Conjecturas

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Use of solvents in the process of elimination of parasitar load in samples of sewage sludge

Uso de solventes no processo de eliminação de carga parasitária em amostras de lodo de esgoto

Bruna Avelar Oliveira1[1], Dilceu Silveira Tolentino Júnior[1]*, Ana Carolina Souza Viana Colen[1], Alexandre Sylvio Vieira da Costa[1], Eliseu Miranda de Assis[2]

ABSTRACT

Among the types of waste, sewage sludge stands out for being an organic and chemical compound with a significant pathogenic load, requiring prior cleaning for commercial use. We evaluated the use of chemical solvents and autoclave sterilization in the elimination of parasite load in sewage sludge samples generated in the drying bed of the Sewage Treatment Station (STS) in the city of Teófilo Otoni, Minas Gerais, Brazil. Samples of sludge treated by the UASB method (Upflow Anaerobic Sludge Blanket) were added with the solvents acetone, chloroform, and hexane and evaluated by direct microscopy and by the method of Hoffmann, Pons, and Janer (HPJ) for the presence of parasites. The tested solvents were not efficient in reducing the parasite load while autoclaving eliminated the presence of parasites in the samples tested.

Keywords: Sludge treatment; Chemical disinfection; Thermal inactivation; Parasites.

RESUMO

Dentre os tipos de resíduos, o lodo de esgoto destaca-se por ser um composto orgânico e químico com carga patogênica significativa, necessitando de limpeza prévia para uso com fins comerciais. Avaliamos o uso de solventes químicos e a esterilização por autoclave na eliminação de carga parasitária em amostras de lodo de esgoto geradas no leito de secagem da Estação de Tratamento de Esgoto (ETE) da cidade de Teófilo Otoni, Minas Gerais, Brasil. Amostras de lodo tratado pelo método UASB (Upflow Anaerobic Sludge Blanket), foram adicionadas dos solventes acetona, clorofórmio e hexano e avaliadas por microscopia direta e pelo método de Hoffmann, Pons e Janer (HPJ) para a presença de parasitas. Os solventes testados não foram eficientes na redução da carga parasitária, enquanto a autoclavagem eliminou completamente a presença de parasitas nas amostras testadas.

Palavras-chave: Tratamento de lodo; Desinfecção química; Inativação térmica; Parasitas.

[1] Federal University of the Jequitinhonha and Mucuri Valleys
[2] Federal Institute of Bahia
*E-mail: dilceujunior@bol.com.br

INTRODUCTION

Waste has always been present in human life, as the history of man is directly related to the increase in problems arising from its poor management as the species evolved. In the Middle Ages, sanitary waste accumulated in the streets and surroundings of cities, contributing to the emergence of diseases, including the proliferation of endemic diseases causing the death of millions of people (FERRONATO and TORRETTA, 2019).

The final disposal of sludge by its own qualitative and quantitative characteristics is one of the main problems involving a sewage treatment plant. As a result, the sewage treatment generates the treated effluent, which is released into rivers, which can generate other by-products such as scum, gases, and sewage sludge, so proper treatment and disposal are necessary to avoid environmental impacts or even harm the sewage collection and treatment system (ANDREOLI, VON SPERLING and FERNANDES, 2014).

Among the various types of waste, sewage sludge stands out for being an organic compound rich in macro and micronutrients, due to its chemical composition and for having a significant pathogenic load. A considerable number of researches have already been developed considering its use in various ways, among these, the use of sewage sludge in the form of biodiesel, use as a substrate in agriculture and civil construction, using its pure or mixed constitution (SCHEER, CARNEIRO and SANTOS, 2010; GOMES et al., 2013; FREITAS et al., 2013; LOPES et al., 2018).

Because it contains pathogens in its composition, its use for commercial purposes requires prior cleaning, and its sterilization can be carried out through various processes, such as composting, solarization, the addition of lime, autoclaving, among others (JUNGA, MACH and MAREEK, 2017).

The sewage treatment process generates a significant amount of sludge in the drying bed in the Sewage Treatment Plants (STPs), being approximately 1% to 2% of the total volume of treated sewage (Nowak, 2006), and representing in terms of expenses, a percentage of up to 80% of the total cost of treatment and final disposal of the sludge (HUDCOVÁ, VYMAZAL and ROZKOŠNÝ, 2019). Even though studies are showing the effectiveness of the application of sludge as an agricultural input, fertilizer, and use in civil construction, its final disposal in landfills is noteworthy due to the challenge of its integration in the production cycle, due to economic factors and feasibility (LAMASTRA, SUCIU and TREVISAN, 2018). In addition, its accumulation in sewage

treatment plants, occupying space and increasing costs with storage and operation of the stations, in addition to the risk of damage to the environment during the rainy season, due to the possibility of being leached (SILVA, RESCK and SHARMA, 2002).

