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Morphological features of thermophilic activated sludge treating food industry wastewater in MBR

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Abstract. Microscopic examination of the activated sludge and morphological characterization of the flocs provides detailed information about the treatment process. The aim of this study is to investigate the morphological parameters of flocs obtained from a thermophilic jet loop membrane bioreactor (JLMBR) in different sludge retention times (SRTs), considering EPS and SMP concentration, hydrophobicity, zeta potential. The results showed that irregularity decreased with the increasing SRT. The compactness value was calculated to be less than 1 for all SRTs. However, the sludge had a more compact structure when the SRT increased. Zeta potential increased whereas hydrophobicity and floc size reduced, with increasing SRT. Furthermore, 2-D porosity calculated using the hole ratio was higher at greater SRTs. Hence, there was a significant correlation between the results obtained using the imaging technique and operation conditions of thermophilic JLMBR.

Keywords: EPS; hydrophobicity; membrane bioreactor; morphological parameters; SMP; thermophilic

1. Introduction

Combining environmental compliance with cost effectiveness calls for new approaches to wastewater treatment. Jet loop bioreactors are known for their efficiency in wastewater treatment and cost-effectiveness compared to the conventional treatment technologies (Hung *et al.* 2006). The prominent advantages of these high-rate reactors can be listed as; having an uncomplicated structure, operation simplicity, well-defined flow regimes, better dispersion impacts, lower power consumption, lower sludge production, and high mass transfer (Hung *et al.* 2006, Farizoglu and Keskinler 2006, Koseoğlu-İmer *et al.* 2011). Among these advantages, the achievement of high dissolved oxygen concentrations by means of the turbulence created throughout the reactor is of particular importance to obtain high removal efficiencies for high strength wastewater (Hung *et al.* 2006). All these features can be further improved by creating thermophilic conditions in these reactors.

Thermophilic aerobic wastewater treatment improves high-rate reactors by providing faster degradation rates, rapid inactivation of pathogenic microorganisms, and low sludge yields. An increase in biodegradation rates would reduce the retention time necessary for organic matter decomposition, thus lowering the capital cost. High biodegradation rates would also improve process stability enabling the system to recover from unexpected conditions. There are, of course, certain disadvantages of thermophilic aerobic processes, such as increased costs in relation to aeration, poor settling characteristics, and foaming problems. In an earlier study, the oxygen requirements for thermophilic processes were estimated to be 14% higher than for conventional aerobic processes (Surucu 1975). To overcome this problem, the use of aggressive aeration equipment and greater tank depths is recommended (Rozich and Colvin 1997). Here, the importance of selecting appropriate aeration equipment comes into prominence as one of the most critical decisions in the design process (Lapara and Alleman 1999). Therefore, jet-loop reactors with a high mass transfer capacity and high turbulence can be offered as suitable media for thermophilic aerobic treatment.

The size and structure of flocs are considered fundamental to the successful operation of industrial unit processes (Waite 1999). In water and wastewater treatment processes, the aim is to remove impurities from water in the form of solid particles. Once the solid particles are produced, they can be separated from water using sedimentation, flotation, filtration, and thickening techniques (Sebayang *et al.* 2013, Adonadaga 2015). The physical characteristics of the floc are therefore essential for determining their removal efficiency. For example, large compact flocs have a high settling rate that results in the treated water having low turbidity during settlement (Wilen *et al.* 2003, Sebayang *et al.* 2013, Adonadaga 2015) whereas correlatively large and porous flocs aid filtration due to high permeability (Bushell *et al.* 2003, Hai *et al.* 2014).

Microscopic examination of the activated sludge and morphological characterization of floc provides detailed information about the treatment process. The flocs with loose and bulging hinder sludge precipitation. Thus, the

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quality of the effluent decreases and the filter gets clogged quickly. With recent developments, image analysis has become a very important tool with a large field of applications due to its ability to reduce the subjectivity of human analysis, the possibility to extract quantitative data, and avoid tedious and highly time-consuming tasks for researchers (Amaral and Ferreira 2005).

In recent years, image analysis has become a fundamental tool with great applications in environmental science. In aerobic activated sludge systems, it has been applied for the morphological characterization of microbial flocs, allowing the estimation of different parameters of the Euclidean geometry (Grijspeerdt and Verstraete 1997, Jin *et al.* 2003, Amaral 2003, Grizzi 2015), the fractal analysis of contour of these aggregates, and other aspects such as detecting and counting filaments (Da Motta *et al.* 2001, Wang and Dentel 2010, Jin *et al.* 2013). These parameters have been correlated with the settling properties of activated sludge, estimated as the sludge volume index (SVI) (da Motta *et al.* 2001, Amaral 2003, Perez *et al.* 2006) in order to monitor filamentous bulking in wastewater treatment plants.

