**Dark Fermentative Hydrogen Production Using Macroalgae (*Ulva* sp.) as the Renewable Feedstock**

Winny Margareta, Dillirani Nagarajan, Jo-Shu Chang, Duu-Jong Lee

**Abstract**

Macroalgae are alternative and sustainable feedstock for biohydrogen production because of their high levels of carbohydrates and low levels of lignin. Macroalgae can be cultivated in non-arable seawater with minimal nutritional requirements, and hence they can overcome issues regarding to land use changes. Macroalgae can be considered as one of the most productive biological systems for carbon capture in order to mitigate global CO2 emissions and reduce global warming. This study is to design a suitable bioprocess to assess the potential of using macroalgae as the fermentation substrate for *Clostridium* sp. for biohydrogen (bioH2) production.

The green macroalgae used in this study (*Ulva* sp.) is composed of 50.3% carbohydrates, 25.7% protein, 2.3% lipid, and 21.7% ash. After harvesting, the macroalgal biomass was subjected to saccharification using acid-hydrothermal pretreatment, followed by dark fermentation with the *Clostridium* sp.. The macroalgae hydrolysate mainly consisted of 21.1% total reducing sugar, 9.8% total nitrogen, and 1.5% total phosphorus. *C. butyricum* CGS5 was used to perform dark fermentation of macroalgae hydrolysate and achieved the highest cumulative H2 production (2340 ml/L), maximum H2 productivity (208.3 ml/L/h), and H2 yield (1.53 mol H2/mol RS) using an initial reducing sugar concentration of 12 g/L at pH 5.5. The optimal fermenting conditions mentioned above were further used for continuous H2 production using macroalgae hydrolysate as the carbon source under different hydraulic retention time (HRT) (Fig. 1). When HRT was gradually shortened from 8 to 4 h, the H2 production rate increased from 429 to 812 ml/L/h, whereas the H2 yield decreased from 1.62 to 0.98 mol H2/mol reducing sugar.

![Fig. 1. H2 production, substrate consumption, and soluble metabolites during H2 fermentation under batch and continuous fermentation using 12 g RS/L macroalgae hydrolysate as carbon source, Endo medium, 200 rpm, and pH control 5.5](image-url)