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Scientific Paper

Microfauna in the activated sludge treatment of the effluent from a soybean processing industry: a form of evaluation and control of the process

Microfauna no tratamento de lodo ativado de efluente de uma indústria de processamento de soja: uma forma de avaliação e controle do processo

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ABSTRACT

Wastewater treatment system by activated sludge, the purification of the organic matter by specific heterogeneous microorganisms, occurs in the presence of oxygen in aeration tanks. The analysis of the microfauna present in the sludge is an important indicator of the performance and efficiency of the activated sludge system. Considering this importance, the objective of this work was to identify the microorganisms which are part of the microfauna present in the aeration tank of the effluent treatment plant of a soybean processing industry in the state of Mato Grosso, evaluating the biological activity of the sludge and the efficiency of the process. Protozoa and bacteria were identified through electronic microscopy and physical-chemical analyses such as pH, dissolved oxygen, nitrogen, phosphorus, BOD, COD, suspended solids, and SVI. During the study, it was possible to identify bacteria related to flake formation, organic matter degradation, nitrification and denitrification, and also microorganisms such as thecamoebas, micrometazoa, fixed ciliates and ciliates free natantes, characterizing the process with good purification and the structure of the sludge flakes between ideal and filamentous bulking with a high sludge age. The identification of the microfauna present was efficient as a bioindicator of the treatment system condition with removal of BOD and COD of 59.75 and 67.09%, respectively.

Keywords: treatment plant; microorganisms; efficiency.

RESUMO

No sistema de tratamento de efluentes por lodo ativado ocorre a depuração da matéria orgânica por microrganismos heterogêneos específicos, em presença de oxigênio nos tanques de aeração. A análise da microfauna presente no lodo é um importante indicador do desempenho e da eficiência do sistema de lodos ativados. Diante disso, o objetivo do trabalho foi identificar os microrganismos que compõem a microfauna do tanque de aeração da estação de tratamento de efluentes de uma indústria alimentícia processadora de soja do estado de Mato Grosso, avaliando a atividade biológica do lodo e a eficiência do processo. Foi realizada a identificação dos protozoários e das bactérias pela microscopia eletrônica, e fizeram-se as análises físico-químicas de pH, oxigênio dissolvido, nitrogênio, fósforo, demanda bioquímica de oxigênio, demanda química de oxigênio, sólidos suspensos e índice volumétrico de lodo. Durante o estudo, foi possível observar as bactérias relacionadas na formação do floco, na degradação da matéria orgânica, na nitrificação e na desnitrificação, e também microrganismos como as tecamebas, micrometazoários, ciliados fixos e ciliados livres natantes, que caracterizam o processo com alta idade de lodo, boa depuração e alta concentração de oxigênio dissolvido. A identificação da microfauna presente mostrou-se eficiente como bioindicadora da condição do sistema de tratamento, observando-se que o sistema da indústria se apresentou com boa depuração e com remoção de demanda bioquímica de oxigênio e demanda química de oxigênio, de 59,75 e 67,09%, respectivamente.

Palavras-chave: estação de tratamento; microrganismos; eficiência.

INTRODUCTION

Effluent treatment systems by activated sludge are based on the purification of organic matter by specific heterogeneous microorganisms, in the presence of oxygen in aeration tanks. This process presents high removal efficiency of organic matter, solids, and other components, in addition to possibility of nutrient removal via nitrification and small area of implantation when

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compared with other treatment processes (DEZOTTI, 2008; MORETTI *et al.*, 2016); therefore, it prevails among the currently employed biological systems (SOWINSKA *et al.*, 2017).

Activated sludge consists of flakes, which are a complex microsystem composed of a mixed population of microorganisms containing many species of viruses, bacteria, protozoa, fungi, metazoa, and algae (NIELSEN; SERVIOUR, 2010; SOWINSKA *et al.*, 2017; SATOH *et al.*, 2021). These microorganisms are important both in terms of their function and competition with filamentous bacteria, which often cause serious problem in the system. Bacteria play the most important role, deteriorating the complex substrates, and are responsible for structuring the flakes. However, protozoa and metazoa contribute to turbidity and Biochemical Oxygen Demand (BOD) reductions of the effluent, mainly in the flocculation process (PAYANDEH; MEHRDADI; DADGAR, 2017).

