Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2014.994107

57 (2016) 4328–4344
February

Taylor & Francis
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Treatment of distillery wastewater in an upflow anaerobic sludge blanket (UASB) reactor

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Received 28 May 2014; Accepted 26 November 2014

ABSTRACT

The treatment of high-strength wastewater from distillery was studied in a bench-scale upflow anaerobic sludge blanket (UASB) reactor. Reactor was operated at different organic loading rates (OLRs) and constant hydraulic retention time for 2 d at mesophilic temperature of 37˚C for a period of about two years (635 d). The maximum COD and BOD removals achieved were 68.35 and 89.11%, respectively, at optimum OLR of 15.34 kg COD/m^3 d. UASB reactor performance was also evaluated in terms of hydrolysis, acidification, and methanogenesis, and the performance values were found to be 33.88, 52.16, and 48.07%, respectively. Total and soluble biodegradability of the high-strength wastewater were 48.09 and 78.06%, respectively, that represents good conversion of soluble substrate to biogas. The average biogas produced was 0.38 m^3/kg COD removed. The COD mass balance of the reactor showed that 51.32, 0.24, 9.46, 1.75, and 37.22% COD was converted into methane (gaseous phase), methane (aqueous phase), sludge, sulfate reduction, and effluent, respectively. The characteristics of the port sludge elaborate the sludge profile in the reactor. FEG-SEM shows the morphology of the sludge granules, showing various colonies of cocci and rods grown on granules surface. ICP-AES indicates that Ca was utilized for the formation of granules and FTIR shows IR spectrum of an alcohol.

Keywords: Bench-scale reactor; Distillery wastewater; UASB reactor; COD mass balance; Reactor processes; ICP-AES; FEG-SEM; FTIR

1. Introduction

Alcohol distilleries are rated as one of the 17 most polluting industries in India. About 90% of the molasses produced in cane sugar manufacture is consumed in ethanol production [1]. The world's total production of alcohol from cane molasses is more than 13 billion liters/annum. There were 319 distilleries in India with an installed capacity of 3.25 billion liters of alcohol, which were generating 40.4 billion liters of distillery spent wash annually as per the figure given by Mohana et al. [2]. The aqueous distillery effluent stream known as spent wash is a dark brown highly organic effluent and is approximately 12–15 times by volume of the product alcohol. Spent wash is

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considered as a high-strength wastewater having high COD and BOD with low pH and dark brown color [3]. This dark brown-colored effluent, when discharged into water bodies without proper treatment, defiles the natural ecosystem [4]. Indian distilleries were stipulated to achieve zero discharge of spent wash to inland surface water by December 2005 [5].

Adequate treatment is, therefore, imperative before the effluent is discharged. Many criteria must be kept in mind while choosing environment-friendly process for treating distillery wastewater. Some criteria that are to be kept in mind are high removal efficiency, low cost with respect to construction, operation, and maintenance, low land requirement, and good possibilities for recovery of useful by-products [6].

There are many high-rate anaerobic reactors which have been checked for high-strength organic wastewaters [7–9]. Some of them like expanded granular sludge bed (EGSB) [10,11], hybrid reactors [12], granular-bed anaerobic baffled reactor [13], upflow blanket filter [14] internal circulation reactor (ICR) [15], fixed bed reactor [16], upflow anaerobic filter [17], and fluidized bed reactor [18] were studied for the treatment of wastewater by different researchers. Each of the reactors have their own limitations such as usefulness at lower temperatures only (4–20˚C) [19] and treatment of relatively very low-strength wastewaters [20], and inadequacy for the removal of particulate organic [21] for EGSB. Cost, maintenance, and more monitoring problems are associated with hybrid reactors. Biofilm formation on carriers poses problems leading to long start-up times, difficulty in control of biofilm thickness, and clogging problem for ICR. More cost, careful monitoring requirement, channeling in reactor, service, and cleaning difficulties are problems in fixed bed reactor. Foaming and flotation due to gas production, flooding problems, erosion by abrasion of particles, particle entrainment, higher initial capital costs, and erosion of internal components are drawbacks of fluidized bed reactor.

Among all these reactors, upflow anaerobic sludge blanket (UASB) has secured an important place due to its usefulness to continuously treat high-strength and non-diluted wastewaters. It can sustain higher organic loading rates (OLRs), and requires shorter hydraulic retention time (HRT) than other reactors. The UASB has many advantages over other treatment units as given by Ma (2002) like; a gas–liquid–solid separator provided near the top of the reactor enables sludge to settle into the blanket [22], biogas to escape into the dome at the top of the reactor, and treated supernatant to flow out of the reactor. It is a compact unit, ideal for economic space utilization. The UASB treatment process requires no external input of energy. The required mixing is achieved by upflowing of wastewater and the rising gas bubbles. The residues (sludge) generated by UASB are less in amount and are well digested compared to other anaerobic reactors, thus requiring reduced sludge handling and causing less odor problems. The UASB reactor has no mechanical or moving parts to wear and tear. Thus, it is virtually maintenance free and involves few operational problems. A biogas, rich in methane, is generated as a valuable by-product. Methane production is about 0.15–0.35 L/g COD destroyed. Both the capital cost and operating cost of a UASB reactor-based treatment plant are significantly less. The UASB reactor is a noiseless, closed, and covered unit that is esthetically very satisfying.

An exhaustive comparison was done by Saleh and Mahmood [23] for various high-rate reactors for the treatment of high-strength wastewaters. They recommended an order for the choice of the reactor based on (i) operating skills (fixed film < UASB < RBC < fluidized bed), (ii) energy consumption (UASB < fixed film < EGSB < fluidized bed < RBC), and (iii) capital cost, land requirement, operation, and maintenance (RBC < fixed film < UASB < EGSB < fluidized bed). Based on these factors, UASB seems to be the best among other high-rate reactors.

The real distillery effluent and raw molasses wastewater were treated by laboratory-scale anaerobic upflow-fixed bed reactor, which found that COD and BOD reductions were more than 75 and 85%, respectively, for both wastewater samples [24]. Treatment of wastewater that contains ethanol and acetate with UASB reactor obtained more than 80% COD removal efficiency [25]. Treatment of winery wastewater was studied on three UASBs. The first reactor was inoculated with granular sludge enriched with *Enterobacter sakazakii* and reached a 90% COD removal within 17 d at HRT of 24 h, the second was inoculated by brewery granules and achieved 85% COD removal within 50 d, and the third was seeded with just sludge and showed the typical problems encountered with conventional sludge seeding and had to be continuously reseeded [26]. Treatment by a UASB reactor resulted in 90% COD removal when sugarcane molasses spent wash was used as a feed [27]. Feed was diluted before treatment due to the presence of sulfur compounds, potassium, calcium, and free hydrogen ions left in solution after pH correction [28]. More than 90% COD removal efficiency was found during study period of three seasons in a UASB plant treating distillery wastewater [29].

Apart from bench-scale studies, many full-scale studies are also available in literatures [7–9] and the treated effluent is discharged for land applications and in open streams. The treated spent wash does not meet Central Pollution Control Board (CPCB) standards of discharge into streams or land applications [30]. It implies that these treatment plants are not working efficiently due to high OLRs. Most of the research works reported in the literature for the treatment of distillery wastewater were either carried out on synthetic spent wash or diluted one, which are generally based on the inlet and treated effluent concentrations. These limitations restrict transformation of those works in the field.

Therefore, a thorough bench-scale study was proposed for getting optimum values for the best performance of the UASB reactors with real spent wash. At the same time, for getting more in-depth removal mechanism, different phases of anaerobic digestion process was studied within the reactor for different parameters. Overall, study focuses on (i) performance of UASB reactor at different OLRs for finding out its optimum value, (ii) stability and effectiveness of the reactor with the emphasis on the removal efficiency of COD, BOD, and TS, (iii) sludge profiling in the UASB reactor, (iv) morphology and other physicochemical properties of the granules, (v) COD mass balance in UASB reactor, (vi) monitoring of different phases of anaerobic digestion in reactor i.e. hydrolysis, acidogenesis and methanogenesis, and biodegradability of distillery wastewater, and (vii) evaluation of biogas production at different OLRs.

