass and waste water and this MEC Technology is closely related to microbial fuel cells (MFC) but operational principle is reverse of MFC where O2 is reduced at the cathode to give H2O and electricity through exoelectrogenic bacteria to oxidise organic matter on anode.(Refer equation(1)) The mechanism involves as the electron moves through external circuits to cathode side and the protons are travelled to cathode via proton conducting membrane (electrolyte) whereas protons and electrons combined in producing the hydrogen.This shows the principles of MEC process in which some eletrochemical pôtential is produced during oxidation in anode side is insufficient to give decreased voltage and required for H2 evolution reaction at the cathode side hence it requires extra voltage (0.2V-1.0V).

Although a circuit voltage requirement >0.13V to produce H2 at the cathode using acetate molecule ,typically >0.3 V are used even with Pt catalysts to increase the production rates (0.001-0.063 liter H2/L.H at 0.2-0.8V) yielding (3.03-3.95 molH2/mol acetate at 0.3-0.8V) shows the energy efficiency ranges from 681%-243%.as a result of energy (evaluated in terms of voltage addition (0.2-0.8V)) contributed by bacterial oxidation of acetate. Lactate,propionate,butyrate or Glucose are also considered as good substrates for H2 production using MEC at 0.6V achieving substrate efficiencies of 91%,89%,80% or 71% respectively.

Among many of bacterias,few individuals-strains can act as anode produce as high as power densities from mixed communities.Nowadays anodes are made of graphite brush,stainless steel-Ni alloys ,electrodeposited NiMo or NiW as well as Ni electrodeposited on carbon paper.Membranes have been eliminated owing to proton diffusion hindering and creating substantial pH difference between the electrodes.one among the example is single chamber MEC(graphite brush anode,0.8V) can produce H2 as rapid as 0.13L.H2/L.h. with a concomittant production of CH4(3.5%).

Kyazze et al producing 1.1molH2/mol acetate since volatile FA important for MEC system then the solid waste is used for bioH2 production.

POSSIBLE DESIGN AND PERFORMANCE OF MEC PROCESS;-

This shows two chambered reactor of two bottles separated by a cation exchange membrane(CEM) producing H2 gas to the top of cathode chamber and then collected.and further optimisation of MEC can be done through different ways such as increasing size comparative to electrode-projected surface area and also using anodic electrode large surface area,and then decreasing distance between electrodes,designing various membrane two or single less chamber MEC using MEC-MFC coupled systems using Dye-sensitized Solar cell(DSSC).In order to validate the above performance of the process,the two chamber MEC constructed with exchange membrane equipped with flow through bioanode and Ni foam cathode had shown a maximum H2 production rate of 50 liters H2 per liter/d. observable at an applied voltage using acetate as substrate.

LIMITATIONS AND OTHER ADVANTAGES OF MEC PROCESS;-

The simplicity and positive advantages of MEC system is considered as an alternative to second stage of hybrid system.

This system is used to reduce the organic content of effluent and possible to obtain theortically 12 Moles Hydrogen per mol of Hexoses**..**Cellulose used as solid substrate and yielded 0.24m3H2/m3./d. whereas starch based solid waste could result in similar yields with this hybrid system..

DARK FERMENTATION:-

It is an Anaerobic process in which organic materials such as glucose,or other hexoses and pentoses derived from carbohydrates decomposed by bacteria into CO2,H2, and low weight organic acid upon treating the Biomasses concentration in water inoculated by anaerobes then cultivated under the influence of parameters such as temperature,partial pressure,metal ions or pH.,reactor type and feed of nutrients to get the efficiency of fermentation and these can be transformed into pyruvate.If the substrate is simple carbohydrates or glycerol ,it can be one stage process.According to Hallenbaeck et al ,the theortical maximum yield is 33% possible from hexoses than 38%in the cases of glycerol .

Many of the studies were performed on dark fermentation process using facultative microorganismes such as Enterobacter aerogenes ,E.Cloacae,E.Coli & Citrobacter intermedius and

obligate anaerobes such as Clostridum bejerinckii,C.paraputrificum,&Ruminococcus albus etc.occuring at higher rate of biological H2 production than photofermentation & photolysis process.

Otherwise,Bacteria producing H2 are from a group of endospore forming rods bacillaceae (genuses Clostridium,Bacillus),Gram +Ve cocci(MicroCoccaceae,Peptococcaceae),Gram+ve Asporogenous rod shaped bacteria(Lactobacillae),Gram -Ve facultative anaerobic -rods(Enterobacteriaceae,Vibrionaceae) and Cocci(Veillonellaceae). etc..Unfortunately,these bacterias produces in large amounts hence not suitable for large scale purposes

INFLUENCING PARAMETERS FOR H2 PRODUCTION;-

The important factors in dark fermentation are to be considered such as temperature,pH,Nutrients,partial pressure of H2,hydralulic retention time(**HRT**) etc..

The supplementation of **nutrients** for bacterial growth is also critical in increase of H2 production in presence of Carbon source. to influence on H2 productivity.

The **pH of redox** environmental system is an essential index for microbial population .The optimal pH is needed below 6.0 for H2 production

.Lin& Lay et al demonstrated that **C/N ratio** of 47 having the productivity and reached rate of 4.8 mol/mol of sucrose and 270 m.molof H2 /L/day respectively.

TEMPERATURE:-

Thermophiles could reach the theortical value of 4molH2/mol glucose which is much higher than Mesophiles (>2mol H2/mol glucose).Normally,operation at high temperature is thermodynamically favourable for increased rate of H2 production and increases in entropy the system makes more energetic avoids the contamination of H2 utilising enzymes & microorganismes.

Tang et al investigated the studies on H2 production with a mixed cultures at 35-45°C yielding of H2 (319 mL H2/Gm of substrates) measuarable in the form of CDD whereas yield decreased to 1821mL/G.substrates while increase in temperature between 35-45°C.

HRT is an important factors in microorganismes selection than shorter HRT that would restrict the growth of methanogenic microorganismes whereas optimum HRT is recommended for a wide variety of substrates for H2 production especially between 8- 14 hours.

According to Woodward et al ,there are three thermodynamically possible dark fermentation pathways from Hexoses;Acetate equation,Butyrate equation,and acetate -ethanol equation .The acetate pathway is one with the highest H2 yield of 4 hydrogen moles /per mole of hexose.

$C_6H_{12}O_6 + 4H_2O >>>>>> 2CH_3COO^- + 2HCO_3^- + 2CO_2 + 4H_2 + 2H^+$

Delt°G= -48KJ/mol-1 .....(1)

$C_6H_{12}O_6 + 2H_2O >> CH_3CH_2CH_2COO + 2HCO_3^- + 2CO_2 + 2H_2$+3H

Delta °G= 137 KJ mol-2 ....(2)

$C_6H_{12}O_6 + 3H_2O >> CH_3COO^- + 2HCO_3^- + 2CH_3CH_2OH + 3H$+

Delta °G= -76KJ mol-1 ..... (3)

STRATEGIES FOR ENHANCING HYDROGEN PRODUCTION PROCESS THROUGH INTEGRATIVE APPROACHES:-

The major drawbacks with the biological conventional H2 process are the low substrates conversion efficiencies and the accumulation of VFA which reflects on very low overall yield..Although the theortical H2 production yield could reach 12 mole H2 /mol glucose in dark fermentation. then H2 production is limited to 4mol H2 /mol glucose,a major technical hurdle for practical application.

The effluents can be additionally treated for further energy generation before disposal ino the environments that would be wise economical factor etc..

INTEGRATIVE APPROACHES;-

To overcome the limitation of several processes,integrated approaches are recommended for the H2 productivity increase in dark fermentation through the use of residual acid rich organic substances from the effluents thereby recovering further energy is possible while in integrating two stage energy producing process.

The examples of numerous secondary processes such as methanogensis for methane,acidogenic fermentation for H2,photobiological processes for H2,MEC for H2,anoxygenic nutrients limiting processes for bioplastics,cultivation of heterotrophic algaes for lipids and MFC for bioelectricity generation were integrated with the primary dark fermentation processes for H2 production.

Dark fermentation coupled with MEC -Process;-

With these integrated approaches,the primary process uses the further substrates for additional energies production and the entire process is more economically feasible and viable especially only coupling with MEC process in association with simultaneous waste water treatments for the wide variety of soluble organic substances.

A two stage process was used to convert acid rich dark fermentation effluents into the substrates for additional H2 production as indicated in **Refer Figure9:1** .The MEC process can be integrated with the dark fermentation process to use acid rich effluents having the concentration off 3000mg/L opertable with a small range of varying applied potential(0.2,0.5,0.6,0.8 & 1.0V) in presence of anaerobic mixed species as a biocatalyst.According to Balud et al ,the maximum hydrogen production rate (HPR) and the cumulative H2 production(CHP)are reported to be 0.53mmol/Hour and 3.6mmol respectively with 49.8% of VFA utilised at 0.6V.

This two stage process approach could be a viable option for H2 production efficiency by 90% realisable through higher substrate conversion efficiency.

***BIOAUGMENTATION;-**

The bioaugmentation strategy can be improved by protecting the single or mixed microflora with the species such as acidogenic bacterias,fermentative H2 producing bacterias , C.Acetobutylicum communities etc..for enhancement of process efficiencies via reactor performance etc...

Advantages &Disadvantages of Dark fermentation;-

Although ,low yields of H2 is possible on substrates in anaerobic conditions where pyruvate enters into the acidogenic pathways coupled with H2 conversion other than volatile fatty acid (VFA) like acetic acid,propionic butyric ,maleic etc..as a disadvantage factor in dark fermentation.owing to the consumption of inorganic & organic compounds with their concurrent reduction &regeneration of reducing powers.

To Conclude, the multidisciplinary fermentation processes can be recommended for biological H2 production efficiently possible through variety of substrates& mixed cultures including usage of waste water as C source considered as ecofriendly and economically feasible solution....

18;4A COMPARATIVE STUDY OF BIOHYDROGEN PRODUCTION WITH PEM TECHNOLOGY PROCESS;- (Research Review)

PEM (PROTON EXCHANGE MEMBRANE PROCESS) -

(WATER ELECTROLYSIS METHOD)

Among many H2 production methods,eco-friendly, and high purity Hydrogen (99.99%) can be obtained from electrolysis of water to produce hydrogen and and oxygenThe basic reaction is described as follows;

H2O +Electricity(237.2kJ /mol) >Heat(48.6kJ /mol) H2 + 1/2 O2

PEM water electrolysis technology method is introduced by General Electric Co,USA in 1966 considered as a favourable method to produce high pure Hydrogen converting renewable energy,similar to PEM fuel cell technology where poly sulfonated membrane (Naflon,Fumapem)used as electrolyte(proton conducteur).This membrane have the different properties with different advantages such as low IrOr gas permeability at the cathode and ,high proton conductivities (0.1+-0.02 µS/cm etc..,

It has another promising advantages such as compact design,high current density(above 2Acm2),high efficiency etc...,producing ultrahigh pure hydrogen.and O2 as a byproduct.The state of the art of elctrocatalysts for PEM are high activity of noble metals such as Pd/Pt as the hydrogen evolution reaction(HER) and IrO2/RuO2 as the oxygen evolution reaction (OER).This makes more expensive than alkaline electrolysis hence the production cost is to be reduced and high efficiency is to be maintained.

PRINCIPLES OF PEM;-

Water is electrochemically split into H2 and O2 at their respective electrodes.(H2 in anode& O2 in cathode)PEM is accrued by pumping water to anode where splitting occurs into O2,protons(H+) and electrons(e-)and These protons are travelled via protons conducting membranes to the cathode side.These electrons exit from anode through external power circuit providing driving force (cell voltage)for the reaction.As described,the protons and electrons recombines to produce hydrogen at the cathode.

.Gibbs free energy for water splitting can be calculated as follows;

delta-G =n FE rev.....(2)

where n=no. of electrons

F=96500(Faradays consatant)

E rev= Reversible Voltage

The reversible voltage can be calculated by following equations:

$$E_{rev} = \frac{\Delta G}{} = 1.23V \quad \text{.....(3)}$$

Some heat energy(entropy) generated at the time of water splliting hence it is more suitable to employ enthalpy(Delta-H) in place of delta-G for potential calculations. .Typically,water electrolysis efficiency(WEE) is calculated by higher heating value(HHV)of hydrogen. owing to supply in liquid cell efficiency towards the cell, calculated by following equations(5)

$$n = VTN / V_{cell} \quad \text{.....(4)}$$

where V**TN**= Thermo neutral volatage

V cell = Cell Voltage

WEE can be calculated by any current densities therefore lower cuirrent density at lower voltages are recommendable to make electrolyser efficiency becomes higher.

FARADAY EFFICIENCY(FE):-

Faraday efficiency is the quantitative analysis useful to determine transportation of electrons quantities in the external circuit to the surface of electrode which conducts electrochemical reactions either OER or HER and other electrochemical reactions in the electrolytes.Therefore FE

can be defined as the ratio between volume of gas value experimentally evolued and theortical clculated volume of gas value as shown in equation -6

n(faraday) (calculated) = VH2 (produced) .....(.5)

The theortical volume of gas can be calculated by 2nd Faraday law based on current density,electrolysis time, and electrode area assumed and calcultable as 100% FE as shown in equation-7.

V H2= VM(l) (10+3 ml/l)(t(60sec)/min)(l/ 2F(C).....(6)

where V**H2**indicates theortical H2 yield,

VMis the ideal gas expression

(VM=R 273+T/P), R indicates ideal gas constants(0.082 atm.K/mol),

P means pressure(atm),t is time(s)

l is the applied current (A) and F indicates the Faraday;s constant(96485C /mol).

The experimental value can be measured by water-gas displacements method or gas chromatograhic analysis.

PEM Water electrolysis cell components:-

The components are membrane electrode assemblies(MEA) ,Current collectors(Gas diffusion layers)and separater plates as described in **FIGURE-6** with a provision of cell separation of two half cells in the middle of electrolyser.

MEA:-

MEA consists of membrane, ionomer solution and anode,cathode electrocatalysts denotes 24% overall cell cost()Membrane is the basic component of PEMWE cell composed of Perfluourosulfonic acid polymer as indicated earlier(Neflon,Fumapem,Flemion,and Aciplex)which have unique properties such as operating at higher densities,&strength,&efficiency,etc..

Ionomers solutions composed of Naflon,ionomers,isopropanol and water followed by sonification for 30 minutes and these slurry is used on electrocatalyst in homogeneous suspension to improve upon ionic tranport properties in the catalytic layers.

CURRENT COLLECTORS;-

In PEM ,the feed water travels through separater plates and diffuses via current collectors(anode & cathode) where H2O molecules decomposed into O2,protons and electrons.In this case,O2 return to out of the cell through electrode surface,current collectors then separator plates whereas the protons are moving from the anode to cathode side through proton conducting membrane and the electrons travels from current collectors ,separator plates than moving to cathode then recombined with protons to produce hydrogen leaving via cathode current collector and separater plates.

SEPARTATER PLATES;-

The separter plates and current collectors represents 48% of overall cell cost and provides required cell voltage .The separater plates are made up of costly titanium,stainless steel and graphite materails and having several operational drawbacks such as corrosion etc. thereby performance of electrolyser decreases;. but gives outstanding strength,high thermal conductivity, etc..

ELECTROCATALYSTS FOR PEM ELECTROCATALYSIS;-

Noble metals are used such as Pt/Pd based catalysts as cathode towards hydrogen Efficiencyreaction(HER) and RuO2/IrO2 catalyst anode for OER(Oxygen efficiency reaction).The first study was conducted by General electric in 1973 obtained the performance of 1.88V at an operating current of 1A cm2 and 2.24 V at 2A cm2 with cell life of 15000Hours without any

degrading performance

Electrocatalyser for HER;

In most of the research studies for the development of electrocatalysts for the cathode,Pt based materials have been used typically as a standard catalyst for HER due to its excellent HER activity and exhibits outstanding ability in acidic environment but highly dispersed carbon supported Pt based carbon nanoparticles materials are currently bench mark catalysts for HER in electrolyser. to enhance the surface area..

Electrocatalyser for OER:-

The metal oxides of RuO2 and IrO2have shown the higher metallic conductivities having the value of 104 /cm/ohms due to metal-metal distance values and radius of cations overlapping inner d-orbital make feasible results enhancing the conductivity. Therefore RuO2 has shown better OER performance among the other metal oxides than IrO2.From economic feasibility point of view along with better stability,it is need to add IrO2 to enhance stability of RuO2..To reduce the cost ,it is required to replace Ir by non-noble metal oxides.by mixing with transition metal oxides with IrO2 and /or RuO2 such as TiO2,MnO2,Ta2O5,Nb2O5,Sb2O5,PbO2 etc.

..SnO2-IrO2-Ta2O5 shows better performance towards acidic environments& the role of Tantalum is to increase the surface area with charge storage capacity.and enhances electrical conductivity.

CONCLUSIONS;-

Among the biological methods of producing biohydrogen,the most efficient and simplest design is the dark fermentation possible through the number of variations such as build a hybrid system with MEC Process.This uses many effluents & waste from food processing industries such as paper,dairy,cellulosic glycerol etc..require a high COD& BOD which threaten the aquatic fauna hence the use of C rich effluents/waste water as fermentable substrates , an attractive promising approach for H2 producion which may solve the dual purpose of waste disposal & clean energy generation.

Attempting for build a hybrid system of biophotolysis,dark and photofermentation or two of combined process give rise to a yield of 12 mole H2 generation per mole of hexoses.The same study in combined process produces 7.1 moles of H2 per mole of glucose with a optimum pH 7.5 ,higher than dark fermentation possible with mixed culture of C.Butyricum,enetrobacter aerogenees,Rhodobacter sp M-19.

To reduce the cost ,it is required to replace Ir by non-noble metal oxides.by mixing with transition metal oxides with IrO2 and /or RuO2 such as SnO2-IrO2-Ta2O5 shows better performance towards acidic environments& the role of Tantalum is to increase the surface area with charge storage capacity.and enhances electrical conductivityHence,particular attention to be given for the electrocatalysts that more efficient in stability at large current densities and efficacity in HER and OER.

Sequesteration of CO2 is one of an option of H2 prodution for viable near-term solution with the global climate change as 4th generation fuels and make challenges over the growing demand for zero emission fuels.

The integrated approach on acid rich reactor effluents with simultaneous recovery of H2 energy may be efficient and economical one in commercialisation of process as discussed earlier.

19;0 BIOBUTANOL

ISOPROPANOL-BUTANOL-ETHANOL (IBE)

FERMENTATION

Biobutanol is considered as superior fuel compared to ethanol in terms of properties such as high miscibility with gasoline or diesel, higher energy density,low octane value etc..Additionally biobutanol and its derivatives used in other industrial applications

The global market was estimated 3.8 millions tons in 2012(7 billion) and the perspective growth from 2013 to 2018 wlll achieve 9.9 billions US dollars.Thereby the butanol production gets more concentrated in various parts of the world.

