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Development of 2,3-Butanediol Production Process from *Klebsiella aerogenes* ATCC 29007 Using Extracted Sugars of *Chlorella pyrenoidosa* and Biodiesel-Derived Crude Glycerol

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Abstract: Expectation for renewable energy is increasing due to environmental pollution such as fossil fuel depletion, CO_2 emission, and harmful gases. Therefore, in this study, extracted sugars of microalgae, which cause algal blooms and crude glycerol, a biodiesel industry byproduct, were used simultaneously to produce 2,3-BDO. The 2,3-BDO production using only extracted algal sugars was about 4.8 g/L at 18 h, and the production of 2,3-BDO using both extracted algal sugar and crude glycerol was about 7 g/L at 18 h. It was confirmed that the main culture with crude glycerol was increased 1.5-fold compared to the case of using only extracted algal sugars. In addition, four components of the main medium (ammonium sulfate, casein hydrolysate, yeast extract, and crude glycerol) were statistically optimized and the concentrations of the medium were 12, 16, 12, and 13 g/L, respectively. In addition, the final 2,3-BDO production was about 11g/L, which 1.6-fold higher than before the optimization process. As a result, it was confirmed that 2,3-BDO production is possible through the simultaneous use of algal sugars and crude glycerol, which can greatly contribute to the development of zero-waste processes.

Keywords: 2,3-butanediol; microalgae; crude glycerol; response surface methodology (RSM)

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1. Introduction

Generally, platform chemical substances have been dependent on the oil-based petrochemical process for decades. However, as environmental problems such as global warming and harmful gas emission are growing severe, development of environment friendly and sustainable biotechnology is direly necessary. Accordingly, the development of bioconversion technology is being activated by using microorganisms and biomass resources [1–5].

In order to replace the existing oil industry, it is essential to secure the economic feasibility and biomass of this bioprocess. Although the present bioprocess carbon source is obtained from starch crops, its potential to replace oil is limited due to problems of limited land use and ethical issues of using fertilizers and food resources [6,7]. Currently, much research is focusing on the second-generation biomass (wood biomass) other than starch resources. Land plants can be used for fermentation of microorganisms because the carbon source can be obtained by dissolving cellulose and hemicellulose [6–9]. However, land plants have a hard structure due to lignin, a need for additional processes to remove

fermentation-inhibiting materials generated in preprocessing, and are difficult to secure economic feasibility due to costly preprocessing and saccharification process [8–11].

Presently, algae—sea plants—are being focused on as the next generation carbon source. Because sea algae also photosynthesize, they have all the merits of land plants [12]. Additionally, it is well known that they grow rapidly because they have higher photosynthesis efficiency than land plants, and they have a 1.5-fold to 2-fold higher carbon dioxide fixation effect [13,14]. *Chlorella*, a microalgae species, is a round algae living in seawater and freshwater, looks similar to *Nannochloropsis*, and is currently used for production of biodiesel due to its high lipid content. However, other than lipids, it also contains sugars that can be fermented, and the contained sugars can be extracted along with lipids [12–16]. These sugars can be used as the carbon source and converted to biofuels and chemical substances.

While crude glycerol research is actively progressing, a European consortium carried out a project titled "Glycerol Biorefinery Approach Towards the Production of High-Quality Products of Industrial Value" with 15 partners from nine countries [17]. The consortium discovered a glycerol technology and developed an effective commercialization solution using biorefinery technology. In addition, crude glycerol is produced as a byproduct in the biodiesel process, which is converted from the lipids of microalgae [18]. Therefore, a zero-waste process is constructed using both crude glycerol and extracted algal sugars, which can be applied to the development of renewable energy [16,18–20].

2,3-BDO, a chemical substance that can be produced by the biomass conversion process through fermentation of microorganisms, has a strong hydrophilic property as a colorless and odorless chiral compound in liquid or crystal form [21–23]. Because it has a low freezing point, −60 °C, it can be used as antifreeze. Moreover, 2,3-BDO is a very important chemical substance that can be used in various areas as 1,3-butadiene that becomes the raw material of synthetic rubbers, as methyl-ethyl-ketone (MEK) that has a high usage in related industries, as acetoin and diacetyl that are used as liquid fuel additives, food additives, or flavoring materials, and as a polyurethane precursor used for manufacturing thermosetting polymer plasticizers, cosmetics, and medicines [24].

The purpose of this study was to establish the optimum condition of collecting extracted algal sugars from microalgae, and thereby produce 2,3-BDO by using *Klebsiella aerogenes* ATCC 29007 that could metabolize extracted algal sugars. In addition, optimum compositions of the main medium were achieved by a statistical method.

2. Materials and Methods

2.1. Materials

Microalgal biomass (*Chlorella pyrenoidosa*) dried powder was purchased from WUDI LV QI BIOENGINEERING Co., Ltd. (Shandong, China). Biodiesel-derived crude glycerol was supplied by Dansuk Industry (Siheung-si, Gyeonggi-do, South Korea). The yeast extract was purchased from Bacto™ (212750). The casein hydrolysate (91079-40-2) and ammonium sulfate (7783-20-2) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Sugar Extraction of Chlorella pyrenoidosa Using Hydrochloric Acid

The components of *C. pyrenoidosa* were analyzed to be 9.94% carbohydrate (55% glucose and 45% galactose), 0.64% fiber, 56.61% protein, 4.55% lipid, and 7.86% ash [15]. The sugar extraction process was performed with 2% hydrochloric acid and a solid–liquid ratio of 100 g/L in an Erlenmeyer flask, using an autoclave (VS-1221-100, Vision science, Daejeon, South Korea) at 121 °C for 15 min, after which the pH was regulated to 7 by using NaOH [12].

