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Production of 5-hydroxymethylfurfural from agarose by using solid acid catalyst in dimethyl sulfoxide

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Abstract

In this study, an effective method for 5-HMF production from agarose, a biomass material derived from red-algae was proposed. 5-HMF was produced from the decomposition of agarose by the catalytic action of a solid acid, Amberlyst-36 in dimethyl sulfoxide (DMSO), which was a polar aprotic solvent apt for providing a micro-aqueous environment. The moisture content in DMSO was found to be the governing factor for 5-HMF production having an optimum level of 7.2 % at which the 5-HMF yield was 62 %, the highest level ever observed in the production of 5-HMF from agarose. DMSO was also found to protect 5-HMF once produced from rehydration in the present system. It was demonstrated in a packed-bed reactor that Amberlyst-36 could be repeatedly used, which was the prime objective of using a solid acid instead of a liquid acid as catalyst.

Keywords: agarose, 5-hydroxymethylfurfural, Amberlyst-36, DMSO, packed-bed reactor
1. Introduction

The chemical and energy industry nowadays heavily depends on fossil resources such as oil, natural gas and coal. However, they have a serious problem of greenhouse gas emission during their conversion to fuel or chemicals and life cycle of these products, let alone their expected depletion in the long run. For these reasons, an alternative renewable resource with no or much reduced net greenhouse emission is in need in many areas of the chemical and energy industry.\textsuperscript{1-4} Biomass is a promising candidate for such purpose, which can be converted to various chemicals and energy material through chemical, biological, and/or enzymatic processes.\textsuperscript{5}

5-Hydroxymethylfurfural (5-HMF), furan derivative is called ‘sleeping giant’ with many potential applications. It can be produced from biomass-derived carbohydrates and have a good potential to be sustainable substitutes for petroleum-based building blocks used in the production of fine chemicals, pharmaceuticals and bio-fuels.\textsuperscript{6-8} Recently many researchers have been interested in producing platform compounds including 5-HMF from biomass.

Since the discovery of 5-HMF at the end of the 19th century, many workers have tried to produce it from various raw materials.\textsuperscript{9} Biomass-derived monosaccharides such as fructose and glucose have been the most commonly studied precursors for the production of 5-HMF by dehydration. However, glucose with an aldose configuration has been found difficult to be converted to 5-HMF. On the contrary fructose, a ketose is readily converted to 5-HMF with a high selectivity.\textsuperscript{10} For this reason fructose has played a role as major material source for 5-HMF production for decades. Fructose, however, cannot be a viable source of 5-HMF synthesis due to its relatively low abundance in nature and high price.
There are emerging interests in producing 5-HMF from polysaccharides in these days. Starch\textsuperscript{11}, cellulose\textsuperscript{12-15}, inulin\textsuperscript{16-19} and cotton seed hull\textsuperscript{20} have been reported to be used as raw material for 5-HMF production. There is, however, a serious moral issue with starch and inulin, of consuming food resource for the production of chemicals\textsuperscript{21}. Another candidate as material source for 5-HMF production is agarose, a galactan, contained in red algae. Agarose comprises over 50 \% of \textit{Gelidium amansii}\textsuperscript{22, 23}, one of the most abundantly available red seaweed species, for example. Only a few cases of HMF production from agarose hydrolysate have been reported to date. Yang et al., obtained 40\% of 5-HMF yield from agarose with metal chloride in aqueous media\textsuperscript{24}, which is the most recognized study of 5-HMF production. In the previous work in our group, 5-HMF and levulinic acid (LA) produced as byproducts during the hydrolysis of agarose to produce galactose by using acid were recovered by nanofiltration and electrodialysis\textsuperscript{25} or by chromatography.\textsuperscript{26} Recently, acid hydrolysis of biomass materials have been increasingly done by using solid acid catalyst instead of liquid acid mainly because it can be recycled saving the material cost for catalyst.\textsuperscript{17, 18, 27-34}

In this study 5-HMF was produced from agarose by using a solid acid catalyst, which can be recycled after the reaction to save material cost and at the same time to eliminate the necessity of subsequent neutralization step, which is mandatory when a liquid acid is used. The reaction was performed in a polar aprotic solvent instead of in aqueous phase to provide a more favorable micro-aqueous environment to 5-HMF formation. The effects of moisture content were investigated to identify the optimum level. The possibility of repeated use of the solid catalyst was also examined.
2. Experimental

2.1. Materials

Agarose (SeaKem® LE) was purchased from Lonza Group Ltd., Switzerland. 5-hydroxymethylfurfural, levulinic acid, Amberlyst-36 (wet type), \( N,N \)-dimethylformamide (DMF), \( N,N \)-dimethylacetamide (DMA) and dimethyl sulfoxide (DMSO, anhydrous \( \geq 99.9 \% \) ) were purchased from Sigma-Aldrich Co. LLC. Formic acid was purchased from Junsei Chemical Co. Ltd., Japan.

