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Aqueous phase catalytic conversion of agarose to 5-hydroxymethylfurfural by metal chlorides

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The production of 5-hydroxymethylfurfural (5-HMF) from agarose catalyzed by metal chlorides was studied in aqueous phase. A series of metal chlorides, including NaCl, $CaCl_2$, $MgCl_2$, $ZnCl_2$, $CrCl_3$, $CuCl_2$ and $FeCl_3$, were comparatively investigated to catalyze agarose degradation for the production of 5-HMF at temperatures of 180 °C, 200 °C and 220 °C, catalyst concentration of 0.5% (w/w), 1% (w/w) and 5% (w/w), time of 0–50 min, and substrate concentration of 2% (w/w). Results revealed that alkali and alkaline earth metal chlorides, including NaCl, $CaCl_2$ and $MgCl_2$, resulted in higher 5-HMF yields from agarose with negligible amount of byproducts, such as levulinic acid and lactic acid, derived from further degradation reactions. 1% (w/w) $MgCl_2$ was the most efficient catalyst among the tested metal chlorides for 5-HMF production from agarose and resulted in both the highest yield of 40.7% and highest selectivity of 49.1% at 200 °C for 35 min. The cleavage of C–O–C bond in agarose with subsequent isomerization of galactose to its ketose was considered as a possible mechanism for formation of 5-HMF under $MgCl_2$ catalyzed conditions.

1. Introduction

The decreasing reserves of fossil fuels and the growing greenhouse gas emissions have caused an increase in bioenergy and biochemical research in recent years.[1] Biomass-derived carbohydrates represent a promising carbon-based alternative as an energy source and a sustainable feedstock. Substantial efforts have recently been devoted towards converting biomass to 5-HMF, an important chemical building block used to make a wide variety of chemicals (*e.g.* plastics, polymers, *etc.*) and fuels that are currently derived from petroleum.[2] However, the current processes of 5-HMF production are primarily dependent on edible monosaccharides such as fructose and glucose.[2,3] Using cellulosic biomass as the feedstock for 5-HMF production has been also extensively studied over the years. However, technical challenges in developing an efficient and economic viable process for commercial application still remain.[2,4]

Recently, polysaccharides from algal biomass were recommended as an alternative feedstock for the large scale production of biofuel and biochemicals because of their substantial abundance, negligible food value, as well as use of non-arable land.[5,6] Agarose, mainly composed of galactose and 3,6-anhydrogalactose,[7,8] is one of major polysaccharides stored in algal biomass.[5,9] For example, the agarose content in *Gelidium amansii* (a red algae species) is around 60–65%.[5] Even though agarose exists in a gel form caused by the intra- and intermolecular hydrogen bonds at room temperature, these hydrogen bonds can be dissociated when the temperature exceeds 90 °C,[7] leaving agarose more susceptible to decomposition. Furthermore, 3,6-anhydrogalactose with highly strained additional cyclic unit in agarose presents relatively high reactivity and could readily lead to the conversion of agarose into degradation compounds.[5]

Various reaction media, including organic solvent/organic-water biphasic systems, ionic liquids and the aqueous phase, were used for 5-HMF production. The use of organic solvents or ionic liquids as reaction medium has received significant attention because relatively high 5-HMF yield of 50–70% can be realized.[10,11] However, these reaction media present some limitations for the large scale production of 5-HMF. Although the 5-HMF yield was increased in the organic solvent dimethyl sulfoxide (DMSO), 5-HMF is highly soluble in DMSO which has a high boiling point, thus prevents economical separation of 5-HMF.[12] Although ionic liquids are reported to enhance the 5-HMF yields, ionic liquids are expensive and require a high level of purity to maintain their physical properties. Thus, industrial applications of ionic liquids are limited.[13,14]

Production of 5-HMF in aqueous phase (*i.e.* water) instead of these expensive and difficult-to-separate solvents will significantly reduce its production cost. The physical properties of water at elevated temperatures (*e.g.* 200 °C) are different from those under ambient conditions.[15] For example, the solubility of organic compounds in water can increase at high temperatures (above 100 °C) due to the lower dielectric constant of water.[16,17]

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Furthermore, protons released from water at high temperatures[18–20] could accelerate the formation of 5-HMF to some extent.[21] However, the degradation of saccharides (*e.g.* fructose and glucose) in water-only conditions proceeds with poor selectivity to 5-HMF production, resulting in many byproducts. Byproducts, such as glycolaldehyde, glyceraldehyde, and lactic acid, were generated from the retro aldol reaction of hexose (*e.g.* glucose and fructose), while others including levulinic acid and formic acid were generated due to the further rehydration of 5-HMF.[18–20] Formation of byproducts significantly reduced the 5-HMF yield.[18–20] Catalysts used for 5-HMF production in aqueous phase were mostly limited to mineral acids (*e.g.* H_2SO_4, H_3PO_4, and HCl),[15,22] organic acids (*e.g.* oxalic acid, ρ-toluenesulfonic acid),[15] H-form zeolites,[23] strong acid cation exchange resins,[24,25] and solid metal phosphates.[26,27] However, mineral acid and organic acid possess the catalytic ability of accelerating the rehydration of 5-HMF thereby limiting its yield;[15,22] heterogeneous catalysts, such as H-form zeolites, strong acid cation exchange resins and solid metal phosphates, are often not suitable to catalyze water-insoluble polysaccharides (*e.g.* cellulose) into degradation products (*e.g.* 5-HMF) due to mass transfer limitation.[28-30]

