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ATP content and biomass activity in sequential anaerobic/aerobic reactors

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Abstract: Specific ATP content of volatile solids was measured to characterize the sludge activity in a sequential anaerobic/aerobic wastewater treatment system, with an upflow anaerobic sludge blanket (UASB) reactor and a three-phase aerobic fluidized bed (AFB) reactor. The wastewater COD level was 2000–3000 mg/L in simulation of real textile wastewater. The ATP content and the specific ATP contents of volatile solids at different heights of the UASB reactor and those of the suspended and immobilized biomass in the AFB reactor were measured. In the UASB reactor, the maximum value of specific ATP (0.85 mg ATP/g VS) was obtained at a hydraulic retention time (HRT) 7.14 h in the blanket solution. In the AFB reactor, the specific ATP content of suspended biomass was higher than that of immobilized biomass and increased with hydraulic retention time reaching a maximum value of 1.6 mg ATP/g VS at hydraulic retention time 4.35 h. The ATP content of anaerobes in the UASB effluent declined rapidly under aerobic conditions following a 2nd-order kinetic model.

Key words: ATP, Biomass activity, Sequential anaerobic/aerobic, UASB reactor, AFB reactor
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INTRODUCTION

Sequential anaerobic/aerobic wastewater treatment has received increasing attention for effective nutrient removal, enhanced degradation of recalcitrant xenobiotics and treatment of high biological oxygen demand (BOD) wastewater (Randall *et al*., 1997; Bhattacharya *et al*., 1996; Malaspina *et al*., 1995). The anaerobic digestion is a feasible unit operation for treatment of high-strength industrial wastewater with less sludge production and less energy consumption (Speece, 1983). An aerobic treatment is an essential step for pollutant biotransformation and process polishing (Zitomer and Speece, 1993). Obligate aerobes depend upon molecular oxygen for their metabolism whereas obligate anaerobes can only grow in an oxygen-free environment. It has been shown that under oxygen-limiting conditions, obligatory aerobic and anaerobic bacteria will survive in a mixed culture (Gerritse *et al*., 1992). Little is know about the change in the activity of anaerobic bacteria when they are exposed to aerobic conditions.

The quantity and activity of microorganisms in a bioreactor are two critical parameters which determine the reactor's performance in wastewater treatment. The conventional method of determining the biomass quantity is to measure the total suspended solid (TSS) or volatile suspended solid (VSS) (Ali *et al*., 1985). The measurement, however, does not distinguish the microbial mass from the insoluble organic wastes such as cellulose and starch and cannot reflect the metabolic activity of the microorganisms. Traditional plate count of microbes cultivated in nutrient agar can give the number of living cells in a water sample but it may underestimate the active cells due to medium selectivity or overestimate them due to growth of inactive cells within the reactor on the nutrient agar. Oxygen uptake rate (OUR) is an *in situ* indication of biomass activity of activated sludge (Jorgensen *et al*., 1992) but is not suitable for anaerobic biomass. The contents of biological compounds, such as proteins, DNA, NADH, and ATP in cellular mass have been used as alternatives to the dry biomass for estimation of biomass concentration (Agar, 1985). Adenosine triphosphate (ATP) exists only in viable cells as energy-storing macromolecules, and disappears quickly with cell death (Holm-Hansen and Booth, 1966). ATP measurement was proposed as a control parameter for aerobic biological processes such as activated sludge process (Roe *et al*., 1982; Kucnerowicz and Verstraete, 1979; Weddle and Jenkins, 1971). ATP was also found to reflect the activity of anaerobic digestion and respond similarly to other activity measurements such as gas production rate (Chung and Neethling, 1988). ATP content of living cells is dependent on environmental conditions, and reflects how active the cellular metabolism is.

