Acidity Tunable Ionic Liquids as Catalysts for Conversion of Agar into Mixed Sugars

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As oil prices spike to a new level, the importance of alternative resources and fuels becoming more apparent and the use of bioethanol as an alternative steadily increases around the world.[1,2] Accordingly, there have been significantly increasing endeavors on the technology development that facilitate the transformation of biorenewables into transportation fuels. To meet this end, many technologies have employed sugar- and corn-based biomass for the industrial production of bioethanol, especially in Brazil and U. S., respectively. While they contributed a lot to the commercialization process, the viability of the so-called 1st generation biofuels is somewhat questionable because of their conflict with food supply. Another key factor influencing biofuel efficacy is whether native ecosystems can be maintained or not. No matter how effective biomass is for producing ethanol, its benefits quickly decrease if all the tropical forests are being razed to make energy crops, leading to another type of a large amount of greenhouse gas (GHG) emission pathway.[3] To solve this crisis, a new type of biomass should be developed and their biofuels should be produced locally in sustainable systems. From this viewpoint, seaweeds (macro algae) can be an excellent alternative raw material as a new marine biomass for biofuel production. Among them, *Gelidium amansii* is one most abundantly available red seaweed (*Rodophyta*) species along the shallow coastal area of many countries. It mainly consists of polysaccharide complexes of fiber and agar whose basic monomer is glucose and galactose residue, respectively.

Generally, there are five major bottom lines for a bioethanol process to be economically viable: the feedstock must be plentiful, inexpensive, in high energy conversion rate, in low demand for food industry, and finally, has to be cultivated in sustainable systems. Accordingly, red seaweed shows very fast growing rate (4 - 6 harvest cycles per year) with high CO_2 fixation ability which is 5 - 7 times higher than that of a land plant. Furthermore, they can be mass-cultivated using sea water and free sunlight without any need of nitrogen-based fertilizer which has been a significant source of GHG that also destroys stratospheric ozone.[4] Red seaweed shows very high carbohydrate content up to 75% (w/w) based on dried sample and does not cause food supply problems at all. In addition, they do not contain any lignin that has to be eliminated prior to hydrolysis step, which has been a major obstacle to increase production cost in lignocellulosic process. Furthermore, the red seaweed has an ability to absorb nitrogen and phosphorous thereby purifying sea water which leads to oceans' sustainability.

Recently, an effort has been made for the utilization of the fiber extracted from *Gelidium amansii*, to the making of high quality pulp & paper.[5] Therefore, the agar will be an inevitable byproduct during the process of manufacturing red algae-based paper in future. In general, lignocellulosic bioethanol process involves a pretreatment step for delignification to remove lignin contents, which results in somewhat complicated and cost-provoking unit process. In contrast, the pretreatment step for obtaining agar from red seaweed is very simple. The agar and fiber residue can be separated from the original substrate, *Gelidium amansii* with boiling water. Utilization of marine biomass strategy therefore reduces the number of required unit process dramatically, leading to cutting down the production cost significantly.

Room temperature ionic liquids have been widely applied for task-specific purposes, especially their economical and promising role as active catalysts in organic synthesis.[6] Despite many efforts that have been devoted to ionic liquids-derived chemical processes, there have been no studies on their catalytic bioconversion process using red seaweed galactan (agar) as a substrate to produce fermentable sugars. In case of agar, the 5-hydroxymethylfurfural (5-HMF) is mainly generated from the degradation of 3,6-anhydrogalactose (AG) due to its acid-labile character.[7] Using a strong inorganic acid such as H_2SO_4 in the saccharification step accelerates the formation of 5-HMF, resulting in pivotal hamper in terms of ethanol fermentation efficacy. Incorporation of ionic liquids with conventional inorganic acid systems may enable to reduce acidity from moderate to weak depending on their type of cation, shedding light on minimizing the generation of 5-HMF.

In this context, various bisulfate-containing acidic ionic liquids as hydrolytic catalysts for the saccharification of agar extracted from *Gelidium amansii* have been investigated and compared with conventional sulfuric acid system from the viewpoint of sugar yields and 5-HMF formation.

