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Evaluation of operational parameters for biodegration of bacterially disintegrated sludge

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ABSTRACT

The present investigation was undertaken to increase the efficiency of sludge solubilization using bacterial pretreatment with a thermophilic protease secreting bacteria, *Bacillus licheniformis* and deflocculation through ethylene diamine tetra acetic acid (EDTA). About 0.4% of EDTA was used to remove the flocs from the sludge. Then, the deflocculated and bacterially pretreated sludge was subsequently followed by aerobic and anaerobic digestion. In comparison with the control, the aerobic digestion showed 43% increase in the reduction of suspended solids (SS) and 13% increase in the solubilization of chemical oxygen demand. Pretreatment of deflocculated sludge along with anaerobic digestion prompt to 34% of SS and 38% of volatile solids reduction, with an enhancement of 65% biogas production. Pretreatment of deflocculated sludge with *B. licheniformis* elevates less energy, which was environmentally sound when compared to other pretreatment techniques.

Keywords: EPS removal; Bacterial pretreatment; Anaerobic digestion; Biochemical methane potential test; Anaerobic digestion; Suspended solids

1. Introduction

One of the most important problem that municipal wastewater treatment plants (WWTPs) has been facing during the last decades is the production of excess sludge, which is to be treated and disposed. Prior to the ultimate disposal, the waste-activated sludge (WAS) needs to be treated for its enormous organic and pathogen microorganism content. Therefore, the management of sludge is one of the most serious and integral problems in the treatment plants. The treatment and discarding cost of leftover biological sludge is as high as 40–50% of the total cost of the operating cost of the WWTPs [1,2]. Sludge decrement has been recognized as an impressive method to alleviate this problem. WAS mainly consists of microbes, and cell walls that act as physical barricade that do not pass intracellular organics to be efficiently biodegraded through digestion. Therefore, to upgrade digestion efficacy, the most sensible approach is to crack the cells by mechanical [3], advanced oxidation process [4], thermal [5], electric alakali [6], microwave [7], combined [8] and biological [9] methods before digestion of sludge. The primary goal of the pretreatment technologies is to agitate sludge flocs.

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A major part of WAS is predominant by extracellular polymeric substances (EPSs), which is secreted by microorganisms. Many polymeric substances, such as polysaccharides, protein, nucleic acid, uronic acid, and humic substances [10,11], are found in EPS. The major components of EPS are carbohydrates and proteins [12]. EPS is considered as the house of microorganisms due to its protective layer for the cells against harsh condition [13]. Thus, EPS plays a major role in biofilm formation and also in physiochemical properties of sludge [14].

Hydrolysis is the primary agent of deflocculation, hydrolysis, and oxidation of sludge flocs. Protease can catalyze and hydrolyze proteins to enhance the lysis efficiency of total sludge, thereby significantly enhancing sludge reduction [15]. For rapid degradation of sludge, a thermophilic aerobic bacteria have been employed [16] due to its fast action. About 40% of sludge solubilization rate was obtained by thermostable enzymes [17]. Thus, degradation of sludge by thermophilic bacteria that secrete protease extracellular enzymes can be fully subjugated in a pretreatment approach [18,19]. A thermophilic bacterial strain identified as *Bacillus licheniformis*, which produces extracellular protease, was isolated from a municipal wastewater treatment plant. Solubilization of sludge was carried out using this bacteria. EPS is removed before the sludge is subjected to solubilization, using EDTA [20]. Removal of EPS enhances the efficiency of bacterial enzymatic pretreatment [21]. The core objectives of the study are (1) to investigate the efficiency of deflocculation and bacterial pretreatment on aerobic degradation of WAS, (2) to evaluate the efficiency of bacterial pretreatment on biochemical methane potential (BMP) assay through kinetic analysis, and (3) to assess the potential of bacterial disintegration on laboratory-scale anaerobic digestion on comparing control and experimental reactors.

