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Catalytic efficiency of sulfuric and hydrochloric acids for the hydrolysis of *Gelidium latifolium* (Gelidiales, Rhodophyta) in bioethanol production

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Abstract

Gelidium latifolium was selected as a potential resource for bioethanol production among 28 tropical red seaweed species candidates due to its high carbohydrate content. This report shows a catalytic efficiency comparison between sulfuric (H_2SO_4) and hydrochloric acid (HCl) as feasible catalysts, which are used for the hydrolysis of *G. latifolium*. H_2SO_4 showed better hydrolysis compared to HCl based on sugar production, catalytic efficiency, and ethanol production. These results are important for future applications of bioethanol production on an industrial scale.

Keywords: *Seaweed, Hydrolysis, Bioethanol, Catalytic, Efficiency*

1. Introduction

Many countries are exploring bioethanol to overcome central issues such as economic growth, poverty, and pollution. Recently, these countries have produced fuel ethanol from land plants including sugarcane, corn, and other land-plant biomass. Some problems arise when we use land plants because the majority are used as food sources. Thus, red seaweed could be a good alternative as a sustainable resource to address the world dependency on land-plant resources for bioethanol production. Since seaweeds have a high polysaccharide content, they are applicable as an alternative resource to produce bioethanol. Some studies reported that the tropical red seaweed *Kappaphycus alvarezii* is a potential candidate for the production of bioethanol because of its high sugar content [1,2]. At this time, the majority of studies on seaweed bioethanol have been performed using farmed seaweed, and few studies have explored natural seaweed potential. However, the utilization of farmed seaweed for bioethanol production will create other problems since they have been utilized as a polysaccharide source for food, pharmaceuticals, and other industries. On an industrial scale, identifying novel seaweed candidates from the wild is critical. Of 28 tropical red seaweed species, the tropical seaweed species *Gelidium latifolium* was selected as a potential candidate due to its high carbohydrate content. Members of the red algal family Gelidiaceae are sources of high-quality agar distributed from intertidal to subtidal habitats as well as in most oceans, including tropical and temperate waters. At this time, the majority of Gelidiaceae are harvested extensively from natural populations. Most resources of temperate *Gelidium* have been obtained from Spain, Portugal, North Africa, and Korea, with the Spanish harvest accounting for 20% of the world total *Gelidium* harvest [3,4]. In tropical areas, *Gelidium*

species are distributed mostly in Indonesia, Mexico, and Vietnam [4]. Indonesia is a potential tropical area that produces *Gelidium* where species are distributed from natural beds on the south coast of Java and Sumatra, and many of the islands of Indonesia that lie between Java and Timor.

Hydrolysis is an important step in the degradation of complex carbohydrates and conversion to simple monomers for bioethanol production. Currently, chemical and enzymatic methods are commonly used for hydrolyzing cellulosic materials in land-plant biomass, which can also be applied in seaweed biomass. When costs are considered, chemical hydrolysis using acid as a catalyst is favorable due to the high costs of enzyme production [5]; dilute acid hydrolysis is a chemical method that has been developed for the pretreatment of lignocellulosic wood and land-plant materials [6–8]. This process has several important advantages including a rapid reaction rate, simple pretreatment, and use of a cheap and easily available acid catalyst [9]. However, weaknesses of acid hydrolysis, such as the possible by-product compounds released, may lower the production of fermentable sugars and inhibit yeast growth [10,11]. Some inhibitors may be present in the sample before hydrolysis, but the majority of inhibitors are formed during the hydrolysis process. Generally, heavier hydrolysis reaction conditions generate more by-product and less fermentable sugar. Hence, the dilute acid hydrolysis reaction should be designed toward increasing the concentration of fermentable sugars and decreasing inhibitors to maintain the concentration of sugar at high levels and inhibitors at a low level. Therefore, in this study, we examined the catalytic efficiency of sulfuric (H_2SO_4) and hydrochloric acid (HCl) as catalysts in the hydrolysis of *G. latifolium*. This study also investigated critical parameters of hydrolysis such as the substrate concentration, type of acid, acid concentration, and hydrolysis time to achieve high fermentable sugar and low by-product formation, which will enhance ethanol production efficiency from *G. latifolium*.

2. Materials and methods

2.1. Study area

The southern part of the Java coastline was selected as the study area due to its high seaweed diversity. Three sampling areas along the southern part of the Java coastline (Sayang Heulang, Menganti, and Kondang Merak beaches) were selected to represent the tropical seaweed diversity in southern parts of West, Central, and East Java.

2.2. Sampling

Samples of seaweed were collected during low water spring tides. Seaweeds were sampled randomly in triplicate with a square metallic frame of 0.25 m^2 within a permanent quadrat [12]. Sampled plots were marked to avoid subsequent resampling.

2.3. Preparation of seaweed samples

Once collected, the seaweed was washed with distilled water to remove dirt, salt, epiphytes, and impurities, then weighed as wet weight. The seaweed was identified and dried under shade drying for 3 days, and dried seaweed was ground into powder using a mortar for further study.