2.2. Reactors and reaction conditions

In beaker experiments, 5 g/L of agarose in 100 mL of DMF, DMA or DMSO was treated with 6 g on dry basis of Amberlyst-36 at 140 °C for 180 min in a 250 mL beaker. The moisture content in the reaction mixture was varied in the range of 1.8 ~ 12.6 % by adding the required amount of distilled water. When the reaction was performed in water for the purpose of comparison, an autoclave was used due to the high vapor pressure of the reaction mixture at 140 °C for 210 min. Experiments were carried out also in a packed-bed differential recycle reactor (Fig. 1). The total liquid volume was 300 mL and 18 g of Amberlyst-36 was packed in the reactor. The reaction temperature was controlled at 140 ± 1.0 °C, and the reaction time was 180 min.

2.3. Amberlyst-36 recycling

At the end of the reaction, Amberlyst-36 was taken out of the reactor and washed by using distilled water with vacuum filtering to remove the reaction products. The washed catalyst
was exsiccated for 24 hours at 100 °C in an oven, and then recycled to the reactor for the next run of reaction.

2.4. Calculations

Agarose is composed of repeating units of dimeric galactosyl-anhydrogalactosyl (G-A) residue. Its decomposition can be expressed as 3 step-reaction: agarose hydrolysis to release galactose and 3,6-anhydro-α-L-galctose(AHG) (Eq. 1); 5-HMF formation from AHG by dehydration (Eq. 2); and rehydration of 5-HMF into LA and formic acid (FA) (Eq. 3) as depicted in Fig. 2.

\[ C_9H_9O_4 \cdot [C_6H_{10}O_5 + C_6H_8O_4]_{n-1} - C_6H_{11}O_6 + (2n-1)H_2O \rightarrow nC_6H_{12}O_6 + nC_6H_{10}O_5 \quad (\text{Eq. 1}) \]

\[ C_6H_{10}O_5 \rightarrow C_6H_6O_3 + 2H_2O \quad (\text{Eq. 2}) \]

\[ C_6H_6O_3 + 2H_2O \rightarrow C_5H_8O_3 + CH_2O_2 \quad (\text{Eq. 3}) \]

The amount of agarose was presented in moles of the dimeric residue of G-A, and galactose yield from agarose was defined on a mole base to be \( \left[ \frac{\text{M galactose}}{\text{M G-A in the feed}} \right] \) as presented in Eq. 4. The molar yields of 5-HMF, LA and FA were defined in the same way.

Galactose yield: \[ \text{Galactose yield: } Y_{\text{Gal}} = \frac{C_{\text{Gal}}/\text{MW}_{\text{Gal}}}{C_{\text{Ag}}/\text{MW}_{\text{G-A}}} \quad (\text{Eq. 4}) \]

where, \( C_{\text{Gal}} \) and \( C_{\text{Ag}} \) are concentrations of galactose produced and agarose in the feed in g/L, respectively, and MW_{Gal} and MW_{G-A} molecular weights of galactose and G-A in g/mole, respectively. The molecular weights of G-A, galactose, 5-HMF, LA and FA are 306, 180, 126, 116 and 46, respectively.

2.5. Analytical procedures

5-HMF was quantified by using a high-performance liquid chromatograph (HPLC) (Dionex...
Inc., Korea) equipped with a Luna 5u C18 column (250 × 4.6 mm, Pheonomenex Inc., USA). Five % (v/v) of acetonitrile in distilled water was used as the mobile phase at a flow rate of 0.8 mL/min and the column temperature was 30 °C. Its detection was done at a wavelength of 280 nm by using a UV detector (Dionex Inc., Korea). LA and FA were analyzed by using the same HPLC with an Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad Laboratories, Inc., USA). Ten mM of H$_2$SO$_4$ was used at a flow rate of 0.6 mL/min and the column temperature was 65 °C. Their detection was done at a wavelength of 210 nm by using the UV detector mentioned earlier. The galactose concentration was measured by using another HPLC (Waters Corp., USA) with a Asahipak NH$_2$P-50 4E column (250 × 4.6 mm, Shodex, Japan). As the mobile phase, 65 % (v/v) of acetonitrile in distilled water was used at a flow rate of 1.0 mL/min. The column temperature was 40 °C. An evaporative light scattering detector (Sedex 75, Sedere, France) was used.
3. Results and discussion

