Selective Conversion of Hemicellulose in Macroalgae *Enteromorpha prolifera* to Rhamnose

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Supporting Information

**ABSTRACT:** Direct hydrothermal conversion (HC) of macroalgae *Enteromorpha prolifera* was conducted over the temperature range of 140−240 °C. At 160 °C, monosaccharides and small molecular acids began to generate. A high yield (18.8%) of monosaccharides was obtained at 180 °C, whereas 29.6% of small molecular organic acids was attained at 200 °C. Formic acid (FA) was then employed as a catalyst, which could selectively catalyze the conversion of hemicellulose at low temperature (94.1%, 140 °C). Rhamnose (45.2%) based on the mass of carbohydrates in *E. prolifera* was produced by the catalysis of 0.7 mL of FA (160 °C, 60 min, 1 g of biomass loading). A low ratio of biomass amount to water was beneficial to the solution of water-soluble components of hemicellulose in *E. prolifera* to get high yields to monosaccharides. HC showed promise to be an applicable and efficient method in the treatment of *E. prolifera* with high conversion of carbohydrates.

**INTRODUCTION**

The rapidly growing demand for fossil fuels and related environmental pollution had raised great concern on the exploitation of green and sustainable resources. Enormous amount of works has been devoted to the utilization of biomass and their derivatives, including the production of chemicals, bio-oils, and bio-based materials. 1−3 Algae as renewable resources were considered to be a promising alternative to replace the fossil resources. 4 The green tide broken out in China’s Yellow Sea near Qingdao in recent years formed a huge number of drifting *Enteromorpha prolifera*, one kind of macroalgae, which flocked and then floated to other maritime spaces, then became barrier, and soon began to decay. The large-scale outburst of *E. prolifera* did harm not only to the coastal ecological environment but also to the seawater quality and human health, which had a strong impact on the local tourism and influenced the economic and ecological balance. 5,6 The effective utilization of *E. prolifera* could not only remit the pressure of environment but also turn waste into wealth. *E. prolifera* had the advantages of no occupation for land resources, no request for fresh water, and so on compared with the terrestrial biomass, as described in the references. 7,8 Moreover, *E. prolifera* was rich in polysaccharides, 9 which was good for the production of value-added chemicals or bio-alcohols. As a new resource and environmental pollutant, *E. prolifera* should be efficiently utilized, which was of great practical significance.

A great number of studies have been focused on the thermochemical conversion (pyrolysis and liquefaction) of algae. 10−12 Pyrolysis characteristics and kinetics of *E. prolifera* were studied, and the results indicated that three stages of dehydration, primary devolatilization, and residual decomposition appeared during pyrolysis, and this kind of alga was suitable for pyrolysis. 13 Similar results were found by other researchers. 14,15 Fast pyrolysis of *E. prolifera* for bio-oil production was conducted in a free-fall reactor at different temperatures ranging from 100 to 750 °C, and the average heat value of the bio-oil obtained was 25.33 MJ/kg, with a higher oxygen content of 30.27 wt %. 5 Hydrothermal liquefaction of *E. prolifera* was investigated at 300 °C, for 30 min with 5 wt % Na2CO3 added, and the highest bio-oil yield was 23.0 wt %. 16 The results showed that the bio-oil was complex in composition and could potentially produce possible value-added chemicals. 16 Another work used alcohols as a solvent to...
produce ester compounds from *E. prolifera* by liquefaction. The bio-oil contained a considerable fraction of light components of biodiesel but still had high oxygen content. Thus, further separation and refining of bio-oil were necessary.\(^{17}\)

Fewer studies have focused on the hydrothermal conversion (HC) of *E. prolifera* to produce value-added chemicals, taking the advantage of the high oxygen content of the material itself. Monosaccharides are expected as renewable platform chemicals, which are versatile precursor to biodegradable plastics, chemicals, fuels, and medicine.\(^{18,19}\) Rhamnose is of great importance in cosmetics industry. For example, in skincare, the primary implication of rhamnose is to form rhamnolipids, which serve as a good emulsifier and surfactant. Rhamnolipids could also be used in antiaging and antiwrinkle products.\(^{20,21}\)

