Optimization of extraction methods for efficiently recovery of metabolites from two variety of freshwater alga (Chlorophyta)

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Abstract
Microalgae have been known to contain large amounts of untapped lipid reserves and several economically important bioactive metabolites. Nevertheless, microalgae has been widely explored only for their oil property that can be transformed into biofuel. However, biofuel production from microalgae is still in its infancy and yet to achieve with the high maintainance and production cost. Oils of microalgae for biofuel production are a low-value with high cost production; ie US$1.13/kg compared with bioactive compounds, ie US$ 300-1500/kg. Therefore, to increase the income with same production cost, exploring the production of bioactive compound concurrently is the only outcome. Hence, extraction method of microalgae that enable preservation of bioactive compounds and recovery of most oil from biomass is of major advantage. In this study, the efficiency of four disruption techniques (sonication, HCl extraction, homogenizing and cryogrinding) were tested on *Chlorella sorokiniana* and *Scenedesmus regularis*. Samples were pre-treated with different drying process before extracted using solvent mixture of methanol and chloroform to extract both oil and bioactive compounds. Particle sizes of disrupted algae were observed ranges from 2.0 – 4.0 µm with HCl extraction and homogenizing techniques reported the highest numbers. Highest recovery yield of aqueous and chloroform portions (45% - 80%) were obtained from HCl extraction in both species. The metabolites data were acquired by using Nuclear Magnetic Resounance (NMR) and the effiency of extraction methods were analysed by applying chemometrics approaches. From the NMR analysis, the improvised extraction method also shows it efficiency in preserving those heat-labile metabolites.

Keywords: Metabolomic, NMR, bioactive compounds

Enzymatic Hydrolysis of Palm Biomass for Fermentable Sugars using Polyethylene Glycol Immobilized Cellulase

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Abstract
In this work, enzymatic hydrolysis using cellulase both in solution and immobilized form was studied to convert lignocellulosic biomass from empty fruit bunch into fermentable sugars. The cellulase was covalently immobilized with activated and functionalized polyethylene glycol via glutaraldehyde coupling. To determine sample enzyme activity, the equivalent reducing sugars released during hydrolysis reaction with free cellulase and immobilized cellulase respectively, were quantified using 3, 5-dinitrosalicylic acid (DNS) method. As a whole, the immobilized cellulase displayed 50% higher efficiency over free cellulase, in reducing sugar recovery during hydrolysis reactions. From the kinetic study, it showed that Michaelis constant (K_m) and limiting velocity(V_{max}) of immobilized cellulase were 179.2 mg/ml and 33.5mg/ml.min respectively, whereas that of free cellulase were 171.8mg/ml and 34.5mg/ml.min respectively. The higher K_m value of immobilized cellulase could be attributed to the polyethylene glycol interference with the binding of cellulase to expose substrate, and enables free interaction of cellulase to hydrolyse cellulose maximally.

Keywords: Kinetic Study, Polyethylene glycol, Hydrolysis, Immobilized Cellulase, Glutaraldehyde.

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