Microscopic analysis of the activated sludge allows both the identification of the microfauna present and the determination of the physical characteristics of the flakes (CYDZIK-KWIATKOWSKA; ZIELIŃSKA, 2016). This microbiological evaluation provides information of the sludge biological activity, which is crucial to properly address any strategy directed to control the operation of the activated sludge system, as well as projections of future performance levels, avoiding sludge quality problems, such as poor sludge settling, compaction, and dewatering (KOIVURANTA *et al.*, 2015; MESQUITA; AMARAL; FERREIRA, 2016; BURGER *et al.*, 2017).

The predominance of one or two species or the rapid depletion in microfauna diversity are relevant indicators of effluent toxicity (KOKINA *et al.*, 2022). Information gathering is particularly important due to the lack of microbiological data (FOISSNER, 2016), especially for industrial effluents from agricultural products, constituting a quick, simple, valuable, and integrative indicative method on the performance of the treatment plant.

The application of microscopic analysis in treatment plants (aerobic, anaerobic or combined systems) can be very useful for understanding the metabolic process of the effluent to be treated, making it possible to identify the causes of operational problems, such as poor settling, by the identification of filamentous microorganisms (SOWINSKA *et al.*, 2017).

Several studies have demonstrated the correlation of the performance parameters of activated sludge wastewater treatment plants through the exploration of microfauna (specific microbial taxa) (FRYER; GRAY, 2012; SANTOS *et al.*, 2014; CASTILLO; MEDINA; CONTRERAS, 2016; CYDZIK-KWIATKOWSKA; ZIELIŃSKA, 2016; AMARAL *et al.*, 2018). As activated sludge microbial communities vary widely based on plant operating technology, influent characteristics, and treatment plant capacity (AMARAL *et al.*, 2018; BEGMATOV *et al.*, 2022), research on the identification of microbial species in activated sludge systems, especially in agricultural industrial sewage stations, is of great importance.

Therefore, the present work had the objective of identifying the microfauna present in the activated sludge of a soybean processing food industry located in Mato Grosso, evaluating the biological activity of the sludge and the efficiency of the process.

MATERIAL AND METHODS

Effluent generation and treatment

The effluent treatment plant (ETP) of the soybean processing industry treats effluents from the soybean protein concentrate processes (SPC), with the production of soybean concentrate protein and molasses, and from the extraction process, which performs the manufacture of "hipro" and "hifiber"soybean meal, soybean lecithin, and degummed oil.

The SPC process effluent consists of caustic soda from Clean-In-Place (CIP) cleaning; micelles (sugars and ethanol) in case of possible pumps leaks in the concentration process; and condensed steam from plant traps.

The effluent from the extraction process consists of condensed steam from the kettle, from the plant traps, and from the drain of the lecithin dryer; oil from possible leaks of oil hydrator; gum from possible leaks in the centrifuge; micelle (oil and hexane) in case of possible pumps leaks in the concentration process.

The treatment of these effluents in the ETP is performed in steps: first the effluent flows into the equalization tank, where homogenization takes place. Then, the effluent is treated by a dissolved air flotation system, which is subsequently sent to the anaerobic lagoon, where a series of reactions are triggered by a diversified culture of anaerobic microorganisms.

The effluent follows from the anaerobic lagoon to the aeration tank, where the aerobic microorganisms of the activated sludge perform the degradation of the dissolved organic load of the effluent. Oxygen, necessary for the respiration of these microorganisms, is supplied by air blowers and distributed in the tank by membrane diffusers.

The existing biomass in the activated sludge system, when observed under the microscope, is variable over time, due to oscillations in the characteristics of the effluent entering the system. It is necessary to have the physical-chemical control of the aeration tank, as well as the microbiological analysis of microfauna present in the sludge, to verify the occurrence of organic overloads, changes in oxygen availability, pH, settling of the sludge, *i.e.*, process stability as a whole (ROCHA *et al.*, 2016).

