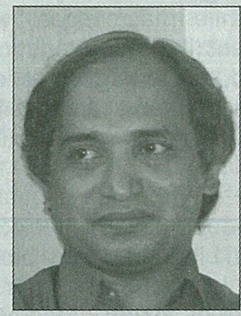
Bioethanol prospects of Algae in Central West Coast of India



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Summary

Dwindling stock of fossil fuel, escalating oil prices, enhanced greenhouse gases and consequent global warming, etc. have necessitated the exploration of cost effective sustainable alternatives. The focus of the current communication is the ethanol prospects of macro algae in Central West coast of India. These resources are renewable and sustainable without competing with either food (first generation biofuel) or land resources (second generation biofuel). Abundant distribution of seaweeds along the coast gives an opportunity to arrive at economically viable biofuel feedstock and also take up seaweed off shore cultivation. These macroalgae accumulate large concentration of carbohydrate and constitute appropriate feedstock for bioethanol manufacture. This involves subjecting the seaweed to hydrolysis to break down the carbohydrate and ferment the sugar using suitable microorganism.

Introduction

Rio Earth Summit in 1992 witnessed the beginning of the international political responses and adoption of the UN Framework on Climate Change (UNFCCC), which aimed at stabilising atmospheric concentrations of greenhouse gases (GHGs) to avoid dangerous anthropogenic interference with the climate system. Recent conference COP21 (http://www.cop21paris.org) unanimously agreed to restrict the increase in the global average temperature to well below 2°C above pre-industrial levels and also make efforts to limit the temperature increase to 1.5°C by reducing the dependence on fossil fuels and cutting the greenhouse gas emissions. This has given impetus to the exploration of viable renewable energy alternatives to meet the energy demand of the burgeoning population. Alternative sustainable energy sources are wind solar, geothermal, hydroelectric, biomass and biofuel (Ghadiryanfar et.al, 2016).

Earlier attempts in this regard were the manufacture of biodiesel and pure plant oil derived from sugarcane, corn, soybean, potato, wheat or sugar beet (first generation biofuel), which proved to be unsustainable due to the competition with human food resources. This conflict, led to new attempts on biofuel derived from lingo-cellulosic biomass (second generation biofuel). This involved direct and indirect land use changes with the energy crop cultivation inducing a significantly high carbon debt and higher water consumption (Dominguez-Faus. et.al, 2009). Conflict with land for cultivation of biofuel feedstock, led to the exploration of viable alternatives focusing on algal biofuels (third generation biofuels).

Table 1 : Comparison of productivities of lignocellulosic biomass and seaweeds

Biomass	Productivity dry g/(m2.year)
Lignocellulosic biomass	
Switchgrass	560 - 2,240
Corn stover	180 - 790
Eucalyptus	1,000 - 2,000
Poplar	300 - 612.5
Willow	46 - 2,700
Switchgrass	560 - 2,240
Seaweeds	
Green seaweeds	7,100
Brown seaweeds	3,300 - 11,300
Red seaweeds	3,300 - 11,300

Sources: Yanagisawa, et al 2011; Ramachandra et al, 2009

Algal feedstock being carbon neutral has proved to be a very promising renewable resource for sustainable energy production. Algae fixes the greenhouse gas (CO_2) and have higher photosynthetic efficiency (6-8%) compared to any terrestrial biomass (1.8-2.2%) (Ramachandra et al., 2009; Aresta, 2005; FAO, 1997). Also, algae feedstock can be grown in fresh as well as marine waters which reduces the need for higher water consumption. Micro algae grown in marine and freshwater ecosystems and macro algae grown in estuaries have proved to be beneficial feedstock for biofuel production. Microalgae grown in marine ecosystem (with higher salinity and silica) accumulate lipid, while macro-algae, which are multicellular with plant like characteristics are rich in carbohydrate and net energy (net energy of 11,000 MJ/t dry algae; Aitken et al., 2014) and are aptly suited for bioethanol conversion (Jin et al., 2013).

