Anaerobic digestion of sulphate-rich post-tanning wastewater at different COD/sulphate and F/M ratios

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Received: 6 November 2017 / Accepted: 3 February 2018 / Published online: 13 February 2018
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Abstract

Anaerobic digestion of post-tanning wastewater was performed in batch anaerobic digester to evaluate the effect of COD/sulphate ratio [0.62, 0.69, and 1.20 (w/w) %] and F/M ratio [0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, and 1.5 (w/w) %] on the removal efficiency of COD. The F/M ratio of 0.3 was found to be the optimum ratio for the removal of COD by 53, 57, and 65%, respectively at COD/sulphate ratio of 0.62, 0.69, and 1.20. The maximum sulphate removal was observed at F/M ratio of 0.2 and the removal efficiency was 48, 50, and 58% at COD/sulphate ratio of 0.62, 0.69, and 1.20, respectively. The removal efficiency of COD and sulphate was increased with increase in COD/sulphate ratio from 0.62 to 1.20 and decreased with increase in F/M ratio from 0.2 to 1.5 in anaerobic digestion of post-tanning wastewater. The maximum concentration of sulphide formation was 784 mg/L at COD/sulphate ratio of 0.62 in anaerobic digestion process and the process was inhibited at this sulphide concentration. The microbial activity in the sludge was evaluated through live and dead cell assay using fluorescent microscopy. The maximum amount of dead microbes was observed in the anaerobic digester, which was operated at COD/sulphate ratio of 0.62 than other studied ratio.

Keywords COD/sulphate ratio · F/M ratio · Post-tanning wastewater · Sulphate-laden wastewater · Live/dead cell assay

Introduction

Many industrial processes such as paper and pulp processing, molasses fermentation, sea-food processing, potato and starch processing, leather manufacturing, edible oil refineries, pharmaceutical, petrochemical processing, and wine distilleries discharge sulphate-laden wastewater. Amongst them, tannery wastewater contains a wide spectrum of normal and synthetic organic compounds with different degrees of biodegradability. However, aerobic and anaerobic biological processes have been applied for the treatment of these wastewater (Mannucci et al. 2010; Chandra et al. 2011). The direct discharge of untreated tannery wastewater creates environmental threat for its high concentration of dyes, poly-phenolic compounds, surfactants, sulphonated oils, chromium salts, chlorides, sulphides, sulphates, and nitrogenous inorganic compounds (Durai and Rajasimman 2011;

Zupancic and Jemec 2010). Post-tanning process involves neutralisation and washing steps, followed by re-tanning with synthetic aliphatic and aromatic organic compounds, dyeing, and fat liquoring of tanned hides/skins. The use of sodium bisulphite and sodium metabisulphite in post-tanning operations is the prime source for the high concentration of sulphate ions in the post-tanning wastewater (Saravanabavan et al. 2003). Moreover, the post-tanning wastewater contains a wide spectrum of aliphatic and aromatic organic compounds, chloride, sulphide, and chromium, besides suspended solids (Haydar et al. 2009). The high concentration of nitrogenous and sulphated phenolic compounds and tanning agents renders post-tanning wastewater more recalcitrant in nature (El-Sheikh et al. 2011).

In recent years, anaerobic digestion (AD) processes have been applied for the treatment of organic rich wastewater discharged from many industries (Lefebvre et al. 2006; Tamilchelvan and Dhinakaran 2012). The application of anaerobic treatment to sulphate-laden wastewater has been found to be challenging due to the formation of hydrogen sulphide (H₂S). The sulphide formation and methane generation take place simultaneously in anaerobic digestion process. Both sulphate-reducing bacteria (SRB) and