2. Materials and methods

2.1. Sludge sampling

From local wastewater treatment plant located in Trivandrum, Kerala, WAS was collected. The initial characteristics of the sludge were measured as per the standard methods mentioned in American public Health Association [22]. The pH of the raw sludge was 6.5, the soluble chemical oxygen demand (COD) was 0.2 g L^{-1}, the total COD was 12 g L^{-1}, the total solids content was 13 g L^{-1}, the volatile solids (VS) content was 8 g L^{-1}, and the suspended solids (SS) content was 10 g L^{-1}.

2.2. Removal of EPS

EDTA concentration was optimized to 0.4%, which is used before pretreatment of sludge for EPS removal. The dosage of EDTA used is indicated as the g equivalent, i.e. 0.4 g of EDTA is added to 100 ml of sludge. At 4˚C, the mixture was kept for 3 h with continuous agitation to confirm good blending. Through EPS removal, the sludge was dispersed and deflocculated, which increases the surface area for bacterial activity. This deflocculated sludge is further subjected to bacterial pretreatment.

2.3. Bacterial pretreatment

Bacillus licheniformis, a thermophilic protease producing bacterium, was used to pretreat the deflocculated sludge. This bacterium was mass cultivated in a nutrient broth at a temperature of 55˚C in a 5 l fermenter, and the cells were harvested after 20 h (early stationary phase). These cells functioned as the inoculum. Bacterial cells at a concentration of 77×10^6 cells mg^{-1} of SS were inoculated. The inoculated sludge was pretreated for 24 h as per the steps followed by Merrylin et al [20], and this pretreated sludge was used for further studies.

2.4. Aerobic digestion

Two 5 l PVC cylinders labeled as experimental reactor (ER) and control reactor (CR) were used for aerobic digestion. ER consists of bacterially pretreated deflocculated sludge and WAS in the ratio of 1:1 [23], whereas CR consists of only WAS. Maximum of 3 mg/l of dissolved oxygen was sustained for good aerobic digestion [24]. Following Kavitha et al. [25], the subsequent experimentation was performed.

2.5. Biochemical methane potential

For samples such as deflocculated, flocculated and control the BMP test was done. The inoculum used in this assay was cow rumen. The inoculum substrate ratio was maintained in the ratio of 3:1, which was selected based on the study of Luna-del Risco et al. [26]. The methodology for BMP assay was followed according to the procedure followed by Uma et al. [27]. The modified kinetics of biogas production was observed using Gompertz equation shown in Eq. (1).

$$B_t = B \times \exp[-\exp[R_b/B \times \exp(\lambda - t) + 1]] \quad (1)$$

where B is the biogas production potential (L/g VS), R_b is the maximum biogas production rate (L/g VS d), and λ is the lag phase (days). Most of the researchers have employed kinetics models for assessing the potential of BMP. Among them, modified Gompertz model is the most widely used model [28]. Gompertz model was known for its simplicity and reliability [29]. The constants B, $R_{b,}$ and λ were determined using the nonlinear Polymath software.

2.6. Semicontinuous anaerobic digestion

Two identical semicontinuous reactors labeled as CR and ER were run. CR consists of raw sludge, and ER consists of pretreated sludge, and the working volume of 3 l was used from the total volume of 5 l. The reactors were inoculated with the activated slurry collected from a local biogas plant in India. Organic loading rate (OLR) of 0.16, 0.26, 0.34, 0.4, 0.5, 0.58, 0.66, and 0.84 kg SS/m^3 d with the solids retention time (SRT) ranging from 30 to 12 d were serially used to examine the act of anaerobic digestion of the pretreated sludge. The first three loadings were carried out with different mixed liquor SS (MLSS) of 5,000, 7,500, and 10,000 mg/L at the constant SRT of 30 d. The rest of the loadings were carried out with different SRT of 25, 20, 17, 15, and 12 d with a constant MLSS of 10,000 mg/L. The CR was also run under the same conditions as that of the ER. Using peristaltic pumps, feeding and removal were done every day in a semi-continuous mode. Liquid displacement method was used to evaluate biogas production, in which the difference in water level in a cylinder related to the reactors was observed. The evacuated liquid is therefore considered to have the same volume as the produced biogas.

