Algal Research 31 (2018) 116–121

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

# Optimization of the levulinic acid production from the red macroalga, *Gracilaria verrucosa* using methanesulfonic acid

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ARTICLE INFO

*Keywords:*
*Gracilaria verrucosa*
Methanesulfonic acid
Platform chemicals
5-hydroxymethylfurfural
Levulinic acid

ABSTRACT

In this study, *Gracilaria verrucosa*, which is a red marine macroalgae and potential renewable resource, and methanesulfonic acid (MSA), which is known to be a strong and biodegradable acid catalyst, are introduced to produce levulinic acid (LA), which is a platform chemical, under hydrothermal conversion. Under the optimized conditions of the MSA-catalyzed hydrothermal conversion of *G. verrucosa*, a 22.02% LA yield based on biomass weight (36.92% based on carbohydrate) was obtained under the conditions of 180 °C, 10% biomass, 0.5 M MSA, and 20 min. In the same conditions, only 0.27% 5-HMF, 1.23% glucose, and 0.47% galactose were obtained. In the relationship of LA yield and combined severity factor (CSF) value, the LA yield was sharply increased to CSF 3.5 and then slightly decreased. Additionally, it fits well with a quadratic polynomial regression model. From these results, the MSA-catalyzed hydrothermal conversion of red-macroalgae *G. verrucosa* as a potential resource was concluded to be a valuable method for platform chemical production.

## 1. Introduction

As alternatives to fossil resources, biomass feedstocks such as sugars, starches, and lignocellulosics, have been investigated. Moreover, bioresource-based products, such as various biofuels, chemicals, and materials, can replace the use of carbon skeletons derived from fossil resources in various industrial fields [1–5].

Among various platform chemicals, 5-hydroxymethylfurfural (5-HMF) and levulinic acid (LA) have reactive groups, which have high potential in the synthesis of versatile chemicals and materials [2,3,5–7]. They can be obtained from many biopolymers, such as sugars, starches, lignocellulosics, and macroalgae, by hydrolysis, dehydration, and subsequent rehydration under acidic processes [1,3,5,6,8]. LA (4-oxypentanoic acid, γ-keto-valeric acid, $pK_a$ = 4.59) is one of the top 12 promising building blocks selected by US DOE [1]. It also has two highly reactive functional groups: carbonyl and carboxy groups [5–7]. These two groups are considered promising organic moieties for synthesis of a wide range of various chemicals, such as tetrahydrofuran, succinic acid, 1,4-butanediol, 1,4-pentanediol, levulinate esters, 5-bromolevulinate, δ-aminolevulinic acid, Nylon 6,6, diphenolic acid, acrylic acid, α-angelicalactone, 2-methyltetrahydrofuran, 5-nonanone, and γ-valerolactone. These chemicals are used as fuels, solvents, chemical intermediates, plasticizers, anti-freezing agents, pharmaceutical agents, herbicides, polymers, resins, and flavoring agents [5–7].

In this study, we introduced *Gracilaria verrucosa*, which is a red marine macroalgae, as a bioresource for the production of platform chemicals, such as 5-HMF and LA. *G. verrucosa* is a Rhodophyta (red macroalgae) and agarophyte. Nowadays, it is commercially cultivated as an important resource for food and agar production in Asian countries [9]. Agar is the major polysaccharide of *G. verrucosa*. It is composed of the main subunits of D-galactose and 3,6-anhydro-L-galactopyranose [10,11]. Recently, it was reported as a potential resource for the production of bioethanol and platform chemicals, such as 5-HMF and LA [12–14].

For the efficient conversion of *G. verrucosa* into 5-HMF and LA, two key steps are required. The first step is the hydrolysis of the polysaccharide of *G. verrucosa* into mono-sugars. The next step is the conversion of mono-sugars into 5-HMF and LA [6,13,14]. Recently, hydrolysis and chemical conversion were investigated using various acid catalysts, such as sulfuric, hydrochloric, phosphoric, and other acids [9,12–14].

In this study, MSA is introduced to produce 5-HMF and LA from *G. verrucosa*. MSA is a strong, biodegradable, non-foaming, non-oxidizing acidic catalyst [15]. It causes fewer environmental problems than strong inorganic acids, such as $H_2SO_4$ and HCl. Moreover, it was recently applied as an interesting alternative to organic and inorganic strong acid catalysts in some reaction fields, such as esterification and alkylation [15–17]. However, there has been no study on the conversion of macroalgae into 5-HMF and LA using MSA as a catalyst.

In this study, the MSA-catalyzed conversion of marine macroalgae

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https://doi.org/10.1016/j.algal.2018.02.004
Received 28 September 2017; Received in revised form 30 January 2018; Accepted 5 February 2018

*G. verrucosa* into platform chemicals, such as 5-HMF and LA, was performed. The effects of reaction parameters for 5-HMF and LA production were investigated. Additionally, the efficiencies of hydrothermal catalytic conversion with MSA were evaluated using the combined severity factor (CSF).

## 2. Materials and methods

### 2.1. Materials

The *G. verrucosa* biomass was collected at Wan-do (Jeonnam, Korea). It was washed with distilled water three times to remove contaminants and salts, and then lyophilized for three days. The freeze-dried biomass was ground and mesh-screened to a particle size below 100 μm and then stored in a sealed bag at room temperature [12]. The MSA (Samchun Pure Chemical Co., Ltd., Korea) was of reagent grade, and the glucose, galactose, LA, 5-HMF, and all other chemicals were of analytical grade.

