Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2015.1029526

57 (2016) 9093–9100
April

Taylor & Francis
Taylor & Francis Group

Evaluation of operational parameters for semi-continuous anaerobic digester treating pretreated waste activated sludge

S. Gopikumar[a], P. Arulazhagan[b], S. Kavitha[a], S. Adish Kumar[a], J. Rajesh Banu[a,*]

[a]*Department of Civil Engineering, Regional Centre of Anna University, Tirunelveli 627007, TN, India, Tel. +91 9444215544; emails: gopikumar.srf@gmail.com (S. Gopikumar), cutekavibio@gmail.com (S. Kavitha), adish2002@yahoo.co.in (S. Adish Kumar), rajeshces@gmail.com (J. Rajesh Banu)*

[b]*Centre of Excellence in Environmental Studies, King Abdulaziz University, Jeddah, Saudi Arabia, Tel. +966 508215360; email: arulazhagan_p@yahoo.co.in*

Received 26 May 2014; Accepted 10 March 2015

ABSTRACT

Experimental analysis of citric acid and bacterial pretreatment on waste activated sludge (WAS) was performed in a semi-continuous anaerobic reactor for assessing the sludge reduction. The sludge pretreatment was carried out by deflocculating the WAS using 50 mM/L citric acid and subjecting to bacterial disintegration using 2 g dry cell weight/L of *Bacillus licheniformis*. The pretreatment resulted in a COD solubilization of about 40%. Anaerobic digestion (AD) of pretreated sludge was carried out in a 3.5 L semi-continuous anaerobic reactor. The AD results reveal that an organic loading rate (OLR) of 1 g/L d operated at 15 d hydraulic retention time was preferably the pertinent OLR for the efficient digestion. AD of pretreated sludge resulted in 43% of suspended solids reduction and 48% of volatile solids (VS) reduction, respectively, with biogas yield of 189.61 mL/g VS added.

Keywords: Waste activated sludge (WAS); Citric acid; Pretreatment; Solids reduction; Biogas

1. Introduction

A large amount of sludge is produced by aerobic biological wastewater treatment processes, and it has become a serious environmental problem. Excess sludge produced by these processes must be disposed of. The disposal may account for up to 60% of the total plant operating costs. New stringent regulations regarding sludge treatment, disposal as well as social and environmental concerns have resulted in a considerable impetus to developing strategies to reduce excess sludge production [1]. Anaerobic digestion (AD) is the widely used biological process for excess sludge reduction and biogas production. AD process can be enhanced by sludge disintegration methods.

Sludge disintegration has been commonly practiced as a pretreatment for sludge reduction. Pretreatment destroys cell walls leading to the solubilization of extracellular and intracellular materials into the aqueous phase. With pretreatment, not only hydrolysis is accelerated by the increase in dissolved components, but the improvement of biodegradability, sludge dewatering and reduction of pathogens and foaming can also be achieved [2]. There are several kinds of pretreatment methods studied so far, which are either physical [3,4], chemical [5], mechanical [6–8] and biological [9–11] in nature, or perhaps a combination of any two of these methods [12–15].

*Corresponding author.

1944-3994/1944-3986 © 2015 Balaban Desalination Publications. All rights reserved.

Compared to other disintegration methods, biological disintegration can effectively solubilize particulate organic matter in the sludge in an energy efficient way. Among the biological disintegration, usage of enzymes has certain advantages such as high solubilization, no drastic alteration in substrate environment and low energy requirement. However, these enzymes are costly and difficult to isolate. Keeping this in mind, in the present study, an extracellular enzyme secreting thermophilic bacteria (*Bacillus licheniformis*) is used. *B. licheniformis* is known for its sludge disintegration potential [9].

Extracellular polymeric substances (EPS) play a crucial role in sludge flocculation. EPS protect the biomass of the flocs by masking them to get directly exposed to harsh environmental conditions [16]. Therefore, it is essential to remove EPS to facilitate efficient sludge disintegration. There are varieties of cation-binding agents used to remove EPS [17]. Among them, citric acid has the following advantages such as easily biodegradable and cost effective. The AD of pretreated sludge was carried out in a semi-continuous anaerobic digester and its disintegration efficiency was evaluated by comparing it with control. The primary aim of this research is to study the effect of this combined sludge disintegration on AD. AD was carried out in semi-continuous anaerobic digesters, and optimum parameters were evaluated on the basis of solids and suspended solids (SS) reduction.