2.7. Analytical parameters

The parameters such as SS, VS, and alkalinity were analyzed according to the standard methods [22]. The pH was measured using a digital pH meter. Using distillation–titration method, volatile fatty acid(VFAs) was examined, and the result was stated in acetic acid. Using Eq. (1), SS reduction was calculated. Baroda gas chromatograph was used to analyze methane present in biogas.

3. Results and discussion

3.1. Aerobic digestion

3.1.1. Effect of aerobic digestion on SS reduction

The effect of aerobic digestion on SS reduction was portrayed in Fig. 1a. From the figure, it is observed that biological pretreatment of deflocculated sludge enhances the sludge solubilization during aerobic digestion. One of the most important directly measurable parameters in assessing digester performance or efficiency is the reduction in sludge mass over a specified period of time [30]. Therefore, SS reduction increases with the increase in sludge retention time (SRT) until it reaches its optimum point. SS reduction of ER was about 59% and in the CR was about 15.3%. Compared to the control, there was a 43% increase of SS reduction in ER. The SS reduction in ER was high due to converting carbonaceous substrates to gaseous end products. Diminution of the SS occurred drastically for the first 6 d and later on when the SRT was increased, the reduction was stabilized. Thus, for an efficient aerobic digestion, 8 d of SRT is sufficient.

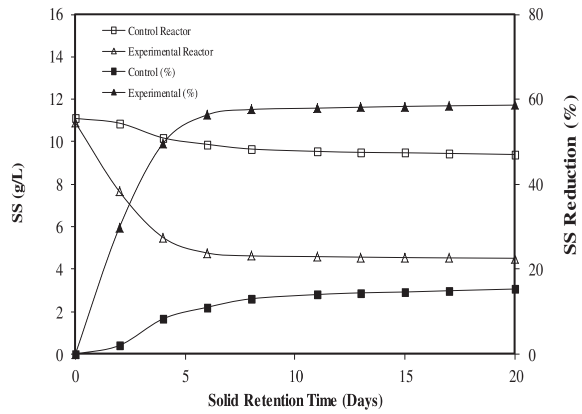

Fig. 1a. Effect of pretreatment on SS reduction during aerobic digestion.

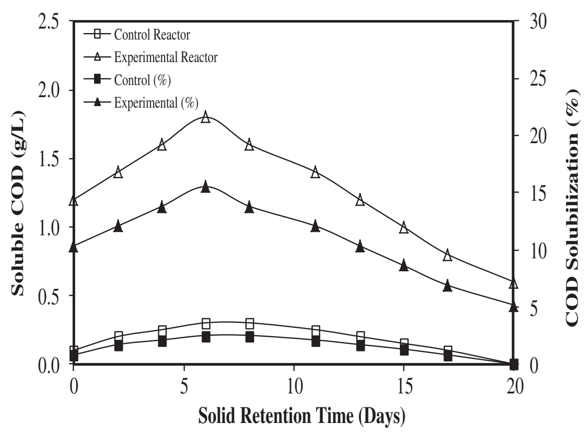

Fig. 1b. Effect of pretreatment on COD solubilization during aerobic digestion.

3.1.2. Effect of aerobic digestion on COD solubilization

The effect of aerobic digestion on COD solubilization was portrayed in Fig. 1b. With the increase in SRT, there was a steady increase in soluble COD reaches a maximum (1.8 g/L) at 6 d and further increase in SRT (after 6 d), there was a reduction in soluble COD release. This may be due to the utilization of the lysed materials as exogenous substrates by the aerobic microbes. When compared with the CR, there was a 13% increase in COD solubilization. Aerobic digester follows first-order kinetics for the substrate removal with a correlation coefficient (R^2) value of less than 90%. The rate constant in the CR was $0.0122\ d^{-1}$ and ER was $0.0443\ d^{-1}$ when time (t) is plotted against the difference in the natural logarithm of the substrate concentration (lnC) and the natural logarithm of the initial substrate concentration (lnC_o). From calculation, the rate constants of ER were four times faster than that of CR. A linear trend shows that substrate concentration is directly proportional to SRT. It is probable that all kinds of aerobic digestion would have exhibited some form of cell lysis (defined as the inability of the organisms to maintain cellular integrity, as a result of extreme environmental conditions, with the subsequent rupture of the cell membrane and release of cellular materials into the bulk liquid of the digester), and hence, the soluble COD concentrations in the ER were always higher than in CR [31].

