Characterization of Thermophilic Aerobic Granular Sludge for the Treatment of Bleached Kraft Pulp Mill Effluent

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The objective of this work was to evaluate the physical characteristics of the thermophilic granular aerobic sludge used in the treatment of bleached kraft pulp mill effluents. Four sequential batch reactors (SBRs) were operated with cycles of 12 hours. Reactor (R1-FSR) with flocculent sludge was used as control. The other three reactors (R2-GSR, R3-GSR$_{+100}$, and R4-GSR$_{+200}$) were operated with granular aerobic sludge. Concentrations 100 and 200 mg. L^{-1} of calcium were applied in the R3-GSR$_{+100}$ and R4-GSR$_{+200}$, respectively. The pH was maintained in the neutral range in all reactors. The experimental plan was carried out for 490-day period, in 5 phases at different temperatures of 35 °C to 55 ºC. All SBRs showed COD removal efficiency above 60% in all temperature ranges. The granule average diameter in the R2-GSR, R3-GSR$_{+100}$, and R4-GSR$_{+200}$ ranged from 5 to 8 mm. The reactor R3-GS$_{+100}$ showed better stability due to the addition of 100 mg. L^{-1} of calcium. The granular sludge sedimentation velocity was 40 m.h-^{1}, which was eight times higher than the flocculent sludge. Thermophilic treatment (55 °C) using aerobic granular sludge proved to be a promising way for treating bleached kraft pulp mill effluent without a prior cooling process.

Keywords: Thermophilic aerobic granules; High-temperature; Integrity coefficient; Industrial wastewater

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INTRODUCTION

Pulp mills demand significant amounts of water and generate high volumes of effluent with high organic matter and high temperatures, requiring biological treatment prior to discharge (Calvo *et al.* 2007; Morais *et al.* 2018; Da Mata *et al.* 2019). The effluent from pulp mills presents potential for biological treatability, for which it is generally applied the activated sludge process an aerobic system, which shows advantages in comparison with anaerobic treatments (Thompson *et al.* 2005). However, the activated sludge used to treat the pulp mills effluent generally is operated at a mesophilic temperature (about 35 °C), which implies the need for effluent cooling, increasing the operating costs of treatment plants due to the high energy consumption required in the cooling stage. Such costs could be avoided by using the treatment system in thermophilic conditions.

Thermophilic biological wastewater treatment has been the subject of studies since the early 1950s (Lapara and Alleman 1999). In this way, researchers have been documenting and encouraging the use of aerobic thermophilic treatment as an alternative to mesophilic processes, especially in industries that generate high-temperature effluent (above 50 °C), thus eliminating the need for cooling systems (Suvilampi and Rintala 2004;

Adav *et al.* 2008). Recent research has shown that thermophilic treatment allows a high rate of removal of organic loads (Halim *et al.* 2016; Ibrahim *et al.* 2017), and the use of the thermophilic condition in biological treatment has been investigated with positive contributions to the pulp and paper industry (Zheng and Liao 2014). However, some biological processes require greater care in the operation, as described by Zheng and Liao (2016), when studying membrane aerated biofilm reactors for thermomechanical pulping pressate (TMP) treatment, such as a promising technology that request a necessary careful biofilm thickness control for achieving better performance.

Some studies have shown that thermophilic conditions can cause negative effects on the microbiology of sludge, interfering with microbial diversity and richness, which can result in a drop in efficiency in the treatment system (Silva *et al.* 2010).

Besides the effects on microbiology, the thermophilic biological treatment has several difficulties for its implementation, due to the negative effect of high temperature on the formation and sedimentability of biological sludge (Hakim *et al.* 2016).

A hypothesis that supports the thermophilic treatment suggests the use of granular sludge, which provides more physical/mechanical resistance on the biological aggregates formed, maintaining the stability of the treatment at high temperature. The use of thermophilic anaerobic granular sludge has been reported earlier than the use of thermophilic aerobic granular sludge; however, a greater consumption rate of organic matter by aerobic way, can be more attractive for treatment of the biggest volume of effluent due to the smaller hydraulic retention time (Van Lier *et al.* 1996; Dutta *et al.* 2014; Soerensen *et al.* 2015). There are useful opportunities of the thermophilic treatment systems applying aerobic granular sludge that proposed a substitution of the mesophilic condition where the flocculent sludge was used (Zitomer *et al.* 2007; Hakim *et al.* 2016).

The first step for the technology application is to produce the biological aerobic aggregates. Granulation is a gradual process of conversion of flocculent sludge into granular sludge through the selection of aggregates by sedimentation. The granulation process can be initiated by bacterial adsorption and adhesion to inorganic, inert, and precipitated materials, as well as by the adhesion of microorganisms to other cells through physicochemical interactions. Some groups of bacteria, such as filamentous bacteria, contribute to and enhance the granulation process, making the granules more resistant (Yu *et al.* 2001; Morais *et al.* 2018).

Aerobic granulation has gained importance in the wastewater treatment field (Ebrahimi *et al.* 2010; Lee *et al.* 2010; Ren *et al.* 2017; Morais *et al.* 2018), although the use of full-scale plants is still incipient. Aerobic granular sludge technology is currently applied on a full-scale level by Royal Haskoning DHV (Nijmegen, Netherlands) in a system called Nereda® (Pronk *et al.* 2015).