2. Materials and methods

2.1. Sludge collection and characterization

The waste activated sludge (WAS) sample for the current study was collected from the return line of an activated sludge treatment plant at Kerala (India) and stored at 4°C. The characteristics of the sludge were as follows: pH was 6.7, soluble COD (SCOD) was 310 mg/L, total COD (TCOD) was 19,300 mg/L, total solids content was 14,932 mg/L, volatile solids (VS) content was 6,400 mg/L, SS content was 8,506 mg/L.

2.2. Sludge disintegration

Sludge disintegration has been carried out in two steps. The first step of sludge disintegration is deflocculation. Citric acid, a cationic binding agent, was used to deflocculate the sludge. Deflocculation experiments were carried out in a series of 1 L conical flasks containing 500 mL of sludge, and were added with a citric acid dosage of 50 mM. Mixtures were kept in a shaker at 150 rpm for 3 h with constant agitation to ensure proper mixing. After deflocculation, pH of the sample was adjusted to 6.5 with a help of 0.1 N NaOH. The second step of sludge disintegration is hydrolysis and was carried out by inoculating the deflocculated sludge with *B. licheniformis* (2 g dry cell weight/L). After inoculation, the contents of the flask were incubated for 24 h at 55°C and 100 rpm. The results of disintegration were demonstrated in terms of SCOD fraction as per Eq. (1) [18]. The pretreated sludge was subsequently subjected to AD in semi-continuous anaerobic digester.

$$\text{SCOD fraction} = \left(\text{SCOD}_{\text{after pretreatment}}/\text{TCOD}_{\text{after pretreatment}}\right) \times 100 \tag{1}$$

2.3. Experimental apparatus

Sludge digestion were carried out in two identical lab scale completely stirred semi-continuous anaerobic reactors (control and experimental) at a mesophilic temperature of 33°C. The total volume of digester is 5 L with a working volume of 3.5 L. Control reactor (CR) was fed with the raw sludge and the experimental reactor (ER) was fed with disintegrated sludge. Mixing was accomplished by the motor (50 rpm) with four blade impeller vertical shaft mounted at the top. The digested sludge removal and disintegrated sludge loading was performed simultaneously with the help of a peristaltic pump. Biogas volume was assessed by water displacement method in which water was displaced in a graduated measuring cylinder linked to the reactors. Displaced liquid volume was converted to total biogas (mL/g VS added). The schematic diagram of anaerobic reactor is depicted in Fig. 1.

2.4. Startup of anaerobic reactor

In ER, the pretreated sludge was inoculated with an active methanogenic bacterial population for the quick startup of the reactor. Digested cow dung slurry from an active biogas plant at a dairy cattle farm was selected as the inoculum [19]. The ER was fed with cow dung slurry and pretreated sludge in the ratio 1:1. Similarly, the CR was fed with cow dung slurry and raw sludge in the ratio 1:1.

2.5. Operational parameters

The organic loading rate (OLR) and the hydraulic retention time (HRT) are mutually dependable variables. Initially, the loading rate of the reactor was increased by fixing HRT to 20 d and increasing the solid concentration in the feed from 5 g to 15 g/L.

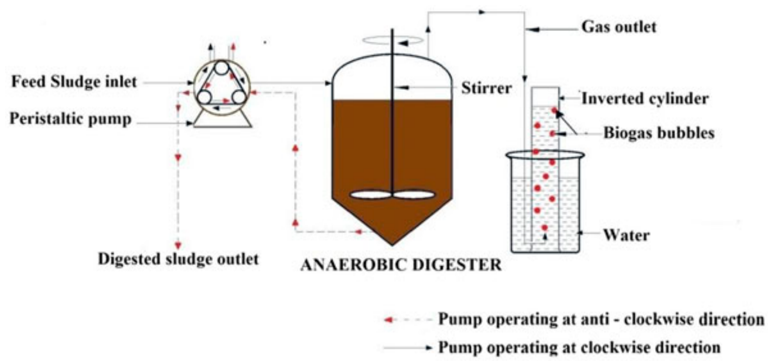

Fig. 1. Schematic diagram of anaerobic digester.

