Concentration of Organic Matter Effect on Activated Sludge PHB Accumulation

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Abstract—**Effective PHB accumulation requires a stoichiometric balance between organic carbon and nitrogen. This balance is often expressed in terms of COD/N ratio of the influent. The objective of the work reported here was to determine the optimal COD and COD/N ratio for high efficient production of PHB. Compared influent initial COD concentrations from 200 to 1000 mg·L-1, results indicated that the COD 600 were more propitious to PHB accumulation. Simultaneity, compared the influent initial COD/N ratios of 100, 200, 300, and 400 respectively, results showed COD/N ratio of 200 was more favorable acetate fully converted into PHB in aerobic open mixed cultures.**

Keywords-Activated sludge; COD; COD/N; PHB accumulation

I. INTRODUCTION

Activated sludge is an inevitable product of biological wastewater treatment processes and which can pollute the environment and affect human health. While under specifically conditions, the activated sludge could be synthesize biodegradable plastics (polyhydroxyalkanoates, PHAs) by numerous microorganisms. PHA is energy and carbon storage material accumulated under unfavorable growth conditions. Under normal growth conditions, PHB content in the cells is usually 2–10% weight of the dry cell weight. However, PHB content can reach up to 80% of the dry cell weight if growth is limited by the depletion of an essential nutritional compound such as nitrogen, phosphorus, sulfur, or magnesium. Therefore, nutrient limitation is often necessary for high yield and productivity of PHB. In literature studies, nutrients were usually fed in the reactor intermittently or constantly in response to dissolved oxygen or pH as a feedback parameter. In these approaches, however, substrate(s) (nitrogen and/or phosphorus) concentration in the culture broth could not be precisely maintained due to the nature of indirect estimation. However, for efficient production of PHB, non-limiting concentration of carbon source and limiting concentration of nitrogen are needed [1]. In order to identify the appropriate feed time, its concentration and addition rate mathematical model-based cultivation approach is very useful to do correlative experiment.

Albuquerque et al [2]found that if the supplementation with a nitrogen source was not sufficient (nitrogen limitation), no stable PHA storing culture could be obtained. On the other hand, other studies with cultures grown on acetate did show that stable PHA producing cultures with fairly high PHA storage capacities can be enriched with nutrient limited feeds[3]. Similarly, Chua[4] achieved a PHA content of 39% of the sludge is dry mass by using a chemically processed wastewater that contained xenobiotic organics at a COD/N (COD: chemical oxygen demand) ratio of 140. The influence of carbon versus nutrient limitation on the enrichment of a PHA producing culture requires thus further investigation. We investigated the influence of the COD to nitrogen ratio (COD/N ratio) of the medium on the performance of a mixed culture in a selection reactor and on the culture's PHA production capacity. We performed culture selection experiments in sequencing batch reactors (SBR) at a range of COD/N ratios from carbon to nitrogen-limited with acetate as the substrate. When a stable reactor operation was obtained the reactor behaviour was documented by measurements of the relevant state variables. The selected cultures were subsequently transferred into nutrient limited fed-batch reactors in order to evaluate the capacity of the selected cultures to accumulate PHA.

II. MATERIALS AND METHODS

A. Operation Conditions

The cyclic activated sludge used in this study was obtained from the secondary sedimentation tank of a sewage treatment plant in WenChang Harbin, China, in which the anaerobic/aerobic disposal rector process was used. The initial natures of sludge were put into two 15L brown sequencing batch reactor (SBR) and sealed. The total reaction process period of 240 min. starting with fill phase for nutrient solution with acetate and nitrate in 1 min. And then including a sequence of anoxic and aerobic phases adjusted to 180 min and 120 min, respectively. The rest of the cycle included the sludge settling phase, and withdrawal phase of the treated effluent. Reactor was mechanically stirred at 200 rpm/min keep DO and was operated at room temperature ($25℃$). After 40 cycles domestication when the phosphate accumulating organisms reach to stability. The phosphate accumulating organisms were transfer to flask carried out different COD concentration and optimal COD/N ratio for high efficient PHB accumulation experiment. The influent COD from 200 to 1000 mg·L-1 was adjust by varying concentrations of sodium acetate. Nitrogen concentration was adjusted by varying concentrations of NH4Cl base on needed COD/N ratio. The nutrient solution was composed of 10g/L KH2PO4, 45g/L MgSO4·7H2O,

18g/L KCl, 60g/L CaCO3, 0.3mL/L trace elements solution according to Vishniac and Santer (1957) and 50 mg/L allylthiourea (to prevent nitrification).