Although several studies have performed an image analysis on activated sludge, to the best of our knowledge, no study has conducted an image analysis on thermophilic activated sludge using a JLMBR. Therefore, the aim of this study was to develop a digital image analysis procedure for the characterization of microbial flocs obtained from a JLMBR. Additionally, the proposed procedure was evaluated with activated sludge in terms of its efficiency in identifying the major relationships between the analyzed morphological parameters.

2. Experimental design

2.1 Wastewater source and characterization

The wastewater used in this study was obtained from a factory producing chips and other potato and corn snacks in Turkey. The factory produces around 400 m^3 wastewater/day/product type. The wastewater produced after peeling and cutting processes was used during the course of the study. The characteristics of the wastewater are given in Table 1. All parameters in the table was analyzed according to APHA (2005). The raw wastewater was transferred to the laboratory in 100 L barrels, which were constantly kept at 4°C.

2.2 Equipment, instrumentation, and analysis

The jet loop bioreactor consists of a cylindrical reactor (outer tube) and a draft channel (inner tube) both made from stainless steel with a conical bottom (Fig. 1). Two glass windows were added to monitor the loop occurring in the reactor. The jet is formed at the jet head where the wastewater and air is introduced at various ratios at the end of a nozzle. In these reactors, circulation is achieved by a liquid jet drive. The liquid is injected into the reactor via a nozzle with a high velocity, causing a fine dispersion of liquid and gaseous phases. The liquid and the gas inside the draft tube flow downwards and after deflection at the bottom of the reactor, the mixture rises up between the outer and inner tubes. At the upper end of the draft tube, part of the fluid is recycled into the draft tube by the sucking action. This results in a homogeneous dispersion of the bubbles and the biomass produced in the biological reaction.

A Microdyn-Nadir (MD 063 TP 2N) tubular microfiltration membrane unit with a pore diameter of 0.2 μm was used. The unit was externally placed in the circulation line of the reactor to provide filtration. The membrane made from polypropylene material which was continuously operated in the system had an effective filtration area of 0.20 m^2. The membranes were cleaned with chemical wash on average every month according to the manufacturer's recommendation. The washing procedure includes these steps; (i) washing with 10% NaOH for half an hour, (ii) washing with distillation water during 10 minutes, (iii) washing with 3% HCl for half an hour, (iv), washing with distillation water until reaching neutral pH. In addition, the unit was equipped with a water circulator that was automatically activated when the temperature of the reactor fell below or rose above the thermophilic temperature of 45±2℃. Due to prove organic loading rate (OLR) of 2.0 kg COD/m^3·day at different SRT,

Table 1 Wastewater characteristics (after peeling and cutting processes)

Parameter	Unit	Value
TCOD	g/L	5.60
BOD_5	g/L	4.60
pH	-	6.80
TKN	g/L	0.22
Ammonia	g/L	0.09
TP	g/L	0.08
Sulphate	g/L	0.05

TCOD: Total Chemical Oxygen Demand; BOD_5: 5-day Biochemical Oxygen Demand; TKN: Total Kjeldahl Nitrogen; TP: Total Phosphate

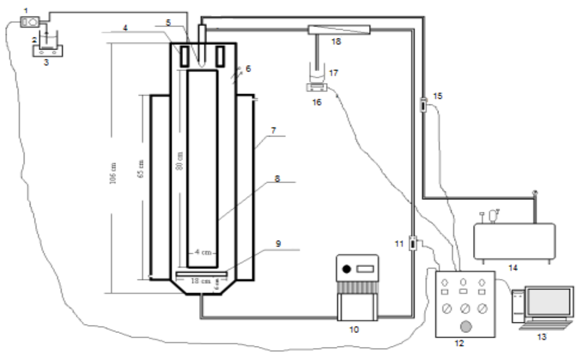


Fig. 1 Schematic depiction of the JLMBR system and its dimensions (1: peristaltic pump, 2: wastewater, 3: stirrer, 4: viewing window, 5: nozzle, 6: pH and DO probes, 7: jacket, 8: draft tube, 9: impact plate, 10: pump, 11: liquid flowmeter, 12: control panel, 13: computer, 14: compressor, 15: gas flowmeter, 16: analytical balance, 17: treated wastewater, 18: membrane)

hydraulic retention time (HRT) was fitted 2.4 day. In this system, transmembrane pressure adjusted 190 kpa and cross-flow rate was 4.5 m/s for all SRT. Membrane fluxes were determined to be 31.78; 34.70; 39.60 and 43.70 $L/m^2 \cdot h$ for the SRTs of 10, 30, 60 and 100 days respectively. During the study, the concentration of dissolved oxygen in the reactor was 2 mg/L on average and the pH was at the neutral level.

