

Analysis of residual sludge stored in UASB of a WWT in Petrolina-PE-Brazil

Análises do lodo residual armazenados em UASB de uma ETE em Petrolina-PE-Brasil

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Abstract

Anaerobic digestion is a process that occurs through microorganisms in an anoxic condition and aims to digest organic matter resulting mainly in biogas. This process is common in wastewater treatment WWTs (Waste Water Treatment), which usually occur in bioreactors. In Brazil the most widespread is the UASB (Upflow Anaerobic Sludge Blanket) reactor due to its temperature conditions, which found in the country an ideal parameter. Archeas make up the microbiota responsible for digestion acting in the final stage of methanogenesis. The studies of these organisms are mainly through metagenomics, because laboratory cultivation is difficult. Therefore, the research aimed to study the physical and molecular parameters of the sludge. Four UASB reactors from WWT Center in Petrolina – Pernambuco- Brazil were evaluated. For the DNA extraction process the adapted protocol was applied, the physical analysis of the solids obeyed the determinations of APHA (2005). DNA extraction was achieved with the modified protocol and demonstrated a

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high concentration of DNA present in the samples, being the 4 most abundant reactor. Physical quantifications of the solids analysis showed that the values found are in compliance with current standards.

Keywords: Anaerobic digestion; Reactor UASB; Organic matter; Archaea; Methagenomic

Resumo

A digestão anaeróbica é um processo que ocorre através de microrganismos numa condição anóxica e tem como objetivo digerir a matéria orgânica resultando, principalmente, em biogás. Esse processo é comum em ETEs (Estação de tratamento de Esgotos) para o tratamento de afluentes domésticos, em que ocorre geralmente em biorreatores. No Brasil o mais difundido é o reator UASB (Upflow Anaerobic Sludge Blanket) devido suas condições de temperatura, que encontraram no país um parâmetro ideal. As arqueas compõem a microbiota responsável pela digestão atuando na etapa final de metanogênese. Os estudos desses organismos são principalmente através de metagenômica, devido ao cultivo em laboratório ser difícil. Diante disso, a pesquisa objetivou realizar o estudo de parâmetros físico e molecular do lodo. Foram avaliados quatro reatores UASB da ETE Centro em Petrolina-Pernambuco-Brasil. Para o processo de extração de DNA foi aplicado um protocolo adaptado, a análise física dos sólidos obedeceu as determinações do APHA (2005). A extração do DNA foi alcançada com o protocolo modificado e demonstrou uma alta concentração de DNA presente nas amostras, sendo o reator 4 mais abundante. As quantificações físicas das análises dos sólidos demonstraram que os valores encontrados estão respeitando as normatizações vigentes.

Palavras-chave: Digestão anaeróbica; Reator UASB; Matéria Orgânica; Archaea; Metagenômica

1. Introduction

One of the consequences generated by population growth is the increase in effluent production, causing greater concern with the mitigation of impacts caused by the final disposal of sewage and its by-products.

According to IBGE 2019, Petrolina fits into the regions that present a population increase, the city has an estimated total of 349,145 people, having presented in the last ten years a population increase of more than 55,000 inhabitants. Its percentage of sanitary sewage has about 72.7% of area met by this demand, in the last study conducted by the institute in 2010. Being the 12th municipality with the largest sanitary sewage area, in the state of Pernambuco.

The Pernambuco Company of Sanitation (COMPESA), among other attributions, is responsible for treating the domestic sewage generated in the residences of the municipalities. The company has a complete treatment, according to the Brazilian Association of Sanitary and Environmental Engineering, which infers about conventional systems as being first established a sewage direction to a Waste Water Treatment (WWT), where they are held there the preliminary (removal of coarse materials), primary (removal of sediment and floating materials), secondary (degradation of dissolved organic matter) and tertiary (nutrient removal and disinfection) steps (Tonetti, 2018).

