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Production of Sugars and Levulinic Acid from Marine Biomass *Gelidium amansii*

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Abstract This study focused on optimization of reaction conditions for formation of sugars and levulinic acid from marine algal biomass *Gelidium amansii* using acid catalyst and by using statistical approach. By this approach, optimal conditions for production of sugars and levulinic acid were found as follows: glucose (reaction temperature of 139.4°C, reaction time of 15.0 min, and catalyst concentration of 3.0%), galactose (108.2°C, 45.0 min, and 3.0%), and levulinic acid (160.0°C, 43.1 min, and 3.0%). While trying to optimize the conditions for the production of glucose and galactose, levulinic acid production was found to be minimum. Similarly, the production of glucose and galactose were found to be minimum while optimizing the conditions for the production of levulinic acid. In addition, optimized production of glucose required a higher reaction temperature and shorter reaction time than that of galactose. Levulinic acid was formed at a high

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reaction temperature, long reaction time, and high catalyst concentration. The combined results of this study may provide useful information to develop more economical and efficient systems for production of sugars and chemicals from marine biomass.

Keywords Marine biomass · *Gelidium amansii* · Response surface methodology · Chemical intermediates · Levulinic acid · Sugar

Introduction

Due to the finite and limited supply of fossil fuel resources, availability of fossil fuels for use as an energy source will be nonexistent in the future. In this regard, biofuels have recently attracted significant attention for reasons associated with energy security, diversity, and sustainability [1, 2]. For long-term applications, biomass must be utilized as the feedstock for biofuel energy production and in the chemical industry [2, 3].

Biofuel is generally defined as energy obtained from biomass, which can occur through biochemical, thermochmical, and various other methods. Biomass can be divided into three classes: sugar-based, starch-based, and wood-based. Sugar and starch-based biomass can easily be converted to biofuels and chemicals; however, conversion of wood-based biomass into biofuels or chemicals, typically, requires additional steps, such as pretreatment processing and saccharification [4]. Until now, only sugar and starch-based biomass has been commonly used for biofuel production. The most common concern related to this system of biofuel production is that as production capacity increases so does competition within the agricultural industry; thus, land resources for food production would become limited as demand for biofuels increase [5]. Consequently, food costs would rise, and biofuel production, by this method, would not be economically feasible. Currently, there is no predictable industrial trend for producing biofuels using wood-based biomass due to the fact that it is still far from commercialization [1, 4, 5].

Development of marine biotechnology from macro- and microalgae was initiated in the middle of the last century. Several applications of this technology have emerged recently, including nutritional additives for food and animal feed, cosmetic additives, bioenergy sources, etc. [6, 7]. Marine algae are divided into macroalgae and microalgae, where macroalgae are mainly divided into three categories: brown, red, and green algae [4]. Marine algae have high carbohydrate content and are easily degradable, making them a potential substrate for biofuel and chemical production [5–8]. Worldwide annual production of marine algae is approximately 14 million tons and is expected to increase to more than 22 million tons by 2020. Compared with other types of land biomass, marine biomass is growing rapidly (four to six times of harvest per year is possible in subtropic regions); it is easy to cultivate using wide arable areas of the sea without the need for high priced equipment. In addition, annual CO_2 absorption by marine biomass is 36.8 ton/ha, which is five to seven times higher than that of wood-biomass [4].

Production of biofuels, such as bioethanol and biodiesel from marine biomass, has recently attracted considerable attention [4, 5, 8]. A significant number of previous studies have examined various pretreatment methods with the aim of enhancing the digestibility of cellulosic materials [9–11]. Nevertheless, pretreatment methods specifically for marine biomass have received very little attention [4, 6]. Despite being members of the same marine algae species, the chemical compositions were found to be different according to harvesting site [4]. Carbohydrate content, which is easily converted to biofuel, was as high as 72–80% in *Gelidium amansii*. Red algae, such as *gracilaria* and *cottonii*, contained

carbohydrate of approximately 74% and 50%, respectively. However, green algae, such as *Codium fragile*, had a carbohydrate content of approximately 59%. Brown algae, such as *Undaria pinnattnda* and *Laminaria japonica*, contained about 41% and 46%, respectively. Noncarbohydrate content was high in green algae, such as *C. fragile*, whereas, *G. amansii* had a low content [4].

G. amansii, a red algae, is widely used in agar production, which is applied in the food and chemical industries. Moreover, its carbohydrate content (glucose, galactose, galactan, etc.) is higher than that of protein, lipid, etc. These carbohydrates can easily be converted to biofuels and chemicals through proper biochemical or chemical processes [12, 13].