Earlier industrial production of butanol was based on starch and molasses with solventogenic Clostridium species to produce mainly butanol and acetone However increasing price of C sources and rapid growth of petrol industries & subsequent high demand of cattle feed ,the biochemical process of ABE fermentation facilities were closed upon considering the feedstock cost accounts around 70% of whole process;According to current situation of butanol industries in china,it costs the petrochemical process still more advantageous (1.52USD/Kg)than fermentation process(1.87USD/Kg)

One of the strategy decrease the cost associated is the use of renewable LCWBmaterials (agricultural waste,paper wastes,wood etc..) which involves integrated important three steps such as pretreatments,hydrolysis and fermentation. using clostridium species ***C.Beijerinkii,C.saccharoperbutylacetonicum.*** etc..

Biobutanol is naturally synthesizable by Clostridium species through Acetone-Butanol-Ethanol(ABE) fermentation process that has been considered as alternative fuel for gasoline due to its more advanced properties over bioethanol.

.The problem associated with the process is high product cost recovery enhanced by low butanol concentration and low butanol yield produced by formation of by-products such as acetone representing 30% total mass in ABE.

Fermentation Process;-

ABE fermentation involves two distinct phases such as acidogenic and solventogenics.During first phase,the organic acid(acetic,butyric) are produced and then followed by assimilation of those acids to convert into solvents (acetone,butanol ,ethanol) during the second phase. The main challenges are to face downstream separation problems(solvent distillation for insitu continuous recovery) other than cell growth inhibition during fermentation generated through inhibitors.Besides the high operating cost already mentioned, the factors such as yield,productivity,low titer and solvent toxicity are the challenges to overcome through metabolic engineering apporoach to make biobutanol economically feasible.

19;0 PRODUCTION OF ISOPROPANOL-BUTANOL-ETHANOL-(ABE) MIXTURE BY ENGINEERED CLOSTRIDIUM ACETOBUTYLICUM XY-16 (Research study)

In this present research investigation,Clostridium Acetobutylicum XY-16 is genetically engineered to produce IBE mixtures through simple introduction of plasmid harboring pS-ADH gene. eliminating acetone as by-products formation that affects the fermentation yield significantly during the manufacture of Acetone-Butanol-Ethanol(ABE).

After the introduction of secondary alcohol dehydrogenase into Clostridium Acetobutylicum XY-16,the study shows that the engineered XY-16 indicates not only complete elimination of acetone and but also converting into Isopropanol which is the indicator of great potential and yield for the production of IBE mixtures.The condition is as follows;

-The optimum **pH level; 4.8**

-Total IBE production increased from 3.88 to 16.09 g/L with final yield of 9.97,4.98 and 1.14g/L for Butanol,Isopropanol,Ethanol respectively.

Furthermore,CaCO3 could play a role as buffering agency and activation of NADPH+ and (NADK).Supplementation of CaCO3(10g/L)further increases improves IBE production to 17.77g/Lwith 10.51,6.02 and 1.24g/L of butanol,Isopropanol and Ethanol respectively.

The results shows the above optimum conditions permit us to accelerate process in a potential way .The analysis of redox cofactor indicates that the availability of NADPH is the main source for the improvement of IBE production.

To better understand basic phenomenon behind the study is the shifting pH medium from ABE to IBE and a decrease of IBE was observed at 5.2- 5.5 thereby the metabolic flux shifted towards acid production rather than solvents resulting high acid production 12.51 g/L of butyric acid and 7.23g/L acetic acid.When pH is 4.8 is controlled,the maximal IBE production of 16.09g/L was obtained of which the concentration of butanol,Isopropanol and ethanol was 9.97,4.98 and 1.14 g/L respectively.This explains the recombinant strain XY-16(pSADH) utilised more glucose molecules at a rate of (0.86g/L/H)efficiently at pH4.8.This shows the recombinant strain not only stimulate cell growth during acidogenesis but also improving IBE production during solventogenesis.

CaCO3 supplementation for High IBE Production;-

Supplementation of CaCO3 at various concentration(2,4,6,8,10 & 12 g/L)in the medium was carried out under batch fermentation process for 72 hours .This shows that increase of dosage upto 2-4gm/L and butanol production will increase to 4.19-7.56g/L leads to rapid cell growth of strain XY-16.The glucose substrate utilisation was observed in an anaerobic condition filled with 80% N2,10%CO2 and 10%H2. .

HIGH -IBE PRODUCTION THROUGH pH REGULATION;-

pH reported to be critical for ABE fermentation that directly influences the production of solvents through regulation of intracellular levels of NAD(P)H.The the batch fermentation were performed at different pH levels of 4.6,4.8,5.0,5.2 & 5.5 in the modified (P2) medium.The supplementation of CaCO3 at a rate of 10g/L may influence a favourable pH range both for cell growth and the solvent profiles of IBE concentration by strain XY-16 capable to carry-out in 5L fermenter.

The research shows that pH value decreased to around 4.9 ,considered as optimal pH for IBE fermentation with the increase of fermentable period.It is reported to be IBE concentration increases by 10% from 3.87 to 17.77g/L with a glucose consumption rate of 0.99g/L/h.

FERMENTATION STRATEGY CONDITIONS;-

Clostridium Acetobutylicum were grown in P2 medium.

KH2PO4	; 0.5 gm	MnSO4.H20	; 0.01 Gm
K2HPO4	; 0.5 gm	FeSO4 .7H2O	; O.O1 gm
CH3COONH4	: 2.2 Gm	NaCl	: 0.O1 gm
MgSO4 .7H2O	: 0.2 gm	Corn Starch Liquor	; 1 gm

The research study was carried out with 10% V/V actively growing suspension inoculated with C.butylicum and allowed to grow in above P2 medium.Then N2 gas is purged to remove O2and pH is controlled and the initial fermentation(2L)of broth was sterlised at 121°C for 15 minutes alongwith glucose sterlisation separately and then it is added to culture medium to final concentration to 60g/L .Temperature was maintained at 37°C with agitation at 120 RPM.

& pH maintained at different level with automatic addition of 2M HCl and 2M NaOH.

Fermentation conducted at 72 hour period of time with different concentration of $CaCO_3$ (2,4,6,8,10 & 12 g/L). $CaCO_3$ sterilised by dry heat sterlisation at 160°C for 30 minutes added to the medium.Precultures grown in YPS medium(10%) transferred into 100 ml Pyrex medium bottles containing 40 mlP2 medium.P2 medium without $CaCO_3$ used as control and above procedure repeated in triplicate.

ANALYTICAL METHODOLOGY;-

-OD 600 analysable using Spectrometers.

-**D**ry Cell weight(DCW) calculated using the formula :-

$$DCW(g/L) = 0.26 * OD\ 600$$

-Glucose analysable using SBA-40C biosensor analyser.

-Acetate,Butyrate,Ethanol,Isopropanol,Acetone,Butanol concentration were measured in duplicate using HPLC analysis(Chromeleon,P680pump,Dionex-USA) equipped with UV & Refractive index (RI)detectors.

-The supernatent filtered by 0.2micrometer Nylon filter before injecting to HPLC -An Aminex HPX-HPX-87H organic acid column (Bio-RadLab-CA)(7,8 * 300mm)maintained at 15°C with 0.05 mM H_2SO_4 as mobile phase and at a flow rate of 0.5mL/min.

-The solvent yield(Isopropanol/Butanol/ethanol)is the amount produced from 1gm totally consumed sugar(Gm/Gm).

-Strains Closridium Acetobutylicum XY-16 screened in laboratory and stored in typical culture center(CCTC no;M2010011).The culturing was done at 37°Cin cuvette.The reaction initiated by addition 50micro liter of alcohol Dehydrogenase.(500units/mL for NAD(H) or Glucose-6-PO_4 Dehydrogenase(70units/mL for NAD(P)H.The absorbence determined at 570nm and the whole experiments done in triplicate.

NAD+ and NADP+/ NADPH ASSAYS;-

, There is a need for pH regulation strategy permits to maximize the IBE production increased upto 16.09Gm/L.The detection level of NADH and NADPH in above strain can be investigated with cell growth during acidogenesis.**Refer TAB-1).**

CONCLUSIONS;-

Clostridium Acetobutylicum XY-16 (pSADH) successfully and metabolically constructed to produce IBE fuel mixtures with complete removal of acetone.The availability of efficient NAD(P)H indicated by perturbation of redox cofactor will enhance the IBE production.Both pH control strategy and Ca CO_3 could facilitate the increase intracellular NAD(P)H level and IBE concentration.Under the optimum pH level 4.8 and subsequent concentration of $CaCO_3$ -10G/L stimulate the enhanced IBE production to 17.77 gm/L from the titer value of 16.09 G/L.

*20;0 ENERGY PRODUCTION **(BIOMETHANE)***

THROUGH ANAEROBIC PRODUCTION (AAP) OF LIVESTOCK MANURE;-

Livestock manure can be a source of energy production.Biogas generated from manure can be used directly in a gas fired combustion engine or microturbine to create electricity.Additional energy in the form of waste heat from turbine operation can be used to create hot water as well as maintain temperature of digester.

ANAEROBIC DIGESTION PROCESS;-

When organic material decompose biologically in the absence of O_2 ,it is referred as anaerobic digestion, occuring the production of CH_4 from manure and the process releases biogas composed of 50-80%CH_4,20-30%CO_2.and traces of gases such as H_2S and moisture.).The overall

conversion process of complex matter into CH4 and CO2 can be divided into four steps namely **Hydrolysis,Acidogenesis,Acetogenesis and Methanogenesis**.This combined processes occur simultaneously producing biogas so that the concentration of these products becomes low at any time.

HYDROLYSIS;-

In anaerobic digestion,hydrolysis is the essential step normally comprises of very large unstable organic moleculesbreaking down into simpler compounds including H2 and acetate may be used for methanogenesis later the molecules are further broken down while acidogenesis to create CH4.

FERMENTATION AND ACIDOGENESIS;-

it needs to proceed towards the stage of Acetogenesis.

Acidogenic microorganismes breaks down further biomass producing acidic environments creates H2,CO2,H2S,shorter volatile fatty acids,carbonic acids,alcohols and other byproducts etc....

ACETOGENSIS;-

Acetogenic bacteria catabolises many products created while acidogenesis into acetic acid,CO2 and H2 then these products are utilised by methanogenes to create CH4.

METHANOGENESIS;-

It is the final stage of the anaerobic digestion where methanogenes produces CH4 obtainable from acetogenesis as well as intermediate products created through hydrolysis.The basic mechanism involves production of methane through acetic acid pathway using following equation;-

CO2 + 4H2 >>>>>>>>>>>>>CH4 + 2H2O

CH3COOH>>>>>>>>>>>>>>> CH4 + CO2

:-FEED STOCKS;-

The following feedstocks can be used as raw materials for generating biogas that can vary in energy production ,indicating as follows;-

-Waste feed -Syrup from ethanol production

- Food Processing waste -Glycerin from biodiesel

-Fats,Oils and Grease -Milk house wash water

-Slaughterhouse waste -Fresh produce waste

-Corn Sillage -Cafeteria waste

In farm based anaerobic digestion,many of the energy dense feedstocks such as food processing waste and ethanol sillage can be added to livestock manure and this could be benefited from increased Gas production.Manure considered as prime source for AAP having a neutral pH and high buffering capacity and naturally occuring mixture of microbes as it provides nutrient, micronutrients and trace metals etc..

CARBO/NITROGEN(C/N) RATIO;-

Anaerobic microorganismes utilises C for energy and N2 for building cell structures.These bacterias utilises C about 30 times faster than N2.assumed to be favourable conditions for optimum growth.If N2 availability is higher (low **C/N** ratio=30/15) signifies C source exhausted then it leads to stopping fermentation. and remaining N2 will convert as NH3.

START-UP DIGESTION PROCESS;-

It is important to operate the process with a number of parameters assure the good start-up leads to anaerobic digestion otherwise it translates highly on interruptive in gas production and ends on failure of the system.

Firstly,digestion tank is filled with H2O about 20-25% Volume.Over 6-8 weeks of period of time,

the amount of fresh manure is to be added gradually leads to constant gas production occuring in the 4th week after start-Up require 2-3 months multiplying bacteria efficiently..It is important note that CO2 or another O2gas is to be purged to decrease start-Up time and reduce the danger of explosion during the start-up phase.The parameters such as temperature,pH,volatile acid,Concentration etc..are set to appropriate levels maintaining pH 7.0...

MIXING IN GAS PRODUCTION;-

.The advantages of mixing is to increase the amount of bio-gas production and to speed up the process of volatile solids breakdown.through the pump and impellers facilitate the agitation of slurry in motion provided process under circulatation. It is reported from the experimental studies that diffrerent mixing does not exhibit an effect on long term performance but proportionately affect the microbial populations involved on wastes break down.So,the objectives are fixed to control the microbial communities affecting the overall stability of the digestion process..

TYPES OF ANAEROBIC DIGESTER;-

There are five types of digesters available such as Covered lagoon,Mixed Plug flow digester,Complete mixed digesters,Fixed film digesters,Temperature phased anaerobic digester,ASBR etc...

The components of typical digestion system include: Manure collection,Anaerobic digester,Effluent storage,gas handling,and gas use-Electricitygenerating equipments etc

In anaerobic digester ,attached and suspended growth system vary on digester size,operating temperature,solid retention time,hydraluic retention time,total solids,concentration of feedstocks fed to digester,biogas production,ease of management & other factors etc....

-These are efficient to perform well with dilute waste streams having 1-5% total solids content.It shows a high gas production rate per volume in comparison to lower gas production through removal solids.

CONCLUSIONS;-

Each system generate biogas indifferent ways according to the management of internal and external factors.Feeding the digester at the proper loading time,adequate mixing of digestate throught the digester profile and maintain appropriate environmental conditions ensure maximum gas production rate over time.Anaerobic digestion in general should compete successive process steps in order to faciltate higher yield of biogas under anaerobic environment. In order to produce effective CH4 yield,residues from processing unit can be utilised having the starch content and higher fat content permit to boost the gas..

Hence bioaugmentation was effective in increase of initial CH4 production rate yielding 21-44% more methane than pretreated birch from LCB. The combined SE and addition of C.Bescii enhances CH4 production yields upto 140% compared to untreated birch whereas SE process contributes to the major share of CH4 enhancement by 118%. Biogas production from LCB considered as a challenging issue once biomass is reduced to recalcitrant nature

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21;0 ADVANTAGES - DISADVANTAGES OF BIOFUELS

It is safe for use in all conventional diesel engines -offer same performance like petroleum diesel . It is non inflammable,non toxic,reduces tail-pipe emissions, visible smoke and noxious fumes,& odours.Since it has low or no S content and it is often used as an additive to ultra-low Sulphur diesel(ULSD) fuel.It has been shown high lubricity than any other fuel.It has high cetane number and produces less particulates-CO and hydrocarbon emissions.It improves the environment quality with a

pleasent fruit odour.It can be produced easily from a variety of raw material of various resources including recycled waste oil . .

Biodiesel is alternative,renewable,and domestic energy source and it is biodegradable,non-toxic and possessing high lubricity in nature that offeres several advantages to the environments and not contributing the net accumulations of GHSEand exhibits low CO2 emissions,low SO2 etc..

ADVANTAGES

The cellulosic biomass considered as a potent feedstock for the generation of biofuels(bioethanol,biodiesel etc.) based on their huge abundence,sustainability and low cost materials.The agricultural residues ,waste papers,municipal wastes are the important substrates considerable in current situation to process them economically.It does not require land developments in fresh water as several strains found to grow in seawater and wastewater.

Bioethanol processingTechnologies are well developed in present generation and may be easier for commercialisation.Though obtainable from main crops like corn,starch materials ,this can be easily processable with above residues substrates for economical eq uity.

Biodiesel ,as a product of cooking oil,palm oil,microalgae etc.. displays a better properties having higher cetane numbers.This implies shorter ignition delay that can affect the quality of combustion. representing clean emission and engine performance.non-toxicity,renewablity,sustainability and acceptability. Other properties such as kinematic viscosity affects the flow,spray,atomisation and combustion process.

Palm biodiesel and by-products acts as a future raw materials due to their availability,cost,abundency, environmentally adoptiveness, and minimum impacts on food chain & security.This biodiesel presents a high flash point indicating good properties for storage and transportation with minimum product yield of 96.5% compiled with EN norms and becomes a more versatile product as solves pour point problem (+15°C permits useable in tropical countries) and low pour points (wintergrade shows temperature from -21°C to 0°C) and can be sucessfully met the seasonal requirements by temperate countries).

DISADVANTAGES;-

The principle disadvantages include high production cost,resulting from high cost of feedstocks,enzymes,detoxifications, and ethanol recovery respectively.This possess a low volumetric energy,density signifies more volume of ethanol consumption/Km(upto50%) compared to conventional gasoline.

The difficulties of using lignocellulosic materials are due to their poor porosity,high crystallinity and lignin contents presence that inhibits the entire processing aspects thereby processing cost will become higher.

Several issues still hamper or not allowing the biofuels production sucessfully such as high demand of energy,biological contaminationhigh loading of enzymes mixture for breaking down the feedstocks expensive treatments for effluents having toxic compounds,high capital investments cost on equipments and qualified workcomeforce etc..;These challenges make the production still not economically competatives especially when compared to fossil fuels production while falling of oil prices.

The oilseed crops s uch as Canola,Sunflower and soyabean require attention for crop production from harvesting to post harvest stages in regards to economic viability.

It leads to deforestation and in other words, biofuel can not be efficient as fossil fuels specifically bioethanol ,as an example.Biomass still generates harmful toxins that can not be released into the

atmosphere as it is combusted damaging bad to health.Biomass plant requires a lot of space,a great deal of land and water for certain biomass crops hence ,large amount of storage place is needed for storing the products.In some occasions,burning biomass is dangerous that releases CO,Nausea,dizziness and in high concentration leads to headaches and premature consequences in the body.

The requirement of thermal energy during pretereatments and distillation process alongwith usage of cocktail enzymes for cellulosic degradation makes the production cost higher whereas the feedstock & capital investment cost are high for biosynthetic fuels while the process cost is compartatively lower as compared to bioethanol.

Bioenergy from animal,& human wastes leads to increase in CH4 gas emission and also harmful to earth O3 layer apart from CO2 emissions during power engine feed.

Biofuels from LCWB may normally require very lengthy processing time of 20-30 days.Though it produces nocive gases into the environments ,the implementation are essentially needed admitting to isolate from the metropolitan areas. and requires vaste processing areas to explore the biomasses.

BIOFUELS IMPACT ON BIODIVERSITY;-

Biofuel production sometimes affect ecological biodiversity while using feedstocks that may require tropical climate.

