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BIOCHEMICAL MODEL FOR ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL

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Abstract—Enhanced biological phosphorus (bio-P) removal from wastewater is a promising technology for which the fundamental mechanisms are still unclear. The purpose of this paper is to present a biochemical model that explains bio-P removal mechanisms occurring under anaerobic, aerobic and anoxic conditions of the process. A bio-P bacterium is referred to as one that can store both polyphosphate and carbon (as poly-β-hydroxybutyrate for example). In this communication, observations from the literature are first reviewed and mechanisms of bacterial bioenergetics and membrane transport are summarized. The model for bio-P metabolism under anaerobic, aerobic and anoxic conditions is then presented. The role of polyphosphate under anaerobic conditions is suggested to be as a source of energy both for the reestablishment of the proton motive force, which would be consumed by substrate transport and for substrate storage. The role of the anaerobic zone is to maximize the storage of organic substrates in bio-P bacteria. For this purpose the supply of readily available substrates should be maximized and the presence of electron acceptors (molecular oxygen or oxidized nitrogen) minimized. Under subsequent aerobic or anoxic conditions, bio-P bacteria will accumulate polyphosphates in response to the availability of electron acceptors (oxygen or oxidized nitrogen) for energy production. Carbon reserves in bio-P bacteria should provide energy for growth and for soluble phosphate accumulation as polyphosphate reserves.

Key words—enhanced biological phosphorus removal, wastewater treatment, polyphosphate, poly-β-hydroxybutyrate, biochemical model, proton motive force, membrane transport

INTRODUCTION

As a viable alternative to chemical phosphorus precipitation, enhanced biological phosphorus (bio-P) removal has received increased attention in the last decade. Bio-P removal is characterized by an efficiency of phosphorus removal in excess of metabolic requirements and of natural precipitation. Effluent phosphorus concentrations of < 1.0 or even $0.3\ mg\ l^{-1}$ are reported (Oldham, 1985; Arvin, 1985). A number of processes can be used for bio-P removal (refer to Marais *et al.*, 1983; Arvin, 1985).

As stated by Melcer (1982) "imperfect understanding of the (bio-P removal) technology did not deter its full scale application, which did not always result in the expected performance". Furthermore, Arvin (1985) suggested that "in addition to better operation management, an improved insight into the process mechanisms is needed to obtain better treatment results". Some fundamental models (Fuhs and Chen, 1975; Nicholls and Osborn, 1979; Rensink *et al.*, 1981; Marais *et al.*, 1983) arose from the recognition that the anaerobic–aerobic sequence was favoring the growth of bacteria that could accumulate phosphate aerobically as polyphosphate granules. Under anaerobic conditions, substrates from the sewage would be stored in carbon reserves using energy from accumulated polyphosphate. Subsequently, this stored carbon would favor growth and accumulation of phosphate by bacteria capable of dual polyphosphate and carbon storage. This scheme has been well accepted among researchers because of its ability to explain many observed phenomena, but no integrated model has yet been proposed to satisfactorily explain the mechanisms involved at a biochemical level. It is the purpose of this paper to propose such a biochemical model based on experimental observations and principles of bacterial bioenergetics and membrane transport.

The experimental basis of the biochemical model proposed here was originally presented by Comeau (1984) and Comeau *et al.* (1985b), and an earlier version of the model was summarized by Comeau *et al.* (1985a).

OBSERVATIONS RELATED TO BIO-P REMOVAL

Anaerobic conditions and phosphate release

The essential prerequisite to induce bio-P removal in an activated sludge treatment plant is the presence of an anaerobic zone upstream of the standard aerobic process. An anaerobic zone is considered to be one in which both dissolved oxygen and oxidized nitrogen (nitrate or nitrite) are absent. The addition to the anaerobic zone of sewage or of simple carbon substrates such as acetate results in phosphate release and has been reported to also result in carbon storage (Fukase *et al.*, 1982).

Figure 1 shows typical data obtained from a batch test in which three samples of aerobic mixed liquor obtained from the University of British Columbia bio-P pilot plant were placed in a stirred but non-aerated container for the duration of the experiment. After preliminary equilibration, a concentrated solution of sodium acetate was injected so that 0, 30 and 60 mg l^{-1} of acetate as HAc was added to the various containers. Four hours later a solution of sodium nitrate was injected such that 10 mg l^{-1} of nitrate as N was added to each container. The evolution with time of soluble phosphorus, oxidized nitrogen and poly-β-hydroxybutyrate (PHB) is shown. For more details on the experimental methods, refer to Comeau *et al.* (1985b). The addition of acetate resulted in phosphate release in proportion to the amount of acetate injected. The time at which the accumulation rate of phosphate from solution decreased substantially corresponded to the disappearance of acetate from solution. With acetate addition, the rate of denitrification was also increased. Concurrently with acetate uptake from solution, carbon storage in the form of PHB took place. With subsequent nitrate addition, phosphate uptake by the biomass was observed until the disappearance of oxidized nitrogen from solution. It also appeared that PHB reserves were consumed. The effects of oxidized nitrogen will be discussed further in the next section which deals with phosphate uptake.

Phosphate release is reported to also be triggered by the addition of other simple carbon compounds such as propionate and glucose (Potgieter and Evans, 1983), by fermented primary sludge (Oldham, 1985), and by septic sewage (Paepcke, 1983). The phosphate released in solution originates from polyphosphate reserves (Marais *et al.*, 1983; Arvin, 1985). The accumulation of PHB following acetate addition was also documented by Deinema *et al.* (1980) and Fukase *et al.* (1982).

