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ATP as an indicator of biomass activity in thermophilic upflow anaerobic sludge blanket reactor

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Abstract: This work investigated the biomass activity in a thermophilic upflow anaerobic sludge blanket (UASB) reactor of wastewater treatment. Synthetic textile wastewater with pH 10—11, COD level of 2000—3000 mg/L was tested. Cellular adenosine triphosphate (ATP) in volatile solids (VS; mg ATP/gVS) was measured and expressed as specific ATP content to compare the biomass activity in up zone and lower zone in UASB reactor. The result shows that the specific ATP content based on total volatile solids (VS) in lower zone (0.046 mgATP/gVS average) is much lower than that in up zone (0.62 mgATP/gVS average) due to high content of inactive biomass and high pH in lower zone. The SATP in up zone increases as HRT increases and approaches to a maximum value of 0.85 mgATP/gVS at HRT of 7h, then decreases. It shows most of the total VS in up zone represent active bacterial biomass at HRT of 7h. Rate of subtract utilization is directly related to the activity of microorganisms in the reactor. The effect of HRT on SATP in lower zone is not as significant as on SATP in up zone. The buffer capacity of the thermophilic UASB reactor is very good. It is the activity of sludge granules in lower zone that give the UASB reactor such a good buffer capacity to the inlet high pH.

Key words: ATP; biomass activity; UASB reactor

Introduction

The quantity and activity of microorganisms presented in a bioreactor are two critical parameters that determine the reactor performance in wastewater treatment. The conventional parameter for biomass quantity measures is total suspended solid (TSS) or volatile suspended solid (VSS; Ali, 1985). The measurement, however, does not distinguish the microbial mass from the insoluble organic wastes such as cellulose and starch and can not reflect the metabolic activity of the microorganisms. Oxygen uptake rate (OUR) is an *in situ* indication of biomass activity of activated sludge (Jφrgensen, 1992) but 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, 1966). ATP measurement has been proposed as a control parameter for aerobic biological processes such as activated sludge process (Roe, 1982; Kucnerowicz, 1979; Weddle, 1971). It has been shown that ATP content per viable cell remained constant over a wide range of growth rate and that ATP could be used as a rapid and convenient indicator of the viable organisms in activated sludge. ATP was also found to reflect the activity of anaerobic digestion and respond similarly to other activity measurements such as gas production rate (Chung, 1988). Although ATP content of living cells is dependent on environmental conditions, it does reflect how active the cellular metabolism is.

The thermophilic upflow anaerobic sludge blanket (UASB) reactor showed a stable performance on hydrolysis and acidogenesis of textile wastewater which contains a large amount of starch and with high influent pH (10—11; Ji, 1998). This study is aimed at investigating the biomass activity in a thermophilic upflow anaerobic sludge blanket (UASB) reactor in treatment of high pH textile wastewater. We assessed the ATP concentrations and specific ATP contents of biomass in up zone and lower zone at different hydraulic retention times in UASB reactor. Effect of pH on ATP or biomass activity was also investigated.

1 Materials and methods

1.1 Thermophilic upflow anaerobic sludge blanket (UASB) reactor

Fig. 1 shows the schematic structure of UASB reactor made of plexiglass.

The reaction section of the UASB reactor with a volume of 3L (ϕ85 × 520 mm) had a water jacket and its temperature was kept at 55℃. The expanded section has a working volume of 4.5L (ϕ150 × 230 mm) for gas-liquid-solid separation. A layer (~10 cm thick) of low-density polyethylene rings ($\rho = 0.95$ g/cm^3, ϕ10 × 10 mm) floated on the water surface to facilitate gas-liquid solid separation. Seven-liter seed sludge (14g dry solid/L) taken from an anaerobic digestion tank of a textile wastewater treatment plant was first cultured at temperature from 25℃ to 55℃ over 10 days under anaerobic conditions and then fed into the UASB reactor. The reactor was then fed continuously with a synthetic starch wastewater. Temperature, pH, biogas production, oxidation-reduction potential (ORP) in the UASB reactor were monitored on-line.

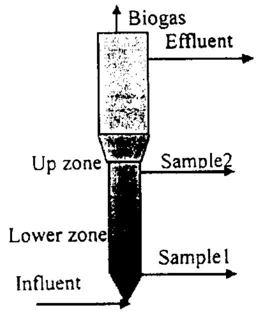

Fig. 1 The schematic structure of upflow anaerobic sludge blanket (UASB) reactor

1.2 Continuous flow operation

A synthetic wastewater at COD concentration of 2000—3000 mg/L was prepared. Its composition is shown in Table 1 and its pH was adjusted to 11 with NaOH. The solution was fed into the reactor system at different flow rate to create different HRT (from 25h to 3h) over a period of 9 weeks. Following each change in HRT, the UASB was run for 3—4 times of the related HRT. The specific ATP content (SATP) of biomass in UASB reactor was measured at a regular time interval along with the measurements of chemical oxygen demand (COD) and suspended solids. The results indicated that a steady state had been reached after a time period of 3—4 times of residence time.

