

# Treatment of Effluent of the Cellulose and Paper Industry Using Aerobic Granular Sludge Thermophilic

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**Abstract**— Large amounts of effluents are generated in pulp and paper mills. In general, these effluents present temperatures above 40 °C, which may preclude biological treatment in mesophilic conditions (35 °C). For this, the present work aimed to produce aerobic granules in mesophilic conditions and, after their stability, leads them to thermophilic conditions. After the thermophilic granules were obtained, analyzes of their maintenance, treatment efficiency and verification of the production of the extracellular polymeric substances (carbohydrates, proteins and humic acids), present in the granules under these conditions were carried out. It was observed that the granules, generated from the biological sludge and the effluent of a pulp and paper industry, can act up to a temperature of 45 °C, presenting a removal rate of around 70%, without great loss of efficiency of the proposed treatment compared to that of mesophilic flocculent sludge.

**Keywords** — Aerobic granules, Paper, Sludge, Thermophilic, Treatment .

## I. INTRODUCTION

The pulp and paper industries use a large amount of water in their processes, around 60 m<sup>3</sup> of water per ton of pulp, and although they present modern and efficient operating techniques for the production of one ton of paper, In the generation of large volumes of effluent. The effluent from pulp and paper mills is the sixth largest polluter because it contains a variety of liquid and solid waste that is discharged into the environment[1].

The development of aerobic granule technology has been studied as a method to improve conventional activated sludge processes, mainly because of its greater efficiency in the removal of organic matter[2].

The low space requirement, simplicity and operational flexibility of granular aerobic sludge systems make it

possible to apply this technology in the treatment of sewage in communities and industries that have little availability of physical space [3].

Aerobic granules have been developed with mixed cultures (such as activated sludge) or defined cultures. Aerobic granulation has been observed in reactors fed with various organic substrates (phenol, ethanol, glucose, acetate, etc.) and industrial wastewater. The microbial structure of the granules is dependent on the inoculums, chemical composition, dissolved oxygen concentration (OD) and the size of the granules. A conceptual granule is composed of three microbial communities, aerobic bacteria that grow on the outside, bacteria that oxidize ammonia in the medium and facultative / anaerobic in the center of the granules [4; 5 and 6].

The microbial community of aerobic granular mud can range from species limited to extremely diverse species and are strongly affected by substrate composition or wastewater, inoculums, and environmental and operational factors [3].

The size of the aerobic granular sludge may influence the distribution of the microbial community structure in the granules. The OD concentration also influences the microbial structure of the granular sludge. OD deficiency favors the growth of different filamentous bacteria during granulation. Different temperatures result in different dominant microorganisms. The microbial community structure at 25 °C exhibits less similarity to those at other temperatures [7].

The process of formation of the aerobic granules is determined by the presence and action of the extracellular polymeric substances (EPS), which act as an important factor in the characteristics of microbial aggregates in effluent treatment processes [8].

The formation of aerobic granules in effluent treatment processes has been the subject of studies, although there

are controversies in the literature on the ideal conditions for their formation. Another aspect not yet well clarified has been the characterization of the microorganisms that aid in the aggregation of the granules.

Few studies exist on the capacity of formation of aerobic granules under thermophilic conditions (temperature between 40 and 60 °C). The search for microorganisms able to aggregate in the form of granules under these temperature conditions can constitute an important advance for the treatment of industrial effluents. The pulp and paper industries generally discharge an effluent stream that is relatively warm (50 °C) when compared to effluents from other industries. Some researchers have attempted to treat pulp and paper effluents at the thermophilic temperature (in which they are discharged) in an attempt to reduce energy costs for treatment. [3; 6 and 9].

Based on this, the objectives of this work were: To produce thermophilic aerobic granules, used in the effluent treatment of the Pulp and Paper Industry; determine the microbial diversity in aerobic granules and quantify the major chemical components of the EPS produced in the process.

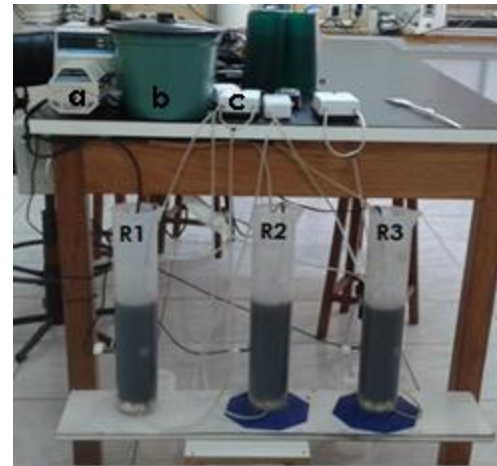
## II. MATERIAL AND METHODS

### 2.1. Production of thermophilic aerobic granules

Three reactors (R1, R2 and R3) with a useful volume of 2 liters were used, each reactor was initially fed with 800 mL of effluent and same volume of biological sludge from the Effluent Treatment Station (ETE) of the Pulp and Paper industry. Papel Celulose Nipo-Brasileira S / A (CENIBRA®). One of the reactors (R1) was maintained under typical process conditions with activated sludge to form flocculent sludge, and the other two reactors (R2 and R3) were operated with parameters that favored the formation of aerobic granular sludge [10].

