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ORIGINAL ARTICLE

Bioethanol production from agarophyte red seaweed, *Gelidium elegans*, using a novel sample preparation method for analysing bioethanol content by gas chromatography

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Abstract

In this study, *Gelidium elegans* is investigated for ethanol production. A combination of factors including different temperatures, acid concentration and incubation time was evaluated to determine the suitable saccharification conditions. The combination of 2.5% (w/v) H_2SO_4 at 120 °C for 40 min was selected for hydrolysis of the seaweed biomass, followed by purification, and fermentation to yield ethanol. The galactose and glucose were dominant reducing sugars in the *G. elegans* hydrolysate and under optimum condition of dilute acid hydrolysis, 39.42% of reducing sugars was produced and fermentation resulted in ethanol concentration of 13.27 ± 0.47 g/L. A modified method was evaluated for sample preparation for gas chromatography (GC) analysis of the ethanol content. A solvent mixture of acetonitrile and iso-butanol precipitated dissolved organic residues and reduced water content in GC samples at least by 90%. Results showed that this method could be successfully used for bioethanol production from seaweed.

Keywords *G. elegans* · Seaweed · Sample preparation · Gas chromatography · Bioethanol

Abbreviations

GC	Gas chromatography
CO_2	Carbon dioxide
NITE	National institute of technology evaluation
YPD	Yeast potato dextrose
CFU	Colony-forming unit
H_2SO_4	Sulphuric acid
FID	Flame ionisation detector
EtOH	Ethanol
Conc	Concentration
ND	Not detected
DW	Dry weight

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Introduction

Biofuels have received great attention as sustainable alternative fuels (Sudhakar et al. 2018). In Brazil, bioethanol is being extracted from the agriculture crops and used as biofuel in the transportation sector (Salles-Filho et al. 2017). Conflicts on the use of land for cultivation of biofuel crops instead of agriculture crops have arisen (Adams et al. 2009). The cultivation of algae to produce feedstock for biofuel production, using non-agricultural land, can avoid this conflict. Algae-based biofuels have high impact, unlike the use of corn or sugarcane (Searchinger et al. 2008; Chia et al. 2018). Algae have potential to reduce greenhouse gases by consumption of CO_2 emitted from power plants and natural gas operations, as indicated by life-cycle assessments (Maranduba et al. 2015; Vuppaladadiyam et al. 2018). An advantage of using seaweed is the low lignin content which improves the enzymatic hydrolysis of cellulose. The carbohydrate content in the red seaweed *G. elegans* was high compared to lipid and protein contents; the amount of carbohydrate was increased to 72.6% from 53.4% by pre-processing, and showed its potential use as feedstock for bioethanol production (Wi et al. 2009). Adams et al. (2009) reported that sugarcane is the most productive terrestrial crop and can produce 6756 L/ha for bioethanol production. However, the

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