ABSTRACT

The demand for energy has been considerably increasing since the last few decades. This trend is expected to continue in the coming years. According to International Energy Outlook, the worldwide consumption of energy is projected to increase by 28% in the years between 2015−2040, while the consumption of petroleum-based fossil fuels will increase from 190 to ~230 quadrillion British thermal units. Furthermore, the consumption of fossil fuels releases approximately 29 gigatons of CO_2 every year into the atmosphere, which in turn contributes to global warming. Considering the increased demand of petroleum-based fossil fuels, their effect on environment and their scarcity in the near future, alternative energy sources look more attractive and practically feasible than ever. Microalgae have emerged as an alternative renewable energy source because of their inherent property to accumulate high amount of lipid (up to 50 % of its dry cell weight (DCW)) which is then used for biodiesel production. Moreover, microalgae-based biofuels (biodiesel) are non-toxic, biodegradable, emit low Sulphur content and release high oxygen (10–45%) as compared to petroleum-based fuels.

In spite of these advantages, microalgae-based lipid and biodiesel production has not yet been done on a commercial scale due to several gaps in technology such as low biomass productivity, high production cost, variable temperature, and light intensities especially in tropical countries, and the use of wastewater as growth media. For the current study, mixotrophic cultivation of microalgae was chosen a route for lipid production due to higher biomass and lipid productivities under this mode of cultivation as compared to autotrophic cultivation. The present study has assessed 3 microalgae species namely *Chlorella sorokiniana*, *Scenedesmus obliquus*, and *Scenedesmus abundans* for mixotrophic cultivation using a different concentration of glucose (0.5−5 g L^{-1}), acetate (0.5−5 g L^{-1}) and mixed substrate (glucose + acetate: 2−6 g L^{-1}). The mixed substrate (glucose:acetate−1:1) was found effective in enhancing biomass productivity (up to 330 mg L^{-1} d^{-1}) of microalgae species by 5.4 times and reduce respiration by up to 40% as compared to when an individual organic carbon source was used. The *C. sorokiniana* accumulated highest amount of lipid (Lipid

productivity: 29.8–36.2 mg L^{-1} day^{-1}) among the three strains. The strategy of the mixing of organic carbon substrate (glucose and acetate) was also found effective in increasing the tolerance of microalgae species up to 6 g L^{-1} from 3 g L^{-1} as in case of individual organic carbon source. The biodiesel properties derived from fatty acid methyl esters (FAME) for individual and mixed organic carbon substrates met standard properties of biodiesel with respect to cetane number (> 47), Iodine value (< 120 g I_2/ 100g), degree of unsaturation and cold filter plug point.

The production of microalgae biomass all over the world was reported to be about 9000 tons per year, with the production cost being anywhere between \$20−\$200 per kilogram. There are two ways to reduce biodiesel production cost. One way is to focus on the production of high-value products i.e., omega fatty acids that have various health benefits. The second way is to use cheap organic carbon sources like wastewater instead of conventional organic carbon sources like glucose and acetate. The former approach was achieved by exposing the microalgae culture to red light spectra in an airlift photobioreactor (PBR) that can alter the biomass productivity and lipid quality of microalgae. The red light was found effective in enhancing biomass, lipid, and omega fatty acids (linoleic and linolenic acid) productivity of *S. abundans* and *C. sorokiniana* by ~2 times in airlift PBR. Mixotrophic condition strongly influenced nitrate consumption, and its early consumption was the key factor in getting higher total lipid content. Among acetate and glucose, the latter was found to be the more effective organic carbon to enhance biomass, lipid, and omega fatty acids productivity of *C. sorokiniana*. While comparing the effect of light spectra, both white and red lights were found to produce biodiesel of appropriate quality in mixotrophic mode (CN: 51–55) for both microalgae species.

The present study used food product processing industrial wastewater for the latter approach. The pre-feasibility study for the cultivation of *Chlorella* species at different dilutions (0, 1.6 and 5x dilution) of synthetic and actual food product processing industrial wastewater reduced the freshwater requirement by 20%–60% and successfully removed COD, TN, and TP from wastewater. An integrated approach had also been studied to produce microalgal biodiesel using both raw (RW) and anaerobic digested wastewater (ADW) of food product processing industry without addition of extra nutrients or carbon source for cultivation. The addition of RW as a carbon source in ADW (RW + ADW), enhanced biomass and lipid productivities to 1.5–2.9 times than that obtained with RW or ADW alone for *C. sorokiniana*, *S. obliquus* and *S. abundans*. The effluent obtained after microalgae growth contains COD (200 mg L^{-1}), TN (23 mg L^{-1}), and TP (8.8 mg L^{-1}) below the discharge limit. The FAME obtained with all three microalgae species for mixed wastewater (RW + ADW) met standard properties of biodiesel. Hence, the integrated approach i.e., the addition of carbon-rich RW into carbon-deficient but nutrient-rich ADW was a suitable solution for both *in situ* wastewater treatment and biomass generation for biofuels.

As the biodiesel production process can only be scaled up in outdoor conditions, achieving high biomass and lipid productivities at uncontrolled environmental conditions especially at high irradiance and high-temperature in Nagpur region is the biggest challenge. Therefore, the present study assessed cultivation of microalgae species in airlift PBR in summer as well as in winter season. The outdoor study in summer season showed photoinhibition effect at high temperatures (47 °C) and irradiance conditions. The microalgae *S. abundans* can be considered to be a thermotolerant species as it can tolerate temperatures up to 45 °C with optimum biomass productivity of 162.5 mg L^{-1} day^{-1}. The fed-batch cultivation strategy accumulated higher lipid content as compared to respective batch cultivation. The fed-batch strategy favorably influenced consumption of acetate in light periods. The respiratory loss of carbon was 60−70% lower in fed-batch cultivation as compared to batch cultivation. Thus, the outdoor microalgae cultivation in summer season would be cost-effective under fed-batch strategy. Similarly, in winter season three microalgae species namely *C. sorokiniana*, *S. obliquus*, and *S. abundans* were successfully cultivated in a mixed substrate (3 g L^{-1} glucose + 3 g L^{-1} acetate). The fatty acid derived biodiesel properties obtained in all runs in summer and winter season also complied with International biodiesel standards (ASTM and EN14214) (CN: 51−63).