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REVIEW: POTENTIAL OF ENDOPHYTIC MARINE FUNGI FOR BIOETHANOL PRODUCTION FROM SEAWEED

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ABSTRACT

Bioethanol can be derived from industrial waste processing that still contains lignin, cellulose and hemicelluloses. Utilization of waste will reduce the impact of competition for food resources. The main components in waste seaweed (cellulose) can be converted into bioethanol. Ethanol that produced using cellulolytic activity, can be used as an alternative renewable fuel. One way to convert the cellulose to ethanol is using enzyme hydrolysis followed by fermentation. Hydrolysis and fermentation process are using potential microbial activity as fungi and yeast. All this time, the terrestrial fungi are commonly used for bioetanol, while endophytic fungi from the marine is still rarely explored although marine fungi that have enzyme activities that can be developed in the manufacture of bioethanol so they have potential to degrade cellulose from seaweed waste. The fuels from commercial macroalgae, a priority to identify microorganism that have a bioactive compund to metabolize major from carbohydrates or cellulose. There were some researchers have developed macroalgae specific enzymes to hydrolyze macroalgal carbohydrates/cellulose. Most of the microorganisms were originated from marine flora and fauna. Their enzymatic functions on macroalgae have been well reviewed in many studies. Once those kinds of microorganisms and enzymes have been identified and developed, they can be applied to the Simultaneous Saccharification and Fermentation (SSF) and Seperate Hydrolysis and Fermentation (SHF) method. Simultaneous Saccharification and Fermentation (SSF) method has many advantages: low contamination, low initial osmotic stress of fermenting microorganisms, and high energyefficiency. It should be noted however, that applying various microorganisms to a fermentation condition as in Simultaneous Saccharification and Fermentation (SSF) method could inhibit enzyme activity and extracellular enzyme secretion [22]. Also, various and complex carbohydrates of macroalgae can lead to low yield of bioethanol in the Simultaneous Saccharification and Fermentation methode. Therefore, it is very important to know the potential of endophytic marine fungi in in the manufacture of bioethanol.

Keywords: Bioethanol, celullose, endhophytic, hemicellulose, fungi, lignin, seaweed waste

Introduction

The expansion in fossil fuel costs, issues of national security, and ecological concerns have prompted a staggering enthusiasm among scientists to grow monetarily reasonable procedures for the creation of elective transportation energizes. Alternative fuel is a renewable source of energy. Biological resources based energy are expected to reduce the problem caused by fossil fuels, such as fuel shortage and environmental pollution since it is more environmentally friendly.

Bioethanol, a renewable source of energy, can be derived from materials that contain sugar, starch, or fiber. Raw materials commonly used as bioethanol include cassava, sugarcane, palm sap, sorghum, sweet potato, canna, seaweed, and others [1]. Bioethanol can be also derived from processing industrial waste. Industrial waste that is still containing lignin, cellulose, and hemicelluloses can be utilized as bioethanol. The Utilization of industrial waste will reduce the impact of competition for food fulfillment. Bioethanol is produced from carbohydrate non-starch content of seaweed such as cellulose and lignin [2]. Bioethanol has been created from green algae include *Ulva* sp. [3, 4], from red algae such as *Kappaphycus* sp. [5], *Gracilaria* sp. [6], and *Gelidium* sp. [7], from brown algae such as *Laminaria* sp. [8] and *Sargassum* sp. [9,10]

Seaweed has the potential as a bioethanol feedstock. Seaweed production in Indonesia has increased, in 2009 reached 2.791.689 tons and 3.399.438 tons in 2010. Content of waste produced by The processing of seaweed is carbohydrate in the form of cellulose. Cellulose contained in the seaweed waste reach 15-25% [11].

One of the components in seaweed (cellulose) is a material that could be changed into bioethanol. One of the methods of converting cellulose to ethanol was used enzyme hydrolysis followed by fermentation. Cellulose degradation and fermentation process can be running due to potential microbial activity, such as fungi and yeast. Cellulose production obtained from selected microorganisms through a screening process.

The ability of fungi in cellulose degradation into sugar aided by its enzymes, such as cellulase and xylanase enzymes. Sugars was produced by fungi and then converted into ethanol through fermentation process. Terrestrial fungi most often used for saccharification, such as *Trichoderma harzianum* were isolated from soil and wood at the Amazon rainforest [12]. Endophytic fungi have more active secondary metabolites [13], so the endophytic fungi from marine organisms and plants also have the potential to degrade cellulose but its existence has not been widely studied further. Therefore, it is very important to know the potential of endophytic marine fungi for bioethanol production with seaweed waste.