3.2. Biochemical methane potential test

Biogas production was gentle at the initial stage due to the specific growth rate of methanogenic bacteria, which has been reported in many studies [32,33]. This could be explained using the concepts of lag time and food to micro-organism (F/M) ratio. At the initial stages, the F/M ratio was high as there was more food (i.e. substrates) and less active bacteria. Hence, microbial consortia had to acclimatize to this new environment for their optimal function [34]. Biogas production in deflocculated and flocculated reached its optimum rate from 7 to 9 d as soon as it has elapsed its acclimatization (Fig. 2). After this, a gradual decrease in biogas generation was noted. However, it took more time to acclimatize in control and for the digestion of substrates and release biogas.

Individual cumulative biogas production data were employed to calculate the three parameters of the modified Gompertz equation, namely maximum biogas production rates (R_b), biogas production potential (B) and lag phase time (λ) for all reactors and are tabulated in Table 1. The cumulative biogas generated from all the reactors studied fitted very well to the

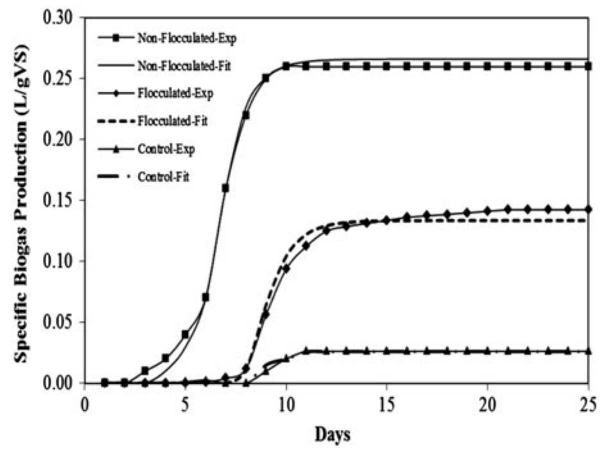

Fig. 2. Modified Gompertz model fit to the experimental data.

modified Gompertz equation with R^2 values ranging from 0.992 to 0.996. At the end of the digestion, it was observed that deflocculated has a high biogas production rate (R_b) of 0.03 L/g VS d with the highest cumulative biogas production potential (B) of 0.26 L/g VS, with a lag phase (λ) of 3.4 d. The currently achieved biogas potential was relatively comparable with the outcomes obtained by Zhen et al. [28], Kavitha et al. [35] and Sowmya et al. [36]. Flocculated have biogas production rate of 0.02 L/g VS d with a biogas production potential of 0.12 L/g VS at a lag phase of 3.5 d. While in control, maximum biogas production rate of 0.009 L/g VS d with the biogas production potential of 0.04 L/g VS.

With reference to the data for R_b and λ (Table 1, Fig. 2), it was observed that after crossing the maximum biogas generation phase, the rate of biogas generation becomes stabilized. This could be due to the slowdown in the biodegradation of complex materials and depletion of remaining readily biodegradable materials. Besides, the remaining readily biodegradable materials of the substrates would have been trapped within cells by cell walls of the organisms. Therefore, they were not easily accessible for microbial degradation [37].