Phosphate release was also observed as a result of the addition of the following chemicals: 2,4-dinitrophenol (DNP), sodium hydroxide (high pH), H_2S gas, and CO_2 gas [see Figs 2(a) and (b)]. In this experiment, samples of aerobic mixed liquor were

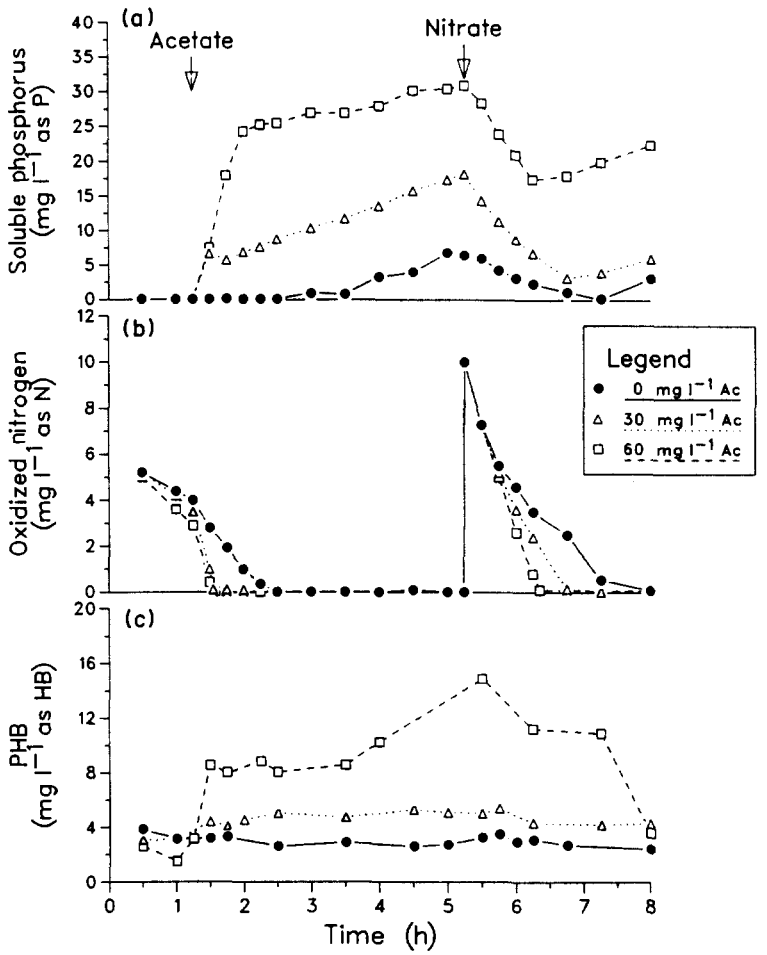

Fig. 1. Effects of various levels of acetate and of nitrate addition on the concentration profile of (a) soluble phosphorus, (b) oxidized nitrogen and (c) poly-β-hydroxybutyrate in batches of mixed liquor (from Comeau *et al.*, 1985b).

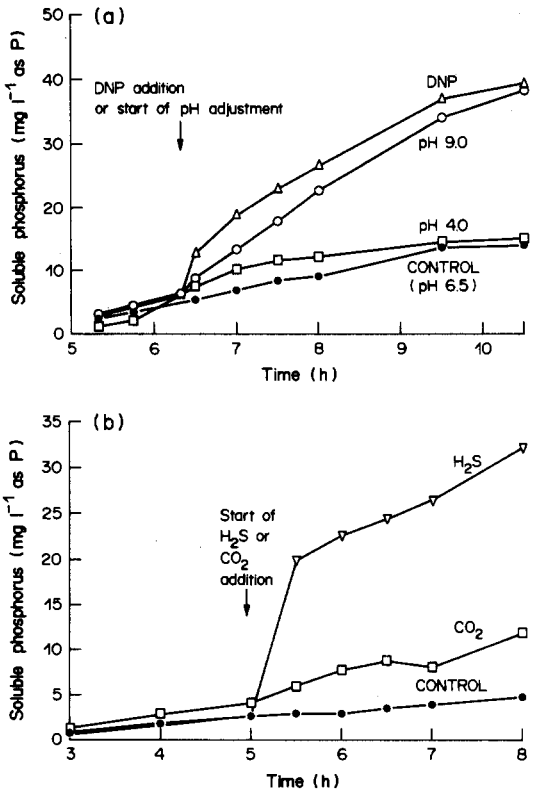

Fig. 2. (a) Effects of 2,4-dinitrophenol (DNP) addition, low and high pH adjustment, and (b) effects of H_2S and CO_2 gas addition on the concentration profile of soluble phosphorus in batches of bio-P mixed liquor (adapted from Comeau *et al.*, 1985b).

maintained under non-aerated conditions until complete denitrification had occurred (Comeau *et al.*, 1985b). From the profile of soluble phosphorus it can be seen that both DNP addition and high pH adjustment resulted in phosphate release, but that a low pH did not result in a phosphorus profile significantly different from the one of the control reactor. Both CO_2 and especially H_2S resulted in anaerobic phosphate release. Fuhs and Chen (1975) and Deinema *et al.* (1985) also observed that CO_2 could induce phosphate release from the biomass. It should be noted that in each of these cases, carbon storage is not expected since these gases cannot be stored readily. Therefore, since polyphosphate degradation was proposed as a mechanism to supply energy for carbon storage (Marais *et al.*, 1983), how can it be explained that the above chemicals also triggered polyphosphate degradation and phosphate release? An explanation based on the hypothesis that polyphosphate reserves can be used to supply energy to maintain the proton motive force of bio-P bacteria will be proposed following a review of bacterial bioenergetics and the presentation of the biochemical model for anaerobic conditions.

Phosphate uptake

Phosphate uptake from solution by a microbial biomass occurs under aerobic conditions. The extent to which the uptake occurs can be correlated to the degree of the previous anaerobic phosphate release (Marais *et al.*, 1983). Fukase *et al.* (1982) have reported that PHB reserves were consumed under aerobic conditions.

