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ANAEROBIC SUBSTRATE UPTAKE BY THE ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL ACTIVATED SLUDGE TREATING REAL SEWAGE

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ABSTRACT

The enhanced biological phosphorus removal (EBPR) activated sludge process is a wastewater treatment process by which not only organic pollutants but also phosphorus are removed. In the EBPR process, it is known that organic matter in the influent is removed in the anaerobic phase of the sequencing anaerobic and aerobic conditions. Although the mechanism of the anaerobic substrate uptake is being revealed, the existing observations are based on the experiments with activated sludge acclimatized with synthetic sewage, or synthetic media. In this study, the anaerobic substrate uptake by EBPR activated sludge treating real sewage was examined. The sludge was obtained from a pilot plant of the University of British Columbia, Canada. And as the substrate, acetate, propionate, lactate, pyruvate, malate, succinate, and fermented sewage were examined. The results clearly showed that most part of the sink of carbon anaerobically taken up is explained by PHA (poly 3-hydroxyalkanoates), and that glycolysis is playing a significant role in the anaerobic uptake of acetate and propionate.

KEYWORDS

Enhanced biological phosphorus removal; anaerobic substrate uptake; poly 3-hydroxyalkanoates; glycolysis; sewage.

INTRODUCTION

The enhanced biological phosphorus removal (EBPR) process is achieving reputation as an economical and reliable option for the removal of phosphorus from wastewaters. Although the process has already got matured technically, we have not yet understood the process very well from the biochemical and microbiological point of view.

One of the most mysterious phenomena observed in the EBPR process is the removal of soluble organic materials by the activated sludge under the anaerobic conditions. This phenomenon, which we refer to as the anaerobic substrate uptake, is explained in the following way (Marais *et al.*, 1983; Comeau *et al.*, 1986; Wentzel *et al.*, 1991). Various kinds of microorganisms are already known to accumulate polyphosphate under the aerobic condition. Some of them, often referred to as the bio-P bacteria, have the ability to use polyphosphate as the energy source under anaerobic conditions to take up organic substrates. If we cultivate a mixed culture under a sequencing anaerobic and aerobic conditions and feed it with organic substrates only in the anaerobic phase of the sequence, the bio-P bacteria usually get dominant in the culture. This is because the bio-P microorganisms have an advantage in the competition toward organic substrates under anaerobic conditions.

Although this explanation gives an answer to the energetic aspect of the anaerobic substrate uptake, and already verified by many researchers, it does not explain the material balance and the redox balance in the anaerobic substrate uptake.

The information on the sink of carbon in the anaerobic substrate uptake used to be very limited in the earlier days. Only PHB (poly 3-hydroxybutyrate) and glycogen were known as the sink. Comeau*et al.* reported the accumulation of PHV(poly 3-hydroxyvalerate) in addition to 3HB (3-hydroxybutyrate) after the anaerobic uptake of various short chain organic acids(Comeau *et al.*, 1987). On the other hand, based on the observation that glycogen in the activated sludge decreased in the anaerobic uptake of acetate, Arun *et al.*(1988) and Mino *et al.*(1987) suggested the significance of glycolysis as the supplier of reducing power in the anaerobic substrate uptake. Satoh *et al.*(1992) identified that the sink of carbon in the anaerobic uptake of acetate and propionate was PHA (poly 3-hydroxyalkanoates) composed of 3HB, 3HV (3-hydroxyvalerate), 3H2MB (3-hydroxy-2-methylbutyrate), and 3H2MV (3-hydroxy-2-methylvalerate)(Satoh *et al.*, 1992). They measured the amount of substrate taken up, PHA in sludge increased, carbohydrates in sludge decreased, and phosphate released. The observed stoichiometric relations among the metabolic substances were in good correspondence with the model which assumes that glycolysis supplies the reducing power necessary for the conversion of acetate or propionate into PHA. The shortcoming about their research was the material they used; they used activated sludge acclimatized not with sewage but with synthetic media which contained acetate, propionate, peptone, and yeast extract. In actual cases, activated sludge is fed with real wastewaters containing various organic compounds. The above idea should be directly applied to explain the anaerobic substrate uptake in practical EBPR processes.

In the present study, we investigated the mechanism of anaerobic substrate uptake by activated sludge which was treating real sewage. The sludge was obtained from the pilot scale EBPR plant of the University of British Columbia (UBC). The organic substrates examined were acetate, propionate, lactate, pyruvate, malate and succinate. As the key metabolic materials which are supposed to be related with the anaerobic substrate uptake, we assumed polyphosphate, PHA, and glycogen.

