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Full Length Research Paper

Production of mono sugar from acid hydrolysis of seaweed

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The purpose of this work was to optimize the process conditions for the saccharification of macroalgae (seaweed) into mono sugar using the following parameters such as: Amount of biomass, catalyst concentration, temperature and reaction time. The major component of *Ulva pertusa* (green seaweed), *Laminaria japonica* (brown seaweed) and *Gelidium amansii* (red seaweed) is carbohydrate which can be converted into variety of sugars using sulfuric acid with hot-compress treatment. Rhamnose (37.89 wt %) and glucose (16.14 wt %) were extracted from dried *U. pertusa*, while galactose (49.32 wt %) and glucose (12.62 wt %) were extracted from dried *G. amansii*. Mannitol (31.53 wt %) was produced from dried *L. japonica*.

Key words: Marine biomass, seaweed, saccharification, sulfuric acid hydrolysis, mono sugars.

INTRODUCTION

Marine biomass (marine algae) has recently received a lot of attention as a renewable source of bioethanol (Okamoto et al., 1994), biomethanol (Bird et al., 1990), biohydrogen (Rupprecht et al., 2006), biodiesel (Nocito, 2008) and biobutanol (DuPont, 2009). Marine algae are categorized as macroalgae (seaweed) and microalgae, where seaweed is mainly divided into three categories: Green, brown and red seaweed (Mchugh, 2003).

Annual CO_2 absorption by marine biomass is 36.8 ton/ha, which is five to seven times higher than wood-biomass (Mchugh, 2003). Seaweed biomass has a higher mass productivity (13.1 kg dry weight m^2 over 7 months) than land plants (0.5 to 4.4 kg dry weight m^2 $year^{-1}$) and worldwide annual production of marine algae is approximately 14 million tons (Mchugh, 2003). Seaweed is easy to cultivate using wide arable areas of the sea by simply tying them to floating anchor lines without any high cost equipment.

Until now, most seaweed research dealt with component analysis because seaweeds contain unique polysaccharides and carbohydrates making them suitable as dried feed, human food, fertilizers and soil amendment agent (Rioux et al., 2007; Choi et al., 2009; Wi et al., 2009). The alginic acid from seaweed is extensively used for producing foods, medicines, cosmetics, dyes, paints, paper manufacturing, interior finishing materials, lubricating oil, etc. (Indergaard and Østgaard,1992).

There are only a few research reports on saccharification of seaweed, and mainly only enzymatic method is examined (Horn et al., 2000; Korenaga, 2000; Isa et al., 2009). Disadvantage of enzyme method is high cost and complexity of the saccharification process (Huimin et al., 2007), and seaweed enzyme cocktail have this problem due to saccharide composition of seaweed.

In this study, acid hydrolysis as a common method of wood biomass saccharification (Martin et al., 2007) was applied in combination with hot-compress treatment. The parameters that had been investigated in this study were amount of biomass, catalyst concentration, temperature and reaction time. It was a good process of seaweed saccharification, because it had advantages of low-cost, short hydrolysis time and simple operation when compared to other saccharification methods.

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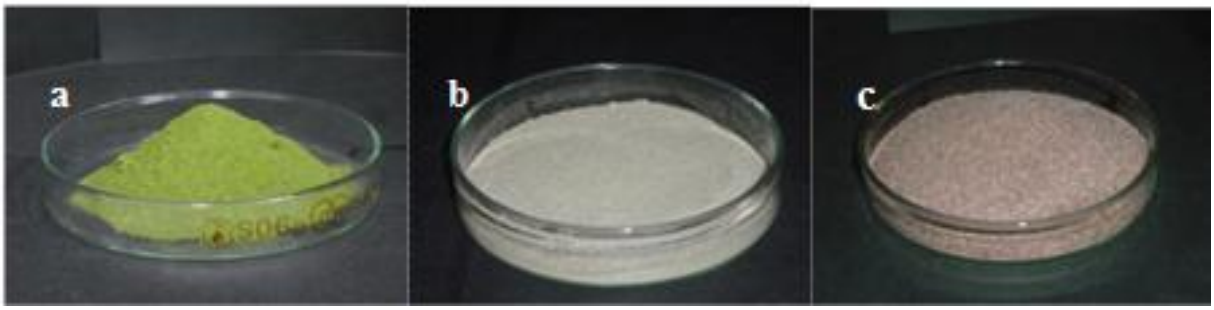

Figure 1. Seaweed powder milled less than 100 mesh sizes. (a) *U. pertusa* (green seaweed), (b) *L. japonica* (brown seaweed) and (c) *G. amansii* (red seaweed).

The purpose of this work was to standardize the reaction conditions for saccharification using sulfuric acid in hot-compress instrument from *Ulva pertusa* ((U. pertusa), brown seaweed of *Laminaria japonica* (*L. japonica*) and red seaweed of *Gelidium amansii* (*G. amansii*) as a representative sea-weed in Japan.

MATERIALS AND METHODS

Raw materials

The *L. japonica* (brown seaweed) of Hokkaido product and *U. pertusa* (green seaweed) and *G. amansii* (red seaweed) of Fukuoka product used in this study were obtained from a supermarket in Japan. The seaweeds were washed with running water for 5 times to remove salts and then dried in the oven at 50°C until constant weight of the sample was obtained. These were milled to less than 100 mesh sizes by a coffee mill as shown in Figure 1.