B. Analytical methods

The concentration of dissolved oxygen DO in the reactor was measured with a DO electrode (Mettler Toledo) as percentage of air saturation. The amount of acid or base dosed for pH control was determined by using HORIBA Compact pH meter (Model B-212, Japan). Carbon dioxide and oxygen partial pressures in the gas entering and leaving the reactor were analyzed in dried gas with a gas analyzer (NGA 2000, Rosemount). Concentrations of COD in reactor samples were measured with standard methods [5]. PHB was measured by the improved method of hypochlorous-natrium chloroform. The amount of PHB was subtracted from the TSS to calculate the concentration of active biomass. The active biomass concentration was converted from g/L into carbon moles per liter (Cmol/L).

III. RESULTS AND DISCUSSION

### A. Confirm the optimal COD concentration

Activated sludge was domesticated in A/O SBR about 40 cycles. After the phosphate accumulating organisms reach to stability, the seeds were transfer to the flask. Adjusted sodium acetate made the COD concentration were 200, 400, 600 and 1000 respectively. As shown in Fig.1, the COD of each fermentation broth decline rapidly from the initial value during the first 5 hours. While 5 days later, the changes of COD values were not significantly. From the Fig.1-(a), we also knew that the COD removal rate could reach to 97.4% after 12 hours culture. However, the initial COD were 400, 600 and 1000, after 12 days culture COD removal rate were 88.6%, 78.8%, 44.6% respectively. Compared with initial COD 200, the initial COD 400, 600 were all with enough carbon substrate for the microbial storage polymers as the energy. Generally the phosphate accumulating organisms need external substrate converted into internal storage products (PHB). Shijian[6] used the influent COD of 308 ± 172 mg/L obtained the favorable nitrogen and phosphorus removal performance. Katarzyna[7]adopt the influent COD of the reactor is 430.0 ± 30.0 mg L-1. In this research, the subsequent experiment was carried out on the COD 600.

B. Confirm the optimal nitrogen concentration

Changes of COD in different nitrogen concentration

When the influent COD of the reactor is 600 mg L-1, adjust nitrogen concentration so as to COD/N ratio reach to 100/1, 200/1, 300/1 and 400/1 respectively. Fig. 2 shows COD variations in different nitrogen concentration wastewater in the reactor during the reaction. Curve profile indicated that the four concentration treatments with the same variation trends. The COD of each treatments decline rapidly from the initial value of 600 to 100 during the first 5h. The variation trend thereafter, declined relatively smooth at about 50 after 5 hours cultured. From the COD variations profile we can conclude that nitrogen concentration effect little on the carbon consume.
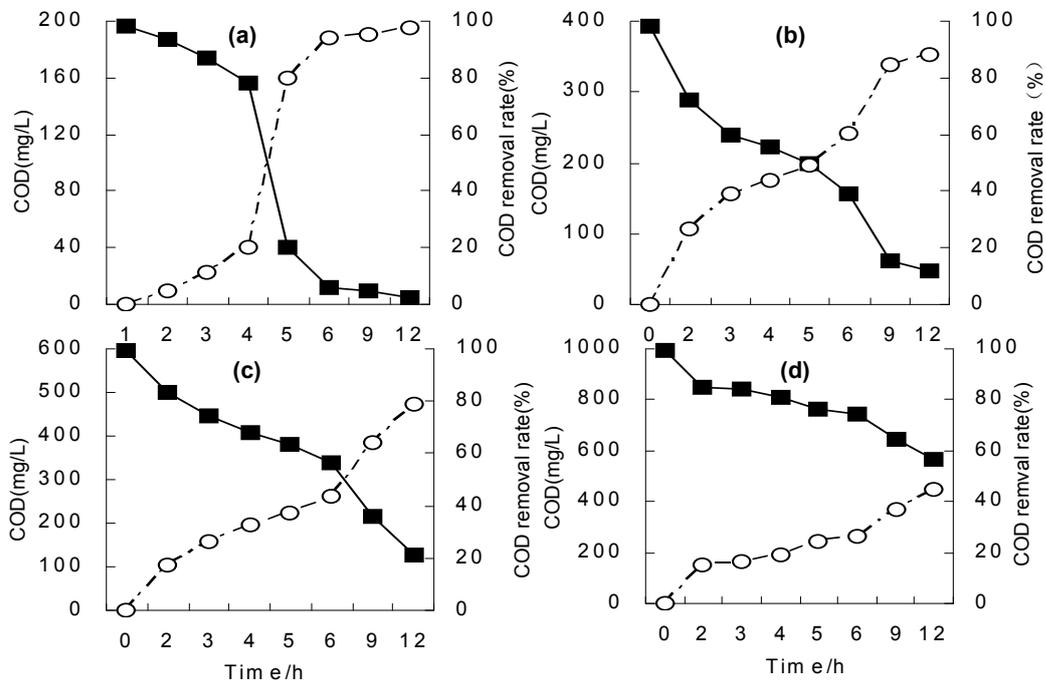
(a) COD=200; (b) COD=400; (c) COD=600; (d) COD=1000;Influent COD(■) ; COD removal rate (○)
Figure 1.Different COD concentration consumed by PAOs