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methane-producing bacteria (MPB) use hydrogen and acetate as the sources for electron donor which leads to a competition for organic substrate (Barrera et al. 2014; Stefanie et al. 1994; McCartney and Oleszkiewicz 1993). Generally Chemical Oxygen Demand (COD)-to-sulphate (SO_4^{2-}) ratio is a parameter widely used to control biological sulphate reduction and as well as the methane production. It has been shown that the competition of SRB was more pronounced at low COD/ SO_4^{2-} ratio in the wastewater (Singh et al. 2014). The consumption of organic substrates in sulphate-laden wastewater is strongly dependent on the COD/ SO_4^{2-} ratio (Sarti and Zaiat 2011). The COD/ SO_4^{2-} ratio of 0.67 was reported to be theoretically optimum ratio for the significant removal of COD by SRB (Rinzema and Lettinga 1988). The relationship between the inoculum quantity and COD was investigated by many researchers to understand the appropriate food (substrate)-to-microorganism (inoculums) (F/M) ratio for the anaerobic digestion of wastewater (Gonzalez-fernandez et al. 2009). The substrate concentration (COD) was considered as an important parameter which influences the efficiency of the anaerobic digestion process (Lianhua et al. 2010; Sanchez et al. 2001). At very low substrate concentration, there is a risk of low metabolic activity and very low quantity of biogas production. In contrast, if the substrate concentration is high that might lead to a substrate overload condition in which intermediate products were accumulated and resulted in product inhibition of the process (Zhang et al. 2014). Hence, an appropriate F/M ratio is required to maintain efficient anaerobic digestion processes for industrial wastewater. Therefore, it is important to evaluate the influence of substrate concentration which plays a critical role on both process stability and methane gas production in the anaerobic treatment of wastewater.

The focal theme of the present investigation was to treat the sulphate-rich post-tanning wastewater (PTWW) by anaerobic digestion process. The variables such as F/M ratio and COD/ SO_4^{2-} ratio were evaluated for the effective treatment of post-tanning wastewater. Furthermore, the presence of high sulphate concentrations in specific wastewater restricts the application of anaerobic treatment technology, because toxic, corrosive, and odorous hydrogen sulphide (H_2S) is produced (Shayegan et al. 2005; Muyzer and Stams 2008; Lens et al. 1998). The toxicity of H_2S on microbial population was analyzed using fluorescent microscopy.

Materials and methods

Wastewater and seed culture

The post-tanning wastewater was collected from a Common Effluent Treatment Plant (CETP), Pallavaram, Chennai. The

anaerobic sludge was collected from the anaerobic digester plant at Perungudi, Chennai and used as an inoculum in the experimental study.

Anaerobic digestion-batch study

The batch experimental anaerobic digestion was performed for post-tanning wastewater with different COD/ SO_4^{2-} ratio (0.62, 0.69, and 1.2). The COD/ SO_4^{2-} ratio (0.62–1.2) was selected, because the wastewater discharged from varied leather manufacturing process (raw-to-finish operation, raw-to-wet blue operation, and post-tanning operation) contains COD/ SO_4^{2-} in the range of 0.62–1.2. The wastewater with different F/M ratios as 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, and 1.5 was used in the present study. The F/M ratio was studied in the wide range to evaluate the sulphide detoxification level. The serum bottles of volume 120 mL were used as anaerobic reactors in batch experimental study with working volume of 80 mL. The serum bottles were closed with rubber cap and aluminum seal was used after purging nitrogen gas at a rate of 15 mL/S (at ambient temperature and pressure, 0.8–1.0 kg/cm²) for 10 min to establish anaerobic condition in the reactor. The batch reactors were monitored for every 12 h time interval up to 108 h, and after every 12 h time interval, the respective anaerobic reactor was removed from the incubator and the wastewater was separated from the sludge and characterized. The experiment repeated with wastewater alone served as blank and the experiment was repeated with sludge alone served as control. The experiments were run in triplicate and the average values were calculated. The characteristics of post-tanning wastewater used for batch study are shown in Table 1.

