



Role of Algae in Bioethanol Production: A Sustainable Biofuel Generation and Modification by Nanotechnology

Eman Tariq^a, Huma Gul^b, Kiran Fatima^{c}*

^a Women University, Swabi (23430), Pakistan

^b Women University, Swabi (23430), Pakista

^c University of Engineering and Technology, Taxila(47080), Pakistan

ABSTRACT

Global issues such as climate change, ozone layer depletion, fuel security, greenhouse effect, and economic complications demand a sustainable alternative to fossil fuels. Bioethanol derived from algal biomass revolutionized the industry and is proven as a clean, renewable, environmentally friendly, biodegradable, and economical fuel. Apart from its advantages the process still faces some shortcomings like laborious and time-consuming production processes, unstable harvesting of algae, and inconsistent production rate. This can be overcome by applying nanotechnology-based additives in the conversion process of biomass. These nanomaterials reduce the production cost and processing time, as well as their toxicity concerns can be overpowered by preparing them with biological methods. This review paper describes the background of algae along with the biofuels produced from algae. Moreover, it also sheds light on the production process of bioethanol including pretreatment, fermentation, and extraction. The authors also describe the involvement of nanomaterials in the various stages of bioethanol production. Future technologies can further work on the limitations of nanomaterials prepared from biological methods, for a more safe and effective bioethanol biofuel.

Keywords: Bioethanol, Algae, Biofuels, Nanotechnology, Biomass

Introduction:

Algae are aquatic organisms with the fastest-growing ability, and due to their photosynthetic capabilities, they possess the potential for biomass production from solar power, in large amounts (Sirajunnisa and Surendhiran, 2016; Suganya et al., 2016). They can tolerate extreme environmental circumstances including temperature, drought, turbidity, and irradiation, also they have a high productivity rate along with no land requirements (Sahoo et al., 2012). They are mainly classified as; macroalgae and microalgae, between them the former one is better because of their high carbohydrate content, increased biomass production, and uncomplicated harvesting process (Sudhakar et al., 2018). Biomass production from fossil fuels badly elevates the degree of atmospheric CO₂ which in turn enhances global warming, this leads to energy sustainability and environmental problems, also the global demand is increasing due to more energy utilization and depletion of resources (Mac Kinnon et al., 2018). It demands a better, sustainable, and economical source of energy. Algae due to its above-mentioned abilities can produce 5-10% more biomass and it is proven as an ecological, affordable, efficient, and biodegradable source (Chen et al., 2013; ElFar et al., 2021). Biofuels produced from algal biomass with properties including sustainability, ozone-friendly behavior, oil-abundant composition, and accessibility, can act as a substitute for transportation fuels that have previously been obtained from fossil fuels. Biofuel is mainly a gaseous or liquid fuel which is predominantly categorized as bioethanol, biohydrogen, and biodiesel. Biofuels derived from algae liberate minimum CO₂ in the environment along with the best production capacity rate (Bellou et al., 2014).

Among these biofuels, bioethanol is the most advantageous one due to its unique characteristics including; biodegradability, non-toxicity, renewability, high octane number, and minimal carbon dioxide and carbon monoxide release (Niphadkar et al., 2018). Due to the unique properties of ethanol, it is being tremendously produced in different countries around the globe, the highest in the US and Brazil (as of 2021). Fig1 represents the percentage of its production in different countries. In the past few years, an immense enhancement was observed in bioethanol production, from 1975 to 2010 it increased from 1 billion liters to 86 billion liters and approximately 160 billion liters by 2020 (Bibi et al., 2017). Bioethanol has multiple applications in different sectors but it has been recognized as an ideal alternative for transportation fuels such as gasoline, and as a result, it reduces the use of crude oil (Sirajunnisa & Surendhiran, 2016).

We can produce bioethanol from different biomass sources like sugar cane, starch, and lignocellulose, but due to its limited yield, uneconomic behavior, and inefficient conduct, they are replaced by algae. Algae has been proven as the best feedstock for bioethanol due to its distinctive characteristics including its ubiquitous nature, high yield, energy-rich products, low lignin, and high carbon content (Kumar et al., 2013; Li et al., 2014; Wu et al., 2014).

