Chapter - 07

Summary

The current study focused on evaluating a sustainable solution for microalgae-based remediation of dairy effluent and revealing the potential of microalgae for remediation of dairy effluent as well as generating biomolecules. Dairy effluent is highly rich in nutrients like; nitrogen (N), (all forms of nitrogen; such as nitrate, nitrite, ammonia, urea), phosphorus (P) in the form of PO_4^{-3} and trace amount of metals. The micro-algal biomass provides multifaceted applications towards bioenergy (lipids precursor for biofuel) as well as biomolecules production. The Considerable research activities were investigating the potential of autochthonous microalgal species that were isolated from dairy effluent, collected from the milk plant named as Parag situated at Mansurpur, in Muzaffarnagar (UP), India. Microalgae strains in order to remediate DWW to address the waste-to-bioproducts economy. Dairy sector, is one of them which generates enormous amount of drained water, rich in nutrients. Several physicochemical & biological techniques used for treatment of dairy waste. Because of the availability of high organic matters, dairy waste proved to be a marvelous medium for the cultivation of microalgae hence it performs a dual role as a bioremediator and as well as a good producer of various valuable compounds like lipids, proteins, carbohydrates, etc. Among these, microalgal lipids have vast applications in human health sector, as nutraceuticals, and pharmaceuticals etc. These are also highly required in the cosmetics, and biofuel field. In present work, we examined the role of autochthonous microalgae which provide a promising source for the remediation of dairy waste water and as well as a nutrient source for producing biomass and accumulated lipids as polyunsaturated fatty acids, (PUFAs), carotenoids (pigments), oxylipins (oxygenated PUFAs) etc., to offer as good feedback for the manufacturing

biodiesel and other commercial applicability. Recently, microalgae have become an amazing and resource-friendly alternative choice for the manufacturing of advantageous bio-products like; lipids, proteins, amino acids, carbohydrates etc. due to the richness of high-value-added primary and secondary metabolites. Microalgae being photosynthetic organism, help in CO_2 sequestration and release of oxygen in the atmosphere (with an increasing efficiency as compared with terrestrial plants). The microalgae biomass can be further processed to obtain value added various metabolites of commercial value.

After screening all the 10 microalgae isolates, based on their growth kinetics, 02 potential strains were selected for this study. Both the selected microalgal isolates were grown for the estimation of nutrient removal capacity (chemical oxygen demand, nitrate, phosphorus), biomass and lipid productivity. In the present study two autochthonous microalgae were isolated from dairy wastewater. Both were analyzed for their bioremediation capacity and production of biomass and lipids simultaneously. Observation made and data collected from the current study clearly illustrates that both microalgae isolates can be effectively used for dairy effluent treatment. Both the microalgae isolate (DWW06 and DWW08) were found to exhibit higher biomass in 75% conc. of dairy waste water.

In the current work, isolated strain, DWW06 was identified as a chlorophyte, *Botryococcus sp.* (Accession no. KF746941.1.) depending on its microscopic examination and 18S rRNA gene sequences. While the another microalga, DWW08 was identified as a chrysophyte, *Poterioochromonas melhamensis* according to its microscopic examination and 18S rRNA gene sequences. Both these isolates were effectively remediated DWW and produced lipids (fatty acids). The growth of microalgae isolates was optimized by using different concentrations of DWW, supplemented with all the nutritive requirements for better progression and flourishment. The

maximum biomass (dry cell weight) reached 1.478 ± 0.003g L^{-1} in 15 days from an inoculum of 0.072 g L^{-1} in case of DWW08, While DWW06 was able to attain 1.385 ± 0.002 g L^{-1} from the inoculum 0.064 g L^{-1} in 75% concentration (75 ml DWW + 25 ml BBM media) Microalgae biomass was found to decrease slightly in DWW after 15 days. It was assumed that biomass concentration directly depends on the consumption of nutrients, in the wastewater. If the nutrients are consumed rapidly then biomass increased progressively. It was found that 75% concentration (75 ml DWW +25 ml BBM media), showed better media for growth than any other concentrations.