Initially all reactors were maintained at 35 °C under aeration in order to maintain the dissolved oxygen content (OD) above 2.00 mg.L<sup>-1</sup>, with reaction cycles of 12 hours, where each cycle 300 ML of treated effluent was withdrawn and 300 mL of new effluent were added. Before addition, the effluent was supplemented with nitrogen and phosphorus to maintain the COD: N: P ratio of 250: 5: 1. After the addition, the aerators were reconnected, starting a new cycle. The pH of the reaction mixture was corrected to maintain in the range of 6.5-7.5. All reactors were operated with a settling time (ST) of 1 hour at the end of each cycle of 12 hours, initially. In the reactor operated for the formation of flocculent sludge (R1), this time was maintained until the end of the experiment. For the reactors operated for the formation of aerobic granular sludge (R2 and R3) the time was decreased, every three days, to 30, 20, 10, 5, 3, 2 and 1

minute (s), the latter being the time indicated for the formation and maintenance of aerobic granular sludge [10]. After the formation of the granules the time of 1 minute for decantation was maintained until the end of the experiment.



*Fig.1: Structure for the operation of the three reactors used to treat paper and pulp industry effluent via flocculent sludge (R1) and mesophilic /thermophilic granular aerobic sludge (R2 and R3). A) Peristaltic pump, for feeding the reactors to each cycle; B) Storage of the effluent to be added before the start of each cycle and c) Aerators, for maintenance of the DO.*

With the aerobic granules formed, the temperature of 35 °C was maintained in the reactors R2 and R3 for one week. After that, the temperature was increased at a rate of 1 °C per day until the next study temperature (40 °C), this temperature was maintained for one week and the increase was increased at the same rate until the Temperature reached 45 °C. The procedure was repeated for temperatures of 50 and 55 °C.

In order to verify the efficiency of the proposed treatment, samples of the treated effluent were collected daily from all three reactors, and chemical oxygen demand (COD) analyzes were carried out.

The analyzes were performed in triplicates, per cycle of each reactor, following the colorimetric method, after closed reflux (APHA method 5220D).

In order to verify the efficiency of the proposed treatment, COD analyzes were performed on the system as well as at the end of each cycle. The difference between the input COD and the treated effluent was defined in percentage values and indicated as a percentage of COD removal.

The processes of formation and maintenance of the granules, as well as the stages of temperature change in order to obtain thermophilic granular sludge, and the COD analyzes, were carried out in the Laboratory of Environmental Analysis of the Pulp and Paper Laboratory of the Federal University of Viçosa (UFV).

## 2.2. Aerobic granular sludge mesophilic

Fifteen samples were collected in each reactor, each sample was related to the target reactor temperatures for the production and analysis of thermophilic aerobic granules. Table 1 shows the relationship between collection points and associated temperatures. Taking into account the behavior of the organic matter removal efficiency (COD) at the temperatures studied in the reactors R2 and R3, those of interest were defined, since in the case of reactors R2 and R3, the target temperatures represent: Point 1 -Formation of the granules and start of treatment with aerobic granular sludge; Point 3-Apex of the treatment, indicated by the rate of removal of organic matter; And Point 5-Temperature in which there is a marked decay of the removal rate. From this definition, 09 samples were selected and submitted to analysis of the structure of the formed granules and analysis of the production and quantification of extracellular polymer substances at each target temperature during the proposed treatment.

*Table 1. Relationship between collect points and sample temperatures of each reactor during pulp and paper industry wastewater treatment by flocculent sludge (R1) and mesophilic and thermophilic granular aerobic sludge (R2 and R3).*

Collect point	Reactor 1 (flocculent)	Reactor 2 (granular)	Reactor 3 (granular)
1	35 °C	35 °C	35 °C
2	35 °C	40 °C	40 °C
3	35 °C	45 °C	45 °C
4	35 °C	50 °C	50 °C
5	35 °C	55 °C	55 °C

## 2.3. STRUCTURAL ANALYSIS OF AEROBIC GRANULAR SLUDGE

The nine samples (Table 1, 3 samples from point 1, 3 of point 3 and 3 of point 5) were submitted to the structural analysis step of the obtained granules, via scanning electron microscopy and confocal microscopy, performed at the Center of Electron Microscopy Of the State University of Santa Cruz (CME-UESC). For this purpose, the samples were fixed with respective fixative buffer for scanning and confocal electron microscopy, and then stored.

