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Bio-butanol Production by *Clostridium saccharoperbutylacetonicum* N1-4 using Selected Species of Brown and Red Macroalgae

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A B S T R A C T

Macroalgae are a promising option because they can be propagated easily along the seaside thus eliminating the need for land and nutrient resources. Hence, different macroalgae were assessed for their potential in butanol fermentation. In this study, four species of brown macroalgae (*Undaria pinnatifida*, *Laminaria japonica*, *Ecklonia stolonifera*, *Hizikia fusiforme*, and *Sargassum fulvellum*) and two species of red macroalgae (*Porphyra tenera* and *Gelidium amansii*) were investigated for the production of butanol by *Clostridium saccharoperbutylacetonicum* N1-4. To hydrolyze the polymeric materials of the algal biomass, dilute acid hydrolysis was carried out using 0.15 M H_2SO_4 followed by thermal pretreatment at 121°C for 1 h. Using 100 g/L of hydrolyzed brown alga, the highest butanol production (5.51 g/L) was observed for *L. japonica*. Other brown and red macroalgae did not exceed the butanol production by *L. japonica*. Moreover, the detoxification of the thermo-chemically pretreated hydrolysate of *L. japonica* using the activated carbon and overliming method, increased the butanol production by 24.14 and 12.16%, respectively. These results showed that macroalgae could be a promising substrate for butanol fermentation that is cheap, easily propagated, and non-terrestrial and non-food competing.

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INTRODUCTION

Many studies have been achieved to explore the potential of different kinds of biomasses in butanol fermentation. This involves the direct conversion of known biomasses such as sugarcane bagasse [1], liquefied corn starch [2], wheat bran [3], cassava starch [4-6], corn starch [5], soy molasses [7], sago starch [8], and kudzu roots [9]. Other utilization also involves the use of carbon-rich effluent or spent biomasses such as spent liquor from spruce [10], corn stalk [11], corn stover, wheat straw, distillers dried grains with soluble [12], corn fiber, wheat bran [3], and potato wastes [13]. But few have taken investigations on the use of macroalgae as feedstock for butanol fermentation [14-16].

It is commonly known that using food crops can be an inappropriate alternative source for biofuel production.

Thus, a search for more suited terrestrial non-food crop sources has been initiated. However, the aspect on additional agricultural land usage which concerns terrestrial plants poses as a major limitation. To resolve this, a third generation biomass for biofuel production was introduced by using macroalgal biomass [17]. Macroalgae is known to have higher growth rate than land biomass because it can be harvested 4-6 times in a year. Cultivation is also not a burden because only sunlight, CO_2, and marine water are needed for its growth [17-18]. On the other hand, land based biomass may need additional fertilizer input and water. It was estimated that an increase of more than 22 million tons (MT) from 14 MT of annual marine algae production will be achieved

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in 2020 [18]. In Korea, current production of marine algae nearly reaches 500,000 tons/year [18]. Since macroalgae does not have lignin but contains high carbohydrates, it is superior for biofuel feedstock than lignocellulosic biomass. Moreover, its CO_2 absorption capacity is about 5-8 times higher (36.7 tons/ha) than the starch and wood-based biomass [18]. CO_2, as a greenhouse gas, is known to contribute to global warming.

In our previous study, we observed that *Laminaria japonica* (a brown macroalgae) produced considerable amounts of butyrate during butyrate fermentation [19]. Based on the thermo-chemically pretreated 100 g/L *L. japonica*, the *Clostridium tyrobutyricum* ATCC 25755 (a known high butyric acid producer) produced 11 g/L butyrate [19]. In this study, similar approach was made, however, instead of butyric acid production we focused on the direct utilization of representative macroalgal biomass into butanol.

