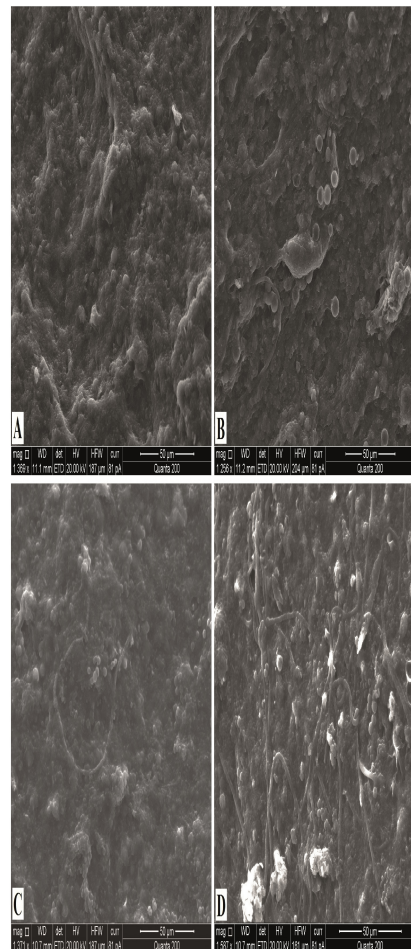
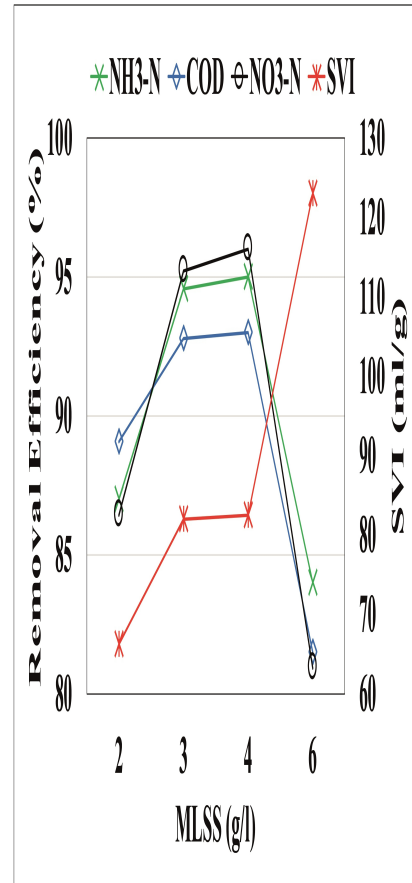


Aerobic-anoxic sequencing batch reactor (AASBR)

different MLSS concentrations (2, 3, 4 and 6 g/l)

+

MLSS (mg/l)	COD		NH ₃ -N		NO ₃ -N	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
2000±200	500±1	54±1	8±1	1.05±0.05	18±1	2.4±0.05
3000±200	501±1	36±1	8.1±1	0.43±0.05	18±1	0.8±0.05
4000±200	499±1	35±1	8.8±1	0.43±0.05	19±1	0.75±0.05
6000±200	507±1	92±1	9.1±1	1.46±0.05	17.8±1	3.38±0.05



The effects of different MLSS concentrations on the sludge characteristics and effluent quality in an AASBR under low temperature

Ali W. Alattabi^{a,c}, Clare B. Harris^b, Rafid M. Alkhaddar^b, Montserrat Ortoneda-Pedrola^b, Ali T. Alzeyadi^a

^a Department of Civil Engineering, Liverpool John Moores University, Henry Cotton Building, Webster Street, Liverpool L3 2ET, UK

^b Department of Civil Engineering, Liverpool John Moores University, Peter Jost Centre, Byrom Street, Liverpool L3 3AF, UK

^c University of Wasit, Wasit, Iraq

Abstract. The aim of this study is to enhance the effluent quality and improve the sludge settleability by determining the effects of the mixed liquor suspended solids (MLSS) on the solid's settling behaviour and the treatment efficiency in an aerobic-anoxic sequencing batch reactor (AASBR). The results obtained from this study revealed that raising the MLSS concentration from 2 to 3 g/l improved the chemical oxygen demand (COD), ammonia-nitrogen (NH₃-N) and nitrate-nitrogen NO₃-N removal efficiency, and led to an increase in the sludge volume index (SVI) value. Moreover, increasing the MLSS concentration from 3 to 4 g/l did not significantly affect the COD, NH₃-N and NO₃-N removal rates or the solid's settling behaviour. However, increasing the MLSS concentration from 4 to 6 g/l significantly reduced the COD and nitrate removal efficiency and the sludge settling rate slowed down. The results proved that the optimal MLSS concentration for COD, NH₃-N and NO₃-N removal is between 3 and 4 g/l. In this range the removal rates for COD, NH₃-N and NO₃-N were 93%, 95% and 96% respectively, and the effluent quality was 35 mg/l, 0.43 mg/l and 0.75 mg/l for COD, NH₃-N and NO₃-N respectively. In addition, a good solid separation occurred during that range with SVI value of 81 ml/g; this finding was supported by a morphological study along with scanning electron microscopy (SEM).