3.3. Semicontinuous anaerobic digestion

3.3.1. Acclimatization of the reactor

The reactor was filled with biologically pretreated sludge at an OLR of 0.16 kg SS/m^3 d at its acclimatization phase and the set up were continuing to operate till a stable period was achieved. Stable periods were set when a constant pH, VFA concentration and

Table 1
Kinetic parameters calculated from the theoretical Gompertz model

S. no.	Experimental condition	Modified Gompertz parameters (Model) R_b (L/gVS d)	B (L/gVS)	λ (d)	R^2	Methane (%)
1	Non-flocculated	0.03	0.26	3.14	0.992	63–65
2	Flocculated	0.02	0.12	3.59	0.990	64–66
3	Control	0.009	0.04	4.21	0.996	58–60

biogas production in the reactor were maintained [38]. With this calculation after 30 d, a steady state was achieved with <10% fluctuations of parameters. After normal operation has been set up, seeding of the fresh solids into the digester and mixing them with the digesting sludge greatly improve the pace of digestion [39].

3.3.2. SS reduction

The effectiveness of sludge stabilization was measured by SS reduction [40]. Fig. 3a shows SS reduction, which is directly proportional to SRT. During the first OLR, there was a 12% SS reduction, and with the increase in OLR to 0.26 kg SS/m^3 d, there was a 18% SS reduction. During the third loading rate, the SS reduction was stabilized and was found to be 19%. The first three loadings were carried out by fixing SRT at 30 d and varying organic load in terms of MLSS. Even though the MLSS was increased, the SS reduction was stabilized at 30 d SRT; hence, the SRT was reduced to 25 d by increasing the OLR to 0.4 kg SS/m^3 d. At 25th, 20th, 17th, and 15th days, the SS reduction was about 22, 27, 32, and 34%, which shows a major decrease with the rise in OLR. To further reduction in SRT, the SS reduction reduced to 33%, which could be due to the overfilling of the reactor, whereas

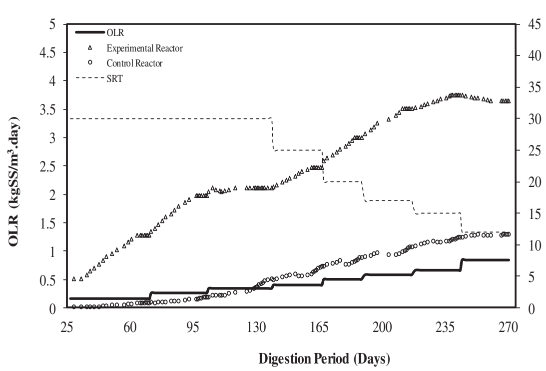

Fig. 3a. Effect of SRT on the removal of SS during the study period.

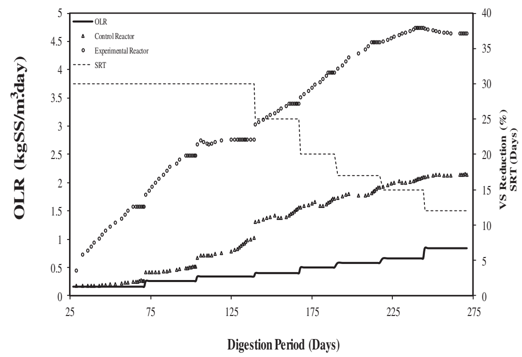

Fig. 3b. Effect of SRT on the removal of VS during the study period.

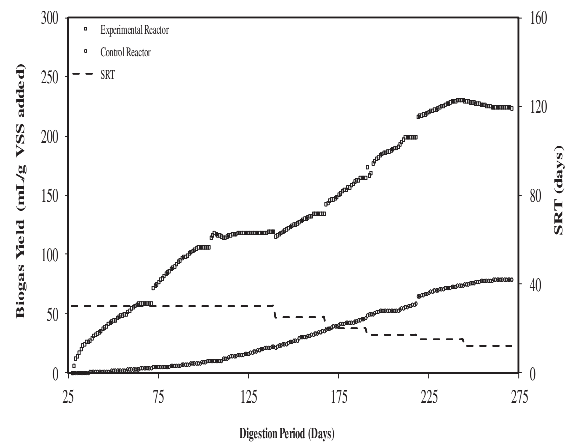

Fig. 3c. Effect of SRT on biogas yield during the study period.