Biobutanol is a renewable energy and it is considered as a potential biofuel. It consists of four carbon backbone, thus having a higher energy content than ethanol. It is well suited to current vehicle and engine technologies and it can be used at higher blend compared to ethanol. It can also be transported through the existing fuel pipelines. Biobutanol production so far has been limited by its low titer and productivity. The feedback effect of solvent, especially butanol, is the most delimiting factor in butanol fermentation and therefore requires more focus. Many researches such as changing the metabolic profile of the clostridium strain [20-21], process modification , in-situ product removal [22], and physiological modification [23-25] were attempted and gained promising results. An alternative approach, however, is to find a cheaper substrate for fermentation. It was found that in a typical industrial plant for ABE fermentation, a total of 60% direct cost can be accounted to the utilization of substrate [26]. Thus, cheaper and readily available brown macroalgae (*Undaria pinnatifida*, *Laminaria japonica*, *Ecklonia stolonifera*, *Hizikia fusiforme*, and *Sargassum fulvellum*) and red macroalgae (*Porphyra tenera* and *Gelidium amansii*) were investigated for the production of butanol by *Clostridium saccharoperbutylacetonicum* N1-4. The study circumvented on the determination of butanol production of *C. saccharoperbutylacetonicum* N1-4 strain on the representative macroalgae. The reducing sugars consumed during fermentation were not determined instead, the total reducing sugar analysis was made. Nevertheless, despite the absence of specific reducing sugar analysis, the objective which is to determine the potential of macroalgae for bio-butanol production, was established and properly investigated. Moreover, overliming and activated carbon method was also applied to detoxify the pretreated hydrolysate of the best macroalga substrate. Application bio-butanol as fuel for renewable energy may preserve our emission free environment.

MATERIAL AND METHODS

Cultivation of *Clostridium saccharoperbutylacetonicum N1-4*

The lyophilized cells of *C. saccharoperbutylacetonicum* N1-4 ATCC 27021^T was purchased from the Korean Collection for Type Culture (KCTC) with a depository number KCTC 5577. Upon arrival, it was suspended in sterile water and streaked on the tryptone-yeast extract-acetate (TYA) agar medium [27]. When sufficient colony is observed on the agar plate, the cells were transferred into the liquid TYA medium to make a pre-culture. Pre-culture was conducted in a 150 mL serum vial while a 500 mL glass bottle (Schott Duran, Germany) was used for the main fermentation. The pre-cultured cells were inoculated into the main culture medium using a 1:10 ratio of inoculum and medium volume right after exponential growth (12-15 h). Both pre-culture and main culture were cultivated at 30°C incubation temperature with 120 rpm mixing.

All vials, bottles, rubber stoppers, inoculating loops, and other materials were sterilized at 121°C for 20 min prior to use. Glucose was autoclaved separately from the rest of the medium components. After autoclaving, glucose and other media components were mixed, capped with a rubber stopper and flushed with filter-sterilized nitrogen gas (passing through a 0.2 µm membrane filter) before inoculation. To ensure anaerobicity, all inoculation steps were carried out in an anaerobic chamber (SK-G002-SFD, Three-Shine, Seoul, Korea) filled with nitrogen gas. Samples were withdrawn from the bottle periodically, centrifuged at 12000 rpm and 4°C for 10 min to obtain the supernatant. The samples were stored in 4°C for no longer than one day prior to analysis.

Macroalgae source and hydrolysate preparation

The brown and red macroalgae were either purchased from the traditional market in Yongin, Korea or bought online (Natural food, www.0808.or.kr). Upon arrival, the macroalgae were washed thoroughly with tap water to remove the remaining salts and air dried for several days. After drying, the macroalgae were chipped at approximately 3 x 3 cm prior to milling. Milling was achieved in order to pulverize the macroalgae at a mesh size below 60. The moisture content of the powdered macroalgae was observed to be at 5.7 to 6.1 wt.%.

Following from the recent study [19], 100 g/L of powdered macroalga was suspended in a 0.15 M H_2SO_4 and autoclaved for 1 h in order to hydrolyze. The autoclaved suspension was cooled to room temperature and aseptically centrifuged at 4°C and 4000 rpm for 20 min to obtain the soluble hydrolysate (supernatant). To adjust the pH of the acidic hydrolysate, 4 M NaOH was gradually added until pH 6.0. Alternatively, a three-fold

(3X) concentrated TYA medium without glucose were separately autoclaved and mixed with the hydrolysate to make 1X strength. Based from previous study, it was found that 60 to 70% of the initial volume was recovered as the hydrolysate, therefore, 3X TYA medium should be made to compensate for the initial concentration of the hydrolyzed macroalgae. The final pH of the mixture was within the range of 6.0 to 6.5.

Post-treatment (detoxification) by activated carbon and overliming

Overliming was carried out by gradually adjusting the pH of the hydrolysate to pH 10.0 using anhydrous calcium hydroxide for 30 min at 50°C with continuous agitation at 200 rpm. The pH of the overlimed hydrolysate was then adjusted to about 6.0 using 4 M HCl solution. Finally, the hydrolysate was collected after centrifugation at 4000 rpm for 10 min. The final mixture was achieved by mixing this hydrolysate to the previously prepared 3X TYA medium.

