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Short Communication

# Alkaline in situ transesterification of *Aurantiochytrium* sp. KRS 101 using potassium carbonate

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## HIGHLIGHTS

- In situ transesterification was successfully conducted to *Aurantiochytrium* sp.
- Potassium carbonate was used as a potent alkaline catalyst for this process.
- The FAME recovery yield resulted in over 90% using dry microalgae.

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## ABSTRACT

The aims of this work were to evaluate $K_2CO_3$ as a potent alkaline catalyst for in situ transesterification of *Aurantiochytrium* sp. KRS 101, one step process in which oil extraction and conversion take place together. This $K_2CO_3$-based in situ transesterification was optimized in terms of recovery yield of fatty acid methyl esters (FAMEs) by way of varying biomass concentration, reaction temperature, reaction time, and catalyst concentration. The optimal condition was achieved at 50 g/L of biomass concentration and 1% of $K_2CO_3$ in the methanol, 25 °C of reaction temperature, and 5 min of reaction time, resulting in the FAME recovery yield over 90%. It was found that $K_2CO_3$ performed better than any other tested catalysts including acids, supporting the notion that $K_2CO_3$ is a promising catalyst, especially for in situ transesterification.



## 1. Introduction

The world needs sustainable energy sources that are free from issues of environmental pollution and energy security (U.S. Department of Energy, 2014). Microalgae are one of such options and are a particularly advantageous source for transportation fuels (Seo et al., 2015). Among them, heterotrophic microalgae, which grow using organic carbon, have gathered increasing attention in the academic and market places in view of fast growth, high biomass and lipid concentrations (Chisti, 2007; Halim et al., 2011; Sung et al., 2014).

The production of the aimed products like biodiesel requires many steps, i.e., cultivation, harvesting, oil extraction, and conversion (Kim et al., 2015a) and it is costly. Though literally all the steps are challenging in terms of economics, downstream processes, from harvesting to conversion, are estimated to account for 60% total production cost (Kim et al., 2015b). Any means of reducing the cost must be developed for the algae-derived biodiesel to be commercialized, and the integration of more than two steps is an obvious strategy.

Lately, in-situ transesterification (also known as direct conversion or direct transesterification), which is a simultaneous process of lipid extraction and oil conversion, has received increasing attention because of its simplicity and efficiency (Park et al., 2015). One determining factor for it is the catalyst, in both cell disruption and oil conversion; either acid or alkaline catalysts are most commonly employed for the purpose. The cell wall of microalgae, mainly composed of cellulose, is not readily disrupted (Fu et al., 2010). Consequently, cell disruption needs not only the chemical catalyst but also thermal or physical means. The resulting lipids can eventually be converted into fatty acid methyl ester (FAME) through acid/alkaline- or whole cell/enzyme-catalysis (Verma et al., 2014). Alkaline catalysts have selective advantages over acidic counterparts. They cause to form less inhibitors in disrupting cell wall (Mosier et al., 2005) and at the same time proceed the transesterification at a much faster rate under milder conditions (Kim et al., 2013). This seemingly ideal treatment, however, has a fatal limitation of saponification which decreases the yield of FAME recovery. As one exception, potassium carbonate

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($K_2CO_3$) was reported to possess such a problem only to a limited extent and yet still superb catalytic efficiency (Baroi et al., 2009). The carbonate is to react with alcohol to produce bicarbonate instead of water, and this bicarbonate product prevents the esters from being hydrolyzed, resulting in overall high FAME recovery (Ejikeme et al., 2010).

The goal of this study is therefore to develop $K_2CO_3$-based in situ transesterification. To this end, a heterotrophic microalgae species *Aurantiochytrium* sp. KRS 101 was used and key process parameters were investigated to obtain optimal conditions for FAME recovery.

## 2. Methods

### 2.1. Microorganism preparation

*Aurantiochytrium* sp. KRS 101, obtained from the Korea Research Institute of Bioscience and Biotechnology (KRIBB, Republic of Korea), was cultivated in a nutrient medium containing the following ingredients: 60 g/L of glucose, 10 g/L of yeast extract, 9 g/L of $KH_2PO_4$, 10 g/L of sea salt, and 10 mg/L of tetracycline, in a 5-L bioreactor for 3 days at 28 °C with shaking at 120 rpm and 0.5 v/v/min of air. The pH of the culture was 4.6. After cultivation, microalgae biomass was harvested by centrifugation and lyophilized.

### 2.2. In situ transesterification

A stock solution of alkaline catalyst (3% (w/v) $K_2CO_3$/methanol) was prepared for in situ transesterification, and different catalyst concentrations were obtained by dilution with methanol. The alkaline catalyst solution was added to a designated amount of dry microalgae powder, both of which were mixed at 700 rpm in a 250-mL Erlenmeyer flask with a screw-cap. Operational variables were as follows: 10–100 g/L of biomass concentrations in the alkaline catalyst solution, 25–60 °C of temperature, 0–3% of alkaline catalyst solution, and 5–60 min of reaction time. Experiments were conducted in triplicate. The chemicals used were methanol (99.9% purity, DUKSAN Chemicals, Korea), hexane (95.0% purity, Samchun Chemicals, Korea), chloroform (99.5% purity, Samchun Chemicals, Korea), sulfuric acid (95.0% purity, Sigma Aldrich, USA), potassium carbonate (99.5% purity, Junsei Chemicals, Japan), and potassium hydroxide (95.0% purity, Samchun Chemicals, Korea), which were all of analytical grade.