Keywords. Filamentous bacteria, Mixed liquor suspended solids, Sequencing batch reactor, Sludge bulking, Synthetic wastewater

1. Introduction

Several factors must be considered when planning to select a wastewater treatment plant; the two most significant ones are capital and operating costs. Considering these two factors, biological wastewater treatment is better than other treatment processes due to its economic advantages [1]. Although conventional biological wastewater treatment has the advantage of converting waste into renewable energy [2], and treats industrial wastewater without producing toxic compound by-products [3], it fails to treat wastewater containing high-strength pollutants or achieve high-quality effluent [4]. The activated sludge process (ASP) is known to be one of the most common conventional biological treatment systems. It could be considered as an environmentally friendly approach, because it uses microorganisms to biodegrade wastewater's constituents. However, it is only used to treat wastewater with low-strength pollutants [5,6]. Therefore, in order to treat industrial wastewater that contains high-strength pollutants, alternatives such as the sequencing batch reactor (SBR) should be considered.

The SBR is a fill and draw activated sludge process that operates in time rather than in space, and which is designed to degrade a wide range and high concentration of industrial wastewater [7-9]. In a single tank, the SBR performs biological treatment along with final clarifier through a timed control sequence. It consists of five basic stages – Fill, React, Settle, Draw and Idle [10]. Its ability to minimise biomass from effluent wastewater means that it is being used by many researchers to achieve good removal of both organic and inorganic compounds. However, although the SBR is widely used for industrial wastewater treatment, its stable operation is still affected by sludge settling problems, such as sludge bulking [11,12].

The settling problems in the SBR and ASP are often correlated with differentiation in growth rate between filamentous bacteria and floc-forming bacteria; this differentiation affects floc structure and

results in settling problems in both the SBR and ASP [13,14]. Sludge bulking is a result of the overgrowth of filamentous bacteria, which can lead to a drop in effluent quality and sludge washout through discharging the treated effluent [15]. Excessive growth of filamentous bacteria can be observed under the microscope by the presence of large and irregular flocs, while pinpoint flocs, which are small and compact flocs, could indicate a lack of filamentous bacteria [16]. Likewise, overproduction of extracellular polymeric substances (EPS) indicates an excessive growth of floc-forming bacteria; thereby a buoyant and weak floc will be formatted, which is also known as zoogloal bulking. On the other hand, dispersed and small flocs could indicate a low amount of floc-forming bacteria [17]. Filamentous bacteria, floc-forming bacteria and other types of bacteria present in the treatment system represent the majority of suspended solid in the system, and, as reported in the previous literature [5,6,12-17], the amount of certain types of bacteria such as filamentous bacteria can directly affect the settling performance of the system. Thus, the amount of suspended solid is a key parameter that can affect the settling behaviour of the system. In the SBR a certain amount of suspended solid is mixed with the incoming wastewater and the combination is called mixed liquor suspended solids (MLSS), which is expressed in milligrams per litre (mg/l). MLSS concentration is a key operational parameter for sequencing batch reactor technology, and should be monitored regularly, as it can directly affect the treatment efficiency. If its value is high, it will lead to sludge bulking and the treatment system will become less efficient. On the other hand, if the MLSS value is low, the energy will be wasted without treating the effluent effectively [18]. Considerable studies have sought to study the effects of the SBR's operating conditions (F/M ratio, dissolved oxygen (DO), temperature, nutrient deficiency) on the effluent quality [4,7,8,9,11,19-25]. Others have attempted to improve the settling performance of the ASP [5,6,12-17]. In this study, the effects of MLSS on both settleability and effluent quality in an AASBR will be investigated through a series of experiments (water quality parameters' removal efficiency, settling performance test, microscopic study with image processing using MATLAB and SEM) in an attempt to improve the settling performance and enhance the effluent quality at the same time.

2. Material and Methods

2.1 Activated sludge source and synthetic wastewater

The activated sludge used in this study was obtained from a wastewater treatment plant called Sandon Docks, located in Liverpool, UK. The influent synthetic wastewater was prepared in deionised water, as shown in Table 1 [26,27]. All reagents used in this study were purchased from Sigma-Aldrich, UK.

Table 1
Composition of synthetic wastewater

Chemicals	Chemical formula	Concentration
Glucose	$C_6H_{12}O_6$	500 mg/l
Magnesium Sulphate Heptahydrate	$MgSO_4 \cdot 7H_2O$	5 mg/l
Sodium Bicarbonate	$NaHCO_3$	200 mg/l
Ammonium Chloride	NH_4Cl	25 mg/l
Potassium Nitrate	KNO_3	25 mg/l
Monobasic Potassium Phosphate	KH_2PO_4	5 mg/l
Iron(III) Chloride Hexahydrate	$FeCl_3 \cdot 6H_2O$	1.5 mg/l
Calcium Chloride Dihydrate	$CaCl_2 \cdot 2H_2O$	0.15 mg/l

2.2 Experimental setup and operation of the lab-scale treatment system (AASBR)

As shown in Fig. 1, four identical reactors used in this study. Each is made of Plexiglas, and has a total volume of 6.5L and a working volume of 5L. Peristaltic pumps were used for filling and withdrawal of the effluent wastewater. Air diffusers were used to supply the reactors with fine bubble air. The mixing was achieved by using an overhead stirrer at a speed of 200rpm. Four electronic sensors (probes) were installed in each reactor to monitor the pH, oxidation-reduction potential (ORP), temperature and dissolved oxygen (DO). The treatment cycle used in this study is 12 to 12.5h: 0.5h fill, 10h react (8h aeration + 2h mixing), 0.5-1h settle, 0.5h draw (withdrawal) and 0.5h idle.

