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

3

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5

6 **Abstract**

7

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

18

19 **Keywords:** agarose, 5-hydroxymethylfurfural, Amberlyst-36, DMSO, packed-bed reactor

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1 **1. Introduction**

2
3 The chemical and energy industry nowadays heavily depends on fossil resources such as oil,
4 natural gas and coal. However, they have a serious problem of greenhouse gas emission
5 during their conversion to fuel or chemicals and life cycle of these products, let alone their
6 expected depletion in the long run. For these reasons, an alternative renewable resource with
7 no or much reduced net greenhouse emission is in need in many areas of the chemical and
8 energy industry.¹⁻⁴ Biomass is a promising candidate for such purpose, which can be
9 converted to various chemicals and energy material through chemical, biological, and/or
10 enzymatic processes.⁵

11 5-Hydroxymethylfurfural (5-HMF), furan derivative is called ‘sleeping giant’ with many
12 potential applications. It can be produced from biomass-derived carbohydrates and have a
13 good potential to be sustainable substitutes for petroleum-based building blocks used in the
14 production of fine chemicals, pharmaceuticals and bio-fuels.⁶⁻⁸ Recently many researchers
15 have been interested in producing platform compounds including 5-HMF from biomass.

16 Since the discovery of 5-HMF at the end of the 19th century, many workers have tried to
17 produce it from various raw materials.⁹ Biomass-derived monosaccharides such as fructose
18 and glucose have been the most commonly studied precursors for the production of 5-HMF
19 by dehydration. However, glucose with an aldose configuration has been found difficult to be
20 converted to 5-HMF. On the contrary fructose, a ketose is readily converted to 5-HMF with a
21 high selectivity.¹⁰ For this reason fructose has played a role as major material source for 5-
22 HMF production for decades. Fructose, however, cannot be a viable source of 5-HMF
23 synthesis due to its relatively low abundance in nature and high price.

1 There are emerging interests in producing 5-HMF from polysaccharides in these days.
2 Starch¹¹, cellulose¹²⁻¹⁵, inulin,¹⁶⁻¹⁹ and cotton seed hull²⁰ have been reported to be used as raw
3 material for 5-HMF production. There is, however, a serious moral issue with starch and
4 inulin, of consuming food resource for the production of chemicals²¹. Another candidate as
5 material source for 5-HMF production is agarose, a galactan, contained in red algae. Agarose
6 comprises over 50 % of *Gelidium amansii*^{22, 23}, one of the most abundantly available red
7 seaweed species, for example. Only a few cases of HMF production from agarose
8 hydrolysate have been reported to date. Yang et al., obtained 40% of 5-HMF yield from
9 agarose with metal chloride in aqueous media²⁴, which is the most recognized study of 5-
10 HMF production. In the previous work in our group, 5-HMF and levulinic acid (LA)
11 produced as byproducts during the hydrolysis of agarose to produce galactose by using acid
12 were recovered by nanofiltration and electrodialysis²⁵ or by chromatography.²⁶ Recently, acid
13 hydrolysis of biomass materials have been increasingly done by using solid acid catalyst
14 instead of liquid acid mainly because it can be recycled saving the material cost for catalyst.^{17,}
15 ^{18, 27-34}

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

23

1 **2. Experimental**

2

3 **2.1. Materials**

4 Agarose (SeaKem® LE) was purchased from Lonza Group Ltd., Switzerland. 5-
5 hydroxymethylfurfural, levulinic acid, Amberlyst-36 (wet type), *N,N*-dimethylformamide
6 (DMF), *N,N*-dimethylacetamide (DMA) and dimethyl sulfoxide (DMSO, anhydrous \geq
7 99.9 %) were purchased from Sigma-Aldrich Co. LLC. Formic acid was purchased from
8 Junsei Chemical Co. Ltd., Japan.

9

10 **2.2. Reactors and reaction conditions**

11 In beaker experiments, 5 g/L of agarose in 100 mL of DMF, DMA or DMSO was treated
12 with 6 g on dry basis of Amberlyst-36 at 140 °C for 180 min in a 250 mL beaker. The moisture
13 content in the reaction mixture was varied in the range of 1.8 ~ 12.6 % by adding the required
14 amount of distilled water. When the reaction was performed in water for the purpose of
15 comparison, an autoclave was used due to the high vapor pressure of the reaction mixture at
16 140 °C for 210 min. Experiments were carried out also in a packed-bed differential recycle
17 reactor (Fig. 1). The total liquid volume was 300 mL and 18 g of Amberlyst-36 was packed in
18 the reactor. The reaction temperature was controlled at 140 ± 1.0 °C, and the reaction time was
19 180min.