Few studies have reported the use of full-scale granular sludge, and normally full-scale systems operate in batch mode, which still acts as a limiting factor for the use in high-flow effluent treatment systems (Giesen and Thompson 2013). The physical instability of the granule structure over the bioreactor operating time is one of the most severe obstacles to a practical application of aerobic granular sludge, especially in continuous-feed flow systems.

An important step for granular sludge technology to be used on a large scale is to develop techniques that give the sludge a greater cohesion and more mechanical resistance to withstand the shear stress of the effluent in the aerated bioreactors. The aerobic granules can be classified according to their capacity to tolerate high shear forces by measuring the integrity coefficient (Ghangrekar *et al.* 2005).

A more complex scenario is observed when aerobic granular sludge is subjected to high temperatures. Little is known about the formation of granules and the effects of high temperatures on the physical characteristics of the granules, as well as the performance of organic matter removal efficiency on the wastewater treatment plant under thermophilic conditions.

The objectives of the present research were to evaluate the physical characteristics of the aerobic granular sludge and the organic matter removal efficiency when treating bleached kraft pulp mill effluents under the thermophilic conditions.

EXPERIMENTAL

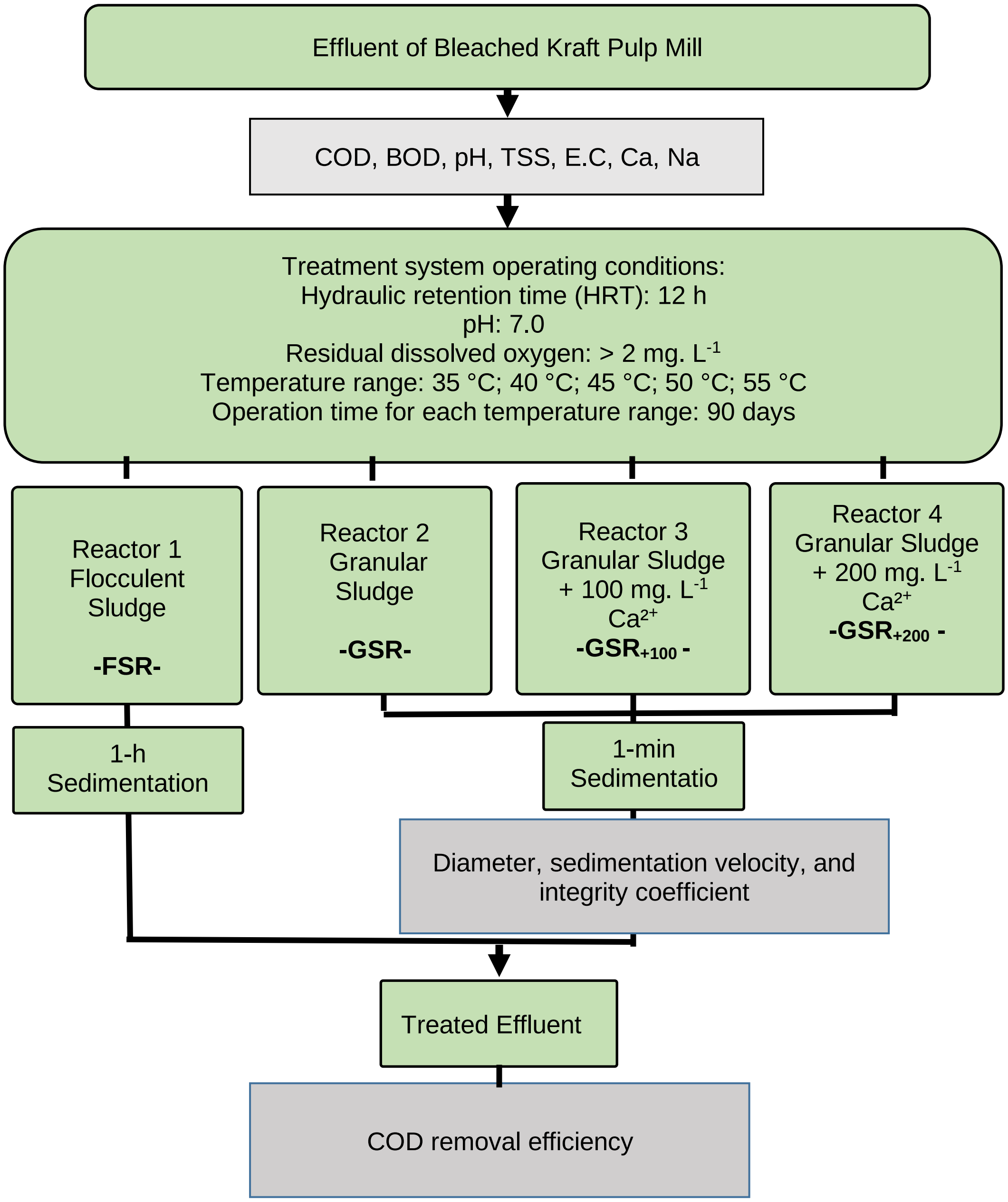

Fig. 1. Experimental design

Materials

The effluent was obtained from a Brazilian eucalyptus bleached kraft pulp mill that has a D_{hot} (EP) DP bleaching sequence. The effluent was collected upstream of the activated sludge treatment system and the biosludge was collected in the sludge recirculation line for further use in the lab sequential batch bioreactors.

