



# Influence of Organic Loading Rate in Acclimatization Phase of Hybrid Upflow Anaerobic Sludge Blanket (UASB) Reactor Treating Distillery Spent Wash

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## ABSTRACT

A lab scale hybrid Upflow Anaerobic Sludge Blanket (UASB) reactor inoculated with anaerobic digested sludge, procured from distillery industry, was used to treat distillery wastewater with low strength of organic loads during acclimatization phase under ambient conditions. The lab scale hybrid UASB reactor with an effective volume of 12.5 L was operated for period of 80 days as a start-up phase at temperature  $30 \pm 5^\circ\text{C}$ , optimal hydraulic retention time (HRT) of 24 h, for various organic loading rates (OLR) viz., 0.25, 0.35, 0.70, 1.10, 1.30, 2.30  $\text{kg COD m}^{-3}\text{d}^{-1}$  respectively. Chemical oxygen demand (COD) removal efficiency achieved was more than 95% and biogas rich in methane content was produced. Sludge granules were observed under scanning electron microscope (SEM). The study revealed that the hybrid UASB reactor would be a feasible, eco-friendly and sustainable treatment system for treating distillery spent wash.

## INTRODUCTION

The distillery waste in the form of spent wash is amongst the worst pollutants produced by any industry both in magnitude and strength ( $\text{BOD}_5$  30,000  $\text{mg L}^{-1}$ ). At present approximately 40.4 billion litres of wastewater for 3.25 billion litres of total alcohol production per annum are been generated from 319 distilleries across India (Pant & Adholeya 2007). This wastewater (spent wash) contains high COD,  $\text{BOD}_5$ , recalcitrant compounds and is dark brown in colour. The dark brown coloured wastewater having very high organic loads with low pH is due to the presence of dissimilar organic compounds (Kaushik & Thakur 2009). It contains a melanoidin substance present because of the reaction formed between amino acids and carbohydrates called as Maillard reactions (Wedzicha & Kaputo 1992). Hence, it has been a major cause of concern in India for the past few decades. Amid the several treatment technologies, biological treatment systems are followed in various tropical and developing countries, because of cost effective and lower sludge production (Singh et al. 2013), particularly anaerobic digestion processes have earned popularity and has been applied for various types of wastewater, mainly the treatment of high strength wastewater as it contains adequate nutrients for microbial growth and recovery of renewable energy (Foresti 2001). Biodegradation of distillery wastewater by anaerobic digestion is an elucidation treatment due to ecofriendly and socioeconomically acceptable technology as its COD:N:P ratio is disturbed by aerobic treatments which require nitrogen and phosphorus in addition (Moletta 2005).

Hybrid UASB reactor extends the advantage of both UASB and anaerobic filters and it was more designed for the handling of soluble and partially soluble organic matters (Vieira & Tilche 1991). The start-up process of hybrid upflow anaerobic sludge blanket (UASB) reactor is optimized by various control factors like wastewater characteristics, nutrients, pH, seed sludge, presence of toxic compounds, and operating conditions like hydraulic retention time (HRT), liquid mixing, organic loading rate, reactor design and upflow velocity ( $V_{up}$ ) (Zhang et al. 2012). The main objective of the present work was to study the start-up time and best possible HRT required for hybrid UASB reactor working under an ambient condition with distillery spent wash and to study the biomass properties of sludge granulation by SEM observation.

## MATERIALS AND METHODS

### Physico-chemical characteristics of distillery spent wash:

Grab samples were collected from a distillery situated at Cuddalore District, Tamilnadu, India. The sampling procedures and preservation of the samples were carried out as per the prescribed standard procedure. The characteristics of distillery spent wash, spent wash diluted with 50% water and spent wash diluted with 75% water were studied and given in Table 1. The samples were analysed for the various physico-chemical parameters such as odour, colour, pH, conductivity, total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), total hardness, chemical oxygen demand (COD), calcium, chlorides, magnesium, phos-

Table 1: Physico-chemical characteristics of distillery spent wash.

