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Enhancement of continuous fermentative bioethanol production using combined treatment of mixed microalgal biomass



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ABSTRACT

The impact of combined sonication, heat and enzyme (SHE) treatment on continuous bioethanol production from mixed microalgal biomass (cyclotella and filamentous) was evaluated in a fermentor. Different pretreatments resulted in varied degrees of cell lysis for microbial fermentation. Filamentous algae were partially damaged under sonication pretreatment, while SHE treatment leads to complete destruction of both cyclotella and filamentous microalgal cells. SHE treatment significantly increased the dissolved carbohydrate concentration (up to 5.8 folds greater than non-pretreated), which enhanced the ethanol production through microbial fermentation. Higher bioactivity of alcohol fermentation by *Dekkera bruxellensis* (yeast) resulted in higher ethanol yield compared to mixed bacterial culture. The cumulative ethanol production after SHE treatment was 1.4 fold higher than with combined sonication and enzyme (SE) treatment using *D. bruxellensis*. These results demonstrate that combined SHE treatment is an effective method for the enhancement of yeast promoted fermentative bioethanol production from mixed biomass.

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1. Introduction

Microalgae have drawn much attention as a potential feedstock for generation of bioethanol, as they possess high amounts of carbohydrates (11–48%) in the form of starch and cellulose, which can be fermented to bioethanol [1–3]. Microalgal carbohydrates lack lignin, which make their conversion to monosaccharides much easier compared to lignocellulosic materials [2,4]. Studies on microalgae based bioethanol production have been focused at the single-cell level using cyclotella species cultured under controlled light [5] and stress [6] conditions. Microalgae represent an exceptionally diverse but highly specialized group of microorganisms categorized into two groups including cyclotella and filamentous, based on their morphology [7].

Cyclotella algae possess soft or firm cell wall, which is composed of four layers containing simple sugars, muramic acid, glutamic acid, diaminopimelic acid, galactosamine, glucosamine and alanine [8]. Demirbas and Demirbas [9] reported that various cyclotella microalgae species have an average carbohydrate content of 15.6% (*w*/w), which can theoretically be a good source for bioethanol production through sugar fermentation. The cell wall of filamentous type microalgae is comparatively more rigid mostly composed of cellulose, while in few species cellulose is absent (e.g., *Volvox* sp.) and other components including pectin, sporopollenin, calcium carbonate or even silica have been

* Corresponding author. *E-mail address:* bhjeon@hanyang.ac.kr (B.-H. Jeon). observed [8]. However, use of pure cultures or strains of microalgae presents difficulties in industrial applications due to contamination and economic issues. The algal growth from natural habitats, which is predominantly mixed cultures of different algae species residing as a consortium can be managed more easily than pure cultures, and can emerge as a more practical approach for commercialization of microalgae-based bioenergy production. Previous research on bioethanol production using microalgae biomass as feedstock has been investigated using single microalgae strains [10,11], but there are no reports on the use of mixed microalgael (cvclotella and filamentous) feedstock.

The rigid cell wall and cell membrane of microalgae inhibit or delay the subsequent biodegradation in the fermentation process. Thus, pretreatment of microalgae biomass should be performed for cell disruption, leading to easy access of the carbohydrates from within the cells [11,12]. Saccharification, the process of breaking down complex carbohydrates into simple fermentable sugars such as glucose and mannose is one of the most crucial steps in microalgae based bioethanol production. Different pretreatment methods including ultrasonication, acid, alkaline and heat have been studied to extract carbohydrates from intact, chemically treated or mechanically ruptured cells [11-13], but the acidic conditions may lead to decomposition of the sugars into undesirable compounds that might inhibit the fermentation process. Although enzymatic hydrolysis is comparatively more expensive than acid hydrolysis [14,15], it is an environmentally benign process and can obtain higher glucose yields without formation of products that interfere with the fermentation process [16,17]. Despite the extensive research,



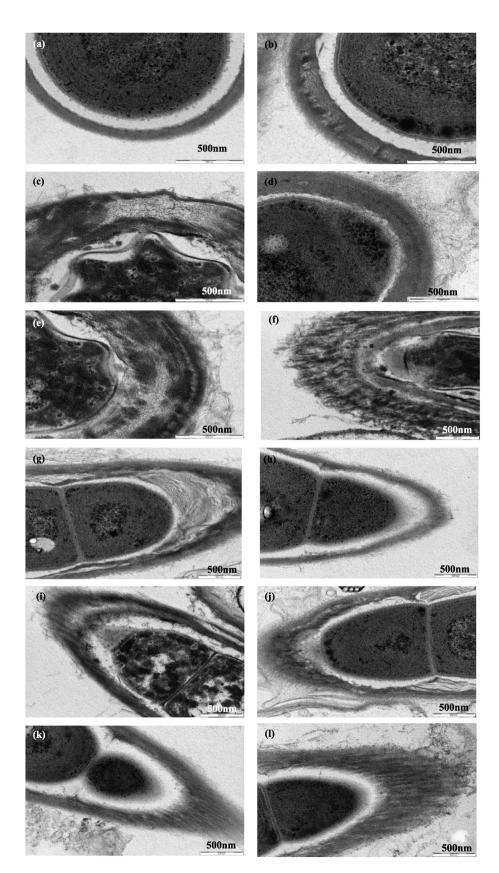


Fig. 1. TEM images showing the destruction of *C. vulgaris* YSL001 (a,b,c,d,e,f), and filamentous *U. belkae* YSL010 (g,h,i,j,k,l) cell wall on algae surfaces and in periplasm. Non-sonicated algal cell (a and g), enzyme treated algal cell (b and h), sonicated algal cell for 15 min (c and i), combined treated (sonication + enzyme) algal cell (d,j), combined treated (sonication + heat) algal cell (e,k), and combined treated (sonication + enzyme + heat) algal cell (f and l).

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