

## Research Article

# Rheology and Microbiology of Sludge from a Thermophilic Aerobic Membrane Reactor

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A thermophilic aerobic membrane reactor (TAMR) treating high-strength COD liquid wastes was submitted to an integrated investigation, with the aim of characterizing the biomass and its rheological behaviour. These processes are still scarcely adopted, also because the knowledge of their biology as well as of the physical-chemical properties of the sludge needs to be improved. In this paper, samples of mixed liquor were taken from a TAMR and submitted to fluorescent in situ hybridization for the identification and quantification of main bacterial groups. Measurements were also targeted at flocs features, filamentous bacteria, and microfauna, in order to characterize the sludge. The studied rheological properties were selected as they influence significantly the performances of membrane bioreactors (MBR) and, in particular, of the TAMR systems that operate under thermophilic conditions (i.e., around 50°C) with high MLSS concentrations (up to 200 gTS L<sup>-1</sup>). The proper description of the rheological behaviour of sludge represents a useful and fundamental aspect that allows characterizing the hydrodynamics of sludge suspension devoted to the optimization of the related processes. Therefore, in this study, the effects on the sludge rheology produced by the biomass concentration, pH, temperature, and aeration were analysed.

## 1. Introduction

The monitoring of biological wastewater treatment processes, whatever its final goal is (assessment of compliance with effluent legal emission values, evaluation of specific stages efficiency, calculation of the maximum treatment capacity and comparison with the actual treated load, mass balance for conventional and emerging pollutants, estimation of energy consumption, etc.), is based on physical, chemical, and biological analyses. Guidelines and technical documents define the main parameters together with the proper analytical methods in case of conventional and/or nonspecific pollutants, such as the COD (chemical oxygen demand), the

BOD (biological oxygen demand), nitrogen and phosphorus compounds, surfactants, oils and greases, and suspended solids. The biological process is based on the activity of different populations of organisms set at growing levels of the detritus food web. Therefore, any tools enabling us to identify and measure the activity of these organisms provide valuable information for improving and strengthening the operation of wastewater treatment plants ([1–4] among others).

The removal capacity of specific pollutants can be assessed by performing the measurement of metabolic activity, for example, by means of ammonia uptake rate (AUR) and nitrate uptake rate (NUR) tests [5–9] and enzymatic activity quantification [10, 11]. Besides, the microscopic observation

of floc characteristics and the identification and quantification of filamentous bacteria allow us to highlight disturbances within their community and to foresee the occurrence of specific dysfunctions, for example, sludge foaming, bulking, and solids washout [4, 12–15]. Ecological features like specific indexes (Sludge Biotic Index (SBI), designed by [1] and Sludge Index (SI), proposed by Grupo de Bioindicación de Sevilla (GBS, Spain) [16–19]) enable plant managers to tune the operation parameters, such as sludge age, hydraulic retention time, and dissolved oxygen concentration, in order to maintain optimum conditions for the sludge biotic components.

Scientific literature reports hundreds of applications of conventional and innovative biological tools, as well as examples of investigations based on ecological criteria [9, 20–27]. Almost all the biological processes have been explored in terms of biomass composition (bacteria, protozoa, metazoan, and fungi) and activity: conventional activated sludge, attached biomass, membrane reactors, and treating either municipal [27–34] or industrial wastewater [35–39]. Likewise, identification and quantification of microorganisms operating under different conditions (aerobic, anoxic, and anaerobic) have been reported [25, 40–46].

Rheological properties are crucial for activated sludge applications in wastewater treatment plants, since they severely impact the flow behaviour and many aspects that interfere with process performance and energy consumption, for example, sludge pumping, bioreactor hydrodynamics, mass transfer efficiency of aeration systems, sludge-water separation via settling, and filtration [47–54]. Due to the observed significant impact of rheology on different processes, a good knowledge of the activated sludge rheological behaviour is of great importance in both optimizing design and operation of sewage treatment plants [55]. Appropriate treatment of wastewater as a result of efficient design and operation would help in environment protection and preservation [56]. This significance motivates the recent experimental investigations and mathematical modelling on activated sludge rheology and on the impact of rheological properties on operating parameters for conventional activated sludge plants (e.g., [57–59]) and for membrane bioreactors MBRs (e.g., [51, 60, 61]).

Basically, rheology of a sludge is defined by its viscous characteristics, which can be determined by the relationship between shear rate and shear stress, obtained through a rheological measurement (which imposes either shear rate or shear stress [62–64] and measures alternatively these parameters). The observed relationship is strictly related to the characteristics of the sludge, such as the relative concentration of water and suspended matter due to the nature of the wastewater and to the treatment process that is subjected to [65].

At lower concentration of the suspended matter, sludge's behaviour can be reasonably approximated to a Newtonian fluid characterized by a linear relation between shear stress and rate of deformation, with the proportionality coefficient being the fluid viscosity. The measured viscosity is rather independent from shear rate, at given values of temperature and pressure. The flow curve is therefore a straight line through the origin of the coordinate axes.

As the particulate concentration increases, the sludge deviates from Newtonian behaviour. For a non-Newtonian liquid, the measured viscosity becomes dependent on the rate of shear, and the so-called *apparent viscosity* is introduced which is defined as the ratio between shear stress and shear rate at a given point of the flow curve. The apparent viscosity allows us to interpret the external and internal interaction forces between the constituents of the sludge that influences its rheological behaviour [51]. A highly concentrated sludge exhibits *yield stress* that is the critical stress triggering plastic deformation of the material continuum. Under the yield stress, no significant flow can be detected. According to [49], the yield stress can be related to reciprocal interactions among the suspended solid particles opposing to the deformation. There are two types of yield stress: *static* and *dynamic*. Static yield stress can be measured in an undisturbed sample, while dynamic yield stress refers to a perturbed sample and is extrapolated from its equilibrium flow curve as shear rates approach zero. It has been reported that the static yield stress was not observed at a mixed liquor suspended solid (MLSS) concentration equal to 16 g/L, probably because at this concentration the static yield stress almost equals the dynamic yield stress [51].

The rheological characterization of non-Newtonian fluids leads to practical difficulties; different measurement protocols and devices (i.e., capillary rheometers as in [66] and rotational rheometers as in [67]) have been adopted to study the rheological properties and a wide variety of models have been proposed. Widely used non-Newtonian relationships between shear stress and shear rate for activated sludge are the Ostwald de Vaele model, the Bingham model, the Herschel and Bulkley model, and the Casson model [49, 68]. All these models belong to the category of time-independent rheological models for which the relation between stress and applied shear rate does not depend upon the duration of shearing.

The properties of activated sludge, such as MLSS concentration, particle size distribution and shape, interaction among particles, flocculation ability, and surface physico-chemical characteristics, all have effects on rheology and, thus, on the kind of model most efficient in describing the sludge rheological profile and on the values of the preferable model parameters [49, 69].

A number of studies demonstrate that also temperature significantly affects the rheological characteristics: sludge becomes progressively more fluid as the temperature increases due to thermal [65, 70, 71]. These studies also prove that temperature irreversibly modifies the sludge structure.

This paper reports the results of an integrated study performed on the sludge deriving from a real scale TAMR, supplied with pure oxygen (being the average dissolved oxygen concentration of the mixed liquor equal to 2.3 mg L<sup>-1</sup>, while minimum and maximum values equal 0.2 and 15.0 mg L<sup>-1</sup>, resp.) located at a facility for the treatment of solid and high-strength liquid wastes. The sludge has been characterized in terms of microbiological and rheological features. Actually, the authors found, previously, links between the amount of filamentous bacteria and zooglyca clusters and viscosity values of mixed liquors from conventional activated plants treating municipal wastewater, thus suggesting the use of rheological

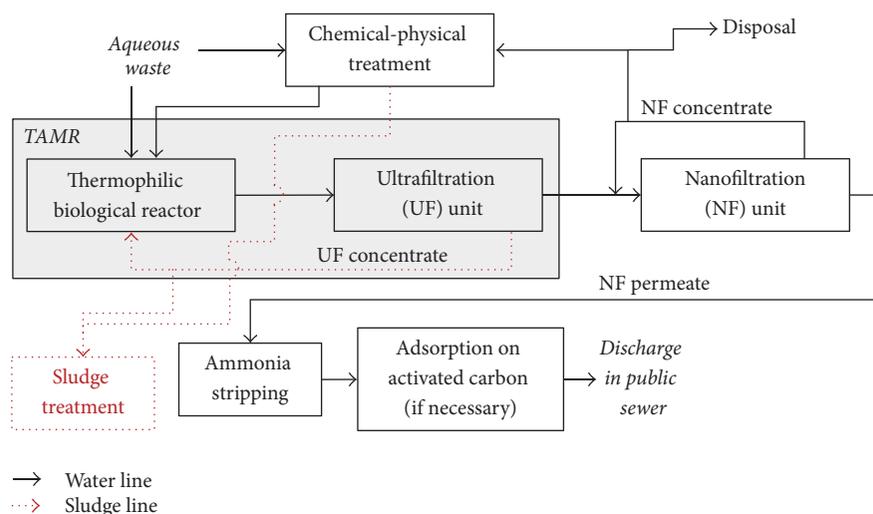


FIGURE 1: Process scheme of the studied facility.

tools to control dysfunctions caused by the proliferation of specific microorganism [72].