5-Hydroxymethylfurfural (HMF) and levulinic acid are versatile biomass-derived platform compounds that can be used to synthesize a broad range of chemicals and are currently derived from petroleum. In addition, liquid fuels derived from HMF using chemical processes are potential alternatives to ethanol, which is obtained through biochemical and chemical processes [1, 14, 15].

In this study, marine biomass *G. amansii* was subjected to the acid hydrolysis method with the goal of assessing whether or not *G. amansii* can be used as a potential resource for the production of sugars (glucose and galactose) and chemical intermediates (HMF and levulinic acid). This study specifically focused on the use of a response surface methodology for optimization of conditions and evaluation of the reciprocal interaction of operation factors related with sugar production processes (glucose and galactose) and chemical intermediates (HMF and levulinic acid) from marine biomass *G. amansii.*

Materials and Methods

Materials

Dried *G. amansii* was obtained from a market in Gwangju, Korea. In order to desalt, biomass was washed with distilled water. *G. amansii* was dried for 2 days at 60°C after washing. The dried sample was processed by grinding and screening to a nominal size through a 20~40 mesh and kept in a sealed bottle. Sulfuric acid (99.8%, Duksan Pure Chemical Co. Ltd., Korea), calcium carbonate (Sigma-Aldrich Co. Ltd), and phosphoric acid (Sigma-Aldrich Co. Ltd) that were used had reagent grade. The used Sugars (glucose, galactose, arabinose, xylose, hemicellulose, etc.), 5-hydroxyl methyl furfural, levulinic acid, and furfural that were used had analytical grade. Sugar, furfural, HMF, and levulinic acid stock solutions were also prepared in deionized water and filter sterilized for analysis.

Experimental Procedure

In a typical batch reactor of stainless steel, 2 g of dry biomass was packed with 40 ml of the desired sulfuric acid concentration. The reaction was initiated by raising the reactor temperature in the oil bath. Approximately 5 min of preheating was necessary to reach the desired temperature. However, the time required to reach the desired temperature varied slightly at each temperature setting. Reaction temperature was controlled using oil bath equipped with a PID (Proportional Integral Derivative) temperature controller. Mixing was done with a magnetic stirrer, spinning at approximately 200 rpm. Once the reaction was complete, the reactor was quickly cooled to room temperature in a water bath. All batch reaction experiments were run as duplicates. Solids were recovered by filtration and centrifugation for 20 min at 15,000 g and then, washed with one tenth volume of sterile

deionized water. The pH of hydrolysate supernatants was adjusted with calcium carbonate to 6.5, and supernatants were then filtered using 0.4 μm filter for further analysis.

Experimental Design

A five-level three-factor central composite rotatable design (CCRD) was adopted for optimization of the experimental design. This method required 20 experiments, which included eight factorial points, six axial points, and six central points to provide information about the interior of the experimental region [16, 17]. Variables, which were selected to assess, decompose the biomass, and their respective levels were as follows: reaction temperature (80–180°C), reaction time (5–55 min), and amount of catalyst (0.3–3.7 wt.%). Table 1 shows coded and uncoded independent factors (X_i), levels, and experimental design.

Statistical Analysis

Experimental data (Table 2) were analyzed using a response surface methodology to fit the following second-order polynomial equation. The fit was done using Design-Expert 7 software (Stat-Ease, Inc., USA). Second-order coefficients were generated via regression without elimination. The response was fit to the factors via multiple regressions, and the quality of the model fit was evaluated using coefficients of determination (R^2) and analysis of variances. The quadratic response surface model was then fit to the following equation:

$$Y = \beta_{k0} + \sum_{i=1}^{3} \beta_{ki} x_i + \sum_{i=1}^{3} \beta_{kii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{kij} x_i x_j \quad (1)$$

where Y is the response factor (concentration), x_i is the ith independent factor, β_0 is the intercept, β_i is the first-order model coefficients, β_{ii} is the quadratic coefficients for the factor i, and β_{ij} is the linear model coefficient for the interaction between factors i and j [16, 17].

