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# Bioethanol from macroalgae: Prospects and challenges

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ABSTRACT

Burgeoning dependence on fossil fuels for transport and industrial sectors has been posing challenges such as depletion of fossil fuel reserves, enhanced greenhouse gas (GHG) footprint, with the imminent changes in the climate, etc. This has necessitated an exploration of sustainable, eco-friendly and carbon neutral energy alternatives. Recent studies on biofuels indicate that algal biomass, particularly from marine macroalgae (seaweeds) have the potential to supplement oil fuel. Marine macroalgae are fast growing and carbohydrate rich biomass having advantage over other biofuel feedstock in terms of land dependence, freshwater requirements, not competing with food crops, which were the inherent drawback of the first- and second-generation feedstock. The present communication reviews the macroalgal feedstock availability, screening and selection of viable feedstock based on the biochemical composition, process involved, scope and opportunities in bioethanol production as well as technology interventions. The prospect of bioethanol production from algal feedstock of Central West Coast of India has been evaluated taking into account challenges (feedstock sustenance, technical feasibility, economic viability) in order to achieve energy sustainability. The green algae exhibited growth during all seasons and highest total carbohydrate was recorded from green seaweed *Ulva lactuca* (62.15 ± 12.8%). Elemental (CHN) analyses of seaweed samples indicate 25.31–37.95% of carbon, 4.52–6.48% hydrogen and 1.88–4.36% Nitrogen. Highest carbon, hydrogen and nitrogen content were recorded respectively from *G.pusillum* (C: 37.95%), *G. pusillum* (H: 6.48%) and *E.intestinalis* (N: 4.36%). Green seaweeds are rich in cellulose content (>10%) compared to other seaweeds (2–10%). Higher cellulose content was estimated in *U.lactuca* (14.03 ± 0.14%), followed by *E. intestinalis* (12.10 ± 0.53%) and C.*media* (10.53 ± 0.17%). Cellulose is a glucan present in green seaweeds, which can easily be hydrolysed through enzyme and subsequently fermented to produce bioethanol. Lower sugar removal in acid hydrolysate neutralization process ($Na_2CO_3$) was recorded in *U.lactuca* (39.8%) and *E.intestinalis* (14.7%). Highest ethanol yield of 1.63 g and 0.49 g achieving 25.8% and 77.4% efficiency in SHF (Separate Hydrolysis and Fermentation) and SSF (Simultaneous Saccharification and Fermentation) process respectively was recorded for green alga *E. intestinalis*.

## 1. Introduction

Fossil fuels such as oil, coal and natural gas are the major commercial energy sources and about 87% of global $CO_2$ emitted due to the anthropogenic activities [1,2] are contributed by utilization of coal (43%), oil (36%) and natural gas (20%). Earth endows finite source of oil reserve and its increased consumption in several sectors has led to increased oil production, exerting pressure on the reserves which is apprehended to peak and no longer suffice the world's demand with the fast dwindling stock [3]. Rising population with higher consumption levels coupled with a fast pace of development have spurred higher exploitation of fossil fuels leading to the escalating prices and resultant greenhouse gases (GHGs) posing problems for planet's climatic stability [4–6]. It is imperative to ensure energy security through the sustainable alternative energy sources [7,8]. Globally, nations are actively addressing the issues concerning greenhouse gases and peak oil crisis through several mitigation measures such as; energy conservation, fuel substitution, incentives for the use of unconventional and renewable oil, and policy reforms such as carbon tax [8]. Therefore, the current focus is on carbon neutral renewable sources, notably photovoltaic, wind,

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hydrogen, etc. These alternative sources were useful in addressing the electricity requirement, but the exploration for viable alternatives to oil in order to meet the requirement of transport sector, etc. is quintessential. Despite the existence of possible solutions such as renewable resources, energy efficient products (CFLs and LEDs) have not been widely adopted due to market barriers. Wind power contributes 2.5% of world electricity output and are weather dependent, susceptible to geographic and climatic changes [9,10]. Dependency on conventional generation coupled with the depleting stock and the enhanced environmental awareness in the public have been the major constraints faced by the land based energy systems [11,12]. Nuclear power witnessed 2% growth in Europe, but encountered resistance with respect to disposal of waste, safety during nuclear accident and declining global uranium stocks. Nuclear disaster at Fukushima Daiichi nuclear power plant in 2011 [13], led Germany to rethink its energy policy [14].

India has been the 3rd largest energy consumer surpassing Russia, China and USA and about 80% of India's energy consumption was contributed by imported crude oil [15], which was estimated to be 213.93 Million Metric Ton (MMT) in the year 2016–17. This is attributed to the poorly endowed natural reserve of hydrocarbon in India, however crude oil production in India is about 36.01 MMT, from the 0.3% oil reserves [15]. India is emerging as the fastest growing economy next to China with the growing energy demand, burgeoning population (at 1.58% annual) and dwindling stock of fossil fuel in next few decades, it is challenging to support this growing economy demand [16]. The total $CO_2$ emission in India accounts for 965.9Tg/yr, with electricity generation (343Tg/yr) and transport (246.23Tg/yr) sectors as the major contributors [17]. The higher level of $CO_2$ emissions necessitates implementation of efficient management strategies to mitigate changes in climate [18]. The new renewable energy resources are being explored to meet the energy demand in all sectors and also research is underway to address the intermittency problems associated with wind and solar based energy systems [19–21].

In this context, studies have shown that biofuels are emerging as promising alternative to liquid fuels. Realizing the potential of biomass, different technologies have evolved towards the conversion of biomass into fuels, popularly known as biofuels [22–24]. Produced from renewable plant sources or other organic wastes, biofuels have the advantages of cutting down carbon emission and dependency on oil [25]. In India, around 80% of rural energy [26] is met by biomass energy consumption, in the form of firewood, agriculture residues, cow dung cake and other natural feedstock [24,27,28]. Fig. 1 represents the share of each country in the global bioethanol production, which highlights that India's share is only 2% [29] despite burgeoning demand for fossil fuel. This emphasizes the need for augmentation with the viable indigenous alternative feedstock to minimize fossil fuel dependence.

Biofuel from first generation feedstock involved food crops like corn and sugarcane which were exploited for biofuel production over three decades, but this technique encountered resistance due to the limited stock and competition with food crops [25]. The inadequacy of first generation feedstock in augmenting the growing energy demand led to the evolution of second generation feedstock involving lignocellulose biomass (Fig. 2). However, biofuel from second-generation feedstock also failed, due to the difficulty in scaling up and process technology involved in the cost-intensive delignification process [30]. Due to this, the cost of production of cellulosic ethanol is two to three fold higher than the price of corn grain ethanol [31]. In the US, it was seen that, the fossil energy required to produce bioethanol from corn, grain, soybean and wood biomass was more than the energy content of the biofuel, while sufficing only 12% of gasoline and 6% of the diesel demand. Though first and second generation feedstock are explored for biofuel production and assessed for carbon sequestration, environmental impacts and production potential only marginally complies with various other sustainability criteria's such as; disruption of global food supply, soil erosion, extensive usage of fertilizers, conversion of ecologically vulnerable wetlands, rainforests, peat lands, savannas into energy crop lands contributing to several magnitude of $CO_2$ [32,33]. GHGs footprint of major cities in India [34], recorded aggregation of carbon dioxide equivalent emission of GHGs in the range of 13,734.59-38,633.2 Gg, with transportation being one of the major sector next to the energy generation. Emergence of a strong global biofuel feedstock is expected to realize a positive balance between energy and ecological footprints [35].

Table 1 illustrates the prospects of algal biomass emerging as an ideal alternative to the first and second generation [37,38]. Though, algae is being utilized as an energy feedstock since 1950s [26], the oil crisis of 1970's spurted the research [39]. Algal feedstock do not require prime agricultural land and can be grown in fresh water, wastewater [40] and saline waters with zero nutrient input and non-interference with the land used for food production [38,41]. Algal biomass have higher photosynthetic efficiency (up to 5%) as compared to terrestrial biomass (1.8–2.2%) [42], and require for their growth light, carbon dioxide and nutrients (such as nitrogen, phosphorus, potassium, etc.), which are maintained through continuous flow of water [42]. Algae have a higher yield per unit area compared to terrestrial plants e.g. brown algae under the cultured condition, yields ~13.1 kg dry weight/$m^2$ over 7 months as compared to sugarcane yield of ~10 kg dry weight/$m^2$/yr [43]. Algae based on their morphology and size are grouped into micro and macroalgae [29]. Microalgae accumulate large quantities of neutral lipids

