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BIOETHANOL PRODUCTION FROM NATURAL SOURCES: A REVIEW

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ABSTRACT **Background:** Bioethanol has been point of attention as the world starts to shift towards the greener aspect of energy source. In present scenario production of bioethanol utilizing natural crop and agricultural waste becomes researcher's desire. Low production cost and high extraction efficiency are now need of the time, especially to obtain 4G bioethanol. Now the field of Genetic engineering requires more devotion towards the genetic manupulation of micro-organism or metabolic engineering of sugar hydrolysing enzyme to ease way more cheaper discovery of fourth generation bioethanol. This work describes the production of bioethanol from natural sources like sugarcane, molases[1G bioethanol] or from lignocellulosic material[2G bioethanol], or microalgal biomass [3G bioethanol] and discusses the procedures of bioethanol production as well as factors hindering the production of bioethanol followed by the additional pieces of information on approaches to evaluate the obtained ethanol. Now Fourth Generation Bioethanol has been selected for the researcher's topic of interest as the commercial production of it can satisfy the huge energy demand of the world with minimal environmental exploitation. **Conclusion:** The article concludes with an urge to discover De Novo technique for Bioethanol production with an appreciable yield percentage to be claimed as potential green fuel of future and it is only possible by introducing more genetically modified strain of algae for high biomass production, by genetical modification as well as metabolic engineering of micro-organism and enzyme respectively.

KEYWORDS : Bioethanol, lignocellulosic material, extraction efficiency, agricultural waste, genetic engineering, De novo technique

BACKGROUND:

Fossil fuels are the major energy source including thermal and chemical energy although it is synonymously engaged with the greenhouse effect. Intellectuals, authorities, and councils are raising eyebrows about the ever-increasing demand for fossil fuel or conventional energy sources even after witnessing the biggest pandemic of the century i.e. COVID outbreak throughout the world. The world now pushes for greener aspects to replace conventional energy sources with renewable ones. In the discussion of green fuel, automatically picture of Bioethanol becomes prominent as the research community believes it is the future fuel. Bioethanol has been considered as the potential replacement of Gasoline as bioethanol contains 35% of liquified oxygen obtained as a result of microbial fermentation of monomeric sugar from sugarcane, molasses or agriculture waste[1][2].

Bioethanol is most reliable in the transport sector and can be blended with gasoline as an octane enhancer(ethyl tertiary butyl ether (ETBE), consisting of 45 % per volume of bioethanol and 55 % per volume of isobutylene). Bioethanol can be mixed with gasoline at the volume fractions of 5, 10 and 85 % with fuel name E5-E85. Without any engine modification, 5-10% mixing of bioethanol with gasoline is possible but 85% bioethanol by volume requires the technology of a Flexible fuel vehicle (FFV) [1].

The uniqueness of bioethanol production is its substrate can be sugar cane or sugar beet or may be a lignin source, and algae also. But the most prominent dilemma in using sugarcane, corn or wheat as a source of first-generation (1G) bioethanol production is their immense dominance in African peninsula as primary food material[5]. Comparatively, second-generation(2G) bioethanol i.e. lignoethanol is easy to procure as switch grass, corn stalk, wood, herbaceous crop, waste paper etc can be used as source material. But another additional problem is the processing cost or production through fermentation remains a potential challenge for the researcher. The sole difference between first-generation and second-generation bioethanol is the different source of sugars. In second-generation bioethanol production, different woody cellulosic or hemicellulosic material leads to the lignoethanol by the process of **S**imultaneous **S**accharification and Fermentation (SSF) of resulting sugar at optimal temperature. Additionally, the cheaper nature of the substrate material allows them to be a better choice of feedstock unlike the source material of first-generation (1G) bioethanol as there is close competition between use as food or feed. In second-generation bioethanol production, the source is reported to emit out lesser percentage of greenhouse gases as compared to first-generation bioethanol production [2].

Still, the challenge remains active as lignocellulose is tough to deconstruct into its respective constituent sugars. The biomass requires to be extensively pretreated in order to remove the lignin and subsequently, the cellulose and hemicellulose are hydrolyzed to fermentable sugars. Despite easy availability as feed stock costly pretreatment process keeps it devoid of rational production of 2G bioethanol. Moreover, the seasonal variation in woody mass and quality of cellulose and hemicellulose material results in fluctuations in the pretreatment process as no such universal pretreatment process exists for the pretreatment of lignocellulosic material. It further deviates the yield percentage as well as performance of production operations.

