

# Influence of Oxygenation on Granulation in Anaerobic Wastewater Treatment

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The emphasis of this study was to put on the ability of bacteria to form granules in anaerobic wastewater treatment. This direction was taken mostly because the success of wastewater treatment in such systems as Upflow Anaerobic Sludge Blanket (UASB) depends on how long the biomass is retained, and this is a function of adhesion of bacteria to reach other to form well-settling granular sludge. The granular sludge will remain in the reactor and will be exposed to the continuous supply of nutrients (wastewater) which is injected into it. The wastewater used in this study was synthetic. It consisted of molasses dissolved in water (100%), and a mixture of molasses and peptone dissolved in water (20 % and 80 %, respectively). Several factors or parameters influence bacteria to form good granules and improve the performance of the digesters. The effects of oxygen on the anaerobes, and the extent to which it acts as an inhibitor or toxic was studied. This was found to be both advantageous and disadvantageous.

**Keywords:** Anaerobic, bacteria, granular, sludge, synthetic wastes, UASB.

## INTRODUCTION

The increasing amounts of wastewaters from industry due to multiplied industrial activities, from households due to increased population, and also from agriculture and agricultural activities has raised much concern on how best to treat the waste before disposal into the natural systems (for example, surface water). So far there exist two technologies, the aerobic and anaerobic wastewater treatment that are being used. However, the low operational costs, the production of methane which can be used as energy source and the lower amounts of sludge produced from the anaerobic systems (Verstraete et al., 1992), have made the anaerobic approach more attractive. At present there is a lot of research going on in the anaerobic wastewater treatment technology.

The anaerobic treatment of wastewater technology has been employed for many years to municipal sewage units. Research has now made it possible to treat both high and low strength industrial wastewaters. In their work, Herbert et al. (1989) reported successful treatment of brewery effluent with organic content. Wastewater from a potato processing plant was treated in a UASB-type (Upflow Anaerobic Sludge Blanket) reactor, achieving a COD (Chemical Oxygen Demand) removal efficiency of 86 % (Van Wambeke et al., 1990). Other industrial wastewaters treated successfully include a petrochemical effluent, with COD removal efficiency of 88 % (Augoustinos et al., 1989).

The conversion of organic matter in the wastes to methane and carbon dioxide involves a number of microorganisms working together. The microbes bring about these

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conversions of organic matter to obtain energy for maintenance and to build more biomass. The breakdown of organic substances is a sequence where metabolites of one species serve as food source for the other species, giving final end-products, apart from methane and carbon dioxide gases, like hydrogen sulphide, hydrogen, and nitrogen. The whole process can essentially be called a food chain (Verstraete et al., 1992). The biodegradation of organic waste is carried out by quite a large and complex bacterial community, each with its own specialization (Ross, 1991). Many diverse genera of aerobic, obligate and facultative anaerobic bacteria are the responsible microbial populations. This population diversity includes several different trophic groups of carbon catabolizing bacteria, each possessing genera with differing cellular makeup and properties (Visser et al., 1991; Ross, 1991).

In the wastewater treatment systems such as UASB, the formation of well-settling biomass in the form of small sphere or granules is a key factor. It means that bacteria can have longer residence times without being washed out from the digester. The treated wastewater comes out more clearly than when there is a lot of badly settling biomass (flocs) and free-living cells which result in turbid effluent (Verstraete et al., 1992). Granules of 0.5-5 mm size are a result of the growth of microbial association.

During biodegradation of the biopolymers, microbes excrete extracellular enzymes to attack the complex molecules. The bacteria can then obtain the energy for their ATP (Adenosine Tri Phosphate). The disappearance of the organic matter follows a first order reaction rate (Verstraete et al., 1992) permitting the bacteria grow. The maximum quantity of bacteria is produced and the conversion of organic matter takes place when bacteria are growing unrestricted in an excess of food (exponential growth). Aggregation and adhesion of mathanogenic consortia or granule-formation is important at this stage

but the current knowledge of this mechanism is very limited. Matson and Characklis (1976) indicated that growth of the microbes is dependent on the diffusibility of the substrate (rate-limiting) in the biofilm. It is therefore important that substrate is available in the right form. Furthermore if a given substrate will penetrate in biological film, two reactions will be occurring simultaneously: an aerobic reaction near the surface and an anaerobic reaction where oxygen does not penetrate. In order to facilitate uptake of the substrate, bacteria produce cell wall-associated or extracellular surface-active agents. Passeri et al. (1992) found that as a result of these biosurfactants, surface tension was reduced from 72 mN/m to 30 mN/m in water.

