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Efficient conversion of glucosamine to ethyl levulinate catalyzed by methanesulfonic acid

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Abstract−This study is focused on the possibility of using crustacean waste shells for sustainable biofuels and chemical production. We investigated the synthesis of ethyl levulinate (EL) from glucosamine by the methanesulfonic acid-catalyzed hydrothermal reaction using Box-Behnken design. In the ethyl levulinate synthesis, higher water content highly inhibited the formation of EL. Among the reaction factors, reaction temperature, catalyst concentration, and reaction time positively affected the outcome more than substrate concentration. The optimized reaction conditions were 200 °C reaction temperature, 60 g/L substrate concentration, 0.75 M catalyst concentration, and 44.9 min. Under these conditions, a 22.76 mol% EL yield was achieved. These results suggest that crustacean waste shells can be used for renewable feedstocks to produce valuable chemicals and biofuels.

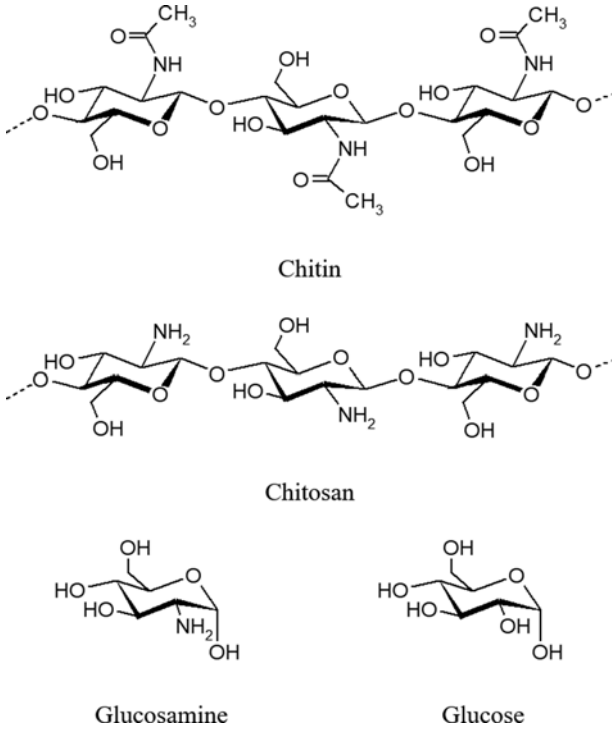
Keywords: Glucosamine, Ethyl Levulinate, Platform Chemical, Methanesulfonic Acid, Acid-catalyzed Hydrothermal Process

INTRODUCTION

Climate change and the depletion of fossil resources have been seriously considered throughout the world. To overcome these problems, renewable energy resources have been introduced and researched. Bioenergy and biomass feedstocks have been regarded as renewable, sustainable, and a replacement for fossil resources [1-3]. Biofuels and chemicals, which originate from various feedstocks such as sugars, starches, lignocellulosics, and macro-/microalgae, have been sustainably converted by biological and/or thermochemical methods [2,4-7]. Recently, chitin/chitosan, derived from marine crustacean shells, has been investigated to produce biofuels and chemicals [8-12].

Chitin and chitosan, which are mainly composed of *N*-acetyl-D-glucosamine and D-glucosamine, respectively, are natural polymers (Scheme 1) [9,10]. These can be recovered from the exoskeletons of crustaceans and insects, and the cell walls of yeasts, fungi, and mushrooms [8-10]. Due to the similarity of the structure of chitin/chitosan with cellulose, chitosan can be partially hydrolyzed by some cellulases [9,13,14]. The amino sugar (2-Amino-2-deoxyglucose) [9,10], a glucosamine, is a monomer of chitosan. Glucosamine and chitosan have been commercially introduced in dietary supplements, cosmetics, pharmaceuticals, agriculture, and environmental applications. Recently, the applications of chitin, chitosan, and glucosamine have been expanded to the production of biofuels and chemicals, which include 5-hydroxymethylfurfural, levulinic acid, monoethanolamine, pyrazine, pyridine, N- and O-containing chemicals [8, 10-14].

Works related to the synthesis of chemical intermediates (so-called versatile platform chemicals) have been reported using various biomass feedstocks by biological or thermo-chemical methods [2-4,10-12,14-20]. Levulinic acid has two functional groups (ketone and carboxylic acid). It is one of the 12 compounds reported by the DOE in 2004 as the "Top Value Added Chemicals from Biomass" [3] and top 10 chemicals revised in 2010 [21]. It



Scheme 1. The structure of chitosan, glucosamine, cellulose and glucose.

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can be converted into various potential chemicals such as hydrocarbon fuels, polymer building blocks, and herbicides [15,19,22].