Subsequent to the aeration tank, the following step is the secondary decanter, where the physical separation between solid and liquid occurs, producing a clarified liquid (final effluent). The sludge from the secondary decanter is recirculated to the aeration tank (increase the solids concentration) denominated biological sludge or will be removed (called exceeding sludge) to maintain the equilibrium of the system.

Identification of microfauna in activated sludge system

The microorganism's identification present in the activated sludge system of the ETP was carried out on 10/13/2017, 10/23/2017, 10/27/2017, and 10/30/2017 at the biology laboratory of *Instituto Federal de Mato Grosso* (IFMT), Sorriso campus.

Samples for microorganism's identification were collected in the aeration tank and stored in polyethylene bottles, which remained open and refrigerated during transportation to the IFMT laboratory, not exceeding 2 hours between collection and analysis.

An aliquot of the mixture (effluent and sludge) was placed on the blade and analyzed on the microscope (Kozo - XJS900) and, when necessary, a methylene blue solution was added for better visualization. The images analyzed, when possible, were recorded by photomicrographs using an Industrial Digital Camera (UCMOS - FMA-050).

Physical-chemical analysis in the activated sludge system

Physical-chemical analyses of the aeration tank were carried out on 10/13/2017 and 10/23/2017, based on the following parameters: dissolved oxygen (DO) was measured with an oximeter (Digimed - DM4P-V1.7), inlet and outlet aeration

tank; pH was monitored with a pHmeter (Gehaka - PG1800). The parameters biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were monitored inlet and outlet of the biological treatment, nitrogen and phosphorus were monitored in the secondary decanter, all only on 10/13/2017.

Sludge volume index (SVI) indicates sludge settleability in the aeration tank and was analyzed only on 11/06/2017. This parameter is calculated based on solids settled by the Cone Imhoff method (V30) and suspended solids (Xe). The SVI was determined according to Equation 1 (VON SPERLING, 2012).

$$SVI\ (mL.g^{-1}) = \frac{V_{30}}{X_e.\ V_c} \quad (1)$$

Where:

V_{30} = volume occupied by the sludge after 30 minutes of sedimentation (mL);

X_e = concentration of solids in the sample, expressed as total suspended solids (TSS) or volatile suspended solids (VSS), ($mg{\cdot}L^{-1}$);

V_c = volume of liquid initially contained in the Cone Imhoff (L).

RESULTS AND DISCUSSION

BOD and COD results of the effluent inlet and outlet of the aeration tank on 10/13/2017 are presented in Table 1. It was observed that the values were low for both BOD and COD, representing low organic load in the tank. The reduction obtained from BOD and COD parameters by biological treatment represented 59.75 and 67.09%, respectively.

The BOD value at the entrance of the aeration tank process was below the value required by the National Environmental Council (*Conselho Nacional do Meio Ambiente* – CONAMA) No. 430 (BRASIL, 2011), which is 120 $mg{\cdot}L^{-1}$ for the discharge of effluents in water bodies. As the industry under study aims at subsequent water reuse in gas scrubbing systems, boiler ash transport and in the future for use in plant cooling towers, the treatment must achieve the largest possible reduction in BOD.

Table 2 shows the DO values in the aeration tank, which remained high during the analyses in relation to the ones recommended in the literature, which is to maintain the DO at 1.5 to 3 $mg{\cdot}L^{-1}$ for activated sludge systems (DEZOTTI, 2008; SOARES *et al.*, 2014). Low DO concentrations can provide the appearance of filamentous bacteria, which can cause sludge bulking (LIU; WANG; CAMPBELL, 2018).

Table 1 – Biochemical Oxygen Demand and Chemical Oxygen Demand results.

Sample	BOD ($mg{\cdot}L^{-1}$)	COD ($mg{\cdot}L^{-1}$)
Inlet aeration tank	39.75	147.70
Outlet aeration tank	16.00	48.60

BOD: Biochemical Oxygen Demand; COD: Chemical Oxygen Demand.

Table 2 – Dissolved Oxygen results.

Sample	DO 10/13 ($mg{\cdot}L^{-1}$)	DO 10/23 ($mg{\cdot}L^{-1}$)
Inlet aeration tank	6.8	4.5
Outlet aeration tank	7.3	6.5

DO: Dissolved Oxygen.

