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ABSTRACT

Phycoremediation of swine wastewaters has been widely reported as an attractive tertiary treatment system, that effectively removes the excessive nutrient loads whilst offering a valuable source of feedstock biomass. Digestate from an upflow anaerobic sludge blanket (UASB, 6%v/v) and a nitrification reactor (NR; 50% v/v) were used as culturing media to microalgae. Experiments were carried out in lab scale photobioreactors (PBRs) using a consortia of *Chlorella* and *Scenedesmus*. Ammonia (44 to 90%) and phosphorus (77%) were efficiently removed from both effluents tested after 4 days. Microalgae biomass harvested from the UASB effluent showed 57, 34 and 1% of proteins, carbohydrates and lipids, respectively. Comparatively, the cellular composition of microalgae grown on NR effluent had lower protein (43%) but higher carbohydrate (42%) contents. Negligible difference in lipid fraction was observed independently of the effluents tested. The results suggest that the biomass harvested from phycoremediation of swine wastewaters can offer a valuable protein and carbohydrate feedstock for nutritional and biotechnological applications.

Keywords: Carbohydrate, lipid, microalgae, protein, phycoremediation

INTRODUCTION

Phycoremediation is the use of microalgae for the tertiary treatment or polishing stage of nutrients removal (N and P) from wastewaters. It offers advantages due to production of valuable microalgae biomass during the treatment process which can be used for animal nutrition, biofuels or other biotechnological applications. Microalgae can accumulate considerable quantities of carbohydrates, proteins and/or lipids (SUN et al., 2014). Microalgae proteins are rich in lysine, which is an essential nutritional supplement for feeding purposes (QUEVEDO et al., 2008; SAFI et al., 2014). Biomass can also be converted into biofuels, such as biogas through anaerobic digestion (PERAZZOLI et al., 2013), biodiesel from lipid transesterification and bioethanol/biohydrogen from fermentation processes (SCRAGG et al., 2003; CRAGGS et al., 2011). The cellular composition of the microalgae, however, can vary largely depending on the culturing media. Thus, determining how the culturing media affects microalgae composition is important because the amount of protein, carbohydrate and/ or lipids produced within cell dictates the rationality behind the feasibility usefulness of the biomass. Therefore, the objectives of this study were to evaluate the potential of algae-based treatment on the removal of ammonia and

phosphorus from swine digestate and to characterize the biochemical compositions of the resulting microalgae biomass.

MATERIAL AND METHODS

A microalgae consortium was obtained from a facultative pond at Embrapa Swine and Poultry wastewater facility. Lab scale 9-L glass reactors (20 cm Ø) were used as photobioreactors (PBR). PBR were kept at room temperature (23°C) and exposed to 40 W fluorescent lamps ($44.8 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) under mixotrophic conditions. Each PBR was prepared with 30% (v/v) inoculum and either 6% v/v UASB or 50% v/v NR diluted effluents. Media was continuously stirred with aquarium pumps (Sarlobetter). Samples were taken over time for ammonia and phosphorus (APHA, 2012) analysis. For determination of cellular composition, microalgae were harvested by physical-chemical coagulation and flocculation process (MEZZARI et al., 2014). The cellular lipid fraction was determined by the ethereal extract (EE) method (AOCS, 2009). Protein content was analyzed by the combustion method (AOAC, 1995). Ash content (CZ) was determined according to the method of BCAA n^o. 36 (2009). Carbohydrate was determined the difference in lipid, protein and ash content (BI, et al., 2013).

RESULTS AND DISCUSSION

Phosphorus was efficiently removed from UASB and NR effluents tested after a period of 4 days of experiment. The removal rates were 90% and 77% for UASB and NR, respectively (Figure 1).

After 4 days, 44% and 77% of the ammonia was removed from the effluents from UASB and NR, respectively (Figures 2 and 3). Nitrite (NO_2^- -N) and nitrate (NO_3^- -N) concentrations increased over time which suggests that nitrification was occurring concomitantly to N assimilation by microalgae (Figure 3) (Mezzari et al., 2013).