### 2.3.1. Scanning Electron Microscopy (SEM)

The granules were fixed in cacodylate-glutaraldehyde buffer (2.5%), followed by washing twice with 0.1M cacodylate buffer. The samples were dehydrated by a series of water-acetone (50-100%), then they were submitted to the critical point, for the removal of all the

water, being mounted in Stubs using carbon double face tapes. The assembled samples were metallized by depositing a thin layer of gold through an evaporation system known as "sputtering" using the Sputter Coater SCD 050 (Bal Tec) apparatus. At the end, the samples were examined with Scanning Electron Microscope (SEM), model Quanta 250 (FEI Company), operated with an acceleration voltage of 20 KV.

### 2.3.2. Confocal Microscopy

The samples were stained in microtubes (1.5 mL) covered with aluminum foil and placed in a shaker rack (100 rpm, 15 min). The dyes used were fluorescein isothiocyanate (FITC) (0.01%), which is reactive to amines and blends all the proteins and amino sugars of cells and EPS. Concanavalin A (ConA) lectin conjugated with Texas red (100 µg.mL<sup>-1</sup>) binds α-mannopyranosyl and α-glucopyranosyl sugar residues. Syte 63 is a nucleic acid permeant dye of cells. After each staining step, the samples were washed with phosphate buffered saline. The samples were visualized in channels with corresponding excitation and emission wavelengths for each dye using the Confocal LSM 700 Microscope.

## 2.4. EXTRACTION AND ANALYSIS OF GRANULAR AEROBIC SLUDGES EPS

For the EPS extraction step, 1 mL of each sample was transferred to microtubes (2.0 mL), centrifuged (11200 g, 4 ° C, 15 min) and the supernatant was stored in a new microtube for further quantification of free EPS. The pellets were resuspended in 20 ml of phosphate buffer, the pH was adjusted to 11 by the addition of 1M NaOH, followed by heating in a water bath (80 ° C, 30 minutes). After this extraction step, the samples were centrifuged (11200 g, 4 ° C, 10 min) and the supernatant stored for further analysis of the bound EPS [5].

The chemical characterization of the extracellular polymeric substances produced was carried out through the analysis of carbohydrate contents [1] proteins and humic acids [12]. The analyzes were performed in triplicates.

## III. RESULTS AND DISCUSSION

### Efficiency of the proposed treatment

The mean values of COD removal were obtained in the R1 reactor (35 °C) at six collection points (Figure 2), each collect point at R1 was related to the collection point at each study temperature in the R2 and R3 reactors, 35, 40, 45, 50 and 55 ° C.

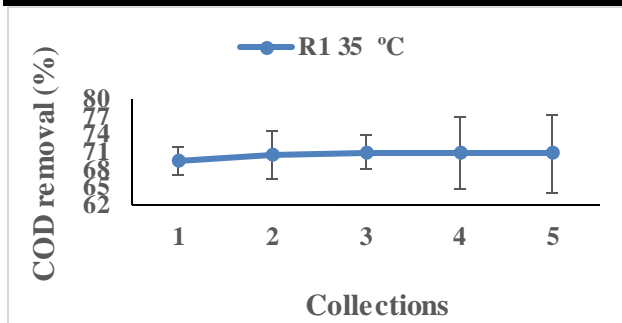


Fig.2: Averages (+ standard deviation) of the COD removal rate at the collection points of study in the reactor R1, with the temperature maintained at 35°C, during treatment of effluent from the pulp and paper industry using mesophilic flocculent sludge.

Fig. 3 shows the results obtained from the analysis of the organic matter removal rate of the collected samples, at the study temperatures, in the reactors R2 and R3.

Based on the Figs. 2 and 3 it was found that there is a difference in efficiency between treatment with granular aerobic sludge and flocculent sludge. Although in the temperatures between 35 and 45 °C the reactors with granular aerobic sludge (R2 and R3) presented a removal rate close to or equal to that found in the treatment with flocculent sludge (R1).

In a study developed by Jungles *et al.*[3] a removal rate of about 75% was obtained using the granular aerobic sludge process in domestic sewage treatment in a sequential batch reactor. A removal rate between 70-75% was verified in an experiment performed by Liu *et al.* [6], during treatment with the same type of domestic effluent. Using the granular aerobic sludge technique in the treatment of effluent from the palm oil industry, Abdullah *et al.* [11] obtained a COD removal rate below

70%. It is noteworthy that, in the works mentioned above, the reactors were operated under mesophilic conditions.