2. Materials and methods

2.1. Experimental set-up

Schematic diagram of bench-scale UASB reactor is shown in Fig. 1. The UASB reactor was made of borosilicate glass with inner diameter of 92.10 mm and a total height of 79.60 cm, height of digestion zone was 59.97 cm, having a capacity of 5 L. Water jacket was given around the reactor of inner diameter 132.10 mm. Six sampling ports were installed along the height of the reactor, starting from a distance of 74 mm at the base of UASB reactor and the regular interval of 100 mm, having diameter of the sludge port as 12 mm and outlet at 47.5 mm below the top of a reactor. Bottom most portions of 46 mm height were made conical and small inert material pebbles were placed for dispersion of the inflow and having an angle of 90°, a feed inlet pipe of 12 mm diameter was provided to avoid chocking during operation. The water jacket also had inlet and outlet pipes at the bottom and top of the reactor, respectively, to circulate the hot water to maintain the temperature of 37˚C inside the reactor.

A three-phase separator was provided in the upper portion of the reactor for separating solid, liquid, and gas. The height of the three-phase separator was 150 mm. Deflectors were having a height of 70 mm and projected horizontally 25 mm from the inner surface of the reactor, making an angle of 35.6° with the vertical inner surface of the reactor. The inverted funnel was fitted on the top of the reactor for the biogas collection. The inverted funnel bottom was placed at 4.8 mm away from the inner surface of the reactor. The height of the inverted funnel was 65 mm. Distillery spent wash was continuously fed to the reactor from the inlet provided at the bottom of the reactor with the help of peristaltic pump (MICLINS INDIA, Chennai, India) to maintain uniform flow. The UASBR effluent was collected in the effluent tank and biogas was collected from the head space on the top of the reactor and passed to biogas collection assembly, having a capacity to collect biogas of 47.32 L. The biogas was measured by the liquid displacement method. The sludge was retained in the UASB reactor. The reactor had a conical base of 4.08 cm high.

2.2. Seeding sludge and distiller’s spent wash

Hot spent wash without dilution was collected from the outlet of a distillery industry, Ahemadnagar, India in polyethylene carboys, cooled by sprinkling cool water over the carboys and transported to the laboratory safely. The raw wastewater characteristics are shown in Table 1. The distillery is molasses based, established in March 1992, with an average operating capacity of 0.450 million liters per day (ML/D) of the effluent coming out of the industry. Sludge from the same UASBR of distillery wastewater treatment plant was used as inoculum. The reactor was seeded with inoculums of about 30% of reactor volume [31]. In the inoculum sludge; total solids, total suspended solids (TSS), and volatile suspended solids were 325.64, 312.04, and 301.80 g/L. Color was dark brown and VSS: TSS ratio was 0.967. VSS: TSS ranging from 0.7 to 0.85 is likely to cause the granulation [32].

2.3. Analytical procedures

Total solids, total dissolved solids (TDS), TSS, and volatile suspended solids were analyzed as per standard methods [33]. The pH was measured with a portable pH meter (pocket sized pH meter, HANNA Instruments). The COD was measured by open reflux process as explained in standard methods [33]. For BOD determination, samples were incubated for 3 d at 27˚C temperature. Conductivity was measured by

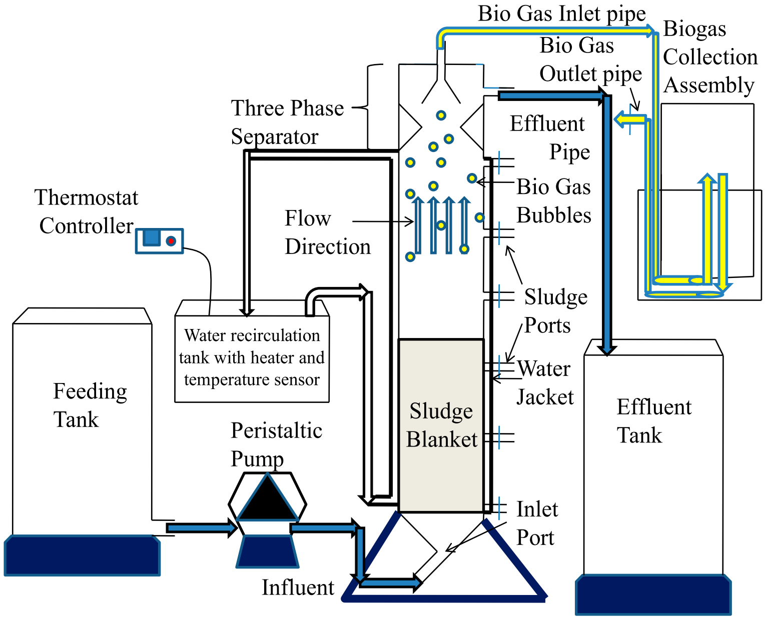

Fig. 1. Schematic diagram of bench-scale UASB reactor.

Table 1
Average composition of the raw distillery spent wash

Parameter	Range	Mean ± SD
pH	3.42–5.84	4.41 ± 0.64
Raw spent wash temperature (°C)	61–93	81.73 ± 11.45
Electrical conductivity (mS/cm)	15.6–26.4	21.56 ± 3.74
Total solids (mg/L)	31,520–126,240	92,640 ± 29,120.62
Total dissolved solids (mg/L)	30,480–106,080	86,240 ± 25,192.10
Total suspended solids (mg/L)	1,040–22,640	4,260 ± 7,064
Biochemical oxygen demand (mg/L)	21,600–35,000	26,466.67 ± 7,414.40
Total COD (mg/L)	68,000–100,000	83,340 ± 9863.63
Soluble COD (mg/L)	32,000–96,000	72,104.87 ± 16,567.09
Dissolved oxygen (mg/L)	0	
Chlorides (mg/L)	7,820–8,800	8146.67 ± 565.80
Sulfates (mg/L)	3,990–4,170	4,110 ± 103.92
Nitrogen (mg/L)	2,200–2,300	2,266.67 ± 57.74
Phosphate (mg/L)	310–370	350 ± 34.64
Potassium (mg/L)	11,290–15,130	13,850 ± 2,217.03
Volatile fatty acids (mg/L)	610–4,715	1,356 ± 1,173.90
Alkalinity (mg/L)	1,140–3,620	2,878.67 ± 654.32

using the conductivity meter (Global Instruments, India).

VFA and alkalinity determination were performed by centrifuging 50 mL of the sample for 10 min at 6,000 rpm. Initial pH of the 25 mL supernatant in a beaker was measured. The pH was decreased to 4.3 by adding 0.1 NH$_2$SO$_4$. The same procedure was continued till the pH decreased to the range of 3.5–3.3. Then sample was gently boiled for three min and then cooled down to room temperature. The pH of the sample was raised to 4.0 and than 7.0 by adding 0.05 N NaOH. Volume of NaOH was measured while increasing the pH from 4.0 to 7.0, and VFA was calculated [34].

2.4. Mode of operation

The UASB reactor was operated in continuous mode. First HRT was long, up to 80 h to start up the anaerobic bioreactor. HRT was then reduced in the stepwise fashion while maintaining a constant incoming substrate concentration. Due to this process, greater reactor stability and superior reactor performance can be achieved [35]. Accordingly, initial HRT was maintained for 3.5 d (81.96 h), then it was fixed to 2 d, flow rate was 0.00146 m^3/d for initial 4 d than it was maintained 0.00254 m^3/d for the whole study. Once in 2 d, 5 L of effluent was withdrawn followed by feeding the UASB reactor with 5 L of influent without disturbing the sludge bed. Upflow velocity of the feed maintained was 0.01–0.02 m/h. Initially, the OLR was maintained at 1.76 kg COD/m^3 d for 4 d. Then it was increased gradually to 8 kg COD/m^3 d within 75 d as per the performance of the reactor. From day 75 to 141, average OLR maintained was 8.07 kg COD/m^3 d; from 142 to 230 d, it was 10.18 kg COD/m^3 d; from 231 to 322 d, it was 12.75 kg COD/m^3 d; and from day 323 to 453, OLR was changed to 15.34 kg COD/m^3 d; further, from days 581, 591, 616, and 634, OLR was increased to 17.83, 18.95, 22.94, and 25.88 kg COD/m^3 d, respectively.