In recent years, metal chlorides were shown effective for transforming saccharides into 5-HMF because they can assist the isomerization of aldose to ketose (*e.g.* glucose to fructose),[31,32] which is a key step for 5-HMF production.[3] In addition, as homogeneous catalysts,[33] metal chlorides possess the potential of being easily separated from reaction products such as 5-HMF through semipermeable membranes. For example, Kotraro *et al.*[34] reported that the use of semipermeable composite membranes for separating organic compounds (molecular weight < 300) such as furan from aqueous inorganic salts (*e.g.* NaCl) containing solutions. Use of $CrCl_3$ for 5-HMF production was reported by various authors. Qi *et al.*[35] reported that 70% glucose was converted into 5-HMF when 99% glucose was degraded with 1.5% (w/w) $CrCl_3$ in ionic liquid solvents. Similarly, Li *et al.*[36] also presented that 0.5% (w/w) $CrCl_3$ led to 60% 5-HMF yield from cellulose substrate in ionic liquid under microwave irradiation. However, with same catalyst ($CrCl_3$) in aqueous phase, the yield of 5-HMF was decreased to around 5%.[37] Catalysis of cellulose by 0.01 M $CrCl_3$ at 180 °C for 120 min led to 5% 5-HMF yield and 30% levulinic acid yield.[37] In addition to $CrCl_3$, several other kinds of metal chlorides were studied for 5-HMF production in aqueous phase. 16.1% 5-HMF yield was achieved when 63% (w/w) $ZnCl_2$ was applied to 5-HMF production from glucose in aqueous phase at 120 °C.[31] The limited 5-HMF yield from saccharides (*e.g.* cellulose and glucose) with $ZnCl_2$ was mainly attributed to the formation of byproduct lactic acid during the process.[38,39] Seri *et al.*[40] reported that a 20% 5-HMF yield was obtained from cellulose using 0.25% (w/w) $LaCl_3$ in aqueous phase at 250 °C within 2.5 min, and 5% levulinic acid was simultaneously formed. De *et al.*[41] applied $AlCl_3$ to catalyze conversion of insulin and starch to 5-HMF. Around 30% 5-HMF yield was obtained from these polysaccharides at 120 °C within 5 min,[41] and byproducts levulinic acid were considered as the cause for limiting the 5-HMF yields.[41] These studies of using transition metal chlorides catalyzed reaction suggested low 5-HMF selectivity due to the formation of undesired byproducts such as levulinic acid[37] and lactic acid.[38] Rasrendra *et al.*[38] reported poor 5-HMF selectivity (around 3–27%) when catalyzing glucose under 140 °C for 6 h. The use of alkali and alkaline earth metal chlorides for 5-HMF production from mono-, oligo-, or polysaccharides were seldom reported. Nevertheless, Liu and Wyman[42] investigated the degradation of xylotriose with 0.8% (w/w) NaCl, $CaCl_2$ and $MgCl_2$ under hot water conditions at 180 °C and found that the rate constants of xylotriose degradation to furfural (k) using $CaCl_2$ and $MgCl_2$ were around 2.5 fold greater than that with water-only treatment. In addition, Sabesan and Spado[43] obtained 66% furfural yield from biomass with 1% (w/w) $CaCl_2$ (160 °C, 30 min, water and THF mixture (1 : 1) system), which was 18% higher than that without $CaCl_2$. The potential of applying alkaline earth metal/alkali metal chlorides on decomposing polysaccharides (*e.g.* agarose) to 5-HMF needs more studies.

Further efforts are needed to realize enhanced selectivity and yield of 5-HMF in aqueous phase using efficient catalysts. The objective of this study was to investigate effects of different groups of metal chlorides, including alkali metal chlorides, alkaline earth metal chlorides, and transition metal chlorides, on the degradation of agarose to 5-HMF, in order to improve 5-HMF yield with less byproducts in aqueous phase using non-food based feedstocks. The mechanism of agarose to 5-HMF with desired metal chlorides was further discussed.

2. Materials and methods

2.1. Feedstocks, catalysts and standards

Agarose (BP160, Fisher Scientific) was purchased from Fisher Scientific, Pittsburgh, PA. Galactan was defined as the unit of agarose and the purity is 98%. The galactan content was determined following the Laboratory Analytical Procedure (LAP) of "Determination of Structural Carbohydrates and Lignin in Biomass".[44] This procedure employed a two-step acid hydrolysis: (1) about 300 mg substrate was placed into a vial and hydrolyzed in 72% (w/w) sulfuric acid at 30 °C for 2 hours; (2) the substrate was further hydrolyzed in 4% (w/w) sulfuric acid at 121 °C for 1 hour. Sugars in the liquid were determined by HPLC. NaCl, $CaCl_2$, $MgCl_2$, $ZnCl_2$, $CrCl_3$, $CuCl_2$ and $FeCl_3$ (Alfa Aesar, Ward Hill, MA) were used as the catalysts for 5-HMF production. All standard chemicals were purchased from Sigma-Aldrich, St. Louis, Mo.

2.2. Experimental methods

Batch tubular reactors (1.27 cm OD × 15.24 cm length, 0.0889 cm wall thickness, Hastelloy C-276) (Swagelok Northwest, Richland, WA) were used in this study. The total volume of each reactor was 13.6 ml with 10 ml of working volume.

0.2 g of substrate was loaded into the batch tubular reactor with 10 ml of water, sulfuric acid (*e.g.* 0.5% (w/w), 1% (w/w), 5% (w/w)) or metal chlorides (*e.g.* 0.5% (w/w), 1% (w/w) or 5% (w/w)), respectively. Reactors were heated to reach the target temperature within 1 min in a 4 kW fluidized sand bath (model SBL-2D, Omega engineering, Inc., Stamford, CT) and treated at target temperatures. The reactions were quenched within 1 min by immersing the batch tubular reactor in cold water bath after being subjected to the target reaction temperature for a specified time. After cooling the tubular reactor, the caps and plugs were removed, samples were pushed out and separated into liquid hydrolyzate and solid residue by vacuum filtration using a 0.22 μm glass fiber filter (Fisher Science, Pittsburgh, PA) for analysis.