With the expansion of cities and the industrial sector, there was an increase in the generation of sewage sludge from urban and industrial waste, being a viable solution to invest in the use of these residues in the soil as fertilizer for plants (SUCIU, LAMASTRA and TREVISAN, 2015).

Nowadays, the most used methods for sewage sludge treatment are physical, chemical, and biological processes. The main objective of this treatment is to generate a more stable product with a smaller volume to facilitate its handling and, consequently, reduce costs in subsequent processes. Usually, the treatment of sludge, after its generation, includes one or more of the following steps: densification, reduction of humidity, stabilization, conditioning, volume reduction, and one of the most important processes is hygiene, which guarantees a low level of pathogenicity (CASSINI, 2003).

Several Brazilian municipalities are collecting and properly treating sewage to dispose of the sludge generated in agriculture. In Brazil, some medium to large cities in the interior, such as Campinas, Franca, Jundiaí, Limeira, Piracicaba, and São José dos Campos, have already started the treatment of their generated sludge. Among the Brazilian capitals that are ahead in this process are São Paulo-SP, Belo Horizonte-MG, Brasília-DF, Campo Grande-MS and Curitiba-PR (BETTIOL and CAMARGO, 2006).

Aiming to contribute as an alternative for disposal and final cost in the treatment of sewage sludge, we propose to reinsert this by-product into the production cycle and evaluate alternative methods to reduce the parasitological load through the use of solvents and saturated steam under pressure, identifying the main ones during this process parasites present in the *in natura* composition of this sludge.

METHODOLOGY

The analyzes were processed in the Laboratory of Water and Soil Chemistry at the Federal University of Jequitinhonha and Mucuri Valleys, at Mucuri Campus, located in the city of Teófilo Otoni, Minas Gerais - Brazil, and analyzed in different laboratories as to the parasitological load. The material studied was the sewage sludge, coming from the drying bed of the Sewage Treatment Plant (STP) of Teófilo Otoni, which uses the

Upflow Anaerobic Sludge Blanket method (UASB) to treat this sewage, whose production is approximately 33.6 tons of dry sludge per month, being generated per year about 403.2 tons of sludge. According to Costa (2020), this STP serves 95% of the total population with about 130 thousand inhabitants (**Figure 1**).

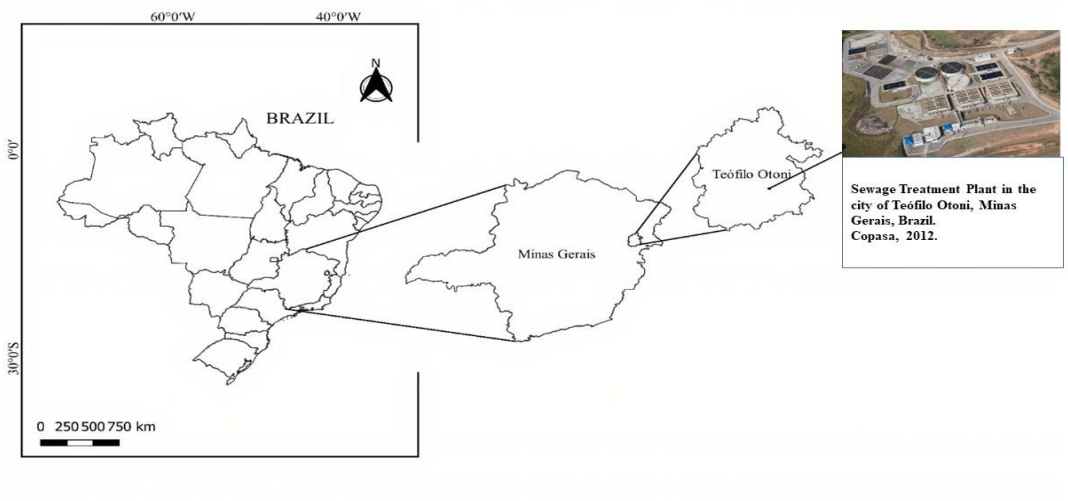

Figure 1. Location map of the Sewage Treatment Plant in the city of Teófilo Otoni, Minas Gerais state, Brazil.