1.3 ATP assay

Table 1 Composition of synthetic starch wastewater

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

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, 1975). 1 ml sludge was removed from the reactor and mixed with 9 ml boiling tris-EDTA solution in about 1 minute to minimize ATP consumption. The extraction was conducted for 90—120 seconds and cooled in an ice bath. The extract solution was adjusted to 10 ml with tris-EDTA solution before ATP assay. 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. 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 five 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 0.5μmol/L ATP.

1.4 Specific ATP in reactor

Samples were collected at two different heights (up zone and lower zone) to measure the distribution of biomass activity in the UASB reactor. For the samples of high biomass concentration, they were diluted by 10 times first before the extraction. The specific ATP defined as mgATP/gDS was calculated from the concentration of ATP and total volatile solids (VS). All measurements were made in duplicate.

1.5 Others

Total solids (TS), volatile solids (VS) and COD was measured following the Standard Methods (Standard methods, 1992).

2 Results and discussion

2.1 ATP, HRT and COD removal

Fig.2(a and b) illustrates the specific ATP contents and ATP concentrations in up zone and lower zone in UASB reactor at different hydraulic retention times. Fig. 3 illustrates the specific COD removal rate and the COD removal rate at different hydraulic retention times. The specific ATP content based on total volatile solids (VS) in up zone is much higher than SATP content in lower zone as illustrate in Fig. 2a. The reason is that the total VS consists of biodegradable matters, dead or living cell mass, and inert solids. The high concentration of particulate matter such as insoluble starch in the feed solution settled from the up zone to the lower zone which caused the volatile solids in lower zone contained a large amount of volatile but inactive solids.

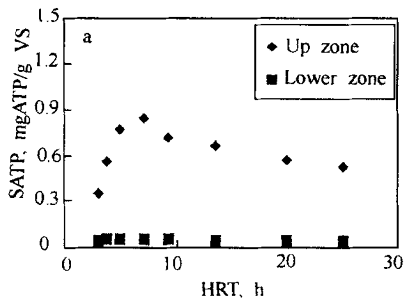

Fig.2a Specific ATP contents of up zone and lower zone at different HRT

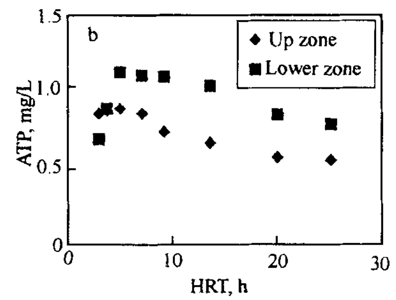

Fig.2b ATP concentrations of up zone and lower zone at different HRT

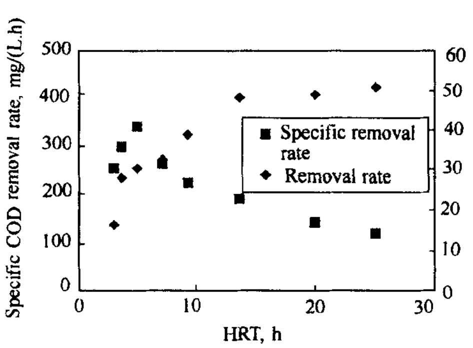

Fig.3 Specific COD removal rate and removal rate at different HRT

The effect of HRT on SATP in lower zone is not as significant as on SATP in up zone. The SATP in up zone increases as HRT increases and approaches to a maximum value of 0.85 mgATP/gVS at a HRT of 7h, then decreases. A long retention time (or low dilution rate) favors the COD removal rate. The COD removal was mainly due to the formation of gaseous methane and carbon dioxide under anaerobic conditions (Ji, 1998). Since methanogenic bacteria grow much slower than acidogenic bacteria, a long retention time favors the formation of methane and hence COD removal. It is interesting to note that both ATP concentration and specific ATP content of biomass in the reactor are not at their maximum levels corresponding to the highest COD removal as shown in Fig. 3. The ATP content of methanogenic bacteria is relatively low compared to the acidogenic bacteria and its cells are in starvation under such long retention times. Actually, no residual starch or glucose was detected in the effluent under the long retention time (>10h) while total fatty acid concentration reached 500 mg/L (Ji, 1998). At shorter retention time (<5h), the COD removal rate and specific ATP content in the reactor decline with HRT. A washout caused by high flow rate (short retention time) might be the reason. This assumption is confirmed by two phenomena. First, the levels of VS in up zone elevate at the short retention time (Table 2) and most of the VS are inactive solids such as dead cells and inert organic solids (insoluble starch). Secondly, a pH drift is observed at short HRT as listed in Table 2. Rate of substrate utilization is directly related to the activity of microorganisms in the reactor. Specific COD removal rate increases as HRT increases, and reaches a maximum value, then decreases. It fits well with the change of ATP content with HRT (Fig.2a).

