Bacterial stress: prerequisite for biological removal of phosphorus

H. A. Nicholls, D. W. Osborn
City Health Department, Johannesburg

When the bacteria normally found in an activated sludge plant are temporarily deprived of one or more key elements such as nitrogen, sulfur, phosphorus, or oxygen, their normal metabolic processes are unable to function and the organism may find itself in a state of severe stress. Some bacteria may well succumb under these conditions, while others may be able to make use of alternative metabolic pathways.

A particular survival mechanism, which will be constantly referred to in this presentation, is the storage of phosphorus in the form of polyphosphate (poly P) and carbon in the form of poly-β-hydroxy-butyrate (PHB) and/or glycogen. They are high energy compounds that are stored as granules and whose presence can easily be demonstrated by staining with specific dyes. These compounds have a low solubility in water and therefore do not affect the osmotic pressure within the bacteria.

Wilkinson, as quoted by Dawes and Senior,[1] has postulated three criteria governing intracellular storage:

- Storage takes place under conditions when the supply of substrate from exogenous sources is in excess of the immediate requirements of the cell.
- The stored compound is utilized when the energy supply from exogenous sources is no longer sufficient for optimal cell maintenance.
- The stored compound is capable of degradation to produce energy in a form suitable for use by the cell.

Harold [2] has shown that the temporary limitation of sulfur or nitrogen can result in the accumulation of polyphosphate granules within the cells of certain bacteria, while Stanier *et al.*[3] have shown that PHB will accumulate when nucleic acid and protein synthesis are impaired. Dawes and Senior [1] have shown that when exogenous carbon is abundantly available and nitrogen is limiting, glycogen will then accumulate in certain organisms.

Bacteria will behave in their own characteristic way to specific stress conditions, and some may find it expedient to accumulate more than one storage material to provide for times of adversity.

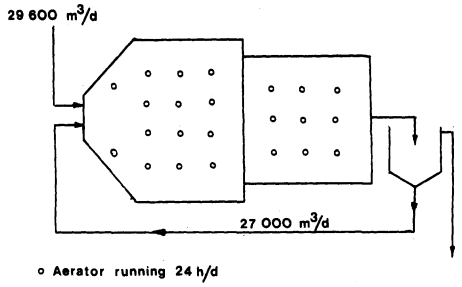
To encourage the storage of both poly P and PHB in a normally operating activated-sludge plant, a temporary stress-producing situation must be introduced, and this is most easily achieved in practice by completely restricting the availability of oxygen and nitrate at certain points in the plant. In such circumstances, aerobic bacteria will be forced to fall back on internally stored energy sources, but facultatively anaerobic bacteria can switch to fermentative metabolic pathways and survive without difficulty. Under such circumstances the latter class of bacteria would therefore not find it necessary to store high energy polymers for survival purposes.

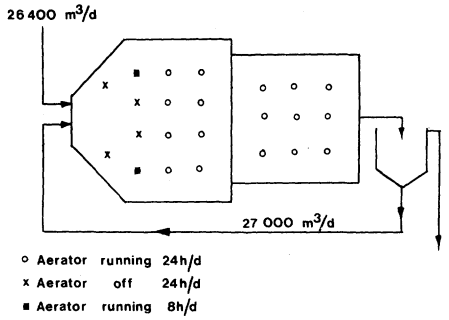
The above concepts appeared to have considerable merit and to develop these ideas further it was decided to modify the operation of two of the existing extended-aeration plants in Johannesburg to provide an anaerobic stress zone.

MODIFICATION OF EXISTING WORKS OPERATION TO PRODUCE ZONES OF BACTERIAL STRESS

The Johannesburg Alexandra Works, commissioned in 1974, was designed as a nitrifying extended-aeration plant having a liquid retention time of 24 hours and a minimum sludge retention time during winter conditions of 8 days. Design capacity was 27 000 m³/d, aeration being conducted by a number of mechanical aerators. The aeration capacity is divided into a primary and a secondary

TABLE I. Effect of introducting a stress zone into the Johannesburg Alexandra Works.