Determination of chemical composition is of great importance since the yields of sugars are calculated based on the compositional analytical data. Normally, acid hydrolysis is a common procedure for determining lignocellulosic biomass, which consists of two-steps: First, hydrolysis with 72% (w/v) H_2SO_4 at 30 °C for 2 h, followed by the dilution into 4% of the reaction mixture and then second hydrolysis at 121 °C for 1 h. The same methodology was applied to the analysis of Glu and Gal, but 1% (w/v) H_2SO_4 was employed for AG at the first hydrolytic step due to its easy degradation mode. The agar ex-
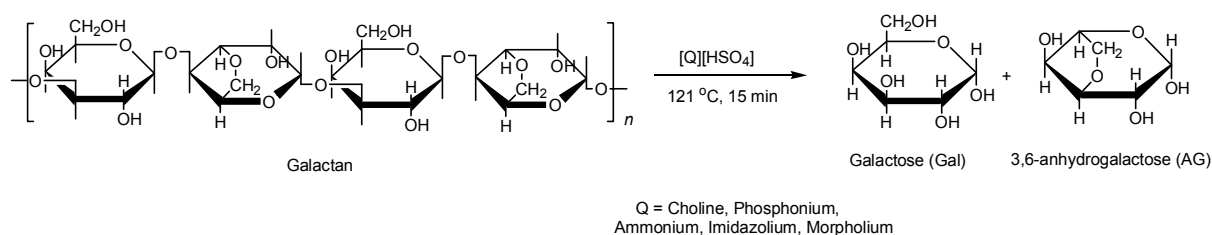
Scheme 1. Structure of galactan polymer and their hydrolyzed products

Table 1. Sugar compositional analysis data for Gelidium amansii, agar, and fiber

| Substrate	Sugar composition (%)			
	[b]Glu	[c]Gal	[d]AG	Total carbohydrate
[a]G.A.	16.6	25.6	33.0	75.2
Agar	[e]3.0	37.5	51.0	91.5
Fiber	92.8	tr.	0	93.2

[a]G.A. = *Gelidium amansii*; [b]Glu = glucose; [c]Gal = galactose; [d]AG = 3,6-anhydrogalactose; [e]Residue during agar extraction from *Gelidium amansii*.

Figure 1. Effect of sulfuric acid loading on the sugar and 5-HMF formation. Conditions: S/L ratio = 5%, Agar = 5 g, DI-H_2O = 100 mL, T = 121 ^{o}C, t = 15 min.

Figure 2. Effect of [Bmim][HSO_4] loading on the sugar and 5-HMF formation. Conditions: S/L ratio = 5%, Agar = 5 g, DI-H_2O = 100 mL, T = 121 ^{o}C, t = 15 min.

tracted from *Gelidium amansii* is generally classified with red seaweed galactan which is composed of repeating agarobiose unit of alternating 1,3-linked-D-galactose and 1,4-linked 3,6-anhydrous-L-galactose residues.[6] Once the agar is hydrolyzed, D-galactose (Gal) and 3,6-anhydrogalactose (AG) are released (Scheme 1). Based on the theoretical structure of repeating agarobiose unit of galactan, equal molar amounts of Gal and AG are expected, but experimental composition ratio of Gal to AG ranges from 0.9 to 1.2 depending on the species.[7-9] The result of the sugar compositional analysis for *Gelidium amansii*, agar, and fiber is summarized in Table 1. The total sugar content of *Gelidium amansii* is found to be 75.2% with AG being the dominant monosaccharide in the cell wall polysaccharide fraction. The total amount of sugar in agar and fiber are 91.5% and 93.2%, respectively. From these analytical results, the theoretical maximum concentration of Gal and AG were found to be 19 and 26 g/L at the S/L (solid/liquid) ratio of 5% and 38 and 51 g/L at 10% of S/L, respectively.

