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## IMMOBILISATION OF ACTIVATED SLUDGE IN A COLUMN TYPE UPFLOW ANAEROBIC SLUDGE BLANKET REACTOR

Juni Agustian\*

### ABSTRACT

Development of immobilised microorganisms in UASB reactor relies on the formation and the stability of highly settleable immobilised microorganisms' sludge. One of the factors that influence these mechanisms is the type of inoculum. This experiment used the activated sludge to develop anaerobic sludge and aimed to observe the process of immobilisation of the activated sludge and to determine the capacity of the formed anaerobic cells. Observation indicated that immobilisation of the activated sludge required proper immobilisation strategy. Concentration of anaerobic sludge were around 17.00 g-TSS/L at pH of 6.60-6.90, which were suitable for the UASB operation, but the efficiencies of decomposition were low (5-37%). Approximately 1 (one) day was required by microorganisms to recover from effects of changes of influent flow rates. The best flow rates for the cultivation were between 0.30-0.50 L/h.

**Keywords:** immobilisation of activated sludge, characteristics of biomass

### ABSTRAK

Pengembangan mikroorganisme terimobilisasi di reaktor UASB bergantung pada pembentukan dan penstabilan lumpur mikroorganisme dengan kemampuan pengendapan yang tinggi. Salah satu faktor yang mempengaruhi mekanisme tersebut adalah jenis inokulum. Eksperimen ini menggunakan lumpur aktif untuk menghasilkan lumpur anaerobik dan bertujuan untuk mengamati peristiwa immobilisasi lumpur aktif dan memperhitungkan kemampuan sel anaerobik yang terbentuk. Observasi mengindikasikan bahwa inokulum lumpur aktif membutuhkan strategi immobilisasi yang tepat. Konsentrasi lumpur anaerobik adalah sekitar 17,00 g-TSS/L pada pH 6,60-6,90, kondisi ini cocok untuk pengoperasian reaktor UASB tetapi efisiensi dekomposisi relatif rendah (5-37%). Dibutuhkan waktu sekitar 1 (satu) hari agar mikroorganisme pulih dari efek perubahan laju alir influen. Laju alir terbaik selama proses tersebut adalah 0,30-0,50 L/jam.

**Kata kunci :** immobilisasi lumpur aktif, karakteristik biomassa

## I. INTRODUCTION

Recent applications of the Upflow Anaerobic Sludge Blanket (UASB) reactors prove their successful operations (Vieira et al. 1994; Fang et al. 1995). They have efficiently treated a lot of types of wastewater strengths such as ethanol distillery, domestic sewage, and instant coffee liquor (Callander et al. 1987; Quarmby and Foerster 1995). So the bioreactors have been accepted as high rate operations for the anaerobic biological treatment of medium to high strength wastewaters (Wiegand and Lettinga 1985). The key of their operations is development of sludge bed of immobilised microorganisms.

Formation and stabilisation of highly settleable sludge containing a lot of either flocks or dense granules or both determine the reactor performance. However, these mechanisms, which depend upon characteristics of wastewaters, operational variables, environmental factors, characteristics of seed material(s) and amount of seed materials, are not well understood yet.

## 2. IMMOBILISATION OF MICRO-ORGANISMS

The mechanisms of granulation are started when a cell attaches to a surface of another cell and/or inert material. Some steps are associated with the

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mechanisms i.e. cells transports, reversible adsorption, irreversible adhesion, and multiplication of cells (Schmidt and Ahring 1996). The attached cells firstly forms granule precursors (small bacterial aggregates that tend to washout from the reactors (Pol et al. 1982) and then grow to microorganisms granules (Callander and Barford 1983).

The process of cells immobilisation either to form loose or dense granules depends upon types of wastewater and operational conditions of the bioreactors. The average sizes are determined by superficial velocities, inorganic elements, hydrogen inhibitors (Uemura and Harada 1995), pH, sulphate inhibitors, effects of shock loading (Iliaszczuk et al. 1994), nutrients supplies, types of nutrients, granules growth rates, shear forces, production of extracellular biopolymers, gas generation, inorganic precipitation (Grotenhuis et al. 1991), types of inoculum and supporting particles (Wiegant and de Man 1980).

Supply of soluble carbohydrates such as glucose molasses accelerates the granulation process not only in medium strength wastewaters, but also in high strength wastewaters (Wu et al. 1987). However, this process might slow down when the influent contains suspended solids (Pohland and Malina 1992). Even distribution of the supplied substrate(s) inside the granules (to reach the microorganisms) and removal of produced gas from the sludge bed are required, but they depend on availability of pores on granule surfaces and the pores size. Insufficient substrate transports to the surface of the granules caused by low substrate penetration (that could initiate bacterial lysis), and increase of bubbles size lead to disintegration of the microorganisms granules (Kosaric et al. 1990; Alpheenaar et al. 1992). Although high loading rates and upflow velocities increase access of the granules to nutrients, washout of the small granules could occur in the reactor and the granules tend to become weaker (Quarby and Forster 1995; Kosaric et al. 1990; Guiot et al. 1988). Distribution of inorganic elements contained in the influent such as Ca, P, S, and Fe helps flocculation of the anaerobic granules (Uemura and Harada 1995; Lettinga et al. 1980).