2.3. Microorganism and Culture Conditions

Klebsiella aerogenes ATCC 29007 was purchased from the American Type Culture Collection (ATCC, Virginia, USA). The seed medium was nutrient broth (5 g/L peptone, 1 g/L meat extract, 2 g/L yeast extract, 5 g/L sodium chloride) and the main culture was

performed with 50 mL of seed culture in a 250 mL Erlenmeyer flask for 12 h at 37 °C in a rotary shaking incubator (VS-8480, Vision science, Daejeon, South Korea) at 180 rpm [25]. Inoculum (3%, *v*/*v*) was inoculated into the main culture when the cell growth reached 2.0 to 2.5 OD_{600} using UV-VIS spectrometer (U-1900, Hitachi, Japan). The main medium to produce 2,3-BDO contained 6.8 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 5.35 g/L $(NH_4)_2SO_4$, 5 g/L yeast extract, 10 g/L casein hydrolysate, 0.28 g/L Na_2SO_4, 0.75 g/L potassium chloride, 0.26 g/L $MgSO_4$, 0.42 g/L citric acid, and 0.3 mL/L trace element solution containing 2.7 g/L $FeCl_3$, 34.2 g/L $ZnCl_2$, 0.85 g/L $CuCl_2$, 0.31 g/L H_3BO_3, and 10 g/L $MnCl_2$. The initial pH of the main medium was adjusted to 5.5. Carbon sources extracted from microalgae were autoclaved at a pressure of 15 psi and 121 °C for 15 min using an autoclave (VS-1221-100, Vision science, Daejeon, South Korea) [26].

2.4. Experimental Design and Statistical Analysis

Central composite rotatable design (CCRD) was performed to improve the 2,3-BDO production. CCRD shows the interaction of independent variables for 2,3-BDO production, which can provide mathematical and statistical models. Four variables were performed with 30 trials at five different levels (−2, −1, 0, +1, +2). Table 1 shows the four variables of CCRD and the five levels of each variable for experimental conditions. The following variables and their concentrations were selected for the study of 2,3-BDO production: ammonium sulfate (X_1), 5–15 g/L; casein hydrolysate (X_2), 7–20 g/L; yeast extract (X_3), 5–15 g/L; and crude glycerol (X_4), 5–15 g/L. The independent variables were coded according to Equation (1):

$$x_i = (X_i - X_0)/\Delta X, (i = 1, 2, 3, \ldots, j) \quad (1)$$

where x_i and X_i are the coded and real values of the independent variable, respectively. ΔX is the step-change value and X_0 is the real value of independent variable at the center point. The response value was evaluated by analysis of variance (ANOVA). Each variable and its interaction were described by the following quadratic Equation (2):

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \sum \beta_{ii} {X_i}^2 \quad (2)$$

where Y is the predicted response, X_i and X_j are the input variables that influence the response variable Y; β_0, β_i, β_{ii}, and β_{ij} are offset term, first order model coefficient, quadratic coefficient for the variable i, and linear model coefficient for the interaction between variables i and j, respectively. Table 2 shows the predicted values and experimental design of thirty time experiments. The maximum value of 2,3-BDO production using optimized medium was taken as the responses of the designed experiments. The experimental design, estimation of the regression equation coefficients, and regression analysis of the data were performed using Design-Expert 7 software (Stat-Ease, Minneapolis, USA) [9].

Table 1. Each level of variable for medium components using central composite rotatable design (CCRD).

Variable (g/L)	Symbol	Levels				
		−2	**−1**	**0**	**1**	**2**
Ammonium sulfate	X_1	5	7.5	10	12.5	15
Casein hydrolysate	X_2	7	10.25	13.5	16.75	20
Yeast extract	X_3	5	7.5	10	12.5	15
Crude glycerol	X_4	5	7.5	10	12.5	15

Table 2. Central composite rotatable design and its response from experimental data for 2,3-butanediol fermentation.

Std.#	Coded Values				2,3-Butanediol Conc. (g/L)	
	X_1	X_2	X_3	X_4	Predicted	Actual
1	−1	−1	−1	−1	6.82	6.73
2	+1	−1	−1	−1	6.64	6.40
3	−1	+1	−1	−1	7.27	7.21
4	+1	+1	−1	−1	7.46	7.56
5	−1	−1	+1	−1	7.15	7.13
6	+1	−1	+1	−1	7.12	7.36
7	−1	+1	+1	−1	9.21	8.99
8	+1	+1	+1	−1	9.56	9.53
9	−1	−1	−1	+1	7.11	7.15
10	+1	−1	−1	+1	7.03	7.40
11	−1	+1	−1	+1	8.64	8.55
12	1	+1	−1	+1	8.93	8.95
13	−1	−1	+1	+1	7.09	7.14
14	+1	−1	+1	+1	7.16	7.24
15	−1	+1	+1	+1	10.23	10.48
16	1	+1	+1	+1	10.68	10.91
17	−2	0	0	0	8.12	8.27
18	2	0	0	0	8.39	8.08
19	0	−2	0	0	4.78	4.64
20	0	2	0	0	8.74	8.72
21	0	0	−2	0	7.81	7.86
22	0	0	2	0	9.89	9.69
23	0	0	0	−2	7.45	7.69
24	0	0	0	2	8.86	8.46
25	0	0	0	0	7.63	7.55
26	0	0	0	0	7.63	7.41
27	0	0	0	0	7.63	7.79
28	0	0	0	0	7.63	7.62
29	0	0	0	0	7.63	7.87
30	0	0	0	0	7.63	7.57

