INTRODUCTION

The slaughterhouse industry poses a significant environmental impact by discharging effluent to receiving waters containing a high concentration of biodegradable organic matter. Aerobic processes are not regarded as a suitable treatment option because of high energy requirements for aeration, limitations in liquid-phase oxygen transfer rates and large quantities of

ABSTRACT

Aims: Present study was done by using upflow anaerobic sludge blanket (UASB) reactor to investigate the effect of influent chemical oxygen demand (COD) and organic load rate on the formation of anaerobic granules in wastewater treatment.

Martials and Methods: Upflow anaerobic sludge blanket reactor with working volume 30 L was studied using actual slaughterhouse wastewater at a hydraulic retention time of 1.24 d and at temperatures in the range of 35°C ± 0.5°C for 320 days. The inoculum was extracted from the anaerobic digester of municipal sewage treatment plant, and the UASB reactor was filled with 8 L of sludge.

Results: The results indicated that under the optimal conditions, about 94.6% of COD could be effectively removed from slaughterhouse wastewater with the UASB. The highest and lowest COD removal efficiency was 40.5 and 94.6% corresponding to influent COD of 1266.8 and 1222.2 mg/L, respectively. The fluctuation of solution pH in UASB operation was in the range of 6.68-8.03. The average of solution pH was 7.46 ± 0.36. The solution pH was gradually improved with UASB operation. Different granule sizes coexisted in the UASB reactor, but granules with the size of 1-3 mm were predominant. The maximum observed size of anaerobic granule was 7 mm.

Conclusion: Application of slaughterhouse wastewater as feed wastewater demonstrated that the slaughterhouse wastewater to be more effective in promoting the formation of anaerobic granules and granule size in UASB reactor.

Key words: Anaerobic granulation, slaughterhouse wastewater, upflow anaerobic sludge blanket
sludge production. Traditional anaerobic processes are also limited by low rates of organic matter removal, long hydraulic retention times (HRT), accumulation of excessive residual organic matter and intermediate products, and large reactor volume requirements.\[^1\]

Since its introduction in the early 1980s, the upflow anaerobic sludge blanket (UASB) system has gone through a lot of improvement in both design and operational details and has been used to treat a variety of industrial effluents.\[^2\] Presently, the UASB reactor is the most popular high-rate anaerobic process for the treatment of organic wastewaters.\[^3\] Their hydraulic design and the formation of granules let these reactors hold a high concentration of active anaerobic biomass in suspension.\[^4\] Also, they attain successful applications due to its simple design, easy construction and maintenance, low operating cost and ability to withstand fluctuations in pH, temperature and influent substrate concentration.\[^5\]

Knowledge of the microbial process and of the operating principles that lead to successful methanogenesis is essential for the industrial use of this advantageous biotechnology. Sludge granulation is undoubtedly one of the most important and interesting phenomena of the UASB process.\[^6\] Anaerobic granules are formed mostly in UASB presses.\[^7\] The formation of granular sludge can be considered as the major reason of the successful introduction of the UASB reactor concept for anaerobic treatment of industrial effluents. This granulation process allows loading rates far beyond the common loading rates applied so far in conventional activated sludge processes. (anaerobic sludge granulation, hulshoff) Anaerobic granular sludge is the core component of a UASB reactor.\[^8\] One major drawback of the UASB reactor is its long start-up period, which generally requires 2-8 months for the development of anaerobic granular sludge. In order to reduce the space-time requirement of bioreactors, eventually leading to a cheaper treatment of high strength wastes, strategies for expediting granular formation are highly desirable.\[^9\]

Sludge granulation is a physical, chemical, and biological process.\[^9\]

Slaughterhouse wastewater contains high amounts of organic matter with a soluble fraction in the range of 40-60%. The suspended and colloidal components in the form of fats, proteins, and cellulose can have an adverse impact on the performance of UASB reactors, leading to deterioration of the microbial activity and washout of active biomass.\[^10\]

Del Nery et al.\[^6\] examined granule characteristics using a UASB reactor treating poultry slaughterhouse wastewater with an average chemical oxygen demand (COD) reduction of 85%. Granules with the size of 0.5-1.5 were found to be predominant.

In a study by Torkian et al., performance of UASB system for treatment of slaughterhouse wastewater was evaluated. The granules were formed after 4 months. They were dark brownish granules with a diameter of 1-4 mm and a settling velocity of 20 m/h.\[^11\]

The aim of this study was to investigate the effect of influent COD and organic load rate (OLR) on the formation of anaerobic granules in slaughterhouse wastewater treatment.