Ethyl levulinate (EL), which is an ester form of levulinic acid, is a versatile chemical with potential usage such as flavors, diesel additives, CO_2 sequestration processes, and γ-valerolacton synthesis [17,19,23,24]. Moreover, the advantages of EL for diesel additives are miscibility, low sulfur content, high lubricity, good flow properties, good flashpoint stability, high anti-knocking index, and reduction of NO_x emission [19]. Due to these versatile applications of EL, the development of eco-friendly and cost-effective processes has been focused on the field of biorefining [15,17,19]. Generally, EL can be synthesized by the esterification of levulinic acid and ethanol under acid catalysts. EL can be obtained by acid-catalyzed ethanolysis from biomass and biomass-derived materials, such as monomeric sugars, polysaccharides, furfuryl alcohol, and chloromethyl furfural [19]. Though, the conversion routes are different, according to substrate properties [19].

The applications of chitin, chitosan, and glucosamine for the conversion into platform chemicals such as 5-HMF and levulinic acid were investigated by several research groups [8-12]. There has been no investigation of ethyl levulinate synthesis from glucosamine. Here we studied the formation of ethyl levulinate from glucosamine as a substrate by the one-pot acid-catalyzed hydrothermal reaction with ethanol media. To optimize and evaluate the effects of four reaction factors and their reciprocal interactions on EL synthesis, we introduced the 3-level 4-factor Box-Behnken design as a statistical approach.

MATERIALS AND METHODS

1. Materials

Reagent grade glucosamine HCl and ethyl levulinate were purchased from Sigma-Aldrich Co. Ltd., USA. Methanesulfonic acid, ethanol, and the other chemicals also were of analytical grade.

2. One-pot Experimental Procedure

For each reaction, the designated amounts of substrate, catalyst, and ethanol were added to a 50 mL-scale stainless steel reactor. The reaction was started when the temperature of reactants in the

Table 1. Experimental design matrix and result for 5-level-4-factor response-surface analysis in Box-Behnken design

Run	Temperature (°C)	Glucosamine amount (g/L)	Catalyst conc. (M)	Reaction time (min)	EL conc. (g/L)	EL yield (mol%)
1	200(1)	100(1)	0.50(0)	30(0)	6.70	10.02
2	200(1)	75(0)	0.75(1)	30(0)	9.20	18.34
3	180(−1)	50(−1)	0.50(0)	30(0)	3.08	9.21
4	190(0)	75(0)	0.50(0)	30(0)	4.79	9.54
5	190(0)	100(1)	0.50(0)	45(1)	4.79	7.17
6	180(−1)	75(0)	0.50(0)	15(−1)	3.82	7.62
7	180(−1)	75(0)	0.75(1)	30(0)	6.15	12.26
8	190(0)	50(−1)	0.75(1)	30(0)	5.72	17.12
9	180(−1)	75(0)	0.50(0)	45(1)	4.11	8.19
10	180(−1)	100(1)	0.50(0)	30(0)	4.72	7.06
11	190(0)	75(0)	0.50(0)	30(0)	4.54	9.06
12	180(−1)	75(0)	0.25(−1)	30(0)	2.51	5.01
13	200(1)	75(0)	0.50(0)	15(−1)	6.40	12.76
14	190(0)	100(1)	0.25(−1)	30(0)	2.75	4.11
15	190(0)	75(0)	0.75(1)	15(−1)	5.45	10.86
16	190(0)	75(0)	0.50(0)	30(0)	4.50	8.98
17	200(1)	75(0)	0.25(−1)	30(0)	2.40	4.78
18	190(0)	75(0)	0.50(0)	30(0)	4.25	8.47
19	190(0)	50(−1)	0.50(0)	15(−1)	3.78	11.32
20	190(0)	100(1)	0.75(1)	30(0)	8.24	12.32
21	190(0)	75(0)	0.75(1)	45(1)	8.49	16.93
22	190(0)	50(−1)	0.25(−1)	30(0)	1.91	5.71
23	190(0)	50(−1)	0.50(0)	45(1)	5.24	15.68
24	190(0)	75(0)	0.50(0)	30(0)	5.76	11.50
25	200(1)	50(−1)	0.50(0)	30(0)	4.82	14.41
26	190(0)	100(1)	0.50(0)	15(−1)	4.20	6.28
27	190(0)	75(0)	0.50(0)	30(0)	5.36	10.69
28	200(1)	75(0)	0.50(0)	45(1)	5.92	11.80
29	190(0)	75(0)	0.25(−1)	15(−1)	2.33	4.65
30	190(0)	75(0)	0.25(−1)	45(1)	2.82	5.62

reactor reached the required temperature. The temperature of the reactants was monitored and controlled by means of a proportional integral derivation (PID) temperature controller. The reactants were mixed by using a magnetic bar and stirrer at about 200 rpm. Upon finishing the reaction, the reactor was quickly cooled using tap-water. The products were recovered by centrifugation at 15,000 rpm for 20 min, and the supernatant was filtered using a 0.2 μm syringe filter for further analysis [11]. To investigate the effects of water content on the synthesis of ethyl levulinate, the reaction was performed under the conditions of reaction temperature 200 °C, 50 g/L glucosamine, 0.5 M methanesulfonic acid, 30 min reaction time, and 0-100% water content. All of the experiments were repeated two or more times and the data are presented as the mean ±SD.