Experimental design

The experimental design is shown in Fig. 1.

Table 1 describes the analytical processes and the frequency of the analysis. The operation period in each temperature phase was 90 days, for a total of 450 days for the 5 phases at different temperature beginning at 35 °C and intercalated by 5 °C every 90 days until 55 °C.

Table 1. Analytical Program

Nature	Analyses	Location	Frequency
Characterization	COD, BOD, pH, TSS, VSS, E.C, Calcium, and Sodium	Input	For every sample
Routine	COD, pH, TSS	Output	Three times a week
Routine	Size Determination (Petri dish sludge photographs)	Granular Sludge	Twice a week
Routine	Granule strength and sedimentation rate test	Flocculent and Granular Sludge	Monthly

COD- Chemical oxygen demand, BOD- Biochemical oxygen demand, TSS- Total suspended solids, VSS- Volatile suspended solids, EC- Electrical conductivity

Experimental apparatus

A sequential batch reactor (SBR) system composed of four reactors (Fig. 2), with a volume of 2000 mL each, was assembled.

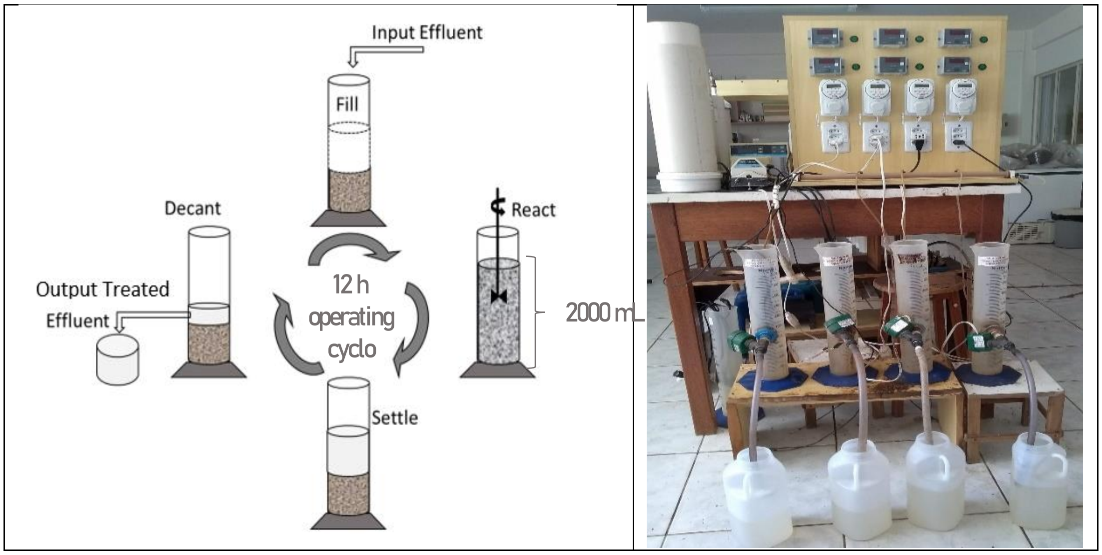

Fig. 2. Sequential batch reactors/ Experimental apparatus

Each reactor was equipped with an air diffusion aeration system and a semi-automatic treated effluent discharge system composed of a timer and a solenoid valve. Effluent feeding was performed *via* a peristaltic pump. In each reactor, a thermostatically controlled heater was installed. The temperatures were programmed for each phase of the test, beginning with a temperature of 35 ℃ and increasing 5 ℃ in each phase up to a temperature of 55 ℃.

The system operated in batch cycles of 12 h each. The pH in each reactor was kept in the neutral range (pH = 7) through the addition of sulfuric acid when necessary. Dissolved oxygen (DO) concentration was always kept above 2 $mg.L^{-1}$ in all reactors.

The bleached pulp mill effluent received the addition of nutrients (N and P) in the ratio of 250:5:1 for COD:N:P (Morais *et al.* 2018), because the effluents are poor in nutrients for the microorganisms.

Reactor R1-FSR with flocculent sludge was operated in flocculent sludge conditions (1-h sedimentation time). Reactors R2-GSR with granular sludge, R3 - GSR_{+100} granular sludge and 100 $mg.L^{-1}$ of additional calcium, and R4-GSR_{+200} granular sludge with 200 $mg.L^{-1}$ of additional calcium were operated under granular sludge formation conditions (1-min sedimentation time). The R3-GSR_{+100} and R4-GSR_{+200} reactors received a fixed dosage of 100 and 200 mg Ca^{2+} L^{-1}, respectively, in the form of calcium chloride. All reactors operated with a 12-hour cycle. Reactor R1-FSR operated with 11 hours of reaction time, which included 5 minutes for filling, 1 hour of sedimentation, and 2 minutes discharge. Reactors R2-GSR, R3-GSR+ 100 and R4-GSR+ 200 had the cycles of 11 hours and 57 minutes of reaction including 5 minutes for filling, 1 minute for sedimentation, and 2 minutes for discharging.