2.5. UASB reactor processes

The percentage of hydrolysis (H), acidification (A), methanogenesis (M), and biodegradability in the UASBR was calculated by (Eqs. (1)–(5)), respectively [36].

$$\text{Hydrolysis (H) (\%)} = \left\langle \frac{\text{CH}_4 \text{ as COD} + \text{Effluent COD}_{\text{soluble}} - \text{Influent COD}_{\text{soluble}}}{\text{Influent COD}_{\text{particulate}}} \right\rangle \times 100 \quad (1)$$

$$\text{Acidfication (A) (\%)} = \left\langle \frac{\text{CH}_4 \text{ as COD} + \text{Effluent VFA as COD} - \text{Influent VFA as COD}}{\text{Influent COD}_{\text{total}} - \text{Influent VFA as COD}} \right\rangle \times 100 \quad (2)$$

$$\text{Methanogenesis (M) (\%)} = \left\langle \frac{\text{CH}_4 \text{ as COD}}{\text{Influent COD}_{\text{total}}} \right\rangle \times 100 \quad (3)$$

$$\text{Biodegradability}_{\text{total}} \text{ (\%)} = \left\langle \frac{\text{CH}_4 \text{ as COD}}{\text{Influent COD}_{\text{total}}} \right\rangle \times 100 \quad (4)$$

$$\text{Biodegradability}_{\text{soluble}} \text{ (\%)} = \left\langle \frac{\text{CH}_4 \text{ as COD}}{\text{Influent COD}_{\text{soluble}}} \right\rangle \times 100 \quad (5)$$

3. Results and discussion

3.1. Reactor startup

The reactor was initially fed with dilute spent wash (COD = 6,000 mg/L) and HRT 81.96 h for 3.5 d. The influent COD concentration was gradually increased from 6,000 to 20,000 mg/L by reducing the dilution factor. From day 3.5, the HRT maintained throughout the study time was 47.11 h. The reactor took about 65 d for startup. Steady-state conditions are generally achieved after 50 d [37,38].

3.2. Monitoring parameters

3.2.1. pH

The pH of the raw spent wash ranged from 3.42 to 5.84. For the stable operating condition of anaerobic reactor, pH of the reactor should be in the range of 6.8–7.4 [39]. The pH of the raw spent wash was increased by the addition of NaOH and adjusted nearer to 7. Fig. 2(a) shows the variation of pH during the study period. Anaerobic techniques are greatly pH reliant. From Fig. 2(a), we can observe that influent pH maintained was between the range of 6.5 and 7.2 and effluent pH was in between 7.8 and 8.2. For acid-forming bacteria, high acid pH is advantageous [40]. The pH of an anaerobic system is naturally maintained between methanogenic ranges to prevent the dominance of the acid-forming bacteria, which may source for VFA build-up. It is important for the reactor for making available sufficient buffering capacity to counteract any ultimate VFA gathering and thus avoiding the increase of local acid zones in the reactor. Sodium bicarbonate is useful to increase the alkalinity; it is the only chemical, which

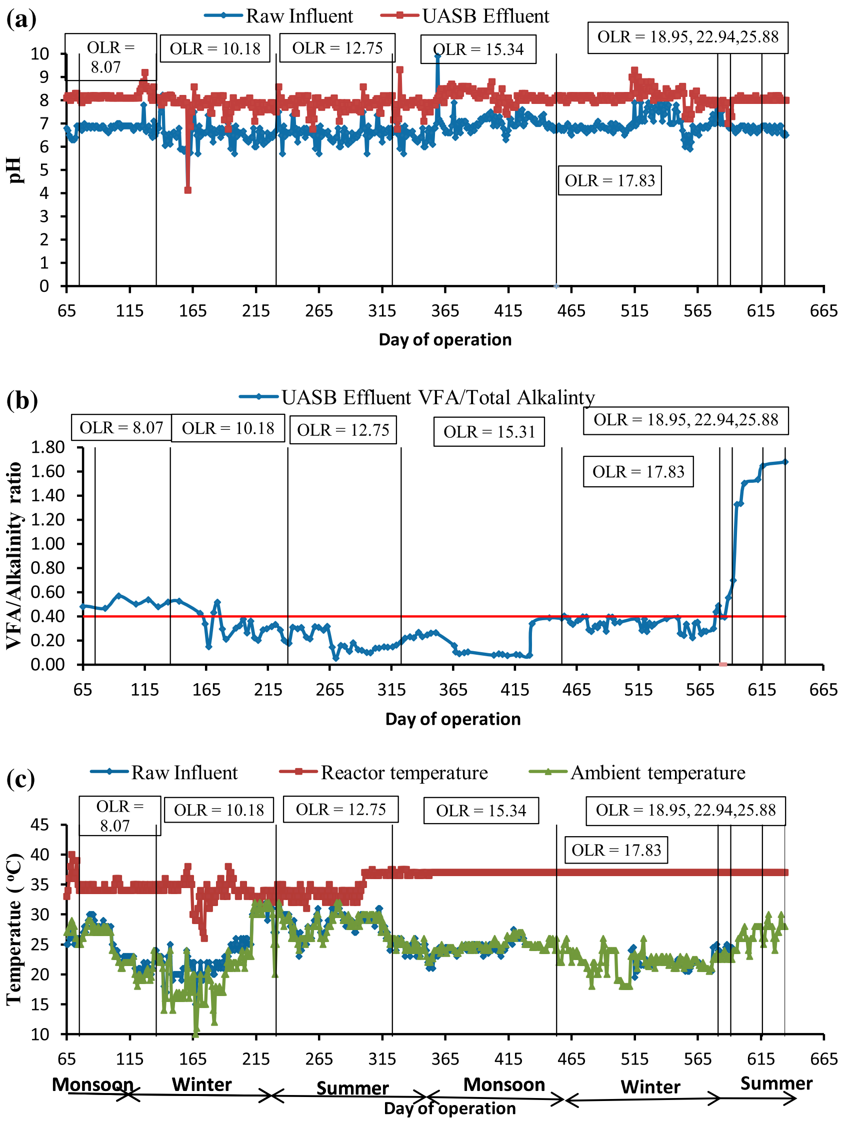

Fig. 2. (a) pH, (b) VFA/alkalinity and (c) temperature variation during reactor operation.

smoothly shifts the balance to the preferred value without troubling the physical and chemical equilibrium of the breakable microbial population [41]. In the studies of dairy wastewater by the use of UASBR, it is found that the pH was increased to 6.61 [42]. In Hybrid UASBR, sago wastewater was digested which gave a neutral pH. There was an increase in pH and alkalinities of the reactor that are assumed to be good sign of performance.

3.2.2. VFA and alkalinity

As shown in Fig. 2(b), the VFA/alkalinity ratio of the reactor ranged in between 0.05–2.0 (Mean ± SD = 0.34 ± 0.29). The reactor stability can also be estimated using VFA/alkalinity ratio. This ratio should be less than 0.4 for the reactor to be stable [43]. The mean value of the VFA/Alkalinity ratio is 0.34, which shows the stable condition of the reactor but from day 588, VFA/alkalinity ratio exceeds the limit of 0.4 and increased up to 1.68 (day 634). In this period, the performance of the reactor was deteriorated which is discussed further. The lower the ratio, the better is the balance of oxygenic and methanogenic bacteria [44]. For a stable digestion, alkalinity should be in the range of 2,000–4,000 mg/L as $CaCO_3$ [39]. Higher alkalinities applied during start-up period provides buffering in the reactor to prevent the scouring of the reactor and speed up the formation of granular sludge. A change in alkalinity indicates the possibility of occurrence of reactor