Whereas, commercialization of 2G bioethanol does require a cost-friendly as well as minimal energy resource-requiring process considering the environmental aspect as well. The problems of high pretreatment procedural cost of 2G bioethanol production can be minimized by adopting algae as feedstock in the production of third-generation bioethanol(3G) as the traceable lignin percentage is nearly zero[2]. So, the destruction and isolation of lignin substances through the pretreatment process no longer remain a necessity. Due to the abundant cultivation and plenty of presence of carbohydrate content in the cell wall of algae, world has adopted the alage as feedstock for the production of 3G bioethanol. Among the different algal species some microalgae (unicellular) as well as macroalgae(multicellular) have been chosen as first-line feedstock based on their appreciable yield percentage of bioethanol production including Chlorococcum infusionum, Chlamydomonas reinhardtii UTEX 90, Chlorella vulgaris among the microalgae and Gelidium elegans, Gracilaria salicornia, Ulva pertusa, Sargassum fulvellum, Undaria pinnitifida, Alaria crassifolia etc among macroalgae. Detailed discussion on algal classes used as feedstock is discussed in table-1 [6].

Table1: Species of Algae used in the 3G bioethanol production [6]

Class of Algae based on number of cell	Species
Micro algae	Chlorococcum infusionum, Chlamydomonas reinhardtii UTEX 90, Chlorella vulgaris

Macro algae	Green: Ulva lactuca, Ulva pertusa
	Red: Kapphaphycus alvarezii, Gelidium amansii, Gelidium elegans, Gracilaria salicornia
	Brown: Laminaria japonica, Laminaria hyperborean, Saccharina latissima, Sargassum fulvellum, Undaria pinnitifida, Alaria crassifolia

The algae feedstock undergoes acid or enzymatic hydrolysis followed by Simultaneous saccharification and fermentation(SSF) or separated hydrolysis and fermentation (SHF) to obtain 3G bioethanol with high yield percentage. The algae feedstock can be hydrolyzed by different methods like acid hydrolysis, and enzymatic hydrolysis. The acid hydrolysis helps to rupture all the bonds between polysaccharide chains and makes it susceptible to be hydrolyzed further to monosaccharide molecules[6].

In enzymatic hydrolysis most employed enzyme is cellulase, further categorized into three subclasses namingly endoglucanases, exoglucanases and β- glucosidases. The common goal of all the three enzyme family is to convert the complex sugars into simple sugar unit or monosaccharides eg. Glucose[8]. After hydrolysis is over, simple sugar undergoes fermentation to result bioethanol by the action of microorganism like Eschericia coli, Saccharomyces cerevisiae, Zymomonas mobili etc.

With the advancement of science, progression also happens in bioethanol production technology, and the genetic modification in algae increases benefit to production cost ratio. The main difference between third-generation (3G) and fourth-generation (4G) biofuel production is that the latter use "cell factory" concept. In 4G biofuel technology algae or phototropic cyanobacterium is driven by the sunlight for the production of bioethanol or biofuel, using CO_2 as source material. Most unique characteristic of the 4G bioethanol production is devoid of essentiality of costly processes like pretreatment and fermentation as the end product will be automatically secreted out without requirement of these two mentioned techniques. So the overall step involved in the biofuel production is also less, so is the production cost. Researchers also find additional interest due to its environmental aspects as the micro-organism minimizes the concentration of emitted CO_2 in the air by availing them as feedstock [9].

Table2: Comparison summary on production of bioethanol of different generation [6]

SL NO	Parameter	First Generation	Second Generation	Third Generation	Reference
1	Resources to be used as feedstock	Edible crops	Non-edible crops (lignocellulosic, forest residues)	Algal biomass	[1],[10],[13],[14],[15]
2	Food stock *vs* feedstock usage competition	Very much dominant as sugarcane or sugar beet is main source of production	Not so much significant as lignocellulose is used as feed	Not significant as algal source is used	
3	Requirements for cultivation land	Grows on arable land	Grows on arable and marginal land	Seawater, freshwater, wastewater	
4	Methodology of processing	Sugar extraction, fermentation, distillation	Pre-treatment, hydrolysis, fermentation, distillation	Hydrolysis, fermentation, distillation	
5	Yield capacity	Low	Medium	High	
6	Environmental aspect	Low contribution to the mitigation of CO_2	High contribution to the mitigation of CO_2	High contribution to the mitigation of CO_2	
7	Features	Relatively simple conversion process	No competition with food resources	High growth rate	
8	Limitations	"Food vs fuel" debate	Recalcitrant structures of the feedstock	Limited investments and difficulties in process design	