Analytical Methods

Sugar composition was determined by hydrolyzing biomass with sulfuric acid, and the production of sugars was analyzed using high performance liquid chromatography as described below [18]. First, 300 mg (±10 mg) of biomass sample was mixed with 3 ml of 72 wt.% sulfuric acid in a shake flask and kept in shaking water bath (30°C, 100 rpm) for 60 min to initiate prehydrolysis. Sugars in the solid samples were determined followed by secondary acid hydrolysis to account for oligomer contents. Second, after the completion of prehydrolysis step, 84 g of deionized water was added to the prehydrolyzed reactant and then, autoclaved at 121°C for 60 min to induce secondary hydrolysis. Following

Table 1 Factors and their levels in the central composite design

Variable	Symbol	Coded factor levels				
		−1.68	−1	0	1	1.68
Reaction temperature (°C)	X_1	79.5	100	130	160	180.5
Reaction time (min)	X_2	4.8	15	30	45	55.2
Catalyst amount (wt.%)	X_3	0.3	1.0	2.0	3.0	3.7

Table 2 Central composite rotatable second-order design, experimental, and estimated data for five-level three-factor response surface analysis

Std	Run	Reaction temperature, X_1[a]	Reaction time, X_2[a]	Catalyst amount, X_3[a]	Concentration (g/l)			
					Glucose	Galactose	HMF	Levulinic acid
5	1	−1.00	−1.00	1.00	1.56	11.54	1.91	0.36
2	2	1.00	−1.00	−1.00	2.43	10.77	0.91	4.96
7	3	−1.00	1.00	1.00	2.58	16.87	3.46	2.04
14	4	0.00	0.00	1.68	2.38	14.28	0.26	5.77
9	5	−1.68	0.00	0.00	0.09	1.66	0.12	0.02
13	6	0.00	0.00	−1.68	1.58	14.23	4.49	0.35
18	7	0.00	0.00	0.00	2.25	14.61	1.43	4.07
11	8	0.00	−1.68	0.00	2.26	17.07	2.85	0.65
12	9	0.00	1.68	0.00	2.23	13.71	0.37	5.36
19	10	0.00	0.00	0.00	2.20	13.43	0.73	4.74
16	11	0.00	0.00	0.00	2.22	14.04	0.83	4.87
4	12	1.00	1.00	−1.00	2.00	5.68	0.64	6.81
6	13	1.00	−1.00	1.00	3.26	8.28	0.34	8.24
1	14	−1.00	−1.00	−1.00	0.44	4.78	0.58	0.02
20	15	0.00	0.00	0.00	2.37	14.61	1.18	4.50
3	16	−1.00	1.00	−1.00	1.14	11.13	2.13	0.21
17	17	0.00	0.00	0.00	2.26	14.17	1.04	4.55
10	18	1.68	0.00	0.00	0.10	0.21	0.10	11.45
8	19	1.00	1.00	1.00	0.96	0.39	0.34	8.66
15	20	0.00	0.00	0.00	2.26	14.17	1.04	4.70

[a] Based on coded factor levels

Std: standard

completion of the secondary hydrolysis step, the temperature of the reactor was immediately lowered to room temperature using a water bath. The pH of the hydrolysate supernatants was adjusted with calcium carbonate to 6.5; supernatants were then filtered (0.4 μm pore size) prior to sugar composition analysis [18].

Concentrations of sugars, organic acids, furfural, HMF, and levulinic acid were determined using an HPLC system equipped with a Supelcogel C-610H column (cross-linked polystyrene divinylbenzene resin, 30 cm × 7.8 mm ID, Supelco, USA) and refractive index detector (RID A10, Shimazu, Japan). Operation conditions were 40°C for oven temperature, 0.05% phosphoric acid as the mobile phase, and a 0.6 ml/min flow rate. In this sugar analysis by HPLC, the retention times of galactose, fructose, and xylose are near, whereas, those of glucose, arabinose, and hemicellulose are clearly separated. So, in this study, the content of galactose, fructose, and xylose are expressed as that of galactose. These results are expressed as the mean values of at least two independent measurements.

Results and Discussion

This study focused on optimization of reaction conditions for production of sugars and chemical intermediates from marine biomass *G. amansii* using an acid catalyst and

statistical methodological approach. In order to construct an experimental model to optimize production of sugars and chemical intermediates, the central composite rotatable design, which is, generally, the preferred design for response surface optimization was selected with five-level and three-factor: reaction temperature, reaction time, and catalyst amount [16, 17].