Physico-chemical analysis of wastewater

The PTWW samples were characterized for physico-chemical parameters following the methods described in the standard methods for water and wastewater analysis (APHA 2005). The sulphide content of the samples was determined by immediately fixing with 2 N zinc acetate at alkaline condition to avoid sulphide oxidation. The wastewater samples after fixation were centrifuged at 5000 rpm for 5 min to separate the precipitate. The supernatant solution was analyzed for sulphate and the precipitate was used for the determination of total sulphides by iodometric method. The COD content was determined (without zinc acetate fixation) after acidifying the sample with 1 N sulphuric acid to strip out sulphides as H_2S by purging nitrogen gas and fixed by the addition of potassium dichromate. The close reflux digestion for the analysis of COD was carried out using HACHs COD digester (DRB 200). The

Table 1 Physico-chemical characterization of initial post-tanning wastewater and treated wastewater at F/M ratio 0.3

Parameters	Sample-1		Sample-2		Sample-3	
	Initial	Treated	Initial	Treated	Initial	Treated
pH	6.88	8.98	7.06	8.34	7.18	7.26
Oxidation reduction potential (ORP), mV	− 303	− 362	324	− 384	− 337	− 413
Chemical oxygen demand (COD), mg/L	3027	1400	3097	1317	3320	1220
Sulphate, mg/L	4853	2670	4480	2380	2753	1153
COD/sulphate ratio	0.62	–	0.69	–	1.20	–
Sulphide, mg/L	43	686	38	648	26	420
Volatile fatty acids (VFA), mg/L	1140	604	1190	550	1110	334

Oxidation–reduction potential (ORP) of the samples was recorded using Orion 920 (thermo-electronic instruments) and pH was measured using a digital pH meter (Hanna Instruments, Model: HI 2212). The generation of methane gas was measured by NaOH displacement method based on Mariette principle, i.e., the volume of 1 N NaOH solutions displaced by an equivalent volume of methane gas generated. The generation of methane gas was recorded on daily basis.

The cumulative methane yield at the given condition was calculated using the following mathematical equation:

$$\eta_{\max} = M_E/S_L \quad (1)$$

where $\eta_{\max}$ represents the cumulative methane yield (L/g-COD removed), M_E is the total volume of methane gas (L-CH₄) collected at the end of experiment, and S_L is the amount of substrate removed (g-COD) at the end of experiment.

Fluorescence microscopic study

The sludge samples after anaerobic digestion for 108 h were collected by centrifugal separation at 5000 rpm for 10 min; then, the pellets were re-suspended in phosphate buffer (pH, 7.0) solution. The suspended sludge samples were diluted to record an optical density (OD @ 600 nm) of 0.5 at wavelength λ_{600} nm in UV–visible spectrophotometer (Varian, Model: Carry 100 conc). The suspended sample of volume 20 μ L was stained by adding SYTO 9 dye of volume 1.25 μ L (3.34 mM in DMSO) and propidium iodide of volume 1.25 μ L (3.34 mM in DMSO) for the fluorescence imaging in visible fluorescence microscopy (Olympus BX-61). The samples were mounted on microscope slides after 5 min of dyes addition using a

blue fluorescence filter sets to capture the fluorescence images of live/dead cells. The SYTO9 stain was used to identify all living cell by green image, while propidium iodide stain was used to identify dead cells captured by red image.

Results and discussion

The treatability of PTWW is highly significant in anaerobic system due to the presence of sulphate (influenced by COD/SO₄^{2−} ratio). In general, the competition exists between MPB and SRB, and the minimum COD/SO₄^{2−} ratio required for the optimum conversion of COD to CH₄ was reported to be 0.67 (Lens et al. 1998). The COD/SO₄^{2−} ratio of the wastewater collected from the CETP was in the range 0.62–1.2 $\pm$ 0.19 (Minimum–Maximum $\pm$ Standard deviation). Hence, in the present investigation, anaerobic batch reactors were operated for 108 h at different COD/SO₄^{2−} ratios (0.62, 0.69, and 1.2) at various F/M ratios in the range 0.2–1.5.

