Carbohydrate Polymers 94 (2013) 561–566

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Hydrolysis of macroalgae using heterogeneous catalyst for bioethanol production

Inn Shi Tan, Man Kee Lam, Keat Teong Lee*

School of Chemical Engineering, Universiti Sains Malaysia, Engineering Campus, Seri Ampangan, 14300 Nibong Tebal, Pulau Pinang, Malaysia

ARTICLE INFO

Article history:
Received 2 October 2012
Received in revised form 5 December 2012
Accepted 17 January 2013
Available online 24 January 2013

Keywords:
Macroalgae
Heterogeneous acid catalyst
Sugars
Fermentation
Third-generation bioethanol

ABSTRACT

Utilization of macroalgae biomass for bioethanol production appears as an alternative source to lignocellulosic materials. In this study, for the first time, Amberlyst (TM)-15 was explored as a potential catalyst to hydrolyze carbohydrates from *Eucheuma cottonii* extract to simple reducing sugar prior to fermentation process. Several important hydrolysis parameters were studied for process optimization including catalyst loading (2–5%, w/v), reaction temperature (110–130 °C), reaction time (0–2.5 h) and biomass loading (5.5–15.5%, w/v). Optimum sugar yield of 39.7% was attained based on the following optimum conditions: reaction temperature at 120 °C, catalyst loading of 4% (w/v), 12.5% (w/v) of biomass concentration and reaction time of 1.5 h. Fermentation of the hydrolysate using *Saccharomyces cerevisiae* produced 0.33 g/g of bioethanol yield with an efficiency of 65%. The strategy of combining heterogeneous-catalyzed hydrolysis and fermentation with *S. cerevisiae* could be a feasible strategy to produce bioethanol from macroalgae biomass.

1. Introduction

Focus on the use of biomass as an alternative energy feedstock to fossil fuels is intensifying in these recent years due to its significant role in reducing CO_2 emissions to the atmosphere when combusted as fuel (Schmidt, Leduc, Dotzauer, Kindermann, & Schmid, 2010). While development of fuels from biomass continues apace, first-generation bioethanol derived from edible crops have come under serious controversy because of food versus fuel feud (Nigam & Singh, 2011). Second-generation bioethanol which is mainly derived from lignocellulosic biomass offers an alternative option due to its abundant availability and do not compete with food production (Jegannathan, Chan, & Ravindra, 2009). Nevertheless, problems in removing lignin from lignocellulosic materials have impeded the commercialization potential of this renewable source (Karthika, Arun, & Rekha, 2012; Zakzeski, Bruijnincx, Jongerius, & Weckhuysen, 2010). Thus, interest has now diverted to third-generation bioethanol which is derived from aquatic sources such as macroalgae (Ross, Jones, Kubacki, & Bridgeman, 2008). Generally, macroalgae grows faster compared to terrestrial crops and does not compete with agricultural land area for mass cultivation. In addition, macroalgae contains high carbohydrate content which is rich in polysaccharides and more importantly, does not contain lignin (Park et al., 2011).

The utilization of macroalgae as bioethanol feedstock is still scattered in literature. Goh and Lee (2010) have reported that if *Eucheuma* spp. is used as a feedstock for bioethanol production, the estimated bioethanol yields could reach up to 110,000 tonnes annually. The carbohydrate is composed of the two monosaccharides: D-galactose and 3,6-anhydro-galactose, 56.2% and 43.8%, respectively (Lin, Tako, & Hongo, 2000). After hydrolysis process, these monosaccharides are suitable to be used as substrate during fermentation process for bioethanol production.

Up to now, the uses of enzyme and homogeneous acid-catalyst have been reported for the hydrolysis of carbohydrates from various biomass to fermentable sugar (Monavari, Galbe, & Zacchi, 2011; Mussatto, Carneiro, Silva, Roberto, & Teixeira, 2011; Seguin, Marinkovic, & Estrine, 2012). However, enzyme catalyzed processes face several challenges such as difficulty in recovering the enzyme from the products and requiring long hydrolysis time. On the other hand, Meinita et al. (2011), Jeong et al. (2012) and Khambhaty et al. (2012) have reported bioethanol production from *Kappaphycus alvarezii* (cottonii) using sulfuric acid as the catalyst. Nevertheless, it was found that the homogeneous acid hydrolysis process also suffers from several problems such as formation of large amount of hazardous compounds and the catalyst cannot be recovered for subsequent use. Therefore, the need of easily separable and reusable heterogeneous acid catalyst is considered essential for the hydrolysis process, in term of economical and environmental perspective. In order to ensure efficient process, the heterogeneous catalysts should be water-tolerant (Busca, 2007), have strong acidic sites and high surface area (Vigier & Jérôme, 2010). Recently, several studies have reported on the use of various types of heterogeneous acid catalyst for the hydrolysis of disaccharides (cellobiose and sucrose) with the aim to ascertain its activity (Dwiatmoko, Choi, Suh, Suh, & Kung, 2010; Nasef, Saidi, & Senna, 2005).

* Corresponding author. Tel.: +60 4 5996467; fax: +60 4 5941013.
E-mail address: chktlee@eng.usm.my (K.T. Lee).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.01.042

Table 1
Properties of Amberlyst (TM)-15 catalyst.