Table 1
Operational parameters of AD and disintegration efficiency of bacterial pretreatment

				Efficiency of bacterial pretreatment	
Digestion period (d)	MLSS (mg/L)	OLR (g/L d)	HRT (d)	SCOD (g/L)	SCOD fraction
51–95	5,000	0.25	20	3.09 ± 0.06	46.4
96–140	7,500	0.37	20	3.86 ± 0.18	44.7
141–185	10,000	0.5	20	5.35 ± 0.21	43.2
186–230	12,500	0.62	20	6.28 ± 0.07	41.3
231–275	15,000	0.75	20	8.04 ± 0.14	40.5
276–320	15,000	0.88	17	8.12 ± 0.19	40.9
321–365	15,000	1	15	7.99 ± 0.25	40.2
366–410	15,000	1.25	12	8.08 ± 0.27	40.7

Later, the increment was done by fixing solid concentration in the feed to 15 g/L and varying HRT. The operational parameter maintained during the study period and the disintegration efficiency of bacterial pretreatment is summarized in Table 1.

2.6. Analytical parameters

The parameters such as pH, SS, VS, SCOD, TCOD, volatile fatty acids (VFA) and alkalinity were evaluated as per standard methods in APHA [20].

3. Result and discussion

3.1. Pretreatment proficiency

The bacterial disintegration efficiency was shown in Table 1. From Table 1, it was evident that an increase in solid concentration marginally decreases the sludge solubilizing potential of bacterial pretreatment. The SCOD fraction during the study period varied in the range of 40–46%. Similar to the present study, 36% of SCOD fraction is achieved using *B. licheniformis* and EDTA [21]. The addition of citric acid resulted in the breakage of divalent cations bridging the flocs and causes deflocculation [22]. This phenomenon paves way for the *B. licheniformis* to efficiently disintegrate the sludge [9]. The disintegration of sludge was attributed to the action of extracellular enzymes secreted by the *B. licheniformis*, which break the cell wall of the bacteria and releases the intracellular content of biomass [23].

3.2. Acclimatization of the reactor

During the acclimatization phase, the disintegrated sludge was fed at an OLR of 0.25 g/L d, and the set up was operated until the biogas produced, VFA concentration and pH in the reactor reached constant levels, without showing symptoms of any process imbalance or failure. Once the reactor attained steady state, the evaluation of operational parameters for the semi-continuous anaerobic reactor was initiated.

3.3. pH

The steadiness of pH in an anaerobic digester is tremendously significant since methanogenesis progress at an elevated rate when the pH is sustained within a range of (7.3–7.8). The variation of pH with digestion period is depicted in Fig. 2. It was observed from Fig. 2 that during every shift to the next lower HRT, the pH dropped. Earlier studies reported that an indiscriminate increment in OLR resulted in a drop of digester pH below 6.6 [24]. A low pH has inhibitory effects on the methanogen [25]. The activity of methanogenic organism will get inhibited at a pH < 6.3 and higher than 7.8 [26]. From the figure, it was evident that no drastic change in pH of the reactor was observed during the study period. The pH of the digested sludge at initial period (50–75 d) of operation was recorded to be 7 for control and 7.45 for ERs, respectively.

From the initial pH, it decreased marginally to 6.9 for control and 7.2 for ERs at the end of the operational period. So, from the above, it can be concluded that the fluctuations observed in the present study was well within the methanogenic range, which proved that the digester could maintain the pH within a neutral range.

3.4. Alkalinity

pH cannot be a valuable computing factor for determining the strength of an anaerobic process, once there is a elevated buffering competence [27]. At this state, alkalinity range discloses probable anaerobic process efficiency directly.

The alkalinity of a balanced process is between 1,000 and 5,000 mg $CaCO_3$/L [28]. Lower values of effluent alkalinity warn about impending reactor failure. The variation of alkalinity throughout the digestion period with increase in OLR is depicted in Fig. 3. With an increase in OLR, an increase in alkalinity of ER was normally due to the better activity of methanogenic bacteria than CR, which can produce alkalinity in the form of carbon dioxide, ammonia and bicarbonate [29]. The alkalinity in an anaerobic digester is proportional to the amount of solids loaded [30]. The alkalinity was observed to be in the range of 3.9–4.5 g/L in the ER and 3.6–4.2 g/L in the CR (Fig. 3).