Effect of F/M ratio on anaerobic digestion of wastewater with COD/SO₄^{2−} ratio of 0.62

The effect of F/M ratio (0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, and 1.5) on anaerobic digestion of post-tanning wastewater at COD/SO₄^{2−} ratio of 0.62 is shown in Fig. 1. Figure 1a shows the COD elimination at different F/M ratios from 0.2 to 1.5. The COD was found to be eliminated from 3027 to 1520, 1400, 1660, 1800, 2000, 2240, 2340, 2430, and 2660 mg L^{−1} with removal efficiency of 49, 53, 45, 40, 33, 25, 22, 19, and 12% at F/M ratios of 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, and control, respectively (Fig. 2). The maximum COD removal was observed by 53% at F/M ratio of

Fig. 1 Effect of F/M ratio —■— Control, —●— 0.2, —▲— 0.3, —▼— 0.5, —◆— 0.7, —▶— 0.9, —◆— 1.1, —●— 1.3, —●— 1.5 on batch anaerobic digestion of post-tanning wastewater at COD/sul-

phate ratio of 0.62, **a** COD removal, **b** sulphate reduction, **c** sulphide formation, **d** pH, **e** VFA

Fig. 2 Percentage COD removal at different COD/sulphate ratio and F/M ratios in anaerobic digestion of post-tanning wastewater

0.3. Figure 1b shows the change in sulphate concentration in batch anaerobic reactor operated at different F/M ratios. The results revealed that the sulphate reduction was from 4853 to 2500, 2670, 2783, 3032, 3453, 3694, 4080, 4252, and 4652 mg L⁻¹ with removal efficiency of 48, 44, 42, 37, 30, 26, 22, 17, and 4% at F/M ratios of 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, and control, respectively (Fig. 3). The sulphate ions were reduced to sulphide ions by SRB in

Fig. 3 Percentage sulphate removal at different COD/sulphate and F/M ratios in the anaerobic digestion of post-tanning wastewater

anaerobic digestion process (Barrera et al. 2014). According to Lens et al. (1998), anaerobiosis is a necessary condition in AD for the reduction of sulphate to sulphide. Figure 1c shows the profile of sulphide generation over the incubation period of 108 h during anaerobic digestion of PTWW at different F/M ratios. The concentration of sulphide was found to be increased from 43 to 744, 686, 630, 558, 480, 400, 348, 256, and 108 mg L⁻¹ at F/M ratios of 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, and control, respectively. The sulphate reduction results in the accumulation of sulphide in anaerobic reactor. The increase in sulphide concentration may be considered to inhibit or cause imbalance in the anaerobic process (Sarti et al. 2010). Hence, the residual COD in the batch anaerobic reactor was found to be increased with decrease in COD/sulphate ratio (Nagpal et al. 2000), which resulted in poor COD removal (53%) at COD/sulphate ratio of 0.62. The methane gas production in the anaerobic digestion with blank samples remained zero. The amount of methane generated from control sample (sludge alone) was subtracted from the methane gas generated in experimental samples. Hence, the data of the blank and control anaerobic digesters are not shown in Fig. 4. The methane production was recorded during AD of PTWW at different F/M ratios and it showed that the methane generation was nil at all F/M ratios (Fig. 4). The SRB grows faster due to availability

Fig. 4 Profile of the methane gas generation in L/g of COD removed at different COD/sulphate and F/M ratios in the anaerobic digestion of post-tanning wastewater