For the activated carbon detoxification, 4 g/L of activated carbon was added to the hydrolysate at pH 3.5. It was agitated at 200 rpm for 30 min at room temperature. The precipitate formed was removed by centrifuging the sample at 4000 rpm for 10 min. To neutralize the solution, 4 M NaOH was carefully added until the pH was around 6.0. Likewise, the mixture was added to the 3X TYA medium.

Analytical methods

Glucose and reducing sugar were analyzed using the dinitrosalicylic (DNS) colorimetric method [28]. Acid (acetic and butyric acids) and solvent (acetone, butanol, and ethanol) concentrations were determined by a gas chromatograph (GC, M600D, Young-Lin, Korea) consisting of an injector (220°C), HP-FFAP (Agilent Technologies, USA) fused-silica capillary column (30 m x 0.2 mm x 0.33 µm), an oven (50°C for 1 min, 50-80°C at 5°C/min, 80-220°C at 15°C/min, and 10 min at 220°C), and a detector (flame ionization detector, 250°C) with helium carrier at 1.5 mL/min. Butanol yield (Y_{BuOH}, g BuOH/g algae) was calculated as the concentration of butanol produced over the amount of macroalga used in the fermentation [27].

RESULTS AND DISCUSSION

***C. saccharoperbutylacetonicum* N1-4 fermentation on glucose**

From the previous account of *C. saccharoperbutylacetonicum* N1-4 fermentation, it was identified that the strain can metabolize glucose up to 60 g/L producing approximately 13 g/L butanol [29]. However, a glucose concentration of above 60 g/L showed a decrease in glucose consumption and solvent production. Optimal glucose consumption was observed at 32.28 g/L with a yield of 0.248 g butanol/g glucose [29].

Brown macroalgae fermentation

Five representative species of brown macroalgae, *U. pinnatifida, L. japonica, E. stolonifera, H. fusiforme,* and *S. fulvellum*, were investigated as substrates for the production of butanol from the fermentation of *C. saccharoperbutylacetonicum* N1-4 ATCC 27021^T. The optimized thermo-chemical pretreatment used to hydrolyze the selected macroalgae in this study was adopted from Song et al. (2010) [19].

From the fermentation of *U. pinnatifida* (Figure 1a), the concentration of reducing sugar noticeably decreased after 60 h of fermentation. During the initiation of the acidogenic stage, a rapid increase in the butyrate and acetate concentrations of the culture was observed after 36 h. The acetate concentration increased from the initial 2 g/L to 2.5 g/L in the medium and the butyrate increased at approximately 2.5 g/L. The resulting increase of the acid concentration led to the metabolic shift of the cell to solventogenesis (biochemial reduction of the produced acids into correponding solvent). In typical glucose fermentation, the acidogenic stage is usually observed after 12 h of fermentation. However, the consumption of the pretreated macroalgal biomass as subtrate by the *C. saccharoperbutylacetonicum* N1-4 ATCC 27021^T was slower than the more reduced substrate such as glucose [27]. The acid production signalled the solventogenic shift to produce acetone and butanol but not ethanol (Figure 1a). No ethanol was formed, which was a typical characteristic of the *C. saccharoperbutylacetonicum* N1-4 ATCC 27021^T strain [29-30]. The overall fermentation from the *U. pinnatifida* produced 1.39 g/L butanol and 2.67 g/L total solvent (Table 3.1).

L. japonica showed a better fermentation profile than *U. pinnatifida* because of the higher solvent production (Figure 1b). The *L. japonica* may not seem to have an active sugar utilization up to 48 h, however, acetate and butyrate levels were gradually increased during the first 20 h of fermentation. A shift in the solventogenic stage was perceived after 24 h of fermentation and lasted to about 90 h of fermentation. Reducing sugars from *L. japonica* were also consumed at a faster rate enabling the cell to produce more solvent. Reducing sugar utilization was around 80%, which was higher than the previous fermentation. Overall, the complete fermentation from this brown macroalga yielded 5.51 g/L and 7.11 g/L butanol and solvent, respectively.

