

## Biological Treatment of Antibiotic Plant Effluent in an UASFF Bioreactor

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**Abstract:** In this research, an up-flow anaerobic sludge blanket fixed film (UASFF) bioreactor was successfully used for antibiotic effluent treatment. The UASFF was a hybrid system combined of two compartments for providing granular and fixed biomasses in a single bioreactor. Sea shell was used as an internal packing for plug flow segment. Prior to packed column, the granulated bed served as anaerobic digester. External granulation was conducted and then transferred to the column at ambient temperature and anaerobic condition. The bioreactor was operated under ambient temperature (24 and 37°C) and its performance was evaluated at various organic loading rates of the antibiotic plant effluent (HRTs of 12, 18, 30 and 40 hours). Beside COD removal, excessive amount of biogas was produced from biodegradation of the industrial wastewater in the UASFF bioreactor.

**Key words:** UASFF • Anaerobic digestion • Antibiotic wastewater • Biogranules • Biogas

### INTRODUCTION

The up-flow anaerobic sludge blanket (UASB) is a popular biological treatment process, fully developed in number of plants for the treatment of industrial effluents [1-6]. The UASB reactor exhibits positive features, such as high organic loadings, low energy demand, short hydraulic retention time (HRT) and easy reactor fabrication [7-10]. The UASB is compared with CSTR; the UASB design relies on granulation of bacterial biomass with enhanced settling properties facilitates to increase retention of microbial consortia. Washout phenomena of the microorganisms before proper granulation is one of the major problems associated with UASB reactors. The main reason for long duration of start up in UASB resulted from washout of the fine granulated sludge [11-13]. The major problem associated with conventional UASB reactors was resolved in this novel UASFF. There were several mechanisms and methods for anaerobic granulation in UASB reactor [14-18]. The up-flow anaerobic sludge blanket fixed film (UASFF) reactor is a hybrid system with combination of up-flow anaerobic sludge blanket and up-flow anaerobic fixed film reactors. The lower part of UASFF reactor is the UASB portion where flocculants and granular sludge are developed. The upper part of UASFF serves as fixed film bioreactor. The advantages of

this new system have high removal efficiency of the anaerobic processes [19-23]. The suspended and colloidal components in the form of fat, protein and cellulose have an adverse impact on the performance of UASB reactors, leading to deterioration of microbial activities and wash out of active biomass [24]. Biogranulation is a process taken by microbial consortia as the cell self immobilization is formed in a sludge granular blanket. The biogranules are dense microbial consortia packed with several bacterial species and typically contain millions of organisms per gram of biomass [25, 26]. Extensive studies were conducted on performance of UASFF reactor for treatment of several industrial wastewaters, such as slaughterhouse, swine and starchy wastewater [27-30].

The objective of the present research was to enhance the treatability of antibiotic plant effluents (APE) in an anaerobic treatment process. One of the advantages of the hybrid systems was to increase solids and microbial detention time. The use of up-flow anaerobic sludge fixed-film (UASFF) reactor for treating APE with a short start-up period and high treatability of APE has been achieved.

### MATERIALS AND METHODS

**Experimental Set Up:** The schematic diagram for the experimental set up is shown in Figure 1. A laboratory-scale, UASFF reactor with temperature controlled system