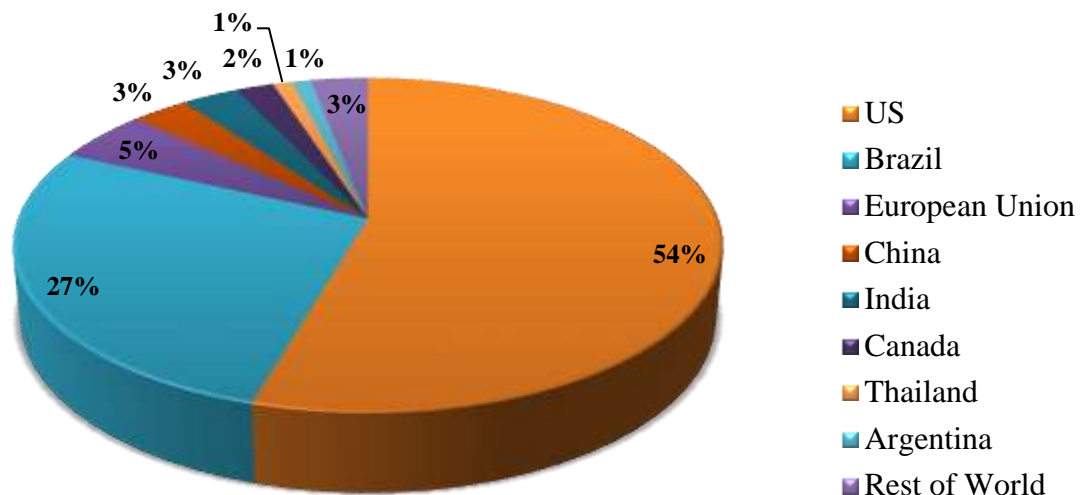


Fig1: Represents the percentage of ethanol production around the globe (RFA, 2021).

Four major operations required for bioethanol production from algae-like cellulosic ethanol are pretreatment fermentation, distillation, and hydrolysis. 7.7 g/L ethanol was extracted from *S. japonica* biomass by fermentation and saccharification giving a 33.3 % theoretical yield (Jang, J.-S. et al., 2012). For effective bioethanol production, recently synthetic yeast was established from algae. For the production of ethanol from algae, differently designed pretreatments help to separate hemicellulose, lignin, and cellulose. In this way, complex carbohydrate molecules of algal cells can be degraded into simple sugars, by water addition or by hydrolysis done by enzyme catalysis. These sugars can be transformed into ethanol by microorganisms and ethanol purification can be done by fuel specifications (Enquist-Newman et al., 2014).

In the last few years, more than a hundred algal fuel corporations have been established globally, but not a single algae fuel has been built for commercial use. Many useful chemicals and residues that contain high cellulose can be extracted from the algae which later can be utilized for bioethanol production. Before commercially producing bioethanol many barriers should be overcome to make it large-scale commercially produced bioethanol (Niphadkar et al., 2018). The present review aims to shed light on the nature of algae as biomass, biofuel production, production of bioethanol from algae, and nanotechnology-based enhancement of the process.

1. Algae:

Algae are eukaryotes that can grow very fast as compared to other biomasses (Singh et al., 2011). Multicellular sex organs are not developed in algae. All types possess chlorophyll but they are covered up by photosynthetic pigment that gives a characteristic color that is responsible for their specific identification (Menetrez, 2012).

1.1 Sources of algae:

Macroalgae are also termed as seaweeds. They are found in sub-tidal and intertidal habitats of coastal areas. Algae can grow easily and rapidly in numerous habitats including freshwater, saline water, and also in municipal wastewater (Harun et al., 2010). Algae are widely distributed throughout the world. They are commonly found along the coastal areas near the seashore and attach to the suitable substratum (Roesijadi et al., 2010).

1.2 Composition of algae:

They are multicellular, consisting of a structure that resembles the roots, leaves, and stems of higher plants. Many species possess a gas-filled structure that helps in providing buoyancy (Chen et al., 2010). Based on pigmentation, brown algae are divided into three classes namely; (i) Phaeophyceae or Brown seaweeds, (ii) Chlorophyceae or Green seaweed, and (iii) red seaweed or Rhodophyceae (Singh et al;2011). Similarly, microalgae are mostly unicellular, photosynthetic, and microscopic (Chen et al., 2010). There are also three classes of microalgae such as; (i) Bacillariophyceae or Diatoms, (ii) Chlorophyceae or Green algae, and (iii) Golden algae or Chrysophyceae (Carlsson et al., 2007). Microalgae have fast and efficient products that can be harvested easily as compared to various feedstocks that's why they meet the demand for enough bioethanol production (Jones and Mayfield, 2012). Micro and macroalgae are considered third-generation regarding biomass production (John et al., 2011). Algae also play a role in the production of polysaccharides and oils that are used for the production of biofuels. They are also capable of removing carbon dioxide (Aresta et al., 2005).