1.1 Biorreactor UASB (Upflow Anaerobic Sludge Blanket)

The first anaerobic treatment systems developed in the late nineteenth century had two main conceptual misconceptions: (1) Sedimentable solids were the main compounds to be removed and (2) the low efficiency was a result of the limited ability of bacteria to metabolize organic components. However, the poor performance was due to structural errors rather than the microbial metabolism mechanism. Thus, in search of improvements for treatment, the upward flow and the retention of sludge that were characteristic of the UASB reactors were created (Haandel et al., 2006).

Currently, various technologies for anaerobic urban wastewater treatment are available, such as septic tank, Imhoff tank, anaerobic pond, anaerobic filter, fluidized bed

reactor (FBR), expanded bed reactor (AEBR), expanded granular sludge blanket reactor (EGSB), sequential batch anaerobic reactor (ASBR) and anaerobic sludge blanket upflow reactor (UASB) (Korsak, 2008). In sewage treatment, sludge is a byproduct and can be converted to organic fertilizer after treatment.

The Upflow Anaerobic Sludge Blanket (UASB) has found wide acceptance in the treatment of domestic sewage in tropical countries such as Brazil and Colombia, where units operate on a large scale (Lew et al., 2003).

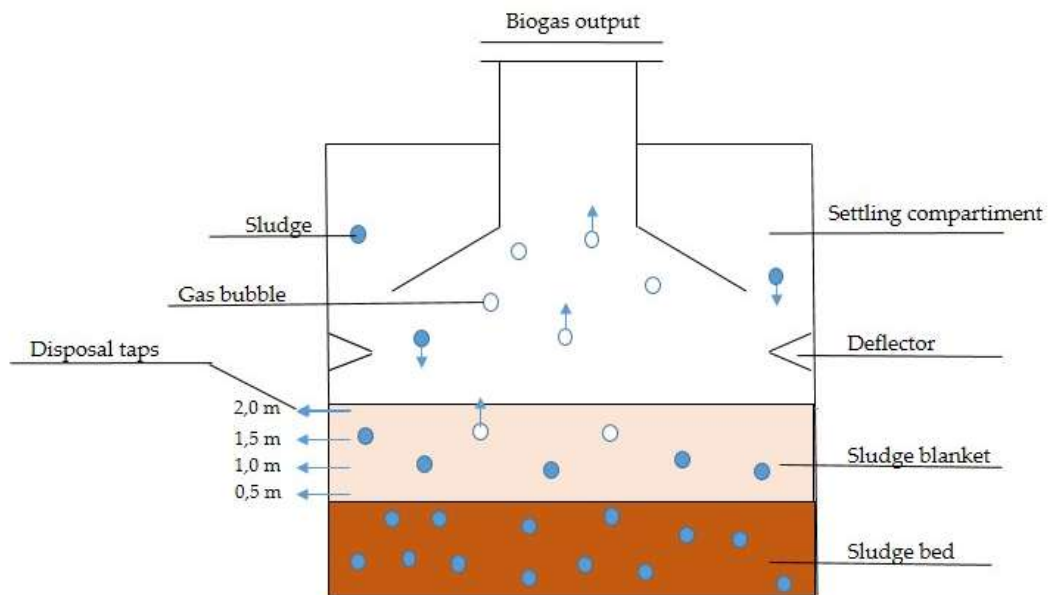
The main advantage is in the climate of these countries, where thermophilic temperatures are present all year, thus allowing the metabolic performance of bacteria and archaea involved in the anaerobic digestion process. Moreover, they present a high constructive, operational and hydrodynamic viability, thus having greater viability when compared to traditional treatment methodologies (Belli Filho et al., 2001).

UASB is a biological treatment system for organic compounds. The reactor consists of a tank divided into digestion zone and sedimentation zone. The effluent to be treated enters the reactor from its base and through an upward movement crosses the sludge blanket where digestion occurs and reaches the sedimentation zone through which it is discharged from the surface (Haandel et al., 2006). When passing through the sludge blanket what occurs is a processing of this residue, in which the present organic compounds are degraded and digested under anaerobic conditions, these results in biogas production and maintenance of an association of microorganisms (Figure 1).