Potential of Endhophytic Marine Fungi

Endophytic microbes were almost found in all plants on earth and they were grown in plant tissue. Endophytic fungi can be isolated from the roots, stems, and leaves. Bacteria and fungi are types of microbes commonly found as endophytic microbes, but the class of fungi was most isolated then class of bacteria. The relationship between microbial endophyte and its host were mutualism symbiotic and pathigenic [14]. The Relationship of mutualism symbiotic was characterized with a mutually beneficial relationship between endophytic microbes and its host.

Fungal endophytes were isolated from all organs of various trees, asymptomatic herbaceous plants, and marine algal thalli such as *Fucus serratus*, *Fucus spiralis*, *Fucus vesiculosus*, *Ceramium* sp. *Laminaria* sp., *Ascophyllum nodosum*, *Enteromorpha* sp, *Halarachnion ligulatum*, and *Plumaria elegans* [13]. Endophytic fungi have been detected and effectively cultured from soil and some of terrestrial plants [12]. These energizing revelations with terrestrial plants make one wonder if marine algae, seagrasses (the main angiosperms plants that become submerged in the ocean) harbor endophytic fungi that produce secondary metabolites. Kobayashi and Ishibashi are of the conclusion that marine endophytic fungi may can create various sorts of compounds from those of terrestrial sources [15]. In addition to secondary metabolites, endophytic marine fungi also produce primary metabolites that have activity, such as enzyme activity. There were two strains of the marine fungi, such as Aspergillus terreus and Mucor plumbeus with cellulase enzyme activity [16].

There were some provisions to isolate endophytic microbes that capable to produce potential bioactive compound, such as:

1. The host plant of endophytic fungi grows in a specific environment.
2. The plant has a history ethnobotanical closely related to the utilization of specific plants by the native people.
3. Host plants are endemic plant
4. The host of endophytic fungi grow in areas that have high biodiversity

The host plants of endophytic fungi must have a certain selection process based on the influence of the environment, harvesting, and history of its host plants [17]. These growths seem to have an ability to create a variety of secondary metabolites showing an assortment of biological activity. Even though the capacity of organisms to create bioactive metabolites is well known, endophytes have not been exploited, perhaps because we are only starting to comprehend their dispersion and biology [18].

The component of cellulose, hemicellulose, and lignin in rotting plants can be degraded by Fungi. The degrade of cellulose in plant by a complex of excreted oxidative and hydrolytic enzymes, for example, cellulases enzyme, hemicellulases enzyme, and ligninases enzyme, while from potential bacteria to degrade lignocellulose can be isolated from soil and compost. The potential filamentous bacteria was from generally of the genus Streptomyces.

There are several microorganisms can degrade cellulose, hemicellulose and lignin called by cellulolytic microorganisms, including from fungi, and bacteria. The potential of cellulolytic fungi including from genera Trichoderma, Penicillium, Aspergillus, and Humicola grisea var thermoidea, while cellulolytic bacteria such as the anaerobic Bacillus, Clostridium, aerobic actinomycetes, and Cellulomonas [19].

Cellulose activity from marine fungal strains were screened (Table 1). Amongst them maximum producer of enzyme was identified as *Mucor plumbeus* and *Aspergillus terreus*. These two strains were explored for the optimization of cellulase enzyme yield. Different agro wastes were screened as carbon source, among them eucalyptus as substrate was found to be a good carbon source for both marine fungal strains. The utilization of agro waste as an enzyme substrate for bioethanol production can reduce the cost of producting bioethanol and facilitate the processing of converting carbohydrate into fermentable sugar. Mucor plumbeus and Aspergillus terreus were optimized with physical (temperature & pH) as well as nutritional parameters like a variable range of nitrogen sources. It was found that the optimum temperature for enzymatic activity was 37 °C & pH 7. Aspergillus terreus and Mucor plumbeus have the potential of converting cellulose in a single-step fermentation process. So far the literature study is concerned most of the studies regarding cellulase production by agro waste degradation have been carried out with terrestrial genera [16].

Tabel 1. Xylanolytic, cellulolytic and ligninolytic activites isolated from marine environment [20].