Table 1 presents the experimental parameter settings and results based on the experimental design. All 20 of the designed experiments were conducted, and results were analyzed via multiregression using Design-Expert 7 software. Coefficients of the full model were evaluated via regression analysis and tested for significance on the basis of the p values after evaluating the coefficients. In the case of glucose formation, two linear coefficients (X_1 and X_3), one quadratic coefficient (X_1^2), and two cross-product coefficients (X_1X_2 and X_1X_3) were found to be significant (Table 3). However, in order to minimize error, all coefficients were considered in the design. The coefficient of determination (R^2) of the model was 0.884, indicating that the model adequately represented the actual relationships among these experimental parameters within the ranges tested. In the case of galactose formation, one linear coefficient (X_1), one quadratic coefficient (X_1^2), and two cross-product coefficients (X_1X_2 and X_1X_3) were ultimately determined to be significant (Table 4). When all coefficients were considered in the design, the coefficient of determination (R^2) of the model was 0.959. In the case of HMF formation, there were no significant model terms (data not shown). When all coefficients were considered in the design, the coefficient of determination (R^2) of the model was 0.501. This result indicates that the model was a poor fit for the actual relationships among these experimental factors within selected ranges. This phenomenon was caused by rapid decomposition of HMF using heat and acid to produce levulinic and formic acid. In the case of high reaction temperature and long reaction time, HMF formation was low. HMF concentration also showed a sharp decrease with an increase in reaction temperature. In low catalyst amounts,

Table 3 Analysis of variance results for the response surface-reduced quadratic model for glucose formation.

Source	Sum of squares	Degree of freedom	Mean square	F value	Prob>F[a,b]
Model	12.447	9	1.383	8.483	0.0012
X_1	0.637	1	0.637	3.909	0.0762
X_2	0.082	1	0.082	0.501	0.4952
X_3	0.990	1	0.990	6.074	0.0334
X_1^2	2.475	1	2.475	15.178	0.0030
X_2^2	0.962	1	0.962	5.898	0.0355
X_3^2	0.299	1	0.299	1.832	0.2057
X_1 X_2	6.655	1	6.655	40.818	<0.0001
X_1 X_3	0.097	1	0.097	0.593	0.4592
X_2 X_3	0.003	1	0.003	0.017	0.8998
Residual	1.630	10	0.163		
Lack of fit	1.614	5	0.323	96.259	<0.0001
Pure error	0.017	5	0.003		
Cor. total	14.078	19			

[a] Prob>F level of significance

[b] Values of Prob>F less than 0.05 indicate that model terms are significant

Table 4 A results for the response surface-reduced quadratic model for galactose formation

Source	Sum of Squares	Degree of Freedom	Mean Square	*F* Value	Prob>*F*[a,b]
Model	528.569	9	58.730	25.829	<0.0001
X_1	34.311	1	34.311	15.090	0.0030
X_2	3.528	1	3.528	1.551	0.2413
X_3	1.691	1	1.691	0.744	0.4086
X_1^2	75.961	1	75.961	33.408	0.0002
X_2^2	51.493	1	51.493	22.646	0.0008
X_3^2	1.829	1	1.829	0.805	0.3908
$X_1\ X_2$	352.993	1	352.993	155.245	<0.0001
$X_1\ X_3$	0.378	1	0.378	0.166	0.6920
$X_2\ X_3$	0.823	1	0.823	0.362	0.5609
Residual	22.738	10	2.274		
Lack of fit	21.788	5	4.358	22.935	0.0019
Pure error	0.950	5	0.190		
Cor. total	551.307	19			

[a] Prob>*F* level of significance

[b] Values of Prob>*F* less than 0.05 indicate that model terms are significant

relatively high amount of HMF was produced. For levulinic acid formation, three linear coefficients (X_1, X_2, and X_3) and two quadratic coefficients (X_2^2 and X_3^2) were ultimately determined to be significant (Table 5). When all coefficients were considered in the design, in order to minimize error, the coefficient of determination (R^2) of the model was 0.961.

Table 5 Analysis of variance results for the response surface reduced quadratic model for levulinic acid formation

Source	Sum of squares	Degree of freedom	Mean square	*F* value	Prob>*F*[a,b]
Model	192.509	9	21.390	30.032	<0.0001
X_1	150.052	1	150.052	210.681	<0.0001
X_2	10.678	1	10.678	14.993	0.0047
X_3	19.731	1	19.731	27.703	0.0008
X_1^2	0.022	1	0.022	0.031	0.8655
X_2^2	1.093	1	1.093	1.535	0.2505
X_3^2	0.000	1	0.000	0.001	0.9817
$X_1\ X_2$	2.269	1	2.269	3.186	0.1121
$X_1\ X_3$	3.696	1	3.696	5.189	0.0522
$X_2\ X_3$	3.439	1	3.439	4.828	0.0592
Residual	5.698	8	0.712		
Lack of fit	5.330	5	1.066	8.696	0.0525
Pure error	0.368	3	0.123		
Cor. total	198.207	17			

