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Short Communication

# Maximization of volatile fatty acids production from alginate in acidogenesis

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## HIGHLIGHTS

- The VFA production from alginate as a feedstock was approached for the first time.
- Optimization of alginate concentration and initial pH was determined using RSM.
- The effect of variables on VFAs production was evaluated.
- Acetic acid was the major component of the alginate fermentation.

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## ABSTRACT

In this study, the response surface methodology (RSM) was applied to determine the optimum fermentative condition of alginate with the respect to the simultaneous effects of alginate concentration and initial pH to maximize the production of total volatile fatty acids (*TVFAs*) and alcohols. The results showed that the alginate fermentation was significantly affected by initial pH than by alginate concentration and there was no interaction between the two variables. The optimum condition was 6.2 g alginate/L and initial pH 7.6 with a maximum *TVFAs* yield of 37.1%. Acetic acids were the main constituents of the *TVFAs* mixtures (i.e., 71.9–95.5%), while alcohols (i.e., ethanol, butanol, and propanol) were not detected.



## 1. Introduction

Fundamental issues in the current energy system such as dramatic increase in fossil fuel prices, sharp depletion of petrol, and climate change, have led scientists to investigate new alternative energy sources. Among these, marine biomass from sources such as microalgae and macro-algae, represent one of the most promising resources due to its cleanliness and sustainability (Kim et al., 2013).

Recently, marine macro-algae, classified as green-, red-, and brown-algae, have attracted attention as biomass resources for biofuels and biomaterials (Chang et al., 2010). For instance, bio-alcohol (Borines et al., 2013) and bio-hydrogen (Shi and Yu, 2006) can be produced from these algae by anaerobic fermentation and bio-oil (Ross et al., 2009) can be produced by pyrolysis. Among the macro-algae, massive brown algae are primarily composed of polysaccharides such as alginate, laminaran, fucoidan, mannitol, and cellulose (Chang et al., 2010) and the feasibility of the fermentative conversion of these polysaccharides into liquid biofuels has demonstrated (Horn et al., 2000). Alginate is a polysaccharide, which accounts for up to 40% of the dry weight in brown algae and is a principal component of the cell wall (Draget et al., 2005; Jung et al., 2013). Although this new marine biomass presents a high potential as a polysaccharide feedstock for liquid biofuels, the use of alginate in fermentation processes is still a challenge due to its low solubility in water and its limited usage as a microbial substrate, compared to terrestrial biomass components (Pawar and Edgar, 2012).

In this study, we investigated the experimental conditions that are necessary to maximize TVFA and alcohol production by the fermentation of alginate. The objective of this study was to identify the optimal conditions (alginate concentration and initial pH) required to maximize the efficiency of the bioconversion of alginate into *TVFAs* and alcohols by anaerobic fermentation.

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## 2. Methods

### 2.1. Feedstock

Sodium alginate (80–120 mPa·s, Wako Pure Chemical Industries Ltd., Japan) was dissolved in distilled water and autoclaved (121 °C for 15 min) then used as a microbial growth substrate. Alginate was the sole carbon source in the medium, which also contained $NH_4HCO_3$, 2.0 g/L; $KH_2PO_4$, 1.0 g/L; $MgSO_4.7H_2O$, 0.01 g/L; NaCl, 0.001 g/L; $Na_2MoO_4.2H_2O$, 0.001 g/L; $CaCl_2.2H_2O$, 0.001 g/L; $MnSO_4.7H_2O$, 0.0015 g/L; and $FeCl_2.4H_2O$, 0.00388 g/L as nutrient additives. The initial pH was adjusted as required using 5 N NaOH or 5 N HCl.

### 2.2. Inoculum preparation and fermentation

Anaerobically digested sludge was obtained from a municipal wastewater treatment plant in Busan, Korea. In order to enhance the activity of VFA-producing bacteria, an acid pre-treatment (2 N HCl) was applied at 35 °C for 24 h (Lee et al., 2009). A continuous anaerobic fermentation was operated in a 3 L bioreactor with a working volume of 2 L at 35 °C and pH was maintained at 5.5 with 5 N NaOH or 5 N HCl. Part of the fermentation broth was removed daily and replaced (the retention time was 1 day) with a fresh feed (Lee et al., 2009). The concentrations of *TVFAs* and alcohols in the system were maintained at 12–14 g/L. The effluent from the inoculum system was used as seed culture (equivalent to 10% of working volume) for a series of 500 mL amber reactors with a working volume of 400 mL.

The alginate fermentation was operated at 35 °C and 120 *rpm*. Chloroform ($CHCl_3$; 100 μM) was used as a methanogen inhibitor from both $H_2/CO_2$ and acetate. It also inhibited acetate consumption by sulfate reducers (Hu and Chen, 2007).