Phosphate uptake has been shown to take place by the consumption of oxidized nitrogen instead of dissolved oxygen as an electron acceptor (see Fig. 1(a); Simpkins and McLaren, 1978; Hascoët *et al.*, 1985). This observation suggests that a subpopulation of the bio-P bacteria is capable of one or both of nitrate reduction (to nitrite) or denitrification (with a gaseous end product).

The detrimental effect of oxidized nitrogen on anaerobic phosphate release and overall phosphorus removal in bio-P treatment plants, is well documented and has led to the development of many processes aiming at a reduction of oxidized nitrogen loadings to the anaerobic zone (e.g. Bardenpho, Phoredox, UCT, A/O, A_2O). Indeed, with nitrate or nitrite present, readily available substrates could be consumed for denitrification instead of being stored by bio-P bacteria.

Co-transport of metallic cations with phosphate

Concurrent phosphate and metal release or uptake was observed by Miyamoto-Mills *et al.* (1983), Arvin and Kristensen (1985), and Comeau *et al.* (1985b). The molar ratios reported are shown in Table 1.

From these observations it appears that the metallic cations K^+, Mg^{2+} and Ca^{2+} are co-transported with phosphate molecules in a total molar ionic charge ratio of about 1.0 irrespective of the origin of

Table 1. Molar ratios of cations co-transported with phosphorus

	Reference				
Cation/P	*	†	‡	‡	‡
K^+/P	0.27	0.23	0.34	0.20	0.23
Mg^{2+}/P	0.26	0.32	0.24	0.28	0.27
Ca^{2+}/P	0.00	0.05	0.06	0.09	0.12
Sum of charges/P	0.79	0.97	0.94	0.94	1.01
Direction of transport	P release	P release	average of P release and uptake	P release	P uptake

*Miyamoto-Mills *et al.* (1983).
†Arvin and Kristensen (1985).
‡Comeau *et al.* (1985b).

the sludge or of the direction of transport. Thus, on average, one charge of a transported phosphate molecule could be neutralized by one of these cations. Because the ratio remains about the same for release and uptake these cations could remain in association with phosphate molecules even in solution.

Bio-P bacteria

The two essential characteristics of bacteria responsible for bio-P removal are proposed to be first, the ability to store polyphosphate and second, the ability to store carbon in a form such as PHB. Such bacteria are referred to as bio-P bacteria in this paper.

It has been demonstrated repeatedly that *Acinetobacter* bacteria are involved in bio-P removal processes (Fuhs and Chen, 1975; Deinema *et al.*, 1980; Brodisch and Joyner, 1983; Hascoët *et al.*, 1985). In addition to *Acinetobacter*, Lotter (1985) found large proportions of the microbial biomass to be composed of *Aeromonas* and *Pseudomonas* which were capable of substantial polyphosphate accumulation. In this paper the term bio-P bacteria is intended to be non-restrictive in defining the species of bacteria that can perform bio-P removal.

In a microbiological review of the *Acinetobacter* genus, Juni (1978) reported that these bacteria were generally not capable of nitrate reduction. Lotter (1985), however, studying sludge from bio-P plants detected many *Acinetobacter* spp that were capable of reducing nitrate to nitrogen gas. As mentioned in the section on "phosphate uptake", phosphate uptake can take place in the presence of nitrate. Thus, it would appear that at least a fraction of the bio-P bacteria, *Acinetobacter* or other genera, are capable of nitrate reduction and possibly denitrification.

BIOCHEMICAL MODEL

Review of microbial processes

Polyphosphate metabolism. The metabolism of polyphosphate in microorganisms has been reviewed by Harold (1966), Dawes and Senior (1973) and Kulaev and Vagabov (1983). Harold (1966) indicated that two fundamental mechanisms of polyphosphate accumulation could be recognized: "luxury uptake" and "overplus accumulation". In the luxury uptake mechanism, deprivation of a nutrient such as nitrogen or sulfur could result in polyphosphate formation. In the overplus mechanism, phosphate deprivation followed by a sudden exposure to phosphate would cause polyphosphate to accumulate. As discussed by Siebritz *et al.* (1983) and Arvin (1985) neither of these conditions can be expected to occur in plants treating municipal wastewaters.

The role of polyphosphate as a phosphate reserve for growth is well recognized but its role as an energy source is not as clearly established. Polyphosphate appears to be synthesized by the transfer of a phosphate from ATP to a growing chain of polyphosphate (Harold, 1966). Polyphosphate degradation usually occurs by simple hydrolysis and results in the loss of the energy contained in the phosphate bond. At low ATP/ADP ratios, however, polyphosphate can transfer its energy back to ATP (Kornberg, 1957; Kulaev, 1975). Varma and Peck (1983) indicated that short and long-chain polyphosphates could serve as energy sources for the anaerobic growth of bacteria. Mino *et al.* (1985a, b) observed the presence of both low and high molecular weight polyphosphate fractions in bio-P sludges. They suggested that the low molecular weight polyphosphates functioned as an energy pool under anaerobic conditions and that high molecular weight polyphosphates served as phosphate reserves for microbial growth. Kulaev and Vagabov (1983) reported on an enzyme in yeasts and fungi that could hydrolyze polyphosphate into smaller fragments. Finally, the phosphorylation of other compounds such as sugars by polyphosphate is also possible although the required enzymes do not occur as widely (Kulaev and Vagabov, 1983). Based on these observations it is conceivable that polyphosphate could phosphorylate various compounds, possibly ADP to produce ATP, under reduced energy conditions in bio-P bacteria.

Poly-β-hydroxybutyrate. Poly-β-hydroxybutyrate (PHB) is a polymer of D(−)-β-hydroxybutyrate. The major pathways of synthesis and degradation of PHB are shown in Fig. 3.