Property	Amberlyst (TM)-15
Surface area (m^2/g)	34.85
Particle size (μm)	600–800
Capacity (meq/gm)	4.2
Average pore dia (Å)	260
Supplier	Rohm & Haas, France

Thus, the objective of this study is to develop a new method of saccharification for raw macroalgae extract using a strong acidic heterogeneous catalyst, Amberlyst (TM)-15. Process variables such as catalyst loading, temperature, biomass concentration and reaction time were thoroughly optimized to attain the highest simple sugar yield. Then, the simple sugar was further explored for possible bioethanol production.

2. Material and methods

2.1. Raw materials and chemicals

Eucheuma cottonii which was used in all the experiments was purchased from Futt Put Enterprise (north coast of Sabah, Malaysia). The *E. cottonii* sample was washed with distilled water, dried at 40 °C, pulverized, and filtered with 300-mesh filters. The sample obtained was analyzed for proximate composition and kept in an air tight container until being used for the preparation of macroalgae extract.

Amberlyst (TM)-15, Amberlyst (TM)-A21, Calcium hydroxide, standard D-galactose, D-glucose and *Saccharomyces cerevisiae* (YSC2, type II) were purchased from Sigma–Aldrich (USA). Sulfuric acid, potassium dihydrogen phosphate and ethanol were purchased from Fisher Scientific (UK). All reagents were of analytical grade. Table 1 shows the properties of Amberlyst (TM)-15 catalyst.

2.2. Preparation of macroalgae extracts

120 g of dry *E. cottonii* (less than 300 mesh) was soaked in 4 L water for 30 min. Then, it was grounded to form pulp, followed by boiling at 90 °C for 2 h. The hot extracts were filtered (45 μm mesh size), dried to a constant weight at 40 °C and pulverized.

2.3. Proximate composition analysis

Crude protein content in *E. cottonii* was determined according to (Method 988.05) Association of Official Analysis Chemists (AOAC, 2000). Crude fat was determined by the Soxhlet extraction method (Method 920.30) (AOAC, 2000). Ash content was determined by heating the samples at 550 °C for 1 h. The total carbohydrate content of *E. cottonii* was determined according to a modified method that is based on the National Renewable Energy Laboratory (NREL, Golden, CO) analytical methods for biomass (Sluiter et al., 2008) using a two-step acid hydrolysis procedure. The sample was initially subjected through a primary 72% sulfuric acid hydrolysis, followed by a secondary dilute-acid hydrolysis. The content of sugar was quantified with high performance liquid chromatography.

2.4. Hydrolysis of E. cottonii

2.4.1. Heterogeneous-catalyzed hydrolysis

The heterogeneous-catalyzed hydrolysis process using Amberlyst (TM)-15 was investigated by varying different process parameters: amount of Amberlyst (TM)-15 (2–5%, w/v), temperature (110–130 °C), biomass loading (solid/liquid ratio: 5.5–15.5%, w/v) and reaction time (0–2.5 h). The dried macroalgae extract was subjected to distilled water to form different solid/liquid ratios. The mixture was then mixed with different amount of Amberlyst (TM)-15 and incubated at different temperature in an autoclave reactor. Stirring speed for all experiments was maintained at 370 rpm while the internal pressure of the autoclave was kept constant at 10 bars. After a specific hydrolysis time, the samples were cooled to room temperature and the residue was separated from the liquid by filtration using filter paper. For comparison, instead of using macroalgae extracts, raw *E. cottonii* (s/l: 12.5) without prior extract (Section 2.2) was directly subjected to hydrolysis. The raw biomass was directly treated with 4% (w/v) Amberlyst (TM)-15 at 120 °C for 1.5 h. All experiments in the current work were performed in triplicates, and the data reported are the average of the three replications.

2.4.2. Homogeneously-catalyzed hydrolysis

The performance of the heterogeneous-catalyzed hydrolysis was compared with conventional sulfuric acid hydrolysis. 12.5% (w/v) macroalgae extract was treated with 0.2 M sulfuric acid at 120 °C for 1.5 h.

2.5. Neutralization of hydrolysate

The acidic hydrolysate was neutralized with appropriate amount of Amberlyst (TM)-A21 or lime until pH 6.3–6.8 was attained. After neutralization, the hydrolysate was filtered and being measured for sugar content and subsequently fermented for ethanol production.

2.6. Bioethanol fermentation by yeast

S. cerevisiae was used for fermentation. The hydrolysate was concentrated by rotary evaporator before transferring to a basal medium which consist 0.175% (w/v) KH_2PO_4 at pH 5. The volumetric ratio of hydrolysate to basal medium used was 1:2. The medium was poured into a 250 mL Erlenmeyer flask and was sterilized at 121 °C for 15 min. The mixture was then incubated in a shaking incubator at 34 °C with a shaking speed of 135 rpm for a total time of 144 h. 1.5 mL of samples were withdrawn at different interval time during fermentation and was centrifuged at 10,000 × *g* for 10 min. The supernatant obtained after centrifugation was then analyzed for bioethanol and residual glucose and galactose content.