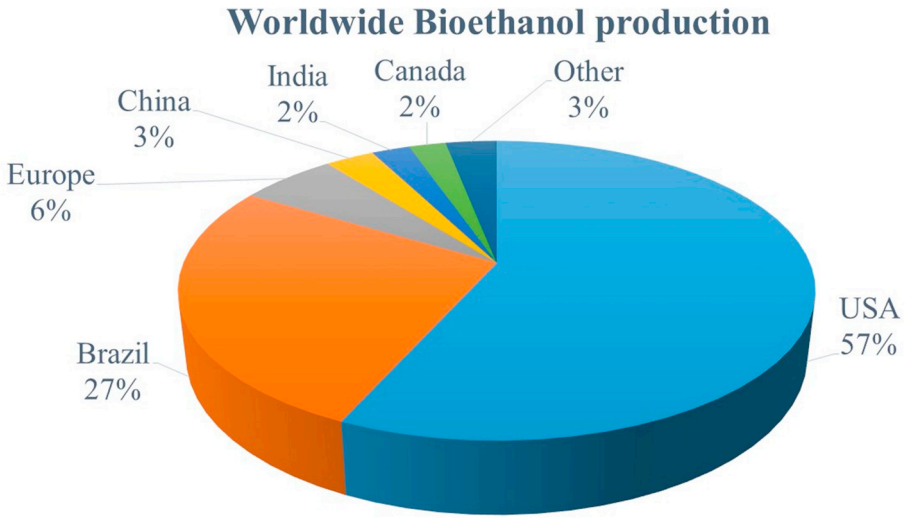


**Fig. 1.** Worldwide bioethanol production.

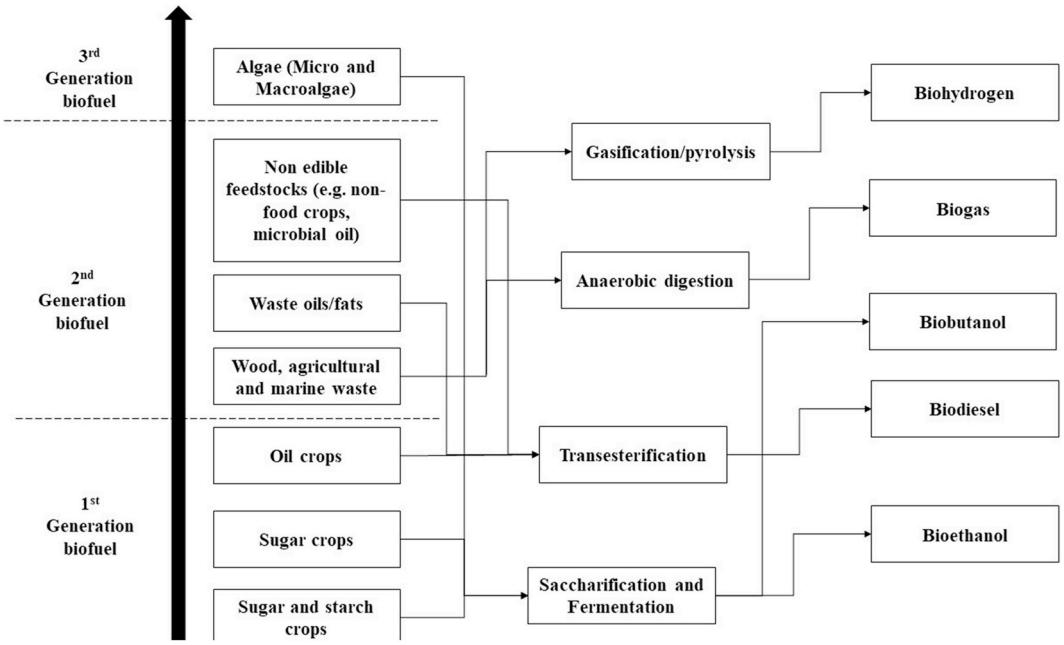


**Fig. 2.** Evolution of biofuel production from feedstocks and technologies.

**Table 1**
Yield and Ethanol production of First, Second and Third generation feedstocks.

| Biofuel | Crop | Yield(ton/ha/yr) | Ethanol (litres/ha/yr) |
|---|---|---|---|
| First generation | Sugarcane | 50–90 | 3500–8000 |
| | Sweet sorghum | 45–80 | 1750–5300 |
| | Sugar beet | 15–50 | 1350–5500 |
| | Fodder Beet | 100–200 | 4400–9350 |
| | Wheat | 1.5-2.1 | 510–714 |
| | Barley | 1.2–2.5 | 300–625 |
| | Rice | 2.5–5.0 | 1075–2150 |
| | Irish potatoes | 10–25 | 1110–2750 |
| | Cassava | 10–65 | 1700–11,050 |
| | Sweet potatoes | 8–50 | 1336–8350 |
| | Grapes | 10–25 | 1300–3250 |
| Second generation | Nipa palm | | 2300–8000 |
| | Maize | 1.7-5.4 | 600-1944 |
| | Sorghum | 1.0–3.7 | 350-1295 |
| Third generation | Algal biomass | 730 | 23400 |

*Source:* [36].

which serves as raw material for biodiesel production [44,45], whereas macroalgae are carbohydrate rich biomass which are useful for bioethanol production. Large scale cultivation of macroalgae in Korea reveals an uptake of 8–10 tonne $CO_2$ per hectare [42].

### 1.1. Potential macroalgal feedstock available

Marine macroalgae or seaweeds establish on hard substratum and grow luxuriantly along nutrient rich coastal zone (Fig. 3). One of the richest seaweed resources in the world is in Nova Scotia/Gulf of St. Lawrence area [46]. Global seaweed distribution can be summarized as: (i) Least flora <200 Spp in latitudes >60° in both hemispheres, (ii) Moderate flora of 600–700 spp. that occur throughout warm and cold tropical and temperate regions, (iii) Highest flora of 900–1100 spp. occur in four regions Southern Australia, Mediterranean, Japan and Philippines.

Seaweed resources and their uses are well established across regions in the world. Red seaweeds are mostly utilized for extraction of hydrocolloid valuing $585 million [47] and source of food (e.g. Salads) valuing $5 billion [48] with Asia as its prime market [43]. Cultivation of macroalgae is a promising option as seventy percent of the Earth surface is covered by water [39,42,49,50], therefore in order to satisfy these industrial demands, macroalgae are cultivated in large scale, mainly of the genus *Laminaria, Undaria, Poryphyra, Eucheuma, Enteromorpha* and *Gracilaria* representing 76% of total macroalgae aquaculture production [51].

In recent years, algal genera of *Kappaphycus, Gelidium, Gracilaria, Sargassum, Laminaria* and *Ulva* (Fig. 2), are the promising potential feedstock for biofuel production in addition to the value added products for phycocolloids extraction, human food, cosmetics, fertilizer and other chemicals [52,53]. These algal feedstock have been chosen considering the availability and assessment of resources around the globe, ease of cultivation and harvesting. However, there is still scope to assess other potential macroalgal species based on their availability, biochemical composition and prospects for cultivation.

### 1.2. Bioethanol production from macroalgal feedstock

Bioethanol from algal biomass is a sustainable and eco-friendly option of renewable biofuel production [39]. Macroalgae or seaweed, saltwater thriving algae have proved to be the viable biofuel feedstock [54] for sustainable biofuel production as it avoids the competition with fresh water, food crops or cultivable land [39,55]. Seaweeds are multicellular marine macroalgae, broadly grouped as green, brown and red based on the pigment present in the thallus. Seaweed consists of carbohydrates (Table 2), which are converted to bioethanol by appropriate microorganisms such as yeast or bacteria. The common processes involved in ethanol production are (i) pretreatment, (ii) hydrolysis and (iii) fermentation.

#### 1.2.1. Pretreatment and hydrolysis for extraction of macroalgal sugar

Different types of biomass contain different amounts of sugars and the complexity of the biomass is reflected between structural and carbohydrate components [62,63]. Plant biomass is mostly composed of lignin (13.6–28.1%), cellulose (40.6–51.2%) and hemicellulose (28.5–37.2%) biopolymer [64], which serves as raw material for production of fuels. However, critical step involved in biofuel production is the conversion of biomass to sugars [65]. It is therefore important to carefully choose the pretreatment process based on the biomass and an optimal pretreatment process towards better yield of sugar with the low energy input [66].

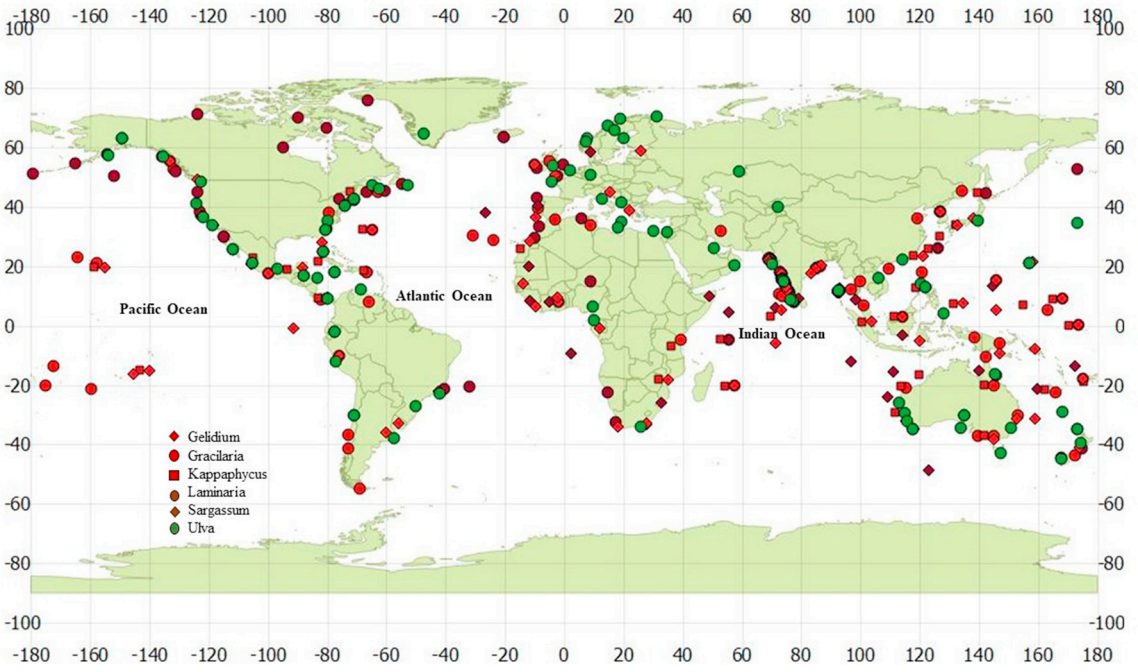


**Fig. 3.** Prominent coastal regions of the world rich in seaweed resources and potential feedstock for bioethanol production.

Pretreatment involves physical, chemical and biological (or combinatorial) process to expose the cell constituents and cell wall materials of feedstock [67]. Physical pretreatment involves reduction in size of the feedstock to increase the surface area for better transport of acid/base catalysts, enzymes and steam to the fibers (cellulose) [68]. Chemical pretreatment involves dilute acid, alkaline, ammonia, organo solvent and other chemicals. Biological pretreatment involves microorganisms like bacteria and fungi (rich in *cellulase* enzyme) to degrade the biomass and release the sugars [69]. Integrated pretreatment involves combination of all the process such as acid catalyzed steam explosion, ammonium fiber explosion (AFEX), acid pretreated enzyme hydrolysis etc [66].

First generation biomass is starch based and requires no stringent pretreatment conditions to extract sugar, whereas lignocellulose biomass is complex in structure due to the presence of biopolymer lignin that embeds cellulose in a matrix resulting in a higher degree of polymerization and crystallization, which is the main factor responsible for recalcitrance [66,70–72] requiring a high cost for delignification process [73]. Therefore, the process of sugar extraction requires severe pretreatment conditions such as steam explosion at 200 °C [74], at 121 °C [75], AFEX, Sulphite pretreatment to overcome recalcitrance of lignocellulose (SPORL) [31], pressurized steam liquefaction [76]. It is seen that alkaline based pretreatment is effective in solubilizing significant portion of lignin from lignocellulose biomass [69]. Lignin was removed from cotton stalk pretreated using sodium hydroxide at high temperature and 96% fermentable sugars were recovered [77,78]. Around 11.4 MMT cotton plant wastes available in India, can generate 3533 billion litres of ethanol considering 90% fermentation efficiency [78]. Removal of 89% lignin and 69.77% hemicellulose in rice husk was achieved through wet air oxidation pretreatment method [79]. Hydrothermal pretreatment of wheat straw was carried out and viewed under scanning electron microscope (SEM), which reveal partial de-fibration of the lignin fibers due to pretreatment, whereas in delignification process lignin appears as layer of globular deposits exposing the cellulose structure [71].

Compared to this, macroalgae with the large concentration of structural polysaccharides (Table 3) and low lignin contents [80] requires mild and low-cost processes for extraction of sugars. The most widely used chemical pretreatment method for macroalgal biomass is dilute acid (Table 4), as it solubilizes hemicellulose and exposes cellulose fibers for further enzyme hydrolysis [68]. The energy consumed in acid pre-treatment is comparatively low as compared to other pre-treatments and higher sugar yields are achieved [69]. Dilute acid concentration for hydrolysis varies based on the feedstock, listed in Table 3. However, limitation of dilute acid pretreatment is the formation of Hydroxymethyl furfurals (HMF) and Levulinic acid (LA) resulting from the degradation of sugars that inhibit the subsequent process (fermentation) in ethanol production [81,82]. These inhibitors are mitigated by neutralization process before fermentation [83,84] or by employing other sustainable alternatives such as biological pretreatment: enzyme hydrolysis [53,85–94].

Enzyme hydrolysis of cellulose is carried out efficiently by *cellulolytic* (*cellulase*) enzyme, which is comprised of exo-, endo-glucanases and cellobiase (β- D-glucosidase) enzymes [71]. *Endoglucanases* cleave cellulose at random sites of β-1, 4-bond and form free reducing ends and short-chain oligosaccharides [84] *Exoglucanases* cleaves the accessible ends of cellulose molecules to liberate glucose and cellobiose. β-D-glucosidase hydrolyses soluble cellobiose and other cellodextrin to produce glucose molecules [95]. Enzyme conversion is substrate specific without any by-product formation. The process could be enhanced [7], by exposing the cellulose fibres through pre-treatment using acid. Enzymatic hydrolysis disintegrates the cellulose and hemicellulose into simple sugars [96]. Along with this, depolymerization of xylan (polysaccharide composed of xylose) can be achieved by dilute-acid pretreatment [88] with about 64% xylose conversion efficiency. Pretreatment techniques include high thermal liquefaction process (HTLP) [97], alkali pretreatment, CaO, Ozonolysis, etc. However it was seen that the acid treated biomass was more susceptible for enzyme attack than HTLP, NaOH, CaO and other pretreatment [87].