3. Design of Experiment and Statistical Analysis

For the experimental design, a 3-level 4-factor Box-Behnken design requiring 30 runs was introduced, using the Design-Expert 9 program (Stat-Ease, Inc., USA) [25]. Experimental factors (and their levels) were as follows: reaction temperature (185-200 °C), substrate concentration (50-100 g/L), catalyst concentration (0.25-0.75 M), and reaction time (15-45 min.). Table 1 presents the coded and real factors (X_i) and their levels. The model was developed via multiple regression and its quality and significance were evaluated, based on the coefficient of determination (R^2) and ANOVA (Table 3) using the Design-Expert 9 program [25].

The model is given by Eq. (1):

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} x_i x_j \quad (1)$$

where Y is the response (EL yield), x_i is the ith independent parameter, β_0 is the intercept, β_i the linear coefficient, and β_{ij} is the interaction coefficient between i and j [25].

4. Analytical Method

The glucosamine concentration was measured using the modified 3,5-dinitrosalicylic acid method [26] with spectrophotometry (Spekol 1300, Analytik Jena, Germany) at 540 nm and glucosamine as standard. The concentration of ethyl levulinate was analyzed by gas chromatography (YL 6100; Young Lin Instrument Co., Ltd., Korea) equipped with an FID and DB-WAX UI capillary column (Agilent Technologies, USA). The temperature of the oven was programmed as follows: 80 °C (1 min), increased to 240 °C (1 min) at a rate of 10 °C/min. Both temperatures of the injector and detector were set to 250 °C.

5. Calculation of Conversion Yield and Statistical Analysis

The conversion yield of glucosamine to EL was calculated by the following equation: conversion yield (%)=product concentration (M)/initial substrate concentration (M)×100. The statistical significance of differences in the experiments was evaluated with one-way analysis of variance (ANOVA) and Duncan's multiple range test ($P<0.05$) using SPSS version 23 (SPSS, Cary, NC, USA).

RESULTS AND DISCUSSION

1. Effects of Water Content

To investigate the effects of water content on the synthesis of ethyl levulinate, the reaction was performed under the conditions of 50 g/L substrate concentration, 0.5 M catalyst concentration at 200 °C for 30 min with water content 0-100%. Fig. 1 shows the effects of water content on ethyl levulinate synthesis. The EL yield was decreased by an increase in water content. The highest EL yield was obtained at 0% water content (anhydrous ethanol). When water was added to the reactant, the EL yield decreased. The esterification of levulinic acid and ethanol can occur more under non-water conditions [27]. Similarly, Xu et al. [27] reported the inhibition of EL yield was observed with increasing water content in the one-pot preparation of EL from cellulose. The highest reported EL yield was under no-water addition conditions. Peng et al. [18] reported similar results in the solid acid catalyzed conversion from glucose. They proposed that the decrease of EL yield was caused by the competition of ethanolysis and hydrolysis reactions under a high-water environment. Comparably, Rataboul and Essayem [28] reported the synthesis of methyl levulinate from cellulose was efficiently conducted in a methanol-water mixture (90 : 10, wt : wt).

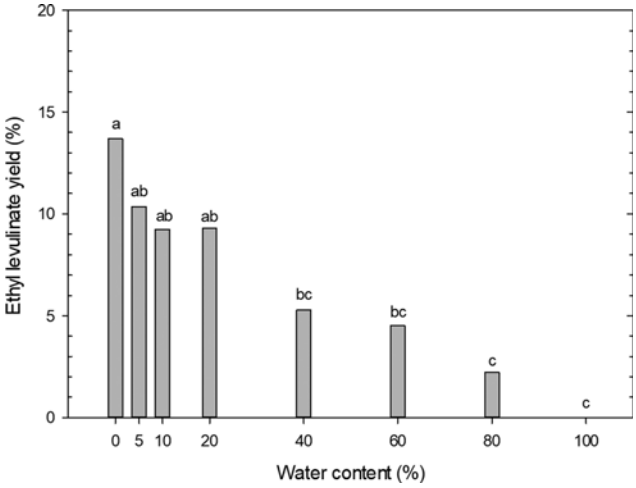

Fig. 1. Effect of water content on ethyl levulinate formation under 200 °C reaction temperature, 50 g/L substrate concentration, 0.5 M catalyst concentration, and 30 min reaction time.