Since in UASB the success of the system depends on granulation or aggregation of bacteria (Morgan et al., 1991) considerable efforts have been made to understand why and how this process occurs. Morgan et al. (1991) further reported that extracellular polymers (ECP) are known to assist in the attachment of bacteria to surfaces, and therefore polymers and granule formation/ structure are inter-related.

The objective of this work was to study of effect of oxygen on the methanogenesis in the anaerobic digestion, given the hypothesis that obligate anaerobes are bacteria which readily grow in anaerobic conditions but are unable to utilize oxygen productively, and, in addition, are inhibited or even killed by oxygen.

## **MATERIAL AND METHODS**

### **Wastewater**

This study was carried out on different types of wastewater (substrate) prepared in the laboratory. The wastewater was prepared by mixing molasses and peptone in the ratio of 20 % to 80 %, giving the final COD of 7500 mg/l.

### Modification of UASB Reactor

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The experiment was conducted in an Upflow Anaerobic Sludge Blanket (UASB). Some modification were made to the UASB (Figure 2.1) where external recirculation was included, hereafter called External Recirculation Upflow Anaerobic Sludge Blanket (ER-UASB). In the normal UASB reactor, the

reactor liquid internally circulated, forming a closed system where oxygen is excluded. In the ER-UASB, the closed system was opened by including an external basin where different degree of oxygenation could take place before allowing the reactor liquid to enter the reactor again.

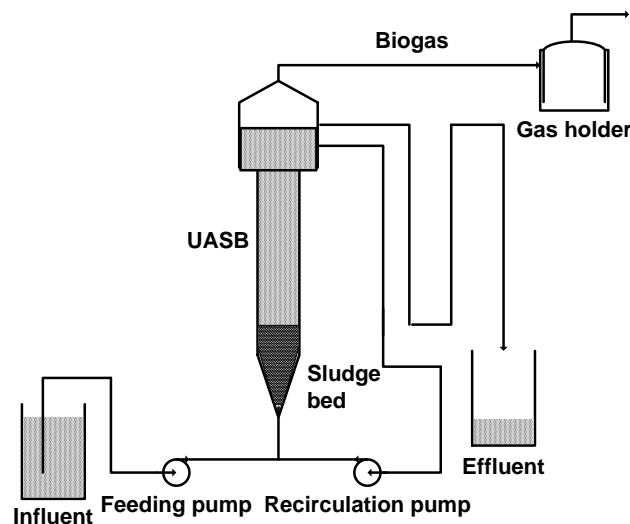


Fig 1. Laboratory-scale UASB reactor

### Effect of Oxygenation on Granulation

Three lab-scale UASB reactors with the same dimensions were set up as shown in Figure 2.2. Each reactor was started with seed granule volume of 110 ml (from reactor using same feed). To each UASB reactor trace elements were added daily. An amount of 60 ml of sodium bicarbonate (105 g/l) was added to maintain pH above 7. The experiment was let to run at 33° C. Each reactor was allowed

different degree of oxygenation as follows: (1) ER-UASB reactor 1: mechanical aeration by pump of liquid in the external recirculation basin; (2) ER-UASB reactor 2: Aeration occurring by diffusion as reactor liquid discharge dropwise over the liquid surface of the external recirculation basin; and (3) ER-UASB reactor 3: Aeration occurring by diffusion at the surface of the external recirculation basin as the reactor liquid discharge is submersed.