Methods

Physicochemical characterization

The physical-chemical parameters analyzed were COD (method 5220D), BOD and DO by respirometry test (method 5220B), solids concentration (methods 2504B, C, D and E), based on methodology described in the Standard Methods for the Examination of Water and Wastewater (2012), as well as the procedures of collection, preservation and pretreatment of the samples (APHA 2012). The pH was measured using the HACH HQ40D multi-parameter.

Calcium, potassium, and sodium were determined by atomic absorption spectroscopy according to TAPPI T266 om-02 (2006).

Analysis of the physical characteristics of aerobic granular sludge

a) Size of the granules

The determination of the size and circularity of the granules was carried out using ImageJ software (NIH, 1.51K, Bethesda, MA, USA) (Rasband 2017). The diameters were calculated as equivalent circular diameters (Lochmatter *et al.* 2013). The images used in the ImageJ software were obtained by diluting the sludge samples, placing them in a glass Petri dish, and photographing them together with a graduated ruler, for the conversion of the scalar factor of the image pixels produced.

b) Sedimentation velocity and stress test

The average sedimentation rate was determined using the sedimentation column assay (Ghangrekar *et al.* 2005).

The procedure consisted of filling a sedimentation column of a diameter of 7.5 cm and a height of 75 cm with water, to which 25 mL of diluted sludge (5:1) was added.

Collection was performed at time intervals of 0.5, 1, 1.5, 3, 7.5, 15, and 60 min. From the collected material, the TSS of each sample was determined to quantify the fraction of settled sludge at each time point.

The average sedimentation velocity was calculated by Eq. 1,

$$v_{mean\ sed..} = \frac{\sum v \times m}{M} \tag{1}$$

where $v_{\text{mean sed.}}$ is the mean sedimentation velocity (m. h^{-1}), v is the sedimentation velocity (m. h^{-1}) of the fraction in the interval, m is the mass (g) of sedimented fraction over time, and M is the total mass (g) of sludge sample.

The stress test was conducted on the fraction of the sludge that settled in 1 min. The granules were transferred to a 250-mL Erlenmeyer flask, and the volume was completed with tap water to a total volume of 150 mL. The sample was placed on a shaker at 200 rpm for a period of 5 min. After stirring, the sample was allowed to rest for 1 min. Then, the total suspended solids of the supernatant and the decanted liquid were measured. The results of the TSS analyses were used to determine the integrity coefficient, which is defined as the ratio of the mass of the supernatant solids divided by the mass of the total solids of the sample. Thus, the lower the integrity coefficient, the greater the strength of the granules (Ghangrekar *et al.* 2005).

Statistical Test

The results were submitted to an analysis of variance and the Tukey test (α=0.05), using the R software (RFSC, v.R 3.3.3. Vienna, Austria) in the data processing.

RESULTS AND DISCUSSION

Characterization of Bleached Kraft Pulp Mill Effluent

The characteristics of the effluent used during the experiment are shown in Table 2.

Table 2. Characterization of the Bleached Kraft Pulp Mill Effluent

Sample	COD (mg.L^{-1})	BOD (mg.L^{-1})	E.C (mS.cm^{-1})	TSS (mg.L^{-1})	VSS (mg.L^{-1})	Ca (mg.L^{-1})	Na (mg.L^{-1})	K (mg.L^{-1})
Effluent	1309 ± 75	530 ± 93	3,2 ± 0,3	185 ± 13	114 ± 20	170 ± 10	603 ± 14	18 ± 4.0

The effluent showed typical characteristics of kraft pulp mill wastewaters, with an average COD of 1309 mg.L^{-1}, and an approximate BOD/COD ratio of 0.4 (Morais *et al.* 2018; Da Mata *et al.* 2019). The effluent had a conductivity value (3.2 mS.cm^{-1}) that was significantly correlated with the presence of calcium, sodium, and potassium salts (170, 603, and 18 mg.L^{-1}, respectively). It is noteworthy that high electrical conductivity can affect the formation of biological aggregates (Da Mata *et al.* 2019). The value found, although high, is within the typical values for this type of effluent.

The calcium concentration was increased in the R3-GSR$_{+100}$ (100 mg Ca L^{-1}) and R4-GSR$_{+200}$ (200 mg Ca L^{-1}) reactors, so that the R3-GSR$_{+100}$ reactor had a total concentration of 270 mg.L^{-1} of calcium, and the R4-GSR$_{+200}$ increased to 370 mg.L^{-1}.

Organic matter removal efficiency

The COD removal efficiency using granular sludge in the sequence batch reactor system is shown in Table 3.