		Nonstress Condition		Stress Condition	
		Feed	Effluent	Feed	Effluent
Total nitrogen	N	42.4	22	34.1	9.7
Ammonia	N	25.6	0.8	17	6.1
Nitrate	N	Nil	18	Nil	1.9
Orthophosphate	P	8.0	8.0	4.7	2.8
Total COD	O	670	80	540	58
Suspended solids		—	24	—	23
pH		7.4	7.1	7.7	7.6
MLSS		5 040		3 900	
Power consumed kWh/d		18 000		13 000	

Notes: Results expressed as mg/l where applicable. Diagram on left = nonstressed condition; right = stress condition.

basin of which the former occupies 60% of the total volume. The plant is schematically represented in the diagrams contained in Table I.

Nicholls [4] modified the operation of this plant by turning off six aerators at the head of the primary aeration basin, thus forcing the normally aerobic bacteria to pass through a zone of quasi anaerobiosis either on their return passage to the plant from the final clarifiers or by recirculation caused by the remaining operating aerators, acting as recycle pumps. This exposure of bacteria to nonoptimal growth conditions, far from having a detrimental effect, appeared to enhance the overall performance of the plant, resulting in 72% removal of total nitrogen, 40% removal of orthophosphate, and 89% reduction in the chemical oxygen demand (COD) concentration. This was achieved without additional construction or modification costs and with a considerable power savings. The results of these experiments are summarized in Table I.

Similar and virtually simultaneous experiments were carried out at the much larger 80 000-m³/d Johannesburg Olifantsvlei Works, which has design parameters virtually identical to the Alexandra Plant. Considerable versatility for experimentation was provided as the total treatment capacity was divided into four equally sized, identical basins, each capable of handling 20 000 m³/d. Furthermore, each basin was divided into four compartments, the one at the inlet end being 40% of the total capacity and the remaining three being of equal size. Provision was also available for the addition of unsettled wastewater to the head of the aeration tank or to a limited number of points downstream of the primary compartment. Areas of oxygen limitation could therefore be created virtually anywhere in the plant by switching off preselected surface aerators. Different aerator performance patterns could be created in different basins and compared with the performance of an unmodified basin.

In summary, it was found that biological uptake of phosphorus was not achieved in the module operating with all its surface aerators running. The best performance was achieved in the unit having most of the aerators in the primary-aeration compartment shutdown. This procedure necessitated all sludge returned from

the final clarifiers having to pass through a zone largely devoid of either dissolved oxygen (DO) or nitrates. Aerobic bacteria passing through this zone were therefore subject to a considerable degree of stress. Sludge tended to accumulate on the floor of this zone, and it was necessary to run the aerators for a period of 2 hours each day to transfer this accumulated mass through the remainder of the system. Venter *et al.*[5] have described these experiments more fully elsewhere, but a typical set of results is given in Table II, from which it will be noted that the introduction of a stress condition for bacteria appears to enhance the biological accumulation of phosphate.

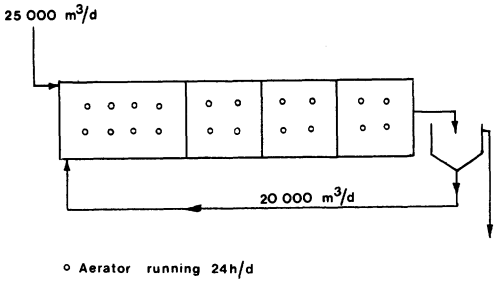
MICROSCOPIC IDENTIFICATION OF POLY P AND PHB IN PHOSPHATE ACCUMULATING SLUDGES

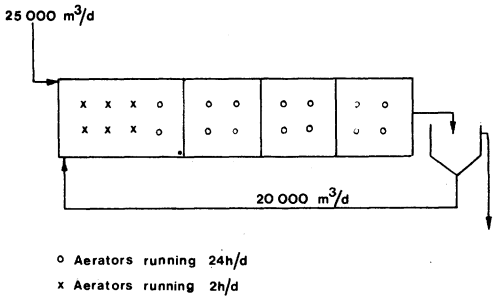
The literature abounds with numerous references to the accumulation of phosphorus as poly P in volutin or metachromatic granules and of PHB as inclusions in the bacterial cytoplasm. Most of the workers in these fields have not been associated with the purification of wastewater, and this is probably the reason that the knowledge has not been applied for the removal of phosphorus and carbon from wastewater effluents at an earlier date. A more comprehensive understanding of the mode of entry of these two elements into the bacterial cell and the reactions that take place intracellularly is obviously necessary if the design of wastewater purification works is to be revised to incorporate nutrient removal.