The conversion of macroalgae to rhamnose is a promising process for the efficient utilization of harmful marine algae, which could be also regarded as a sustainable and important source. Fractional hydrothermal of biomass was explored in our previous work.\(^{22}\) Different components in material had different solubilities in water or organic solvent. Nearly, all of the cellulose in the selected biomass types were dissolved and degraded into oligomers with molecular weight of 200–400 Da under the NaCl–H\(_2\)O system. Another work about selective conversion of lignin in corncob residue to monophenols with high yield and selectivity showed the possibility of fractional HC of actual biomass.\(^{23}\)

Here in, the study on the hydrothermal process of *E. prolifera* was conducted from 140 to 240 °C. The effect of different temperatures on the distribution of liquid products and the property of solid residue was discussed. Formic acid (FA) was employed as a catalyst in the present system to catalyze the conversion of *E. prolifera* to produce monosaccharides because carbohydrate biomass could produce FA with high yield as reported.\(^{24,25}\) Thus, the use of FA might inhibit the further decomposition of monosaccharides and realize the in situ catalytic process. The reaction temperature and time, effect of dosage of FA, and the biomass loading on the production of monosaccharides were also discussed. We attempted to search a rather splendid method for the utilization of *E. prolifera* and produce more value-added chemicals.

**RESULTS AND DISCUSSION**

**Characterization of *E. prolifera*.** The contents of different elements in *E. prolifera* obtained were the followings: carbon 32.44%, hydrogen 5.36%, and nitrogen 0.91%. Others\(^{a}\) is mainly composed of oxygen and ash, which include inorganic compounds as confirmed by ICP (Al: 1.14 wt %; Ca: 9.92 wt %; Mg: 4.62 wt %; Na: 1.79 wt %; and Fe: 1.49 wt %). A small amount of lipids 1.5% was extracted from the material, and a lower content of proteins (5.7%) was calculated by the nitrogen content from elemental analysis correspondingly. The most abundant component in *E. prolifera* was carbohydrates (42.4%), which then subdivided into hemicellulose (23.1%), cellulose (13.8%), and some water-soluble carbohydrates. Others\(^{b}\) includes vitamin, amino acid, and other bioactive components in *E. prolifera*. Ash (25.1%) and volatiles (62%) were also detected. These results showed that *E. prolifera* was lipid less and with high percentage of carbohydrates (Table 1). Thus, further study was performed to convert the carbohydrates in order to obtain monosaccharides.

**Conversion of Hemicellulose and Cellulose without and with FA.** The HC of *E. prolifera* without FA catalyst was conducted at 140–240 °C as a control group. The residue obtained was analyzed using chemical titration and element analysis. The conversion of hemicellulose and cellulose and the yield of residue after reaction were first investigated. As shown in Figure 1, the conversion of both hemicellulose and cellulose augmented with the increase of temperature. As for hemicellulose, a higher conversion (70.8%) was achieved at a relatively lower temperature of 140 °C and it changed in a small scale (70.8–92.9%) when the temperature increased from 140 to 240 °C, which demonstrated that HC was suitable for the conversion of hemicellulose at relatively low temperature. The conversion of cellulose was much lower than that of hemicellulose at lower temperature (140–200 °C; 20.7–58.6%), but it increased obviously from 58.6 to 91.9% with temperature rising from 200 to 240 °C. The increase of temperature led to great decomposition of cellulose, especially when the temperature was higher than 200 °C. It was shown that a higher temperature was required to reach a higher conversion of cellulose. Nevertheless, 92.9% of hemicellulose and 91.9% of cellulose were converted at 240 °C. The fractional conversion of hemicellulose and cellulose would be realized by controlling temperature, that is, lower temperature was beneficial to the conversion of hemicellulose. The residue deceased from 41.6 to 34.5% and from 140 to 160 °C and

![Figure 1](https://example.com/figure1.png)

**Table 1. Characteristics of *E. prolifera***

| elemental analysis (wt %) | biochemical composition (wt %) | proximate analysis (wt %) |
|--------------------------|--------------------------------|--------------------------|
| C                        | carbohydrates                 | moisture                 |
| 32.44%                   | 42.4%                         | 4.1                      |
| H                        | proteins                      | 5.7                      |
| 5.36%                    | 5.7%                          | 13.8                     |
| N                        | lipids                        | 1.5                      |
| 0.91%                    | 1.5%                          | 3.3                      |
| others\(^{a}\)           | others\(^{b}\)                | ash                      |
| 60.88%                   | 23.5%                         | 25.1                     |

\(^{a}\)Calculated by difference, others = 100 − C − H − N. \(^{b}\)Others = 100 − carbohydrates − lipids − proteins − moisture − ash. FC = 100 − moisture − volatiles − ash.