The AASBR was filled with 1.5L activated sludge and 3.5L synthetic wastewater. Air was supplied at the rate of 1LPM and pH was maintained at between 6.5 and 7.5. Temperature was maintained at 12 ± 1 °C. To acclimatise the microorganisms, the treatment reactor was aerated for 20 days. After that, the synthetic wastewater was added to the reactor. The SBR reactors (R1, R2, R3 and R4) were operated with MLSS concentrations of 2, 3, 4 and 6 g/l, and the samples were taken and analysed from each reactor for influent and effluent respectively; the effect of MLSS on settling performance and effluent quality was studied.

The AASBR operation was carried out as follows: the synthetic wastewater was added to the treatment reactors through peristaltic pumps in the first 0.5 h (fill stage). Then, the aeration was introduced to the reactors for 8 h followed by 2 h mixing stage (react stage). Settling is the third stage of AASBR operation, and it was achieved by turning off the aeration and mixing for 0.5-1 hr. The fourth stage (draw or decant) was to discharge the treated wastewater from the reactor via peristaltic pumps, and it was achieved in 0.5 h. The idle stage is the last stage of the AASBR; in this stage, a certain amount of sludge was discharged from the treatment reactor to keep the system under the targeted MLSS concentration. Then the cycle above was repeated twice a week for a period of 4 months.

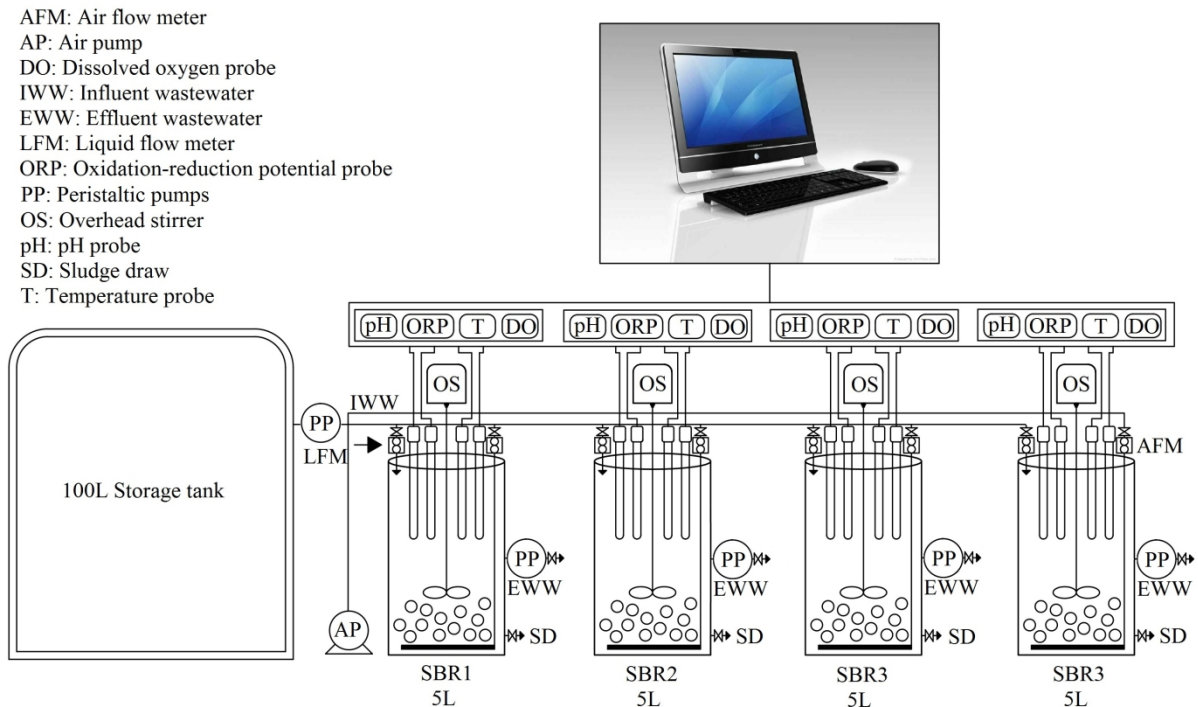


Figure 1. The configuration of laboratory-scale SBRs (SBR1, SBR2, SBR3 and SBR4)

2.3 Analytical methods

Influent and effluent samples were taken from the reactors twice a week and filtered through 0.45 μm filter paper. The concentrations of COD, nitrogen compounds ($\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$), MLSS and SVI were measured by following the procedures in the standard methods [28].

2.3.1 Morphological study and image analysis

To study the sludge characteristics, a morphological study using a light microscope AX10 (Zeiss, Germany) with colour video camera (PixeLINK, Canada) followed by image analysis was performed. A100x magnification was used. Samples were taken from the reactors 2 days per week to study the sludge characteristics of each reactor with different MLSS concentration. For each sample, 2 microscope slides were used and for each slide a 10 μL of the sample was applied on the slide using a micropipette [16]. To avoid bias, a total of 100 images were captured for each sample (50 images per slide). A quantitative study for the captured images was conducted by studying the ratio of filament length per MLSS value (TL/MLSS) and the ratio of filament length per the sample volume (TL/ Vol), and these were achieved by the method used in Mesquita et al. [29]. Image acquisition, background removal, filamentous segmentation and debris elimination were carried out using MATLAB 9 (The Mathworks, Natick, USA), and Mesquita et al.'s [29] procedure was followed.

2.3.2 SEM observation

In addition to microscopic study of the sludge, SEM analysis was conducted to find the effect of MLSS on sludge characteristics and settleability performance. SEM analysis was carried using INCA x-act, OXFORD Instruments, UK. The method from Kalab et al. [30] was performed to prepare the samples for SEM analysis.