Sludges taken from the aforementioned two Johannesburg works were microscopically examined at weekly intervals for the presence of poly P, PHB, and glycogen. Specific staining techniques [6-8] were used to differentially color each of the above compounds, and rough quantitative assessments were made. Both poly P and PHB were found to be present in abundance (Figures 1 and 2) in what appeared to be the same type of bacteria. Glycogen was seen in some species of protozoa but not in bacteria. For this reason, glycogen will not be considered further.

The apparent simultaneous appearance of both poly P and PHB in a large number of the bacteria present in these plants was considered to be particularly significant and sug-

TABLE II. Effect of introducting a stress zone into the Johannesburg Olifantsvlei Works.





		Nonstress Condition		Stress Condition	
		Feed	Effluent	Feed	Effluent
Total nitrogen	N	46	24	31	9
Ammonia	N	28	1	16	3
Nitrate	N	Nil	19	Nil	5
Orthophosphate	P	6.0	6.3	4.1	1.9
Total COD	O	560	100	420	52
Suspended solids		—	34	—	17
pH		7.8	7.1	7.3	7.3
MLSS		3 850		4 090	
Power consumed kWh/d		13 000		9 000	

Notes: All results expressed as mg/l where applicable; sludge age not strictly controlled. Diagram on left = nonstress condition; right = stress condition.

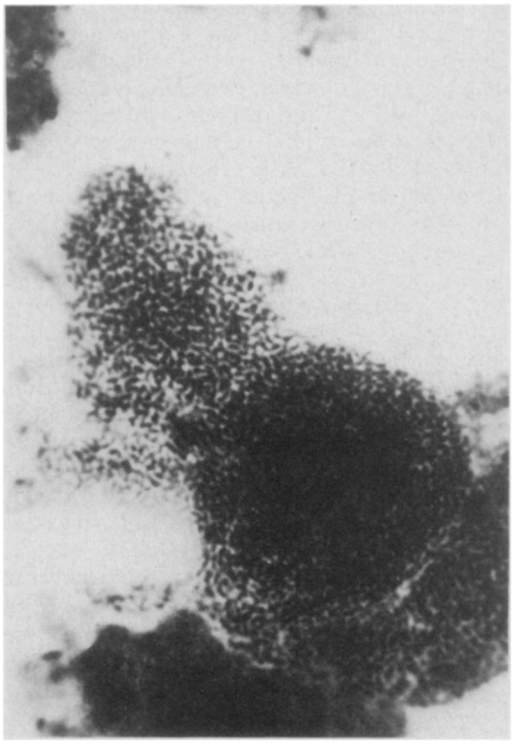


FIGURE 1. Colony of bacteria stained with sudan black to show poly-β-hydroxybutyrate accumulation (magnification 800x).

gestive that the PHB and poly P may play a mutually interdependent role in assisting aerobic bacteria to survive through a period of anaerobiosis. Table III describes these observations in somewhat greater detail.

The remainder of this dissertation is therefore devoted to consideration of the mode of accumulation and utilization of both poly P and PHB, the reasons why both are stored in certain bacteria, and the probable enaction of these stored materials during conditions of stress to provide a survival mechanism for aerobic bacteria in oxygen-limiting circumstances.

FACTORS AFFECTING THE ACCUMULATION OF POLYPHOSPHATES (POLY P)

Research on a whole front has dealt with the capability of bacteria to store phosphorus as polyphosphates.[1, 2, 9] The role of stress has been commented on by the present authors in a previous paper.[10] Other authors have come to general agreement that accumulation of poly P is by two main mechanisms, "overplus" and "luxury uptake."

"Overplus accumulation" (translated by Liss and Langen [11] from the German "überkompensation") occurs when certain bacteria are temporarily deprived of adequate supplies of phosphorus and then subsequently re-exposed to an abundance of this element. This reaction is typified by the findings of Harold [2] and depicted in Figure 3(a), which shows that when an aerobic culture of *Aerobacter aerogenes*, grown in a phosphate deficient medium, is suddenly brought into contact with a phosphate-rich medium, poly P accumulates very rapidly.

Nesmeyanova *et al.*,[12] in an extension of Harold's work, demonstrated in controlled growth experiments using *Escherichia coli* that poly P, amounting to 0.2 to 0.4% of the dry bacterial mass, was accumulated at the end of the lag phase and the start of the log growth phase. Data from their experiments are depicted in Figure 4. It is significant to note that the reduction of stored poly P is accelerated under conditions of rapid growth, which also requires the creation of proportionately large quantities of nucleic acid.