Algal cell wall is composed of cellulose Iα (triclinic crystalline form) unlike the cellulose Iβ (monoclinic crystalline form) in plant cell wall. Cellulose Iα consists of weaker hydrogen bonds resulting from spatial arrangement of individual cellulose chains, resulting in easy access to endocellulases enzymes during enzyme hydrolysis [98]. Most common categories of enzymes considered for cell wall depolymerization are cellulases, hemicellulases and accessory enzymes [99], produced from wood-rot (soft rot) fungi such as *Trichoderma, Penicillium*, and *Aspergillus* [100]. The production costs of these enzymes are relatively higher.

**Table 2**
Detailed characteristics of different types of Seaweeds.

| Characteristics | Green seaweed | Red seaweed | Brown seaweed |
|---|---|---|---|
| Number of species recorded | 6032[a] | 7105[b] | 2039[c] |
| Habitat | Freshwater and Marine | Strictly marine | Strictly marine |
| Photosynthetic pigment present | Chlorophyll *a, b*, carotene and Xanthophyll | Phycoerythrin | Fucoxanthin |
| Photosynthetic rate (μmol $CO_2$/h) g/dry | 30 to 1786 | 20–1808.7 | 100–500 |
| Productivity [dry g/($m^2$ year)] | 7100 | 3300–11300 | 3300–11300 |
| Nature of cell wall | Cellulose, pectin rarely hemi-cellulose | Cellulose and pectic material with polysulphate esters | Cellulose with alginic acid and fucocinic acid |
| Sexuality | Isogamy to oogamy | Advanced and complex (oogamous) | Isogamy to oogamy |
| No. of flagella and their insertion | 2 or 4, equal anterior, whiplash | Absent | Only in reproductive cells, 2 unequal, lateral whiplash and tinsel |
| Cell structure | Eukaryotic | Eukaryotic | Eukaryotic |
| Phycolibins | Absent | Allophycocyanin, r-Phycoerythrin r-Phycocyanin | Absent |
| Carotenoids | α-, β-, γ-carotene | α-, β- carotene | α-, β-, ε- carotene |
| Xanthophylls | Lutein Prasinoxanthin | Lutein | Fucoxanthin, Violaxanthin, Diadinoxanthin, Heteroxanthin, Vacheriaxanthin |
| Carbohydrate (%) | 30–60 | 30–50 | 20–30 |
| Protein (%) | 10–20 | 6–15 | 10–15 |
| Lipid (%) | 1–3 | 0.5-1.5 | 1–2 |
| Ash (%) | 13–22 | 5–15 | 14–28 |
| Photosynthetic reserve* (Stored food) | Starch | Floridean starch (intermediate between true starch and dextrin) | Laminarin and mannitol (hexahydride alcohol) |

*Source:* [50,54,56–61].

**Table 3**
Sugar profile of Macroalgae.

| | Green seaweeds | Red seaweeds | Brown seaweeds |
|---|---|---|---|
| Structural polysaccharide | Cellulose | Cellulose, lignin | Cellulose, Alginate |
| Storage polysaccharide | Starch, Ulvan, Mannan | Agar, Carrageenan | Fucoidan, laminarin |
| Monosaccharides | Glucose, Mannose, Rhamnose, Xylose, Galactose | Glucose, Galactose, Agarose | Glucose, Galactose, Fucose, Xylose |
| Sugar alcohol | | | Mannitol |
| Sugar Acid | Uronic acid, Glucuronic acid | | Uronic acid, Mannuronic acid, Glucuronic acid, Alginic acid |

Source [47–50]:

Commercial industrial enzymes are produced from aerobic fungi *Trichoderma reesei,* which produces over 100 g per liter of crude cellulase enzyme with higher specific activity, achieved by genetic engineered strains [101]. Most common enzymes employed for seaweed hydrolysis are commercial enzymes such as Cellulase, Celluclast 1.5 L, Viscozyme L, Novozyme 188, Termamyl 120 L, β-glucosidase, Multifect, Meicelase, Amyloglucosidase etc operated at pH 4.5–5.5 and temperature 35–55 °C, incubation time varies based on the algal feedstock [56,71,89,90,102–109].

Cellulase producing microbes have been screened and isolated from various sources such as soil from forest and nature reserves, hot water springs, marine bacteria [90] compost, sewage, animal manure and bovine rumen [91]. Enzymatic hydrolysis has been done conventionally at <50 °C, resulting in lower sugar yield [95]. Therefore, research is under progress for isolating efficient cellulolytic enzyme systems from a wide variety of bacteria, fungi, aerobes, anaerobes, mesophiles, thermophiles and thermo-stable microbes [92,93,96] which can overcome low sugar yield for biofuel production. *Cellulase* from thermophilic and psychrophilic microbes are preferred as they are resistant to high and low temperatures respectively [91]. Thermo-stable enzymes increase solubility of reactants and products, facilitating easy recovery of end products [96] while reducing hydrolysis time, decreasing contamination and cost of energy.

Marine fungus *Cladosporium sphaerospermum* was isolated to extract cellulase enzyme and used to hydrolyze *U. pertusa* biomass, which yielded 112 mg/g of reducing sugar at pH 4 and temperature 25 °C for 42 h [94]. Similarly, marine bacteria was isolated from degrading *U. lactuca* to extract cellulase enzyme, which is tolerant to high salt concentration and alkaline pH [86]. Polysaccharolytic enzymes extracted from the gut of the abalone *Haliotis midae* degraded the polysaccharides laminarin, carboxymethylcellulose (CMC), alginate, agarose and carrageenan [109].

### 1.2.2. Fermentation of macroalgal sugars

Macroalgal biomass contain different types of polysaccharides, exclusively composed of glucose i.e., glucans. Main glucans present in green: cellulose and starch; red: cellulose and floridean starch; brown: cellulose and laminarin [46,50,61]. Non-glucans are sulphated polysaccharides such as agar, carrageenan and alginate. In order to obtain higher ethanol, hydrolysis of glucan as well as non-glucan with the fermentation of the resulting sugars is essential [60]. Sugar released from the pretreatment process has been fermented using microorganisms such as yeast, bacteria, and fungi, which ferment these sugars to produce ethanol as a by-product [41,110]. *Saccharomyces cerevisiae* is the commonly used yeast microorganism for fermentation as it readily ferments glucose [111]. However, pretreatment releases mixed sugars namely; glucose, galactose, mannitol, rhamnose and xylose. Due to the lack of xylose transport system, *S.cerevisiae* is not capable of utilizing xylose [112]. Its uptake takes place through glucose transport system and is regulated by the concentration of glucose. At only low concentration of glucose, xylose is consumed by the yeast [113]. As a result, studies related to isolation of wild yeast strains from various sources is done that can ferment both hexose and pentose sugars yielding higher ethanol. Bacteria, yeast and fungi are explored for xylose fermenting organisms, and mostly preferred organisms are bacteria and yeast as fungi are too slow for competitive industrial process [7].

Single or combination of strains are being attempted for utilization of sugars. Laminaran and mannitol obtained from *L. hyperborea* were subjected to fermentation using one bacterium (*Zymobacter palmae* T109) and three yeast strains (*Pichia angophorae, Pacchysolen tannophilus and Kluyveromyces marxianus*). It was seen that only *P. angophorae* is capable of fermenting laminaran and mannitol at higher oxygen transfer rate to produce 0.43 g ethanol/g substrate [114]. Utilization of mannitol by *Zymobacter palmae* resulted in the production of 0.37 g ethanol/g mannitol [115], however mannitol was utilized at lower oxygen rate in fermentation media. Mannitol was effectively fermented by *E.coli* KO11 for production of 0.41 g ethanol/g mannitol [116]. Similarly, glucuronic acid fermentation was attempted using *Pachysolen tannophilus* and *E.coli.*

Bioethanol production from all forms of macroalgal biomass; wet, dried and residues (after extraction of hydrocolloid) was attempted. Residues after extraction of hydrocolloids are rich in cellulose, which have been utilized for bioethanol production. Floating residue of

**Table 4**
Bioethanol production from macroalgal biomass.

| Green Seaweeds | Pretreatment conditions | Enzyme hydrolysis conditions | Yeast/Bacterial strain and Fermentation process | Reducing Sugar g/L | Ethanol yield g/g | Theoretical yield (%) | Reference |
|---|---|---|---|---|---|---|---|
| *E.intestinalis* | Hydrothermal process (75 mM for 90min) | Celluclast 1.5 L and Viscozyme L (55 °C, 120 rpm for 54 h) | *Saccharomyces cerevisiae* KCTC 1126 (pH 5.5, 30 °C, 220 rpm for 12 h) | 40.4 | 0.21 | 41.74 | [120] |
| *U.fasciata* | H2SO4 (0.1% at 100 °C for 1 h) | Cellulase 22119 (Sodium acetate buffer pH 4.8 at 45 °C for 36 h) | *Saccharomyces cerevisiae* ($10^9$ CFU/ml 28 °C, 120 rpm for 48 h) | 20.6 | 0.45 | 88.24 | [89] |
| *U.lactuca* | | Cellulase isolated from *Cladosporium sphaerospermum* (pH 4, 25 °C, 42 h) | *Saccharomyces cerevisiae* MTCC180 (28 °C for 12 h) | 112mg/g | 0.47 | 92.16 | [94] |
| *U.pertusa* | Citric acid buffer (0.1 M sterilized using autoclave) | Meicelase (combined saccharification) (pH 5.5, 50 °C, 100 rpm for 120 h) | *Saccharomyces cerevisiae* IAM4178 (30 °C for 36 h) | 59.1 | 0.47 | 91.24 | [70] |
| | HTLP + Enzyme (150 °C, 15 min) | Cellulase & Amyloglucosidase (pH 4.8, 50 °C, 150 rpm for 24 h) | *Saccharomyces cerevisiae ATCC24858* (pH 5.5, 150 rpm, 30 °C for 24 h) | 26 | 0.48 | 93.51 | [97] |
| *Red Seaweeds* | | | | | | | |
| *G. elegans* | | Meicelase (pH 5.5 at 50 °C for 120 h) | *Saccharomyces cerevisiae* IAM4178 (30 °C for 36 h) | 49 | 0.38 | 73.63 | [70] |
| *G. amnasii* | $H_2SO_4$ (56–168mM,45–240min) | Enzyme Viscozyme L (0.024 FBG/ml) | Scheffersomyces stipitis (pH 5.5, 30 °C, 200 rpm) | 43.5 | 0.47 | 92.40 | [108] |
| | $H_2SO_4$ (2%, 150 °C for 4 h) | | Brettanomyces custersii KCCM11490 (pH 4.8–5.5, 27–30 °C) | 42.2 | 0.38 | 74.51 | [126] |
| *G. verrucosa* | $H_2SO_4$ (1.5%, at 80 °C for 2 h) | | | 87 | 0.43 | 84.29 | [60] |
| | 373 mM $H_2SO_4$ | Celluclast 1.5L and Viscozyme L (pH 5, 45 °C, 150 rpm for 72 h) | *Saccharomyces cerevisiae KCTC1126* (pH 5, 30 °C, 150 rpm for 114 h) | 20.4 | 0.48 | 94 | [110] |
| *Gracilaria* sp. | $H_2SO_4$ (0.1 N,121 °C for 30min) | Commercial enzyme (pH 4.5, 50 °C) | *Saccharomyces cerevisiae* (30 °C for 48 h) | 11.46 | 0.42 | 82.80 | [132] |
| *K. alvarezii* | Soaked in 1.6 L distilled water for 30 min and boiled at 90 °C for 1 h | Celluloclast 1.5 L & Novozyme (pH 5, 50 °C, 150 rpm for 24 h) | *Saccharomyces cerevisiae* (pH 5,35 °C, 130 rpm for 6 h) | 79.2 | 0.25 (SHF)<br>0.27 (SSF) | 49<br>52.9 | [88] |
| | $H_2SO_4$ (0.2 M, 130 °C for 15min) | | Commercial brewer's yeast (30 °C 120 rpm pH 5 for 72 h) | 20.4 | 0.21 | 41.18 | [133] |
| | $H_2SO_4$ (0.9 N, 100 °C for 1 h) 5 cycles | | *Saccharomyces cerevisiae* NCIM (5% v/v, 30 °C 150 rpm, pH 6.4–6.8 for 48 h) | 51.9 | 0.42 | 82.36 | [117] |
| *P. palmata* | Acid hydrolysis | | | 21.84 | 0.173 | 33.92 | [133] |
| *Brown Seaweeds* | | | | | | | |
| *A. crassifolia* | Citric acid buffer (0.1 M sterilized using autoclave) | Meicelase (5g/l at 50 °C for 120 h) | *Saccharomyces cerevisiae* IAM 4178 (30 °C for 36 h) | 66.3 | 0.38 | 75 | [70] |
| *L. hyperborea* | Extracted in water at 65 °C | | *Pichia angophorae* | 30 | 0.43 | 84.31 | [114] |
| | Extracted in water 121 °C for 20min | | *Zymobacter palmae* (pH 6, 30 °C) | 3.8 (mannitol) | 0.38 | 74.51 | [115] |
| *S. sagamianum* | | | *Pichia stipitis* (pH 5, 200 rpm) | 19.8 | 0.35 | 69.32 | [125] |
| *S. janponica* | $H_2SO_4$ (1 mM, 121 °C, for 120min) | Cellulase and cellobiase (pH 4.8, 50 °C, 150 rpm for 48 h) | *Saccharomyces cerevisiae* (pH 6.5, 30 °C for 36 h) | 34 | 0.41 | 80.74 | [103] |
| | Acid hydrolysis (0.1 N, 121 °C for 15min) | Celluclast 1.5L, Viscozyme L, Novoprime 959, Novoprime 969 or AMG 300L (50 °C, 150 rpm for 24 h) | *E.coli KO11 (*30 °C for 24 h) | 30.54 | 0.41 | 80.39 | [116] |
| | $H_2SO_4$ (40 mM, 121 °C for 60min) | Novozyme (Termamyl 120L) | *Pichia angophorae* KCTC 17574 (5% 30 °C at 200 rpm, 136 h) | 45.6 | 0.16 | 33.3 | [52] |
| | Shredding and enzymatic (23 °C for 30min) | | *Ethanol Red yeast* (32 °C) | 35 | 0.45 | 88.24 | [126] |
| *U. pinnatifida* | Dilute acid (5% H2SO4, 120 °C for 24 h) | Celluloclast 1.5L & Novozyme188 (pH 4.6, 45 °C) | E.coli (pH 7170 rpm, 37 °C for 12 h) | 20 | 0.144 | 28.2 | [107] |