During Ethanol production,plant releases liquid & air polluants leads to health problems for local people.Hence,ethanol industries need to meet the environmental issues such as reduce climate change(GHGE),sustainability,energy water preservation,co-products generation,,utilisation of waste water treatments etc..to avoid pollution.Water usage for ethanol feedstocks is questionable as it affects water availability to human and other factors like waste water treatment before disposal. From the productional plants,feedstocks leads to depreciation of roads and other socio-economic impacts.

22; 0 <u>COST BREAK-DOWN CALCULATIONS &ANALYSIS OF BIOETHANOL PRODUCTIVITIES FROM LIGNOCELLULOSIC FEEDSTOCKS OVER OTHER STARCH MATERIALS(CORN ETC...)</u>

Though the yield obtainable from lignocellulosic materials reported to be lower in compared to corn ethanol, the biomass shows 0.51 wt% productivity of bioethanol for every one ton of feedstock possible.

In the case of Wet milling of processing ,it shows 2.7 gallons per bushel of corn whereas dry milling produces 2.8 gallons of ethanol per bushel of corn.This indicates higher ethanol production obtainable from corn owing to the presence of higher content of starch and the matured technology being practiceable to update the process on today makes the processing cost more viable and feasible.

<u>Techno-Economic Analysis of Lignocellulosic Ethanol;-</u>

<u>Sceanerio 1</u>

A economic feasibility report of a typical enzymatic hydrolysis based ethanol implantation (USA)using the feedstocks (<u>80% hardwood,&20% maples)</u>are given herein showing the size of plant capacity 25 millions gallons per year.The process is a SHF based having a pretreatment with dilute acid prehydrolysis , with on-site enzyme production,CO2 recovery,&Furfural production and a sugar solution (after saccharification)concentrated using a multieffect evoporater.The economic feasibility analysis is performed (on IRR)showing ethanol selling price as 2.06$ /Gallons.(US

$0;54/L)set to 10%.

Sceanerio2

In the case of hybrid poplar wood chips as feedstocks,the process comprises seven main areas including feedstock handling & drying,gasification,gas clean-up,& conditioning,alcohol synthesis and alcohol separation.and the economic evaluation is based on levelized production cost terming(Minimum ethanol selling price(MESP))or product value(PV).This gives US$ 1.07/gallons ethanol and then design case is such as to meet target with a discount rate of 10%.

Upon analysing different locational ethanol production, the raw material plays an important role other than method of processing strategy.The contribution made by feedstock production to ethanol production cost increases from one MYPP(Multi Year Program Plan) to other due to progress in understanding & estimating feedstock production & logistics.The cost breakdown of ethanol production is indicated in (Tab-14.0)

Other changes occur in US Energy policy which reinforces the role of biofuels. set by RFS,EISA etc..

The simulation of 3 starch feedstocks with SSF process included having the mass flow of 30675 Kg/h(Refer Tab-.14.1) gives simulation of four lignocellulosic feedstock ethanol with a mass flow of 35556Kg/h such as sugarcane bagasse,paperwaste,paper waste & use of SSF process converting starch into EtOH & molecular sieves & recovering it .The second one includes wood chips conversion to EtOH by dilute acid pretreatments & SSCF process. followed by azeotrophic distillation.For this,only main equipemnts is taken into account for analysis of operating & capital costs annually and then it comes to 106,9$/tons for starch & corresponding costs for biomass estimated to be 120,5$/ton.

The results obtained above are considerably higher for lignocellulosic ethanol due to complex technology involvements more specifically pretreatments reactor operations other than azetrophic operations in comparison with liquefaction& saccharification of starch.

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CONCLUSIONS

This bioenergy project shows the tactic approach on every successive generation of biofuels,the number of net emisssion falls into the atmosphere. This contributes acquiring the strategy of zero emission technologies possible with second & third generation biofuels compared with technologies of first generation..

To overcome the depletion strategy of fossil fuels,the biofuels production(bioethanol etc.;) are profondly studied in multidisciplinary criteria towards the benefits of GHGE relevant to the performance of emission and to neutralise for pollution free environments through CO2 sequsteration in view of carbon neutrality. and to reduce the impact of global warming.

This project study does not propose first or primary generation biofuels from the food crops as it adversely affects the food chains .Therefore second and third generation bioenergies may be highly recommended from raw materials such as Lignocellulosic wood materials,perennial crops,waste Cooking oil,micro-algaes biomasses etc..as the only ideal substitute ,widespread,abundant,inexpensive and sustainable resources.

This explains herbaceous feedstocks showing lower ethanol yield due to high moisture content Whereas sugarcane bagasse shows pêrspective strategy for tropical sugar producing countries.The simulation shows the use of paper waste (newspaper,waste paper of chemical pulp)

can be the potential feedstock for bioethanol taking into consideration for its higher cellulose content but it may be suggested that the usage of raw material such as municipal solid waste etc. are to be investigated thoroughly for bioethanol yield in order to maintain the bioethanol strategy.

Waste management is one of the leading strategies (from banana and citrus wastes, total pineapple waste etc..)for sustainable environment in bioethanol .This is equivalent to 50%w/w of total production of bioethanol worldwide. Hence CBP is the most adoptive solution for utilisation of these wastes require in combined strategy to obtain biofuels in profitable manner.

Cassava starch used as biomass where Zymomonas mobilis act as important ethanologenic organism at pH5.0 at 30°C yielded maximum of EtOH Conc.13.3g/L at a substrate conc of 1g/L. which is equivalent to 0.51 g EtOH /g sugar whereas 0.57 g EtOH/g of polysaccharide Xylans and glucans.This shows that the above feedstock is economically feasible with the species..

Integration of different biofuels production systems and the use of low cost feed-stocks could help in decrease of facilities cost and attract more investments.

The production plants regularly produces co-products which increases the plant profits during bioethanol production offers a potential and equity. . **Immobilization** , a possible technique enhances the activity and prolonged usability more than 90%even after 15 sucessive cycles .10%increase in saccharification efficiency is possible than normal.

Laccases are one among the potential pretreatments agents in removal of lignin compounds in biofuel and act as a biotechnological tool for removing phenolic inhibitors to arrive an optimal results and adptation of biorefinery concept.

Inappropriate ratio of Beta Glucosidases will lead to accumulation of cellobioses that inhbits the activity of cellulases as it catalyses the rate limiting step in breakdown of cellulose molecules.Hence strategy is developed to maximise the saccharification .through inclusion of endo1,4 beta Xylanases ,beta Mannosidases,beta Mannases,Pectinases,Beta Glucosidases,L-Arabinofuranosidases etc..in appropriate levels necessarily required.in a cocktail mix.(as in CBP).

Genetic engineering approach on biofuels yield ;

It is important to add up on my thesis work stating that research study on yeast cell engineering (Sacchromyces Cerevisiae) or genetically improved E.Coli or Zymomonas mobilis may influence on ethanol production improvements that may be well considered not only for generating efficient biofuels through synthetic pathways but also to reduce toxic product inhibition,tolerance towards osmotic condition (ethanol conc) and to widen the substrate range. such as high solids loading at the beginning, and high temperatutre profile in simultaneous saccharification stage.etc...

This shows broad substrate specificity on various biomass materials towards the sustainable improved yield of biofuels than producing other compounds such as organic acid, lycopene,enzymes,vitamins,HMF,Furfural etc..after purification.

Inhibitors formation & Detoxification strategy;-

Detoxification strategy phenomenon are suggested to inhibit

the effects of inhibitors formation, and the encapsulation of yeast can be viable solution to stabilise the productivity of alcohol compared to freely suspended cells. or developing microorganismes in static or set cultivation medium where fixed condition (temp/pressure/aeration) to be regulated.

The other alternative solution is to increase the cell concentration (Immobilisation)or by

Genetic engineering or modification of cells makes them forming non-toxic that exerts a negative effects on bioethanol yield. Ion exchange,Biocatalyst & liquid-liquid extraction etc.; are also recommended as the better option for detoxification of hydrolysates**.**

Fermentation Strategy;-

This project proposes an improved fermentation strategy of all chemically treated susbstrates via microbial saccharification where cellulose conversion into glucose can be done in presence of lower lignin contents. by Neutralisation method ,a better detoxification strategy employable (.saccharification optimiseable with 20 UFP/gm db.in pH5.5 after 36 hours at 30°C)**.**

< **.**Both the processes SHF & SSF are complementary to one another as combination can be used for economic assessment & optimisation of production process.

In SSCF,employing the mixed microbes involved in fermentation of hexose & pentoses sugars are limited by the respective ability usually grow faster resulting higher rate of conversion from hexoses.It is reported to be the reduction in glucose inhibition owing to the nature of two different or one recombinant microorganismes activity performance whereas SHF process suffers inhibition while xylose assimilation occurs in glucose & ethanol concentration

CBP strategy shows 60% conversion of Xylan by CelA in native switchgrass showing its potential as an industrial process possible using mild or no pretreatment.This shows difference in activity translates to a seven fold increase in activity for CelA at the molecular level.

HPLC equipped with Bio Rad Aminex HPX-87H method can be applied to check the progress of fermentation gives the data and characterisation of fermentation broth based on standards for ethanol,butanol,and other inhibitors such as lactic acid, acetic acid etc. as well as data on glucose and xylose.

Regenerated Cellulose.-

The route proposed by this project study(Route-1-5) ,regenerated cellulose can be produced through tailor made approach upon treating lignocellulosic feedstocks through acidic hydrolysis process Via dilute H_2SO_4 and part of hydrolysates transformable into bioethanol via saccharolytic enzyme processing.

According to XRD-investigational study,regenerated cellulose may be recommended as precursor in acidic solution for many industrial products and precipitatable amorphised flocs having Cll type polymorph characterstics with 64-65% H_2SO_4 having low crystallinity (X=25-30%) and low DP (40-50%) and results shows to find a optimal conditions for the production of amorphised cellulose in commercial scale as raw materials for biofuels.

This cellulose is easier for transportation involves normal implementation strategy other than adoptation of lignocellulosic material processing require large space area for storage in remote place and surroundings..

Microalgaes

Microalgaes emerges as one among the promising feedstocks such as surplus utilisation of corncobs harvest for bioethanol and soyabean,rapeseed,palmoil etc.. for biodiesel relates to the consumption of 1⁄3 of harvest in USA and other parts of the world resulting significant increase in global grain prices and thereby microalgae acts as stimulant that replaces the main raw material food crisis.

Bioethanol fermentation also generates large amounts of CO_2 as by-product which can be recycled for cultivation of carbohydrates rich algaes and then residual biomass used in anaerobic digestion for methane production.

The highlighting features of microalgaes cultivation is the limited investment requirement for biofuel production through waste water remediation that may be considerable as one of the best option for sustainablity and low emitting biofuels strategy also the technologies available for processing algaes considered to be green process and selling algaes bio-oil will become acceptable (2USD per L) other than 1USD(fossil fuels)flows in the next decades that contributes 75% of world market.

The study may also insists on making effort through goverrnment towards the exploitation of microalgal productivity through effective design of Photobioreactor in order to make it viable and industrially feasible by CO2 sequesteration and waste water remediation where acquiring the abundence of light available to the land in regards to underutilisation status due to lack of bioreactors which,in turns offering a huge environmental benefits throughout the year .This stands upon economic-feasible strategy for the biofuel generation..

The highest lipid accumulation have been achieved with **N.Oculta,T.Suecica,L.Galbasa and P.Lutheri** ranges from 37.3, to 23.6, with slight reduced cell growth of 0.64- 0.38 g/L culturing under deficiency conditions of 10-65 g/L KNO3,3-7.5g/L NaHPO4 and 2.5 g/L FeCl 3**.This shows that** The reactor conditions,nutritional manipulations and culture conditions are all effective factors to improve upon the productivities of microalgae cultivations for lipids at optimum photoperiod and light intensity.

-<u>CO2 Sequesteration & waste remediation;-</u>

Microalgae production needs to be done on very large scale to make it profitable based on low cost media differes from,culture media laboratory

and evolution of large scale processes and implication of nutrient recycling in biorefineries.

Waste water are considered to be one of best options for sustainablity,zero emissions production of biofuels as sourcing nutrients for biomass production than potable water or sea water remarkably adds up the cost of biomass.

In such circumstances,This projet may also sugggest that integrating municipal waste water treatments supplementing with the use of seawater containing anaerobically digested piggery wastes are presentable for cultivation of Arthrospira(Spirulina) and bio gas production,Biodiesel,Hydrogen and other high value added chemical products possible through cost effective harveting methods such as ECH,Bio-flocculations etc .

The waste management point of view,Methanol is to be recycled and able to recover at a maximum level before disposal into waste water stream .This make the process easier access permission from the pollution control board.

WCO

This can be used as potential feedstock and secondary raw material to the biodiesel if converted can satisfy to a larger extent the world demand of biodiesel

Palm oil,WCO are recommended as a a potential feedstocks for biodiesel and the conversion through transesterfication is possible by simpler ultrasonification methods showing the yield as 90% FAME.. where the process require lower ethanol and less catalyst and consume 1KW energy for scale-Up (0.1 cts. to 1 /L/gallon).

Post processing the biodiesel,a complicated step involving separation of ester phase from the reaction mixture having the difference of densities arises between methanol,soaps,FFA, moisture or more phases..The centrifugal systems can help this in continual operations

A novel method is highly suggestable to reduce the normal process water content usage

(3-10 liters of H2O required for 1 liter of biodiesel) but it can be performed through Microfiltartion followed by sand filtration,Activated carbon etc..showing 15% lower water consumption through dilution rate with make-Up water to purification having 1000 ppm in the final product& removes excessive colours .To solve this,Vacuum drier,& falling film evoporater are mostly used to remove water contents than removal of S compounds ,odours operatable under low pressure.

Glycerol refining;

In future,non-ester side streams to be treated in parallel to overall biodiesel process such as recyclage the glycerol,excess methanol to be recycled estimated to be 10% by weight of input reactants.within the system and waste water stream..

Purification is the Ultra-necessary step normally recommended in conjunction with multiple washing method other than traditional techniques .This referred to Ionic liquid or Supercritical CO2,ion Exchange resin techniques ,Organic /Inorganic membranes technologies highly recommended on the basis of feedstocks. Ion Exchange resin play an important role on removal of minerals,catalysts glycerol and other impurities through cation,anions & mixed bed system at the final polishing stage .In the case of heterogeneous system,.PD 206 &BD10 dry cationic bed are recommended for purification of biodiesel from WCO & rapeseed oil alongwith soap & glycerol removal in contrary to methanol.This shows an opportunity to recover maximum amount of above by-products.

PolySulfone(PS) & PolyAcrylonitrile(PALN) etc. are the successful membrans used for implementation of UltraFiltration (ethersulfone)& MicroFiltration Cellulose ester membrane systems for the glycerol separation having 0.02wt% to 0.009% limit thus finally conforms EN & ASTM standards.

Genetic Manipulation for improving oil contents;-.

The overexpression of genes or enzymes of biosynthetic pathways,suppression,blocking or knock-out of genes of competitaive pathways, regulation of pass pathways, multigenes approaches etc..could find a suitable solution for synthesis,storage & profile of lipids as per the adoptivity of microorganismes into the environments.This results in change in production rates both for biofuels energy and neutraceutical purposes.

The above strategy are realisable through the expression of two key genes ACC1 & DGAT1 in oleagineous yeast (yarrow Lipolytica)by 2 times & 4 times respectively whereas the overexpression in combined form results in 5 times greater lipid accumulation than control indicates their synergistic effect .

Cyanobacteria,a blue green algaes consider as a model organismes known for genetic recombination to find a change in metabolic pathways in which lipids not in accumulation in contrary to carbohydrates production as secondary metabolites.

Yarrow Lipolytica , an indiustrial yeast is well studied strain for genetic manipulations and unique ability to grow on hydrophobic substrates.produces EPA yield of 161.04mg/g/day .

Whenever the suppression or activation of genes are required in genes modification,the methods such as mutagenesis,homologus recombination,the use of micro RNA (miRNA) and short interfering RNA (siRNA) can be practiced based on type of microorganisms,the strains,their genetic profiles and the desired results etc...

Renewable diesel;-

In view of upgrading the biodiesel purification,the deoxygenation pathways appears to be promising route for the Renewable diesel transformation.using Silica,Alumina,Zeolithes and fluid

cracking catalysts.This will enhance the cetane numbers by catalytic deoxygenation but this pathways can not be used for biodiesel due to the involvements of nature of extra steps, increase in capital& opeartional costs etc..Hence,proper combined status process are essentially required to obtain a robust biodiesel purification.

To obtain new quality category diesel called as Renewable diesel,the current commercial approach comprises of a two step process involves an initial (Hydro)-deoxygenation step followed by (Hydro-Isomerisation)process ensuring a high cetane number,excellent cold flow properties and environmental friendliness of the obtained renewable diesel than petroleum diesel & biodiesel.

Traustochytrids(fungus like clade of stramenpiles),a good source of DHA has been recommended for commercial production through improved technology requiring 25-30°C temperature for optimal growth and reduced temperature 15°C enhances DHA production at the reduced growth level.

Schizochytrium Spp. shows total lipids contents of 35-40%w/w as DHA in contrary to Aurantiochytrium Sp.T66 (marine ATCCPRC 276))in heterotrophic condition yielding dry cells weight (10.38g/L) and total lipids(4.98 g/L) while using forest biomass hydrolysate (30gm/L glucose).This shows 25.98% DHA constitution as compared to bioreactor cultivation obtaining the growth level 11.24 g/L and a total lipids of 5.90 g/L and DHA content of 35.76%realisable as it appears to be great potential in valorising the sustainable resources for commercial DHA production

Recovering value-added Byproducts including PUFA ,helps in reduce the overall production cost.This could acquire omega-3-FA production from diminishing fish-stock creates long term persistent problem in production from global aquatic ecosystem through replacement of use of fish oil.

Red Microalgaes are the potential sources of many ***AntiViral*** compounds often referred to sulphated Polysaccharides(mainly Xylose/Glucose/Galactose units) showing features capable to interfere with protein-protein interaction phenomenon through against two Rhabdoviruses such as VHSV and ASFV. Dunaliella sp.,extracts found to be inactivated the initial viral functions after stage **.**

BioHydrogen;-

To overcome the limitation of several processes,integrated approaches are recommended to increase in dark fermentation through the use of residual acid rich organic substances from the effluents uses the further substrates for additional energies H2 production and the entire process is more economically feasible and viable especially only coupling with MEC process in association with simultaneous waste water treatments for the wide variety of soluble organic substances with mixed culture of C.Butyricum,enterobacter aerogenees,Rhodobacter etc **..** thereby recovering further energy is possible upon integrating two stage energy producing process.

The examples of numerous secondary processes such as methanogensis for methane,acidogenic fermentation for H2,photobiological processes for H2,MEC for H2,anoxygenic nutrients limiting processes for bioplastics,cultivation of heterotrophic algaes for lipids and MFC for bioelectricity generation were integrated with the primary dark fermentation processes for H2 production.