The effect of various sulfuric acid concentrations on the product distribution is evaluated at the S/L ratio of 5% at 121 ^{o}C for 15 min in autoclave. The results in Figure 1 show that the concentration of Gal increases very slowly with increasing amount of sulfuric acid up to 6.1 mmol, giving 18.6 g/L (98% yield), whereas the AG concentration decreases sharply due to its easy conversion into 5-HMF, resulting in concentration around 7.5 g/L at 4.1 mmol of sulfuric acid. According to a preliminary study, the 5-HMF concentration above 5 g/L plays a crucial role in inhibiting fermentation process when using red seaweed hydrolysates.[10] Therefore a different strategy has been undertaken to reduce the formation of 5-HMF. Due to the aforementioned acid-labile character of AG, an acidic ionic liquid was tested, which may provide a milder condition than sulfuric acid and to study what differences this acidic ionic liquid may impart on product distribution such as Gal, AG, and 5-HMF formation. The same hydrolysis was performed in the presence
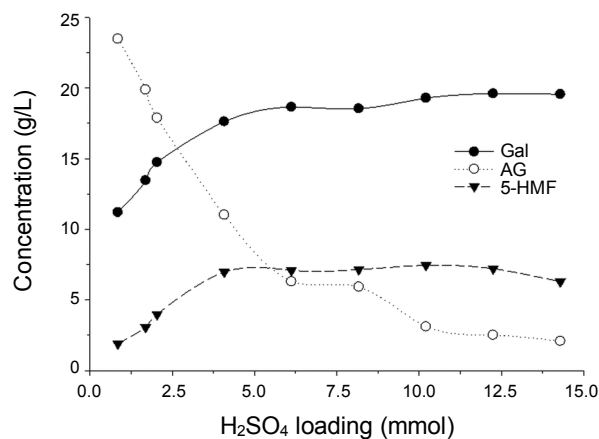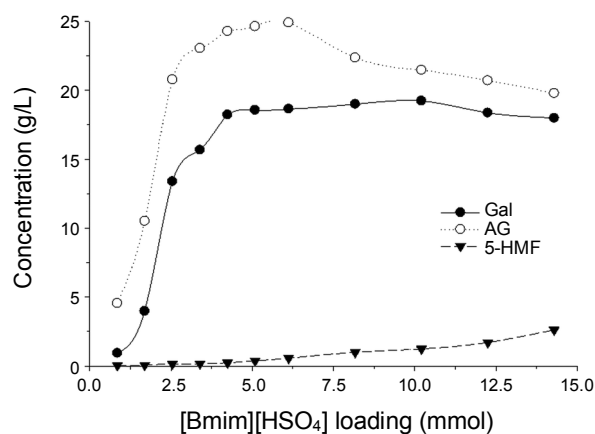
of [Bmim][HSO_4] as a model compound and the results illustrated in Figure 2 show that Gal concentration increased spontaneously with the increase in [Bmim][HSO_4] up to 4.2 mmol, reaching 18.2 g/L of Gal (96% yield). In addition, AG yields concomitantly increased with the increase in [Bmim][HSO_4] concentration, leading to 24.9 g/L (96% yield) at 6.1 mmol of catalyst loading. It is also worth noting that the formation of 5-HMF was very negligible, ranging from 0.03 to 0.37 g/L at the same amount of ionic liquid loading, suggesting 95 ~ 99% reduction effect in the formation of 5-HMF compared with H_2SO_4.

To test other ionic liquids in order to find out a more effective
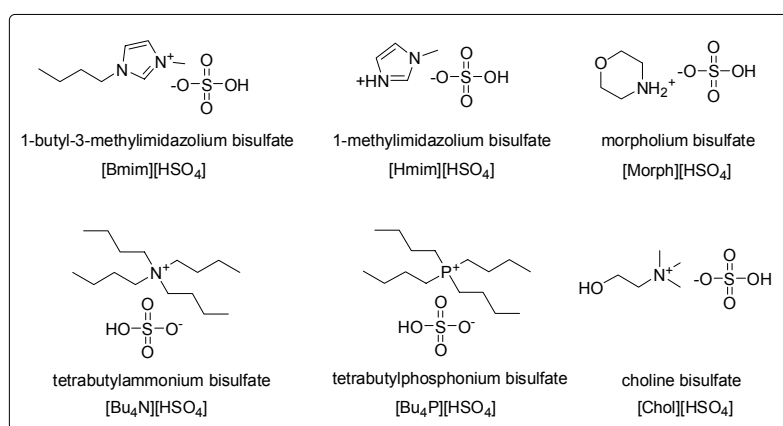
Scheme 2. Various bisulfate ionic liquids as catalysts for the agar saccharification

Table 2. Effect of various bisulfate ionic liquids on the product distribution[a]

