Potentiality of Vietnam Green Seaweed for Bioethanol Production

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Abstract

The Paris Conference on Climate Change in 2015 called on all nations to work together to reduce climate change. The producing biofuels from seaweed biomass is a potential research to contribute to reducing climate change. In Vietnam, green seaweed is a large biomass source, their suitable for ethanol production. This is a solution to create clean fuels, reducing environmental pollution and increase economic benefits for the coastal people. Therefore, this review article provides an overall perspective required for the composition of species, the aquaculture, the chemical composition and the process of saccharification and fermentation to produce ethanol from green seaweeds.

Keywords: Green seaweed, chemical composition, cultivation, fermentation, hydrolysis, bioethanol

1. Introduction

The* Paris Conference on Climate Change in 2015 called on all nations to work together to reduce climate change. From 2015 to 2020 the providing $ 100 billion each year for developing countries that use renewable amount to reduce greenhouse gas emissions into the environment to against climate change, Vietnam needs to respond to this call. Producing ethanol fuel from seaweed is a new research. Vietnam has a long coastline that contain diversity seaweed sources and producing ethanol fuel techniques are not too complicated and investment not too big. So this is a potential and suitable technology for Vietnam. The green seaweed (GS) is a suitable biomass source for biofuel production [1]. This article have an overview of the species composition of Vietnam GS, aquaculture GS and chemical composition GS and the process of GS hydrolysis and alcohol fermentation.

The Vietnam GS are diverse composition species and large biomass. That is estimated about 2 million dry tons are produced each year from 137 species of green seaweed [2, 3]. However, this biomass source has not been used properly, only a few species are researched and processed biological products, the others is apoptosis in the nature to environmental pollution. The GS is a tropical species that are good photosynthesis to produces large biomass, especially some species such as: *Ulva (U.), Chaetomorpha (C.), Cladophora (C.), Enteromorpha (E.)* are fast growing which are suitable for aquaculture research. The chemical composition of GS are composed mainly of carbohydrates 40-70%, the main carbohydrates are cellulose, starch, ulvan, agar that are suitable for alcohol production [4]. The articles hydrolysate and ethanol fermentation from GS such as: *U. pertusa, U. lactuca, U. fasciata, C. linum, C. socialis, E. torta* .... Therefore, the ethanol producing research from GS is an appropriate solution to create clean fuels, solve environmental pollution and increase economic benefits for coastal people.

2. Species composition and chemical composition of green seaweed

2.1 Green seaweeds species common in Vietnam

According to results of survey GS resources have indicated the common GS species of Vietnam in Table 1 that contain survey parameters of GS species for ethanol production. Survey parameters include natural habit, frequencies encountered, weight, coverage [1, 2]

Tab.1 shows that have 42 common Vietnam GS species life in sea, shrimp pond, ponds, salt ponds and water treatment ponds... The genus of *Monosstroma, Rhizoclonium, Valonia, Codium, Caulerpa, Cladophoropsis, Bryopsis* and *Halimeda* are only in sea coastal areas. Which have low weight 130 g/m2, the frequency encountered 10-20% the coverage is low 10-30%.

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Table 1. Selection of green seaweed of Vietnam for ethanol production