TAMRs operate at 45°C and are profitably applied for the treatment of high-strength wastewater deriving from food processing, pulp, and paper and pharmaceutical factories [73–76]. Recently, their extension to sludge treatment has been explored [77]. In effect, an aerobic thermophilic biomass is characterized by a lower yield, hence a decrease in specific sludge production (down to 0.02 kg SSV/kg COD<sub>removed</sub>). Furthermore, other valuable advantages consist in faster chemical reaction rates, increased organics solubility, high process stability, that is, the prompt activity recovery after operational changes, capability of degrading high salinity and biorecalcitrant wastewater, and possibility of integrating the thermophilic stage, as a pretreatment into a combined scheme, thus improving the removal extent of organic substances [76, 78, 79]. Nevertheless, despite the aforementioned advantages, thermophilic processes are still rarely adopted at the real scale [80, 81].

This research was aimed, therefore, at exploring the rheological and microbiological features of TAMR biomass, which remain almost obscure, in spite of the increasing knowledge gained about its excellent performances.

## 2. Materials and Methods

A synthetic description of the studied waste treatment facility, together with sampling and analytical procedures, is presented.

**2.1. The Waste Treatment Facility.** The facility is located in Northern Italy and treats 60,000 t/y of solid and liquid high-strength COD wastes (except mutagens and carcinogens); since fifteen years ago, this technology has been investigated in detail by [80].

Figure 1 depicts the scheme of the unit fed with liquid wastes: the main phase consists in the TAMR (see [80] for a complete description).

Before entering the biological tank, metals are removed by means of a chemical-physical treatment which leads to their precipitation as ammonium salts, hydroxides, and phosphates (pH equal to 11 units). The TAMR surface is 267 m<sup>2</sup> wide; net volume is about 1000 m<sup>3</sup>.

Pure oxygen is supplied through static mixers, yielding oversaturation of the sewage, which is recirculated within them. Ultrafiltration unit includes two parallel lines consisting in a feeding pump, a recirculation pump, and three channels of ceramic membranes (each containing 99 tubular membranes having 25 channels). Pores size allows retaining molecules larger than 0.3 microns and with a molar mass higher than 300 kDa. Minimum and maximum operation pressure values are 3 and 5 bar, respectively.

The average concentrations of pollutants inlet to the TAMR are 25,000 mg COD L<sup>-1</sup>, 1200 mg TN L<sup>-1</sup>, and 700 mg TP L<sup>-1</sup>. The TAMR performances, in terms of COD, TN, and TP removal yields, are 78%, 80%, and 90%, respectively. The high removal yields for TP, as reported by [79], are due to the chemical precipitation: this phenomenon could be due to the dosage of lime (in the chemical-physical treatment before the TAMR), but the most important aspect is ascribed to the aeration of reactor that promotes the phosphorus crystallization.

**2.2. Characterization of Sludge.** Samples taken from the TAMR were submitted to investigations aimed to define their microbial profiles and rheological behaviour.

**2.2.1. Microbial Community Investigation.** Mixed liquor samples were taken from TAMR and freighted immediately to the laboratory at 4°C. After adding absolute ethanol (Sigma Aldrich), (1:1) mixed liquor was pipetted onto a glass slide and allowed to dry at 46°C for 15 minutes; afterwards, samples were submitted to *fluorescent in situ hybridization* (FISH), according to prescriptions of VIT® (Vermicon AG, Munich, Germany) gene probe technology, based on fluorescent labelled DNA probes. Only viable microorganisms

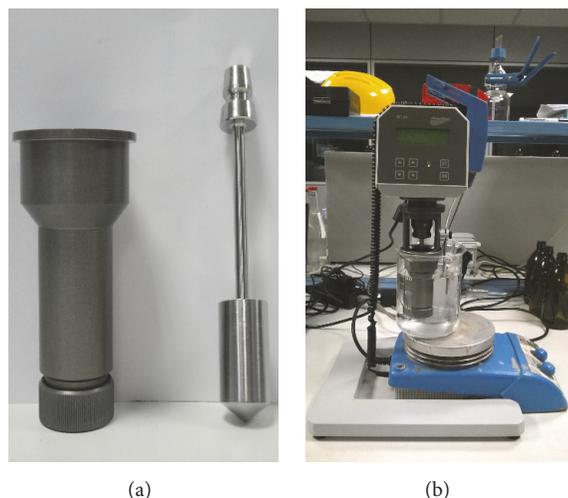


FIGURE 2: Experimental apparatus: coaxial cylinder CC25DIN (a) and rheometer RC20 (b).

are identified, quantified, and even visualized in their environment. The major advantage of this method is that the analysis is based on the stable genetic material of the cells and not dependent on phenotypic features which may be quite variable among bacteria. Moreover, as the target of the gene probes is the rRNA of the cells, only viable bacteria can be detected. As fluorescent signal intensities are correlated with the rRNA content within the cells, a further result on the physiological activities of the detected cells can be obtained. The detection limit is about  $1000 \text{ cells mL}^{-1}$ . A process of image analysis enabled the quantification of single populations, by comparing the signals of each group with the fluorescence emitted by the all the viable microorganisms.

Besides, observation of floc features and identification and abundance assessment of filamentous bacteria were performed according to [12]. In particular, the analyses were performed by using a Zeiss Axiostar Plus microscope under phase contrast illumination. Raw samples were firstly examined: afterwards, due to their conspicuous density, they were diluted with the supernatant (1:10 and 1:100) in order to enable the visualization of single flocs. Their morphology was registered (according to [12]), by considering the shape (irregular, round), the firmness, and the mean diameter. For this purpose, 10 microscopy fields were considered as replicates; flocs diameter was measured by employing a graduated slide as a reference. This tool was used also for measuring the length of filamentous bacteria. Likewise, the effects of filaments on floc structure, their abundance (with respect to each floc), and the presence of other important factors, such as free cells in suspension, inorganic/organic particles, and zoogloas, were recorded.

Mixed liquor samples were also submitted to the analysis of the microfauna for the calculation of the Sludge Biotic Index (SBI) according to [1] (microscopic observation under direct illumination; magnification: 100x).

**2.2.2. Rheological Tests.** The rheological experiments were carried out using rheometer RC20 (RheoTec) with a configuration CC25DIN of coaxial cylinders (Figure 2(a)): spindle

radius: 12.5 mm; internal radius of the measuring cylinder: 13.56 mm.

The working principle of the instrument is based on this aspect: the sliding of the sludge in the cavity between the coaxial cylinders, due to the spindle rotation with a fixed share rate, while the external cylinder is held, requires a torque that is measured by the instrument. Several rotational tests with different controlled shear rate (CSR) were performed to obtain the rheological equation:

$$\tau = f(\dot{\gamma}), \quad (1)$$

where  $\tau$  is the shear stress [Pa] and  $\dot{\gamma}$  is the share rate [ $\text{s}^{-1}$ ].

The temperature was controlled by the use of water in a beaker placed on a heating magnetic stirrer (Figure 2(b)).

In Table 1, the details of rheological test are summarized (the pH and temperature values represent the average data recorded during the tests). The tests were carried out on two samples of thermophilic biomass with different concentrations of total solids (TS). The pH values are between 6.6 and 9.1 (sulphuric acid and soda were used) and the temperature is close to  $45^\circ\text{C}$ .

The biomass samples withdrawn from the TAMR were delivered to the laboratory within 120 min after sampling. They were stored (for 180 min) at  $45^\circ\text{C}$  under different conditions: a first subsample was not submitted to aeration; a second subsample was kept in aeration conditions by an air compressor at lab scale ( $1 \text{ L}_{\text{air}} \text{ min}^{-1}$ ); only for the tests on sample B, a third subsample was aerated with an air flow of  $0.5 \text{ L}_{\text{air}} \text{ min}^{-1}$ .

Each rheological test was performed with fixed shear rates that were maintained for 300 seconds; shear rate was increased step by step as reported in Table 1. Shear stress and apparent viscosity were recorded every 30 s (ten data sets for each share rate).

Both the sludge samples presented in Table 1 were tested starting from an imposed shear rate of  $100 \text{ s}^{-1}$  with a step increment of  $100 \text{ s}^{-1}$  (or  $50 \text{ s}^{-1}$  in some cases). Anyway, the adopted rheometer was unable to resolve the shear

TABLE 1: Rheological tests performed: operative conditions.

Test #	Operative conditions			Shear rate [s <sup>-1</sup> ]	
	Average pH	Average temperature [°C]	Aeration		
Sample A (150 gTSL <sup>-1</sup> )	1	6.7	44.5	N	700, 800, 900, 1000
	2	6.7	45.2	Y	700, 800, 900, 1000
	3	7.7	45.1	N	700, 800, 900, 1000
	4	8.3	45.3	Y	750, 800, 900, 1000
	5	9.0	45.1	N	750, 800, 850, 900, 1000
	6	9.1	44.9	Y	800, 850, 900, 1000
Sample B (190 gTSL <sup>-1</sup> )	7	7.0	45.4	N	500, 600, 750, 900, 1000
	8	6.7	45.3	1/2	500, 600, 750, 900, 1000
	9	6.6	45.0	Y	500, 600, 750, 900, 1000
	10	7.8	44.9	N	500, 600, 750, 900, 1000
	11	8.3	45.2	1/2	500, 600, 750, 900, 1000
	12	8.4	45.3	Y	500, 600, 750, 900, 1000
	13	8.9	45.0	N	500, 600, 750, 900, 1000
	14	9.0	45.3	1/2	500, 600, 750, 900, 1000
	15	9.1	45.0	Y	500, 600, 750, 900, 1000

N: no aeration; 1/2: limited aeration (0.5 L<sub>air</sub> min<sup>-1</sup>); Y: full aeration (1 L<sub>air</sub> min<sup>-1</sup>).

stress for values below 700 s<sup>-1</sup> for the lower concentration (150 gTSL<sup>-1</sup>) and, respectively, for values below 500 s<sup>-1</sup> for the higher concentration (190 gTSL<sup>-1</sup>).