2.3. Analytical methods

2.3.1. Liquid analysis

HPLC analysis. Galactose, 5-HMF, levulinic acid, formic acid, glyceraldehyde, dihydroxyacetone and lactic acid in aqueous solution were analyzed through Waters HPLC system (model 2695) equipped with a 410 refractive detector and a Waters 2695 auto-sampler using Waters Empower Build 1154 software (Waters Co., Milford, MA). Bio-Rad Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA) were operated under 65 °C for separation and quantification of compounds. Mobile phase was 0.005 M H_2SO_4 with flow rate of 0.6 ml min^{-1}.

GC-MS analysis. The collected samples were diluted with acetone then analyzed with an Agilent gas chromatography mass spectrometer (GC-MS; GC, Agilent 7890A; MS, Agilent 5975C) equipped with a DB-5MS column (30 m × 250 μm × 0.25 μm).[45] The oven temperature was programmed from 40 °C to 300 °C at a ramping rate of 10 °C min^{-1}. Both the initial and final temperatures were held for 5 minutes. The flow rate of the carrier gas (helium) was 1.3 ml min^{-1}.

2.3.2. Solid analysis. The agarose in solid residue after separation was analyzed based on a standard analysis procedure developed by National Renewable Energy Laboratory (NREL).[44] This procedure was a two-step acid hydrolysis: the sample was first treated with 72% (w/w) H_2SO_4 at 30 °C for 1 h; the reaction mixture was subsequently diluted to 4% (w/w) H_2SO_4 and autoclaved at 121 °C for 1 hour. The sugars in liquid after this two step procedure were then determined by HPLC.

2.4. Calculation

The overall reactions include: galactan (galactan is defined as the unit of agarose) to galactose; galactose to 5-HMF; 5-HMF to levulinic acid and formic acid; galactose to glyceraldehyde, dihydroxyacetone or lactic acid. These reactions can be expressed as eqn (1)–(4), respectively:

$$C_6H_{10}O_5 + H_2O \rightarrow C_6H_{12}O_6 \tag{1}$$

$$C_6H_{12}O_6 \rightarrow C_6H_6O_3 + 3H_2O \tag{2}$$

$$C_6H_6O_3 + 2H_2O \rightarrow C_5H_8O_3 + CH_2O_2 \tag{3}$$

$$C_6H_{12}O_6 \rightarrow 2C_3H_6O_3 \tag{4}$$

The yields of degradation compounds were based on the original amount of galactan. Concentrations (g l^{-1}) of galactose, 5-HMF, levulinic acid, formic acid, glyceraldehyde, dihydroxyacetone and lactic acid measured by HPLC were converted to yields as a percent of the theoretical maximum, as follows:

Galactose yield:

$$Y_{\text{Ga}}\ (\%) = \frac{C_{\text{Ga}} \times \text{MW}_{\text{Gan}}}{C_{\text{Gan}} \times \text{MW}_{\text{Ga}}} \times 100\% \tag{5}$$

5-HMF yield:

$$Y_{\text{HMF}}\ (\%) = \frac{C_{\text{HMF}} \times \text{MW}_{\text{Gan}}}{C_{\text{Gan}} \times \text{MW}_{\text{HMF}}} \times 100\% \tag{6}$$

Levulinic acid yield:

$$Y_{\text{LA}}\ (\%) = \frac{C_{\text{LA}} \times \text{MW}_{\text{Gan}}}{C_{\text{Gan}} \times \text{MW}_{\text{LA}}} \times 100\% \tag{7}$$

Formic acid yield:

$$Y_{\text{FA}}(\%) = \frac{C_{\text{FA}} \times \text{MW}_{\text{Gan}}}{C_{\text{Gan}} \times \text{MW}_{\text{FA}}} \times 100\% \tag{8}$$

Glyceraldehyde yield:

$$Y_{\text{Gly}}\ (\%) = \frac{C_{\text{Gly}} \times \text{MW}_{\text{Gan}}}{C_{\text{Gan}} \times (2 \times \text{MW}_{\text{Gly}})} \times 100\% \tag{9}$$

Dihydroxyacetone yield:

$$Y_{\text{Dih}}\ (\%) = \frac{C_{\text{Dih}} \times \text{MW}_{\text{Gan}}}{C_{\text{Gan}} \times (2 \times \text{MW}_{\text{Dih}})} \times 100\% \tag{10}$$

Lactic acid yield:

$$Y_{\text{Lac}}\ (\%) = \frac{C_{\text{Lac}} \times \text{MW}_{\text{Gan}}}{C_{\text{Gan}} \times (2 \times \text{MW}_{\text{Lac}})} \times 100\% \tag{11}$$

In these equations, C_{Gan} is the initial concentration of galactan; C_{Ga} is the concentration of galactose; C_{HMF} is the concentration of 5-HMF; C_{LA} is the concentration of levulinic acid; C_{FA} is the concentration of formic acid; C_{Gly} is the concentration of glyceraldehyde; C_{Dih} is the concentration of dihydroxyacetone; C_{Lac} is the concentration of lactic acid. MW is the molecular weight: $\text{MW}_{\text{Gan}} = 162$; $\text{MW}_{\text{Ga}} = 180$; $\text{MW}_{\text{HMF}} = 126$; $\text{MW}_{\text{LA}} = 116$; $\text{MW}_{\text{FA}} = 46$; $\text{MW}_{\text{Gly}} = 90$; $\text{MW}_{\text{Dih}} = 90$; $\text{MW}_{\text{Lac}} = 90$.

The agarose conversion was evaluated as below:

$$X = \frac{Y_0 - Y_r}{Y_0} \times 100\% \tag{12}$$

X is the conversion of agarose, Y_0 (g) is the initial weight of agarose, Y_r (g) is the agarose in solid residue.

The selectivity of degradation compounds from agarose was calculated as below:

$$S = \frac{Y}{X} \times 100\% \tag{13}$$

S is the selectivity of product; Y is the yield of product; X is the conversion of agarose.