20

21 **2.3. Amberlyst-36 recycling**

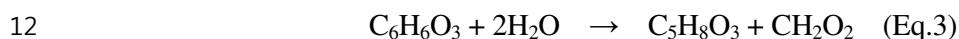
22 At the end of the reaction, Amberlyst-36 was taken out of the reactor and washed by using
23 distilled water with vacuum filtering to remove the reaction products. The washed catalyst

1 was exsiccated for 24 hours at 100 °C in an oven, and then recycled to the reactor for the next
2 run of reaction.

3

4 **2.4. Calculations**

5 Agarose is composed of repeating units of dimeric galactosyl-anhydrogalactosyl (G-A)
6 residue.³⁵ Its decomposition can be expressed as 3 step-reaction: agarose hydrolysis to release
7 galactose and 3,6-anhydro- α -L-galactose(AHG) (Eq. 1); 5-HMF formation from AHG by
8 dehydration^{23,36} (Eq. 2); and rehydration of 5-HMF into LA and formic acid (FA) (Eq. 3) as
9 depicted in Fig. 2.



13 The amount of agarose was presented in moles of the dimeric residue of G-A, and galactose
14 yield from agarose was defined on a mole base to be [M galactose/M G-A in the feed] as
15 presented in Eq. 4. The molar yields of 5-HMF, LA and FA were defined in the same way.

$$16 \quad \text{Galactose yield: } Y_{\text{Gal}} = \frac{C_{\text{Gal}}/\text{MW}_{\text{Gal}}}{C_{\text{Ag}}/\text{MW}_{\text{G-A}}} \quad (\text{Eq. 4})$$

17 where, C_{Gal} and C_{Ag} are concentrations of galactose produced and agarose in the feed in
18 g/L, respectively, and MW_{Gal} and $\text{MW}_{\text{G-A}}$ molecular weights of galactose and G-A in g/mole,
19 respectively. The molecular weights of G-A, galactose, 5-HMF, LA and FA are 306, 180, 126,
20 116 and 46, respectively.

21

22 **2.5. Analytical procedures**

23 5-HMF was quantified by using a high-performance liquid chromatograph (HPLC) (Dionex

1 Inc., Korea) equipped with a Luna 5u C18 column (250 × 4.6 mm, Phenomenex Inc., USA).
2 Five % (v/v) of acetonitrile in distilled water was used as the mobile phase at a flow rate of 0.8
3 mL/min and the column temperature was 30 °C. Its detection was done at a wavelength of 280
4 nm by using a UV detector (Dionex Inc., Korea).³⁷ LA and FA were analyzed by using the same
5 HPLC with an Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad Laboratories, Inc., USA).
6 Ten mM of H₂SO₄ was used at a flow rate of 0.6 mL/min and the column temperature was
7 65 °C. Their detection was done at a wavelength of 210 nm by using the UV detector
8 mentioned earlier.³⁸ The galactose concentration was measured by using another HPLC (Waters
9 Corp., USA) with a Asahipak NH₂P-50 4E column (250 × 4.6 mm, Shodex, Japan). As the
10 mobile phase, 65 % (v/v) of acetonitrile in distilled water was used at a flow rate of 1.0 mL/min.
11 The column temperature was 40 °C. An evaporative light scattering detector (Sedex 75, Sedere,
12 France) was used.
13

1 **3. Results and discussion**

2

3 **3.1. Selection of solvent**

4 Agarose was treated with Amberlyst-36 in water and three different polar aprotic solvents
5 of DMF, DMA and DMSO. When the reaction took place in water, 5-HMF yield increased in
6 the beginning to reach rather a low value of 17 % and then decreased as 5-HMF was
7 rehydrated into LA and FA (Fig. 3a). The galactose yield was found to monotonically
8 increase to reach 80 %. On the contrary, 5-HMF yield monotonically increased while
9 galactose yield showed a maximum when the polar aprotic solvents were used as exemplified
10 by the case with DMSO (Fig. 3b). The moisture content in DMSO was 5.4 % (w/w). Among
11 these three solvents, DMSO showed the highest 5-HMF yield of 50 % (Table 1). For this
12 reason, DMSO was chosen for the subsequent experimental study. The higher yield with
13 DMSO might have been due to its own catalytic activity as already reported.³⁹ However, no
14 noticeable amount of 5-HMF was formed when DMSO was used without Amberlyst 36 in
15 this study (data not presented).

16 One thing to note is that a much higher amount of galactose is produced in aqueous phase
17 than in DMSO. In addition, no noticeable decrease in galactose yield with time is observed,
18 while galactose yield decreased is significant after 120 min in DMSO. The reaction products in
19 DMSO were analyzed by thin layer chromatography and HPLC (Fig. 4) to identify the cause
20 for such loss of galactose. The chromatogram showed that the reaction mixture contains
21 oligomeric compounds of neoagarotetraose and neoagarohexaose, galactose, and an unknown
22 compound smaller than galactose. Considering that the two oligomers were from partial
23 hydrolysis of agarose, it was speculated that galactose formed from agarose hydrolysis was

1 degraded into a low molecular weight compound. An experiment was performed with
2 galactose in DMSO to prove such hypothesis. The results showed that as galactose
3 disappeared the same unknown compound was formed (Fig. 5). In a previous study by other
4 group, galactose was found to be converted to glyceraldehyde, dihydroxyacetone or lactic
5 acid²⁴. However, in this study, the unknown compound from galactose could not be identified.