-Sequesteration of CO2 is one of an option of H2 prodution for viable near-term solution with the global climate change as 4th generation fuels and make challenges over the growing demand for zero emission fuels.

<u>Advantages & disadvantages of dark fermentation in H2 prodn</u>;-

Although ,low yields of H2 is possible on substrates in anaerobic conditions where pyruvate enters into the acidogenic pathways coupled with H2 conversion other than volatile fatty acid (VFA) like acetic acid,propionic butyric ,maleic etc..as a disadvantage factor in dark fermentation.owing to the consumption of inorganic & organic compounds with their concurrent reduction &regeneration of reducing powers.

Future aspects of Biohydrogen results;

In order to increase the production of biohydrogen,intensive research are suggested on advancement of the process such as fermentative and Biophotolysis through development of genetic engineered microbes and also ,bioreacor design improvement and engineering the hydrogenases enzyme produces essentially required in future.

- ***Biomethane;-***

Biogas referred as Biomethane to be launched in a most securised way through modernisation of process .In order to viabilise the project towards the pollution free environments ,the measures & controls must be exploited that are inevitable in maintain the strategy.

Biobutanol;-

Biobutanol, a higher second largest fermentative fuel product is possible to produce with engineered C.Acetobutylicum XY16,harbouring through pSADH gene favouring efficient catalysis resulting alcohol mixtures had a direct end usage as fuel,solvent and chemical intermediates

Besides the high operating cost of the factors such as yield,productivity,low titer and solvent toxicity are the challenges to overcome through metabolic engineering approaches to make biobutanol economically feasible.due to the formation of acetone as by-products representing 30% as ABE..For this purpose,Clostridium Acetobutylicum XY-16 (pSADH) successfully and metabolically constructed to produce IBE fuel mixtures (BIobuatnol)with complete removal of acetone using starch based feedstocks such as microalgaes,lignocellulosic materials etc .

FUTURE SUGGESTION FOR SCALE UP ON BIO ENERGY PRODUCTION;-

Researches to be undertaken and needs to be addressed in large scale system in algal biofuels upgrading through:

-Scale-Up process over bioreactors and open ponds in order to limit the tackover the invading weedy local algaes.

-Scale-Up the Sustainable nutrient source for culture stability

-System level productivity analysis is required for standardise.

-Water preservation management and scaling & Cost reduction

-setting Norms & Standards to improve upon product quality & safety issues towards handling, transport & Usage and environmental health etc..

-An alternative to searching for a novel microbial pathway ,there is a need to use independent culture techniques such as metagenomics,,bioengineered novel strain etc..through genetic tools such as MAGE,CRISPR/Cas,ZFN,TALEN etc helping to improve GMO with desired industrial characterstics.

-Encouraging enterprises to implement the process and to realise the applications.

ECOLOGICAL &ENVIRONMENTAL IMPACTS (EEI) OF BIOENERGY

The total impacts influences the overall net positive and negative effects of bioenergy may be regulated on the basis of feed stock type,type of production system,conversion technologies,transportation and distribution systems.The above strategy will be successful only if it may have a diversified focus on EEI dispostif such as land use ,GHGE,climate change,wildlife and

biodiversity,invasive&transgenic plants,marginal lands& water quality and quantity helps in resolving on multitactic approach.

There is a need to develop sustainability factors through Environmental,Economic and social aspects. according to the Hypothesis indicated.So ethanol industries need to address the ethical and environmental issues such as climate change reduction(GHGE),energy and water conservation and waste water managements.

The conclusion of the project shows that assessments needs to be made on the basis of each type of biomass materials,locations and extraction techniques & other recent technological innovation to be applied for successive biofuel prodution in a sustainable way. Certainly this provide a status and maximise the productivity which will reflect on acquiring clean water,Clean air,clean energy etc..

Table 1.0 The physico-chemical properties of ethanol and gasoline

Properties	**Units**	**TestMethods**	**Ethanol**	**Gasoline**	**References**
Molecularformula	-	-	C2H5OH	C4-C12	
Composition(C,H, O)	(Mass%)	ASTM D5291-02	52,13,35	86,14,0	(Mohebbietal., 2018)
Densityat15ºC	(Kg/L)	ISO12185	0.79	0.73	(S.H.Park,Yoon,&Lee,2014)
Boilingpoint	(ºC)	-	78.3	27to 225	(Hedfi,Jedli,Jbara, &Slimi,2014)
Auto-ignitiontemperature	(ºC)	-	360	228 to470	(Balki, Sayin,&Canakci, 2014)
Flashpoint	(ºC)	ASTMD93	21.1	-45to -38	(H.Liuetal., 2014)
Lower heartingvalue	(MJ/kg)	ASTMD240	27.0	43.5	(Elfasakhany,2016)
Octanenumber	VM	ASTM D2699	108	95	(Mařík,Pexa,Kotek, &Hönig,2014)
Cetanenumber	-	ASTMD2700	11	0to10	(RajeshKumar &Saravanan,2016)
Latent heat ofvaporization	(KJ/kg)	-	838	223.2	(Thangaveletal.,2016)
Stoichiometric air/fuelratio	w/w	-	9.0	14.7	(Guet al., 2012)
Viscosityat20^{o}C	(mm^{2}/s)	-	1.19	0.37 to0.44	(Mohebbi et al., 2018;Yücesu,Topgül,Çinar,&Okur,2006)
Saturationpressureat 38 o_C	(KPa)	-	13.8	31	(Thangaveletal.,2016)
FlammabilityLimit,20 °C	(vol%)	-	3.3 to19	1.0 to8.0	(Ulrik,Troels,&Jesper,2009)
Aromatics	(%v/v)	-	0	33.3	(Costagliolaetal.,2016)
Enthalpy of formationLiquid	(kJ/mol)	-	*-224.1*	*-259.28*	(Masumetal.,2013)
Gas			*-234.6*	*-277*	

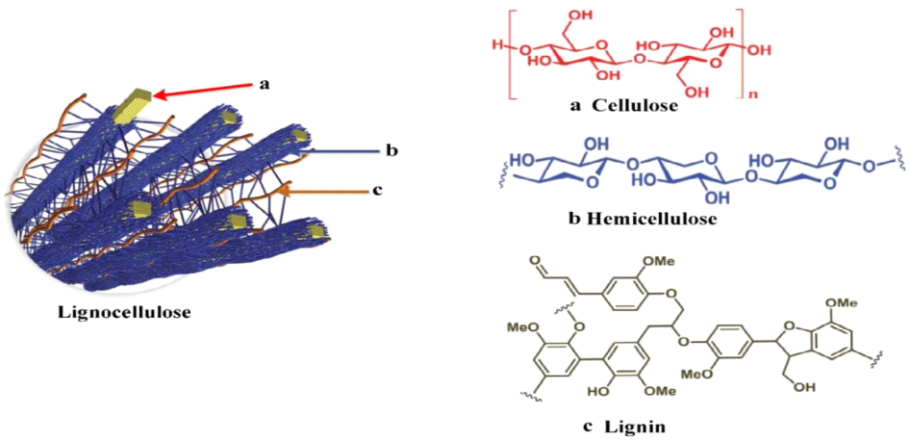


Figure 1 Lignocellulosic biomass (a) Cellulose; (b) Hemicellulose; (c) Lignin (Brandt, Gräsvik, Hallett, & Welton, 2013; Kobayashi & Fukuoka, 2013).

Bioethanol Production Techniques from Lignocellulosic Biomass as Alternative Fuel:

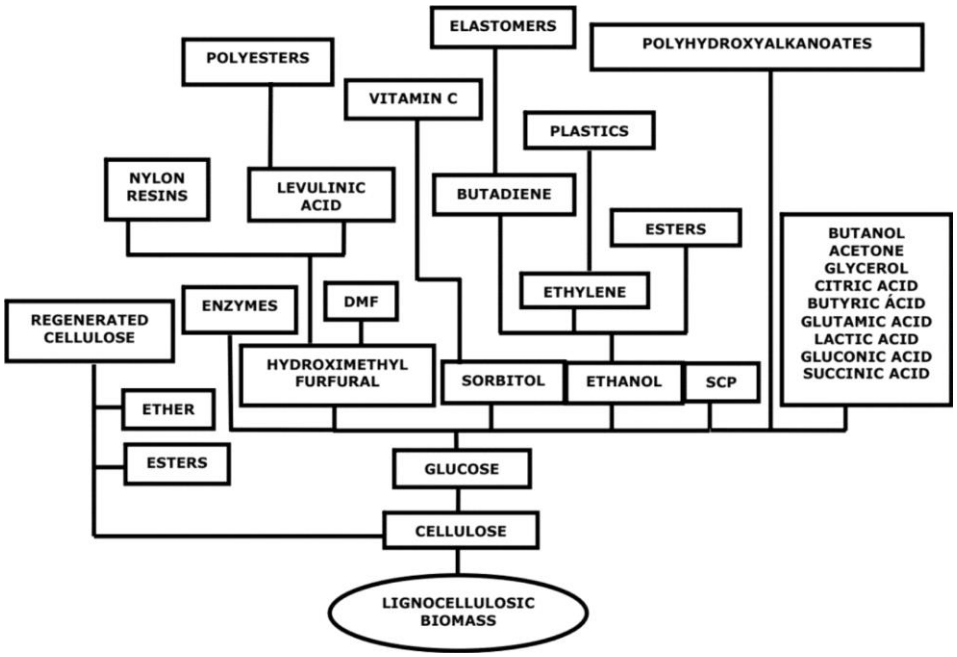


Figure1. 2 Schematic concepts of biorefinery from lignocellulosic biomass composition (cellulose products) (Pereira et al., 2008).

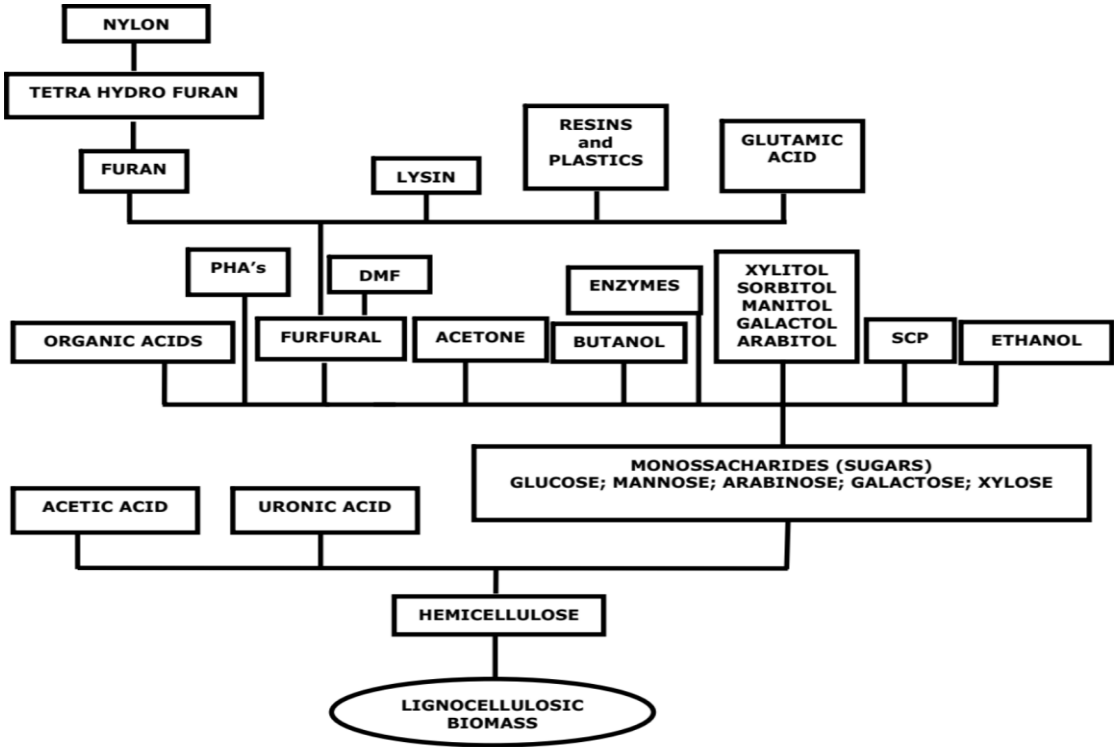


Figure1. 3 Schematic concepts of biorefinery from lignocellulosic biomass composition (hemicellulose products) (Pereira et al., 2008).

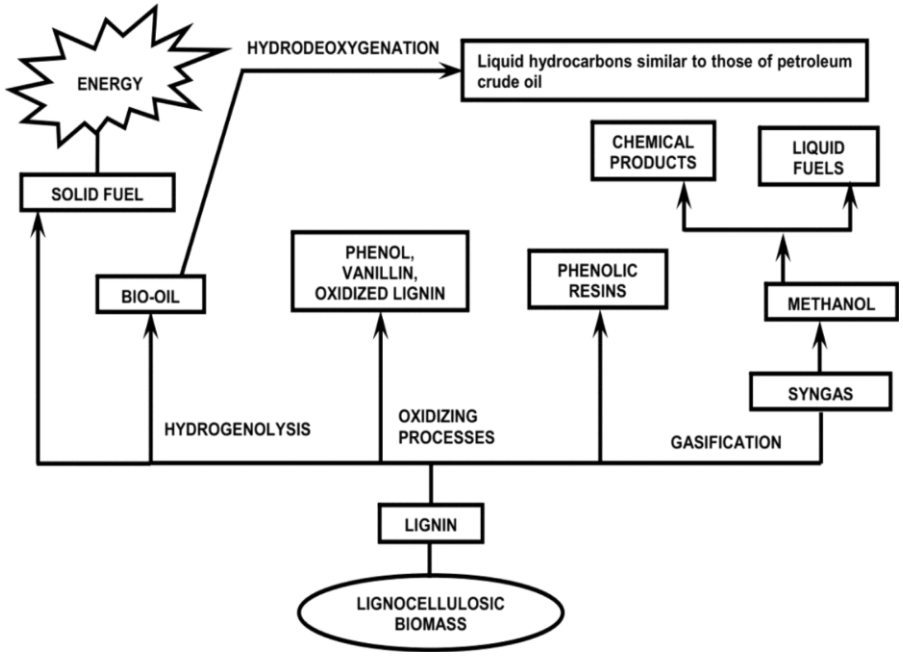


Figure1. 4 Schematic concepts of biorefinery from lignocellulosic biomass composition (lignin products) (Pereira et al., 2008).

Table 2.0 Advantages and disadvantages of different pretreatment processes for lignocellulosic biomass materials.

PRETREATMENTMETHOD	PROCESS	ADVANTAGES	DISADVANTAGES	REFERENCE
Physical	Mechanical: Physicalreduction in substrateparticle size bygrinding,milling, etc.	Reduces cellulosecrystallinity anddegree ofpolymerization Reduced theparticle size toincreaseaspecific surfacearea	Power consumptionusually higher thaninherent biomassenergy	(Balat,2011)

Physico-chemical	Steam explosion:Substrate particlesrapidly heated byhigh-pressure saturatedsteam. Explosivedecompression causedby quick release ofpressureacidsreleasedtoaid inhemicellulose hydrolysis.	Cost-effectiveCauses lignintransformationand hemicellulose solubilizationHigh yield ofglucoseand hemicellulosein the two-stepprocess	Partial hemicellulosedegradation Toxic compoundsgeneration Acidic catalystneeded to make theprocess efficient withhigh lignin contentmaterial	(Brodeur et al.,2011)
	Ammonia fiberexplosion (AFEX):Substrate is exposedtohotliquid ammoniaunder high pressure.Pressure is releasedsuddenlybreaking open biomassstructure.	Increasesaccessible surfacearea Fewer inhibitorsformation Does not require asmall particle sizeofbiomass	Very high pressurerequirementsExpensive Not very effectivefor the biomass withhighlignincontent	(Gumisiriza,Hawumba,Okure,&Hensel,2017)
	CO2 explosion:Injected to thebiomass reactor invery high pressureand heated at hightemperature.	Increasesaccessible surfacearea Non-flammabilityDonotforminhibitory compounds Availability atrelativelylowcost	Very high pressurerequirements A portion of xylanfractionlost It can emit the CO2emission to theatmosphere	(Maurya et al.,2015;Sebayang et al., 2016)

PRETREATMENTMETHOD	PROCESS	ADVANTAGES	DISADVANTAGES	REFERENCE
		Easy recoveryafter extractionandenvironmental acceptance		
Chemical	Acid: Addition ofdilute or concentratedacid solutions result inhemicellulosehydrolysis (H2SO4,HCl,HNO3,H3PO4).	High glucose yieldHighconcentrationcan bedoneatroom temperature Solubilizeshemicellulose	High operational andmaintenance costsCorrosive Formation ofinhibitorsConcentratedacids aretoxic and hazardous	(A. K. Kumar &Sharma, 2017)
	Alkali: Addition ofbase causes swelling,increasing the internalsurface of cellulosewhich provokes ligninstructure disruption(NaOH, KOH, Lime,Mg(OH)2,NH4OH).	Decreased cellulosecrystallinity anddegree ofpolymerization Can be done atroom temperatureEfficientremoval oflignin	Relatively expensiveNot used for largescaleplant Irrecoverable saltsformed andincorporated intobiomass	(Bali et al., 2015;Rabemanolontsoa&Saka, 2016)
	Ozonolysis: Powerfuloxidant, soluble inwater and is readilyavailable.	Reduces lignincontent Does not producetoxicresidues No requirement ofchemical additivesOperationat ambient temperature andpressure	Relatively expensivedue to a large amountofozone generated Highly reactive,flammable,corrosiveand toxiccharacteristics ofozone	(Travaini,Martín-juárez,Lorenzo-hernando, &Bolado-rodríguez,2016)

	Ionic Liquids (ILs):Organic saltscomposed of organiccations and eitherorganic or inorganicanions([EMIM][Ac], [BMIM][Cl]).	Highly efficient(over 80 %saccharificationyield) Environmentalfriendly chemicalsMinor degradation ofrawmaterials Negligiblepr oductionof inhibitory compoun ds	Veryexpensive Has negative effectson cellulose activityand affect the finalyield of cellulosehydrolysis Consume muchwater	(KeikhosorKarimi et al.,2013)
Biological	Fungiandactinomycetes:Microorganismsdegrade and alterbiomassstructure (white-, brown-, soft-rotfungi).	Low energyconsumption Simple equipmentdegrades ligninandhemicelluloses	A rate of hydrolysisis verylow Low degradation rateto attain a highdegree of lignindegradation	(P. Kumar et al.,2009)

PRETREATMENTMETHOD	PROCESS	ADVANTAGES	DISADVANTAGES	REFERENCE
		Relativelyinexpensive Does not causecorrosion to theequipment Low production ofinhibitors		

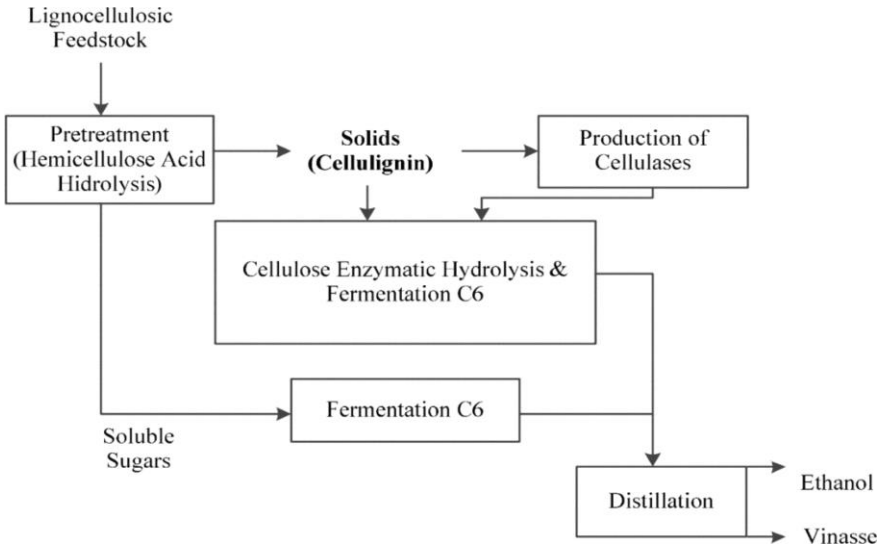


Figure 1.5 The schematic process of the simultaneous saccharification and fermentation (SSF) (Sebayang et al., 2016).