1.3 Cultivation of algae:

They can be cultivated in the pond ecosystem (Hase et al., 2000). The open pond includes lakes and lagoon etc. Mostly shallow ponds, raceway ponds, tanks, and circular ponds are utilized for algal cultivation. The advantage of this system is its easy construction and operation as compared to closed ponds. Similarly many types of photo-bioreactors are used for the manufacturing of various products of algae. Vertical alveolar panels as well as flat plate reactors are utilized for the cultivation of the mass of various algae (Hoekema et al., 2002).

1.4 Environmental factors:

Temperature is a crucial factor that impacts the growth of algae, nutrient requirement, biochemical composition, and cell size. They can grow under varied temperature ranges depending upon their strain, region, and season. Their growth rate is enhanced with the enhancement in temperature (Renaud et al., 2002). At optimal temperatures, algal growth resulted in minimal cell size. The non-optimal temperature results in a decrease in the usage of nitrogen and carbon (Darley and Darley, 1982). Similarly, the intensity of the light also impacts algal growth by affecting its photosynthetic activity (Stockenreiter et al., 2013). The pH also contributes to the metabolism of algae because it is responsible for determining carbon dioxide and nutrient availability (Chen and Durbin, 1994).

1.5 Algae as biomass:

Algae are rich in carbohydrates including sugar and starch that are used to produce biofuels by the process of fermentation. They have minimal levels of hemicellulose, and do not possess lignin that's why they can be hydrolyzed and fermented efficiently (Choi et al., 2012).

Algae have been constructively utilized for biofuel production and due to this algal carbohydrates are utilized for the fermentation of bioethanol, just after the process of saccharification because of the lack of lignin (John et al; 2011). Processing of oil into biodiesel can be done by transesterification. Similarly, the biomass of algae can be utilized for the generation of power and heat by utilizing anaerobic digestion as well as pyrolysis (Bridgewater, 2004).

Generally, macroalgae have three types i.e., red, brown, and green algae (Yanagisawa et al., 2011). Sea mustard which is a type of brown algae (*Undaria pinnatifida*) and kelp (*Saccharina japonica*) are considered very important biomass for biofuel production due to their enhanced productivity of cultivation as compared to all the three macroalgae types (Aizawa et al., 2007). Brown algae contain the highest concentration of carbohydrates thus they are utilized as suitable biomass for bioethanol production (Adams et al., 2009). Therefore brown algae are considered the most sustainable and renewable biomass for chemicals and bioethanol production as they contain the highest concentration of carbohydrates.

It has been found that in brown algae laminarin, alginate, and mannitol are present as superabundant sugars. Glucose, and mannitol from brown algae are used effectively for the fermentation of bioethanol (Lee et al., 2013). Brown algal cell wall contains alginate which is a polysaccharide (Kennedy and Panesar, 2006). Alginate exists as an insoluble salt of calcium and is the most abundant carbohydrate known for bioethanol production (Chee et al., 2011). In brown algae, alginate represents 60% of the total sugars. Alginate extraction involves alkaline extraction which in turn requires the conversion of calcium alginate into alginic acid, which is then converted into a soluble form of Sodium alginate (Hernández-Carmona et al., 1999). Similarly, the alginate is converted into unsaturated uronate monosaccharide by degrading enzyme alginate lyase (Ochiai et al., 2010). Many kinds of alginate lyases catalyze the process of breaking glycosidic bonds in alginate and have undergone cloning and characterization (Kim et al., 2011). Alginate lyase contributes to a 30% yield when used as a biocatalyst in the process of saccharification that is based on alginate lyase (Park et al; 2012).

Similarly, the extract of *Laminaria Hyperborea* contains Laminarin and mannitol that are allowed to ferment for bioethanol production by using *Zymobacter Palmae* under low concentrations of oxygen (Horn et al., 2000). After chemoenzymatic saccharification, the hydrolysates of *Laminaria japonica* contain mannitol that has been utilized for bioethanol production (Kim et al.; 2011). Similarly, the biomass of *S. Japonica* also produced 7.7g/L ethanol using the method of saccharification and fermentation simultaneously. For brown algae, the ratio of sugar from mannitol to alginate is 8:5 (Wargacki et al., 2012). Therefore both of these sugar components are utilized simultaneously in bioethanol production. A synthetic platform has been developed from brown algae for the production of bioethanol in an efficient way (Enquist-Newman et al.; 2014). Generally, red, brown, and green algae produce 0.045-0.236g/g of bioethanol regarding their dry mass (Meinita et al., 2013).