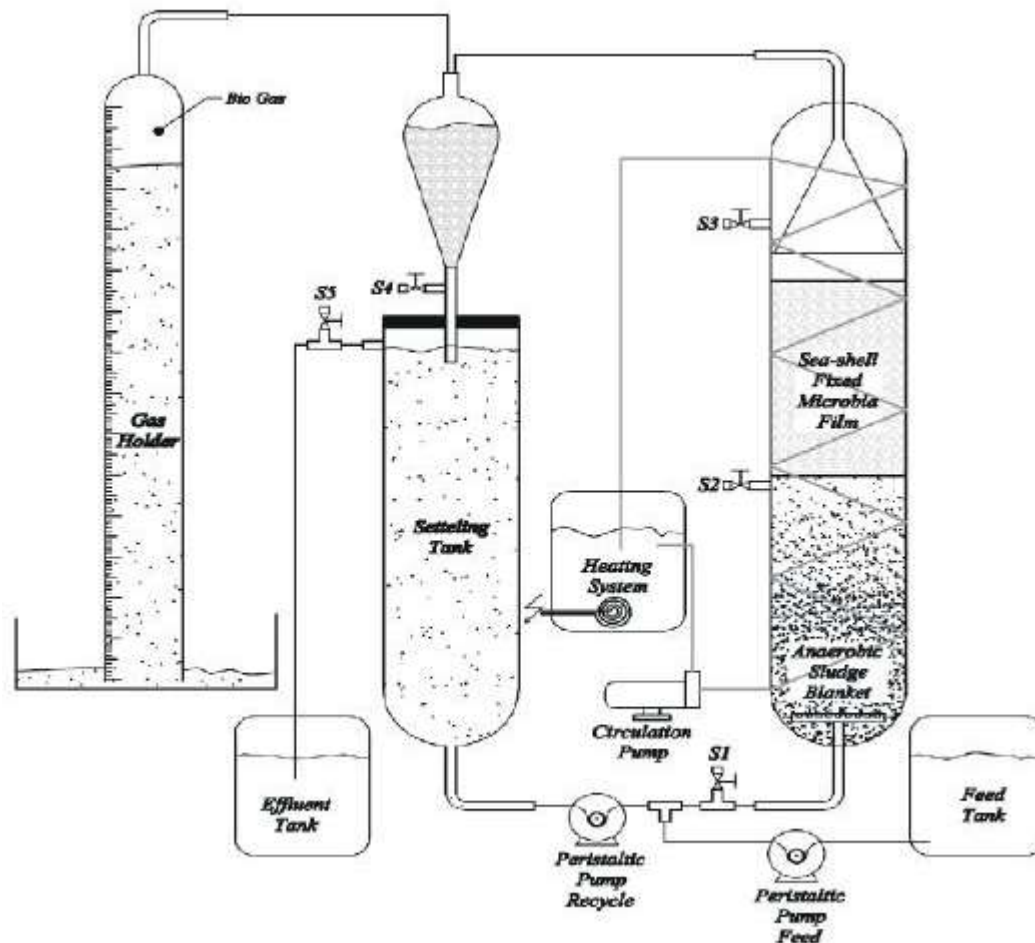


Fig. 1: Schematic diagram of UASFF experimental set up

was used in this study. The system was designed to behave plug flow, therefore height per diameter ratio was set 20:1. The bioreactor column was fabricated with Pyrex glass. The column with an internal diameter and height were 6.5 and 132cm, respectively. The UASFF reactor was operated under isothermal conditions (37°C). The temperature was maintained by circulating hot water through the bioreactor jacket. The circulated flow in the jacket was sent to a water bath to ensure isothermal operation. APE as substrate was continuously fed to the reactor using a peristaltic pump (Thomas, Germany) and the effluent leaves from the top of the column through the settling chamber. In order to distribute feed uniformly into the reactor, an influent liquid distributor was mounted in the base of the column. The top of the UASFF was connected to a water displacement gas tank to measure volume of produced biogas. The produced gas bubbles were raised from the bottom to the top of the bioreactor column and stored in the gas holding tank.

**Antibiotic Plant Effluent:** The UASFF was fed using fresh APE. The APE samples were weekly taken from the near by antibiotic plant industry. The wastewater was stored in a cool room at 4°C before use. Different dilutions of APE were prepared using distilled water. The pH of the feed was measured to be 6.9. The characteristics of the supplied APE are summarized in Table 1. The APE is mostly contained organic solvents such as methylene chloride, methyl and ethyl acetoacetates which were originated from amoxicillin plant.

**Seed Sludge:** The initial inoculums for seed culture were the mixture of influent and sludge from the bed of an anaerobic digestion bioreactor which was obtained from the antibiotic treatment plant.

**Reactor Operation:** The reactor contained acclimated sludge which was previously formed in the reactor. The system was started with an initial loading rate of 10.43 g COD/l.d and a HRT of 30 hours. The HRT was maintained

Table 1: Characteristics of APE

Content	Dose
COD	30000-35000 mg/l
Methylene chloride	450-600 mg/l
Butyl acetate	650-750 mg/l
Ethyl acetate	300-400 mg/l
Isopropyl alcohol	400 mg/l
Dimethyl acetamite	1000 mg/l
Acetone	400 mg/l
Penicillin	Less than 60 mg/l
Sefixcin	Less than 60 mg/l
Amoxicillin	Less than 60 mg/l
Cefalexcin	Less than 60 mg/l
Methyl aceto-acetates	Trace
Ethyl aceto-acetates	Trace
Turbidity	Trace
Odor	Strongly odor
Color	Yellow – Brown
pH	6.8-7.0

constant through out the start up period. The influent COD concentration was initially fed at 5215 mg/l and then the COD concentration was stepwise increased to 20400 mg COD/l from 7 to 16 days. The start-up period of the bioreactor in continuous mode was achieved in a short duration of about 16 days and influent feed concentration of 20400 mg COD/l. Subsequently, both OLR and HRT were altered by increasing influent COD and hydraulic flow rate fed to the reactor. Temperature was maintained at 37°C. There was no control of pH to regulate the pH of the reactor since the pH was remained relatively stable through out the experiments that was due to biocarbonate alkalinity production during the course of anaerobic reactions.