2.4. Acid hydrolysis

Acid hydrolysis was performed to break down carbohydrates into mono-sugars. HCl and H_2SO_4 were used as catalysts in acid hydrolysis. To further characterize acid hydrolysis, four parameters were selected: substrate concentration (0–15%), acid concentrations (0.1–1.0 M), hydrolysis time (10–90 min), and hydrolysis temperature (0–140°C). Dried seaweed powder (5 g) with 50 mL of different concentrations of HCl and H_2SO_4 in a 250-mL flask was hydrolyzed in an autoclave at different temperature condition and different times. After hydrolysis, the residue was separated from the solution by centrifugation at 3000 rpm for 25 min. The upper yellowish liquid (hydrolysate) was then isolated and analyzed for sugar and by-product content.

2.5. Fermentation of hydrolysates

Prior to fermentation, the hydrolysate sample was neutralized at pH 5 using 10 N NaOH. The ethanol fermentation process used the *Saccharomyces cerevisiae* ATCC 20062 to produce ethanol. Based on our previous study, it was the best yeast and able to ferment red seaweed hydrolysates [1,10,11]. The strains were maintained in yeast medium containing 10 g yeast extract, 6.4 g urea, and 20 g glucose per liter. The basal medium was adjusted to pH 5 with NaOH and HCl [13]. The fermentation broth consisted of the hydrolysate, basal medium, and buffer solution at a ratio of 1:1:1 [1]. The fermentation was conducted with 3 mL of broth in an 8-mL bottle for 72 h in a shaking incubator at 130 rpm and 30°C with gentle shaking. Samples for measuring sugar and ethanol concentrations were obtained at specific times during fermentation, which were then analyzed using high-performance liquid chromatography (HPLC) and gas chromatography (GC).

2.6. Analysis of sugars, by-products, and ethanol

The total carbohydrate content of dried seaweed samples was determined using the phenol–H_2SO_4 method [14]. The moisture content was measured by weight after drying 3 g of seaweed samples at 105ºC for 18 h until they reached a stable weight. The content of reducing sugar was analyzed using the dinitrosalicylic acid (DNS) method [15]. The monosaccharide content (galactose) and by-products [5-hydroxymethylfurfural (5-HMF) and levulinic acid] were measured using HPLC with an IOA 1000 column organic acid (7.8 mm × 300 cm, Alltech, Portland, ME, USA) equipped with a refractive index detector and maintained at 60ºC. The mobile phase was 0.005 N H_2SO_4 at a flow rate of 0.3 mL min^{-1}. The ethanol content was measured using GC (6890N, Agilent Technologies, Santa Clara, CA, USA) with a 2B-WAX column (Agilent Technologies). The injection volume was 2 µL with an inlet split ratio of 30:1. The initial and maximum oven temperatures were 35ºC and 200ºC, respectively.

2.7. Catalytic efficiency

The acid catalyst efficiency according to [16] was calculated using the equation below:

$$E = \frac{X}{\sum I}$$

where X is the main product wanted (galactose and glucose) and ∑I is the sum of the concentration of by-product compounds (5-HMF and levulinic acid).

3. Results and discussion

G. latifolium, an agarophyte, possessed the highest amount of carbohydrate among the 28 tropical red seaweed species tested (Table 1). It also showed the highest carbohydrate content (59.87 ± 0.34%, w/w). The carbohydrate fraction can be depolymerized into sugars, which act as a primary carbon source for microbial biocatalysts and ethanol production. Total carbohydrate

content describes the overall carbohydrate content in the form of monosaccharides, oligosaccharides, and polysaccharides. *G. latifolium* consists primarily of cellulose and agar, whose basic monomer is galactose and glucose; agar is the major polysaccharide present in *G. latifolium*. In general, this polysaccharide form is most commonly found in seaweed species of the orders Gracilariales and Gelidiales. The agar content in *Gelidium s*pecies is more stable compared to other types of red seaweed, and the gel strength generatedby *Gelidium* is higher than that of other types of red seaweed [17]. Chemically, agar is composed of α(1-4)-3,6-anhydro-L-galactose and β(1-3)-D-galactose with esterified sulfate [18–20]. The 3,6-anhydro bridges are known to be acid-labile; i.e., they are very prone to be decomposed into 5-HMF and subsequently into organic acids such as levulinic acid and formic acid, which act as inhibitors during the fermentation process [21]. In this hydrolysis study, we compared two different acid catalysts (HCl and H_2SO_4) during the hydrolysis of *G. latifolium* and identified the optimal hydrolysis conditions for maximal sugar production and low by-product formation.

3.1. The effect of substrate concentration on sugars and by-product production

The effect of substrate concentration on the acid hydrolysis of *G. latifolium* was investigated at different substrate concentrations (1–15 g 100 mL^{-1}) using 0.2 M H_2SO_4 or HCl at 130°C for 15 min. Fig. 1 shows the effect of different substrate concentrations on the formation of galactose (Fig. 1a), glucose (Fig. 1b), 5-HMF (Fig. 1c), and levulinic acid (Fig. 1d). A different pattern at the optimal *G. latifolium* concentration was observed for H_2SO_4 and HCl hydrolysis. During H_2SO_4 hydrolysis, the highest galactose yield (34.43 ± 3.31 g L^{-1}) was obtained at a *G. latifolium* concentration of 12% with an H_2SO_4 concentration, temperature, and reaction time of 0.2 M, 130°C, and 15 min, respectively. Furthermore, the highest glucose formation was observed under the same substrate concentration (2.40 ± 0.48 g L^{-1}). The maximum concentration of 5-HMF (5.7 ± 0.48 g L^{-1}) and levulinic acid (2.56 ± 0.08 g L^{-1}) coincided with the highest galactose and glucose production. A further increase in galactose and glucose resulted in an increase in 5-HMF and levulinic acid formation, which peaked at a substrate concentration of 12%. On the other hand, during HCl hydrolysis, the highest formation of galactose (15.20 ± 0.80 g L^{-1}), glucose (1.51 ± 0.01 g L^{-1}), 5-HMF (2.36 ± 0.12 g L^{-1}), and