Table 3. COD Removal Efficiency at Different Temperatures

Parameter	COD Removal Efficiency (%)				
Operating Temperature	35 ºC	40 ºC	45 ºC	50 ºC	55 ºC
R1-FSR	69 ± 3.2	65 ± 9.2	67 ± 5.2	67 ± 5.9	66 ± 10.1
R2-GSR	67 ± 5.2	65 ± 7.4	67 ± 8.4	68 ± 7.3	64 ± 7.8
R3-GSR$_{+100}$	68 ± 5.1	67 ± 8.1	65 ± 9.5	68 ± 5.5	64 ± 9.4
R4-GSR$_{+200}$	68 ± 4.2	66 ± 4.7	67 ± 8.4	64 ± 10.5	65 ± 7.5

R1-FSR = Flocculent sludge reactor; R2-GSR = Granular sludge reactor; R3-GSR $_{+100}$ = Granular sludge reactor 100 mg.L^{-1} Ca^{2+}; R4-GSR $_{+200}$ = Granular sludge reactor 200 mg.L^{-1} Ca^{2+}, n = 46

The overall removal efficiency was above 60% under all tested conditions. The efficiencies achieved for COD removal are similar to those reported in the literature for bleached pulp mill effluents (Kamali and Khodaparast 2015; Morais *et al.* 2018; Da Mata *et al.* 2019).

The SBR performance did not present statistically significant variation between the average COD removal efficiency of the different reactors, within the same temperature range.

Performance was similar among the flocculent (R1-FSR) and granular (R2-GSR, R3-GSR $_{+100}$, and R4-GSR $_{+200}$) sludge reactors. The addition of calcium was not found to have any adverse effect on organic load removal efficiency (Morais *et al.* 2018).

Figure 3 presents the results of the overall reactor efficiency over the experiment period, in the different temperature phases.

The treatment maintained a COD removal efficiency of over 60% at all temperatures. The COD removal efficiency above 60% at high temperatures was also observed in the use of granular sludge (Song *et al.* 2009).

Temperature transition can negatively affect biological activities. It was observed that after each temperature transition, there was an efficiency drop of up to 30%, but the removal efficiency returned gradually to maximum after a short period of adaptation at the new temperature. It is noteworthy that the recovery of COD removal efficiency in the temperature transition from 50 to 55 °C was longer, only returning to total recovery after approximately 20 days, while in the other temperature ranges the recovery of efficiency occurred over a medium period of 10 days.

The drop in efficiency can be attributed to the fact that high temperatures may cause adverse effects on the microbial sludge community and sludge formation (Hakim *et al.* 2015; Ibrahim *et al.* 2017).

At no time did the addition of calcium influence the reactors' performances regarding the COD removal.

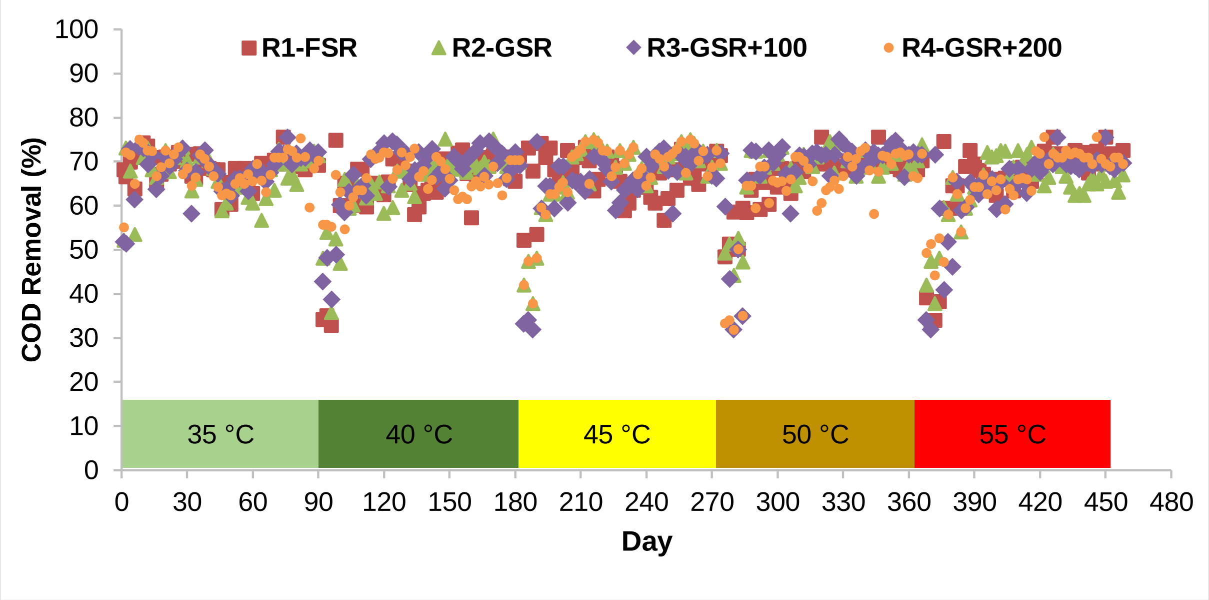

Fig. 3. Performance of reactors in soluble COD removal

The efficiency of COD removal shows that it was possible to treat the effluent in all conditions tested. Thus, it was possible to observe that the effluent treatment of the cellulose mill can be carried out in thermophilic conditions, suggesting a viable alternative for the industry, with a significant reduction in the installation and operation costs of the effluent treatment system. Above all, meeting the standards for launching environmental standards.

The thermophilic treatment with the granular sludge combines the advantages of the characteristics of the granular sludge (excellent sedimentation rate, less area requirement, greater robustness to shock loads) with the benefits of high temperature operation (higher degradation rate, less generation sludge), being an attraction for the use of technology on an industrial scale.