Table 2
Average characteristics of effluent

	Effluent							
Parameter	HRT = 47.112 h							
OLR (kg COD/m^3 d)	8.07 ± 0.34	10.18 ± 0.24	12.75 ± 0.15	15.34 ± 0.73	17.83 ± 0.30	18.95 ± 0.30	22.94 ± 0.95	25.88
Dilution factor	1:5	1:4	1:3	1:2.33	1:1.85	1:1.5	1:1.22	1:1
Period of operation (days)	75–141	142–230	231–322	323–453	454–581	582–591	592–616	617–634
Duration (days)	66	88	91	130	127	9	24	17
Avg. reactor temp. (°C)	34.45 ± 0.57	33.68 ± 2.03	34.18 ± 1.75	36.98 ± 0.18	37	37	37	37
pH	8.20 ± 0.22	7.81 ± 0.47	7.84 ± 0.28	8.07 ± 0.35	8.16 ± 0.32	7.69 ± 0.31	8.01 ± 0.16	8.08 ± 0.08
Elect. conductivity (mS/cm)	9.29 ± 0.25	11.12 ± 2.09	12.38 ± 0.81	12.66 ± 1.50	15.79 ± 2.88	22.80 ± 1.01	21.48 ± 1.71	21.16 ± 1.94
COD_t (mg/L)	5,861.88 ± 1,141.69	8,018.73 ± 3,432.78	7,892.94 ± 2,076.28	9,522.72 ± 3,311.36	13,143.27 ± 3,724.21	13,000 ± 2,033.06	27,680 ± 3,593.33	40,200
COD_s (mg/L)	4,008 ± 1,297.53	6,406.68 ± 2,991.80	6,820.72 ± 2,177.80	5,566.35 ± 1,127.57	7,126.69 ± 2,225.02	10,050 ± 1,454.88	23,820 ± 3,609.99	35,200
COD_p (mg/L)	1,853.58 ± 1,186.17	1,612.05 ± 1,254.45	1,072.22 ± 511.93	3,956.37 ± 2,863.60	6,016.57 ± 2,542.89	2,950 ± 1,100.00	3,860 ± 676.76	5,000
% COD_t removal	62.82 ± 8.08	59.73 ± 17.64	68.47 ± 8.28	68.35 ± 10.91	62.42 ± 10.73	64.98 ± 5.98	38.69 ± 5.91	20.87
% COD_s removal	28.57 ± 13.91	44.21 ± 19.23	57.21 ± 9.52	67.04 ± 5.45	69.76 ± 10.88	65.08 ± 12.08	35.42 ± 5.60	21.08
% COD_p removal	82.35 ± 10.08	79.40 ± 17.97	88.05 ± 6.78	68.12 ± 23.06	41.12 ± 25.68	42.21 ± 22.75	52.84 ± 11.97	19.35
BOD (mg/L)	3,938 ± 680.31	2,226.32 ± 1,322.39	1,392.86 ± 648.62	1,652.38 ± 936.41	2,282.94 ± 1,036.74	3,500 ± 1,757.84	7,114.29 ± 1,435.77	7,825 ± 250
% BOD removal	35.50 ± 9.04	70.96 ± 17.11	84.02 ± 6.43	89.11 ± 4.65	86.65 ± 6.19	79.72 ± 10.28	47.50 ± 16.23	21.53 ± 3.12
TS (mg/L)	15,750 ± 3,090.83	11,553.68 ± 2,794.53	12,273.08 ± 2,657.90	16,426.61 ± 5,169.70	21,752.94 ± 4,232.27	34,066	24,800	34,408 ± 4,041.47
TDS (mg/L)	13,692 ± 2,526.72	8,833.68 ± 3,252.02	9,234.62 ± 2,706.28	13,368.21 ± 4,796.46	16,993.24.70 ± 3,238.4	19,101	20,981	27,655.33 ± 2,423.22
TSS (mg/L)	2,058 ± 1,181.11	2,720 ± 1,302.87	3,038.46 ± 546.32	3,648.39 ± 2,024.94	4,759.70 ± 3,194.42	14,965	3,819	6,752.67 ± 3,003.74
VFA (mg/L)	2,239.17 ± 157.81	1,776.14 ± 556.44	1,144.23 ± 426.46	1,348.13 ± 1,238.40	3,840.64 ± 216.23	1,350.45 ± 1,685.39	4,900 ± 122.47	5,200
Alkalinity (mg/L)	4,390 ± 121.98	5,908.18 ± 1,276.01	6,366.85 ± 914.53	6,080.17 ± 2,034.40	11,323.64 ± 1,053.06	3,831.52 ± 4,737.18	3,360 ± 270.19	3,100
Biogas (m^3/kg COD removed)	0.02 ± 0.01	0.10 ± 0.05	0.26 ± 0.05	0.38 ± 0.09	0.70 ± 0.15	0.74 ± 0.09	0.61 ± 0.18	0.03
Hydrolysis (%)	3.65 ± 2.35	15.69 ± 9.81	30.83 ± 8.88	22.02 ± 10.06	57.43 ± 26.59	60.82 ± 17.67	58.76 ± 11.23	62.67
Acidification (%)	7.95 ± 5.54	31.47 ± 10.75	51.67 ± 7.19	47.02 ± 6.72	77.78 ± 6.71	76.70 ± 4.50	45.24 ± 6.06	29.75
Methanogenesis (%)	12.96 ± 4.41	30.42 ± 10.11	47.09 ± 4.80	46.69 ± 5.90	68.23 ± 5.02	67.75 ± 3.51	39.71 ± 5.15	26.15
$Biodegradability_{total}$ (%)	12.96 ± 4.41	30.42 ± 10.11	47.09 ± 4.80	46.69 ± 5.90	68.23 ± 5.02	67.75 ± 3.51	39.71 ± 5.15	26.15
Biodegradability soluble (%)	36.85 ± 17.11	56.65 ± 22.95	76.50 ± 13.78	85.10 ± 10.01	100.14 ± 14.98	86.18 ± 19.96	48.92 ± 7.73	29.79

upset. At that time, OLR must be checked. Here, in this reactor, no significant fluctuation in the effluent alkalinity was observed, meaning the reactor was performing well (Table 2). The alkalinity of the influent was 1,140–3,620 mg/L.

3.2.3. Temperature

The temperature of the influent varied in between 15 and 32℃ and that of the reactor effluent varied in between 26 and 40℃ (34.92 ± 1.97) (Fig. 2(c)). Anaerobic digestion is robustly influenced by temperature and can be grouped under one of the following categories; psychrophilic (0–20℃), mesophilic (20–42℃), and themophilic (42–75℃). In the mesophilic condition, the bacterial metabolism and augmentation decreases by one half after each 10℃ jump down below 35℃ [41]. Thus, for a given amount of digestion to be attained, the lesser the temperature, the longer is the digestion time.

Anaerobic bacteria have a characteristic that their decomposition rate is slow at temperatures lower than 15℃. It is advisable to safeguard the anaerobic sludge for much more time, not losing much of its action. This is beneficial in the anaerobic treatment of wastewater from seasonal industries such as sugar mills. For the full period of study, the electrical conductivity of the effluent was more than that of the influent; this was because of the mineralization of the substrate which took place that led to the increase in the conductivity of the effluent.

3.3. Overall UASBR performance

The reactor was operated at 37 ± 3℃ and started at initial OLR of 1.76 kg COD/m^3 d. From day 75, OLR was increased to 8.07 kg COD/m^3 d and fed concentration of 15,000 mg COD/L at HRT 47.11 h (Fig. 3(a)). From days 75 to 141 (66 d), COD removal was about 63%. From the beginning, the COD$_{particulate}$ (COD$_p$) removal was more (82.35%) than COD$_{total}$ (COD$_t$) (63%) and COD$_{soluable}$ (COD$_s$) (28.57%) (Table 2). From day 141, the COD influent concentration was increased to 20,000 mg/L (OLR 10.18 kg COD/m^3 d) by increasing the COD concentration to 1.33 times. Fig. 3(c) shows the sudden drop in the COD removal. This may be due to shock loading of increase in the influent COD concentration (Fig. 3(b)), which lowered efficiency and after about 7 d, the reactor again attained its previous COD removal efficiency. Reactor regained its stability due to the self-regulation capability inherent in the biological system, making it possible for the microbial consortium to acclimate itself to the increased OLR [45]. Shock load can result in the failure; the system can rapidly be restored to