It is worth noting that in the present study a limited number of shear rates has been investigated (i.e., four values for tests 1–4 and 6, and five in case of tests 7–15). This aspect, in principle, may represent an inherent limitation of the work that, however, has the merit to provide a first insight into the rheological behaviour of a highly concentrated thermophilic sludge. Furthermore, the preliminary findings (see Section 3.2) show that the trend of the flow curves exhibits a certain degree of reliability, as confirmed by the regression coefficient displayed in Table 3. Anyway, a more refined investigation by increasing the range of explored shear rates and the amount of samples will be useful to clarify some issues concerning the effects of aeration on the rheological behaviour and will be included in the next steps of the research.

### 3. Results and Discussion

This chapter gathers the information obtained from the microbiological and the rheological analyses and sets them in the scientific literature context.

**3.1. Microbiological Features of Thermophilic Sludge.** The sludge appeared extremely thick, due to the great suspended solids concentration maintained in the TAMR (150–200 g L<sup>-1</sup>). Figure 3 is a micrograph (100x magnification) of the mixed liquor, characterized by a huge amount of inorganic particles (clusters of salts and hydroxides). Flocs are almost absent and bacterial cells are free in the liquor.

Based on the characterization rules of [12], the mean floc diameter was <150 μm. Flocs could be classified as irregular

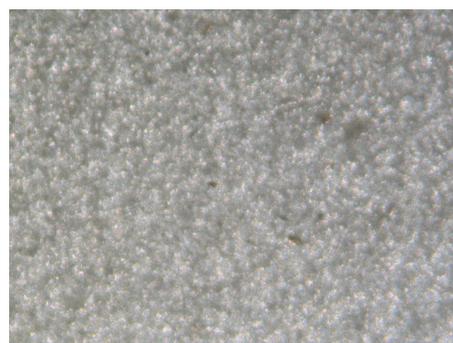


FIGURE 3: Micrograph of the raw sample (magnification: 100x).

and diffuse. Filamentous bacteria abundance belonged to class I (few, which means that filaments are observed only in an occasional floc), according to the distinction proposed by [12], who identified six different cases, based on the average number of filamentous bacteria located in a single floc. Neither zoogreas nor spirochaetes were observed. Likewise, Protozoa and Metazoa were completely absent (as noticed by [78]); therefore, the Sludge Biotic Index (SBI) could not be calculated.

These findings tally with the outcome reported in the scientific literature: a poor (or even lacking) flocculation is usually seen to occur under thermophilic conditions [78, 82–84].

About the amount of filamentous bacteria, however, [83] observed filamentous abundances ascribable to class IV (very common) and class V (abundant), while [85] reported two cases, where filaments reach class IV, with their growth being inversely proportional to the hydraulic retention time.

The paper [78] puts forward several hypotheses to explain the lack of flocculation: firstly, the absence of floc forming microorganisms (such as zoogreas, not found also in the present case, as previously mentioned); secondly, the impossibility to reach the physiological state enabling the flocculation; finally, the establishment of medium conditions interfering with flocculation and coagulation. Further assumptions are made by authors in [84] who ascribed the scarce flocculation to the high shear sensitivity of the thermophilic floc, which becomes prone to erosion; they also suggest a possible influence of decreased cell hydrophobicity.

Table 2 presents the results of FISH analyses, while Figure 4 highlights the percentages of detected taxa over the total bacterial population. Only three major bacteria groups were found with higher shares (Betaproteobacteria, Gammaproteobacteria, and Cytophaga-Flexibacter). Two further major groups (Deltaproteobacteria and Chloroflexi) were quantified with very low shares and the other groups were completely absent, indicating very limiting conditions for typical wastewater treatment plant bacteria. The FISH analyses confirmed the nearly total absence of filamentous bacteria. Furthermore, physiological groups like nitrifying bacteria were completely absent. Such a profile is quite unusual for a mesophilic activated sludge and can be attributable to temperature conditions and wastewater characteristics, which trigger a very special composition of the population. Actually, the above-mentioned factors, together with plant running strategies, can be nowadays linked with the growth of specific bacterial populations and the achievement of a particular physiological state [86–88]; on the other hand, this is even more true in the case study, where temperature is constantly maintained at 45°C, and the influent sewage consists in high-strength COD wastes.

Betaproteobacteria were the dominant group in the sludge sample. They were detected with a remarkable high share of 77% of the total viable bacteria. No filaments or ammonium oxidizing bacteria belonging to this class were observed, as found by [78]. Almost the complete population was formed by members of the  $\beta$ 1-group. All organisms of this group showed bright fluorescence signals indicating a good physiological status of the cells. Levels up to 25% of this group are considered as normal in industrial wastewater treatment plants. Gammaproteobacteria were measured with an unusual high share of 15% in comparison to typical wastewater sludge values. In effect, normally, this group does not exceed a share of 10%. Here, they appeared mainly as thin long rod-shaped bacteria with middle fluorescence signals indicating a moderate physiological activity of the cells. No characteristic filaments of this group like *Thiothrix* or Eikelboom type 021N were found. The authors in [88] postulated that these cocci possess weak flocculation properties (at least a subgroup of Gammaproteobacteria): also their abundance might be related to the presence of small flocs.

The share of members of the Cytophaga-Flexibacter sub-phylum was 4% of the total viable flora, which corresponds more or less to typical values in activated sludge, which can reach a percentage of 10%. The thin rod-shaped cells showed low fluorescence signals. Filaments like *Haliscomenobacter hydrossis* were not detected.

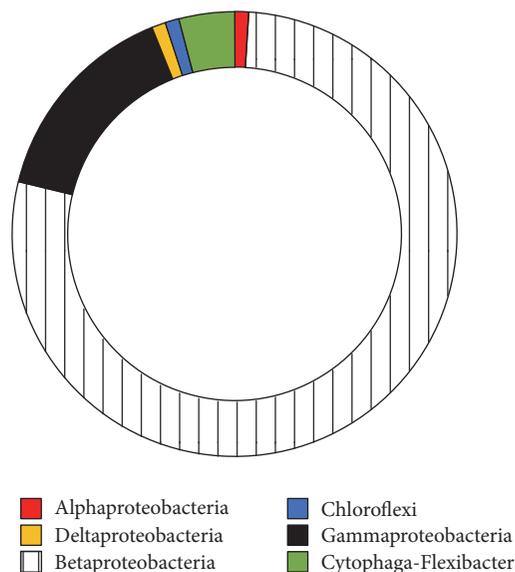


FIGURE 4: Pie chart of the detected taxa shares.

Alphaproteobacteria were detected with a share of only 1% and appeared completely as coccus-shaped uniform cells. Shares of up to 20% of this group are quite normal for industrial activated sludge.

Members of the *Chloroflexi* genus were measured with a very low share of <1% in the sample. The single filaments had a length of about 50  $\mu\text{m}$ . In wastewater treatment plants, 10–20% of this group are quite common. *Chloroflexi* bacteria in WWTPs showed increased shares over the last years; they cause bulking and foaming events if huge abundances are reached.

Deltaproteobacteria, including most sulphate-reducing bacteria, were represented as uniform single cells, with a share of <1%. Values of up to 8% for this group can be found in the mixed liquor of plants treating industrial wastewater. All other main bacteria groups including filamentous bacteria were not detected.

High values for total cell counts (dead and alive) were determined, with  $2.5 \cdot 10^{10} \text{ mL}^{-1}$  and total viable cell counts with  $5.0 \cdot 10^9 \text{ mL}^{-1}$ , which is larger than the total viable cell counts of up to  $1-3 \cdot 10^9 \text{ mL}^{-1}$  usually detected in industrial wastewater activated sludge treatment plants.

Figures 5–7 clearly show the structure of the sludge, consisting basically in clusters of salts and hydroxides and free cells.

### 3.2. Rheological and Statistical Analysis of Activated Sludge.

Several analytical models have been proposed to mimic the rheological behaviour of biological sludge, each with different degree of complexity depending on the number of the parameters contained [68]. Most of these models can be mathematically described by a power-law curve including an offset to account for yield stress.

In the paper [51], the rheological characteristics of an activated sludge sampled in a pilot MBR system, with a MLSS concentration varying between  $2.74 \text{ g L}^{-1}$  and  $16 \text{ g L}^{-1}$ , were

TABLE 2: Results of the application of the VIT gene probes.