The microbiota established under the structural conditions of this type of reactor compresses into dense flakes or granules that are suspended by self-adhesion. At the bottom of the reactor there is the accumulation of sludge layers allowing the biomass to settle in high quantity and present high activity. This configuration allows the reactor to have a high organic load and an adequate hydraulic detention time (Campos, 1999).

(Continue...)

Figure 1 - Representative scheme for the UASB reactor. Source: Own Authorship



Reactors feature a highly structured community of microorganisms that play a crucial role in the anaerobic digestion in UASB reactors. However, the variation of operational parameters, such as temperature, solid retention time and pH can interfere in the composition of microorganism groups, compromising the whole digestion process. For example, the reduction in pH that is caused by the accumulation of volatile fatty acids, in turn, due to the compromised population of acetogenic bacteria, results in the negative performance of methanogenic archaea and makes methane production unfeasible, since these microorganisms work within a pH range of 6.5 to 8.5, but with an optimal range of 7 to 8 (Mes et al., 2003; Weiland, 2010; Shah et al., 2014).

Temperature is a fundamental parameter to determine the ability of bacteria to act in the degradation process, given the anaerobic condition presented by the UASB reactor. Since these reactors are usually under mesophilic or thermophilic conditions, the temperature requirements of the sludge population must be achieved. A thermophilic sample kept at low temperature for analysis of its activity will present a time deficit for population acclimatization to be thermophilic test (Kaviyarasan, 2014).

For optimal reactor operation it is important to consider the excess sludge accumulation inside the reactor, which can cause a large loss of solids to the settling

compartment. Thus, generating release of solids together with the liquid effluent. Occasionally, the reduction of the treatment efficiency caused by the presence of particles of organic material (Chernicharo, 2007).

It is important to perform analyzes of the sludge mass present in the reactors and their specific methanogenic activity in order to determine the frequency of excess sludge discharge from the reactors to optimize the operation at the WWT and to lead to increased effluent quality (Chernicharo, 2007)

Recent studies that deal with this theme were developed: Vasconcelos et al., 2019 aimed to assess microbial structure, diversity, productivity, and stability and the influence on the performance of an anaerobic reactor; Thiyagu & Sivarajan, 2019 isolated and characterized novel bacterial strain present in a lab scale hybrid UASB reactor.

1.2 Anaerobic Digestion

The anaerobic digestion process consists of a coordinated activity of different groups of microorganisms in which these populations interact to promote stable and self-regulating degradation of organic matter in four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis producing main product biogas (Mes et al., 2003; Appels et al., 2008; Weiland, 2010); a mixture consisting mainly of methane and carbon dioxide gases, and small amounts of hydrogen sulfide and ammonia, highly polluting (Anderson et al., 2003).

Regarding the main global flows of bioelements, methane formation is one of the most important biological processes on earth (Garcia, 2009). Anaerobic microorganisms mineralize organic materials entering anoxic environments (Conrad, 1999).

The methanogenesis step corresponds to the final process of anaerobic digestion of organic matter under limiting sulfate conditions. This shows the important role of these organisms in the recycling of carbon components (Reis, 2012). In addition, it is important to note that methanogens have created most of the natural gas (fossil fuel) reserves that are captured as energy sources for domestic or industrial use. On the other hand, methanogenesis also has a severe effect on global ecology. Methane is the most important greenhouse gas after carbon dioxide and contributes to 16% of the greenhouse effect (Deymer, 2000).

In the hydrogenotrophic archaea group, they produce methane from the reduction of carbon dioxide and hydrogen. There is a prevalence of bacteria that use carbon dioxide and hydrogen in the methanogenesis process in reactors with high rate of organic compounds of agricultural origin, according to Bauer (2008).

Anaerobic digestion is performed by microorganisms in an anaerobic environment, whose decomposition of organic matter through microbial activity allows its conversion into products such as CO₂ and CH₄ gases, components of biogas (Craveiro et al., 1982).