This study is aimed at investigating the biomass activity in sequential anaerobic/aerobic reactors by measuring ATP content of biomass. We assessed the distribution of ATP content in an upflow anaerobic sludge blanket (UASB) reactor and a three-phase aerobic fluidized bed (AFB) reactor at different hydraulic retention times. We also measured the activity change of anaerobic bacteria when they were exposed to aerobic conditions and estimated the effect of anaerobic biomass from UASB on biomass activity in AFB reactor.

MATERIALS AND METHODS

Sequential UASB and AFB reactors

Fig.1 shows the UASB and AFB reactors which were made from glass. The small section of the UASB reactor had a working volume of 3 L (Φ85×520 mm) and the expanded section had a working volume of 4.5 L (Φ150×230 mm) for gas-liquid-solid separation. A layer (~10 cm thick) of

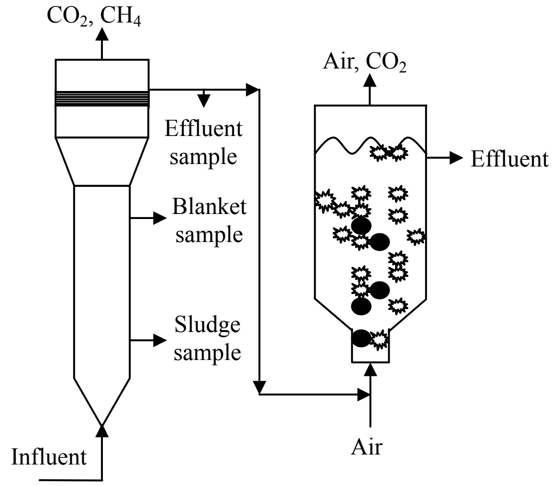


Fig.1 The schematic combination of a UASB reactor and a AFB rector

low-density polyethylene rings (ρ = 0.95 g/cm^3, Φ10×10 mm) floating on the water surface facilitated gas-liquid solid separation. Seven-liter seed sludge (14 g dry solid/L) taken from an anaerobic digestion tank of a textile wastewater treatment plant was transferred to the UASB reactor. Temperature, pH, biogas production, oxidation-reduction potential (ORP) of the UASB reactor were monitored on-line.

The effluent of the UASB reactor was fed into an aerobic fluidized bed (AFB) reactor made from a glass column (Φ150×600 mm) with a working volume of 6.6 L. Polyurethane sponge cubes (5×5×5 mm) were used as the immobilization carriers of microorganisms. The static volume of total carriers was 50% of the reactor working volume. The carriers were fluidized in the reactor by air that was introduced at the reactor bottom and dispersed with a gas diffusing stone. Temperature, pH, dissolved oxygen (DO) concentration and oxidation-reduction potential (ORP) of the AFB reactor were monitored on-line.

Continuous flow operation

A synthetic wastewater at COD concentration of 2000–3000 mg/L was prepared. The wastewater composition is shown in Table 1. The solution was fed into the sequential reactor system at nine hydraulic retention times (from 1.4 h to 20 h for UASB and from 1.3 h to 16.7 h for AFB) over a period of 9

Table 1 Composition of synthetic starch wastewater (Malaspina *et al.*, 1995)

Component	Concentration (mg/L)
Starch	2000–3000
NH_4HCO_3	1000
KH_2PO_4	150
K_2HPO_4	350
$MgCl_2·6H_2O$	100
$CoCl_2·6H_2O$	0.125
$CaCl_2$	260
$MnSO_4·4H_2O$	15
$FeSO_4·7H_2O$	25
$NiCl_2·6H_2O$	80
$ZnCl_2$	6

weeks. Following each change in hydraulic retention time, the culture was allowed to stabilize for 3–4 times of retention time. The specific ATP contents (SATP) of the solids in UASB and AFB reactors were measured at regular time intervals. The results indicated that a steady state had been reached after a time period of 3–4 times of retention time.

