

Mixotrophic and heterotrophic cultivation of *Auxenochlorella protothecoides* in chicken manure extract supplemented with sodium acetate: effect of substrate C/N ratio on biochemical composition

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Introduction

Recovery of nutrient and energy from agro-industrial wastes is gaining attention in the context of circular economy. Substantial amounts of chicken manure (CM) are annually generated in the frame of livestock production (Yin et al., 2021). Microalgae are advantageous microorganisms which can grow in various substrates under mixotrophic or heterotrophic conditions. Nitrogen source and carbon to nitrogen (C/N) ratio in the culture medium influence the biomass growth and biochemical composition (Chalima et al., 2017). In this study, *Auxenochlorella protothecoides* was cultivated in a protein-rich extract of CM supplemented with sodium acetate in order to simulate a volatile fatty acid (VFA) rich substrate after CM acetogenic fermentation. The effect of different C/N ratios and light conditions (heterotrophy and mixotrophy) on the biomass productivity and biochemical composition as a potential feed supplement was examined.

Materials & Methods

The extraction of proteins from CM, collected from a poultry farm in Megara region (Attika), was carried out for 24 h adding 250 g CM in 1 L of 0.1 M NaOH.

The properties of CM filtrate were: 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 O₂/L, 528.1 ± 5.6 mg/L NH₄-N, 7.7 ± 0.2 mg/L NO₃-N, 13.4 ± 0.2 mg/L PO₄-P, 20.6 ± 0.2 g/L total solids, EC = 11.68 mS/cm and pH= 9.55.

Biomass growth and biochemical composition was examined at the following C/N ratios (w/w): 10/1, 10/2, 10/4 and 10/6 expressed as total VFAs to extracted CM proteins. The experiment was carried out using 200 mL sterile CM medium and 20 mL inoculum under aseptic conditions, LED panel illumination (5000 lux), photoperiod of 16:8 h, temperature 26 ± 2 °C. The cultures were agitated with filtered-sterilized air (0.2 L/min).

Results & Discussion

- ❖ Final percentage removal of proteins from the growth medium (CM extract) amounted to 22-41% (Fig. 1a).
- ❖ Each culture was harvested after achieving a VFA removal > 90% (Fig. 1b).
- ❖ The lowest C/N ratio 10/6 prolonged the culture duration (10-11 d) due to a slower VFA removal (Fig. 1b).
- ❖ The net optical density ($OD_{Total} - OD_{supernatantCM}$) of biomass was observed to increase upon time reaching a plateau at the end of each experimental run.
- ❖ Based on dry weight, the highest biomass concentration was attained in the mixotrophic (1.78 g/L) and heterotrophic (1.34 g/L) cultures at ratio 10/6, while the lowest in the mixotrophic (0.74 g/L) and heterotrophic (0.86 g/L) cultures at ratio 10/2 (Fig. 1c).