Through anaerobic digestion it is possible to obtain biogas and biofertilizer as products (Granzoto et al., 2016). Kong et al. (2018) conducted research to analyze the methanogenic community during anaerobic digestion in different substrate media and amount of organic matter. For this, three reactors were collected and were evaluated in several parameters as the performance of the bioreactor in performing anaerobic digestion. Thus, it was observed the action of the archaea, mainly methanogenic, acting in the digestion process and generating an efficient functioning under the conditions of the evaluated anaerobic reactors.

Anaerobic digestion depends mainly on two conditions: substrate and microbiota, according to Liu et al. (2008). Their study sought to evaluate the biogas process by quantifying the methane that is produced by inducing change in the substrate composition. It was observed that a change in substrate composition through inoculum leads to changes such as increased or decreased methane production and also generating change in microbial community composition.

1.3 Archaea Domain

Archaea was presented to the scientific community as a third domain in the tree of life (Woese and Fox 1977; Fox et al 1977). In addition to the Eukarya and Bacteria domains. However, it was not until the 1990s that archaeas were validated as a third domain based on molecular analysis of a small universal ribosomal RNA subunit (SSU rRNA) by comparative genomic analysis (Woese and Kandler and Wheelis, 1990; Gribaldo and Brochier -Armanet, 2006).

The classification of bacteria and archaea is currently based on genetic and phenotypic information and is restricted to cultivated strains, which is a slow, difficult and especially challenging procedure to obtain pure strains of microorganisms that have complex metabolic requirements (Yarza et al., 2014).

According to Forterre (2002) the first Archaea domain organisms was found in environments of extreme conditions, presenting extreme pH, high temperatures, salinity and acidity, leading to infer the group as belonging to this type of environment. Studies that are more recent have led to another view, as they are also found in less extreme environments.

Eme (2015) points out the studies of Delong in 1998 as pioneers in the investigation of archeas in mild environments. They used the Polymerase Chain Reaction (PCR) technique and found the presence of archaeobacteria in coastal marine environments in Antarctica. The author also states in her study that the first sequenced genome of archaea was *Methanococcus jannaschii*, published in 1996.

Currently their distribution may be proportional to that of bacteria since they also inhabit extreme environments. Therefore, DeLong (1998) states the archaea as ubiquitous microorganisms that will present themselves in extreme and mild environments.

Methanogenic archeas are those that produce methane (Özcan, 2012). There are also archeas that may have characteristics that classify them into more than one group, for example, Methanothermaceae family archaea, which are methanogenic and hyperthermophilic (Liu and Whitman, 2008).

The study by Gomaa and Abed (2017) found the predominance of methanogenic sewage archaea, where an analysis of the production of biomethane and other products was performed, in which these bacteria are unique in the performance of this degradation of the phylum Euryarchaeota with different genera, result obtained through the sequencing of the 16S rRNA gene from samples collected from sewage sludge.

A research developed by De Vrieze et al. (2017) evaluates the microbiota activity of methanogenic archaea in a digester with substrate salinity control. The study concluded that there is a dependence on saline conditions for an efficient performance of these bacteria in the degradation of organic matter and methane production, demonstrating that in high

saline conditions there is a change in the composition of the microbial community. Thus, it is an important parameter in the control of methanogenic activity.

Microbial community enrichment in UASB reactor was evaluated by Cerrillo et al. (2016). With the purpose of using methanol and acetate for enrichment to observe the methanogenic activity of the arches, specific Methanogenic Activity, Polymerase Chain Reaction in real-time (qPCR) and sequencing assays were used. The result obtained was a community made up of metilotropic arcqueas (Methanomethylovorans and Methanolobus genus), demonstrating as the main reaction the direct conversion of methanol to CH₄. Research indicates the inoculum for possible use in the enrichment of methanogenic archaeobacteria, resulting in improved performance of biogas (CH₄ and CO₂) production.

The work aimed to analyze molecular and solids (values of total solids, volatile total solids and fixed total solids) of the residual sludge resulting from the domestic sewage treatment of a Petrolina-PE.