The values of the nutrients, nitrogen and phosphorus, at the outlet of the aeration tank (decanter) on day 10/13, were elevated (Table 3). In aerobic processes, a minimum BOD5: N:P ratio of 100:5:1 is required, the nitrogen requirement is 0.8 $mg{\cdot}L^{-1}$, and the phosphorus is 0.16 $mg{\cdot}L^{-1}$.

The phosphorus value of this study (11.50 $mg{\cdot}L^{-1}$) was similar to that found by Rocha *et al.* (2016), in a study in the ETE of an agroindustry in Santa Catarina, that ranged from 7.21 to 10.67 $mg{\cdot}L^{-1}$, whereas for nitrogen the value found (44 $mg{\cdot}L^{-1}$) was much higher in relation to these authors', ranging from 2.8 to 16.40 $mg{\cdot}L^{-1}$.

The pH in the aeration tank on day 10/13 was 8.1 and on day 10/23, it was 7.6. Both results were within the ideal range, since most microorganisms develop well in a pH environment between 6-9 (MARX *et al.*, 2012).

For the SVI analysis, in 11/06, values of 22 $mL{\cdot}L^{-1}$ of settling solid and 323 $mg{\cdot}L^{-1}$ of total suspended solids were obtained. Thus, it obtained an SVI of 68.11 $mL{\cdot}g^{-1}$, classifying the effluent with good settleability according to Dezotti (2008). The SVI classification is based only on the literature, as it is not an official analysis, being therefore necessary to analyze other factors for the classification of the settleability.

Regarding the analysis of activated sludge flakes, there were no variations during the period analyzed, remaining dispersed and without filaments (Figure 1) and low concentration of total suspended solids (323 $mg{\cdot}L^{-1}$). Dispersed flakes are an important indicator of inadequate settleability conditions of the sludge and, consequently, inadequate clarification (WANG *et al.*, 2018).

Another factor that may contribute to the poor quality of the flakes is the presence of chemical compounds in the effluent (PENG *et al.*, 2021), such as hexane from the soybean extraction process and SPC ethanol. These compounds affect not only the microbial activity, but also the bioflocculation phenomenon, resulting in the formation of small flakes (TAMBURUS *et al.*, 2020).

Flocculation can also be affected by excess nutrients, as this environment impairs the flocculation performed by bacteria.

In the identification and quantification of the bacteria present in the activated sludge (Table 4), it was possible to verify the presence of heterotrophic, autotrophic nitrifying, filamentous bacteria, and bacilli.

Table 3 – Nutrients results.

Sample	Total nitrogen ($mg{\cdot}L^{-1}$)	Total phosphorus ($mg{\cdot}L^{-1}$)
Outlet aeration tank	44	11.5

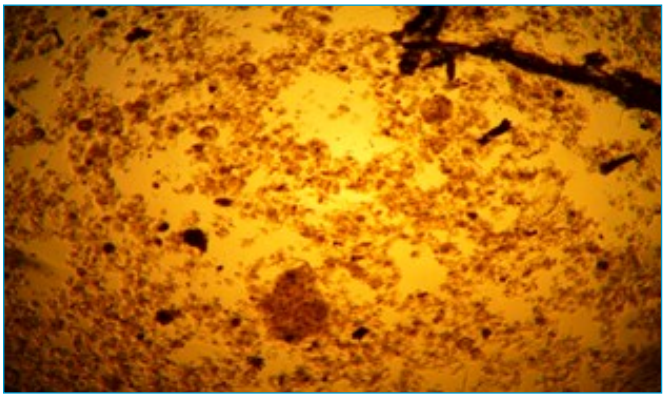

Figure 1 – Activated sludge flakes (5x).

Heterotrophic bacteria, such as *Zooglea*, *Pseudomonas*, *Flavobacterium*, *Achromobacter*, and *Chromobacterium*, were present in greater quantity than autotrophic bacteria, since the former generally have higher growth and cellular production rates when compared to autotrophic nitrifying bacteria (GERARDI, 2006; SHENG *et al.*, 2018).