ATP assay

ATP was extracted from living cells by boiling a biomass sample in a tris-EDTA solution containing 20 mmol/L tris [tris(hydroxymethyl) methylamine] and 2 mmol/L EDTA (ethandiaminotetra-acetic acid) in double-distilled water at pH 7.75 (Lundin and Thore, 1975). ATP content was determined based on luciferin-luciferase reaction (Leach, 1981). The luminescence intensity from the reaction was proportional to ATP concentration in the assay solution and measured with a monolight 2010 bioluminometer (Analytical Luminescence Laboratory, USA). A calibration curve was prepared from ATP disodium salt in double distilled water. The overall uncertainty of the assay including both extraction and reaction, quoted as the standard deviation of four separate determinations, was 14% at the level of 0.5 μmol/L ATP. The uncertainty of the reaction, quoted as the standard deviation of five determinations, was 3% at the level of 0.5 μmol/L ATP.

Specific ATP

Samples were collected at three different heights to measure the distribution of biomass activity in the UASB reactor: one in the sludge bed, one in the blanket solution and one in the effluent. High biomass concentration samples were first diluted by 10 times before the extraction. The specific ATP defined as mg ATP per gram dry solids was calculated from the concentration of ATP and total volatile solids (VS).

The ATP content of suspended biomass in the AFB reactor was determined as described above. For immobilized biomass, several sponge cubes were collected from the reactor, washed with distilled water to remove suspended cells attached on the carrier surface, and placed in a test tube filled with water. By squeezing the sponges, the immobilized biomass in the sponge cubes was released into the water. An aliquot of the released biomass was used for ATP determination and the rest for volatile solids determination. All measurements were made in duplicate.

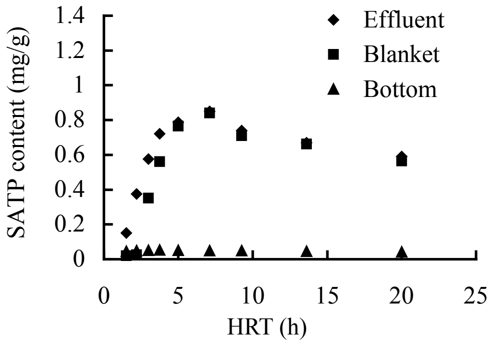
Others

The measurement of total solids (TS) and volatile (suspended) solids (VS(S)) followed that in the Standard Methods (1992).

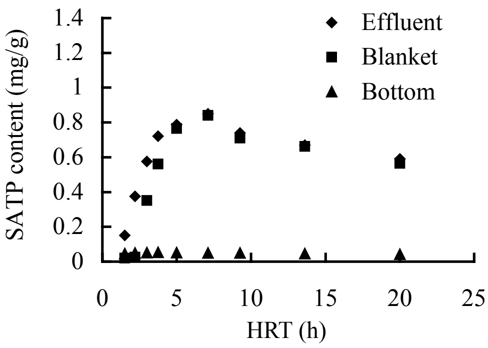
RESULTS AND DISCUSSION

ATP contents in UASB reactor

Fig.2a and 2b show the specific ATP contents and ATP concentrations in sludge bed, blanket solution and effluent of the UASB reactor at different hydraulic retention times. The specific ATP content based on total volatile solids (VS) in blanket solution was at the same level as that in the effluent, and much higher than the SATP content in the sludge bed as shown in Fig.2a. However, the ATP concentration based on the reactor volume of the sludge bed was in the same range of the blanket solution and effluent (Fig.2b) due to the high volatile solids concentration in the sludge bed (Table 2). The average specific ATP content in the sludge bed was around 0.047 mg ATP/g VS. The total VS consisted of biologically decomposable matters, dead or living cell mass, and inert solids. Due to the insoluble starch's high concentration of particulate



(a)



(b)

Fig.2 (a) Specific ATP contents (b) ATP concentrations at different hydraulic retention times in UASB reactor

Table 2 Variation of sludge profile at different hydraulic retention times (HRT) in UASB (VS: mg/L)