### 2.3. Experimental design and selection of variables

Response surface methodology (RSM) was used to determine the effects of alginate concentration and initial pH. It was applied to evaluate the relative significance of the experimental variables and to find the optimum conditions within the design boundary of the independent variables, under which TVFA and alcohol yields were maximum. The experiment was based on the central composite in cube design (Montgomery et al., 2011) and consisted of a $2 \times 2$ orthogonal design (alginate concentration and initial pH) in order to minimize the number of trials needed to obtain statistically valid results (Table 1). The ranges of independent variables were set 4.0–9.0 g of alginate/L and pH 6.0–10.0 based on preliminary results (data not shown). Each trial with a center point (i.e., 6.5 g of alginate/L and initial pH 8.0) was replicated 3 times as previously described. This type of design was used to minimize the number of trials needed to obtain statistically valid results (Song et al., 2007).

A sequential procedure of collecting data, estimating polynomials, and checking the adequacy of the model was applied. The method of least squares was used to estimate the parameters in the approximating polynomials. For the statistical analysis, Minitab software (version 15.1.1.0, Minitab Inc., State College, Pennsylvania, USA) was applied to establish the experimental design and to test complex polynomials to model the data.

**Table 1**
Experimental design and observed total volatile fatty acids (*TVFAs*) production in the anaerobic alginate fermentation.

| | Trials | Independent variables | | *TVFAs* yield(%) |
|---|---|---|---|---|
| | | Alginate concentration (g/L) | Initial pH | |
| Linear design | 1 | 4 | 6 | 30.9 |
| | 2 | 9 | 6 | 24.0 |
| | 3 | 4 | 10 | 15.1 |
| | 4 | 9 | 10 | 20.7 |
| | 5[a] | 6.5 | 8 | 34.7 ± 0.2 |
| Quadratic design | 6 | 2.9 | 8 | 23.9 |
| | 7 | 10.0 | 8 | 21.6 |
| | 8 | 6.5 | 5.2 | 13.6 |
| | 9 | 6.5 | 10.8 | 8.0 |
| Validation | 10 | 6.2 | 7.6 | 37.0 ± 0.1 |

[a] Center point was repeated by three times.

### 2.4. Analytical methods

Liquid and gas samples were taken daily for analysis. The liquid samples were centrifuged for the detection of VFAs (C2–C6) and alcohols (ethanol, butanol, and propanol) at 3000 *rpm* for 10 min. The VFAs profile was detected by UV/VIS detector at 210 nm, and alcohols were determined by Refractive Index detector using HPLC (Ultimate 3000, Dionex, USA) with column Aminex HPX-87H. Every analysis was performed at 65 °C under isocratic condition with 2.5 mM $H_2SO_4$ as mobile phase. Total organic carbon (TOC) was analyzed by a TOC analyzer (TOC–VCPH, Shimadzu, Japan). The volatile solids (VS) concentration was determined according to the procedures in Standard Methods (APHA-AWWA-WEF., 1998). The carbohydrate concentration was determined using the phenol–sulfuric acid method (Dubois et al., 1956) and pH was monitored by a pH meter (Istek, model 720P, Korea).

Gas samples for hydrogen were analyzed by GC-HP5890 with a packed column Hayesep Q (SS, 1.8 m × 1/8″, and 80/60 mesh) and a thermal conductivity detector (TCD) of 90 °C, 35 °C, and 120 °C. And methane and carbon dioxide were measured by GC-HP5890 with a flame ionization detector of 180 °C, 35 °C, 280 °C, and 350 °C using Ni catalyst and a packed column Porapak Q (SS, 2 m, 1/8″, 80/100 mesh).

### 2.5. Calculation

The yield of *TVFAs* (g carbon in *TVFAs*/g carbon in substrate) was calculated as the amount of carbon in the *TVFAs* produced divided by the amount of soluble carbon in the substrate feed.

$$TVFAs \text{ yield } (\%) = \frac{n_{TVFAs}}{n_{\text{alginate}}} \quad (1)$$

where: $n_{TVFAs}$ = the carbon amount of *TVFAs* produced as observed (mole carbon). $n_{\text{alginate}}$ = the carbon amount of alginate feeding as obtained (mole carbon).

## 3. Results and discussion

In this study, acetic-, butyric-, propionic-, lactic-, and valeric acids were the bioconversion metabolites, whereas alcohols (i.e., ethanol, butanol, and propanol) were not detected. In individual VFA, acetic acid was the main constituent with 71.9–95.5% of the *TVFAs*. Ethanol production from lignocellulosic biomass which has similar chemical structure with alginate has been reported (Nakashima et al., 2011). In contrast, in our study, the utilization of alginate as acidogenic feedstock did not lead to the production of alcohols.

A total of 11 trials, including a center point, were run to approximate the response surface for *TVFAs* production. To find the maximum bioconversion efficiency, increasingly complex equations from linear to quadratic were sequentially tested to model the data obtained from the trials in Table 1. When the data were analyzed using the various models, the *P*-value of regression was significant