Environmental pH should be maintained at favourable conditions for microorganisms. This is essential to cultivate an well-adapted granular sludge. It could be set at 7.0-7.5, but around 6.5-7.5 could be used because they still exhibit optimal activity (Wu et al. 1987). The pH between 5.0-6.5 is required for the acidogenesis step whether the methanogenesis needs above 6.5 (Pohland and Malina 1992).

The preferable material for an inoculum is granular sludge, but non-granular sludge could be used (Singh et al. 1998). Contents of calcium ions, filamentous microorganisms, and polysaccharides, and low capillary suction time increase immobilisation and performance of cells (Adebowale 1990). Activated sludge that contains higher methanogenic microbes could be used as the starting inoculum as well as cow manure, digested sewage sludge and mesophilic granular sludge (Wiegant and de Ma 1980; Wu et al. 1987).

The quantity of inoculum is around 40% of the reactor volume (Fang et al. 1995; Adebowale 1990). Higher quantity such as 75% could be inoculated (Fang and Cui 1993; Kwong and Fang 1996), but it leads to decrease of the granule sizes (Adebowale 1990).

### 3. AIMS OF EXPERIMENT

The experiment observed the process of immobilisation of activated sludge and to account for the formed anaerobic cells in decomposing sugar-containing influent in column type lab scale UASB reactors.

## 4. MATERIALS AND METHODS

### 4.1 Experimental Set-up

Equipment of the system consisted of an influent tank (20-L), a column type reactor (plastic, 6.4-L, working volume 5.5-L, ID 90-mm), a plastic gas funnel, a settler (1-L), an effluent reservoir (20-L), and a water column (5.0-L). Peristaltic pumps with speed adjuster were applied. A pH electrode was fixed in the middle part of the reactor. The diagram

of the UASB process used in the experiment was illustrated in Figure 1.

The reactor had a flat top- and bottom-head. Two lines were fitted on each head. At the bottom-head, a line was used as substrate port and another was drain port, whilst the top-head consisted of a main gas line from the gas funnel and an additional gas line. Three sampling ports were placed at the same distance i.e. 200-mm measured from the bottom head.

A spherical substrate distributor that had 50-mm diameter (ratio to column inside diameter was 0.55) and 10-mm height was used. It had 8 holes with 5-mm diameter along its circumference with a same distance from each other.

A gas collector made from plastic funnel, which had a diameter of cone around 80-mm, was placed on topside of the reactor (ratio to column diameter was 0.90) and 10-mm in diameter of neck installed at the height of 250-mm from the top. A 15-mm width and 5-mm height flat circular baffle was installed at 30-mm from the cone edge.

#### 4.2 Inoculum and Medium of Experiment

Activated sludge taken from a wastewater plant in Greater Manchester area was used to develop granules. It was allowed to adapt for several days aerobically to the medium. The inoculum was filtered to remove large materials, and then measured before it was transferred to the reactor.

Defined medium was based on MacLeod composition (MacLeod et al. 1990) except source of

sucrose was supplied by table sugar. The medium contained a substrate and sufficient amount of nutrients.

#### 4.3 Analysis of Experiment

The reactor was operated under various influent-loading rates. The influent was pumped at initial loading rate of 0.1 L/h and then increased. Visual and quantitative observations were carried out. Sludge quantities and dissolved solids contents, reactor pHs, and sucrose concentrations were respectively measured using the APHA 2540-D standard method (APHA 1985), Biolab 104/01/90 MKZ pH-meter, and RFM-390 digital refractometer. Visual observations were focused on the substrate distributor through sludge bed profile, and hydrodynamic conditions of the gas-liquid surface.

### 5. RESULTS AND DISCUSSION

#### 5.1 Strategy of Immobilisation

The process was started by transferring 2.40-L of the inoculum ( $\pm$  44% of the reactor working volume) from the top sampling port. Then influent was pumped at the initial loading rate, and was changed gradually during one month of the reactor operation.

Visual observation on the above strategy showed that loose aggregates contained in the inoculum tended to float. Lifting pressure created by upflow of the medium could cause this phenomenon. The initial upflow of the medium was set at a minimum condition based on specific activity of the inoculum to prevent washout of sludge. But this upflow

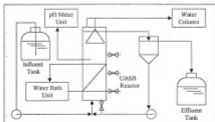


Figure 1. Diagram of the UASB system.

created heavy washout of sludge, so the initial measured biomass concentration available in the reactor was low ( $\pm 3.50$  g-TSS/L).