2.3 Determination of floc morphology

The development of image processing technology in recent years has led to a wide range of applications in different areas. In this study, an automatic image processing method was used for the characterization of activated sludge morphology.

Image collection: To collect each image, three samples were taken at a single point on the bioreactor to minimize experimental errors in every sludge retention time (SRT) because the activated sludge mixture was homogeneous with high turbulence in the thermophilic JLMBR. The samples were dropped onto the lam with a caliber automatic micropipette (tipped wide to spoil the large-scale floc) and the lamella closed the lam before the images were taken. Approximately 50 images were obtained from each sample.

Image processing: The images of the activated sludge taken from the bioreactor were obtained with a laboratory microscope (Nikon Eclipse). The microscope had an imaging system including a camera, so the images could be stored on a computer. It has been reported that 50-100 times magnification is sufficient to observe the activated sludge containing floc and filament (Liwarska-Bizukojc 2005). Therefore, the magnification of the microscope was set to 100 times. The digital photos were stored in the JPEG format (1280×1024 pixels). The image processing of flocs and other bacteria was carried out using the 'ipwin32' program. The measurement of the relevant objects consists of several stages depending on the object. The processed images were analyzed in terms of certain Euclid geometry parameters and the microbial floc fractal dimension was determined.

2.4 Morphological parameters

After obtaining images according to the procedures described above, certain morphological parameters (such as convexity, compactness, roundness, two-dimensional porosity, fractal geometry, and equivalent diameter) were analyzed. The morphological parameters of the activated sludge floc can be divided into two groups. The first consists of parameters providing information on the floc size such as area, average examination area, perimeter, and equivalent sphere. The average examination area is a basic shape analysis parameter that is easily determined by the condensation of the number of pixels and scaling factor. Other size parameters are calculated based on the average examination area.

For example, the equivalent diameter (D_{eq}) is calculated from the average examination area as follows

$$D_{eq} = 2.\sqrt{\frac{Area}{\pi}} \qquad (1)$$

Flocs can be classified into the following three groups depending on their size; small (<100 μm), medium (100 μm-500 μm) and big (>500 μm) (Nisar *et al.* 2012). Another parameter generally used to describe floc concentration and size is work area (F_A), which is the ratio of the area of the floc in the image to the total image area (Nisar *et al.* 2012).

The second group of morphological parameters describes the shape of floc. The advantage of measuring these parameters is that they are not associated with location, size, and orientation of the displayed objects. They allow the examination of objects that have the same shape but are dissimilar in other respects. Generally, they provide information about the roundness (RD) and regularity of the flocs. Here, the most commonly used parameters are RD and roundness index (C_x). These parameters indicate the size of the measured floc and are similar to each other. The RD value ranges from 0 to 1 with the RD of a fully round object being zero. The C_x is also equal to 1 if the object is completely round. When RD decreases, the C_x value increases. RD is calculated from the average examination area as follows

$$RD = \frac{4.Area}{\pi.Length} \qquad (2)$$

Compactness, convexity, and RD are used to define the shape and amount of microbial flocs. Compactness is expressed as the ratio between the area of an object and circle area with the same perimeter. This parameter is 1 for a round object and smaller than 1 for a non-round object. RD is also 1 for a round object but greater than 1 for objects of a different shape as mentioned above.

Convexity refers to the ratio of between the convex perimeter of an object and its perimeter. The convex perimeter is the perimeter of the outline convex of an object. In this study, these two lengths were measured by ipwin32 software. The convexity value is 0 for an irregular object and 1 for a convex object.

This study also included the 2D porosity parameter. This was estimated from the hole ratio parameter which is the ratio of the object area excluding holes to the total area of the object. This is calculated from the ratio between total hole in the object and the area of the object, expressed as follows

$$\text{2-D porosity} = 100 \times (1 - \text{hole ratio}) \qquad (3)$$

Fractal Geometry: The floc fractal dimension is used to characterize bacterial flocs (Zhao *et al.* 2013). It is a measurement of circumference irregularity. In some studies, this parameter has been used to characterize the contour disorder of the microbial flocs in the water and wastewater processes (Perez *et al.* 2006, Zhao and Chen 2011). In the current study, the floc fractal dimension was calculated using the box counting method (Perez *et al.* 2006, Yingyi *et al.* 2012). The value of this parameter ranges from 1 to 2. The objects, having disordered contour, give small values and the uniformity grows as the value increases (Perez *et al.* 2006, Zhu *et al.* 2015).