Entry	Catalyst	Product distribution				
		Gal		AG		5-HMF
		g/L	yield (%)	g/L	yield (%)	g/L
1	H_2SO_4	35.6	93.7	35.9	70.4	7.5
2	[Bmim][HSO$_4$]	29.5	77.6	27.8	54.5	0.8
3	[Hmim][HSO$_4$]	29.3	77.1	32.3	63.3	1.0
4	[Morph][HSO$_4$]	8.6	22.6	31.1	61.0	0.5
5	[Chol][HSO$_4$]	36.6	96.3	51.1	99.9	1.3
6	[Bu$_4$P][HSO$_4$]	35.6	93.7	48.1	94.3	1.6
7	[Bu$_4$N][HSO$_4$]	33.7	88.7	47.1	92.4	2.0

[a]Conditions: Catalyst = 3.0 mmol, S/L ratio = 10%, Agar = 10 g, DI-H_2O = 100 mL, T = 121 °C, t = 15 min.

hydrolytic system, a series of bisulfate ionic liquids (Scheme 2) were synthesized according to a literature method.[11] These were then applied for the catalytic agar saccharification at the S/L ratio of 10% at 121 °C for 15 min in autoclave and their performance was compared with H_2SO_4 hydrolytic result. The molar amount of all the catalysts was set to 3.0 mmol and the results are shown in Table 2. As already seen in Figure 1, H_2SO_4 delivered 7.5 g/L of 5-HMF which is sufficiently high to the degree of hampering the fermentation. The ring structure in cationic part, imidazolium- and morpholium-based bisulfate ionic liquids (Table 2, entries 2-4) afford relatively low Gal and AG concentration with negligible amount of 5-HMF in less than 1 g/L. Interestingly, non ring structural ammonium-, phosphonium-, and choline-based ionic liquids (Table 2, entries 5-7) show fairly high Gal and AG yields with a very small amount of 5-HMF formation in less than 2 g/L at the same amount of catalyst loading. Among them, the [Chol][HSO$_4$] ionic liquid synthesized from the reaction of sulfuric acid and commercially available choline chloride provides the best result that shows very high Gal as well as AG yields at the same catalyst loading.

From these results, the activity of ionic liquids influencing Gal and AG yields are classified into, high and low depending upon the cation type of ionic liquids used. The reason for the suppression of 5-HMF generation as well as the well-balanced
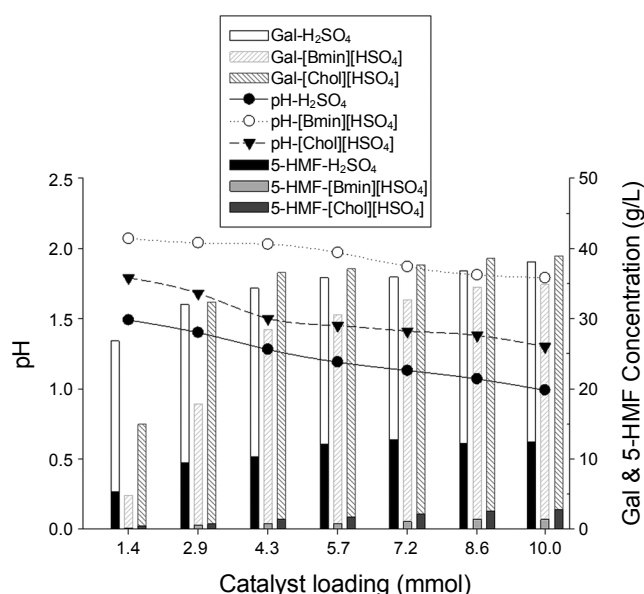
Figure 3. Effects of various catalysts loading on the pH and galactose concentration. Conditions: S/L ratio = 10%, Agar = 10 g, DI-H_2O = 100 mL, T = 121 °C, t = 15 min.

sugar distribution without the degradation of AG to form 5-HMF using these ionic liquids cannot be fully explained at the moment, but it is likely that the type of cation of ionic liquids plays a significant role in regulating or, at least, reducing brønsted acidity of bisulfate anion ([HSO$_4$]⁻). This is partially supported by simple measurement of pH value using the same molar amount of several ionic liquid catalysts, which may help to derive some clues from the acidity-reactivity correlations.