Microalgae cellular composition can vary depending on changes in pH, light, temperature as well as variations in nutrients concentrations (BRENNAN, OWENDE; 2010). Microalgal biomass harvested from the UASB effluent showed 57, 34 and 1% of proteins, carbohydrates and lipids, respectively (Figure 4). The biochemical composition of microalgae grown on NR culturing media showed 43, 42 and 1% of proteins, carbohydrates and lipids, respectively (Figure 5). The increased concentration of nitrogen (NH_3 -N) in both effluents tested led to substantial production of proteins and carbohydrates and low production of lipids as discussed elsewhere (JIAN-MING, et al., 2010; HO, et al., 2013).

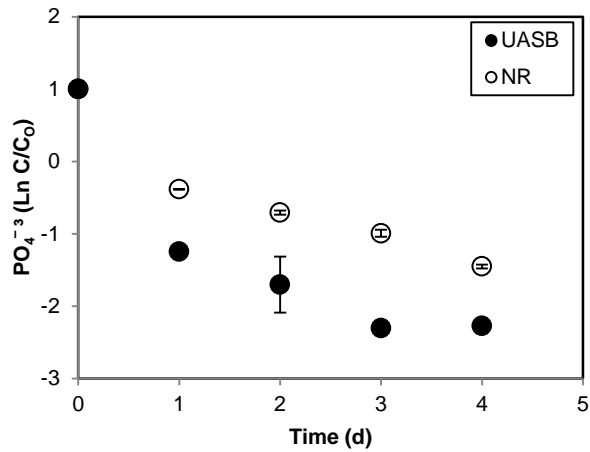


Figure 1. Phosphorus concentration profile over time. Initial PO_4^{3-} concentrations (C_0) were 10.3 and 11.5 mg. L^{-1} for UASB and NR, respectively.

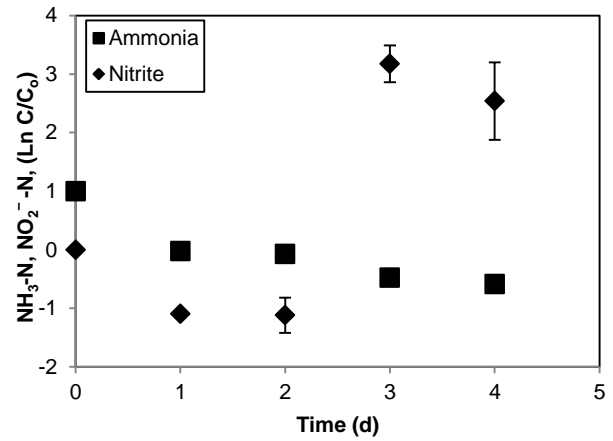


Figure 2. Ammonia concentration profile over time. Initial concentrations (C_0) were 45.4, 0.1 mg L^{-1} for $\text{NH}_3\text{-N}$ and $\text{NO}_2^-\text{-N}$ respectively.

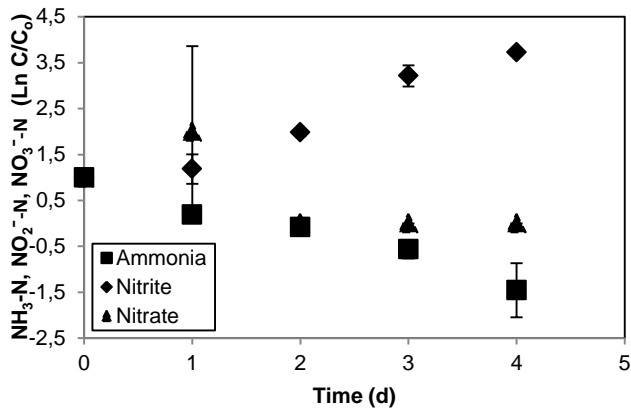


Figure 3. Ammonia concentration profile over time. Initial concentrations (C_0) were 32.8, 0.5 e 0.2 mg.L^{-1} for ammonia, nitrite e nitrate, respectively.

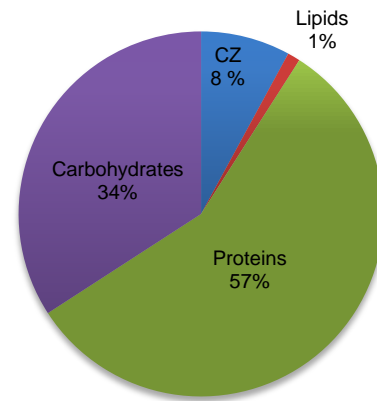


Figure 4. Biochemical constitution of microalgae grown in diluted (6% v/v) UASB effluent.