3. Results and discussion

3.1. Catalytic effects of metal chlorides on the products distribution and 5-HMF yield from agarose

The effects of 0.5–5% (w/w) metal chlorides including alkali metal chlorides (NaCl), alkaline earth metal chlorides ($CaCl_2$ and $MgCl_2$), and transition metal chlorides ($ZnCl_2$, $CuCl_2$, $FeCl_3$

and $CrCl_3$), on the products distribution from 2% (w/w) agarose were investigated at varied temperatures ranging from 180–220 °C. Water-only and dilute sulfuric acid treatments were used as references. The 5-HMF yields from agarose catalyzed by these metal chlorides during the time course of 0–50 min were also investigated. In addition, the 5-HMF selectivity obtained by varied metal chlorides and the reference conditions (water-only and dilute sulfuric acid) was also studied.

3.1.1. Effects of temperature. Fig. 1 shows the effects of 1% (w/w) metal chlorides on the distribution of products decomposed from agarose at 180 °C, 200 °C and 220 °C for 30 min. Transition metal chlorides $ZnCl_2$ and $CrCl_3$ resulted in various compounds such as galactose, 5-HMF, lactic acid, glyceraldehyde and dihydroxyacetone, which was similar to water-only treatment. In addition to these compounds, $CrCl_3$ led to the formation of levulinic acid. Results suggested that transition metal chlorides $ZnCl_2$ and $CrCl_3$ could catalyze the degradation process *via* both reactions of dehydration[18] and retro aldol[38] to these multiple compounds. Rasrendra *et al.*[38] reported that multiple products, including lactic acid and 5-HMF, were observed from the decomposition of glucose in aqueous phase at 140 °C for 6 h using 6.8% (w/w) $ZnCl_2$ and 6.1% (w/w) $CrCl_2$. Levulinic acid yields were reported at 6.1% (w/w) using $CrCl_2$ only. Temperature played an important role in determining the distribution of these degradation compounds from agarose catalyzed by $ZnCl_2$, $CrCl_3$, as well as that under water-only conditions. When temperature was raised from 180 °C to 200 °C, galactose yields decreased sharply from 22.6% to 2.7% with $ZnCl_2$, and from 5.3% to 0.3% with $CrCl_3$, while the yields of 5-HMF catalyzed with $ZnCl_2$ and $CrCl_3$ increased from 15.9% and 9.8% at 180 °C to the highest yields of 20.9% and 12.9% at 200 °C, respectively. The yields of byproducts also increased as temperature was elevated from 180 °C to 200 °C, thus limiting the production of 5-HMF. When the temperature was further raised to 220 °C, 5-HMF yields with $ZnCl_2$ and $CrCl_3$ decreased; the yields of byproducts such as lactic acid declined as well; whereas the yields of glyceraldehyde and dihydroxyacetone continuously increased, which further reduced the 5-HMF yield. The distribution of galactose, 5-HMF, and various byproducts under water-only conditions showed a similar trend as when catalyzed by $ZnCl_2$ and $CrCl_3$ over the temperature range of 180 °C to 220 °C. However, results indicated both slower formation and degradation of such compounds. Other transition metal chlorides used in this study, including $CuCl_2$ and $FeCl_3$, resulted in low yields of galactose (2.2–19.4%) and significant quantity of levulinic acid (27.3–41.2%), but negligible amount of 5-HMF at all tested temperatures. The decomposition of agarose catalyzed by H_2SO_4 showed similar pattern of products distribution compared with $CuCl_2$ and $FeCl_3$: only galactose (0–8.5%) and levulinic acid (38.6–40.8%) were observed at temperatures ranging from 180–220 °C. The formation of diverse byproducts (*e.g.* lactic acid, levulinic acid, *etc.*) catalyzed by transition metal chlorides contributed to the relatively lower or negligible 5-HMF yield. Temperature (180–220 °C) had negligible effects on the types of generated products when using transition metal chlorides for the agarose degradation. However, temperatures influenced the reaction rates of these degradation products, thereby impacting the yields of 5-HMF and overall byproducts.

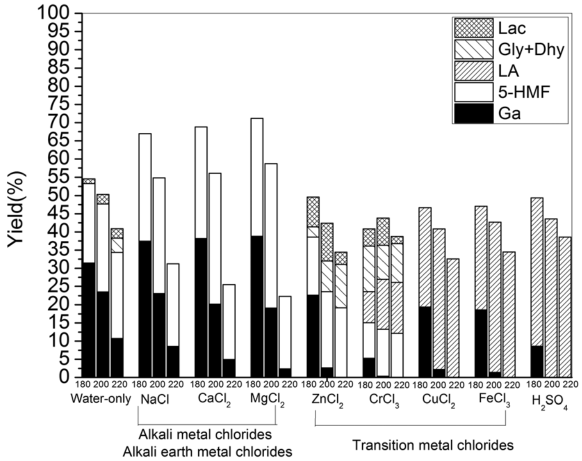

Fig. 1 Distribution of agarose decomposition products by various metal chlorides, water-only, H_2SO_4 treatments under different temperature (180 °C, 200 °C and 220 °C). Conditions: 30 min, 1% (w/w) metal chlorides, water-only or 1% (w/w) H_2SO_4. Lac: lactic acid; Gly: glyceraldehyde; Dhy: dihydroxyacetone; LA: levulinic acid; 5-HMF: 5-hydroxymethylfurfural; Ga: galactose.

Addition of alkali and alkaline earth metal chlorides NaCl, $CaCl_2$ and $MgCl_2$ merely led to 5-HMF and galactose at all tested temperatures. The distribution of galactose and 5-HMF decomposed from agarose catalyzed by NaCl, $CaCl_2$ and $MgCl_2$ over the three temperatures (180 °C, 200 °C and 220 °C) followed a similar trend. At 180 °C, 29.3–32.5% 5-HMF and 37.5–38.8% galactose yields were obtained with these alkali and alkaline earth metal chlorides. As temperature increased to 200 °C, 5-HMF yield increased to 31.7–39.5%, while the galactose yield decreased to 19.1–23.1%. Yields of 5-HMF and galactose sharply decreased to 19.7–22.5% and 2.4–8.5%, respectively, when temperature was further raised to 220 °C. The reduced yield of 5-HMF could be attributed to the formation of humins, which were observed by Patil and Lund[46] as the decomposed product of 5-HMF under 135 °C in aqueous phase. The highest 5-HMF yields obtained with NaCl, $CaCl_2$ and $MgCl_2$ were 31.7%, 35.8% and 39.6% at 200 °C, respectively.