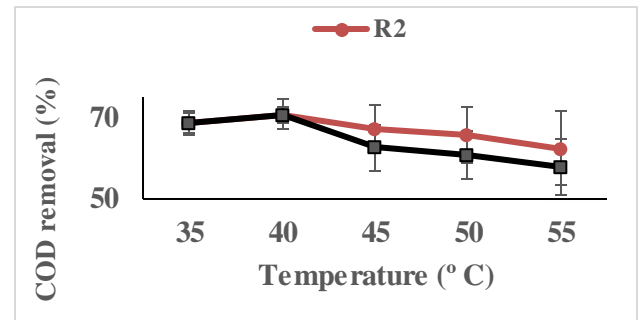


Fig.3: Averages (+ standard deviation) of the COD removal rate at the study collection points in the R2 and R3 reactors, with temperature change, during treatment of cellulose and paper industry effluent using mesophilic and thermophilic granular aerobic sludge.

Thus, in the case of effluent from the pulp and paper industry, which has a higher recalcitrant potential than the effluents studied above, the removal rate (45 °C) of COD between 65-70%, obtained during the execution of this experiment, can be considered as a parameter to prove the efficiency of the treatment with aerobic granular thermophilic sludge.

Above the temperature of 50 °C, the efficiency dropped by 10 percentage points and the final temperature of study (60 °C) decreased by 20 points, characterizing a very low rate for the execution of the process. Thus, the maximum temperature for treatment in thermophilic conditions, without losing much of the efficiency of the treatment, was 45 °C.

#### STRUCTURAL ANALYSIS - SEM

Samples submitted to analysis by scanning electron microscopy produced the following images (Figures 4 to 6).

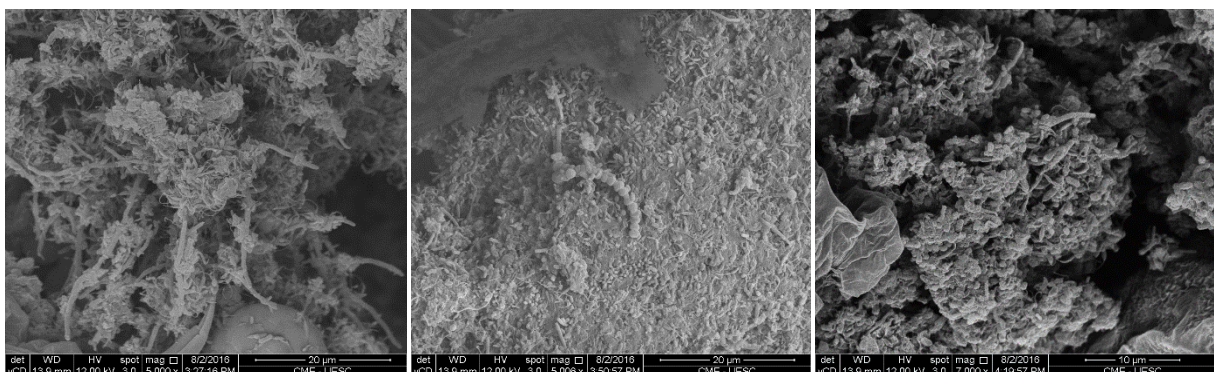


Fig.4: Microscopic structure of the flocculent sludge samples obtained from the reactor R1 operated at 35 °C using scanning electron microscopy.



