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BIOETHANOL PRODUCTION FROM *Chara globularis* USING YEAST AND YIELD IMPROVEMENT BY OPTIMIZATION OF CONDITIONS

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The rising population, depletion of petroleum-based fossil fuel and atmospheric contaminations by fossil fuel combustion have opened avenues for alternative, eco-friendly and renewable energy sources. Bioethanol is an alternative and renewable substrate which has drawn attention due to environmental concerns and energy security. This study aimed to determine the best bioethanol producing freshwater flora abundantly available in the Northern Province of Sri Lanka using *Saccharomyces cerevisiae* and optimize the fermentation conditions to enhance the ethanol yield from *Chara globularis*. When freshwater flora such as *C. globularis, Cabomba caroliniana, Spirodela polyrhiza, Salvinia minima, Salvinia natans, Wolffia arrhiza* and *Wolffia globosa* were hydrolyzed with 1 M sulfuric acid solution, and the amount of reducing sugar was determined, *C. globularis* produced a higher amount of reducing sugar than other species tested. When pre-treatment of *C. globularis* was done with 1 M acid solutions (sulfuric acid, nitric acid and hydrochloric acid) and alkaline solutions (sodium hydroxide and potassium hydroxide), a higher amount of reducing sugar was obtained with sulfuric acid. When alcohol was produced from *C. globularis* using *S. cerevisiae* after three different hydrolysis methods, namely acid hydrolysis (1 M sulfuric acid), enzymatic hydrolysis (1% alpha-amylase) and a combination of chemical and enzymatic hydrolysis (1 M sulfuric acid and 1% alpha-amylase), a combination of chemical and enzymatic hydrolysis gave higher ethanol yield; thus this combination hydrolysis was selected. The conditions for fermentation of *C. globularis* substrate using *S. cerevisiae* were optimized sequentially by changing one factor at a time while keeping the other variables constant. After the optimization of fermentation time (24 h), operating temperature (35 °C), rotation speed (200 rpm) and sulfuric acid concentration for combined pre-treatment (0.75 M) with an inoculum size of 100 g/L, bioethanol yield was increased by two times compared with the non-optimized condition.

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