TT	Species		Natural habit	Frequency encountered (%)	Weight (dry g/m^2)	Coverage degree (%	Selection
1	*Monostroma nitidum*		Sea	10,7	130±15	30	-
2	*Ulva*	*lactuca*	Sea	53,6	130±17	55	+
3		*papenfussii*	Shrimp pond, Sea	57,1	220±22	70	+
4		*reticulata*	Sea	64,3	210±18	60	+
5		*clathrata*	Sea	17,9	60±12	10	-
6	*Enteromorpha*	*torta*	Water ditch	100,0	280±25	65	+
7		*flexuosa*	Shrimp pond	85,7	190±21	55	+
8		*compressa*	Salt pond	64,3	120±14	50	+
9		*intestinalis*	Water ditch	53,6	230±25	45	+
10	*Rhizoclonium*	*kerneri*	Shrimp pond	25,0	40±5	20	-
11		*kochianum*	Shrimp pond	21,4	45±5	20	-
12		*grande*	Shrimp pond	28,6	35±5	15	-
13	*Chaetomorpha*	*gracilis*	Shrimp pond	35,7	180±14	40	-
14		*area*	Water treat pond	75,0	300±21	80	+
15		*linum*	Shrimp pond	71,4	340±26	60	+
16		*antennina*	Shrimp pond	57,1	260±16	35	-
17		*capillaris*	Water treat pond	67,9	210±14	65	+
18		*ligustica*	Shrimp pond	64,3	190±16	45	+
19		*javanica*	Pond	60,7	150±15	40	+
20		*crassa*	Sea	35,7	120±10	40	-
21	*Cladophora*	*albida*	Sea	35,7	30±3	10	-
22		*crispula*	Shrimp pond	75,0	190±16	55	+
23		*flexuosa*	Pond	67,9	110±8	40	+
24		*laetevirens*	Sea	28,6	65±4	20	-
25		*papenfussii*	Sea	32,1	60±5	15	-
26		*prolifera*	Sea	25,0	60±5	35	-
27.		*socialis*	Pond	96,4	120±8	55	+
28	*Valonia*	*aegagropila*	Sea	21,4	20±3	10	-
29		*fastigiata*	Sea	21,4	25±2	10	-
30	*Boodlea composita*		Sea	25,0	35±3	5	-
31	*Cladophoropsis*	*membranacea*	Sea	17,9	35±3	25	-
32		*adhaerens*	Sea	14,3	20±2	25	-
33	*Codium*	*adhaerens*	Sea	21,4	20±2	15	-
34		*repens*	Sea	21,4	20	15	-
35	*Caulerpa*	*peltata*	Sea	17,9	50±3	40	-
36		*racemosa*	Sea	17,9	110±6	45	-
37		*sertularioides*	Sea	17,9	60±5	40	-
38		*taxifolia*	Sea	17,9	50±4	35	-
39	*Bryopsis*	*indica*	Sea	17,9	30±2	10	-
40		*hypnoides*	Sea	17,9	30±2	10	-
41	*Halimeda*	*gracilis*	Sea	17,9	80±4	25	-
42		*opuntia*	Sea	17,9	85±4	25	-

Note: (+) select species seaweeds, (-) not select species seaweeds.

Table 2. Chemical composition of green seaweed species for ethanol production

Green seaweed		Polysaccharide	Protein (%)	ASH (%)	Lipid (%)	Total carbo-hydrates (%)
Ulva	*lactuca*	Cellulose (40-42%), Starch (4-6%), Ulvan (10-12%), Agar (4-5%)	20,24	17,97	3,24	56,06
	papenfussii		21,35	15,93	3,32	56,40
	reticulata		22,52	14,58	3,28	56,71
Enteromorpha	*flexuosa*		16,83	12,98	2,9	65,59
	intestinalis		19,96	15,84	2,69	60,92
	torta		14,67	14,64	1,98	65,06
	compressa		14,66	12,17	3,83	67,24
Chaetomorpha	*area*		15,68	11,80	2,43	67,58
	capillaris		13,70	11,96	2,03	69,83
	javanica		13,45	17,91	2,32	64,21
	ligustica		15,68	15,32	3,71	63,24
	linum		21,01	10,46	2,53	64,20
Cladophora	*socialis*		16,13	8,72	2,24	70,88
	flexuosa		17,06	8,8	2,34	69,99
	crispula		18,84	8,6	1,70	65,07

The *Ulva* genus is widely distributed along the coastal provinces, extending from Vung Tau to Quang Ninh. The *Ulva* thallus is small size and attached to the hard bottoms, so it is difficult to exploit it, and it is only exploited at the crop end when the old *Ulva* thallus it exit to the hard bottoms and *Ulva* quality is reduce. The *Chaetomorpha, Enteromorpha and Cladophora of genus* are widely distributed in brackish water areas, such as estuaries and aquaculture ponds. These are not attached to the hard bottoms and life float a depth of less than 1m, thus easy exploitation of source. In addition, *Chaetomorpha, Enteromorpha, Cladophora* were successfully cultivated [1, 2]

