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Valorization of poultry manure extract enriched with sodium acetate in mixotrophic cultivation of *Auxenochlorella protothecoides*

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Abstract Poultry manure (PM) is byproduct of intensive agricultural production and can be used as a low-cost microalgae growth substrate due to its nutrient and protein-rich content. Microalgae are capable to grow rapidly and thrive in different substrates, thus serving as a valuable source of biomass. In this study, *Auxenochlorella protothecoides* was cultivated in PM extract supplemented with sodium acetate in order to simulate a volatile fatty acid (VFA) rich substrate after PM thermophilic acetogenic fermentation. The effect of different VFA concentrations (0-30g/L) in the substrate on biomass growth and biochemical composition under mixotrophic conditions was evaluated. *A. protothecoides* was capable to acclimatize and multiplied in the PM substrate, ultimately achieving the highest biomass concentration at 20 g/L VFA. Proteins were the most abundant component accounting for 38.1-43.9% w/w in the dry biomass, while carbohydrates and lipids followed, comprising 31.5-36.3% and 10.9-14.1%, respectively. The addition of VFA enhanced the protein, carbohydrate and lipid productivity achieving the maximum values 43.9%, 36.3%, 14.1% respectively at 20 g/L VFA. The results indicate that VFA-enriched PM extract can potentially be used as a sustainable and cost-effective growth medium for microalgal cultivation, while the biochemical composition of algal biomass makes it a promising animal feed supplement.

Keywords: poultry manure, mixotrophy, volatile fatty acids, microalgae, *Auxenochlorella protothecoides*

1. Introduction

Food and energy demands expand rapidly along with the human population growth causing increasing organic waste flows. Significant amounts of poultry manure (PM) are generated annually due to intensification of agricultural production systems, adversely impacting air and water resources (Singh et al., 2010) and posing health hazards (Gržinić et al., 2023). Poultry manure is rich in micro- and macronutrients but also can contain various contaminants (Mata-Alvarez et al., 2014). Thus, if not properly managed, PM can lead to to environmental pollution, soil degradation and spread of diseases (Bolan et al., 2010). Therefore, finding sustainable processes for the valorization of the agricultural wastes or byproducts into added-value products, would prevent aquifer depletion and promote circular bioeconomy.

Coupling the bioremediation of agricultural waste streams with microalgae can provide low-cost and enviromental friendly solutions to the rising demands (Patel et al., 2020). Several microalgae species have been used for the treatment of raw as well as anaerobic digested agricultural wastewaters (Gupta and Pawar, 2018). PM treatment under anaerobic fermentation can result in the bioconversion of organic matter into volatile fatty acids (VFA) and inactivation of pathogens, where the VFA-rich liquid fraction can be used as organic and inorganic nutrient source. Thus, this PM nutrients and protein-rich substrate renders it ideal as a cost-effective microalgae growth medium in order to promote waste-to-bioenergy processes.

Microalgae cultivation own the advantages of rapid growth rates as well as higher photosynthetic efficiencies and nutrient accumulation compared with other terrestrial plants or microorganisms (Perez-Garcia et al., 2011). Microalgae are considered robust and versatile microorganisms, as they have the ability to grow under autotrophic, heterotrophic or mixotrophic conditions (Silva et al., 2021). Therefore, they can be used as a valuable source of biomass for various applications, including biofuel production, animal feed, and wastewater treatment (Chisti, 2007). Mixotrophic cultivation conditions could improve the growth rates and enhance the microalgal productivity by metabolize organic and inorganic carbon concurrently and therefore provide reasonably wide-ranging applications in environmental bioremediation (Patel et al., 2021).

In this study, Auxenochlorella protothecoides was cultivated in PM extract supplemented with sodium acetate in order to simulate a volatile fatty acid (VFA) rich substrate after PM thermophilic acetogenic fermentation. The aim of this study was to evaluate the effect of different VFA concentrations (0-30g/L) in the medium under mixotrophic conditions on biomass growth rates and productivity, nutrient removal efficiency as well as on biochemical composition.