HRT	10 h	5 h	2.2 h	1.4 h
Sludge bed	40.3	40.0	37.8	37.8
Blanket	0.99	1.13	30.2	34.5
Effluent	0.91	0.95	1.29	1.49

matter in the feed solution and the sedimentation of large granules from the blanket solution, volatile solids in the sludge bed contained a large amount of volatile but inactive solids. Up to 40% to 50% of the VS in the anaerobic sludge was not active biomass estimated by Chung and Neethling (1990). The maximum SATP value of the blanket solution (0.85 mg ATP/g VS) in this study was in close agreement with values obtained by Chung and Neethling (1990). They estimated the biomass VS using a kinetic model of anaerobic sludge, and reported that the viability of the anaerobic sludge based on the biomass fraction of VS ranged from 0.76 to 0.99 mg ATP/g VS. It showed that most of the biomass in the blanket was active at hydraulic retention time 7.1 h.

At short hydraulic retention times, the living cells metabolism should reach its maximum and specific ATP content of biomass should approach a constant as reported by Chung and Neethling (1988). The decrease in specific ATP contents under hydraulic retention time at 7.1 h was probably due to the fact that the levels of VSS in the blanket solution and effluent rose at the short hydraulic retention times (Table 2); and that most of the VSS were inactive solids such as dead cells and inter organic solids (insoluble starch). The effect of hydraulic retention time on SATP in the sludge bed was not as significant as that on the SATP in blanket solution. The SATP in the sludge bed slightly decreased at long hydraulic retention time.

ATP contents in AFB reactor

The immobilized biomass growth was affected by the external substrate flux into the carriers. At short hydraulic retention time (high organic loading rate), the substrate flux into the carriers was also high, leading to high substrate concentration and metabolic activity within the carriers. This was confirmed by the fact that the specific ATP content of the immobilized biomass was increased from 0.6 mg ATP/g VS to 1.21 mg ATP/g VS as hydraulic retention time decreased in the AFB reactor (Fig.3). The specific ATP content of the suspended biomass was higher than that of the immobilized biomass at the same hydraulic retention time. In addition to the possible mass transfer resistance as described above, other biological factors might also contribute to

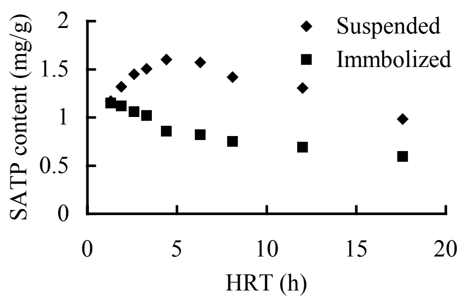


Fig.3 Specific ATP contents at different hydraulic retention time in AFB reactor

this phenomenon. The suspended biomass present in the AFB reactor came from the attrition of the immobilized biomass, the reproduction of suspended microbes and the effluent from the UASB reactor. The specific ATP of those anaerobic microorganisms in the UASB effluent might change significantly under anaerobic conditions. The specific ATP content increased from 0.98 mg ATP/g VS and reached a peak value of 1.6 mg ATP/g VS at hydraulic retention time 4.34 h, and decreased. This result did not agree well with the data reported by Gaikas and Livington (1993). They found that the specific ATP content of suspended biomass in a single three-phase-air-lift reactor increased with decrease of hydraulic retention time and approached a maximum.