3.1.2. Effects of catalyst loading. The distribution of degradation products from agarose with metal chlorides was also investigated under different catalyst loadings. The effects of concentrations of the metal chlorides (0.5% w/w, 1% w/w and 5% w/w), including NaCl, $CaCl_2$, $MgCl_2$, $ZnCl_2$, $CrCl_3$, $CuCl_2$ and $FeCl_3$, as well as H_2SO_4 treatment (0.5% w/w, 1% w/w and 5% w/w) on degradation of agarose at 200 °C for 30 min are shown in Fig. 2. Only galactose and 5-HMF were obtained from agarose using alkali and alkaline earth metal chlorides (NaCl, $CaCl_2$ and $MgCl_2$) over the three catalyst loadings (0.5% w/w, 1% w/w and 5% w/w). Galactose yields decreased with the elevated loading of these alkali and alkaline earth metal chlorides. 5-HMF yields from agarose using these three catalysts increased to the highest value, which were 31.7%, 35.8%, 39.6%, respectively, when loading was increased from 0.5% (w/w) to 1% (w/w). As the catalyst loading was further increased to 5% (w/w), the 5-HMF yield decreased sharply.

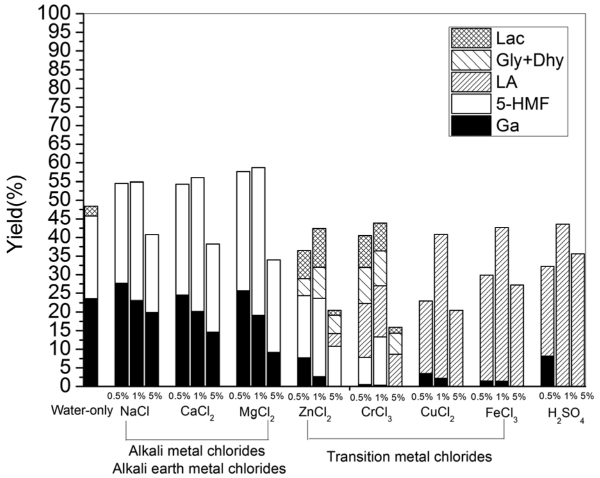

Fig. 2 Distribution of agarose decomposition products by various metal chlorides, water-only and H_2SO_4 treatments under different loading (0.5% (w/w), 1% (w/w), 5% (w/w)). Conditions: 200 °C for 30 min. Lac: lactic acid; Gly: glyceraldehyde; Dhy: dihydroxyacetone; LA: levulinic acid; 5-HMF: 5-hydroxymethylfurfural; Ga: galactose.

Transition metal chlorides with loadings of 0.5% (w/w), 1% (w/w) and 5% (w/w) led to various kinds of byproducts. Besides galactose and 5-HMF, 0.5% (w/w) transition metal chlorides $ZnCl_2$ and $CrCl_3$ generated byproducts such as lactic acid, glyceraldehyde, and dihydroxyacetone. Moreover, levulinic acid was also observed with 0.5% (w/w) $CrCl_3$. The increased catalyst loading presented insignificant impact on the types of compounds generated from agarose, whereas had significant influence on their yields. Galactose yield was reduced sharply from 0.5–7.7% to negligible amounts as the loading of $ZnCl_2$ and $CrCl_3$ was raised from 0.5% (w/w) to 5% (w/w). In contrast, the yields of 5-HMF and other products catalyzed by $ZnCl_2$ and $CrCl_3$ increased when the catalyst loading was enhanced from 0.5% (w/w) to 1% (w/w), and reached their highest yields. 1% (w/w) loading of $ZnCl_2$ and $CrCl_3$ resulted in the highest 5-HMF yields of 19.0% and 12.9%, respectively. Further increasing the catalyst loading from 1% (w/w) to 5% (w/w) led the yields of 5-HMF and those byproducts to decline significantly. Other transition metal chlorides $CuCl_2$ and $FeCl_3$ as well as H_2SO_4 merely resulted in less than 3.5% yield of galactose and a considerable amount of levulinic acid (19.5–41.3% yield) over the three tested catalyst loadings (*i.e.* 0.5%, 1% and 5% w/w), while 5-HMF was negligible. It was found that the loading of metal chlorides and H_2SO_4 had a minor impact on the variety of degradation compounds from agarose. However, the catalyst loading had evident effects on the distribution of these degradation compounds, thereby leading to different 5-HMF yield.

3.1.3. Effects of reaction time. The time courses of 5-HMF yields from agarose by 1% (w/w) metal chlorides, water-only and 1% (w/w) H_2SO_4 at 200 °C for 0–50 min are shown in Fig. 3. Results showed that catalytic reaction with transition metal chlorides $CuCl_2$ and $FeCl_3$ led to similar trends as 1% (w/w) H_2SO_4, and the highest yield of around 5% 5-HMF was obtained at 5 min, and then rapidly decreased. 5-HMF yields with transition metal chlorides $ZnCl_2$ and $CrCl_3$ were relatively higher, which were 22.6% at 25 min and 12.9% at 30 min, respectively. However, similar to $CuCl_2$ and $FeCl_3$, the 5-HMF yield catalyzed by $ZnCl_2$ and $CrCl_3$ declined sharply as reaction time was prolonged. These observations suggested that transition metal chlorides catalyzed 5-HMF production were sensitive to reaction time, which required the reaction system with better control of residence time (*e.g.* flowthrough reactor).