levulinic acid (1.94 ± 0.34 g L^{-1}) occurred under the following conditions: 10% *G. latifolium*, 0.8–0.2 HCl, 130°C, and 15 min. Based on our results, the maximum galactose and glucose yields obtained during HCl hydrolysis were lower than that observed for H_2SO_4 hydrolysis (2.18 ± 0.34 g L^{-1}). The decreased yield of mono-sugars from both H_2SO_4 and HCl hydrolysis coincided with the higher concentration (>15% *G. latifolium* for H_2SO_4 hydrolysis and >10% *G. latifolium* for HCl hydrolysis), which indicates that the acid could not properly hydrolyze the substrate at high substrate concentrations.

3.2. The effect of the acid concentration on sugars and by-product production

Fig. 2 shows the effects of acid concentration on the formation of sugars and by-products using 5 g substrate/100 mL and 0.2 M H_2SO_4 or HCl at 130°C. Different acids concentrations (0–1 M) were applied during H_2SO_4- and HCl-catalyzed hydrolysis using 5% *G. latifolium* at 130°C for 15 min. Both hydrolysis reactions showed interesting patterns. During H_2SO_4 hydrolysis, an increase in acid concentration up to 0.2 M resulted in a sharp increase in galactose and glucose formation. During HCl hydrolysis, an increase in acid concentration up to 0.4 M led to a sharp increase in glucose formation. Galactose increased steadily when the HCl concentration reached 0.8 M. During 0.2 M H_2SO_4 hydrolysis, the galactose and glucose concentrations were 10.56 ± 0.01 g L^{-1} and 0.64 ± 0.07 g L^{-1}, respectively. However, during HCl hydrolysis, galactose concentrations during 0.8 M HCl hydrolysis reached 7.77 ± 0.24 g L^{-1} and hydrolysis at a glucose concentration of 0.4 M HCl attained 0.46 ± 0.01 g L^{-1}. The sugar content gradually decreased when the acid concentration was higher than the optimal concentration. During H_2SO_4 and HCl hydrolysis, a higher acid concentration (>0.2 M) decreased 5-HMF formation. In addition, no 5-HMF was observed at high acid concentrations, i.e., concentrations higher than 0.4 M for H_2SO_4 hydrolysis and 1 M for HCl hydrolysis. These results may have been due to the formation of levulinic acid from 5-HMF, which is converted from mono-sugars. However, the highest 5-HMF concentration during H_2SO_4 hydrolysis (3.49 ± 0.06 g L^{-1}) was higher than the highest 5-HMF concentration during HCl hydrolysis (0.82 3 ± 0.06 g L^{-1}). The levulinic acid content increased with an increased concentration of both H_2SO_4 and HCl. The highest concentrations of levulinic acid during H_2SO_4 and HCl hydrolysis were 6.78 ± 0.13 g L^{-1} and

2.39 ± 0.12 g L^{-1}, respectively. The major repeating unit of agar content in *G. latifolium* is agarobiose, a disaccharide composed of 1,3-linked-D-galactose and 1,4-linked 3,6-anhydrous-L-galactose. As the 3,6-anhydro bridges are acid-labile, the 3,6-anhydrogalactose residues are converted into either galactose residues or degradation products such as 5-hydroxy-methylfurfural under these conditions. Sugars can be degraded to furfural (which is formed from pentoses) and 5-HMF (which is formed from hexoses). Compound 5-HMF can be further degraded, forming levulinic acid.

3.3. The effect of hydrolysis time on sugars and by-product production

The effect of hydrolysis time on the formation of sugar and by-products using 5 g substrate/100 mL and 0.2 M H_2SO_4 or HCl at 130°C is shown in Fig. 3a–d, respectively. The maximum formation of galactose and glucose occurred at a hydrolysis reaction time of 15 min for both H_2SO_4 (0.63 ± 0.02 g glucose L^{-1} and 10.23 ± 0.37 g galactose L^{-1}, respectively) and HCl (0.37 ± 0.03 g glucose L^{-1} and 3.8 ± 0.25 g galactose L^{-1}, respectively). As shown in Fig. 3c, the concentration of 5-HMF, which is a degraded product of sugar, peaked at 15 min for both H_2SO_4 hydrolysis (3.59 ± 0.03 g 5-HMF L^{-1}) and HCl hydrolysis (0.80 ± 0.11 g 5-HMF L^{-1}). During H_2SO_4 hydrolysis, the formation of 5-HMF at 15 min (3.59 ± 0.03 g 5-HMF L^{-1}) was significantly lower than that at 30 min (1.50 ± 0.11 g 5-HMF L^{-1}). The formation of levulinic acid at 15 min (0.88 ± 0.08 g levulinic acid L^{-1}) was significantly lower than that for 90 min (0.31 ± 0.05 g levulinic acid L^{-1}), which suggests that hydrolysis for 5 min using 0.2 M H_2SO_4 was not sufficient to degrade the sugars into 5-HMF and levulinic acid. A longer hydrolysis time resulted in an increased degradation of sugars. The maximum sugars and by-products produced during H_2SO_4 hydrolysis were higher than that observed for HCl hydrolysis.

