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Solids reduction and microbial protein measurement in biodigester used in cassava wastewater treatment

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Resumo: O trabalho teve por objetivo avaliar a eficiência de remoção de sólidos e quantificar a proteína microbiana em biomassa residual proveniente de um biodigestor utilizado no tratamento de efluente de mandioca. Avaliou-se o efluente na entrada e na saída para determinar sua eficiência e apenas o da saída do biodigestor para a quantificação de proteína microbiana. O teor proteico total na biomassa seca foi determinado pelo método de Kjeldahl, adotando-se o fator de 6,25 para a conversão a partir dos teores de nitrogênio total. Determinou-se a massa seca e quantificou-se o teor de proteína presente na mesma. Houve remoção de turbidez, sólidos totais e sólidos voláteis de 73,97, 64,37 e 76,04%, respectivamente. A massa seca média foi de 2,1 $kg.m^3$, com cerca de 54,73% de proteína bruta. Considerando a vazão de efluentes média da amidonaria de 90 $m^3.h^{-1}$, estima-se uma produção de proteína bruta média de 124 $kg.h^{-1}$. Com os resultados obtidos, pode-se visar o aperfeiçoamento e otimização do processo para produção de proteína unicelular com qualidade alimentícia.

Palavras-chave: Biomassa residual, sólidos voláteis, teor proteico.

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Abstract: This study aimed at evaluating the efficiency of solids removal and quantifying microbial protein in residual biomass from a digester used to treat cassava effluent. The effluent was evaluated in the inlet and outlet to determine its effectiveness, but, in order to measure microbial protein, only the digester output was used. The total protein content in dry biomass was determined by Kjeldahl method, adopting the factor of 6.25 for the conversion from the total nitrogen. Was determined dry weight and quantified the protein content present in the same. Turbidity, total solids and volatile solids were removed in 73.97, 64.37 and 76.04%, respectively. The average dry weight was 2.1 kg m^3 with almost 54.73% crude protein. The average effluent flow of cassava was considered as 90 m^3 h^{-1}; thus, average crude protein production was estimated in 124 kg h^{-1}. According to the obtained results, the improvement and optimization of the process are aimed to produce a single-cell protein with food quality.

Keywords: Residual biomass, volatile solids, protein content.

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1. Introduction

Extraction of cassava starch aims to acquire a product with high purity, which must have low concentrations of proteins, lipids, ash and fiber. The optimization of the extraction process provides increased in the manufacturing and generates a residue with a high content of fibers, which can be exploited as a source of dietary fiber. The extraction of cassava starch consists of the following steps: washing, disintegration, fiber separation from soluble material and drying. During this process, there is a great generation of effluent, which is composed of water from raw material and the residual processed water, with high organic load ((Leonel *et al*., 2001).

Anaerobic digestion is a biological process in which the absence of oxygen, facultative or strictly anaerobic bacteria degrade complex organic compounds, which undergo a series of sequential oxidative processes that are converted to methane, carbon dioxide and other mineralized by-products (Bassin and Dezotti *et al*., 2008). The physicochemical parameters from medium and environment are determining factors in microbial growth. The total solids comprise organic and inorganic substances, which are dissolved and suspended in the liquid (Sampaio *et al*., 2007).

Volatile solids determine the organic compounds fraction while fixed solids indicate mineral solids content (Diesel *et al*., 2002). Kuczman *et al*. (2007) stated that the highest concentration of volatile solids will lead to greater production of biogas. And after anaerobic digestion, the effluent that comes out reactor also drags portions of biomass with important nutrients, such as microbial protein that has been highlighted since it is an unconventional alternative protein source.

Many agroindustrial by-products can be used as substrates for a single-cell protein production. They are easy to be acquired and an alternative source of low commercial value such as wooden processing effluent (Reffatti *et al*. 2007), cassava processing (Lupatini *et al*. 2013) and industrial waste of fruits (Albuquerque, 2003). Albuquerque (2003) points out that the combination of wastewater treatment and protein production has been a solution for industries that requires alternatives to reduce the cost of treating its residues.

The production of a single-cell protein from industrial wastes ejectment reaches its most profitable levels because their raw materials are cheaper and different, especially, if they can be used in the same place where they are produced, therefore, there is a great saving with transportation costs. Thus, according to this context, this study aimed at evaluating the efficiency of solids removal and microbial protein rate in residual biomass from an anaerobic digester used to treat an effluent from cassava.