**Analytical Methods:** A colorimetric method using closed reflux system was developed for measurement of COD. Spectrophotometer (Unico2100, USA) at wavelength 600 nm was used to measure the light absorbance of COD samples. Based on standard method, potassium hydrogen phthalate (KHP) standard solution was prepared in the range of 0 to 1000 mg/L as the wastewater sample had a high COD range. Therefore 425 mg of KHP was dissolved in 500 ml of distilled water. KHP has a theoretical COD value of 1.176 mg O<sub>2</sub>/mg. Thus, the standard KHP solution, 1000 mg/l has a theoretical COD value of 1176 mg O<sub>2</sub>/l. The standard KHP solution was used for the preparation of COD calibration curve [31]. The biogas was measured by displacement of water in the gas tank and pH of the effluent was measured by a pH meter, HANNA Model 21 (Italy).

## RESULTS AND DISCUSSION

The UASFF experiments for treating APE were continuously conducted for duration of more than nine months. Organic loading rates were stepwise increased and the bioreactor performance was evaluated. The effects of HRT and temperature on treatability of APE and COD removal was investigated during the course of the experiment. Figure 2 presents the SEM monograms of the biogranule samples. The microbial core generated by the consortium of microorganisms is shown in magnifications of 5000 and 10000. The texture of biogranules was hard and the release funnels and cavities on the granules surface are naturally generated by exiting biogas flow produced from the anaerobic activities in the core of the granules. It enhanced substrate mass transport (convective) from outside to inside of the granules [32]. Also some parts of the SEM monograms are shown that the microbial cores were dominated by the mixture of bacteria, that means the consortium of bacteria creating the biogratules of UASFF.

Figure 3 shows the COD removal with respect to HRT at different temperatures (24 and 37°C). The percentage of COD removal was stable and steady for high COD concentration of about 30000 mg/l. As the HRT was increased from 12 to 40 h, the COD removal was also increased from 20 to 50 percent. The percentage of COD removal was compared to similar system using palm oil mill effluent with COD concentration of 37400 mg/l at HRT of 40h, 75% removal rate was reported [16], while using APE with concentration of 30000 mg/l at the same HRT, 49% removal was obtained. The reason for lower COD removal was justified, since the feed from antibiotic effluent was more toxic than the palm oil mill effluent. At low HRT, the removal of COD was not appreciable since the anaerobic digestion and the process of hydrolysis and acidification may required longer duration for digestion. The hybrid system was able to handle high loading rate with OLR of 60000mg/l.d. The obtained data showed that the COD removal rate was increased when the HRT increased, indicating that the readily biodegradable fraction of COD or soluble BOD in APE is low [33, 34]. Also maximum COD removal rate was obtained as HRT increased to 40 hours.

In UASFF process, biogas was generated. The gas analysis showed that the percentage of methane was increased with respect to HRT. It was attributed to the methanogenic bacteria activities which are slow in growth and metabolic reaction relative to other species [35, 36]. Figure 4 depicts the biogas production rate at