As described by Dawes and Senior (1973) the synthesis of PHB is unique among energy storage compounds since it does not require direct participation of ATP, provided that a source of acetyl CoA is available. Reducing power in the form of NADH is essential, however, and PHB formation may be regarded as a quasi-fermentation process permitting the reoxidation of NADH into NAD^+. Such a process is particularly useful under conditions of oxygen limitation which prevent the reoxidation of NADH by the electron transport chain (refer to the next section). Accumulation of PHB as high as 50% of the cell dry weight has been reported (Dawes and Senior, 1973).

PHB degradation will occur when the internal concentration of both NAD^+ and of CoA are high while the concentration of acetyl CoA is low. For example, PHB will be degraded in the presence of oxygen when external carbon sources are limited.

Bioenergetics: the proton motive force. A major aspect of bacterial bioenergetics is concerned with the maintenance by bacteria of a proton motive force (pmf). The pmf is a chemiosmotic gradient across the bacterial cytoplasmic membrane that can be considered to comprise two distinct components. One component is an electrical potential (expressed as interior negative) arising from a net negative charge on the cytoplasmic side of the cell membrane as compared to the outside. The other component is a pH gradient (interior alkaline) caused by the relative alkalinity of the bacterial cytoplasm. Translocation

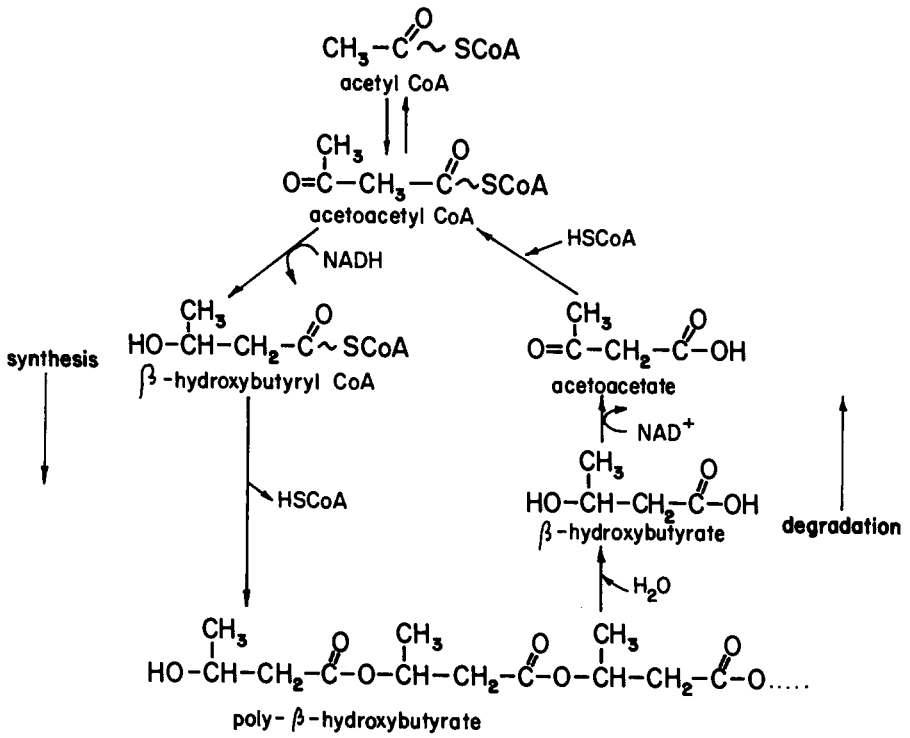

Fig. 3. Poly-β-hydroxybutyrate metabolic pathways (adapted from Stanier *et al.*, 1976).

of protons outside the cell membrane thus increases both components of the pmf (Harold, 1977; Brock *et al.*, 1984).

Major roles of the pmf are in the production of ATP by the membrane-bound ATP-ase enzyme complex, and for the transport of substrates as discussed in the next section.

Three major mechanisms are used by most bacteria to translocate protons and maintain a pmf (see Fig. 4). The first one is of major importance and makes use of the cytoplasmic membrane-bound electron transport chain to expel H^+ from the cell when carbon substrates and an electron acceptor, mainly oxygen or oxidized nitrogen, is present. This process is called aerobic or anaerobic respiration respectively. Substrates can be processed via glycolysis and/or the tricarboxylic acid cycle to produce NADH which then acts as a donor for the electron transport chain to result in proton explulsion. In the absence of electron acceptors, this mode of proton expulsion will be inoperative and the accumulation of NADH will inhibit further NADH production from metabolic pathways. Under such conditions, a second mechanism which consists of ATP breakdown at the ATP-ase site can be used to translocate protons. This process is essentially a reversal of the production of ATP from the pmf. A third mechanism makes use of the enzyme NADH-transhydrogenase to break down NADH into NAD^+ in order to translocate H^+ (Harold, 1977).

Bacteria will tend to maintain a fairly constant value for their pmf. If the external pH is decreased, for example, the high H^+ concentration outside the cell will cause the pH gradient to increase. To maintain a constant pmf the charge gradient could be reduced by cation expulsion. Potassium can be used for that purpose since its concentration in cells is relatively high (200 mM in *Escherichia coli*). Conversely, at a high external pH, cation accumulation by the cell would result (Bakker and Mangerich, 1981.

The various components of the pmf can be neutralized individually or simultaneously by the addition of toxicants. To neutralize only the pH gradient, acetate or other weak acids can be used (Kaback, 1976). Indeed, such acids form a neutral molecule before diffusing through the membrane. Once in the cell, the acids dissociate because of the relatively high pH in the cytoplasm of the cell (e.g. pH 7.6; Schuldiner and Padan, 1982) and remain in their ionic form, thus trapped inside. For each molecule of acetate taken up, one H^+ is removed from outside and released inside. Consequently the pH gradient will be decreased.

