

## Optimization of Bioethanol Production from *Chara globularis* Using *Saccharomyces cerevisiae*

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Ethanol is one of the important alternative energy sources to fossil fuels. Since Sri Lanka has a huge amount of inland water resources and plenty of diverse under-utilized flora which are rich in cellulosic substrates, the possibility of using inland plant resources as substrates for bioethanol production is being researched. This study was aimed to comparatively assess the ethanol production from different fresh water flora in the Northern Province of Sri Lanka and to optimize the conditions for a higher yield from selective spp. Fresh water flora such as *Chara globularis*, *Salvinia*, *Wolffia*, *Spirodela polyrhiza*, *Lemna minor*, and *Cabomba caroliniana* were used as substrates for ethanol production using baker's yeast, *Saccharomyces cerevisiae*, in a liquid fermentation system. Thirty grams of dried plant samples were treated with 50 mL of 3% (v/v) H<sub>2</sub>SO<sub>4</sub> for 15 minutes and the amount of ethanol produced was determined using an ebulliometer. Among the plant substrates used, a significantly higher amount of bioethanol (0.25%) was produced from *C. globularis*. Thus *C. globularis* was selected for further studies. When *C. globularis* was pretreated with 50 mL of different 3% (w/v) alkaline solutions (NaOH and KOH) and 3% (v/v) acid solutions (H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub> and HCl), a significantly higher amount of ethanol (0.25%) was obtained with 3% H<sub>2</sub>SO<sub>4</sub>. Therefore, H<sub>2</sub>SO<sub>4</sub> was selected as the best hydrolyzing agent for further study. When H<sub>2</sub>SO<sub>4</sub> pretreating concentration (1-10%) was optimized, a significantly higher yield of ethanol (0.3%) was obtained at 4% H<sub>2</sub>SO<sub>4</sub>. When *C. globularis* was acid hydrolyzed for different time periods (15 min, 30 min and 45 min), a significantly higher ethanol yield (0.4%) was obtained at 30 min incubation period. When the amount of ethanol produced by *C. globularis* pretreated with 50 mL of 4% H<sub>2</sub>SO<sub>4</sub> for 30 minutes was measured at every 12 hours, the highest amount of ethanol (0.5%) was produced at 60 hours of fermentation time. When fermentation was carried out with baker's yeast inoculated peptone, yeast extract and nutrient (PYN) medium at room temperature and pH 7.0 and under the optimized conditions, the amount of ethanol produced from *C. globularis* was two times higher than the non-optimized conditions. Further optimization studies are underway in order to enhance the final ethanol yield.

**Keywords:** Bioethanol, *Chara globularis*, Fermentation, Pretreatment, *Saccharomyces cerevisiae*