3.4. Effect of hydrolysis temperature on acid hydrolysis

The effect of the hydrolysis temperature on acid hydrolysis using 5 g substrate (100 m L^{-1}) and 0.2 M H_2SO_4 or HCl for 15 min is shown in Fig. 4. An increase in sugar and by-product formation was observed with an increase in hydrolysis temperature (0–130°C). The optimum

hydrolysis temperature (130°C) during both H_2SO_4 and HCl hydrolysis resulted in the maximum formation of sugars and by-products.The maximum concentrations of galactose, glucose, 5-HMF, and levulinic acid during H_2SO_4 hydrolysis were 10.47 ± 0.38 g L^{-1}, 0.61 ± 0.03 g L^{-1}, 3.45 ± 0.03 g L^{-1} and 1.52 ± 0.07 g L^{-1}, respectively. For HCl hydrolysis, the maximum concentrations of galactose, glucose, 5-HMF, levulinic acid, and reducing sugar were 3.79 ± 0.37 g L^{-1}, 0.38 ± 0.02 g L^{-1}, 0.81 ± 0.07 g L^{-1}, and 1.26 ± 0.05 g L^{-1}, respectively. Thus, a higher temperature results in improved polysaccharide degradation relative to low temperatures. However the higher temperature (>130^{0}C) results in decrease of galactose, glucose and 5-HMF. This may be due to the degradation of galactose to other by-product compounds. The main by-products compounds of sugar degradation are 5-HMF and levulinic acid; 5-HMF is a by-product of hexoses such as glucose and fructose [26].

3.5. Comparison of catalyst efficiencies

The catalyst efficiency parameter (E) can be used to determine the efficiency and effect of acid catalysts used during hydrolysis processes. H_2SO_4-based catalysis resulted in a higher catalyst efficiency (4.2 by galactose and 4.5 by glucose and galactose) than that by HCl (3.5 by galactose and 3.9 by glucose and galactose), suggesting that H_2SO_4 was more efficient at hydrolyzing *G. latifolium* (Table 2). Table 2 also shows a comparison of the catalyst efficiency parameter (E) on *G. latifolium* and our previous study on *K. alvarezii*. As limited catalyst efficiency studies are available on agarophytes, we compared the catalyst efficiency of *G. latifolium* with carrageenophytes and land plants. Based on the catalyst efficiency parameter (E), acid hydrolysis of *K. alvarezii* is less efficient compared to *G. latifolium*. Thus, the catalyst efficiency parameter (E) depends on the seaweed material, and acids may show different catalytic efficiency performances on different raw materials.

3.6. Ethanol production

In this study, we measured the time course of the main ethanol fermentation from the *G. latifolium* hydrolysate resulting under the optimal H_2SO_4 and HCl hydrolysis conditions. As

shown in Fig. 5, the hydrolysates resulting from H_2SO_4 and HCl hydrolysis showed different performances for ethanol production. The maximum final ethanol produced from the optimum H_2SO_4 hydrolysate of *G. latifolium* was 1.38 g L^{-1} in 24 h. Alternatively, the fermentation of the HCl hydrolysate of *G. latifolium* containing optimal galactose yielded 0.59 g L^{-1} of ethanol in 18 h. In addition, contamination with undesired fermentation inhibitors such as 5-HMF and levulinic acid must be minimized. However, the ethanol production obtained in this study was comparatively higher than those reported in earlier studies on agarophytes for bioethanol production [1,22]. As the fermentations reported in the present study have not been optimized, subsequent fermentations will further increase the ethanol yield by improving the efficiency of the fermentation. Due to structural differences between seaweeds and land plants, seaweeds are capable of producing high yields of material when compared to even the most productive land-based plants. Seaweeds can be compared with the other important bioethanol feedstocks. Theoretically, 1 kg of galactose yields 0.511 kg of ethanol and the fermentation efficiency is assumed to be 0.90. Thus, the ethanol yield from seaweeds can be estimated. Detailed information is provided in Table 2.

We observed that less ethanol was produced when HCl was used as a catalyst. Three possibilities exist to explain this observation: (a) The reduced degradation of monosaccharides in HCl must primarily be due to the weaker nature of this acid compared to H_2SO_4. Generally, the degradation of monosaccharides in acid proceeds from protonation of the carbonyl group. Some acid-catalyzed reactions can proceed with HCl, but others will require a stronger conjugate base to abstract a proton and regenerate the catalyst. Since HCl is weaker than H_2SO_4, the degradation of each monosaccharide was much slower in HCl than in H_2SO. (b) During acid hydrolysis of polysaccharides, the acid catalyzed not only the hydrolysis of polysaccharides into monosaccharides, but also the further degradation reaction of monosaccharides into HMF and levulinic acid. According to [26], such compounds include 5-HMF and levulinic acid, which are generated during acid hydrolysis at high temperatures and may negatively impact cell growth and ethanol production. (c) The final sample may contain by-products, which can result from fermentation processes. Based on the formula $C_2H_5OH + HCl \rightarrow C_2H_5Cl + H_2O$, HCl may produce ethyl chloride or chloroethane.