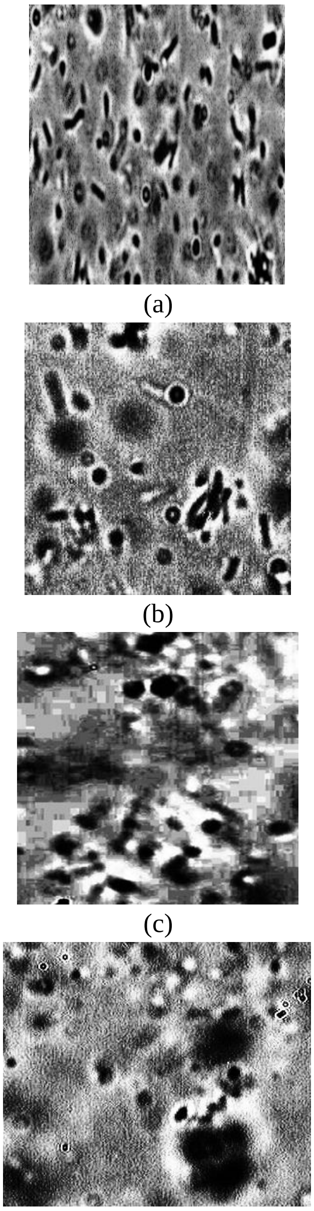
Additionally, the microbial adhesion to hydrocarbons method was used to analyze the relative hydrophobicity (Hsu *et al.* 2013). The particle size and zeta potential were measured using the Mastersizer 2000 model (Malvern) when the system reached a steady state for different SRTs. Each sample was measured three times. There is no standard method for the acceptance analysis of extracellular polymeric substance (EPS). Therefore, EPS was detected by formaldehyde as the most widely used extraction method in the current literature (Tinggang *et al.* 2008). In addition, the Lowry method was used for protein analysis, and the phenol sulfuric acid method was utilized for carbohydrate analysis (Koseoglu-Imer *et al.* 2011).

3. Results and discussion

Due to the effects of shear force on the metabolism and physical properties of sludge flocs (Liu *et al.* 2005, Zhang and Liu 2011, Jalili *et al.* 2015), high turbulence in JLMBR affects sludge flocs. With the increasing shear force, the compactness, roundness, and decrement rates are observed to increase (Tay *et al.* 2001). Liu *et al.* (2005) reported that particle size increased from 37 μm to 59 μm when the mixing speed increased from 50 rpm to 400 rpm. However, further increasing the stirring speed (800 rpm) decreased the particle size to 36 μm. Weak or strong mixing reduce the size of sludge flocs. Therefore, the floc size of activated sludge in a JLMBR is much smaller than in conventional membrane bioreactors due to high turbulence. Liu *et al.* (2005) reported that SVI decreased from 165 mL/g to 124 and finally 66 mL/g when the mixing speed increased from 50 rpm to 200 and reached 400 rpm. When the stirring speed was 800 rpm, SVI increased again (193 mL/g). Sludge particle size and precipitation are usually very similar; however, in cases where they are different, this situation is associated with the stirring speed. The particle size of sludge in the JLMBR was small because of high turbulence, so the precipitation of sludge was fairly bad.

Sludge particle size is associated with zeta potential, cell surface hydrophobicity, and EPS production rate. When the hydrophobicity and production of carbohydrates in EPS increase, the sludge particle size also increases. However, the sludge particle size and cell surface hydrophobicity decrease with the increased EPS production. Cell surface hydrophobicity is the most important driving force in bringing together all the cells (Krasowska and Sigler 2014, Das 2014).

Increasing SRT in a membrane bioreactor changes sludge morphology in the form of reduced floc size, compression, aggregates, and the proliferation of organisms. There is no clear relationship between SRT and floc size. However, floc size distribution is more stable at high SRTs (Liu *et al.* 2003, Sun and Liu 2013). Fig. 2 presents sludge flocs images obtained at different SRTs in the JLMBR.