11% SS reduction was observed in CR. Thus, there was 65% increment in SS reduction in the ER compared to the CR.

3.3.3. VS Reduction

The VS reduction was taken into account as well as to evaluate the reactor performance and stability of the digestate. The amount of stabilization is often

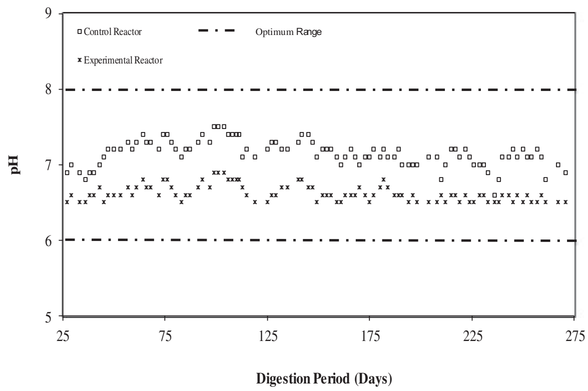

Fig. 3d. Variation of pH in the anaerobic reactors during the study period.

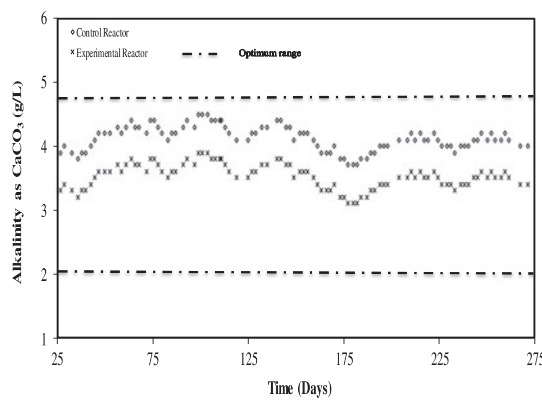

Fig. 3e. Variation of alkalinity in the anaerobic reactors during the study period.

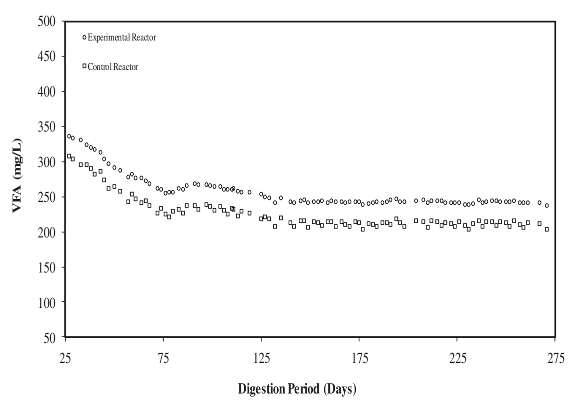

Fig. 3f. Variation of VFA in the anaerobic reactors during the study period.

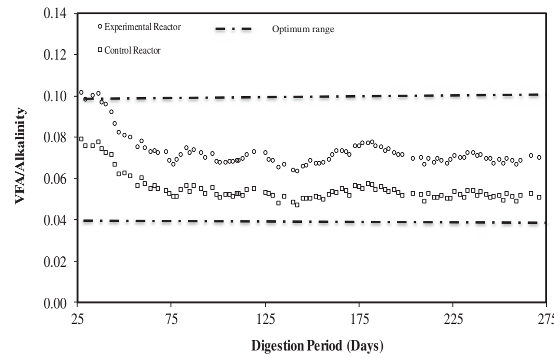

Fig. 3g. Variation of VFA/alkalinity ratio in the anaerobic reactors during the study period.

stated as the percent reduction in VS [41]. Fig. 3b shows that the rise in OLR led to an increase in VS reduction. VS reduction was stabilized each time before switching to the next OLR. During 30 d SRT, about 22% of VS reduction was observed. Further decrease of SRT to 25 d, with the increase of OLR to 0.4 kg SS/m^3 d, led to 27% of VS reduction, which shows a major reduction with the increase in OLR. The SRT was decreased further to 20 d, and the VS reduction was 32%. When the OLR was shifted from 0.5 to 0.58 kg SS/m^3 d, there was 36% of VS reduction. With a further fall of SRT from 17 to 15 d, a stabilized VS reduction was observed. Alike SS reduction, nearly maximum of 38% of VS reduction of was found at an OLR of 0.66 kg SS/m^3 d. Above 37% of VS removal was observed with the raise of OLR beyond 0.66 kg SS/m3. Similarly, 38% and 40% of VS reduction was obtained by Lin et al. and Diclehan [42,43] through biological pretreatment of sludge during anaerobic digestion.