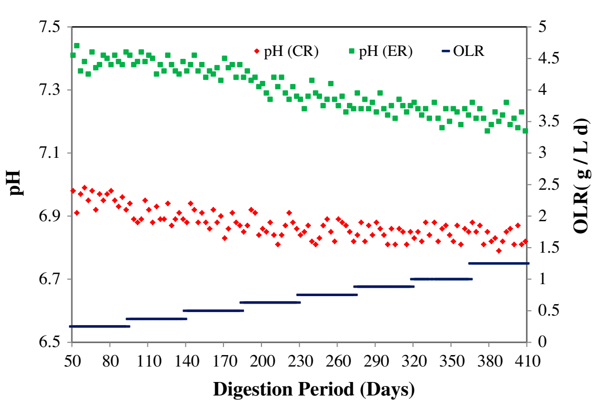

Fig. 2. Variation of pH in the anaerobic reactors during the study period.

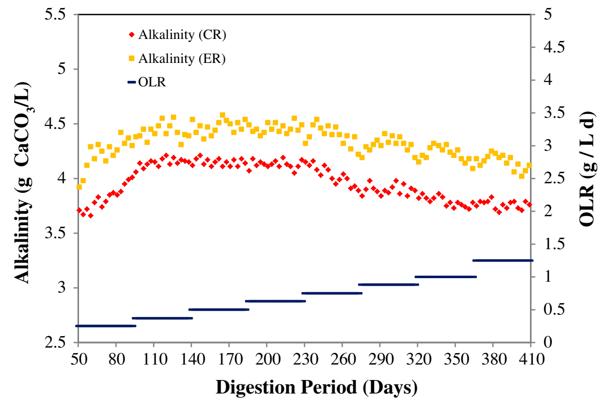

Fig. 3. Variation of alkalinity in the anaerobic reactors during the study period.

3.5. Volatile fatty acids

Generally, VFAs are the useful indicators of AD process performance and stability or imbalance [24]. The presence of higher quantities of VFAs has been reported to be inhibitory to methanogenic activity. The profile of VFA production during AD is depicted in Fig. 4.

From the figure, it was noted that the concentration of VFA increased slightly from 308 to 310 mg/L in CR and 310 to 315 mg/L in ER during the initial period of reactor operation (0.25 g/L d). The VFA concentration begins to decline when the OLR was increased from 0.25 to 0.37 g/L d. To maintain a favourable environment in the anaerobic reactor, VFA production and utilization were balanced by a change in the methanogenic bacterial composition, which varies based on the OLR [31]. The declining profile of VFA was found to be stabilized at an OLR 0.75 g/L d. The VFA concentration in control and ER reached 272 and 282 mg/L, respectively, at day 410 (with an increase in OLR) indicating healthy AD. So, it can be concluded that VFA does not act as a rate limiting step in the present study.

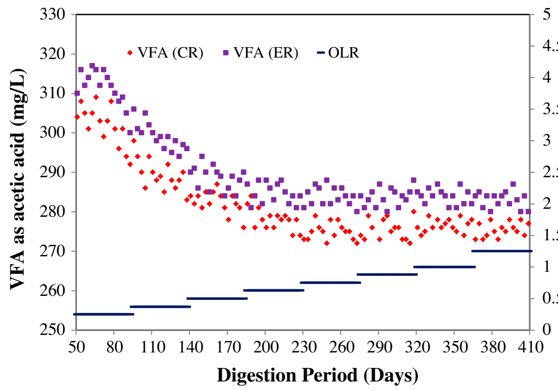

Fig. 4. Variation of VFA in the anaerobic reactors during the study period.

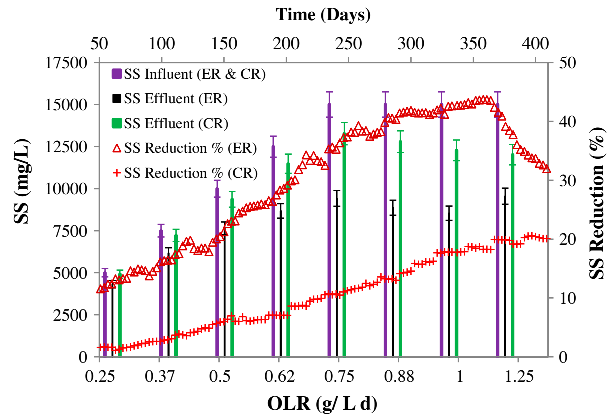

Fig. 5(a). Influence of OLR on SS removal during the study period.