2.5. Analytical Methods

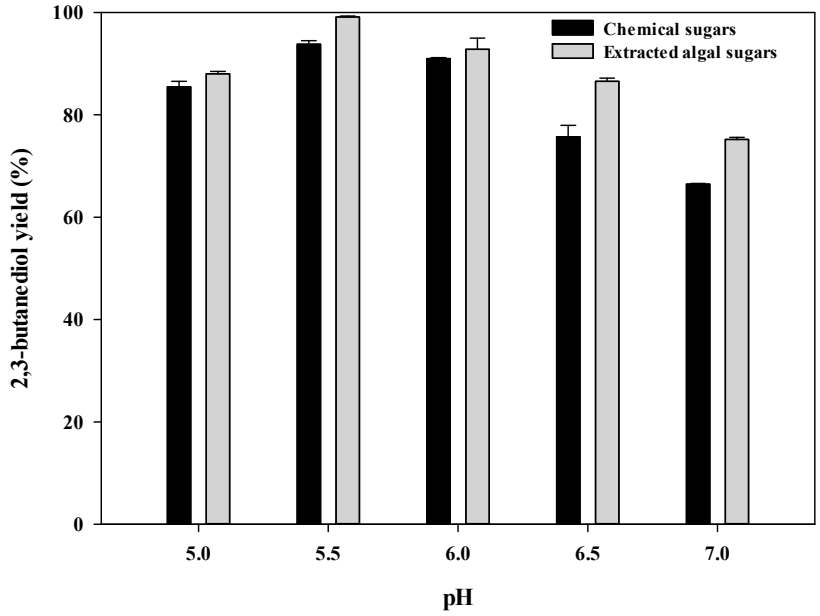
The concentrations of the medium components and 2,3-BDO were analyzed using high-performance liquid chromatography (HPLC) by an Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad, USA) and a refractive index detector (RID–10A, Shimadzu, Japan). The column temperature and detector was maintained at 55 °C and the mobile phase in the column was 0.005 N H_2SO_4 at a flow rate of 0.6 mL/min [8,13,14,19,26].

3. Results and Discussion

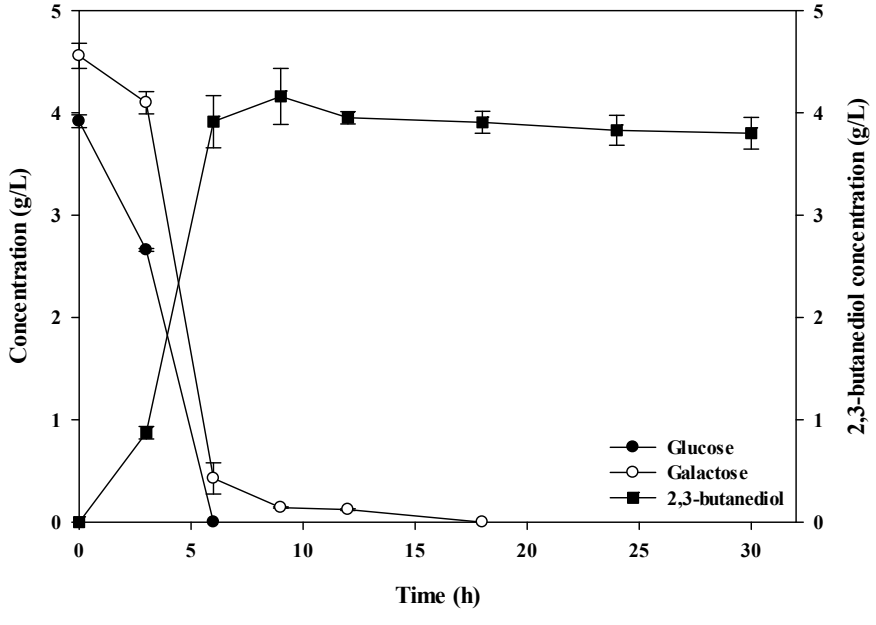
3.1. Comparison of Chemical Sugars and Extracted Algal Sugars for 2,3-BDO Production

