

Utilization of poultry manure wastewater by mixotrophic cultivation of microalga *Auxenochlorella protothecoides*

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Objectives

Auxenochlorella protothecoides was cultivated in poultry manure extract supplemented with sodium acetate in order to simulate a volatile fatty acid (VFA) rich substrate after PM **thermophilic anaerobic fermentation**. Valorization of the agricultural wastes or byproducts into added-value products would prevent aquifer depletion and promote **circular bioeconomy**.

The aim was to evaluate the effect of **different VFA concentrations (0-30g/L)** under mixotrophic conditions on biomass productivity, nutrient removal efficiency as well as on biochemical composition.

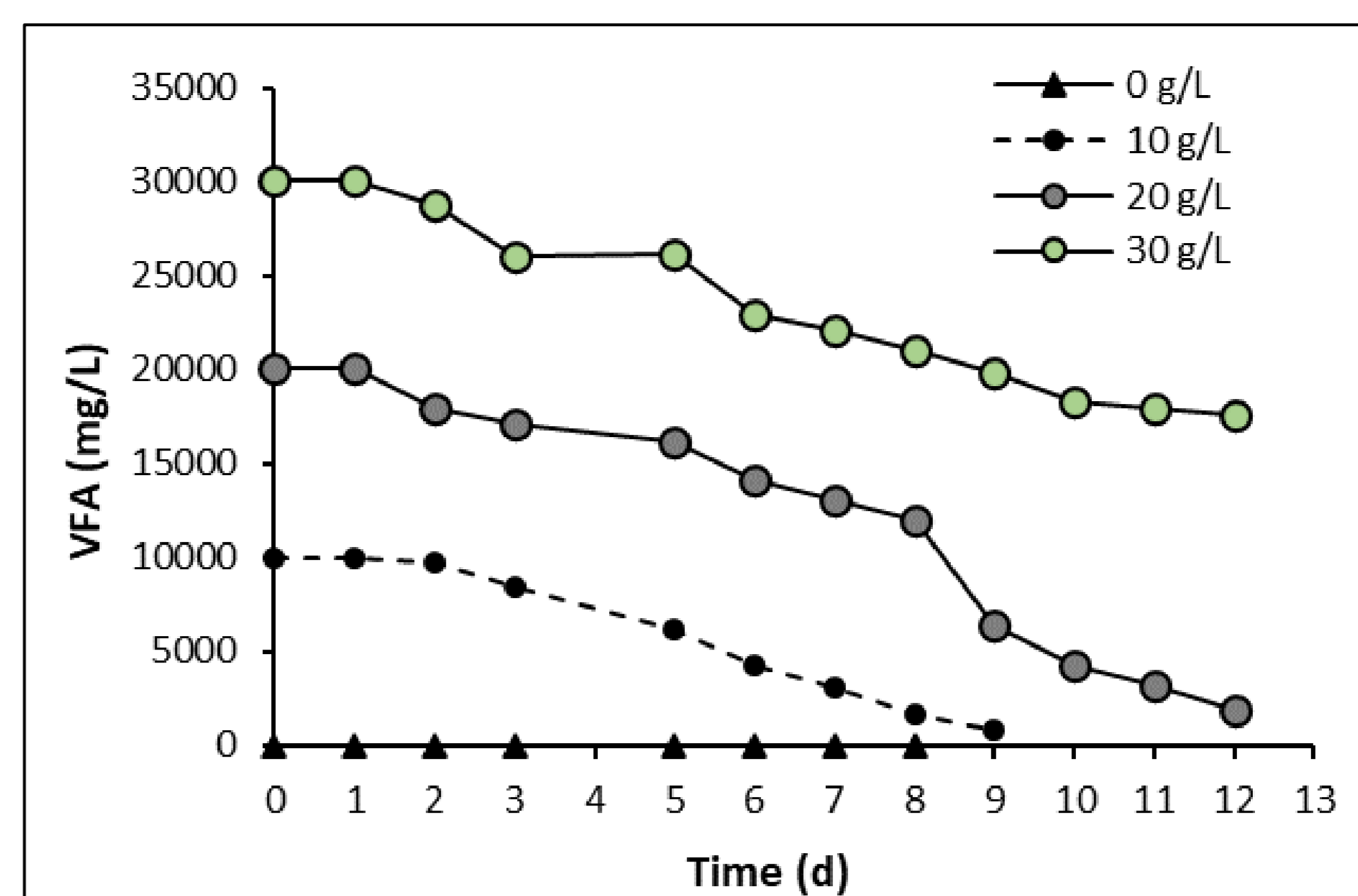


Figure 1. VFA removal efficiency during the cultures of *A. protothecoides* at different VFA concentrations.

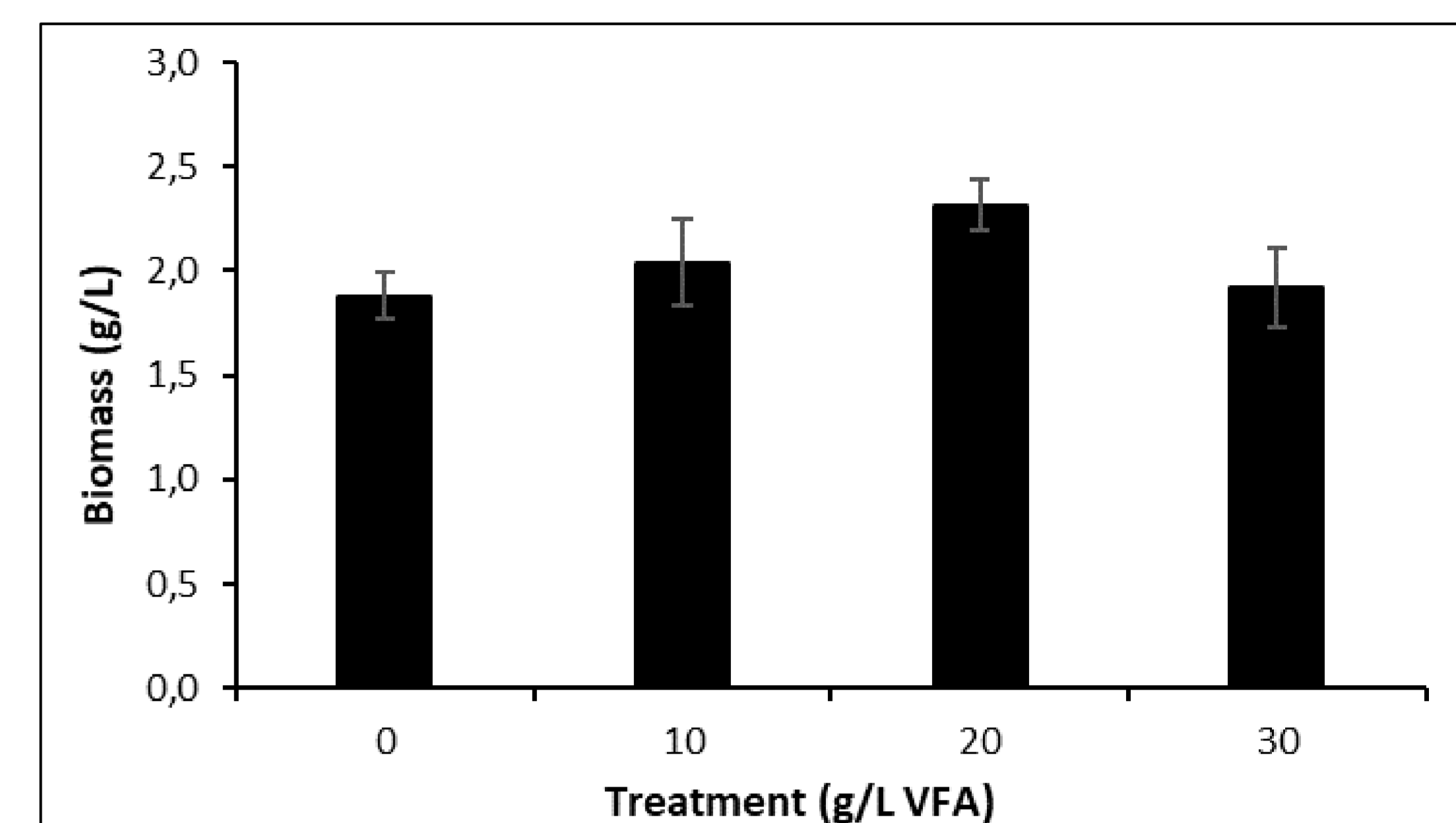
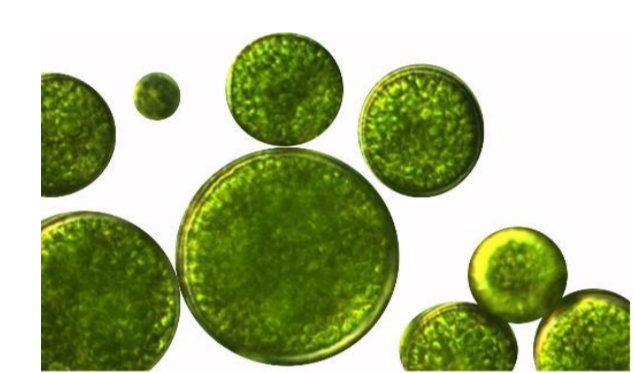


