

Study of Algal Biofilm to Enhance Biomass and Lipid Accumulation

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Abstract

Microalgal biomass for the production of biodiesel is emerging at the forefront in the relevant research realm. They are considered the most versatile biomass due to certain characteristics such as (1) the ability to convert carbon dioxide (CO₂) into biomass through transesterification, (2) short life-cycle, (3) high growth rate, and (4) microalgae cultivation that does not compete for fertile fields with food crops. Biodiesel production can be improved by increasing biomass and lipid yield (LY), as well as optimizing downstream processing. Studies have shown various approaches to increase microalgae biomass and lipid production. High biomass productivity (BP) and LY depends on the potential of strains and cultivation strategy. The goal of this thesis is to investigate the potential of microalgae species that are native to North-Eastern, India for biodiesel production and to assess their ability to treat wastewater and produce biomass that can be used for animal feed. The freshwater microalga, *Scenedesmus* sp. DDVG I was selected as a potential strain for biomass production. The freshwater cyanobacterium, *Limnothrix* sp. DDVG II was used as an auto-flocculating strain. The Bioenergy Lab isolated both strains from a swampy region of the Indian Institute of Technology Guwahati (IITG), Indai. DDVG I and DDVG II microalgal isolates were molecularly characterized using 18S and 16S rRNA gene sequence analysis. The sequences were submitted to NCBI GenBank as MN630585 and MN630310, respectively. DDVG I and DDVG II strains grew the fastest in a normal BG11 medium at pH 7 and 27 °C, with specific growth rates of 0.12±0.005 d⁻¹ and 0.11±0.00 d⁻¹, respectively. Further supplementation of the medium with 1.05 g/L urea, 0.04 g/L K₂HPO₄, and 6 g/L glucose resulted in increased biomass and LY of up to 8.5 g/L & 39.5% for DDVG I and 3.3 g/L & 19.9% for DDVG II, respectively.

Another method for increasing biomass recovery from culture broth is to use microalgae as the biofilm. The extracellular polymeric substance (EPS) is a critical intrinsic component for the growth of microalgae as a biofilm. In this study, three distinct extraction procedures were used to evaluate the generation of EPS from DDVG I and DDVG II. We observed negligible EPS yield in DDVG I over 24 days of cultivation. However, DDVG II produced significant EPS with a maximum output on the 12th day of culture. Furthermore, by exposing EPS to various substrata, the adhesive

properties of the material as well as the formation of microalgal biofilms were confirmed with maximum adhesion on rough polylactic acid (PLA) sheet.

Nitrogen shortage is the most prevalent technique for lipid accumulation; nonetheless, this strategy severely limits cell development. Some researchers demonstrated the enhancement of biomass and biomass and lipid production under the combined effects of low nitrogen (N) and high phosphorus (P). However, the selection of a balanced N:P ratio remains ambiguous among microalgal species and is subject to debate. We investigated the effect of N:P ratios ranging from 2:1 to 44.11:1 with supplementation of glucose in the BG 11 medium. The experiment was carried out by exposing DDVG I, to these diverse conditions in heterotrophic and mixotrophic modes. Under mixotrophic mode, the N:P of 100:50 or N-deficient P-over excessive ($N_{def} P_{oexcs}$) condition had the highest growth rate of $0.15 \text{ d}^{-1} \pm 0.01$, with a maximum BP and LY of 41.1 0.5 mg/L.d and $39.1 \text{ 1.2\% dry cell weight (DCW)}$, respectively. To improve the harvesting efficiency, we co-cultured DDVG I and DDVG II mixotrophically under different inoculum ratios in the $N_{def} P_{oexcs}$ condition. The results showed that the 1:1 co-culture system achieved the highest biomass harvesting efficiency of $99.9 \pm 0.5 \%$, with increased BP and LY up to $42.0 \pm 0.9 \text{ mg/L.d}$ and $40.2 \pm 1.0 \%$ DCW, respectively. The fatty acid methyl ester (FAME) compositions of co-culture 1:1 was also suitable for biodiesel production, with high levels of C18:2, C16:0, C18:1, and C18:0. The biodiesel derived from the co-culture 1:1 also met standard specifications for biodiesel production.

To test the feasibility of employing wastewater as a low-cost nutrient, we cultured DDVG I in primary municipal wastewater (PMWW) from Minneapolis, USA, in heterotrophic and mixotrophic modes. The experiment was expanded to a 3-L bubbling bioreactor. Over 10 days in the mixotrophic mode, DDVG I exhibited superior BP (0.069 g/L. d), LY (22.5%), and FAME content (22.04% in DCW). The biomass had a high essential amino acid content (159.8 mg/g DCW) and an EAAR of 0.45. The chemical oxygen demand (COD) and total nitrogen (TN) by *Scenedesmus* sp. were 75.6% and 99.8% respectively. Meanwhile, ammonia nitrogen ($\text{NH}_3\text{-N}$) and total phosphorus (TP) were removed at up to 100%.

Additionally, this study demonstrated the growth of microalgae in low strength domestic wastewater (DWW). Urea was added to the DWW at two different levels to balance its nutritional content before being utilized to grow microalgae. The results showed that the optimal nutritional state (N₂₅₀/P₅) consisted of TN of 250 mg/L and TP of 5 mg/L. In this ideal medium, DDVG I increased COD, TN, and TP removal by up to 89.5%, 99.98%, and 99.1%, respectively. Further, co-cultivation of DDVG I and DDVG II in N₂₅₀/P₅ medium achieved 99.5% biomass harvesting efficiency with efficient wastewater treatment. Their corresponding biodiesel met the required standards as well. The analysis of the biomass's essential amino acid content and in vitro digestibility demonstrated its utility as a feed supplement. Overall, this study showed a future for a cleaner environment and greener energy production.