Parameters	Units	Spent wash	Dilution 50%	Dilution 75%
Colour	-	Dark brown	Brown	Light brown
Odour	-	Unpleasant	Offensive	Offensive
pH	-	3.8	3.9	4.0
Conductivity	dSm <sup>-1</sup>	45.5	29.2	18.1
Total solids	mg L <sup>-1</sup>	140260	74220	33558
Total dissolved solids	mg L <sup>-1</sup>	112400	57840	26542
Total suspended solids	mg L <sup>-1</sup>	27860	16380	7016
Total hardness	mg L <sup>-1</sup>	23400	14400	5500
Chemical oxygen demand	mg L <sup>-1</sup>	162000	114480	92650
Calcium	mg L <sup>-1</sup>	2975	1692	684
Chlorides	mg L <sup>-1</sup>	10650	7806	3954
Magnesium	mg L <sup>-1</sup>	2380	1436	788
Potassium	mg L <sup>-1</sup>	10820	7120	3823
phosphate	mg L <sup>-1</sup>	2100	1400	840
Sulphates	mg L <sup>-1</sup>	3015	1785	690

phate, potassium and sulphates etc.

**Seed sludge inoculum:** Anaerobic digested sludge collected from a distillery unit was used as inoculum. The anaerobic digested sludge used as inoculum was sieved with a mesh of 1 mm to remove large debris and inert impurities. The initial concentration of seed sludge was 234 g on VSS basis. Primarily, about 30% reactor volume was filled with seed sludge.

**Feed wastewater:** Spent wash collected from a molasses based distillery was used as the substrate. The spent wash was diluted with tap water to obtain the desired concentration and neutralized with NaOH to acquire the pH within 6.5 to 8.5 range before feeding.

**Nutrients:** The nutrients for biomass growth contained the subsequent composition (g/L) of NH<sub>4</sub>Cl - 0.5, K<sub>2</sub>HPO<sub>4</sub> - 0.25, MgCl<sub>2</sub>·6H<sub>2</sub>O - 0.3, CaCl<sub>2</sub>-0.005, CuCl<sub>2</sub> - 0.0105, which were added to the substrate to maintain a C:N:P ratio of 100:5:1 (Mullai et al. 2013). All chemicals used were of analytical grade.

**Experimental setup:** A laboratory scale hybrid UASB reactor system was made of acrylic tube with an internal diameter of 0.10 m and overall height of 1.85 m as shown in Fig. 1. The Gas Liquid Solid Separator (GLSS) was installed at the top of the hybrid UASB reactor, which effectively separates the treated effluent, sludge granules and biogas with the help of Fujino's spiral media (size 26 mm, surface area 500 m<sup>2</sup>/m<sup>3</sup> and void ratio 87%) floating against a screen at 1.6 m height and the effective volume of 12.5 L. The substrate was fed into the reactor using a peristaltic pump (Model: pp-30-EX, Miclins) which can maintain a constant flow rate in the range within 5 mL h<sup>-1</sup> to 20 L h<sup>-1</sup>, available with timer and LED display for a flow rate of function and time. The inlet

and outlet pipes were connected to a water seal arrangement to prevent the escape of gas through the effluent. The gas collected is measured by the water displacement method and the reactor was operated at the ambient condition.

**Experimental procedure:** Hybrid UASB reactor filled with anaerobic sludge was fed with wastewater, enriched with nutrients. The nutrients added with the substrate were utilized by the inoculated facultative anaerobes for incubation under an anaerobic condition at ambient temperature for 80 days. The initial observation of the reactor stability was monitored by visually comparing the difference in colour changes in the inlet and outlet effluent and biogas production. During the initial stage, biomass gets acclimatized to the new environment by adapting itself with the substrate. The biogas production was very meagre at initial stage, may be due to the adaptation of the microorganisms with biomass (i.e., log phase to lag phase), as substrate utilization increased, biogas production also gradually increased (Acharya et al. 2008). The pH range of the influent was maintained within 6.5 to 8.5 by neutralizing and desired influent concentration by diluting the substrate. The hybrid UASB reactor was operated on a continuous basis at a desired retention time and the influent was pumped into the reactor with an essential upward flow rate of 550 mL hr<sup>-1</sup> using a peristaltic pump.