To affect both the charge and the pH gradient, 2,4-dinitrophenol can be used. This inhibitor shuttles H^+ across the membrane such that the gradient of H^+ is dissipated. Since H^+ movement influences both the pH gradient (since pH represents the free proton concentration) and the charge gradient (since protons are charged) both of these will be affected by 2,4-dinitrophenol. Inhibitors like 2,4-dinitrophenol are called "uncouplers" since they uncouple ATP formation at the ATP-ase site from respiration

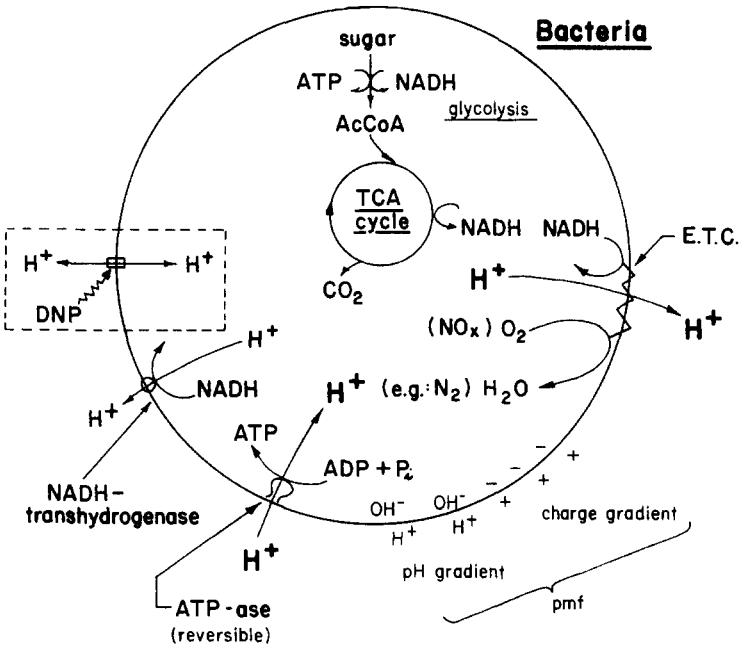

Fig. 4. Overview of bacterial bioenergetics—the proton motive force (pmf). AcCoA, acetyl CoA; DNP, 2,4-dinitrophenol; E.T.C., electron transport chain; H^+, proton; NADH, reduced nicotinamide adenine dinucleotide; NO_x, oxidized nitrogen; Pi, phosphate; TCA, tricarboxylic acid (cycle). The pmf is composed of a pH and of a charge gradient across the bacterial inner membrane. Substrates such as sugars are degraded by glycolysis and the TCA cycle to produce NADH which is used at the electron transport chain in presence of oxygen or oxidized nitrogen (which are electron acceptors) to expel protons. ATP is produced by consuming the proton gradient. ATP breakdown and NADH can be used to produce a pmf. Toxicants such as 2,4-dinitrophenol can dissipate one or the two components of the pmf.

(which uses oxygen or oxidized nitrogen at the electron transport chain) by dissipating the pmf.

The concept of reduction of the pmf by acetate, a high pH or 2,4-dinitrophenol will be used in the discussion section to explain anaerobic phosphate release resulting from the addition of these chemicals.

Bacterial membrane transport. Bacteria have a cytoplasmic membrane that acts as a permeability barrier for hydrophilic and charged molecules. A peptidoglycan layer that surrounds the cytoplasmic membrane confers rigidity and shape to the bacteria. In gram-negative bacteria, an additional outer membrane serves as a barrier to large hydrophilic and to hydrophobic molecules (Hancock, 1984).

There are three kinds of bacterial membrane transport: passive diffusion, facilitated diffusion and active (energized) transport (Harold, 1977; Brock *et al.*, 1984). Passive diffusion is a transport mechanism by which neutral molecules tend to equilibrate across the membrane. Water, oxygen and carbon dioxide are transported by passive diffusion across the cytoplasmic membrane.

In the case of facilitated diffusion, the permeating molecule combines with a membrane carrier and is transported inside the cell along its concentration gradient. An example of facilitated diffusion is the non-specific bacterial porins which are channels of the outer membrane.

There are three well-recognized categories of active transport: ATP-dependent transport, group translocation and transport coupled to the pmf. For active transport a specific carrier is required for each solute. The solute can be accumulated such that its internal concentration exceeds its external concentration.

In ATP-dependent transport, the hydrolysis of ATP drives the internal accumulation of solutes such as negatively charged amino acids. In group translocation, the solute is modified during its transport such as sugars by phosphoenolpyruvate. In transport coupled to the pmf, cations, anions or neutral molecules can be brought through the cytoplasmic membrane. Cations, such as potassium can accumulate in the cell in response to the charge gradient (interior negative). Anions such as phosphate can be co-transported with protons or other cations such that the molecule is neutral or carries a net positive charge when it crosses the membrane. For neutral molecules, such as sugars or amino acids, the carrier proteins effectively transfer a positively charged molecule where protons are bound to the carrier for its activation.

Postulated model for anaerobic conditions

The postulated model for anaerobic metabolism of bio-P bacteria will be presented with acetate as a substrate because of the experience of the authors

with this compound. It was reviewed that in the absence of oxygen and oxidized nitrogen, acetate appeared to be stored as PHB while polyphosphate reserves were degraded and phosphate molecules released in solution. First, the reduction of the pmf by acetate diffusion into bacteria will be mentioned. Mechanisms available to bio-P bacteria to reestablish the pmf will then be discussed. Finally, sources of energy for the storage of acetate as PHB will be suggested. Figure 5 summarizes the major concepts of the postulated model.