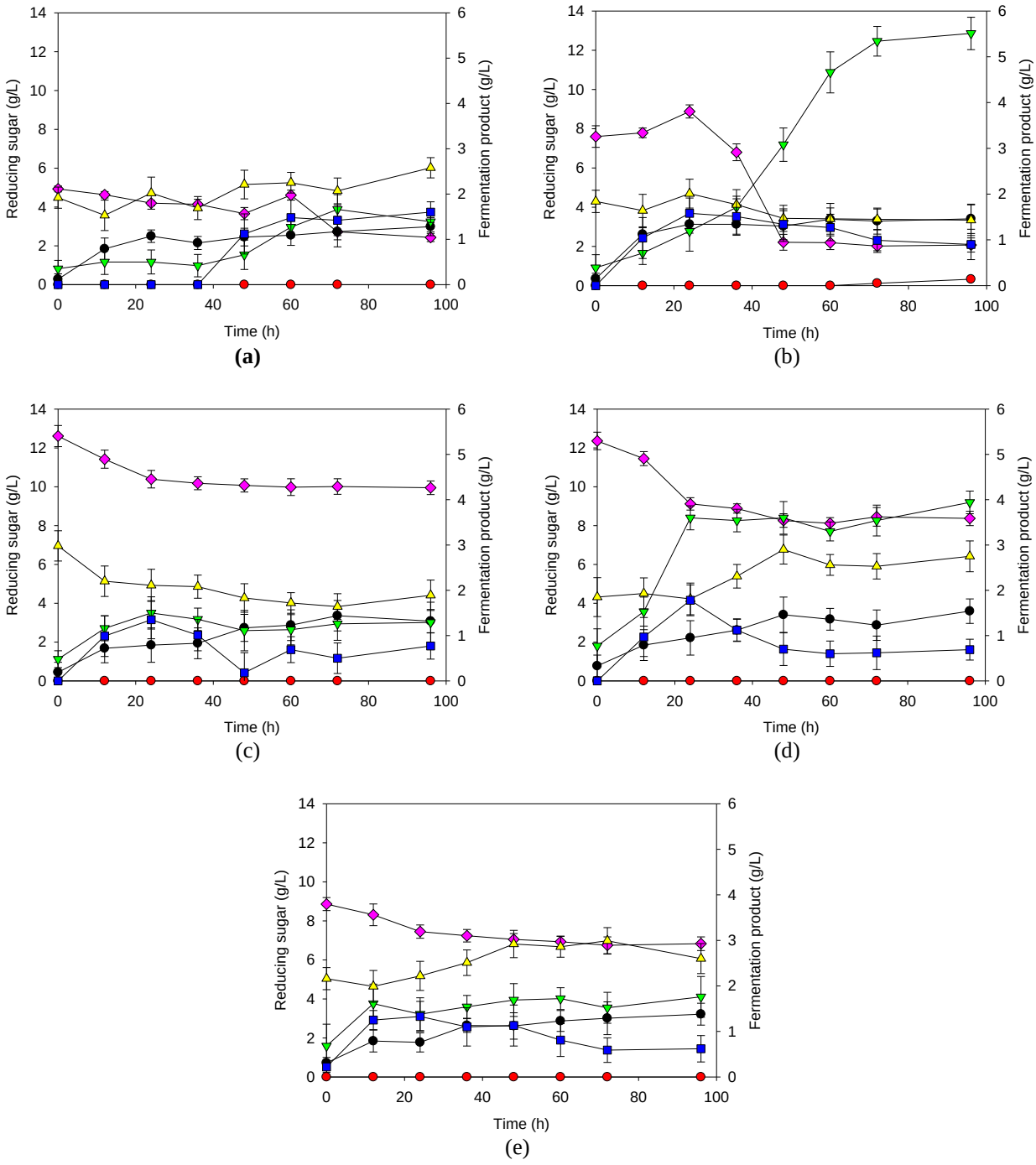


Figure 1. Butanol fermentation from the representative brow macroalga: (a) *U. pinnatifida*, (b) *L. japonica*, (c) *E. stolonifera*, (d) *H. fusiforme*, and (e) *S. fulvellum*. Note: Symbols represent (—◆—) reducing sugar, (—●—) acetone, (—●—) ethanol, (—▼—) butanol, (—▲—) acetate, and (—■—) butyrate. Bar represent standard error for three replicates.