Recently, Lee et al. reported the significant effect of casein hydrolysate on microbial growth and metabolite production. In addition, the casein hydrolysate was supplemented in the main medium to improve the consumption of crude glycerol from *K. aerogenes* [26]. Our present study shows that 2,3-BDO could be produced using extracted algal sugar, and the 2,3-BDO production capacity of chemical sugar and extracted algal sugar were compared in the medium containing casein hydrolysate and crude glycerol (Figure 1). Figure 1A shows the effect of pH for 2,3-BDO production from *K. aerogenes* ATCC 29007 using chemical sugars and extracted algal sugars. The extracted algal sugars were composed of glucose and galactose, and the concentration of sugars were confirmed to be about 4~5 g/L, respectively. The highest yield of 2,3-BDO was confirmed at pH 5.5, at which time the yield was converted to about 99%. The fermentation process using algal sugars confirmed that the total conversion yield of 2,3-BDO was higher than the chemical sugars process. Figure 1B shows the 2,3-BDO production using chemical sugars during 30 h, when

using glucose (3.9 g/L) and galactose (4.5 g/L). In the 2,3-BDO production process using chemical sugars, glucose was consumed within 6 h and galactose was consumed within 18 h. The 2,3-BDO production was about 4.1 g/L within 10 h. Figure 1C shows the 2,3-BDO production for 30 h using glucose (5.7 g/L) and galactose (4.1 g/L) from the extracted algal sugars. The extracted algal sugars took more time to uptake than chemical sugars. The fact that the extracted algal sugars have more inhibitors than chemical sugars confirmed the delay in the sugar consumption time for the microorganism. Nevertheless, the 2,3-BDO concentration was confirmed to be as high as when using chemical sugar. As a result, the 2,3-BDO production was about 4.8 g/L within 20 h. In this study, the possibility of production of 2,3-BDO was confirmed through the extracted algal sugar, and the production capacity as much as chemical sugar was confirmed by controlling the culture conditions.

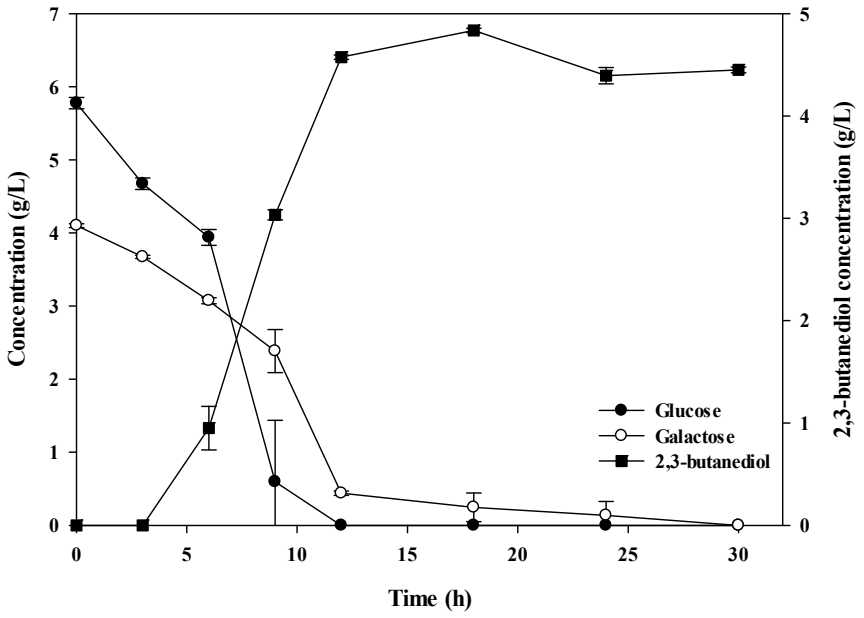


(**A**)



(**B**)

Figure 1. *Cont.*



(**C**)

Figure 1. The fermentation of *K. aerogenes* ATCC 29007 using chemical sugars and extracted algal sugars for 2,3-BDO production. (**A**) Comparative experiment by pH concentration, (**B**) 2,3-BDO production using chemical sugars, and (**C**) 2,3-BDO production using extracted algal sugars.

3.2. Improvement of 2,3-BDO Production Using Extracted Algal Sugars and Biodiesel-Derived Crude Glycerol

Previous studies reported that 2,3-BDO has been investigated from various strains and many substrates [10,21,23,24,27,28]. Among them, 2,3-BDO research was performed using biodiesel-derived crude glycerol from *K. aerogenes* ATCC 29007. The substrate concentration was significantly limited for fermentation with only extracted algal sugars. The high concentration algal sugars increased the viscosity of the solution, which limited microbial growth due to the osmotic pressure. Therefore, the production of 2,3-BDO was increased by adding biodiesel-derived crude glycerol. Figure 2A shows the effect of crude glycerol concentrations for 2,3-BDO production from *K. aerogenes* ATCC 29007 using extracted algal sugars at 24 h. According to previous reports, *K. aerogenes* ATCC 29007 at a concentration was 40 g/L or more had a substrate inhibition effect by crude glycerol [22]. Therefore, 2,3-BDO production was examined at a crude glycerol concentration of 5 g/L to 30 g/L. The production of 2,3-butanediol was the highest when using 30 g/L of crude glycerol. However, the uptake time of all the substrate was 24 h, which was two-fold longer than the average fermentation time of 12 h. For the efficiency of the overall process, 2,3-butaendiol has to be fermented in a shorter time and in higher yield, which was confirmed at 10 g/L of crude glycerol concentration. The 2,3-BDO conversion rate was about 84% (*w*/*w*) at 10 g/L crude glycerol.