2.2 Chemical composition of the green seaweed species selected for ethanol production

The large GS species biomass suitable for ethanol production were selected and determined chemical composition in Tab. 2 [5]

The polysaccharides composition GS include cellulose, starch, ulvan and agar in which cellulose and starch are the two main constituents that are 40-50 % of dry weight. Thus, when GS will be hydrolysis has large amounts of glucose [4]. So GS is a good material for ethanol production. Tab. 2 shows that the polysaccharides composition GS are different with lignocellulose of plant biomass on land. GS lack hemicellulose and lignin, which are essential for structural support in most terrestrial plants such as: *Ch. linum* have the cell wall has an outer lamellar part mainly made of highly crystalline cellulose and an inner amorphous matrix made of a complex branched polymer of galactose, with some xylose [6] *U. fasciata* does not contain lignin in their cell wall [7]. The wood biomass contains about 50% cellulose, lignin 23-33%, hemicellulose 15-20% [8], the bagasse contains about 44.7% w cellulose, 24.6% w lignin, 28.5% w hemicellulose [9], the straw contains 37.8% w cellulose, 5.0% w lignin, 21.6% w hemicellulose [10].

Example, the sugar composition of *C.linum* seaweed was 65.16% glucose, 18.56% rhamnose and 11.58% galactose. The *C. linum* has a high content of 6 carbon sugar, which accounts for 96% of sugar content and 3-4% of 5 carbon sugar. This indicates that *C.linum* is easy hydrolysate and fermentation to ethanol production, thus indicating that this material is superior to other cellulose-rich materials such as wood, bagasse and straw [11]. Because of the wood, straw and bagasse materials when are hydrolysis to 5 carbon sugar that yeast is difficult to use. According to some studies when hydrolysis of wood to 60% glucose and 40% xylose [8], hydrolysis of bagasse yields 70% glucose and 30% xylose [9], the hydrolysis of straw to 75% glucose and 25% xylose [10].

3. Cultivation of green seaweed species suitable for ethanol production

Vietnam is located in the tropical climate so it is very suitable for the growing of GS. However, the cultivation of GS in Vietnam is still limited, only few species are cultivated to providing in food that is high economic value such as *Caulerpa lentifera, Caulerpa taxifolia....* and the others *U. lactuca, U. reticulata and*

Table 3. The harvested yield of the green seaweeds is rotational cultivation in ponds

Months	1	2	3	4	5	6	7	8	9	10	11	12
Species	*Enteromorpha torta*			*Chaetomorpha linum*						*Cladophora socialis*		
Season	3 crop time (28 day/ season)			4 crop time (35-42 day/ season)						2 crop time (35-42 day/ season)		
Harvesting yield	17,23 fresh tons			15,34 fresh tons						11 fresh tons		
Total harvesting Yield	3 x 17,23+4 x 15,34+2 x 11=117,82 fresh tons /ha/year=27,01 dry tons/ha/year (dry/fresh=1/5)											

U. papenfussii have not seed production. In addition, there is a research on the GS cultivation for ethanol production. The cultured species are *C. linum, E. torta and C. socialis*. During the growing of them was determined of weight fluctuations a life cycle and harvested yield of each species [1,12,13]

The aquaculture conditions *C. linum* and *C. socialis* is easier to do than *E. torta.* The right temperature for *E. torta* grows from 18-24ºC, while the temperature grows in *C. linum* is 30-40ºC and *C. socialis* is 22-35ºC. The season in the year of *E. torta* is January-March, *C. linum* is April - October and seaweed *C. socialis* in October this year to March next year. The growing season of *E. torta* is shorter than *C. linum* and *C. socialis*. These species have huge reserves, them exploited from the wild and aquaculture up to 2.5 million tons/ year. The aquaculture time is 7 to 9 weeks and the harvesting time is from 4^{th} to 5^{th} weeks. The total yield is 18-27 tons/ha. The comparing total harvesting yield of other biomass such as: acacia hybrid wood will be 8-9 tons dry/ha [14], 6 dry tons/ha of rice straw [10], 5 dry tons/ha of bagasse [15]. Indicate that, the growth and development of GS are better cultivation time and harvesting yield than biomass other. These seaweed species provide year-round ethanol production.