E. stolonifera fermentation (Figure 1c) showed higher rate of production than *U. pinnatifida* (Figure 1a). Previous fermentations were observed to produce butanol after 24 h, however, this macroalga had shown an earlier shift to solventogenesis. It can be clearly identified that butanol concentration started to elevate after 12 h as depicted in Figure 1c. The production of butanol resulted in the increased utilization of reducing sugar of *E. stolonifera*. The reducing sugar was utilized continuously during fermentation and started to cease after 48 h. From

this period, no consumption of sugar was observed, leaving about 66% of unconsumed reducing sugar. Previous fermentations (Figures 1a and 1b) also appeared to incompletely utilize the reducing sugar in the hydrolysate. Higher concentration of initial reducing sugar in the *E. stolonifera* hydrolysates were obtained, however, it did not signify higher butanol production in comparison to *L. japonica* fermentation (Figure 1b). Butyrate concentration increased up to 2 g/L during the first 24 h and concomitantly consumed after this period. The consumption of butyrate in the medium may indicate corresponding butanol production, however, acetate utilization is more directly involved in acetone production [31]. Likewise, the fermentation did not produce certain level of ethanol possibly because of the physiological characteristics of the *C. saccharoperbutylacetonicum* N1-4 ATCC 27021^T and the low concentration of reducing sugars available from *E. stolonifera*. From the fermentation of the *E. stolonifera*, butanol and solvent production were 3.94 g/L and 5.48 g/L, respectively. So far, this fermentation yielded the second highest butanol production next to the *L. japonica* fermentation (Figure 1b).

For the butanol fermentation of *H. fusiforme* (Figure 1d), acidogenesis was observed up to 24 h because of the continuous increase of butyrate concentration in the medium during fermentation. However, the acetate production was observed to decrease during fermentation. Butanol concentration also increased during this period approximately reaching the maximum concentration at the end of acidogenesis stage. Butanol production ceased after 24 h, accumulating a concentration of 1.29 g/L from the initial 100 g/L *H. fusiforme* hydrolysate. Reducing sugar was consumed gradually during the first 24 h of fermentation, utilizing about 2 g/L from the initial 12.4 g/L reducing sugar. The acetone tended to increase gradually until the fermentation was finished at 96 h. The final acetone and total solvent concentration were 1.32 and 2.61 g/L, respectively. Similarly, no formation of ethanol was observed in this fermentation. Remaining acids in the medium was still high indicating an incomplete utilization of the reducing sugar. This phenomenon was also observed from previous fermentations (Figure. 1). The *H. fusiforme* was almost similar to the *U. pinnatifida* fermentation in terms of butanol and solvent production (Table 1).

The *S. fulvellum* was the last brown macroalga tested for butanol fermentation (Figure 1d). The thermochemical pretreatment of the macroalga produced an approximately 9 g/L of reducing sugar. However, only minimal concentrations (~1 g/L) of the reducing sugar was utilized during fermentation (Figure 1d). The acidogenic and solventogenic stage were observed to be similar to the fermentation of *E. stolonifera* (Figure 1c). Butyrate increased up to 1.25 g/L during the first 24 h of fermentation and the acetate concentration remained high throughout the fermentation. Similar to the previous fermentations, ethanol was also not produced. However, butanol and acetone gradually increased producing 1.76 g/L and 3.14 g/L, respectively. In comparison, butanol and solvent production from the *S. fulvellum* were higher than the *U. pinnatifida* (Figure 1a) and *H. fusiforme* (Figure 1d) fermentations.

TABLE 1. Summary of brown alga fermentation

Brown Macroalgae	Reducing sugar, initial (g/L)	Butanol (g/L)	Solvent (g/L)[a]	Butanol yield (g/g)[b]
U. pinnatifida	4.93±0.15	1.39±0.22	2.67	0.014
L. japonica	7.60±0.55	5.51±0.35	7.11	0.055
E. stolonifera	12.36±0.54	3.94±0.45	5.48	0.040
H. fusiforme	12.61±0.45	1.29±0.25	2.61	0.013
S. fulvellum	8.86±0.34	1.76±0.44	3.14	0.018

[a]Sum of the final concentration of acetone, butanol, and ethanol concentration.
[b]Calculated from the final concentration of butanol divided by the initial concentration of macroalgae.

From the fermentation of the five representative brown macroalga, it can be seen that the *L. japonica* yielded the highest butanol concentration (Table 1). Noting from the previous study of Song et al. [19], the butyrate fermentation from *L. japonica* had also gained the highest yield. Although, it was presented from Figure 1 and Table 1 that *E. stolonifera* had the highest reducing sugar, this did not correspond to the higher solvent production. From Table 1, *E. stolonifera* was only second to *L. japonica* in terms of solvent and butanol production. In comparison to the remaining brown macroalgae fermentations, *U. pinnatifida*, *H. fusiforme*, and *S. fulvellum*, the butanol production from these brown macroalgae produced butanol and solvent within the range of 1.30-1.80 g/L and 2.60-3.15 g/L, respectively. *S. fulvellum* released almost similar concentration of reducing sugar compared to *L. japonica*, however, it was accounted that the butanol and solvent production from this brown macroalga were the lowest among the brown macroalgae tested.