of high sulphate in wastewater and utilizes hydrogen and acetate as the sources for electron donor (Barrera et al. 2014). This leads to a competition between SRB and MPB for organic substrates, and thereby, high sulphide production was facilitated in the present investigation. Sulphide is the most toxic in its undissociated form, because the neutral molecules permeate the cell membrane with ease (Stefanie et al. 1994). McCartney and Oleszkiewicz (1991, 1993) reported that methanogens were more sensitive to an increase in the total sulphide concentration and also observed that methanogenic activity was inhibited by about 50% at 100 mg L⁻¹ of free-H₂S. The activity of methanogenic bacteria was very low in batch anaerobic reactor due to the toxicity of H₂S, and thereby, the methane production was very low in the present study. The cumulative yield of methane and COD elimination was retarded at low COD/SO₄²⁻ ratio, due to the formation of exceedingly high concentration of H₂S in the reactor; this corroborates with Jeong et al. (2008). The accumulation of H₂S in the batch anaerobic reactor altered the pH profile in batch reactor, as shown in Fig. 1d. The pH was increased gradually from 6.88 to 8.8, 8.98, 8.68, 8.38, 8.1, 7.98, 7.89, 7.81, and 7.71 over the incubation period at F/M ratios of 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, and control, respectively. The generation of methane gas depends on the conversion of substrate into volatile fatty acids (VFAs) and followed by the conversion of VFA into methane by methanogenic bacteria. The solution pH increased due to the accumulation of H₂S in the batch anaerobic reactor and affected the activity of methanogens resulted in poor methane generation. Figure 1e shows the elimination of VFA concentration in batch anaerobic reactor from 1140 to 615, 604, 643, 724, 774, 814, 830, 850, and 884 mg L⁻¹ at F/M ratios of 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, and control, respectively. The increase in reactor pH and accumulation of H₂S due to reduction of sulphate by SRB in the reactor inhibited the activity of methanogenic bacteria resulted in very low elimination of VFA.

Effect of F/M ratio on anaerobic digestion of wastewater with COD/SO₄²⁻ ratio of 0.69

The performance of AD treating post-tanning wastewater with COD/SO₄²⁻ ratio of 0.69 at different F/M ratios (0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, and 1.5) is shown in Fig. 5. The methane yield was measured at different time intervals for

different F/M ratios and the data are presented in Fig. 4. The maximum methane gas generation was found to be 0.03, 0.05, 0.035, and 0.01 L/g of COD removed at F/M ratios of 0.2, 0.3, 0.5, and 0.7, respectively, at the incubation period of 108 h. The methane production was nil at F/M ratio of 0.9, 1.1, 1.3, and 1.5, it may be attributed to substrate inhibition at high F/M ratio. The COD removed was from 3097 to 1397, 1317, 1587, 1787, 2010, 2250, 2337, 2417, and 2600 mg L⁻¹ with removal efficiency of 54, 57, 48, 42, 35, 27, 24, 21, and 16% for F/M ratios of 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, and control, respectively (Figs. 2, 5a). The maximum COD removal was 57% and methane generation was 0.05 L/g of COD removed at F/M ratio of 0.3. Figure 5b shows the sulphate profile over the incubation period in batch anaerobic reactor operated at different F/M ratios. The results revealed that the sulphate reduction was from 4480 to 2230, 2380, 2520, 2666, 3080, 3321, 3684, 3804, and 3997 mg L⁻¹ with percentage removal efficiency of 50, 46, 43, 40, 31, 25, 17, 15, and 10% for F/M ratios of 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, and control, respectively (Fig. 3). The reduction of sulphate to sulphide by SRB was found to be increased from 38 to 710, 648, 600, 516, 416, 346, 222, 176, and 80 mg L⁻¹ at different F/M ratios of 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, and control, respectively (Fig. 5c). The sulphide formation was accompanied with pH profile in the batch anaerobic reactor, as shown in Fig. 5d. The pH was increased gradually from 7.06 to 8.24, 8.34, 8.15, 8.05, 7.86, 7.88, 7.85, 7.85, and 7.8 over the incubation period of 108 h for F/M ratios of 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, and control, respectively. Figure 5e shows destruction of VFA concentration in anaerobic batch reactor from 1190 to 590, 550, 655, 685, 710, 754, 796, 850, and 880 mg L⁻¹ at F/M ratios 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, and control, respectively. The increase in reactor pH and accumulation of H₂S due to reduction in sulphate by SRB in the reactor inhibited the activity of methanogenic bacteria and thus resulted in low elimination of VFA.