Macro-algae or seaweeds have higher potential to produce sustainable bioenergy and biomaterials and do not require land or freshwater for their cultivation. (Lobban et.al., 1985). Macro-algae are currently used for hydrocolloids, fertilizers and to some extent as animal feed (Bixler and Prose, 2011; McHugh, 2003). Despite all these environmental and economic merits of macroalgae, challenges are experienced during extraction of biofuel as macro-algae have unique carbohydrate architecture, distinctively different from terrestrial biomass (Roseijadi et al., 2010; sze, 1993). Though macro-algae are ideally suited for biofuel such as biogas, bioethanol, etc., attempts towards economically efficient technological solutions of biofuel production are still at infancy (Bastianoni S et al., 2008). Marine macro-algae are broadly classified as (i) brown algae (Phaeophyceae), (ii) red algae (Rhodophyceae), and (ii) green algae (Chlorophyceae). Table 2 lists number of species and characteristics, which are distinctly different with regard to their photosynthetic reserve and cell-wall polysaccharides. Abiotic parameters of habitat (namely light, temperature, salinity, nutrient, pollution, water motion, etc.) play a vital role in algae's growth, pigment and also other chemical constituents. Macro-algae are vertically distributed from the upper zone (close to the sea surface) to lower sub-littoral zone to optimally use natural light and the pigment absorb selectively light at specific wavelength (Guiry 2012).

1.1 Chemical composition of Macroalgae:

Chemical composition of macro-algae include lower contents of carbon, hydrogen, and

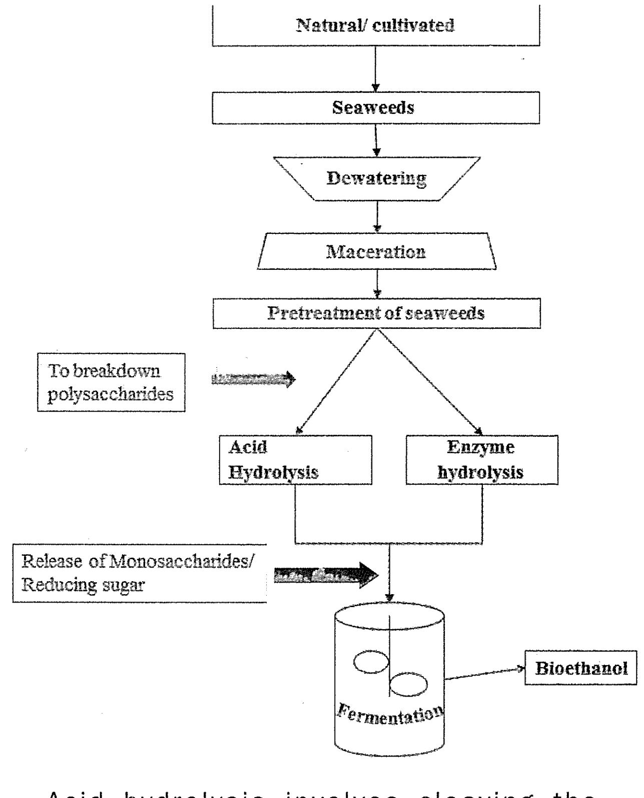
Table 2 : Characteristics of Green, Red, and Brown seaweed

	Chlorophyta	Rhodophyta	Phaeophyta
Number of species recorded	6032a	7105b	2039c
Photosynthetic pigment	chlorophyll a, chlorophyll b, carotin, xanthophyll	Phycoerythrin	Fucoxanthin
Habitat	Freshwater and Marine	Strictly marine	Strictly marine
Reproduction	Asexual and sexual	Asexual and Sexual	Asexual and sexual
Photosynthetic reserve*	Chlorophyta accumulates starch as their photosynthetic reserve.	Carbohydrate reserves of red algae are floridean starch (intermediate between true starch and dextrin)	Carbohydrate reserve is called laminarin and mannitol (hexahydride alcohol)

Source: a A. Pascher, 1914; b Algaebase.org; c Kjellman, 1891 *Smith,1938 oxygen and higher contents of nitrogen and sulfur compared than that of land-based, lignocellulosic biomass. Macroalgae have complex carbohydrates, consist of various neutral sugar and sugar acids which are also found in terrestrial plants. Along with these sugars, macro algae also contains acidic (phycocolloids) half ester sulphate groups attached to hydroxyl group of sugar. These sugars have identical chemical constituents with different spatial arrangements. Linkage of these sugars gives rise to vast number of polysaccharides with different shapes and different properties. These sugars are food reserves and constituents of cell walls and exists as mucilage or gels.