2. Optimization of EL Synthesis

In statistical experimental design, response surface methodology (RSM) can be analyzed for the effects of reaction factors and their reciprocal interactions on the optimization of chemical or biochemical reactions [25]. In this study, the optimization of reaction conditions and their reciprocal interactions for the synthesis of ethyl levulinate (from glucosamine) was performed and evaluated via a statistical approach and methanesulfonic acid-catalyzed hydrothermal process. To optimize and evaluate the synthesis of EL, a 3-level 4-factor (reaction temperature, substrate concentration, catalyst concentration, and reaction time) Box-Behnken design was applied [25].

Table 2 lists the 30-experimental conditions and their final results for the 3-level 4-factor response surface analysis. The obtained EL yields were 4.11-18.34 mol% (1.91-9.2 g/L EL concentration). The highest EL yield, 18.34 mol%, was achieved at 200 °C, 75 g/L glucosamine, 0.75 M catalyst, and 30 min (Run 2). In contrast, the lowest EL yield was obtained at 190 °C, 100 g/L glucosamine, 0.25 M catalyst, and 30 min (Run 14).

Table 2. ANOVA for Response Surface 2FI model of ethyl levulinate production

Source	Sum of squares	df	Mean square	*F*-value	*p*-Value (Prob>F)
Model	417.32	10	41.732	29.675	2.E-09
X_1 : Temperature (°C)	43.23	1	43.227	30.738	2.E-05
X_2 : Substrate conc. (g/L)	58.44	1	58.440	41.556	4.E-06
X_3 : Catalyst conc. (M)	280.01	1	280.005	199.111	2.E-11
X_4 : Reaction time (min)	11.79	1	11.794	8.387	9.E-03
$X_1 X_2$	1.27	1	1.265	0.900	4.E-01
$X_1 X_3$	9.94	1	9.938	7.067	2.E-02
$X_1 X_4$	0.58	1	0.580	0.412	5.E-01
$X_2 X_3$	2.56	1	2.558	1.819	2.E-01
$X_2 X_4$	3.02	1	3.021	2.148	2.E-01
$X_3 X_4$	6.49	1	6.492	4.616	4.E-02
Residual	26.72	19	1.406		
Lack of fit	20.06	14	1.433	1.075	5.E-01
Pure error	6.66	5	1.332		
Cor total	444.04	29			

Table 3. Comparison of ethyl levulinate production from different substrate

Substrate	Reaction condition	Product yield***	References
Corn stover	MI**, 190 °C, 15 g/g liquid-to-solid mass ratio, 2.84 wt% sulfuric acid, and 30.4 min	15.5 wt% (58.1 mol%) EL	[24]
Corn stover	CH*, 190 °C, 15 g/g liquid-to-solid mass ratio, 2.84 wt% sulfuric acid, and 30.4 min	5.9 wt% (22.1 mol%) EL	[24]
Wheat straw	CH*, 183 °C, 19.8 mass ratio of liquid-to-solid, 2.5% sulfuric acid, and 30.6 min	17.91 wt% (51.0%) EL	[17]
Cellulose Starch Glucose Fructose	CH*, 160 °C, 0.5 mmol substrate, 4.5 ml ethanol, 0.2 mmol $AlCl_3$ as catalyst, and 10 min	11.3% 18.8% 23.3% 25% EL	[16]
Furfuryl alcohol	CH*, 150 °, Furfuryl alcohol 1 g, ethanol 19 g, glucose-derived carbonaceous catalyst 0.5 g, and 60 min	67.1% EL	[31]
Glucosamine	MI**, 200 °C, 0.26 mmol $SnCl_4 \cdot 5H_2O$, 100 mg glucosamine and 20 mL water	32.0 wt% LA	[32]
Glucosamine	CH*, 200 °C, 125 g/L glucosamine, 0.3 M sulfamic acid, 15 min	33.76 mol% LA	[12]
Glucosamine	CH*, 200 °C, 0.75 M methanesulfonic acid, 60 g/L glucosamine, and 44.9 min	22.76 mol% EL	This work

*CH : conventional heating
**MI : microwave irradiation
***EL (ethyl levulinate), LA (levulinic acid)

The obtained data were evaluated and analyzed via multiple regression analysis and ANOVA for the model equation and its significance using Design-Expert 9 software. Table 3 presents the results of ANOVA for the model equation of ethyl levulinate synthesis. The *F*-value of the model was evaluated to 39.68, which implies that the model is significant. The coefficients of the model were analyzed using RSM, and the values are presented in Table 3. The significance of model terms was evaluated by "Prob>F" values. Generally, lower values than 0.05 are significant. In this model, four linear coefficients (X_1, X_2, X_3, X_4) and two interaction coefficients (X_1X_3, X_3X_4) were evaluated for significance. The ANOVA of the regression model represented that the model was significant with a low probability value (*P*=1.72E-09). Moreover, the coefficient of determination (R^2) of the model was 0.940, which indicates the high quality of the model for reaction factors and EL yield (Fig. 2). Also, the values of "Predicted R^2" and "Adjusted R^2" were