3.1. Selection of solvent

Agarose was treated with Amberlyst-36 in water and three different polar aprotic solvents of DMF, DMA and DMSO. When the reaction took place in water, 5-HMF yield increased in the beginning to reach rather a low value of 17 % and then decreased as 5-HMF was rehydrated into LA and FA (Fig. 3a). The galactose yield was found to monotonically increase to reach 80 %. On the contrary, 5-HMF yield monotonically increased while galactose yield showed a maximum when the polar aprotic solvents were used as exemplified by the case with DMSO (Fig. 3b). The moisture content in DMSO was 5.4 % (w/w). Among these three solvents, DMSO showed the highest 5-HMF yield of 50 % (Table 1). For this reason, DMSO was chosen for the subsequent experimental study. The higher yield with DMSO might have been due to its own catalytic activity as already reported. However, no noticeable amount of 5-HMF was formed when DMSO was used without Amberlyst 36 in this study (data not presented).

One thing to note is that a much higher amount of galactose is produced in aqueous phase than in DMSO. In addition, no noticeable decrease in galactose yield with time is observed, while galactose yield decreased is significant after 120 min in DMSO. The reaction products in DMSO were analyzed by thin layer chromatography and HPLC (Fig. 4) to identify the cause for such loss of galactose. The chromatogram showed that the reaction mixture contains oligomeric compounds of neoagarotetraose and neoagarohexaose, galactose, and an unknown compound smaller than galactose. Considering that the two oligomers were from partial hydrolysis of agarose, it was speculated that galactose formed from agarose hydrolysis was
degraded into a low molecular weight compound. An experiment was performed with galactose in DMSO to prove such hypothesis. The results showed that as galactose disappeared the same unknown compound was formed (Fig. 5). In a previous study by other group, galactose was found to be converted to glyceraldehyde, dihydroxyacetone or lactic acid. However, in this study, the unknown compound from galactose could not be identified.

3.2. Effects of moisture content

Theoretically speaking, an environment with no water is desirable for 5-HMF production since it is formed through dehydration of AHG and it is degraded into LA ad FA by rehydration. However, AHG is generated from agarose hydration, which is favored in an environment with high moisture content. It could be easily expected that the optimum moisture content for 5-HMF formation would exist considering such contradictory effects of water. For the control of initial moisture content in the reaction mixture, Amberlyst-36 purchased in a wet form was dried before being used for reaction. It was desiccated for 24 hours in a furnace at 100 °C until no weight change was observed. The necessary amount of distilled water was added for the adjustment of moisture content at various levels from 1.8 to 12.6 % (w/w). As expected, the 5-HMF yield increased as the moisture content increased to reach a maximum of 62 % at 7.2 % moisture content, and then decreased (Fig. 6). At this moisture content, LA yield was 11 %. The FA yield was no longer presented since it was identical to LA yield.

It was to notice that the LA yield was limited under a low level of 13 % in DMSO regardless of moisture content in the range tested, while it was as high as 50 % in aqueous solution. Mushrif et al. studied the molecular dynamics of solvent effects in the selective conversion of fructose to 5-HMF and provided a theoretical basis for the effects of DMSO.
solvation in protecting 5-HMF from rehydration.\textsuperscript{40} According to their theoretical and simulation work, DMSO was found to solvate C1 and C2 carbons of 5-HMF, and thus to protect the bond between them from being cleaved during the process of rehydration. Such a shielding effect of DMSO against 5-HMF rehydration well explains the limited LA yield observed in this study.