Analysed target microorganism(s)	Physiological features	Share of each group in relation to the overall bacteria population (%)	
		Species	Group
<b>Alphaproteobacteria</b> (amount of filamentous bacteria)	Group with high amount of heterotrophic organisms (e.g., genera <i>Paracoccus</i> , <i>Sphingomonas</i> , <i>Rhizobium</i> , <i>Caulobacter</i> , and <i>Rhodospirillum</i> )		1 (n.d.)
<i>Alysiosphaera</i>	Group consisting of filamentous bacteria similar to <i>Nostocoida limicola</i> II, with typical chain structures. Responsible for bulking sludge in industrial wastewater treatment plants	n.d.	
<b>Betaproteobacteria</b> (amount of filamentous bacteria)	Group consisting of the $\beta$ 1-group (many filamentous bacteria) and of the $\beta$ 2-group (most ammonia oxidizing bacteria). In municipal WWTPs, mostly the dominating group (e.g., genera <i>Burkholderia</i> , <i>Sphaerotilus</i> , <i>Alcaligenes</i> , <i>Thiobacillus</i> , and <i>Nitrosomonas</i> )		77 (n.d.)
$\beta$ 1-group of the Betaproteobacteria (amount of filamentous bacteria)	Group consisting of floc forming bacteria and <i>S. natans</i> related filamentous bacteria	76 (n.d.)	
<b>Gammaproteobacteria</b> (amount of filamentous bacteria)	Group consisting of heterotrophic, mixotrophic, and autotrophic microorganisms (e.g., genera <i>Acinetobacter</i> , <i>Aeromonas</i> , <i>Pseudomonas</i> , <i>Vibrio</i> , and <i>Thiothrix</i> )		15 (n.d.)
<i>Acinetobacter</i>	Filamentous and nonfilamentous bacteria. Common in municipal and industrial WWTPs with a relatively high sludge age. Favoured by scarce dissolved oxygen concentration	n.d.	
<i>Thiothrix</i>	Filamentous sulphur bacterium, favoured by nutrient deficiency. Adapted to low dissolved oxygen concentration	n.d.	
<i>Eikelboom type 021N</i>	Filamentous sulphur bacterium common in municipal and industrial WWTPs. Favoured by nutrient deficiency. Adapted to low dissolved oxygen concentration	n.d.	
<b>Deltaproteobacteria</b> (amount of filamentous bacteria)	Group including most sulphate reducing bacteria (e.g., families Desulfobacteraceae, Desulfobulbaceae, and Desulfovibrionaceae)		<1 (n.d.)
<b>Chloroflexi</b> (amount of filamentous bacteria)	Group of filamentous organisms with increasing impact on municipal and industrial WWTPs, due to their contribution to foaming and bulking events, very stable against mechanical stress (genus <i>Herpetosiphon</i> , type 1851, type 0803, and type 0092 and many other filaments still unknown)		<1 (n.d.)
<i>Eikelboom type 1851</i>	Filamentous bacterium; contribution to sludge bulking. Favoured by nutrient deficiency	n.d.	
<i>Herpetosiphon aurantiacus</i>	Filamentous bacterium. Aerobic and anaerobic conditions	n.d.	
Cytophaga-Flexibacter subphylum (amount of filamentous bacteria)	Group containing filamentous and floc forming bacteria. Mainly in WWTPs with nutrients removal (e.g., genera <i>Cytophaga</i> , <i>Flavobacterium</i> , and <i>Flexibacter</i> ),		4 (n.d.)
<b>Haliscomenobacter hydrossis</b>	Filamentous bacterium; contribution to sludge bulking. Favoured by high sludge age, low dissolved oxygen concentration, and high ammonia concentration	n.d.	
<b>Planctomycetes</b> (amount of filamentous bacteria)	Group, mainly in municipal WWTPs; members of this group are involved in the ANAMMOX process. Adapted to low dissolved oxygen concentration (e.g., genera <i>Planctomyces</i> , <i>Pirellula</i> , <i>Isosphaera</i> , and <i>Scalindua</i> )		n.d.
Candidatus <i>Nostocoida limicola</i> type III	Filamentous bacterium; contribution to sludge bulking	n.d.	

TABLE 2: Continued.

Analysed target microorganism(s)	Physiological features	Share of each group in relation to the overall bacteria population (%)	
		Species	Group
<b>Actinobacteria</b> (amount of filamentous bacteria)	Group of GRAM positive bacteria with a high DNA GC content. Several problematic filamentous bacteria like <i>Microthrix parvicella</i> and nocardioforms. In WWTPs with nutrients removal (also biological phosphorous removal)		n.d.
<i>Microthrix parvicella</i>	Filamentous bacterium; in WWTPs with nutrients removal. Contribution to sludge bulking, floating, and foaming. Strong hydrophobic characteristics	n.d.	
Nocardioforms	Group containing filamentous and floc forming bacteria. Contribution to sludge bulking, floating, and foaming. Strong hydrophobic characteristics	n.d.	
Candidatus <i>Nostocoida limicola</i> type II	Typical chain structures. Contribution to sludge bulking. In municipal WWTPs	n.d.	
<b>Firmicutes</b> (amount of filamentous bacteria)	Group of GRAM positive bacteria with a low DNA GC content. Several fermentative bacteria like the genera <i>Streptococcus</i> , <i>Bacillus</i> , and <i>Lactobacillus</i>		n.d.
Candidatus <i>Nostocoida limicola</i> type I	Filamentous bacterium. Contribution to sludge bulking	n.d.	
<b>Candidatus TM7</b>	Group recently detected, including filamentous bacteria like Eikelboom type 0041		n.d.
<b>Group of ammonia oxidizing bacteria (AOB)</b>	For example, genera <i>Nitrosomonas</i> and <i>Nitrosococcus</i>		n.d.
<b>Group of nitrite oxidizing bacteria (NOB)</b>	For example, genera <i>Nitrobacter</i> and <i>Nitrospira</i>		n.d.
<b>Phosphate accumulating organisms (PAO)</b>	Responsible for the biological phosphorus removal (e.g., Candidatus <i>Accumulibacter phosphatis</i> )		n.d.
<b>Glycogen accumulating organisms (GAO)</b>	Candidatus <i>Competibacter phosphatis</i>		n.d.

examined. These authors showed that *Herschel-Bulkley* model provides suitable fitting of experimental data for shear rate above  $25 \text{ s}^{-1}$ .

Proper fitting of the experimental data analysed in this work (MLSS concentrations in the range  $150\text{--}190 \text{ g L}^{-1}$ ) was obtained with *Herschel-Bulkley* model which is recognized to be more efficient in describing the overall rheological behaviour of sludge with high solid concentration [65]:

$$\tau = \tau_y + K\dot{\gamma}^n. \quad (2)$$

In (2), the two fitting parameters are the consistency coefficient  $K$  and the flow behaviour index  $n$ . As explained in the following, yield stress  $\tau_y$  was not considered as a fitting parameter of the flow curve.

Several statistical descriptors can be adopted to measure the accuracy of prediction of rheological models and the reliability of their estimated parameters [65]. In this work, the coefficient of determination (or regression coefficient)  $R^2$  and the standard error of the estimate  $\sigma_{\text{est}}$  in (3) were adopted according to [51]

$$\sigma_{\text{est}} = \left[ \sum_{i=1}^N \frac{(y_i - y_{\text{pre}})^2}{N - 2} \right]^{1/2}. \quad (3)$$

In (3),  $N$  denotes the number of samples included in the population of the stochastic variable  $y$  (i.e.,  $N$  is the number of measures contained in the data set of each test carried out at a given shear rate, temperature, pH, and aeration), while  $y_{\text{pre}}$  is the corresponding predicted value obtained through the following linear regression equation:

$$\begin{aligned} y &= ax + b, \\ y &= \log(\tau - \tau_y), \\ x &= \log(\dot{\gamma}), \\ a &= n, \\ b &= \log(K). \end{aligned} \quad (4)$$

The linearized flow equation for the regression analysis, denoted by the first equation of (4), contains three rheological parameters of the sludge that are the consistency coefficient  $K$ , the flow behaviour index  $n$ , and the yield stress  $\tau_y$  that may be, in general, different from zero.

The method of ordinary least squares (OLS) was adopted for linear regression for fitting the dependence of shear stress versus shear rate.

As well known, only two of the three above-mentioned model parameters can be reliably estimated through linear

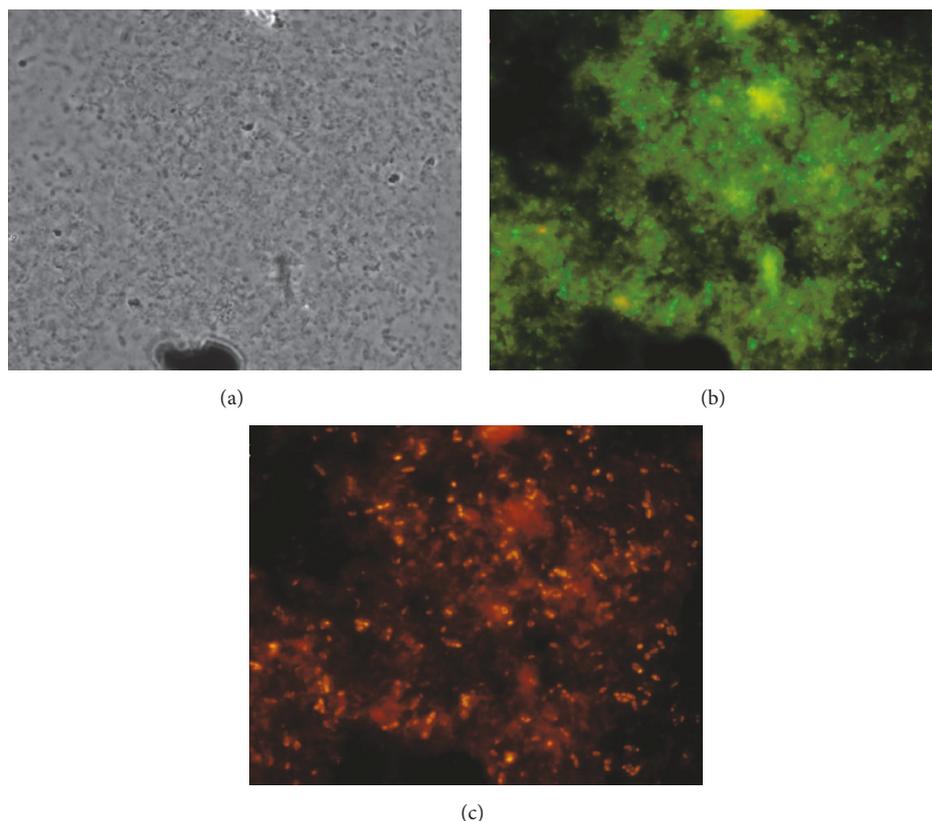


FIGURE 5: Micrographs (magnification: 1000x) of the flocs under phase contrast and epifluorescence. Comparison of the same floc area. Hybridization target: viable bacteria and Betaproteobacteria. (a) Phase contrast. Identical microscopic fields under fluorescence: (b) detection of all viable bacteria and (c) analysis with a specific probe for Betaproteobacteria.