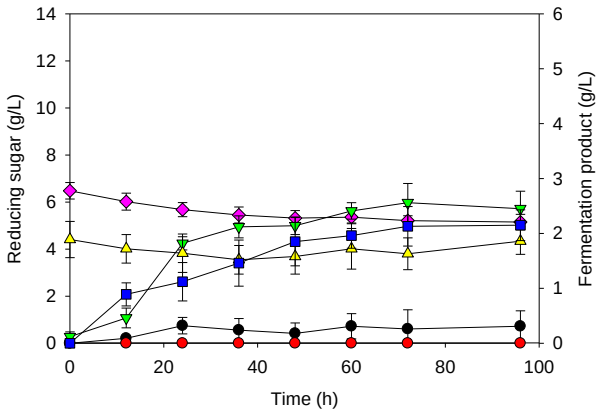
Red macroalgae fermentation

To compare the ability to produce butanol from the fermentation of other macroalgae species, two representative red macroalgae were also subjected to similar fermentation (Figure 2). Comparing from the previous brown macroalgae fermentations, the *Poryphyra* sp. (Figure 2a) fermentation profile was close

to the fermentation of the representative brown macroalgae (Figure 1). However, the fermentation of *G. amansii* had almost no metabolic activity (Figure 2b). Early solventogenesis was observed in the fermentation of *Poryphyra* sp. (Figure 2a) because of the early formation of butanol and acetone. After 12 h, butanol concentration exponentially increased up to 24 h. A gradual increase of butanol formation occurred at this stage until a maximum butanol concentration was reached at 72 h. Butanol concentration was recorded at 2.56 g/L and a minimal acetone production of 0.37 g/L (Figure 2a, Table 2). Butyrate should decrease at the event of solventogenesis, however, the fermentation of *Poryphyra* sp. appeared to increase until the end of fermentation (Figure 2a). The low utilization of the reducing sugar must be the limiting factor in the conversion of the butyric acid into butanol. Thus, there was still a higher butyrate concentration (2.5 g/L) in the fermentation broth. Consequently, acetate (1.6 g/L remaining acetate) was also not consumed during fermentation.

G. amansii did not provide a good fermentation profile (Figure 2b), however, its reducing sugar concentration obtained after the acid hydrolysis was similar to *Poryphyra* sp. (Figure 2a). The *G. amansii* might produce higher level of inhibitory compounds than the other hydrolyzed macroalage (Figure 1, Figure 2a). As seen in Figure 2b, the reducing sugar was slightly consumed during fermentation. Butanol concentration gradually increased but it was observed to be only 0.62 g/L after the whole fermentation period. Acetone and ethanol were also not formed during the fermentation. The butyrate level seemed to increase in concomitant to the increased butanol concentration, however, the concentration of acetate decreased but no acetone in the fermentation broth was detected.

The summary of red alga fermentation was tabulated in Table 2. Comparing with the brown macroalgae fermentation (Table 1), the representative red macroalga did not exceed the fermentation of *L. japonica* (Figure 1b) and *E. stolonifera* (Figure 1c). The fermentation of *Poryphyra* sp. (Figure 2a, Table 2) was the highest among the three brown macroalgae (Figures 1a, 1d, and 1e; Table 1), however comparing closely, it was about two times lower than the butanol production of *L. japonica* (Figure 1b, Table 1). *G. amansii* fermentation had the lowest produced butanol from the red alga tested, which maybe accounted to the production of higher concentration of inhibitors. As have been reported, polyphenols especially tannins are the growth inhibitory compounds associated in the composition of macroalgae [32]. Furthermore, excessive pretreatment might have decomposed the reducing sugars into non-utilizable and inhibitory compounds such as 5-hydroxymethylfurfural and other phenolic compounds.



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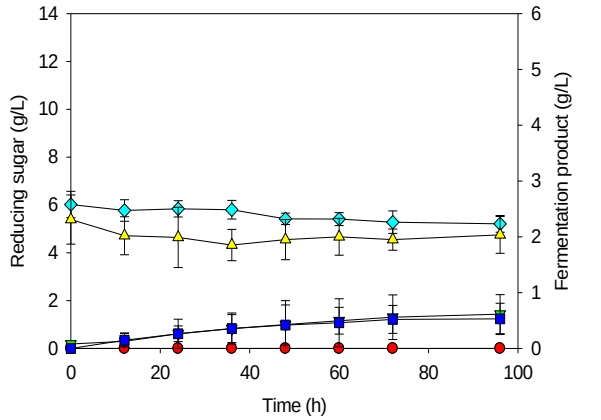


(b)

Figure 2.Fermentation from the representative red macroalga: (a) *Poryphyra* sp. and (b) *G. amansii*. Note: Symbols represent (—◆—) reducing sugar, (—●—) acetone, (—●—) ethanol, (—▼—) butanol, (—△—) acetate, and (—■—) butyrate. Bar represent standard error for three replicates. Bar represent standard error for three replicates.

