MICROALGAE OBTAINED FROM SWINE WASTEWATER TREATMENT AS SOURCE OF AMINOACIDS AND OMEGA-3

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ABSTRACT: The use of microalgae to remove nutrients from swine wastewater effluents (i.e. phycoremediation) has been extensively reported as effective tertiary treatment or polishing treatment to remove nutrients. One of the greatest advantages of this biological treatment is in the production of valuable microalgae biomass that can be considered as food supplement for animal nutrition. In this work, two different swine wastewater effluents [i.e., digestate from an Anaerobic Upflow Sludge Blanket (UASB), and effluent from an air-sparged nitrification-denitrification reactor] were tested for the growth of microalgae and the produced biomass compared for its protein, aminoacids and fatty acids contents. The biomass from both effluents tested had high amino acids content: arginine (2.6%), isoleucine (2.1%), histidine (1%), leucine (4.1%), lysine (2.5%), methionine (0.8%), phenylalanine (2.6%), threonine (2.4%), tryptophan (0.5%), valine (2.8%). Stress-induced conditions such as limiting the concentration of nutrients in microalgae culture medium contributed to the production of lipids and polyunsaturated fatty acids (5.3%, mainly omega-3) and unsaturated fatty acids (10 %). Overall, the results suggest that microalgae biomass obtained from the phycoremediation of swine wastewater that are rich in nutrients lead to production of a low cost microalgae biomass rich in aminoacids and fatty acids which are of paramount importance as supplemental diet for the maintenance of healthy animal growth.

Keywords: microalgae, wastewater, aminoacids, fatty acids, animal nutrition.

INTRODUCTION

The swine farming in Brazil plays an important role in the country's economy (ABPA, 2017). To warrant the growth of healthy animals, careful attention is constantly being placed on the right formulation of the animal diets offering adequate nutritional balance. Protein sources are usually expensive and its costs continue to increase considerably over the years. Therefore, it is wise to consider new alternative sources of proteins that are cheaper, abundant, and compatible with animal feed formulations. Microalgae are known to accumulate considerable quantities of proteins, lipids (SUN et al., 2014) and associated essential amino acids and omegas (LUM et al., 2013). Studies have shown that the use of microalgae in animal feed can replace up to 50% of protein in existing diets (HARUN, et al., 2010) and help to improve animal's immune system, intestinal function, appetite with weight gain (SATHASIVAM, et al., 2017). Microalgae biomass could be cheaply and promptly obtained from different types of reactors, including the ones built to treat wastewater effluents. Therefore, we hypothesize that it may be possible to combine the production of microalgae biomass rich in protein and lipids with swine wastewaters treatment (i.e., phycoremediation) (PRANDINI, et al, 2016). It is worth mentioning that variations in nutrient concentration present in different types of wastewater can affect the biochemical composition of the biomass (MICHELON, et al., 2015). Thus, associating the composition of wastewater with amino acids and or fatty acids contents in microalgae can guide us in the production of biomasses that better suit our expectations regarding to the minimum amount of protein or lipid contents from the biomass. Therefore, the objective of this study was to compare the amino acids and fatty acid concentrations in microalgae grown in two different types of swine wastewater effluents, i.e., digestate from anaerobic digester and effluent from a nitrification-denitrification reactor, as well as under nitrogen and phosphorus limited conditions

MATERIAL AND METHODS

Microalgae consortia were obtained from a facultative pond at EMBRAPA Swine and Poultry, Concórdia, Brazil. The inoculum was composed by microalgae consortium dominated by *Chlorella* spp. Experiments were conducted in 12-L glass photobioreactors (PBR) filled with non-sterile 6% v v^{-1} UASB digestate effluent diluted in water. PBRs were inoculated with 70 mg dry weight microalgae L^{-1} (or 30 % v v^{-1} of inoculum). PBRs were maintained at room temperature (23ºC) under mixotrophic conditions (44.8 µmol