2. Methods and Material

2.1 Sampling

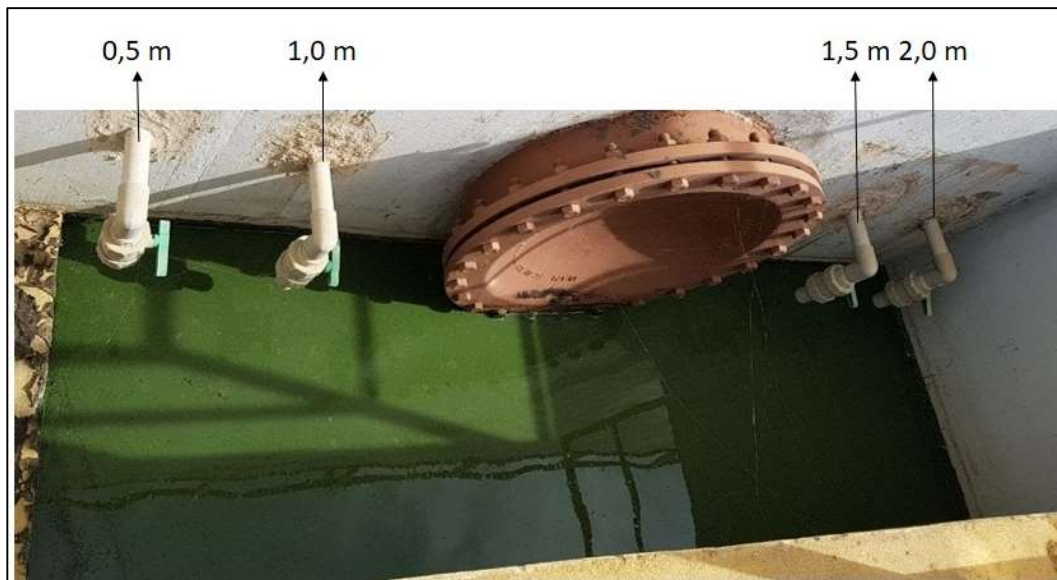
The Pernambucana Sanitation Company (COMPESA) Center Sewage Treatment in Petrolina, being one of its duties the collection and treatment of domestic sewage. WWT Centro has a structure of four UASB reactors that perform an important stage of the treatment of liquid waste. Each reactor has a size of 2,100 m³ and has been in operation since September 2014. The station has an average input flow of 250 L/s, this material is distributed among the 4 reactors giving an average value of 62.5 L/s for each UASB reactor.

The reactors were identified by R1, R2, R3 and R4. Sample collection was performed obeying the depths of 0.5 m, 1.0 m, 1.5 m and 2.0 m (Figure 2) presented in each of the reactors. Thus, from each reactor four samples were collected, except from reactor R3 where the material corresponding to depth 0.5 m did not pass the tap to be released and collected.

15 samples were analyzed. The volume was dispensed into falcon tubes, 90 ml for physical analysis and 10 ml for molecular analysis. Molecular analyzes were performed at the Laboratory of Genetics and Biotechnology - UNIVASF, Campus of Agricultural Sciences. The

solids analysis was performed at the Environmental Engineering Laboratory - UNIVASF, Campus Juazeiro.

Figure 2 - UASB WWT Centro Petrolina PE reactor taps to dispense residual sludge generated in the digestion process. Source: Personal Archive



2.2 Genomic DNA Isolation

The extraction and purification of the genomic DNA was performed by a modified saline protocol from Lucena, 2008. The quality of the extracted genetic material was verified by 1.0% agarose gel, in 0.5 X TBE buffer, stained with ethidium bromide and visualized under UV light in transilluminator. DNA was quantified on a mini-1240 UV-Vis UV spectrophotometer at 260 nm wavelength to measure the absorbance of nucleic acids and 280 nm for proteins.