Biotechnological strategies for modification of algae:

There is a need for improvement in the productivity parameter to increase its economic viability. The approaches applied for this purpose are medium optimization techniques, environmental specifications, and genetic manipulations. The first two techniques fail to meet the expectations of scientists regarding algal biorefinery, but the third one successfully overpowers all the drawbacks (Alexandrov et al., 2015). Genetic manipulation by biotechnological strategies can be achieved by genetic engineering tools or synthetic biology tools. Genetic engineering is an alternative potential approach used to increase the algal carbohydrate, and lipid content, comprised of two integral steps: selection and use of genetic engineering tools (Jagadevan et al., 2018). Selection is done to screen the best possible algae concerning its characteristics for genetic manipulation while the engineering tools may involve different selection markers, promoters, CRISPR (clustered regularly interspaced short palindromic repeats), ZFN (zinc-finger nucleases), and TALEs (transcription activator-like effectors), etc. (Banerjee et al., 2016a; Banerjee et al., 2016b; Sirajunnisa and Surendhiran, 2016). In synthetic biology, we reprogram algae for the reconstruction of its biological pathways and this can be done by digestion and ligation, or by homologous

recombination. Concerned risks may include, the chances of instability in modified algae and their harmful release in the environment, also risk assessment should be done before their release in industries (Apel et al., 2017).

2. Biofuels from algae:

At the present moment, when the world's energy demand is increasing and the resources are decreasing, biofuels can act as a better replacement for the mitigation of energy demands (Kumar, M. et al., 2019; Kumar and Thakur, 2018). Algae are a novel source for biofuel production due to their innate quality of being renewable as well as their capacity for biomolecule synthesis (Paramesh et al., 2018). Biofuel is considered a clean, environment-friendly, and renewable fuel, in which energy production occurs through the stabilization of carbon (Nematian & Barati, 2019). These renewable biofuels mainly include biodiesel, biohydrogen, and bioethanol (Fig 2), now we are going to discuss these biofuels separately.

2.1 Biodiesel:

Biodiesel is a non-toxic, safe, economical, sulfur-free, and renewable fuel with a high octane number, produced by transesterification from oils or lipids (Paramesh et al., 2018). Principally biodiesel is comprised of monoalkyl esters which are derived from long-chain fatty acids. First and second-generation biodiesels have been proven inappropriate due to their unfavorable resources and low quality (Sharma et al., 2014). Third-generation biodiesels, derived from algae, have distinctive characteristics like high oxygen content, elevated combustion rates, ecological, and zero carbon emission. This happens because of the algae's high oil content, better photosynthetic rates, low area requirement, high biodegradability, and rapid growth (Nematian and Barati, 2019). Algal biodiesels have better cold flow qualities due to more unsaturation, but simultaneously they release hydrogen peroxide, as well as they are 10 to 20% more viscous than the conventional ones. The latter two mentioned properties are not feasible, also they have long-term storage problems. To overcome these limitations, there is a need to work more on these algae-based biodiesels (Kumar and Thakur, 2018).

2.2 Biohydrogen:

Biohydrogen is a viable high-energy fuel and can be used as an attractive alternative due to its extraordinary characteristics including renewability, being environmentally friendly, economical, and less energy demand (Saratale et al., 2019). It is produced from algae by a process named biophotolysis (Sirawattamongkol et al., 2020). Due to the remarkable nature of algae biohydrogen possesses some exceptional qualities over conventional fuels including; easy and rapid cultivation and production methods, reduced CO₂ emission, effective waste management ability, and high protein and carbohydrate content with zero lignin (Kumar, D. et al., 2019; Kumar, M.D. et al., 2019). Further genetic manipulation of hydrogenases in algae enhances biohydrogen production up to 30-fold (Yang et al., 2019).

2.3 Bioethanol:

Bioethanol is a carbon-neutral algae-derived power source, considered a promising alternative because of its biodegradable nature which ultimately minimizes the liberation of greenhouse gases and reduces carbon emission in the transport sector (Kumar et al., 2020). Its synthesis is done by simple microbial fermentation by using several chemical or biological approaches, and algae are considered an ideal feedstock because of elevated carbon and sugar content, high yield, and reduced lignin concentration (Ciani et al., 2016; Kumar et al., 2016).