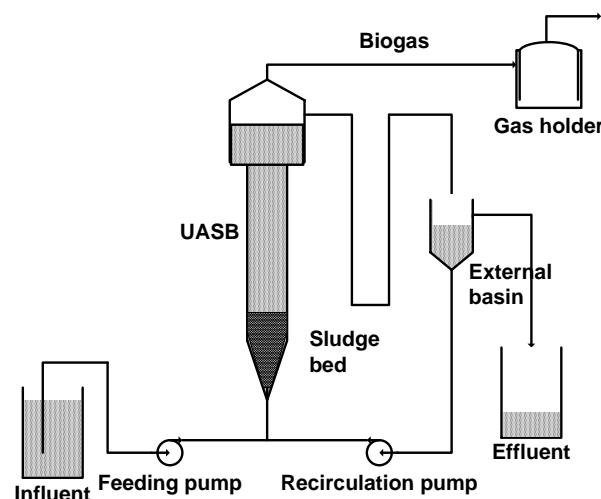


Fig 2. Laboratory-scale ER-UASB reactor

### Determination of Chemical Oxygen Demand (COD)

The COD measurement were carried out by taking 1 ml of effluent (before feeding to reactors), 2 ml of effluent (after reactor digestion) into 100 ml digestion tubes. To these were added 0,4 g of mercury sulphate (for complexing all interfering chloride). This was followed by addition of 20 ml of distilled water. A volume of 10 ml of 0,25 N  $K_2Cr_2O_7$  and 30 ml of  $H_2SO_4$  containing  $Ag^+$  were next added. A blank (20 ml of distilled water), with same chemical was also prepared. These were digested for 2 hours at 150°C. The cooled digested solution was titrated with iron ammonium sulphate, using 0.5 ml ferroin as indicator.

### Determination of Volatile Fatty Acids

Filtered sample (2 ml) was mixed with 0.5 ml of 50 %  $H_2SO_4$ . To the mixer, 0.4 g NaCl and 0.2 ml of internal standard for gas

chromatography measurement was added. This was followed by addition of 2 ml diethylether. Everything was mixed by rotating the tube for 5 minutes, then centrifuged for 3 minutes at 3000 rpm. The etheric layer was transferred to a vitatron tube to which was added a pinch of  $CaCl_2$  (for drying). Of this solution, 0.8  $\mu$ l was injected into the gas chromatography (GC, Carlo Erba Fractovap 4160).

### Determination of Volatile Suspended Solids

Measured volume (100ml) of sample from effluent was centrifuged at 5000 rpm for 10 minutes. After decanting the centrifuged sample, it was weighed and dried in an oven at 105°C in a crucible for 24 hours, followed by equilibration in a dessicator for 2 hours. After weighing again, the crucible was put in the oven and ashed at 450° C for 4 hours, and then equilibrated in dessicator for 2 hours and weighed.

removal efficiency in UASB reactor 2 and 3 continued to increase and finally attained an efficiency of about 92 % when the reactors achieved steady state. UASB reactor 1 could only achieve an efficiency of about 90 % at steady state. The mechanical aeration was stopped in this reactor at the end of period 2 when biogas nearly came to a stand still. Biogas production picked up again when the oxygenation was put at a minimum.

## RESULTS AND DISCUSSION

### Effect of Oxygenation on COD Removal

It is shown in Figure 3.1 that there was an increase in the efficiency of COD removal in all the three UASB reactors during period 2 (day 6-47). The first period (day 0-5) was actually not significant in COD removal as the reactors were acclimatizing. However, the efficiency of COD removal dropped markedly at the end of period 2 due to the effect of active aeration in UASB reactor 1, whereas the upward trend in COD removal efficiency in UASB reactor 2 and UASB reactor 3 continued. The efficiency of COD removal could be seen to increase again when at the start of period 3 (day 48-72), the mechanical aeration was stopped in UASB reactor 1 and dripwise aeration was adopted. The drop in COD removal efficiency could be attributed to the probable inhibition of methanogens. During this period the COD

### Effect of Oxygenation on Residual Volatile Fatty Acid (VFA)

The probability of inhibition of methanogens resulting in decrease of COD removal efficiency during period 2 could further be seen from the increase in the residual VFA in the UASB reactor 1 (Figure 3.2). The residual VFA in UASB reactor 2 and 3 remained low and continued decreasing as the reactors approached steady state conditions. Reduction of amount of oxygenation in UASB

reactor 1 resulted in reduction of residual VFA suggesting the removal of inhibition.

### Effect of Oxygenation on Biomass Production

Of interest was the behaviour of biomass in the reactors. When the production of biogas nearly stopped in UASB reactor 1 during period 2, it would be expected that biomass growth had slowed down. Contrary to this idea, overall biomass (total sludge-bed) growth was more at all times in UASB reactor 1 than those in the other two reactors (Figure 3.5). What was more notable was the amount of sludge washout in UASB reactor 1. There was at all times loss of sludge in this reactor until beginning of period 3 (day 48-72) when the

washout was as much as in the UASB reactor 2 and 3 due to the interruption of vigorous aeration. There was no significant difference in sludge washout in UASB reactor 2 and 3 (Figure 3.3).

Visual observations of the biomass bed in the reactors during the experiment distinguished two layers. The layer for large granules (bottom) and the layer for small granules (top). Figure 3.5 shows the total layer (large granules and small granules). There was fluffy material (fine flocs) in UASB reactor 3, but these were completely absent in the UASB reactor 1 and 2, perhaps due to the higher aeration. Figure 3.6 shows the profiles of a daily oxygen to sludge ratio (OSR) during experimental period.