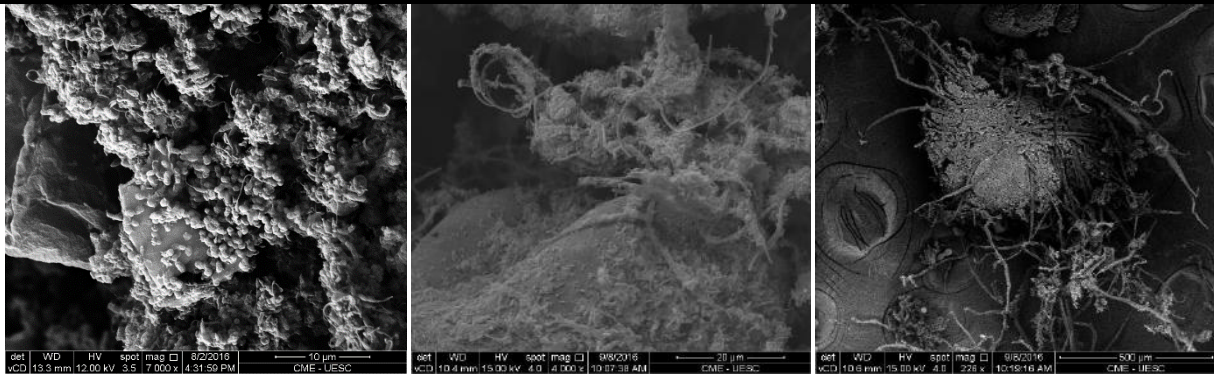


Fig.5: Microscopic structure, using scanning electron microscopy, of aerobic granular sludge samples obtained in reactor R2 operated at different temperatures for thermophilic conditions. a) at 35 °, b) at 45 ° C; And c) at 55 ° C.

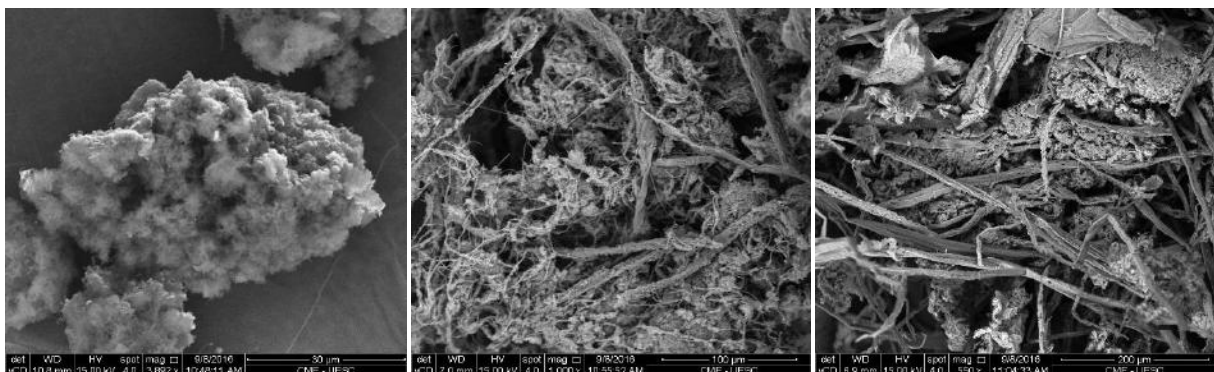


Fig.6: Microscopic structure, using scanning electron microscopy, of aerobic granular sludge samples obtained in reactor R3 operated at different temperatures for thermophilic conditions. A) at 35 °, b) at 45 ° C; And c) at 55 ° C.

In the reactor operated under conditions of flocculent sludge production (R1), maintained at 35 ° C during the experiment (Figure 4), it was observed that there was no change in the structure of the flakes produced and that they had the same configuration of aggregation during development of the experiment.

In the reactors operated under conditions for the production of thermophilic aerobic granules (R2 and R3), it was observed that in both, aerobic granules were produced at 35°C (Figures 5a and 6a). However, when raising the temperature, for operation in thermophilic conditions, it was observed that filamentous bacteria were produced, even on a small scale (Figure 5b and 6b). However, a high production of filamentous bacteria was observed (Figures 5c and 6c).

The behavior shown in the reactors that operated under conditions for the production of thermophilic aerobic granules (R2 and R3) indicate that the appearance and high reproduction of the filamentous bacteria may have contributed to the decay of the treatment efficiency through aerobic granular thermophilic sludge. One of the factors that may have led to such production is due to the change in temperature, as in the present study. With this,

there was also a change in the microbial population present in the formation of the granules.

The appearance of filamentous bacteria is not a problem in itself, as they aid in the initial stage of aggregation for the formation of thermophilic aerobic granules. However, their proliferation is extremely detrimental during treatment, since, in a high population density, these bacteria hinder the sedimentation process of the biomass or granules present in the reactors [13].

#### STRUCTURAL ANALYSIS - Confocal Microscopy

Taking into account that the FITC dye binds to proteins and other amino-compounds, in addition to being able to bind to groups of proteins and glycoconjugates associated with cell walls, Syto 63 was used as a counter-agent to distinguish cells of extracellular polymer substances [5 and 14].

During the observations in the confocal microscope LSM 700 it was verified that the images stained with the ConA dye presented the same behavior as the FITC, that is, the polysaccharides stained with ConA are also stained with the FITC, since it also has glycoconjugates besides Proteins [5 and 14].