4. Conclusions

Our results show that red seaweeds selected from the tropical region (namely *G. latifolium*) is a good source for bioethanol production. Utilization of tropical red seaweed as a potential source for bioethanol production has many advantages due to its availability, which does not rely on the season. Until now, the agarophyte *G. latifolium* has not been cultivated. Domestication of these species is required since no cultivation techniques are available, and the development of seaweed culture will be the next challenge to compensate for the lack of seaweed biomass. Our finding on hydrolysis optimization revealed that the optimal hydrolysis condition for *G. latifolium* was using H_2SO_4 with a substrate concentration, acid concentration, reaction time, and reaction temperature of 12%, 0.2 M, 15 min, and 130ºC, respectively. H_2SO_4 was a superior hydrolysis method compared to HCl hydrolysis based on sugar production (34.43 ± 3.31 g galactose L^{-1} and 2.40 ± 0.02 g galactose L^{-1}), catalytic efficiency (4.5 g g^{-1} galactose and 4.2 g g^{-1} glucose), and ethanol production (1.38 g L^{-1}). The best hydrolysis method of *G. latifolium* produces a high-quality hydrolysate that ensures its fermentability to produce ethanol. Further studies on fermentation and microorganism selection are required to increase our understanding about the potential of bioethanol production from the tropical red seaweed *G. latifolium*.

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Table 1. Proximate chemical composition of the different seaweeds. Carbohydrates, lipids, and ash are presented relative to the total dry weight. Data represent the mean values of triplicate determinations ± standard deviation.

No.	Species	Carbohydrate % (w/w)	Lipid % (w/w)	Ash % (w/w)
Sayang Heulang Beach				
1	*Acantophora muscoides*	48.28 ± 0.04	0.36 ± 0.14	13.00 ± 0.45
2	*Gracilaria foliferas*	41.39 ± 0.23	0.35 ± 0.05	13.77 ± 0.12
3	*Gracilaria verrucosa*	51.46 ± 0.09	0.85 ± 0.03	11.73 ± 0.42
4	*Gracilaria salicornia*	45.21 ± 0.17	0.86 ± 0.03	11.87 ± 1.93
5	*Gelidium latifolium*	59.87 ± 0.34	1.40 ± 0.16	10.47 ± 0.62
6	*Anotrichium tenue*	40.02 ± 0.19	1.47 ± 0.12	14.40 ± 2.33
Menganti Beach				
1	*Gelidium amansii*	40.73 ± 0.09	0.19 ± 0.07	22.57 ± 4.17
2	*Gracilaria textoriii*	37.86 ± 0.19	0.35 ± 0.00	23.17 ± 1.92
3	*Gracilaria debilis*	42.73 ± 0.43	0.19 ± 0.03	24.13 ± 1.05
4	*Rhodymenia sonderi*	47.39 ± 0.18	0.45 ± 0.09	20.50 ± 2.38
5	*Gracilaria verrucosa*	33.07 ± 0.30	0.57 ± 0.08	15.87 ± 2.15
6	*Grateloupia indica*	28.94 ± 0.41	0.68 ± 0.12	21.57 ± 2.84
7	*Laurencia elata*	* ± *	* ± *	* ± *
8	*Crytonemia undulata sonder*	35.54 ± 0.59	1.12 ± 0.27	17.17 ± 2.55
9	*Gracilaria corticata*	39.24 ± 0.21	0.53 ± 0.11	15.70 ± 2.65
Kondang Merak Beach				
1	*Rhodymenia sonderi*	41.63 ± 0.16	0.54 ± 0.24	24.03 ± 2.68
2	*Gracilaria debilis*	23.80 ± 1.56	0.27 ± 0.02	26.43 ± 3.18
3	*Gracilaria corticata*	23.22 ± 0.47	0.55 ± 0.09	35.93 ± 3.47
4	*Gelidiela acerosa*	36.98 ± 0.31	0.26 ± 0.05	21.07 ± 3.30
5	*Gracilaria salicornia*	26.97 ± 1.34	0.46 ± 0.09	10.47 ± 1.60
6	*Amphiroa sp.*	43.28 ± 0.40	0.56 ± 0.09	12.33 ± 0.94
7	*Gelidium latifolium*	31.07 ± 0.30	0.52 ± 0.15	11.80 ± 0.51
8	*Hypnea asperi*	* ± *	* ± *	* ± *
9	*Botryocladia leptopoda*	32.99 ± 0.31	0.70 ± 0.10	10.57 ± 0.71
10	*Acanthopora muscoides*	33.88 ± 0.32	0.56 ± 0.25	20.40 ± 2.93
11	*Meristotheca populosa*	26.03 ± 0.24	0.16 ± 0.05	18.33 ± 6.07
12	*Scinaia hatei*	22.36 ± 2.02	0.15 ± 0.06	9.63 ± 1.43
13	*Tolypiocladia glamerulata*	* ± *	* ± *	* ± *