The nitrifying bacteria present in the system were *Nitrosomonas* and *Nitrobacter*, which perform the ammonia oxidation to nitrate. This process is divided into two stages, in the first one, ammonia oxidizing bacteria (*Nitrosomonas*) produce nitrite, which in turn will be oxidized to nitrate by nitrite oxidants (*Nitrobacter*) (SANTOS *et al.*, 2020).

In relation to the bacteria that can perform denitrification, which occurs when nitrites and nitrates are reduced to gaseous nitrogen (SANTOS *et al.*, 2020), one can mention those of the genera *Achromobacterium*, *Bacillus*, *Pseudomonas*, and *Chromobacterium* that were visualized in this study.

However, the denitrification process occurs under anoxic conditions, *i.e.*, in the absence of oxygen and in the presence of nitrates (DEZOTTI, 2008). The system under study does not have the adaptation for this condition and it is not observed that this process can be occurring in the decanter, since it does not have the flotation of the sludge in the secondary decanter due to the release of gaseous nitrogen.

As previously seen, the value of nitrogen in the secondary decanter was high, 44 mg L^{-1}, contributing to the hypothesis that, although there are bacteria that perform denitrification, nitrogen removal may not be happening or not be efficient, emphasizing that the nitrogen value of the raw effluent was not analyzed in the present study.

Regarding filamentous bacteria, only the genus *Beggiatoa* was found in few quantities. A high number of filamentous bacteria, in relation to flocculant bacteria, can impact the physical properties of flakes and, consequently, the settleability of the flakes in the secondary clarifier (BURGER *et al.*, 2017).

Gerardi (2006) also emphasizes that insufficient amounts of filamentous bacteria result in the formation of very small flakes, which are dispersed in the liquid phase. Thus, the fact that the flake of the present study is dispersed, as previously analyzed, justifies this.

The presence of free ciliates, fixed ciliates, rotifers, thecamebas, worms, and unicellular algae were observed in the three-day microbiological characterization concerning protozoa and micrometazoa in the aeration tank, and only on 10/30 was the presence of microcrustaceans and flagellates observed.

The free ciliates (Figure 2) found are free ciliates natantes, which are associated with effluents with a high concentration of free bacteria, and should not be dominant in a fully operational system, and the crawlers that are the predators of the flake, are characteristic of a system with good operational conditions and efficient removal of organic matter (ZHOU *et al.*, 2008; PONCE-ROBLES *et al.*, 2018).

Among the crawlers, it was possible to visualize ciliate similar to *Aspidisca sp.*, an organism that feeds on bacteria and suspension particles and can indicate the possible occurrence of the nitrification process in the system, appearing under conditions of low organic load and high oxygen concentration (PONCE-ROBLES *et al.*, 2018; WALCZYŃSKA *et al.*, 2018).

These free ciliates were present in small quantities in the three analyses, since these protozoa are more frequent in the process implantation phase, and the ETP in study has been operating for about 5 years.

Small amounts of pedunculated or fixed ciliates were also identified (Figure 3). Among them, it was possible to identify the protozoan similar to *Vorticella*, which occurs in systems with good efficiency and well-oxygenated sludge (LEE *et al.*, 2004; PAYANDEH; MEHRDADI; DADGAR, 2017; PONCE-ROBLES *et al.*, 2018; JIANG *et al.*, 2021).

Ciliates similar to *Acineta sp.*, which are organisms that have a fixed peduncle to the biological flake, are present in activated sludge systems with advanced age, good quality sludge, and low organic load (SILVA; SANTOS; CHAVES, 2019).

Table 4 – Bacteria identification in aeration tank.