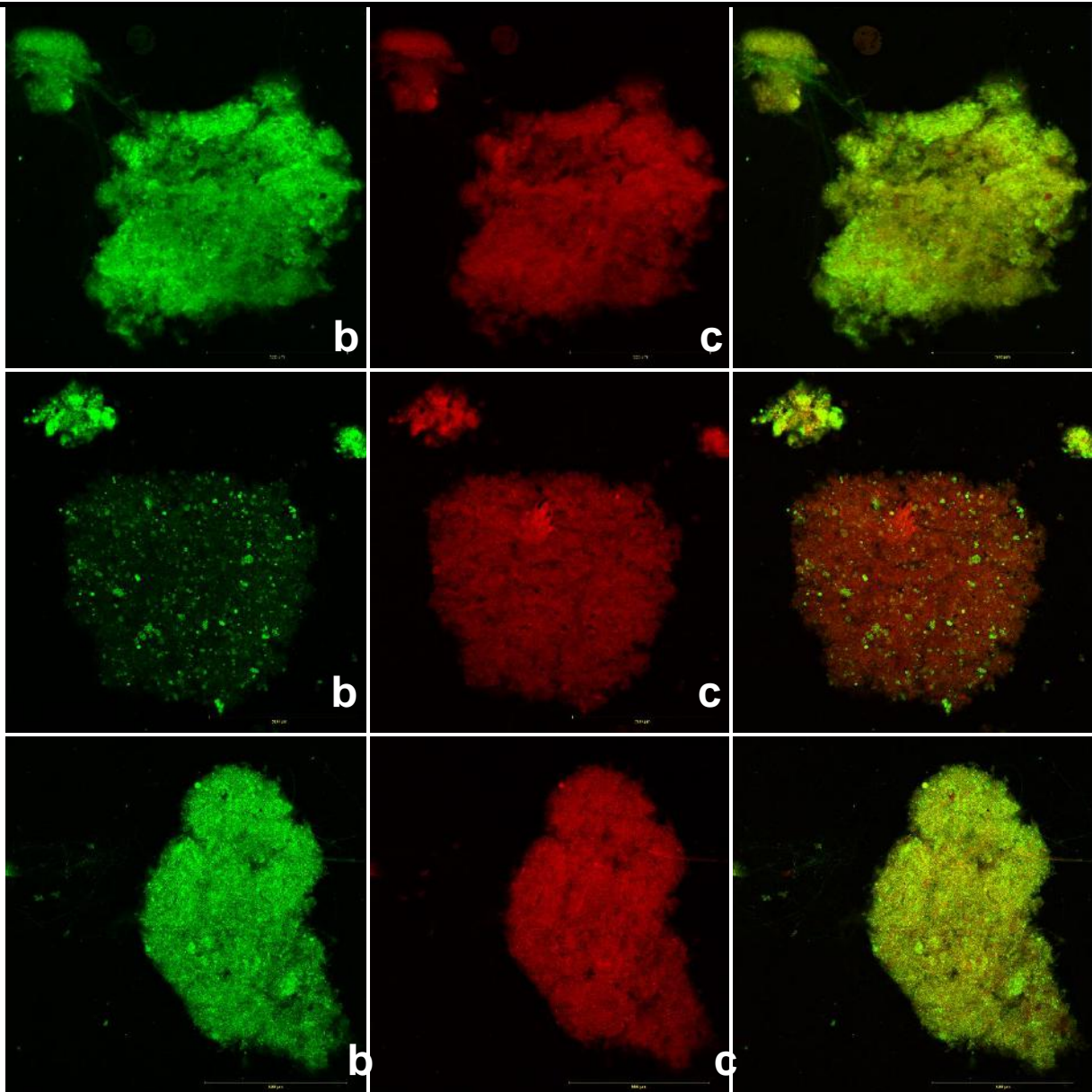
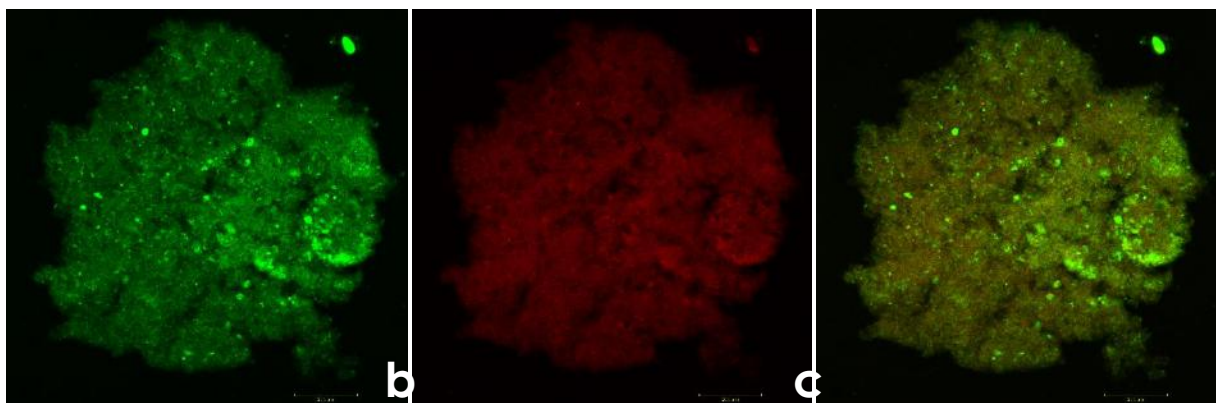