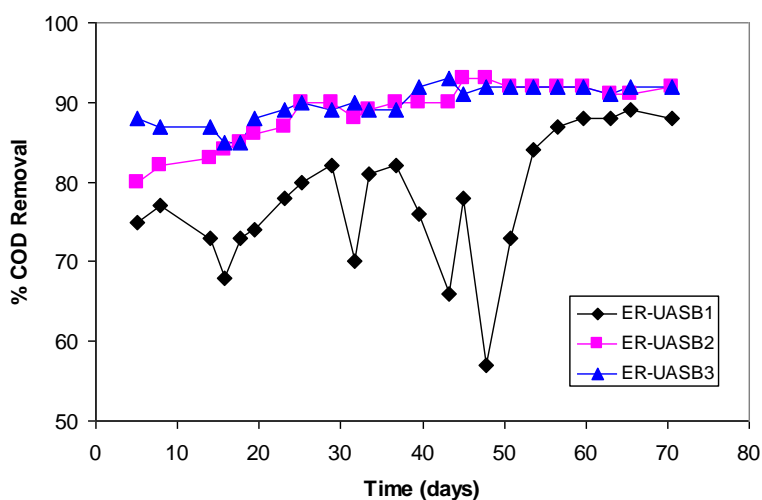


Fig 3. Profiles of soluble COD removal

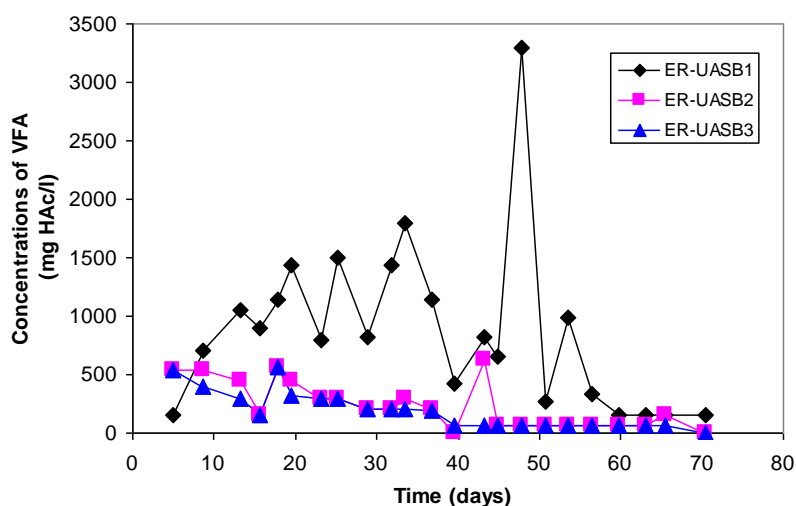


Fig 4. Profiles of residual VFA

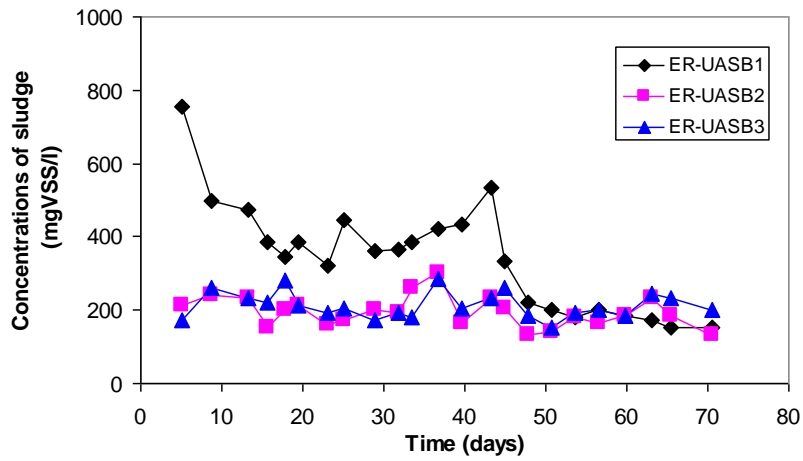


Fig 5. profiles of sludge washout

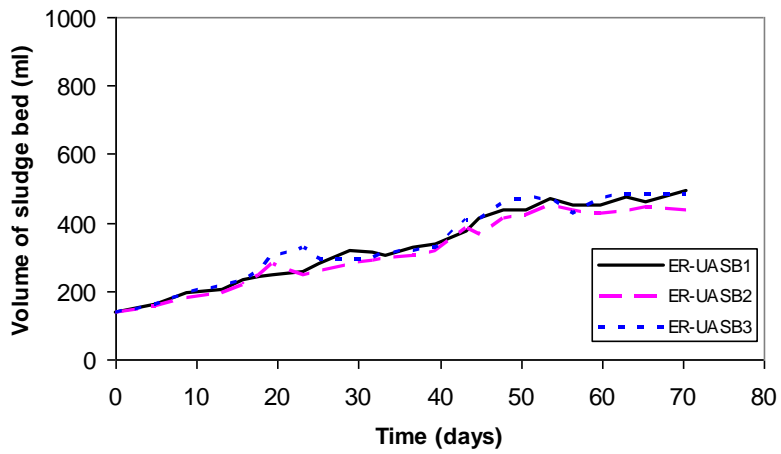


Fig 5. Profiles of granular sludge-bed

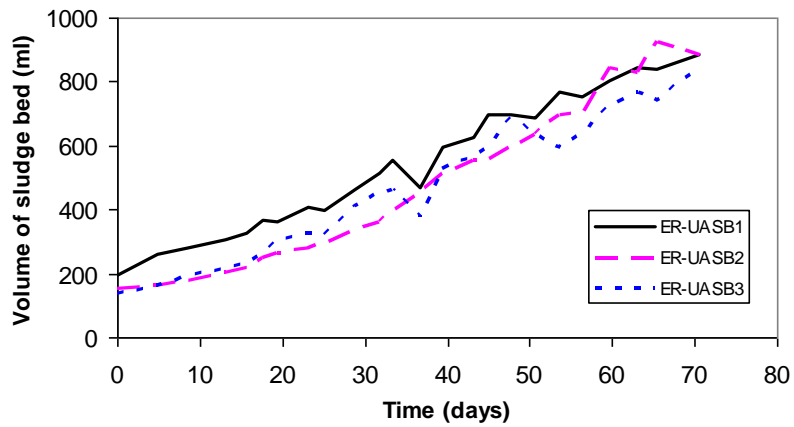


Fig 6. Profiles of total sludge-bed

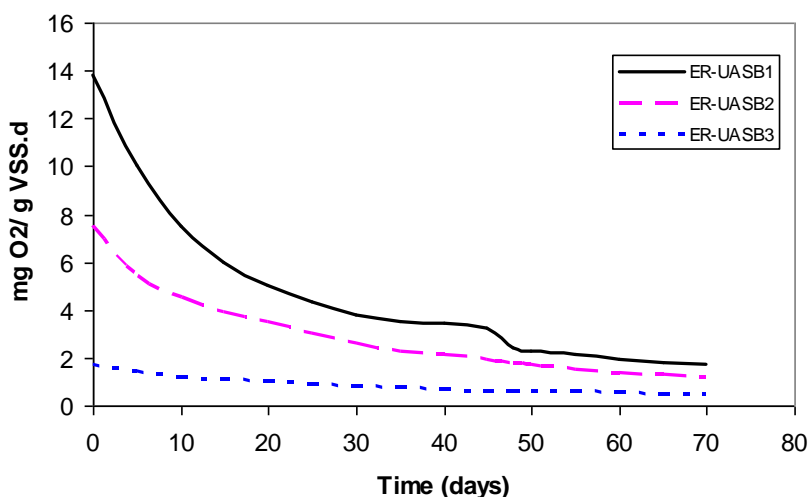


Fig 7. Profiles of daily oxygen to sludge ratio

## CONCLUSIONS

The importance of this study to control the operations of anaerobic wastewater treatment systems cannot be emphasized more. As has been already mentioned success of these systems really depends on the aggregation of bacteria into compact granules. From the study of different degrees of aeration it has been shown that at proper range of oxygenation i.e., by means of the daily oxygen to sludge ratio (OSR) ( $\text{mgO}_2/\text{g VSS.d}$ ), the ER-UASB reactor can be operated and at the same time maintain its methanogenic consortia.

To build up biomass (granules) in either a conventional UASB reactor (closed system) or an ER-UASB reactor during start up period, temporary application of extra oxygenation might accelerate a colonization of facultative microbes as a first step, before a fully methanogenic step is applied later.

## ACKNOWLEDGEMENT

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## LIST OF ABBREVIATIONS

ATP	Adenosine Tri Phosphate
COD	Chemical Oxygen Demands
ECP	Extracellular polymers
ER-UASB	External Recycle - Upflow Anaerobic Sludge Blanket
OSR	daily oxygen to sludge ratio
UASB	Upflow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solids

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