2. Materials and Methods

2.1. Algal strain, culture conditions and growth medium

The algal strain of *A. protothecoides* was provided by the Culture Collection of Algae and Protozoa SAMS Limited Scottish Marine Institute. The stock cultures were grown in substrate composed of 10 mL/L BG-11, 0.5 g/L K_2HPO_4, 5 mL/L $MgSO_4$, 1 mL/L trace elements solution, 1 g/L peptone and 6.83 g/L CH_3COONa (as equivalent to 5 g/L acetic acid) with LED panel illumination and a light-to-dark cycle of 16:8. The culture was maintained in 500 mL Duran flasks each containing 200 mL of the substrate and 50 mL of the inoculum under axenic conditions and constant magnetic agitation. The inoculum sub-culturing was carried out systematically every week for maintenance. Proteins were extracted from PM by adding 250 g PM in 1 L of the optimum concentration of 0.1 M NaOH, after comparative extraction assays with deionized water (DW) and 0.1-0.5 M NaOH. After 24 h, the extract underwent centrifugation and subsequently filtered using Buchner funnel. The PM extract was used as growth medium and consisted of 6307.1 ± 288.7 mg/L proteins, 5003.1 ± 138.0 mg/L VFAs, 425.3 ± 12.0 mg/L carbohydrates, COD of 27.5 ± 2.6 g O_2/L, 528.1 ± 5.6 mg/L NH_4-N, 7.7 ± 0.2 mg/L NO_3-N, 13.4 ± 0.2 mg/L PO_4-P, 20.6 ± 0.2 g/L total solids, EC = 11.68 mS/cm and pH= 9.50.

2.2. Experimental set-up

Biomass growth and biochemical composition was examined at four different VFAs concentrations under mixotrophic cultivation conditions. Specifically, the PM extract was 6-fold diluted and supplemented with the appropriate CH3COONa mass in order to achieve a VFA concentration of 0, 10, 20 and 30 g/L VFAs as an equivalent acetic acid in each substrate. As the aim of the study was to access the feasibility of the microalgal strain to assimilate to PM VFA-rich substrate, no extra nutrients were added. The experiment was carried out using 200 mL sterile PM growth medium and 20 mL inoculum in Duran flasks with LED panel illumination 5000 lux and photoperiod of 16:8 h under aseptic conditions. The cultures were aerated with 0.2 L/min filter sterilized air, while the temperature was 26 ± 2 °C.

2.3 Analyses

Biomass was harvested from the growth medium through centrifugation (5000 rpm for 10 min), washed twice with DW (15 mL) and then dried overnight in an oven at 60 °C in order to determine the cell dry weight per L. Biomass concentration was also daily monitored by measuring the total optical density OD_T of the culture and subtracted the OD_{PM} of the supernatant, both at wavelength of 750 nm. The concentration of proteins, carbohydrates, lipids in the lyophilized biomass as well as of total VFAs in the liquid substrate were measured spectrophotometrically (Cadas 50, Dr.Lange GmBH, Germany).

3. Results and discussion

The harvest of each culture was carried out once VFA removal rates exceeded 90%. Specifically, the cultures at 10, 20, 30 g/L VFA lasted 6, 12, 18 days respectively. However, high VFA concentration (30g/L) prevent removal efficiency which was observed to reach a plateau after day 12 (Figure 1a). In line with this, Chalima et al., (2019) reported that high initial propionic acid concentration restrained its utilization and consequently the growth of heterotrophic marine microalga.

The maximum biomass concentration was reached at 20 g/L VFA (2.88 g/L), while the lowest at the 0 g/L (1.05 g/L) (Figure 1b). These results indicate that *A. protothecoides* was capable to adapt and reproduce in the PM substrate, which is consistent with previous study reported enhanced biomass productivity in VFA-rich substrate under fermentation processes by microalgae (Chalima et al., 2017). Nevertheless, biomass at 30g/L was found to be lower than 20 g/L VFA (Figure 1b) which is consistent with Bumbak et al., (2011) and Chen and Johns (1994) findings that high organic substrate concentration (e.g., acetate) can be prohibitive to microalgae growth.

Regarding the biochemical composition, proteins were found to be the most abundant substance in dry biomass (38.1-43.9% w/w), followed by carbohydrates (31.5-36.3%) and lipids (10.9-14.1%). Protein, carbohydrate and lipid productivity was enhanced by the addition of VFA compared to the control (0 g/L), achieving the highest values 43.9%, 36.3%, 14.1% respectively at 20 g/L VFA (Figure 1c). In accordance to that, microalgae cultivation in VFA-rich substrates have been demonstrated to promote efficient nutrient accumulation and trigger protein, lipid and carbohydrate deposition (Fei et al., 2015, Guihéneuf and Stengel, 2015)

4. Conclusions

Auxenochlorella protothecoides was cultivated under mixotrophic cultivation, with PM extract enriched with sodium acetate serving as a source of organic carbon, inorganic compounds and proteins. The results demonstrate that the poultry manure VFA-enriched substrate could enhance the microalgal growth and promote the accumulation of proteins, carbohydrates and lipids. The concentration of 20 g/L VFA was found to be the most productive PM extract with the highest biomass yield and nutrient augmentation. Overall, this study indicates that microalgae cultivation in VFA-rich PM extract has potential to be used for simultaneous waste nutrient removal and biomass production based on its high nutrient removal efficiency as well as its high growth rate and productivity. Hence, poultry manure can be valorised as a low-cost and sustainable feedstock for microalgal cultivation. This could provide significant implications for the development of high-value bioproducts such as animal feed supplements from microalgae.