To correlate the findings, a separate hydrolysis reaction was carried out in the presence of three types of representative catalysts (H_2SO_4, [Bmim][HSO$_4$], and [Chol][HSO$_4$]) at the S/L ratio of 10% at 121 °C for 15 min in an autoclave. The results illustrated in Figure 3 indicate that H_2SO_4 shows the lowest pH, [Chol][HSO$_4$] is located at the midpoint, and [Bmim][HSO$_4$] reveals the highest value in the pH determining curve. The lowering in acidity upon incorporation of ionic liquids can be ascribed to a depletion of the proton in sulfuric acid to give the

bisulfate-based ionic liquids. Accordingly, H_2SO_4 and [Chol] [HSO$_4$] show good activity for the formation of Gal in the overall catalyst loading and [Bmim][HSO$_4$] shows the lowest Gal release. Although H_2SO_4 shows a high yield towards Gal, the generation of 5-HMF is significant, probably due to the acid labile-induced degradation of AG to 5-HMF. This can be attributed to the brønsted acidity of H_2SO_4. In contrast, [Chol][HSO$_4$] ionic liquid which shows a lower brønsted acidity than H_2SO_4 in terms of pH, shows very high Gal yield with reduced 5-HMF formation. It demonstrates that [Chol][HSO$_4$] has an acidity enough to depolymerize galactan to afford high yields of Gal and to prevent AG from degradation to 5-HMF. The AG yields were found to be more than 95% in the presence of [Chol] [HSO$_4$] (data not shown in Figure 3). Further investigation on the effect of different type of cation (ring structure *vs.* non-ring structure) on the acidity-reactivity relationship is under progress.

To summarize, various factors affecting yields of Gal, AG, and 5-HMF formation during saccharification were investigated using agar as a substrate in the presence of several bisulfate-based acidic ionic liquids as catalysts. The result was compared with employing sulfuric acid from the viewpoint of sugar yields and 5-HMF formation. [Bmim][HSO$_4$], [Hmim][HSO$_4$], [Morph] [HSO$_4$], [Bu$_4$N][HSO$_4$], [Bu$_4$P][HSO$_4$], [Chol][HSO$_4$] showed moderate to high yields of Gal and AG with a remarkable decrease in 5-HMF formation compared with sulfuric acid. Among them, [Chol][HSO$_4$] ionic liquid was found to exhibit the highest yield of sugars with an acceptable concentration of 5-HMF that does not inhibit the fermentation process.

Experimental Section

Hydrolytic Reaction. The representative agar saccharification reaction is as follows: A mixture of 10 g of extracted agar from *Gelidium amansii* and a 100 mL of aqueous solution containing acidic ionic liquids were charged in an autoclave. The hydrolytic reaction was carried out at a temperature of 121 $^{\circ}$C for 15 min. Then, the pH of the hydrolysates was adjusted to 5.5 by adding $CaCO_3$. After this pre-treatment, the liquid was separated by centrifugation and analyzed for sugar concentration.

Synthesis of Choline bisulfate ([Chol][HSO$_4$]).[12] The representative synthesis of Choline bisulfate is as follows: Choline chloride (50 g) was charged into a 500 mL round-bottom flask and a stoichiometric amount of dilute sulfuric acid (50%, 35.93 mL) was added drop wise very slowly and then the mixture was stirred for 24 h at room temperature. The resulting solid was washed repeatedly with ethyl acetate to remove non-ionic residues followed by drying in vacuum to give a white solid. The resulting ionic liquid was further purified by recrystallization with methylene chloride. ^{1}H NMR (200 MHz, D$_2$O) δ 1.78 (s, 1H), 3.61 (d, 3H), 7.12 (d, 2H), 8.38 (d, 2H). IR (KBr, cm^{-1}: 3040, 1220, 1036. Anal. Calcd. for C$_5$H$_{15}$NO$_5$S: C, 29.84; H, 7.51; N, 6.96; O, 39.75; S, 15.93. Found: C, 32.72; H, 7.33; N, 7.60; O, 28.95; S, 19.17. MS (EI) *m/z*: 201.07 (M$^+$).

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12. The supporting information on the synthesis and characterization of other bisulfate-based acidic ionic liquids is available on request from the correspondence author (Fax: +82-41-589-8580, E-mail: yjkim@kitech.re.kr).