(a) (b) (c) (d)

Fig. 2 Floc Microscope Images in different SRT ((a) 10, (b) 30, (c) 60 and (d) 100 day) (size 1280×10204)

In the literature, there are conflicting opinions regarding the effect of SRT or food/microorganism (F/M) ratio on floc size (Andreadakis 1993, Li and Ganczarczyk 1993, Barbusiński and Kóscielniak 1995). Li and Ganczarczyk (1993) and Barbusiński and Kóscielniak (1995) found that F/M was one of the most important factors affecting floc size distribution with larger flocs being present at high F/M ratios (low SRTs). Li *et al.* (2011) reported that the mean size of the aerobic granules in different reactors increased from 1.2 to 4.5 mm nearly linearly with the F/M ratio applied to their reactors. In contrast, Andreadakis (1993) reported that the average floc size (20 μm) obtained from SRT 1.1 d was smaller than that of SRT 4.2 and 17.4 d (35 and 45 µm, respectively).

The stability of floc size distribution at different SRTs can be explained with their growth rate. There are more substrates for the growth of microorganisms at low SRTs. The substrate is limited to sludge growth at a high SRT and the death phase creates a more stable form of biomass.

In MBRs, floc size is closely related to stability of sludge flocs and the applied shear force (Lin *et al.* 2014). The average floc size in conventional MBR systems is within the range of 5-240 μm (Iorhemen *et al.* 2016). However, floc size distribution at the SRTs of 100, 60, 30, 10 d were 6.0-15.0 μm, 7.897-7.968 μm, 8.249-9.411 μm, and 8.303-9.852 μm, respectively, and the mean particle sizes were 6.98 μm, 7.923 μm, 8.769 μm, and 9.18 μm, respectively.

Floc friction increases with the increase in mixed liquor suspended solids (MLSS) concentration, which causes a decrease in the particle size. Therefore, in this study, the particle size was found to be quite low at each SRT. Finally, the average particle size decreased and biomass flocs were small when the SRT increased (MLSS concentration increased). Additionally, changing the EPS amount also affected the particle size with the increasing SRT. Floc collapsed and the particle size was reduced with the reduced bound EPS, which has also been previously implicated in the literature (Liu *et al.* 2003, Jang *et al.* 2006, Lin *et al.* 2014, Iorhemen *et al.* 2016).

Microorganisms have a net negative surface charge at physiological pH values under normal conditions (Tuson and Weibel 2013). EPS can change the negative charge on the cell surface by forming a bridge between two neighboring cells (Lorite *et al.* 2011). EPS is usually formed of sludge floc, but at high concentrations, EPS is weak, thus loosely bound. As a result, poor bioflocuIation occurs making it difficult to separate sludge from water. In brief, the accumulation of EPS results in poor precipitation of aerobic sludge. The EPS and soluble microbial product (SMP) concentrations in the JLMBR were analyzed for different SRTs (10, 30, 60 and 100 d) and the results are given in Table 2.

The hydrophobicity of a microorganism surface is also considered to be significant for the flocculation of activated sludge and sludge precipitation. High hydrophobicity increases the adhesion between sludge flocs and reduces turbidity (Liao *et al.* 2003). Li *et al.* (2016) reported that hydrophobicity accelerated sludge precipitation. There is a positive correlation between hydrophobicity and the occurrence of sludge floc (Xie *et al.* 2010, Li *et al.* 2016). Similarly, Overmann and Pfenning (1992) found a relationship between flocs and hydrophobicity, with the latter folding the proteins on the cell surface.

The hydrophobic interaction is a short-range interaction having effect over a distance of at maximum three layers of water molecules, in total approximately 1 nm. The hydrophobic interaction (or hydrophobic effect) is an entropic phenomenon: water molecules, when faced with non-polar molecules or hydrophobic domains re-orientate in such a way that they can participate in H-bond formation more or less as in bulk water (Vogelaar *et al.* 2005). This is entropically very unfavorable since it disrupts the existing water structure and imposes a new and more ordered structure on the surrounding water molecules. The net result is a clotting of hydrophobic domains and/or hydrophobic molecules in order to minimize the entropy loss of the water molecules. At higher temperatures, due to the increased randomization of water, structure formation around a non-polar molecule plays a less important role and the entropy gain due to the clotting of hydrophobic domains becomes smaller. In review performed by Liu and Fang (2010) was reported that the strains of *Aeromonas salmonicida* would lose surface protein, A-protein, at elevated temperature, reducing the strain hydrophobicity by 10 to 30%. The strains of *Candida albicans* had lower hydrophobicity of 9% at 37℃, when compared with 57% for those grown at 25℃. Therefore, hydrophobicity of thermophilic activated sludge is less than that of mesophilic activated sludge.