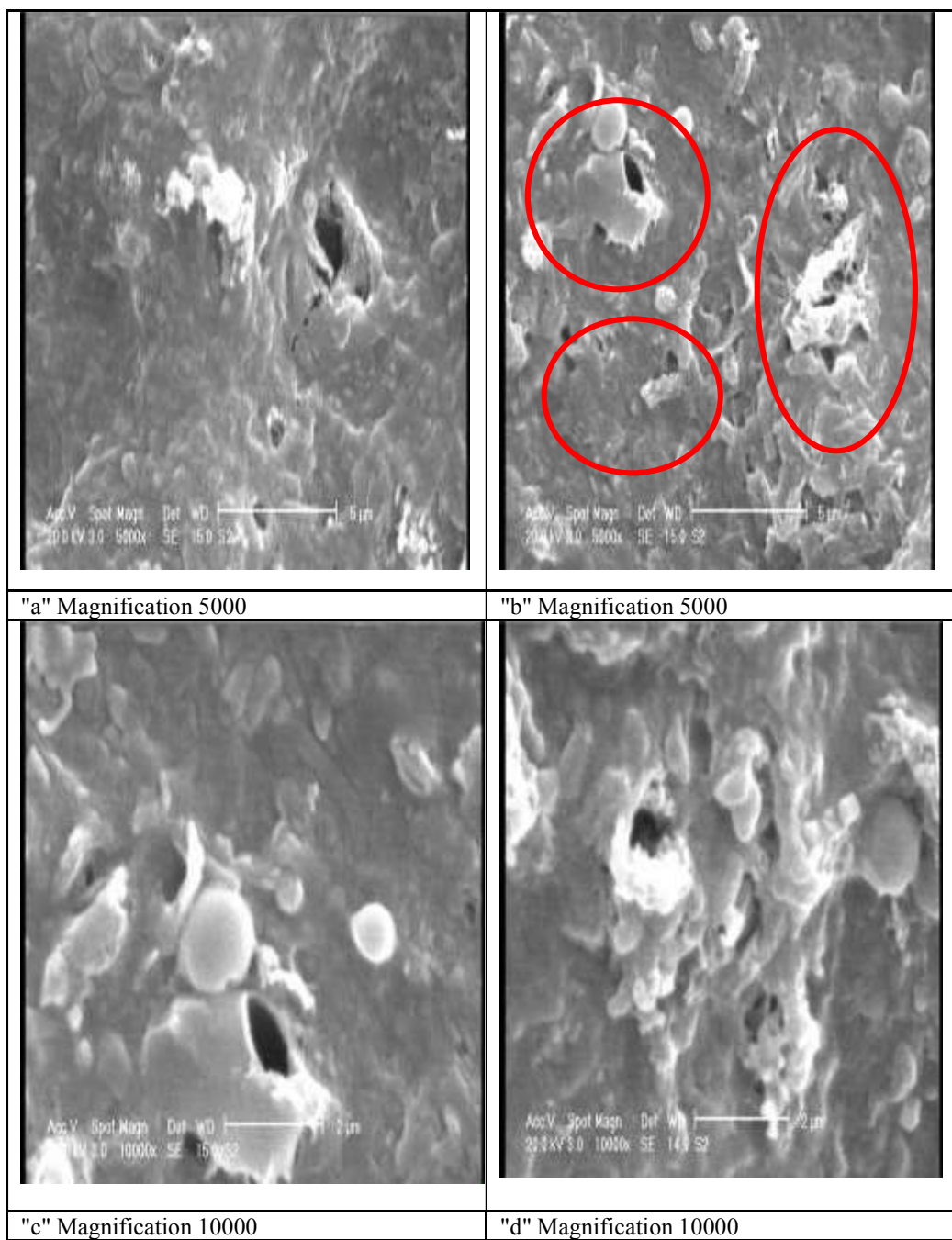


Fig. 2: The SEM monograms of the biogranule samples obtained from the column

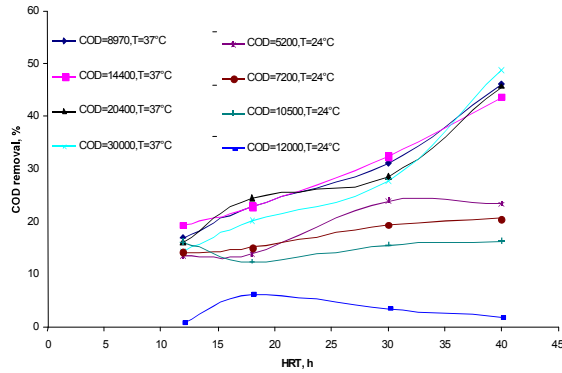


Fig. 3: COD removal vs HRT at 24 and 37°C

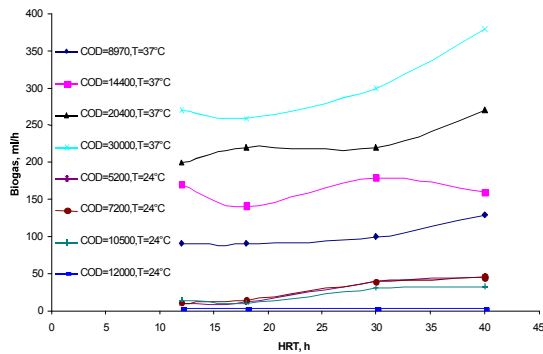


Fig. 4: Biogas Production vs HRT at 24 and 37°C

various feed COD concentrations with respect to HRT. At 37 °C, the rate of biogas production was also increased as the concentration of COD or the organic load was increased whereas inverse results obtained for the condition with temperature of 24 °C. It might be due to inhibiting impact of the COD concentration on methanogenesis activities at lower temperature [37]. At atmospheric pressure and room temperature, maximum volume of biogas, about 380 ml/h (9.12l/d) was produced