To be stored as PHB, substrates must first be transported into the cell. Several monocarboxylic acids are transported neutrally across the membrane if an appropriate pH gradient exists (Kaback, 1976). In solution, at a pH >6.5, more than 99% of the acetate is in the ionic form. Nevertheless the residual amount of acetic acid can be utilized for electroneutral transport. With acetate diffusion into the cell followed by acetate dissociation, a decrease in the pH gradient of about one H^+ for each acetate transported is expected. This decrease in the pH gradient will reduce the pmf which cells tend to maintain at a fairly constant level (Bakker and Mangerich, 1981; Schuldiner and Padan, 1982). In addition, unless bacteria regenerate the pH gradient, acetate uptake by the pH gradient-dependent mechanism, and consequently the ability to increase carbon storage as PHB, will quickly cease.

Three mechanisms presented in the section on bioenergetics are available to bacteria to reestablish the pH gradient. Under aerobic conditions, H^+ can be expelled by the electron transport chain. Under anaerobic conditions, however, when no electron acceptor (oxygen or oxidized nitrogen) is present this mechanism cannot be functional for bio-P bacteria.

Another way to eject H^+ involves breaking down ATP by the membrane-bound ATP-ase enzyme (Harold, 1977). With acetate as substrate, however, ATP could not be regenerated by fermentation and any cellular ATP would be rapidly depleted.

A third mechanism involves the utilization of NADH at the membrane-bound transhydrogenase enzyme to expel H^+ (Harold, 1977). NADH could be provided from feeding acetyl CoA into the tricarboxylic acid cycle as is done by anaerobic bacteria. Acetyl CoA, in turn, could be produced from the energization of acetate by polyphosphate as will be discussed later. This mechanism may be available to bio-P bacteria and should be kept in mind in future investigations. However, the utilization of acetyl CoA to produce NADH for the regeneration of the pH gradient does not give a direct role to polyphosphate reserves.

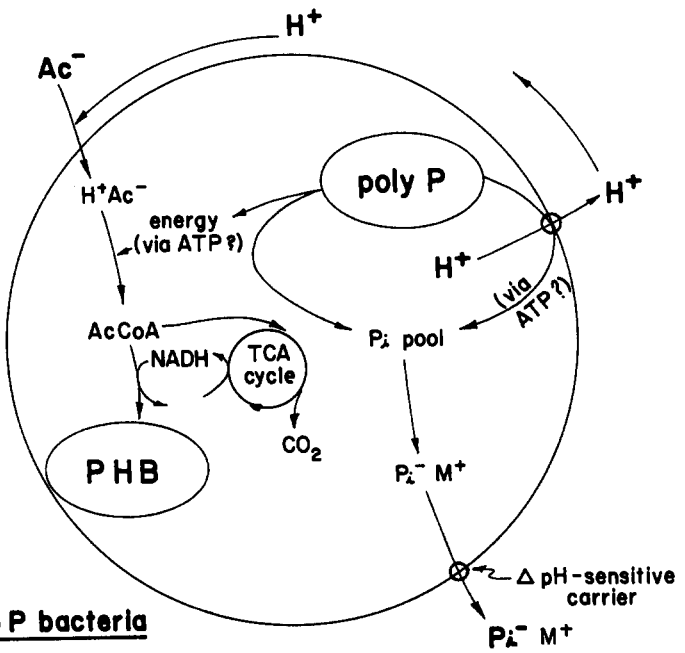

Fig. 5. Postulated model for *anaerobic* metabolism of bio-P bacteria. Ac^-, acetate; AcCoA, acetyl CoA; H^+, proton; HAc, acetic acid; M^+, metallic cation; NADH, reduced nicotinamide adenine dinucleotide; PHB, poly-β-hydroxybutyrate; Pi, phosphate; polyP, polyphosphate; TCA, tricarboxylic acid (cycle). Under anaerobic conditions, substrates (such as acetate) diffusion as neutral molecules will decrease the pH gradient of bacteria. Bio-P bacteria will be able to utilize their polyphosphate reserves to reestablish the pH gradient possibly directly or via the production of ATP. Polyphosphate can also provide energy for the formation of acetyl CoA. Storage as PHB of acetyl CoA requires NADH which the TCA cycle can produce anaerobically. Phosphate expulsion takes place because of the excess of phosphate molecules accumulating in the cell. A pH gradient-sensitive carrier could "sense" that phosphate cannot be used for synthesis. Metallic cations are co-transported with phosphate.

Finally, as a fourth mechanism it is proposed that polyphosphate is used to expel protons across the cytoplasmic membrane by a translocating enzyme. As mentioned in the section on "bioenergetics: the proton motive force" ATP breakdown can be used for this purpose by reversing the normal function of the ATP-ase enzyme (Harold, 1977). ATP could be synthesized from polyphosphate under anaerobic conditions as a result of the low intracellular ATP/ADP ratio (Kornberg, 1957; Kulaev, 1975). Alternatively, an enzyme similar to the ATP-ase could use polyphosphate directly for proton translocation. It is possible that such an enzyme would be induced under "stringent" conditions of energy limitation when the synthesis of other enzymes is repressed, as is the case for enzymes produced when bacteria are starved for amino acids (Cozzone, 1981; Nierlich, 1978). In fact, Barsky *et al.* (1975) and Moyle *et al.* (1972) reported that pyrophosphate is used by some bacteria for proton translocation. Thus, it is conceivable that longer chains of polyphosphate could play a similar role to pyrophosphate, although such enzymes have yet to be detected. Mino *et al.* (1985a, b) reported that both low and high molecular weight polyphosphates occurred in bio-P bacteria and they suggested that the low molecular weight polyphosphate functioned as an energy pool under anaerobic conditions.