Bacteria	Quantity (number of bacteria m L^{-1})
Nitrosomonas	3.1×10^3
Nitrobacter	4.8×10^4
Zooglea	2.7×10^4
Pseudomonas	5.2×10^3
Achromobacterium	6.3×10^4
Chromobacterium	2.9×10^5
Beggiatoa	1.1×10^1
Bacillus	7.7×10^5
Flavobacterium	2.1×10^3

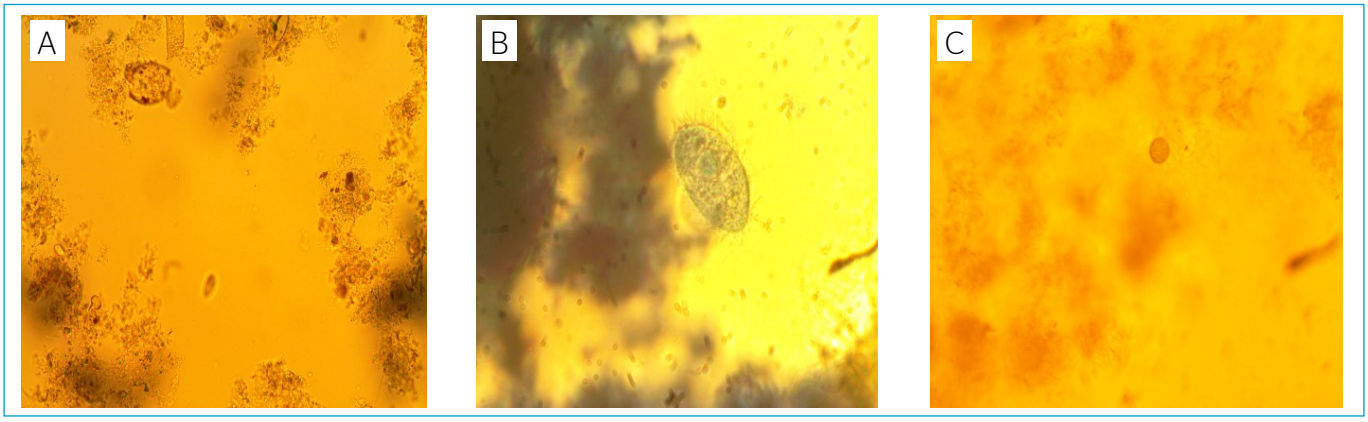

Figure 2 – (A) Free ciliates natantes (20x), (B) Crawling ciliates (40x; methylene blue), (C) Crawling ciliate similar to Aspidisca sp. (20x).

Among the micrometazoa, small amounts of rotifers and worms were visualized in all sludge analyses (Figure 4). The observed worms were similar to the *Gastrotricha phylum* family, which occur in nitrification activated sludge systems, since they are susceptible to ammonia toxicity (JENKINS; RICHARD; DAIGGER, 2003).

The rotifers present in the system were similar to the *Philodina roséola*, *Rotaria citrinus* and *Epiphanes senta* genera, which are efficient enough in the consumption of bacteria dispersed or adhered to the flakes, as well as of small particles of organic load (METCALF & EDDY, 1991; AZEVEDO *et al*., 2022).

According to Bento *et al*. (2005), Ponce-Robles *et al*. (2018), and Azevedo *et al*. (2022), these micrometazoa present slow growth and reproduction rates, consequently high cellular detention time, and therefore higher ages of the sludge. These factors are evidenced in the aeration tank of this study, since it was designed for a high sludge age, ranging from 15 to 20 days.

Another microorganism identified was the microcrustacean similar to *Daphnia magna* (Figure 5), known as water flea, measuring from 0.5 to 5 mm. These microorganisms are considered filtering organisms, their legs are consisted of bristles which act as sieves that retain algae, bacteria, and small particles of organic material from the water, acting on the aquatic food chain as the primary consumer among the metazoans (LAITANO; MATIAS, 2006).

The presence of microscopic green algae and unicellular algae was also visualized (Figure 6) mainly in recirculation sludge, which although not common in

Figure 5 – Microcrustacean similar to Daphnia magna (40x).