\(\text{HC} = 100 \times \text{HC of actual biomass}\)
hemicellulose was maintained at a relatively high level (94%) from 140 to 180 °C, whereas the conversion of cellulose increased from 6 to 48.3%. When temperature rise to 150 °C, glucose derived mainly from cellulose could be observed. It could be found that the rise of temperature leads to the increase of conversion of cellulose, as mentioned in the HC of *E. prolifera* without FA. Compared with the result of reaction without FA at 140 °C, the conversion of hemicellulose increased from 70.8 to 94.1%, which indicated that the conversion of hemicellulose was promoted by FA. FA could catalyze the conversion of hemicellulose selectively at relativity low temperature. When increasing the temperature to 160 °C, the conversion of hemicellulose increased from 88.1 to 97.1%, whereas the conversion of cellulose varied from 22.7 to 26.4%. At 180 °C, the conversion of cellulose was further increased to 48.3%, which is higher than that without FA (36.5%). The reason for the high conversion of hemicellulose might be ascribed to the H⁺ in water formed by the ionization of FA, which would attack the O atom in glycosidic bond and then the O atom was protonated. The positive charge was transferred to C atom along with the broken of C–O bond. After then, the C⁺ was attacked by H₂O and an OH⁻ was obtained to form new polysaccharides, with the release of H⁺. This process was circulated, and the hemicellulose was gradually degraded.²⁸,²⁹ As for cellulose, when the reaction temperature was above 160 °C, the acidic environment formed by FA could also promote the conversion of it. Overall, the total conversion of carbohydrates increased with the presence of FA.

**Formation of Monosaccharides in Liquid Products.**

The amounts of monosaccharides and small molecular weight products without FA as catalyst are listed in Table 3. The yield of glucose, xylose, and rhamnose was first increased and then decreased with rising temperature. No glucose was detected at 140 and 160 °C. As the temperature rise to 180 °C, the amount of rhamnose increased from 1.58 to 10.92%. Also, the amount of xylose increased to 4.98%, and 2.87% of glucose was obtained. The formation temperature of glucose was higher than that for xylose and rhamnose. More monosaccharides (18.77%) were obtained at 180 °C. It was demonstrated that 180 °C was suitable for the production of monosaccharides.

Further increase of temperature to 200 °C, the amount of xylose and glucose decreased and rhamnose was not detected. Glucose was not detected above 220 °C, and when the temperature increased to 240 °C, none of the monosaccharides was detected. These could be ascribed to the fact that higher temperature promoted the conversion and/or decomposition of monosaccharides to form a variety of products, including hydroxymethyl furfural (HMF), acetic acid (AA), lactic acid, and so on. Among these monosaccharides, rhamnose obtained at 180 °C (10.92%) was the most abundant among the monosaccharides in the absence of FA catalyst.

The HC of *E. prolifera* with FA as a catalyst was conducted at different temperatures with 0.7 mL of FA. The amounts of monosaccharides obtained are shown in Table 4. As could be seen, the addition of FA greatly promoted the generation of rhamnose. At 140 °C, 4.9% of xylose and 18.8% of rhamnose were obtained, pretty higher than those obtained without FA. When temperature increased, the yield to monosaccharides increased. The highest yield of 41.7% rhamnose was obtained at 160 °C, whereas there was only 5.22% of rhamnose obtained without FA. The variation of xylose and rhamnose behaved coincidently. Higher temperature (180 °C) leads to further conversion of xylose and rhamnose, resulting in decreased