Polyphosphate breakdown will result in phosphate accumulation in the cell. Although bacteria have inorganic phosphate pools (Rae and Strickland, 1975), like any other unused metabolite, phosphate would build up to a certain level above which it would be released to their surroundings along its concentration gradient. To avoid wasting inorganic phosphate, this compound could be "sensed" by the cell to be non-essential under anaerobic conditions by having a phosphate carrier protein that would be pH gradient-sensitive. With a reduced pH gradient, as is the case for bio-P bacteria under conditions of energy limitation, inorganic phosphate could not be used for synthetic processes and would be released from the cell if the intracellular concentration exceeded a certain level. Conversely, under conditions of favorable pH gradient (such as under aerobic conditions), phosphate release by this carrier would not occur. For the carrier enzyme to be pH gradient-sensitive would not be a unique phenomenon. For example, transport proteins have been reported to respond to changes of the pH gradient for inward transport of potassium (Bakker and Mangerich, 1981), to changes of the charge gradient for outward movement of sodium (Sorensen and Rosen, 1982), and to changes of the pmf for sugar uptake (Peterkofsky and Gazdar, 1979; Reider *et al.*, 1979).

Therefore, phosphate release in itself is suggested to play a passive role in bio-P removal and the rate of phosphate release would reflect the rate of polyphosphate utilization by bio-P bacteria. It is also expected that the maximum extent of acetate accumulation by bio-P bacteria would be limited by the availability of polyphosphates that can be used to reestablish the pH gradient and allow more acetate uptake. Indeed, Comeau *et al.* (1985b) showed that when more than a given amount of acetate was added to a batch of bio-P sludge, no further phosphate release was observed and the excess of acetate remained in solution.

Another use for the energy produced by polyphosphate is for the storage of acetate as PHB (see Fig. 3). Acetyl CoA can be produced either directly from acetate or via the formation of acetyl phosphate. In both cases, energy in a form such as ATP is required. The enzyme that catalyses the reaction from acetyl phosphate to acetyl CoA is called phosphotransacetylase (Thauer *et al.*, 1977). Lotter (1985) reported a significant activity of the enzyme phosphotransacetylase in full-scale bio-P treatment plants. ATP could be generated from polyphosphate under conditions of low energy but it is conceivable that polyphosphate may be involved directly in the energization of acetate.

Finally, a source of NADH is required to produce acetoacetyl CoA from acetyl CoA (see Fig. 3). Feeding some acetyl CoA into the TCA cycle could provide NADH at a rate just sufficient to resupply the amount utilized.

Postulated model for aerobic (and anoxic) conditions

Phosphate uptake with molecular oxygen (aerobic conditions). The mechanisms explained below are summarized in Fig. 6.

Upon entering the aerobic zone, bio-P bacteria will have accumulated PHB reserves and contain reduced amounts of polyphosphate. With oxygen available they will generate energy by electron transport phosphorylation coupled to ATP formation at the ATP-ase enzyme site (see the section on "bioenergetics: the proton motive force").

With the availability of oxygen, the internal ATP/ADP ratio will increase and the formation of polyphosphate from ATP should take place. As external carbon-containing substrates are consumed by the biomass, bio-P bacteria are expected to degrade their own PHB reserves to produce energy (and possibly to also obtain carbon for synthetic processes). The presence of PHB in bio-P bacteria should help them to grow and rebuild their polyphosphate reserves by taking up soluble phosphate from solution.

Phosphate uptake with oxidized nitrogen (anoxic conditions). Some bio-P bacteria appear to be capable of utilizing oxidized nitrogen (nitrate or nitrite) as an electron acceptor in the absence of oxygen (see "bio-P bacteria"). The only difference to be expected from the utilization of dissolved oxygen for phosphate accumulation, is in the terminal electron acceptor of the electron transport chain for the production of energy. Thus, nitrate-reducing or denitrifying bio-P bacteria are expected to take up phosphate from

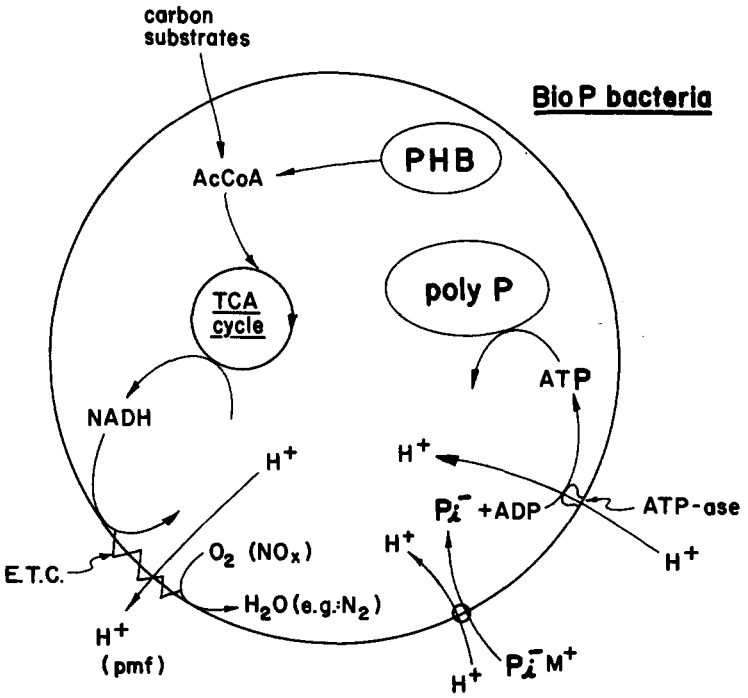

Fig. 6. Postulated model for *aerobic* (and *anoxic*) metabolism of bio-P bacteria, AcCoA, acetyl CoA; E.T.C., electron transport chain; H^+, proton; M^+, metallic cation; NADH, reduced nicotinamide adenine dinucleotide; NO_x, oxidized nitrogen; PHB, poly-β-hydroxybutyrate; pmf, proton motive force; polyP, polyphosphate; TCA, tricarboxylic acid (cycle). Consumption of external or stored substrate in presence of oxygen (or oxidized nitrogen under anoxic conditions) will allow bio-P bacteria to produce a proton motive force (pmf). The pmf can be used, in particular, for phosphate transport and ATP production. ATP is then utilized for growth but also for polyphosphate storage as a result of the availability of soluble phosphate and of energy. Metallic cations are co-transported with phosphate molecules.