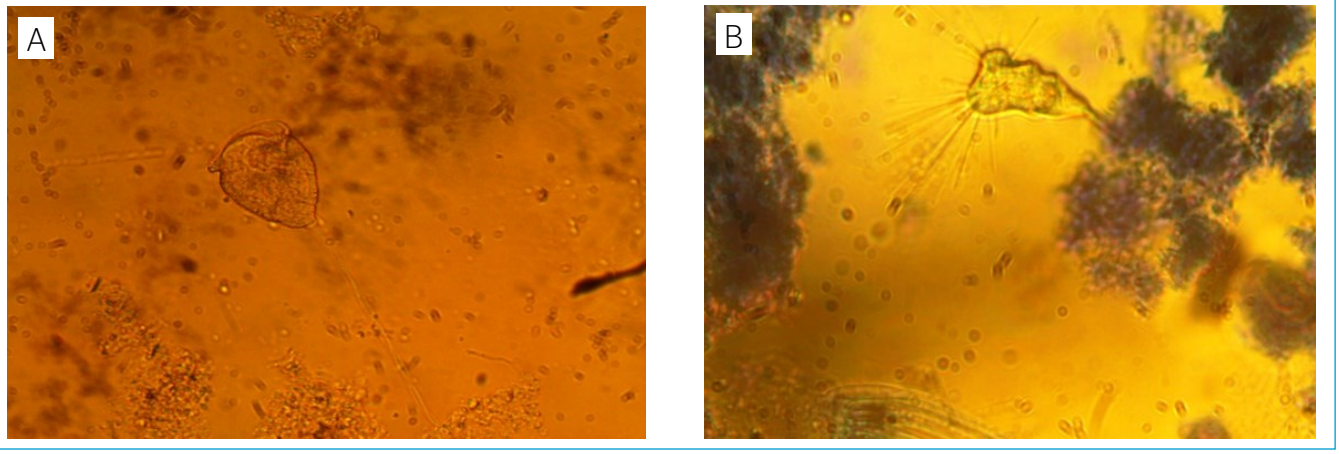

Figure 3 – (A) Pedunculated ciliate similar to Vorticella sp. (40x), (B) Pedunculated ciliate similar to Acineta sp. (40x; methylene blue).

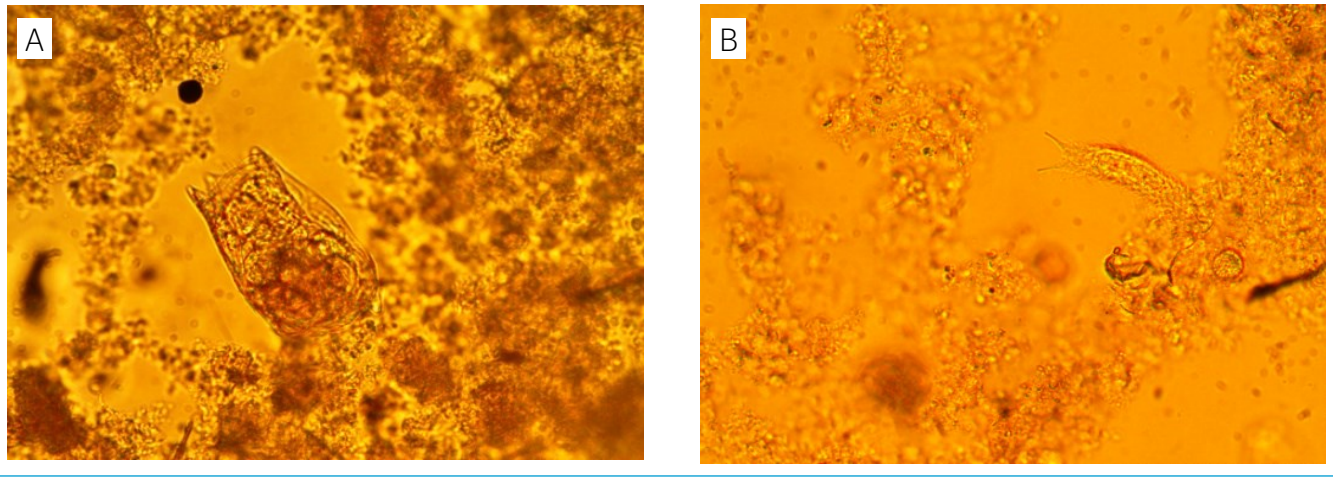

Figure 4 – (A) Rotifer similar to Philodina roséola (40x), (B) Worm similar to Gastrotricha family (20x).

