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# Optimization of bioethanol production from *Chlorella* sp. isolated from Sri Lankan fresh water habitats

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Bioethanol is an alternative energy source that can be used to replace fossil fuel. It is produced by the fermentation of various feedstock such as edible crops and lignocellulosic biomass that contain fermentable sugars. However, this production is challenging as these feedstocks contain high amounts of cellulose and lignin, which is difficult to digest during bioethanol production. To overcome these challenges researchers are now focusing on the use of microalgae as the feedstock. Effective carbon dioxide fixation, rapid growth, non-competition for arable land and potable water, potentially high carbohydrate and lipid accumulation along with less lignin content have made microalgae an ideal feedstock for bioethanol production. However, little or no evidence has been found on the production of bioethanol from microalgae in Sri Lanka. Therefore, experiments were carried out to produce bioethanol from microalgae found in freshwater habitats in Sri Lanka and to optimize bioethanol production. Microalgae were collected from selected natural ponds, identified and cultivated in 1.5 L culture bottles. Cultivation media (Bold's Basal medium, Bolds Basal medium + Compost extract and Modified R medium) and harvesting method (Centrifugation, gravity filtration, vacuum filtration, coagulation and flocculation) were optimized to obtain a high yield of microalgae biomass. Pre-treatment condition (Sulphuric acid concentration – 1%, 10%, 70% and reaction time - 30 minutes, 6 hours, 1 day) and fermentation time (2 days, 1 week, 2 weeks, 3 weeks) were optimized to release high amount of sugar and ethanol. Molisch's and Fehling's tests were used to detect released sugar. The dinitrosallicylic acid (DNS) method was used for the quantitative determination of released sugar. Ebulliometer and Gas Chromatography with Flame-Ionization Detector (GC-FID) were used for the characterization and quantitative determination of generated bioethanol. The isolated microalgal strain was identified as *Chlorella* sp. using identification keys for freshwater microalgae. *Chlorella* sp. grown in Modified-R-medium yielded the highest algal biomass of 1 g/L. Coagulation and flocculation method, which was selected as the best method to harvest microalgae yielded biomass of about 1 g/L. $H_2SO_4$ acid concentration of 70% which was the highest acid concentration used yielded the highest sugar concentration of 0.423 ± 0.01 g/L. The reaction time for acid hydrolysis was selected as 6 hours as it yielded the highest sugar concentration of 0.425 ± 0.008 g/L. The optimized results of fermentation time showed that two-week fermentation period yielded the maximum bioethanol of 0.554 ± 0.007 g/g of sugar. These values were further confirmed by GC-FID analysis which also gave a bioethanol content of 0.514 g/g of sugar. The results indicate that the *Chlorella* sp. inhabiting Sri Lankan freshwater habitats contain a considerable amount of fermentable sugars which can be used to produce bioethanol.

**Keywords:** Bioethanol, *Chlorella*, Fermentation, Microalgae, Pre-treatment