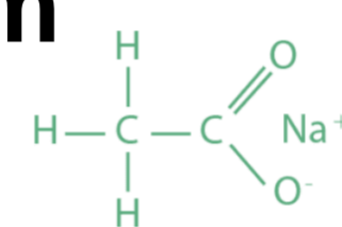
Figure 2. Biomass yield of *A. protothecoides* cultivated in PM extract at different VFA concentrations.

Methods



i. *A. protothecoides* was provided by the Culture Collection of Algae and Protozoa SAMS Limited Scottish Marine Institute.

ii. PM growth medium dilution to achieve 6 g/L proteins and enrichment with **0, 10, 20 or 30 g/L sodium acetate** as equivalent acetic acid.



iii. Use of 200 mL sterile PM medium and 20 mL *A. protothecoides* inoculum in 500 mL Duran flasks.

iv. Each culture was harvested after achieving VFA removal rates higher than 90%.



v. Biomass dry weight, Proteins, Carbohydrates, Lipids.

Cultivation conditions

LED panel illumination 5000 lux, Photoperiod 16:8 h, Temperature 26 ± 2 °C, Filtered sterilized air 0.2 L/min, aseptic conditions.

Results

❖ High VFA concentration (30 g/L) inhibit removal efficiency which plateaued after day 9 (Figure 1).

❖ Based on dry weight, the maximum **biomass concentration** was reached at 20 g/L VFA (2.31 g/L), while the lowest at the 0 g/L (1.88 g/L) (Figure 2).

❖ **Proteins** were the most abundant component accounting for 39.1-44.8% w/w in the dry biomass, while **carbohydrates** and **lipids** followed, comprising 32.6-37.4% and 10.2-13.1%, respectively (Figure 1c).

❖ Protein, carbohydrate and lipid productivity was enhanced by the addition of VFA compared to the control (0 g/L), achieving the highest values 44.8%, 37.4%, 13.1% respectively at **20 g/L VFA** (Figure 3).

Conclusions

➤ Poultry manure VFA-enriched wastewater 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.

➤ Microalgae cultivation in VFA-rich PM extract has potential to be used for simultaneous waste nutrient removal and biomass production due to its capacity of efficient nutrient removal, coupled with its robust growth rate and productivity.

➤ Hence, the utilization of poultry manure wastewater as a low-cost and sustainable resource for microalgal cultivation holds potential implications for the development of high-value bioproducts, including animal feed supplements derived from microalgae.