6

7 **3.2. Effects of moisture content**

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

20 It was to notice that the LA yield was limited under a low level of 13 % in DMSO
21 regardless of moisture content in the range tested, while it was as high as 50 % in aqueous
22 solution. Mushrif *et al.* studied the molecular dynamics of solvent effects in the selective
23 conversion of fructose to 5-HMF and provided a theoretical basis for the effects of DMSO

1 solvation in protecting 5-HMF from rehydration.⁴⁰ According to their theoretical and simulation
2 work, DMSO was found to solvate C1 and C2 carbons of 5-HMF, and thus to protect the
3 bond between them from being cleaved during the process of rehydration. Such a shielding
4 effect of DMSO against 5-HMF rehydration well explains the limited LA yield observed in
5 this study.

6

7 **3.3. Recycling of Amberlyst-36**

8 The main advantage of using a solid acid catalyst like Amberlyst-36 is its reusability,
9 which can greatly contribute to the process economy as discussed earlier in the section of
10 Introduction. For this reason, the number of repeated use of Amberlyst-36 can be an
11 important factor affecting the process economy. Recycling test was performed by reusing the
12 spent Amberlyst-36 after washing and desiccating for the two subsequent runs of experiment.
13 Table 2 shows that the 5-HMF yield decreased from 62 % in the fresh or first run to 55 % in
14 the third run, showing the feasibility of repeated use of Amberlyst-36 in the view point of
15 activity. It was found, however, that its repeated use would be not feasible due to its structural
16 weakness. As shown in Fig. 7, Amberlyst-36 beads were found to have been almost
17 completely broken down after three times of usage due to attrition caused by mixing during
18 the reaction. The loss in activity of Amberlyst-36 can be explained in two parts. The first one
19 is the detachment of sulfonic acid groups on the bead surface and the second is the loss of
20 powdery Amberlyst-36 during the recovery and washing steps between runs. Such a
21 structural fragileness will be the main hurdle for Amberlyst-36 to be used in a mixed reactor
22 clearly suggesting the choice of other attrition-free type of reactors. In this study, a packed-
23 bed differential recycle reactor was used to avoid the structural disintegration of Amberlyst-

1 36. In a previous study by other group to produce 5-HMF from glucose by using solid acid
2 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
3 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
4 robust and could be reused up to five times.²⁷

6 **3.4. 5-HMF production in packed-bed reactor**

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

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

23 The main objective of employing a packed bed reactor is to protect the catalyst from the

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

11

1 **4. Conclusions**

2

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

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15

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1 **Notes and references**

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- 9 1. J. N. Chheda, G. W. Huber and J. A. Dumesic, *Angewandte Chemie*, 2007, 46, 7164-
10 7183.
- 11 2. D. M. Alonso, J. Q. Bond and J. A. Dumesic, *Green Chemistry*, 2010, 12, 1493.
- 12 3. A. A. Rosatella, S. P. Simeonov, R. F. M. Frade and C. A. M. Afonso, *Green*
13 *Chemistry*, 2011, 13, 754.
- 14 4. J. C. Serrano-Ruiz and J. A. Dumesic, in *Catalysis for Alternative Energy Generation*,
15 Springer, 2012, DOI: 10.1007/978-1-4614-0344-9_2,#Springer.
- 16 5. B. Kamm, *Angew Chem Int Ed Engl*, 2007, 46, 5056-5058.
- 17 6. M. Bicker, J. Hirth and H. Vogel, *Green Chemistry*, 2003, 5, 280-284.
- 18 7. E. L. Kunkes, D. A. Simonetti, R. M. West, J. C. Serrano-Ruiz, C. A. Gärtner and J. A.
19 Dumesic, *Science*, 2008, 322, 417-421.
- 20 8. J. Lewkowski, *Arkivoc*, 2001, 17-54.
- 21 9. J. Lewkowski, *Archive for Organic Chemistry*, 2001, 17-54.
- 22 10. B. F. M. Kuster, *Starch - Stärke*, 1990, 42, 314-321.
- 23 11. S. Hu, Z. Zhang, J. Song, Y. Zhou and B. Han, *Green Chemistry*, 2009, 11, 1746.