The authors (2022).

The sewage sludge was collected and treated to eliminate possible pathogens present in the material, considering that the anaerobic reactor system at the Teófilo Otoni STP does not eliminate all the contaminants. The sewage sludge samples were collected in a single step, placed in two plastic bags of 100 liters, with about 20 kilos of sludge, and subsequently sealed. Then these samples were taken to the laboratory of the Federal University of the Jequitinhonha and Mucuri Valleys, where they were processed. The handling of the waste was done using personal protective equipment (PPE), and the other equipment involved in the evaluation followed the manufacturers' guidelines.

The amount of 100 grams of sludge was weighed and separated for the addition of solvents. Then the samples were filtered on cotton and vacuum. For every 100 grams resulting from the filtration, 400 ml of Hexane, 400 ml of Chloroform, and 100 ml of Acetone were added. After this process, the sludge filtered with each solvent was analyzed to verify its potential for sterilization.

The samples (acetone sludge, chloroform sludge, hexane sludge, crude sludge, and autoclave-sterilized sludge) were removed about 50 grams and analyzed by direct

microscopy and by the Hoffmann, Pons, and Janer method, by two different laboratories of the city of Itaobim, state of Minas Gerais, Brazil.

The Hoffman, Pons, and Janer method was chosen because it is currently the most used method of sedimentation by gravitational force in human laboratory routine in Brazil, and because it is more efficient in the detection of heavy eggs such as those of cestodes (*Taenia spp*.) and trematodes (*Schistosoma spp*.), and some laboratories also use it for the observation of light eggs such as *Trichuris trichiura*, *Ancylostoma duodenale* and often even for the detection of Giardia spp. It is a simple method of execution, it does not change the viability of the eggs, but it is a technique that requires a longer time for its execution and reading and accumulates a lot of debris, making it difficult to observe and identify the parasites (FERREIRA, 2012; GONÇALVES et al., 2012; 2016; KATAGIRI and OLIVEIRA-SEQUEIRA, 2007).

The direct fresh examination and the Hoffman, Pons, and Janer technique, when used together, increase the probabilities of real and reliable diagnoses, especially in the diagnosis of helminth eggs and cysts, but each one separately has greater sensitivity in specific cases (CUNHA e RODRIGUES JÚNIOR, 2021).

In autoclaving, the sample with 4 kg of sludge was placed in bags suitable for autoclaving and subjected to a temperature of 121°C for 30 minutes, with a pressure of 1.5 kgf/cm². It is noteworthy that the temperature and time standards recommended by Galvão, Silva and Teixeira (2013), were considered to carry out this process to eliminate any parasitological load. It is also worth noting that for each parasite, the time and temperature for elimination are different, ranging from 20° to 60° degrees, lasting between 30 and 60 minutes. Due to this factor, it was decided to use the standard temperature already established for the total elimination of the parasitological load.

The remaining samples with approximately 8 kg of raw sludge were processed, adding the solvents acetone, chloroform, and hexane. Afterward, these samples were crushed and sieved to reduce their particles, with the objective of uniformity. For this process 5.0 mm mesh sieves were used.

RESULTS AND DISCUSSION

The following parasites were found in the analyzed raw sludge samples: adult and larval forms of *Strongyloides Stercoralis*, larval forms, and eggs of *Necator Americanus* and *Ancylostoma Duodenale*, eggs of *Enterobius Vermicularis* and *Ascaris Lumbricoides* using the method of Hoffmann, Pons, and Janer (HPJ) and direct microscopy (**Table 1**).

Table 1. Parasites found in samples of raw sludge and samples added to common disinfectant solutions, from the sewage treatment plant (STP) in the city of Teófilo Otoni, in May 2016.

Organisms	Raw sludge samples with 50 grams	50 gram samples of sludge tested with 400 ml of chloroform	50 gram samples of sludge tested with 400 ml of hexane	50 gram samples of sludge tested with 100 ml of acetone	100 gram samples of autoclaved sewage sludge
Strongyloides Stercoralis larvae	+	+	+	+	-
Necator Americanus eggs	+	+	+	+	-
Necator Americanus larvae	+	+	+	+	-
Ancylostoma Duodenale eggs	+	+	+	+	-
Ancylostoma Duodenale larvae	+	+	+	+	-
Strongyloides Stercoralis adults	+	+	+	+	-
Enterobius Vermicularis eggs	+	+	+	+	-
Ascaris Lumbricoides eggs	+	+	+	+	-

Analysis method: HPJ and direct microscopy; (+) Positive in the sample, (-) Negative in the sample

Source: The authors (2022).