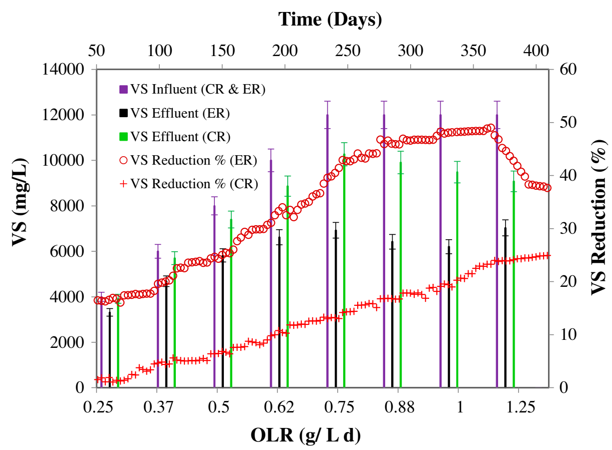

Fig. 5(b). Influence of OLR on VS removal during the study period.

3.6. Solids reduction

Suspended solids reduction is a sign of the sludge steadiness, and it is employed for measuring the efficiency of a method in stabilizing sludge [32]. Fig. 5(a) exhibits the overall reactor performance in terms of the reduction of SS with digestion time. At individual HRTs, steady states of operations were retrieved and the results presented are an average of five consecutive consistent readings. From the figure, it was observed that during the first OLR, there was a 14% reduction in SS with an increase in OLR to 0.37 g/L d, there was 17% reduction in the SS. At the third loading rate of 0.5 g/L d, the SS reduction was increased to 24%. OLRs of 0.62 and 0.75 g/L d had 31 and 37% reduction in the SS. The first five loadings were carried out with the HRT of 20 d with varying MLSS.

In the rest of the studies, the MLSS concentration in the feed was kept constant at 15,000 mg/L, as sludge thickening above 15,000 mg/L is not practically feasible. The HRT was decreased further to 17 d, thereby increasing the OLR to 0.88 g/L d. The SS reduction was approximately 41%, which indicates significant reduction with the increase in the OLR. The HRT was decreased further to 15 d, and the SS reduction was 43%. With a further decrease in HRT, the SS reduction decreased to 36%, which may be due to the overloading of the reactor. In case of the CR, SS reduction was 20% for the highest OLR of 1.25 g/L d. From the above result, it was evident that bacterial pretreatment was responsible for 54% increment in the amount of SS reduction in the ER compared to that of the CR. This finding indicates that the biological pretreatment with *B. licheniformis* has a greater advantage over the non-pretreated sludge (control). It was also observed that the maximum SS reduction was achieved when the loading rate was 1 g/L d, and the reduction percentage declined with further increase in the OLR.

Fig. 5(b) exhibits the overall performance efficiency in terms of reduction in VS with digestion time. From the figure, it was observed that with the increase in OLR, there was an increase in VS reduction. During 20 d HRT, the VS reduction was found to be 42%. When the HRT was decreased further to 17 d (OLR 0.88 g/L d), the VS reduction was about 46%, which indicates significant reduction with the increase in OLR. Further decrease in HRT from 17 to 15 d, the VS reduction was stabilized. Similar to SS reduction, maximum VS reduction of about 48% was obtained at an OLR of 1 g/L d. Increase in OLR beyond this lead to a reduction in VS removal efficiency and was found to be 41%. The present results showed that the biological pretreatment potential of sludge significantly improved AD when compared to control (24%). This elevated efficiency was attributable to the pretreatment, which cleaves the sludge biomass for rapid consequent degradability. It ultimately facilitated the decay response which led to biodegradation of much more organics in the reactor. The volatile solid removal in ER during stable operation period (OLR 0.25–0.75 g/L d) was 71% higher than that in CR.

3.7. Total biogas yield

Fig. 6 shows the comparison of biogas yield at different HRTs. During each phase of the OLR, the total biogas production in the digester showed appreciable increases until a stage when methanogenesis could not work fast enough to convert acetic acid to methane. Fig. 6 revealed the difference in biogas production between experimental and control was found to be 51.5%. In contrast to the present study, works with physical and chemical pretreatment showed higher levels of biogas production in the range of 84–88% [33].