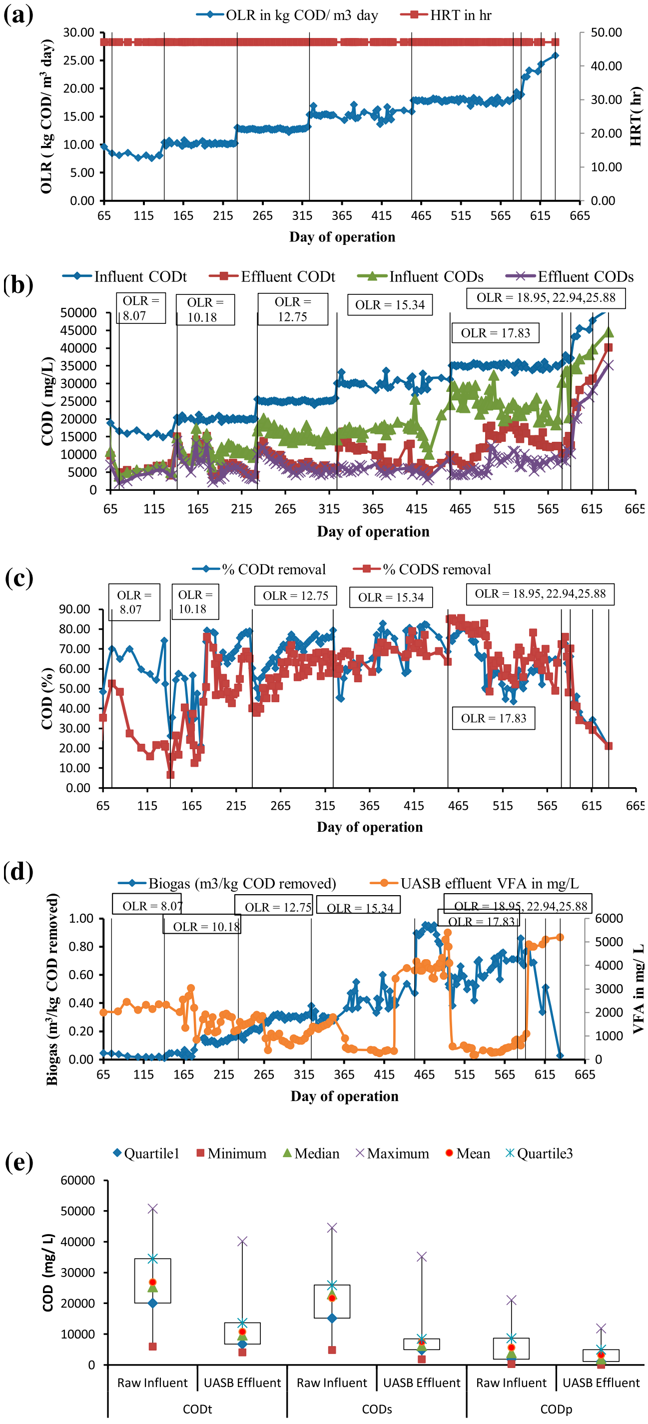

Fig. 3. UASBR performance: (a) Variations in operating variables like HRT and OLR; (b) Influent and effluent COD$_t$ and COD$_s$; (c) COD$_t$ and COD$_s$ percent removals; (d) biogas production; and (e) box plot of Influent and effluent COD$_t$, COD$_s$, and COD$_p$.

normal [46]. In general, it was observed that, with every change in OLR, there was drop in the COD_t and COD_s removal. It took around 7–12 d to regain its past COD removal efficiency and afterward, the percentage of COD_t and CODs removal was increased gradually. Reactor was operated from days 75 to 141 (66 d). OLR was further increased on days 231, 323, 454, 582, 592, and 617 by 1.25, 1.20, 1.16, 1.06, 1.21, and 1.12 times, respectively. From Table 2 and Fig. 3(b)–(c), it is seen that the average COD_t removal increased up to an increase in avg. OLR 15.34 kg COD/m^3 d (day 453) and afterward removal efficiency of the reactor started decreasing with increasing OLR. This may be the maximum limit of the UASB reactor to treat the COD. For the OLR 22.94 and 25.88 kg COD/m^3 d, the avg. COD removal efficiency was to its minimum up to 38.69 and 20.87%, respectively. For OLR 12.75 and 15.34, the avg. COD removal efficiency was to its maximum up to 68.47 and 68.35%, respectively, so we can say this is the maximum capacity of the reactor to treat COD. Avg. COD_s removal showed increasing trend up to OLR 17.83 kg COD/m^3 d (day 581) (Fig. 3(c)), and further it was decreasing as the OLR increased, because organic load in the reactor exceeded the capacity of the micro-organisms to treat it. These results show the capacity of the microbial consortium to adapt to potentially organic effluents in the UASB reactor. Avg. COD_p removal increases up to OLR 12.75 and further starts decreasing as the OLR increases.

The overall COD removal of the reactor was in the range of 20.87–82.74, 6.53–85.52, and −26.67–99.32% for COD_t, COD_s, and COD_p, respectively. The box plot in Fig. 3(e) shows the COD_t of influent, effluent from columns 1 and 2, respectively, columns 3 and 4 show COD_s of influent and effluent, respectively, and last two columns 5 and 6 show COD_p of influent and effluent, respectively. In which COD_t and COD_s removal were more than COD_p as the degradable matter converted into the soluble form and then degraded. Effluent BOD concentration and percentage BOD removal followed the same trend as that of COD (Fig. 4). The BOD percentage removal increases as an increase in OLR up to 15.34 kg COD/m^3 d and then starts decreasing as the OLR increases; the reason behind it is the same as that for the decrease in COD removal (Table 2). The overall BOD removal ranged in between −11.76 and 95.83%.

Distillery spent wash contains very high concentration of solids and the major part of it is in dissolved form so, in box plot, Fig. 5 columns 3 and 4 show the difference in the mean value as well as columns 1 and 2 of total solids which also show the difference in the mean value. At different OLRs; the TS, TDS, and TSS concentrations in the effluent varied from 6,200 to 38,133 mg/L, 4,340 to 30,750 mg/L, and 220 to 14,965 mg/L, respectively (Fig. 5). The overall percentage removal of the reactor was in the range of 6.83–58.26% (35.22 ± 13.16) and 4.23–70.64% (38.81 ± 17.53) for total solid and TDS, respectively. The effluent TSS concentration was increasing day by day due to increase in sludge washout, and sludge washout increase due to increase in OLR (Table 2).

In the study period, average biogas production in case of OLRs 8.07, 10.18, 12.75, 15.34, 17.83, 18.95, 22.94, and 25.88 kgCOD/m^3 d were 0.02, 0.10, 0.26, 0.38, 0.70, 0.74, 0.61, and 0.03 m^3/kgCOD removed (Fig. 3(d)). The biogas volumetric production was increased with increasing OLR. This higher biogas production may be due to the higher concentration of organic matter in the wastewater. But biogas production starts decreasing from day 617 because the COD removal was very less in this OLR 25.88 kg COD/m^3 d range. The overall average biogas production was 0.38 m^3/kg COD removed, the same result is shown in Fig. 10 also 51.32% COD is utilized for the production of biogas. Methane content in the biogas at different OLRs varied from 65 to 75% and the remaining was considered as carbon dioxide.

Table 2 compiled all performance monitoring data for various OLRs. From Table 2, it is clear that during

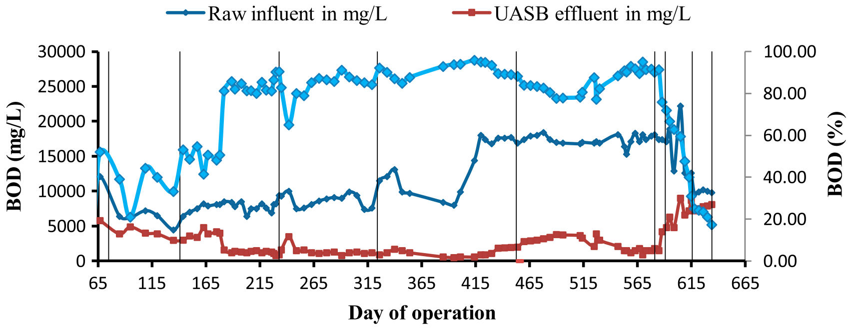

Fig. 4. UASBR performance: Influent and effluent BOD, BOD percent removals.

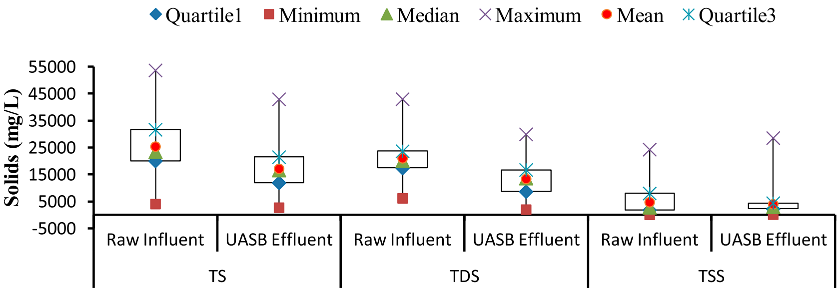

Fig. 5. UASBR performance: box plot of Influent and effluent total solids, TDS, and TSS.