- 1 12. Y. Su, H. M. Brown, X. Huang, X.-d. Zhou, J. E. Amonette and Z. C. Zhang, *Applied*
2 *Catalysis A: General*, 2009, 361, 117-122.
- 3 13. F. Jiang, Q. Zhu, D. Ma, X. Liu and X. Han, *Journal of Molecular Catalysis A:*
4 *Chemical*, 2011, 334, 8-12.
- 5 14. X. Qi, M. Watanabe, T. M. Aida and R. L. Smith, *Cellulose*, 2011, 18, 1327-1333.
- 6 15. N. Shi, Q. Liu, Q. Zhang, T. Wang and L. Ma, *Green Chemistry*, 2013, 15, 1967.
- 7 16. S. Hu, Z. Zhang, Y. Zhou, J. Song, H. Fan and B. Han, *Green Chemistry*, 2009, 11,
8 873.
- 9 17. F. Ilgen, D. Ott, D. Kralisch, C. Reil, A. Palmberger and B. König, *Green Chemistry*,
10 2009, 11, 1948.
- 11 18. X. Qi, M. Watanabe, T. M. Aida and R. L. Smith Jr, *Green Chemistry*, 2010, 12, 1855.
- 12 19. J. N. Chheda, Y. Roman-Leshkov and J. A. Dumesic, *Green Chemistry*, 2007, 9, 342.
- 13 20. S. Wang, Y. Du, P. Zhang, X. Cheng and Y. Qu, *Korean Journal of Chemical*
14 *Engineering*, 2014, DOI: 10.1007/s11814-014-0143-y.
- 15 21. T. Damartzis and A. Zabaniotou, *Renewable and Sustainable Energy Reviews*, 2011,
16 15, 366-378.
- 17 22. J.-H. Park, J.-J. Yoon, H.-D. Park, Y. J. Kim, D. J. Lim and S.-H. Kim, *International*
18 *Journal of Hydrogen Energy*, 2011, 36, 13997-14003.
- 19 23. B. Kim, J. Jeong, S. Shin, D. Lee, S. Kim, H.-J. Yoon and J. K. Cho, *ChemSusChem*,
20 2010, 3, 1273-1275.
- 21 24. L. Yan, D. D. Laskar, S.-J. Lee and B. Yang, *RSC Advances*, 2013, 3, 24090.
- 22 25. J. H. Kim, J. G. Na, J. W. Yang and Y. K. Chang, *Bioresource Technol*, 2013, 140, 64-
23 72.

- 1 26. E. Valentin, H.-G. Nam, P.-H. Kim, H. W. Joo, H. J. Shim, Y. K. Chang and S. Mun,
2 *Separation and Purification Technology*, 2014, 133, 297-302.
- 3 27. H. Yan, Y. Yang, D. Tong, X. Xiang and C. Hu, *Catalysis Communications*, 2009, 10,
4 1558-1563.
- 5 28. Y. Yang, X. Xiang, D. Tong, C. Hu and M. M. Abu-Omar, *Bioresour Technol*, 2012,
6 116, 302-306.
- 7 29. Y. Yu, X. Xi, L. Jia, Q. Wei-Yan, Y. Hong-Peng, L. Gui-Ying and H. Chang-Wei,
8 *CHEM. RES. CHINESE UNIVERSITIES*, 2009, 25, 234-238.
- 9 30. X. Shen, Y. X. Wang, C. W. Hu, K. Qian, Z. Ji and M. Jin, *ChemCatChem*, 2012, 4,
10 2013-2019.
- 11 31. A. Takagaki, M. Ohara, S. Nishimura and K. Ebitani, *Chemical communications*,
12 2009, DOI: 10.1039/b914087e, 6276-6278.
- 13 32. A. J. Crisci, M. H. Tucker, J. A. Dumesic and S. L. Scott, *Topics in Catalysis*, 2010,
14 53, 1185-1192.
- 15 33. K.-i. Shimizu, R. Uozumi and A. Satsuma, *Catalysis Communications*, 2009, 10,
16 1849-1853.
- 17 34. M. Ohara, A. Takagaki, S. Nishimura and K. Ebitani, *Applied Catalysis A: General*,
18 2010, 383, 149-155.
- 19 35. K. t. Nijenhuis, in *Thermoreversible Networks*, Springer Berlin Heidelberg, 1997, vol.
20 130, ch. Agarose pp. 194-202.
- 21 36. B. Yang, G. L. Yu, X. Zhao, G. L. Jiao, S. M. Ren and W. G. Chai, *Febs J*, 2009, 276,
22 2125-2137.
- 23 37. Y. H. Li and X. Y. Lu, *Journal of Zhejiang University. Science. B*, 2005, 6, 1015-1021.

- 1 38. K. Lourvanij and G. L. Rorrer, *Industrial & Engineering Chemistry Research*, 1993,
2 32, 11-19.
- 3 39. R. M. Musau and R. M. Munavu, *Biomass*, 1987, 13, 67-74.
- 4 40. S. H. Mushrif, S. Caratzoulas and D. G. Vlachos, *Physical Chemistry Chemical*
5 *Physics*, 2012, 14, 2637.