*Not available

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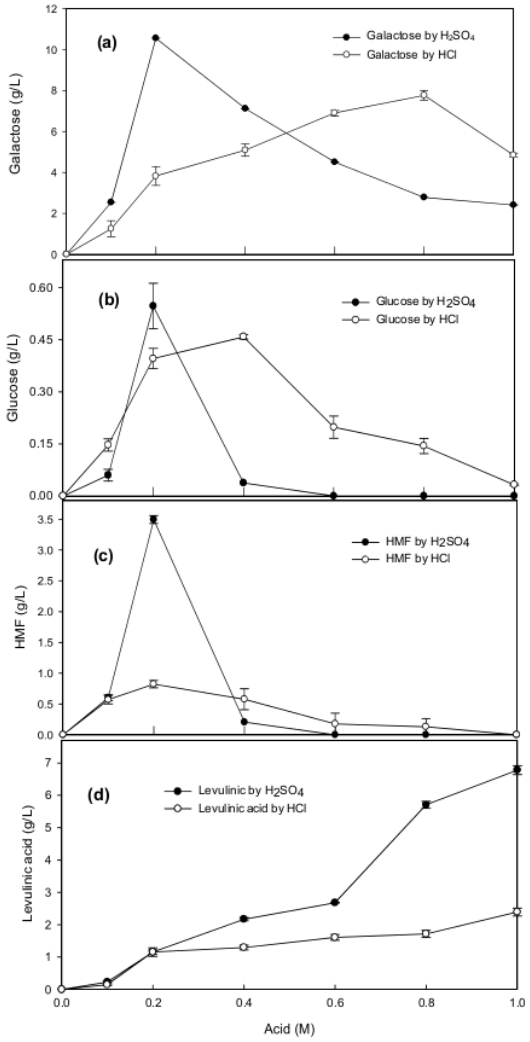

Fig. 2. Effect of acid concentration on sugars and by-products during acid hydrolysis. (a) Glucose, (b) galactose, (c) 5-HMF, and (d) levulinic acid. The hydrolysis was performed by diluting 5% of *G. latifolium* hydrolysate samples in 100 mL of different concentrations (0–1M) of H_2SO_4 and HCl at 130°C for 15 min. All values were calculated against the dry weight of tissues after removing the moisture content. Values represent the mean ± SD ($n \geq 3$) and error bars are standard deviations.

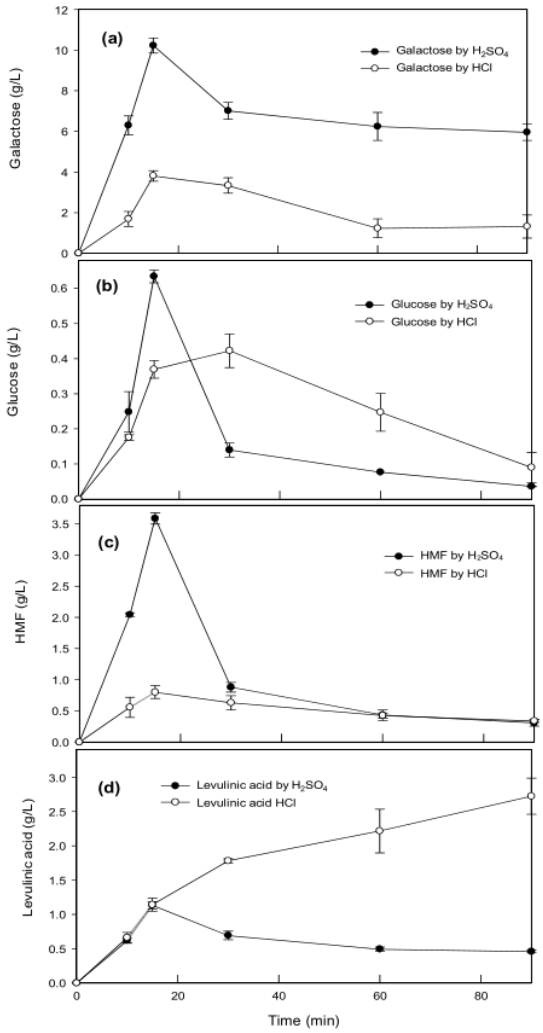

Fig. 3. Effect of hydrolysis time on sugars and by-products during acid hydrolysis. (a) Glucose, (b) galactose, (c) 5-HMF, and (d) levulinic acid. Hydrolysis was performed by diluting 5% *G. latifolium* hydrolysate samples in 100 mL of 0.2 M H_2SO_4 and HCl at 130°C for different hydrolysis times (0–90 min). All values were calculated against the dry weight of tissues after removing the moisture content. Values represent the mean ± SD ($n \geq 3$) and error bars are standard deviations.