regression. Therefore, in the following statistical analysis, it was assumed as fitting parameters the flow behaviour index  $n$  and the consistency coefficient  $K$  that are included in the two parameters  $a$  and  $b$  of the linear equation (see (4)). However, the proper value of yield stress should be estimated in order to fit experimental data in all testing conditions.

In some works, the yield stress  $\tau_y$  has been determined through graphical extrapolation of the flow curve [51]. Owing to the above-mentioned lack of experimental data below the shear rate of  $500 \text{ s}^{-1}$ , in the present study, an alternative procedure for the estimate of  $\tau_y$  was adopted, as explained below.

In each test, when carrying out the linear regression of the experimental data with respect to the unknown parameters  $K$  and  $n$ , the yield stress was considered as a deterministic parameter ranging in the interval  $[0.0\text{--}3.9] \text{ Pa}$  at steps of  $0.05 \text{ Pa}$ : for each of these values statistical estimates of both  $a$  and  $b$  were obtained along with the corresponding value of the regression coefficient  $R^2$ . The value of the yield stress that, for each test, maximizes the regression coefficient was assumed as an estimate of  $\tau_y$ .

The obtained results of the statistical analysis are summarized in Table 3 for both concentrations of suspended solid.

The results obtained for both MLSS concentrations show that the flow behaviour index  $n$  was greater than one; at the lower MLSS concentration of  $150 \text{ g L}^{-1}$ , the sludge exhibited

shear-thickening (dilatant) behaviour, with the estimated yield  $\tau_y$  stress equal to zero.

For all the tests carried out, the apparent viscosity increased, at any shear rate, with the solid content; moreover, the differences in apparent viscosity measured at different shear rates increased with solid content, in accordance with [89]. This rheological behaviour was different from the shear-thinning (pseudo-plastic) behaviour noticed by [51] for MBR sludge samples with suspended solid concentration not exceeding  $16.0 \text{ g L}^{-1}$ . In the present study, however, the MLSS concentration was about one order of magnitude higher. The interactions between the sludge particles were expected to be even more intense and therefore one might have expected a nonzero yield stress [89]. In fact, as the particle concentration increases, they became progressively more close to each other, thus leading to a rapid growth of the number of interactions that opposes to deformation.

Anyway, such a dilatant behaviour may be related to the fact that in this work the sludge samples were tested at very high temperature of about  $45^\circ\text{C}$ , which is unusual if one considers that generally the testing temperature does not exceed  $35^\circ\text{C}$  [68].

In order to point out possible temperature effects on the obtained results, the work by [65] can be considered which investigated the rheology of mixed primary and secondary sludge as a function two critical parameters affecting flow

TABLE 3: Summary of rheological tests and results of linear regression analysis.

Test #	Average $T$ [°C]	Average pH [—]	Aeration	MLSS [gTS L <sup>-1</sup> ]	$\tau_y$ [Pa]	$n$ [—]	$K$ [Pa s <sup><math>n</math></sup> ]	$R^2$ [—]	$\sigma_{est}$ [—]
1	44.5	6.7	N	150	0.0	2.760	$1.992e-8$	0.997	0.0296
2	45.2	6.7	Y		0.0	2.909	$6.805e-9$	0.971	0.0944
3	45.1	7.7	N		0.0	2.976	$4.474e-9$	0.965	0.1066
4	45.3	8.3	Y		0.0	3.120	$1.642e-9$	0.967	0.0905
5	45.1	9.0	N		0.0	2.138	$1.125e-6$	0.923	0.0711
6	44.9	9.1	Y		0.0	2.925	$4.080e-9$	0.953	0.0706
7	45.4	7.0	N	190	2.35	5.307	$3.824e-16$	0.986	0.2107
8	45.3	6.7	1/2		1.95	3.901	$6.532e-12$	0.992	0.1146
9	45.0	6.6	Y		2.05	3.508	$9.316e-11$	0.989	0.1199
10	44.9	7.8	N		1.90	4.433	$1.60e-13$	0.998	0.0646
11	45.2	8.3	1/2		1.65	3.452	$1.711e-10$	0.994	0.0875
12	45.3	8.4	Y		1.70	3.590	$6.253e-11$	0.992	0.1091
13	45.0	8.9	N		1.40	4.585	$6.285e-14$	0.998	0.0651
14	45.3	9.0	1/2		1.60	5.468	$1.469e-16$	0.998	0.0791
15	45.0	9.1	Y		1.25	5.221	$8.227e-16$	0.998	0.0852

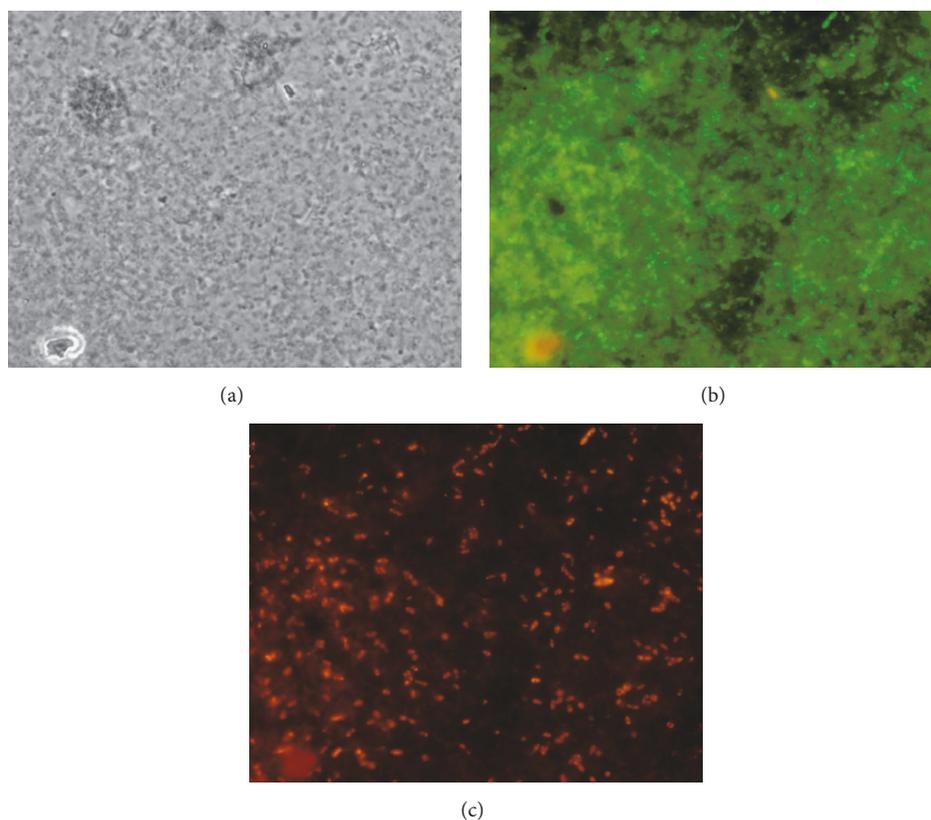


FIGURE 6: Micrographs (magnification: 1000x) of the flocs under phase contrast and epifluorescence. Comparison of the same floc area. Hybridization target: viable bacteria and  $\beta 1$ , Betaproteobacteria. (a) Phase contrast. Identical microscopic fields under fluorescence: (b) detection of all viable bacteria and (c) analysis with a specific probe for  $\beta 1$ -group of Betaproteobacteria.

behaviour: temperature, ranging between 25°C to 55°C, and solid concentration, varying between 4.3% and 9.8% that is quite below the total solid content investigated in this work.

These authors have shown that (for a given solid concentration) yield stress depends exponentially on the inverse

of the temperature. Therefore, an increase of the testing temperature seems consistent with a relevant reduction of the yield stress.