2. Material and methods

The evaluated effluent came from an anaerobic digester, with continuous process, tubular model and 19000 m^3 averaged volume. It was used as part of the effluent treatment from a producing starch industry in Western Paraná. The hydraulic retention time (HRT) of the anaerobic digester is

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10.5 days, whereas the daily flow of wastewater industry is about 1800 m^3. The collections were carried out from May to July 2013, every other day at the digester inlet and outlet. There were 30 samples (total) for each collection point.

After each collection, the samples were identified, stored and carried to the laboratory for analyses of pH, electrical conductivity (EC) and turbidity. While the samples were kept refrigerated at 4 °C for a maximum period of 7 days to determine total solids (TS), fixed solids (FS) and volatile solids (VS) (APHA, 2005). The samples were acidified with sulfuric acid to pH less than 2 and kept under refrigeration at 4 °C for a maximum period of 28 days to determine total nitrogen and its subsequent conversion to crude protein (CP) (APHA, 2005). The analyses were carried out at the Laboratory of Analytical Chemistry and Environmental Analyses, at Federal Univesity of Paraná (UFPR) – Palotina – PR, Brazil.

In order to separate biomass and determine dry weight, 50 ml aliquots were homogenized and centrifuged at 3400 rpm for 10 minutes. The supernatant was discarded and the biomass was dried in a circulating oven and air renewal at 55 °C (in order to do not cause protein denaturation) until constant weight (Pelizer, 1999). This analysis was obtained in triplicate and subsequently evaluated protein content. The total protein content in dry biomass was determined by Kjeldahl method (APHA, 2005). The 6.25 factor was adopted to convert it up from total nitrogen contents (IAL, 2008). Equation 1 shows the percentage calculus of protein in dry biomass from the total nitrogen.

$$TP(\%) = TN(\%) \times f \qquad (1)$$

were,
TP: total protein
TN: total nitrogen
f: conversion factor (6.25)

3. Results and discussion

Based on the descriptive analysis data (Table 1), there was greater amplitude in the input data for all parameters. Moreover, it is observed that the input parameters showed a high coefficient of variation that exceeded the values found in the output parameters. The water electrical conductivity depends on the amount of dissolved salts and it was nearly ratable to its quantity (de Pádua and Ferreira, 2006). The electrical conductivity showed an averaged value of 2675 μS cm^{-1} in the input and 2895 μS cm^{-1} in output. Probably, there was an increase on dissolved salt amount during the anaerobic digestion process, such as sodium and potassium.

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Table 1. Descriptive analyses of pH, EC, turbidity, total, fixed and volatile solids in the inlet and outlet of a biodigester

Parameter	N	Average	S.D.*	C.V.** (%)	Minimum	Maximum
Inlet						
pH	30	5.94	0.50	8.41	5.28	7.23
EC (µS cm^{-1})	30	2675.14	1981.80	74.08	6.43	8350.00
Turbidity (UNT)	30	2190.40	1131.82	51.57	73.00	4550.00
TS (mg L^{-1})	30	8682.58	3599.45	41.46	297.50	13487.50
FS (mg L^{-1})	30	2082.63	1314.33	63.11	90.00	5285.00
VS (mg L^{-1})	30	6595.82	2856.77	43.31	207.50	9958.00
Outlet						
pH	30	6.13	0.25	4.02	5.59	6.75
EC (µS cm^{-1})	30	2895.37	1025.99	35.44	1442.00	6220.00
Turbidity (UNT)	30	532.97	195.49	36.68	161.50	935.00
TS (mg L^{-1})	30	3337.63	570.29	17.09	2108.00	4462.50
FS (mg L^{-1})	30	1652.28	396.07	23.97	1046.00	2282.50
VS (mg L^{-1})	30	1685.35	506.91	30.08	527.50	2266.00

*S.D – standard desviation **C.V – coefficient of variation

At the input, it was obtained 5.94 pH and for output, the answer was 6.13. The pH value and stability in the reactor are extremely important because it can be developed a high rate of methanogenesis only when pH remains in a narrow range, although it can be obtained methane when pH ranges from 6.0 to 8.0. However, values below 6.0 and above 8.3 should be avoided since they can completely inhibit the forming methane bacteria activity (Chernicharo, 1997). Turbidity showed average values of 2190 TNU in the input and 532 TNU in the output, with a considerable reduction. The days without production were excluded, since there was no representative flow rate, so, it is concluded that there was a 73.97% average removal and 11% standard deviation, with minimum and maximum values of 42.28 and 88.34%, respectively.