2.3 Solids Analysis

Analysis was performed according to the Standard Methods for the Examination of Water and Wastewater (Apha, 2005). The triplicate experiment was determined for each sample collected. For the 90 ml collected, 30 ml triplicates were performed. The initial stage consisted of weighing the crucible (P1), then the sludge was dispensed in the crucible, taken

to a plate at 100 °C and weighed (P2), finally the crucible was carried to muffle at 550 °C and weighed again (P3). In order to obtain the values of total solids, volatile total solids and fixed total solids, as follows:

Total Solids:

$$TS \text{ a } 100 \text{ }^{\circ}\text{C (mg/L)} = \frac{(P2 - P1) \times 1000 \times 1000}{V}$$

Fixed Total Solids:

$$FTS \text{ a } 550 \text{ }^{\circ}\text{C (mg/L)} = \frac{(P3 - P1) \times 1000 \times 1000}{V}$$

Volatile Total Solids:

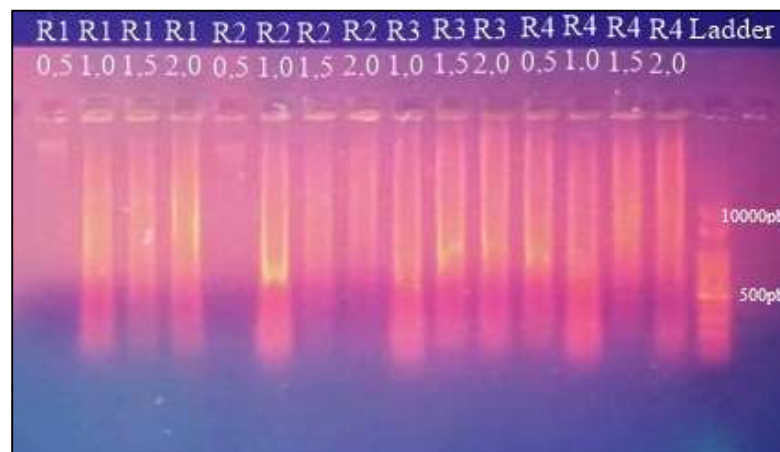
$$VTS = TS - FTS$$

3. Results and discussion

3.1 Genomic DNA Extraction

The execution of this step was performed in the 15 collected samples, by applying the adapted protocol in which it was possible to obtain electrophoresis visualization (Figure 3) of the extracted DNA for each depth of the four reactors in which the samples were collected.

Figure 3 - Agarose gel 1% electrophoresis, performed with DNA extracted from UASB sludge. R1-Reactor 1; R2 - Reactor 2; R3- Reactor 3; R4- Reactor 4; the numbers mean the four depths analyzed



Source: Own authorship

A high concentration of DNA can be observed in most samples. Regarding the bands that have a weaker gel presentation, probably due to the physical condition with little organic matter, which these samples had at the time of collection. It was noticeable the most liquid aspect found in samples R1 (0.5) and R2 (0.5).

According to Blagodatskaya et al. (2003), in a study on soil microbial composition, found a proportional decrease between extracted DNA and microbial biomass, concluding that the amount of DNA from similar samples has the same relationship, where the amount of extracted DNA may reflect the biomass microbial present.

Moreover, it is possible to infer that in samples such as raw sludge, they need reagents with high performance in the washing process of the collected material, in order to have a better purification and DNA isolation, consequently better delineated bands in the gel.

One of the biggest difficulties in extracting DNA from environmental samples, according to Head et al. (1998) is not having a priori assured the amount of microbial DNA and because there was no adequate performance for complete breakdown of cells of the sample microorganisms.