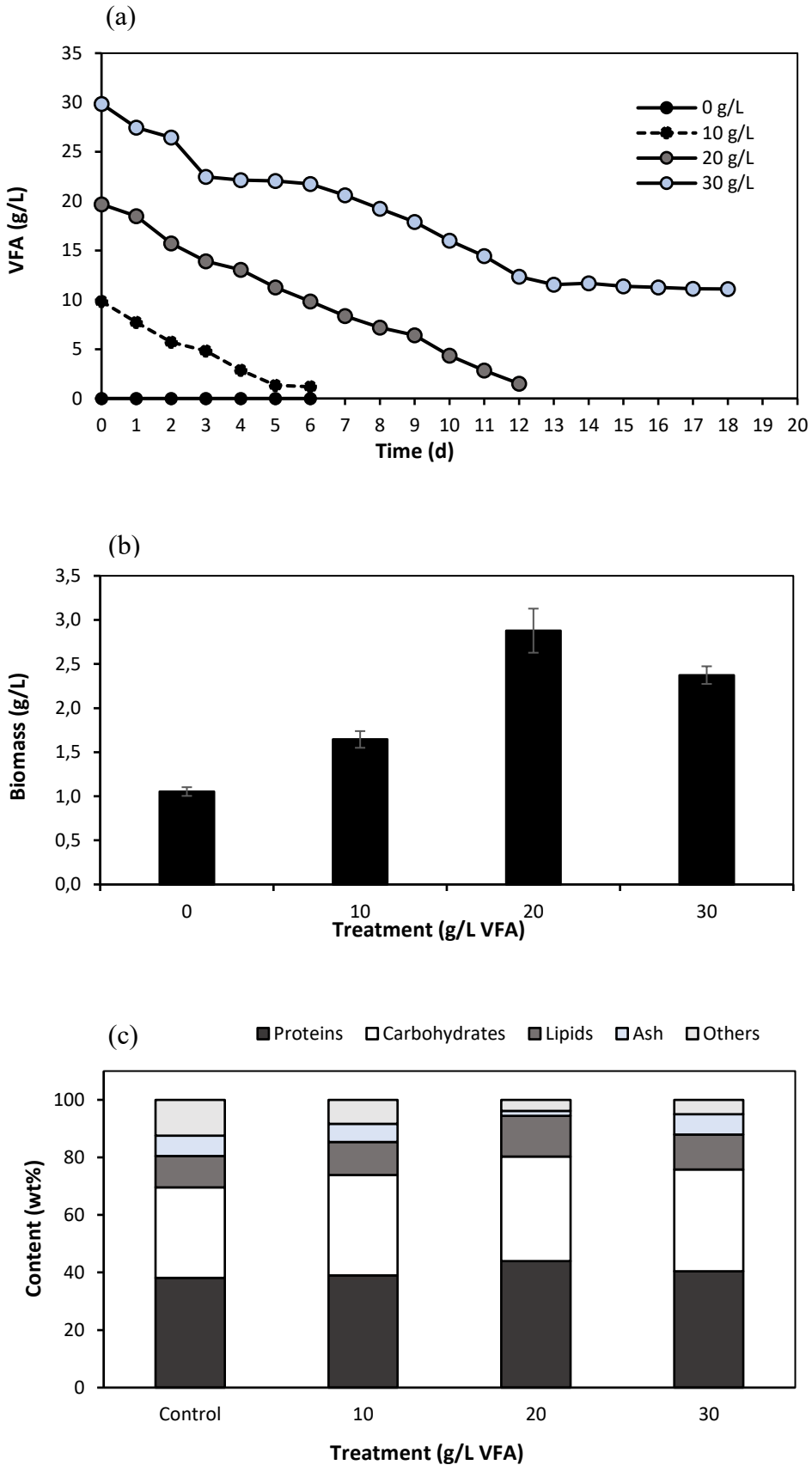

Figure 1. (a) VFA removal efficiency during the cultures (b) biomass yield and (c) biochemical composition of *A. protothecoides* cultivated in PM extract at different VFA concentrations.

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