Table 2 EPS and SMP Concentrations for different SRT

SRT	EPS (mg/L)		SMP (mg/L)			
	Carbohydrate	Protein	Carbohydrate		Protein	
			Reactor	Effluent	Reactor	Effluent
10	194.02	455.95	201.08	17.32	478.13	105.44
30	183.48	379.80	193.26	16.24	427.05	97.30
60	171.01	270.75	182.27	15.13	313.50	52.50
100	163.84	168.76	176.35	11.67	275.10	23.23

According to the results obtained from the thermophilic JLMBR for different SRTs (10, 30, 60, 100 d), hydrophobicity was reduced when the SRT increased. The low hydrophobicity value indicates that the sludge had a hydrophilic structure. One of the causes of poor settlement properties of thermophilic sludge in the JLMBR was the low value of hydrophobicity. In the literature, it has been reported that the protein and carbohydrate content of EPS is influenced by the bacterial effects on hydrophobicity (Drews 2010, Deng *et al.* 2014, Zhang *et al.* 2015b). When the carbohydrate content of EPS is higher than the protein, the structure of sludge is hydrophilic (low hydrophobicity), whereas higher protein content results in hydrophobic sludge (high hydrophobicity). While hydrophilic molecules are polar or charged, hydrophobic molecules are nonpolar (Jin *et al.* 2003, Chen *et al.* 2014). Nonpolar molecules are mixed with water less than polar molecules. Therefore, the hydrophobicity of sludge provides holding the flocs together in water (Jin *et al.* 2003). In JLMBRs, the sedimentation properties of thermophilic activated sludge are also poor due to much lower hydrophobicity compared to the conventional MBR system.

The increase in the zeta potential value of sludge was due to the type and concentration of EPS. It is known that protein is a hydrophobic EPS component whereas carbohydrate is hydrophilic. Hydrophobicity also changes depending on the changes in the type and concentration of EPS at different SRTs. This change was observed when hydrophobicity was reduced with the increasing SRT. Although both protein and carbohydrate concentrations decreased, protein underwent the highest reduction as the SRT increased. Thus, the ratio between protein and carbohydrate (P/C) decreased by increasing the SRT. According to Li *et al.* (2003), Sponza (2003), and Deng *et al.* (2014) decreasing the P/C ratio leads to reduced hydrophobicity.

The surface characteristics of microorganisms in wastewater treatment are important for the flocculation of activated sludge and solid-liquid separation (Xie *et al.* 2010, Yu *et al.* 2017). These characteristics provide information about hydrophobicity and surface charge (Xie *et al.* 2010). Surface charge and zeta potential have great importance for the formation and stability of activated sludge floc. Microorganisms generally have negative zeta potential (Xie *et al.* 2010). The zeta potential of the sludge in JLMBR were measured at the SRTs of 10, 30, 60, and 100 d, -32.0

mV, -38.4 mV, -42.3 mV, and -43.1 mV, respectively (Table 3). These values indicated that the sludge was negative charged and the surface charge increased with the increasing SRT. Lee *et al.* (2003) and Li *et al.* (2016) reported the same result in their study.

Some researchers have drawn attention to the important role of carbohydrates in the flocculation process (Xie *et al.* 2010, Nouha *et al.* 2015). Several studies have reported that the protein ratio of EPS was higher than carbohydrates in an activated sludge system and the EPS protein played an important role in flocculation (Liao *et al.* 2003, Zhang *et al.* 2015a).

The relationship between positively charged proteins and negatively charged carbohydrates affects the zeta potential of active sludge flocs and the hydrophobicity of sludge (Xie *et al.* 2010). A high P/C ratio generates a less negative charge. A high zeta potential is associated with a high floc index. Cells with a high negative surface charge have lower power flocs and weak flocculation.

Floc diameter decreased with the increasing SRT (Ke and Junxin 2009). Accordingly, the equivalent diameter decreased (Table 4). The increased MLSS concentration with higher SRT resulted in an increase in cellular density, thus reducing the particle size. The equivalent diameter, which provides information about the particle size, was also smaller. The decreased equivalent diameter is associated with the reduction of the dependent EPS components. However, the floc size was also affected by hydrodynamic conditions. The friction of the particles with each other increased due to the increased cellular density with higher SRT. With the reduced F/M ratio at higher SRT, the EPS was produced less, on the grounds that it was likely used as energy source for cell maintenance. Therefore, flocculation was also reduced due to the decreased amount of carbohydrates in the EPS.