MATERIALS AND METHODS

The activated sludge was sampled from the end of the aerobic zone of the pilot plant of the University of British Columbia, and was subjected to the batch experiments to clarify the mechanisms of the anaerobic substrate uptake.

The influent for the UBC pilot plant was taken from the sewage pipe running near the plant once a day, and stored in a tank. The sewage was pumped up into a fermentation tank which was to enrich volatile fatty acids in the sewage, followed by a primary sedimentation tank where suspended solid in the fermented sewage was separated. The effluent of the primary sedimentation tank was introduced into the anaerobic zone of the EBPR process of the UCT (University of Cape Town) configuration. Although the operational conditions such as the SRT and the HRT were changed from time to time in the research period, the operational conditions were basically the same as described by Comeau *et al.* (1987).

TABLE 1 BATCH EXPERIMENTS CONDUCTED

Date	MLSS (mg/l)	P content (% of MLSS)	Substrate (Conc.)	Condition
Apr.9, 1994	1150	2.7	acetate (30mg/l)	2 hours Anaerobic
Apr18, 1994	1250	3.3	acetate (37mg/l) propionate(32mg/l)	3 hours Anaerobic
May 24, 1994	1800	2.9	acetate (38mg/l) propionate (28mg/l) malate (28mg/l)	3 hours Anaerobic
May 26, 1994	1700	2.9	lactate (34mg/l) pyruvate (35mg/l) succinate (33mg/l)	3 hours Anaerobic
July 2, 1994	2000	2.8	acetate (29mg/l) propionate (32mg/l) fermented sewage	2 hours Anaerobic + 3 hours Aerobic

Batch experiments conducted are listed in Table 1. The batch experiments were conducted with 500ml or 2500ml triangular flasks (Comeau *et al.*, 1987). The activated sludge was sampled from the end of the aerobic zone of the pilot plant, and placed in the flask. In order to remove oxygen in the head space, the triangular flask was purged with nitrogen gas. The flask was sealed with a gum cap, and was left stirred by a magnetic stirrer for about 6 hours in order to remove dissolved oxygen, nitrate, and nitrite in the sludge mixed liquor. Then, the sample before feeding was taken, and concentrated substrate was injected through the septum on the gum cap with a syringe. When fermented sewage was used as the substrate, sludge mixed liquor was condensed twice by sedimentation, and it was mixed with the effluent of the primary sedimentatin tank at a volumetric ratio of 1 to 1. For the sampling, a nitrogen-gas filled balloon attached to a syringe needle was inserted through the septum on the cap to make internal pressure higher, and small amount of sludge mixed liquor was driven out through the sampling line from inside of the reactor. The supernatant sample for the analyses of supernatant TOC (total organic carbon) and phosphate was obtained by centrifugation of the sludge mixed liquor. In order to take samples for the analyses of carbohydrates and PHA quantitatively and rapidly, the sludge mixed liquor running out from the sampling line was received with a 5.82ml (± 1.5%) narrow sampling glass tube. One tubeful or two of the received sample was immediately replaced into a sample storage tube containing a few drops of 1N sulfuric acid to stop the bacterial activity. Each of the batch experiment was accompanied by a control experiment in which substrate was not added. In the anaerobic-aerobic batch experiments conducted on July 2, 1994, the condition was turned to aerobic after two hours of the substrate injection. In the aerobic condition, oxygen was supplied by surface aeration. About 300ml of the mixed liquor was replaced into a 2500ml triangular flask with the cap opened, and the mixed liquor was stirred by a magnetic stirrer.