The nematodes identified in the table 1 are from different life cycles and species, both share both the free-living form in soil and aquatic environments, as well as the human and animal parasitic form. Of the identified stages, the egg is the most challenging for public health, given that helminth eggs are an important concern for the reuse of wastewater and sludge for agriculture and aquaculture, there is still little information about its behavior during different treatment processes (JIMENEZ, 2007). Study conducted by Santos et al. (2012) when analyzing the parasitological load in effluents from a wastewater treatment plant in the city of Piracicaba, state of São Paulo, Brazil, identified the presence of eggs of *Taenia spp*, *Toxocara* and also of *Ascaris spp*, the only evolutionary stage that presented similarity with the results of the analysis of sewage sludge from the city of Teófilo Otoni, which confirms that each region has its own identity and particularity, with a different parasitic ecosystem.

The microorganisms found in sewage sludge can be saprophytes, diners, symbionts, and parasites. Those of greatest medical interest are parasites as they are capable of causing diseases in humans and animals. For this, several factors must be considered among which are the infective dose, pathogenicity, environmental factors, and host susceptibility. For an infection to spread, and infecting dose of the pathogen must be able to pass from the excreta of the infected individual or the infection reservoir to the mouth or other port of entry of a susceptible individual (WRIGHT *et al*., 2018). It is known that the main sources of pathogenic bacteria in the present station come from hospital waste and from inadequate treatment of the local urban eviction (BRETTAR and HÖFLE, 2008).

Although the experimental bacteriological study has not been conducted to identify different forms of bacteria in the sewage treatment plant of Teófilo Otoni, it is believed that they are present and that the highest concentration of gram-positive bacteria is of the Bacillus genus. This finding was identified by Faria *et al*., 2006 in the sewage sludge from the Franca and Barueri treatment plants in the state of São Paulo.

The bacteria *Salmonella spp*, *S. Typhi* and *Paratyphi*, *vibrio cholerae* and *escherichia coli* (MAIER, 2009; SILVA *et al*., 2010) and hepatitis A viruses and enteroviruses (PRADO, GASPAR and MIAGOSTOVICH, 2014) represent health risks and may be present in these wastes, although they have not been identified in this analysis, this possibility is real with impacts on human and animal health. These pathogens can access the drinking water distribution system of the studied municipality through fecal contamination by sewage discharge. Although they are considered important, their pathogenic loads are considered relatively sensitive to disinfection, unlike helminth eggs (WHO, 2006).

Some human parasitic protozoa such as *Cryptosporidium spp*, *Entamoeba histolytica*, *Giardia Lamblia*, *Balantidium coli*, and *Toxoplasma gondii* are transmitted from one host to another by ingesting their resistance forms which are called cysts. Because its cellular structure consists of oocysts, disinfection is less likely, at the usual concentrations and contact times used in water treatment processes (GHENGHESH et al., 2016; ROUSSEAU et al., 2018). Protozoan cysts are lighter than helminth eggs and therefore take longer to settle, making them more apt to be found in liquid effluent (KIM et al., 2009).

The chemical treatment proposed was not effective in reducing or eliminating the parasite load constituted by different stages and species of helminths contained in the analyzed samples. *Ascaris* eggs are the best indicator of parasites of this group in biosolids, as they are the most resistant to chemical and mechanical influences of all eggs and cysts. Therefore, its inactivation indicates the destruction of all other parasite eggs. It is believed that this resistance of the *Ascaris* eggshell to chemical agents is generally quite high, and is due to the outer layer that can fix and protect against lack of moisture, the intermediate layer that has a support and to the inner layer formed by the vitelline membrane that provides impermeability to its inner content (NAIDOO et al., 2016).

Although its outer layer is composed of protein, being permeable only to oxygen and soluble materials such as lipids (QUILÈS, BALANDIER and CAPIZZI-BANAS, 2006), solvents based on acetone, hexane and chloroform were not effective in terms of their sterilizing potential on parasites contained in the respective tested samples. Acetone showed no effective physical or chemical action as it is a volatile liquid with a lower density than water. Hexane was not very reactive as an inert solvent in an organic reaction. As for chloroform, a volatile solvent, slightly soluble in water, it also showed the same performance as the other solvents tested, with a worse indicator because it is a carcinogen, in addition to compromising the respiratory and nervous system of those who handle it without due care to its manipulation (AGUWA et al., 2020).