3.3. Recycling of Amberlyst-36

The main advantage of using a solid acid catalyst like Amberlyst-36 is its reusability, which can greatly contribute to the process economy as discussed earlier in the section of Introduction. For this reason, the number of repeated use of Amberlyst-36 can be an important factor affecting the process economy. Recycling test was performed by reusing the spent Amberlyst-36 after washing and desiccating for the two subsequent runs of experiment. Table 2 shows that the 5-HMF yield decreased from 62 % in the fresh or first run to 55 % in the third run, showing the feasibility of repeated use of Amberlyst-36 in the view point of activity. It was found, however, that its repeated use would be not feasible due to its structural weakness. As shown in Fig. 7, Amberlyst-36 beads were found to have been almost completely broken down after three times of usage due to attrition caused by mixing during the reaction. The loss in activity of Amberlyst-36 can be explained in two parts. The first one is the detachment of sulfonic acid groups on the bead surface and the second is the loss of powdery Amberlyst-36 during the recovery and washing steps between runs. Such a structural fragileness will be the main hurdle for Amberlyst-36 to be used in a mixed reactor clearly suggesting the choice of other attrition-free type of reactors. In this study, a packed-bed differential recycle reactor was used to avoid the structural disintegration of Amberlyst-
36. In a previous study by other group to produce 5-HMF from glucose by using solid acid catalysts of $\text{SO}_4^2-/\text{ZrO}_2$ and $\text{SO}_4^2-/\text{ZrO}_2-\text{Al}_2\text{O}_3$ in a magnetically stirred glass reactor, it was observed that the yield was about 34%, and that $\text{SO}_4^2-/\text{ZrO}_2-\text{Al}_2\text{O}_3$ was found structurally robust and could be reused up to five times.\textsuperscript{27}

3.4. 5-HMF production in packed-bed reactor

As shown in Fig. 1, the reaction mixture was recycled through the catalyst packed in the glass tube reactor. In this situation, the recycle flow rate should be high enough to minimize or eliminate external mass transfer problem, which can negatively affect the reactor performance. The 5-HMF yield showed practically no difference in the tested range of recycle flow rate of 300 ~ 1200 mL/min (Table 3). This meant 300 mL/min of recycle flow rated was high enough not to cause a mass transfer problem and thus the recycle flow rate was fixed at 300 mL/min in the subsequent experiments.

Effects of the initial moisture content were investigated and compared with those from the beaker experiments (Fig. 8). The optimum moisture content was found to be 7.2 % (w/w) again in consistency with the results in beaker. However, 5-HMF yield in the packed-bed reactor was 4~9% lower than that in beaker. Such results were rather unexpected considering that a mixed-flow reactor like beaker and a packed-bed reactor are in principle the same in terms of reaction performance except for the flow pattern inside the reactor. Such higher catalytic activity in beaker experiment might be explained, at least partially, by that the breakage of catalyst beads due to attrition in this type of mixed-flow reactor offers an increased exposure of sulfonic acid groups to the reaction mixture.

The main objective of employing a packed bed reactor is to protect the catalyst from the
detrimental effects of attrition and thus to substantially improve its reusability or lifespan. For the purpose of investigating the performance of Amberlyst-36 as it was repeatedly used, consecutive runs of experiments were carried out without changing the catalyst. After each run the reaction mixture was completely drained from the reactor and a fresh reaction mixture was added into the reactor for the next run. A sharp drop in 5-HMF yield was observed after the 1st run (Fig. 9). It converged from 53% in the 1st run to about 34% after the 4th run. The abrupt drop after the 1st run was purely speculated to be due to the loss of sulfonic acid groups on the bead surface caused by the flow-induced shear force. The stabilized performance of Amberlyst 36 after the 4th run supports its good reusability although no long-term reusability test has been done.
4. Conclusions

Production of 5-HMF through the hydrolysis of agarose, a renewable biomass material, is one of promising alternatives to chemicals production from fossil resources such as petroleum and coal. It was demonstrated herein that DMSO, one of the polar aprotic solvents, played a role as effective medium for 5-HMF production by providing a micro-aqueous environment. The moisture content in DMSO was found to be the governing factor for 5-HMF production having an optimum level of 7.2 % at which the 5-HMF yield was 62 %. DMSO was also found to repress 5-HMF rehydration, which would lower its yield. It was proved that Amberlyst-36, the solid acid catalyst used in this study could be repeatedly used when a packed-bed reactor was used, which was the prime objective of using a solid acid instead of a liquid acid as catalyst.

Acknowledgments

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Notes and references

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Figure captions

Fig. 1 Schematic diagram of packed-bed differential recycle reactor system

Fig. 2 Pathway of galactose, 5-HMF, levulinic acid and formic acid formation from agarose

Fig. 3 Galactose, 5-HMF, levulinic acid and formic acid formation from agarose
(a): in aqueous solution (5 g/L of agarose in distilled water, 60 g/L of Amberlyst 36, 140 °C, 210 min), (b): in DMSO (5 g/L agarose in DMSO, 60 g/L of Amberlyst 36, 140 °C, 180 min).