The RD parameter is used to determine the shape of a particle (Perez *et al.* 2006). If the parameter is equal to or greater than 1, this indicates a circular shape. When the RD values for different SRTs were examined (Table 4), it was observed that the round shape was not a predominant structure for all SRTs. Due to the high speed mixing and shear originating from running principle of jet loop reactor, the jet, leaving the nozzle, and slamming, to impact plate, crash and break the flocs. The effect of crashing and friction of MLSS was enhanced by decline of floc flexibility with decreasing EPS and SMP. Hence the increased SRT enhanced the value of RD causing the structure of the particles to move away from roundness. Compactness is defined as the ratio between the area of the objects and the area of a circle with the same perimeter. This parameter takes the value of 1 for circular objects and a value lower than 1 for noncircular objects (Amaral and Ferreira 2005). In this study, the compactness values were found to be small than 1. It can be said that the compactness of the sludge increased with the increased SRT. The diameter of flocs declined due to decreasing EPS and SMP. Additionally, decreasing of P/C ratio of EPS and SMP resulted in increasing of zeta potential and forming of steady and compact flocs. Similarly, Liao *et al.* (2006) observed round and more compact flocs in the activated sludge at high SRTs.

Convexity is a parameter providing information about the irregularity of flocs. It ranges from 0 for irregular objects to 1 for convex objects (Liwarska-Bizukojc 2005). In this study, irregularity was observed to decrease with the increasing SRT in the thermophilic JLMBR; in other words, the convexity value was closer to 1 as shown in Table 4. The cohesion of activated sludge flocs and their ability to retain colloidal particles is governed by both electrostatic and hydrophobic interactions (Yin *et al.* 2010). The forms adhered to the periphery of floc declined, since the electrostatic interactions outweighed owing to decreasing of P/C and hydrophobicity. However, other studies have indicated that the floc structure is much more irregular at low SRTs (Liao *et al.* 2001).

Table 3 Particle size, zeta potential and hydrophobicity in the different SRT

SRT (d)	Zeta potential (mV)	Particle size (µm)	Hydrophobicity (%)
10	-32.0	9.180	2.95
30	-38.4	8.769	2.67
60	-42.3	7.923	2.17
100	-43.1	6.980	1.38

Table 4 Microbial morphology analysis results belonging to different SRT

SRT (d)	Value	Roundness	2D porosity	Equivalent diameter	Compactness	Convexness	Fractal Geometry
100	min.	1.10	0.00	1.17	0.46	0.66	1.00
	max.	5.60	9.40	3.10	1.00	0.98	1.87
	ave.	2.43	2.33	1.79	0.77	0.84	1.36
60	min.	0.99	0.00	1.25	0.43	0.58	1.00
	max.	5.23	7.40	3.82	0.97	0.97	1.81
	ave.	2.14	1.45	1.86	0.75	0.83	1.34
30	min.	0.82	0.00	1.25	0.42	0.55	1.00
	max.	5.13	7.30	4.36	0.95	0.96	1.58
	ave.	1.8	1.12	1.98	0.7	0.82	1.32
10	min.	0.80	0.00	1.95	0.40	0.52	1.00
	max.	4.98	5.00	5.12	0.91	0.91	1.41
	ave.	1.59	0.9	2.1	0.61	0.81	1.30

The fractal dimension of floc contour was also employed to characterize the bacterial aggregates. This parameter has also been used in other studies to characterize the contour irregularity of microbial aggregates generated in water and wastewater treatment processes. The values for this parameter are between 1 and 2, with the smaller values being observed in objects with an irregular contour and higher values in highly irregular objects (Perez *et al.* 2006, Zhu *et al.* 2015).

There was a positive correlation between fractal dimension and SRT in the thermophilic JLMBR. When the SRT was increased, the value of fractal geometry parameter increased; in other words, irregularity was reduced. The reduction of the floc size due to the increased MLSS and decreased EPS and SMP resulted in increased fractal geometry. It was also enhanced by increasing of zeta potential and compactness. Similarly, Massé *et al.* (2006) reported that fractal geometry increased with the increasing SRT.