2.7. Analytical method

The sugar concentration was quantified with an Agilent series 1200 infinity high performance liquid chromatography (HP-LC) system equipped with a 385-ELSD and a 300 mm × 7.7 mm Hi-Plex Ca column. The mobile phase used is deionized water at a flow rate of 0.6 mL min^{-1} and injection volume of 20 μL. Purified nitrogen was used as carrier gas (70 psi) for the detector. The spray chamber temperature was set at 40 °C whereas detector temperature at 80 °C. Samples were diluted 100 times with deionized water and filtered with 0.20 μm syringe filter (Nylon membrane, Fisher Scientific) prior to HP-LC analysis. The presences of various compounds in the sample were identified by comparing the retention time of individual peaks with those of standard compounds (glucose and galactose, Sigma). Calibration curve for different types of sugars were obtained for quantitative analysis. The sugar yield was calculated as:

$$\text{Yield}\% = \frac{\text{Concentration (g/l) of sugar at time of } t}{\text{Initial concentration (g/l) of substrate}} \times 100\% \quad (1)$$

Bioethanol concentration was quantified by gas chromatography (GC) using a 5890 Series II chromatography equipped with

Table 2
Composition of the macroalgae species *Eucheuma cottonii*.

Component	Composition % (w/w)
Carbohydrate	35.2
Protein	2.2
Lipid	3.7
Ash	26.1
Moisture	21.7
Others	11.1

flame ionization detector (FID) (Hewlett Packard, Palo Alto, CA). The column used was 2.0 m in length and 0.2 cm ID, 80/120 mesh Carbopack B-DA/4% Carbowax 20M (Supelco, USA). The operating condition was as follows: detector temperature of 225 °C; injector temperature of 225 °C; oven temperature was programmed to increase from 100 (2.0 min) to 175 °C at 10°CC min^{-1} For each GC analysis, 2 μL of sample was injected. Helium was used as the carrier gas while 0.5% (v/v) 2-pentanone was used as internal standard. Bioethanol yield and percent theoretical yield were calculated based on the following equations, respectively (Keating, Robinson, Bothast, Saddler, & Mansfield, 2004):

$$Y_{P/S} = \frac{[EtOH]_{max}}{[Sugar]_{ini}} \quad (2)$$

$$Y_{\%T} = \frac{Y_{P/S}}{0.51} \times 100\% \quad (3)$$

where $Y_{P/S}$ = ethanol yield (g/g), $[EtOH]_{max}$ = highest ethanol concentration achieved during fermentation (g/L), $[Sugar]_{ini}$ = total initial sugar concentration at onset of fermentation (g/L), $Y_{\%T}$ = percent theoretical yield (%), 0.51 is the maximum ethanol yield per unit of hexose sugar from glycolytic fermentation (g/g), P = product and S = substrate.

At least three samples were used in all analytical determinations, and data are presented as the mean of three replicates.

3. Results and discussion

3.1. Raw material composition

Table 2 shows the composition of *E. cottonii* used in this study. The carbohydrates content in the biomass is 35.2% (w/w), which consists of 25% (w/w) galactose and 10.2% (w/w) glucose. The remaining components are protein, lipid, ash, moisture, and other, which occurred to be 2.2%, 3.7%, 26.1%, 21.7% and 11.1%, respectively.

3.2. Hydrolysis of E. cottonii

3.2.1. Effect of the catalyst loading

The effect of catalyst loading on hydrolysis of *E. cottonii* extract is shown in Fig. 1. It can be seen that the yield of galactose generally increases with time for all catalyst loading. This result indicated that Amberlyst (TM)-15 has the potential to hydrolyze galactans extracted from *E. cottonii* to galactose. The type of galactans in *E. cottonii* are mainly κ-carrageenans that can be extracted using hot water (Estevez, Ciancia, & Cerezo, 2004). The structure of κ-carrageenans was reported as alternating 3-linked β-D-galactose 4-sulfate and 4-linked 3,6-anhydro-α-D-galactose units connected through glycosidic linkages (Estevez, Ciancia, & Cerezo, 2000) which contains the basic unit of D-typed galactose that can be fermented by yeast (Meinita et al., 2011). Fig. 1 also shows that higher loading of Amberlyst (TM)-15 accelerates the hydrolysis rate of macroalgae extract, which can be explained due to the increase in the total number of available active catalytic sites for the reaction (Rinaldi, Meine, vomStein, Palkovits, & Schüth, 2010). The highest

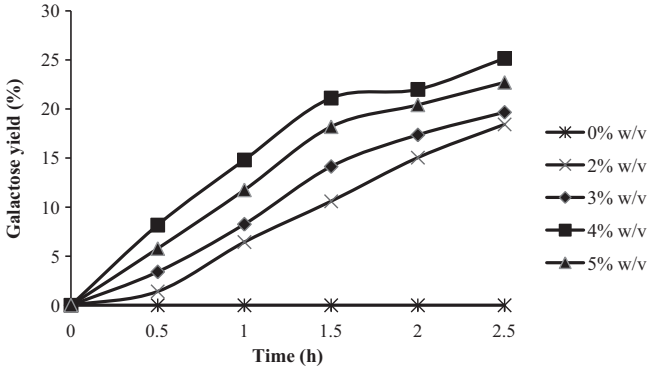

Fig. 1. Effects of catalyst concentrations on the hydrolysis of seaweed extract in the presence of a heterogeneous catalyst Amberlyst (TM)-15. (Conditions: s/l ratio: 5.5%, reaction temperature 110 °C, reaction time: 0–2.5 h.)