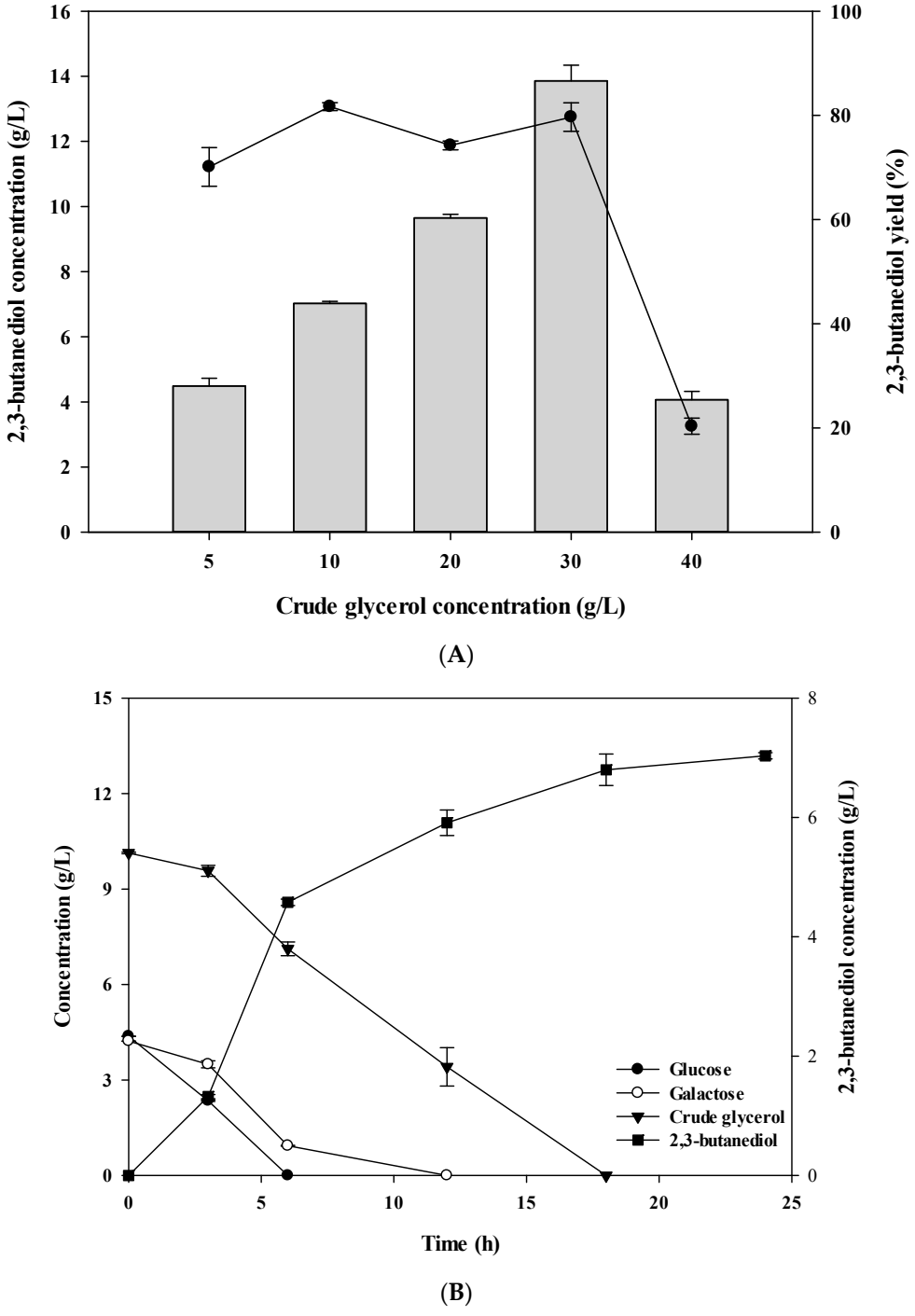


Figure 2. Production of 2,3-BDO from *K. aerogenes* ATCC 29007 using extracted algal sugars and biodiesel-derived crude glycerol. (**A**) Comparative experiment by crude glycerol concentration and (**B**) 2,3-BDO production using extracted algal sugars and crude glycerol.

Figure 2B shows the 2,3-BDO production using extracted algal sugars and crude glycerol for 25 h, which used glucose (4.3 g/L), galactose (4.7 g/L), and crude glycerol (10 g/L). In the 2,3-BDO production process using extracted algal sugars, glucose was consumed within 6 h and galactose was consumed within 12 h. In addition, all the crude glycerol was completely taken up in 18 h. The 2,3-BDO production was about 7 g/L at 18 h, which was increased by 3 g/L compared to when crude glycerol was not added. In addition, it was confirmed that the 2,3-BDO production was converted to about 80% compared to

the total substrate. These results confirmed the potential for 2,3-BDO production capacity by mixing the extracted algal sugar and crude glycerol.