Supernatant TOC was measured by a Shimazu TOC-500 analyzer. Supernatant phosphate was analyzed by the automated ascorbic acid method. Total phosphorus of the mixed liquor was digested by the potassium persulfate method, and quantified by the automated ascorbic acid method. Instead of analyzing glycogen in the microorganisms selectively, we measured total carbohydrates in the sludge by the anthrone method (Gaudy and Gaudy, 1981). The methanolysis-GC method based on the method by (Braunegg *et al.*, 1978; Comeau *et al.*, 1988) was employed for the analysis of PHA with a minor improvement. The acidified mixed liquor previously described was centrifuged, and the sludge pellet at the bottom of the tube was lyophilized. To the tube, 2ml of acidified methanol (10% sulfuric acid) containing benzoic acid as the internal standard and 2ml of chloroform were added. The tube was closed with a teflon-lined screw cap, mixed well, and heated for 20 hours in an oven at 105°C. After cooled down to the room temperature, the tube was opened and 1ml of 14% aqueous ammonia solution was added into it. The tube was vigorously shaken, centrifuged, and the lower phase of chloroform solution was replaced into another tube. To purify the chloroform solution, 0.5ml of water was added into the tube, the tube was shaken again, and centrifuged. A Hewlett-Packard gas chromatograph 5880A equipped with a DB-1 column (internal diameter 0.32mm, film thickness 0.25μm, and length 30m) and a flame ionization detector was employed for the GC analysis. The purified chloroform phase of 0.5μl

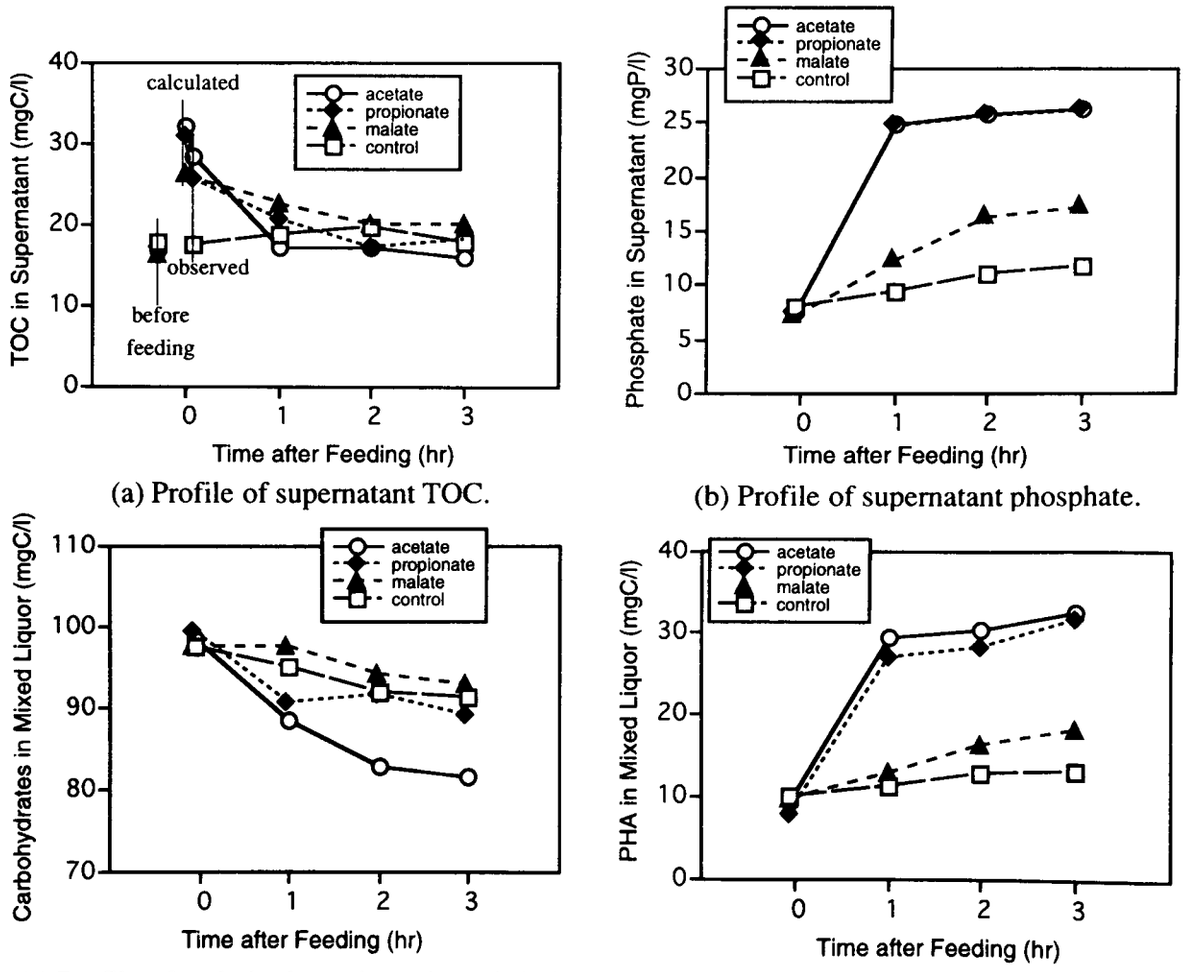
was injected with a split ratio of 1 to 10. Helium gas of 2ml/min was used as the carrier gas. The injector temperature was 225°C, and the detector temperature was 280°C. The column temperature was 70°C in the initial 4 minutes after the injection, then increased at a rate of 8°C/min to 118°C and then 30°C/min up to 200°C. The column temperature was held at the final temperature of 200°C for 3 minutes. Commercial sodium 3-hydroxybutyrate was used as the standard of 3HB in PHA. Sodium 2-hydroxyvalerate was used as the quantitative standard of 3HV in PHA. Purified PHA sample obtained from laboratory EBPR sludge which had taken up propionate under anaerobic condition was used as the standard of 3H2MV after determination of its composition by proton NMR spectrometry.