Determination of proximate composition

Dietary fibre was determined according to the A.O.A.C. method with some modifications (Prosky et al., 1988), while carbohydrate content was determined by calculating the percentage difference from all the other constituents. Neutral sugars (NS) in the hydrolysates were quantified by HPLC. A manual colorimetric procedure for measuring ammonium nitrogen in Kjeldahl digester was used for the determination of total nitrogen and protein content, which was calculated using a nitrogen conversion factor of 6.25 (Baethgen and Ally, 1989). Fat content was determined by the Soxhlet system (AOAC, 1997). Total ash was determined by calcination in muffle furnace at 550°C until constant weight was obtained (AOAC, 1997).

Sulfuric acid hydrolysis

In order to determine appropriate biomass concentration in catalyst solvent for saccharification, about 3 to 10% (w/v) of seaweed was treated with 0 to 25% (v/v) H_2SO_4 at 120°C and for 30 min by autoclaving (HA-300M, Hirayama Japan), and to determine the effect of temperature and time course on the saccharification of seaweed, 3 to 5% (w/v) seaweed was treated with 0.5 to to 5% (v/v) H_2SO_4 at 120 to 150°C and 0.5 MPa for 0 to 2 h by hot-compress instrument. Reaction temperature was carried out at 120, 130, 140 and 150°C, respectively. As soon as the reaction temperature reached the desired values, initial sampling was done at 0 h. Sampling was conducted for 2 h at 15 and 30 min intervals (0, 15, 30, 45, 60, 90 at 120 min). As shown in Figure 2, hot-compress instrument is equipped with controller box and 2L reactor. Heat controller (a) and temperature controller (b) are connected to maintain the reaction temperature. The sampling was carried out aseptically using a sterile sampler in the sampling port (e) connected to a N_2 gas reservoir to maintain pressure. Once the reaction was completed, the hydrolysed biomass was discharged through the draining port (g). The slurry solution obtained from the acid saccharification was then centrifuged at 3500 rpm for 5 min to remove the solid residue. The supernatant obtained from the hydrolysis was neutralized with 10% (v/v) ammonium hydroxide (NH_4OH) and stored at -20°C prior to analysis.

Analysis

The concentration of mono sugar was measured by a HPLC. The Shimpack SPR-Ca column (Shimadzu, Japan) at 80°C with IR-detector was used. The mobile phase was distilled water at a flow rate of 0.5 ml min^{-1}. The samples were filtered with 0.45 µm of cellulose acetate filter and 10 µl of injection volume was added. Biomass conversion ratio was worked out as:

$$\frac{\text{Sugars formed (g)}}{\text{Biomass (g)}} \times 100 \quad (1)$$

Percentage conversion of carbohydrate to mono sugars was worked out as:

$$\frac{\text{Total mono sugars produced (g)}}{\text{Total carbohydrate (g)}} \times 100 \quad (2)$$

RESULTS AND DISCUSSION

Proximate composition *of U. pertusa, L. japonica* and *G. amansii*

The results of proximate composition are shown in Table 1, although the amounts of seaweeds component varies according to the season, age of population, species, geographic location and temperature, generally (Kaehler and Kennish, 1996; Graham and Wilcox, 2000). The

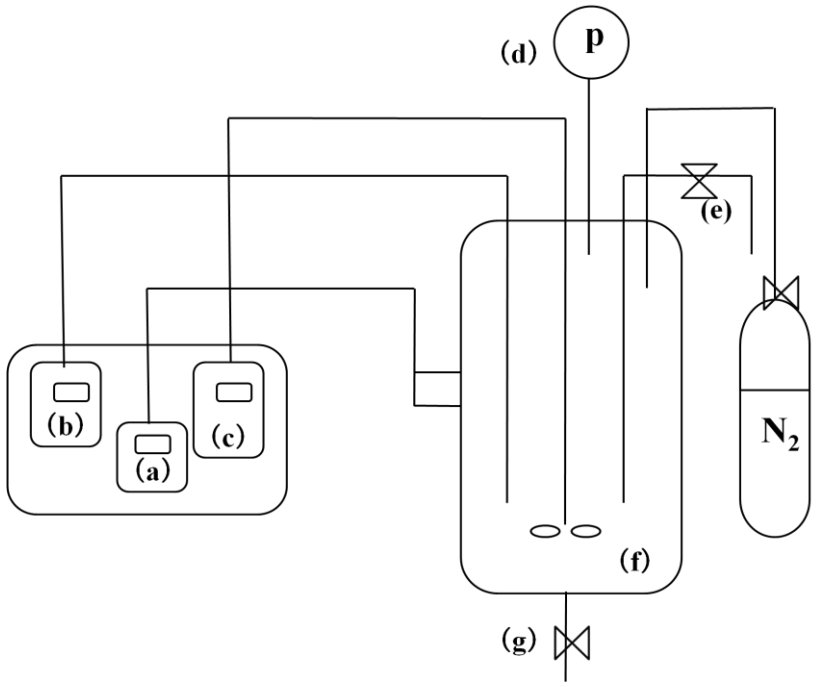

Figure 2. Schematic diagram of hot-compress treatment instrument: (a) Heat controller, (b) temperature controller, (c) agitator controller, (d) compressor gauge (e) sampling port, (f) 2L-reactor vessel and (g) draining port.

Table 1. Proximate composition of seaweed.