5-HMF yields catalyzed by alkali and alkaline earth metal chlorides NaCl, $CaCl_2$, $MgCl_2$, and water-only treatments increased steadily over time, then declined slowly at around 35–40 min. The highest yields of 5-HMF catalyzed by NaCl, $CaCl_2$ or with water-only treatment were 32.8%, 37.2% and 27.4% at 40 min, respectively. $MgCl_2$ resulted in the highest yield of 5-HMF 40.7% at 35 min. Results implied that alkali and alkaline earth metal chlorides (NaCl, $CaCl_2$ and $MgCl_2$) behaved with suitable catalytic activity for the conversion of agarose to 5-HMF, while simultaneously maintaining the relative stability of 5-HMF as reaction prolonged.

3.1.4. Comparison of the highest 5-HMF yield with varied metal chlorides and the corresponding selectivity. 5-HMF yields catalyzed by metal chlorides (NaCl, $CaCl_2$, $MgCl_2$, $ZnCl_2$, $CrCl_3$, $CuCl_2$ and $FeCl_3$) were optimized under temperature 180–220 °C, time 0–50 min and catalyst loading 0.5–5% (w/w), respectively. Table 1 summarized and compared the highest 5-HMF yields obtained with varied metal chlorides, water-only and H_2SO_4 treatment. In addition, the corresponding 5-HMF and byproducts selectivity as well as the agarose conversion were also listed and discussed.

Results (Table 1) demonstrated that addition of alkali and alkaline earth metal chlorides (NaCl, $CaCl_2$ and $MgCl_2$) led to higher 5-HMF yields than transition metal chlorides ($ZnCl_2$, $CrCl_3$, $CuCl_2$, $FeCl_3$), water-only and H_2SO_4. Particularly, $MgCl_2$ resulted in the highest 5-HMF yield of 40.7%. The relatively higher 5-HMF yields with alkali and alkaline earth metal chlorides (NaCl, $CaCl_2$ and $MgCl_2$) compared with transition metal chlorides ($ZnCl_2$, $CrCl_3$, $CuCl_2$, $FeCl_3$), water-only and H_2SO_4 could be attributed to fewer byproducts formed under tested conditions. The corresponding 5-HMF selectivity with varied

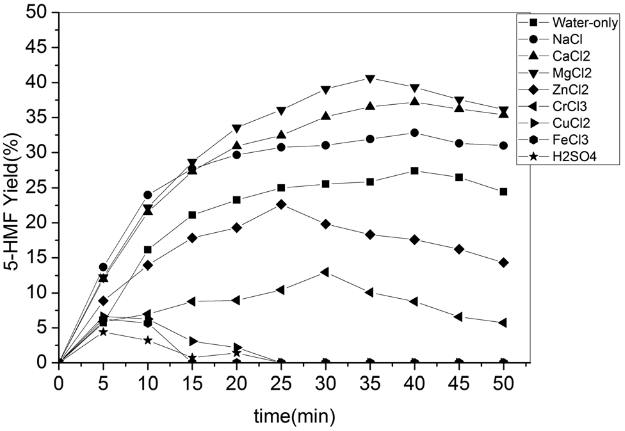

Fig. 3 Time course of 5-HMF yield decomposed from agarose by water-only, 1% (w/w) dilute sulfate acid and 1% (w/w) various metal chlorides treatments at 200 °C.

Table 1 The highest 5-HMF yield and product selectivity from agarose degradation by various metal chlorides, H_2SO_4 and water-only treatment[b]

				Selectivity				
Catalyst	Conditions[a]	5-HMF yield (%)	Agarose conv. (%)	5-HMF	Ga	LA	Gly + Dhy	Lac
$MgCl_2$	200 °C/1% (w/w)/35 min	40.7	82.9	49.1	20.5	0	0	0
$CaCl_2$	200 °C/1% (w/w)/40 min	37.2	85.3	43.6	21.1	0	0	0
NaCl	200 °C/1% (w/w)/40 min	32.8	81.1	40.5	25.2	0	0	0
None	220 °C/1% (w/w)/20 min	29.5	79.3	37.2	20.5	0	0	6.2
$ZnCl_2$	200 °C/1% (w/w)/25 min	22.6	92.1	24.5	4.2	0	12.1	15.8
$CrCl_3$	200 °C/1% (w/w)/30 min	13.0	100	13.0	0.5	13.7	9.4	7.5
$CuCl_2$	200 °C/0.5% (w/w)/10 min	8.2	91.6	9.0	31.9	20.4	0	0
$FeCl_3$	200 °C/0.5% (w/w)/10 min	7.7	93.8	8.2	29.1	26.9	0	0
H_2SO_4	200 °C/0.5% (w/w)/5 min	7.1	90.3	7.9	28.2	22.7	0	0

[a] Conditions to reach the highest 5-HMF yields. [b] Gal: galactose; LA: levulinic acid; Gly: glyceraldehyde; Dhy: dihydroxyacetone; Lac: lactic acid.

metal chlorides listed in Table 1 suggested that alkali and alkaline earth metal chlorides (NaCl, $CaCl_2$ and $MgCl_2$) with fewer byproducts resulted in higher 5-HMF selectivity than transition metal chlorides ($ZnCl_2$, $CrCl_3$, $CuCl_2$, $FeCl_3$), water-only and H_2SO_4. 5-HMF selectivity obtained using $MgCl_2$ resulted in the highest value of 49.1%. Such selectivity was comparable to the 5-HMF selectivity (40–53%) obtained from glucose in acidic biphasic systems, as reported by Dumesic and coworkers.[47] In comparison, the considerable selectivity for byproducts (*e.g.* lactic acid) formed *via* retro–aldol reaction could serve as a possible explanation of the relatively low 5-HMF selectivity from water-only or $ZnCl_2$ treatment. For example, lactic acid selectivity for $ZnCl_2$ catalyzed reactions was 15.8%, while the corresponding 5-HMF selectivity was merely 24.5%. Using $CuCl_2$, $FeCl_3$ and H_2SO_4 led to 20.4–26.9% selectivity toward levulinic acid through further rehydration of 5-HMF, while their selectivity toward 5-HMF didn't exceed 9.0%. $CrCl_3$ resulted in all byproducts listed in Table 1 with the selectivity ranging from 7.5–13.7% for each compound, consequently leading to 13.0% 5-HMF selectivity.