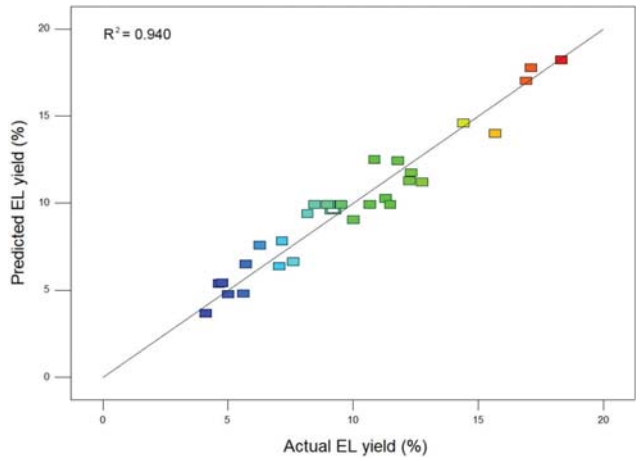

Fig. 2. Relationship between the predicted and actual values of ethyl levulinate formation. Different small letters indicate that it is significantly different with each water content (P<0.05, Duncan's test).

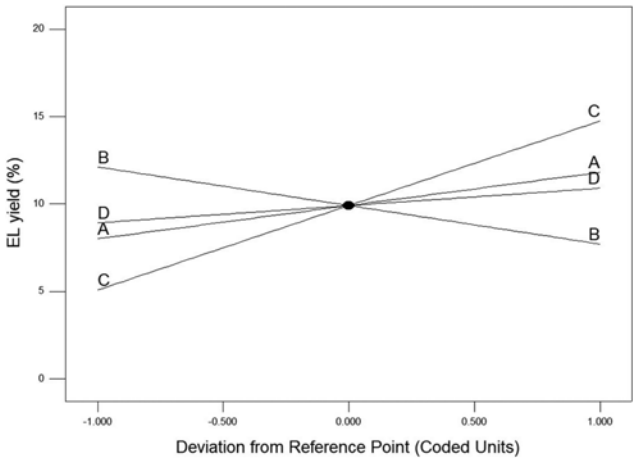

Fig. 4. Perturbation plot for comparing the effects of reaction temperature, substrate concentration catalyst concentration and reaction time on ethyl levulinate formation from glucosamine. Reaction temperature (A), substrate concentration (B), catalyst concentration (C) reaction time (D).

0.836 and 0.908, respectively. This represents a reasonable agreement for the model [25]. The "Adeq Precision" value, 20.25, indicates the signal-to-noise ratio, which is greater than 4 [25]. According to the above evaluation of ANOVA, this model can be used for design applications.

The final model equation, based on actual values, was obtained as

$$Y=-22.714+0.119\,X_1+0.473\,X_2-101.066\,X_3+0.552\,X_4-0.002\,X_1X_2 +0.630\,X_1X_3-0.003\,X_1X_4-0.128\,X_2X_3-0.002\,X_2X_4+0.340\,X_3X_4 \quad (2)$$

where Y is the response (ethyl levulinate yield (mol%)), and X_1, X_2, X_3 and X_4 are the actual values of the independent factors: reaction temperature (°C), substrate concentration (g/L), catalyst concentration (M), and reaction time (min), respectively.

Fig. 3 presents the effects of reaction factors on the model equation according to the coded values for EL synthesis from glucosamine. The factors of X_1, X_3, X_4, X_1X_3, and X_3X_4 had positive effects. X_3 (catalyst concentration), the most significant factor, affected the yield the most. Other factors had negative effects. Especially, X_2 (substrate concentration) negatively affected the EL synthesis the most.

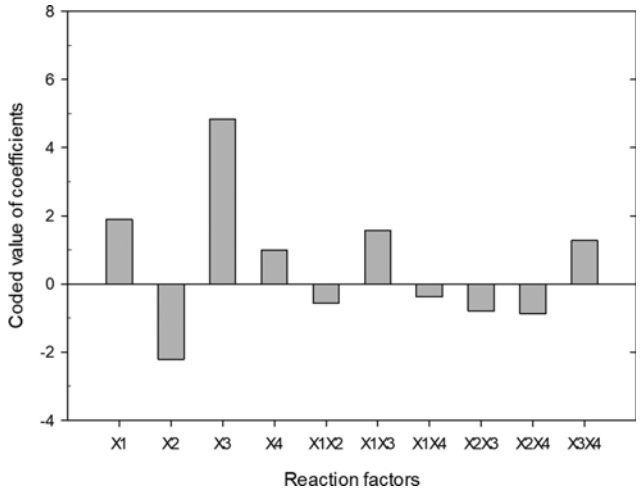

Fig. 3. Effect of explanatory factors on ethyl levulinate yield. X_1 (Temperature (°C)), X_2 (Biomass conc. (g/L)), X_3 (Catalyst conc. (M)), X_4 (Reaction time (min)).

