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Novel protocols for biomass harvesting, lipid extraction, and biodiesel synthesis from Microalgae, *Chlorella* sp.

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At present, there is a huge interest in utilizing microalgae fatty compounds as a source of energy for the production of biodiesel. The small size and thick-walls of microalgae have made it difficult to harvest from cultures and extract produced lipids. For the same reason, most of the published protocols in the literature, still give unsatisfying results. Therefore, a study was carried out to develop novel protocols for harvesting biomass and extraction of lipids for the efficient synthesis of biodiesel. *Chlorella* sp. was cultivated in commercial-grade Albert's solution for mass production of microalgae. The ECF (Electrocoagulation and Flocculation) technique was used for harvesting biomass. A novel Aluminium and hybrid (Aluminium-Carbon) electrode systems were also employed and optimized for harvesting microalgae. Two systems were evaluated by two-factor factorial ANOVA design to determine the significance of variation due to the electrode system and distance between electrodes. Harvested microalgae were dewatered and dried. A newly developed sand crushing-heating method was adopted to extract lipids from the microalgal biomass. Factorial analysis of variance was used to determine the significance of variation due to sand to sample ratio and amount of solvent in the extraction procedure. To synthesize biodiesel, extracted lipids were trans-esterified by a novel, stoppered bottle-mixing method and optimized a by trial and error method. The resulting fatty acid methyl esters (Biodiesel) were subjected to *GC-MS* to analyse the fatty acid profile. Out of the methods tested, the pure Aluminium electrode system was recognized as the best ECF technique for harvesting microalgae after statistical analysis. According to the data analysis, the optimum distance between two electrodes was 3.0 mm and harvested 4.0 liters of culture within 5.75 ($\pm$ 0.7) minutes. There was sufficient evidence of interaction effect between the electrode system and distance between electrodes at $\alpha = 0.05$. The newly developed sand crushing-heating method was employed to extract lipids from microalgae with a low amount of impurities compared to conventional chemical extraction methods such as the Floch method (Chloroform-Methanol, 1:2 v/v) and its modifications. There was sufficient evidence of interaction effect between sand to sample ratio and Hexane volume using 0.05 of significance level. The optimum sand to sample ratio was found to be as 2:1 and the optimum volume of Hexane was found as 15.00 mL per 1.00 g of sample. Results indicated that the stoppered bottle mixing method could convert crude, extracted algal oil (172 ± 10 mg/g) into biodiesel efficiently ($\approx$ 95%). According to the fatty acid profile, oleic acid (38.64%), linoleic acid (36.58%), palmitic acid (11.28%), and stearic acid (5.53%) were observed as major fatty acid components present in lipids of *Chlorella* sp. This study highlights the advantages of developed novel protocols, both in terms of efficiency and purity.

Keywords: Microalgae, Biofuel, Transesterification, Electrodes

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