galactose yield of 25.2% was attained at a catalyst loading of 4% (w/v). However, the galactose yield decreased when the amount of catalyst loading was increased beyond 4% (w/v). The reaction produces a negative sugar output rate as degradation is evident. This is because higher acid concentration might degrade the sugar compounds and produce more by-product inhibitors such as 5-hydroxy-methyl-furfural and organics acids (Park et al., 2011). In order to further elucidate on the role of catalyst, hydrolysis was carried out without catalyst, whereby no reducing sugar was detected even after 2.5 h of reaction (Fig. 1). The results indicated that catalyst plays a crucial role in hydrolyzing the galactans to galactose. The mechanism which is widely accepted for the acid catalyzed hydrolysis of carrageenan is based on the protonation of the glycosidic oxygen. High temperature and acidic medium are reported to promote hydrolysis of the acid-sensitive (1 → 3) glycosidic linkages (Khambhaty et al., 2012). 4% (w/v) of Amberlyst (TM)-15 was chosen as the optimal catalyst loading.

3.2.2. Effect of the reaction temperature

The effect of reaction temperature was also investigated in this study. According to Fig. 2, an increase in temperature from 110 to 120 °C resulted in a sharp increase in the formation of galactose. This shows that temperature has a significant effect on the hydrolysis yield of macroalgae extract. Higher temperature was also reported to result in improved hydrolysis rate relative to low temperature (Meinita, Hong, & Jeong, 2012). After 1.5 h at 120 °C, the highest galactose yield of 27.7% was attained. However, beyond 1.5 h, there is a slight drop in the yield of galactose. This is probably caused by conversion of galactose to other chemicals such as HMF or levulinic acid (Jeong & Park, 2010). For the hydrolysis conducted

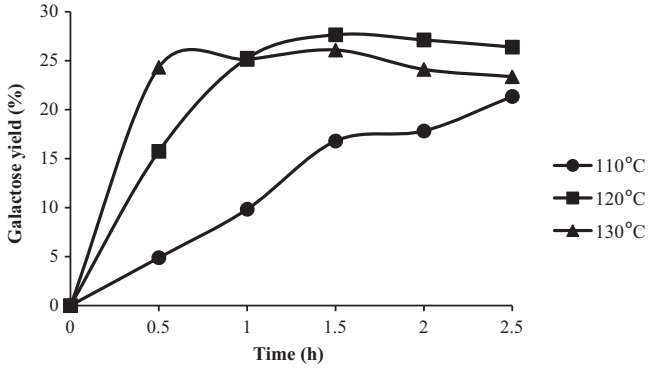

Fig. 2. Effect of reaction temperature on the hydrolysis of seaweed extract in the presence of a heterogeneous catalyst, Amberlyst (TM)-15. (Conditions: s/l ratio: 5.5%, catalyst loading: 4%, w/v, reaction time: 0–2.5 h.)

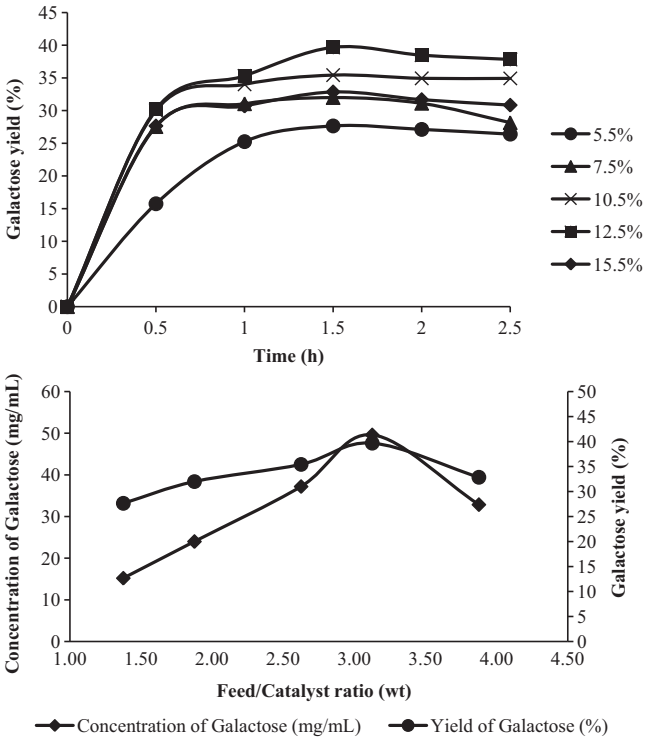

Fig. 3. Effect of biomass concentration on the hydrolysis of seaweed extract in the presence of a heterogeneous catalyst, Amberlyst (TM)-15. (Conditions: reaction temperature: 120 °C, catalyst loading: 4%, w/v, reaction time: 0–2.5 h). (A) Solid/liquid ratio (5.5–15.5%). (B) Concentration (mg/mL) and yield (%) of galactose after 1.5 h.

at 130 °C, it can be seen that initially, at 0.5 h, the galactose yield obtained surpassed the yield obtained at 110 °C and 120 °C. However after 0.5 h, instead of increasing, the galactose yield was found to stabilize at 25% with a slight drop, then after. This result indicates that extended reaction time or high reaction temperature can cause a negative effect on the galactose yield due to degradation of sugars in hydrolysis reactions and formation of undesirable by-product (Harun & Danquah, 2011b). In addition, Amberlyst (TM)-15 may also be deactivated at higher hydrolysis temperature due to low thermal stability of ion-exchange catalyst. Furthermore, high temperature could also cause the reduction of catalyst surface area as well as the number of H^+ active site (Morales, van Grieken, Martín, & Martínez, 2010). Therefore, 120 °C was chosen as the optimum hydrolysis temperature for this study.