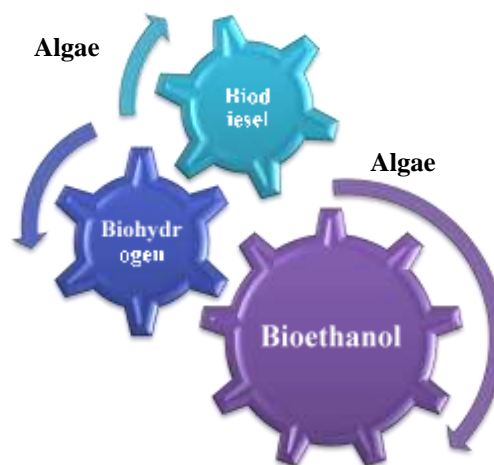


Fig 2: Represents the different types of algal biofuels.

3. Bioethanol production from algae:

It is well-thought-out as a hygienic and environmental fuel because of its biodegradable and non-toxic nature, due to this it is a perfect alternative to gasoline (Hong et al., 2014; Tamayo and Del Rosario, 2014). It can be extracted from lignocellulosic biomass including corn starch or sugar cane. They are economically cheap and also impact food prices. They can produce high amounts of polysaccharides which can be hydrolyzed enzymatically or chemically and physically and converted into bioethanol by fermentation (Chaudhary et al., 2014). The whole process is shown in Fig 3.

3.1 Pre-treatment:

3.1.1 Physical treatment:

Autoclave and ultrasonication using steam pressure methodology are broadly utilized in pretreatment for liberating carbohydrates. (Nahak et al., 2011) examined the impact of steam detonation on seaweed, its explosion was sustained at 120 °C for 1 min at 15 psi and chemical pretreatment was done by utilizing nitric acid/acetic acid and diluted sulfuric acid, it was concluded that the yield of carbohydrates was elevated after physical treatment. Recently, (Kim et al., 2015) proved that autoclaving is the most advanced and generated maximum output from red seaweed *Gelidium amansii*.

3.1.2 Chemical treatment:

This encompasses alkali and acid procedures and these techniques are widely used because the lowermost acid concentrations can hydrolyze the cell wall the most (Cho et al., 2013). (Fakhrudin et al., 2014) testified that elevated sugar levels were gained by 3% sulphuric acid. (Ho et al 2013) described that 1% sulfuric acid was more productive with 93.6% on *Chlorella vulgaris* than miscellaneous enzyme cellulase/amylase treatment – 90.4%. (Harun et al., 2011) briefed that the alkaline pre-treatment method showed to be a promising option to pre-treat microalgal biomass for bioethanol production.

3.1.3 Enzymatic treatment:

Acid and alkali-based methods are quicker, informal, and inexpensive as compared to others, acids can cause sugar breakdown making undesirable components that hinder the fermentation procedure (Harun et al., 2010). Alkalis are not eco-friendly and are costly. In disparity, the enzymatic method is an ecologically friendly process and produces elevated glucose levels (Rabelo et al., 2009).

3.2 Fermentation:

Fermentation-consuming microorganisms will employ sugars including, glucose, galactose, mannose, xylose, and arabinose. For the preparation of ethanol from hexoses, *Saccharomyces cerevisiae* is the most well-known microbes (Sulfahri et al; 2011). Researchers examined bioethanol production through fermentation using *Z. mobilis* and *S. cerevisiae*. The results showed that *Z. mobilis* was able to survive at high concentrations compared with *S. cerevisiae*. Some researchers applied ethanol-genic *E. coli* KO11 to algae fermentation (Kim et al., 2011).

3.3 Extraction of bioethanol:

This step is the separation and purification of ethanol after the fermentation process. It is achieved by boiling for the reason that the boiling point of water (100°C) is higher than the boiling point of ethanol (78 °C), ethanol vaporizes before water. However, due to being an azeotrope mixture, a high quantity of energy is used for distillation. To separate azeotrope mixtures, an agent which changes the azeotrope structure must be added to the mixture. The added substance changes the volatility of the mixture by carrying out the molecular attractions in the mixture. The distillation column which has two streams at the top and bottom separates most of the bioethanol from the mixture. Generally in plants, the regaining of bioethanol in distillation columns is fixed to be 99.6% due to the reduction of loss of bioethanol (Hassan et al., 2013).