*L. japonica* was subjected to acid pretreatment followed by enzyme hydrolysis, an ethanol yield of 14 g/L was obtained from 34 g/L of reducing sugar achieving 41.2% conversion efficiency [103]. Similarly, *K. alvarezii* dried residues after extraction of sap were utilized for production of bioethanol [117]. Wet biomass of *G. amansii* was used as bioethanol feedstock, *Brettanomyces custersii* KCTC 18154P strain was utilized for fermentation of the hydrolysate due to the ability of the strain in exhibiting co-fermentability. Utilization of raw or wet macroalgal biomass is not feasible for bioethanol production due to high viscosity of the medium for fermentation [118]. In green seaweeds, studies have focused on conversion of cellulose and starch to bioethanol. Whereas conversion of other sulphated polysaccharides such as Ulvan to produce ethanol is yet to be explored [60]. Non availability of natural strains capable of fermenting alginate, a major polysaccharide of brown algae [60], makes it difficult to achieve higher ethanol production.

Fermentation is carried out in two process, Separate Hydrolysis and

Fermentation (SHF) and Simultaneous Saccharification and Fermentation (SSF) [119]. SHF involves hydrolysis and fermentation performed sequentially, whereas SSF involves performing simultaneous hydrolysis and fermentation [74]. *Saccharina japonica, Undaria pinnatifida and Poryphyra* were subjected to SSF using *Pichia angophorae* KCTC strain and obtained 7.7 g/L of ethanol [52]. SHF process is faster but presence of inhibitors resulting from acid pretreatment has significant impact on yeast microorganisms. SSF is preferred over SHF as the sugars released are readily metabolized by yeast microorganisms, which results in a faster ethanol production rate and lower capital costs. SSF has a drawback due to the difference in temperature optima of *cellulase* (50 °C) and fermenting microorganism (35 °C). SHF and SSF of *Enteromorpha intestinalis* or *Ulva (Enteromorpha) intestinalis* produced 8.6 g/L and 7.6 g/L with 30.5% and 29.6% fermentation efficiency respectively. Conversion of ethanol to acetic acid by yeast and suboptimal temperature of 30 °C than the optimum temperature of 55 °C for enzyme activity was attributed to the lower ethanol yield in SSF [120].

Higher temperature shortens the exponential phase of the yeast cell [121] affecting the ethanol production. However, this has been overcome through thermotolerant yeast strains or cell immobilization technique which allows higher processing temperatures [120–123]. Thermotolerant yeast species such as *Candida tropicalis* and *Kluyveromyces marxianus* (38–45 °C) are mainly utilized to produce bioethanol from lignocellulosic biomass [123,124].

Bioethanol production from macroalgae utilized commercial yeast strains such as *S. cerevisiae* KCTC 1126 [110,111], MTCC 180 [60], IAM 4178 [70], ATCC 24858 [97], KCTC 17574 [52], *Pichia stipitis* [125], *Pichia angophorae* [114], *Scheffersomyces stipitis* [108], *Brettanomyces custersii* KCCM 11490 [126], Ethanol red yeast [36] and bacterial strains such as *Zymobacter palmae* [115] and *Escherichia coli* SJL2526 [107]. Fermentation of macroalgal polysaccharides is carried out at pH 4.5–6.8 and temperature 25–30 °C and the incubation time is largely strain dependent. The yeast growth rate is dependent on temperature and fermentation time [131]. However, exponential phase of yeasts are shortened at large temperatures and pH < 4, requiring longer incubation for higher ethanol production, as reported in *S.cerevisiae* BY4742 [64]. Shorter fermentation time causes inadequate growth of microorganisms resulting in inefficient fermentation [132].

In order to optimize ethanol yield and improve substrate utilization range [129], studies focused on immobilization of yeast cells [121-130]. Immobilized yeast cells have enhanced the ethanol productivity and reusable for 15 cycles with bacterial cellulose-alginate sponge [121]. Free and immobilized strains were used for molasses fermentation. Free cells were unable to ferment at temperatures greater than 38 °C, compared to immobilized yeast. Immobilized yeast strains exhibited both psychrophilic and thermo-tolerant characteristics, suitable for fermentation in a wide range of temperatures [131] and increased ethanol yield and higher cellular stability, while reducing downstream processing expenses [132]. Fermentation of *U.lactuca* biomass done using immobilized *Saccharomyces cerevisiae* strain, yielded ethanol (concentration of 12 g/g of sugar) with conversion efficiency of 47.1% [130]. Table 4 summarizes ethanol yield from the three types of macroalgae along with the process conditions and strains utilized for hydrolysis (pretreatment) and fermentation. Fermentation of red seaweed *Gracilaria* using free yeast cells yielded 0.41 g/g of ethanol and immobilized yeast cells yielded 0.42 g/g achieving 80 and 82.8% fermentation efficiency [132].

Studies emphasize on production of bioethanol from readily available carbohydrates of brown and red seaweeds, but utilization of red and brown seaweeds such as *Kappaphycus, Gelidium, Gracilaria, Sargassum,* and *Laminaria* have the likelihood to override the existing multi-billion dollar hydrocolloid industry [89]. This can be addressed in two ways: (i) utilization of cellulosic rich residue after hydrocolloid extraction, (ii) exploration of green seaweeds which are abundantly recorded from various estuaries and abandoned aquaculture ponds across maritime states in India [134]. Green seaweeds exhibit characteristics of a potential feedstock for biofuel production by their cosmopolitan distribution, wide environmental tolerance, higher growth rates and year around productivity [135]. In India, seaweeds are seldom consumed as a food source, and the suitability for biofuel production is still underexplored as several species accumulate different levels of carbohydrate. Seaweeds contain low amounts of polysaccharides composed of glucose, highlighting the need for ethanol production from carbohydrates including sulphated polysaccharides, sugar acids and sugar alcohols. Not all the reported microorganisms are capable of fermenting these sugars and a major limitation is lack of tractable microorganisms that can efficiently ferment all sugars extracted from seaweed into ethanol. Isolation of yeast strains to ferment both pentose (C5) and hexose (C6) sugars are vital for achieving high ethanol yield. In this backdrop, the current study explores bioethanol prospects from viable feedstock habituated in the west coast of India, which involves:

(i) screening and prioritizing potential macroalgal feedstock for bioethanol production based on the biochemical composition;
(ii) comparative performance analysis of chemical and biological pretreatment method for extraction of sugar from macroalgal biomass; and
(iii) bioethanol potential assessment of green seaweeds and comparative analysis of ethanol yield across macroalgal species.

## 2. Materials and methods

### 2.1. Macroalgal resource assessment and screening of potential feedstock for biofuel production

Macroalgal sampling has been done monthly during low tide period. Due to the immersion and emersion periods in rocky shore, a 0.25 $m^2$ random quadrats sampling was carried out. Algal biomass within the quadrat were handpicked, species-wise sorted and washed in seawater to remove adhering epiphytes. Collected samples were identified using standard identification keys [61,136] and standing biomass was determined, which is expressed in g/$m^2$. In areas, where seaweeds were not accessible, only qualitative data such as the presence of seaweed species were recorded with location details using high spatial resolution virtual remote sensing data (Google Earth).

### 2.2. Biochemical composition along Central West Coast of India

Species samples were washed thoroughly in seawater, followed by tap water to clean debris of extraneous material. Cleaned seaweeds were sun dried in the shade for 2–3 days, followed by drying in the hot air oven, below 60 °C till constant weight is attained. The dried material was pulverized and sieved to obtain a uniform particle size. The powder was preserved in a zip lock pouches for subsequent analysis of total carbohydrate [137], cellulose [138], protein [139], and lipid [140] contents. Carbon, hydrogen and nitrogen were estimated using CHN elemental analyser. These samples were analyzed in triplicates and the mean values were considered for further calculations and results are expressed in mean ± SD.

### 2.3. Macroalgal pretreatment process

Sugar from macroalgae was extracted using boiling water, sonication, dilute acid and alkali pretreatment. Seaweed was boiled in a 10 ml water for 30 min, sonication was carried out using an ultrasonic bath (frequency 35 kHz) for 30 min. Dilute acid pretreatment involved 0.7 N $H_2SO_4$ and alkali pretreatment was carried out using 5 N NaOH for 30 min. Neutralization was carried out for *U.lactuca* and *E.intestinalis* acid hydrolysate using $Na_2CO_3$, NaOH, Activated charcoal and $Ca(OH)_2$. The sugar removal (%) by these agents was calculated as per equation (Eq. (1)).

$$\% \ Sugar \ removal = \frac{(A - B)}{A} X \ 100 \quad (1)$$

where, A is the amount of reducing sugar (mg/g) before treatment, and B is the amount of reducing sugar (mg/g) after treatment [141].