OLR 12.75 and 15.34 kg COD/m^3 d, the maximum average percentage COD$_t$ removal was 68.47 and 68.35%, respectively. The maximum average percentage BOD removal was 89.11% during OLR 15.34 kg COD/m^3 d. The maximum COD$_t$ removal during this OLR was 82.74%. The average percentage COD$_s$ removal during OLR 15.34 kg COD/m^3 d was 67.04% that was also high compared with other OLRs results. Percentage COD removal starts declining from this OLR, so OLR 12.75 kg COD/m^3 d can be considered as the optimum OLR during the study period of 634 d.

The percentages of hydrolysis (H), acidification (A), methanogenesis (M), and biodegradability in reactor were calculated by the (Eqs. 1–5). Calculations of CH$_4$ as COD and VFA as COD were done on the assumptions that in the biodegradation of the organic wastewater, 4 g of COD was converted to 1 gm of methane and ratio of VFA/COD equals 1.28 [47–49]. Some researchers have adopted this ratio as 1.44 [50]; or 1.24–1.40 [51].

As shown in Fig. 6, due to fluctuation in the inlet concentration, there is fluctuation in the hydrolysis throughout the period. The percentage hydrolysis is more sensitive to high loading rates than percentage acidification and percentage methanogenesis [52]. Increase in OLR up to 10.18 kg COD/m^3 d results shows low hydrolysis, acidification, and methanogenesis due to start-up period and low concentration of COD, but was increased after day 141. Hydrolysis, acidification and methanogenesis were increasing as the OLR was increasing. The low percentage hydrolysis of the entrapped solids allows the solids to accumulate in sludge bed at higher OLR [52]. From day 617 the reactor process were showing the declining trend due to decrease in the removal of COD.

Fig. 6 shows that the conversion of organic matter in distillery spent wash to VFA and the conversion of VFA to CH$_4$ increased with increasing OLR. The results clearly showed that the methanogenesis was limiting the overall conversion of organic matter to methane as the effluent contains a high amount of VFA throughout the period of study, more than 200 mg/L [53,54]. These excess acids are coming out with the effluent (Table 2) so the acidification was higher throughout the period of study. From (Eq. 2) if effluent VFA increased, acidification increases.

In Fig. 3(d), from day 350 to 427 effluent VFA was decreased and converted in to methane which is reflected in Fig. 6, during same period methanogenesis

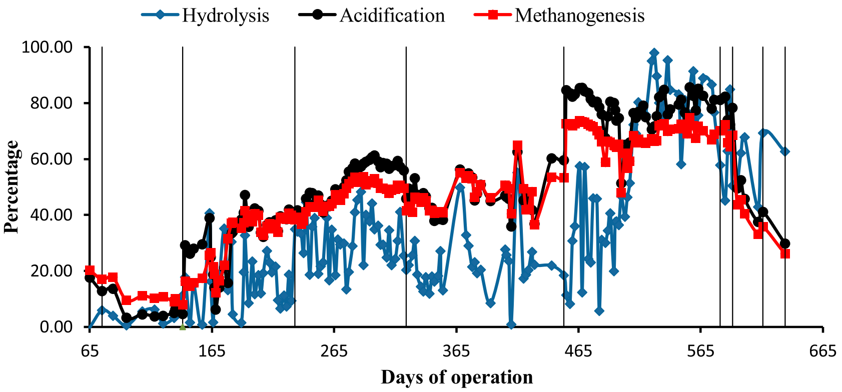

Fig. 6. Percentage hydrolysis, acidification, and methanogenesis.

was increased and likely same as acidification. During the higher methanogenesis; stabilization of accumulated sludge takes place [36]. After day 350 due to better methanogenic conditions in the UASB reactor hydrolysis can be enhanced due to improving the contact between substrate and the hydrolytic enzymes due to biogas production [55]. During period of day 500–592 VFA increases at the same time biogas production was also increased shows that wastewater was converted to VFA at the same time VFA is converted to methane. In the period of day 592–616 VFA production decreases at the same time biogas production also decreased and further from day 616 biogas production nearly stopped due to excess OLR, VFA production was increased and methanogenesis was decreased.

The period in between 290 to 324 day percentage methanogenesis slightly decreased but the acidification increased. This shows that conversion of wastewater to VFA was taking place but conversion of VFA to CH_4 decreased, at the same time effluent VFA was also showing the increasing trend (Fig. 3(d)).

From Fig. 3(d) during the period of day 316 to 350 VFA was more, this may be due to VFA accumulation due to increase in OLR and it took time for the consumption of VFA to methane. The overall hydrolysis, acidification, methanogenesis, biodegradability (total) and biodegradability (soluble) were 33.88 ± 24.41, 52.16 ± 21.22, 48.07 ± 17.13, 48.09 ± 17.11 and 78.06 ± 24.66 respectively (Fig. 6). The good results of acidification and methanogenesis may be due to good performance towards COD removal and due to high sludge residence time and hydraulic residence time. The results showed higher biodegradability of the reactor. While treating catalytically oxidized olive mill wastewater by hybrid UASB reactor, 32.6 ± 11, 49.4 ± 14, 59.4 ± 12.5, 68% hydrolysis, acidification, methanogenesis and biodegradability respectively [56].

3.4. Sludge profile

Fig. 7(a) shows the graph plotted for total solids concentration verses no of ports. Total solids concentration shows a decreasing trend from port 1 to port 6. At the lower three ports, due to sludge accumulation, TS is more, and further ports-treated effluent is present. Fig. 7(b) shows variation in TDS along the sludge ports; in the bottom, the 1st port TDS is more, which may be due to its entrapment in the sludge present at the lower portion of the reactor. From port 2, TDS decreases slightly in the upward direction of the reactor. Fig. 7(c) shows variation in TSS along the sludge ports. TSS show an increasing trend up to port 2, then decrease fast up to port 4, and then decrease gradually up to port 6. TSS gets accumulated in the sludge which is present up to the 3rd port. Fig. 7(d) shows variation in total volatile solids along the sludge ports that are distributed near about uniformly throughout the reactor. Fig. 7(e) shows variation of volatile

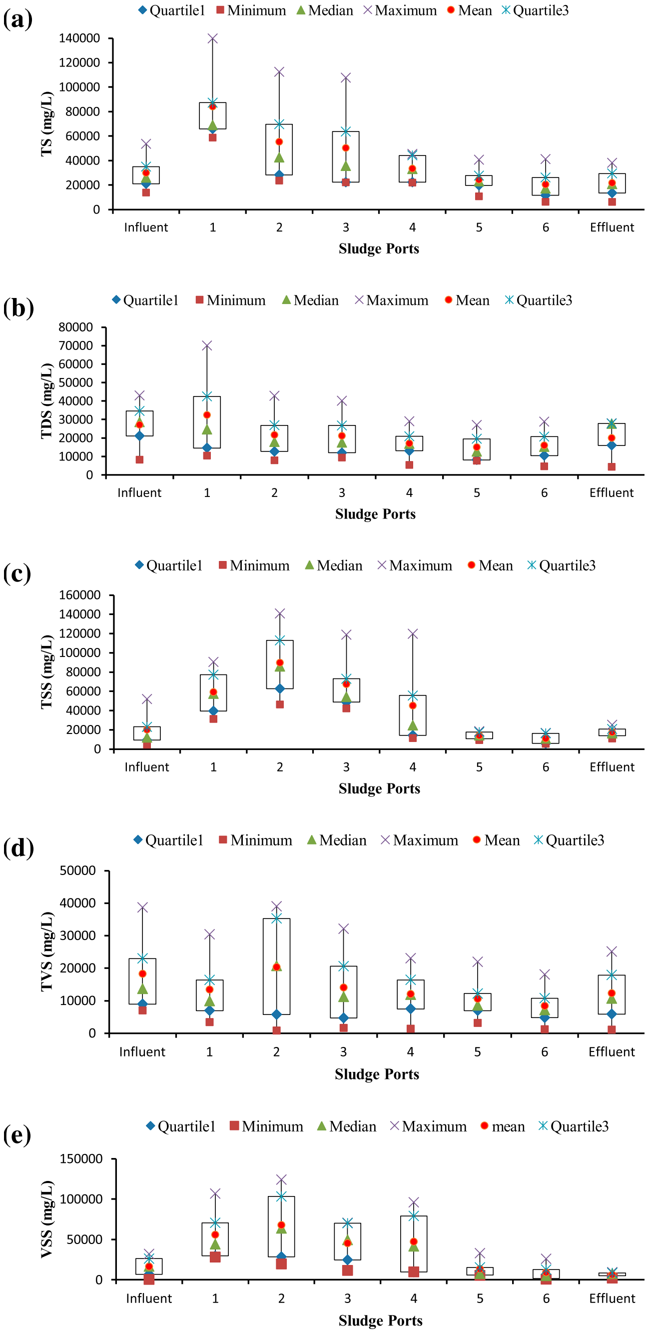

Fig. 7. Variation of (a) total solids, (b) TDS, (c) TSS, (d) total volatile solids, and (e) VSS concentration along the sludge ports in UASB reactor.

suspended solids along the sludge ports which show the same trend as that of TSS. The organic matter and micro-organisms concentration are more in the lower ports.