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Figure captions

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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)

	Solvent	Max. yield (%)			
		5-HMF	Galactose	LA	FA
1 ^a	H ₂ O	17	80	50	49
2 ^b	DMF	1	8	N/A	N/A
3 ^b	DMA	48	24	N/A	N/A
4 ^b	DMSO	50	29	8	8

^a Reaction time: 210 min

^b Reaction time: 180 min

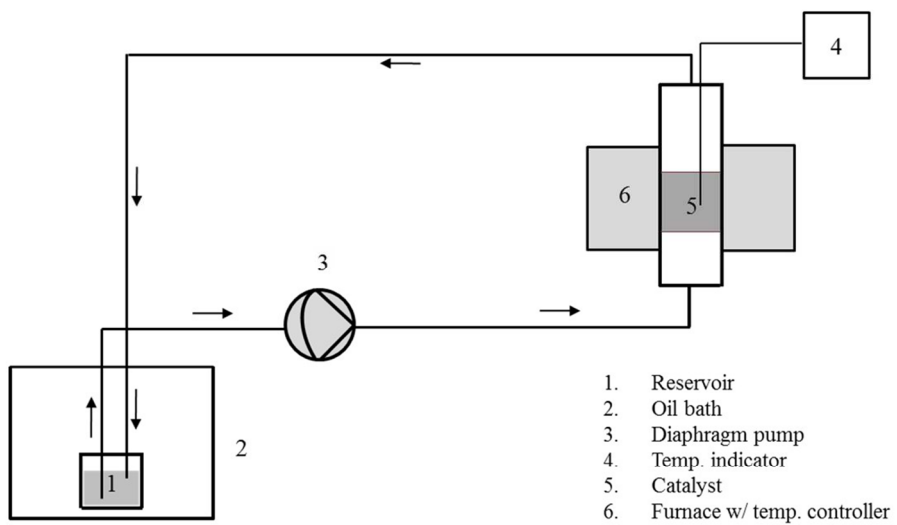
*N/A: not available

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)

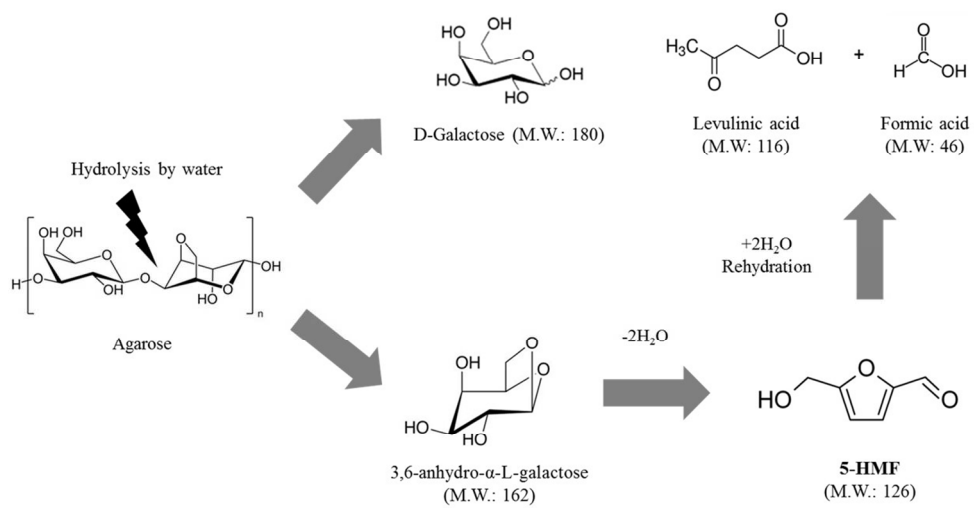
	1st run	2nd run	3rd run
5-HMF yield (%)	62	57	55
LA yield (%)	10	12	11

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)