4. Technology of ethanol production from green seaweed

4.1 Hydrolysis of green seaweed

Clean processing of green seaweed

After harvest, GS must be cleaned prior to ethanol production. Steps of cleaning seaweed are salt separation and separation of debris such as rock, sand, snails or rubbish, then wash them with water after dried them to a moisture content of 10-15%. The mechanical processing, GS will be milled by grinding machines to the size of GS as 0.5-5mm. This processing makes the hydrolysis easy [16, 17]. After clean processing of GS, they are hydrolysis that converts polysaccharide to monosaccharide, which are used for fermentation. GS is hydrolyzed by two methods. The acid hydrolysis method, GS of carbohydrate is effectively hydrolyzed to produce monosaccharide by dilute sulfuric acid at high temperature. The four factors influencing this process are reaction temperature, reaction time, acid concentration and seaweed weight. The enzyme hydrolysis method, GS will be pretreated with acid then them hydrolyzed by enzyme [18].

The green seaweed hydrolysis by acid

Under the action of acid the polysaccharide of seaweed will be chopped to oligosaccharide or monosaccharide. The H^+ ions of the acid interact directly with the polysaccharide at the linkage linking the monosaccharide to the oligosaccharide or monosaccharide. Hydrolysis of acid produces a solution of sugar required for ethanol fermentation [18]. There are some seaweed species that are efficiently hydrolyzed by acid such as *U. pertusa* has 59% w of carbohydrate that is hydrolyzed at 3% v/v H_2SO_4, 120°C, 30 minutes, the result is 91% of hydrolyzed yield. The hydrolyzed solution is contained 37% of rhamnose and 16.4% of glucose [19]. *U. lactuca* has 56% w of carbohydrate that is hydrolyzed by 1M H_2SO_4, 100°C, 3 hours, the result is 90% of hydrolyzed yield. The hydrolyzed solution is main contain of rhamnose and glucose [20]. *C. linum* has 64% w of carbohydrate that is hydrolyzed at 3% v/v H_2SO_4, 120°C, 54 minutes, the result is 89% of hydrolyzed yield. The hydrolyzed solution is contained 18% of rhamnose and 65% of glucose and 11% of galactose [11]. *C. socialis* 70% w of carbohydrate that is hydrolyzed at 4% v/v H_2SO_4, 120°C, 60 minutes, the result is 89% of hydrolyzed yield. The hydrolyzed solution is contained 18% of rhamnose and 27% of glucose and 49% of galactose [21]. Thus, different seaweed species have different hydrolysis conditions. The GS usually hydrolyzed conditions as H_2SO_4 of 1-5% v/v, temperature of 100-120^{O}C, time from 30

minutes to 2 h. Compared hydrolyzed conditions with other materials such as soft wood is 1/3 of rate subtrate, 10g/l of H_2SO_4,228ºC in 11 minutes[22]. According to Lynd, the hard wood is milled to a size of 10 mm and then they are hydrolyzed in 75% v/v of H_2SO_4 at 50°C, then diluted to 20-30%v/v and continue hydrolyzed in 1 h to create a mixture of glucose and xylose and wood residue, then recover sugar and continue to hydrolyse the residue twice. During this hydrolysis to sugar is 70% of glucose and 30% of xylose [8]. According to Pattana 's research, bagasse is hydrolyzed with 3% v/v of H_2SO_4 at 120 ° C in 2 h to produce sugar for lactic acid fermentation [23]. The hydrolyzate process of GS is simpler than lignocellulose materials and GS hydrolyzate solution has mainly 6 carbon sugar as glucose, rhamnose, galactose which are suitable sugars for fermentation.