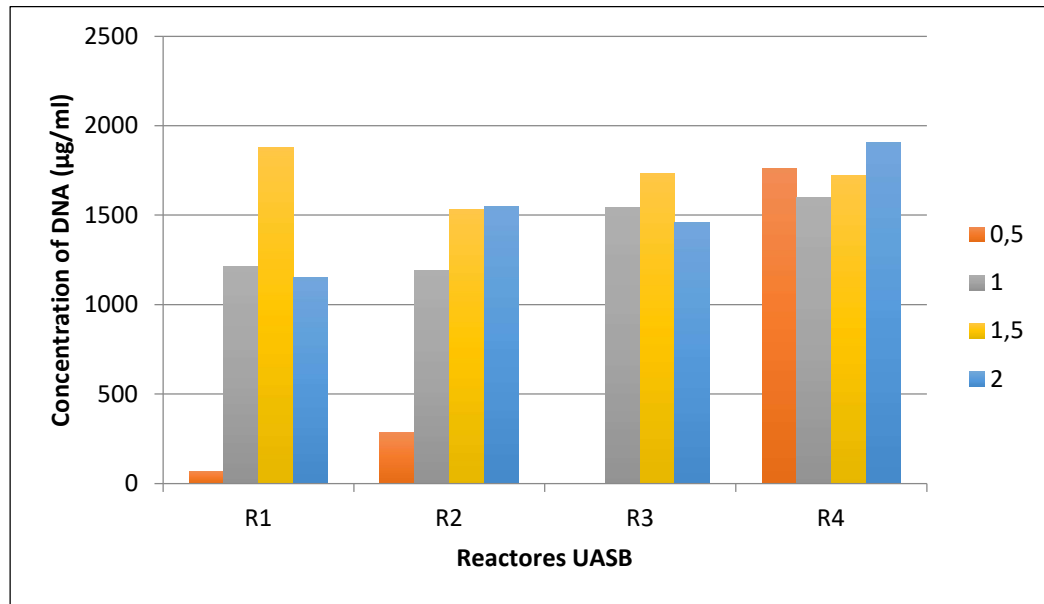
Xavier (2018) performed the extraction of genomic DNA from the sludge, also generated by COMPESA. The resulting gel in its analysis corresponded to a material with high DNA concentration present in the samples, where the less dense samples generated a better defined band when visualized with 1% agarose gel electrophoresis. Thus, corroborative with the current study with a similar pattern in DNA electrophoresis.

3.2 Spectrophotometer Quantification of samples

The spectrophotometric analysis process, in which the 15 samples were diluted 50X and subjected to UV light with wavelengths 260 nm and 280 nm, initially demonstrated in obtaining DNA concentration (260 nm) (Figure 4) a high result concentration of the genetic material, these data being corroborative for the quantification in agarose gel electrophoresis.

(Continue...)

Figure 4 - DNA concentration obtained by spectrophotometry at 260 nm absorbance. Numbers on the right represent the four depths (in meters) of the reactors.



Source: Own authorship

As expected, DNA concentrations for samples R1 (0.5) and R2 (0.5) were lower compared to the same depth in reactor 4 as for all other samples. This result proves to be reliable as it follows the pattern presented in the electrophoresis gel.

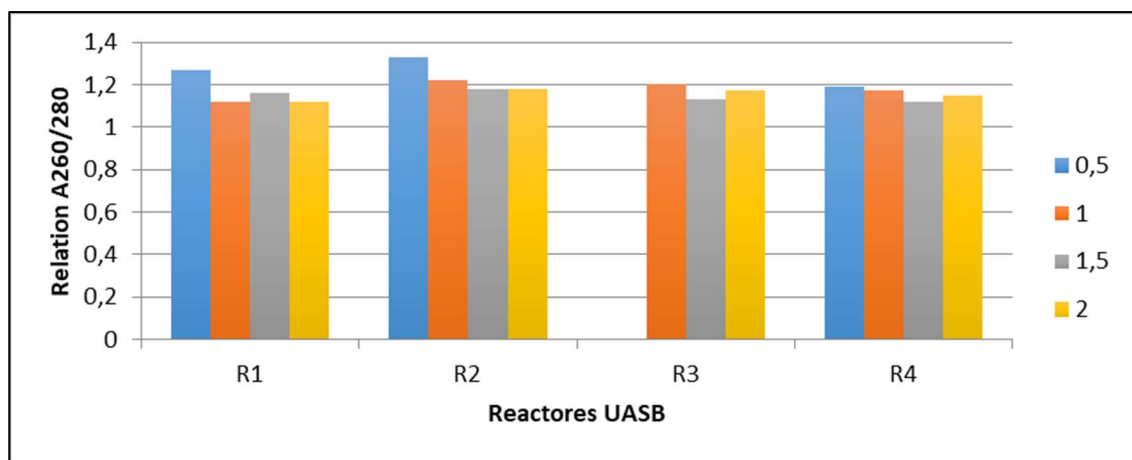
The average value of DNA concentration obtained between the depths of R1 was 1,079.6 µg / ml, being the lowest concentration. R2 had 1,139.4 µg/ml concentration, while R3 had a value of 1,580.8 µg / ml. The highest average observed was R4 with 1,748.8 µg / ml.