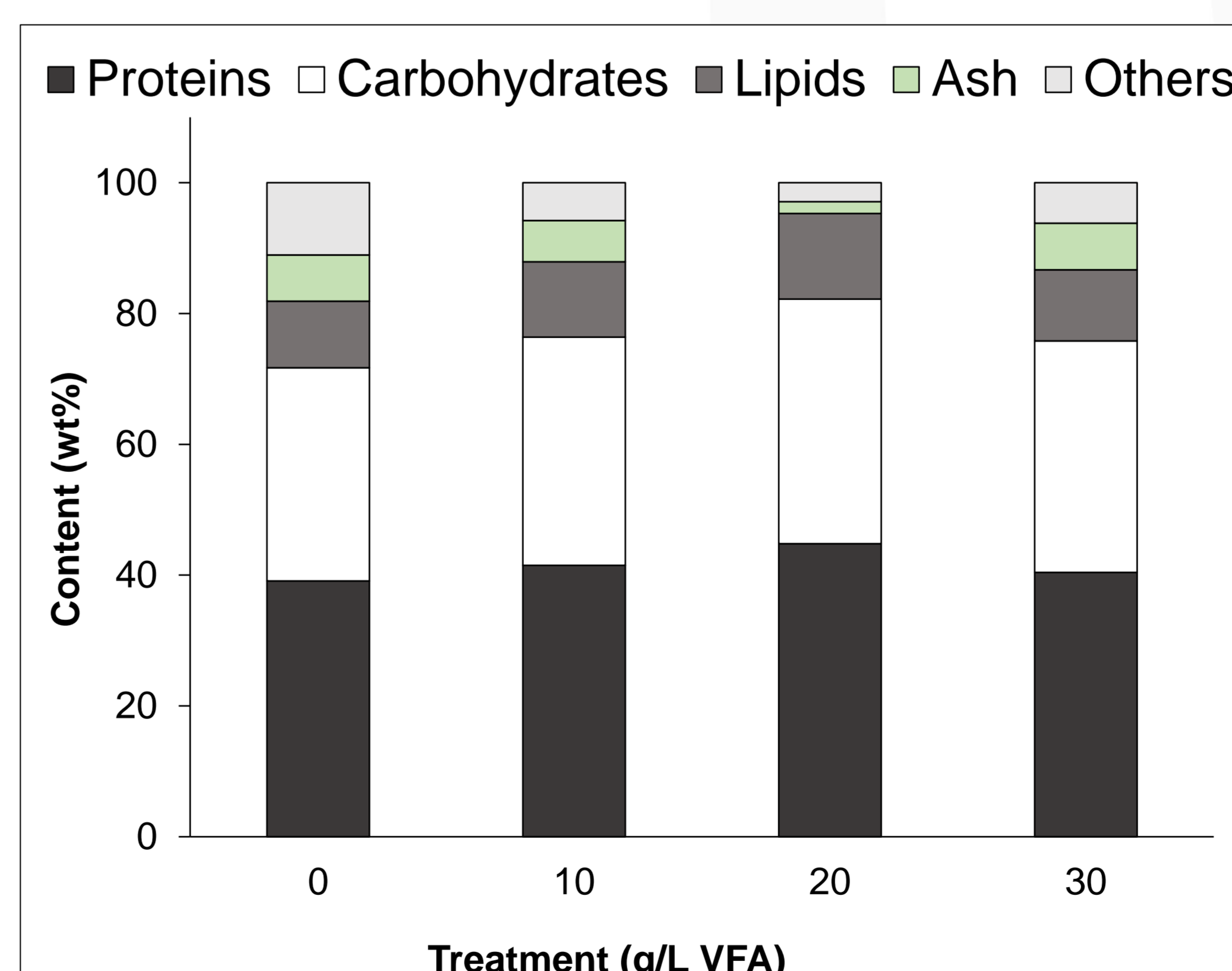


Figure 3. Biochemical composition of *A. protothecoides* cultivated in PM extract at different VFA concentrations.

Parameter	PM Growth medium
pH	9.30
EC (ms/cm)	10.28
Proteins	6007.1 ± 128.7
COD (g O ₂ /L)	25.5 ± 2.1
Total solids (w/w%)	21.6 ± 0.3
VFA (mg/L)	5003.1 ± 128.0
NH ₄ -N (mg/L)	488.1 ± 5.1
PO ₄ -P (mg/L)	12.4 ± 0.2