3. Results and discussion

The influent and effluent concentrations of COD, $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ under various concentrations of MLSS are illustrated in Table 2.

Table 2

The influent and effluent concentrations of COD, $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$

MLSS (mg/l)	COD (mg/l)		$\text{NH}_3\text{-N}$ (mg/l)		$\text{NO}_3\text{-N}$ (mg/l)	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
2000 \pm 200	500 \pm 1	54 \pm 1	8 \pm 1	1.05 \pm 0.05	18 \pm 1	2.4 \pm 0.05
3000 \pm 200	501 \pm 1	36 \pm 1	8.1 \pm 1	0.43 \pm 0.05	18 \pm 1	0.8 \pm 0.05
4000 \pm 200	499 \pm 1	35 \pm 1	8.8 \pm 1	0.43 \pm 0.05	19 \pm 1	0.75 \pm 0.05
6000 \pm 200	507 \pm 1	92 \pm 1	9.1 \pm 1	1.46 \pm 0.05	17.8 \pm 1	3.38 \pm 0.05

3.1 MLSS effects on COD removal efficiency

MLSS concentration effects on COD removal are shown in Figure 2. The results obtained from this study revealed that raising the MLSS from 2 to 3 g/l improved the removal efficiency for COD; it was raised from 89.1% to 92.8%. Moreover, increasing the MLSS from 3 to 4 g/l did not significantly affect this removal efficiency. However, increasing the MLSS from 4 to 6 g/l reduced the COD removal efficiency to 82.7%. This showed that higher MLSS concentration reduced the SBR's performance in relation to organic degradation. The results obtained from this study agree with those of Wanner et al.

[31], who stated that the removal efficiency for COD was related proportionally to the concentration of MLSS. However, Watanabe et al. [32] stated that increasing the concentration of MLSS from 4.5 to 5 g/l had no impact on the removal efficiency for COD. The results also agree with Tsang et al. [33], who stated that the effluent quality drops under high concentration of MLSS in the system. The results from this study suggest that an MLSS concentration between 3 and 4 mg/l is the ultimate range in which the COD removal is at its peak value in the AASBR system.

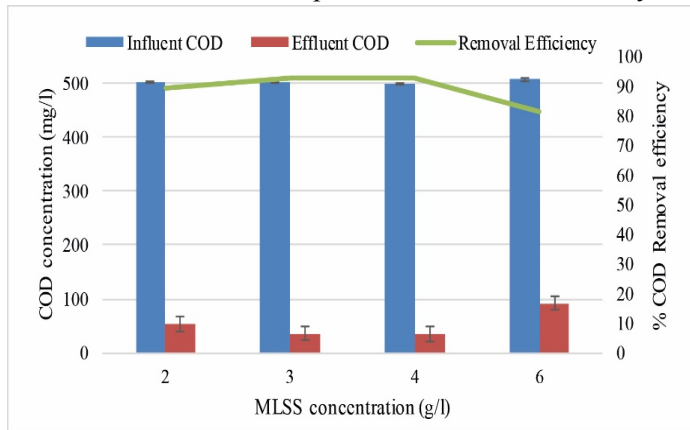


Figure 2. The effect of MLSS on COD removal

3.2 MLSS effects on nitrogen compound removal efficiency

Figure 3(a, b) shows the removal efficiency for $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ along with their influent and effluent concentrations during different MLSS concentrations. The results obtained from this study revealed that raising the MLSS from 2 to 3 g/l improved the removal efficiency for $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$; it was raised from 87% to 94.6% for $\text{NH}_3\text{-N}$ and from 86.4% to 95.2% for $\text{NO}_3\text{-N}$, with effluent quality of 0.43 and 0.85 for $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ respectively. Moreover, increasing the MLSS from 3 to 4 g/l did not affect this. However, increasing the MLSS from 4 to 6 g/l reduced the $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ removal efficiency; it dropped from 95% to 84% for $\text{NH}_3\text{-N}$ and from 96% to 80.9% for $\text{NO}_3\text{-N}$. By using the same technology with 24 h HRT, [11] achieved 96% removal efficiency for $\text{NH}_3\text{-N}$ and 92.5% removal efficiency for $\text{NO}_3\text{-N}$. On the other hand, using different technologies, [34] achieved 98% removal efficiency for $\text{NH}_3\text{-N}$ with effluent quality less than 3 mg/l by using a membrane-aerated biofilm reactor (MABR). Moreover, [35] achieved 93.4% removal efficiency for $\text{NH}_3\text{-N}$ with effluent concentration of 9.4 mg/l by using an up-flow anaerobic sludge blanket (UASB). The results obtained from this research proved that the AASBR operated with MLSS concentration between 3 and 4 mg/l with aeration and mixing offered complete nitrification and denitrification and achieved high removal efficiency of nitrogen compounds ($\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$).