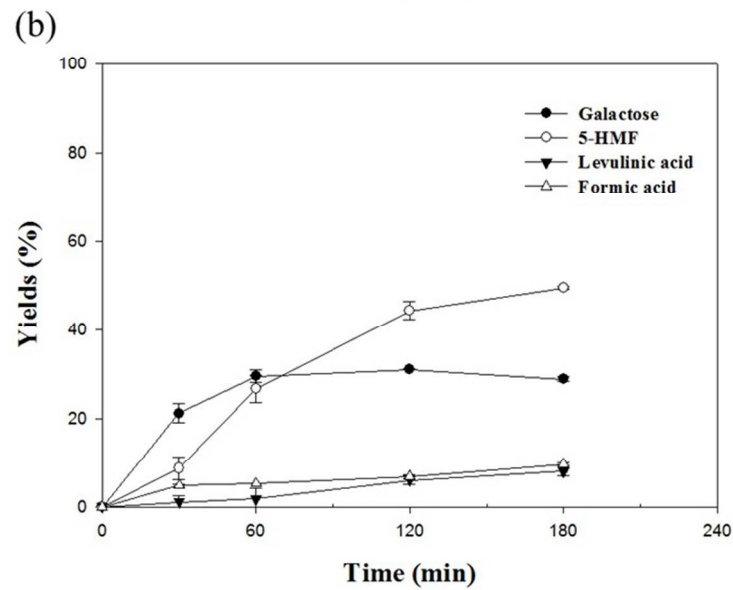
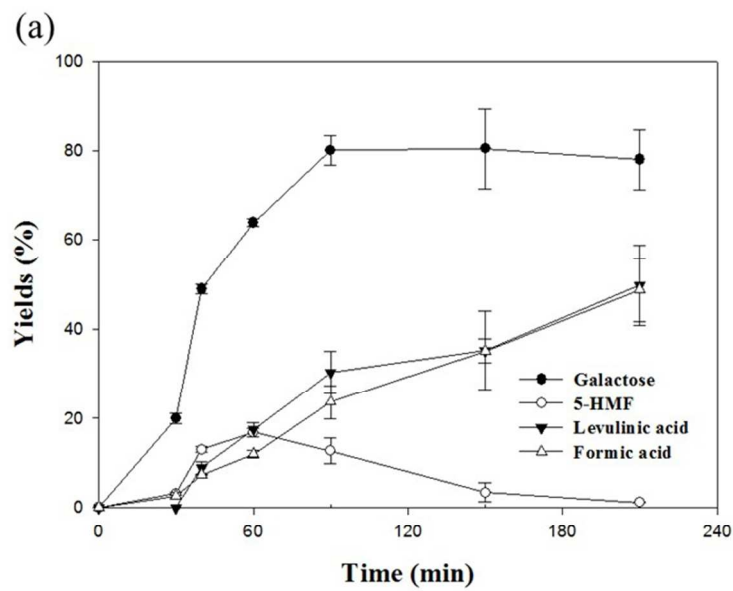
	Flow rate (mL/min)			
	300	600	900	1200
5-HMF yield (%)	53 ± 0.4	53 ± 0.2	51 ± 1.6	52 ± 0.3



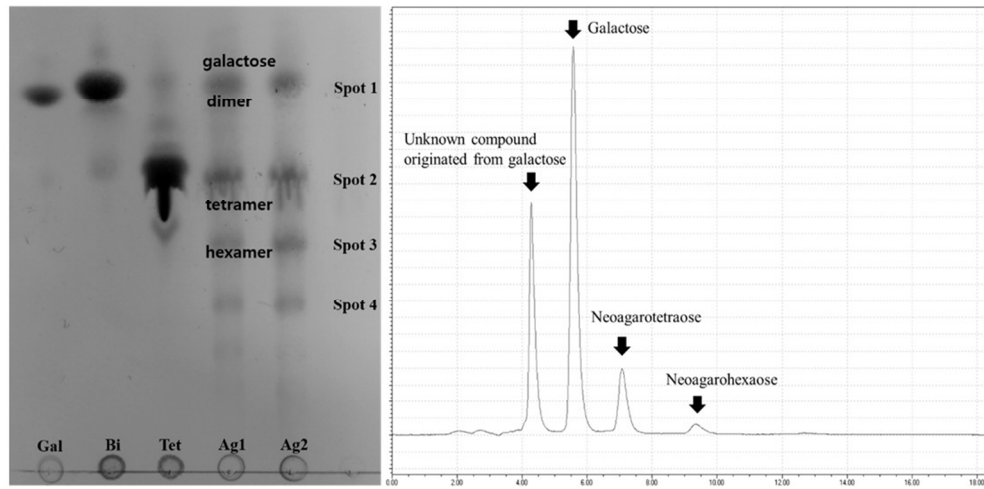
320x190mm (96 x 96 DPI)