3.3. Statistical Optimization of Medium Components to Improve 2,3-BDO Production from K. aerogenes ATCC 29007

In order to increase the production of 2,3-BDO using the medium efficiently, components of the main medium such as casein hydrolysate, ammonium sulfate, yeast extract, and crude glycerol were selected as statistical variables through basic experiments. In addition, the maximum point was statistically optimized using RSM [9,19,26]. The optimal concentration of each medium component to increase 2,3-BDO production was investigated using the CCRD of analysis of variance (ANOVA) by Design-Expert software. Table 3 shows the results of ANOVA for the response surface quadratic model, which confirmed the importance of the model selected, such as *f*-value, *p*-value, coefficient of determination, and coefficient of variation. The model F-value comparing the model variance of the residual error variance is 45.57, which indicated the model's significance. The F-value of the lack-of-fit (LOF), which means the variance of the data around the fitted model was 2.93 [9,19,26]. The coefficient of variation (CV) was 3.26%, which indicates accuracy. These statistical results confirmed the high reliability of the relationship between the experimental data and the predicted data.

Table 3. Result of statistical analysis and analysis of variance (ANOVA) for response surface quadratic model.

(A) ANOVA for the Selected Model					
Source	Sum of squares	DF	Mean squares	*f*-Value [a]	*Pr* > *F* [b]
Model	42.57	14	3.04	45.57	<0.0001
Lack of fit	0.85	10	0.085	2.93	0.1236
Error	0.15	5	0.029	-	-
Corrected total	43.57	29	-	-	-
(B) Statistical Analysis of Factors					
	Factor		Mean square	*f*-value [a]	*p*-value [b]
	X_1		0.10	1.56	0.2309
	X_2		23.57	353.30	<0.0001
	X_3		6.49	97.33	<0.0001
	X_4		2.98	44.69	<0.0001
	X_{11}		0.66	9.86	0.0067
	X_{22}		1.31	19.61	0.0005
	X_{33}		2.55	38.18	<0.0001
	X_{44}		0.47	6.99	0.0184
	X_{12}		0.14	2.07	0.1708
	X_{13}		0.024	0.36	0.5591
	X_{14}		0.009	0.14	0.7178
	X_{23}		2.60	38.94	<0.0001
	X_{24}		1.15	17.30	0.0008
	X_{34}		0.12	1.80	0.1998

Coefficient of variation (CV) = 3.26, coefficient of determination (R^2) = 0.9770. X_1: ammonium sulfate, X_2: casein hydrolysate, X_3: yeast extract, X_4: crude glycerol. [a] The F-value indicates how well the factors describe the variation in the data about its mean. [b] *Prob* > *F* (*p*-value) is the probability of obtaining a result at least as extreme as the one that was actually observed.

The estimated response model equation (based on Equation (2)) for 2,3-BDO production by *K. aerogenes* ATCC 29007 was predicted by the polynomial Equation (3):

$$Y = 7.63 + 0.066X_1 + 0.99X_2 + 0.52X_3 + 0.35X_4 + 0.093X_1X_2 + 0.039X_1X_3 + 0.024X_1X_4 - 0.40X_2X_3 + 0.27X_2X_4 - 0.087X_3X_4 + 0.15{X_1}^2 - 0.22{X_2}^2 + 0.3{X_3}^2 + 0.13{X_4}^2 \quad (3)$$

The coded values of ammonium sulfate, casein hydrolysate, yeast extract, and glycerol were appointed by X_1, X_2, X_3, and X_4, respectively. The plotting of three-dimensional response curves was confirmed from the optimum level of each of the variable, and Figure 3 shows the effect of the interaction on 2,3-BDO production.