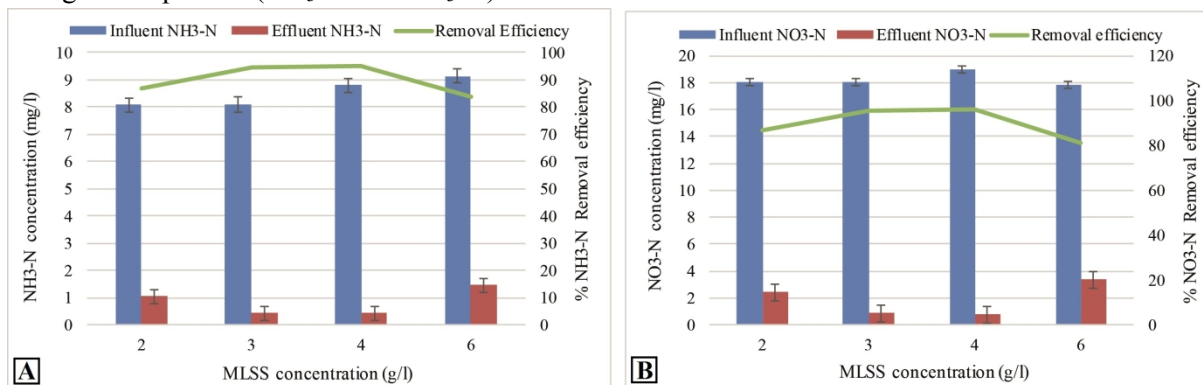


Figure 3. The effect of MLSS on (a) $\text{NH}_3\text{-N}$; (b) $\text{NO}_3\text{-N}$ removal

3.3 MLSS effects on sludge settleability

The impact of MLSS on sludge settling behaviour was studied using four different MLSS concentrations. SVI was measured regularly to monitor the sludge settleability. Figure 4(a) shows a proportional relationship between SVI value and MLSS concentration. Raising the MLSS from 2 to 3 g/l promoted an increase in the value of SVI, from 63.8 ml/g to 81.8 ml/g, and after that it did not change even when the concentration of MLSS increased from 3 to 4 g/l. In addition, the SVI value rose to 129.3 ml/g when increasing the MLSS from 4 to 6 g/l. This agrees with [33], who stated that the effluent quality was negatively affected by increasing the MLSS concentration. The results of this study also agree with [33], who recorded 52.7 ml/g SVI when operating the SBR system with MLSS of 4.5 g/l. Figure 4(b) shows the relationship between filamentous bacteria growth and MLSS concentration, and the results show that, the greater the MLSS concentration, the more abundant the filamentous bacteria, and this can be clearly seen through the SEM pictures in Figure 5. This agrees with [36], who studied the effect of filamentous bacteria on settleability through image analysis, and stated that filamentous bulking occurred when the MLVSS increased in the treatment reactor. Meanwhile, [37] stated that good sludge settleability could occur when the value of SVI was under 150 ml/g, and at that range the filamentous bacteria could appear in low to moderate numbers. In the same vein, [38] reported that SVI raised markedly when the filamentous length increased in the system. Although the presence of filamentous bacteria in the ASP is desired, an excess amount could cause sludge settling problems [39]. It can be seen from the results of this study that MLSS concentration of 2 g/l proved to be the best concentration for solid settling performance, but not the best for effluent quality. Thus, an MLSS range of between 3 g/l and 4 g/l is better for AASBR operation to ensure a good settling performance and to enhance the effluent quality at the same time.

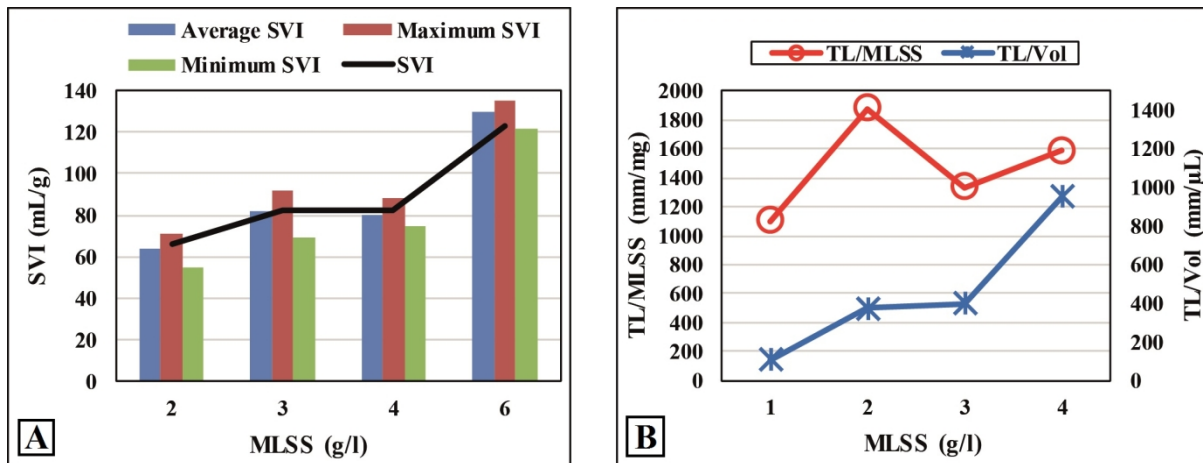


Figure 4. The effect of MLSS on (a) SVI; (b) Filamentous length (TL/MLSS and TL/Vol)