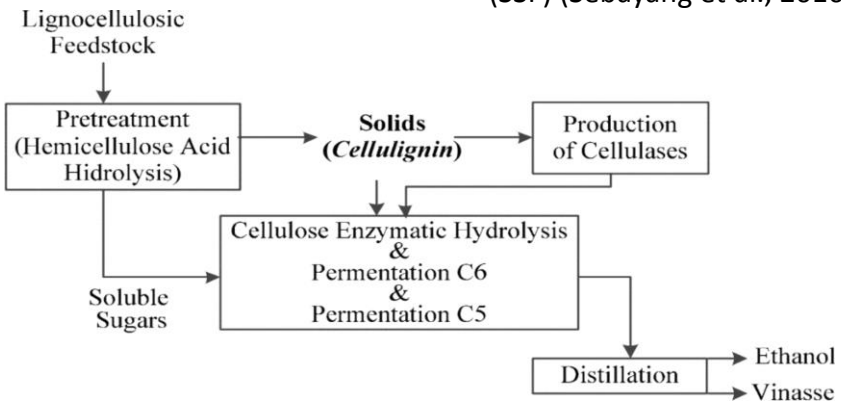


Figure 1.6 The schematic process of the simultaneous saccharification and co-fermentation (SSCF) (Sebayang et al., 2016).

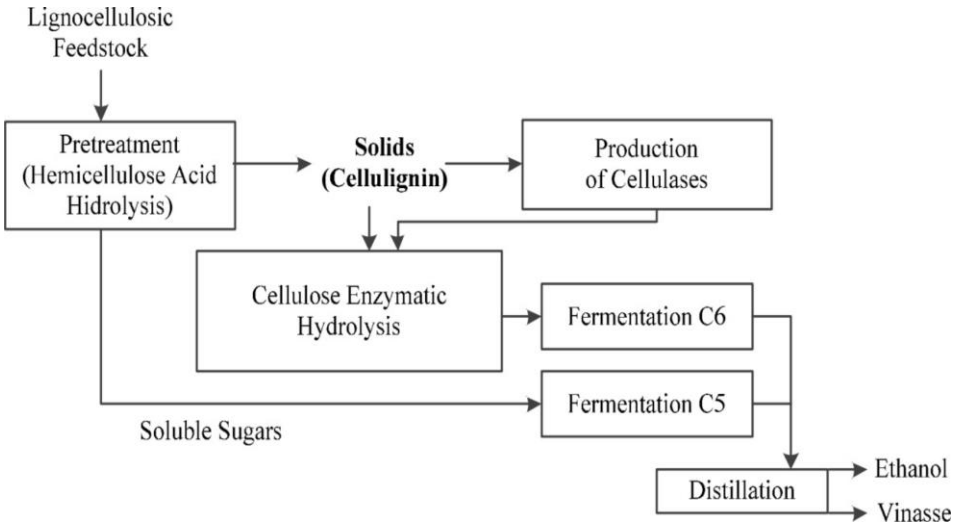


Figure1. 7 The schematic process of the separate hydrolysis and fermentation process (SHF) (Sebayang et al., 2016).

Table 3.0 Advantages and disadvantages of separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and simultaneous saccharification and co-fermentation (SSCF).

Fermentationprocesses	**Advantages**	**Disadvantages**
Separate hydrolysis andfermentation(SHF)	Ability to carry out each step underoptimal conditions, i.e., enzymatichydrolysis at 45 °C to 50 °C for betterperformance and fermentation at 30 °Cfor optimizing sugar utilization (MohdAzhar et al., 2017; Tengborg, Galbe, &Zacchi, 2001). SHF is more efficient than SSF whenbioethanol production is carried outusing cellulosic biomass (Cotana et al.,2015; Wirawan, Cheng, Kao, Lee, &Chang, 2012). The yeast produced during the SHFprocess can be recycled afterfermentation of the hydrolysate, whichisnotpossibleinSSF(Olofsson, Bertilsson,&Lidén,2008).	Inhibition of cellulase and β-glucosidase enzymes by glucosereleased during hydrolysis,which calls for lower solidsloadings and higher enzymeloadings to achieve reasonableyields(Balat, 2011).

Simultaneoussaccharification andfermentation(SSF)	Lower enzyme requirements; higherproduct yields; lower requirements forsterile conditions since glucose isremoved immediately and bioethanol isproduced; shorter process time; and lessreactorvolume (Sun&Cheng,2002). The immediate consumption of sugarsby the microorganism produces lowsugarconcentrationsintheferm entor, whichsignificantlyreducesenzyme	Theconditionsof SSFaremoredifficult to optimize (Krishna,Reddy,&Chowdary, 2001). DuringSSFthereleaseofsugaris not controlled, as all thecellulase enzymes are added atonce (Erdei, Frankó, Galbe, &Zacchi, 2012).

Fermentationprocesses	**Advantages**	**Disadvantages**
	inhibition compared to SHF (SchellMarkF.;Tucker,Melvin P., 1999). This process is often effective whencombined with dilute acid or hightemperature hot-water pretreatment(Balat, 2011). Accept the mode of improvementwhichcombinesthecellulaseenzymesand fermenting microbes in one vesseltoimprovethebioethanolproduction economics(Y. Yu, Lou,&Wu, 2008).	

Simultaneoussaccharification and co-fermentation(SSCF)	Reduced capital costs (Wingren, Galbe,&Zacchi, 2003). Continuous removal of end-products ofenzymatic hydrolysis that inhibitcellulases or β-glucosidases (Olofssonetal.,2008). Higher ethanol productivity and yieldthan separate hydrolysis andfermentation (Alfani, Gallifuoco,Saporosi, Spera, &Cantarella, 2000;Tomás-Pejó, Oliva, Ballesteros, &Olsson,2008). Maintains glucose at low levelsallowingefficient co-fermentationof glucose and xylose (Öhgren et al.,2006).	At high water insoluble solids(WIS) content, the ethanol yielddecreases due to an increase inmass transfer resistance andinhibitors concentration (Hoyer,Galbe,&Zacchi, 2009).

Table 4.0 Different methods, conditions and their effects for bioethanol from various biomasses (reported between the years 2013 to 2019).

Biomass	**Pretreatmentconditions**	**Hydrolysisconditions**	**Sugar yield**	**Fermentationconditions**	**Results:Ethanolyield**	**Remarks**	**Reference**
Energygrass	Alkali:100ml of 1 % (w/v)NaOHat 121 °Cfor 1h	Enzymatichydrolysisby Cellic®CTechwith 0.1g/g grassat 50 °C, 150rpm, pH4.8 for 72 h	467.9mg/g	-	-	Ozonolysisisan efficientpretreatmentmethod forenergygrasses,resulting inup to 51 %delignification.	(Panneerselvam, Sharma-Shivappa, Kolar, Clare,&Ranney,2013)
	Ozonolysis:performed for2h at a flowrate of 0.25l/min		431.9mg/g	-	-		

Corn stover	DA:H2SO4of 5CL with 895.5 kg of^{H2O} at 160 °Cfor20minand N-CR	Biomass: 29 kgglucan, CTec2:583g, HTec2:287gat	65g/L of glucose[a]and 4g/L of xylosein 72 h	*S. cerevisiae*strain 424A(LNH-ST), 0.28 g dry-cell-wt./L andoperated	14kg	AFEX produces highdigestiblesubstrates, highfermentation	(Uppugundlaetal.,2014)

Biomass	**Pretreatmentconditions**	**Hydrolysis conditions**	**Sugar yield**	**Fermentationconditions**	**Results:Ethanolyield**	**Remarks**	**Reference**
		50°Cwith pH4.8 for 72 h		for 120 h(SSF)		metabolicyieldwith98%.	
	IL: [C2mim][OAc] of 900 CL at140°Cfor 180 minand CR	Biomass: 31.7 kgglucan, CTec 2:371g,HTec 2:314g,MultifectPectinase:266gat 50 °C withpH4.8 for 72 h	72 g/L ofglucose[b]and35 g/L ofxylosein72 h		21.2kg		
	AFEX: Anhydrous ammonia of100 CL with^{60} kg of ^{H2O}at140°C,300 psi for 15 minand CR	Biomass: 33.5 kgglucan,CTec 2:670g,HTec 2: 167.5g,MultifectPectinase:	60 g/L ofglucose[c]and29 g/L ofxylosein72 h		20.5kg		

		167.5 g at50 °C withpH4.8 for 72 h					
Sugarcanebagasse	Acid: H2SO4of 1 % (w/v),1:10 solid-liquid ratio at121 °C for 20min.	Hydrolyzed by dilutedacid(2.0 % of^{H2SO4})at 155ºCfor 10min	Glucose 22.74g/L,nox ylose	*S. cerevisiae*st ain NRRLY-712 4at30 °C, 200 rpm for72 h	16.8 g/Lconc., 0.38 g/gand 0.23 g/L/hproductivity	This processgeneratesinhibitorycompounds,and thedetoxification wasrequired forremovingthosecompoundsfoundinthe hydrolysate.	(Bardone etal.,2014)
Switchgrass	IL: Pretreatedwith[C2C1Im][OAc] at 100 °Cfor 3h	Hydrolysis bycellulaseof*novozyme*HTec2at 0.3 % w/w(g	20g/Lglucose	*S.cerevisiae* strainBY4741 at30 °C, 200 rpmfor20h	85.7g	IL pretreatmentdemonstratedhigher bioethanolyields.	(Papa etal.,2015)

Biomass	**Pretreatmentconditions**	**Hydrolysisconditions**	**Sugar yield**	**Fermentationconditions**	**Results:Ethanolyield**	**Remarks**	**Reference**
		enzyme/gxylan),30 min,2 h,6					

		h, 24h and 48h					
Wheatstraw	Ozonolysis: Pretreated for1 and 7hat 0.6 l/min flowrate withambientconditions	Enzymatichydrolysiswasperformed for72h	Glucose of49 % and xyloseof 9.14%	(SSF) wasperformed for140 h	12.9 g/Land67% conc.	Resultsshowed thatozone (orPAP) notonlydegradedlignin butalso had aneffect onepicuticularwaxes on wheatstraw.	(Kádár et al.,2015)
Ricestraw	BP: Pretreatedsubstrates in 30mLof 50mMsodium citratebuffer(pH=4.8)	Hydrolysiswasconductedusing 90 %v/v Cellic®CTec2and10 % v/v Cellic®HTec2 and 30FPU/gcellulase and50 IU/g -glucosidase at45Cand 120 rpmfor 72 h.	69.5 % ofhydrolysisyield	*S. cerevisiae*(CCUG 53310)at37Cand130 rpm for 24 hthrough(SHF)	206 g	Increasing theporosity of thesubstrate byhemicelluloseremoval could bethe maineffectiveparameter in thistype ofpretreatment. However,enzymatichydrolysis andethanol production processesneedto beimproved.	(Bahmani etal.,2016)

Bananapeels	MP and SE:pretreated withautoclaved at 15psi pressure for 30min, knife millingwith 2 cm to 4 cmanddried at 60°C	0.5 % (v/v) to2.5%(v/v) dilutedsulfuric acid70 °Cand 110 °C, pH7 for 10 min to30 min	11g/Lglucose and 5.5g/Lxylose	*S. cerevisiae*strain at 30 °C,200rpmfor24h	45.088 % ofbioethanol	The waste(banana peels)from the FPI maybring seriousenvironmentalproblems. Thiscan be minimizedbytheproduction ofethanol.	(Gebregergs,Gebresemati,&Sahu,2016)
Unripebananapeel	MP: Dried at 60 °Cfor 24 h, electricgrinder and sievedthrough meshnumber 36 (0.45mm).	Hydrolyzed^{byH2SO4} ^{1} %(v/v)at 120°C,100 kPa for 10min	49.2 % (w/w) ofsugarrelease	*S. cerevisiae*(NCIM 3095,NCIM 3570 andNCIM 3059) at30°C,pH5,150 rpmfor36 h	35.5g/L,1.5 g/L/hproductivity	*S. cerevisiae*NCIM 3095 wasfoundtobethe best strain forproduction ofethanolcomparedto the othertwostrains.	(Waghmare &Arya,2016)
Elephantgrass	MP: Dried at 60 °Cfor 3 days, 4 % to20 % (w/v) in aconcomitant ballmilling treatment /triturated with forage chopper(0.5cmto2cm).	124.43U/mL, 6.16 U/mLand 893.55U/ml of b-glucosidases,endoglucanases andxylanasesat	12.47g/L	*S.cerevisiae* CAT-1at28 °C for 48 h	6.1g/L	High ethanolyield is not onlyto do withbiomass butdepends onenzymaticand fermentationprocesses. There	(Menegol,Fontana,José,Dillon, &Camassola,2016)

Biomass	Pretreatmentconditions	Hydrolysisconditions	Sugar yield	Fermentationconditions	Results:Ethanolyield	Remarks	Reference
		50 °C,pH 4.8,150 rpm for 1hto6h				is a need todevelopequipmentfor suchpurposes.	
Pinewood	Alkali:Performedwith0-2% w/v NaOHat100-180 °Cfor 1hto5h.	Enzymesmixture(90 % Cellic®CTec2and10 % Cellic®HTec2)at1.5 FPU/gsubstrate ofcellulaseat45 °C,pH 4.8,120rpm for 72 h	83.5%±0.3 % glucoseyield	*S. cerevisiae*under anaerobicconditionsfor24 h	76.9%to 78.0 % and0.609 g/L/h± 0.015 g/L/hproductivity	Productionofbioethanolrequirescheaprawmaterialswhich caneffectivelyenhance themanufacturingcosts. Usingchemicals for neutralization isunavoidable.	(Safari,Karimi, &Shafiei, 2017)
Cottonstalks	Alkali:NaOH(0.5 % to 4.0 % w/w)and the biomassloading (10 % to25 %)at120°C for20min Acid:H2SO4(0.5 % to 4.0 % w/w)and the biomassloa	Hydrolysisbycellulaseof*P. janthinellum*and 20 FPU/gsubstrate ofcellulaseat50 °C,200 rpmfor48 h	25.59 g/L ofglucose andhydrolytic efficiencyof 80%	4 % (wet wt/v) *S.cerevisiae*RRP-03Nat 30°C ± 2 °C for 48 h,(SHF)	9g	Alkalipretreatmentofcotton stalkseffectively de-lignified thebiomass and ahydrolyticefficiencyof80 % wasattainedwith acombinat	(Christopher,Mathew,Kiran Kumar,Pandey, &Sukumaran,2017)

	ding (10 % to25 %)at120°C for20min					ion ofcommercialand in-housecellulases.	
*Agave tequilana*bagasse	SE(AP): Pretreated atelevatedtemperatures(160 °C to 240 °C) nochemicals required[but H2O]	Hydrolyzedusing Cellic®CTec3 of 25FPU/gofglucan at50 °C, pH4.8, 200rpmfor 72h	131 g/L±1.7 g/L glucoseand81.5 %± 1.7% hydrolysisyield[d]	*S. cerevisiae*ATCC 4126 at30 °C, pH 5.5,100rpm for24h (SHF)	65.26 g/L and95 % of thetheoretical value	AP can be anefficient andrelatively simplemethod for*Agave tequilana*that can beincorporatedina 2[nd]GEPP.	(Rios-González etal.,2017)
Bananapeels (*Tabasco* variety)	Acid and MP:[H2SO4(0 % v/v,0.5 % v/v, 1%v/v),autoclaved at121 °C, 103kPa] for15min,milledby mechanicalgrinding(1mm).	15 FPU/g (Cellulclast1.5L)10%, 15%w/w), and20%(w/w)pretreatedbananapeel	32g/Lglucose	*Kluyveromycesmarxianus*at42 °C, 150 rpm for24h	21 g/L	The bananapeelparticle sizecontrol is not ofgreat importance for thesaccharificationof thislignocellulosic material.	(Palacios etal.,2017)
A.tequilana	AFEX: 1000kg solid, milling DM,Ammonia (2 kgNH**3**/kg DM) with2kgofH2Oat102 °C to 120 °C for30minto	Cellic®CTec3 andHTec 350°C, pH4.8,250 rpm, and72 h	252kgglucoseand 109.8kgxylose	*Saccharomycescerevisiae* 424A(LNH-ST)at30 °C,150rpm, pH 5.5 for 72 h,(SHF	154 kgethanol	The amount ofenzyme loadingused in thisexperiment ishigher;identifying therightcombination ofaccessory enzymes in thefuturewill	(Flores-Gómez et al.,2018)
A.salmiana			301.4 kg glucoseand107kgxylose		176 kgethanol		

	38min			)		further reduce theenzymel oading.	
Agave bagass e	SE/HP(AP): performedat 180 °C for 20 min, 40min,and50 min	*Novozym es*using 20FPU/g of a substrate as	12.42 g/L glucoseat18 0 °C, 15.31 g/LXylo oligosacchar i	*Saccharo mycescere visiae* PE-2at30° C,150 rpmfor12 h	98.5 %[f],99.5 %[e],55.02 g/L of ethanolc oncentrat ion	The resultsho wed adecrease inthe ethanolcon c.on	(Aguilar et al.,201 8)