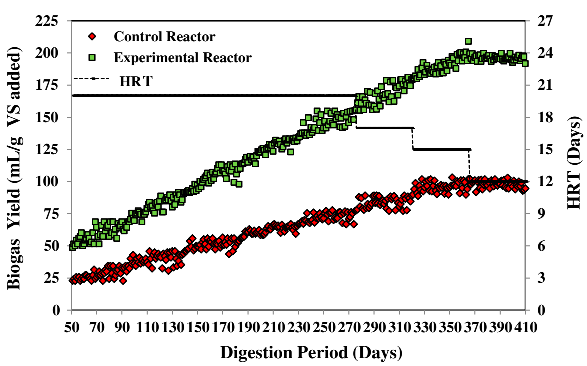

Fig. 6. Effect of HRT on biogas yield during the study period.

However, these pretreatment techniques had intensive energy demands and high operation costs [3].

Biogas production was higher at the OLR 1 g/L d, but with an increase in OLR to 1.25 g/L d, it did not increase proportionally to the solids loaded. This may have been due to the lack of critical requirement of the inoculum to take the load of the additional OLR [34]. The higher gas production in the bacterially pretreated sludge was due to the more hydrolysed organic material, which was immediately used by anaerobic bacteria and eventually facilitated the digestion processes. With a decrease in the HRT from 15 to 12 d, there was stabilization in biogas production. A shorter HRT results in less biogas production [3]. Therefore, by considering the biogas production during the study period, an HRT of 15 d was observed to be an appropriate retention time for effectual sludge degradability. Thus, the reactor efficiency and the digestion efficiency as a function of the OLR and HRT were found to be optimal at OLR of 1 g/L d and 15 d. The cumulative biogas produced calculated from the Fig. 6 during the entire reactor operation period of the ER was 109 L, and the cumulative biogas produced during the entire reactor operation period of the CR was 54 L.

The biogas production of ER increased significantly to 50.4% compared to CR. Table 2 summarizes the biogas productivity at different OLRs. The most favourable loading rates and retention time for anaerobic degradation depends on the feature of the substrate and the required effectiveness of the overall process. From Table 2, it was observed that the biogas production increased with increase in OLR. In the lower OLR-0.25 g/L d, the biogas production in control and ER was found to be 27.74 and 59.39 mL/g VS. A low organic loading does not afford a satisfactory amount of biogas, but would make the reactor unreasonably bulky. Therefore, the biogas production was lower at

Table 2
Influence of OLR on the biogas productivity

		ER		CR	
OLR (g/L d)	HRT (d)	Average biogas (mL)	Total biogas (mL/g VS added)	Average biogas (mL)	Total biogas (mL/g VS added)
0.25	20	41 ± 6	59.39	19 ± 3	27.74
0.37	20	84 ± 11	80.74	41 ± 5	39.61
0.5	20	144 ± 17	102.95	71 ± 7	51.27
0.62	20	219 ± 29	125.21	109 ± 14	62.48
0.75	20	305 ± 33	145.67	151 ± 18	72.14
0.88	17	412 ± 47	167.85	206 ± 24	83.78
1	15	530 ± 64	189.61	268 ± 32	95.85
1.25	12	684 ± 58	196.02	341 ± 29	97.81

0.25 g/L d. At OLR 1 g/L d, the biogas production was found to be 95.85 and 189.61 mL/g VS for control and ER. Further increase in the OLR to 1.25 g/L d resulted in stabilized biogas production due to higher biomass dosage and lower mass transfer rate of food to the bacteria [35] which limits the biogas production. Based on the above facts, it could be concluded that the OLR 1 g/L d was considered to be optimum for effective biogas production.

4. Conclusion

In the present study, the mesophilic lab scale reactors ER and CR were operated for more than a year with pretreated (citric acid 50 mmol/L and *B. licheniformis* 2 g/L dry cell weight) WAS. COD solubilization during bacterial pretreatment varied from 40 to 46%. It was also confirmed that COD solubilization by pretreatment increases the sludge biodegradability. When the pretreated sludge was further subjected to semi-continuous AD, the observed VS and SS reduction were 48 and 43%, respectively, with a maximum total biogas production of 189.61 mL/g VS added at 15 d HRT operated at an OLR of 1 g/L d, which is considered to be the optimum parameter for the efficient operation of the reactor.

Acknowledgements

The authors are grateful to the Department of Biotechnology, India for economic support (BT/PR13124/GBD/27/192/2009) from the Rapid Grant for Young Investigators scheme.

References

[1] C. Libing, Y. Sangtian, X. Xin Hui, S. Xulin, J. Benjamin, Progress and perspectives of sludge ozonation as a powerful pretreatment method for minimization of excess sludge production, Water Res. 43 (2009) 1811–1822.