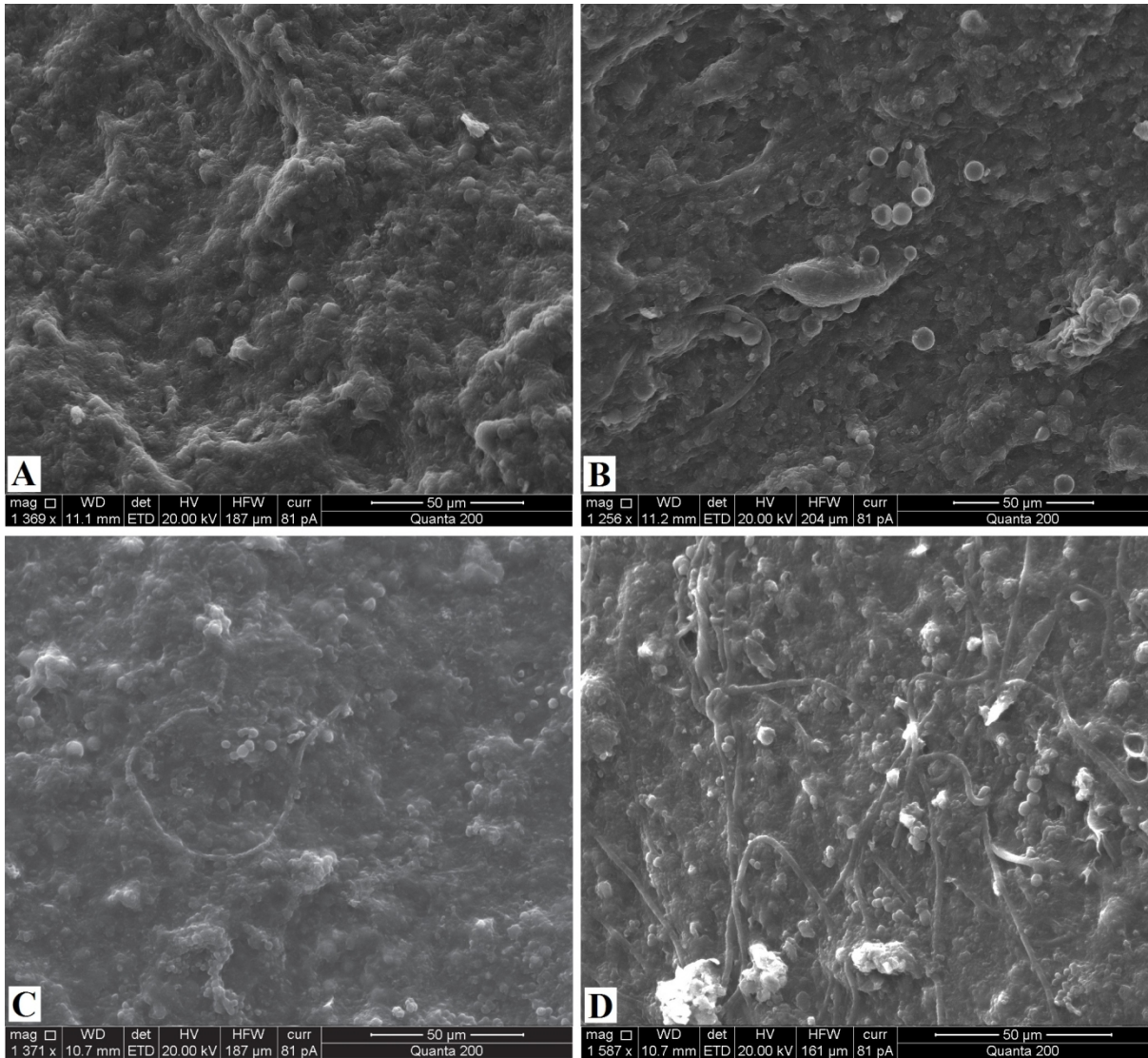


Figure 5. SEM image of the sludge (a) 2 g/l MLSS; (b) 3 g/l MLSS; (c) 4 g/l MLSS; (d) 6 g/l MLSS

3.4 The pH, DO and temperature monitoring

Figure 6 shows the monitoring of pH, ORP and DO throughout the AASBR cycle in the range of MLSS between 3 and 4 g/l. The pH, ORP and DO values at the end of the 12 h HRT treatment cycle are between 6.7-7.8, 149-165 mV and 4.5-6.5 mg/l respectively. It can be seen that there is no clear fluctuation in the pH profile, while a complete degradation of COD and nitrogen compounds can be indicated by increasing the DO profile due to bacterial respiration. The ORP profile was increased in the same pattern as the DO profile because ORP and DO are related to each other in a linear formula [40].

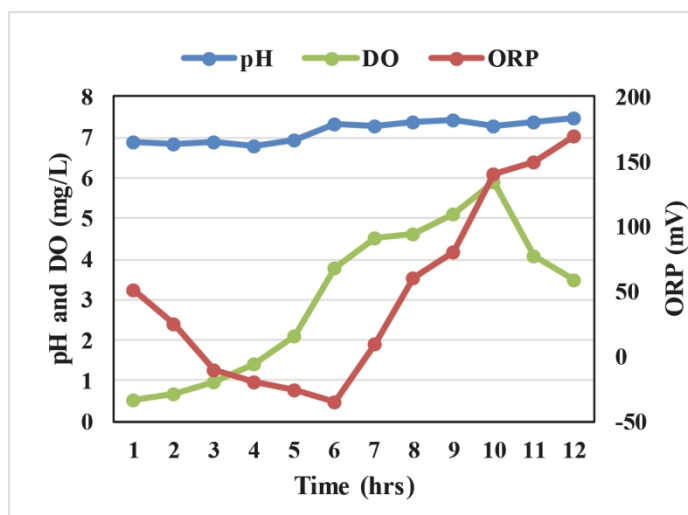


Figure 6. pH, DO and ORP profiles during the 12 h HRT

4. Conclusion

In this research, the effects of MLSS on both settleability and effluent quality in an AASBR was investigated. The removal efficiency for COD and nitrogen compounds ($\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$) was determined, settling performance was tested by measuring the SVI value, and a microscopic study with image processing using MATLAB was conducted as an attempt to improve the settling performance and enhance the effluent quality at the same time. MLSS was proven to be an important parameter affecting the treatment efficiency of the AASBR system. The optimum MLSS range obtained from this study is from 3 to 4 g/l; it can reduce COD levels by up to 93%, $\text{NH}_3\text{-N}$ levels by up to 95% and $\text{NO}_3\text{-N}$ levels by up to 96 %; with effluent quality of 35 mg/l, 0.43 mg/l and 0.75 mg/l for COD, $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ respectively. Additionally, a good settling behaviour accrued in this range; SVI value was recorded to be 81 ml/g in the MLSS range of between 3 to 4 g/l.