Biomass	**Pretreatmen tconditions**	**Hydrolys is conditio ns**	**Sugar yield**	**Fermentati onconditio ns**	**Results:E thanolyie ld**	**Remarks**	**Referenc e**
		loading ofcellulo se at150rp m,pH 4.8,180° C for 20 minunde rIRandNI R	des and 65.87 % ofIR	under (PSSF)an d(SSSF)	and 90.84 %yiel d	a kinetic profile,due to ethanoleva porationdu ring theproduct ionprocess, and theSSSF processwas completed after72 h.	
G.verruc osa	Acid:12%(w /v) *G.verrucosa* with 0.2 M H2SO4at130°Cfor1 5min	Celluclast 1.5L, Viscozym eL, and CellicCTe c2 at50 °C, 150 rpm for 48 h	50.7 g/Lmonosa ccharides	*Pichia stipitis*and*K luyveromyc esmarxianu s* at 150rpmat3 0°C	29.0 g/Lethan ol,0.81 g/L/h producti vity	*P. stipitis* showedmo re efficientcell growthand bioethan olproductivi tythan *K.marxian us.*	(Sukwon g etal.,20 18)

Bananapeels	Acid: pretreatedusingHCl,pH5.0.	Xylanase 1.99IUm/L, FPase 2.0 IUm/L,pectinase 4.0IUm/L,substrate(2.5 % to 20 %) at(60 °Cto90 °C), pH9.0, 150rpmfor (1 hto4h).	37.06mgm/LORSat 70°C	*Geobacillus stearothermophilus* strain HPA19at37°Cfor30h	21.1 g/L, eff.of 76.5% at 30h	Itisgoodtoknow the suitableratio ofcellulolyticandhemicellulolyticenzyme fordifferent substrates toproduce maximumreductionsugars.	(Prakash et al.,2018)
Orangepeel	MP: milled with agrinding machineand dried	Cellulase 1.06U/mL, 337.42 U/mL,and1.36 U/mLat37 °Cfor 18h	20g/Lglucose	*S. cerevisiae* genomeviatheCRISPR-Cas9approachat30 °C, 180 rpm for60 h(SSF)	7.53 g/L	The engineered strains mayprovide avaluable materialfor thedevelopment oflignocellulosic ethanol.	(Yang et al.,2018)
Sunflowers talk	IL:[Bmim]Cl10 % to 25 % (w/w)pH5.0, 60°Cfor 24h Alkali:NaOH0.2 % to 2.0 %, (w/v),pH5.0, 60 °Cfor 24h	cellulase 20 FPU, and 400IU ofxylanase/gbiomassat50 °Cfor 72	302.4 mg/gglucose, 107 mgxylose, 114 mg/greducingsugars	*P. oxalicum* PN8(SSF)	(0.078 g/gbiomass) ofethanol	Results showedthat thecombined IL andalkalipretreatmentcauses moredrastic alterationsin the biomassultr	(Nargotra, Sharma,Gupta, Kour,&Bajaj,2018)

	IL and Alkali:NaOH 0.5 % w/vand[Bmim]Cl(25 %,w/w)90°C for2h	h				astructure ascompared to ILalone or alkalipretreatment.	
Empty palmfruit bunchfiber	Alkali-thermal:NaOH, driedsample (20%w/v)at105 °Cfor 24 h, autoclave(121°C, 15psi,60 min)	(Cellulclast1.5L),20 FPUto100 FPU and -glucosidase(Novozyme188;40CBU) for 72 h	82.2%fermentablesugar conversion	*S.cerevisiae* W303-1A strainat30°C,200 rpm for 28 h,(SHF)	33.8±0.5 g/L ethanol with 1.57 g/L/hproductivity	Separatehydrolysis andfermentationusing hydrolysate are usefulforproducingbioethanol with highproductivity.	(S.Kim,2018)
Matooke peels	MP: Dried at 58 °Cfor 83 h, 0.2 mm to2 mm after millingand grinding withanelectric grinder	0.5%(v/v) to 2.5 % (v/v) of^{H2SO4}, 50 °Cto90°C± 1 °C at 20 minto60min	77.03 g/Lreducingsugars	*S. cerevisiae*NCIM 3570, at29°Cto39 °C±1 °C, 165 rpm,pH5.0forabout 10hto30h	71.54g/L	Utilizing thiswaste biomassfor bioethanolproductionthrough a biotechnological	(Yusuf &Inambao, 2019a)

Biomass	**Pretreatmentconditions**	**Hydrolysis conditions**	**Sugar yield**	**Fermentationconditions**	**Results:Ethanolyield**	**Remarks**	**Reference**
		with gentle shaking				process not onlyhelps to reduceenvironmentalpollution but alsoreduces dependence	

						onoil-producing countries.	

(FIGURE-1.1 CHEMICAL STRUCTURE LCWB)

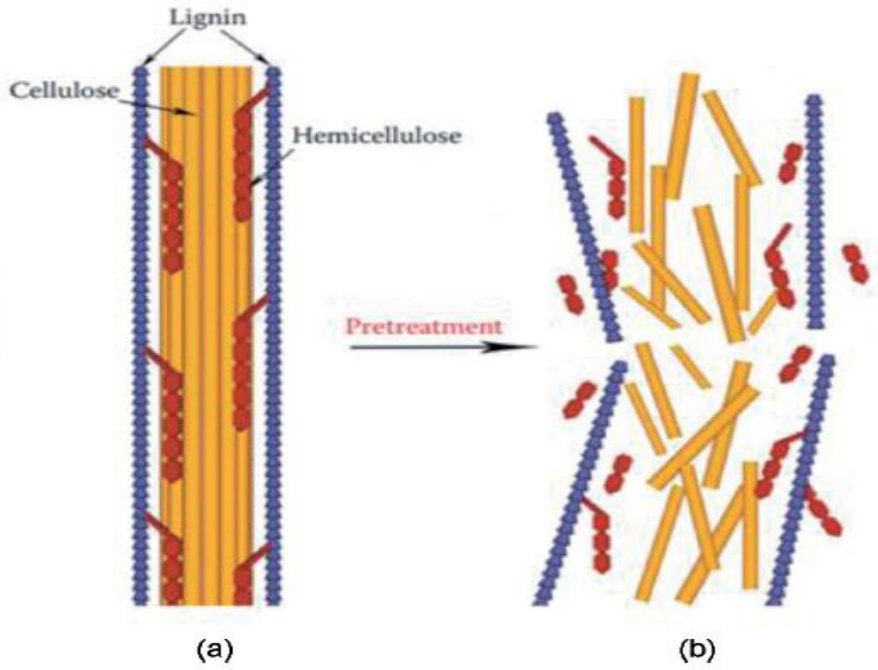


Figure 1.
Effect of pretreatment on the lignocellulosic biomass [16]. (a) Lignocellulosic biomass before pretreatment, and (b) Lignocellulosic biomass after pretreatment.

Algae	Bioethanol yield (%)	Ref.
Nannochloropsis Oculata	3.68	[9]
Tetraselmis suecica	7.26	[9]
Scenedesmus dimorphus	49.7	[10]
Porphyridium cruemtum (seawater)	65.4	[11]
Porphyridium cruemtum (fresh water)	70.3	[12]
Padina Tetrastromatica	16.1	[12]

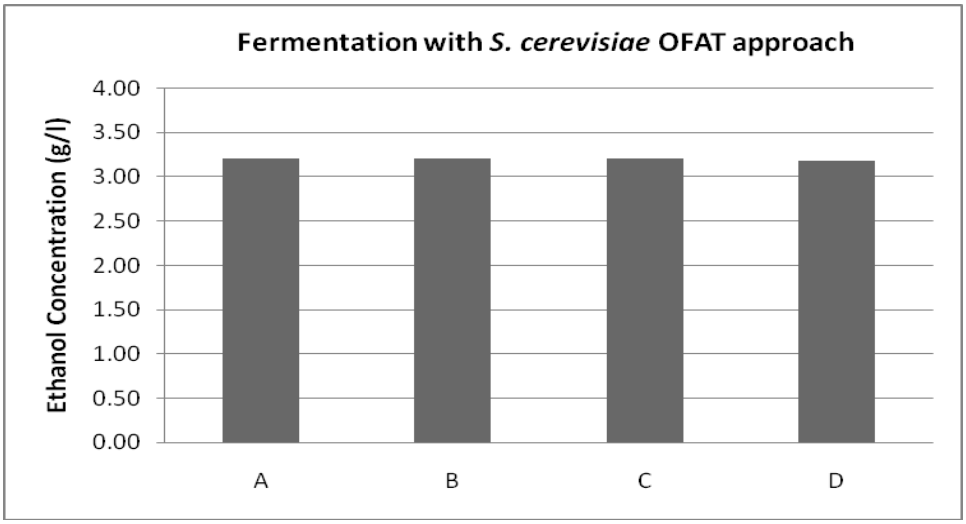
Table 1.
Yield of difference species of algae.

Figure 1: Effect of different concentration of alkali on holocellulose enrichment in Rice Husk at different time intervals

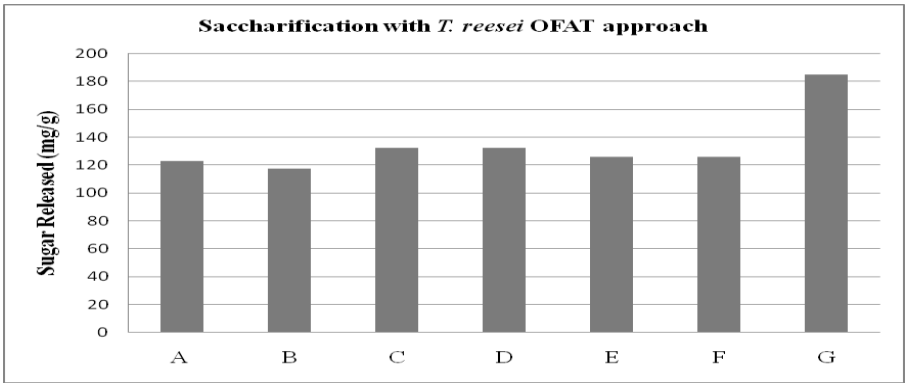
Figure 2: Effect of different concentration of Na-chlorite on holocellulose enrichment in Rice Husk at different timeinterval

	Microorganism Concentration (5ml)	Substrate Consistency(5%)	Temperature(30^oC)	AgitationRate (200 rpm)	pH (5.5)	Surfactant Concentration (1.0v/v)
Correlation coefficient (r)	0.934	0.918	0.949	0.942	0.958	0.930

(Figure 2. 1): Rice husk saccharification where A is Time (7 days), B is Substrate (5 %), C is Microorganism Concentration (5 ml), D is Temperature (30^oC), E is Agitation Rate (200 rpm), F is pH (5.5) and G Tween 80 (1% v/v



(Figure 3.0): Fermentation with *S.* cerevisiae where A is Time (6 days), B is pH (6.0), C is Temperature (30^OC), D is Concentration of Soybean meal (1.20 % (v/v))



Chlorite as shown in figures 1 and 2.

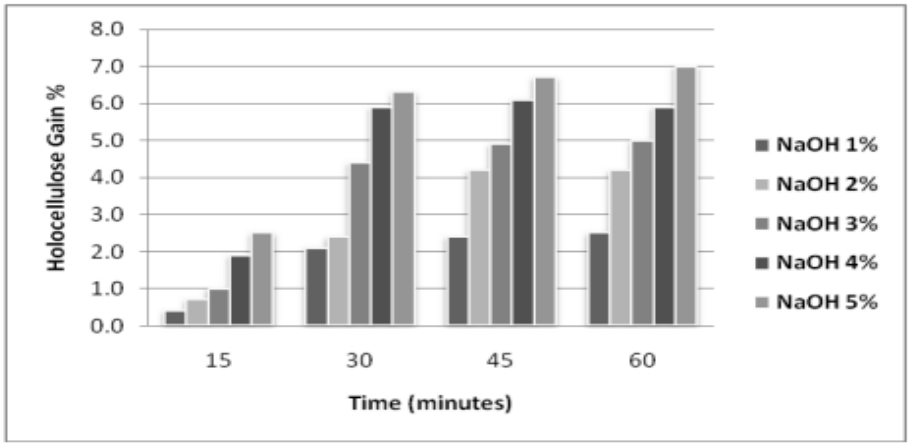


Figure 1: Effect of different concentration of alkali on holocellulose enrichment in Rice Husk at different time intervals

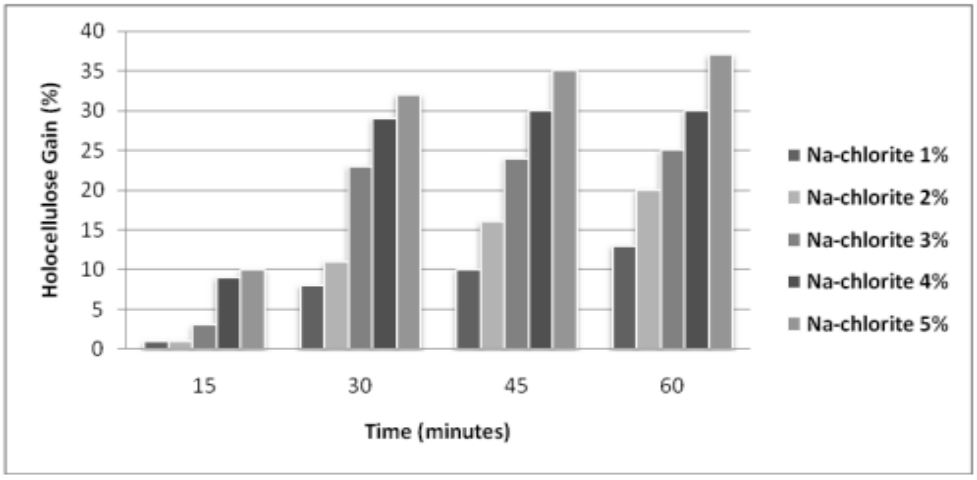


Figure 2: Effect of different concentration of Na-chlorite on holocellulose enrichment in Rice Husk at different time intervals

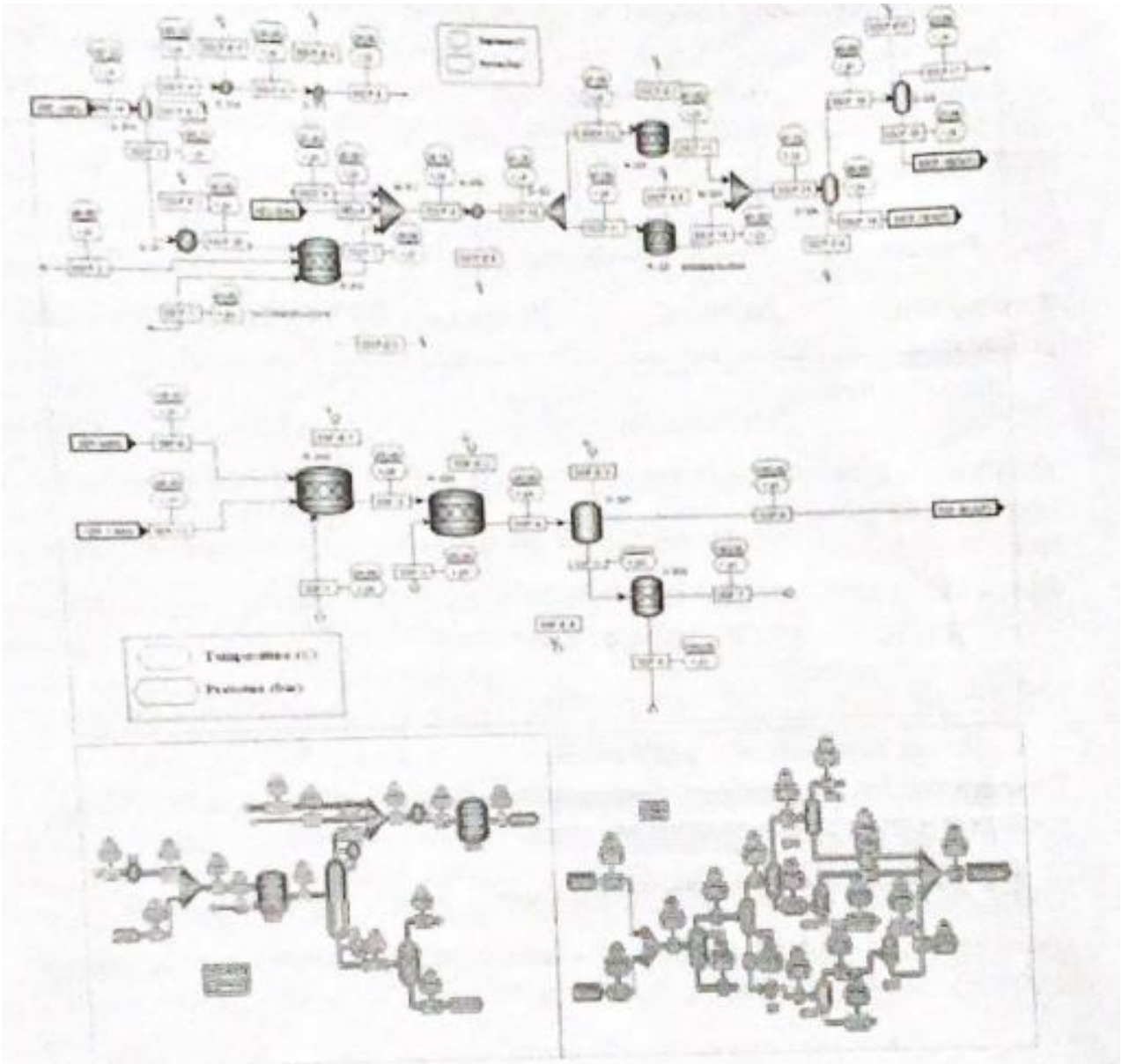
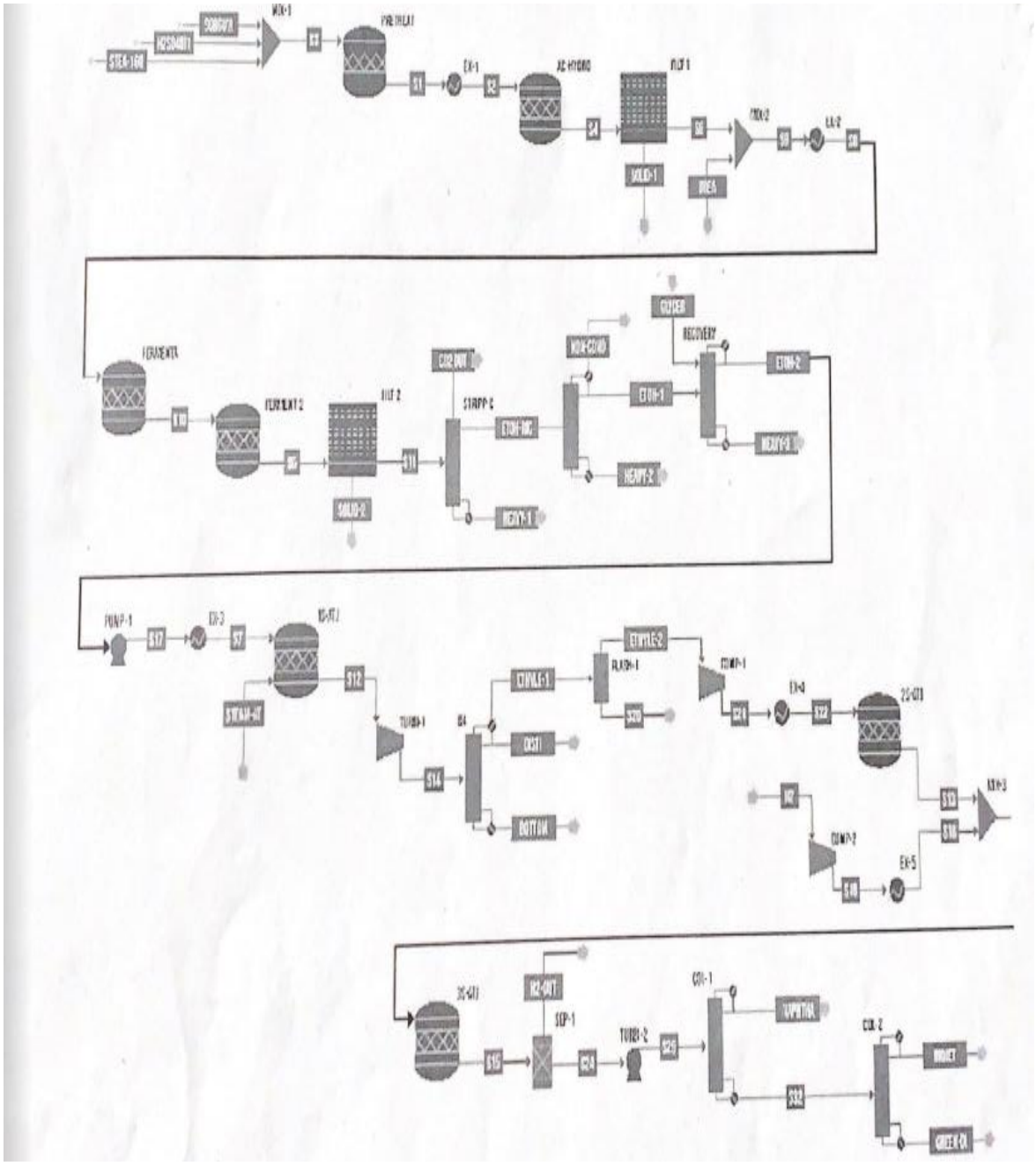


Fig. 1. Simulation of routes evaluated for microalgal bioethanol production SSCF (upper), SSF (medium) and SHF (lower).