3.2.3. Effect of the biomass concentration

Fig. 3(A) shows the effect of different biomass concentrations on the formation of galactose. When hydrolysis was conducted at 120 °C for 2.5 h, the galactose yield was found to increase gradually with an increase in biomass concentration up to 12.5% (w/v); beyond that loading, there was reduction in galactose yield. This result is expected because, at fixed catalyst loading, increasing biomass concentration resulted in more carbohydrate available for hydrolysis. In order to facilitate a more meaningful result, the data in Fig. 3(A) was used to plot the correlations between the concentration and yield of galactose to the weight ratio of biomass to catalyst as shown in Fig. 3(B). This will allow the analysis on the effective biomass to catalyst ratio so that suitable amount of biomass can be used for fixed amount of catalyst. Note that biomass concentration of 5.5%, 7.5%, 10.5%, 12.5% and 15.5% correspond to 1.38, 1.88, 2.63, 3.13 and 3.88 biomass/catalyst ratio, respectively. At 1.5 h, the highest galactose yield of 39.7% was obtained at 3.13 g biomass/g catalyst ratio and the lowest yield of 27.7% was obtained at 1.38 g biomass/g catalyst ratio. The results can be explained by the total amount of carbohydrates present in the biomass. Higher biomass loading constituted higher carbohydrates content with more fermentable sugars that can be hydrolyzed by the catalyst Amberlyst (TM)-15 (Harun & Danquah, 2011a). One interesting observation can be seen from Fig. 3(B). The gradient for the concentration of galactose in the reaction mixture curve is much steeper than the galactose yield. This indicates that with an increase in biomass loading/biomass to catalyst ratio, the increase in galactose yield is not as fast as the increase in the concentration of galactose in the reaction mixture. This result reveals that there may be a retention of sugars in the biomass due to higher hydrolysate viscosity (Miranda, Passarinho, & Gouveia, 2012). From these results, it could be concluded that the optimum conditions for hydrolysis of *E. cottonii* extract were: 4% (w/v) of Amberlyst (TM)-15, 12.5% (w/v) of biomass concentration, reaction temperature at 120 °C and reaction time of 1.5 h.

3.3. Comparison with other hydrolysis methods

The effectiveness of Amberlyst (TM)-15 (a type of solid catalyst) was compared to a more common type of catalyst, 0.2 M H_2SO_4 (liquid catalyst) in the hydrolysis of *E. cottonii*. The results of the comparison as shown in Table 3 clearly indicate the superiority of Amberlyst (TM)-15 as compared to H_2SO_4. For *E. cottonii* with extract, the yield of galactose obtained using Amberlyst (39.7%) was slightly higher than those using H_2SO_4 (34.6%). Recently, Khambhaty et al. (2012) have reported the production of bioethanol from *E. cottonii* extracts, in which the hydrolysis process was catalyzed by 0.9 N sulfuric acid. The maximum reducing sugar yield was merely 26.2% (Table 3). This comparison shows that Amberlyst 15 is a far more superior catalyst than sulfuric acid and has the same activity as sulfuric acid. The acid resins were merely an acidifier of the aqueous slurries, having a similar effect as an aqueous solution of sulfuric acid (Rinaldi, Palkovits, & Schüth, 2008). In addition, Amberlyst (TM)-15 is more environmental friendly because it can be easily separated from the products after hydrolysis process and can be recycle use.

One additional experiment was conducted using the optimum condition obtained for *E. cottonii* extract but using the un-treated macroalgae (fresh seaweed) and the results are shown in Table 3. Based on the result attained in Table 3, the formation of reducing sugars obtained from fresh *E. cottonii* (24.4%) was relatively lower than that of *E. cottonii* extract (39.7%). This is because pre-extraction of carrageenans from macroalgae can remove impurities that can hinder the hydrolysis of galactans, thus facilitate hydrolysis process and subsequently increase the sugar concentration. In addition to that, fresh *E. cottonii* was also subjected to H_2SO_4 and as expected, a lower yield of 20.6% was obtained as shown in Table 3. All the results show the superiority of Amberlyst (TM)-15 for the hydrolysis of *E. cottonii* extract.

3.4. Neutralization

The hydrolysate obtained after hydrolysis of *E. cottonii* extract needs to be neutralized before fermentation (Canilha, de Almeida e Silva, & Solenzal, 2004). During the hydrolysis reaction, it does not only de-polymerize the polysaccharide, but it also hydrolyzes the sulfate group attached to the galactans. Thus, the concentration of total dissolved solids in the hydrolysate before neutralization is as high as 69,000 mg/L. In this study, the effectiveness of two types of neutralization agent was tested; Amberlyst (TM)-A21 and lime (Table 4). After neutralization, concentration of total dissolved solids with Amberlyst (TM)-A21 and lime were 3280 mg/L and 7320 mg/L, respectively. Although the sulfate group could potentially be neutralized by the addition of lime, however it resulted

Table 3
Comparison with other hydrolysis methods of *Eucheuma cottonii*.