Fig. 4 shows the perturbation plot for comparing the effects of all factors (based on coded units) on the synthesis of EL from glucosamine. The EL yield was linearly enhanced by increasing temperature within the tested ranges. Moreover, increasing the catalyst concentration led to a sharp linear increase in EL yield. Also, the EL yield was slightly enhanced over time. In addition, increasing the substrate concentration caused a linear decrease in EL yield.

Fig. 5 presents the effects and reciprocal interactions of reaction factors for EL synthesis from glucosamine. Fig. 5(a) presents the effects and their reciprocal interactions between reaction temperature and substrate concentration at constant conditions of 30 min and 0.5 M catalyst concentration. At a constant substrate concentration, the EL yield linearly increased with an increase in reaction temperature. The EL yield decreased with increasing substrate concentration. Increasing reaction temperature can accelerate the conversion rate of glucosamine and the esterification rate [15]. Tiong et al. [15] similarly reported that a high EL yield was obtained at a higher reaction temperature and high ethanol-to-substrate ratio in the conversion of oil palm biomass. Fig. 5(b) shows the effects of reaction temperature, catalyst concentration, and their reciprocal interactions on EL yield at a constant 75 g/L substrate and 30 min. At a constant reaction temperature, the EL yield sharply increased with increasing catalyst concentration. At a high catalyst concentration, the EL yield increased more than that at a low catalyst concentration. In a similar work, Chang et al. [17] reported that the synthesis of EL from wheat straw can be accelerated under highly acidic conditions. However, in highly acidic conditions, undesirable side-reactions such as the formation of diethyl ether or the condensation of ethanol can occur [17,29]. Fig. 5(c) presents the effects of temperature, time, and their reciprocal interactions on