Regarding the process efficiency, average removals of total and volatile solids were obtained as 64.37 and 76.04%, respectively. Since volatile solids represent the organic fraction, that is, the biodegradable solids matter. Almost 75% total solids in the input part consist of volatile solids. Although in the output, the average value is about 50% volatile solids on total solids. Concerning concentration, there was an average removal of 6463 mg L^{-1} total solids and 5762 mg L^{-1} volatile solids. Torres (2009) evaluated the performance of a system without phase separation and also composed of anaerobic reactors with different support means. So, based on the organic loads evaluation, total and volatile solids removals were higher than 79.1 and 89.6%, respectively.

Volatile solids are the most important substrates for methanogenic bacteria. Thus, volatile solids are directly responsible for biogas production. Hence, the greater volatile solids concentration on daily diet of a digester is, the greater the capacity of a biogas digester will be (Sanchez et al.,

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2005). Since it is a real-scale digester, with uniformity in relation to its structure, concentration and flow rate of effluent due to the different operations that occurred during its industry processing, and does not have control in relation to external factors such as temperature, it can be stated that there was some efficiency on total and volatile solids removal, with higher answers when compared to other authors data, who were mentioned above, with reactors at laboratory or pilot scale.

The samples were centrifuged and dried in order to get dry biomass values (Figure 1). Samples 23 and 24 did not show representative dry mass for calculation. An intense rainfall was an important factor that was observed some days before and may have interfered in these answers, associated to the fact that that there was no industrial processing of cassava.

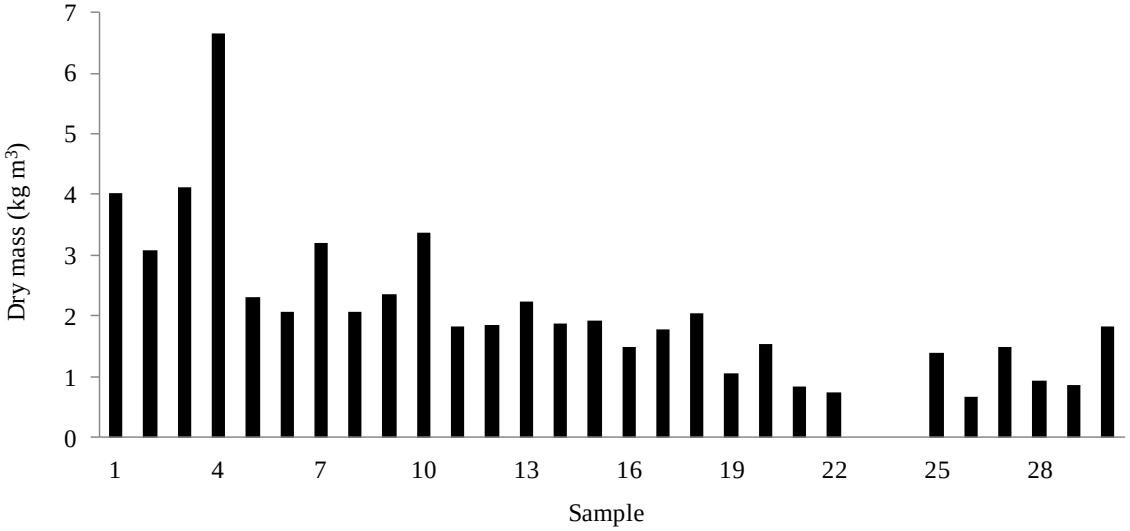

Figure 1. Dry weight from centrifugation and samples drying

The dry mass average was 2.1 kg m^3, with 0.67 kg m^3 as minimum value and 6.66 kg m^3 as the maximum one with 59.76% CV. Lupatini et al. (2013) used two reactors with different support means and different pH in maintenances. These authors obtained the following dry weight averages for Reactor 1 and Reactor 2: 0.249 kg m^3 and 0.629 kg m^3, respectively. The protein determination was obtained in two phases from 15 samples each (Figure 2). The second phase data were affected due to operational problems on important equipment during the sample digestion phase, so, these data were not used. From the total nitrogen content for samples 1-15 were determined crude protein (Figure 3).