However, the galactose selectivity for alkali and alkaline earth metal chlorides (NaCl, $CaCl_2$ and $MgCl_2$) catalyzed reaction was maintained on a relatively high level (20.5–25.2%) compared with some transition metal chlorides (*e.g.* $CrCl_3$) (0.5%), thus reducing the 5-HMF selectivity. Furthermore, the limited 5-HMF selectivity could be also a consequence of the formation of aforementioned (Section 3.1.1) insoluble humins.[46]

The agarose conversion with alkali and alkaline earth metal chlorides (NaCl, $CaCl_2$ and $MgCl_2$), transition metal chlorides ($ZnCl_2$, $CrCl_3$, $CuCl_2$ and $FeCl_3$) were obtained 81.1–100% (Table 1) under conditions for achieving the highest 5-HMF yield, which demonstrated the agarose's susceptibility to decomposition with both alkali and alkaline earth metal chlorides (NaCl, $CaCl_2$ and $MgCl_2$) and transition metal chlorides ($ZnCl_2$, $CrCl_3$, $CuCl_2$ and $FeCl_3$). Different from agarose, when using metal chlorides to hydrolyze cellulose in aqueous phase under 180 °C over 120 min, alkali and alkaline earth metal (*e.g.* NaCl, $CaCl_2$ and $MgCl_2$) showed negligible catalytic ability on cellulose conversion while transition metal chlorides (*e.g.* $CrCl_3$, $FeCl_3$ and $CuCl_2$) converted cellulose efficiently.[37]

3.2. Kinetic analysis of 5-HMF formation from agarose with $MgCl_2$

$MgCl_2$ appeared to result in the highest 5-HMF yield and selectivity among various tested metal chlorides under experimental conditions. A kinetic analysis of 5-HMF production from agarose was performed with 1% (w/w) $MgCl_2$ at a temperature range of 180–220 °C within 50 min.

A pseudo-homogeneous irreversible first order reaction model[48] was used to describe the degradation of agarose to 5-HMF with low substrate concentration (2% (w/w)). This model was also used to describe cellulose (0.8–2.4% (w/w)) degradation to 5-HMF and levulinic acid in aqueous phase with dilute acid.[22]

Results indicated that when agarose was decomposed with $MgCl_2$, 5-HMF and galactose were major products without formation of other byproducts, such as levulinic acid, lactic acid, *etc.* Therefore, the reaction pathway of agarose decomposition in $MgCl_2$ solution can be simply summarized as follows:

$$\text{Agarose} \xrightarrow{k_1} \text{Galactose} \xrightarrow{k_2} \text{5-HMF} \xrightarrow{k_3} \text{Degradation products } (e.g.\ \text{Humins})$$

Based on the reaction pathway, the kinetic model equations were developed to describe the agarose degradation process as follows:

$$dA/dt = -k_1 A \tag{14}$$

$$dG/dt = k_1 A - k_2 G \tag{15}$$

$$dM/dt = k_2 G - k_3 M \tag{16}$$

where A (glucan equivalents, g), G (glucan equivalents, g) and M (glucan equivalents, g) represent the weight of agarose, galactose and 5-HMF, respectively. k_1 (min^{-1}), k_2 (min^{-1}) and k_3 (min^{-1}) are rate constants of agarose hydrolyzed into galactose, galactose dehydrated into 5-HMF, and 5-HMF condensed to humins, respectively. Table 2 shows the value of the rate constants increased with reaction temperature. The rate constant k_3 was lower than both k_1 and k_2 over the tested temperatures (180–220 °C), which was especially apparent at 200 °C (*i.e.* $k_3 = 0.0399$ min^{-1}, $k_1 = 0.0937$ min^{-1}, $k_2 = 0.0729$ min^{-1}).

Results implied that the formation of 5-HMF was much more feasibly than its degradation under such conditions, thus resulted in 5-HMF yield maintaining at a relatively high level (*e.g.* 40.7%). The Arrhenius equation was also established:

$$k = A\exp(-E_a/RT) \tag{17}$$

where k (min^{-1}) is the rate constant, A (min^{-1}) is the pre-exponential factor, E_a (kJ mol^{-1}) is the activation energy, R is universal gas constant (8.3143×10^{-3} kJ mol^{-1} K^{-1}), T (K) is temperature.

The activation energy values of the degradation of agarose, galactose and 5-HMF calculated based on eqn (17) were 67.5 kJ mol^{-1}, 104.6 kJ mol^{-1} and 105.6 kJ mol^{-1}, respectively. The lower activation energy for agarose degradation compared with its monomer and 5-HMF indicated the requirement of relatively lower temperature for agarose degradation in 1% (w/w) $MgCl_2$, which consequently could be favorable for the stability of the released 5-HMF.

3.3. Postulated mechanism of agarose to 5-HMF catalyzed by $MgCl_2$

In order to gain more insights into the mechanism of 5-HMF formation from agarose with $MgCl_2$ treatment, the formation of degradation products derived from $MgCl_2$ treatments (200 °C, 1% (w/w), 35 min) was further verified by GC-MS analysis.

The GC-MS spectra showed that 5-HMF was the dominant compound from agarose catalyzed by $MgCl_2$, and no levulinic acid from further rehydration of 5-HMF or compounds from retro aldol of galactose were detected (Fig. 4). Trace amount of furyl hydroxymethyl ketone ($C_6H_6O_3$), an isomer of 5-HMF was observed. Formation of this isomer can be explained as a C3 carbonyl and C6 hydroxy group intermolecular closure, while the formation of 5-HMF is caused by the pathway of C2 and C5 ring closure.