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**Table 2. Conversion of Hemicellulose and Cellulose with a FA Catalyst**

| Temperature (°C) | Hemicellulose (%) | Cellulose (%) | Residue (%) |
|------------------|-------------------|---------------|-------------|
| 140              | 94.1              | 6.0           | 38.0        |
| 150              | 94.5              | 12.2          | 36.4        |
| 160              | 97.1              | 26.4          | 33.0        |
| 180              | 97.5              | 48.3          | 27.5        |

*Reaction conditions: 0.7 mL of FA; 60 min; 3 g of biomass.*

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**Table 3. Amounts of Monomers in Liquid Products without FA**

| Temperature (°C) | Glu (%) | Xyl (%) | Rha (%) | Lac (%) | FA (%) | AA (%) | HMF (%) | Furfural (%) | Yields (%) |
|------------------|---------|---------|---------|---------|--------|--------|---------|-------------|------------|
| 140              | 0.00    | 0.11    | 1.58    | 0.00    | 2.02   | 0.00   | 0.00    | 0.00        | 3.71       |
| 160              | 0.00    | 1.89    | 5.22    | 0.00    | 10.45  | 0.00   | 0.13    | 0.18        | 17.87      |
| 180              | 2.87    | 4.98    | 10.92   | 0.00    | 20.55  | 1.61   | 0.74    | 1.99        | 43.66      |
| 200              | 1.34    | 0.65    | 6.29    | 0.00    | 17.93  | 5.34   | 4.46    | 1.54        | 37.55      |
| 220              | 0.00    | 0.46    | 0.00    | 6.80    | 12.85  | 4.10   | 4.53    | 0.84        | 29.58      |
| 240              | 0.00    | 0.00    | 0.00    | 9.13    | 7.58   | 2.17   | 0.46    | 0.39        | 19.72      |

*All yields were calculated based on the carbohydrates in *E. prolifera* Reaction conditions: 100 mL of water; 60 min; 3 g of biomass. Glu: glucose; Xyl: xylose; Rha: rhamnose; Lac: lactic acid; FA: formic acid; AA: acetic acid; HMF: hydroxymethyl furfural.*

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**Table 4. Amounts of Monosaccharides in Liquid Products with FA**

| Temperature (°C) | Glu (%) | Xyl (%) | Rha (%) |
|------------------|---------|---------|---------|
| 140              | n.d.    | 4.9     | 18.8    |
| 150              | 2.7     | 7.5     | 31.0    |
| 160              | 11.6    | 9.4     | 41.7    |
| 180              | 17.6    | 6.0     | 14.1    |

*Reaction conditions: 0.7 mL of FA; 60 min; 3 g of biomass. nd, not detected.*
Further studies about the conversion of cellulose were quite low under these conditions. Nevertheless, the monosaccharides increased from 26.4 to 48.3%, whereas the extension of reaction time did not affect the conversion of hemicellulose. Monosaccharides (18.8%) were obtained when the minimum mass of biomass was used. The yield of glucose, xylose, and rhamnose was increased with the addition of FA. Further studies about the conversion of hemicellulose at low temperature were mainly xylose and rhamnose. The yield to xylose and rhamnose increased with the augment of FA added. It was ascribed to the acidic environment caused by FA, which promoted the breakdown of polysaccharides. A higher yield of rhamnose was obtained at 0.7 mL of FA. When the dosage of FA was 0.9 mL, the yield to xylose and rhamnose decreased. An underlying reason might be the fact that the higher acidic solvent promoted the further reaction of monosaccharides. The conversion of hemicellulose increased to above 90% with the FA. Nevertheless, the yield to xylose and rhamnose increased (Table S1). An underlying reason might be the fact that the conversion of cellulose was increased from 15.8% to 29.3% at 15 min (Table S2). Increasing reaction temperature from 140 °C to 160 °C, the content of oligomers decreased to 11.9%, whereas the conversion of cellulose increased from 26.4 to 48.3%. Thus, some of the oligomers form macromolecular compounds with Mw > 1000 Da by polymerization at 180 °C. Meanwhile, some other oligomers would be decomposed into small molecular compounds (Table S5).