Raw material	Reaction conditions					Yield of carbohydrates (%)	References
	Temperature (°C)	Type of catalyst	Catalyst loading	Biomass loading	Reaction time (h)		
Heterogeneous acid catalyst							
Seaweed extract	120	Amberlyst (TM)-15	4% (w/v)	12.5% (w/v)	1.5	39.7	This work
Fresh seaweed	120	Amberlyst (TM)-15	4% (w/v)	12.5% (w/v)	1.5	24.4	This work
Homogeneous acid catalyst							
Seaweed extract	120	H_2SO_4	0.2 M	12.5%	1.5	34.6	This work
Seaweed extract	100	H_2SO_4	0.9 N	5.0%	5 cycles (1 cycle/h)	26.2	Khambhaty et al. (2012)
Fresh seaweed	120	H_2SO_4	0.2 M	12.5%	1.5	20.6	This work

Table 4
Data on neutralization of acidic hydrolysate with Amberlyst-A21 and lime.

Sample	TDS (ppm)	Conductivity (mS)	pH	Sugar (mg/mL)	Color of solution
Hydrolysate without treatment	69,000	137.9	0.80	19.0	Deep yellow
Hydrolysate after treated with Amberlyst (TM)-A21	3280	6.54	6.40	15.0	Yellowish
Hydrolysate after treated with lime	7320	14.67	6.70	18.0	Brown

to the formation of inorganic salt. High concentration of salt in the hydrolysate can hamper fermentation process (Khambhaty et al., 2012). Thus an additional step is required to reduce the level of dissolved salts before fermentation. Instead, when using Amberlyst (TM)-A21 (a weak basic ion-exchange resins), the sulfate group is removed while leaving the sugar concentration relatively unaffected. The role of anion-exchange resins is to substitute the sulfonic anions with hydroxyl ions that can lead toward neutralization. In addition, treatment of the acidic hydrolysate with ion-exchange resin can be one of the most efficient methods for removing inhibiting compounds prior to fermentation (Nilvebrant, Reimann, Larsson, & Jönsson, 2001).

3.5. Fermentation

The utilization rate of fermentable sugar toward bioethanol concentration was monitored during the fermentation process in order to understand the relationship between both variables. As shown in Fig. 4, there were significant differences in the fermentation performance with different inoculum levels. Bioethanol production rate increases with increase in inoculum levels up to certain level and the bioethanol production rate decreases beyond that level. The lowest yeast inoculum levels of 8 mg/mL resulted in very low consumption of sugar resulted with a long lag phase which produces lower bioethanol concentration of 3.48 mg/mL after 144 h. Higher bioethanol production was obtained with an inoculums amount of 16.0 mg/mL. The bioethanol production rate in the early phase of the culture was relatively slow but increased after 24 h and reached a maximum after 72 h of fermentation. The increase in inoculum levels increases bioethanol yield (65%) due to better utilization of sugars and this results in extracellular bioethanol production (Nurgel, Erten, Canbaş, Cabaroğlu, & Selli, 2002). However, after a certain point level, further increase in inoculum levels did not improve the bioethanol production (Fig. 4(C)). The high amount of inoculums can adversely affect bioethanol production due to the fact that high inoculums level decreases the viability of yeast population (Arifa Tahir, 2010). A decrease in bioethanol production was observed after exponential phase due to the depletion of galactose in the fermentation broth. As a result, the produced bioethanol acts as a carbon source and inevitably being consumed by yeast in the same manner as the original simple sugar, thus decreasing the bioethanol concentration during fermentation (Harun & Danquah, 2011b).