It is worth noting that in all tests with suspended solid concentration of MLSS = 150 g L<sup>-1</sup> when imposing a shear

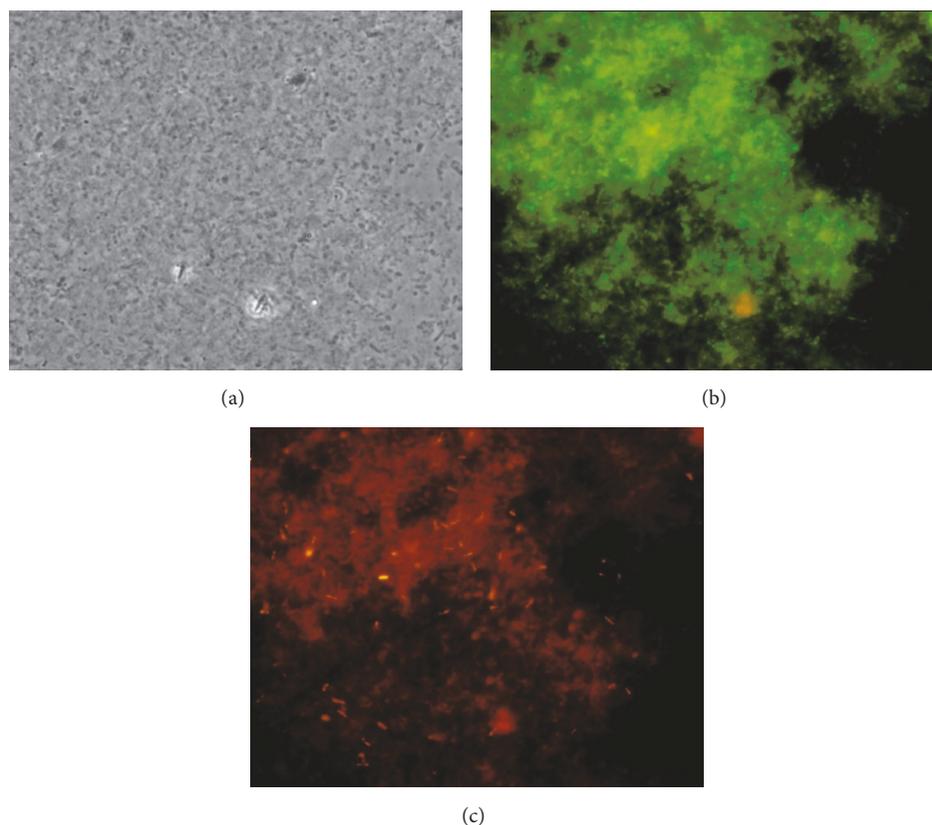


FIGURE 7: Micrographs (magnification: 1000x) of the flocs under phase contrast and epifluorescence. Comparison of the same floc area. Hybridization target: viable bacteria and Gammaproteobacteria. (a) Phase contrast. Identical microscopic fields under fluorescence: (b) detection of all viable bacteria and (c) analysis with a specific probe for Gammaproteobacteria.

rate below  $100 \text{ s}^{-1}$  the measured shear stress was under the minimum value that can be resolved by the rheometer (see Section 2.2.2). Indeed, the assumption of a zero yield stress appears quite plausible.

At this stage of investigation, the flow properties of all the sludge samples were tested at a shear rate greater than or equal to  $500 \text{ s}^{-1}$ . This is due to technical reasons related to the modelling of particular operating conditions of concern in the treatment plant. From a theoretical point of view, the experimental points below the shear rate value of  $500 \text{ s}^{-1}$  may influence the extrapolation of the yield stress for the analysed samples; for this reason, further studies will be carried out to investigate this aspect.

In the work by [65], it has been noticed that (for a given temperature) yield stress increases almost linearly with solid concentration. This seems in accordance with the experimental results obtained for the higher solid concentration (MLSS) of  $190 \text{ g L}^{-1}$  that showed significant shear stress values even at the lower shear rate (see Figures 9(a) and 9(b) and Table 3). In this case, proper fitting of the experimental points requires nonzero yield stress to be considered in the mathematical model of the flow curve in (2).

Suitable values were obtained for the regression coefficient in all tests. The best fitting of the adopted mathematical model is obtained for the higher MLSS concentration of

$190 \text{ g L}^{-1}$ , as confirmed by the values of  $R^2$  for the tests from 7 to 15 in Table 3.

Concerning the lower MLSS concentration of  $150 \text{ g L}^{-1}$ , the minimum value of  $R^2 = 0.923$  was obtained for the test number 5 and was however combined with a relatively small value of the standard error of the estimate  $\sigma_{\text{est}}$ . In the remaining tests at the same concentration, the regression coefficient was significantly higher, and for the test case number 1, the best fitting was obtained.

In the paper by [51], it has been shown that, by varying the mathematical model to represent the rheological behaviour of the sludge sample, the correlation coefficient may fall below 0.9 and  $\sigma_{\text{est}}$  may rise to 0.9 at high concentrations of suspended solids. Further investigations may thus be carried out to assess if the result obtained for test number 5 might be influenced by the selected mathematical model. Anyway, the interpolating curve obtained for test number 5 (see Figure 8) seems quite acceptable for technical purposes in the engineering management of treatment plants where several sources of uncertainty may commonly affect the predictions.

Figures 8 and 9 show, for each rheological test, the flow curves (blue) calculated from the linear regression; the corresponding experimental data are plotted with red plus markers along with symmetric error bars  $2 \cdot \sigma_\tau$  long, where

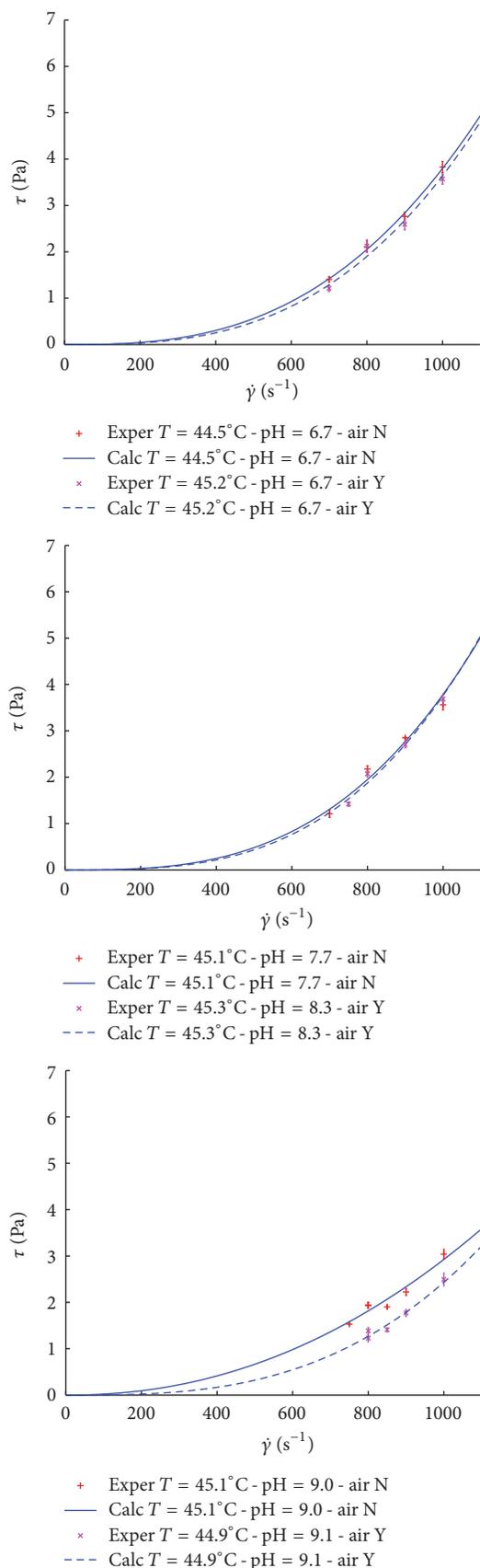


FIGURE 8: Shear stress versus rate of deformation (MLSS 150  $g L^{-1}$ ): comparison between experimental and calculated values.

$\sigma_{\tau}$  is the standard deviation of the shear stress measurements obtained with the rheometer at each shear rate value.

Figure 8 plots the results obtained for the MLSS concentration of 150  $g L^{-1}$ . For a given pH value, the aeration of the sludge sample (air Y) led, in general, to a reduction of apparent viscosity (hence, of the shear stress) at any shear rate with respect to the corresponding test on non-aerated samples (air N). This behaviour is clearly evident in the case with pH around 9.0 (lower panel) where, in the investigated shear rate range, the aeration of the sample produced a significant reduction of the shear stress that reaches 0.55 Pa at some shear rates between 700  $s^{-1}$  and 1000  $s^{-1}$ . At smaller pH values, the Y and N curves are very close to each other and the above-mentioned shear stress reduction may be caused by interpolation uncertainties connected to the low number of experimental points, rather than being connected to the aeration. This aspect deserves further investigation.