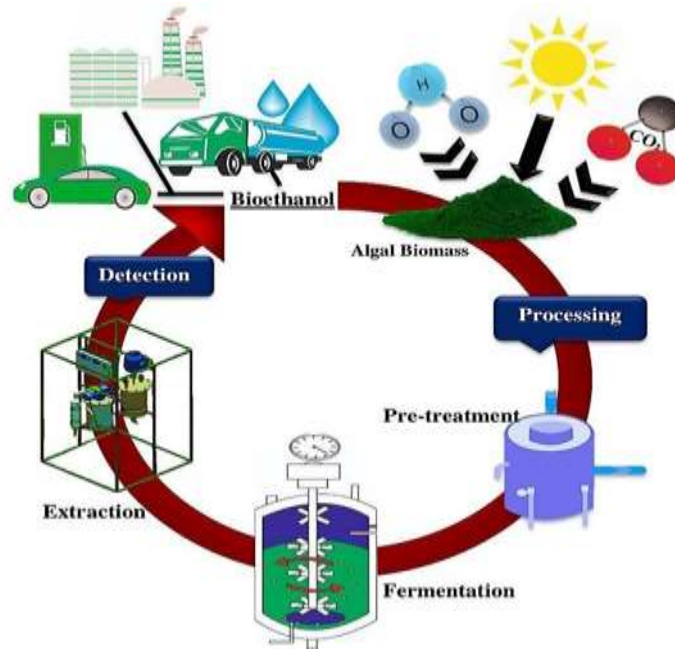


Fig3: Represents the whole process involved in the bioethanol production from algae.

Table 1: Represents the nutrient content, pre-treatment conditions, fermentation agents and conditions, and total yield, concerning the different algal strains.

Algae Strain	Content			Pretreatment conditions	Fermentation (agent and conditions)	Yield in % or g/g or g/L	References
	Carbohydrate content %	Lipid content %	Protein content %				
<i>E.intestinalis</i>	42.8	1.3	31.6	Hydrothermal process (75 mM for 90min)	Saccharomyces cerevisiae (pH 5.5, 30°C, 220 rpm for 12 h)	41.74%	(Cho et al., 2013; Kim et al., 2014)
<i>U.fasciata</i>	43.0	-	-	H ₂ SO ₄ (0.1% at 100°C for 1 h)	Saccharomyces cerevisiae (109 CFU/ml 28°C, 120 rpm for 48 h)	88.24%	(Hebbale et al., 2019; Trivedi et al., 2013)
<i>U.lactuca</i>	11.6–13.2	9.6–11.4	11.4–12.6	Washed, 130 °C/20 min	Saccharomyces cerevisiae (28°C for 12 h)	92.16%	(Kumar et al., 2016; Suganya et al., 2016; Trivedi et al., 2011)
<i>U.pertusa</i>	59.07 ± 0.20	2.39 ± 0.10	6.30 ± 0.25	Citric acid buffer (0.1 M sterilized using autoclave)	Saccharomyces cerevisiae (30°C for 36 h)	91.24%	(Jang, S.-S. et al., 2012; Yanagisawa et al., 2011)
<i>G. amansii</i>	77.2	1.1	13.1	H ₂ SO ₄ (56–168mM, 45–240min)	Scheffersomyces stipitidis (pH 5.5, 30°C, 200 rpm)	92.40%	(Jang, S.-S. et al., 2012; Kim et al., 2011)