Table 5 The correlation with other parameters of morphological parameters

r^2	Roundness	2 D porosity	Equivalent diameter	Compactness	Convexness	Fractal Geometry
SRT	(+) 0.989	(+) 0.966	(-) 0.935	(+) 0.822	(+) 0.978	(+) 0.978
MLSS	(+) 0.987	(+) 0.880	(-) 0.988	(+) 0.911	(+) 0.993	(+) 0.993
F/M	(+) 0.938	(+) 0.994	(-) 0.839	(+) 0.691	(+) 0.911	(-) 0.911
EPS	(-) 0.999	(-) 0.915	(+) 0.977	(-) 0.890	(-) 0.999	(+) 0.999
SMP	(-) 0.977	(-) 0.819	(+) 0.980	(-) 0.905	(-) 0.970	(+) 0.970
P/C_{EPS}	(-) 0.994	(-) 0.955	(+) 0.943	(-) 0.830	(-) 0.981	(+) 0.981
P/C_{SMP}	(-) 0.972	(-) 0.819	(+) 0.963	(-) 0.876	(-) 0.954	(+) 0.954
ZP*	(-) 0.856	(-) 0.642	(+) 0.952	(-) 0.998	(-) 0.898	(+) 0.898
PS*	(-) 0.995	(-) 0.960	(+) 0.932	(-) 0.811	(-) 0.973	(+) 0.973
Hyd.*	(-) 0.971	(-) 0.986	(+) 0.898	(-) 0.768	(-) 0.954	(+) 0.954

*ZP: Zeta potential PS: Particle size, Hyd: Hydrophobicity (%)

The number of pores was also measured at different SRTs during the floc imaging process and a reduction was observed with the increasing SRT. The flocs became more compact as the number of loose floc and pores in the floc decreased, resulting from decreasing the EPS, SMP and P/C ratio. Therefore, 2-D porosity calculated using the hole ratio was higher at greater SRTs.

The studies in the literature have showed that some of the parameters of image analysis could be a good indicator for biomass (Liwarska-Bizukojc 2005). A relationship has also been established between image analysis parameters and standard parameters in wastewater treatment. Grijspeerdt and Verstraete (1997) found a linear correlation between floc area and activated sludge. However, they reported that this correlation was not detected at very high concentrations of activated sludge (MLSS>4 g/L). In this study, a linear correlation between these parameters was observed at high MLSS concentrations (r^2=0.991). Liwarska-Bizukojc and Bizukojc (2005) also confirmed this correlation (Liwarska-Bizukojc 2005).

Table 5 presents the linearity between the morphological and other parameters. The + sign indicates a positive correlation between the two parameters whereas the - sign indicates negative linearity, which means that when one of the parameter decreases, the other tends to increase.

As clearly seen in Table 5, there was a good correlation between the results obtained using the imaging technique and other parameters. This also shows that the imaging technique can be used to make assessments about the activated sludge.

4. Conclusions

The image analysis has become a very important tool with a large field of applications due to its ability to reduce the subjectivity of human analysis, the possibility to extract quantitative data, and avoid tedious and highly time-consuming tasks for researchers. For this purpose, morphological parameters of thermophilic activated sludge in JLMBR were investigated for different SRTs, taking into consideration EPS and SMP, particle size, and zeta potential. The results showed that irregularity decreased with the increasing SRT due to decrease in EPS and SMP. The compactness value was calculated to be less than 1 for all SRTs. However, the sludge had a more compact structure and higher zeta potential when the SRT increased. In addition, as the SRT increased, the particles moved away from RD and the equivalent diameter decreased. There was a positive correlation between fractal dimension and SRT in the thermophilic JLMBR. The hydrophobicity of thermophilic aerobic sludge was reduced when the SRT increased. Considering relationship between morphological parameters and particular size, zeta potential, hydrophobicity, EPS and SMP, the morphological parameters might affect characterization of membrane fouling such as cake porosity and cake compressibility. Thus, new investigations aiming to explain the relationship between morphological features and membrane fouling in MBR can be useful to control membrane fouling.

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Appendix

Symbols

A	Average examination area
C_x	Roundness index
De	Equivalent diameter
F_A	Work area
RD	Roundness

Abbreviations

BOD_5	5-day Biochemical Oxygen Demand
EPS	Extracellular Polymeric Substance
F/M	Food/Microorganism
HRT	Hydraulic Retention Time
JLMBR	Jet Loop Membrane Bioreactor
OLR	Organic Loading Rate
MATH	Microbial Adhesion to Hydrocarbons
MBR	Membrane Bioreactor
MLSS	Mixed Liquor Suspended Solids
P/C	Protein and Carbohydrate Ratio
RD	Roundness
SRT	Sludge Retention Time
SVI	Sludge Volume Index
TCOD	Total Chemical Oxygen Demand
TKN	Total Kjeldahl Nitrogen
TP	Total Phosphate