Organism	Source*	Xylanase (nmol min^{-1} ml^{-1})	CEA** cellulolytic	CEA** Ligninolytic
Gliocladium sp	A	40,9	+	+
Ascomycetes	B	48,5	-	-
Sordaria funicola	B	50,1	+	+
Gongronella sp	B	574,0	-	+
Aigialus grandis	C	58,5	+	+
Aigialus mangrovei	C	222,0	+	+
Lophiostoma mangrovei	C	103,6	+	+
Halosarpheia ratnagiriensis	C	23,4	+	+
Zalerion varium	C	33,4	+	+
Hypoxylon oceanicum	C	177,0	+	+

*A=Mangrove soil, B=Decaying mangrove leaves, C=Decaying mangrove wood

**organisms were inoculated into cellulose azure agar (CEA)

Enzymatic complex, which on the whole have particularity for the b-1,4-glucosidic bonds produced by cellulolytic organism. They have been named as exoglucanases or cellobiohydrolases (EC 3.2.1.91) and endoglucanase (EC 3.3.1.4). The cellobiohydrolases or CBH that shows a higher proclivity for crystalline cellulose, remove the disaccharide cellobiose from the reducing and the non-reducing ends of the cellulose chain. The endoglucanases or EG, with a higher proclivity for the amorphous side of the cellulose, advance a random internal attack in the cellulose chain, delivering cello-oligosaccharides, which are the substrate for the CBH enzymes.

Cellobiose, that is water-dissolvable, is hydrolyzed into two molecules of glucose by the b-glucosidase (EC 3.2.1.21), otherwise called enzyme of cellobiose [21].

Potential of Seaweed for Bioethanol

Seaweed can be used as raw material in producing a bioethanol [22,23]. Seaweed contain a measures of biomass as cellulose and starch which can be converted to simple sugar such as glucose used in fermentation. The glucose can be changed over to bioethanol by a fermentation method. Seaweed can be used for bioethanol fermentation by converting their stock material to fermentable sugars. The close absence of lignin can be hydrolized by enzyme into simple cellulose.

There are several types of seaweed has been used as bioethanol feedstock, such as *Ulva lactuca, Gracilaria verrucosa, Kappaphycus alvarezii* [24-26] with enzymatic or acid treatment. The red algae for example, *Gelidium amansii*, which is made out of cellulose, glucan, and galactan, also can fill as a potential feedstock for bioconversion to ethanol [8]. The utilization of red algae as a raw material for bioenergy production is greater than other source of biomass [27]. Red algae can create bioethanol yield of 4% up to 43 % [8, 25-29]. On research Borines et al., *Sargassum* spp. used to manufacture bioethanol contains such alpha-cellulose 20,35%, hemicelluloses 25,73%, holocellulose 46,08% and mannitol 5,04% [28]. *Ulva lactuca* has biochemical composition to manufacture bioethanol contains such as galactosa 1%, glucose 8,2%, and xylose 4,5% [24].

The use of seaweed feedstock for bioethanol production continuously worried a competition between the availability of raw materials for food, feed, and energy source [30]. This makes problems continuously, so we need raw materials that do not make competition with food sources, such as sewage. Potential sources of raw material are abundant availability, low cost, have not been widely exploited people and structures containing simple sugars that can be converted into ethanol, such as lignocelluloses materials as seaweed processing waste. Utilization of alga waste, that contains cellulose (30%) and a bit of cellobiose (2,2%). In the fact, 277,5 mg g-1 of glucose can be reached with enzymatic hydrolysis methods that it following mild acid pre-treatment using sulfuric acid (0.1 % w/v, 121^{o}C, 1 h) [29]

Lignocellulosic material was derived from plants with the main components lignin, cellulose, and hemicellulose. Abundant availability especially as agricultural, farm, and forestry waste, making this material potentially as a source of energy through the conversion process, either the physical, chemical, and biological. Contains three components of lignocelluloses which include cellulose (30-50%), hemicelluloses (15-35%), lignin (13-30%) [31].

Cellulose is a composer compound 40% - 50% of the wood in the form of microfibrils cellulose, where hemicellulose is a matrix compounds among the microfibrils cellulose. Cellulose is an organic substance which most abundant in nature. Cellulose is insoluble in water and can not be digested by the human body. Cellulose dominate carbohydrates derived from plant (almost 50 %). Cellulose can be hydrolyzed to glucose using water media and assisted with acid or enzyme catalyst. Further glucose will be fermented into ethanol.