**Analytic procedure:** During the operation of the hybrid UASB reactor pH, gas production and COD were monitored daily, whereas VFA, TSS and VSS were analysed weekly. All determinations were performed according to Standard Methods (APHA 1998). COD was quantified by open reflux method and biogas production by water displacement method. SEM analysis of the sludge was carried out using JEOL-JSM 6610LV (Dept of Manuf. Engg., Annamalai University).

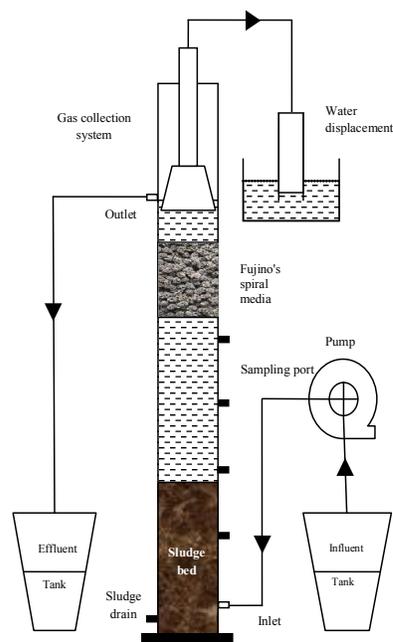


Fig. 1: Schematic view of laboratory scale hybrid UASB reactor.

## RESULTS AND DISCUSSION

### Startup Regime of Hybrid UASB Reactor

The start-up of hybrid UASB reactor is a complicated process and a number of factors, including wastewater characteristics, nutrients, pH, acclimatization of seed sludge, presence of toxic compounds, and operating conditions such as hydraulic retention time, liquid mixing, organic loading rate, reactor design, and upflow velocity affect the growth of sludge bed (Patel & Madamwar 2002, Zhang et al. 2012). Initially, the seed sludge (distillery anaerobic digested sludge) had a TSS content of about  $484 \text{ g L}^{-1}$  and VSS content of about  $234 \text{ g L}^{-1}$  and VSS/TSS ratio of 0.48. Primarily, the colour transformation of the seed sludge was observed in the form of spore forming cell to an active vegetative cell which was achieved through specific nutrients (Hawkes et al. 2002). During the start up regime sludge granulation started after a period of 45 days and it was compared with initial seed sludge.

**Reactor performance:** The hybrid UASB reactor was operated for a total start-up period of 80 days at ambient temperature. During the initial stage of the start-up, at an OLR of  $0.25 \text{ kg COD m}^{-3} \text{ d}^{-1}$  solids, washout was quite high, which was reflected by an increase in SS concentration and thereafter slowly the solids washout decreased gradually and COD removal efficiency was more than 95% at an HRT of 24 h. The OLR was increased stepwise by increasing the

substrate concentration viz., 0.35, 0.70, 1.10, 1.30 and  $2.30 \text{ kg COD m}^{-3} \text{ d}^{-1}$  maintaining a constant HRT of 24 h. COD removal efficiency of more than 95% was considered as the threshold level for the operation of the hybrid UASB reactor. The OLR was progressively increased as the COD removal efficiency achieved was more than 95%.