Reactor 4 (R4) showing an average concentration of DNA, related to its depths, higher than the average concentration of the other reactors analyzed, it is possible to suggest to this result, the condition of the sample when collected is more constituted of organic matter, probably a higher biomass present.

A study by Bianco (2015) in which we collected sludge from reactors for analysis, including quantification of DNA present in the sample by spectrophotometry. In the analysis, the obtained was 2600 µg / ml of DNA concentration. When calculating A260 / A280 that corresponds to protein contamination, a value of 1.69 was obtained, demonstrating that possibly the proteins were not completely degraded during the extraction process.

In the samples obtained in the present work, the evaluation to obtain the protein concentration present in the sample was also performed, using the ratio between A260/A280 (Figure 5), in values that show less than 1.8 mean protein contamination. The results obtained for this study reveal values below 1.8 in all samples, thus indicating protein contamination.

Figure 5 - Data on the relationship between A260 / A280 absorbance grades that demonstrate protein contamination. Numbers on the right represent the four depths (in meters) of the reactors.



Source: Own authorship

A study by Xavier (2018) has shown results in agreement with that observed in the analysis of DNA purity in the samples. Their data show values below 1.8 for all reactors. Unlike the current study, in the extraction process the author describes that lysozyme was not used to assist in protein breakdown. Therefore, there are other factors limiting the DNA purification process that can be attributed to the washing process of samples that require stronger organic reagents such as phenol.

3.3 Physical Analysis of Solids

In the solids quantification, the corresponding samples for each reactor were analyzed (Table 1). For the total solids (TS), which correspond to the collected raw material, the reactor sample 2 corresponded to the largest mass obtained with a value of 356, 65 mg/L

represented by the collection at a depth of 1.0 m. The lowest TS value was obtained in reactor 1 with a mass of 181, 52 mg/L, and its collection was performed at a depth of 1.0 m.

The average value between the reactors for TS was 247, 26 mg/L, for the total fixed solids (FTS) the average was 70, 17 mg/L and lastly the volatile total solids variable (VTS) presented an average value of 177, 18 mg/L.

Table 1 - Quantification of solids of the four reactors by gravimetry

	Sample R1	Sample R2	Sample R3	Sample R4
Total Solids (mg/L)	181,52	356,65	265,16	185,71
Fixed Total Solids (mg/L)	39,86	167,17	43,14	30,49
Volatile Total Solids (ml/L)	141,53	189,31	222,66	155,22

Source: Own authorship

Wastewater from domestic sewage has a high amount of suspended solids, as well as being rich in organic material, nutrients and salts, which if improperly disposed, constitute a high pollutant potential for soil or water (Matos et al., 1999). Release of this wastewater into untreated watercourses can result in serious environmental problems (Von Sperling, 1996).

Solids can act indirectly on aquatic life, preventing light from entering, inducing water heating which consequently decreases the amount of dissolved oxygen in the medium. Therefore, according to CONAMA (Brasil, 2005), the concentration of total dissolved solids must be less than 500 mg / L for effluent discharge into water bodies. The measurements in this study showed that this parameter was achieved.

4. Conclusions

It was possible to establish a methodology for the extraction of sludge DNA in UASB reactors, efficiently with protocol adaptation, without the use of expensive reagents, generating a more accessible cost for the elaboration of the process and avoiding the use of reagents considered toxic.

In the physical analysis of the sludge the collected material presented values within the required parameters. Further studies using samples from all depths of each reactor may more objectively establish the relationship between the amount of organic matter and the amount of DNA present in the sample.

The study then consisted of applying initial analysis to determine and understand the parameters of the sludge microbiota, in view of the influence of the microbiome on the digestion process performance, which in turn results in how the domestic sewage is being treated so that the impact of the effluent arrival to the water bodies is minimal or none.

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