Lignin, on the other hand is a stringent compound surrounding and amplifying the cell walls. Lignin is a polymer of organic matter that is much and important in the world of plants. Lignin composed of phenolic polymer tissue. It makes the cellulose and hemicellulose fibers tissue more strong [32]. Lignin content can obstruct bioconversion of lignocellulosic to ethanol. Lignin protects cellulose, thus cellulose hydrolyzed to glucose difficulty. A Pretreatment process is needed. This protector breaks cellulose until easily hydrolyzed without much loss polysaccharides. In the saccharification of bioethanol, the highest value of convertion from alga to glucose occured in the treatment without pretreatment because the low lignin content in seaweed. Conversion value of lignicellulose into glucose without pre-treatment method is less than 13% [33]. Pre-treatment showed an increase in total soluble sugar content. It selectively removes the lignin with an increase in accessibility of ethanol using sugars [34]

Hemicellulose is polysaccharide having a molecular weight smaller than cellulose. Hemicellulose molecules more easy to absorb water and plasticity. Hemicellulose composed of various types of sugar. Five neutral sugars are glucose, mannose, galactose, xylose, and arabinose (pentosan).

Seaweed Waste into Bioethanol

Production of ethanol from cellulose materials requires several stages before entering into fermentation to produce ethanol. This is due to the structure of cellulose is more complex and should be simplified to ethanol fermentation process can be optimum [31]. Stages of bioethanol production using seaweed [25]. Starches must be hydrolyzed to fermentable sugar before they can be used. The constraint in process of enzymatic saccharification is the presence of lignin content in agricultural biomass. Therefore, although cellulose or starch material are less expensive than sugar-based substrates, there must be a conversion from cellulose or starch to fermentable sugar. Biological treatment showed an increase the ethanol purity grade, volume, and fermentation speed yield after 3 days of fermentation. This is contrasts with physical treatment in producing an ethanol yield.

The enzymatic hydrolysis can be completed independently from the alcoholic fermentation, a procedure known as Separate Hydrolysis and Fermentation (SHF), and the other procedures can run together as Simultaneous Saccharification and Fermentation (SSF) [35]. Hydrolysis process in the SHF method, should be possible at a temperatures as high as 50^{o}C in order to maintain enzyme stability to expand rates and reduce bacterial contamination. The SHF Process facilitates the separation of the sugar syrup from hydrophobic lignin that can be used for solid fuel production. Nevertheless, in this process lead to accumulation of the glucose that can inhibit the activity of endo-glucanases, exo-glucanases and β-glucosidase enzyme thereby affecting the yield and the reaction rates [19].

Ethanol production by SSF method will be faster than SHF method because glucose will be fermented simultaneously into ethanol. In addition, it can reduce the occurrence of contamination due to ethanol formation, the presence of anaerobic conditions, and continuous glucose conversion. The SSF method is present a lower cost as just a single reactor. In this context, it is fascinating to take note of that the ethanol that accumulates in the medium does not significantly influence the action of catalysts. The trouble of this procedure identifies with the diverse ideal temperature for enzymatic hydrolysis (45–50^{o}C) and alcoholic fermentation (28–35^{o}C) [19]. A bioethanol yield of 90,9% was obtained for the SSF Process [36]. Ethanol can be produced by fermentation of glucose using saccharophilic yeast strains such as *Saccharomyces cerevisiae* or some yeasts with fermentative potential [37]. There are yeast with fermentative potential from retrieved from Arabica coffee wet processing waste. The isolates yeast from Arabica coffee pulp showed a different pattern of ethanol production amount 5,01 -6,20%, while ethanol production of 4,5% for S.cereviviae [38]

Conclusion and Future Prospect of Bioethanol

Macroalgae (seaweed) can accumulate carbohydrates or cellulose as a significant bit of their biomass. The significant expense of carbohydrates or cellulose depolymerizing enzyme catalysts for pre-treatment of algal biomass makes the expense of algal bioethanol several folds higher. At present, these enzymes are delivered in microbial bioreactors for commercial use. Further research is required on screening of high cellulose or starches accumulating macroalgae from corresponding water bodies or to create proficient macroalgae using genetic designing or genetic engineering.