Composition ($g \cdot g^{-1}$ dry basis)	*U. pertusa* (green seaweed) (%)	*L. japonica* (brown seaweed) (%)	*G. amansii* (red seaweed) (%)
Carbohydrate[1]	59.07±0.2	54.50±0.09	71.43 ± 0.08
Dietary fiber	56.84±0.26	28.91±0.12	67.10 ± 0.04
Protein	6.30±0.25	7.40±0.06	10.47 ± 0.05
Lipid	2.39±0.1	1.37±0.01	0.74 ± 0.04
Ash	22.86±0.39	28.33±0.01	2.82 ± 0.03
Water	9.38±0.25	8.4±0.06	14.55± 0.06

Based on % dry weight; [1]100 - (protein + lipid + ash + water); values are means ± standard deviation (n = 3); Conversion factor of nitrogen and protein are 6.25.

focus of this study is a comparative investigation of carbohydrate and dietary fiber concentration for each *U. pertusa, L. japonica* and *G. amansii*, which are very common seaweed in Japan. The highest carbohydrate concentration was detected in *G. amansii* followed by *U. pertusa* and *L. japonica*, respectively.

Saccharification of *U. pertusa, L. japonica* and *G. amansii* by sulfuric acid hydrolysis

This study focused on optimization of mono sugar extract from *U. pertusa, L. japonica* and *G. amansii* by acid hydrolysis under high pressure and temperature. In order to optimize the production of mono sugars, four factors such as biomass concentration, catalyst concentration, reaction temperature and reaction time were investigated.

Table 2 shows mono sugar composition of carbohydrate from *U. pertusa, L. japonica and G. amansii*. Seaweed carbohydrate fraction constitutes various neutral and acid sugars which are found in land plants as hexose and pentose sugars (Percival, 1979), but in this study, it was confirmed that glucose, galactose, mannitol and rhamnose were the four types of mono sugars. Total carbohydrate contents of *U. pertusa, L. japonica* and *G. amansii* were 59.07, 54.50 and 71.43%, respectively. Content of other principles in the seaweed in this study agreed with the values previously reported (Manivannan et al., 2009). Conversion to mono sugars was about 91.46, 57.07 and 80.71%, respectively for the three

Table 2. The main mono-sugar composition of the seaweed hydrolysates.

Parameters	*U. pertusa* (green seaweed)		*L. japonica* (brown seaweed)	*G. amansii* (red seaweed)	
Mono-sugar	**Rhamnose**	**Glucose**	**Mannitol**	**Galactose**	**Glucose**
Conversion ratio of biomass to sugar (%)[1]	37.89±0.56	16.14±0.43	31.53±0.08	49.32 ± 0.2	12.62 ± 0.27
Biomass concentration(w/v)	3%	3%	3%	3%	5%
H_2SO_4 concentration(v/v)	5%	20%	0.5%	3%	3%
Percentage conversion to monosugars[2]	91.46		57.07	80.71	

[1]Worked out as (1). [2]Worked out as (2). Seaweed was treated with H_2SO_4 (v/v) at 120°C and 30 min by autoclave.

seaweeds.

Based on the results, it was found that *G. amansii*, *U. pertusa* and *L. japonica* were good sources for the commercial production of mono sugars. These rich sources of carbohydrates could be utilized as substrates for the fermentative production of commodity chemicals like organic acids. Mono sugars like glucose, galactose and mannitol could be converted into lactic acid, while rhamnose could be fermented into succinic acid.

U. pertusa (green seaweed)

As shown in Figure 3, the appropriate concentration of catalyst and biomass under 120°C for 30 min were investigated. High rate of production of rhamnose was observed with 1 to 5% (v/v) H_2SO_4 at 3% (w/v) substrate. Maximum of 38% of sugar conversion ratio was obtained from 3% (w/v) *U. pertusa* using 5% (v/v) H_2SO_4 (Table 2). But with the optimal glucose production obtained at 3% (w/v) of the biomass concentration with 15 to 20% (v/v) concentration of sulfuric acid, the conversion ratio of glucose from 3% (w/v) of *U. pertusa* was 16%. It could be seen that effective conditions to achieve maximum sugar conversion of 43% were 3% (w/v) substrate with 3 to 5% (v/v) H_2SO_4 for selective conversion to rhamnose and 15 to 20% (v/v) H_2SO_4 for selective conversion to glucose. Thus, the H_2SO_4 concentration shift can be effective for selective extraction of rhamnose and glucose.

Figure 4 shows the effects of reaction temperature and reaction time on saccharification to produce mono sugar under optimal biomass (3%, w/v) and catalyst (5%, v/v) concentration in previous experiment. Figure 4 (a) and (c) indicated that the shorter the reaction time, the higher the mono sugar production at 140 and 150°C, however mono sugar production fluctuated around 30 min at 120 and 130°C. The highest production of rhamnose and total sugar was obtained at 140°C and 0 h reaction time (the initial time reached 140°C). The maximum production of rhamnose was up to about 13 g/L at 140°C for 0 h of saccharification process from 3% *U. pertusa*. Further increasing the reaction time resulted in lower rhamnose production. This may be because prolonging the reaction time at higher temperature might have caused decomposition of sugars.

L. japonica (brown seaweed)

It was confirmed through HPLC that mannitol was obtained from *L. japonica*. Figure 5 shows that the optimal mannitol production was possible with 0.5 to 1% (v/v) concentration of H_2SO_4 at 3% (w/v) and the *L. japonica* concentration at 120°C for 30 min. The lower concentration of biomass and higher catalyst concentration above 3% (v/v) of H_2SO_4 resulted in the smaller conversion ratio. In this appropriate condition, 32% of mannitol conversion ratio was obtained from 3% (w/v) of *L. japonica,* while the percentage conversion to mannitol at 120°C was 57.07 (Table 2).