Physical characteristics of granular aerobic sludge at different temperatures

The diameter and sedimentation velocity of the granules were determined (Table 4).

Reactors R2-GSR, R3-GSR$_{+100}$, and R4-GSR$_{+200}$ presented predominantly average diameters between 4 and 8 mm, similar to those reported in studies that point out granular sludge as a viable alternative for industrial and municipal effluent treatment (Zitomer *et al.* 2007; Ebrahimi and Gabus 2010; Wilson *et al.* 2013; Pronk *et al.* 2017; Morais *et al.* 2018). A statistical comparison between the average diameters recorded in each temperature range and between the different granular aerobic sludge reactors showed no significant variation, with a confidence level of 95%. This shows that the diameter of the granules remained similar in the R2-GSR, R3-GSR$_{+100}$, and R4-GSR$_{+200}$ reactors, regardless of temperature variation.

The main physical characteristics of granular aerobic sludge were maintained throughout the entire treatment and under the different operating conditions of the SBRs: *i.e.*, there was no significant deterioration of the granules when operating the SBRs in the different temperature ranges from 35 °C to 55 °C.

Table 4. Diameter, Sedimentation Rate, and Granule Integrity Coefficient

Temperature	Treatments	Diameter (mm)	Sedimentation Rate ($m.h^{-1}$)	Integrity Coefficient x 100
35 °C	R1-FSR	-	4.70 ± 1.40	-
	R2-GSR	7.17 ± 3.14	40.21 ± 1.80	4.70 ± 1.93
	R3-GSR$_{+100}$	7.80 ± 3.82	41.55 ± 0.85	0 ± 0
	R4-GSR$_{+200}$	7.81 ± 3.8	41.53 ± 2.95	9.64 ± 5.48
40 °C	R1-FSR	-	3.93 ± 0.81	-
	R2-GSR	6.29 ± 4.00	39.36 ± 2.36	12.24 ± 4.45
	R3-GSR$_{+100}$	5.83 ± 3.21	41.81 ± 1.99	0 ± 0
	R4-GSR$_{+200}$	8.30 ± 9.51	41.21 ± 3.92	13.62 ± 13.37
45 °C	R1-FSR	-	3.81 ± 0.83	-
	R2-GSR	6.74 ± 4.77	38.58 ± 0.19	18.02 ± 12.23
	R3-GSR$_{+100}$	7.56 ± 5.19	40.55 ± 3.69	2.49 ± 1.04
	R4-GSR$_{+200}$	5.36 ± 3.85	44.415 ± 1.47	18.95 ± 5.84
50 °C	R1-FSR	-	5.08 ± 0.69	-
	R2-GSR	6.60 ± 4.61	42.50 ± 1.51	14.69 ± 7.51
	R3-GSR$_{+100}$	8.08 ± 5.25	42.06 ± 1.16	1.85 ± 2.62
	R4-GSR$_{+200}$	7.65 ± 5.21	41.29 ± 7.04	20.39 ± 6.51
55 °C	R1-FSR	-	5.47 ± 0.75	-
	R2-GSR	6.74 ± 4.77	41.69 ± 0.25	6.73 ± 5.11
	R3-GSR$_{+100}$	7.57 ± 5.25	42.01 ± 1.09	1.39 ± 1.96
	R4-GSR$_{+200}$	6.32 ± 4.66	41.29 ± 4.04	17.95 ± 0

R1-FSR= Flocculent sludge reactor; R2-GSR = Granular sludge reactor; R3-GSR$_{+100}$ = Granular sludge reactor + 100 $mg.L^{-1}$ Ca^{2+}; R4-GSR$_{+200}$ = Granular sludge reactor + 200 $mg.L^{-1}$ Ca^{2+}

Similarly to what was observed on granules that had comparable average diameter at all temperatures (Table 4), it was also observed that the addition of calcium had no effect on the size of the granules, *i.e.*, the reactor without adding calcium, had a mean diameter similar to the R3-GSR$_{+100}$ reactor with the addition of 100 mg. L^{-1} of calcium, as well as the average diameter found for the R4-GSR$_{+200}$ reactor with the addition of 200 $mg.L^{-1}$ calcium.

The granule size has been reported as being an important parameter, but the average diameter has a wide range, varying from 0.2 mm up to 16 mm (Zheng *et al.* 2006; Li *et al.* 2008; Liu *et al.* 2012; Morais *et al.* 2018). In contrast to what has been shown in previous studies, that granules of 0.6 to 1.8 mm may be more efficient for biological treatment (Liu and Tay 2015), and that large granules are not desirable due to instability and smaller surface area and may reduce substrate removal and biomass growth rates (Liu and Tay 2006; Pronk *et al.* 2015). It was observed in this work that the granules formed, besides being stable even with temperature variations, did not present a reduction in the rate of removal of the organic load. The use of real industrial substrate with high readily assimilable organic content is emphasized, which contributes to the formation of large granules. The maintenance and durability of the diameter and mass of the granulate can also be verified indirectly by the sedimentation rate.