### 2.4. Enzyme hydrolysis

Enzyme was isolated from marine bacteria *Vibrio parahaemolyticus* [90] and hydrolysis was carried out for direct biomass and acid pretreated biomass for *U.lactuca* and *E.intestinalis* to demonstrate the effect of acid pretreatment in enzyme hydrolysis. Enzyme hydrolysis was carried out at 55 °C up to 48 h and reducing sugar was estimated every 6 h.

### 2.5. Bioethanol production from selected macroalgae

Dried biomass (5% w/v) was pretreated using dilute (0.7 N) $H_2SO_4$ to release sugars and estimated for reducing sugars by 3, 5 dinitrosalicylic acid method [142]. Acid hydrolysate neutralized using $Na_2CO_3$. Released fermentable sugars were subjected to fermentation using cashew yeast (CY) and toddy yeast (TY) at 30 °C for 18 h in Separate hydrolysis and fermentation (SHF). Saccharification and Fermentation (SSF) was carried out using 2% (w/v) pretreated biomass and 6% (v/v) enzyme and yeast were added to the medium and fermented using CY and TY at 55 °C for 18 h. Co-fermentation of algal hydrolysate was also carried out for SHF and SSF. Ethanol was estimated using GC-FID. Bioethanol production from all the macroalgal biomass was estimated based on published literatures and the probable relationship of ethanol yield and multivariate is determined through regression analyses.

## 3. Results and discussions

### 3.1. Scope for bioethanol production from macroalgal biomass

The availability of spectrum of species amenable for biofuel production is one of the unique aspect of algae when compared to other advanced feedstock [35]. Production of different types of biofuels (bioethanol, diesel, bio-oil, and bio-hydrogen) is optimized by prioritizing species.

Characteristics of prioritized macroalgal feedstock for sustainable biofuel production are (i) availability throughout the year (during all seasons) and (ii) presence of higher quantum of sugar.

#### 3.1.1. Macroalgal feedstock availability and selection

Indian coast has about 1153 marine algal species belonging to 271 genera, of which 60 species are commercially important. Rocky beaches, mudflats, estuaries, coral reefs and lagoons are the preferred habitats for macroalgae. Indian coast harbors predominantly intertidal and subtidal algal communities [143]. Macroalgal feedstock (for bioethanol production) distribution along the Indian Coast and islands were compiled from various secondary data sources [144–150] along with the locations based on the current field investigations is represented in Fig. 4. Seaweed species belonging to Laminaria genera (Brown algae or Phaeophyta) are not recorded in India and these are algae of cold waters and certain brown algae of orders Dictyotales and Fucales (e.g. *Sargassum*) are distinctly warm-water plants [151] are abundantly spread and are recorded from Indian Coast. *Kappaphycus alvarezii*, a red algae is native of Indonesia and Philippines and introduced in India for commercial cultivation purpose [152], which has now colonized in various parts of Gulf of Mannar.

Setting up of bio refinery necessitates easier cultivation strategies apart from ensuring the availability of feedstock during all seasons. During the current field investigations covering 24 months, 25 seaweed species belonging to 19 genera were recorded from intertidal zone.

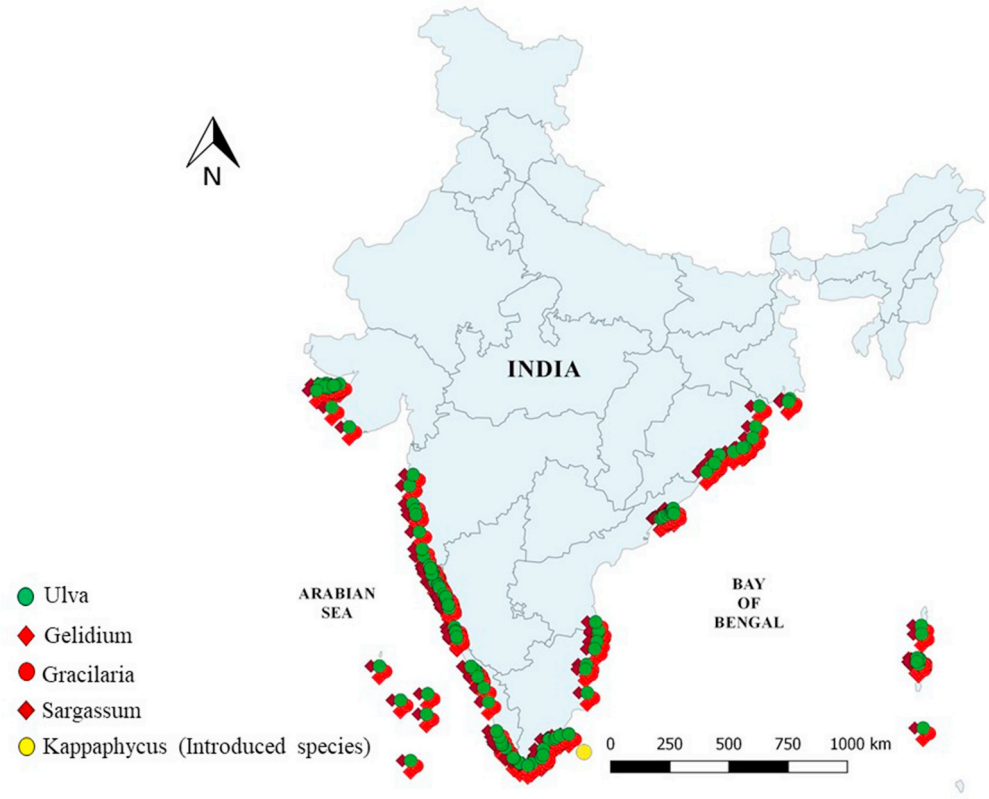


**Fig. 4.** Distribution of potential macroalgal feedstock of Indian coast.

Among these, eight seaweed species abundantly grows during post-monsoon season in the Central West Coast. These species include *Enteromorpha intestinalis* (938.5 g/m$^2$), *Ulva lactuca* (1024.5 g/m$^2$), *Chaetomorpha media* (441.8 g/m$^2$), *Gracilaria corticata* (1039.5 g/m$^2$), *Gelidium pusillum* (205.4 g/m$^2$), *Grateloupia lithophila* (196.6 g/m$^2$), *Sargassum ilcifolium* (1175.7 g/m$^2$) and *Padina tetrastromatica* (1506.7 g/m$^2$), which were considered for further analysis of biochemical composition (Fig. 5). It was seen that green seaweeds; *U.lactuca* and *E. intestinalis*, and red seaweed *G.corticata* occurred in sufficient quantity during all seasons. Occurrence of *Ulva* and *Enteromorpha* in all the seasons is attributed to their euryhaline nature, whereas *G.corticata* species are strictly marine and are restricted to open ocean environment [153]. Year round optimal biomass production of these macroalgal species, overcomes the seasonal constraints faced by the first and second generation biofuel feedstock [45].

### 3.1.2. Biochemical composition of selected seaweeds

Macroalgal species with the higher quantum of sugar plays an important role as the composition of sugar influences the ethanol yield. Biochemical composition of chosen macroalgae were carried out focusing mainly on higher carbohydrate content which is an essential parameter for prioritizing feedstock for bioethanol production (Table 5). Algal biomass are composed of large quantities of carbohydrates in the cell wall mostly in the form of structural (cellulose) and storage (starch) polysaccharides, that serve as substrate for fermentation. Highest total carbohydrate was recorded from green seaweed *Ulva lactuca* (62.15 ± 12.8%) followed by *G. lithophila* (60.5 ± 11.4%), *G.pusilum* (50.1 ± 3.4%) and *E.intestinalis* (40.1 ± 14.6%). Among these, *G.lithophila* and *G.pusilum* have lower biomass production as compared to *Ulva* and *Enteromorpha,* which makes them unsuitable as feedstock for biofuel production. Protein ranged from 3.7 ± 0.94 to 27.3 ± 15.21%. Highest protein content was recorded from C. *media* (27.3 ± 15.21%) followed by *E. intestinalis* (20.4 ± 0.67%) and *U. lactuca* (17.3 ± 1.68%). In green seaweed Ulva, protein concentration ranged from 9 to 33%. Lowest protein recorded was from *G. lithophila* (3.7 ± 0.94%). In general, protein fraction is lower in brown seaweeds (3–15% DW) compared to green or red seaweeds (10–47% DW) similar to the earlier report [154]. Lipid ranged from 0.5 ± 0.2 to 6 ± 4.4%. Highest lipid was estimated in *U. lactuca* (6 ± 4.4%) followed by *G. pusillum* (3.7 ± 1.4%), *P. tetrastromatica* (2.8 ± 0.3%). Lowest lipid content estimated in *G. corticata* (0.5 ± 0.2%). Transesterification of lipids extracted from *Enteromorpha* biomass yielded 90.6% biodiesel [135], which highlights that *Enteromorpha* as a promising feedstock for biodiesel production.

Elemental analysis of the seaweeds indicates 25.31–37.95% of carbon, 4.52–6.48% hydrogen and 1.88–4.36% Nitrogen. Highest carbon, hydrogen and nitrogen content were recorded respectively from *G. pusillum* (C: 37.95%), *G.pusillum* (H: 6.48%) and *E.intestinalis* (N: 4.36%) respectively. Higher cellulose content was estimated in *U.lactuca* (14.03 ± 0.14%), followed by *E.intestinalis* (12.10 ± 0.53%) and C.*media* (10.53 ± 0.17%) least cellulose content was estimated in *G. corticata* (0.87 ± 0.07%). Cellulose is a glucan present in green seaweeds, which can be easily hydrolysed by using enzyme and subsequently fermented to produce bioethanol. Green seaweeds are rich in cellulose content (>10%) [37,89,105] when compared to red and brown seaweed (2–10%).