On the other hand, Figure 6 shows the effects of reaction temperature and time for saccharification of *L. japonica* under optimal biomass concentration (3%, w/v) and H_2SO_4 concentration (1%, v/v) in previous experiment. The highest mannitol production of 9.74 g/L was obtained at 140°C and 30 min. Figure 6 shows that the trend of mannitol concentration increased with reaction temperature, but mannitol concentration at 150°C and 30 min was lower than that at 130°C. Degradation of mono sugar might have occurred quickly under high temperature of over 150°C after 15 min.

Gelidium amansii (red seaweed)

It was confirmed through HPLC that galactose and glucose were the mono sugars obtained from *G. amansii*. Figure 7 shows the effect of biomass and catalyst concentration on sacchrification of *G. amansii* at 120°C for 30 min. The lower concentration of biomass, the higher conversion ratio and higher catalyst concentration resulted in the conversion ratio reduction above 3% (v/v) of H_2SO_4 which is similar to *U. pertusa*. In addition, mono sugar degradation started from low concentration of H_2SO_4 (>5%, v/v), relatively. The conversion ratio to glucose was high at high concentration of biomass, and mono sugar

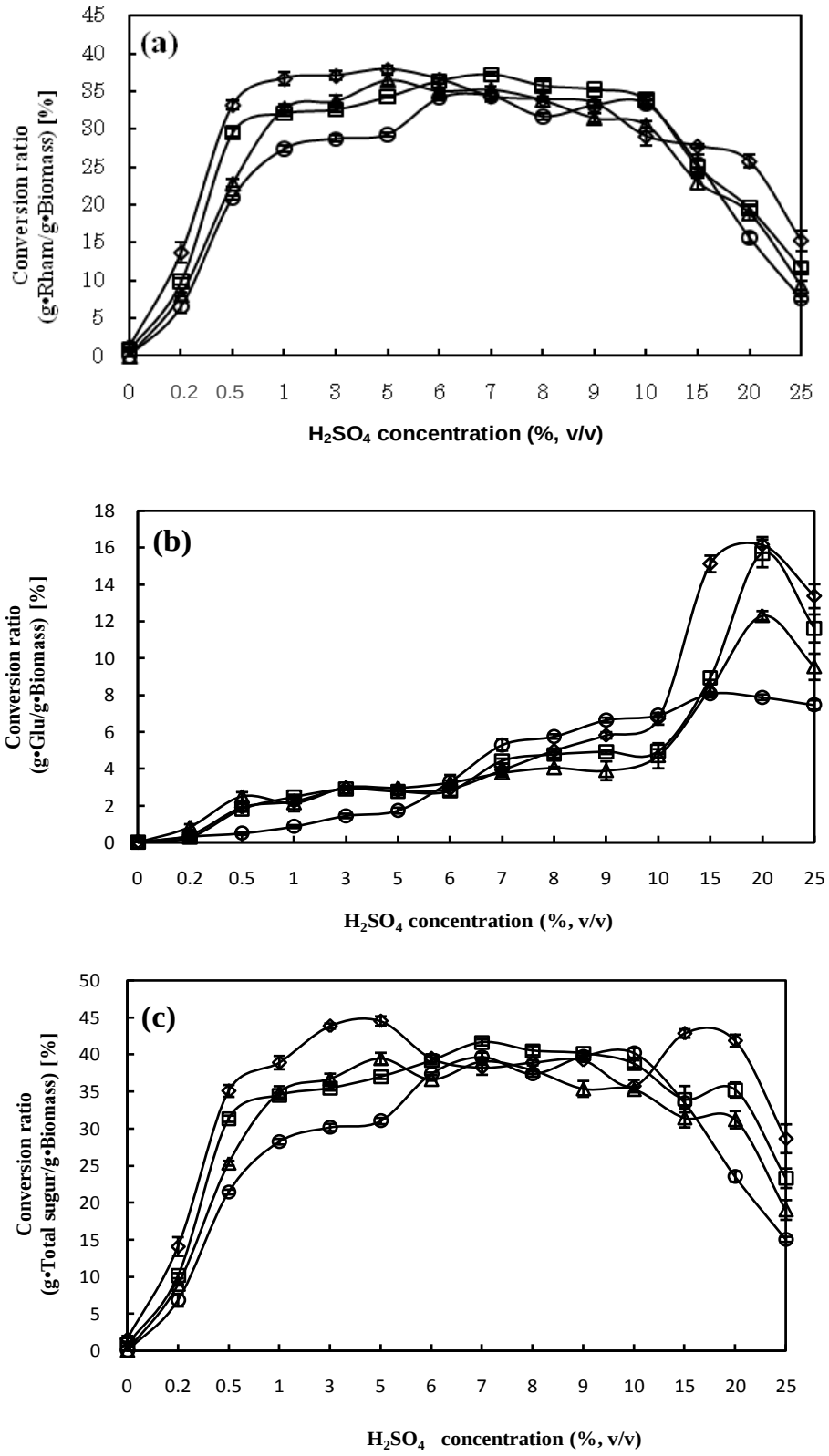

Figure 3. Effect of biomass and catalyst concentration on saccharification of *U. pertusa.* (a) The rhamnose conversion ratio. (b) The glucose conversion ratio. (c) The total (rhamnose and glucose) conversion ratio. *U. pertusa* (3% (◇), 5% (□), 7% (△) and 10% (○), w/v)) was treated at 120°C by autoclaving in the H_2SO_4 (v/v) range of 0 to 25%. The unit of conversion ratio is sugar obtained (g) / biomass (g).