The sedimentation rate values were close to those typically found in the literature (Dahalan *et al.* 2015; Morais *et al.* 2018). Sedimentation rate is one of the most important characteristics of granular sludge, which allows the effluent to be clarified quickly after treatment, making it an attractive alternative for replacement of conventional activated sludge techniques (Zitomer *et al.* 2007; Zhiwei *et al.* 2009; Ebrahimi *et al.* 2010).

The average sedimentation velocity measured for granular sludge under different temperature conditions was approximately 40 $m.h^{-1}$, while the flocculent sludge presented an average sedimentation velocity around 5 $m.h^{-1}$. The granular sludge reached sedimentation velocities 8 times higher than the flocculent sludge. Typical granular sludge sedimentation velocity values have been reported with values similar to those measured in this paper (Zitomer *et al.* 2007; Dahalan *et al.* 2015; Morais *et al.* 2018). This sedimentation rate can be attributed to the size of the granules and their cohesive formation.

Recent studies consider the disaggregation of the granules to be one of the main factors that could limit their use in large-scale plants. Recent articles have shown the use of agents that confer greater cohesion to the aggregate, helping the granules to withstand higher shear stresses caused by the flow of water and turbulence caused by the aeration in the reactors (Zitomer *et al.* 2007; Adav *et al.* 2009; Lee *et al.* 2010). The study by Morais *et al.* (2018) demonstrates that it is possible to optimize the strength of granules with the addition of calcium, allowing for further exploration of the use of granular aerobic sludge.

The maintenance of granular cohesive physical strength plays an important role in ensuring their morphological functionality and settling velocity. In addition, a low strength of the granules results in loss of solids and a poor final effluent quality. The shear strength was evaluated by the integrity coefficient, which showed a significant positive effect of the addition 100 $mg.L^{-1}$ of calcium. The effect of calcium on the R3-GSR_{+100} and R4-GSR_{+200} reactors are shown in Table 4.

Positive effects of the addition of calcium on the granule strength were observed (Tay *et al.* 2001; Vogelaar *et al.* 2002; Zhang *et al.* 2013; Hao *et al.* 2016). It is noteworthy that the R3-GSR_{+100} reactor, which received 100 $m.L^{-1}$ of calcium, presented the lowest integrity coefficient, *i.e.*, smaller solids losses under shear forces.

A low integrity coefficient implies greater cohesion, and indicates that the aerobic granule formed and maintained in the R3-GSR_{+100} reactor showed greater mechanical stability, that is, with an integrity coefficient tending to zero (Lee *et al.* 2010; Zhang *et al.* 2017; Morais *et al.* 2018). Lee *et al.* (2010) explain that the increase in granule strength occurs due to the calcium precipitation inside the granules and the increased polysaccharide content, forming a more resistant core.

The addition of calcium was favorable to the mechanical stability of the granules only in R3- GSR_{+100}, which received the addition of 100 $mg.L^{-1}$. For the R4-GSR_{+200} reactor, which also received additional calcium (200 $mg.L^{-1}$), it was observed that the integrity coefficient was above 9, which indicates an important fraction of disaggregate granules (Ghangrekar *et al.* 2005).

Divalent metal ions like calcium have a positive effect on granulation. The metal ions are recognized as enhancing granulation, providing the rapid formation of granules and giving them greater cohesion (Li *et al.* 2009; Song *et al.* 2009; Wilén *et al.* 2018). The mechanisms of enhancement of granulation may involve several functions, where inorganic ions can neutralize the negative charge on the surface of bacteria and enhance aggregation. The precipitates formed by the inorganic ions serve as nuclei to accelerate the microbial aggregation, and inorganic ions form an ionic bond on the surface of the particles and act as a cation bridge to enhance granulation (Yu *et al.* 2001; Wilén *et al.* 2018).

The sludge of the R3-GSR$_{+100}$ reactor was more cohesive, showing the lowest integrity coefficient. The application of 100 mg.L^{-1} of calcium assisted the formation and stability of the granules. The results observed for the calcium application were similar to those in the studies by Morais *et al.* (2018), Zhang *et al.* (2013), and Hao *et al.* (2016), which also explain that the high concentration of calcium can have negative effects. This situation is related to reactor R4-GSR$_{+200}$, where the application of calcium was two times higher than the calcium applied in reactor R3-GSR$_{+100}$ and did weaken the granules.