However, the phenomenon could also be caused by condition of the inoculum. Microscopic observation as drawn in figure 2 (left) indicated that the inoculum i.e. the activated sludge contained highly motile microorganisms so the inoculum was very loose. This condition supplemented a previous explanation (Wu et al. 1987) that the activated sludge is unstable and has a low density.

The strategy of immobilisation could influence this phenomenon as well. There is no clear explanation on how to start the reactor. Although incubating anaerobically the fresh activated sludge is suggested (Wu et al. 1987) that could increase the start-up time, no description of which was flowed initially to the reactor whether the substrate or the sludge or both was stated. Some strategies that could be very effective such as feeding the inoculum and medium

simultaneously or filling the medium into a certain quantity afterwards pumping the inoculum should be considered (Agustian 2000).

The heavy washout of microorganisms from the sludge bed at this period caused scum layer formation above gas separator. This layer could block gas movement from gas-solution surface so that collection of the gas would be low. However, it could be overcome by using of specific designs as suggested by Lettinga and Pol (1986) or illustrated by Fang et al. (1995).

## 5.2 Characteristics of Biomass

Development of the anaerobic sludge during the experiment was described in Figure 3. At the end of the experiment period, the sludge concentrations were around 17.00 g/L, which were suitable to initiate full operations of the UASB reactor (Wu et al. 1987; Lettinga et al. 1980; Singh et al. 1998). But in the first two weeks, the concentrations were

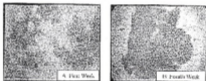


Figure 2. Comparison of the sludge condition (100 x magnifications).



Figure 3. Profile of the sludge concentrations.

low, i.e., below 10.00 g/L. The results indicated that immobilisation of the aerobic inoculum and growth of the anaerobic microorganisms occurred in the reactor.

In the experiment, minimal composition of the nutrients was presence in the medium. Double distilled water was used to dissolve the medium from the first day of the reactor operation until the day 18<sup>th</sup>. Analysis of the anaerobic sludge as described in Figure 3 and 4 showed that the sludge growth rate was low and the formed granules were brittle. To improve the biomass growth from the day 19<sup>th</sup> tap water containing some amounts of inorganic minerals (CEC 1980a) was used. The cell concentrations increased significantly.

Sedimentation of inorganic elements within the granule bed improved biomass flocculation.

During pumping of medium, the flow rates were changed gradually. The changes influenced the biomass sludge in which significant reductions of the concentrations occurred. Approximately 1 (one) day was required by the sludge to adapt to the changes as described in Figure 3. It was found that the flow rates of 0.30-0.50 L/h (equal to 1,500-2,000 g-COD/h) were the best leading rates in cultivation of the anaerobic sludge. Although the cell concentrations achieved the required concentrations for start-up of the UASB reactor, efficiencies of chemical decomposition were low. The efficiencies of sugar removal during the first three days as illustrated in Figure 5 were around 50%. Quick

fermentation of substrate occurred in order to form suitable environment for the process. However, in other periods, the efficiencies were only 5-37%. The results if compared to the general performance of the UASB reactors, which are around 75-85% COD reduction (Lettinga et al. 1980; Metcalf and Eddy 1991), were not good. These low performances could be caused by short adaptation of the sludge to the loading rates. Similar to the previous explanation, the decomposition efficiencies decreased when the influent rates were increased, but the sludge only required a short period, i.e., 1 (one) day to recover from the effect.

Bioreactor pH was found in the range of 6.60-6.90 as figured in Figure 6. The starting pH was alkaline, i.e. 8.46, that decreased rapidly during the immobilisation caused by substrate fermentation. Increase of the reactor pH occurred when the flow rates were changed. There were no supplies of acid or alkaline solution to regulate the pH because it had been in the recommended values for the UASB process besides alkalinity of medium.

## 6. CONCLUSIONS

- Successfulness of the UASB process in immobilising the activated sludge, which is loose and has a low density, depended upon the strategy of immobilisation. The poor strategy leads to heavy washout of the microorganisms and formation of scum layer.

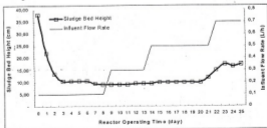


Figure 4. Profile of the sludge bed height.

- Proper formulation of the medium improved immobilisation of the activated sludge and growth of the anaerobic microorganisms in UASB reactor. The sludge concentrations and the reactor pH were around 17.00 g/L and 6.60-6.90 suitable to initiate full-operations of the UASB process. However, the efficiencies of substrate decomposition were low (5-37%).
- Approximately 1 (one) day was required by the microorganisms to recover from the effects of changes of influent flow rates in which the anaerobic sludge must adapt to the new rates. The best flow rates for the cultivation were between 0.30-0.50 L/h (~ 1,500-2,000 g-COD/h).

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Figure 5. The efficiencies of substrate degradation.



Figure 6. Profile of the bioreactor pH.

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