[a] Prob>*F* level of significance

[b] Values of Prob>*F* less than 0.05 indicate that model terms are significant

Final predictive response model equations (based on the coded value) for the production of sugars and levulinic acid were as follows:

$$Glucose: Y = 2.25 + 0.22X_1 - 0.077X_2 + 0.27X_3 - 0.56X_1X_2 - 0.35X_1X_3 - 0.19X_2X_3 - 0.68X_1^2 + 0.082X_2^2 - 0.014X_3^2$$

$$Galactose: Y = 14.21 - 1.59X_1 - 0.51X_2 + 0.35X_3 - 3.08X_1X_2 - 2.54X_1X_3 - 0.48X_2X_3 - 4.95X_1^2 + 0.16X_2^2 + 0.24X_3^2$$

$$Levulinic\ acid: Y = 4.54 + 3.31X_1 + 0.88X_2 + 1.20X_3 + 0.052X_1X_2 + 0.37X_1X_3 + 7.05E^{-3}X_2X_3 + 0.42X_1^2 - 0.54X_2^2 - 0.52X_3^2$$

where Y is the response factor, concentration (g/l). X_1, X_2, and X_3 are the actual values of the independent factors, reaction temperature (C), reaction time (min), and catalyst amount (wt. %), respectively.

Figure 1 shows the effects of reaction temperature, reaction time, and the reciprocal interaction of these factors on formation of sugars and levulinic acid at a catalyst concentration of 2.0% (*w*/*v*). As shown in Fig. 1(a), glucose formation increased linearly with an increase in reaction temperature when reaction time was relatively short. At a low reaction temperature, glucose concentration increased with longer reaction time. However, at high reaction temperatures, glucose concentration decreased at long reaction times. In conditions of high reaction temperature and long reaction time, glucose formation was relatively low. This may have resulted from decomposition of glucose to other chemicals. As is shown in Fig. 1(b), galactose formation linearly increased up to 130°C; however, at higher reaction temperatures of over 130°C, galactose formation was inhibited when reaction time was longer. This may be due to the decomposition of galactose to other chemicals such as HMF or levulinic acid. Galactose formation increased linearly with

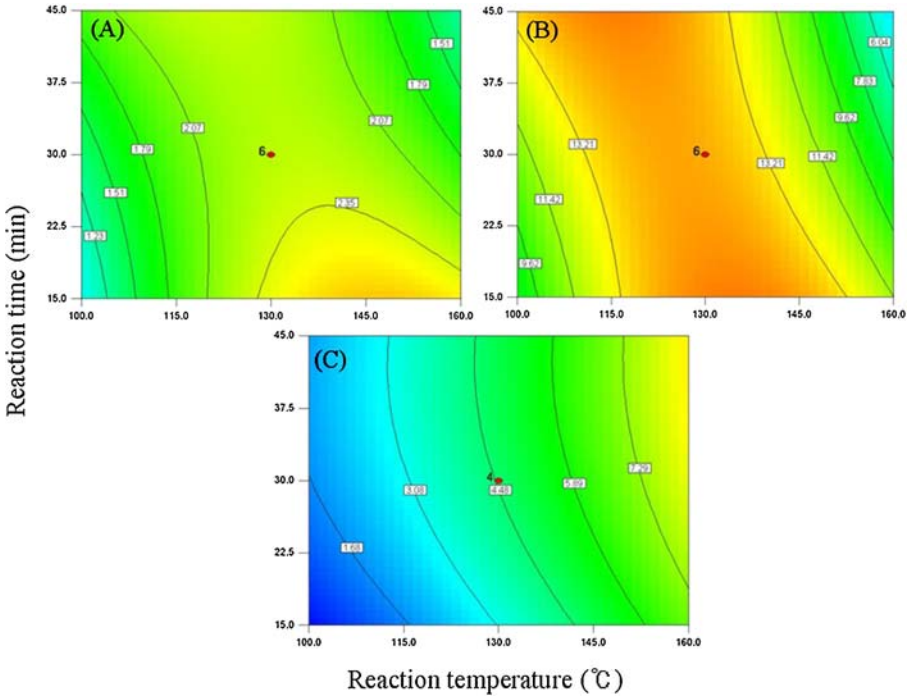

Fig. 1 *Contour plots* representing the effect of reaction temperature, reaction time, and the reciprocal interaction of these factors on the formation of sugars and chemical intermediates at a constant catalyst concentration of 2.0% (*w*/*v*). **a** Glucose, **b** galactose, and **c** levulinic acid