m^{-2} s^{-1}) and continuous agitation. Experiments were also conducted using a pilot scale 500-L reactor placed inside a greenhouse under natural light 321.5 (± 411.4) µmol m^{-2} s^{-1} and temperature controled 25°C conditions. Culture medium in the reactor was kept under continuous agitation using a submersible aquarium pump. After 11 days following inoculation, the growth medium containing the microalgae biomass was harvested via centrifugation (EVODOS, T10, Netherlands). The harvested biomass was immediately frozen (-40 °C) and lyophilized (Model 030-JJ LJI Scientific) for further analyses. For evaluating the effects of nitrogen and phosphorus starvation, briefly, microalgae biomass was harvested, after cells were resuspended in fresh 500-L nutrient-free water. To verify the influence of phosphorus depletion, nutrient-free water was artificially amended with either 50 mg $N–NO_3$ L^{-1}. To avoid nutrient exacerbation during the tests, N was continuously monitored and replenished accordingly. The concentration of amino acid was determined according to Hagen et al. (1989) and White et al. (1986) using a HPLC (Shimadzu, LC-20A). The concentration of tryptophan was obtained according to Lucas and Sotelo (1980) using a spectrophotometer (Femto, 700 Plus). Lipids were analyzed as esters using a FID-equipped gas chromatography (Varian, CP-3800) as previously described (AOCS, 2013).

RESULTS AND DISCUSSION

The effects of two different wastewater effluents on the concentration of amino acids and fatty acids contents in microalgae were compared (Table 1). The obtained amino acids concentrations were compared to current amino acid concentrations required to feed swine at different stages of animal growth. The use of nutrient-rich digestate effluent led to proliferation of microalgae with the following concentrations of amino acids (as % of total protein content) arginine (2.6), isoleucine (2.1), histidine (1), leucine (4.1), lysine (2.5), methionine (0.8), phenylalanine (2.6), threonine (2.4), tryptophan (0.5), valine (2.8). The concentration of amino acids obtained surpassed the concentrations of amino acids typically utilized in the animal diet (NRC, 1998; ROSTAGNO, 2017). Among these amino acids, lysine deserves special attention considering its importance in pig nutrition where it can serve as building block for the production of proteins, peptides and non-peptide molecules. A deficiency in lysine impairs the immunity system and increase the susceptibility of infectious diseases in animals (MORALES et al., 2015). After lysine, threonine is the second most important amino acids to be considered. This latter amino acid is involved in the adequate function of digestive and immune systems (WANG et al., 2006). Microalgae grown in the digestate (effluent of UASB) also showed high concentrations of tryptophan (0.5%, Table 1), an amino acid that plays a major role in the synthesis of proteins, in the weight gain and in feed efficiency (PASTUSZEWSKA et al., 2007). A noticeable higher concentration of threonine (2.4%) was observed in microalgae grown in wastewater under limited concentration of phosphorus as nutrient for microalgae (Table 1).

The lipid and fatty acid contents increased in microalgae grown in N and P limited conditions (Table 5). The increase in lipid content was mainly associated with the accumulation of monounsaturated fatty acids (MUFAs, by up to 4.7%), polyunsaturated (PUFAs, by up to 5.2%) and unsaturated fatty acid (UFAs, by up to 10%). Among all fatty acids studied here, the omega-3 polyunsaturated deserves especial attention since its use in animal feed was reported to decrease serum levels of triglycerides during swine gestation, improving piglet birth weights (POSSER et al., 2018).

CONCLUSIONS

The importance of this research is three fold. First, phycoremediation, which is recognized as an efficient polishing step for swine wastewater treatment, can generate high yields of valuable microalgae biomass. Second, the produced microalgae is attractive as a feedstock rich in amino acids and fatty acids that can be utilized as supplement in animals diets. Third, the concentrations of amino acids and/or fatty acids in the biomass can be adjusted during microalgae growth by controlling nitrogen and phosphorus present in the wastewater as culturing medium. Therefore, we have total flexibility to produce a biomass that provides protein or lipid contents that best suit the nutritional demands. Overall, these results served to improve our current understanding of microalgae biochemical changes as a functional of the growth conditions and how these changes ultimately affect the production of amino acids and fatty acids that can be further explored for supplementation of animal diets.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support provide by CAPES Foundation, Ministry of Education of Brazil and SISTRATES-BNDES (Project No 23.17.00.023.00.00).