RESULTS AND DISCUSSION

Anaerobic Batch Experiments

The results of the batch experiments conducted on May 24, 1994 with acetate, propionate, and malate as the substrates are shown in Fig.1, and those conducted on May 26, 1994 with lactate, pyruvate, and succinate are shown in Fig. 2.

The substrate concentration in the supernatant monitored by the measurement of TOC is shown in Fig. 1 (a) and Fig. 2 (a). Succinate, pyruvate, and lactate disappeared from the supernatant within about 5 minutes after the injection of the concentrated substrate. Most part of acetate and propionate had disappeared after 1 hour of the injection. In comparison to other substrates, the uptake of malate was slower. The release of phosphate, shown in Fig. 1 (b) and Fig. 2 (b), was the maximum with acetate



(a) Profile of supernatant TOC.

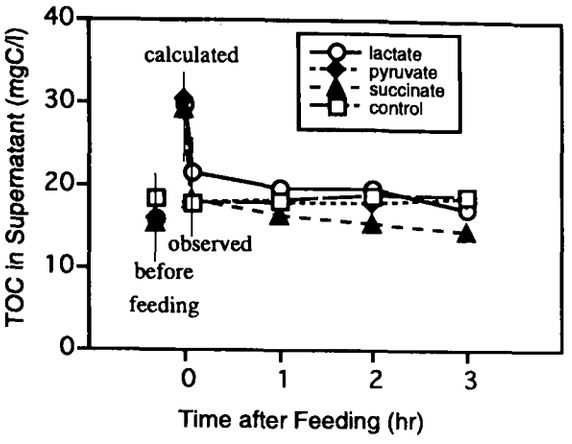
(b) Profile of supernatant phosphate.

(c) Profile of carbohydrates in activated sludge.

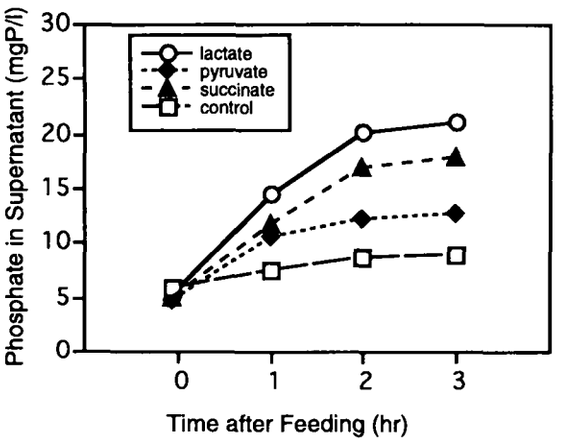
(d) Profile of PHA in activated sludge.

Fig. 1. Anaerobic batch experiments conducted on May 24, 1994 with acetate, propionate, and malate as the substrate.