Genetic designing techniques ought to be activated to deliver all enzymes include cellulase and amylase within the algae or for creating enzyme of cellulolytic in bioreactors. Moreover, future research on the upregulation of carbohydrates or cellulose biosynthesis pathway enzymes for increased polysaccharides will also possibly increment algal bioethanol production. The possibility of competition between various pathways for carbon metabolism, including starch biosynthesis and capacity, may constrain ethanol production. There must be a condition to create ethanol simultaneously with photosynthesis and keep away from the steps of accumulation of starch and change back to sugar for ethanol. In this methodology, there might be a hindrance by collected ethanol towards the metabolic activity of algae and hence could decrease the productivity. This requires the need for ethanol tolerant algae for effective ethanol creation [32].

The fuels from commercial macroalgae, a priority to identify microorganisms that have a bioactive compound to metabolize major from carbohydrates or cellulose. There were some researchers have developed macroalgae specific enzymes to hydrolyze macroalgal carbohydrates/cellulose [32]. Most of the microorganisms were originated from marine flora and fauna. Their enzymatic functions on macroalgae have been well-reviewed in many studies. Once those kinds of microorganisms and enzymes have been identified and developed, they can be applied to the Simultaneous Saccharification and Fermentation (SSF) method [32]. Simultaneous Saccharification and Fermentation (SSF) method has many advantages: low contamination, low initial osmotic stress of fermenting microorganisms, and high energy efficiency. It should be noted however, that applying various microorganisms to a fermentation condition as in Simultaneous Saccharification and Fermentation (SSF) method could inhibit enzyme activity and extracellular enzyme secretion [39]. Also, various and complex carbohydrates of macroalgae can lead to a low yield of bioethanol in the Simultaneous Saccharification and Fermentation methode [32].

In the process of bioenergy production, it is necessary improving microbial capacities molecularly through biotechnology to modify the physicochemical properties of macroalgae, such as macrolagae metabolism engineering through biosynthesis pathways so as to increase their polysaccharide synthesis and productivity [40,41]**.** This methodology could significantly improve seaweed productivity and contribute to the success of marine biorefinery. There have been several attempts that have been made in development of molecular biotechnology in macroalgae, such as gene mapping [42], the addition and introduction of foreign genes in macroalgae cells [43], and selection of promoters [31]. The bioethanol plant under consideration has been divided into varoius parts operating component preferences [44]

References

[1] E. Hambali , Mudjadlipah S, Tambunan A.H, Pattiwiri A.W, and Hendroko R, *Bioenergy Technology.* Jakarta : Agromedia Library, 2007.

[2] F. Offei , Mensah M, Thygesen A, and Kemausour, “Seaweed bioethanol production : a process selection review on hydrolysis and fermentation,” Fermentation 4, pp. 99-117, 2018.

[3] N. Trivedi , Gupta V, Reddy CRK, and Jha B, “Enzymatic hydrolysis and production of bioethanol from common macrophytic green alga Ulva fasciata Delile,” *Bioresource Technology* 150, pp. 106-112, 2013

[4] N. Trivedi , Reddy CRK, Radulovich R, and Jha B, “Solid state fermentation (SSF)-derived cellulase for saccharification of the green seaweed Ulva for bioethanol,” *Algal Res.* 9, pp. 48-54, 2015

[5] P.I. Hargreaves , Barcelos CA, da Costa ACA, and Pereira N, “Production of ethanol 3G from *Kappaphycus alvarezii* : evaluation of different process strategies,” *Bioresource Technology* 134, pp. 267-263, 2013

[6] A. Chirapart , Praiboon J, Puangsombat P, Pattanapon C, and Nunraksa N, “Chemical composition and ethanol production potential of Thai seaweed species,” *J. Appl. Phycol.* 26, pp. 979-986, 2014

[7] J.H. Park , Hong JY, Jnag HC, Oh SG, Kim SH, yoon JJ, and Kim YJ, “Use of *Gelidium amansii* as a promising resource for bioethanol : a practical approach for continuous dilute-acid hydrolysis and fermentation,” *Bioresource Technology* 108, pp. 83-88, 2012

[8] N.J. Kim , Li H, Jung K, Chang HN, and Lee PC, “Ethanol production from marine algal hydrolysates using *Escherichia coli* KO11,” *Bioresource Technology* 102, pp. 7466– 7469, 2011

[9] S.M. Lee , and Lee JH, “Ethanol fermentation for main sugar components of brown-algae using various yeasts,” *J. Ind. Eng. Chem.* 18, pp. 16-18, 2012.