[2] K. Uan Do, T.T.H. Nguyen, J. Rajesh Banu, K. Kyeongjin, H. Jin, I.T. Yeom, Effects of thermochemical pretreatment on the biodegradability of sludge from a biological wastewater treatment system, Maejo Int. J. Sci. Technol. 4 (2) (2010) 250–260.

[3] R. Uma Rani, S. Adish Kumar, S. Kaliappan, I.T. Yeom, J. Rajesh Banu, Low temperature thermo-chemical pretreatment of dairy waste activated sludge for anaerobic digestion process, Bioresour. Technol. 103 (2012) 415–424.

[4] R. Uma Rani, S. Adish Kumar, S. Kaliappan, I.T. Yeom, J. Rajesh Banu, Impacts of microwave pretreatments on the semi-continuous anaerobic digestion of dairy waste activated sludge, Waste Manage. 33 (2013) 1119–1127.

[5] K. Uan Do, J. Rajesh Banu, S. Kaliappan, I.T. Yeom, Influence of the thermochemical sludge pretreatment on the nitrification of A/O reactor with the removal of phosphorus by simultaneous precipitation, Biotechnol. Bioprocess Eng. 18(2) (2013) 313–320.

[6] A. Zhou, C. Yang, F. Kong, D. Liu, Z. Chen, N. Ren, A. Wang, Improving the short-chain fatty acids production of waste activated sludge stimulated by a bi frequency ultrasonic pretreatment, J. Environ. Biol. 34 (2013) 381–389.

[7] R. Uma Rani, S. Kaliappan, S. Adish Kumar, J. Rajesh Banu, Combined treatment of alkaline and disperser for improving solubilization and anaerobic biodegradability of dairy waste activated sludge, Bioresour. Technol. 126 (2012) 107–116.

[8] T. Poornima Devi, A. Vimala Ebenezer, S. Adish Kumar, S. Kaliappan, J. Rajesh Banu, Effect of deflocculation on the efficiency of disperser induced dairy waste activated sludge disintegration and treatment cost, Bioresour. Technol. 167 (2014) 151–158.

[9] J. Merrylin, S. Kaliappan, S. Adish Kumar, I.T. Yeom, J. Rajesh Banu, Effect of extracellular polymeric substance removal on sludge reduction potential of *Bacillus licheniformis*, Int. J. Environ. Sci. Technol. 10(1) (2013) 85–92.

[10] S. Kavitha, C. Jayashree, S. Adish Kumar, I.T. Yeom, J. Rajesh Banu, The enhancement of anaerobic biodegradability of waste activated sludge by surfactant mediated biological pretreatment, Bioresour. Technol. 168 (2014) 159–166.

[11] S. Kavitha, S. Adish Kumar, S. Kaliappan, I.T. Yeom, J. Rajesh Banu, Improving the amenability of municipal waste activated sludge for biological pretreatment by phase-separated sludge disintegration, Bioresour. Technol. 169 (2014) 700–706.

[12] A.M. Yeneneh, K.S. Tushar, C. Siewhui, H.M. Ang, A. Kayaalp, Effect of combined microwave-ultrasonic pretreatment on anaerobic biodegradability of primary, excess activated and mixed sludge, Comput. Water Energy Env. Eng. 2 (2103) 7–11.

[13] S. Kavitha, C. Jayashree, S. Adish Kumar, S. Kaliappan, J. Rajesh Banu, Enhancing the functional and economical efficiency of a novel combined thermo chemical disperser disintegration of waste activated sludge for biogas production, Bioresour. Technol. 173 (2014) 32–41.

[14] M. Suresh Karthik Kumar, T. Krishna Kumar, S. Adish Kumar, I.T. Yeom, J. Rajesh Banu, Effect of alkaline and ozone pretreatment on sludge reduction potential of a membrane bioreactor treating high strength domestic waste water, Desalin. Water Treat. (2014) 1–8, doi: 10.1080/194439942014.923336.

[15] J. Rajesh Banu, P. Arulazhagan, S. Adish Kumar, S. Kaliappan, A.M. Lakshmi, Anaerobic co-digestion of chemical and ozone pretreated sludge in hybrid upflow anaerobic sludge blanket reactor, Desalin. Water Treat. 54 (2014) 1–10, doi: 10.1080/194439942014.912156.