### 2.2. Batch experimental procedure

The batch experiment was conducted as follows. The specified amount of biomass and MSA solution were inserted into a 50-mL stainless steel reactor. Before reaction, the reactant was mixed by magnetic stirring for 10 min for sufficient soaking. The reaction was initiated when the specified temperature of the reactor in the oil bath reached the desired set point. The oil bath and reactor were monitored and controlled by a PID temperature controller. A preheating time of approximately 5 min was required. During reaction, reactant mixing was performed with a magnetic stirrer at 200 rpm. Upon completion of the reaction, the reactor was quickly cooled to room temperature by dipping it into tap water. The product solution was recovered by centrifugation at 17,000 rpm for 10 min, and then filtered using a 0.2-μm syringe filter for HPLC analysis [12].

### 2.3. Conversion of G. verrucosa with MSA

The MSA-catalyzed conversion of *G. verrucosa* was performed in conditions involving four reaction factors: reaction temperature (160–210 °C), catalyst concentration (0-1 M), biomass concentration (2.5–17.5%), and reaction time (0–60 min) (Table 1). All of the experiments were conducted twice or more; the data are presented as the average ± SD.

### 2.4. Effect of CSF

The effect of the MSA-catalyzed hydrothermal reaction was investigated by examining the CSF on 5-HMF and LA production from *G. verrucosa*. The CSF is defined as the combined severity of the reaction, which is a function of the reaction conditions (temperature, time, and acidity of solution) [11,13]. $CSF = \log\ [t\ \exp(T - T_{ref})/14.75] - pH$, where $T(t)$ is the reaction temperature (°C), $T_{ref}$ is the reference temperature (i.e., 100 °C), $t$ is the reaction time (min), and 14.75 is the fitted value of the arbitrary constant. Prior to reaction, the pH of the solution was measured at room temperature [11,13].

**Table 1**
Reaction condition for levulinic acid production from *G. verrucosa* using methanesulfonic acid.

| Reaction factor | Reaction condition | | | | Replication |
|---|---|---|---|---|---|
| | Temperature (°C) | Biomass conc. (%) | Catalyst conc. (M) | Time (min) | |
| Temperature | 160–210 | 5 | 0.2 | 30, 60 | $n$ = 2 or 3 |
| Catalyst conc. | 180 | 5 | 0–1 | 30, 60 | n = 2 or 3 |
| Biomass conc. | 180 | 2.5–12.5 | 0.5 | 30 | $n$ = 3 |
| Reaction time | 180 | 10 | 0.5 | 0–90 | n = 2 |

### 2.5. Analysis

#### 2.5.1. Quantitative analysis of carbohydrate compositions

Carbohydrate composition was determined by the NREL standard protocol [18]. Biomass (0.3 g) and $H_2SO_4$ (72% (*w/w*), 3 mL) was mixed for 1 h at 30 °C in water bath. The mixture was diluted to 4% $H_2SO_4$ with distilled water, and then autoclaved for 1 h at 121 °C. After cooling to room temperature, hydrolysates were neutralized and filtered for sugar analysis. The sugar concentration was measured by DNS method and HPLC analysis.

#### 2.5.2. Product analysis

The concentration of total reducing sugar was analyzed using modified DNS method using spectrophotometry (Spekol 1300, Analytik Jena, Germany). The concentrations of glucose, galactose, 5-HMF, and LA were analyzed using an Agilent 1100 HPLC system (Agilent, San Jose, CA, USA) with a Bio-—Rad Aminex-87H column and a refractive index detector. The HPLC operation conditions were an oven temperature of 65 °C, 5 mM of sulfuric acid as the mobile phase, and 0.6 mL/min flow rate [12].

### 2.6. Calculation of product yield

The product yields of glucose, galactose, 5-HMF and LA based on biomass weight (or carbohydrate weight) were calculated by the following equation: Yield (%) = concentration of product (g/L)/concentration of initial biomass (or carbohydrate) (g/L) × 100. The results were recorded as the average ± standard deviation (SD).

### 2.7. Statistical analyses

The statistical significance of levulinic acid yield was evaluated by one-way analysis of variance (ANOVA) and Duncan's multiple-range test ($P < 0.05$) using the SPSS software (ver. 23; SPSS Inc., Chicago, IL, USA).

## 3. Results and discussion

### 3.1. Carbohydrate composition analysis

The component and content of *G. verrucosa* were analyzed. It is contained about 59.64 ± 0.71% (*w*/w) carbohydrate on the basis of biomass weight. The carbohydrate mainly composed of glucose (16.38 ± 0.61%) and galactose (34.98 ± 0.96%). In addition, some trace amounts of arabinose and mannose were determined.

### 3.2. MSA-catalyzed hydrothermal reaction of G. verrucosa

In this study, MSA-catalyzed hydrothermal reaction was conducted to produce 5-HMF and LA from *G. verrucosa*. For the optimization of the 5-HMF and LA production, the effects of reaction factors, such as reaction temperature, MSA concentration, biomass concentration, and reaction time, on the conversion of *G. verrucosa* were investigated.

Fig. 1 shows the effect of reaction temperature on the conversion of *G. verrucosa* into 5-HMF and LA, and was investigated for various temperatures in the range of 160–210 °C under the 5% (*w*/w) biomass, 0.2 M MSA, and 30 and 60 min condition. As shown in Fig. 1(A), the 5-HMF yield was lower than 4% in all tested temperature conditions. The highest 5-HMF yield, 3.81%, was achieved at 170 °C and 30 min. At higher temperatures than 170 °C, the 5-HMF yield decreased with increasing temperature and reaction time. At over 200 °C, 5-HMF was nearly not detected. The low 5-HMF yield occurs as the 5-HMF formed into LA and FA at high temperature [5,12,15].