### 3.1.3. Reducing sugar extraction using pretreatment methods

Chosen seaweeds composed of all the three types of algae (green, red and brown) with varied amounts of sugar and the complexity of the seaweed is reflected between structural and carbohydrate components [105,155,156]. It is therefore important to carefully choose the pretreatment process based on the biomass and an appropriate efficient pretreatment process to achieve a high yield of sugar for low energy input. The reducing sugar was determined using various pretreatment method as shown in Fig. 5. Pretreatment using boiling water yielded very low reducing sugar and it was observed that red and brown algae formed gel during this pretreatment process due to the presence of sugars like Agar, Carrageenan and Alginate which possess hydrocolloid properties [70,157,158]. It is seen that, liquid hot water pretreatment releases most of oligosaccharides [159]. *U. lactuca* biomass was subjected to four different pretreatments namely; ethanol organ solvent, alkaline, liquid hot water and ionic liquid treatments. Organo solvent and liquid hot water treatment produced highest glucan recovery of 80.8 g/100 g DW and 62.9 g/100 g DW respectively [160]. Sonication pretreatment also yielded lower reducing sugar, since sonication is most commonly used in extraction of lipid from microalgae [161], highest fatty acid composition of C16:0 and C18:1 was achieved in *Spirogyra* sp. employing sonication as cell disruptions technique [40]. Higher reducing sugar was extracted from all types of seaweeds during the dilute acid pretreatment. Dilute acid reduces the degree of polymerization resulting in recovery of 80–90% of hemicellulose exposing cellulosic fraction accessible to enzyme digestion [162]. Pre-treated *Palmaria palmata,* a red alga was exposed to acid hydrolysis, generated 218 mg/g of reducing sugar [133] and *Grateloupia lithophila* gave 191 mg/g of reducing sugar in the current study. Hence, it is feasible to obtain fermentable sugars with lower inhibitor concentration using extremely lower acid concentration [163]. Benefits of treating biomass with extremely low acid concentration simplifies downstream process such as neutralization and waste treatment. Reducing sugar of 14.7 g/L was obtained from *E.intestinalis* by dilute acid concentration of

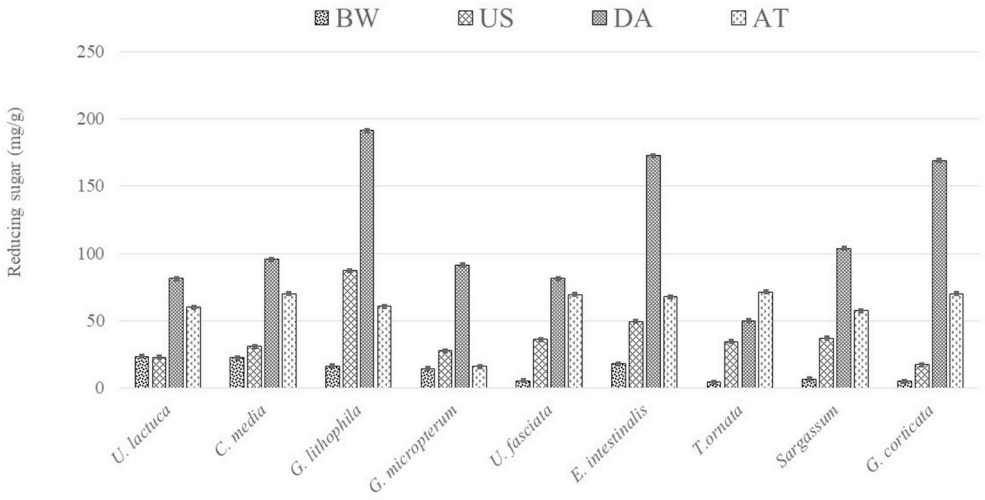


**Fig. 5.** Reducing sugar (mgg$^{-1}$) extracted using various pretreatment methods for selected seaweeds.

**Table 5**
Biochemical composition (%) of seaweeds along Central West Coast of India.

| Seaweeds | Total carbohydrate | Soluble carbohydrate | Insoluble carbohydrate | Protein | Lipid | Cellulose | C | H | N |
|---|---|---|---|---|---|---|---|---|---|
| *U.lactuca* | 62.15 ± 12.8 | 5.5 ± 0.07 | 56.5 ± 12.7 | 17.3 ± 1.68 | 6 ± 4.4 | 14.03 ± 0.14 | 25.31 | 5.44 | 2.61 |
| *E.intestinalis* | 40.1 ± 14.6 | 7.5 ± 0.02 | 32.5 ± 14.5 | 20.4 ± 0.67 | 2.8 ± 0.1 | 12.10 ± 0.53 | 33.00 | 6.44 | 4.36 |
| *C.media* | 25.5 ± 11.2 | 5.3 ± 0.02 | 20.1 ± 11 | 27.3 ± 15.21 | 0.6 ± 0.01 | 10.53 ± 0.17 | 30.14 | 5.31 | 3.28 |
| *G.corticata* | 28.2 ± 11.1 | 13.4 ± 0.01 | 14.7 ± 11 | 14.4 ± 1.33 | 0.5 ± 0.2 | 0.87 ± 0.07 | 26.46 | 5.01 | 1.89 |
| *G. pusillum* | 50.1 ± 3.4 | 4.7 ± 0.01 | 45.4 ± 3.4 | 8 ± 2.04 | 3.7 ± 1.4 | 1.55 ± 0.05 | 37.95 | 6.48 | 3.45 |
| *G. lithophila* | 60.5 ± 11.4 | 14.8 ± 0.01 | 45.7 ± 11 | 3.7 ± 0.94 | 2.4 ± 1.3 | 6.23 ± 0.16 | 29.60 | 6.15 | 2.52 |
| *S. ilcifolium* | 26.4 ± 13.4 | 3.6 ± 0.01 | 22.7 ± 13 | 12.4 ± 0.67 | 1.9 ± 0.01 | 1.30 ± 0.09 | 26.20 | 4.52 | 1.88 |
| *P.tetrastromatica* | 32.5 ± 13.23 | 3.5 ± 0.02 | 28.9 ± 13.2 | 9.7 ± 1.76 | 2.8 ± 0.3 | 1.48 ± 0.07 | 30.68 | 5.54 | 2.35 |

75 mM $H_2SO_4$ [120]. Sequential acid hydrolysis was carried out to concentrate the sugar: hydrolysate of first cycle was utilized as hydrolyzing liquid for the 2nd cycle and up to 5th cycle [117] and generated 72 g/L of reducing sugar at the end of 5th cycle during 0.9 N $H_2SO_4$ hydrolysis from the seaweed granules. However, loss of liquid volume encountered in the hydrolysate that is attributed to the sorption loss by residue. Pulverized wet biomass of *Gelidium amansii,* red seaweed was subjected to continuous acid hydrolysis which yielded higher galactose and lower inhibitor concentration than batch reactor [126].

Utilization of red and brown seaweeds biomass for bioethanol production can lead to debate on hydrocolloid versus fuel affecting the existing multibillion hydrocolloid industry [89]. Therefore, for further processes of detoxification, enzyme hydrolysis and fermentation, two seaweeds *Ulva lactuca* and *Enteromorpha intestinalis* were selected as both the species satisfy the criteria of potential feedstock for bioethanol production such as; annual availability, carbohydrate rich biomass, producing higher reducing sugar concentration, ease of harvest by mechanical means, amenable to transplanting and reproducing prolifically in given environment [89].

*3.1.3.1. Detoxification of acid hydrolysate.* Detoxification of inhibitors (HMF and LA) resulting from acid hydrolysis was carried out using activated charcoal [164], which removed 38.8% LA and 70.37% HMF. However activated charcoal also removed sugars 14.5% glucose and 20.3% galactose present in the hydrolysate [141]. In the current study, highest sugar removal of 63.5% and 52% was recorded from activated charcoal for *U.lactuca* and *E.intestinalis* acid hydrolysate respectively. Similarly, $Ca(OH)_2$ was used for neutralizing the hydrolysate, which led to gypsum (calcium sulphate) formation, which was removed through filtration [117] also calcium ions catalyzes alkaline degradation of mono-saccharides in the over liming process, resulting in removal of fermentable sugars [141]. Similar results were observed in the current study, where Ca $(OH)_2$ treatment removed sugar from *U.lactuca* (56.1%) and *E.intestinalis* (23.3%) acid hydrolysate. Least effect of sugar removal was recorded in $Na_2CO_3$ neutralization process (Table 6) *U.lactuca* (39.8%) and *E.intestinalis* (14.7%).

### 3.2. Enzyme hydrolysis

Enzyme hydrolysis of *U.lactuca* and *E.intestinalis* yielded lower reducing sugar, compared to pretreated biomass during the incubation period. In order to overcome recalcitrance in second generation feedstock, the biomass was pretreated prior to enzyme hydrolysis. The pretreatment removed the lignin and hemicellulose exposing the cellulose fibers for enzyme hydrolysis [64,66,99,165]. Pretreated biomass is then subjected to enzyme hydrolysis, where *cellulase* enzymes bind to cellulose and disintegrate it into simple sugars (glucose). Algal biomass in the current study was pretreated using dilute acid and then subjected to enzyme hydrolysis (Fig. 6a and b). Pre-treatment breaks down the crystalline cellulose structure, the major constituents in the biomass cell walls and maximize enzymatic conversion to reducing sugars [53,105,155,166]. Despite longer incubation period for sugar release, enzyme hydrolysis is widely preferred due to the absence of any inhibitors formation [70].

### 3.3. Bioethanol production from selected macroalgae

Production of bioethanol from carbohydrate rich biomass is economical and sustainable. However, investigations are focused on improving the yield of bioethanol from seaweeds using appropriate microorganisms that have capabilities to convert all sugars present in the seaweeds and achieve ethanol yield of 0.47 g/g reducing sugar [89,167,168]. SHF and SSF process was carried out for *E.intestinalis* and *U. lactuca* feedstock.

#### 3.3.1. SHF and SSF

Macroalgal feedstock *E.intestinalis* (EI) and *U.lactuca* (UL) were pretreated using 0.7 N and 0.5 N $H_2SO_4$ respectively. Seaweed hydrolysate was neutralized and then subjected to fermentation using CY and TY strain and conversion efficiency calculated assuming the sugar available in the hydrolysate are fermentable (Table 7). Higher ethanol yield of 1.63 g and 25.8% efficiency was recorded for EITY combination, whereas ULCY produced lower ethanol yield of 0.37 g achieving 12.1% efficiency. Co-fermentation of *E.intestinalis* hydrolysate yielded 0.8 g with 21.7% efficiency, whereas *U.lactuca* yielded 0.63 g/g achieving 20.4% efficiency [120]. Pretreated *E.intestinalis* to 75 mM $H_2SO_4$ and subjected to SHF and obtained ethanol of 8.6 g/L (0.86 g) achieving 30.5% efficiency at 48 h. At the end of fermentation 10 g/L of reducing sugar remained unutilized indicating presence of non-fermentable sugar not consumed by the yeast *S.cerevisiae. U.fasciata* enzyme hydrolysate was subjected to fermentation and 1.28 g of ethanol yield was produced achieving 88.27% efficiency indicating higher reducing sugar conversion efficiency and ethanol yield than those reported for red and brown seaweeds [169].

SSF of *E.intestinalis* produced 7.6 g/L with 26.9% efficiency, lower yield was attributed to the suboptimal temperature of 32 °C which is unsuitable for enzyme hydrolysis [120]. In this study, SSF process (Table 8) produced higher efficiency when compared to SHF. The

**Table 6**
Comparison of reducing sugar removal after neutralization process.

| | Ulva lactuca | | | Enteromorpha intestinalis | | |
|---|---|---|---|---|---|---|
| | Before treatment (mg/g) | After treatment (mg/g) | % Sugar removal | Before treatment (mg/g) | After treatment (mg/g) | % Sugar removal |
| $Na_2CO_3$ | 206.34 | 124.12 | 39.8 | 201.09 | 171.53 | 14.7 |
| NaOH | | 113.78 | 44.9 | | 153.88 | 23.5 |
| Activated Charcoal | | 75.29 | 63.5 | | 96.50 | 52.0 |
| $Ca(OH)_2$ | | 90.51 | 56.1 | | 154.18 | 23.3 |