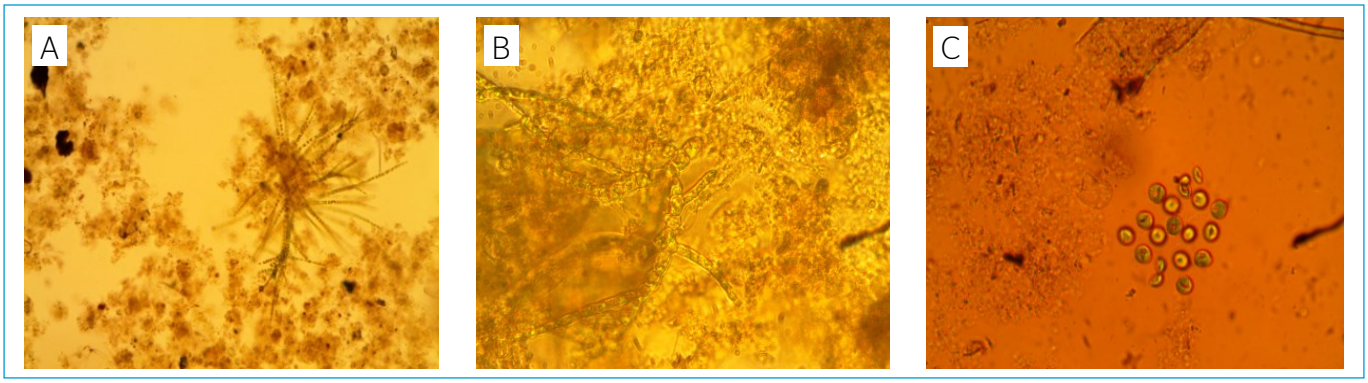

Figure 6 – (A) Green algae (20x), (B) Green algae (40x), (C) Unicellular algae (20x).

the process due to turbidity, their appearance, according to Nunes (2012), may be due to the recirculation of the sludge from the final settler to the aeration tank.

Regarding amoebas, thecamoebas were seen in abundance in relation to other microorganisms, present in sludges with low organic load, with nitrogen removal, and with high available oxygen content (MADONI, 1994; AZEVEDO *et al.*, 2022).

CONCLUSIONS

The identification of microfauna in activated sludge indicated the presence of heterotrophic, autotrophic nitrifying, filamentous bacteria, and bacilli. Heterotrophic bacteria were more predominantly present than autotrophic ones.

The presence of dispersed flakes without filaments and the low concentration of total suspended solids were observed, being important indicators of sludge settling inadequate conditions and the presence of chemical products in the soybean industry effluent.

The occurrence of protozoan similar to Vorticella indicated a high degree of biological stability of the system, with good efficiency and well-oxygenated sludge.

The presence of ciliates similar to Acineta sp., micrometazoans, and thecamebas indicated high cell retention time in the process (advanced age sludge), good quality, and low organic load.

The microorganism's identification demonstrated that protozoa are great bioindicators of the condition present in biological reactors. These biological parameters are important in the evaluation and characterization of the raw effluent to be treated, proving that the system has high sludge age, low organic load, and high dissolved oxygen and nitrogen concentration.

The industry's biological process was efficient, with good purifying conditions and BOD removal, since the system works with low BOD and COD values, and consequently with low loads and low suspended solids, so that it is normal to have identified low amounts of microorganisms.

Global and systemic evaluation of the sludge is important in the qualitative analysis of the system's microbiota. The presence of a single microfauna species should often not be used as an indicative of process performance.

The immediate characterization of the treatment system's purification conditions can be obtained by the qualitative analysis of the sludge, including the general aspects of the flakes concomitantly with the identification of the dominant species.

AUTHOR'S CONTRIBUTIONS

Seidel, L.B.: conceptualization, data curation, formal analysis, investigation, methodology, writing — original draft, writing — review & editing. De Carli, E.M.: supervision, data curation, formal analysis, investigation, methodology. Santos, B.K.: data curation, formal analysis, investigation, methodology, supervision, validation, visualization. Dornelas, K.C.: visualization, writing — original draft, writing — review & editing. Bongiovani, M.B.: conceptualization, project administration, data curation, software, formal analysis, investigation, methodology, writing — original draft, writing — review & editing.

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