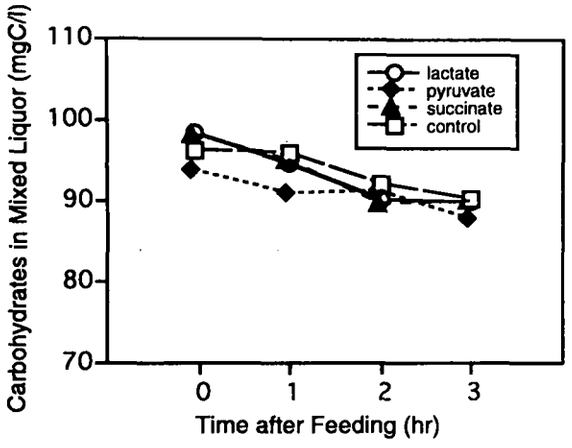
and propionate followed by lactate, succinate, malate, and pyruvate. The concentration of carbohydrates in the sludge, shown in Fig. 1 (c) and Fig. 2 (c), decreased slightly when propionate was used as the substrate, and significantly when acetate was used. The reduction of the carbohydrates in the mixed liquor was the same level as that observed in the control experiments when other substrates were added. The concentration of PHA in the sludge dramatically increased when acetate or propionate was added, and gradually increased when other substrates were added, as shown in Fig. 1 (d) and Fig. 2 (d). The content of the accumulated PHA after feeding is shown in Fig. 3. When acetate was added, 3HB was accumulated mainly, and 3HV was also accumulated slightly. When propionate was added, 3HV and 3H2MV were accumulated where 3HV was more than 3H2MV. In the experiments with lactate, malate,



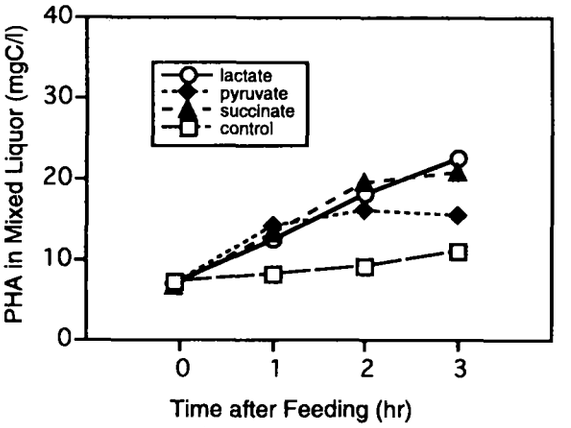
(a) Profile of supernatant TOC.



(b) Profile of supernatant phosphate.



(c) Profile of carbohydrates in activated sludge.



(d) Profile of PHA in activated sludge.

Fig. 2. Anaerobic batch experiments conducted on May 26, 1994 with lactate, pyruvate, and succinate as the substrate.

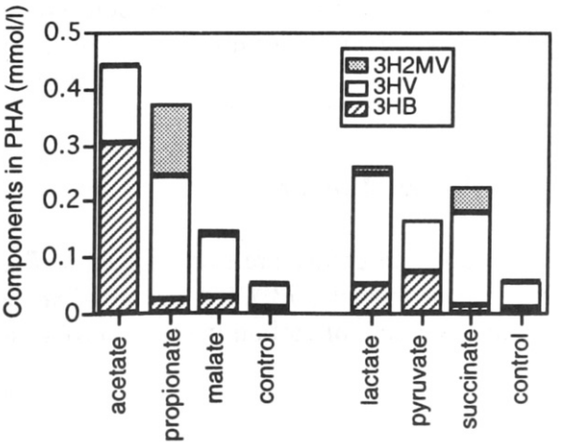


Fig. 3. The content of the accumulated PHA within 3 hours from substrate injection.

and succinate, 3HV was mainly accumulated with the minor component of 3HB in the case of lactate and malate, and 3H2MV in the case of succinate. In the case of pyruvate, about the same amount of 3HB and 3HV were accumulated. And in the control experiments, 3HV was accumulated mainly.

Anaerobic Uptake of Acetate and Propionate

According to the model described by Mino *et al.* (1987), Arun *et al.* (1988), and Satoh *et al.* (1992), the stoichiometric relations among acetate or propionate taken up to glycogen consumed to PHA accumulated is 6 to 1 to 4. The stoichiometric relations in the batch experiments were calculated and compared with the theoretical in Table 2. In the calculation of the observed stoichiometric relations, the effect of acetate or propionate was extracted by the subtraction of the changes in the control experiments from the changes in the acetate- or propionate-added experiments. The observed values were fairly in good relation with the theoretical ones, although the degree of correspondence was not as good as the reported values obtained on activated sludge acclimatized with synthetic wastewater (Satoh *et al.*, 1992). Nonetheless, the significance of glycolysis in the anaerobic substrate uptake was demonstrated clearly.