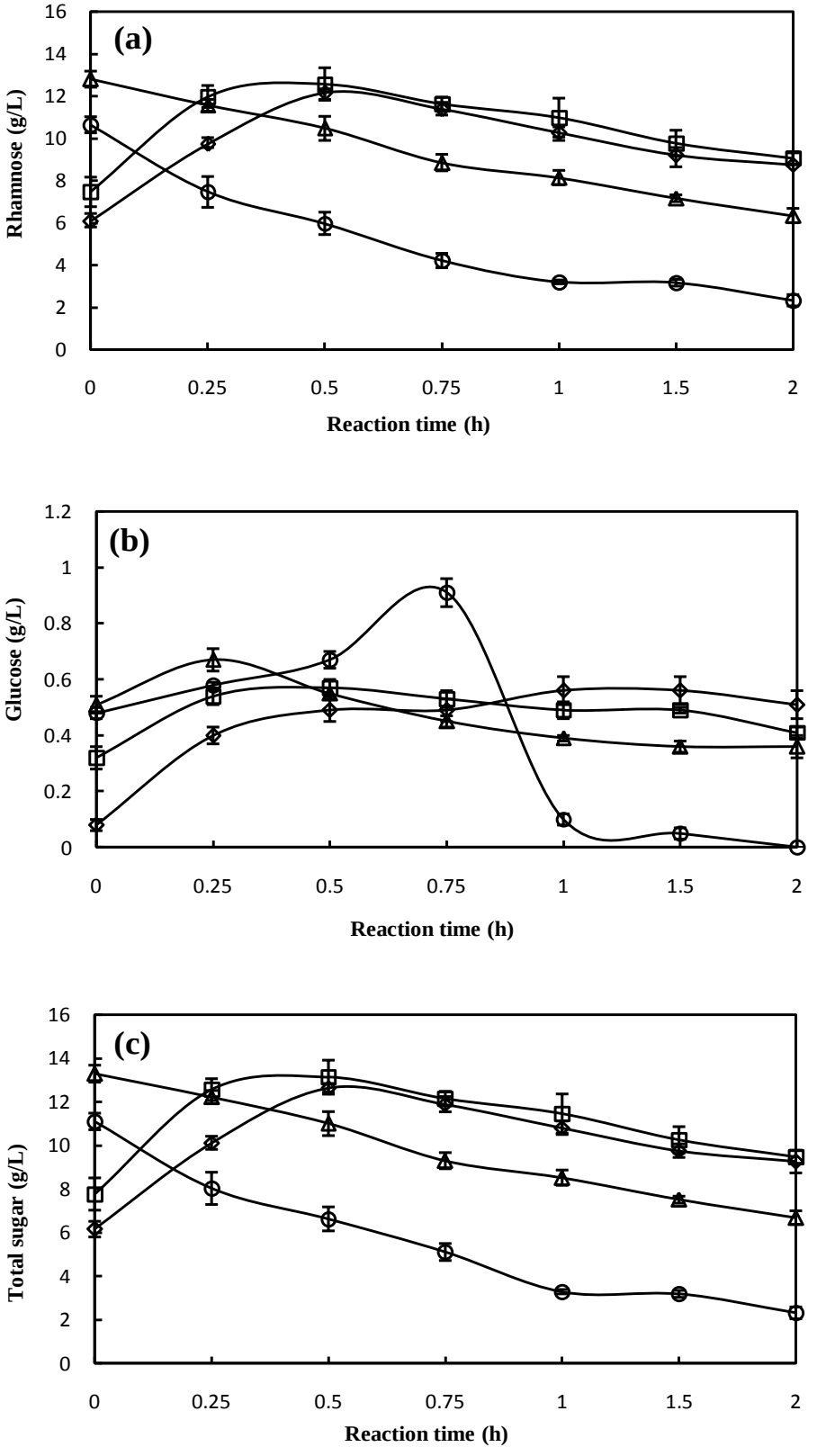

Figure 4. Effect of temperature (120°C (◇), 130°C (□), 140°C (△), 150°C (○)) and time course on the saccharification of *U. pertusa.* (a) Rhamnose (b) glucose (c) total sugar (rhamnose+glucose). *U. pertusa* (3%, w/v) was treated with 3% of H_2SO_4 (v/v) at 0.5 MPa by hot-compress treatment instrument.

