Abstract

In order to develop the 3rd generation microalgal biomass based biofuels, a holistic approach is quintessential towards achieving feedstock, process, environmental and economic sustainability. Present study implements the idea of strategic cultivation of microalgae with simultaneous waste utilization for subsequent downstream processing of biomass constituents into biofuels. In this study, some important physicochemical parameters that critically affect biomass production and lipid synthesis by *Chlorococcum infusionum* were investigated. The medium was formulated and optimized for microalgal biomass cultivation using Plackett–Burman (PB) design and statistical model based Response surface methodology (RSM). The optimized medium resulted in nearly 2.5 fold increase in biomass concentration when compared to the unoptimized Bold Basal Medium (BBM). Maximum lipid accumulation of up to 40±1.8% of dry cell weight (w/w) was achieved under nitrogen limiting condition without affecting biomass concentration as opposed to 11.8±0.7% (w/w) lipid content obtained in control experiments using BBM. The microalga, when cultivated in different types of wastes viz. municipal, domestic, industrial effluents and poultry litter waste (PW), caused significant reduction in pollutant load. Substantial increase in biomass concentration by 1.9 fold and lipid content by about 2 fold, as compared to the corresponding values in BBM under non-limiting conditions, was achieved when industrial effluent was supplemented judiciously with the solid waste, PW. Further, these mixed solid and liquid wastes were used as culture media for microalgal growth in airlift photobioreactor at optimal light intensity of 100 μmol m^{-2} s^{-1} and air flow rate of 1500 mL min^{-1}. Wastewater effluents supplemented with PW (10 g L^{-1}) resulted in higher biomass concentration. The experimental data of microalgal growth, when fitted to the logistic growth model, showed very good correlation with the values predicted by the model. In order to improve the biomass concentration further, feeding strategy involving intermittent addition of PW (10gL^{-1}) at regular time intervals was developed. The addition of PW at 3 days time interval resulted in enhanced biomass concentration up to 1.67±0.03 g L^{-1}. Lipid content, for biofuels application, was also measured to be 25.4±1.5% (w/w). Significant lipid accumulation of up to 45.2±1.6% (w/w) was obtained when subjecting the biomass to nitrogen limiting condition. The fatty acid profile of microalgal lipid indicated high percentage of C_{16}–C_{18} fatty acids largely comprising of C16:0 and C18:1 that are considered favourable for good-quality biodiesel. Subsequently, a downstream processing strategy was designed to convert efficiently the biomass feedstock into biofuel products. Harvesting efficiency of the self-flocculating *Chlorococcum* sp. was enhanced to nearly 94% by treating biomass containing slurry with $FeCl_3$ under acidic condition (pH 4) for 30 min. Of various methods for lipid recovery from *Chlorococcum* biomass, the most effective method involved cell disruption with a bead-beater, followed by lipid extraction with chloroform/methanol (2:1, v/v) resulting in lipid recovery of 96.2±2.9%. The extracted lipid was converted into fatty acid methyl esters (biodiesel) with a conversion of 95±3.1% after standardizing the acid based transesterification process. Additionally, the lipid-extracted residual biomass was used as sugar feedstock for bioethanol production owing to its significant carbohydrate content. The optimal acid hydrolysis condition carried out at 121 °C for 30 min in an autoclave resulted in effective conversion (89.6±3.0%) of total carbohydrates into fermentable sugars. The fermentability of reducing sugars to bioethanol using *S. cerevisiae* was assessed in terms of ethanol concentration, which was found to be 4.1±0.2 g L^{-1}. Thus, the thesis highlights the importance and necessity of strategic production of microalgal biomass by utilizing mixed wastes (solid and liquid) as substitute for expensive media, followed by bioconversion of the biomass constituents into biofuel products in a biorefinery model for sustainable development of bio-based economy in the near future.

Keywords: Microalgae; Biorefinery; *Chlorococcum infusionum*; Plackett-Burman design; Medium optimization; Biomass; Lipid; Reducing sugars; Wastewater remediation; Downstream processing; Pretreatment; Biodiesel; Bioethanol; Sustainability