1.2 Biofuel from Macroalgae: Production of Bioethanol from macroalgae involves (i) pre treatment (maceration, etc.), (ii) breaking polysaccharide into simple sugar (reducing sugar) through acid or enzyme hydrolysis and (iii) fermentation, illustrated in Figure 1. Breaking down of polysaccharide into simple sugar (reducing sugar) involves treating the biomass with acid or enzyme hydrolysis. Diluted-acid hydrolysis is a typical physiochemical method to treat raw algal biomass with 0.3-0.9N H_2SO_4 at 100-140°C. (Meinita et al., 2012; Park et al., 2012). However, acid concentration and hydrolysis time influences the yield of reducing sugars. Enzymes such as cellulase and cellobiase (Ge et al., 2011; Yanagisawa et al., 2011), or macro algae specific enzymes such as laminarinase and agarase have been used and most of these enzymes showed low hydrolysis efficiency (Adams et al., 2011). Hence, for effective hydrolysis to reduce sugars, both chemical and enzymatic hydrolyses have been employed (Adams et al., 2011; Ge et al., 2011; Jang et al., 2012)

Figure 1 : Schematic representation of bioethanol production from macro algae or seaweed



Acid hydrolysis involves cleaving the polysaccharide's glyosidic bond to release monosaccharides. But, acid hydrolysis decomposition also releases undesirable compounds, such as Fufural, 5-hydroxymethylfufural (HMF), levulinic acid and caffeic acid, which inhibit subsequent fermentation. These compounds are derived from xylose and galactose in macroalgal biomass, can be detoxified using activated charcoal treatments (Meinita et al., 2012). Metal contents in macroalgal biomass are (0.5-11% wt)

Table 3 : Seaweed polysaccharides

Seaweeds	Polysaccharides/phycocolloids	Monosaccharides
Chlorophyceae (Green)	Amylose, amylopectin Cellulose, complex hemicellulose Glucomannans, Mannans Pectin, sulfated mucilages (glucuronoxylorhamnans) Xylans	Glucose, Mannose, Rhamnose, Xylose, Uronic acid, Glucuronic acid
Rhodophyceae (Red)	Agars, agaroids Carrageenans, cellulose Mannans, Xylans, rhodymenan	Glucose, galactose, Agarose
Phaeophyceae (Brown)	Alginates, cellulose complex sulfated heteroglucans Fucose containing glycans Fucoidans, Glucuronoxylofucans Laminarans	Glucose, galactose, fucose, xylose, uronic acid, mannuronic acid, Guluronic acid

Source: Sudhakar, 2013 ; Percival et.al, 1967 higher than terrestrial biomass (1-1.5% wt) (Lee and Lee, 2012; Ross et al., 2008), which inhibits microbial fermentation during pretreatment. In contrast to this, during enzyme hydrolysis there is no undesirable compounds as enzyme activity is specific to type of polysaccharides (Nguyen et al., 2009). Simple sugar resulting from hydrolysis is subjected to fermentation using various organisms particularly yeasts microorganisms, to produce ethanol. In order to produce bioethanol cost-effective manner, efforts are in progress to screen microorganisms (Table 4) that possess the ability to directly convert polysaccharides (including glucans) into ethanol. Table 4 also lists species wise quantum of ethanol production, while Table 5 lists microorganisms (to convert sugar into ethanol) for different macro algae.

Table 4 : Yield and concentration of sugar and ethanol produced by hydrolysis of Macro algae

Seaweed group	Seaweed species	Hydrolysis	Fermentation	Ethanol concentration (g/L)
Red algae	Gelidium amansii	Acid +enzyme	Scheffersomyces stipites	20.5g/l of sugar
	Palmaria palmata	Acid	S. cerevisiae	17.3 mg/g of sugar
	Kappaphycus alvarezii	Acid	S. cerevisiae	64.3g/l of sugar
	Gracillaria verrucosa	Acid+enzyme	S. cerevisiae	0.43g/g of sugar
Green algae	Ulva pertusa	Enzyme	S. cerevisiae	18.5
		Acid +enzyme	S. cerevisiae	27.5
	Enetromorpha instestinalis	Enzyme		20.1 g/L Sugar yield
	Ulva fasciata	Acid +enzyme	S.cerevisiae MTCC No.180	0.45 g/g
	Ulva reticulate	Enzyme	S.cerevisiae WL P099	90 L/t dried biomass
Brown algae	Sargassum sagamianum	Thermal hydrolysis	Pichia Stpitis CBS 7126	0.386g/g reducing sugar
	Undaria pinnatifida	Thermal acid hydrolysis	Pichia angophorae KCTC 17574	9.42 g/L
		Acid + enzymatic	Pichia angophorae KCTC 17574	12.98 g/L
	Saccharina japonica	Acid + enzymatic	Saccharomyces cerevisiae DK 410362	6.65 g/L
		Thermal acid hydrolysis	Pichia angophorae KCTC 17574	0.169 g/g reducing sugar
		Engineered microbial enzyme	Engineered BAL1611	0.41 g/g reducing sugar
	Laminaria digitata	Shredding and enzymatic	Pichia angophorae	167 mL/kg algae
	Laminaria japonica	Thermal liquefaction	Pichia stipitis KCTC7228	2.9 g/L using 100 g/L algae
		Acid + enzymatic	Ethanologenic strain E. coli Ko11	0.41 g/g reducing sugar
		Acid + enzymatic	Saccharomyces cerevisiae	143 mL/kg floating residues
	Sargassum sagamianum	Thermal liquefaction	Pichia stipitis CBS 7126	0.43–0.44 g/g reducing sugar
	Saccharina latissima	Shredding and enzymatic	Saccharomyces cerevisiae Ethanol red yeast	0.45% (v/v)
	Dilphus okamurae	Enzymatic	Mixture of B5201 (Lactobacillus), Y5201 (Debaryomyces I) and Y5206 (Candida I)	0.04g 100 m/l 0.03g 100m/l
	Sargassum fulvellum	Acid+ enzymatic		0.0596 0.0215
	Alaria crassifolia	Enzymatic		0.244