FIGURE -1.9) SSCF BIOETHANOL COMPUTER MODELLING



FLOW DIAGRAM FOR THE COMPLETE PROCESS (FIGURE-1.8)

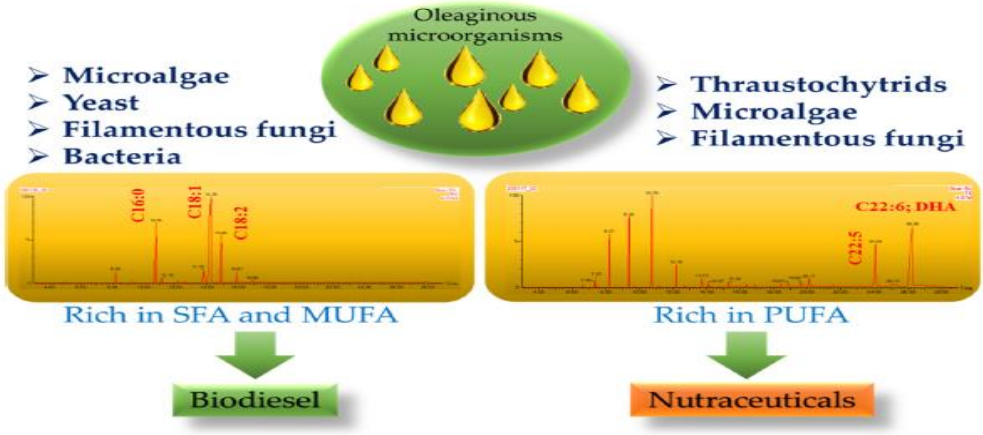


Figure 1. On the basis of the fatty acid profiles, oleaginous microorganisms can be used for biodiesel production or nutraceuticals. Some oleaginous microorganisms such as microalgae, yeast, fungi, and bacteria are rich in saturated and monounsaturated fatty acids in their oils, while some of them are a good source of polyunsaturated fatty acids such as thraustochytrids and microalgae.

(FIGURE-1)

Table 1. A list of oleaginous microorganisms cultivated on various sources and their lipid content.

Oleaginous Microorganisms	Substrates	Lipid Content (%, *w/w*)	References
	Microalgae		
Scenedesmus sp	Photoautotrophic (modified Chu 13 medium) + bubbled with simulated biogas (CO_2:CH_4 40:60)	34.10	[113]
Chlorella protothecoides	Glucose	49	[114]
Tetraselmis elliptica	Photoautotrophic (Flory medium)	14	[115]
C. vulgaris NIES-227	Heterotrophic cultivation on glucose under nitrogen limitation	89	[116]
Auxenochlorella protothecoides	Organosolv pretreated birch biomass hydrolysates	66	[71]
Auxenochlorella protothecoides	Organosolv pretreated spruce biomass hydrolysates	63	[71]
Botryococcus braunii	Photoautotrophic (modified Chu 13 medium)	28	[117]
Chlamydomonas reinhardtii, CC1010	Photoheterotrophic (TAP^{N-} + 0.1% glucose)	59	[118]
	Yeast and filamentous fungi		
Cryptococcus sp. (KCTC 27583)	Pretreated banana peel	34	[119]
Rhodosporidium kratochvilovae HIMPA1	Cassia fistula L. fruit pulp	53.18	[42]
	Hemp seed aqueous extract	55.56	[44]
	Phenol 1 g/L + Glucose (7%)	64.92	[120]
	Hydrophobic waste (clarified butter sediment waste medium	70.74	[7]
Trichosporon fermentans CICC 1368	pre-treated waste sweet potato vines under simultaneous saccharification and fermentation (SSF)	36	[121]
Rhodosporidium toruloides	Brewers' spent grain	56	[45]
Lipomyces starkeyi	Xylose and glucose	48	[122]
Rhodotorula glutinis	Monosodium glutamate with glucose	20	[123]
Cryptococcus curvatus	Waste cooking oil	70	[124]
	Glucose	53	
Lipomyces starkeyi CBS 1807	Sweet sorghum stalks juice	30	[82]
Fusarium oxysporum	Sweet sorghum stalks (12% *w/w* solid load)	22	[125]
	Glucose	42	
	Fructose	26	
	Sucrose	49	
	Glucose, fructose and sucrose mixture	53	
Fusarium equiseti UMN-1	Glucose	56	[126]
Sarocladium kiliense ADH17	Glucose and glycerol	33	[127]
Mortierella alpina LP M 301	Glucose with potassium nitrate	31	[103]
Microsphaeropsis sp.	Corncob waste liquor	22	[96]

(TAB-7.0)

Table 2. A list of oleaginous microorganisms with their EPA and DHA content.

Oleaginous Microorganisms	Substrate	DHA Concentration (%, Total Lipid)	EPA Concentration (%, Total Lipid)	References
Thraustochytrids				
Aurantiochytrium sp. ATCC PRA-276	Glucose (30 g/L)	5.5	-	[185]
		12.5	-	
Aurantiochytrium sp. KRS101	Orange peel extract glucose (5.9 g/L), fructose (5.6 g/L), organic acids	14.31	-	[186]
	5 g/L glucose + orange peel extract glucose (5.9 g/L), fructose (5.6 g/L), organic acids	14.18	-	
Aurantiochytrium sp. KRS101	Modified basal medium glucose (60 g/L)	19.88	-	[187]
Schizochytrium limacinum SR 21	Glucose (90 g/L)	14.72	-	[188]
	Glycerol (100 g/L)	18.38	-	
Aurantiochytrium 4W-1b	Glucose (30 g/L)	27.9	-	[189]
Aurantiochytrium SW1	Fructose (70 g/L)	25	-	[190]
Aurantiochytrium sp. YLH70	High-fructose corn syrup	46.3	-	[191]
Schizochytrium limacinum SR21	Organosolv-pretreated spruce hydrolysate (60 g/L glucose)	66.72	-	[192]
Aurantiochytrium sp. ATCC PRA-276	Organosolv-pretreated birch hydrolysate (30 g/L glucose)	35.76	-	[23]

(TAB-7.1)

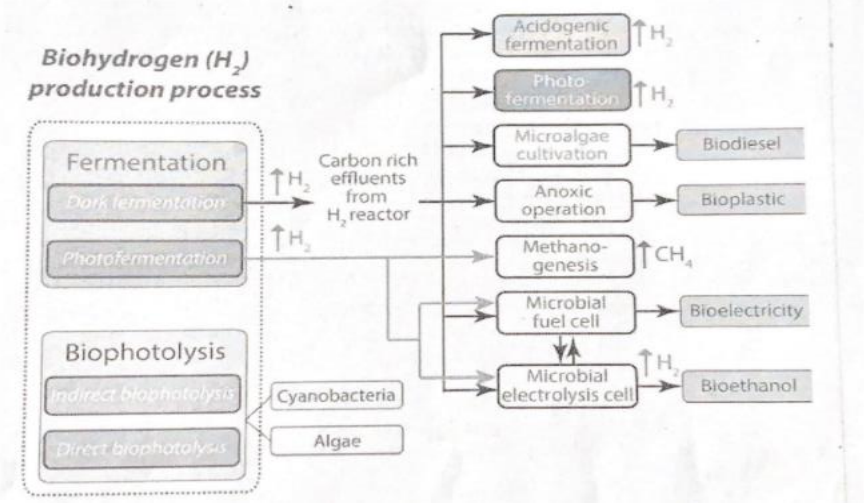


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Figure 3

Schematic representation of the primary biological routes integrated with various secondary processes for effective H_2 production.

TAG-8.0 H2 PRODUCING CATALYSTS

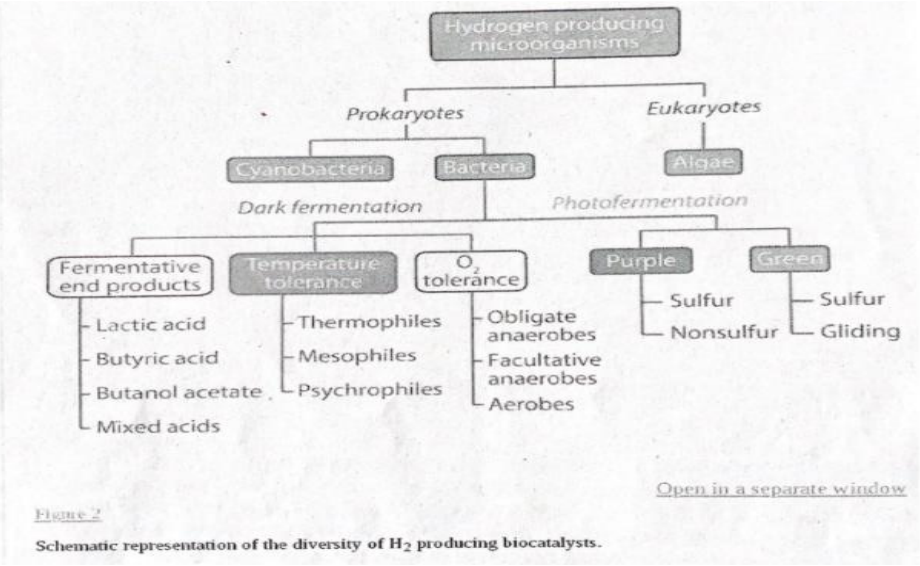

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Figure 2

Schematic representation of the diversity of H_2 producing biocatalysts.

SECONDARY PROCESS FOR H2 FIG-9.2

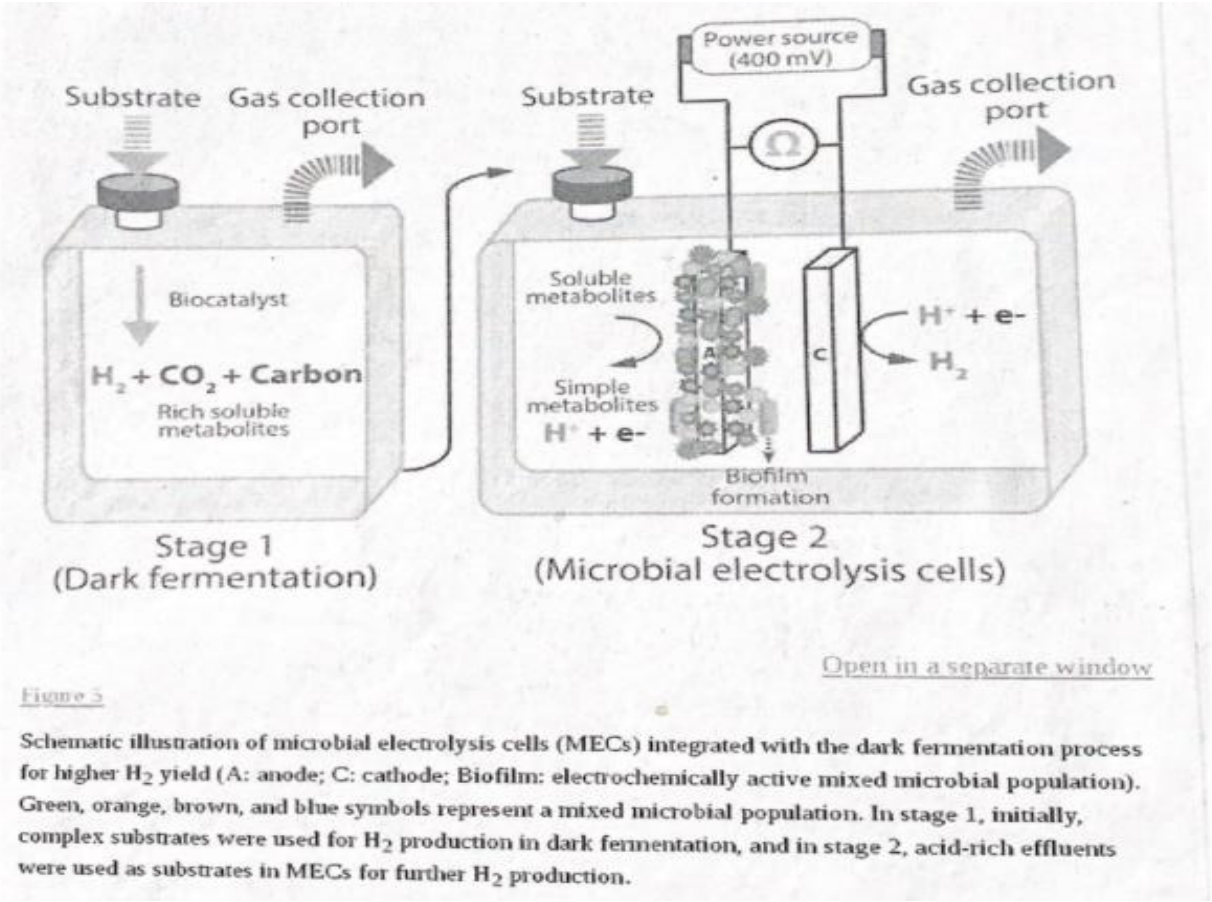

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Figure 3

Schematic illustration of microbial electrolysis cells (MECs) integrated with the dark fermentation process for higher H_2 yield (A: anode; C: cathode; Biofilm: electrochemically active mixed microbial population). Green, orange, brown, and blue symbols represent a mixed microbial population. In stage 1, initially, complex substrates were used for H_2 production in dark fermentation, and in stage 2, acid-rich effluents were used as substrates in MECs for further H_2 production.

Integrated Approaches on Dark fermentation with MEC Fig.9.1

Table 1

Biological pathways for H_2 production and the technical limitations.

Type of Bioprocess	Technical Challenges
Dark fermentation	• low substrate conversion efficiency • low H_2 yield • thermodynamic limitations • mixture of H_2 and CO_2 gases as products, which require separation
Photofermentation	• requirement of an external light source • the process is limited by day and night cycles, with sunlight as the light source • low H_2 yield caused by extremely low light conversion efficiency
Direct biophotolysis	• O_2 generation caused by the activity of PS II • requirement for customized photobioreactors • low H_2 yield caused by extremely low light conversion efficiency
Indirect biophotolysis	• lower H_2 yield caused by hydrogenase(s) • requirement of an external light source • total light conversion efficiency was very low

FIGI

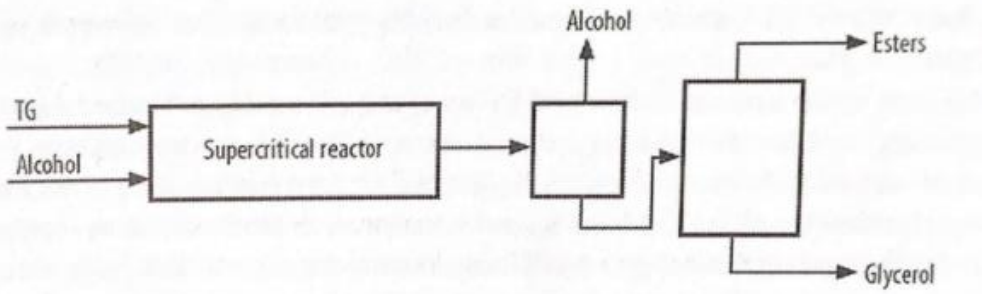


FIGURE 20.7

Supercritical esterification process.

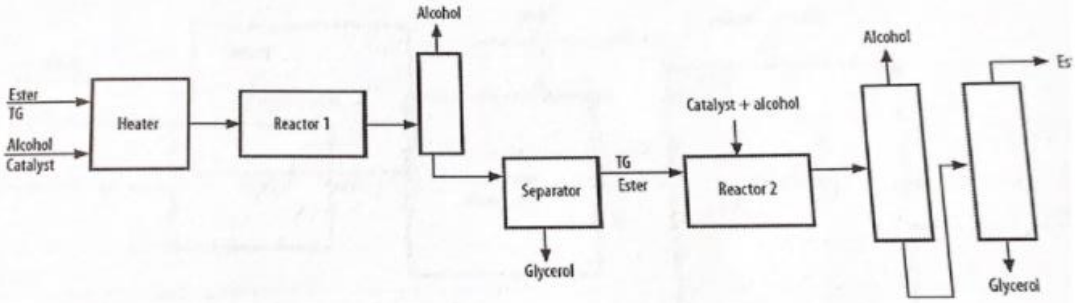


FIGURE 20.2

Plug flow reaction system.