Effect of F/M ratio on anaerobic digestion of wastewater with COD/SO₄²⁻ ratio of 1.2

Figure 6 shows the performance of AD of post-tanning wastewater with COD/SO₄²⁻ ratio of 1.2 at different F/M ratios (0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, and 1.5). The maximum methane generation was observed to be 0.150, 0.180, 0.160, 0.09, and 0.04 L/g of COD removed over the incubation period of 108 h at F/M ratios 0.2, 0.3, 0.5, 0.7, and 0.9

Fig. 5 Effect of F/M ratios — Control, — 0.2, — 0.3, — 0.5, — 0.7, — 0.9, — 1.1, — 1.3, — 1.5 on batch anaerobic digestion of post-tanning wastewater at COD/sul-

phate ratio of 0.67, **a** COD removal, **b** sulphate reduction, **c** sulphide formation, **d** pH, **e** VFA

Fig. 6 Effect of F/M ratios — Control, — 0.2, — 0.3, — 0.5, — 0.7, — 0.9, — 1.1, — 1.3, — 1.5 on batch anaerobic digestion of post-tanning wastewater with COD/

sulphate ratio of 1.2 **a** COD removal, **b** sulphate reduction, **c** sulphide formation, **d** pH, **e** VFA

(Fig. 4). The methane production was observed to be nil at F/M ratio of 1.1, 1.3, and 1.5. The maximum COD reduction was found to be from 3320 to 1140, 1220, 1430, 1740, 2060, 2240, 2480, 2550, and 2660 mg L⁻¹ with removal efficiency of 63, 65, 56, 47, 37, 32, 25, 23, and 19% at F/M ratios of 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, and control, respectively (Figs. 2, 6a). The volume of methane gas production was decreased in AD at F/M ratio other than 0.3 for post-tanning wastewater with different COD/SO₄²⁻ ratios. This illustrates that the optimum F/M ratio for the treatment of post-tanning wastewater is 0.3. The maximum methane gas generation was recorded during the incubation period of 60–72 h with COD removal efficiency in was in the range 54–63% for the COD/SO₄²⁻ ratios exceeded 0.67. The COD removal efficiency decreased, and consequently, methane production was reduced sharply with COD/SO₄²⁻ ratio less than 0.67. The COD removal was 53% for the COD/SO₄²⁻ ratio less than 0.62. A similar observation was recorded by Jeong et al. (2008) for the waste activated sludge operated at different COD/SO₄²⁻ ratios and observed that the maximum methane production was recorded at low COD/SO₄²⁻ ratios. Furthermore, COD removal efficiency was increased with increase in COD/SO₄²⁻ ratios in the range of 0.62–1.2. This corroborates with the reports of Sarti and Zaiat (2011); Kawai et al. (2014); Izumi et al. (2010); Liu et al. (2009); Jeong et al. (2008); Zhang et al. (2007) for AD of sulphate-laden wastewater. Figure 6b shows the change in sulphate concentration in anaerobic batch reactor operated at different F/M ratios. The sulphate elimination was from 2753 to 1133, 1153, 1328, 1502, 1604, 1830, 1966, 2184, and 2390 mg L⁻¹ with the removal efficiency of 58, 58, 51, 45, 41, 33, 28, 20, and 13% at F/M ratios 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, and control, respectively (Fig. 3). The conversion of sulphate to sulphide by SRB was found to be increased from 26 to 460, 420, 400, 380, 320, 214, 188, 90, and 60 mg L⁻¹ in the batch anaerobic reactor operated at different F/M ratios (Fig. 6c). The results illustrate that the increase in COD/SO₄²⁻ ratio from 0.62 to 1.2 increased the removal of sulphate and decreased the accumulation of sulphide at the F/M ratio of 0.3. This corroborates with the findings of Jeong et al. (2008) reported that the formation of H₂S was decreased with increase in COD/SO₄²⁻ ratio from 1.1 to 116 and the removal of sulphate ion was decreased with increase in F/M ratios. Archilha et al. (2010) reported that the production of sulphide was found to be high for the wastewater operated at low COD/SO₄²⁻ ratio. The AD of PTWW with COD/SO₄²⁻ ratio of 1.2 showed H₂S formation