Raw materials for the production of Bioethanol of different generations:

Raw materials for production of 1G Bioethanol:

Different types of biomass has significant potential for the production of bioethanol e.g: sugarcane, sugarbeet, corn, wheat, molasses. But the problem is the competition between their use as food or feed material especially the dominance of sugarcane and wheat as primary food material in the African continent. Although sugarcane as raw material provides certain advantages like it is non-expensive, and does not require pretreatmental procedures. Sugar syrup and granulated sugar are also another substrates used as the feedstock of bioethanol production throughout whole year, and molasses, one of the bulk byproduct of sugar industry can be utilized by the yeast as substrate to extract bioethanol[1].

Raw material containing starch:

Grain crops like barley, wheat and root tuber crops like cassava, potato can be used as raw material as they are large reservoir of starch. Starch is basically mixture of polyglucans i.e. amylose(linear chain) and amylopectin(branched chain). The hydrolysis of starch is done by the action of α-amylase(obtained from genetically modified strain of Escherichia coli and bacillus subtilis)[15,16]. The isolated starch further bioprocessed to derive bioprocessed materials or biofuel. In USA corn starch alone is the 95% bioethanol producer, whereas barley, wheat, whey, beverage residue make up the rest percentage[11]. Cassava tuber contains almost 80% starch(mass percentage) and is a potential source for production of 1G bioethanol. Pretreatment of cassava tuber for bioethanol production involves steps like cleaning, peeling, chipping, drying, and then processed for bioethanol production.

Raw material containing sugar:

Generally, sugarcane and sugar beet are considered the most sugar-containing crops in the world as the sugarcane itself satisfies the $2/3^{rd}$ of the world's total sugar requirements whereas sugarbeet fulfills the rest $1/3^{rd}$ portion[1][17]. The most amazing matter is that sugar-containing raw materials do not involve any pretreatment procedures and can be easily hydrolyzed by the Saccharomyces species utilizing enzyme invertase[18]. Only a single extra step required to extract sugar from sugarcane or sugarbeet is milling, although waiver in pretreatment procedures allows 1G bioethanol production at a cheaper cost. Still, rational competition in being used as food stock or feedstock pushes researchers to opt for relatively less sought feedstock like lignocellulose.

Raw materials for 2G Bioethanol production:

Lignocellulose sources like crop residue(corn stover, wheat straw, rice hulls), cellulose waste(paper pulp), herbaceous biomass(alfa-alfa hay, switchgrass) are better choice as feedstock due to their easy worldwide availability as well as no competence as food-stock. Lignocellulosic material contains on an average 43% cellulose, 27% lignin, 20% hemicellulose and 10% other components[1]. A thorough gravimetric analysis suggests the mass fraction of lignin, cellulose, hemicellulose in wheat straw is attention-worthy including the appreciable extraction efficiency of sugar from different parts[discussed in table-3&4].

Table3: Chemical components present in different parts of wheat straw [19]

SL NO	Component	% of Straw dry weight				Reference
		Leaf	Internode	Leaf base	Node core	
1	Lignin	15.3	14.2	14.1	16.7	[19] [20]
2	Hemicellulose	32.4	33.8	34.2	32.7	
3	Cellulose	37.7	44.8	32.7	37.5	

Pretreatment on the lignocellulosic raw material:

Lignocellulosic material has to undergo pretreatment procedures as the biomass that contains complex sugar needs to be converted into simple sugar molecules to be digested by the microorganisms. Pretreatment

procedures are divided into four major categories i.e., physical pretreatment, chemical pretreatment, Physicochemical pretreatment and biological pretreatment.