The current results show that the biological pretreatment is beneficial as it significantly improved anaerobic digestion with only 17% VS reduction in the CR. Thus, the maximum performance was due to the pretreatment, which accelerates decomposition reaction and led to faster subsequent degradation. The subtract for anaerobic bacteria is obtained from the organic matter released during disruption of sludge floc [44].

3.3.4. Total biogas production

Fig. 3c shows the profile of biogas yield. After acclimatization, digestion was initiated (27th d) with a 30 d SRT and lasted up to 139 d. During that period, biogas production was found to be 120 mL/g VSS added, then second SRT (25 d) was run until 167 d, and the total biogas produced was 135 mL/g VSS added. The reactor was run until 190 d at an SRT of 20 d, and the total biogas produced was 165 mL/g VSS added. The SRT was decreased further to 17 d, and the total biogas produced was 199 mL/g VSS added. During the SRT of 15 d, the reactor was run until 244 d, and the total biogas produced was 231 mL/g VSS added, and then finally when the SRT was decreased to 12 d, biogas production also reduced and the total biogas produced was 224 mL/g VSS added. In ER, the cumulative biogas produced was 66 l for the whole reactor operation period and that in the CR was 17 l. Compared to the control, the overall methane production of the pretreated sludge was increased by 64%. It was also observed that the daily biogas production rate appreciably raises from 30 to 15 d SRT consecutively, while the rate decreased from 15 to 12 d SRT. As the SRT decreases, the OLR spontaneously increases, which led to the over loading phenomenon. Thereby, 15 d of SRT was considered to be a proper retention time for effective sludge digestion.

3.3.5. pH

During the startup of the digesters, the pH was in the range of 6.5–7, which is the favorable pH for the growth of methanogenic bacteria [45]. Fig. 3d shows that the pH in both the digesters was maintained within the range by high organic loading. High production of volatile fatty acids was also observed in pretreated sludge due to high pH in the CR than that of the ER. From the results, it can be concluded that pH did not play any major role in the decrease of solid removal at higher loading rate.

3.3.6. Alkalinity

Vlyssides and Karlis [46] reported that the concentration of bicarbonate alkalinity controls anaerobic digestion. Methane production is inhibited due to the production of volatile acids during digestion, which decreases in pH. According to Metcalf and Eddy [41], the total alkalinity of a well-established digester ranges from 2,000 to 5,000 mg/L. Using $CaCO_3$,the alkalinity was measured in both the reactors that vary within the range of 3,100–4,500 mg/L [47]. Fig. 3e indicates that adequate alkalinity was present to maintain optimum conditions for methanogenesis. The trends are similar to the trends in pH, and the causes of the fluctuation are also similar.

3.3.7. Volatile fatty acids

Among all VFAs, acetic acid is considered most favorable for methane production. Above 70% of digestion are influenced by acetic acid [48], which is considered as a good indicator of anaerobic reactor performances. In this study, according to Fig. 3f, VFA decreased with the decrease in SRT and was within the range of 238–248 mg/L. It was also observed that there is 17% increase in pretreated sludge compared to control. A similar increase in VFA was observed by Ghosh et al. [49] when biologically pretreated sludge is subjected to anaerobic digestion.

3.3.8. VFA/alkalinity ratio

Hampannavar and Shivayogimath [50] reported the need of equilibrium between alkalinity and VFA concentrations for normal digestion. Gerardi [51] suggested that the ratio should be sustained within the range of 0.1 to increase the digestion. Therefore, VFA/alkalinity ratio was within the range of 0.06–0.09, which indicates the good working status of the anaerobic reactor as shown in Fig. 3g.