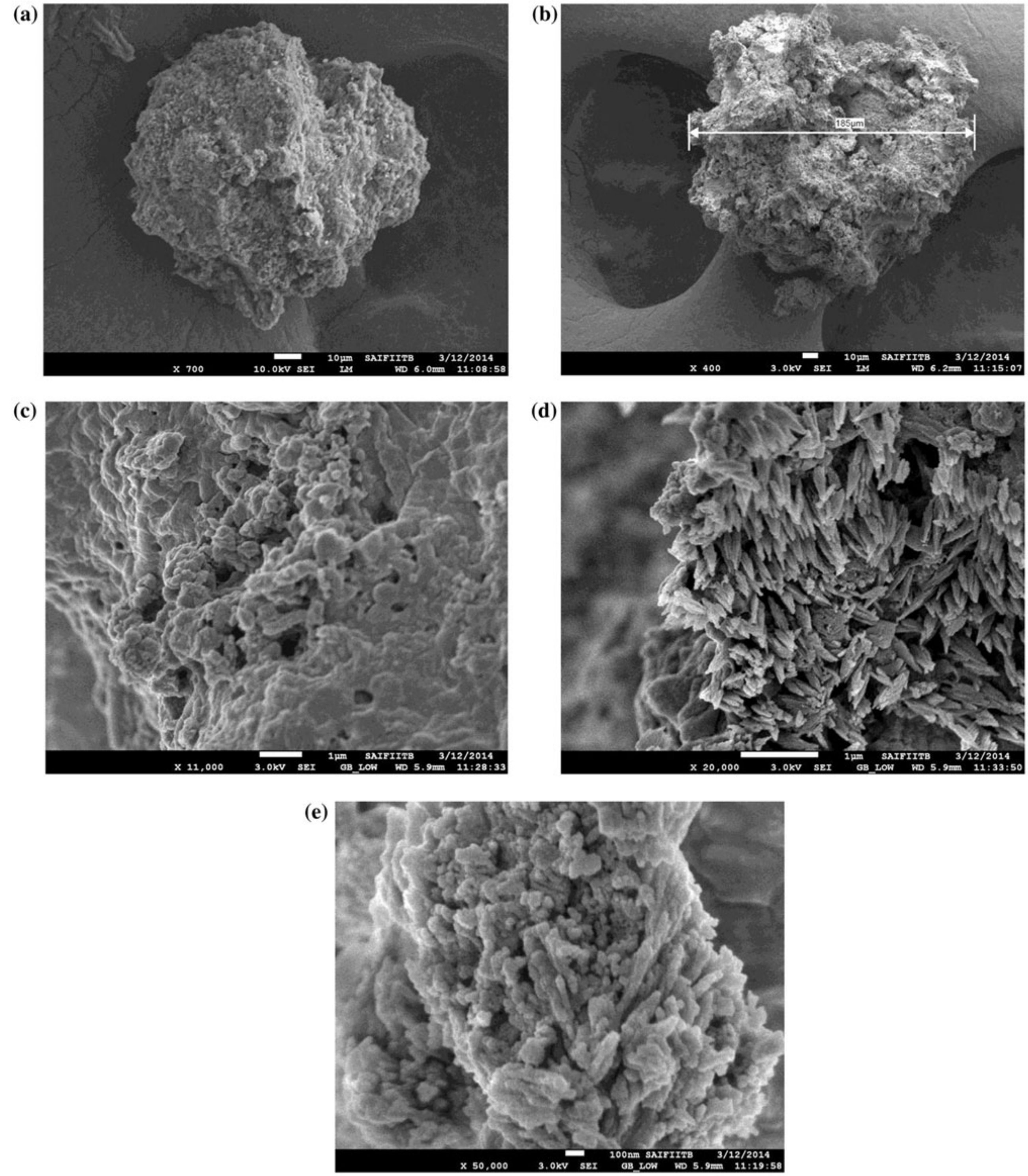

Fig. 8. (a–e) SEM photographs of granular sludge.

3.5. FEG-SEM examination of sludge granules

Field-emission gun scanning electron microscope image of sludge granules shows the rough and uneven surface and spherical shape (Fig. 8(a) and (b)). Granules show some of the cavities in it for the escape of gases (Fig. 8(c)). Fig. 8(d) shows various colonies of cocci and rods grown on granules surface. Fig. 8(e) shows the association of bacterial cells; here, filamentous bacterium was similar in appearance with Methanosaeta. Similar results were noted while studying characteristics of methanogenic granules on glucose in UASB reactor [57].

3.6. Mineral composition of the ash of the granular sludge

Characteristics of the seed sludge and the chemical composition of the substrate govern the chemical composition of the granular sludge [58]. The mineral composition of the granular sludge is shown in Table 2 analyzed by Inductive Coupled Plasma–Atomic Emission Spectrometer. Here, if we compare the concentration of Na, Mg, and Ca, i.e. 532.81, >202.79, and 0.13 ppm, respectively, Ca concentration was found to be very less. It indicates that Ca was utilized for the formation of granules. It also satisfies the assumption that Ca forms granules by neutralizing negative charge on the surface of the bacterial cell as a result of strong van der Waals forces or function as cataonic bridges between bacteria [59]. The higher concentration of Fe 49.99 ppm satisfies assumption that the granulation process is accelerated by forming bridges between negatively charged groups on cell surfaces and linking extracellular polymers. Ca and Fe play important roles in microbial aggregation [60]. Cobalt was internalized by the microbial species and may be found in the form of corrinoides in the cells. It also increased the methanogenic activity [61] (Table 3).

Table 3
Elemental composition of the granular sludge by ICP-AES

Element	Concentration in granular sludge (ppm)
Na	532.81
K	>1,777.40
Mg	>202.79
Ca	0.353
Co	0.13
Fe	49.99
Si	18.99
Al	0.59
Ti	0.26
S	>226.83
Cr	0.353

3.7. FTIR analysis

Fig. 9 shows the Fourier transform infrared spectrometer imaging of the granular sludge sampled on day 568. IR is most useful in providing information about the presence or absence of specific functional groups. The main absorbance bands identified in the spectra were; w (wavelength) 3429.12 cm^{-1} in between w 3,650 and 3,000 cm^{-1} (alcohol O–H bonds, infrared spectrum of ethanol, CH_3CH_2OH). This broad band covers the range of about w 3,000–3,650 cm^{-1}. This spectrum matches with the IR spectrum of an alcohol [62], which interpreted that the most prominent band in alcohol is due to the O–H bond, and it appears as a strong, broad band covering the range of about 3,000–3,700 cm^{-1}. Alcohols have a very distinct strong and broad shape, when we see this sort of elongated U shape around this region (w 3,650–3,000 cm^{-1}); there is an alcohol group [63]. Another important three small peaks were observed at w 2925.46, 2861.87, and 2526.82 cm^{-1} (Carbon—Hydrogen/alkyl (C–H)). These peaks matched with the think book [64], for interpretation of Carbon–Hydrogen /Alkyl (C–H The next major peak is at wavenumber 1657.75 cm^{-1} between IR frequency 1,680 and 1,640 which is of C=C—stretch in alkenes. The very complex-looking region below 1,500 wavenumbers is called the fingerprint region and can mostly be ignored.