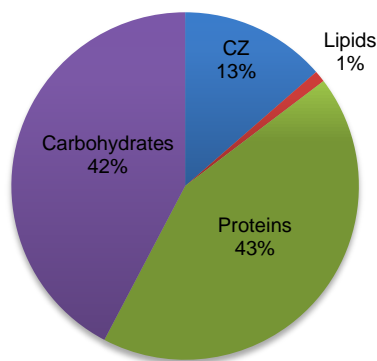


Figure 5. Biochemical constitution of microalgae grown in diluted (50% v/v) NR effluent.

CONCLUSIONS

The results demonstrated that ammonia (44 and 90%) and phosphorus (77%) removal from photobioreactors were satisfactory. The effects of N-rich effluents affected lipid accumulation within cells. This phenomena is particularly interesting to improve our current understanding of metabolic and physiological variations in microalgae as a result of changes in culturing media, specially from wastewater effluents. Microalgae biomass from tertiary treatment processes are rich in proteins ($\geq 43\%$) and carbohydrates (≥ 34) but low in lipid content. Thus, whereas biodiesel production from such biomass seems unreasonable, its use for animal food or bioethanol production may be interesting and should be further investigated.

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REFERENCES

- AOAC - Association of Official Analytical Chemists. Official methods of analysis of the Association of Official Analytical Chemists. 16th ed. 1995.
- AOCS - American Oil Chemists' Society. **Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction**. Official Procedure Am 5-04, 2009.
- APHA - Standard Methods for the Examination of Water and Wastewater, 22nd Ed, American Public Health Association/American Water Works Association/Water Environment Federation. Washington DC, USA, (2012).
- BCAA - Brazilian Compendium of Animal Nutrition. Method n^o 36. Ash or mineral matter, p. 137, 2009.
- BI, Z. et al. Characterization of microalgae for the purpose of biofuel production. **American Society of Agricultural and Biological Engineers**, v. 56, n. 4, p. 1529-1539, 2013.
- BILAD, M. R.; et al. Membrane technology in microalgae cultivation and harvesting: a review. **Biotechnology advances**, v. 32, n. 7, p. 1283-1300, 2014.
- HO, S. H., et al. (2013). Characterization and optimization of carbohydrate production from an indigenous microalga *Chlorella vulgaris* FSP-E. **Bioresource technology**, 135, 157-165.
- JIAN-MING L.V., et al. Enhanced lipid production of *Chlorella vulgaris* by adjustment of cultivation conditions. **Bioresource technology** v. 101, p. 6797-6804, 2010.
- KUHAD, R. C. et al. Microorganisms as an alternative source of protein. **Nutrition reviews**, v. 55, n. 3, p. 65-75, 1997.
- MEZZARI, M. P. et al. Assessment of N₂O emission from a photobioreactor treating ammonia-rich swine wastewater digestate. **Bioresource technology**, v. 149, p. 327-332, 2013.
- MEZZARI, M. P. et al. Assessment of a tannin-based organic polymer to harvest *Chlorella vulgaris* biomass from phycoremediation process treating swine wastewater digestate. **Water Science and Technology**, v. 70, n. 5, p. 888-894, 2014.
- PERAZZOLI, S. et al. Biogas production from microalgae biomass. III International Symposium on Agricultural and Agroindustrial Waste Management, 2013.
- QUEVEDO, C. O. et al, Growth *Scenedesmus sp in* different culture mediums for microalgal protein production. **Vitae**, v. 15, n. 1, p. 25-31, 2008.
- SAFI, C. et al. Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review. **Renewable and Sustainable Energy Reviews**, v. 35, p. 265–278, 2014.
- SCRAGG, A. H. et al. The use of a fuel containing *Chlorella vulgaris* in a diesel engine. **Enzyme and Microbial Technology**, v. 33, n. 7, p. 884-889, 2003.
- SUN, X., et al. Effect of nitrogen-starvation, light intensity and iron on triacylglyceride/carbohydrate production and fatty acid profile of *Neochloris oleoabundans* HK-129 by a two-stage process. **Bioresource technology**, 155, 204-212, 2014.