below 460 mg L⁻¹ at all F/M ratios in the present investigation. The pH of the wastewater increased from 7.18 to 7.39, 7.26, 7.46, 7.53, 7.6, 7.64, 7.68, 7.71, and 7.75 at F/M ratios 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, and control, respectively, over the incubation period of 108 h (Fig. 6d). The pH of the PTWW remained constant during the AD process and it was maintained at pH 7.0 in all over the process and it supported the growth of methanogenic bacteria and resulted in methane generation of 0.180 L/g of COD removed at F/M ratio of 0.3. Figure 6e shows the reduction in VFA concentration in batch anaerobic reactor was from 1110 to 350, 334, 414, 474, 514, 544, 614, 654, and 734 mg L⁻¹ at F/M ratios of 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, and control, respectively. The VFA reduction was very low at COD/SO₄²⁻ ratio of 0.62 and 0.69 due to the increase in reactor pH and accumulation of H₂S. The conversion of sulphate to sulphide by SRB in the reactor inhibits the growth of methanogenic bacteria. The result revealed that the increase in pH above 8.0 in anaerobic system recorded a less amount of methane gas formation at COD/SO₄²⁻ ratio of 0.62. Hence, the optimum pH for the anaerobic treatment of PTWW with the maximum yield of methane gas was observed to be 7.0. A similar observation was reported by Speece (1996) that the optimum pH range for methanogenic activity was 6.5–8.2. The maximum methane gas generation was found to be 0.180 L/g of COD removed at COD/SO₄²⁻ ratio 1.2 and at F/M ratio of 0.3, and the corresponding solution pH was within the range of 7.18–7.26. This suggests that the solution pH ≥ 7.0 was appeared to be effective for the maximum yield of methane gas. According to Guerrero et al. (2013) the methane production was decreased up to 73% by increasing the sulphate concentration or decreasing the COD/SO₄²⁻ ratio in PTWW.

The change in ORP of wastewater operated at different COD/SO₄²⁻ ratios in anaerobic reactor

The oxidation and reduction potential of the anaerobic reactors operated at different COD/SO₄²⁻ ratio was recorded and analyzed to evaluate COD removal efficiency, as shown in Fig. 7. The ORP is a measure of the degree of metabolic reactions that take place during the anaerobic digestion process. Figure 7a shows ORP of the wastewater with COD/SO₄²⁻ ratio of 0.62, varied from – 302 to – 362 mV at F/M of 0.3 and the ORP for the COD/SO₄²⁻ ratio of 0.69 was varied from – 324 to – 375 mV at the same F/M ratio (Fig. 7b). Figure 7c shows the change in ORP of wastewater with COD/SO₄²⁻ ratio of 1.2 was found

Fig. 7 Profile of ORP for the anaerobic treatment of for post-tanning wastewater with different F/M ratios — **Control**, — **0.2**, — **0.3**, — **0.5**, — **0.7**, — **0.9**, — **1.1**, — **1.3**

, — **1.5** at **a** COD/sulphate ratio of 0.62, **b** COD/sulphate ratio of 0.69, **c** COD/sulphate ratio of 1.2

to decrease from -336 to -413 mV. Dolla et al. (2006), Khanal et al. (2003a, b) and Chuang et al. (2005) have determined an optimum ORP range of -250 to -300 mV for the growth and survival of SRB and MPB. The results confirm that the methanogenic reaction was active enough in lower the reduction potential of -300 mV in anaerobic digestion of PTWW, indicated the survival of strict

anaerobes under the reductive environment owing to the formation of fragmented organic compounds. These results showed that the wastewater with $\text{COD}/\text{SO}_4^{2-}$ ratio higher than 0.67 was favorable for COD and sulphate reduction simultaneously by transferring the electrons to sulphate during the H_2S production (Velasco et al. 2008; Gonzalez-Sanchez and Revah 2007).