TABLE 2 STOICHIOMETRIC RELATIONS AMONG THE METABOLIC SUBSTANCES

Date (y/m/d)	Apr.9	Apr.18	Apr.18	May 24	May 24	July 2	July 2	Theoretical
Substrate	acetate	acetate	propionate	acetate	propionate	acetate	propionate	
Substrate Taken Up	6	6	6	6	6	6	6	6
Phosphate Released	4.9	7.6	8.8	4.2	8.3	5.6	6.2	3~4 or larger
Carbohydrates Consumed	0.9	1.2	0.3	1.5	1.3	1.2	0.7	1
Total PHA	3.2	3.6	4.1	3.4	5.4	2.6	3.4	4

Anaerobic Uptake of Lactate, Pyruvate, and Succinate

From Fig. 2 (a) and (d), lactate and succinate seem to have been taken up by microorganisms in the very short time of initial 5 minutes, and gradually converted to PHA in the following 2 to 3 hours. The release of phosphate also continued with the accumulation of PHA. Pyruvate also disappeared from the supernatant within the initial 5 minutes, PHA accumulation completed in the initial 1 hour, and phosphate was released significantly only in the initial 1 hour. On the other hand, from Fig.1 (a) and (d), the removal of malate from the supernatant was accompanied by the accumulation of PHA and release of phosphate. The reduction of carbohydrates in sludge in the anaerobic uptake of these substrates was at the same degree as that in the control experiment.

The results indicate that there are microorganisms that can take up lactate, pyruvate, or succinate in a very short time without the consumption of polyphosphate, and accumulate them in a form other than PHA. And successively, the accumulated material is gradually converted to PHA. It is difficult to tell whether the accumulator of PHA here is the same as the microorganisms that take up lactate , pyruvate, or succinate. Judging from the observation on carbohydrates in sludge, these substrates were probably utilized by the bio-P bacteria directly, without the existence of fermentative bacteria which converts these substrates to acetate and propionate. This is because anaerobic uptake of acetate or propionate should have caused noticeable decrease in sludge carbohydrates. But the release of phosphate can be explained

Carbon Balance in the Anaerobic Substrate Uptake

The carbon balances in the anaerobic substrate uptake are summarized in Table 3. The recovery ratios of carbon were 71% for acetate, 100% for propionate, 73% for lactate, 47% for pyruvate, 76% for malate, 61% for succinate. And the most important sink of carbon in the anaerobic substrate uptake was found to be PHA.

TABLE 3 CARBON RECOVERY RATIO IN THE ANAEROBIC SUBSTRATE UPTAKE

		substrate taken up (mgC/l) A	carbohydrates in sludge consumed (mgC/l) B	PHA accumulated (mgC/l) C	carbon recovery ratio C/(A+B)
Apr 9, 1994	acetate	9	8	13	0.74
	control		4	1	0.22
Apr 16, 1994	acetate	11	9	15	0.71
	propionate	15	4	20	1.01
	control		2	1	0.27
May 24, 1994	acetate	16	17	23	0.69
	propionate	13	10	24	1.03
	malate	6	5	9	0.76
	blank		6	3	0.51
May 26, 1994	lactate	12	8	15	0.73
	pyruvate	12	6	8	0.47
	succinate	15	8	14	0.61
	blank		6	4	0.62
July 2, 1994	acetate	11	10	14	0.69
	propionate	14	7	20	0.96
	fermented sewage	6	9	15	0.97
	control		3	4	1.23

Anaerobic-Aerobic Batch Experiments

The results of the anaerobic-aerobic batch experiments are shown in Fig. 4. In all of the anaerobic-aerobic batch experiments including the control experiment, phosphate was removed from the supernatant, PHA decreased, and carbohydrates in sludge increased under the aerobic condition succeeding the anaerobic condition. We may well interpret the observation that bio-P bacteria fueled the PHA and regenerated polyphosphate and glycogen in preparation for the next anaerobic substrate uptake.