[10] M.G. Borines , de Leon RL, and Cuello JL, “Bioethanol production from the macroalgae *Sargassum* spp.,” *Bioresource Technology* 138, pp. 22-29, 2013.

[11] G.S. Kim , Myung K.S, Kim Y.J, Oh K.K, Kim J.S, Ryu H.J, and Kim K.H, *Methode of producing biofuel using sea algae*. Seoul : World Intelectual Property Organization, 2007.

[12] P.S Delabona , Farinas C.S, da Silva M.R, Azzoni S.F, and Pradella J.G.C, “Use of a new *Trichoderma harzianum* strain isolated from the amazon rainforest with pretreated sugar cane bagasse for on-site cellulase production,” *J. Bioresource Technology,* vol.107, no.1 pp. 517–521, 2012

[13] B. Schulz , Boyle C, Draeger S, Rommert AK, and Krohn K, “Endophytic fungi: a source of novel biologically active secondary metabolites,” *Mycol. Res.* 106, pp. 996-1004, 2002

[14] G.A. Strobel , “Endophytic fungi: News source for old and new pharmaceuticals,” *Pharmaceutical News,* vol. 3, no.11, 2003.

[15] J.Kobayashi, and Ishibashi, “M. Bioactive metabolites of symbiotic marine microorganisms,” *Chemical Review* 93, pp. 1753-1769, 1993.

[16] T. Padmavathi , Agarwal P, and Nandy V, “Exploring marine fungal strains for cellulase production,” *Annals of Biological Research* 3, pp. 3602-3613, 2012 .

[17] U.F. Castillo , Strobel GA, Ford EJ, Hess WM, Porter H, Jensen JB, Albert H, Robinson R, Condron MA, Teplow DP, Steven D, Yaver D, and Munumbicins, “wide-spectrum antibiotics produced by Streptomyces NRRL 30562, endophytic on Kennedia nigricans,” *Microbiology* 148, pp. 2676, 2002

[18] T.S. Suryanarayanan, Thirunavukkarasu N, Govindarajulu MB, Sasse F, Jansen R, Murali TS, “Review: Fungal endo-

phytes and bioprospecting," *Fungal Biology Review* 23, pp. 9-19, 2009.

[19] C.R. Soccol , Vandenberghe LPS, Medeiros ABP, Karp AG, Buckeridge M, Ramos LP, Pitarelo AP, Leitao VF, Gottschalk LMF, Ferrara MA, Bon EPS, de Moraes LMP, Araujo JA, and Torres FAG, "Bioethanol from lignocelluloses: Status and perspectives in Brazil," *Bioresource Technology* 101, pp. 4820-4825, 2010.

[20] C. Ranghukumar , Ranghukumar S, Chinnaraj A, de Souza TM, and Reddy CA, "Laccase and other lignocelluloses modifying enzymes of marine fungi isolated the coast of India," *Botanica Marina* 37, pp. 515-52, 1994.

[21] Y.I.P. Zhang , and Lynd LR, "Toward an aggregated understanding of enzymatic hydrolysis of cellulose: non-complexed cellulase systems," *Biotechnol. Bioeng* 88, pp. 797–824, 2004

[22] M. J, Hessami, Cheng, S. F., Ambati, R. R., Yin, Y. H., and Phang, S. M., "Bioethanol production from agarophyte red seaweed, Gelidium elegans, using a novel sample preparation method for analysing bioethanol content by gas chromatography," *Biotech*, vol. 9, no. 1, pp. 25, 2019.

[23] E. T, Kostas, White, D. A., and Cook, D. J, "Bioethanol Production from UK Seaweeds: Investigating Variable Pre-treatment and Enzyme Hydrolysis Parameters," *BioEnergy Research*, pp. 1-15, 2019.

[24] H. Wal , Sperber Bram LHM, Tan BH, Bakker Robert RC, Brandenburg W, and Contreras AML, "Production of acetone, butanol and ethanol from biomass of green seaweed *Ulva lactuca," Bioresource Technology* 128, pp. 431-437, 2013

[25] S. Kumar , Gupta R, Kumar G, Sahoo D, and Kuhad RC, "Bioethanol production from *Glacilaria verrucosa*, a red alga, in a biorefinery approach," *Bioresource Technology* 135, pp. 150-156, 2013.

[26] Y. Khambhaty , Mody K, Gandhi MR, Thampy S, Maiti P, Brahmbhatt H, Eswaran K, and Ghosh PK, "*Kappaphycus alvarezii* as a source of bioethanol," *Bioresource Technology* 103, pp. 180-185, 2012.