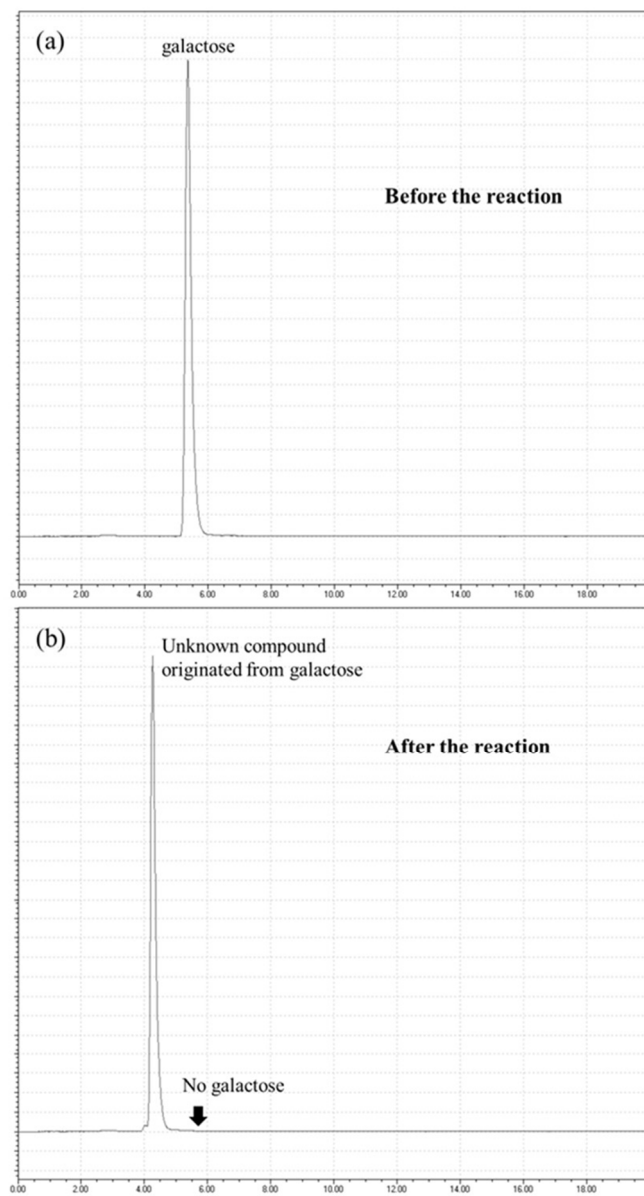
338x190mm (96 x 96 DPI)



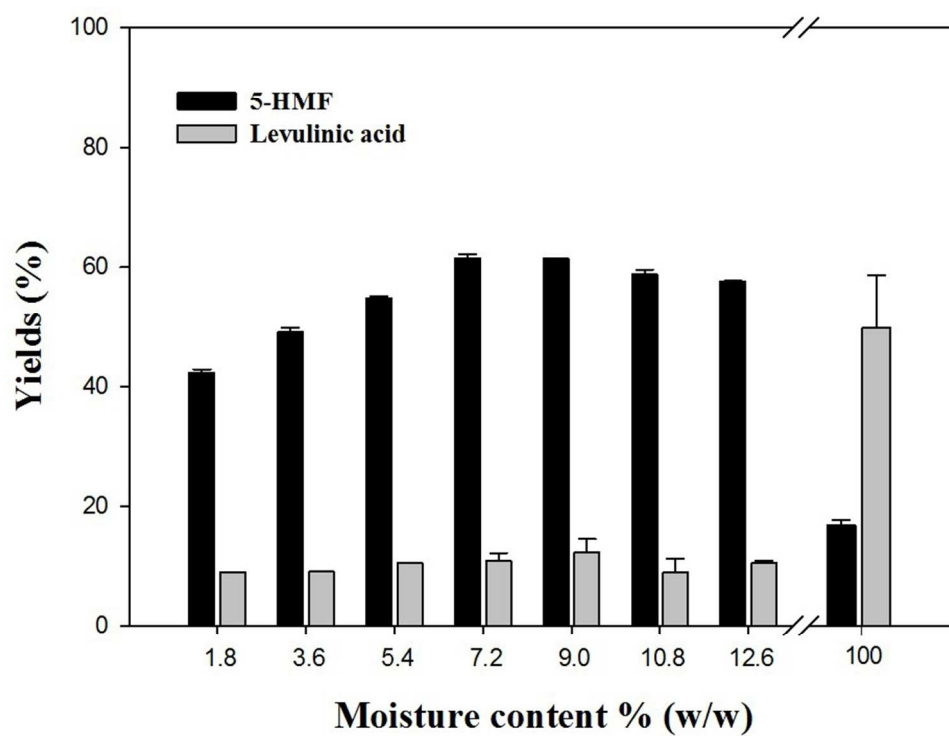
190x300mm (96 x 96 DPI)



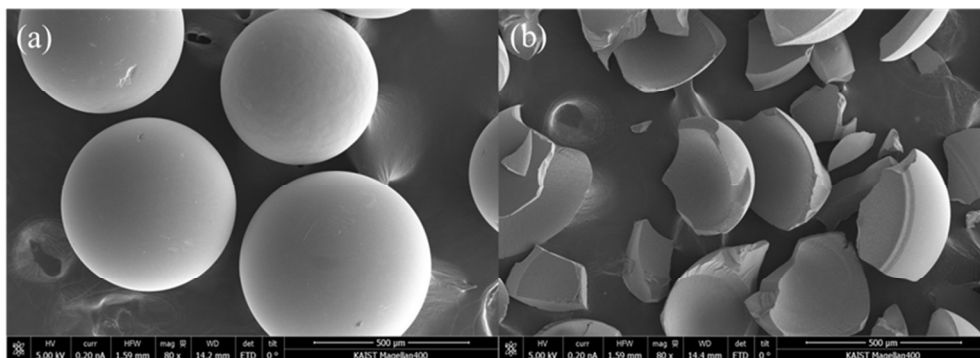
300x150mm (96 x 96 DPI)