**MATERIALS AND METHODS**

**Upflow anaerobic sludge blanket reactor**

A Plexiglas laboratory scale UASB reactor [Figure 1] with working volume of 30 L was used in this study (internal diameter of 20 cm and a height of 100 cm). The reactor was operated under the mesophilic condition, and temperature was kept at 35°C ± 0.5°C by means of an external temperature controlled heater in the bath. Solution pH was kept in the range of 6.8-8. Four sampling ports were installed along the vertical central line of the reactor, and a gas-liquid separator was installed in the top of the reactor. HRT was 1.24 day, and sludge retention time was 95 days. During the operation period, pH, COD, volatile suspended solid were measured routinely. Examinations were done on the influent and effluent wastewater. Analyses were carried out according to the standard methods for the examination of water and wastewater.\[^12\]

**Inoculum**

The inoculum was extracted from the anaerobic digester of municipal sewage treatment plant. The anaerobic sludge was rinsed with deionized water for several times to removing and debris. At first, UASB reactor was filled with 5 L of sludge. The inoculum was containing 19 g/L of mixed liquor volatile suspended solids.

**Feeding wastewater**

Synthetic wastewater was used in this experiment to keep the influent stable. Raw blood with a COD of about 150,000 mg/l was collected from a slaughterhouse and then was diluted to desired concentration. The wastewater was introduced to
the reactor by a peristaltic pump. NaOH 0.25 N was added to the reactor’s influent flow to maintain the optimum pH. The wastewater was continuously fed into the reactor.

The OLR varied from 0.5 to 8 kg COD/m³ day. It was gained by diluting the wastewater. The operation temperature and pH were kept on 35°C and 6.8-8.03 respectively. The operational parameters are provided in Table 1.

**Determination of granule characteristics**
For scanning electron microscope (SEM) image, the granules were placed in 3% glutaraldehyde for 24 h then they were washed with phosphate buffer and finally dehydrated using serial dilution of ethanol 30%, 50%, 70% and 100%. Prior to scanning, they were coated with gold.[13]

To determine the settling velocity of granules, a 50 cm high and 5 cm diameter cylinder was used. The granule’s formula was determined by COSTECH CHNOS instrument.

**RESULTS**

The UASB performance in COD removal was evaluated for 323 days. The COD removal efficiency as a function of influent COD and OLR showed in Figure 2. As seen in Figure 2, maximum and minimum COD removal efficiency was 40.5 and 94.6% corresponding to influent COD of 1266.8 and 1222.2 mg/L, respectively.

Figure 3 has been plotted to show the variation of solution pH during UASB operation. It is observed that at high OLR (6.34 g/L.d), the lowest solution pH (6.67) was obtained. And also, the low influent OLR resulted in high solution pH in effluent wastewater.

Granulation of the anaerobic sludge in the UASB reactor was satisfactorily achieved under different operational conditions (Influent COD and OLR). The SEMs of extracted anaerobic granules from UASB reactor are shown in Figure 3.

Also, the characteristic of extracted anaerobic granules from UASB reactor are presented in Table 2.

**DISCUSSION**

As shown in Figure 2, during UASB reactor operation, the influent COD concentration gradually increased from 1.22 g/L to 10.45 g/L for soluble COD. In addition, the volumetric COD loading rate fluctuated of 0.44 g/L.d to 8.36 g/L.d. The COD reduction was 65.81 ± 16.2 on average (15.2-94.5) base on influent and effluent soluble COD. By operating UASB reactor at constant HRT of 1.24 d, the result showed a satisfactory performance, achieving a COD removal of 65.8% on average. At operation days 1-160d (influent COD: 1.36 ± 0.1 g/L) with increasing operation day the COD removal efficiency was improved. After this point, by ascending influent COD (2.27 g/L), COD removal efficiency promptly depleted from 91% to 44% at day of 166.

During the operational period, variation of effluent solution pH was monitored and depicted in Figure 3. By operating...
UASB operation for 320 d, the fluctuation of solution pH was in the range of 6.68-8.03. The average of solution pH was 7.46 ± 0.36. The solution pH was gradually improved with UASB operation. As seen in Figure 3, the influent wastewater exhibited high buffering capacity and no need to pH adjustment.

Different granule sizes coexisted in the reactor, but granules with the size of 1-3 mm were predominant. The maximum observed size of anaerobic granule was 7 mm. It seems that the slaughterhouse wastewater to be more effective in promoting the formation of anaerobic granules and granule size in UASB reactor. SEM examination further revealed that the granules in UASB were mainly composed of Methanothrix Spp. [Figure 4c].

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