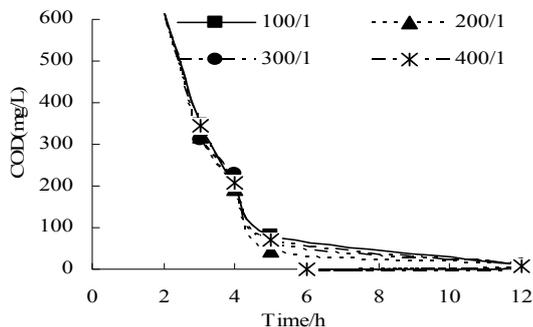*PHB accumulate yield in different nitrogen concentration*

Figure 2.Changes of COD in different nitrogen concentration conditions

Results of PHB accumulation contents in different COD/N ratio conditions are shown in Fig.3. From the figure we can see the dynamic curve of four concentration treatments were nearly the same tendency, namely, they were all increased in the initial stage and then slowly decreased. And the maximum was all appeared on the 4th day. While different COD/N ratio conduce the difference of PHB accumulate yield. Obviously, PHB contents were highest happened in the influent initial COD/N ratios of 200 gCOD/gN. And then, PHB accumulation ability was the influent initial COD/N ratios of 100, 300, and 400 respectively. And the maximum of four concentration influent were COD/N ratios of 200, 300, 100, and 400, respectively. The above results illuminated that the PHB optimal accumulate yield were the influent were COD/N ratios of 200. This result was the same as the Chua[4], who achieved a PHA content of 39% of the sludge dry mass by using a chemically processed wastewater that contained xenobiotic organics at a COD/N ratio of 140. While lots of researches[8] carry out in low COD/N ratios of 3.84, 2.93 and 1.54 gCOD/gN, results found higher COD/N ratio favorable acetate fully convert into PHB. PHB storage yield dropped down with increasing influent nitrate concentrations, indicating that higher portions of acetate were diverted to simultaneous the phosphate accumulating organisms direct growth.
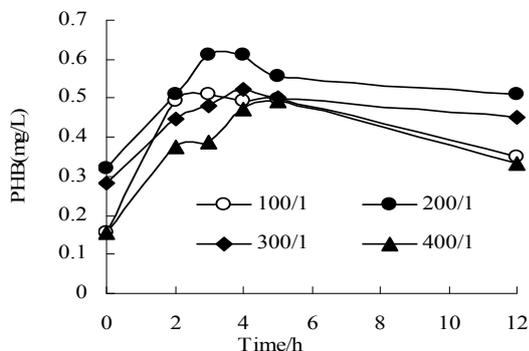
IV. CONCLUSIONS

Effective PHB accumulation requires excess of organic carbon, compared the influent initial COD 200, 400, 600 and 1000. Results showed optimal influent wastewater COD concentration was 600. Compared the influent initial COD/N ratios of 100, 200, 300, and 400 respectively, results showed COD/N 200 were more favorable acetate fully converted into PHB.

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Figure 3. Changes of PHB content in activated sludge in different nitrogen concentration conditions