Table4: Sugar obtained from different parts of wheat straw by acid hydrolysis [20]

SL NO	Component	% of Straw dry weight				References
		Leaf	Internode	Leaf base	Node core	
1	Glucose	33.2	39.4	28.2	33.4	[20]
2	Xylose	21.1	23.8	20.4	23.0	
3	Arabinose	4.9	3.8	10.4	4.3	

Physical pretreatment methods on lignocellulosic raw materials:
Physical pretreatment methods involve milling (impact or attrition mechanism), irradiation (gamma rays, microwave) or newly adopted extrusion method where better control over shear rate and efficient mixing is also possible[21]. But in conventional pretreatment process reduction of particle size as well as obtaining optimal size of particles both make the physical pretreatment procedure a bit expensive [1].

Chemical pretreatment methods on lignocellulosic raw materials:
Chemical pretreatments include acid hydrolysis (sulphuric acid, phosphoric acid or nitric acid), alkaline hydrolysis(sodium or potassium hydrolysis or with ammonium sulphite), gaseous pretreatment (nitrogen dioxide, sulphur dioxide) or even pretreated by oxidation (Oxygen, hydrogen peroxide). Sometimes organic solvent like methanol, ethanol, ethylene glycol etc, and imidazolium ionizing liquid are also utilized in pretreatment process. Acid hydrolysis is done in purpose of solubilizing hemicellulose to enable the cellulose more digestible by the enzymes[22]. Sodium hydroxide, calcium hydroxide, ammonium sulphite induced alkaline hydrolysis is done at relatively lower temperature and pressure to solubilize lignin components[23] [21]. Organic solvent involved chemical pretreatment procedures helps in obtaining more accessible cellulose for metabolism by the different micro-organism and finally result in fermented bioethanol. But in organosolv method, accurate proportion of solvent: water needs to be maintained and precise drainage facility of the solvent residue from the reactor is an obvious requirement[24].

Physicochemical pretreatment methods on lignocellulosic raw materials: Techniques like wet oxidation, explosion, microwave destruction, liquid hot water extraction, ultrasound-assisted destruction are among the most adopted physiochemical pretreatment methods. The physicochemical pretreatment processes are cost-effective, thus followed in the industry-level production of bioethanol from lignocellulosic raw material. Different methods of physicochemical pretreatment procedures are listed below[table-5]

Biological pretreatment methods on lignocellulosic raw materials:
Biological pretreatment as the name suggests has the green edge i.e. environment-friendly method as it does not involve any chemical, additionally the energy input is much lower. In this technique diferent genera of brown, white and soft rot fungi degrade ligin and hemicellulose, although not effective in the cellulose disruption [1][33]. But in the biological pretreatment process the enzyme is required for the hydrolysis of the lignocellulosic feedstock to convert it into fermentable sugar. The cellulase enzyme is used to digest cellulose to be converted into simple sugar that can be utilized by the fungi to result bioethanol production [21].

Raw material for 3G Bioethanol production:
In the production of third-generation bioethanol algae is believed to be potential feedstock due to the easy conversion probability of biomass into energy despite the dependence of the biomass production is associated with technology as well as marine environment [6][7]. The algal biomass is preferred because of several advantageous edges it has in being feedstock for bioethanol production. Like, high growth rate obviously increases the bioethanol yield to manifold, and not so much debate over food vs feed usage of algal biomass.

Table5: Physicochemical pretreatment procedure of ligno cellulosic raw material [25]

SL	Name of the Pretreatment process	Reagents	Procedural description	Purpose	References
1	Wet oxidation	Oxygen as oxidizer	Dried and milled lignocellulosic material requi	Wet oxidation helps to fractionate the lignocellulosic	[25],[26],[27]
		Sodium carbonate	red; for 6g bio mass 1L water is added; in mixture sodium carbonate added and the pre treatment is maintained at 12 bar pressure 195^{0}c for 10-20minutes	material by solubilizing hemicellulose and removing lignin	
2	Steam explosion	Hydrothermal energy or steam	Two step process; at first step at 180^{0}c temperature hemicellulose portion is removed and then at second st age at higher tem perature (210^{0}c) cellulose is broken into carbohy drate linkage	Cost effective method to obtain carbohydrate linkage from complex lignocellulosic structure	[25],[28],[29]
3	Ammonia Fiber Explosion[AFEX]	Liquid anhydrous ammonia	The biomass is mixed with liquid ammonia and kept on high pressure at 60^0-100^{0}c and then rapidly depressurized	This method results ammonolysis of glucuronic cross-linked bonds and partial decrystallization of cellulose structure; rapid expansion of ammonia gas leads to the swelling of biomass feedstock to disrupt lignin-carbohydrate linkage	[25],[30]
4	Super critical fluid pretreatment	Carbon dioxide at supercritical state exhibits excellent potential and with water(steam) for ms carbonic acid	Biomass is exposed to the rapidly released super critical carbon dioxide that disrupts the cellulose and hemicellulose structure; lower temperature aids the stability of the released simple sugar and prevents degradation also	The disruption of cellulose and hemicellulose provides the easy access to the enzyme to facilitate hydrolysis	[25],[31]
5	Liquid Hot Water pretreatment	Liquid hot water	The hot water at high pressure and in liquid state is intended to disintegrate and separate the lignocellulosic matrix	This method helps employs autohydrolysis and more importantly completely solubilize hemicellulose and separate it from rest of the solid matrices	[25],[32]