TABLE 2. Summary of red alga fermentation

Red macroalgae	Reducing sugar, initial (g/L)	Butanol (g/L)	Solvent (g/L)[a]	Butanol yield (g/g)[b]
Poryphyra sp.	6.48±0.35	2.56±0.32	2.87	0.025
G. amansii	6.15±0.55	0.62±0.35	0.62	0.006

[a]Sum of the final concentration of acetone, butanol, and ethanol concentration.

[b]Calculated from the final concentration of butanol divided by the initial concentration of macroalgae.

Effect of post-treatment

The production of butanol was shown to favor *L. japonica* (Figure 1a) as the best substrate compared to the other species of brown and red macroalgae tested. To investigate further for the possible presence of inhibitory compounds in the macroalga hydrolysate, a detoxification scheme using an activated carbon and overliming methods were introduced (Figure 3). The carrying out of this post treatment resulted in the fermentation shown in Figure 3. Comparing closely at the concentration of the reducing sugar from the post treated hydrolysate to the fermentation without post treatment (Figure 1b), a reduction of 55 to 57% of the recovered reducing sugar from *L. japonica* was obtained (Figure 3). The utilization of reducing sugar, however, was more active in this fermentation than the previous fermentation without post treatment (Figure 1b). Furthermore, fermentation occurred at a much faster rate reaching approximately 48-60 h of the total fermentation time (Figure 3). Low concentration of initial reducing sugar was measured in the hydrolysate medium, however, this did not correspond to a lower solvent production. Butanol concentration reached 6.18 g/L and 7.22 g/L using the activated carbon and overliming methods, respectively. At the same initial concentration of powdered *L. japonica*, the overliming method (Figure 3b) yielded 14.4% higher than the activated carbon post treatment (Figure 3b). Thus, it can be concluded that overliming is the more suitable detoxification of the thermo-chemically pretreated brown alga.

In summary, post treatment further improves the production of butanol from the selected macroalga. In comparison, overliming was better than activated carbon method. Butanol and solvent increased by 31.03 and 25.47%, respectively, compared to an untreated hydrolysate using an overliming method. Butanol productivity was also enhanced after detoxification procedure was applied proceeding pretreatment.

In comparison to the other literatures employing the use of macroalgae as substrates for butanol fermentation (Table 4), *L. japonica* with or without an additional overliming post treatment in this study yielded higher butanol production. A higher solvent concentration was achieved in the study of van der Wal (2013) [16], but it could be noted that the addition of glucose in their fermentation medium might possibly led to the increased solvent production.

CONCLUSION

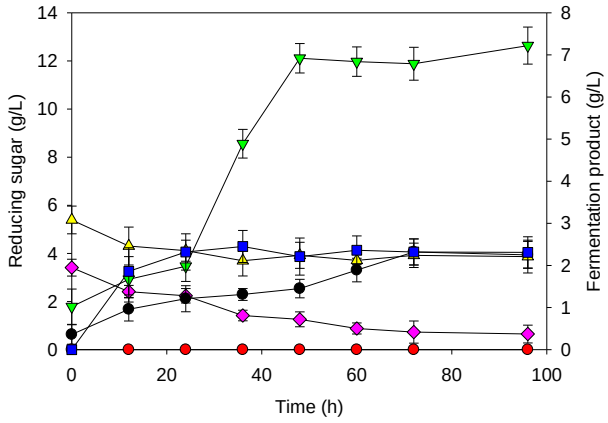
Among the macroalgae tested for butanol fermentation, *L. japonica* produced the highest butanol production (5.51 g/L) followed by *H.* Fusiforme, S. *fulvellum*, *U. pinnatifida*, and *E. stolonifera* in decreasing order. The red macroalgae tested did not exceed the butanol production from *L. japonica* although it was thought that the red macroalgae contain easily digestible polymeric materials. Detoxification using activated carbon and overliming methods further increased

TABLE 3. Summary of the effect of post-treatment

L. japonica	Reducing sugar, initial (g/L)	Butanol (g/L)	Solvent (g/L)[a]	Butanol yield (g/g)[b]
Activated carbon	3.24±0.35	6.18±0.44	7.58	0.062
Overliming	3.41±0.25	7.22±0.43	9.54	0.072

[a]Sum of the final concentration of acetone, butanol, and ethanol concentration.