Influence of anaerobes on ATP contents in AFB reactor

In sequential anaerobic/aerobic treatment, the end products of anaerobic fermentation including anaerobic microorganisms were directly fed into the aerobic reactor. The activity of anaerobes under aerobic conditions might affect the specific ATP of suspended solids in the AFBR and was investigated in a batch experiment. One liter blanket solution was collected from the UASB reactor under air-free conditions. Air was introduced into the solution through a gas diffusing stone and the dissolved oxygen was maintained above 5 mg/L. SATP, ORP, and COD were determined at different time intervals. The results of the experiment are depicted in Fig.4. The ATP content declined rapidly during the first hour aeration (70% drop) and approached a constant (90% drop). Dissolved oxygen (DO) and oxidation-reduction potential (ORP) in the solution increased to their maximum values of 5 mg/L and 70 mv after 30 min, which were in the same range with those in the AFBR, but decrease of COD value in the effluent was negligible. When the microorganisms were subjected to a physiological shock created by oxygen stress, they were not able to produce the essential energy and have to use their ATP reserves for maintenance. The decrease of ATP concentration [ATP] with time was in accordance with a 2nd-order kinetic model:

$$-\frac{\mathrm{d}[ATP]}{\mathrm{d}t}=k_d[ATP]^2 \quad (1)$$

$$[ATP]=\frac{[ATP]_0}{k_d t[ATP]_0+1} \quad (2)$$

Where k_d refers to the specific reaction rate and its value of 1.44 (L/mg ATP·h) was determined with a linear Eq.(2) as shown in Fig.5. The data fitted well a 2nd-order kinetic model (R^2=0.9919). By using this ATP decaying model, the ATP concentration of the active anaerobes in the AFBR was calculated at different hydraulic retention times (not shown here). The results showed that the remnant of the ATP concentration of anaerobic bacteria in the AFBR was very low and could be neglected compared with the ATP concentration of suspended biomass in the AFBR. However, The presence of large amount of anaerobic biomass in the AFB reactor led to a low specific ATP content of the whole biomass, in particular at a short retention time.

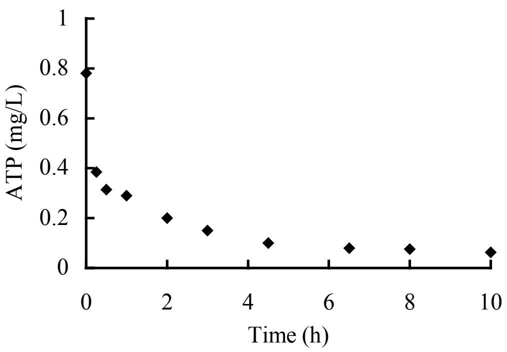


Fig.4 Decrease of ATP content of anaerobes under aerobic condition

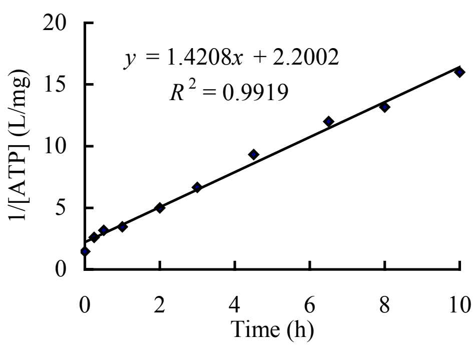


Fig.5 Fitting the ATP decrease with a second order kinetic model

CONCLUSION

The following conclusions can be drawn from the sequential anaerobic/aerobic (UASB/AFBR) treatment of a simulated textile wastewater:

1. Specific ATP content in the blanket solution of the UASB reactor was at the same level as that in the effluent, and much higher than that in the sludge bed. The low level of specific ATP content in the sludge bed was due to the high content of inactive volatile solids in the sludge.

2. Specific ATP contents in the blanket solution and effluent increased with decrease of hydraulic retention times, but dropped rapidly at hydraulic retention times under 7.14 h due to the increase of VSS at short hydraulic retention times. The effect of hydraulic retention time on the specific ATP content in the sludge bed was not significant.

3. In the AFB reactor, the specific ATP content of the suspended biomass was higher than that of the immobilized biomass at the same hydraulic retention time.

4. The influence of anaerobic bacteria on ATP content in the AFB reactor was very low and could be neglected compared with the ATP concentrations of suspended biomass in the AFBR.

5. ATP is a good indicator of biomass activity in UASB and AFB reactors.

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