<i>G. verrucosa</i>	43 ± 2	-	-	373 mM H ₂ SO ₄	Saccharomyces cerevisiae (pH 5, 30°C, 150 rpm for 114 h)	94%	(Kumar et al., 2013; Nguyen et al., 2017)
<i>Gracilaria sp.</i>	76.67	1.2	16.0	H ₂ SO ₄ (0.1 N, 121°C for 30min)	Saccharomyces cerevisiae (30°C for 48 h)	82.80%	(Wu et al., 2014)
<i>K. alvarezii</i>	64	0.75 ± 0.22	5.74 ± 0.89	Soaked in 1.6 L distilled water for 30 min and boiled at 90°C for 1 h	Saccharomyces cerevisiae (pH 5.35°C, 130 rpm for 6 h)	49%	(Abd-Rahim et al., 2014; Meinita et al., 2012; Trivedi et al., 2013)
<i>L. hyperborea</i>				Extracted in water 121°C for 20min	Zymobacter palmae (pH 6, 30°C)	74.51%	(Ramachandra and Hebbale, 2020)
<i>Ulva rigida</i>	53 ± 1	1.2 ± 0.2	23.4 ± 0.51	Enzymatic hydrolysis under mild sonication (37 °C, 3 h)	Saccharomyces cerevisiae (37 °C, pH 5.0, 3 h)	333.30 ± 4.7 mg/g	(El Harchi et al., 2018; Korzen et al., 2015)
<i>Spirulina maxima</i>	13–16	6–7	60–71	-	-	79.5%	(Nautiyal et al., 2014; Suganya et al., 2016)
<i>Sargassum latifolium</i>	20.1	4.2	5.7	Acid treatment followed by fungal saccharification	Saccharomyces cerevisiae (30 °C, pH 6, 150 rpm, 96 h)	0.29 g/g	(Soliman et al., 2018)
<i>Chondrus crispus</i>	21.8 ± 1.57	0.48 ± 0.25	19.9 ± 0.27	-	Saccharomyces cerevisiae (30 °C, 120 rpm, 120 h)	13.0 g/L	(Kostas et al., 2016)
<i>Dunaliella tertiolecta</i>	-	70.6-71.4	-	Chemo-enzymatic saccharification	S. cerevisiae(200 rpm and 30°C for 12 h)	0.14 g /g	(Lee et al., 2013)
<i>Chlorella sp.</i>	27.0	28-32	-	2% HCl and 2.5% MgCl ₂ at 180 °C for 10 min	S. cerevisiae Y01	91%	(John et al., 2011; Zhou et al., 2011)
<i>Eucheuma cottonii</i>	70.0	-	-	Enzymatic treatment	S. cerevisiae	5.47 mg/mL	(Fakhrudin et al., 2014)
<i>Chlorella sorokiniana</i>	18	19.0–22.0	-	5 mol/L NaOH at 90 °C for 30 min		0.23g/L	(Hernández et al., 2015; Kumar et al., 2016)
<i>L. digitata</i>	46.6	1.0	12.9	-	Pichia angophorae(24 °C, pH 4, 69 h)	8.86 ± 0.05 µL/mL	(Adams et al., 2011; Kostas et al., 2017)
<i>Scenedesmus obliquus</i>	10–17	12–14	50–56	2% H ₂ SO ₄ at 121 °C for 20 min	Z. mobilis (30 °C)	94.1%	(Ho et al., 2017; Suganya et al., 2016)

<i>Scenedesmus dimorphus</i>	21–52	16–40	8–18	Acid and autoclave	<i>Z. mobilis</i>	0.202 g/g	(Ho et al., 2013; Suganya et al., 2016)
<i>Chlorella vulgaris</i>	12–17	14–22	51–58	Enzymatic treatment(1% H ₂ SO ₄ at 121 °C for 20 min)	<i>Z. mobilis</i> (30 °C)	87.59%	(Ho et al., 2013; Suganya et al., 2016)
<i>Chlorella pyrenoidosa</i>	26	2	57	-	-	2.90–3.64 g/L	(Kumar et al., 2016; Suganya et al., 2016)
<i>Ascophyllum nodosum</i>	44.66	2.99	5.24	Microwave-Assisted Acid Hydrolysis	<i>Scheffersomyces stipites</i> (30 °C, 100 rpm, 3–150 h)	2.40 g/L	(Yuan and Macquarrie, 2015a, b)
<i>Spirulina platensis</i>	8–14	4–9	46–63	-	-	79.5%	(Nautiyal et al., 2014; Suganya et al., 2016)
<i>Synechococcus sp.</i>	15	11	63	freezing at – 20 °C; 0.1 g/L lysozyme addition for 3 h at 37 °C	<i>S. cerevisiae</i>	90%	(Möllers et al., 2014; Suganya et al., 2016)
<i>Chlorococcum infusionum</i>	32	-	-	Alkaline pre-treatment(0.75% (w/v) NaOH at 120 °C for 30 min)	<i>S. cerevisiae</i> (30 °C for 72 h)	0.26 g/g	(Harun et al., 2011)
<i>Chlamydomonas reinhardtii</i>	59.7	-	-	3% H ₂ SO ₄ at 110 °C for 30 min	<i>S. cerevisiae</i> (288C at 30 °C for 24 h)	100%	(Liang et al., 2009)
<i>Porphyridium cruentum</i>	40–57	9–14	28–39	-	-		(Sarkar & Bhattacharyya, 2012)
<i>Sargassum sp.</i>	41.81	0.75 ± 0.02	10.25	Acidic pre-treatment	<i>S. cerevisiae</i>	0.112 g/g	(Borines et al., 2013)
<i>Laminaria japonica</i>	54.5 ± 0.09	1.37 ± 0.01	7.40 ± 0.06	Acidic and enzymatic pre-treatment	<i>S. cerevisiae</i> (0.375 g/L) 30°C, pH 6.5, 36 h	81%	(Jang, S.-S. et al., 2012; Wu et al., 2014)