Fig.7: Confocal microscopy (500  $\mu$ m) of aerobic granular sludge sample from reactor R2, operated at: 1) 35 °C; 2) 45 °C and 3) 55 °C. (A) FITC dye-green colored beads, (b) red-colored Syto 63 dye; And c) Overlapping of FITC and Syto 63 dyes.





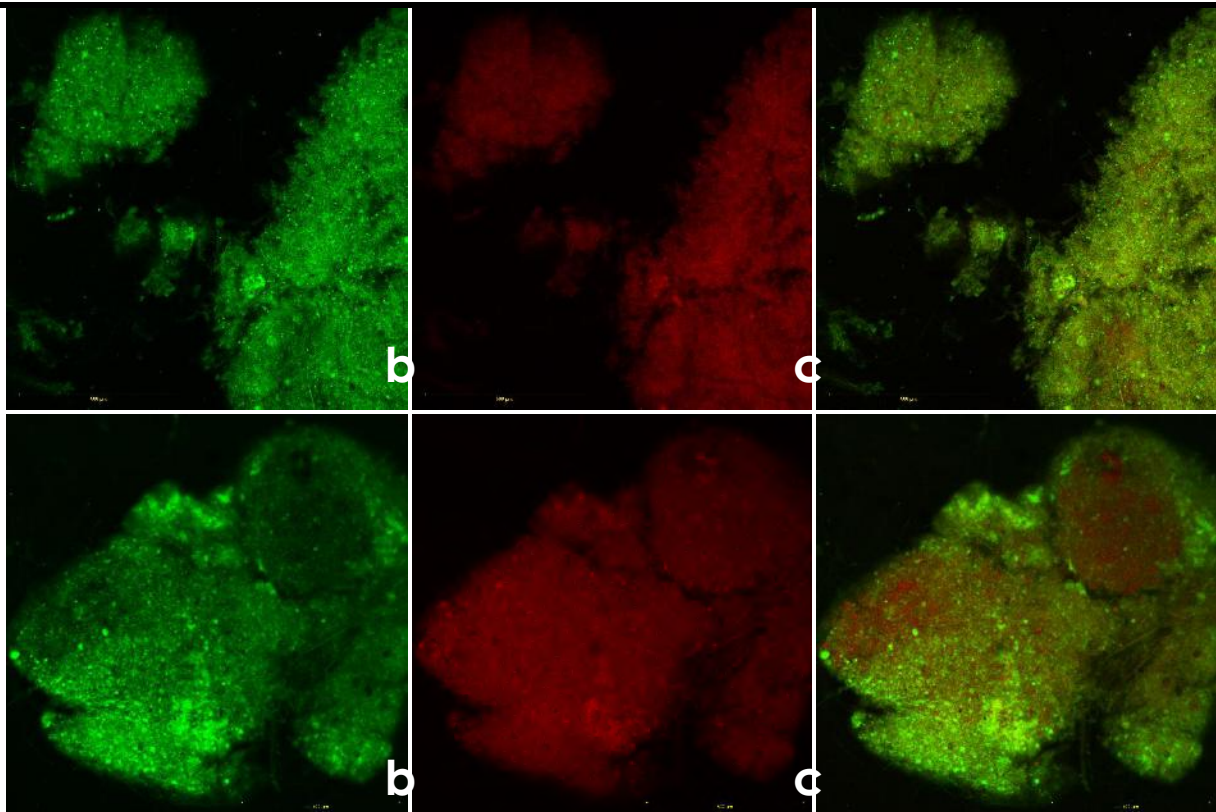


Fig.8: Confocal microscopy (500  $\mu\text{m}$ ) of aerobic sludge sample from reactor R2, operated at: 1) 35 °C; 2) 45 °C and 3) 55 °C. (A) FITC dye-green colored beads, (b) red-colored Syto 63 dye; And c) Overlapping of FITC and Syto 63 dyes.

On the basis of Figures 7 and 8, we can verify that both reactors (R2 and R3) behaved in a similar way, especially when the images obtained from samples of the same study temperature are analyzed.