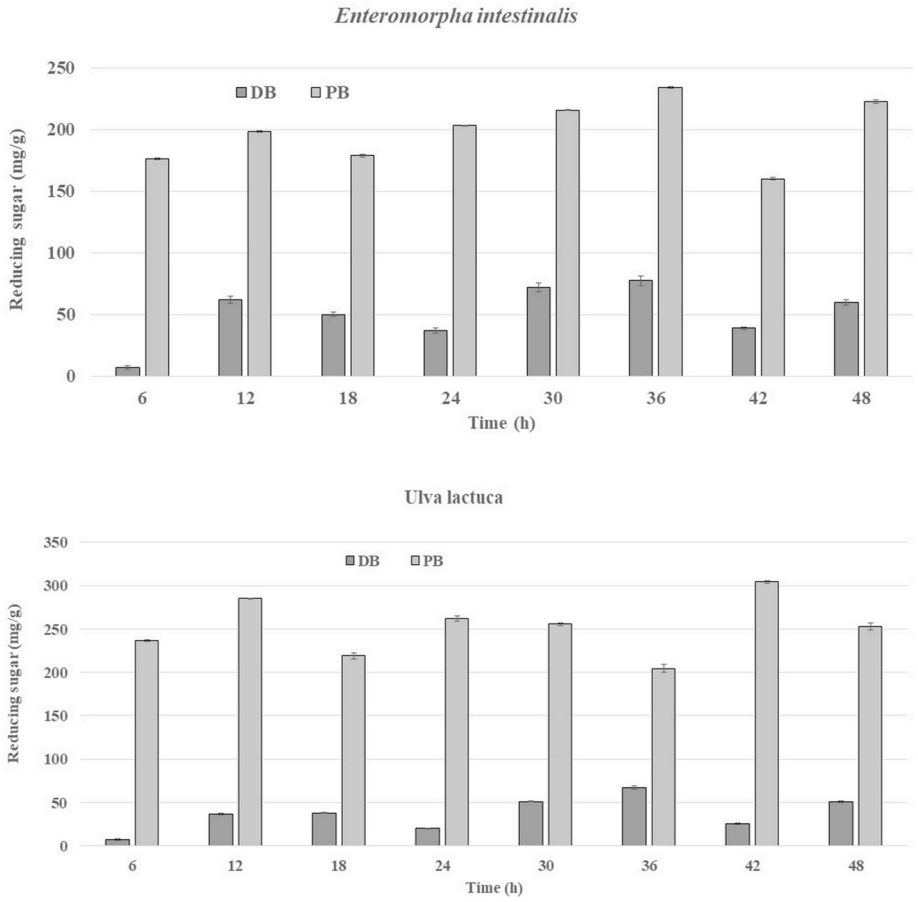


**Fig. 6.** a) Enzyme hydrolysis for direct biomass (DB) and acid pretreated biomass (PB) for *E.intestinalis.* b) Enzyme hydrolysis for direct biomass (DB) and acid pretreated biomass (PB) for *U.lactuca.*

**Table 7**
Fermentation of macroalgal feedstock by the process of separate hydrolysis and fermentation (SHF) and Separate hydrolysis and Co-fermentation (SHCF).

| Macroalgal feedstock | Process | Combinations | Biomass (g) DW | Initial sugar (g) | Final sugar (g) | Fermented sugar (g) | Ethanol yield (g) | Theoretical yield | Efficiency (%) |
|---|---|---|---|---|---|---|---|---|---|
| *Enteromorpha intestinalis* | SHF | EICY | 50 | 22.5 | 10.71 | 11.79 | 1.40 | 6.01 | 23.4 |
| | | EITY | | | 10.13 | 12.37 | 1.63 | 6.31 | 25.8 |
| | SHCF | EITYCY | | | 15.09 | 7.41 | 0.82 | 3.78 | 21.7 |
| *Ulva lactuca* | SHF | ULCY | | 19.2 | 13.17 | 6.03 | 0.37 | 3.07 | 12.1 |
| | | ULTY | | | 12.72 | 6.48 | 0.52 | 3.31 | 15.6 |
| | SHCF | ULTYCY | | | 13.10 | 6.10 | 0.63 | 3.11 | 20.4 |

CY: Cashew yeast; TY: Toddy yeast

**Table 8**
Fermentation of macroalgal feedstock by the process of Simultaneous Saccharification and Fermentation (SSF) and Simultaneous Saccharification and Co-fermentation.

| Macroalgal feedstock | Process | Combination | Biomass (g) DW | Cellulose (g) | Final sugar (g) | Fermented sugar (g) | Ethanol yield (g) | Theoretical yield | Efficiency (%) |
|---|---|---|---|---|---|---|---|---|---|
| *Enteromorpha intestinalis* | SSF | EICY | 20 | 1.8 | 0.34 | 1.46 | 0.25 | 0.74 | 33.3 |
| | | EITY | | | 0.56 | 1.24 | 0.49 | 0.63 | 77.4 |
| | SSCF | EITYCY | | | 0.52 | 1.28 | 0.27 | 0.65 | 41.8 |
| *Ulva lactuca* | SSF | ULCY | | 1.7 | 0.54 | 1.16 | 0.33 | 0.59 | 54.9 |
| | | ULTY | | | 0.54 | 1.16 | 0.39 | 0.59 | 65.5 |
| | SSCF | ULTYCY | | | 0.66 | 1.04 | 0.60 | 0.53 | 113.0 |

CY: Cashew yeast; TY: Toddy yeast

combination of EITY produced higher ethanol yield of 0.49g/g achieving 77.4% efficiency. Simultaneous saccharification and co-fermentation (SSCF) of *E. intestinalis* produced 0.27 g/g of ethanol with 41.8% efficiency whereas *U.lactuca* produced efficiency of 113% indicating fermentation of sugars other than glucan. *Candida shehatea, Scheffersomyces stipitis (Pichia stipitis),* and *Pacchysolen tannophilus* are the most promising yeast species for conversion of Xylose [112], which can be used in combination with *S.cerevisiae* in SSCF process to obtain

higher ethanol yield [104]. subjected cellulosic residue of *Kappaphycus alvarezii* along with galactose synthetic medium to SSCF using single strain *S.cerevisiae* CBS1782 and recorded 64.3 g/L of ethanol indicating utilization of galactose sugar along with glucose. Co-fermentation of corn stover using *S.cerevisiae* and commercial strain TMB3400 yielded 40g/L or ethanol with 59% theoretical efficiency [74]. *S.cerevisiae* and *S. stipitis* were used for SSCF of Rice straw and produced 15.2g/L of ethanol [170].

Seaweeds contain low amounts of polysaccharides composed of glucose. Production of ethanol, therefore, needs to be from carbohydrates including sulphated polysaccharides, sugar acids and sugar alcohols. However, inability of microorganisms in fermenting all sugars present in seaweeds into ethanol is a major drawback. Therefore, isolation of yeast strains to ferment both pentose (C5) and hexose (C6) sugars are vital for achieving higher ethanol yield.

However, recent studies are focusing on non-controversial cellulosic residue following extraction of hydrocolloid from seaweed biomass [60]. But the lower cellulose content of residue prevents it from being a viable feedstock option considering the emergent demand for bioethanol. This necessitates the selection of seaweed species with higher cellulose content together with higher growth rate for sustainable bioethanol production [89,171]. Red algae has more agar and carrageenan and brown algae has more algin and lower cellulose compared to green seaweeds, that have cellulose making up to 70% of their dry weight (as Cladophorales and Ulvales) [176].

It is evident from the experimental results, that green algae are apt as potential feedstock due to higher carbohydrate and cellulose contents. Higher growth rates have been recorded for green seaweeds ranging from 19.15 to 24.25% when compared to red macroalgae (3–8%) [89,177,178]. Green seaweeds have production potential that is 2–20 times that of conventional terrestrial energy crops [179]. Red and brown seaweed's lower cellulose content of residue and lower growth rate prevents it from being a viable feedstock to meet the growing demand for bioethanol. Comparison of ethanol yield from other potential macroalgal feedstock are represented in Table 9.

### 3.3.2. Multivariate analysis of process condition for bioethanol production

Downstream process of bioethanol production from macroalgal biomass is dependent on various factors such as dilute acid pretreatment conditions (acid concentration, temperature, incubation time), enzyme hydrolysis conditions (pH, temperature and incubation time), reducing sugar and fermentation conditions (temperature and incubation time). In order to understand influence of each of these factors in ethanol production, multivariate regression analysis (Table 10) was performed and the probable relationship is given by equation (2). Overall this model explains 97.3% variation in the data.

$$Y = -2.75 - 4.09(X_1) + 0.12(X_2) - 0.13(X_3) + 10.61(X_4) - 0.31(X_5) - 0.04(X_6) + 0.32(X_7) - 1.23(X_8) - 0.12(X_9) \quad (2)$$

Where.

**Table 9**
Comparison of reducing sugar, ethanol yield and percent theoretical yield from various macroalgal biomass.

| Seaweed species | | Reducing sugar g/l | Ethanol yield g/g | %Theoretical yield | References |
|---|---|---|---|---|---|
| Green Seaweeds | *Chaetomorpha media* | 27.79 | 0.057 | 10.15 | * |
| | *Ulva fasciata* | 21.82 | 0.43 | 83.66 | [89] |
| | *Ulva lactuca* | 1.12 | 0.39 | 77.03 | [94] |
| | *Ulva lactuca* | 35.43 | 0.23 | 45.62 | * |
| | *Ulva pertusa* | 43 | 0.43 | 84.36 | [70] |
| | *Ulva pertusa* | 26 | 0.48 | 93.51 | [97] |
| | *Enteromorpha intestinalis* | 48.96 | 0.25 | 49.37 | * |
| | *Enteromorpha intestinalis* | 45.56 | 0.21 | 41.74 | [120] |
| Red Seaweeds | *Gelidium elegans* | 49 | 0.38 | 73.63 | [70] |
| | *Gelidium amansii* | 43.5 | 0.47 | 92.40 | [108] |
| | | 42.2 | 0.38 | 74.51 | [126] |
| | | 7.93 | 0.42 | 82.34 | [172] |
| | | 7.93 | 0.48 | 93.46 | |
| | *Gracilaria verrucosa* | 34.63 | 0.43 | 84.31 | [60] |
| | | 19.70 | 0.43 | 84.31 | [110] |
| | *Gracilaria corticata* | 57.90 | 0.01 | 0.98 | * |
| | *Gracilaria salicornia* | 13.8 | 0.079 | 15.49 | [173] |
| | *Gracilaria* sp. | 11.46 | 0.42 | 82.35 | [132] |
| | *Grateloupia lithophila* | 44.45 | 0.09 | 17.85 | * |
| | *Gelidium pusillum* | 36.96 | 0.04 | 8.49 | * |
| | *Eucheuma cottonii (Kappaphycus alvarezii)* | 11 | 0.45 | 89.13 | [88] |
| | | 81 | 0.45 | 88.24 | [104] |
| | | 20.4 | 0.21 | 41.18 | [164] |
| | | 51.9 | 0.42 | 82.36 | [117] |
| | *Palmaria palmata* | 21.84 | 0.173 | 33.92 | [133] |
| Brown Seaweeds | *Alaria crassifolia* | 67.20 | 0.38 | 74.40 | [70] |
| | *Laminaria hyperborea* | 30 | 0.43 | 84.31 | [114] |
| | *Padina tetrastromatica* | 17.9 | 0.01 | 2.53 | * |
| | *Sargassum sagamianum* | 25.9 | 0.386 | 75.69 | [116] |
| | *Sargassum sagamianum* | 2.55 | 0.89 | 174.55 | [174] |
| | *Sargassum sagamianum* | 19.8 | 0.35 | 69.32 | [125] |
| | *Sargassum ilcifolium* | 27.04 | 0.05 | 9.60 | * |
| | *Sargassum fluvellum* | 9.6 | | | [54] |
| | *Saccharina japonica* | 6.72 | 0.343 | 67.25 | [163] |
| | *Saccharina japonica* | 34 | 0.41 | 80.74 | [103] |
| | *Saccharina japonica* | 45.6 | 0.17 | 33.11 | [52] |
| | *Saccharina japonica* | 75 | 0.34 | 67.45 | [116] |
| | *Saccharina latissima* | 35 | 0.45 | 88.24 | [36] |
| | *Undaria pinnatifida* | 42.9 | 0.22 | 43.05 | [120] |
| | *Undaria pinnatifida* | 20 | 0.144 | 28.24 | [107] |
| Seagrass | *Zosteria marina* | 92 | 0.059 | 11.5 | [175] |

*Current work.