The green seaweed hydrolysis by enzyme

At the present, the biomass enzymatic hydrolysis has been studied by many authors. In this process, the biomass will be pretreated then to add enzymes to do biomass saccharification. *Ulva spp.* is pretreated at 120ºC in 20 minutes and then added 1% v/w cellulase enzyme (Acremonium trade name has 322 FPU/g of active), 5% w/v of polysaccharide conc., the hydrolysis process is at 50ºC in 72 h to 95% of glucose = 47 g/l and then fermented by *S. cerevisiae IR-2* at 30°C in 32 h to 20 g ethanol/l. [24]. *U. pertusa* is mixed at a rate of 75g dry/l and pre-treated with 0.1 M citric acid, at 121ºC in 20 minutes, then they are hydrolyzed by enzyme Meicelase has 73,3 U of cellulase and 227 U of cellobiase (Merck, Germany). The hydrolysis is done rate of 20g seaweed/ g enzyme in120 h at pH = 5.5, at temperature 50ºC. The results is 43 g glucose/l of hydrolyzed solution then that is fermented by *S. cerevisiae IAM 4178* to 18.5 g/l of fermented yield [25]. *E. intestinalis* was pretreated by 75 mM of H_2SO_4 conc., at 120°C in 60 min then by addition of 1% v/w of Celluclast 1.5 L and Viscozyme L hydrolyzed to 40.7 g/l of sugar and then fermentation with *S.cerevisiae* yeast. The fermented yield are 8.6 g ethanol/l of separate hydrolysis and fermentation (SHF) in 24 h and 7.6 g ethanol/l of simultaneous saccharification and fermentation (SSF) in 120 h [26] *C. linum* is pretreated by some method such as high temperature of 190-200°C in 5 minutes, exploded at 1.9 MPa and plasmaized in 48 h. Then addition enzyme Celluclast 1.5 L at a dose of 15 FPU/g, subtrate conc. of 10 g/00 ml hydrolyzed in conditions at 50°C, in 24 h and then reduced heat to 32ºC and addition of 20 FPU/g and 2 g/l yeast prepared and fermented in 200 h. The result of this study is fermented yield 15-18 g ethanol/100 g dried seaweed [7]. Another study, *C. linum* was hydrolysed by the Vicozyme L enzyme. The 100 g/l of *C. linum* conc. is pretreated by 0.3%v/v of H_2SO_4 conc., at 120°C, in15 minutes. Then saccharification of *C. linum* is add 42.5U/ g of Viscozyme L enzyme conc. to done in 33 h, at 50ºC of temperature, pH 5.2. This process to 50 g/l sugar of hydrolysis solution, the main sugar was 20.6 g/l of glucose, 8.05 g/l of galactose and 2.46 g/l of cellobiose [27]. While the treated lignocelluloses of bagasse is complex, they were pretreated in conditions as: rate of NaOH 0.1 g/ l bagasse, at 121°C in 1 h, then they were hydrolyzed follow to conditions such combining enzyme conc. as: 5U of endoglucanase, 10U of CMC ase exoglucanase, 30U of betaglucosidase and rate of 1/15 dry substrate, pH 4.8, temperature of 50ºC in 48 h, stirring of 150 rounds /min, after the hydrolysis add 70 U/g of laccase enzyme was broken phenol. The results of this study were 330 g glucose/kg bagasse, and then fermentation was 120 g ethanol/kg bagasse of Yield [15].