**pH:** The pH of the raw distillery spent wash was 3.80, and it was neutralized by the addition of NaOH and adjusted to a range of 6.5 to 8.5. Influent pH was in the range of 5.34-7.68 and the effluent pH of the reactor was in the range of 6.28-8.3 for various concentrations of the spent wash as shown in Fig. 2. The pH range in the effluent indicate that the reactor was secure. The generally accepted pH range for optimal efficiency was 6.5-7.6 (Jayantha & Ramanujam 1996). Rajeshwari et al. (2000) and Somasiri et al. (2008) reported that for an optimum operational performance or optimum anaerobic treatment pH should be in the range of  $6.5 < \text{pH} < 8.2$ .

**Effect of OLR on COD removal efficiency:** The hybrid UASB reactor was started initially with an initial OLR of  $0.25 \text{ kg COD m}^{-3} \text{ d}^{-1}$  and fed concentration of  $260 \text{ mg L}^{-1}$  at 24 h HRT, operated at ambient temperature. COD removal efficiency was variable during the initial period and slowly acclimatized and reached the steady state, and thereafter for various organic loading rates of 0.35, 0.70, 1.10, 1.30 and  $2.30 \text{ kg COD m}^{-3} \text{ d}^{-1}$ , the removal efficiency increased gradually to more than 95% at a constant HRT of 24 h for 80 days respectively as shown in Fig. 3. The transformation of the spore-forming cell to an active vegetative cell was achieved through specific nutrients that were present in the nutrient medium apart from a carbon source, glucose (Hawkes et al. 2002).

**Effect of OLR on VFA:** Anaerobic reactor unsteadiness is generally manifested by an identification and speedy increase in volatile fatty acid concentration (Bal & Dhagat 2001). Volatile fatty acids values obtained with the effect of organic loading during the startup phase of the hybrid UASB reactor operation are shown in Fig. 4. The reactor was in continuous operational mode, and because of recalcitrant nature of distillery spent wash, which requires longer periods of biomass adaptation and start up. The performance of the reactor for various OLRs of 0.25, 0.35, 0.70, 1.10, 1.30 and  $2.30 \text{ kg COD m}^{-3} \text{ d}^{-1}$ , the VFA concentration was very high as  $780 \text{ mg L}^{-1}$  initially but slowly decreased to  $148 \text{ mg L}^{-1}$  inferring a state of stability in the reactor. Rajeshwari et al. (2000) indicated that for a stable condition in a reactor, the concentration of VFA should be less than  $250 \text{ mg L}^{-1}$ .

**Effect of OLR on biogas production:** The cumulative biogas production was measured regularly during the course of the study. During the first 35 days of start-up phase, biogas



Fig. 2: pH of influent and effluent.

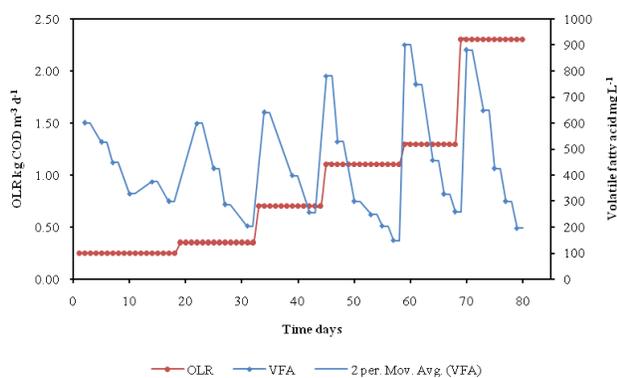


Fig. 4: Effect of OLR on VFA.

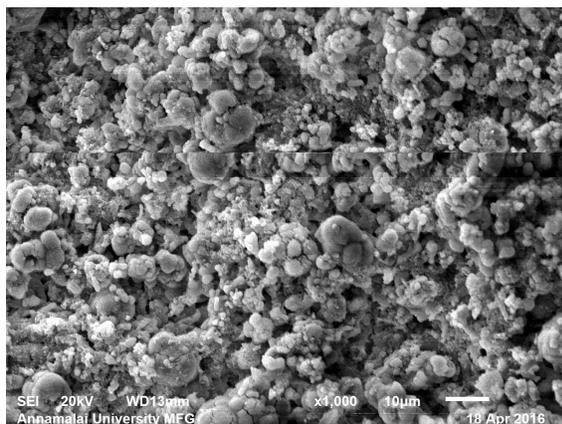


Fig. 6: SEM examination of sludge granules.