### 2.3. Determination of biomass FAME content and FAME recovery yield of the in situ transesterification from gas chromatography (GC) analysis

After the in situ transesterification, a treated sample was cooled down to ambient temperature, and hexane (biomass solution to hexane volumetric ratio of 5:4 (v/v)) was added and the mixture was mixed at 700 rpm for 1 h. Afterwards, distilled water was added to each mixture and then centrifuged at 4000 rpm for 5 min to separate phases, the upper layer containing hexane and fatty acid methyl esters (FAMEs) from the lower layer comprised of water and alcohol. The upper phase was finally analyzed to measure FAMEs. The recovery yields of FAME were calculated by the following equation.

$$\text{FAME recovery yield} = \frac{\text{The amount of obtained FAME}\left(\text{mg}\frac{\text{FAME}}{\text{mg}}\text{cell}\right)}{\text{The FAME content of algal cell}\left(\text{mg}\frac{\text{FAME}}{\text{mg}}\text{cell}\right)} \times 100(\%) \quad (1)$$

The original FAME content of dry *Aurantiochytrium* sp. was analyzed by a gas chromatography (GC) (HP5890; Agilent, CA) according to a slightly modified Folch procedure (1957). 10 mg of a freeze-dried sample was vortexed for lipid extraction in the presence of chloroform:methanol (2:1, v/v) and transesterification proceeded with methanol (reactant) and sulfuric acid (catalyst) at 100 °C for 20 min. The average FAME content in the dry biomass was calculated by the following equation and the value was found to be 418.1 mg FAME/g cell.

$$\text{FAME content} = \frac{\text{FAME weight (mg)}}{\text{Dry cell weight (mg)}} \times 100(\%) \quad (2)$$

## 3. Results and discussion

### 3.1. Effects of the biomass concentration in the alkaline catalyst solution and temperature on FAME yield

To obtain optimal reaction conditions with respect to amounts of biomass and alkaline catalyst, biomass concentrations in the alkaline methanol solution ($K_2CO_3$/methanol) were varied, from 10 to 100 g/L (Table 1). FAME recovery was reported to increase as the ratio decreased (Park et al., 2015). For a fixed condition of 1% of $K_2CO_3$/methanol, 60 °C, and 60 min, the recovery remained high up to the concentration of 50 g/L, and afterward dropped. The maximum FAME recovery yield (93.8%) was obtained at 25 g/L; the other concentrations exhibited differences less than 1%. Consequently, optimal ratio of biomass concentration in $K_2CO_3$/methanol was regarded as 50 g/L.

It is generally known that elevated temperatures lead to enhanced FAME yield (Lotero et al., 2005). Interestingly, however, this study with *Aurantiochytrium* was not the case; even room temperature brought about cell disruption and FAME conversion in a very effective manner (Table 1), yielding 92.3% FAME recovery yield which was nearly the same as that at 60 °C. This exceptionally high effectiveness was likely to arise from the weak nature of the cell wall of *Aurantiochytrium* sp. (Kim et al., 2015c). Besides, the alkaline-catalyzed transesterification requires far lower temperature than the acid counterpart (Ejikeme et al., 2010) and there-

**Table 1**
FAME recovery yield depending on various operational conditions.

| Condition | Value | FAME recovery yield (%) |
|---|---|---|
| Biomass concentration (g/L)[a] | 10 | 93.54 ± 1.28 |
| | 25 | 93.81 ± 2.54 |
| | 50 | 92.14 ± 1.01 |
| | 75 | 55.48 ± 9.44 |
| | 100 | 19.50 ± 7.18 |
| Temperature (°C)[b] | 25 | 92.28 ± 6.52 |
| | 60 | 92.14 ± 6.69 |
| $K_2CO_3$ concentration (%)[c] | 0 | 0.00 ± 0.00 |
| | 0.1 | 0.27 ± 0.34 |
| | 0.5 | 8.28 ± 1.16 |
| | 1.0 | 91.70 ± 4.12 |
| | 2.0 | 93.37 ± 3.17 |
| | 3.0 | 93.26 ± 1.72 |
| Reaction time (min)[d] | 5 | 90.82 ± 3.10 |
| | 10 | 89.64 ± 5.52 |
| | 20 | 92.10 ± 5.16 |
| | 30 | 89.95 ± 2.18 |
| | 60 | 93.06 ± 3.09 |

[a] Operational conditions: 1% $K_2CO_3$/methanol, 60 °C, 60 min.
[b] Operational conditions: 1% $K_2CO_3$/methanol, 50 g/L of biomass concentration, 60 min.
[c] Operational conditions: 50 g/L biomass, 25 °C, and 60 min.
[d] Operational conditions: 1% $K_2CO_3$/methanol, 50 g/L biomass in the $K_2CO_3$/methanol, 25 °C.