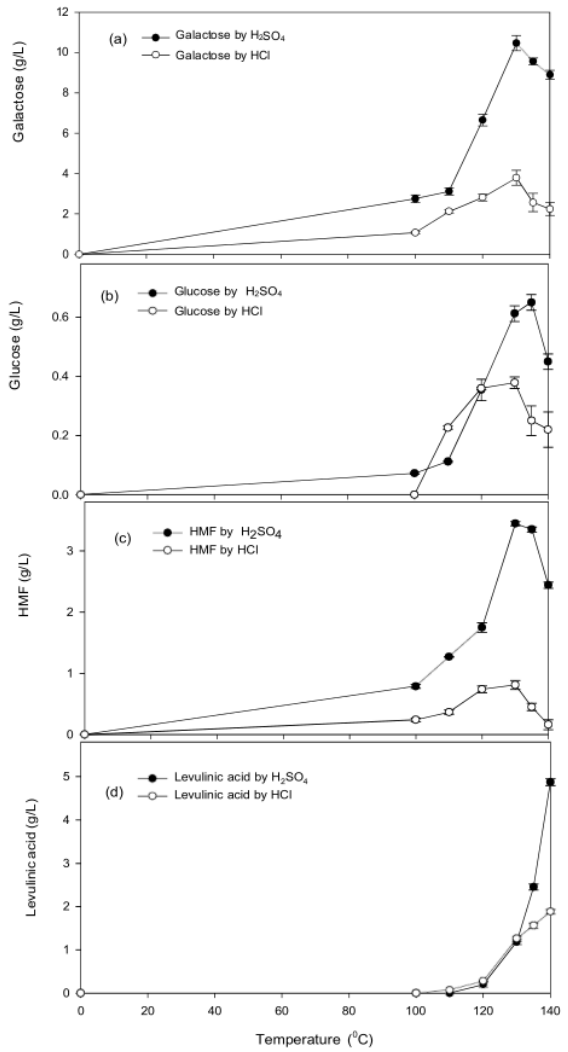

Fig. 4. Effects of temperature on sugars and by-products in acid hydrolysis. (a) Glucose, (b) galactose, (c) 5-HMF, and (d) levulinic acid. The hydrolysis was performed by diluting 5% *G. latifolium* hydrolysate samples in 100 mL of 0.2 M H_2SO_4 and HCl at different temperatures (0–140°C) for 15 min. All values were calculated against the dry weight of tissues after removing the moisture content. Values represent the mean ± SD ($n \geq 3$) and error bars are standard deviations

Table 2 Comparison of catalytic efficiency (E) of the acid catalyst during the hydrolysis of seaweed material.

Raw material	Acid catalyst	Optimum hydrolysis condition	Catalytic efficiency (E)	EtOH * (g/L)	Reference
Seaweed					
Gelidium latifolium	H_2SO_4	0.2 M H_2SO_4 130°C 15 min	4.5	18.20	This study
Gelidium latifolium	HCl	0.2 M HCl 130°C 15 min	3.9	8.58	This study
Kappaphycus alvarezii	H_2SO_4	0.2 M H_2SO_4 130°C 15 min	4.3	11.85	[11]
Kappaphycus alvarezii	HCl	0.2 M HCl 130°C 15 min	2.5	6.60	[11]
Land-plant					
Sorghum straw	H_2SO_4	6% H_2SO_4 100°C 60 min	2.07	3.11	[23]
Sorghum straw	HCl	6% HCl at 122°C for 70 min	2.7	1.75	[16]
Sugar cane bagasse	H_2SO_4	2% H_2SO_4 122°C 24 min	3.02	1.38	[24]
Sugar cane bagasse	HCl	2% HCl 128 °C 51 min	2.54	1.73	[25]

*EtOH was theoretically estimated by calculating galactose and glucose content in the sample. Fermentation efficiency was assumed 90%

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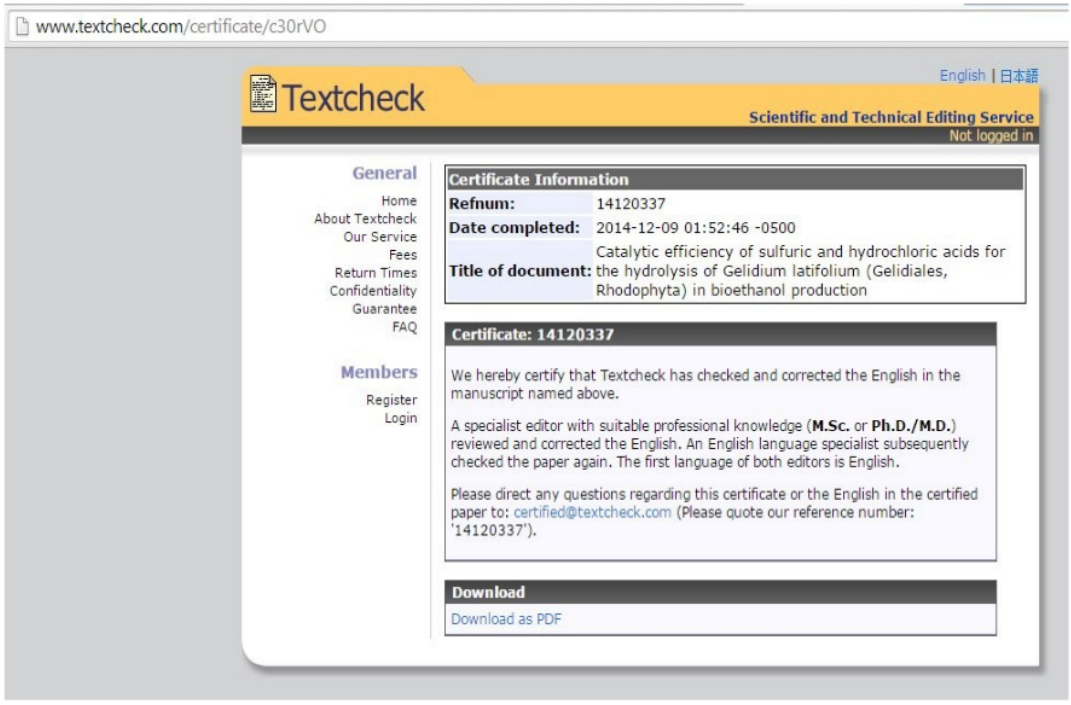