Table 2 Variation of sludge profile at different HRT in UASB

	HRT=20h		HRT=10h		HRT=7h		HRT=3h	
	VS,g/L	pH	VS,g/L	pH	VS,g/L	pH	VS,g/L	pH
Lower zone	40.30	11.2	40.00	10.7	37.80	10.9	37.80	10.8
Up zone	1.21	7.0	1.30	6.8	1.50	6.5	2.50	7.9

The maximum SATP value in up zone (0.85 mgATP/gVS) in this study is in close agreement with values obtained by Chung *et al*. (Chung, 1990). They estimated the biomass VS using a kinetic model of anaerobic sludge, and reported that the viability of the anaerobic sludge based on biomass fraction of VS ranged from 0.76 to 0.99 mgATP/gVS. It shows most of the total VS in up zone represent active bacterial mass at HRT of 7h.

2.2 ATP and pH

pH is a principal operation parameter for biological wastewater treatment. Since the bacteria are highly sensitive to pH. To simulate the real wastewater of textile plant, the pH of inlet solution was kept at 10—11. The high pH in up zone at short HRT was caused by unbalanced neutralization by the fatty acids formed under anaerobic conditions, which might lead to low the cell activity and specific ATP content. A batch test was conducted to confirm this assumption. Sludge sample was taken from the up zone of UASB reactor and injected into several bottles immediately under air-free conditions. The solution pH was adjusted to 4—10 with HCl or NaOH solution from which the dissolved oxygen had been removed by nitrogen stripping. ATP was determined for the sample taken from each bottle after 2h. The result is shown in Fig. 4, that demonstrates the ATP concentration increases considerably with pH, when pH< 7, but decreases while pH>7. The average specific ATP content in lower zone is around 0.046 mgATP/gVS, which is too low compared with the SAPT of up zone (0. 62 mgATP/gVS average). Up to 40% to 50% of the VS in anaerobic sludge were not active biomass estimated by Chung and his coworkers (Chung, 1990). In addition to the relative high content of inactive volatile solids in lower zone, which might cause the relative low specific ATP, the high pH (10—11) in the feed solution is another reason that lead to low specific ATP content. The SATP in up zone remains at a constant high level (as listed in Fig.2a) corresponding to a relatively stable pH (6—7.5) at HRT>5h (Table 2). But the ATP concentration in lower zone is in the same range with that in up zone (Fig.2b) due to the high volatile solids concentration in lower zone (Table 2). It means that the lower zone in UASB reactor has an excellent capacity in neutralization of the high pH wastewater fed into the reactor bottom. The buffer capacity of UASB reactor is very good even though the influent pH is maintained around 11 as shown in Table 2. The biomass in lower zone of UASB reactor either has been adapted to a high pH environment or the local pH within the granules was much lower than the pH in bulk solution. It is the activity of sludge granules that give the UASB reactor such a good buffer capacity to the high pH inlet flow. At a reasonable loading (HRT>5), the UASB can treat a high pH wastewater directly without preneutralizaiton. It may save a significant amount of acid. The key point to maintain a high COD removal rate and high cell acidity is to neutralize the solution with formed fatty acid. Otherwise, the metabolic activity of cells would deteriorate quickly.

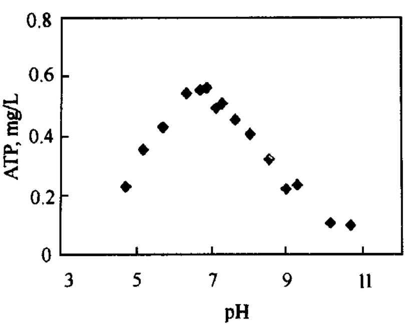

Fig.4 Effect of pH on ATP concentration

3 Conclusion

The thermophilic upflow anaerobic sludge blanket (UASB) reactor shows a stable performance on treatment of high pH textile wastewater. The specific ATP of biomass in up zone increases as

HRT increases and approaches to a maximum value of 0.85 mgATP/gVS at a HRT of 7h, then decreases. It fits well with the change of specific COD removal rate with HRT. Most of the total VS in up zone represent active bacterial biomass at HRT of 7h. The SATP in lower zone is much lower than that in up zone due to high content of inactive biomass and high pH. The SATP in up zone remains at a constant high level to a relatively stable pH (6—7.5) even though the influent pH is maintained around 11. It means that the sludge bed has an excellent capacity in neutralization of the high pH wastewater fed into the reactor bottom at a reasonable loading. It is concluded that the specific ATP content can reflect the metabolic activity of microorganisms and is a rapid and convenient indicator of biomass activity in UASB wastewater treatment system.

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