Financial calculations for SvL to get the net cash flow for the project

Fixed investments			
Machinery			1,117,218,789
Buildings			302,781,211
Total investment			**1,420,000,000**
Operation Costs	**Units**	**SEK/unit**	**Total cost**
Cereals kg	590,000,000	1	590,000,000
Labour (36 employees *40h/week*46 week/year)	66,240	300	19,872,000
Chemicals, enzymes, yeast			28,044,710
Electricity KWh (fermentation & distillation)	39,583,601	0.622	24,621,000
Electricity (animal feed)	35,600,000	0.622	22,143,200
Steam Process MWh (fermentation & distillation)	293,800,000	0.13	37,500,000
Steam (animal feed)	293,100,000	0.13	37,600,000
Water total m^3 supply of fresh water	529,230	4.9	2,593,227
Treatment of wastewater	105,846	9.8	1,037,291
Labour cost transportation (feed and bioethanol)	154,323	180	27,778,140
Cost for diesel for transportation	914,000	9.3	8,481,920
Maintenance building and machinery (6%)			85,200,000
Various costs e.g. insurance			44,243,574
Total operation costs			**929,115,062**
Revenues	**Units**	**SEK/unit**	**Total revenues**
Bioethanol 220 000 m^3	220,000,000	5.5	1,210,000,000
Feed	180,000,000	1	180,000,000
Total revenues			**1,390,000,000**
Net cash flow (revenues-costs)			**460,884,938**

Table 4.2 The estimated total operation costs, revenues and fixed investment in buildings and machinery for SvL at market prices.

The Swedish University of Agricultural Sciences, Johanna Larsson, 2007

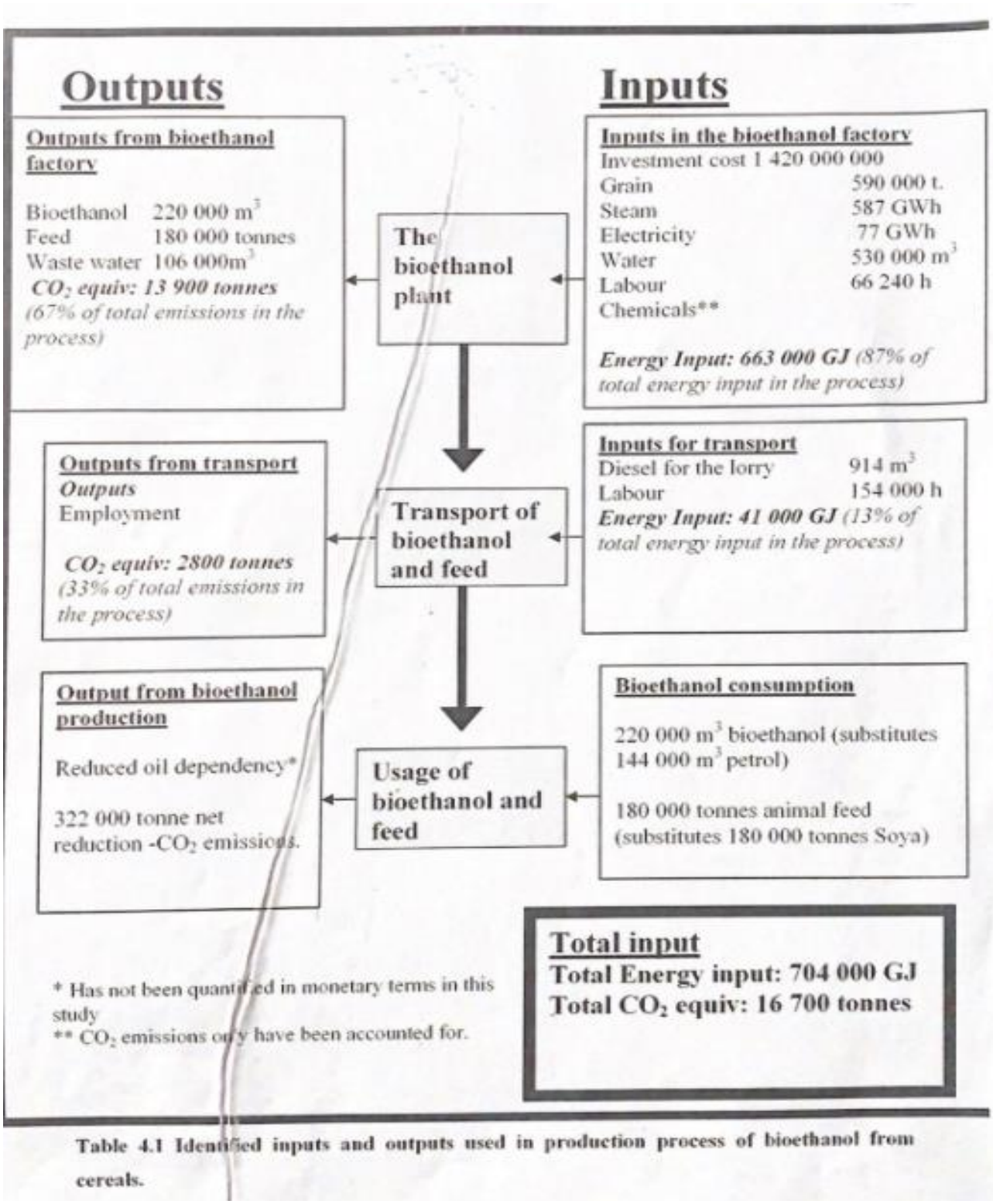
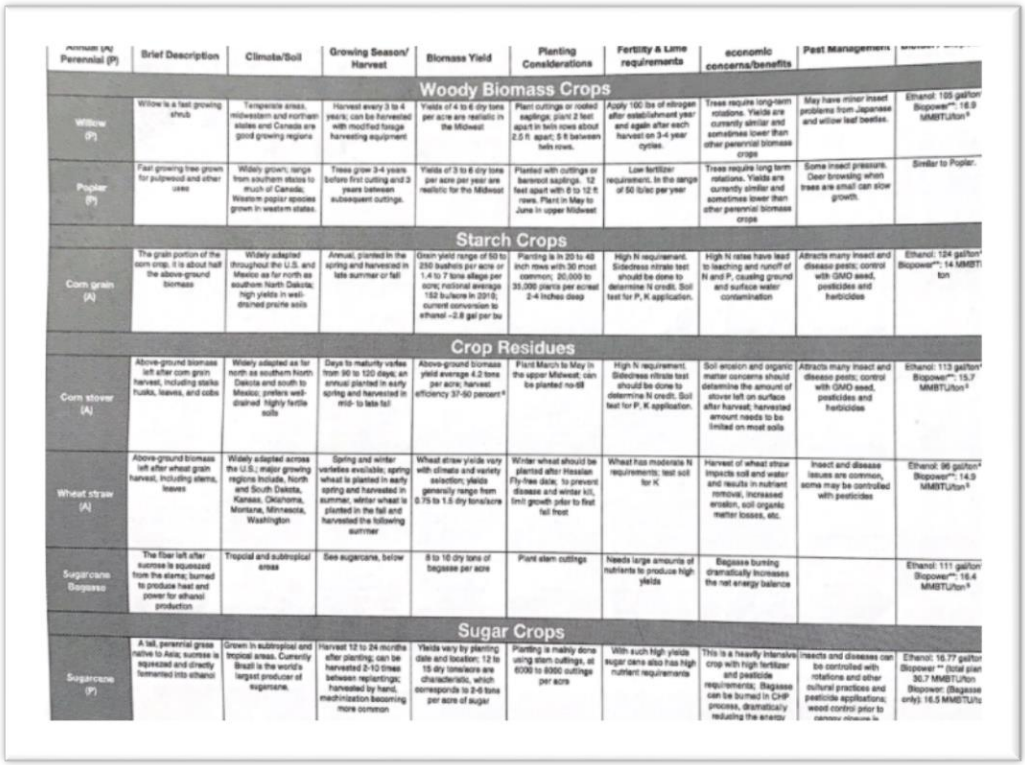


Table 4.1 Identified inputs and outputs used in production process of bioethanol from cereals.

Annual (A) Perennial (P)	Brief Description	Climate/Soil	Growing Season/ Harvest	Biomass Yield	Planting Considerations	Fertility & Lime requirements	Environmental and economic concerns/benefits	Pest Management	Biofuel / Biopower
Woody Biomass Crops									
Willow (P)	Willow is a fast growing shrub	Temperate areas, midwestern and northern states and Canada are good growing regions	Harvest every 3 to 4 years; can be harvested with modified forage harvesting equipment	Yields of 4 to 6 dry tons per acre are realistic in the Midwest	Plant cuttings or rooted saplings; plant 2 feet apart in twin rows about 2.5 ft apart; 5 ft between twin rows.	Apply 100 lbs of nitrogen after establishment year and again after each harvest on 3-4 year cycles.	Trees require long-term rotations. Yields are currently similar and sometimes lower than other perennial biomass crops	May have minor insect problems from Japanese and willow leaf beetles.	Ethanol: 105 gal/ton[2] Biopower**: 16.9 MMBTU/ton[3]
Poplar (P)	Fast growing tree grown for pulpwood and other uses	Widely grown; range from southern states to much of Canada; Western poplar species grown in western states.	Trees grow 3-4 years before first cutting and 3 years between subsequent cuttings.	Yields of 3 to 6 dry tons per acre per year are realistic for the Midwest	Planted with cuttings or bareroot saplings. 12 feet apart with 8 to 12 ft rows. Plant in May to June in upper Midwest	Low fertilizer requirement. In the range of 50 lb/ac per year	Trees require long term rotations. Yields are currently similar and sometimes lower than other perennial biomass crops	Some insect pressure. Deer browsing when trees are small can slow growth.	Similar to Poplar.
Starch Crops									
Corn grain (A)	The grain portion of the corn crop. It is about half the above-ground biomass	Widely adapted throughout the U.S. and Mexico as far north as southern North Dakota; high yields in well-drained prairie soils	Annual, planted in the spring and harvested in late summer or fall	Grain yield range of 50 to 250 bushels per acre or 1.4 to 7 tons silage per acre; national average 152 bu/acre in 2010; current conversion to ethanol ~2.8 gal per bu	Planting is in 20 to 40 inch rows with 30 most common; 20,000 to 35,000 plants per acre at 2-4 inches deep	High N requirement. Sidedress nitrate test should be done to determine N credit. Soil test for P, K application.	High N rates have lead to leaching and runoff of N and P, causing ground and surface water contamination	Attracts many insect and disease pests; control with GMO seed, pesticides and herbicides	Ethanol: 124 gal/ton[4] Biopower**: 14 MMBTU/ton
Crop Residues									
Corn stover (A)	Above-ground biomass left after corn grain harvest, including stalks husks, leaves, and cobs	Widely adapted as for north as southern North Dakota and south to Mexico; prefers well-drained highly fertile soils	Days to maturity varies from 90 to 120 days; an annual planted in early spring and harvested in mid- to late fall	Above-ground biomass yield average 4.2 tons per acre; harvest efficiency 37-50 percent[5]	Plant March to May in the upper Midwest; can be planted no-till	High N requirement. Sidedress nitrate test should be done to determine N credit. Soil test for P, K application.	Soil erosion and organic matter concerns should determine the amount of stover left on surface after harvest; harvested amount needs to be limited on most soils	Attracts many insect and disease pests; control with GMO seed, pesticides and herbicides	Ethanol: 113 gal/ton[4] Biopower**: 15.7 MMBTU/ton[3]
Wheat straw (A)	Above-ground biomass left after wheat grain harvest, including stems, leaves	Widely adapted across the U.S.; major growing regions include, North and South Dakota, Kansas, Oklahoma, Montana, Minnesota, Washington	Spring and winter varieties available; spring wheat is planted in early spring and harvested in summer; winter wheat is planted in the fall and harvested the following summer	Wheat straw yields vary with climate and variety selection; yields generally range from 0.75 to 1.5 dry tons/acre	Winter wheat should be planted after Hessian Fly-free date; to prevent disease and winter kill, limit growth prior to first fall frost	Wheat has moderate N requirements; test soil for K	Harvest of wheat straw impacts soil and water and results in nutrient removal, increased erosion, soil organic matter losses, etc.	Insect and disease issues are common, some may be controlled with pesticides	Ethanol: 96 gal/ton[4] Biopower**: 14.9 MMBTU/ton[3]
Sugarcane Bagasse	The fiber left after sucrose is squeezed from the stems; burned to produce heat and power for ethanol production	Tropical and subtropical areas	See sugarcane, below	8 to 16 dry tons of bagasse per acre	Plant stem cuttings	Needs large amounts of nutrients to produce high yields	Bagasse burning dramatically increases the net energy balance		Ethanol: 111 gal/ton[4] Biopower**: 16.4 MMBTU/ton[3]
Sugar Crops									
Sugarcane (P)	A tall, perennial grass native to Asia; sucrose is squeezed and directly fermented into ethanol	Grown in subtropical and tropical areas. Currently Brazil is the world's largest producer of sugarcane.	Harvest 12 to 24 months after planting; can be harvested 2-10 times between replantings; harvested by hand, mechinization becoming more common	Yields vary by planting date and location; 12 to 15 dry tons/acre are characteristic, which corresponds to 2-6 tons per acre of sugar	Planting is mainly done using stem cuttings, at 6000 to 8000 cuttings per acre	With such high yields sugar cane also has high nutrient requirements	This is a heavily intensive crop with high fertilizer and pesticide requirements; Bagasse can be burned in CHP process, dramatically reducing the energy	Insects and diseases can be controlled with rotations and other cultural practices and pesticide applications; weed control prior to canopy closure is	Ethanol: 16.77 gal/ton[1] Biopower ** (total plant): 30.7 MMBTU/ton Biopower: (Bagasse only): 16.5 MMBTU/ton



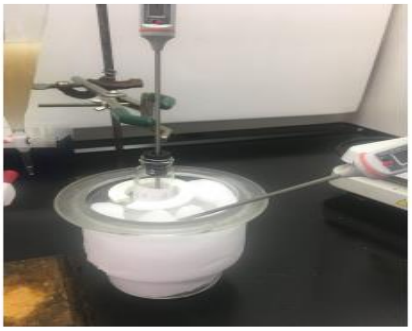


Figure 9: Cooling bath used to conduct cloud and pour point tests on fuel samples

Figure 10: Biodiesel refinery apparatus used to recover excess alcohol and purify esters

Figure 5: Heating of vegetable oil prior to the addition of alcohol

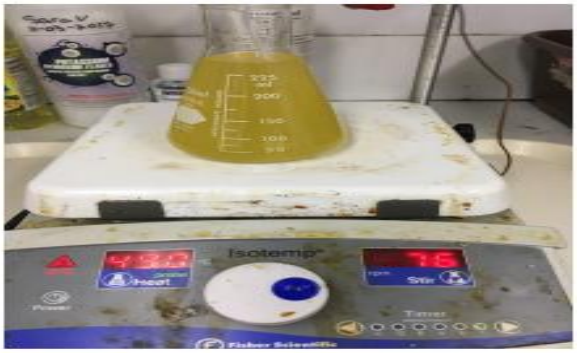


Figure 6: first stage transesterification process of methanol based biodiesel

Figure 7: Second stage transesterification separation of methyl esters and glycerol

Figure 13: Saponification of 2-butanol with vegetable oil and KOH

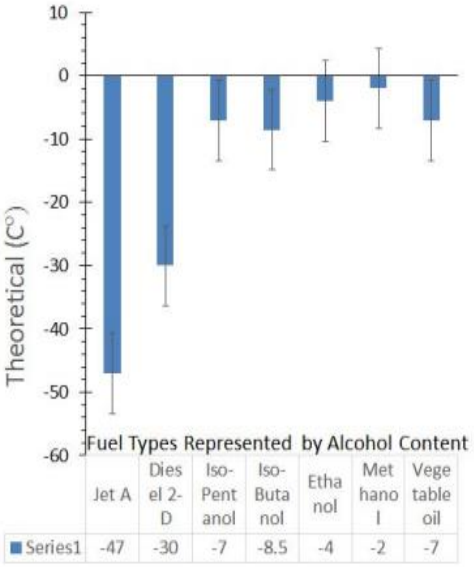


Figure 12: Theoretical Pour Points for Various Fuels

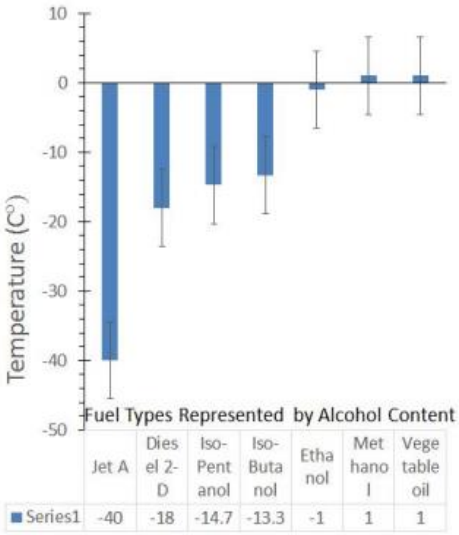


Figure 11:Theoretical cloud points for various fuels

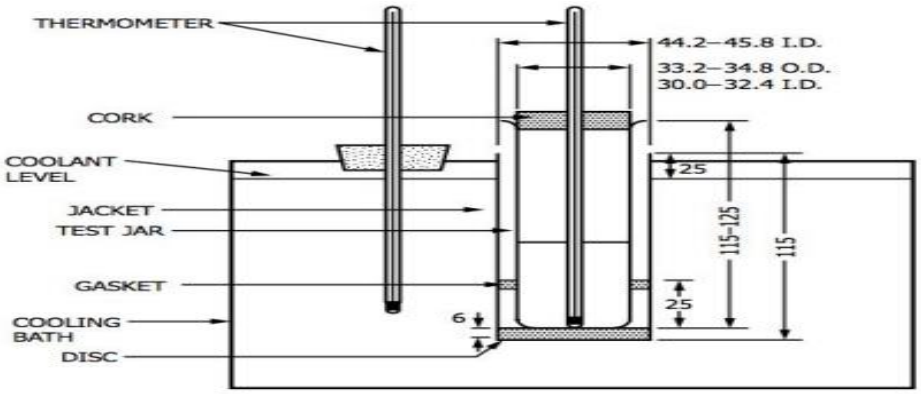


Figure 8: ASTM D5551 apparatus schematic for cloud point test methods

	NaOH	KOH
Price (US$/ton)	400	770

Table 1: Comparison of different catalysts prices in dollars over ton

	Ester Yield wt%	Product Yield wt%
NaOH	94.0	85.3
KOH	92.5	86.0

Table 2: Comparison of different catalysts used in the transesterification of UFO (temperature of 70 °C, reaction time of 30 min, methanol/oil molar ratio of 7.5:1)

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