**Table 10**
Coefficients and SE values of multivariate regression analysis.

| Process | Process condition | Coefficients | Standard Error |
|---|---|---|---|
| Dilute acid pretreatment | Concentration | −4.09 | 1.14 |
| | Temperature | 0.12 | 0.29 |
| | Incubation Time | −0.13 | 0.67 |
| Enzyme hydrolysis | pH | 10.61* | 2.44* |
| | Temperature | −0.31 | 0.16 |
| | Incubation Time | −0.04 | 0.02 |
| | Reducing sugar | 0.32* | 0.04* |
| Fermentation process | Temperature | −1.23 | 2.09 |
| | Incubation Time | −0.12* | 0.02* |

*$p < 0.05$.

Y: Ethanol g/L, $X_1$: Acid concentration (mM), $X_2$: Acid hydrolysis Temperature ($^oC$), $X_3$: Acid hydrolysis incubation time (min), $X_4$: buffer pH, $X_5$: Enzyme hydrolysis temperature ($^oC$), $X_6$: Enzyme hydrolysis incubation time (h), $X_7$: Reducing sugar in (g/L), $X_8$: Fermentation temperature ($^oC$), $X_9$: Fermentation time (h)

This analysis highlights that process conditions such as; acid pretreatment temperature, buffer pH and reducing sugar concentration affects the ethanol yield (Eq. (2)). The increase in hydrolysis temperature during acid catalyzed reaction would also enhance the sugar production and decomposition [180,181]. Pretreatment temperature plays a vital role in sugar release as higher temperatures often leads to the formation of inhibitor, which is detrimental to the fermentative microorganisms leading to reduced ethanol yields [69,181]. Enzymes consists of ionic groups on their active sites which varies along with the pH resulting in changes in the activity of the enzyme, its structure, reaction rate and the product formation [182]. Therefore, pH of the reaction medium or buffer needs to be optimum for higher enzyme activity [183,184]. Fermentation process is slower in low sugar medium, whereas the process increases in the medium containing 15–20 g/L of sugar and remains stable up to 200 g/L. Medium with 200 g/L to 300 g/L of sugar concentration lowers the growth of yeast microorganisms [185–187]. However, higher concentration of fermentable sugars yield higher ethanol, whereas non-fermentable sugars (pentose) can affect the fermentation yield due to lack of transport system in yeasts.

## 4. Scope and opportunities for macroalgal cultivation

Macroalgal cultivation can be attempted in India by taking the advantage of 2.172 million $km^2$ exclusive economic zone (including Andaman and Nicobar islands) as well as abandoned aquaculture ponds. Aghanashini estuary (Lat 14.391°–14.585° N; Long 74.304°–74.516° E) situated in Kumta taluk, Karnataka consists of such aquaculture ponds called *gazni,* which serves as potential site (Fig. 7) for macroalgal cultivation [134]. *U.lactuca* and *E.intestinalis* abundantly grows in this estuary during all the season and *E.intestinalis* naturally occurring in *gazni* ponds are mostly discarded as waste. *Ulva* and *Enteromorpha* genus possess a blade-like or filamentous morphology, which can tolerate wide range of environmental conditions such as temperature upto 40 °C [51] and tolerate salinities in the range of 10–60 ppt [188] and resulting in higher [135].

The spatial extent of *gazani* lands in Aghanashini estuary (Fig. 7) is about 2000 ha (much of it not being used for rice cultivation now) and area of 1000 ha could be considered for algal cultivation during the late monsoon months and subsequently, these lands may be used for shrimp culturing or utilized for other forms of fisheries. The algal production happens indeed without any external input of nutrients, and therefore the *gaznis* hold very good potential for ethanol production, prior to the commencement of fishery operations, and before salinity rises substantially with the cessation of the rainy season. The algal harvests can be effectively made from any *gazni* at biweekly intervals, two weeks' time considered fairly good for harvestable regrowth. It means effectively 1000 ha area are available for algal harvests every week. The lean season for fishery (of prawns and fish mainly) alone needs to be utilized for this purpose, so that prospects for ethanol production, by no means, could come in the way of food production. These 1000 ha of aquaculture areas could be effectively available for algal harvests during the off-season, which might benefit about 2500 small farmers, who were once rice cultivators, who had permitted their erstwhile fields to be converted into shrimp farms, which are not in suitable condition for reintroduction of rice without making substantial structural changes. These *gazni* lands also exhibit positive correlation with water temperature, air and salinity which influences the growth of diatoms such as *Pleurosigma balticum, Melosira* sp., *Nitzschia sigma* and *Nitzchia* spp. that are potential candidates for biodiesel production [189].

Cultivation of *Ulva* and *Enteromorpha* is estimated to produce annual biomass of 45 t/ha [179] and 100 t/ha [51] respectively, which is 2–20 times the production potential of first and second generation feedstock and 3 times the production of brown algae in temperate waters [179]. Commercial cultivation of these seaweeds largely focuses on high-value food (aonori) and aquaculture feed production. Ammonium are abundantly present in aquaculture effluent, which is readily assimilated by *Enteromorpha* and *Ulva* in the range of 50–90 μmol N/g DW h, which makes it feasible to cultivate these algae in aquaculture effluents qualifying as species for bioremediation process [190,191]. However, sporadic sporulation of *Ulva* leads to loss of biomass due to which attempts of cultivating at large scale has failed, also environmental factors responsible for these sporulation have not yet been completely explored

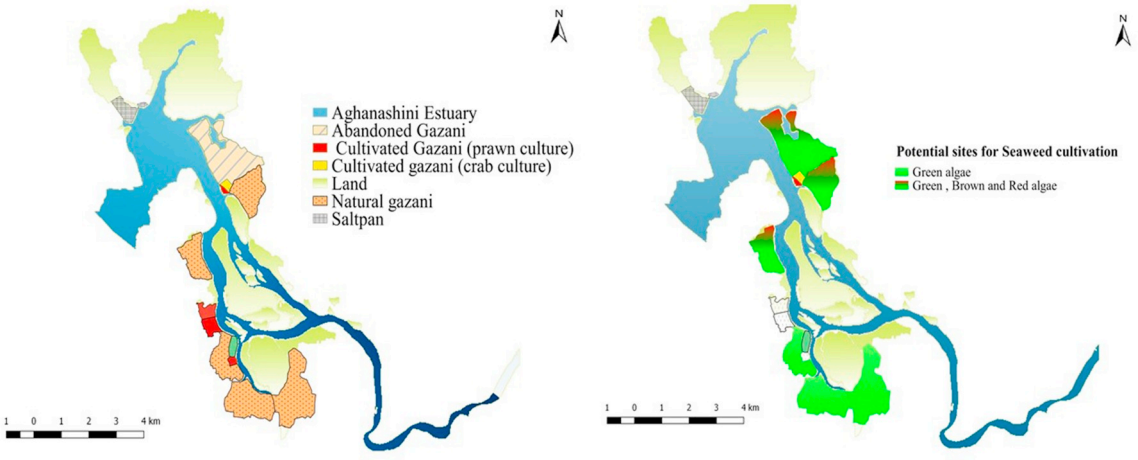


**Fig. 7.** Potential sites for macroalgal cultivation in Aghanashini Estuary.

[190]. This can be overcome by technique [135], involving artificial seeding under controlled conditions. This allows control over higher seeding density and consequently biomass production. Seeding density of 621,000 swarmers/m and nursery period of five days were quantified as key factors affecting the growth and biomass yield of *Ulva* sp. This study also highlights the shorter nursery period and culture cycles required for green seaweeds when compared to red and brown seaweeds.

Large-scale cultivation of macroalgae in open ocean eliminates the need for external inputs such as $CO_2$ cylinders, monitoring of temperature or pH alteration. However in controlled conditions, all these parameters play a crucial role in sustaining biomass productivity. Algae acquire carbon in the form of $CO_2$ or bicarbonates, at pH 8, seawater has alkalinity of 2.3 meq which allows only 0.5 mM of $CO_2$ or 6 mg/l of C to be absorbed from the seawater. In order to increase the level of $CO_2$ absorption pH needs to be increased to nearly 9 which is not feasible as it slows down the photosynthesis [192].In a study [193], brown seaweeds (*Sargassum* sp.) were cultivated at different pH and temperature and was observed that higher biomass productivity obtained at ambient pH of 7–8.2, indicating that low pH values affected the growth of the algae.

The seaweed industry in India is still at infancy, functioning more like a cottage industry. Seaweeds are collected from the natural stock mainly for the production of agar without any regulations [194]. Macroalgal biomass in addition to being potential feedstock for bioethanol production, also serves as a good food supplement. *Enteromorpha* was tested as nutritional supplement in preparation of Indian snacks, which resulted in higher iron and calcium content along with increase in proteins, vitamins and dietary fibres [195]. Macroalgal protein, dietary fibres and phytochemicals are utilized in order to enhance the nutritional quality of the food products. Complete utilization of macroalgal biomass into producing biofuel and value added products has potential to ensure India's energy and food security [134]. The study presents the scope to utilize macroalgal biomass in producing value added products in addition to bioethanol production, bringing resilience to the Industry and improving their economics and usefulness. Prospects of bioethanol with the value added products would support the livelihood of rural population, while aiding as the potential feedstock for biorefinery [36, 196].

## 5. Conclusion

The growing need to cater the energy demand coupled with the urge to mitigate GHG footprint in the energy sector has necessitated inventorying of renewable and sustainable energy alternatives. Green seaweeds have the potential to serve as a renewable and sustainable feedstock for bioethanol production. Dilute acid, $H_2SO_4$ is an appropriate pretreatment method for extraction of sugars from algal biomass. Algal biomass pretreated using dilute acid yields higher sugar during enzyme hydrolysis by exposing cellulose fibres for enzyme degradation. Neutralization using $Na_2CO_3$ exhibited lower sugar removal for *U.lactuca* (39.8%) and *E.intestinalis* (14.7%). Green seaweeds *U.lactuca* and *E. intestinalis* are viable feedstock for bioethanol production due to higher carbohydrate content of 62.15% and 40.1% respectively. Saccharification and Fermentation (SSF) process yielded higher conversion efficiency compared to SHF (Separate hydrolysis and fermentation) process and SSF achieved higher efficiency for *U.lactuca* indicating fermentation of more than one sugar present in the medium. Toddy yeast strain exhibited higher efficacy in fermentation of *E.intestinalis* hydrolysate in both SHF and SSF process achieving 25.8% and 77.4% efficiency respectively. Macroalgal biomass has potential to produce various value added products of commercial significance in addition to the production of biofuels. The study highlights the scope for bioethanol from macroalgae and also the availability of potential sites for cultivation. The comprehensive knowledge of macro algal resources distribution, seasonality and biochemical composition through field survey and choice of appropriate enzyme and yeast would aid in realizing the path of the sustainable, economical and commercially viable biofuel.

## Data and accessibility

Data used in the analyses are compiled from the field. Data is anlysed and organized in the form of table, which are presented in the manuscript. Also, synthesized data are archived at http://wgbis.ces.iisc.ernet.in/energy/water/paper/researchpaper2.html#ce, http://wgbis.ces.iisc.ernet.in/biodiversity/

## Competing interests

We have no competing interests either financial or non-financial.

## Authors contribution

Ramachandra T V: data analysis and interpretation of data; revising the article critically for important intellectual content; final editing Deepthi H: Isolation and characterisation, Design of the fermentation experiment, Carrying out experiments, analysis and interpretation of data; and Paper writing.

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## Research ethics

The publication is based on the original research and has not been submitted elsewhere for publication or web hosting.

## Animal ethics

The research does not involve either humans, animals or tissues.

## Permission to carry out fieldwork

Our research is commissioned by the Ministry of Science and Technology (NRDMS Division), Government of India and hence no further permission is required as the field work was carried out in the non-restricted areas/protected areas.

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