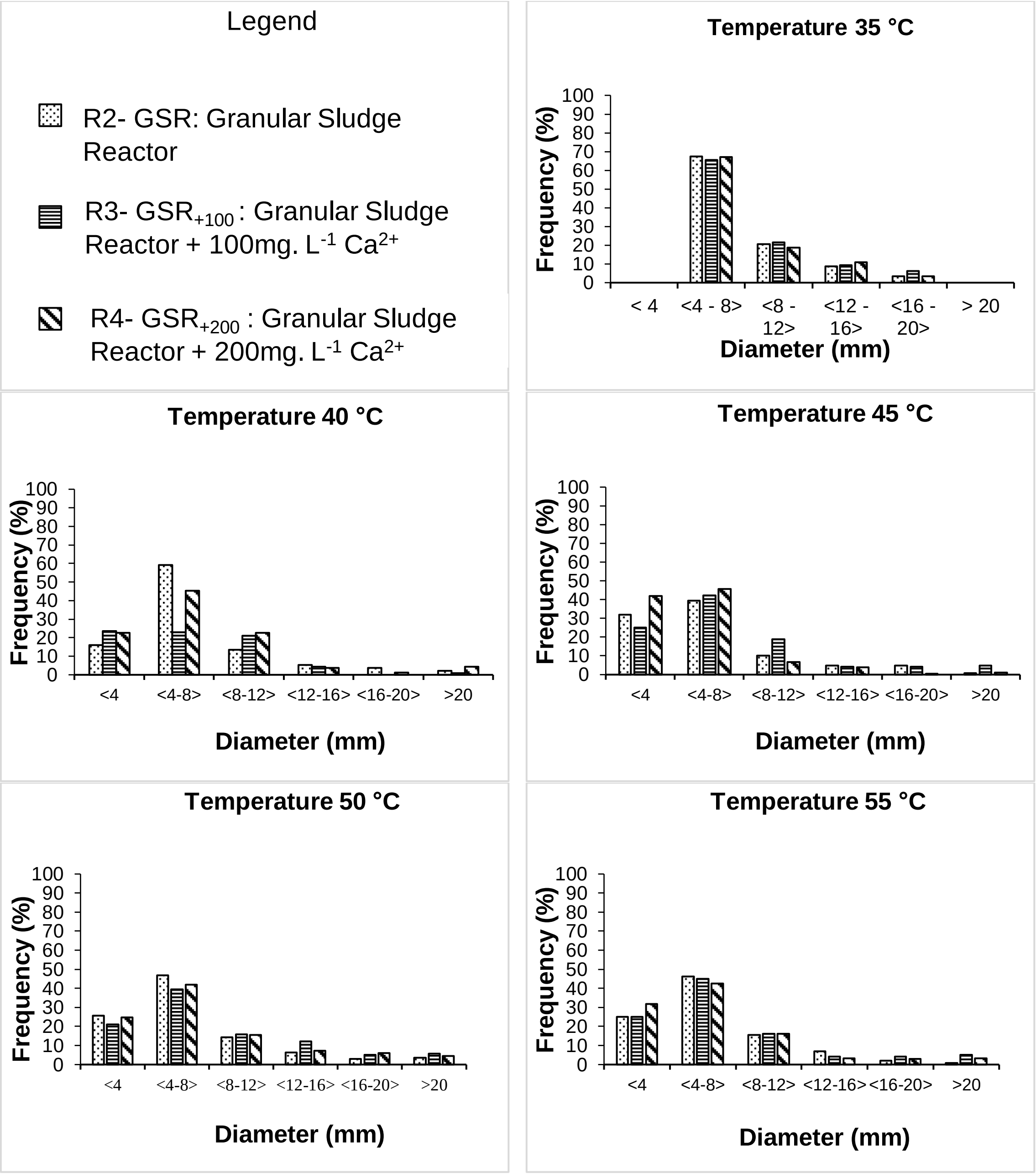

Fig. 4. Size distribution of granules at different temperatures

The evaluation of the physical characteristics of the granular sludge showed that the sludge remained stable in thermophilic conditions, turning the use of this technology in an industrial scale more attractive. It was also possible to observe that the operating conditions used in the experiments were adequate in maintaining the physical characteristics of the thermophilic aerobic granules. The application of calcium positively contributed to the maintenance of the physical properties of granular sludge.

The frequency of occurrence of granules according to their particle size class (diameter) is shown in Fig. 4, in the temperatures ranging from 35 °C to 55 °C.

At 35 °C, the R2-GSR, R3-GSR$_{+100}$, and R4-GSR$_{+200}$ reactors presented a granule diameter greater than 4 mm.

From the temperature increase above 40 °C, it was noted that there was a disturbance in granule formation, and an upward trend of granules smaller than 4 mm. This effect occurred mainly in the R4-GSR$_{+200}$ reactor, in the temperature range between 50 and 55 °C. However, the frequency of granules classified between 4 and 8 mm was not affected, continuing to show the highest proportions of distribution. In contrast, the frequency of granules larger than 8 mm was reduced, but without establishing a clear pattern.

The consistency of granules over the complete range of temperatures shows that the treatment of bleached kraft pulp mill effluent generated at elevated temperatures can be treated by a thermophilic aerobic system. This can be widely used as an alternative for economizing energy, eliminating the need for effluent cooling systems (Suvilampi and Rintala 2004; Ibrahim *et al.* 2017).

CONCLUSIONS

1. The viability of maintaining granular sludge under thermophilic conditions was demonstrated. Granular aerobic sludge was subjected to different temperatures in the range of 35 °C to 55 °C. This granular aerobic sludge showed a COD removal efficiency higher than 60% in all temperature ranges, including 55 °C.
2. The granules were maintained throughout the experimental period presented typical values of aerobic granular sludge, with an average diameter ranging from 4 to 8 mm.
3. The sedimentation velocity of the granular sludge was determined to be 40 m.h^{-1} and was 8 times higher than the sedimentation velocity of the flocculent sludge.
4. The addition of 100 mg.L^{-1} of calcium contributed positively to make the granules more cohesive and to increase their mechanical strength.

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