Biofuel production from microalgae:
The unicellular microscopic organism are generally found in marine or fresh water and notably their existence is spread out into more than 3,00,000 species which is actually much more than the plant species[34]. The microalgal source is identified as one of the potential providers of feedstock due to their rapid conversion ability of sunlight into energy sources as compared to higher plant. Additionally, as the unicellular organism grows in aqueous suspension, it can access water, carbon dioxide as well as nutrients in more efficient way[35] **[36]**. Table -6 suggests the significant worth of microalgae as biofuel source. % dry wight of oil content in microalgae can be upto 80% and the mass doubling time is too short **[36].**

Table 6: % dry weight of lipid content in different microalgae [7] [36]

Sl	Name of microalgae	% dry weight	SL	Name of microalgae	% dry weight
1	Botryococcus braunii	25-75	8	Nannocloris sp	20-35
2	Chlorella sp	28-32	9	Nannochloropsis sp	31-68
3	Crypthecodinium cohnii	20	10	Neochloris oleoabundans	35-54
4	Cylindrotheca sp	16-37	11	Nitzschia sp	45-47
5	Dunaliella primolecta	23	12	Phaeodactylum tricomutum	20-30

6	Isochrysis sp	25-33	13	Schizochytrium sp	50-77
7	Monallanthus salina	20	14	Tetraselmis sueica	15-23

Production of Microalgal biomass:

Although biomass production from microalgae requires a few hours only, production technology is expensive indeed. The growth of microalgae requires adequate light, carbon dioxide and nutrients; temperature needs to be controlled in efficient manner as the regime should be between 20^0-30^0c. To reduce the cost of biomass production, process needs to rely on natural sunlight, and the nutrient supply remains continued by cultivating the freshwater-microalgal habitat in animal wastewater, industrial wastewater or municipal wastewater[37]. In case of harbouring marine microalgae the sea water is fortified with commercial phosphate, nitrate salts and some other micronutrients required for the growth[38]

Fourth Generation Biofuel production:

Third-generation bioethanol or biofuel is obtained from the algal biomass, but it has some limitations with costly production and efficient harbouring requirements. In the production of fourth-generation biofuel, the genetically modified algae[GM algae] is utilized for the enhanced biofuel production. The genetical modification is done to improve photosynthetic efficiency, enhance sunlight penetration into dense microalgae culture in object to use the truncation chlorophyll antena of chlororplast[39] [40]. Photoinhibition is another problem associated with algae, but through genetical modification, it can be countered[41]. In genetically modified algae the photosynthetic efficiency has been improved by the expansion of absorbing spectrum range of microalgae in photosynthesis[42]. Improvement of photosynthetic efficiency is also possible by minimizing the light absorption and manipulation of pigments besides the reduction of size of chlorophyll antenna[43]. Metabolic engineering is done to maximize the biomass of microalgae by enhancing the lipid and carbohydrate content in the cell cytoplasm[44] [45]. Some microalgal strains with specific genetic modification in Fourth Generation Biofuel production is discussed in table-7.