3.3.9. Assessment of the semicontinuous anaerobic reactor

The results obtained after reactor stabilization is summarized in Table 2. The optimal loading rates and retention time for anaerobic digestion will depend upon the quality of the feedstock and on the desired efficiency of the overall process. A high VFA production is obtained with high organic loading, which results in pH reduction, and will have adverse effect on methanogenic bacteria. Adequate quantity of biogas will not be produced at low organic loading and unnecessarily will create a large digester. Too short SRT will not provide enough time for anaerobic bacteria, particularly for methane-producing bacteria, whereas too long SRT will result in an unnecessary accumulation of assimilated materials in the digester, and structure of a digester will be large.

A range of 10–60 d of SRT will be optimal for suspended growth digesters, whereas attached growth and high rate digesters can run at a too shorter SRT [52]. From the results, it is distinguished that the OLR of 0.66 kg SS/m^{3a} d operated at 15 d SRT is the most pertinent OLR for the effective and economic functioning of the digester. OLR and retention times reported in the various studies on MSW digestion have ranged from 0.07 to 0.35 lb VS/ft^3 d and from 10 to 30 d, respectively. Most of these studies have included co-digestion with raw sewage sludge. Uma et al. [27]

Table 2
Digester's performance after stabilization

Parameters	Experimental reactor						Control reactor					
SRT (d)	30	25	20	18	15	12	30	25	20	18	15	12
OLR (kg SS/m^3 d)	(a) 0.16 (b) 0.26 (c) 0.33	0.4	0.5	0.58	0.66	0.84	(a) 0.16 (b) 0.26 (c) 0.33	0.4	0.5	0.58	0.66	0.84
Feed pH	6.9	6.9	6.9	6.9	6.9	6.9	7.5	7.5	7.5	7.5	7.5	7.5
Outlet pH	6.7	6.7	6.6	6.6	6.6	6.5	7.2	7.2	7.2	7.2	7.1	6.9
Alkalinity (g/L)	3.7 ± 0.5	3.3 ± 0.5	3.3 ± 0.5	3.5 ± 0.5	3.5 ± 0.5	3.4 ± 0.5	4.3 ± 0.5	3.9 ± 0.5	3.9 ± 0.5	4.1 ± 0.5	4.1 ± 0.5	4 ± 0.5
SS removal (%)	19	22	27	32	34	33	3	6	8	9	11	12
VS removal (%)	22	27	32	36	38	37	7	12	13	15	16	17
Methane production (mL)	134 ± 10	183 ± 10	277 ± 10	351 ± 10	461 ± 8	449 ± 8	25 ± 10	49 ± 10	78 ± 10	104 ± 10	149 ± 11	159 ± 11
Methane (%)	64	67	68	64	65	63	58	58	62	60	59	62
Biogas yield (mL/g VSS added)	120	135	165	199	231	224	22	36	47	59	75	79
VFA production (mg/L)	248 ± 15	243 ± 15	243 ± 15	244 ± 13	244 ± 13	238 ± 13	220 ± 15	215 ± 15	213 ± 15	209 ± 14	214 ± 14	203 ± 14

have reported OLRs of 3.55–5.9 g VS/L d with a retention time of 20, 15, and 12 d, and it was shown that the operation of the reactor was safe at the SRT of 15 d while using dairy sludge.

4. Conclusion

Ultimately, it was concluded that there is 43 and 13% increase in SS reduction and COD solubilization, respectively, compared to the control during aerobic digestion. BMP results also indicated that there is more gas production and organic matter stabilization in the reactor fed with deflocculated-pretreated sludge compared with the flocculated pretreated sludge and CR. Thus, for a semicontinuous digester deflocculated-pretreated sludge joining with anaerobic digestion led to 34–38% of SS and VS reduction, respectively, with a 65% improvement in biogas production when operated at an efficient OLR of 0.66 kg SS/m^3 d.

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