3.8. COD mass balance

The UASBR COD mass balance was worked out by equalizing the influent COD with the summation of (a) effluent COD, (b) COD converted into methane, (c) COD converted into methane dissolved in effluent, (d) COD converted into sludge, and (e) COD utilized for sulfate reduction. The influent and effluent COD differences were calculated as unaccounted COD.

Methane COD was calculated by (Eq. 6) [65]

$$\text{Methane COD (kg/day)} = 780 \times Y \times V/(273 + t) \tag{6}$$

where Y is the % of methane content in the biogas, V is the volume of the biogas produced in m^3, and t is the temperature in ℃.

Methane in the dissolved form with the effluent was calculated using (Eq. 7)

$$CH_4\text{lost} = 4 \times C_{\text{equil}} \times \text{flow} \tag{7}$$

where C_{equil} is the concentration of gas dissolved in the liquid at equilibrium (mg/L),

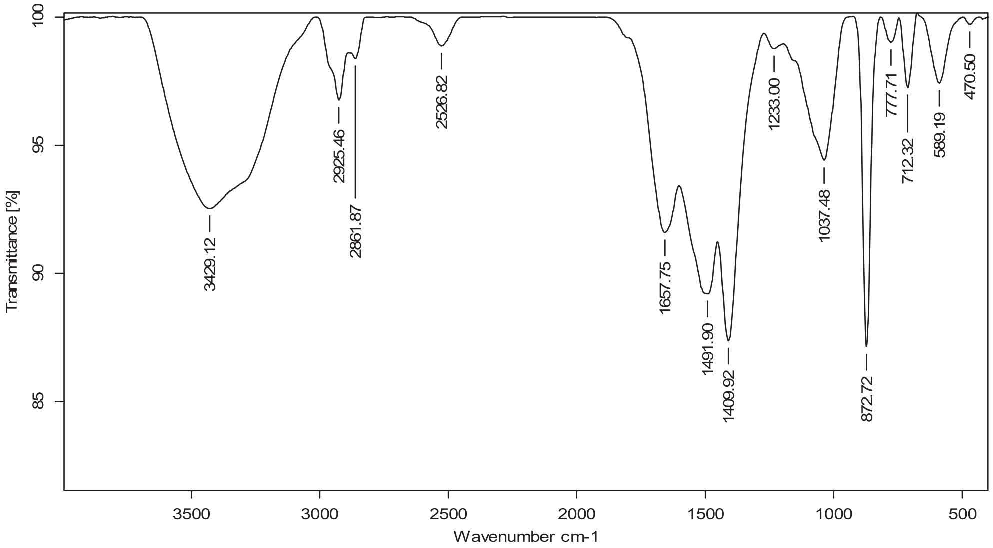

Fig. 9. Evolution of the FTIR Spectra of the granular sludge during reactor operation.

$$C_{\text{equil}} = K_{\text{H}} \times P_{\text{gas}}$$

where K_{H} is the Henry's law constant [65]

$$K_{\text{H}} = 0.384 \times t + 36.44$$

where t is the temperature and P_{gas} is the partial pressure of the gas above the liquid (0.7–0.8).

Biomass COD was calculated using the (Eq. 8) [65]

$$\begin{aligned} \text{COD kg/day} = {} & \text{COD(kg/day)}_{\text{removed}} \\ & \times Y\ (\text{kg VSS})_{\text{biomass produced}}/(\text{kg COD})_{\text{reduced}} \\ & \times F(\text{kg COD/kg VSS}) \end{aligned} \tag{8}$$

where Y is the biomass yield coefficient. F is the COD equivalent of per unit mass of microorganisms (kg COD biomass per kg of biomass), and the value of Y for the present study has been taken as 0.1 and F has been taken as 1.5.

COD utilized for sulfate reduction was calculated using assumption, each 1.5 g sulfate reduction is equivalent to 1 g COD.

COD mass balance was calculated using (Eq. 6 - 8) and above assumption of sulfate reduction. Fig. 10 shows that COD converted into methane was 51.32% in gaseous phase > sludge COD 9.46% > COD utilized for sulfate reduction was 1.75% > COD converted into methane in aqueous phase was 0.24%. COD unaccounted was 5.67%. COD of the effluent was 37.22%. By treating low-strength synthetic wastewater with

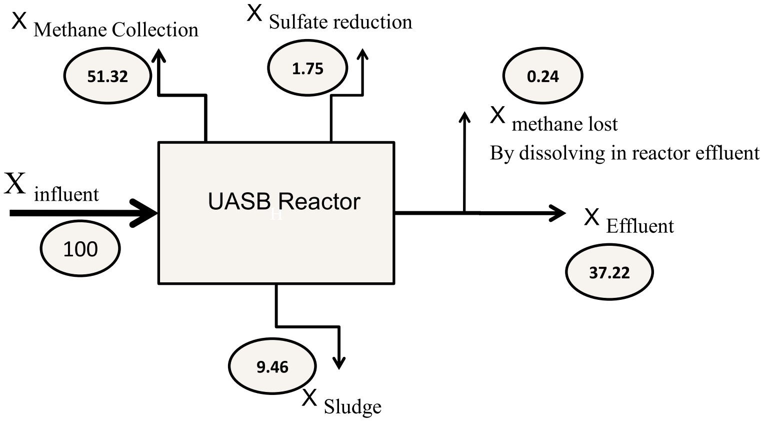

Fig. 10. COD mass balance of UASB reactor.

UASB reactor, 63% of COD was converted into methane [66]. 35–45% of COD conversion into methane for municipal wastewater and COD unaccounted was found to be 11.5–14.3% [67]. 31–55% COD conversion into methane was found while treating slaughter house wastewater using an Anaerobic Baffled Reactor (ABR) [68].

4. Conclusions

(1) The optimum OLR range for UASB Reactor can be 12.75–15.34 kg COD/m^3 d, in that 15.34 kg COD/m^3 d OLR can be considered as optimum because of maximum BOD removal (89%). This can be a maximum capacity of the reactor to treat distillery wastewater. UASB reactor performance gets deteriorated after exceeding the OLR of 15.34 kg COD/m^3 d.

(2) Biogas production was very high as organic matter is highly available in distillery spent wash. The biogas produced was 0.38 m^3/kg COD removed at an average.

(3) The distillery spent wash characteristics showed high organic content and acidic nature.

(4) The COD$_t$ removal was about 68.47% at OLR of 12.75 kg COD/m^3 d. COD$_p$ removal was more than COD$_s$ for maximum period of operation.

(5) BOD removal increased as the OLR increased. Percentage BOD removal ranged from 35% to 89%. After reaching an optimum OLR, BOD reduction was also decreased.

(6) COD and BOD reductions were increased with the increasing age of the inoculum.

(7) The reactor process calculations showed acidification, and the methanogenesis were the dominating processes in the UASB reactor.

(8) The sludge profile shows that TSS and VSS concentration were high in the bottom three ports, which is obvious as the sludge blanket is available in the lower portion of the reactor.

(9) FEG-SEM shows cavities for the escape of biogas as well as various colonies of cocci and rods, filamentous bacterium of Methanosaeta. ICP-AES shows that Ca and Fe play important roles in microbial aggregation. FTIR analysis shows alcohol O–H bonds, infrared spectrum of ethanol, CH_3CH_2OH.

(10) COD Mass balance calculations showed utilization of influent COD as follows: COD converted into methane (gas phase) > COD as effluent > COD converted into sludge > COD converted into sulfate reduction > COD as CH_4 in the effluent (aqueous phase). The percentage of incoming COD converted into CH_4 was 51.32% (gas phase) and 0.24% (aqueous phase).

(11) Study of OLR showed that UASB reactor was capable of sustaining the high organic loads up to 15.34 kg COD/m^3 d.

Acknowledgment

The first author acknowledges the financial support from NDMVP Samaj's College of Engineering, Nashik and Pune University, Pune. He is also thankful to Mr N. V. Tapkire (WWTP inchage Niphad SSK - Distillery Division), Mr S. D. Bothe (WWTP inchage Kopargaon SSK - Distillery Division), and Mr S. D. Shinde for their time-to-time co-operation and support during the study period.

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