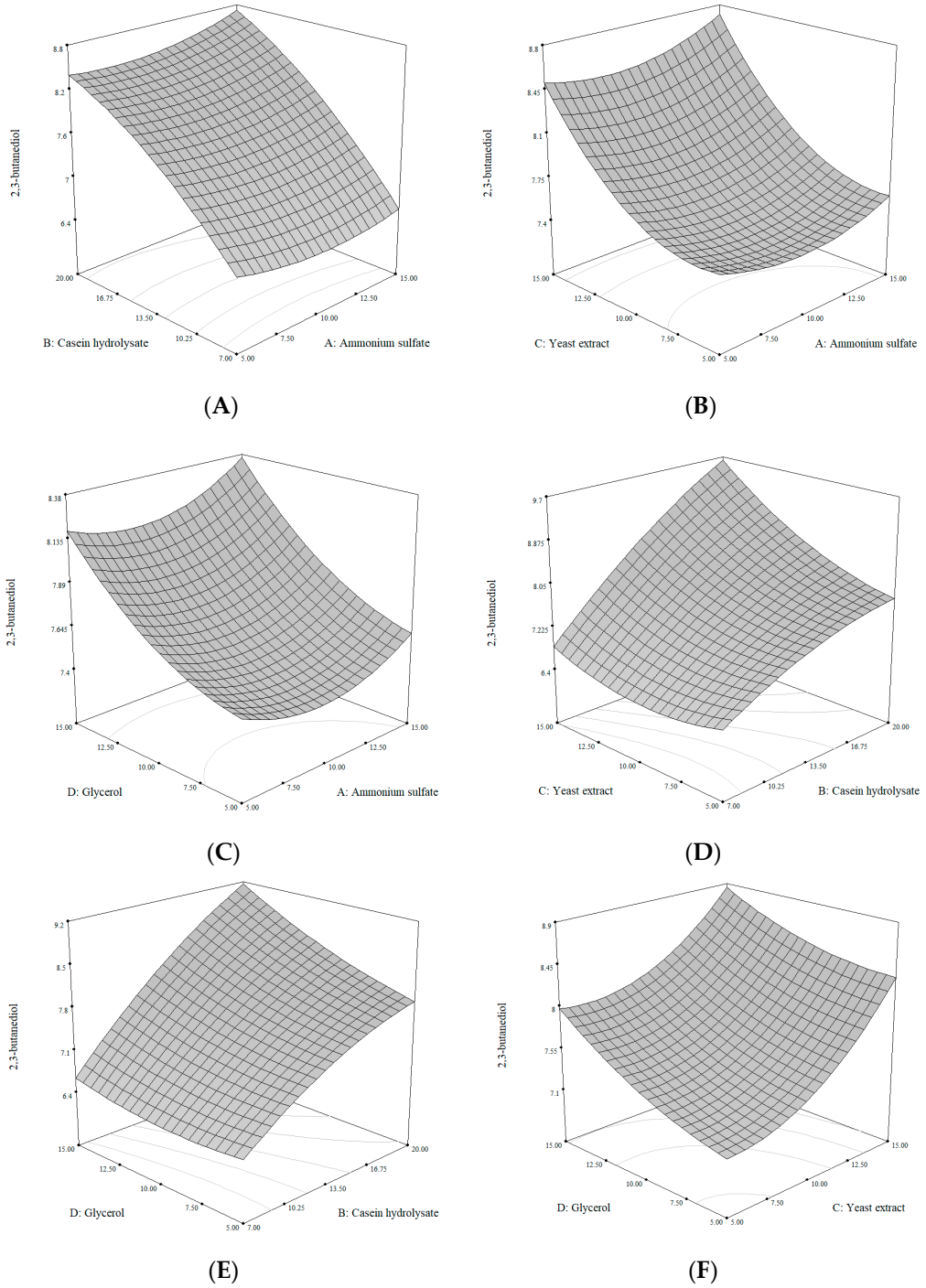


Figure 3. Response surface plots showing the interaction of each variable in 2,3-BDO production. (**A**) Effects of ammonium sulfate and casein hydrolysate, (**B**) effects of ammonium sulfate and yeast extract, (**C**) effects of ammonium sulfate and crude glycerol, (**D**) effects of casein hydrolysate and yeast extract, (**E**) effects of casein hydrolysate and crude glycerol, and (**F**) effects of yeast extract and crude glycerol on 2,3-BDO concentration.

Figure 3A shows the interaction of ammonium sulfate and casein hydrolysate on 2,3-BDO production. 2,3-BDO production was maximized at high casein hydrolysate

concentration, which was slightly increased as the ammonium sulfate concentration increased [26,29]. Figure 3B shows the interaction of ammonium sulfate and yeast extract on 2,3-BDO production. As the yeast extract concentration increased, the 2,3-BDO production also increased, and the increase of ammonium sulfate had no significant effect on the production of 2,3-BDO. The effect of ammonium sulfate and crude glycerol on 2,3-BDO production is shown in Figure 3C. The 2,3-BDO concentration was significantly affected by increasing concentrations of casein hydrolysate, yeast extract, and crude glycerol. However, microbial growth was reported to be inhibited in crude glycerol over 40 g/L and casein hydrolysate over 20 g/L, which was an important key to selecting the concentration range [26,29]. Figure 3D shows the interaction of casein hydrolysate and yeast extract on 2,3-BDO production. Although yeast extract had no significant effect at low casein hydrolysate concentrations, high casein hydrolysate concentration and yeast extract concentration rapidly increased 2,3-BDO due to their synergistic effect [26,29,30]. Figure 3E,F shows the interaction of crude glycerol with casein hydrolysate and yeast extract on 2,3-BDO production. The 2,3-BDO production was maximized in concentrations within the optimal range of crude glycerol and the other variables of ammonium sulfate, casein hydrolysate, and yeast extract. The 2,3-BDO production was not significantly affected by the variables at either the lowest casein hydrolysate concentration or the lowest yeast extract concentration. However, 2,3-BDO concentration and cell growth were clearly improved when increasing the concentration of either casein hydrolysate or yeast extract. These results confirmed the effect of casein hydrolysate and yeast extract on the growth of microorganisms and the 2,3-BDO production.

The optimization medium was achieved using Design-Expert 7 software based on solving the regression model [9,25]. In the conditions of numerical optimization, each variable was maximized for the concentration of 2,3-BDO within the specified concentration range. The statistical variables and the results (predicted value and experimental data) are shown in Table 4.

Table 4. Numerical optimization of medium components to maximize 2,3-butanediol production by regression model.

Components	Goal	Importance	Predicted Value (Coded Value)	Experimental Data (g/L)
Ammonium sulfate (X_1)	In range	3	12.6	12.0
Casein hydrolysate (X_2)	In range	3	16.0	16.0
Yeast extract (X_3)	In range	3	12.0	12.0
Crude glycerol (X_4)	In range	3	13.0	13.0
2,3-butanediol	Maximize	5	8.72	10.89

The predicted values of medium components were decided as 12.6 g/L for ammonium sulfate (X_1), 16 g/L for casein hydrolysate (X_2), 12 g/L for yeast extract (X_3), and 13 g/L for crude glycerol (X_4). In this condition, the predicted value of 2,3-BDO production was 8.72 g/L. The optimum components of the medium were applied to validate the predictive model in main fermentations of *K. aerogenes*.

