Hydrolysis of carbohydrates in marine *Tetraselmis* sp by acid and enzymatic pre-treatments to obtain substrate for ethanol production

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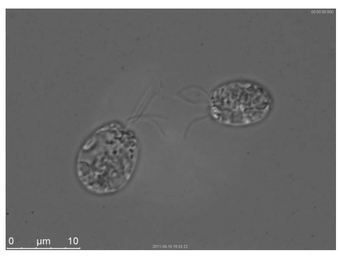
Introduction

Microalgae has been said to have a realistic potential as biomass source for a sustainable large-scale production of bioethanol. Using marine algae, which are able to grow in sea water, will be highly advantageous because globally the freshwater supply is limited (John *et al*., 2011).

The genus *Tetraselmis* is one of the most widely used microalgae in mariculture for feeding marine herbivores, due to its ability to grow under a wide range of physical and chemical environmental conditions. This microalgae has also potency as source of bioethanol production because it has a high carbohydrate content which accounts for 241.8 µg/ ml (Laurenco *et al.*, 1997).

Since the highly complex carbohydrates are entrapped in the rigid cell wall (Libessart *et al*., 1995; Choi et al., 2010), it is essential to perform pre-treatment and hydrolysis steps to break down the cell wall and release these carbohydrates from the biomass and convert them into monosaccharides prior to the fermentation process. Hence, in this research we will study different methods for pre-treatment to open up the cells and a following enzymatic hydrolysis for a *Tetraselmis* sp. isolated from the west coast of Java island, Indonesia.

Method



The species used in this work was isolated from the west coast of Java Island in Indonesia. Samples with 500 µl of cell suspension were taken from the culture. The pellet after centrifugation at 13,500 rpm for 5 minutes was diluted in sulfuric acid with a concentration of either 3%, 5%, or 8% and incubated at 100°C for either 30 minutes or 60 minutes. The best treatment was followed by enzymatic hydrolysis using either 1 µl or 10 µl of each cellulase and β-glucosidase (Celluclast and Novozyme 188, both Novozymes A/S) for 3 days to release fermentable sugars . The sugars were analyzed by HPLC. From the same culture the dry weight was measured.

Result and Discussion

The results showed that the highest glucose level of 0.022 g/l was obtained using 3% sulfuric acid (v/v) incubated at 100°C for 60 minutes (Table 1). After treatment with 10 µl of Celluclast and 10 µl of Novozyme 188 for 3 days, the amount of glucose released was increased to 0.16 g/l (0.06 g glucose/ g biomass) which account for 53% of its total carbohydrate content (Table 2). However, further studies are needed to be done to find the best combination of sulfuric acid and enzymatic treatments in order to get optimum glucose release from the biomass of the microalgal strain.

Table 1. Sugars (g/l) released by sulfuric acid pre-treatments.

Sulfuric Acid	3%	5%	8%	3%	5%	8%
Treatment Time	30 min			60 min		
Sugar	0.018	0.021	0.010	0.022	0.019	0.013

Table 2. Sugars (g/l) released by enzymatic hydrolysis with different levels of cellulose degrading enzymes

Enzyme	1 µl	10 µl
Sugar	0.0023	0.1649

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