There was also a decrease in the production / availability of proteins and glycoconjugates on the surface of the granules when comparing Figures 7.1 and 8.1 with 7.3 and 8.3, when the granules were at 35 °C and 55 °C, respectively.

In summary, we can define that the FITC dye stains the extracellular polymeric substances while Syto 63 blends only the cells, being permeable to the nucleic acids.

#### EXTRACELLULAR POLYMERIC SUBSTANCES

The nine samples, referring to the temperatures and points of study, were submitted to the analysis of carbohydrates, proteins and humic acids. The result is shown in Table 2.

Table.2: Quantification of the extracellular polymer substances in the sample samples of each reactor during pulp and paper industry effluent treatment by flocculent sludge (R1) and thermophilic mesophilic aerobic granular sludge (R2 and R3).

Collect point/ Temperature	Carbohydrates ( $\text{mg.L}^{-1}.\text{g}^{-1}$ )	Proteins ( $\text{mg.L}^{-1}.\text{g}^{-1}$ )	Humic acids ( $\text{mg.L}^{-1}.\text{g}^{-1}$ )
R1 35 °C (1)	$4,75 \pm 0,46$	$0,38 \pm 0,04$	$0,32 \pm 0,04$
R1 35 °C (2)	$3,03 \pm 0,01$	$0,37 \pm 0,04$	$0,32 \pm 0,04$
R1 35 °C (3)	$2,31 \pm 0,43$	$0,39 \pm 0,03$	$0,34 \pm 0,03$
R2 35 °C	$3,11 \pm 0,25$	$0,26 \pm 0,03$	$0,31 \pm 0,03$
R2 45 °C	$5,65 \pm 0,22$	$1,02 \pm 0,51$	$0,32 \pm 0,51$
R2 55 °C	$5,10 \pm 0,07$	$0,57 \pm 0,19$	$0,30 \pm 0,19$
R3 35 °C	$2,65 \pm 0,75$	$0,34 \pm 0,02$	$0,31 \pm 0,02$
R3 45 °C	$4,59 \pm 0,31$	$0,64 \pm 0,22$	$0,33 \pm 0,22$
R3 55 °C	$2,39 \pm 0,27$	$0,37 \pm 0,05$	$0,30 \pm 0,05$

The polymeric substances analyzed are of paramount importance for the formation and maintenance of granular aerobic granules [15].

The polymeric polysaccharides in EPS mainly serve as adhesives favoring the union of the bacteria forming a micro-colony. These polysaccharides under the surface of the small granular particles can serve as bridges or connect other smaller granules to form larger granules [16 and 6].

Proteins are hydrophobic constituents of EPS. The high-protein substances are responsible for the formation, structural stability of the granules and contribute to the reduction of scale in processes to membranes [4; 5 and 17].

Humic acids are related among the polymeric substances produced during the formation of aerobic granules. They act principally on the structural part of the granules [7].

Table 2 shows that, in the reactors operated under conditions of formation / maintenance of granular aerobic sludge (R2 and R3), there was an increase in the production of the three EPS when analyzing the quantification of 35 °C with that of 45 °C. At the temperature of 55 °C there was a reduction in the availability of these substances, in some cases being below that which had been quantified at the beginning of the experiment at 35 °C.

This behavior may be associated with the need for greater EPS action, since they act to maintain the structure and protection of the granules [6].

The values of the quantification confirm the observations made by confocal microscopy, namely, an increase in the EPS availability at 45 °C, in the reactors R2 and R3, with subsequent decay in the samples collected when the reactor was operated at a temperature of 55 °C.

The EPS values at 55 °C, associated with the proliferation of filamentous bacteria, as observed in scanning electron microscopy, indicate that the granule structure was lost and thus the loss of the treatment efficiency under thermophilic conditions above 45 °C.

#### IV. CONCLUSION

Aerobic granules were produced in reactors, which were operated for the production and maintenance conditions of the same. Such granules were initially produced under mesophilic conditions (35 °C) and subsequent raising of the temperature in order to obtain thermophilic operating conditions.

Under these conditions the granules were maintained, but the efficiency of the treatment presented decay in terms of the organic matter removal rate (COD). Microscopic and EPS data indicated that temperature increase, alteration of the microbial population and reduction in the production of EPS components may have influenced this decay.

Thus, the present work concluded that the aerobic granules, formed from the biological sludge and the Effluent of the ETE of a pulp and paper industry, can act in mesophilic conditions (at 35°C), as well as in thermophilic conditions, where the maximum temperature Not exceed 45 °C, without loss of treatment efficiency.

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