increased reaction time when reaction temperature was low. However, at long reaction times, galactose production showed a sharp decrease at a high reaction temperature. Similar to glucose production (Fig. 1(a)), galactose formation was shown to increase in a linear fashion at a high reaction temperature and long reaction time. These results may be caused by degradation of galactose to other chemicals. As shown in Fig. 1(c), levulinic acid formation increased linearly as reaction temperature increased for a given reaction time. At a given reaction temperature, longer reaction times enhanced levulinic acid formation. In conditions of high reaction temperature and long reaction time, levulinic acid formation was high. These results may be caused by conversion of levulinic acid from HMF. Combined results presented in Fig. 1 and Table 1 clearly demonstrate that glucose and galactose formed directly from marine biomass by way of an acid catalyst and heat. In addition, these results show that levulinic acid was converted from HMF derived from sugars.

Figure 2 shows the effects of reaction temperature, catalyst amount, and the reciprocal interaction of these factors on formation of sugars and chemical intermediates at a constant reaction time of 30 min. As shown in Fig. 2(a), glucose formation increased linearly as reaction temperature was increased from 130 to 145°C. After which, any further increase in reaction temperature resulted in a decrease in glucose formation at the given catalyst concentration. However, when reaction time was lower than 30 min, a saddle-type glucose formation pattern was observed in the experiments, where reaction temperature and catalyst amount were varied. At low reaction temperature, glucose formation increased linearly as catalyst concentration increased. When both reaction temperature and catalyst concentration were high, glucose formation was low. As shown in Fig. 2(b), galactose formation showed

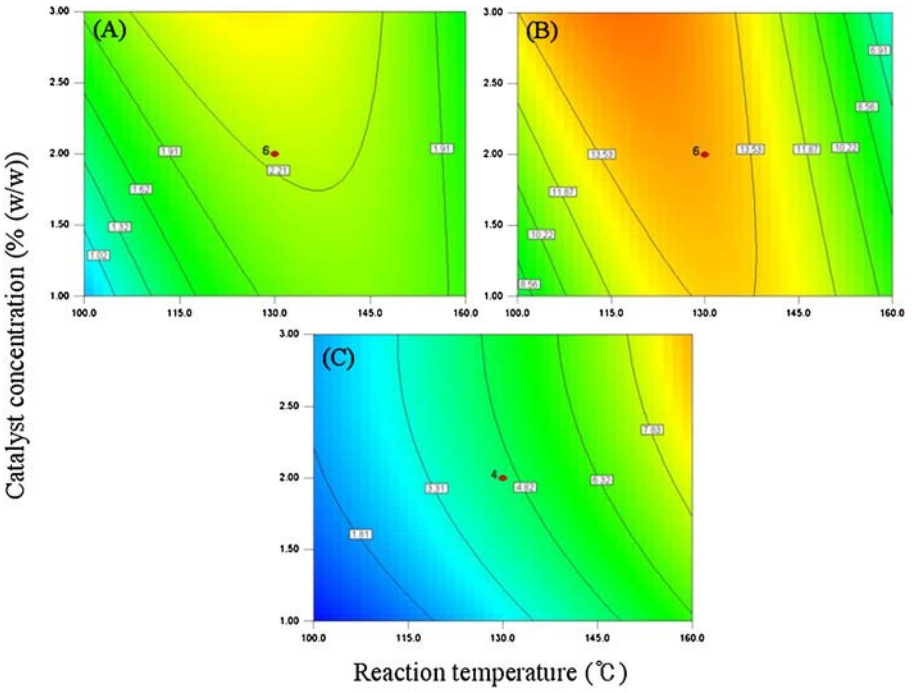

Fig. 2 *Contour plots* representing the effect of reaction temperature, catalyst amount, and the reciprocal interaction of these factors on the formation of sugars and chemical intermediates at a constant reaction time of 30 min. **a** Glucose, **b** galactose, and **c** levulinic acid

a saddle-type pattern in the experiments where reaction temperature and catalyst amount were varied. When catalyst concentration was low, galactose formation decreased linearly as the reaction temperature increased from 130 to 140°C; however, a further increase in reaction temperature resulted in a decrease in galactose formation. At low reaction temperature (100°C), galactose production linearly increased as catalyst concentration increased. At high reaction temperature (160°C), galactose formation slightly increased with an increase in catalyst concentration. When both reaction temperature and catalyst concentration were high, galactose formation was diminished. As shown in Fig. 2(c), levulinic acid formation increased linearly as the reaction temperature was increased at a given catalyst concentration. For a given reaction temperature, an increase in catalyst concentration resulted in a linear increase in levulinic acid production. In conditions of high reaction temperature and high catalyst concentration, levulinic acid formation was high. These results may have been caused by formation of levulinic acid from HMF, which is converted from monosugars, as described in Fig. 1.