4.2 Ethanol fermentation from seaweed hydrolyzed solution

The biomass was hydrolyzed by acid or enzyme to hydrolysis solution which is fermented to ethanol by yeasts. This fermentation depends on the composition of fermented broth and the quality of yeast strains. In groups of microorganisms to ethanol-producing, *Saccharomyces cerevisiae* (*S. cerevisiae*) is the most widely used microorganism. *S. cerevisiae* yeast can ferment sugars such as glucose, galactose, manose, double sugars such as sucrose, maltose [4,26]. The GS has content 30-40%w of protein and mineral, when GS were hydrolyzed to nutrient medium of solution which is suitable of fermentation, so not adding nutrients to the fermentation broth. Besides the GS has less 5carbon of (3-4% w) when GS was hydrolyzed by acid no produces toxins to effective yeast such as Furfural, 5-HMF (5-hydroxyl methyl furfural). And especially the GS contain very little lignin, so does not require hydrolysis of lignin. The component of hydrolyzed GS is full of nutrients, non-toxic, so the hydrolysis solution is the suitable medium for yeast to grow and develop. Therefore, the ethanol fermented studies from hydrolysis solution of GS such as: Study on fermentation of *C. linum* hydrolyzed solution with H_2SO_4 acid. Fermentation conditions: Red Ethanol yeast, sugar conc. 53 g/l, pH 4.3, at temperature 27ºC, in76 h to 18.2 g ethanol/l the same 182 g ethanol /1 kg dry seaweed [11]. The fermentation of *Ulva spp.* hydrolyzed solution by the Acremonium enzyme.

Fermentation conditions: *S. cerevisiae IR-2* yeast, sugar conc. of 47 g/l, pH 4.5, temperature 30°C in 48 h to 20 g ethanol/l [24]. The fermentation of *U. pertusa* hydrolyzed solution by the Meicelase enzyme. Fermentation conditions: *S. cerevisiae IAM 4178* yeast, sugar conc. of 43g glucose/l, pH 5.5, temperature 30°C in 72 h to 18.5 g ethanol/l the same 185g ethanol/ 1kg seaweed [25].The fermentation of *E. intestinalis* hydrolyzed solution by the Viscozyme L and Cellulase enzyme. Fermentation conditions: *S.cerevisiae* KCTC 1126 yeast, sugar conc. of 40,7 g/l, pH 5.5, temperature 30°C in 48 h to 8,6 g ethanol/l [26].The fermentation of *U. fasciata* hydrolyzed solution by the Cellulase 22119 and Viscozyme L enzyme. Fermentation conditions: *S.cerevisiae* (MTCC No. 180) of yeast, sugar conc. of 21g /l, pH 4.8, temperature 28°C in 48 h to fermented yield of 88.2% [6].The fermentation of *C. linum* hydrolyzed solution by the Viscozyme L enzyme. Fermentation conditions: Red Ethanol yeast, sugar conc. of 50 g /l, pH 4.5, temperature 30°C in 96 h to to 14.4 g ethanol/l the same 144g ethanol/ 1kg seaweed [27]. *C. linum* was pretreated by various methods, after hydrolyzed preliminarily with Celluclast 1.5 L in 24 h, then saccharification simultaneous fermentation in conditions as: *S. cerevisiae ATCC 96581* yeast and conc.of 2g/l, seaweed conc. of 100 g /l, pH 5.5, temperature 32°C in 200 h to 18 g ethanol/l the same 180g ethanol/ 1kg seaweed [7]. The above studies show that, fermentation conditions of different GS species it depend on the saccharification and fermentation of method and the sugar composition in the hydrolyzed solution. The method used by many authors is hydrolyzed GS by enzyme and then fermentation. The ethanol content produced from GS is high to140-180 g ethanol /1kg of seaweed, while the hydrolysis and fermentation of bagasse to 120-125 g ethanol/kg [15] and 160 g ethanol/1kg of straw [8].

5. Conclusions

The above studies have shown the promising potential of Vietnam green seaweed for ethanol production. The Vietnam green seaweed has a large they are successful aquaculture to provide enough raw materials for ethanol production. Their chemical composition is high sugar and low lignin, hydrolyzed solution does not inhibitor to yeast. Ethanol production techniques from green seaweed are not too complicated, in which the method of hydrolyzing green seaweed by enzyme and then ethanol fermentation is the suitable choice to application in Vietnam.

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