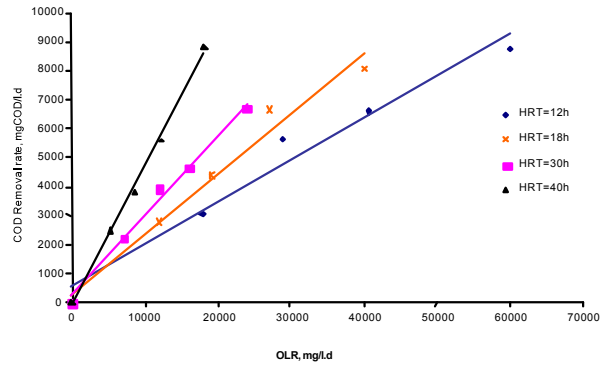


Fig. 5: COD removal rate vs OLR at various HRT and at 37°C

with COD concentration of 30000 mg/l at 40 hours hydraulic retention time.

Figure 5 shows the COD removal rate with respect to OLR at various HRT of 12 to 40 hours. As the HRT increased from 12 to 40 hours the slope of the line fitted with the experimental data was also increased. That means at high HRT the removal rate was high. The maximum removal rate was obtained with HRT of 40 hours at low OLR.

Figure 6 presents the biogas production rate with respect to COD removal rate. As the COD removal rate increased the volume of biogas generated was increased. At low COD removal rate the volume of biogas generated at 30 and 40 hours were about the same while the COD removal rate was increase, appreciable amount of the biogas was also increased. At high COD removal rate, the maximum volume of biogas generated at 40hours was 380ml/h.

Table 2 shows the performance of UASFF operated at 37°C for HRT of 30 and 40 hours. The COD removal, OLR, pH and biogas productions are also given in this

Table 2: Performance of UASFF at various HRT

HRT=30h, T=37°C						
COD (in) mg/l	COD (out) mg/l	COD Removal %	OLR mg/l.d	COD Removal Rate mg/l.d	Biogas ml/h	pH
8970	6175	31.16	7176	2236	100	7
15100	10207	32.40	12080	3914	180	6.8
20150	14379	28.64	16120	4617	220	6.8
30000	21650	27.83	24000	6680	300	6.9
HRT=40h, T=37°C						
COD (in) mg/l	COD (out) mg/l	COD Removal %	OLR mg/l.d	COD Removal Rate mg/l.d	Biogas ml/h	pH
8970	4820	46.27	5382	2490	130	6.8
14400	8120	43.61	8640	3768	160	6.7
20400	11080	45.69	12240	5592	270	6.7
30000	15350	48.83	18000	8790	380	6.8

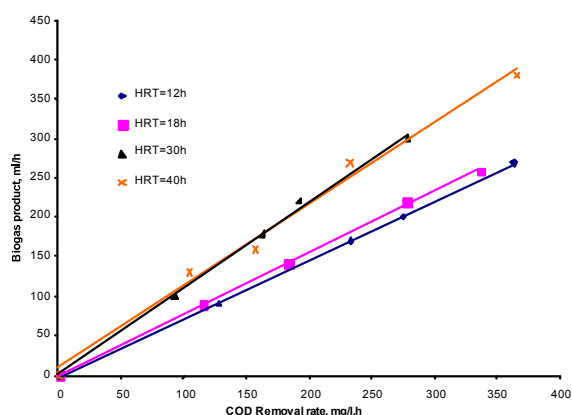


Fig. 6: Biogas Product vs. COD removal rate at 37°C

table. The COD concentration of feed and the treated APE leaving the UASFF at 37°C are summarized.

## CONCLUSIONS

This study reveals that the treatability of APE using UASFF bioreactor. In this novel hybrid bioreactor, the high organic load and COD concentration of APE was successfully treated. The hybrid system efficiency was improved by gradually increasing the HRT and stepwise increasing the organic load. The results showed that by increasing the HRT the treatability of APE was more effective than gradual increase in the inlet COD concentration of feed while running the bioreactor at suitable conditions. At HRT of 40h, 37°C column temperature and COD concentration of 30000 mg/l, COD removal of about 50% was achieved.

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