7.1. Performance evaluation of isolated (DWW06) Botryococcus sp. (Accession no. KF746941.1.)

DWW06 (*Botryococcus sp.*) isolate was achieved 1.385 ± 0.005 g L^{-1} biomass (DCW) from the inoculum 0.064 g L^{-1} in 75% conc. (75 ml DWW+25 ml Bold's Basal media) in 15 days of batch culture. Which was followed by 1.288± 0.004 g L^{-1} 50% concentration (50 ml DWW+50 ml BBM media), and 1.250± 0.004 g L^{-1}100 % DWW. While 25% concentration (25 ml DWW+75 ml BBM media), and Control (BBM media) were produced the similar biomass i.e.,1.122 ± 0.004 g L^{-1}. The biomass productivity of DWW06 88.00 ± 0.003mg/L/d in 75% concentration of DWW. While, the specific growth rate was estimated 0.201 mg/L/d. This strain showed high nitrate removal efficiency 84.96% (from 95.60 to 14.43 $mgL^{-}1$) with 5.41 $mgL^{-1}d^{-1}$, after 15 days of batch culture. The phosphate removal efficiency of this strain (DWW06) was 86.45 % (from 88.52 to 11.99 mgL^{-1}), with 5.10 $mgL^{-1}d^{-1}$ removal rate, after 15 days. The experimental results highlighted the COD removal was 86.45% (from 2398 .00 to 324.92 mgL^{-1}) with 138.20 $mgL^{-1}d^{-1}$ removal rate, after 15 days' cultivation, in 75% conc. of dairy waste water. The maximum lipid content of DWW06 was 29.80±0.002 gL^{-1} in 75 % DWW which followed by 50% DWW (27.70± 0.003 gL^{-1}), 100% DWW

(26.88±0.003 gL^{-1}), 25% DWW (24.35± 0.002 gL^{-1}), and in control (BBM medium) was 21.45±0.002 gL^{-1}. The lipid productivity of DWW06 was 26.22 ± 0.004 gL^{-1} d^{-1} higher in 75% DWW. While, lipid profile through GC-MS of DWW06 (*Botryococcus sp.*) was C16:0 (10.46 %), C16:1 (30.17%), C18:0 (8.28%), C18:1 (14.51%), and C18:2 (12.04%) C20:0 (4.66%) fatty acids.

7.2. Performance evaluation of isolated (DWW08) Poterioochromonas melhamensis (UPMC-A0073)

DWW08 (*Poterioochromonas melhamensis*) isolate was achieved 1.478 ± 0.003 g L^{-1} biomass (DCW) from the inoculum 0.072 g L^{-1} in 75% conc. (75 ml DWW+25 ml Bold's Basal media) in 15 days of batch culture. Which was followed by 1.265± 0.004 g L^{-1} 50% concentration (50 ml DWW+50 ml BBM media), and 1.145± 0.004 g L^{-1}25% concentration (25 ml DWW+75 ml BBM media), and Control (BBM media) was produced biomass i.e.,1.128 ± 0.004 g L^{-1}. While 100% DWW was producing1.056 ± 0.004 g L^{-1}dry cell weight. The biomass productivity of DWW08 93.73 ± 0.006 mg/L/d in 75% concentration of DWW. While, specific growth rate was estimated 0.204 mg/L/d. This strain represented higher nitrate removal efficiency 87.45% (from 95.60 to 11.99 mgL^{-1}) with 5.57 $mgL^{-1}d^{-1}$, after 15 days of batch culture. The phosphate removal efficiency of this strain (DWW08) was 88.90 % (from 88.52 to 9.77 mgL^{-1}), with 5.25 $mgL^{-1}d^{-1}$ removal rate, at the end of batch culture. The experimental results represented that the COD removal was 88.84% (from 2398.00 to 267.61 mgL^{-1}) with 142.03 $mgL^{-1}d^{-1}$ removal rate, after 15 days' cultivation, in 75% conc. of dairy waste water. The maximum lipid content of DWW08 was 31.61±0.003 gL^{-1} in 75 % DWW which followed by 100% DWW (28.46±0.003 gL^{-1}), 50% DWW (26.50± 0.002 gL^{-1}), control (BBM medium) was 23.43±0.004 gL^{-1} and in 25% DWW (22.60± 0.004 gL^{-1}). The lipid productivity of DWW08 was 29.61 ± 0.003 gL^{-1} d^{-1} higher in 75% DWW. Whereas, the lipid profile through GC-

MS of DWW08 (*Poterioochromonas melhamensis*) was C14:1 (3.40%), C14:0 (5.17%), C16:0 (6.46 %), C16:1 (38.17%), C18:0 (15.52%), C18:1 (15.71%), C18:2 (06.04%), C18:3 (02.78%), and C20:1(3.26%) fatty acid.

In conclusion, our studies, for microalgae biomass and remediation purposes, 75% dairy waste water with BBM media provided better results. Remediation of dairy effluent by cultivating the microalga *Poterioochromonas melhamensis,* was the first report of dairy waste water remediation, as far as we informed. Its culture consumed the heavy load of pollution as nutrients and remedied the DWW. Simultaneously, produced valuable lipids for commercial use. it was found to be the most encouraging candidate for bioremediation and lipid production. while the another isolate *Botryococcus sp.* efficiently qualified as the candidate strains combined results for growth (biomass), lipid accumulation with dairy effluent remediation. The lipids of *Botryococcus sp.* also showed the suitability for commercial applications.