Table 5 : Macro algal biomass wise polysaccharides, sugars and organisms (to convert sugars into ethanol)

Biomass	Polysaccharides	Sugar	Organisms
Green seaweeds	Glucan	Glucose	S.cerevisiae
	Ulvan	Xylose	Xylose-fermenting yeast
			Xylose-utilizing S.cerevisiae
			Ethanologenic E.coli
			Clostridium beijerinckii, Clostridium Saccharoperbutylacetonicum
		Glucuronic acid	P. tannophilus Ethanologenic E.coli
Brown seaweeds	Glucan	Glucose	S. cerevisiae
			Pichia angophorae
			Ethanologenic E.coli Ko11
			Ethanologenic E.coli BAL 1611
		Mannitol	Zymobacter palmae
			Pichia angophorae
			Ethanologenic E.coli KO11 developed by integrating zymomonas mobilis ethanol production genes into the pflB gene
			Ethanologenic E. coli BAL1611
	Alginate	Uronic acid	Ethanologenic sphingomonas sp. A1
			Ethanologenic E coli BAL BAL 1611
Red seaweeds	Glucan	Glucose	S. cerevisiae
	Agar, Carrageenan	Galactose	S. cerevisiae, Brettanomyces custersii KCTC 18154P
		3,6-anhyrdogalactose	NR
Engineered strain			
Red seaweed	Agar, carrageenan	Galactose, or simultaneous co-fermentation of galactose and cellobiase	Saccharomyces cerevisiae (engineered for improved galactose fermentation)
Brown seaweed	Glucan	Glucose, mannitol	Escherichia coli (engineered for alginate metabolism)
	Alginate	Glucose, mannitol and alginate	Escherichia coli Ko11

Source: Yanagisawa,2013; Kim N.J. et al.,2011

1.3 Scope for value added products: In India, seaweeds grow abundantly in south coast of Tamil Nadu, Gujarat coast, Lakshadweep and Andaman-Nicobar Islands. Luxuriant growth of seaweeds is also found at Mumbai, Ratnagiri, Goa, Karwar, Varkala, Vizhinjam, Vishakapatnam, Pulicat lake and Chilka Lake. Kaliaperumal, et al., 1992; 1996, recorded about 271 genera and 1053 species of marine algae belonging to four groups of algae namely Chlorophyacea, Phaeophyceae, Rhodophyceae and Cyanophycease from Indian waters. Seaweeds as a food source is used seldom in India, but freshly collected and cast ashore seaweeds are used as manure for coconut plantation either directly or in the form of compost in coastal areas of Tamil Nadu and Kerala. Seaweed manure has been found superior to farm yard manure. It is seen that plants absorb, high amount of water soluble potash, other minerals and trace elements present in seaweeds which aids in controlling mineral deficiency diseases. Also the nature of soil and moisture retaining capacity is improved due to carbohydrates and other organic matter present in the marine algae. Macroalgae in India are used as raw material for manufacture of agar, alginates and liquid seaweed fertilizer. (Chennubhotla et.al. 1978).