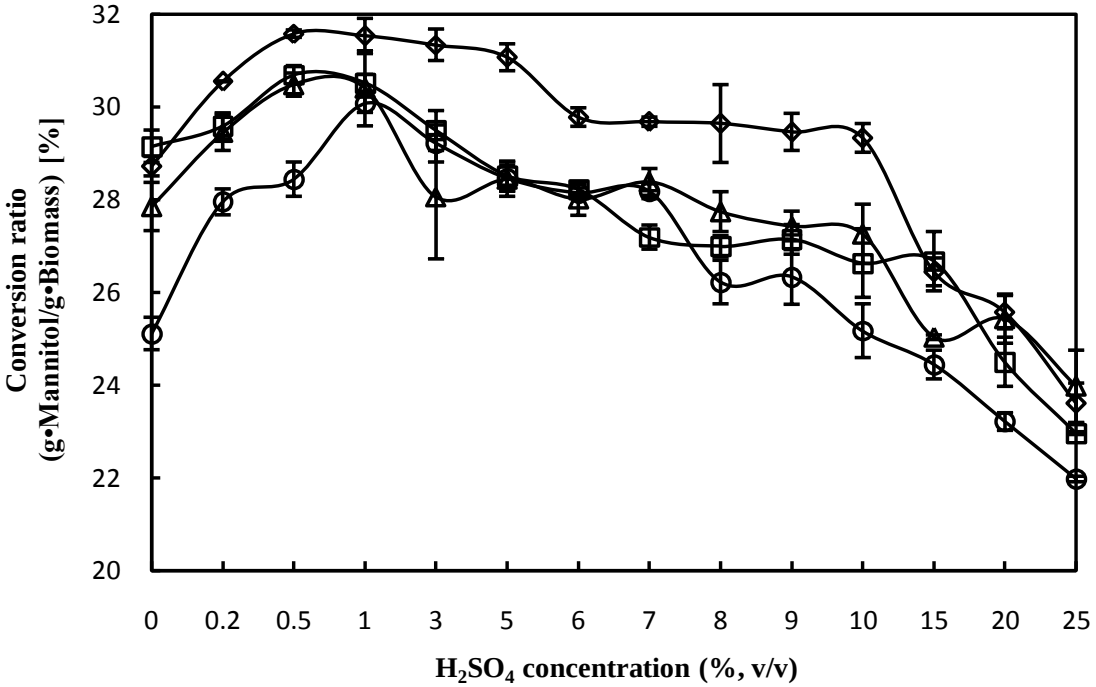

Figure 5. Effect of substrate and catalyst concentration on saccharification of *L. japonica.* Mannitol conversion ratio from *L. japonica* in the H_2SO_4 (v/v). *L. japonica* (3% (◇), 5% (□), 7% (△) and 10% (○), w/v)) was treated at 120°C by autoclaving in the H_2SO_4 (v/v) range of 0 to 25%. The unit of conversion ratio is sugar obtained (g) / biomass (g)

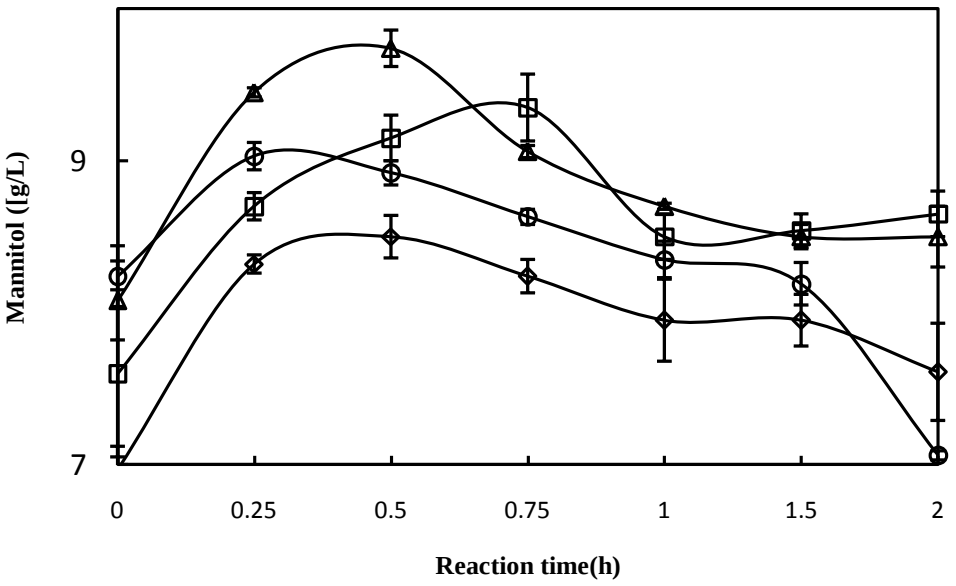

Figure 6. Effect of temperature (120°C (◇), 130°C (□), 140°C (△) and 150°C (○)) and time course on the saccharification of *L. japonica. L. japonica* (3%, w/v) was treated with 1% of H_2SO_4 (v/v) at 0.5 MPa by hot-compress treatment instrument.

degradation occurred as the catalyst concentration was increased from 3 and 7% (v/v). But in terms of production of total sugar, optimal conditions of conversion ratio were 3% (w/v) of the biomass and 3% (v/v) of H_2SO_4. Under

