ENHANCED BIOMASS AND LIPID PRODUCTION FROM OLIVE PROCESSING WASTEWATER USING *SCENEDESMUS OBLIQUUS* IN A TWO-STAGE CULTIVATION STRATEGY UNDER SALT STRESS

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ABSTRACT

Current demands for sustainable supply of food and biofuels increase rapidly worldwide necessitating exploitation of renewable bioresources [1]. Microalgae cultivation has gained significant attention as an advanced feedstock for the production of value-added products [2]. Generation of valuable metabolites from microalgae, such as polyunsaturated fatty acids, avoiding significant loss of biomass is essential to improve the economic efficiency of the process necessitating optimization of growth conditions [3]. Application of stress conditions during microalgae cultivation could serve as an advanced strategy to enhance biosynthesis of bioactive compounds; however, application of stress could hinder biomass growth [4]. Two-stage cultivation, via application of conditions triggering a cellular stress response over the second stage, comprises an efficient strategy towards enhancement of the lipid content in microalgae alleviating growth rate decrease [5].

This study focused on enhancing mixotrophic cultivation of *Scenedesmus obliquus* by optimizing C/N and C/P ratios in synthetic media. Salinity stress effects on algal biomass and lipid production were explored, with nutrient removal capacity assessed during cultivation in olive processing wastewater-based media using a two-stage cultivation strategy. The study investigated the accumulation of glycerol, proline and reactive oxygen species (ROS) to evaluate cellular responses to salinity stress.

Under a C/N ratio of 40/1, *S. obliquus* exhibited biomass and lipid productivity of 0.15 g L^{-1} d^{-1} and 34.1 mg L^{-1} d^{-1}, as well as efficient glucose removal. A two-stage cultivation strategy using 0, 10, 20 and 30 g L^{-1} NaCl during the second stage increased biomass productivity (0.14-0.19 g L^{-1} d^{-1}), as compared to batch mode (0.11 g L^{-1} d^{-1}). Lipid productivity increased by 4.2%-156.9% following 3 and 6 d upon the onset of the second stage as compared to batch conditions. Utilization of reducing sugars from olive processing wastewater reached 53.6%-61.2%. Proline, glycerol and ROS accumulated significantly employing 20 and 30 g L^{-1} NaCl, indicating cellular stress, which was not notably stimulated using 10 g L^{-1} NaCl.

The approach established improved the overall productivity of the microalgae, highlighting the potential of the bioprocess towards valorization of olive processing wastewater. Thus, an effective biosystem was developed that could serve the dual aim of olive processing wastewater treatment as well as microalgal biomass and lipid production.

KEYWORDS: *Scenedesmus obliquus*, Olive processing wastewater, Lipids, Two-stage cultivation, Salt stress

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