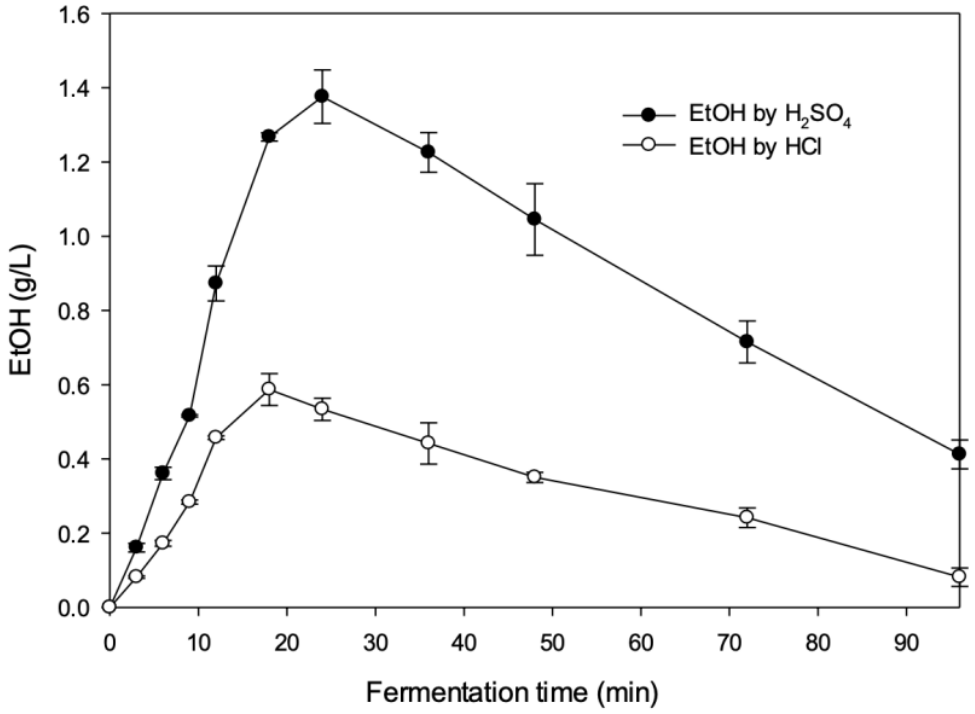

Fig. 5. Ethanol concentration during the fermentation of *G. latifolium* hydrolysate following the optimal condition for HCl and H_2SO_4 hydrolysis. Fermentation was performed in a 135- mL flask at 30°C and 120 rpm for 96 h. All samples were taken aseptically at different times. Values represent the mean ± SD ($n \geq 3$) and error bars are standard deviations.

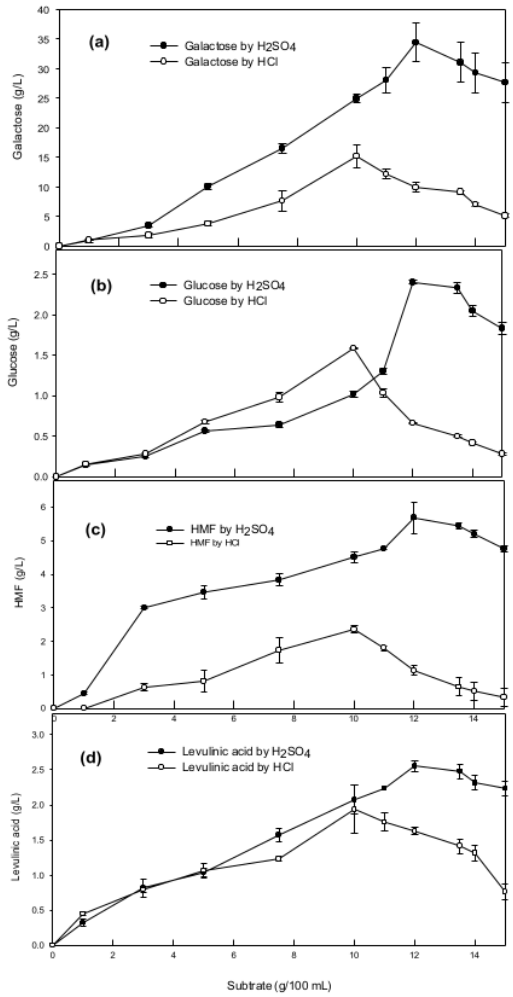

Fig. 1. Effect of substrate concentration on sugars and by-products during acid hydrolysis.
(a) Glucose, (b) galactose, (c) 5-HMF, and (d) levulinic acid. Hydrolysis was performed bydiluting different concentrations (0–15%) of *G. latifolium* hydrolysate samples in 100 mL of 0.2 M H_2SO_4 and 0.2 M HCl at 130°C for 15 min. All values were calculated against thedry weight of tissues after removing the moisture content. Values represent the mean ± SD ($n \geq 3$) and error bars are standard deviations

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