ABSTRACT

Microalgae are the third generation biodiesel feedstock, which have high photon-to-biomass conversion efficiency as well as high biomass production per unit area. In addition, microalgae can be cultivated round the year in non-fertile lands, which make them advantageous than their plant competitors. In oleaginous microalgae, the accumulation of lipid is observed to be a stress-linked phenomenon. The nutrient-starvation is one of such popular strategies used to boost the intracellular lipid accumulation (% dcw) in microalgae. However, under autotrophic cultivation, application of such stresses resulted in significant lowering of biomass yield consequently declining the total lipid yield obtained for biodiesel production. Furthermore, two-stage cultivation adopted to overcome this problem led to several technical and financial complexities. In this respect, single-phase mixotrophic cultivation could serve as an energy- and cost-efficient strategy to target the quantitative enhancement of the total lipid. Hence, in this study, the two independent strategies, viz., mixotrophy and nutrient deficiency have been combined to target the maximization of the triacylglycerols (TAGs) accumulation. Two different oleaginous microalgal strains, viz., *Chlorella minutissima* UTEX 2219 and *Chlorella minutissima* MCC 27 were evaluated for enhanced lipid accumulation. In addition, a phyco-myco co-cultivation was developed under laboratory conditions and evaluated as an alternative biodiesel feedstock. The phyco-myco co-cultures were prepared by the amalgamation of the individual microalgal strains selected in this study with an oleaginous filamentous fungus, *Aspergillus awamori*. Subsequently, the N 11 medium was optimized for both the monoalgal culture and phyo-myco co-culture individually targeting the maximization of total lipid yield (mg L^{-1}) under mixotrophic growth. Further, biodiesels were produced from both the feedstocks by utilizing the celite-immobilized lipase as a biological catalyst for transesterification.

Limited nitrate feeding and supplementations of glucose and acetate as mix-carbon source have been identified as crucial parameters and optimized for enhanced lipid accumulation in *C. minutissima* using CCRD-RSM. In comparison to the individual optimization of the biomass yield (BO) and lipid content (LCO), the simultaneous production of biomass and lipid content and their optimization (SBLCO) was found to be the most efficient strategy to achieve the maximum lipid productivity of 108.81 mg L^{-1} d^{-1}, which was about 10 folds higher than that of the autotrophically cultivated *C. minutissima* MCC 27 culture. Under the optimized conditions of 5.96 g L^{-1} glucose, 4.12 g L^{-1} acetate, 0.73 g L^{-1} nitrate, and 10 days of incubation, the maximum lipid yield obtained was 1129.75 mg L^{-1} in *C. minutissima* MCC 27. On the other hand, the phyco-myco co-culture led to substantial enhancements of biomass and lipid accumulations in both *C. minutissima* UTEX 2219-*A. awamori* and *C. minutissima* MCC 27-*A. awamori* co-cultures. Glycerol and potassium nitrate were observed to be the most effective C- and N-source for phyco-myco co-cultures. A 2.6-3.9 folds increase of biomass (g L^{-1}) and 3.4-5.1 folds increase of total lipid yield (mg L^{-1}) were observed in the co-cultures in presence of glycerol in comparison to the axenic monocultures. The critical parameters of the phyco-myco co-culture have been subsequently optimized via CCRD-RSM approach. The biomass optimized conditions (BO) of 17.31 g L^{-1} glycerol, 1.50 g L^{-1} nitrate, and 4.93 pH on the 8th day of incubation resulted in the maximum biomass yield of 4.42 g L^{-1} along with the maximum total lipid yield of 1353.85 mg L^{-1} in the *C. minutissima* MCC 27 and *A. awamori* co-culture. It was approximately 3.9 folds higher biomass yield compared to autotrophically grown *C. minutissima* MCC 27 monoalgal culture while 1.4 folds higher than the mixotrophic *C. minutissima* MCC 27 culture grown in optimized N 11 medium utilizing SBLCO strategy. The step-wise optimization of lipase-mediated transesterification led to a maximum of 91.52 ± 2.28 % and 93.25 ± 1.93 % FAME conversions in *C. minutissima* MCC 27 monoculture and *C. minutissima* MCC 27 and *A. awamori* co-culture, respectively. The fuel properties of the biodiesels produced from *C. minutissima* MCC 27 monoculture and *C. minutissima* MCC 27 and *A. awamori* co-culture showed comparable results, which fall within the specified limits of various biodiesel standards such as ASTM D6751-15ce1, EN 14214:2012, and IS 15607:2005. As a whole, this work suggests that phyco-myco co-culture between *C. minutissima* MCC 27 and *A. awamori* can be a suitable alternative for biodiesel production owing to the enhanced lipid yield at an earlier incubation time against the monoalgal culture of *C. minutissima* and the resultant biodiesel is suitable for fuel application.

Keywords: Microalgae, Phyco-myco co-cultivation, Mixotrophy, CCRD, Lipase, Biodiesel