### Table 5. Distribution of Molecular Weight in Liquid Products with FA

| T (°C) | Mw > 1000 | 1000−300 | <300 |
|--------|-----------|----------|------|
| 140    | 44.4      | 15.6     | 40   |
| 150    | 33.0      | 17.6     | 49.4 |
| 160    | 9.6       | 19.2     | 71.2 |
| 180    | 13.3      | 11.9     | 74.8 |

*Reaction conditions: 0.7 mL of FA; 60 min; 3 g of biomass.*

**Impact of Dosage of FA on the Production of Monosaccharides.** The effect of the amount of FA on the production of monosaccharides was conducted at 140 °C for 60 min with 3 g of biomass. The liquid products contained mainly xylose and rhamnose. The yield to xylose and rhamnose increased with the augment of FA added. It was ascribed to the acidic environment caused by FA, which promoted the breakdown of polysaccharides. A higher yield of rhamnose was obtained at 0.7 mL of FA. When the dosage of FA was 0.9 mL, the yield to xylose and rhamnose decreased. An underlying reason might be the fact that the higher acidic solvent promoted the further reaction of monosaccharides. The conversion of hemicellulose increased to above 90% with the addition of FA. Nevertheless, the conversion of cellulose was quite low under these conditions.

**Impact of Reaction Time on the Production of Monosaccharides.** Further studies about the effect of reaction time (15−75 min) were conducted with 0.7 mL of FA and 3 g of biomass at 160 °C. The conversion of hemicellulose reached to 96.2% at a short time of 15 min, and the extension of time could not promote the conversion of hemicellulose obviously (from 96.2 to 98.2%). The conversion of cellulose at 15 min was about 15.8% and then maintained at about 25% between 30 and 60 min, reaching to 29.3% at 75 min (Table S2). Increasing reaction temperature from 160 to 180 °C, the conversion of cellulose increased from 26.4 to 48.3%, whereas the extension of reaction time did not affect markedly (about 25%). The yield to xylose and rhamnose showed the tendency of first increasing and then decreasing from 15 to 75 min. At 60 min, a higher yield of 41.7% to rhamnose was obtained. The yield to glucose kept increasing with reaction time, indicating that more time was required for cellulose conversion (Table S3). Before 75 min, the extension of time could promote the decomposition of compounds with Mw > 1000 Da to small molecular compounds (Figure S2). After 75 min, the yield to rhamnose decreased, along with the content of macromolecular compounds increased (polymerization occurred).

**Impact of Biomass Loading on the Production of Monosaccharides.** Different biomass loadings (1−5 g) were investigated (Figure 2, Table S4). Hemicellulose (99.7%) and cellulose (33.2%) were converted when 1 g of biomass was used. Still, the conversion of hemicellulose was much higher than that of cellulose. A relative high yield of 45.2% to rhamnose was obtained when the minimum mass of biomass was used. The yield to glucose, xylose, and rhamnose was augmented with the decrease of biomass. When the amount of E. prolifera was 5 g, the yield of glucose and xylose decreased to 4.9 and 8.6%, but still a relatively high amount of rhamnose 32.9% was obtained. Hemicellulose contained dissociative −OH, which makes it easy to dissolve in water. The first stage is the dissolving out of water-soluble fraction, then the complex phase hydrolysis of insoluble polysaccharides to form oligosaccharides. The last stage is the dissolution of oligosaccharides in homogenous phase to form monosaccharides. Because of the limitation of the equilibrium concentration of products in the process of HC, the low ratio of biomass amount to water was beneficial to the solution of water-soluble components of hemicellulose in E. prolifera to get high yields to monosaccharides. Further work will be performed on the separation of different monosaccharides to get pure products in the future.

**CONCLUSIONS**

Monosaccharides (18.8%) were obtained at 180 °C. At 200 °C, 29.6% of acids was attained. Rhamnose (45.2%) was produced by the catalysis of 0.7 mL of FA. FA could selectively catalyze the conversion of hemicellulose at low temperature (94.1%, 140 °C). Considering the output of products and no need for desiccation, HC was maintained to be a more applicable and efficient method in the efficient utilization of E.
**Element Analysis.** For the determination of carbon, hydrogen, and nitrogen contents in the raw material and residue, a FLASH 1112 SERIES Element Analyzer was employed. The samples were all dried at 80 °C under vacuum condition before test.

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