production was very less. But with the formation of granular sludge, biogas production picked up. From the Fig. 5, it is clear that biogas production is closely related to the OLR, increasing rapidly with the operational time of 80 days. The specific biogas yield during the startup phase was 100 mL d<sup>-1</sup>, indicating highly active granules.

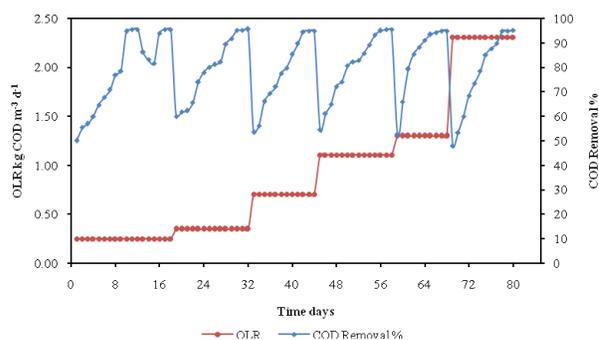


Fig. 3: Effect of OLR on COD removal efficiency.

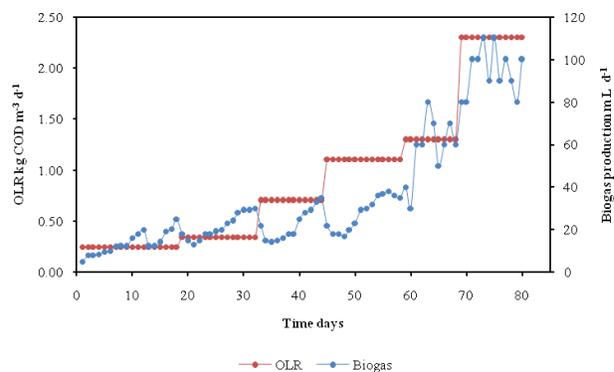


Fig. 5: Biogas production rate in hybrid UASB reactor.

### SEM Examination of Sludge Granules

The granular sludge was sampled from the hybrid UASB reactor to isolate the prominent microorganisms responsible for degradation of the substrate. Examination of sludge granules shown in Fig. 6 reveals that most granules appeared to be compact with fewer cavities. The bacterial organisms are rod shaped and organisms such as proteobacteria were found to be the dominant species in the granules from the SEM observation.

### CONCLUSIONS

The start-up of UASB reactor is the time required for the reactor to achieve an equilibrium condition that depends on concentration of wastewater i.e., organic load. The main objective to be achieved in the first start-up of high rate anaerobic reactors is to accomplish a satisfactory and consistent immobilization of anaerobic organisms. The study concluded that the distillery anaerobic digested sludge can be used as inoculum and distillery spent wash as the substrate. The effluent pH range of the reactor indicate that the reactor was secure. The COD removal efficiency was fluctuating for the first 18 days and slowly acclimatized

and found to be more than 95% for various OLRs ranging from 0.25 to 2.30 kg COD m<sup>-3</sup> d<sup>-1</sup> at an constant HRT of 24h. During the initial stage of start up process, volatile fatty acid concentration was very high as 780 mg L<sup>-1</sup> and slowly decreased to 148 mg L<sup>-1</sup> inferring a state of stability in the reactor. The biogas production was very less in the initial stage but increased gradually to 100 mL d<sup>-1</sup>. Maximum COD removal efficiency of more than 95% was attained at an HRT of 24h implying it to be optimistic. Examination of sludge granules showed that bacterial organisms are rod shaped and organisms such as proteobacteria were found to be the dominant species in the granules from the SEM observations.

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