As can be seen in table 1, the only test capable of causing the death of the parasites tested by heat treatment was autoclaving, and as the concentration reached by the process for viable helminths indicated levels below those detectable, it is possible to guarantee that all others pathogens present in the autoclaved raw sludge sample were eliminated.

Autoclaving is a method that involves the death or thermal inactivation of pathogens without necessarily disintegrating the cells completely. It is also likely that the destruction of these complex molecules takes place by reaction between a complex molecule in the organism and vapor. As in the case of oxidation, one of the reactants (steam) is present in excess, the reaction will be bimolecular, but of first order. Consequently, it can be expected that, whatever the actual mechanism of the thermal sterilization reaction, it will be characterized, at least, by first-order kinetics (BARBOSA et al., 2020). The criteria adopted for non-viable eggs after autoclaving are poorly defined structures, cytoplasm vacuolization, cell condensation, egg in unicellular stage with

granular and vacuolated cytoplasm, in addition to contraction, rupture, and loss of membrane continuity (RAYNAL, VILLEGAS and NELSON, 2012).

The use of any product resulting from sewage sludge is only possible after its safety and innocuousness acquired through the process of disinfection or sterilization for use in agriculture are certified. For this purpose, the final product must be free of pathogens as mentioned in the text above. In this sense, the temperatures necessary for its elimination by autoclaving are death in 30 minutes at a temperature between 55-60 °C (*Salmonella sp*); kill in 60 minutes at 55°C (*Escherichia coli*); kill in 60 minutes at 55°C (*Shigella sp*); death in a few minutes at a temperature of 55º C (*Taenia Saginata*); death in 50 minutes at 45º C (*Necator Americanus*); death in less than 60 minutes at a temperature greater than 50º C (*Ascaris lumbricoides* eggs), according to Xu *et al*., 2010 and Impe *et al*., 2018.

The removal of parasites is usually obtained from the sedimentation of protozoan cysts and helminth eggs. But, some factors can still affect the action of effluent disinfectants in liquid mass as an initial mixture, contact time, concentration, type of chemical agent or intensity, and nature of the physical agent, temperature, number, and type of organisms involved (SENGUPTA, 2011).

If this strategy of autoclaving sludge by-products became a methodology used on a large scale for its biological decontamination in our country, it would certainly not be considered prohibited the application of this material derived from domestic sewage for the cultivation of flooded crops such as rice, vegetables such as lettuce, cabbage, cauliflower, and other vegetables, tubers and other roots for human consumption (BRASIL, 2006).

However, even with good practices for the decontamination of this sewage sludge for reuse in agriculture, there is a need for incentives from regulatory bodies, so that industries are aware of their role in society, seeking to invest and insert their operations in equipment and materials that allow the biological decontamination of this material, minimizing environmental impacts with sustainable and safe actions.

Thus, the method of sterilization of sewage sludge presented here must be analyzed with criteria, to adopt the most viable practice in a technical, economic, and environmental way. The present study has some limitations as the presence of bacteria and viruses was not evaluated in the samples. In addition, due to the high amount of sludge waste produced by the treatment plant, the costs and economic feasibility of

autoclaving disinfection were not evaluated, since this process consumes water and electricity.

CONCLUSION

The chemical treatment proposed with the use of acetone, chloroform, and hexane solvents were not effective in reducing or eliminating the parasite load in the tested samples. Autoclaving remains an efficient method for eliminating parasites in sewage sludge samples.

New tests with different solvents or solutes are necessary to enable the introduction of sewage sludge into the production chain in places where autoclaving is not accessible.

Therefore, it is essential to make society aware of sustainable practices, to protect the environment as well as public health, develop and improve cost-effective technologies as an alternative integrated management of sectors such as environment, agriculture, and health to expand and share responsibilities.

Due to the scarcity of studies that address new methods of eliminating the parasitic load contained in sewage sludge samples used in agriculture, it is evident that the present study is a pioneer in proposing this possibility and alternative. In addition, we suggest that further studies in this direction be encouraged, since the negative pressure method proved to be important for the elimination of different evolutionary forms of parasites in sewage sludge samples, becoming a study that may provide parameters for new studies in this area of knowledge.

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