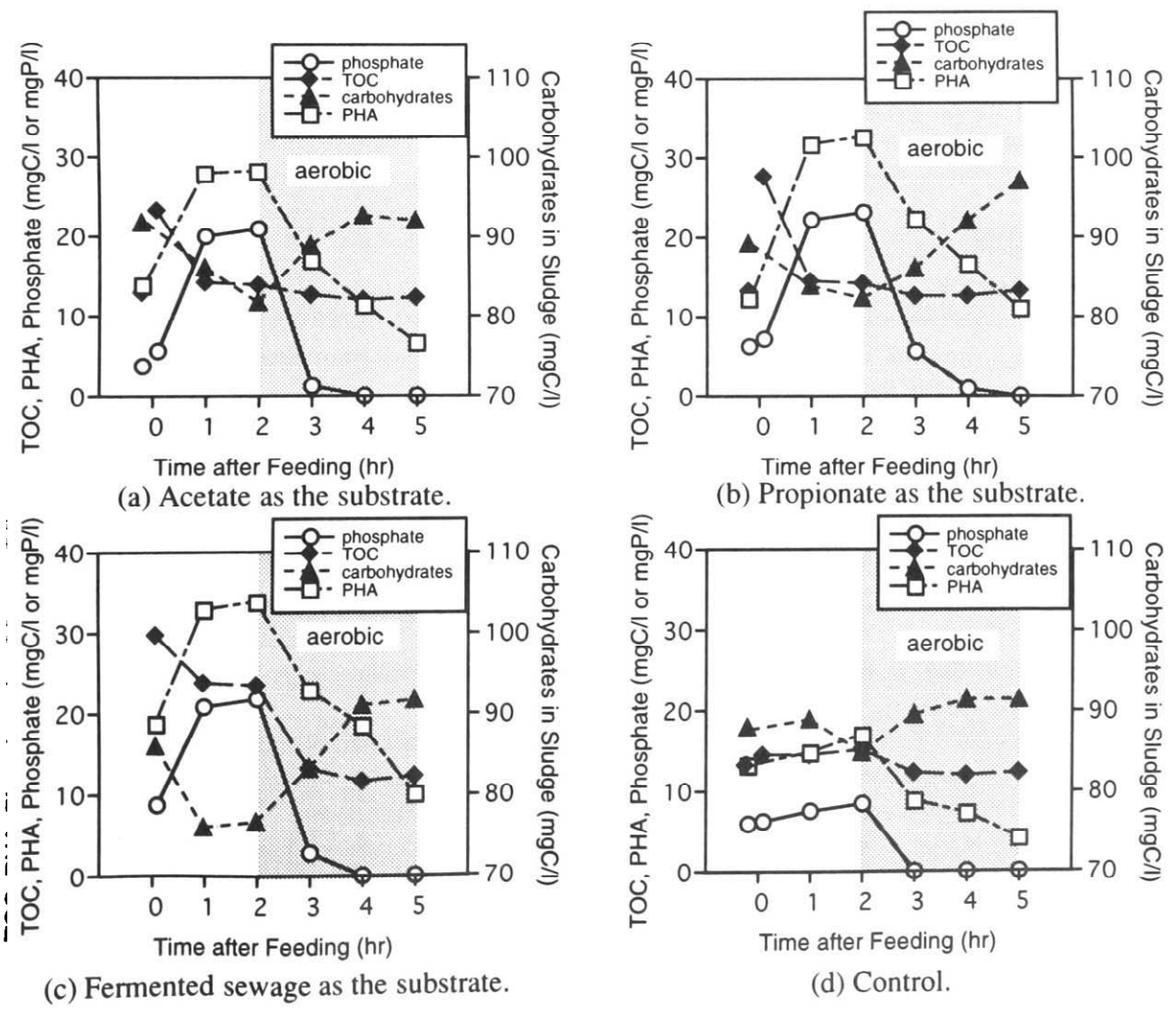


Fig. 4. Anaerobic-aerobic batch experiments conducted on July 2, 1994.

The results obtained in the batch experiment with fermented sewage as the substrate was similar to that obtained with acetate and propionate. Under the anaerobic condition, supernatant TOC was partially removed, phosphate was released, carbohydrates in sludge decreased, and PHA was accumulated in sludge. Under the next aerobic condition, phosphate was removed from the supernatant, PHA decreased, and carbohydrates in sludge increased.

CONCLUSIONS

More than 70% of the carbon sink in the anaerobic substrate was explained by PHA except for the experiments with pyruvate and succinate. The carbon recovery ratio in the anaerobic uptake of pyruvate was 47%, and that of succinate was 61%.

When acetate, propionate, or fermented sewage was the substrate, carbohydrates in sludge clearly decreased in the course of anaerobic substrate uptake, and it increased in the following aerobic condition. The observation suggests that carbohydrates in sludge, most probably glycogen, is playing an important role in the anaerobic uptake of these substrates.

At this moment, information on the behavior of carbohydrates in sludge in full scale EBPR processes is extremely limited. For a better understanding of the EBPR process, extentensive investigation is required.

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