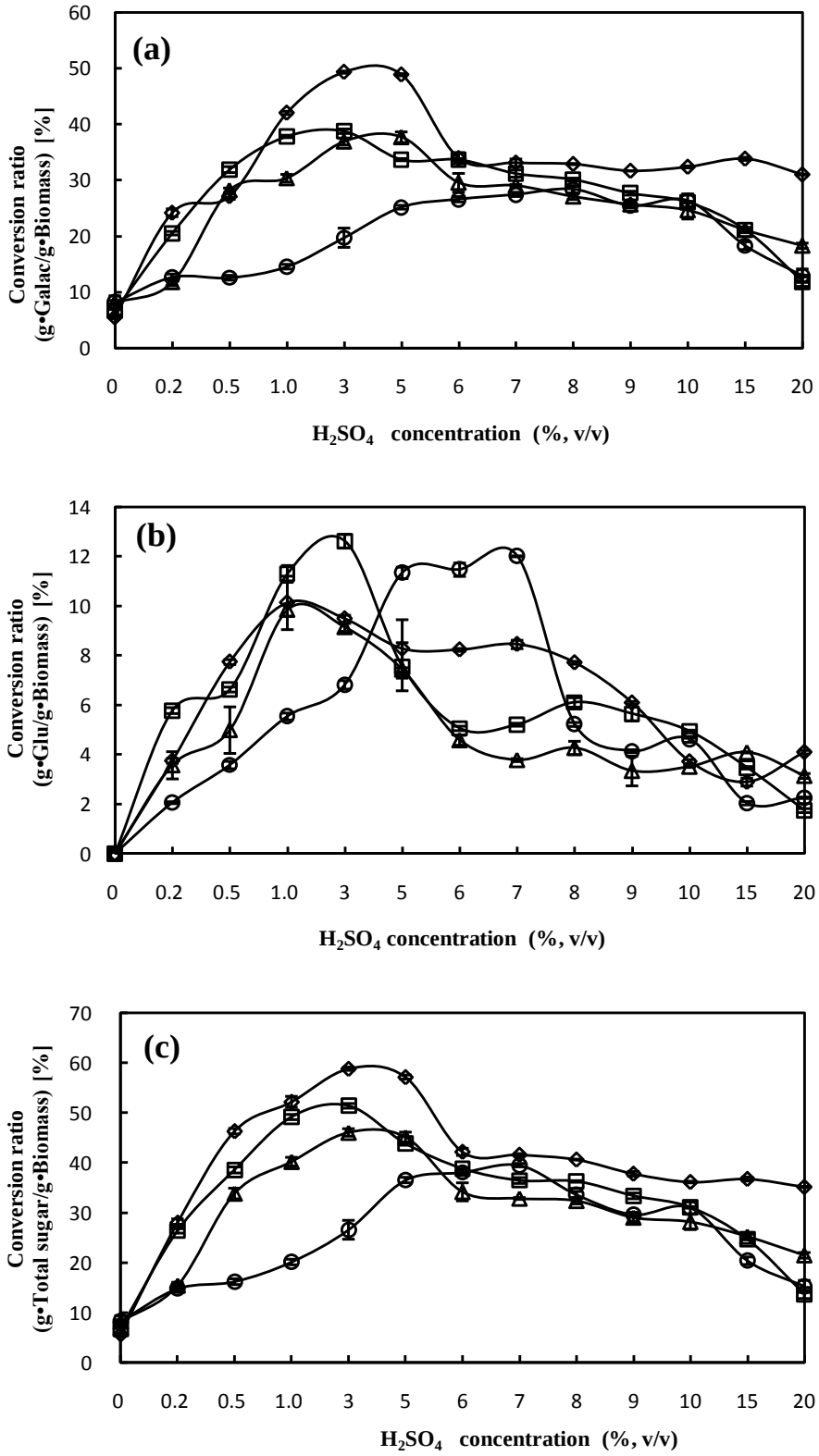

Figure 7. Effect of substrate and catalyst concentration on saccharification of *G. amansii*. (a) The galactose sugar conversion ratio. (b) The glucose conversion ratio. (c) The total (galactose+glucose) conversion ratio. *G. amansii* (3% (◇), 5% (□), 7% (△), 10% (○), w/v)) was treated at 120°C by autoclaving in the H_2SO_4 (v/v) range of 0 to 25%. The unit of conversion ratio is sugar obtained (g) / biomass (g).