Table 2 Rate constant of each degradation step of agarose with 1% (w/w) $MgCl_2$[a]

	Temperature (°C)		
Parameters	180	200	220
k_1 (min^{-1})	0.0426	0.0937	0.1822
k_2 (min^{-1})	0.0327	0.0729	0.3133
k_3 (min^{-1})	0.0145	0.0399	0.1416
$R_{\mathrm{Aga}}{}^2$	0.977	0.959	0.961
$R_{\mathrm{Ga}}{}^2$	0.947	0.972	0.969
$R_{\mathrm{5\text{-}HMF}}{}^2$	0.976	0.960	0.944

[a] Aga: agarose; Ga: galactose.

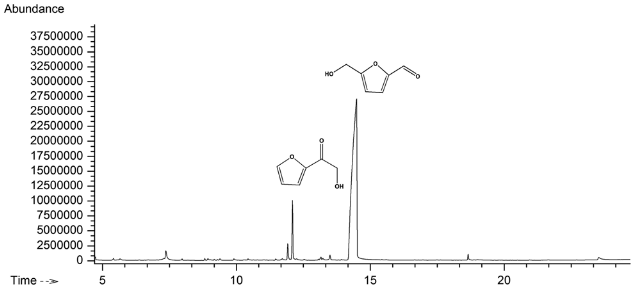

Fig. 4 Product profiles in hydrolysates from treatments with 1% (w/w) $MgCl_2$ at 200 °C for 35 min analyzed by GC-MS.

The reaction mechanism for $MgCl_2$ catalyzing agarose to 5-HMF in aqueous phase is proposed in Fig. 5. It can be anticipated that Mg^{2+} ions ($MgCl_2$) would be able to coordinate with the oxygen atom of C–O–C bond and subsequently catalyze the cleavage of the C–O–C bond acting as Lewis acid. Coordination of Mg^{2+} ions ($MgCl_2$) to the oxygen atom of C–O–C in sugars (*e.g.* sucrose) in aqueous phase has also been supported with IR and ^{13}C NMR spectra.[49] Once agarose is degraded into galactose, magnesium ions from $MgCl_2$ coordinate with oxygen atom of the hemiacetal portion of galactose and the closest hydroxyl group to form an enediol intermediate. This leads to the isomerization of galactose to its ketose form, which can be easily dehydrated to 5-HMF. Although other alkali and alkali earth metal ions, including Na^+ ions and Ca^{2+} ions, appeared to interact with oxygen atom in sugar (*e.g.* oxygen atom of C–O–C bond),[49] thereby enhancing the agarose to 5-HMF reactions as compared to water-only operations, Mg^{2+} ions may possess better activity due to its higher charge density (charge-to-size ratio) than Na^+ ions and Ca^{2+} ions.[49]

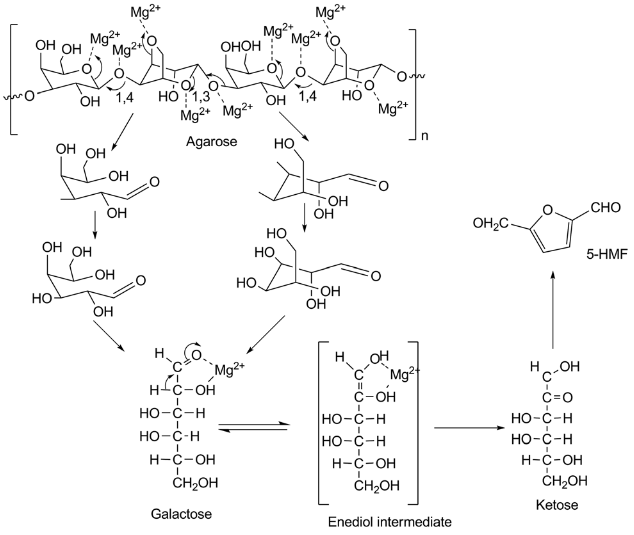

Fig. 5 Proposed reaction mechanism for Mg^{2+} catalyzing the decomposition of agarose to 5-HMF.

Results implied that Mg^{2+} ions under the tested conditions possess suitable properties for 5-HMF production: Mg^{2+} ions can promote the cleavage of C–O–C bond of agarose and enhance the subsequent isomerization of galactose to its ketose. Additionally, our results also suggested that Mg^{2+} ions have little ability to induce the retro aldol reaction of galactose or further rehydration of 5-HMF to levulinic acid, *etc.*, thereby maintaining a relatively higher 5-HMF yield with few byproducts (as depicted in Fig. 4).

4. Conclusion

Metal chlorides had different effects on catalyzing agarose degradation in aqueous phase. The addition of alkali and alkaline earth metal chlorides was found to result in the formation of only galactose and 5-HMF while the addition of transition metal chlorides led to the formation of various degradation products, including levulinic acid, lactic acid, *etc.* Although temperature and catalyst loading had significant effects on reaction rates and the products distribution, the variety of agarose degradation products catalyzed by these metal chlorides was dependent on the intrinsic properties of the catalysts used. Alkali and alkaline earth metal chlorides NaCl, $CaCl_2$ and $MgCl_2$ resulted in higher 5-HMF yields and selectivity than transition metal chlorides $ZnCl_2$, $CuCl_2$, $FeCl_3$ and $CrCl_3$, water-only or dilute sulfuric acid treatments. The addition of 1% (w/w) $MgCl_2$ was the most favorable additive among the tested metal chlorides, resulting in 40.7% of the 5-HMF yield and 49.1% 5-HMF selectivity at 200 °C within 35 min. The mechanism of the $MgCl_2$ catalyzed reaction of agarose to 5-HMF was proposed to proceed through assisting the cleavage of C–O–C bond of agarose and accelerating the subsequent isomerization of galactose to its ketose. The specific ability of $MgCl_2$ in aqueous phase is thought to be the driving force for favoring the 5-HMF formation, while diminishing the generation of undesired byproducts such as levulinic acid, lactic acid, *etc.*

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