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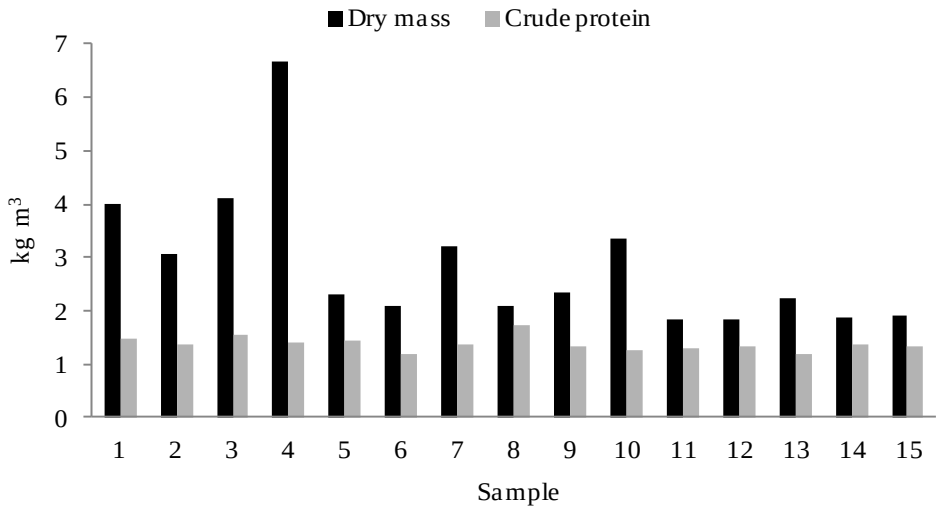

Figure 2. Comparison of dry matter and crude protein concentrations

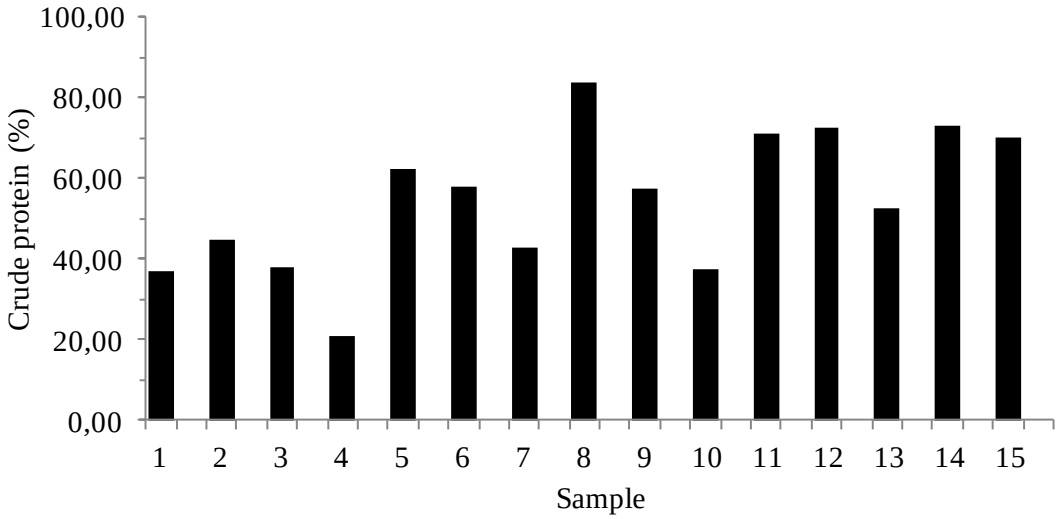

Figure 3. Crude protein content determined from dry biomass

The average crude protein content, from the obtained dry mass, is 54.73% with 17.68% standard deviation and 32.30% coefficient of variation, whose minimum and maximum values were 20.91% and 83.89%, respectively. Based on the average percentage of dry matter present in protein, it can be stated that for every 1 kg of dry mass, there are nearly 547 g crude protein. Bajpai and Bajpai (1986) obtained biomass with 66% and 45% protein, respectively, from hydrolyzed cellulosic materials with Candida utilis yeast. Lupatini et al. (2013) used two reactors to treat wastewater from a starch factory and determined crude protein contents of 50.5% and 45% for reactors 1 and 2, respectively.