Assessment of live and dead cells from the sludge produced in the anaerobic reactor

The present investigation was focused on to study cell viability through live and dead cell assay. The conventional volatile solid estimation cannot differentiate live cells and dead cells. Hence, the present study included the live and dead

cell assay. The bacterial cell viability in sludge was identified using Fluorescence microscopic LIVE/DEAD assay (Arivizhivendhan et al. 2015a, b; Biggerstaff et al. 2006). Figure 8 shows the fluorescence images of dead and live cells in the anaerobic reactor operated at COD/SO₄²⁻ ratio of 0.62. The increase in F/M ratio from 0.2 to 1.5 increased the red colour images corresponding to dead cell over the live

Fig. 8 Fluorescence image of dead and live cells in anaerobic digested PTWW with COD/sulphate ratio 0.62 **a** F/M ratio 0.3, **b** F/M ratio 0.7, **c** F/M ratio 1.1, **d** F/M ratio 1.5

cells confirmed that the increase in F/M ratio decreased the growth viability due to substrate inhibition (Attene-ramos et al. 2007) and sulphide toxicity as shown in Fig. 8a–d. The AD of PTWW at COD/SO₄²⁻ ratio of 0.62 was characterized by low methane gas generation and high H₂S production (426–156 mg/L) and accumulation of VFA at F/M ratio of 0.2–1.5 accompanied with high rate of microbial death in

the process. The AD of PTWW at COD/SO₄²⁻ ratio of 0.69 showed less methane gas generation and H₂S production from 278 to 146 mg/L at F/M ratios of 0.3–1.5. The AD of PTWW at F/M ratio of 0.3 showed the process stabilization and enhanced methane formation and sustainability of live microbes in the process, as shown in Fig. 9a. The anaerobic digestion of PTWW at F/M ratio from 0.7 to 1.5 showed

Fig. 9 Fluorescence image of dead and live cells in anaerobic digested PTWW with COD/sulphate ratio 0.69 **a** F/M ratio 0.3, **b** F/M ratio 0.7, **c** F/M ratio 1.1, **d** F/M ratio 1.5

Fig. 10 Fluorescence image of dead and live cells in anaerobic digested PTWW with COD/sulphate ratio 1.2 **a** F/M ratio 0.3, **b** F/M ratio 0.7, **c** F/M ratio 1.1, **d** F/M ratio 1.5

increase in dead cell concentration; this may be due to the substrate inhibition, as shown in Fig. 9b–d. Figure 9d shows that there are some live cells surrounded by dead cells in the sludge collected at F/M ratio of 1.5. However, anaerobic digestion at COD/SO₄²⁻ ratio of 1.2 showed more live cell count at F/M ratio of 0.7, as shown in Fig. 10a, b. The clustering of dead cells at F/M ratio of 1.1 and 1.5 may be due to the substrate inhibition effect (Fig. 10c, d).

Conclusion

The post-tanning wastewater collected from a Common effluent treatment plant (CETP) dedicated for tanning cluster was characterized by COD/SO₄²⁻ ratios from 0.62 to 1.2 ± 0.19 . The anaerobic digestion of PTWW operated at F/M ratio of 0.3 was the optimum to remove COD and F/M ratio of 0.2 was the optimum for sulphate removal at

all COD/SO₄²⁻ ratios. Anaerobic digestion of wastewater at COD/SO₄²⁻ ratio of 0.62 showed H₂S generation at all F/M ratios (0.2–1.5). The cell toxicity was observed in the anaerobic digestion process due to increase in sulphide concentration. Anaerobic digestion of wastewater at COD/SO₄²⁻ ratios of 0.69 and 1.2 showed less process inhibition and the microbial cells survived in the process. Hence, the wastewater with COD/SO₄²⁻ ratio of 0.62 and less than that requires an effective pre-treatment method prior to anaerobic biological treatment.

Acknowledgements The author M. Mahesh is thankful to Council of Scientific and Industrial Research (CSIR)—Central Leather Research Institute (CLRI), India, for awarding Senior Research Fellowship (Grant number 31/6(429)/2017-EMR-I). The authors are grateful to Director, CSIR–CLRI, India, for granting permission to carry out this research work.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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