Figure 3 shows the effects of reaction time, catalyst concentration, and the reciprocal interaction of these factors on formation of sugars and chemical intermediates at a constant reaction temperature of 130°C. As shown in Fig. 3(a), glucose concentrations increased to a low reaction time (15 min) at any of the tested catalyst concentrations, which ranged from 1% to 3%. At low catalyst concentration, reaction time was only slightly influenced by the increase in glucose concentration. However, at high catalyst concentration, glucose formation showed a sharp decrease at a longer reaction time. These combined results

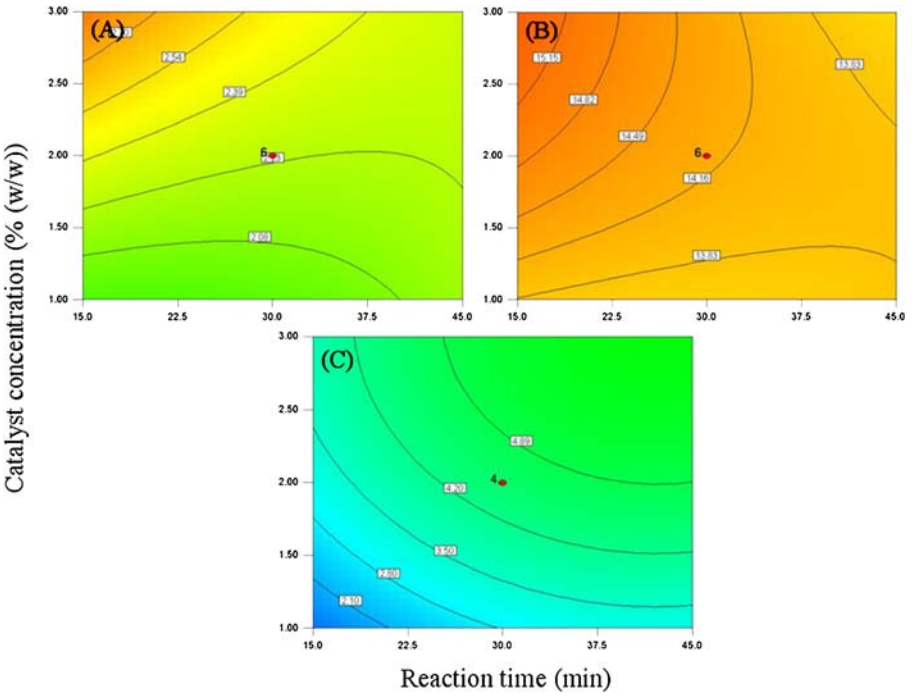

Fig. 3 *Contour plots* representing the effect of reaction time, catalyst concentration, and the reciprocal interaction of these factors on the formation of sugars and chemical intermediates at a constant reaction temperature of 130°C. **a** Glucose, **b** galactose, and **c** levulinic acid

suggest that a low reaction time and high catalyst concentration were optimal for glucose formation at a constant reaction temperature of 130°C. As shown in Fig. 3(b), increased galactose concentrations resulted in a low reaction time at any of the tested catalyst amounts, ranging from 1% to 3%. Reaction time was barely influenced by a decrease in galactose formation at low catalyst concentration. However, at high catalyst concentration, galactose formation showed a sharp decrease at a long reaction time. As was observed for glucose formation (Fig. 3(a)), a low reaction time and high catalyst concentration were optimal for galactose formation. As shown in Fig. 3(c), the formation of levulinic acid increased linearly as catalyst concentration increased at a given reaction time. At the given catalyst concentration, an increase in reaction time enhanced levulinic acid formation. Levulinic acid formation was high when the catalyst concentration was over 2.2%, and the reaction time was over 30 min. This result may be caused by conversion of glucose, galactose, and HMF to levulinic acid.