Reffatti et al. (2007) evaluated single-cell protein production from lignocellulosic agricultural residues and characterized it from a bioreactor with the following data: 17.25% protein, 79.45% moisture, 3.31% ash, 3.71% fiber, 0.90% fat, with a biomass yield per liter of nearly 8 g L^{-1} effluent. Suhet and Fioreze (2011) worked with pineapple industry wastes to obtain a single-cell protein production and the protein content values ranged from 12 to 13%. Several authors have

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reported protein content data from 15 to 60%. This means that according to this study, it can be stated that the single-cell protein value was high (54.73%). This index depends primarily on two factors: type of micro-organism and the nature of the substrate used. It is possible to quantify the crude protein from a digester when considering average effluent flow of a producing starch industry as 90 m3 h-1 (Figure 4)

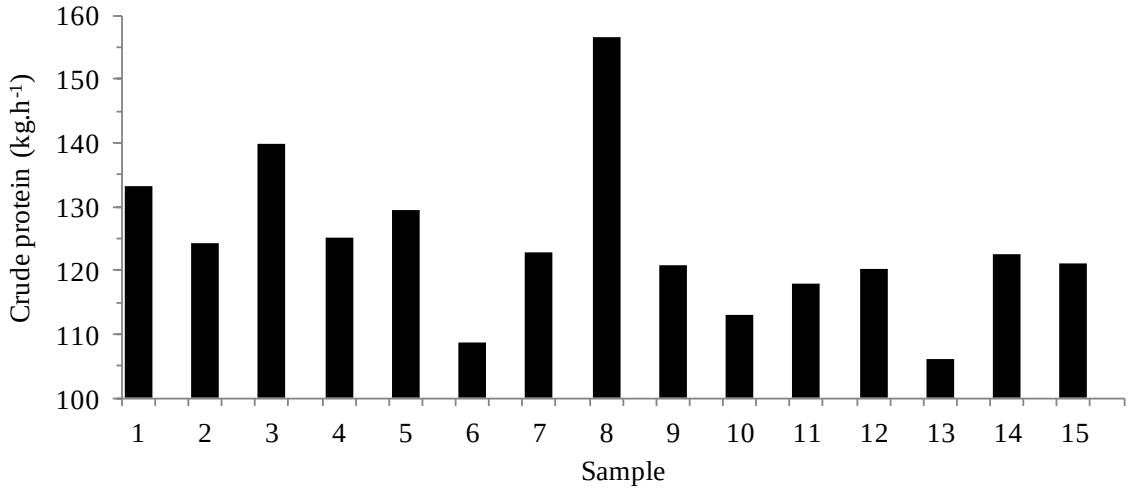

Figure 4 Crude protein (kg h^{-1}) estimation for the studied producing starch industry

The average estimation of crude protein production is almost 124 kg h^{-1}, with 12.49 kg h^{-1} standard deviation and 10.05% coefficient of variation with 106.27 and 156.75 kg h^{-1} CP as minimum and maximum values, respectively. Lupatini et al. (2013) estimated crude protein production during the wastewater treatment from a starch industry in Toledo city. It was pointed out that up from its 560 m^3 day^{-1} flow, it could be obtained nearly 170 kg CP per day. Since the studied biodigester has not undergone any form of operational control concerning factors such as temperature and uniformity of load application. There was not either any addition of nutrients to produce mainly crude protein and it was obtained from the biogas production process, crude protein estimation of the concerned producing starch industry is considered satisfactory.

4. Conclusion

Regarding the process efficiency, during the studied period, there were removals of turbidity, total solids and volatile solids, whose answers were 73.97, 64.37 and 76.04%, respectively. Volatile solids represent the organic fraction, that is, biodegradable material of solids. Almost 75% of total solids in the input area consist of volatile solids. On the other hand, in the output, the average value is about 50% volatile solids on total solids.

The producing starch industry recorded 90 m^3 h^{-1} average flow of effluent, thus, it was estimated approximately 124 kg h^{-1} crude protein production.

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According to the obtained results, it is aimed both improvement and optimization of the process for single-cell protein production with food quality in future trials.

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