Table 6 : Distribution of Seaweed species in Uttara Kannada district

SEAWEED SPECIES	KARWAR	ANKOLA	KUMTA	HONAWAR	BHATKAL
Amphiroa fragillissima (Linnaeus) Lamouroux	+			+	+
Bangia autopurpurea var.fuscopurpurea (Dillwyn) C.Agardh	+				
Caulerpa peltata J.V. Lamouroux	+			+	+
Caulerpa racemosa (Forsskal) J. Agardh				+	+
Caulerpa scalpelliformis (R.Brown ex Turner) C. Agardh				+	+
Caulerpa sertularioides (S.G.Gmelin) M.A.Howe				+	+
Caulerpa taxifolia (Vahl) C.Agardh				+	+
Caulerpa verticillata J. Agardh				+	+
Chaetomorpha linum (Muller) Kutzing	+			+	+
Chaetomorpha media (C. Agardh) Kutzing	+		+	+	+
Dictyopteris australis (Sonder) Askenasy	+			+	+
Dictyota bartayresiana Lamouroux	+		+	+	+
Dictyota dichotoma (Hudson) Lamouroux	+			+	+
Enteromorpha clathrata (Roth) J.Ag	+	+	+		
Enteromorpha flexuosa (Wulf) J.Ag.	+	+	+	+	
Enteromorpha intestinalis (Linnaeus) Nees	+	+	+	+	
Gelidium micropterum Kutzing	+	+	+	+	+
Gelidium pusillum (Stackhouse) Le Jollis	+	+	+		
Grateloupia filicina (Wulf.) Ag.	+	+	+	+	+
Grateloupia indica Borgesen				+	+
Grateloupia lithophila Borgesen			+		+
Gracilaria corticata J. Agardh	+	+	+	+	+
Hypnea valentiae (Turner) Montagne	+	+	+	+	+
Jania adherence Lamouroux	+		+		
Laurencia cartilaginea Yamada	+			+	+
Laurencia obtusa (Hudson) Lamouroux	+				
Laurencia papillosa (C. Agardh) Greville	+		+	+	+
Padina gymnospora (Kutzing) Sonder	+	+	+	+	
Padina tetrastromatica Hauck	+			+	+
Porphyra vietnamensis T.tanaka & Dham-Hoang Ho	+			+	+
Sphacelaria furcigera Kuetz	+			+	+
Spatoglossum asperum J. Agardh	+				+
Sargassum cinereum J. Agardh	+	+	+	+	+
Sargassum ilicifolium (Turner) C. Agardh	+		+	+	+
Sargassum polycystum C. Aga	+			+	+
Sargassum tenerrimum J. Agar	+			+	+
Sargassum wightii Greville	+				+
Stoechospermum marginatum (C. Agardh) Kutzing	+			+	+
Ulva fasciata Delile	+	+	+		
Ulva lactuca Linnaeus	+	+	+	+	
Ulva rigida C. Agardh					+

Source: Agadi, 1985; Untawale et al., 1989; NAAS 2003, Venkataraman and Wafar, 2005; Kaladharan, 2011; * http://www.niobioinformatics.in/seaweed/index.htm

Table 7 : Distribution of potential Seaweeds along West Coast of India as a feedstock for Biofuel production

SEAWEED	GUJARAT	MAHARASHTRA	GOA	KAR	KER
Amphiroa fragillissima	+	+	+	+	
Acanthophora specifera	+	+		+	+
Bangia fuscopurpurea		+	+	+	+
Bryopsis plumosa	+	+	+	+	
Caulerpa peltata	+	+	+	+	
Caulerpa racemosa		+		+	
Caulerpa scalpelliformis	+	+		+	
Caulerpa sertularioides		+		+	
Caulerpa taxifolia		+		+	
Caulerpa verticillata				+	
Chaetomorpha linum	+	+	+	+	
Chaetomorpha media	+	+	+	+	
Cheilosporum spectabile	+	+	+	+	+
Cladophora fascicularis	+	+	+		+
Dictyopteris australis	+	+	+	+	
Dictyota bartayresiana	+	+	+	+	
Dictyota dichotoma	+	+	+	+	
Enteromorpha clathrata	+	+	+	+	
Enteromorpha flexuosa	+	+	+	+	+
Enteromorpha intestinalis		+	+	+	+
Gelidium micropterum	+	+	+	+	
Gelidium pusillum	+	+		+	
Grateloupia filicina		+	+	+	
Grateloupia indica	+	+	+	+	
Grateloupia lithophila		+	+	+	
Gracilaria corticata	+	+	+	+	
Gracilaria verrucosa	+	+	+		
Hypnea musciformis	+	+	+	+	
Hypnea pannosa	+	+		+	
Hypnea valentiae		+	+	+	
Jania adherence		+	+	+	
Laurencia cartilaginea		+	+	+	
Laurencia obtusa	+			+	
Laurencia papillosa	+	+	+	+	
Padina gymnospora	+	+	+	+	
Padina tetrastromatica	+	+	+	+	
Porphyra vietnamensis		+	+	+	
Sphacelaria furcigera	+	+	+	+	
Spatoglossum asperum	+	+	+	+	
Sargassum cinereum		+	+	+	
Sargassum ilicifolium	+	+	+	+	
Sargassum polycystum	+	+	+	+	+
Sargassum tenerrimum	+	+	+	+	
Sargassum wightii		+	+	+	+
Stoechospermum marginatum		+	+	+	
Ulva fasciata	+	+	+	+	+
Ulva lactuca	+	+	+	+	+
Ulva reticulata	+	+	+	+	+
Ulva rigida	+			+	