Harold and Sylvan,[13] using *A. aerogenes*, have shown that the phosphorus so released is trapped as ribonucleic acid (RNA).

Medvecsky and Rosenberg [14] using radioactive phosphorus with *E. coli*, have postulated that all cellular phosphorus requirements from exogenous sources pass through the polyphosphate storage pool and that an external source of energy is required for this process.

Observations by the authors, using a pilot plant with an anaerobic/anoxic/aerobic basin configuration, confirm that aerobic bacteria

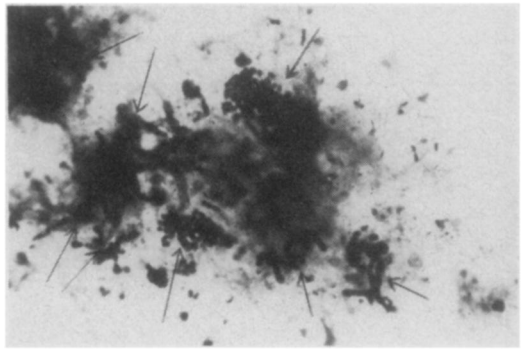


FIGURE 2. Colony of bacteria stained with methylene blue to show polyphosphate accumulation (magnification 800x).

TABLE III. Bacteria accumulating storage compounds isolated from treatment plants removing phosphorus in Johannesburg.

Bacterium	Obligate Aerobe	Facultative Anaerobe	Chemical Stored		Numbers of Organisms Present
			Poly P	PHB	
Acinetobacter	×	–	×	×	abundant
Nocardia	×	–	×	×	scanty
Beijerinckii	×	–	×	×	very scanty
Azotobacter	×	–	×	×	very scanty

leaving the stress condition very rapidly take phosphorus in an overplus-type reaction immediately on entering the aerobic zone, where stress conditions are nonexistent and phosphorus together with an abundant source of energy is available (Table IV).

The deprivation of phosphorus necessary for the overplus mechanism to occur is probably achieved in strict aerobes by curtailing the transfers of exogenous phosphorus into the cell.

Phosphorus can enter the cell by a number of different mechanisms: free diffusion, assisted diffusion, active transport, and group translocations.

The first two processes are slow and require no expenditure of chemical energy from within the cell. For diffusion to occur, a concentration gradient must exist where phosphorus is transferred from a higher concentration to a lower concentration. Therefore, if the phosphorus concentration in a growth medium is higher than that inside the bacterial cell, phosphorus will diffuse in. In activated sludge, particularly in those plants removing soluble phosphorus where the phosphorus concentration within the cell will be much greater than that in the surrounding liquid, there will be a tendency for phosphorus to diffuse out of the cell.