Fig. 4 Analysis of agarose hydrolysis products: (left) Thin-layer chromatography: Gal, Galactose; Bi, 4-β-galactobiose; Tet, 3α,4β,3α-galactotetraose; Ag1, agarose hydrolysis sample 1; Ag2, agarose hydrolysis sample 2. (right) Chromatogram of high performance liquid chromatography

Fig. 5 High performance liquid chromatography analysis of galactose degradation by Amberlyst-36 in DMSO: (a) before, (b) after the reaction. (5 g/L of galactose in DMSO, 60 g/L of Amberlyst 36, 140 °C, 180 min)

Fig. 6 5-HMF and levulinic acid yields at various levels of moisture content. (5 g/L of agarose in distilled water, 60 g/L of Amberlyst 36, 140 °C, 180 min)

Fig. 7 Scanning electron microscope (SEM) images of Amberlyst 36:
(a) before use (x100), (b) after the 3rd run (x100)

Fig. 8 Comparison of 5-HMF yields in beaker and packed-bed reactor. (5 g/L of agarose in DMSO, 60 g/L of Amberlyst 36, 140 °C, 180 min)

Fig. 9 Results of reusability test in packed-bed reactor. (5 g/L of agarose in DMSO with 7.2 % moisture content, 60 g/L of Amberlyst 36, 140 °C, 180 min)
Table 1 Agarose hydrolysis by Amberlyst-36 in aqueous solution and polar aprotic solvents
(5 g/L of agarose, 60 g/L of Amberlyst 36, 140 °C)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>5-HMF</th>
<th>Galactose</th>
<th>LA</th>
<th>FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>17</td>
<td>80</td>
<td>50</td>
</tr>
<tr>
<td>2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>DMF</td>
<td>1</td>
<td>8</td>
<td>N/A</td>
</tr>
<tr>
<td>3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>DMA</td>
<td>48</td>
<td>24</td>
<td>N/A</td>
</tr>
<tr>
<td>4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>DMSO</td>
<td>50</td>
<td>29</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Reaction time: 210 min

<sup>b</sup> Reaction time: 180 min

*N/A: not available*
Table 2: Reusability test in beaker (5 g/L of agarose in DMSO with 7.2 % moisture content, 60 g/L of Amberlyst 36, 140 °C, 180 min)

<table>
<thead>
<tr>
<th></th>
<th>1&lt;sup&gt;st&lt;/sup&gt; run</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; run</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; run</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HMF yield (%)</td>
<td>62</td>
<td>57</td>
<td>55</td>
</tr>
<tr>
<td>LA yield (%)</td>
<td>10</td>
<td>12</td>
<td>11</td>
</tr>
</tbody>
</table>
Table 3 5-HMF yield at different recycle flow rates (5 g/L of agarose in DMSO with 7.2 % moisture content, 60 g/L of Amberlyst 36, 140 °C, 180 min)

<table>
<thead>
<tr>
<th>Flow rate (mL/min)</th>
<th>300</th>
<th>600</th>
<th>900</th>
<th>1200</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HMF yield (%)</td>
<td>53 ± 0.4</td>
<td>53 ± 0.2</td>
<td>51 ± 1.6</td>
<td>52 ± 0.3</td>
</tr>
</tbody>
</table>
1. Reservoir
2. Oil bath
3. Diaphragm pump
4. Temp. indicator
5. Catalyst
6. Furnace w/ temp. controller
Hydrolysis by water

D-Galactose (M.W.: 180)

Levalinic acid (M.W.: 116)
Formic acid (M.W.: 46)

Agarose

3,6-anhydro-α-L-galactose (M.W.: 162)

-2H₂O

Rehydration

5-HMF (M.W.: 126)

338x190mm (96 x 96 DPI)
galactose
dimer
tetramer
hexamer

300x150mm (96 x 96 DPI)
(a) galactose

Before the reaction

(b) Unknown compound originated from galactose

After the reaction

No galactose

220x400mm (96 x 96 DPI)
The bar graph illustrates the yields (%) of 5-HMF and Levulinic acid in relation to moisture content (% w/w). The x-axis represents moisture content, while the y-axis shows yields. The graph indicates that 5-HMF yields increase with increasing moisture content, whereas Levulinic acid yields decrease.
The graph shows the change in 5-HMF yield (%) with moisture content (% w/w). The line with dark dots represents the Packed-bed differential recycle reactor, while the line with open circles represents the Beaker. The data points indicate a slight increase in yield with increasing moisture content, with the Packed-bed reactor showing a marginally higher yield compared to the Beaker at all moisture content levels.