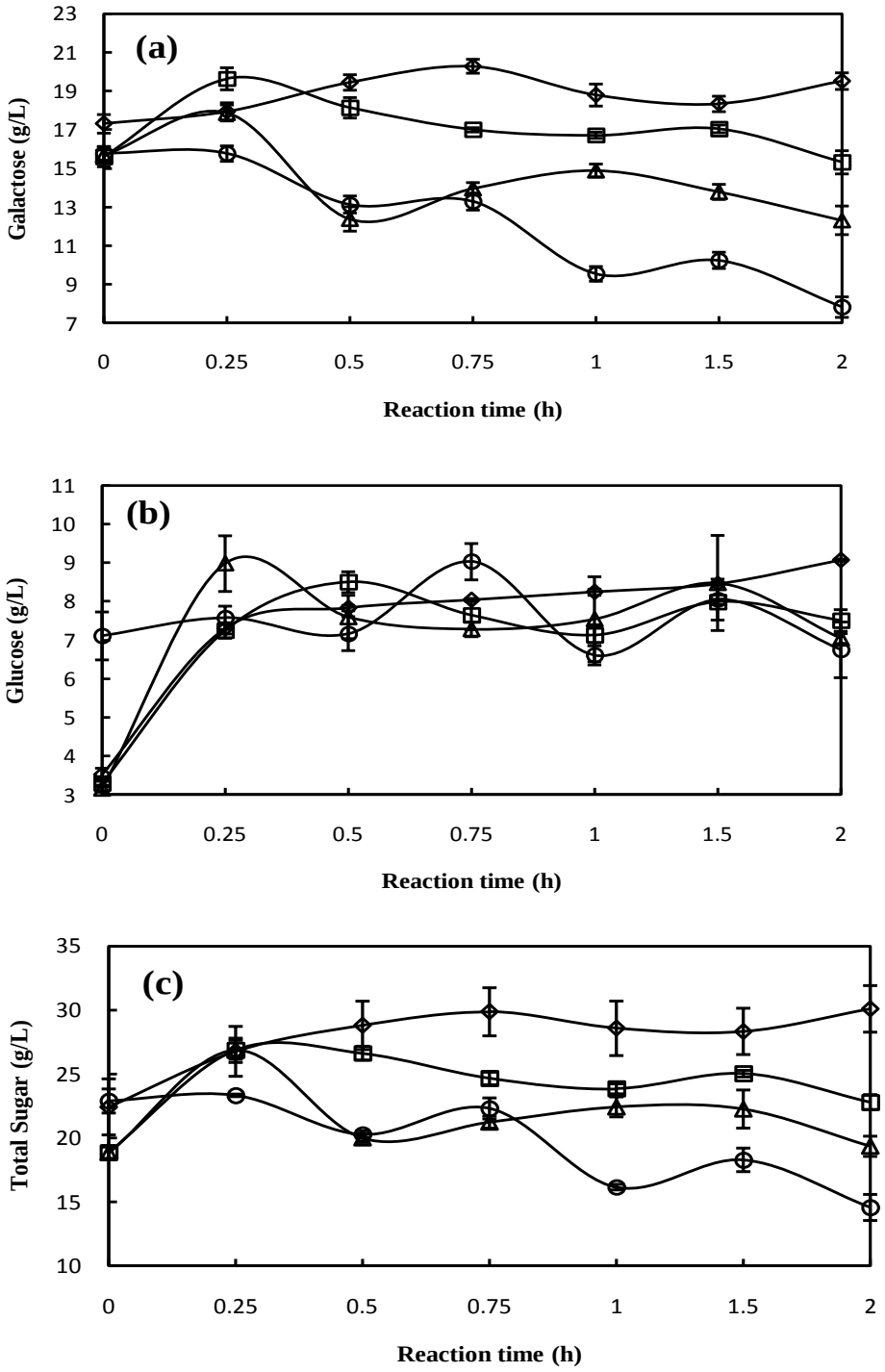

Figure 8. Effect of temperature (120°C (◇), 130°C (□), 140°C (△) and 150°C (○)) and time course on the saccharification of *G. amansii.* (a) Rhamnose (b) glucose (c) total sugar (rhamnose+glucose). *G. amansii* (3%, w/v) was treated with 3% of H_2SO_4 (v/v) at 0.5 MPa by hot-compress treatment instrument.

these conditions, 49% of galactose and 13% of glucose were produced from *G. amansii*. Total mono sugar production in this study is comparable to previous reported value of acid hydrolysates of hardwood, agricultural and agro-industrial residues (Neureiter et al., 2002; Martin et al., 2007).

Figure 8 shows the effects of temperature and time on saccharification to produce mono sugar under optimal

concentration (3%, w/v of biomass and 3% (v/v) of H_2SO_4). From the results, the highest galactose production was obtained at 120°C and at 45 min or 130°C and at 15 min.

The maximum mono sugar production was achieved with low reaction temperature of 120°C and time above 30 min. Results obtained in the condition of higher temperature of 150°C with holding time of 15 min is also similar. However, prolonging the holding time up to 2 h, decreased the mono sugar concentration. There was little effect of reaction time on total sugar production especially after 15 min. Therefore, shorter reaction time is appropriate to optimize total sugar production. Highest glucose production was achieved either at 140°C with holding time of 15 min or at 150°C with holding time of 45 min. Profile of galactose production showed similar trend. Considering above, in order to maximize total mono sugar (both glucose and galactose) production, hydrolysis at 130°C for 15 min using 3% (w/v) of *G. amansii* and 3% (v/v) of H_2SO_4 would be optimal.

So far, we have obtained effective reaction condition for saccharification using sulfuric acid in hot-compress treatment from *U. pertusa, L. japonica* and *G. amansii*. Seaweeds sugars could be easily extracted from the milled seaweed under optimal reaction condition. The results of this experiment will simplify the process of seaweed saccharification.

In addition, further research shall be conducted to identify toxic compounds in the acid hydrolysate such as furans, aliphatic acids (Taherzadeh et al., 1999) and phenolic compounds (Palmqvist and Hahn-Hagerdal, 2000). The removal of these inhibitors is very important in improving the efficiency of fermentation.

Conclusion

Seaweed carbohydrates could be effectively hydrolyzed to mono sugars using sulfuric acid and the results of this study indicate optimal saccharification condition with four-factors: Reaction temperature, reaction time, catalyst concentration and substrate concentration. Without any pretreatment, mono sugar can be produced effectively under moderate conditions of reaction temperature under 150°C optimizing catalyst concentration or reaction time. *U. pertusa* had 59.07% carbohydrate, while the saccharified mash had 37.89% rhamnose and 16.14% glucose. *L. japonica* had 54.50% carbohydrate and yielded 31.53% mannitol. *G. amansii* had 71.43% carbohydrate, while the saccharified mash had 49.32% galactose and 12.62% glucose. Seaweeds have high carbohydrate content and could be efficiently saccharified to monosugars, which could serve as good substrates for the fermentative production of organic acids. Besides, the protein nitrogen and ash in the hydrolysate might be favorable ingredients supporting fermentation. Seaweeds could also serve as non-feed alternative substrate for the production of value added products such as biofuel or bioplastics, especially in view of their abundance and low cost.

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