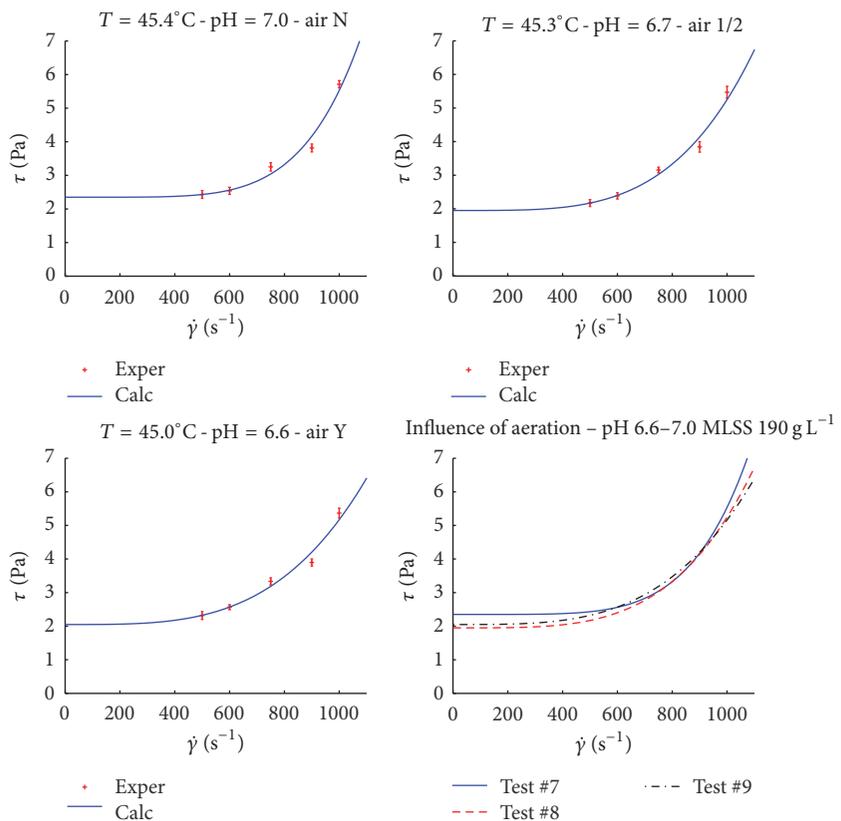
Obtained results seem however reasonable, since aeration of the sludge may lead to bubble entrapment into the bioaggregate that increases the mean distance between suspended solid particles and consequently weaken the interactions between them; this lowers both apparent viscosity and shear stress at any shear rate. Such an effect is clearly evident at the highest investigated pH (lower panel).

Figures 9(a), 9(b), and 9(c) show the flow curves obtained for the higher MLSS concentration of 190  $g L^{-1}$ . In this case, the effect of aeration on the rheology depends on pH condition, as described below.

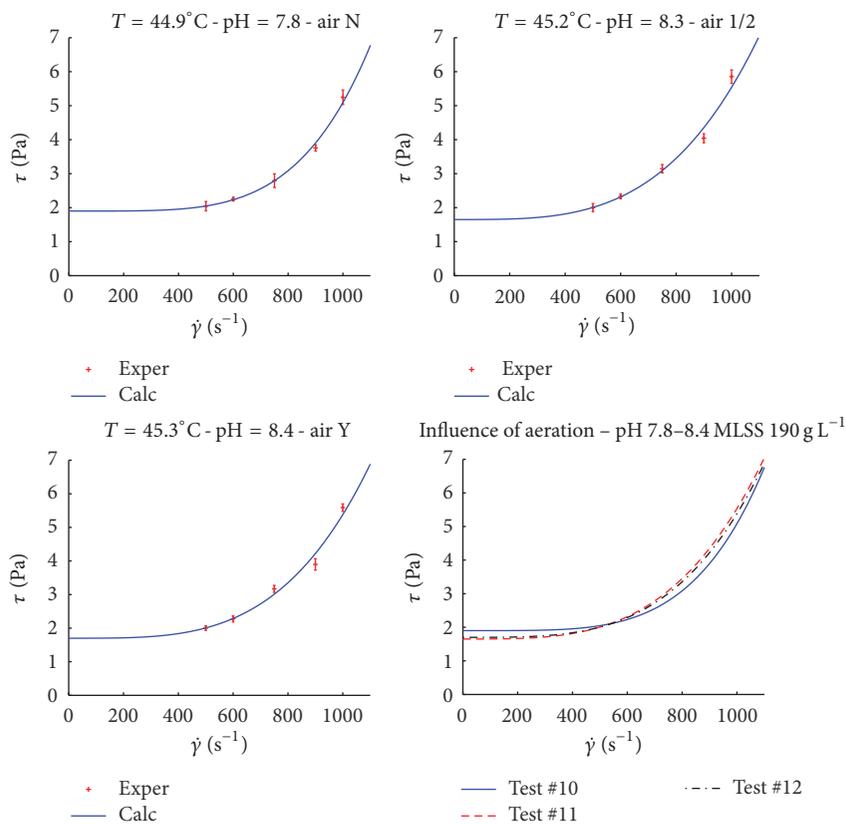
In those tests where pH ranged between 6.6 and 7.0 (Figure 9(a)), the effect of aeration (tests 8 and 9) led to a reduction of shear stress and apparent viscosity when the rate of deformation was below 600  $s^{-1}$  or above 900  $s^{-1}$ ; within the range (700–900)  $s^{-1}$  the flow curves for tests 7 (air N) and 8 (half aeration flow) were very close to each other and below the flow curve estimated for test case number 9 corresponding to fully aerated condition (air Y). This shear stress increment induced by aeration in the shear rate range (700–900)  $s^{-1}$  is quite scarce and apparently in contrast with the findings obtained for the 150  $g L^{-1}$  sample showing that the Y curves are generally below the corresponding N curves at any shear rate (Figure 8).

Increasing the pH around 7.8–8.4 (Figure 9(b)), it could be noticed that if the shear rate was below 540  $s^{-1}$  the aeration of sludge samples (tests 11 and 12) yielded reduction of the shear stress and apparent viscosity with respect to nonaerated sample (test 10); above this shear rate value of 540  $s^{-1}$ , an opposite effect was detected since the shear stress and apparent viscosity became greater in the aerated samples. In both cases, the shear stress differences between these flow curves are quite small and may be related to the interpolation procedure rather than reflecting the actual rheological behaviour.

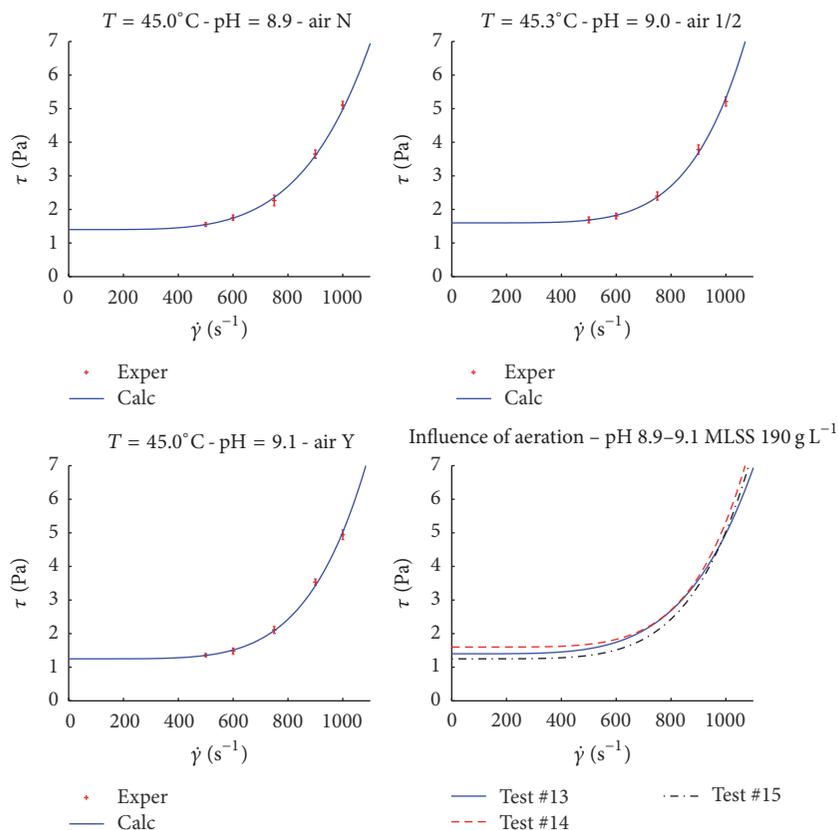
Considering the maximum value of the pH = 9.0 (Figure 9(c)), the shear stress and apparent viscosity for the aerated sample at full flow rate (test 15, air Y) were smaller than those obtained for the nonaerated sample (test 13, air N) when the shear rate was below 970  $s^{-1}$ . Above this shear rate value, the shear stress was greater for the full aerated sample.



(a) Shear stress versus rate of deformation (MLSS  $190 \text{ g L}^{-1}$ ). Lower right-hand panel compares calculated values for pH condition around 6.6–7.0



(b) Shear stress versus rate of deformation (MLSS  $190 \text{ g L}^{-1}$ ). Lower right-hand panel compares calculated values for pH condition around 7.8–8.4



(c) Shear stress versus rate of deformation (MLSS  $190 \text{ g L}^{-1}$ ). Lower right-hand panel compares calculated values for pH condition around 8.9-9.1

FIGURE 9

On the contrary, the flow curve of the half aerated sample (test 14, air 1/2) never fell below the flow curve obtained from the nonaerated sample.

Based on the observation of the interpolated flow curves, the influence of aeration on the rheological behaviour of the samples appears to be not univocal: the reduction of the apparent viscosity does not take place at any shear rate for every investigated pH value. Furthermore, in those cases, where the obtained shear stress differences are extremely slight, such a discrepancy may be also ascribed to possible uncertainties caused by the reduced number of experimental samples available for interpolating the flow curves. Hence, further investigations are required so as to assess the influence of aeration with a higher degree of reliability.