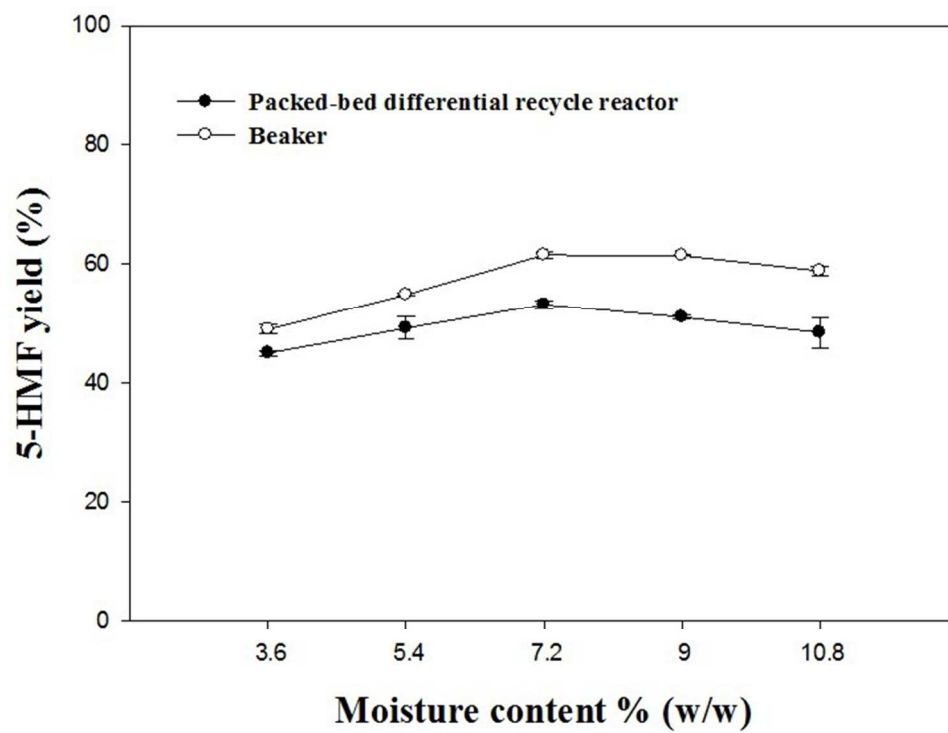
220x400mm (96 x 96 DPI)



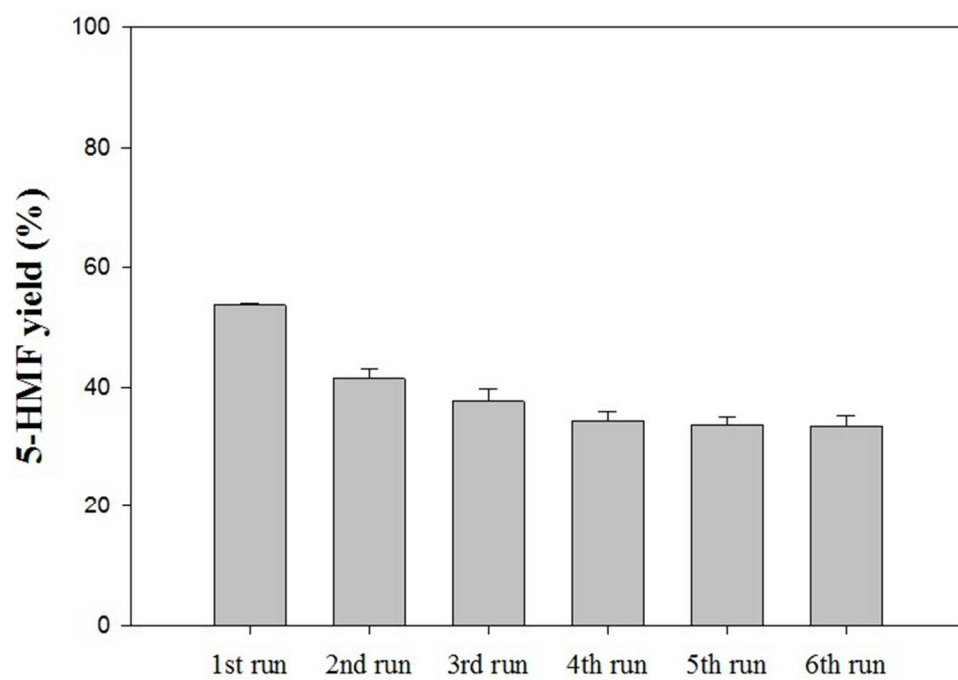
254x190mm (96 x 96 DPI)



400x150mm (96 x 96 DPI)



200x150mm (96 x 96 DPI)



200x150mm (96 x 96 DPI)