Acknowledgement

The first author is highly grateful for the financial support for this research from the University of Wasit, Iraq, and the Ministry of Higher Education and Scientific Research, Iraq.

References

1. A. Mittal, Biological wastewater treatment, *Water Today*, (2011) 32–44.
2. J.H. Tay, K.Y. Show, D.J. Lee, Z.P. Zhang, Anaerobic granulation and granular sludge reactor systems, in H.H.P. Fang (ed.), *Environmental Anaerobic Technology: Application and New Developments*, London, UK: Imperial College Press, (2010) 113–136.
3. B.E.L. Baeta, D.R.S. Lima, S.Q. Silva, S.F. Aquino, Evaluation of soluble microbial products and aromatic amines accumulation during a combined anaerobic/aerobic treatment of a model azo dye, *Chemical Engineering Journal*, 259 (0) (2015) 936–944.
4. R.A. Hamza, O.T. Iorhemen, J.H. Tay, Advances in biological systems for the treatment of high-strength wastewater, *Journal of Water Process Engineering*, 10 (2016) 128–142.
5. M.C. Sarraguc, A. Paulo, M.M. Alves, A.M.A. Dias, J.A. Lopes, E.C. Ferreira, Quantitative monitoring of an activated sludge reactor using on-line UV–visible and near-infrared spectroscopy, *Analytical and Bioanalytical Chemistry*, 395 (4) (2009) 1159–1166, <http://dx.doi.org/10.1007/s00216-009-3042-z>.

6. Y.J. Chan, M.F. Chong, C.L. Law, An integrated anaerobic–aerobic bioreactor (IAAB) for the treatment of palm oil mill effluent: start-up and steady state performance, *Process Biochemistry*, 47 (3) (2012) 485–495.
7. R.P. Oliveira, J.A. Ghilardi, S.M. Ratusznei, J.A.D. Rodrigues, M. Zaiat, E. Foresti, Anaerobic sequencing batch biofilm reactor applied to automobile industry wastewater treatment: volumetric loading rate and feed strategy effects, *Chemical Engineering and Processing*, 47 (2008) 1374–1383.
8. D.Y. Ma, X.H. Wang, C. Song, S.G. Wang, M.H. Fan, X.M. Li, Aerobic granulation for methylene blue biodegradation in a sequencing batch reactor, *Desalination*, 276 (2011) 233–238.
9. G. Durai, M. Rajasimman, N. Rajamohan, Aerobic digestion of tannery wastewater in a sequential batch reactor by salt-tolerant bacterial strains, *Applied Water Science*, 1 (2011) 35–40.
10. United States Environmental Protection Agency (USEPA), *Wastewater technology fact sheet sequencing batch reactors*. Washington, DC, USA: USEPA (1999).
11. H.A. Hasan, S.R.S. Abdullah, A.W.N. Al-Attabi, D.A.H. Nash, N. Anuar, N.A. Rahman, H.S. Titah, Removal of ibuprofen, ketoprofen, COD and nitrogen compounds from pharmaceutical wastewater using aerobic suspension-sequencing batch reactor (ASSBR), *Separation and Purification Technology*, 157 (2016) 215–221.
12. J. Guo, Y. Peng, Z. Wang, Z. Yuan, X. Yang, S. Wang, Control filamentous bulking caused by chlorine-resistant Type 021N bacteria through adding a biocide CTAB, *Water Research*, 46 (19) (2012) 6531–6542.
13. I. Turtin, A. Vatansver, F.D. Sanin, Phosphorus deficiency and sludge bulking, *Environmental Technology*, 27(6) (2006) 613–621, <http://dx.doi.org/10.1080/09593332708618677>.
14. D.P. Mesquita, O. Dias, R.A.V. Elias, A.L. Amaral, E.C. Ferreira, Dilution and magnification effects on image analysis applications in activated sludge characterization, *Microscopy and Microanalysis*, 16 (05) (2010) 561–568.
15. J. Guo, S. Wang, Z. Wang, Y. Peng, Effects of feeding pattern and dissolved oxygen concentration on microbial morphology and community structure: The competition between floc-forming bacteria and filamentous bacteria, *Journal of Water Process Engineering*, 1 (2014) 108–114.
16. D.P. Mesquita, A.L. Amaral, E.C. Ferreira, Identifying different types of bulking in an activated sludge system through quantitative image analysis, *Chemosphere*, 85 (4) (2011) 643–652, <http://dx.doi.org/10.1016/j.chemosphere.2011.07.012>.
17. E. Koivuranta, T. Stoor, J. Hattuniemi, J.Niiniemi, On-line optical monitoring of activated sludge floc morphology, *Journal of Water Process Engineering*, 5 (2015) 28–34.
18. Partech, Mixed liquor suspended solids (MLSS) [Online] (2014). Available on: <http://www.partech.co.uk/measurements/mlss/>. [Accessed 30/07/16].
19. J. Chudoba, J. Blaha, V. Madera, Control of activated sludge filamentous bulking – III. Effect of sludge loading, *Water Research*, 8 (4) (1974) 231–237.
20. J.C. Palm, D. Jenkins, D.S. Parker, Relationship between organic loading, dissolved oxygen concentration and sludge settleability in the completely mixed activated sludge process, *Journal of the Water Pollution Control Federation*, 52 (10) (1980) 2484–2506.
21. B.Q. Liao, H.J. Lin, S.P. Langevin, W.J. Gao, G.G. Leppard, Effects of temperature and dissolved oxygen on sludge properties and their role in bioflocculation and settling, *Water Research*, 45 (2) (2011) 509–520.
22. A.M.P. Martins, J.J. Heijnen, M.C.M. van Loosdrecht, Effect of dissolved oxygen concentration on sludge settleability, *Applied Microbiology and Biotechnology*, 62 (5) (2003) 586–593.
23. S. Knoop, S. Kunst, Influence of temperature and sludge loading on activated sludge settling, especially on *Microthrix parvicella*, *Water Science and Technology*, 37 (4–5) (1998) 27–35.
24. J.H. Guo, Y.Z. Peng, S.Y. Wang, X. Yang, Z.W. Wang, A. Zhu, Stable limited filamentous bulking through keeping the competition between floc-formers and filaments in balance, *Bioresource Technology*, 103 (1) (2012) 7–15.
25. Y.Z. Peng, C.D. Gao, S.Y. Wang, M. Ozaki, A. Takigawa, Non-filamentous sludge bulking caused by a deficiency of nitrogen in industrial wastewater treatment, *Water Science and Technology*, 47 (11) (2003) 289–295.
26. S.R.P. Shariati, B. Bonakdarpour, N. Zare, F.Z. Ashtiani, The effect of hydraulic retention time on the performance and fouling characteristics of membrane sequencing batch reactors used for the treatment of synthetic petroleum refinery wastewater, *Bioresource Technology*, 102 (17) (2011) 7692–7699.
27. D. Zhao, C. Liu, Y. Zhang, Q. Liu, Biodegradation of nitrobenzene by aerobic granular sludge in a sequencing batch reactor (SBR), *Desalination*, 281 (2011) 17–22.
28. American Public Health Association (APHA), *Standard methods for the examination of water and wastewater*, 18th ed., Washington, DC, USA: APHA (2012).
29. D.P. Mesquita, O. Dias, A.L. Amaral, E.C. Ferreira, A comparison between bright field and phase contrast image analysis techniques in activated sludge morphological characterization, *Microscopy and Microanalysis*, 16 (2010) 166–174.