By contrast the latter two processes are

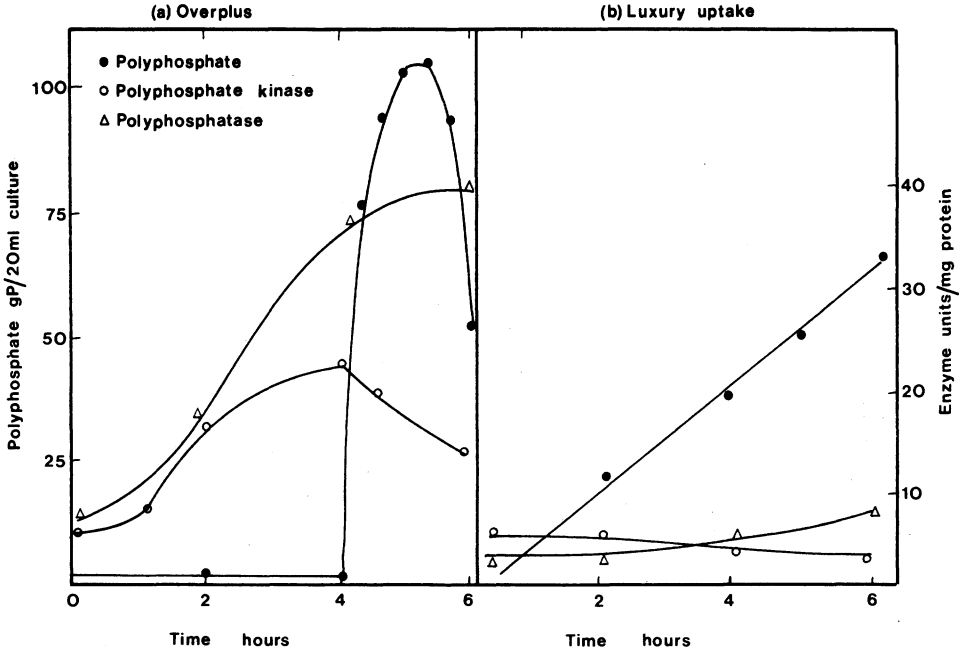
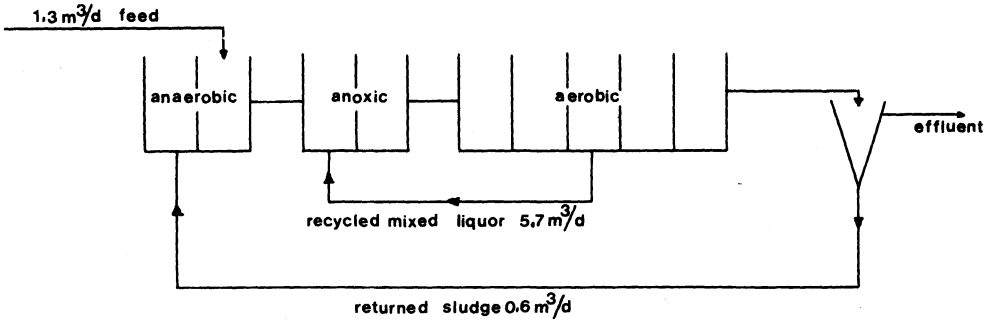


FIGURE 3. Patterns of polyphosphate accumulation in *Aerobacter aerogenes*. (a) Overplus—Cells were placed in medium devoid of phosphate at Oh; phosphate was restored at 4 hours. (b) Luxury uptake—Cells were placed in medium devoid of sulfur at Oh.

TABLE IV. Typical results from a pilot plant with anaerobic/anoxic/aerobic configuration.



Test		Feed	Anaerobic Stress		Anoxic Stress			Aerobic Nonstress			Effluent
Total phosphate	P	20.5									4.2
Orthophosphate	P	10.6	25.2	16.3	7.0	7.2	6.2	4.9	4.1	3.0	3.0	4.0
Ammonia	N	13.9	3.2	6.1	3.4	1.9	1.1	1.4	1.2	0.7	0.9	0.7
Nitrate	N	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	0.3	1.6	1.6
Total COD	O	1070										—
Soluble COD	O	185										60
Suspended solids		680										20.5
MLSS			14 700	7 600						7 400		
Liquid retention time (hr)				2.45		3.15			6.45			
Sludge age days						12						

Note: Results expresses as mg/l where applicable.

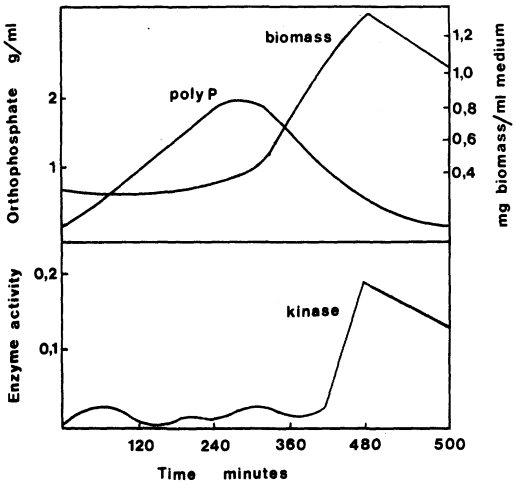


FIGURE 4. Polyphosphate accumulation and kinase activity at different stages in the growth of *Escherichia coli.*

rapid and require adenosine triphosphate (ATP) for their execution. Under conditions of stress where the availability of ATP will be severely limited, a reduction in the amount of exogenous phosphorus passing into the cell is expected. Carberry and Tenney [15] and Yall *et al.*[16] have shown, using P^{32}, that there is a continual movement of P^{32} into and out of the cell under nonstress conditions, where more phosphorus is transported into the cell using active transport than is diffused out of the cell. Under stress conditions the reverse is expected to be true and the net result is deprivation of phosphorus in the cells of strictly aerobic organisms. The overplus accumulation of phosphorus is depicted diagrammatically in Figure 5.