[b]Calculated from the final concentration of butanol divided by the initial concentration of macroalgae.



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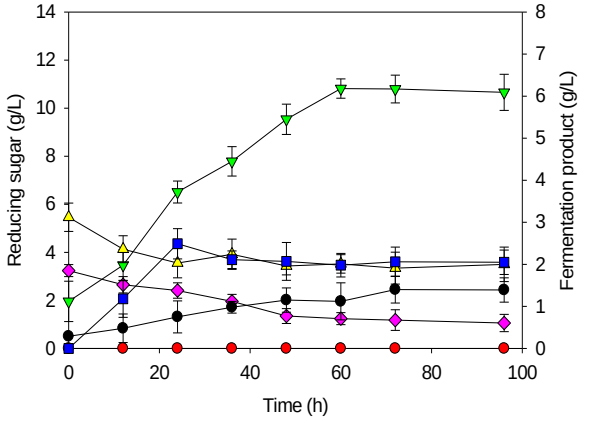

(b)

Figure 3. Effect of post-treatment with (a) overliming, and (b) activated carbon method in the butanol fermentation of *L. japonica*. Note: Symbols represent (—◆—) reducing sugar, (—●—) acetone, (—●—) ethanol, (—▼—) butanol, (—△—) acetate, and (—■—) butyrate. Bar represent standard error for three replicates.

Table 4. Macroalage as substrates for butanol fermentation

Macroalgae	Microorganism	Pretreatment	Butanol (g/L)	Solvent (g/L)[a]	Butanol yield (g/g)[b]	Reference
Saccharina spp.	*C. acetobutylicum* ATCC 824	Dilute acid, mild temperature	-	-	0.12	[13]
U. lactuca	*C. beijerinckii*, *C. saccharoper-butylacetonicum*	Thermo-chemical (dilute acid)	4	-	-	[14]
U. lactuca	*C. beijerinckii*, *C. acetobutylicum*	Hot-water, fermentation with glucose	-	18, 9	-	[15]
L. japonica	*C. saccharoper-butylacetonicum* N1-4	Thermo-chemical (dilute acid); with overliming	5.51; 7.22	7.11; 9.54	0.055; 0.072	This study

[a]Final concentration of acetone, butanol, and ethanol concentration.
[b]Calculated from the final concentration of butanol divided by the initial concentration of macroalgae.

overliming methods further increased the butanol production from *L. japonica* by 24.14 and 12.16%, respectively. This showed that inhibiting substance might be present in the hydrolytic solution of the macroalgae. Optimization of the pretreatment conditions can be further introduced to maximize the production of butanol from these representative macroalgae species. However, evidence showed that *L. japonica* itself might have been the better substrate compared to the other seaweeds. This study showed the potential of macroalgae as substrate for butanol fermentation.

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Persian Abstract

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چکیده

ماکروجلبک¬ها با توجه به اینکه به راحتی در کنار دریا رشد می¬کنند و نیاز به زمین زراعی و تغذیه خاصی ندارند گزینه امیدبخشی هستند. گونه¬های مختلف ماکروجلبک¬ها به منظور تولید بیوبوتانول مورد ارزیابی قرار گرفتند. در این مطالعه چهار گونه از ماکروجلبک¬های قهوه¬ای و دو گونه از ماکروجلبک¬های قرمز مورد استفاده قرار گرفتند. به منظور هیدرولیز پلیمرهای موجود در سلول ماکروجلبک هیدرولیز اسید رقیق مورد استفاده قرار گرفت. بیشترین مقدار تولید بیوبوتانول از گونه *L. Japonica* بدست آمد که مقداری برابر / گرم بر لیتر بوده است. همچنین پیش تیمار اسید رقیق و استفاده از فرآیند ترموکمیکال با استفاده از کربن فعال بازدهی تولید را ۲۴/۱۴ ٪ و ۱۲/۱۶ ٪ افزایش داد. نتایج این تحقیق نشان از آن دارد که ماکروجلبک¬ها می¬توانند جز منابع امیدبخش جهت تولید سوخت زیستی به حساب آیند.