[27] S.G. Wi , Kim HJ, Mahadevan SA, Yang DJ, and Bae HJ, "The potential value of seaweed Ceylon moss (*Gellidium amansii*) as an alternative bioenergy rersource, " *Bioresource Technology* 100, pp. 6658-6660, 2009

[28] M.G. Borines , de Leon RL, and Cuello JL, "Bioethanol production from the macroalgae *Sargassum spp*.," Bioresource Technology 138, pp. 22-29, 2013

[29] K.P. Candra , Sarwono, and Sarinah, "*Eucheuma cotonii* from bontang sea water," *Journal of Coastal Development* 15, pp. 45-50, 2011.

[30] H. Y. Lee, ., Lee, S. E., Jung, K. H., Yeon, J. H., and Choi, W. Y, " Repeated-batch operation of surface-aerated fermentor for bioethanol production from the hydrolysate of seaweed Sargassum sagamianum," *Journal of microbiology and biotechnology*, vol. 21, no. 3, pp. 323-331, 2011

[31] R.P. John , Anisha GS Nampoothiri KM, and Pandey A, "Micro and macroalgal biomass: a renewable source for bioethanol," *Bioresource Technolgy* 102, pp. 186–193, 2011.

[32] K.A. Jung , Lim SR, Kim Y, Park JM, "Potential of macroalgae as feedstock for biorefinery," *Bioresource Technology* 135, pp.182-190, 2013.

[33] M. Yanagisawa , Nakamura K, Ariga O, and Nakasaki K, "Production of high concentration of Bioethanol from seaweeds that contain easily hydrolyzable polyssacharides," *Process Biochemistry* 46, pp. 2111-2116, 2011

[34] Kirti, Patil RS, and Londonkar, "Bioethanol production from agro waste-Pigeon pea (*Cajanus cajan (L.) Mill sp.*) stalk using solid state fermentation," WSN 117, pp. 59-81, 2019

[35] Y. Cho,., Kim, M. J., and Kim, S. K, "Ethanol production from seaweed, Enteromorpha intestinalis, by separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) with Saccharomyces cerevisiae," *Ksbb Journal*, vol. *28, no.* 6, pp. 366-371, 2013.

[36] I.S. Tan , and Lee KT, "Enzymatic hydrolysis and fermentation of seaweed solid waste for bioethanol production : an op-

timization study," *Energy*, pp. 1-10, 2014

[37] M. R. Swain, , Natarajan, V., & Krishnan, C., "Marine enzymes and microorganisms for bioethanol production," *Advances in food and nutrition research,* vol. 80, pp. 181-197, 2017.

[38] M.G. Borines , de Leon RL, and Cuello JL, "Bioethanol production from the microalgae *Sargassum spp,*" Bioresource Technology 138, pp. 22-29, 2013.

[39] C. Reddy , Gupta M, Mantri V, and Jha B, "Seaweed protoplasts: status, biotechnological perspectives and needs," *J. Appl. Phycol* 20, pp. 619–632, 2008.

[40] H. Takeda, ., Yoneyama, F., Kawai, S., Hashimoto, W., and Murata, K, "Bioethanol production from marine biomass alginate by metabolically engineered bacteria," *Energy & Environmental Science*, vol. *4*, no. 7, pp. 2575-2581, 2011.

[41] A. J. Wargacki, Leonard, E., Win, M. N., Regitsky, D. D., Santos, C. N. S., Kim, P. B., and Lakshmanaswamy, A., "An engineered microbial platform for direct biofuel production from brown macroalgae," *Science*, *335,* pp. 308-313, 2012

[42] Sun, C., Chen, Y. J., Zhang, X. Q., Pan, J., Cheng, H., & Wu, M., Draft genome sequence of Microbulbifer elongatus strain HZ11, a brown seaweed-degrading bacterium with potential ability to produce bioethanol from alginate. *Marine genomics*, *18*, (2014) 83-85.

[43] D. Renn, "Biotechnology and the red seaweed polysaccharide industry: status, needs and prospects," *Trends in Biotechnology*, vol. 15, no. 1, pp. 9-14, 1997.

[44] N.K. Sharma , and Kumar S, "Reliability, MTTF and cost analysis of bioethanol plant by orthogonal matrix method," WSN 113, pp. 87-97, 2018