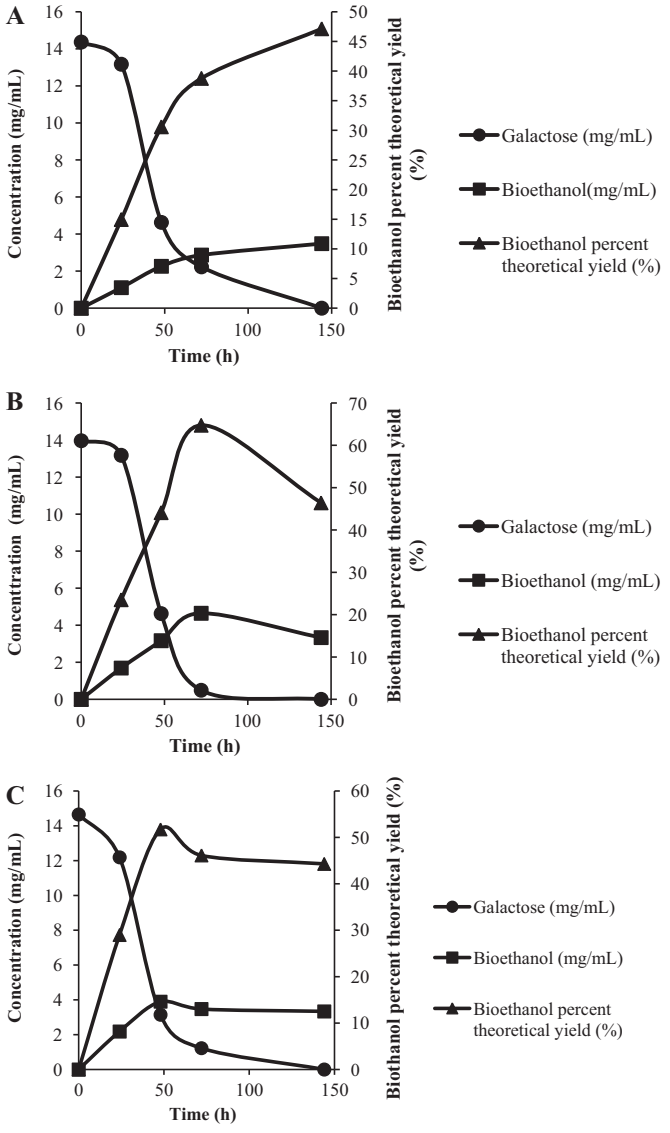

Fig. 4. The effect of different *Saccharomyces cerevisiae* inoculums amounts on bioethanol production. (A) 8 mg/mL (B) 16 mg/mL (C) 24 mg/mL.

4. Conclusion

From the results, it could be concluded that *E. cottonii* could be a potential feedstock for bioethanol production. Heterogeneous catalyst Amberlyst (TM)-15 can act efficiently as solid acid catalyst in the hydrolysis of sugar. This simple but effective method could facilitate an energy-efficient and cost-effective conversion of macroalgae biomass into bioethanol.

Acknowledgements

The authors would like to acknowledge the funding given by Universiti Sains Malaysia (Research University Grant No. 814187 and Postgraduate Research Grant Scheme No. 8045036) and Mybrain15 MyPhD scholarship funded by the Ministry of Higher Education Malaysia for this project.

References

AOAC. (2000). *Official method of analysis* Maryland, USA: Association of Official Analytical Chemists, Inc.

Arifa Tahir, M. A. T. f. (2010). Effect of cultural conditions on ethanol production by locally isolated *Saccharomyces cerevisiae* BIO-07. *Journal of Applied Pharmacy*, *3*(2), 72–78.

Busca, G. (2007). Acid catalysts in industrial hydrocarbon chemistry. *Chemical Reviews*, *107*, 5366–5410.

Canilha, L., de Almeida e Silva, J. B., & Solenzal, A. I. N. (2004). Eucalyptus hydrolysate detoxification with activated charcoal adsorption or ion-exchange resins for xylitol production. *Process Biochemistry*, *39*, 1909–1912.

Dwiatmoko, A. A., Choi, J. W., Suh, D. J., Suh, Y. W., & Kung, H. H. (2010). Understanding the role of halogen-containing ionic liquids in the hydrolysis of cellobiose catalyzed by acid resins. *Applied Catalysis A: General*, *387*, 209–214.

Estevez, J. M., Ciancia, M., & Cerezo, A. S. (2000). The system of low-molecular-weight carrageenans and agaroids from the room-temperature-extracted fraction of *Kappaphycus alvarezii*. *Carbohydrate Research*, *325*, 287–299.

Estevez, J. M., Ciancia, M., & Cerezo, A. S. (2004). The system of galactans of the red seaweed, *Kappaphycus alvarezii*, with emphasis on its minor constituents. *Carbohydrate Research*, *339*, 2575–2592.

Goh, C. S., & Lee, K. T. (2010). A visionary and conceptual macroalgae-based third-generation bioethanol (TGB) biorefinery in Sabah, Malaysia as an underlay for renewable and sustainable development. *Renewable and Sustainable Energy Reviews*, *14*, 842–848.

Harun, R., & Danquah, M. K. (2011a). Enzymatic hydrolysis of microalgal biomass for bioethanol production. *Chemical Engineering Journal*, *168*, 1079–1084.

Harun, R., & Danquah, M. K. (2011b). Influence of acid pre-treatment on microalgal biomass for bioethanol production. *Process Biochemistry*, *46*, 304–309.

Jegannathan, K. R., Chan, E.-S., & Ravindra, P. (2009). Harnessing biofuels: A global Renaissance in energy production? *Renewable and Sustainable Energy Reviews*, *13*, 2163–2168.

Jeong, G.-T, & Park, D. -H. (2010). Production of sugars and levulinic acid from marine biomass *Gelidium amansii*. *Applied Biochemistry and Biotechnology*, *161*, 41–52.

Jeong, T. S., Choi, C. H., Lee, J. Y., & Oh, K. K. (2012). Behaviors of glucose decomposition during acid-catalyzed hydrothermal hydrolysis of pretreated Gelidium amansii. *Bioresource Technology*, *116*, 435–440.

Karthika, K., Arun, A. B., & Rekha, P. D. (2012). Enzymatic hydrolysis and characterization of lignocellulosic biomass exposed to electron beam irradiation. *Carbohydrate Polymers*, *90*, 1038–1045.

Keating, J. D., Robinson, J., Bothast, R. J., Saddler, J. N., & Mansfield, S. D. (2004). Characterization of a unique ethanologenic yeast capable of fermenting galactose. *Enzyme and Microbial Technology*, *35*, 242–253.