Table 6 shows the optimized results for formation of glucose, galactose, and levulinic acid from marine algae *G. amansii* in the tested range. Glucose was produced to 2.92 g/l under optimized conditions such as a reaction temperature of 139.4°C, reaction time of 15.0 min, and catalyst concentration of 3.0%. Under these conditions, galactose and levulinic acid was produced to 14.66 and 4.97 g/l, respectively. Based on optimized conditions of reaction temperature of 108.2°C, reaction time of 45.0 min and catalyst concentration of 3.0% and galactose of 16.12 g/l was produced, and the amount of glucose and levulinic acid formed were 2.45 and 3.08 g/l, respectively. Levulinic acid production was optimized under the following reaction conditions: reaction temperature of 160.0°C, reaction time of 43.1 min, and catalyst concentration of 3.0%. In these conditions, levulinic acid was produced to 9.74 g/l. However, glucose and galactose formation were 1.03 and 1.83 g/l, respectively. Using the above optimization conditions for glucose and galactose production, levulinic acid formation was minimized. In addition, under optimized conditions for levulinic acid formation, glucose and galactose concentrations were minimized. Overall, optimized conditions for glucose production required a higher reaction temperature and shorter reaction time than that of galactose. Levulinic acid production was optimal when reaction temperature was high, reaction time was long, and catalyst concentration was high.

Based on the overall results, sugars used as a substrate for biofuel (bioethanol, biobutanol, biogas, etc.) production can be obtained from marine biomass *G. amansii*. Moreover, further study will be performed to produce biofuels from sugars derived from marine biomass. Also, chemical intermediates (HMF and levulinic acid) produced from marine biomass will be used as platform compounds for use in synthesis of a broad range of chemicals currently obtained from petroleum. Our results may provide useful information in regard to development of more economical and efficient systems for production of sugars and chemicals from marine biomass.

Table 6 The optimized results of the response surface methodology of marine algae *G. amansii* acid hydrolysis

Products	Reaction temperature (°C)	Reaction time (min)	Catalyst concentration (%)	Product concentration (g/l)
Glucose	139.4	15.0	3.0	2.92
Galactose	108.2	45.0	3.0	16.12
Levulinic acid	160.0	43.1	3.0	9.74

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References

1. Hoekman, S. K. (2009). *Renewable Energy*, *34*, 14–22.
2. Bicker, M., Kaiser, D., Ott, L., & Vogel, H. (2005). *Journal of Supercritical Fluids*, *36*, 118–126.
3. Jeong, G. T., & Park, D. H. (2008). *Applied Biochemistry and Biotechnology*, *148*, 131–139.
4. PCT, WO (2008) 105618 A1
5. Schenk, P. M., Thomas-Hall, S. R., Stephens, E., Marx, U. C., Mussgnug, J. H., Posten, C., et al. (2008). *Bioenergetic Research*, *1*, 20–43.
6. Muffler, K., & Ulber, R. (2005). *Advances in Biochemical Engineering/Biotechnology*, *97*, 63–103.
7. Matsunaga, T., Takeyama, H., Miyashita, H., & Yokouchi, H. (2005). *Advances in Biochemical Engineering/Biotechnology*, *96*, 165–188.
8. Horn, S. J., Aasen, I. M., & Østgaard, K. (2000). *Journal of Industrial Microbiology and Biotechnology*, *25*, 249–254.
9. Saxena, R. C., Adhikari, D. K., & Goyal, H. B. (2009). *Renewable and Sustainable Energy Reviews*, *13*, 167–178.
10. Baek, S. W., Kim, J. S., Park, Y. K., Kim, Y. S., & Oh, K. K. (2008). *Biotechnology and Bioprocess Engineering*, *13*, 332–341.
11. Chandra, R. P., Bura, R., Mabee1, W. E., Berlin1, A., Pan, X., & Saddler, J. N. (2007). *Advances in Biochemical Engineering/Biotechnology*, *108*, 67–93.
12. Encyclopedia of herbs and plants. Available from: http://herbs-and-plants.blogspot.com/2009/03/gelidiaceae.html. Accessed May 2009
13. Chae, SW (2009) M.S. Thesis, Chonnam National University, Gwangju, Korea
14. Su, Y., Brown, H. M., Huang, X., Zhou, X.-D., Amonette, J. E., & Zhang, Z. C. (2009). *Applied Catalysis A, General*, *361*, 117–122.
15. Lecomte, J., Finiels, A., & Moreau, C. (1999). *Industrial Crops and Products*, *19*, 235–241.
16. Jeong, G. T., & Park, D. H. (2009). *Applied Biochemistry and Biotechnology*, *156*, 431–441.
17. Jeong, G. T., Yang, H. S., & Park, D. H. (2009). *Bioresource Technology*, *100*, 25–30.
18. National Renewable Energy Laboratory (NREL) (2006) Biomass analysis technology team laboratory analytical procedure: determination of structural carbohydrates and lignin in biomass