Table7: Description of Genetical modification in Microalgae strain [39]

Microalgae strain	Genetic modification	Culture condition	Cultivation system	Reference
Phaeodactylum tricornutum	Overexpressing heterologus genes	Mixotrophic, heterotrophic, photoautotrophic	Several closed photobioreactors and raceway pond	[46]
Chlamydomonas reinhardtii	High-lipid accumulating mutant(CC-4333)	Mixotrophic	Centrifuge tubes culture	[47]
Chlamydomonus reinhardtii	Optimizing thioesterase gene and integrating into the chloroplast genome	Photoautotrophic	Open raceway pond	[48]
Acutodesmus dimorphus	Adding enhanced fatty acid biosynthesis, and recombinant green fluorescence protein (GFP) expression	Photoautotrophic	Carboys, hanging polybags and outdoor airlifted pond	[49]
Pseudochoricystis ellipsoidea	New genus, pseudochoricystis ellipsoidea(MBIC 11204)	Mesotrophic	An outdoor raceway pond	[50]
Chlorella sorokiniana	Antenna size mutation	Photoautotrophic	A simulated outdoor microalgal raceway pond	[51]
Scenedesmus obliquus	The overexpression plasmid	Autotrophic	Tubular photobioreactor and open pond outdoor system	[52]

Procedure:

General biorefinary process involves several sequential steps including

i. Pretreatment and preparation of biomass
ii. Seperation of biomass component
iii. Fermentation
iv. Product purification. Schematic representation is shown in Figure1.

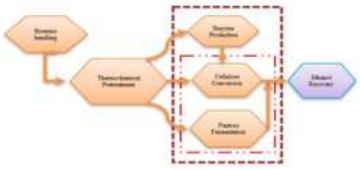

Figure1: General steps of Bioethanol production from natural biomasses [10]

First generation Bioethanol production steps:

First generation bioethanol production from starch containing raw material may involve two methods: dry grind and wet milling. Here yeast like Saccharomyces cerevisiae, Saccharomyces pastorianus etc hydrolyzes starch present in the biomass. Dry milling is widely used because of its cost effective nature and the whole biomass is milled with the help of hammer mill or roller mill followed by mixing with water. Then the watery mass is cooked in a jet cooker at 80^0-90^0c for 10-15 minutes. Secondary liquefaction is done in presence of α-amylase at 95^0c for 90 minutes. Then the mixture is cooled down at 60^0c followed by further mixing with glucoamylase for hydrolysis of sugar. The hydrolysed sugar undergoes metabolism by yeast to result bioethanol production. Generally, two methods are adopted in the hydrolysis and fermentation process i.e. **S**imultaneous **S**accharification and Fermentation[**SSF**] & **S**eperated **H**ydrolysis and **F**ermentation [**SHF**][12].

Wet milling process is more beneficial as the process adds some important co-products e.g: fibre, germ, starch and gluten before fermentation happens, the wet milling process is economically feasible also. Wet milling process needs clean, steeped, degermed corn for obtaining the germ for corn oil extraction. Subsequently, corn is defibrated to obtain fibers and gluten, also starch molecules are separated as well. Rest steps are same like the previous method, i.e. saccharification, fermentation, distillation and ethanol dehydration [53].

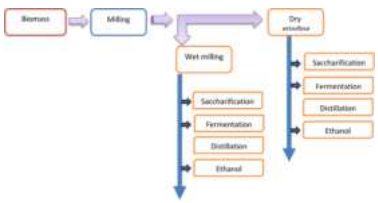

Figure2: Steps of 1G Bioethanol production from Starch containing biomasses

Second generation Bioethanol production from Lignocellulosic raw material:

Lignocellulosic biomass firstly pretreated in purpose of removal of lignin and hemicellulose components. The polymeric material is destructed by the acid, alkaline or enzymatic hydrolysis to be converted into simple sugar. Subsequently, the simple sugar molecule is fermented by yeast (Saccharomyces cerevisiae) into bioethanol and lastly, separation is done to isolate concentrated bioethanol[54] [55]. Here, we need to discuss that Saccharomyces can metabolize monosaccharides and disaccharides like Glucose, Fructose, Maltose, sucrose but not pentose sugar like Xylose or Arabinose. In purpose of metabolizing the latter one pentose metabolizing micro-organisms like Pichia stiptis, Candida shehatae etc are required after pretreatment and hydrolysis process are finished[56].