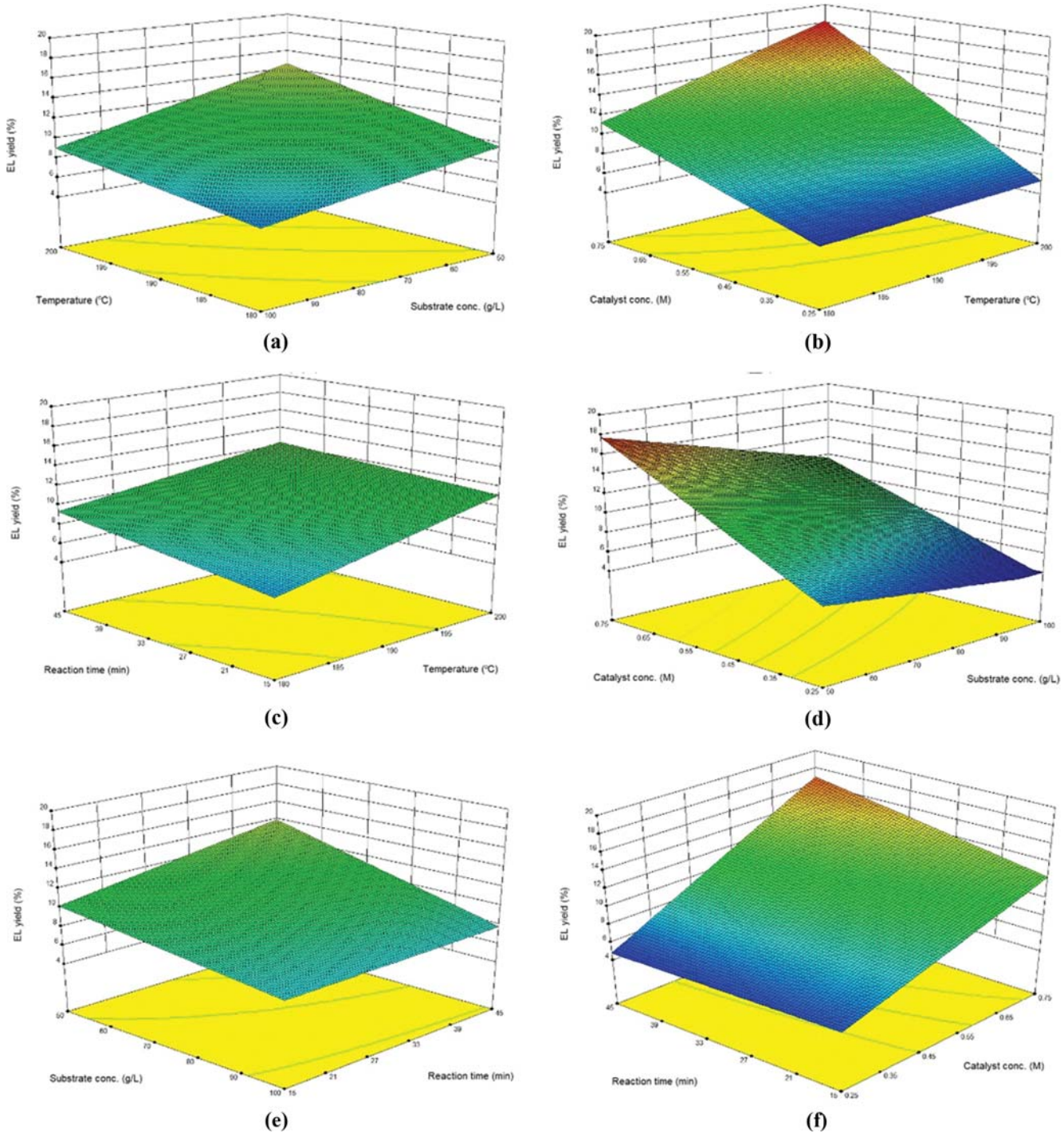

Fig. 5. Response-surface plot representing effects of reaction temperature, substrate concentration, catalyst concentration, reaction time, and their reciprocal interaction on ethyl levulinate formation from glucosamine under constant reaction conditions. (a) At a constant 0.5 M catalyst and 30 min; (b) at a constant 75 g/L substrate and 30 min; (c) at a constant 75 g/L substrate and 0.5 M catalyst; (d) at a constant 190 °C and 30 min; (e) at a constant 190 °C and 0.5 M catalyst; (f) at a constant 190 °C and 75 g/L substrate.

the EL yield at a constant 75 g/L substrate and 0.5 M catalyst. An increasing reaction temperature slightly enhanced the EL yield during the reaction. Also, the EL yield increased at a higher reaction temperature. Fig. 5(d) presents the effects of substrate concentration, catalyst concentration and their reciprocal interactions on the EL yield at a constant 190 °C and 30 min. The synthesis of EL sharply increased with catalyst concentration throughout the tested substrate concentration range. In contrast, increasing substrate concentration decreased the EL yield. Fig. 5(e) presents the effects of substrate concentration, reaction time, and their reciprocal interactions on the EL yield at a constant 190 °C and 0.5 M catalyst. A high EL yield was obtained at lower substrate ranges within the

designed reaction time range. There was a higher EL yield for a longer reaction time at a constant substrate concentration. Tiong et al. [15] reported that a high ethanol-to-substrate ratio in oil palm biomass conversion caused a low EL yield. A constrained mass transfer rate is caused by excess ethanol, which dilutes the reactants and limits the esterification reaction [15,17]. Fig. 5(f) presents the effects of catalyst concentration, reaction time, and their reciprocal interactions on the EL yield at a constant 190 °C and 75 g/L substrate. A higher EL yield was achieved under a higher catalyst and longer reaction time conditions. Both the catalyst concentration and the reaction time highly influenced the increasing EL yield. Overall, the synthesis of EL from glucosamine by the methanesulfonic acid-catalyzed hydrothermal reaction in ethanol solution could be improved under higher temperature, higher amounts of catalyst, and longer time within the designed ranges. In contrast, the substrate concentration negatively affected the EL yield within the tested ranges. In similar work, Zhang et al. [24] reported that high EL yields were achieved under high temperature and catalyst concentration in the production of EL from corn stover under microwave irradiation using sulfuric acid as a catalyst. In this work, the optimized conditions of EL synthesis from glucosamine were as follows: 200 °C reaction temperature, 60 g/L substrate concentration, 0.75 M catalyst concentration, 44.9 min reaction time in ethanol solution. Under these conditions, a 22.76 mol% EL yield was obtained.

Several works have proposed mechanisms for the formation of EL from levulinic acid, furfuryl alcohol, chloromethyl furfural, fructose, glucose, cellulose, and lignocellulosic biomass [15,19]. A trial with glucosamine has not been reported, while the routes of glucosamine conversion to levulinic acid (LA) were proposed by several studies [10-12,30]. The conversion routes of glucosamine were several steps as follows. First, similar to glucose, glucosamine (pyranose form) was isomerized to the furanose form. Second, the furanose form's -NH_2 group was deaminated under acidic conditions. Third, the consequent dehydration and keto-enol tautomerization formed 5-hydroxymethylfurfural (5-HMF). Finally, 5-HMF was rehydrated to LA and formic acid under acidic conditions [10-12,30]. Moreover, the conversion route of EL from LA is known as the esterification of LA and ethanol [19]. Overall, the synthesis routes of EL from glucosamine may be sequential routes, such as the sequential reactions of isomerization, deamination, dehydration, keto-enol tautomerization, rehydration, and esterification [10-12,19,30].