Figure 10 shows how pH conditions of the nonaerated samples influence the calculated flow curves for suspended solid concentrations of  $150 \text{ g L}^{-1}$  (left side panel) and  $190 \text{ g L}^{-1}$  (right side panel).

In the case of  $\text{MLSS} = 150 \text{ g L}^{-1}$ , it can be seen that increasing the pH value from 6.7 (test 1) to about 7.7 (test 3) caused a negligible variation of the shear stress and apparent viscosity took place at any shear rate. Further increase of the pH value to 9.0 (test 5) induced, at shear rates lower than  $730 \text{ s}^{-1}$ , a negligible increment with respect to previous curves of both shear stress and apparent viscosity, while above

$730 \text{ s}^{-1}$  the shear stress decreased progressively together with the shear rate growth.

Considering the case  $\text{MLSS} = 190 \text{ g L}^{-1}$ , the observed behaviour was monotonic because, increasing pH values from 7.0 (test 7) to 8.9 (test 13), the shear stress and apparent viscosity diminished at any shear rate below  $1000 \text{ s}^{-1}$ .

Figure 11 shows the influence of pH on the rheological behaviour of aerated sludge samples.

For the case  $\text{MLSS} = 150 \text{ g L}^{-1}$  (left side panel), it can be seen that passing from pH = 6.7 (test 2) to pH = 8.3 (test 4) a negligible variation of both shear stress and apparent viscosity took place. At pH = 9.1 (test 6), the corresponding flow curve was always below the other two curves, resulting in a significant reduction of shear stress at higher shear rate values.

The analysis of the aerated samples with  $\text{MLSS} 190 \text{ g L}^{-1}$  (right side panel) showed that, increasing the pH value with respect to test 9, the reduction of the shear stress was significant at shear rates below  $800 \text{ s}^{-1}$ , while above this value the flow curves corresponding to pH = 8.4 (test 12) and pH = 9.1 (test 15) tended to grow faster and overcome the curve corresponding to pH = 6.6 (test 9).

Figure 12 shows the influence of pH on the rheological behaviour of the denser sludge samples ( $\text{MLSS} 190 \text{ g L}^{-1}$ ) for half the aeration flow rate. The increases of pH value from 6.7 (test 8) to 8.3 (test 11) and 9.0 (test 14) led to a reduction of the

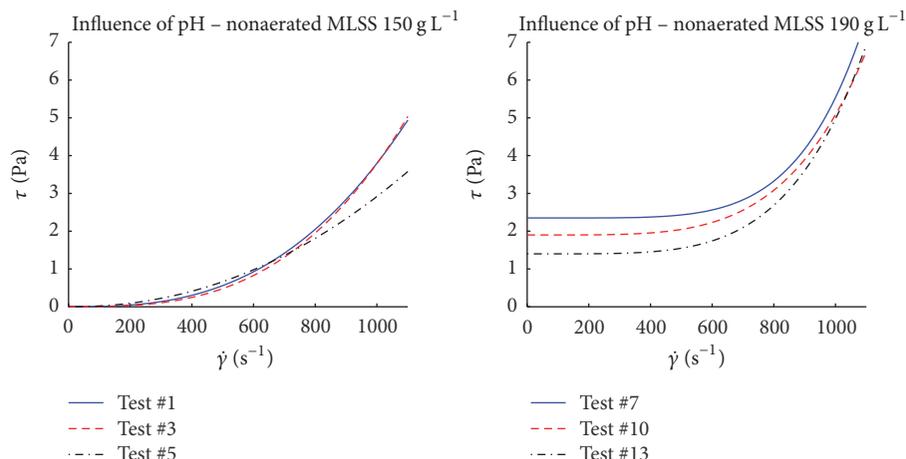


FIGURE 10: Shear stress versus rate of deformation: influence of pH for non-aerated samples.

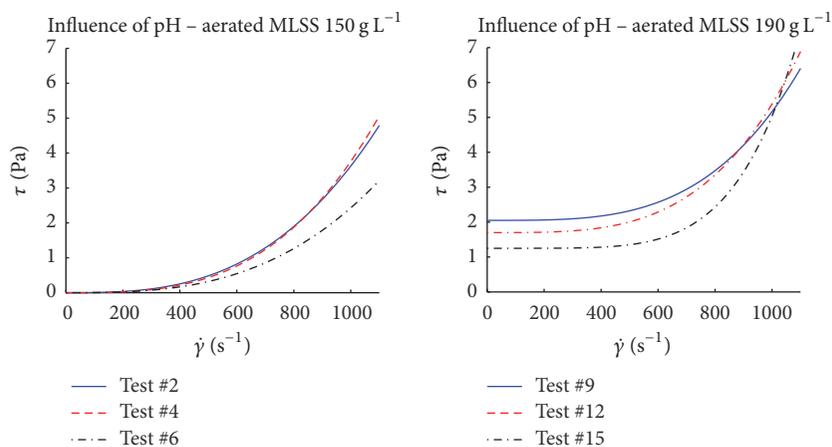


FIGURE 11: Shear stress versus rate of deformation: influence of pH for aerated samples.

shear stress (and following apparent viscosity) if the shear rate was below  $700 \text{ s}^{-1}$  and  $980 \text{ s}^{-1}$ , respectively.

The pH influence on rheological behaviour might be ascribed to the bacteria aggregation extent: [90] showed that, for the activated sludge, both the values of  $\tau_y$  and viscosity increase progressively with pH, until it reaches a value of 7, thus indicating that the strongest flocs cohesion occurs under weakly acidic conditions (pH ranging from 6 to 7). Paradoxically, however, changes of the zeta potential as a function of pH highlight that it is strongly negative for values from 6 to 7, with the isopotential conditions being quite far.

In a subsequent study [59], some tests on activated sludge from both aeration tanks and laboratory scale pilot plants were carried out using rotational rheometer. Although the MLSS concentration was lower than  $10 \text{ g L}^{-1}$ , a similar influence of pH on the mechanical properties of sludge was found. In particular, the yield stress  $\tau_y$  decreased after chlorination, denoting a possible deflocculation.

The samples studied in [59, 90] have a MLSS concentration significantly lower than those tested in the present research: further analyses will be necessary to explore the behaviour of a highly concentrated sludge.

## 4. Conclusions

The population profile differed clearly from a typical profile of municipal as well as industrial wastewater treatment plants. Notable is the missing of several of the major bacteria groups and the low diversity within the present major bacteria groups. Beside the considerably dominating Betaproteobacteria, only a few other main bacteria groups were present showing only very limited diversity within the groups. The remaining organisms seemed to be adapted to the extreme conditions applied and the obtained population profile was very different from typical profiles from other WWTPs with “normal” temperature conditions. All in all, the extreme temperature conditions seem to trigger the surviving of the (fittest) “most adapted” organisms to the conditions applied. Also, the almost complete absence of filamentous bacteria is a typical feature of thermophilic aerobic sludge treatment and can lead to sludge losses due to the inability to form stable sludge flocs.

This early phase of the study was focused on a limited number of shear rate values: nevertheless, the first results provide a valuable insight into the rheological behaviour of

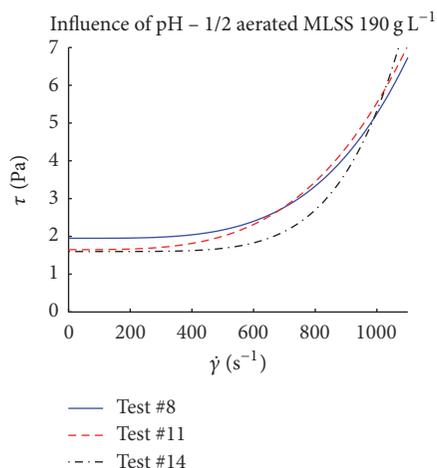


FIGURE 12: Shear stress versus rate of deformation: influence of pH for 1/2 aerated samples.

a highly concentrated thermophilic sludge. Furthermore, the trend of the flow curves shows a certain degree of reliability, as corroborated by the regression coefficients.

The influence of some relevant operative parameters (i.e., pH, temperature, biomass concentration, and aeration within the biological reactor) on the rheological behaviour of the activated sludge was confirmed, thus providing valuable guidelines for an efficient management of the wastewater treatment process. The effects of aeration were sometimes not univocal and reveal an opposing behaviour depending on the values of the other parameters. This aspect may be related to possible uncertainties connected to the low number of samples available for the interpolation of the flow curves, thus resulting in small discrepancy between them. Therefore, it will be better investigated within a future study.

The rheological behaviour affects significantly the treatment processes of TAMR systems because, with respect to conventional processes involving activated sludge, the major part of the energy demand is connected to the use of membranes and is strongly affected by the suspended solid concentration and the biomass viscosity.

The analyses carried out show that the rheological behaviour of TAMR biomass is mainly affected by MLSS concentration.

The sludge exhibited shear-thickening (dilatant) behaviour; at MLSS concentration of  $150 \text{ g L}^{-1}$ , the estimated yield  $\tau_y$  stress was equal to zero, but when MLSS concentration reached  $190 \text{ g L}^{-1}$ , the interactions between the sludge particles were expected to be even more intense and therefore a nonzero yield stress was observed. As a consequence, increasing the biomass concentration, up to  $190 \text{ g TS L}^{-1}$ , could provide important information, in terms of rheological behaviour, for the optimal management of thermophilic process.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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