solution in the presence of oxidized nitrogen. However, bio-P bacteria that cannot use oxidized nitrogen as an electron acceptor are expected to release phosphate into solution under the same conditions. The net effect on phosphate accumulation or release under anoxic conditions should depend on the relative mass and activity of these groups of bio-P bacteria.

DISCUSSION

A biochemical model that integrates observations from the literature and principles of bioenergetics and membrane transport has been presented. It was essentially proposed that polyphosphate reserves are used as an energy source for the transport and storage of acetate under anaerobic conditions (in absence of both dissolved oxygen and oxidized nitrogen). Although the model was developed with acetate, it is expected that the model should hold true for other similar simple carbon substrates. The availability of the carbon reserves for the exclusive use of bio-P bacteria should result in their growth and proliferation, and in the accumulation of polyphosphate reserves under aerobic or anoxic conditions.

In developing the model, the energetic role of polyphosphate for the storage of acetate was deduced from the pathway of poly-β-hydroxybutyrate (PHB) synthesis (see Fig. 3). The second role of polyphosphate as a source of energy for the transport of carbon substrates was supported by the release of phosphate into solution when chemicals that affect the proton motive force (pmf) of bacteria are added. The addition of acetate, 2,4-dinitrophenol, or sodium hydroxide to anaerobic batches of bio-P mixed liquor resulted in phosphate release [see Figs 1(a) and 2(a)]. H_2S and CO_2 also had a similar effect [see Fig. 2(b)].

Acetate addition dissipates the pH gradient component of the pmf (see the section on "bioenergetics: the proton motive force"). The observation that phosphate is released into solution as a result of acetate addition suggested that polyphosphate reserves supply energy for the reestablishment of the pH gradient. However, acetate storage into PHB also requires energy. Thus, it was decided to test whether phosphate release would take place if only the pmf was affected by the addition of chemicals.

The addition of 2,4-dinitrophenol, which dissipates the pmf by the equilibration across the cytoplasmic membrane of the H^+ concentration, resulted in phosphate release. With the addition of sodium hydroxide to maintain a high pH, it is the pH gradient that was reduced because of the lower external H^+ concentration (see the section on "bioenergetics: the proton motive force"). Again, phosphate release into solu-

tion was observed. Thus, it would appear that polyphosphate reserves can be used to produce energy to reestablish the pH gradient. It should be noted, however, that Potgieter and Evans (1983) reported that a low pH resulted in phosphate release and that increasingly higher pH conditions resulted in less and less release and even phosphate uptake at pH values of 8 or 9. Different experimental conditions due to the sewage, to the microbial biomass characteristics, or to chemical reactions may explain this discrepancy.

The effects on the pmf of H_2S or CO_2 is poorly documented but it is known that the dissociation and equilibration of these gases with their ionic species will cause a pH decrease in solution. We observed, however, that a low pH maintained by the addition of HCl did not result in significant phosphate release [see Fig. 2(a)]. Therefore, it can be speculated that the diffusion of H_2S or CO_2 and their subsequent intracellular dissociation could decrease both the outer and inner pH to such as extent that the pH gradient would be reduced. The fact that phosphate release was observed following the addition of these gases [see Fig. 2(b)], provided support to the hypothesis that polyphosphate reserves produced energy to reestablish the reduced pH gradient.

From the postulated model, the role of the anaerobic zone of a bio-P treatment plant emerges as one in which bio-P bacteria should maximize their carbon storage. For this purpose, conditions ensuring the addition of a maximum of simple carbon substrates as well as a minimum of oxygen and of oxidized nitrogen should be provided. Maximizing the addition of simple carbon substrates could be achieved by the addition of fermented primary sludge, septic sewage, favorable industrial wastes or acetate salts. The operation of a primary sludge fermenter should probably be done in such a way as to minimize H_2S and CO_2 gas production. Minimizing oxygen entrainment could be favored by the avoidance of vortexing as created by too vigorous mixing, or of pumping sewage or return sludge with screw or air lift pumps. Minimizing oxidized nitrogen addition could be achieved by the denitrification of the mixed liquor and of the return sludge prior to recycling into the anaerobic zone.

In the subsequent aerobic zone, bio-P bacteria would consume their internal carbon reserves and externally available substrates to produce energy that would result in phosphate accumulation and storage as polyphosphate.

Under anoxic conditions (absence of dissolved oxygen but presence of oxidized nitrogen), phosphate uptake was reported as well as phosphate release if the concentration of added carbon substrate is high enough [see Fig. 1(a)]. Thus, it was proposed that only a fraction of the bio-P bacteria have the ability to use oxidized nitrogen instead of dissolved oxygen for energy production and phosphate uptake, and that the fraction of the bio-P bacteria that cannot use oxidized nitrogen will release phosphate as they accumulate carbon reserves. Therefore, the net effect of phosphate uptake or release in an anoxic zone should depend on the respective mass and activity of these groups of bio-P bacteria.

Finally, the observation that K^+, Mg^{2+} and Ca^{2+} release or uptake were directly correlated to phosphate release or uptake by the biomass, led us to suggest that these metallic cations were co-transported across the inner bacterial membrane with phosphate molecules.

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