[16] T. Gayathri, S. Kavitha, S. Adish Kumar, S. Kaliappan, I.T. Yeom, J. Rajesh Banu, Effect of citric acid induced deflocculation on the ultrasonic pretreatment efficiency of dairy waste activated sludge, Ultrason. Sonochem. 22 (2015) 333–340.

[17] J. Wawrzynczyk, M. Recktenwald, O. Norrlow, E.S. Dey, Solubilization of sludge by combined chemical and enzymatic treatment, Afr. J. Biotechnol. 6 (2007) 1994–1999.
[18] J. Kim, C. Park, T.H. Kim, M. Lee, S. Kim, S.W. Kim, Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge, J. Biosci. Bioeng. 95 (2003) 271–275.
[19] M. Brambilla, F. Araldi, M. Marchesi, B. Bertazzoni, M. Zagni, P. Navarotto, Monitoring of the startup phase of one continuous anaerobic digester at pilot scale level, Biomass Bioenergy 36 (2012) 439–446.
[20] APHA, Standard Methods for the Examination of Water and Wastewater, twenty-first ed., American Public Health Association, Washington, DC, 2005.
[21] S. Gopi Kumar, J. Merrylin, S. Adish Kumar, S. Kaliappan, I.T. Yeom, J. Rajesh Banu, Effect of cation binding agents on sludge solubilizing potential of bacteria, Biotechnol. Bioprocess 17 (2012) 346–352.
[22] W. Joanna, R. Michael, N.E.S. Olof, Solubilisation of sludge by combined chemical and enzymatic treatment, Afr. J. Biotechnol. 6 (2007) 1994–1999.
[23] S. Kavitha, S. Adish Kumar, K.N. Yogalakshmi, S. Kaliappan, J. Rajesh Banu, Effect of enzyme secreting bacterial pretreatment on enhancement of aerobic digestion potential of waste activated sludge interceded through EDTA, Bioresour. Technol. 150 (2013) 210–219.
[24] J. Rajesh Banu, S. Kaliappan, I.T. Yeom, Two stage anaerobic treatment of dairy wastewater using HUASB with PUF and PVC carrier, Biotechnol. Bioprocess Eng. 12 (2007) 257–264.
[25] S. Chuthathip, D. Supusanee, Continuous Co-Digestion of Agro-Industrial Residues, M.S. thesis, University of Boras, Sweden, 2012.
[26] C.P.L. Grady, G.T. Daigger, H.C. Lim, Biological Wastewater Treatment, second ed., Marcel Dekker Inc, New York, NY, 1999.
[27] L. Bjornsson, M. Murto, B. Mattiasson, Evaluation of parameters for monitoring anaerobic co-digestion process, Appl. Microbiol. Biotechnol. 54 (2000) 844–849.
[28] N.Q. Ren, A.J. Wang, The Method and Technology of Anaerobic Digestion, third ed., Chem. Ind. Press, Beijing, 2004, pp. 30–31.
[29] I.S. Turovskiy, P.K. Mathai, Wastewater Sludge Processing, Wiley, New Jersey, NJ, 2006.
[30] Metcalf and Eddy, Wastewater Engineering Treatment and Reuse, fourth ed., McGraw-Hill Inc, New York, NY, 2004.
[31] I. Siegert, C. Banks, The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors, Process Biochem. 40 (2005) 3412–3418.
[32] M. Gholamreza, H. Asilian, A. Jamal, Effect of ozonation on reduction of volume and mass of waste activated sludge, J. Appl. Sci. Res. 4 (2008) 122–127.
[33] H. Zhang, Sludge Treatment to Increase Biogas Production, Trita-LWR Degree Project, Water System Technology, Department of Land and Water Resources Engineering, Royal Institute of Technology (KTH), Stockholm, 2010, pp. 10–20.
[34] R.D. Kritane, P.C. Suryawanshi, M.R. Patil, A.B. Chaudhari, R.M. Kothari, Optimization of organic loading rate for different fruit waste during biomethanization, J. Sci. Ind. Res. 68 (2009) 252–255.
[35] J. Merrylin, S. Adish Kumar, S. Kaliappan, I.T. Yeom, J. Rajesh Banu, Biological pretreatment of non-flocculated sludge augments the biogas production in the anaerobic digestion of the pretreated waste activated sludge, Environ. Technol. 34 (2013) 2113–2123.