30. M. Kalab, A.F. Yang, D. Chabot, Conventional scanning electron microscopy of bacteria, *Infocus*, 10 (2008) 44-61.
31. J. Wanner, K. Kucman, P. Grau, Activated sludge process combined with biofilm cultivation. *Water Research*, 22 (2) (1998) 207–215.
32. Y. Watanabe, S. Okabe, T. Arata, Y. Haruta, Study on the performance of an up-flow aerated biofilter (UAB) in municipal wastewater treatments. *Water Science and Technology*, 30 (11) (1994), 25–33.
33. Y.F. Tsang, F.L. Hua, H. Chua, S.N. Sin, Y.J. Wang, Optimization of biological treatment of paper mill effluent in a sequencing batch reactor, *Biochemical Engineering Journal*, 34 (3) (2007) 193-199.
34. X. Wei, B. Li, S. Zhao, L. Wang, H. Zhang, C. Li, S. Wang, Mixed pharmaceutical wastewater treatment by integrated membrane-aerated biofilm reactor (MABR) system: A pilot-scale study, *Bioresource Technology*, 122 (2012) 189–195.
35. Z. Chen, H. Wang, N. Ren, M. Cui, S. Nie, D. Hu, Simultaneous removal and evaluation of organic substrates and NH₃-N by a novel combined process in treating chemical synthesis-based pharmaceutical wastewater, *Journal of Hazardous Materials*, 197 (2011) 49–59.
36. M. da Motta, M.N. Pons, N. Roche, Study of filamentous bacteria by image analysis and relation with settleability, *Water Science and Technology*, 46 (1-2) (2002) 363-369.
37. B. Jin, B.M. Wilen, P. Lant, A comprehensive insight into floc characteristics and their impact on compressibility and settleability of activated sludge, *Chemical Engineering Journal*, 95 (1-3) (2003) 221–234.
38. M. Sezgin, Variation of sludge volume index with activated sludge characteristics, *Water Research*, 16 (1) (1982) 83–88.
39. M.H. Gerardi, *Settleability Problems and Loss of Solids in the Activated Sludge Process*, Hoboken, New Jersey, USA: John Wiley & Sons (2002).
40. N. Kishida, J. Kim, M. Chen, H. Sasaki, R. Sudo, Effectiveness of oxidation-reduction potential and pH as monitoring and control parameters for nitrogen removal in swine wastewater treatment by sequencing batch reactors, *Journal of Bioscience and Bioengineering*, 96 (3) (2003) 285–290.

- The optimum MLSS range obtained from this study is 3 to 4 g/l.
- COD, NH₃-N and NO₃-N removal efficiencies were 93%, 95% and 96% respectively.
- SVI value was recorded to be 81 ml/g in the MLSS range of between 3 to 4 g/l.