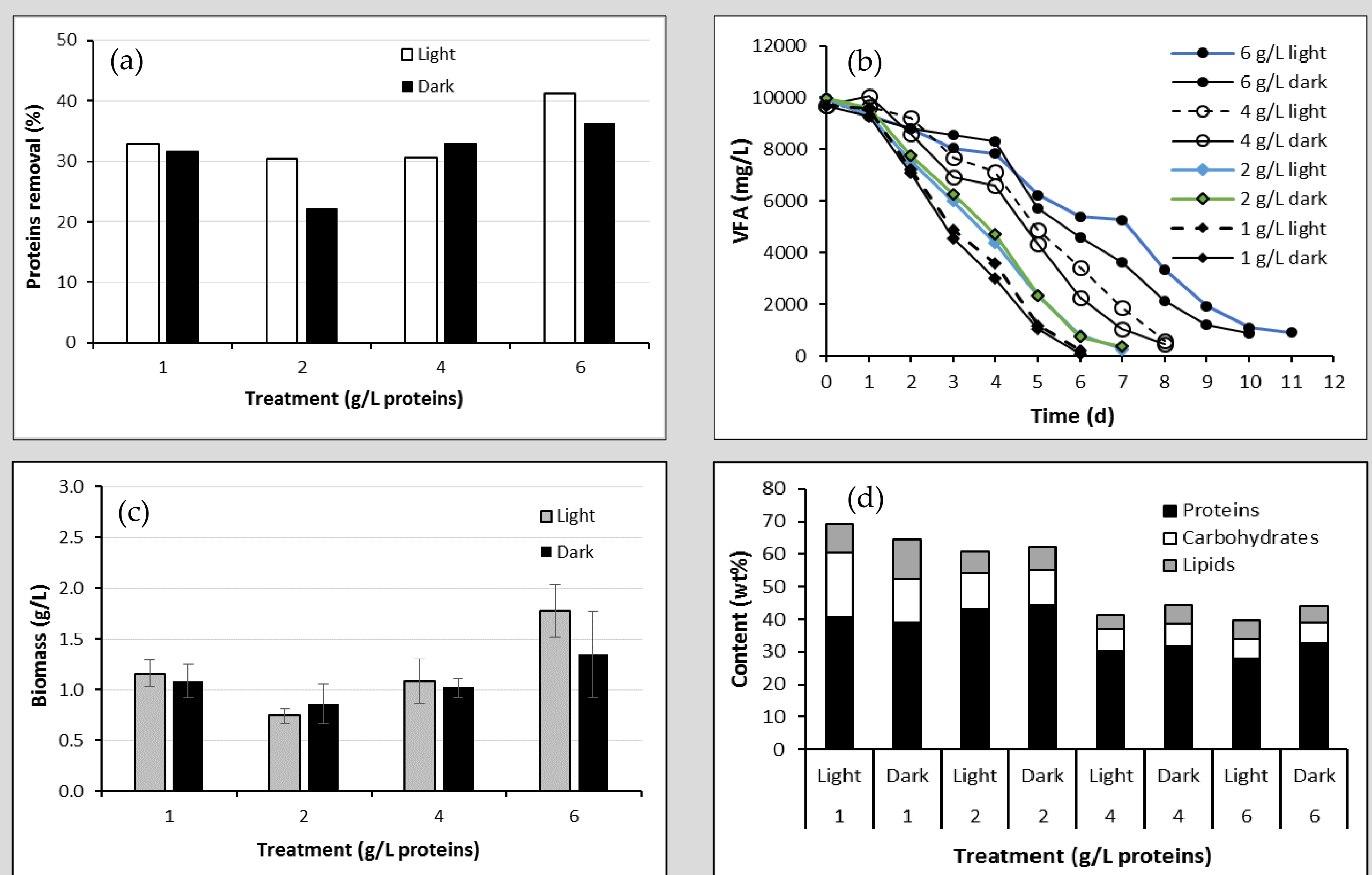


Figure 1: (a) Removal of proteins from the CM extract at the end of each culture, (b) Change of VFA concentration during the cultures, (c) biomass yield and (d) biochemical composition of *A. protothecoides* cultivated in CM extract at different C/N ratios.

These results indicate that *A. protothecoides* was capable to proliferate in the CM substrate, which is consistent with a previous study reported an efficient culture of marine microalgae under regulated C/N ratios (Li et al., 2020).

Proteins were found to be the major substance (28.1-44.2% w/w) in the dry biomass followed by carbohydrates (5.9-19.5%) and lipids (4.4-11.9%) (Fig. 1d).

The highest protein content was observed at ratios 10/2 (42.9-44.2%) and 10/1 (38.8-40.8%), while the lowest at ratio 10/6 and 10/4 under mixotrophic conditions (28.1% and 30.3%, respectively). At C/N ratio 10/1, carbohydrate and lipid concentrations were higher compared to the other ratios (Fig. 1d). In accordance to that, nitrogen-restricted cultures have been demonstrated to trigger lipid and carbohydrate deposition (Guihéneuf & Stengel, 2015).

Conclusions

Alkaline extract of CM supplemented with sodium acetate (simulating a VFA enrichment) was used as a source of proteins, organic carbon and inorganic nutrients for *A. protothecoides* cultivation. The results indicate that the used medium stimulated the microalgal growth and accumulation of proteins, while at high C/N ratio (10/1) carbohydrates and lipids were promoted.

References

- Chalima, A., Oliver, L., Fernández de Castro, L., Karnaouri, A., Dietrich, T., Topakas, E. 2017. Utilization of volatile fatty acids from microalgae for the production of high added value compounds. *Fermentation*, 3(4), 54.
- Guihéneuf, F., Stengel, D.B. 2015. Towards the biorefinery concept: Interaction of light, temperature and nitrogen for optimizing the co-production of high-value compounds in *Porphyridium purpureum*. *Algal research*, 10, 152-163.
- Li, S., Ji, L., Chen, C., Zhao, S., Sun, M., Gao, Z., Wu, H., Fan, J. 2020. Efficient accumulation of high-value bioactive substances by carbon to nitrogen ratio regulation in marine microalgae *Porphyridium purpureum*. *Bioresource technology*, 309, 123362.
- Yin, D.-m., Mahboubi, A., Wainaina, S., Qiao, W., Taherzadeh, M.J. 2021. The effect of mono-and multiple fermentation parameters on volatile fatty acids (VFAs) production from chicken manure via anaerobic digestion. *Bioresource Technology*, 330, 124992.

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