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Table 1. Amino acids concentration profile present in the microalgae biomass. Data shown as percentage of the total protein content.

Amino Acids (%)	UASB	NR	P Limited	N and P limited	[Starting	Growing	Finishing]*
Aspartic acid	4.71	3.02	4.01	1.52	-	-	-
Glutamic acid	5.01	3.24	4.91	1.99	-	-	-
Serine	1.88	1.36	2.07	0.83	-	-	-
Glycine	2.73	2.10	2.87	1.10	-	-	-
Histidine	0.99	0.60	0.57	0.27	0.47	0.38	0.27
Arginine	2.58	1.96	2.59	0.97	0.64	0.47	0.32
Threonine	1.99	1.53	2.37	1.04	0.99	0.80	0.58
Alanine	3.28	2.59	4.00	1.69	-	-	-
Prolina	1.85	1.40	2.42	0.96	-	-	-
Tyrosine	1.42	1.04	1.30	0.56	-	-	-
Valine	2.66	1.85	2.82	1.07	1.02	0.82	0.59
Methionine	0.82	0.43	0.67	0.31	0.41	0.34	0.26
Cysteine	0.70	0.37	0.68	0.27	-	-	-
Isoleucine	1.73	1.41	2.06	0.80	0.80	0.65	0.47
Leucine	3.51	2.55	4.15	1.68	1.41	1.14	0.82
Phenylalanine	2.12	1.48	2.57	0.90	0.71	0.58	0.42
Lysine	2.52	1.29	2.29	0.92	1.46	1.17	0.85
Tryptophan	0.49	0.23	0.33	0.21	0.28	0.24	0.17

* Nutritional requirements of whole male swine of high genetic potential (NRC, 1998; Rostagno, 2017).

Table 2. Fatty acids concentration profile present in the microalgae biomass. Data shown as percentage of the total lipid.

Fatty acids (%)	UASB	NR	P limited	N and P limited
Myristic Acid (C14:0)	0.01	0.01	0.02	0.09
Myristoyl Acid (C14:1)	0.04	0.02	0	0.01
Pentadecanoic Acid (C15:0)	0.01	0.01	0.01	0.02
Palmitic Acid (C16:0)	0.23	0.33	0.84	5.27
Palmitoleic Acid (C16:1n7)	0.16	0.04	0.01	0.04
Margaric Acid (C17:0)	0.01	0.01	0.02	0.05
Estearic Acid (C18:0)	0.03	0.05	0.03	0.44
Oleic Acid (C18:1n9c)	0.08	0.14	0.2	4.63
Linoleic Acid (C18:2n6c)	0.13	0.2	0.16	1.32
Linolenic Acid (C18:3n6)	0.01	0.02	0.02	0.05
Linolenic Acid (C18:3n3)	0.47	0.48	1.03	3.87
Arachic acid (C20:0)	-	-	-	0.02
Eicosatrienoic acid (20:3)	-	-	0.01	0.02
Behenic Acid (C22:0)	-	0.01	0.01	0.04
Erucic Acid (C22:1n9)	-	-	-	0.01
Eicosapentaenoic acid (C20:5n3)	-	-	-	0.01
Lignoceric Acid (C24:0)	-	-	0.01	0.01
Σ MUFAs	0.29	0.2	0.23	4.75
Σ PUFAs	0.63	0.71	1.22	5.27
Σ UFAs	0.92	0.91	1.45	10.03
Σ SFAs	0.31	0.42	0.95	5.96
Σ ω-3	0.48	0.49	1.04	3.9
Σ ω-6	0.15	0.22	0.18	1.37
Σ ω-9	0.09	0.14	0.21	4.7

Monounsaturated (MUFAs), polyunsaturated (PUFAs), unsaturated (UFAs) and saturated (SFA) fatty acid.