Source: http://www.niobioinformatics.in/seaweed/index.htm

2. Seaweed resources in West Coast of Karnataka

Karnataka has a coastline of about 320 km starting from Talapadi in the south to Karwar in the north. Ecology of tidal pond in Mavinahole estuarine creek, Karwar was studied in 1979 by Bopaiah and Neelakantan (1982). Table 6 lists taluk-wise distribution of seaweed species in Uttara Kannada district, which are mostly confined to rocky shores. 43 species of marine algae in the littoral zone of the entire Karnataka coast was reported earlier (Agadi, 1985). NAAS (2003) reported 39 species of seaweeds from Karnataka coast, 39 species of seaweeds from Karnataka coast (Venkataraman and Wafar, 2005; Kaladharan, 2011) and Untawale et al. (1989) reported 65 species belonging to 42 genera from the northern Karnataka coast alone.

Uttara Kannada district is endowed with four productive estuaries namely Kali estuary in Karwar, Gangawali estuary in Ankola, Aghanashini estuary in Kumta, Sharavathi estuary in Honnavar. Aghanashini estuary situated in Kumta taluk on the rive Aghanashini, this estuarine region extends from the river mouth to about 25 km upstream. The Aghanashini estuary has several mudflats and small islands and network of drainage canals called kodis. Farmers of this region traditionally cultivated a variety of salt tolerant rice- "kagga" in large expanses of the reclaimed backwaters, called gaznis, also known as Kharlands or Khajans. In these gazni land, farmers practice alternate rice cultivation and prawn filtration. There are few abandoned gazni in these estuarine region which could serve as a potential site for seaweed cultivation. (Suryanath, 1985). Table 7 lists the distribution of seaweeds along west Coast of India with the wide scope for biofuel production. There is a potential to develop large scale cultivation of seaweeds in west coast of India with optimization of existing labour intensive cultivation and harvesting technologies to reduce cost and energy demand. Extraction of value added products from macro algal biomass along with bioethanol production, further boosts the livelihood of local people while meeting the energy demand.

Prospects of Bioethanol from Macroalgae

Considerable work has been carried out with respect to commercial production of agar and algin from macro algae in India. Different microorganisms are being employed for effective conversion of seaweed polysaccharides as well as of fermentation processes, in order to commercialize macroalgae based fuels, a priority needs to be put on identifying microorganisms that metabolizes macroalgal carbohydrates. Alginate and Ulvan are macroalgae specific carbohydrate which are not readily metabolized by commercially applied fermenting microorganisms such as saccharomyces cerevisiae (Wegeberg and Felby, 2010). To overcome these constraints, macroalgae specific enzymes were developed to hydrolyze macroalgal carbohydrates (Erasmus et al., 1997; Jang et al., 2012). An attempt was made to cultivate red algae Kappaphycus alvarezii along Mandapam coast and demonstrated commercial scale production of bioethanol. Over the past twenty years, large scale cultivation of carrageenophytes (Khambhaty, 2012). In India, edible seaweeds such as Gracillaria edulis, Caulerpa spp., Poryphyra etc. can be cultivated along with biofuel feedstock seaweeds, in estuarine areas and coastal inundated waters. Appropriate technology for large scale seaweed cultivation is imperative to meet the growing energy demand. Implementing seaweed cultivation combined with post harvesting processing units could bring economic returns to seaweed cultivators.

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