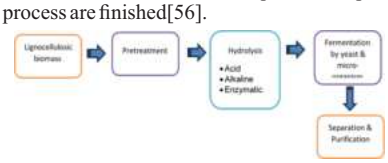

Figure3: Steps of 2G Bioethanol production from Lignocellulose containing biomasses

Third-Generation Bioethanol production from Microalgae

biomass:

Algal feedstock proves its worth to be a potential source of third-generation bioethanol production and in the extraction process thermo-chemical(air, combustion gas and CO_2) or biological method is adopted[57]. But the bioethanol or biofuel production process may vary based on the nature of algal feedstock used. The first step of the production is the drying of the crude extract obtained from the fresh microalgae in purpose of preventing gel formation of the crude extract[58][59]. The size reduction of the dried crude extract is performed to convert it into powdered form that is required for the hydrolysis step. As the size reduction increases the effective surface area, the rate of hydrolysis as well as fermentation are boosted amazingly. Hydrolysis process results the depolymerization of complex algal cell wall to expose cellular components like alginates, fucans, laminarin, carragenans and so on[60]. The hydrolysis-resulted simple sugar can be easily converted into bioethanol by introducing yeast or micro-organism in to the fermentation media and two methods of fermentation are generally followed e.g. Separated Hydrolysis and Fermentation [SHF], Simultaneous Saccharification and Fermentation[SSF][61]. The bioethanol is recovered after purification by following distillation method.

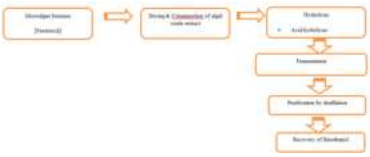

Figure4: Steps of 3G Bioethanol production from Microalgae biomass[6]

Fourth Generation Biofuel production:

Better biofuel production from algal biomass requires the process like genetical modification so that photosynthetic efficiency is improved, sunlight penetration to the dense microalgae culture becomes more feasible, photoinhibition gets reduced, and so on. After the genetical modification is successfully executed, the GM algae are cultivated in suitable cultivation conditions like photoautotrophic, heterotrophic, mixotrophic or may be in photoheterotrophic condition. The obtained algal biomass or microalgal cake undergoes extraction process [liquefaction, torrefaction, pyrolysis] and subsequently fermented to result biofuel with an excellent yield as compared to third generation biofuel [39]

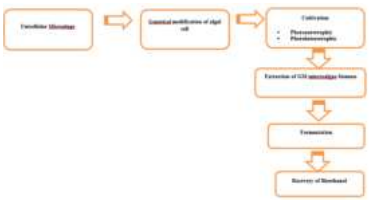

Figure4: Steps of 4G Bioethanol production from GM Microalgae [39]

Future prospect:

Biofuel production at large scale is one of the desperate needs of the time as we are on the verge of replacing conventional fossil fuel considering the immense negative impact of the latter, so the fourth generation biofuel[FGB] has been accepted as the hopeful sunshine for the researchers. The promising future of biofuel production purely depends on the genetic and metabolic engineering of sugar metabolising micro-organisms as well as genetical engineering of non-food crops to enhance the production of biomass at a gigantic scale. Some sort of effort is also focused on the reduction of biomass production cost as well as finding cost effective feedstock destruction methods. Metabolic engineering on hydrolysing enzymes can also miniaturize the baggage of conversion cost[5]. For the advancement on the GM algae assisted biofuel production commercialization of cultivation is very much needed, but the commercial cultivation of GM algae is closely associated with risk of deliberate and unintended release of modified strain in the environment. Researcher community is focused on the enhancement of algal strain development through genetic modification of algae. But some challenges have become prominent with the emergence of hope like the risk of the introgression of GMO strains into natural environment, ample investment dedicated to the research, high operational cost of operating photobioreceptor. So, research institutes must focus on the cost effective commercial production of FGB and should concentrate their study pattern on designing of photobioreactor as well as genetic modification strategies[39].

CONCLUSION:

As the time is to bid bye to the conventional fossil fuel and to welcome highly promising bioethanol or biofuel, the world identifies the GM algal biomass as the most acceptable feedstock for biofuel production. Now biofuel dedicated researchers are concerned about the continual improvement of methodologies to enhance the yield of biofuel from GM algal biomass. In a certain context, Fourth generation biofuel or FGB is now the topic of interest of world and continuous effort is made on the cost-effective commercialization of FGB while minimizing the harmful environmental impact.

Abbreviation:

1G = First generation
2G = Second generation
3G = Third generation
4G = Fourth generation
FFV = Flexible Fuel Vehicle
FGB = Fourth generation biofuel
GM = Genetically modified
SSF = Simultaneous Saccharification and Fermentation

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