4. Moderation by nanotechnology:

Nanotechnology is a discipline in which we deal with matter at the nanoscale (1–100 nm), this nano size is responsible for the unique characteristics of nanomaterials as compared to the bulk materials (Reijnders, 2012). This has directed the attention of scientists towards the use of nanotechnology for the enhancement of conventional technologies. As well as the utilization of nanomaterials can be instrumental in meeting the satisfaction of the world's energy demands. Various types of nano-additives including nanoparticles, nanofibers, nanotubes, nanowires, nanopores, nanoclusters, nanocomposites, nanorods, nanosheets, and metal oxides can play their remarkable role, both directly or indirectly in the production of bioethanol (Kushwaha et al., n.d.; Verma et al., 2013).

Undoubtedly bioethanol production by algae is a bio-efficient process but it still faces some limitations including water requirement for algal growth, inconsistent production and unstable harvesting of algae at an industrial scale, and uneconomic behavior during algal production (Hossain et al., 2019; Reijnders, 2012).

These limitations can be overcome by using nanomaterials, which act by immobilizing enzymes, this will result in the feasible conversion of materials, cost reduction, and the recyclable nature of enzymes (Antunes et al., 2017). This will improve the effectiveness and efficiency of the process and further enhance the rate of reaction by acting as catalysts (Chaturvedi et al., 2012; Singh and Tandon, 2014). At the same time, the magnetic properties of nanoparticles can reduce toxicity concerns (Leo and Singh, 2018). They can also be used in the detection of intermediates and end products (Kushwaha et al., 2018). These properties will lead to an enhancement in the total yield of bioethanol (Duraiarasan et al., 2016). **Fig 4** shows the different types of nanomaterials which can be used as a modifier, in bioethanol production.

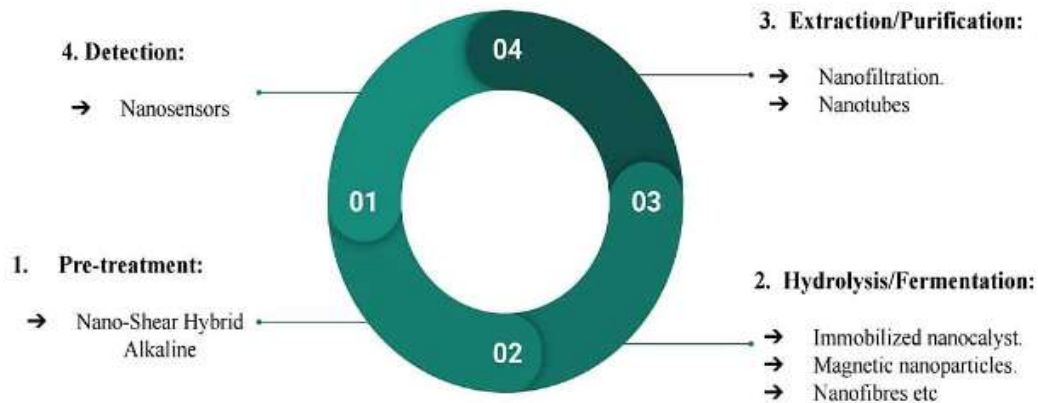


Fig 4: Shows the various nanomaterials used in bioethanol production (Leo and Singh, 2018).

Nanomaterials have a negative impression due to their costly and toxic nature, this toxic nature is a potential hazard for both humans and the environment (Rodríguez-Couto, 2019). To avoid these undesirable consequences we should prepare nanomaterials with biological methods, this approach is environment-friendly and has been proven promising for both humans and the environment (Saratale et al., 2018).

Conclusion:

In the era of ultra-modern industrialization, algal biomass appears as a golden substitute for fossil fuels. Its sustainable and renewable by-products including biodiesel, biohydrogen, bioethanol, etc. made the procedure more efficient. Bioethanol is the most viable biofuel and is produced by the pre-treatment process followed by reduced energy-intensive fermentation and purification. Undoubtedly this process is eco-friendly and feasible, but due to its arduous nature, the process can be innovated by nanotechnology-based additives. Nanomaterial-enhanced algal bioethanol is more economical and efficient, also the addition of nano additives makes the conversion process less laborious and time-saving. Although nanomaterials show toxic behavior towards humans and the environment to some extent, this can be overcome by preparing nanomaterials with biological methods instead of physical or chemical methods. In the future, more work is required on the limitations of biological methods based nano additives, for the sake of acquiring the most appropriate and safe nanomaterials concerning the algal bioethanol production.

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