3. Effects of Combined Severity Factor on Ethyl Levulinate Formation

The combined severity factor (CSF) is defined as an equation combined with the reaction conditions such as temperature, time, and pH of the solution (catalyst activity). The CSF can be applied to lignocellulosic biomass pretreatment and acid-catalyzed hydrothermal reactions [6,11]. The application of CSF in EL synthesis has not been reported. Fig. 6 plots the distribution of the EL yield according to the CSF values of the reaction conditions. In CSF value ranges, a high CSF indicates harsh reaction conditions including higher temperature, higher catalyst concentration (or activity), and longer time [11,12]. For EL synthesis, the EL yield increases with an increasing combined severity factor. There are similar patterns

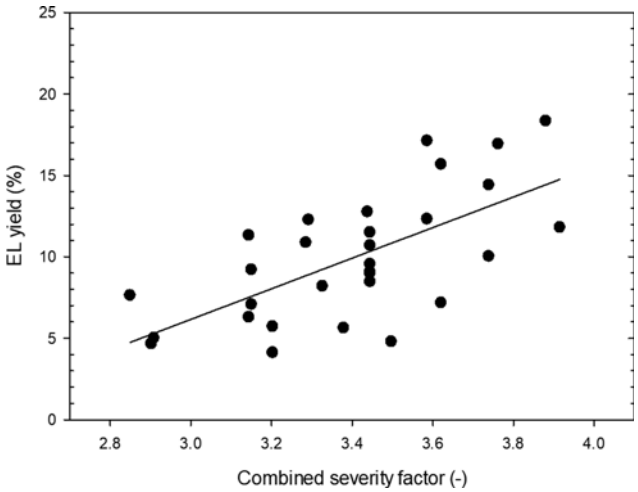

Fig. 6. Effect of combined severity factor on the formation of ethyl levulinate from glucosamine.

that are related to the LA conversion from chitosan and glucosamine [11,12].

4. Comparison of Ethyl Levulinate Formation from Various Substrates

Comparable results for EL synthesis from various substrates are presented [11,16-18]. Zhang et al. [24] reported the EL production from corn stover under microwave irradiation. At 190 °C, 15 g/g liquid-to-solid mass ratio, 2.84 wt% sulfuric acid, and 30.4 min, the EL yield was reported as 15.5 wt% (58.1 mol%). Under the same conditions with conventional heating, only a 5.9 wt% (22.1 mol%) EL yield was obtained. Chang et al. [17] reported a 17.91 wt% (51.0%) EL yield from wheat straw under the optimized conditions of 183 °C, 19.8 mass ratio of liquid-to-solid, 2.5% sulfuric acid, and 30.6 min. Quereshi et al. [16] reported the EL production from various biorenewable feedstocks such as cellulose, starch, glucose, and fructose using metal salt catalysts. The EL yield of cellulose, starch, glucose, and fructose was 11.3%, 18.8%, 23.3%, and 25%, respectively, under the conditions of 160 °C, 0.5 mmol substrate, 4.5 mL ethanol, 0.2 mmol $AlCl_3$ as a catalyst, and 10 min. Zhao et al. [31] reported the EL production from furfuryl alcohol using the environmentally benign and low-cost catalyst in ethanol. The EL yield was 67.1% under the condition of 150 °C, furfuryl alcohol 1 g, ethanol 19 g, glucose-derived carbonaceous catalyst 0.5 g, and 60 min. From this work, the highest EL yield, 22.76 mol%, was achieved from 200 °C, 60 g/L glucosamine, 0.75 M methanesulfonic acid, and 44.9 min in ethanol solution. Nevertheless, a comparison of EL yields obtained from this work and the literature was difficult due to the differences of the substrate, catalyst, and reaction conditions [11]. There was no synthesis of EL from glucosamine at that time. Nevertheless, several studies of the conversion of glucosamine to LA have been reported [12,32]. Omari et al. [32] reported the LA production (32.0 wt% yield) from glucosamine as a substrate under the conditions of 100 mg glucosamine, 0.26 mmol $SnCl_4 \cdot 5H_2O$, and 20 mL water at 200 °C for 30 min-microwave irradiation. Also, Kim et al. [12] reported a 33.76 mol% LA yield at 200 °C, 125 g/L glucosamine, 0.3 M sulfamic acid as catalyst, and 15 min. From these LA synthesis results (from glucosamine), the EL yield and reaction

conditions may be indirectly estimated because the esterification yield of EL from LA is higher than with other reactants.

CONCLUSIONS

This work focused on the synthesis of ethyl levulinate from glucosamine using the response surface methodology with the methanesulfonic acid-catalyzed hydrothermal reaction. Glucosamine is a monomer from crustacean waste shells. As an optimized result, the highest EL yield, 22.76 mol%, was obtained at 200 °C, 60 g/L glucosamine, 0.75 M methanesulfonic acid, and 44.9 min. These results will contribute to the fields of renewable bioresources and the production of chemicals and biofuels.

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