The global market of 2,3-BDO and its derivatives was expected to be about 32 million tons per year, with sales of about $43 billion. The 2,3-BDO market is rapidly growing all over the world. For the production of 2,3-BDO used in various fields, the highly efficient technologies such as fermentation, extraction, and purification are required [27]. The minimum selling price of 2,3-BDO product using microbial fermentation from sugar costs about $5.26 /kg. In addition, the production cost of 2,3-BDO is significantly affected by the cost of the main carbon source and other media components and the fermentation efficiency [31]. Mass production is necessary to reduce cost, and technology readiness level (TRL) has been analyzed to evaluate mass production and industrialization. TRL is a measure of the maturity of a technology, and this study could be evaluated at three levels.

All studies were conducted at laboratory scale, and it is important for the fermentation scale-up to have low cost and high efficiency through optimization of the medium [32]. The most important factor for scale-up is the economic feasibility, and this study can reduce the cost because enzyme treatment is not needed after acid hydrolysis [33]. Priya et al. reported about 99% purity of 2,3-BDO can be recovered by using a downstream process such as membrane, sugaring-out, and salting-out. More scientific research is needed for the efficient extraction process of 2,3-BDO, thus achieving the cost reduction and higher purity in the near future [34].

Therefore, the schematic diagram of mass balance that can be the basis for efficient 2,3-BDO production processing of algal biomass is summarized in Figure 4. On the basis of 1000 g microalgal biomass, about 100 g of the extract could be recovered through extraction process, and approximately 63.5 g hydrolysate, 4.6 g oil, and 31.9 g others were contained in the liquid fraction. In acid hydrolysis, approximately 66.5% of the hydrolysate conversion was obtained by the hydrochloric acid pretreatment of the microalgal biomass; thus, about 9.9 g carbohydrate could be recovered from the process. In addition, 9.9 g carbohydrate was composed of 5.7 g of glucose and 4.2 g of galactose.

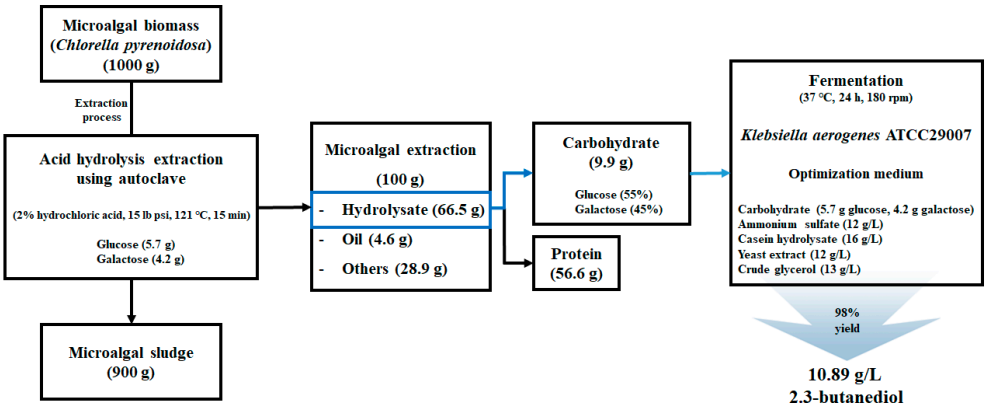


Figure 4. Schematic diagram of mass balance for 2,3-butanediol based on microalgal biomass.

Finally, in the fermentation process, about 10.89 g/L of 2,3-BDO was achieved, which shows about 1.6-fold higher than the value before optimization. As a result, it was proved that the medium optimization using response surface methodology (RSM) could be effectively applied to microbial fermentation. Therefore, this study could provide a guideline for improving 2,3-BDO production through the biochemical process using microalgal biomass and crude glycerol.

4. Conclusions

In this study, it was confirmed that the extracted algal sugars could be used as a substrate for the production of 2,3-BDO. In addition, biodiesel-derived crude glycerol was simultaneously used as substrate for 2,3-BDO, which means both extracted sugars and biodiesel-derived crude glycerol could be simultaneously applied to a waste-zero biorefinery. In addition, the major medium components (ammonium sulfate, casein hydrolysate, yeast extract, and crude glycerol (biodiesel-derived)) were optimized to improve the 2,3-BDO production using RSM.

Optimum composition of the main medium was as follows: 12 g/L ammonium sulfate, 16 g/L casein hydrolysate, 12 g/L yeast extract, and 13 g/L biodiesel-derived crude glycerol, which was carried out based on the extracted algal sugar. Through this optimization, maximum production of 2,3-BDO was found to be 10.89 g/L. As a result, using the optimum medium containing extracted algal sugar could increase cell growth and 2,3-BDO production. Especially, 2,3-BDO was increased around 1.6-fold compared with the medium before optimization. The production of 2,3-BDO using extracted algal sugar achieved was as effective as fermentation using chemical sugars, which might be

useful information for future application of algal sugar. Moreover, production of 2,3-BDO-containing crude glycerol could be recognized as the zero-waste process by the use of biodiesel byproducts. Therefore, these results confirm the promising potential for using extracted algal sugar and biodiesel-derived crude glycerol in biorefining.

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