Khambhaty, Y., Mody, K., Gandhi, M. R., Thampy, S., Maiti, P., Brahmbhatt, H., Eswaran, K., & Ghosh, P. K. (2012). *Kappaphycus alvarezii* as a source of bioethanol. *Bioresource Technology*, *103*, 180–185.

Lin, L.-h., Tako, M., & Hongo, F. (2000). Isolation and characterization of iota-carrageenan from *Eucheuma serra* (Togekirinsai). *Journal of Applied Glycoscience*, *47*, 303–310.

Meinita, M., Hong, Y.-K., & Jeong, G.-T. (2012). Comparison of sulfuric and hydrochloric acids as catalysts in hydrolysis of *Kappaphycus alvarezii* (cottonii). *Bioprocess and Biosystems Engineering*, *35*, 123–128.

Meinita, M., Kang, J. -Y., Jeong, G. -T., Koo, H., Park, S., & Hong, Y. -K. (2011). Bioethanol production from the acid hydrolysate of the carrageenophyte *Kappaphycus alvarezii* (cottonii). *Journal of Applied Phycology*, 1–6.

Miranda, J. R., Passarinho, P. C., & Gouveia, L. (2012). Pre-treatment optimization of Scenedesmus obliquus microalga for bioethanol production. *Bioresource Technology*, *104*, 342–348.

Monavari, S., Galbe, M., & Zacchi, G. (2011). Influence of impregnation with lactic acid on sugar yields from steam pretreatment of sugarcane bagasse and spruce, for bioethanol production. *Biomass and Bioenergy*, *35*, 3115–3122.

Morales, G., van Grieken, R., Martín, A., & Martínez, F. (2010). Sulfonated polystyrene-modified mesoporous organosilicas for acid-catalyzed processes. *Chemical Engineering Journal*, *161*, 388–396.

Mussatto, S. I., Carneiro, L. M., Silva, J. P. A., Roberto, I. C., & Teixeira, J. A. (2011). A study on chemical constituents and sugars extraction from spent coffee grounds. *Carbohydrate Polymers*, *83*, 368–374.

Nasef, M. M., Saidi, H., & Senna, M. M. (2005). Hydrolysis of sucrose by radiation grafted sulfonic acid membranes. *Chemical Engineering Journal*, *108*, 13–17.

Nigam, P. S., & Singh, A. (2011). Production of liquid biofuels from renewable resources. *Progress in Energy and Combustion Science*, *37*, 52–68.

Nilvebrant, N.-O., Reimann, A., Larsson, S., & Jönsson, L. (2001). Detoxification of lignocellulose hydrolysates with ion-exchange resins. *Applied Biochemistry and Biotechnology*, *91-93*, 35–49.

Nurgel, C., Erten, H., Canbaş, A., Cabaroğlu, T., & Selli, S. (2002). Influence of *Saccharomyces cerevisiae* strains on fermentation and flavor compounds of white wines made from cv. Emir grown in Central Anatolia, Turkey. *Journal of Industrial Microbiology Biotechnology*, *29*, 28–33.

Park, J.-H., Hong, J.-Y., Jang, H. C., Oh, S. G., Kim, S.-H., Yoon, J.-J., & Kim, Y. J. (2011). Use of Gelidium amansii as a promising resource for bioethanol: A practical approach for continuous dilute-acid hydrolysis and fermentation. *Bioresource Technology*, *108*, 83–88.

Rinaldi, R., Meine, N., vom Stein, J., Palkovits, R., & Schüth, F. (2010). Which controls the depolymerization of cellulose in ionic liquids: The solid acid catalyst or cellulose? *ChemSusChem*, *3*, 266–276.

Rinaldi, R., Palkovits, R., & Schüth, F. (2008). Depolymerization of cellulose using solid catalysts in ionic liquids. *Angewandte Chemie International Edition*, *47*, 8047–8050.

Ross, A. B., Jones, J. M., Kubacki, M. L., & Bridgeman, T. (2008). Classification of macroalgae as fuel and its thermochemical behaviour. *Bioresource Technology*, *99*, 6494–6504.

Schmidt, J., Leduc, S., Dotzauer, E., Kindermann, G., & Schmid, E. (2010). Cost-effective CO_2 emission reduction through heat, power and biofuel production from woody biomass: A spatially explicit comparison of conversion technologies. *Applied Energy*, *87*, 2128–2141.

Seguin, A., Marinkovic, S., & Estrine, B. (2012). New pretreatment of wheat straw and bran in hexadecanol for the combined production of emulsifying base, glucose and lignin material. *Carbohydrate Polymers*, *88*, 657–662.

Sluiter, B.H.A., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2008). Determination of structural carbohydrates and ligin in biomass. NREL/TP-510-42618.

Vigier, K., & Jérôme, F. (2010). Heterogeneously-catalyzed conversion of carbohydrates. In A. P. Rauter, P Vogel, & Y Queneau (Eds.), *Carbohydrates in sustainable development II* (pp. 63–92). Berlin/Heidelberg: Springer.

Zakzeski, J., Bruijnincx, P. C. A., Jongerius, A. L., & Weckhuysen, B. M. (2010). The catalytic valorization of lignin for the production of renewable chemicals. *Chemical Reviews*, *110*, 3552–3599.