"Luxury uptake" occurs when an essential element other than phosphorus is limited, but sufficient energy is available to transfer phosphorus into the cell. Harold's [2] findings in this regard are recorded in Figure 3(b) and can be regarded as typical of the reactions taking place under nutrient limiting conditions. When related to the Johannesburg works, such reactions could be expected to occur under conditions of endogenous respiration occurring at the end of the aeration tank (see Figure 6). It will be noted that under these conditions, the rate of poly P accumulation is relatively slow.

The enzyme phosphate kinase plays a major role in the conversion of orthophosphate to poly P, the form in which it is stored. This is achieved by catalyzing the transfer of the terminal phosphate group of ATP to poly P.[13] Under conditions where ATP has been reduced to adenosine diphosphate (ADP), as in a stress situation, the storage of poly P is strongly inhibited.[2]

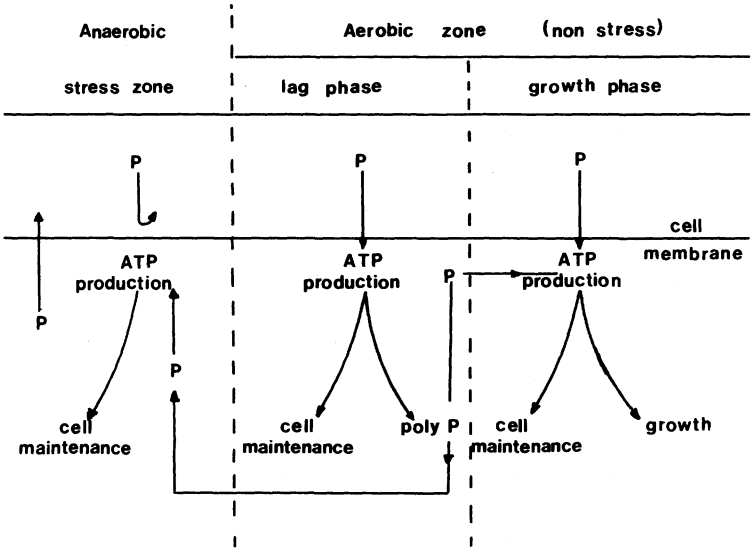


FIGURE 5. Simplified biochemical pathways involved in the movement of phosphorus within the cell during overplus accumulation.

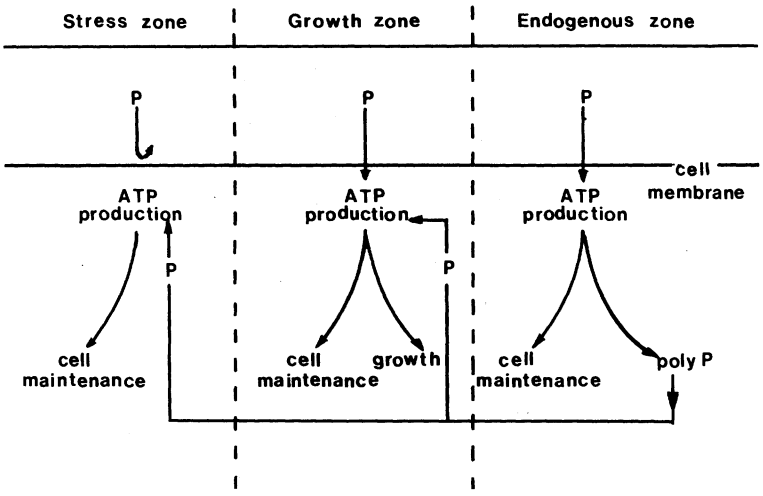


FIGURE 6. Simplified biochemical pathway involving the movement of phosphorus within the bacterial cell during luxury uptake.

Nesmeyanova *et al.*,[17] while unfortunately not continuing their experiments into the endogenous phase that could be of interest to workers operating plants with long sludge ages, were able to show that the concentration of kinase, and hence the ability to store poly P, reached a maximum just before the onset of endogenous respiration. It is also of interest to note that the activity of kinase is regulated by the intracellular level of orthophosphate and is independent of the exogenous level.[18]

The accumulation of poly P is seen as a device to provide a balancing mechanism to attenuate any sudden variations of intracel-

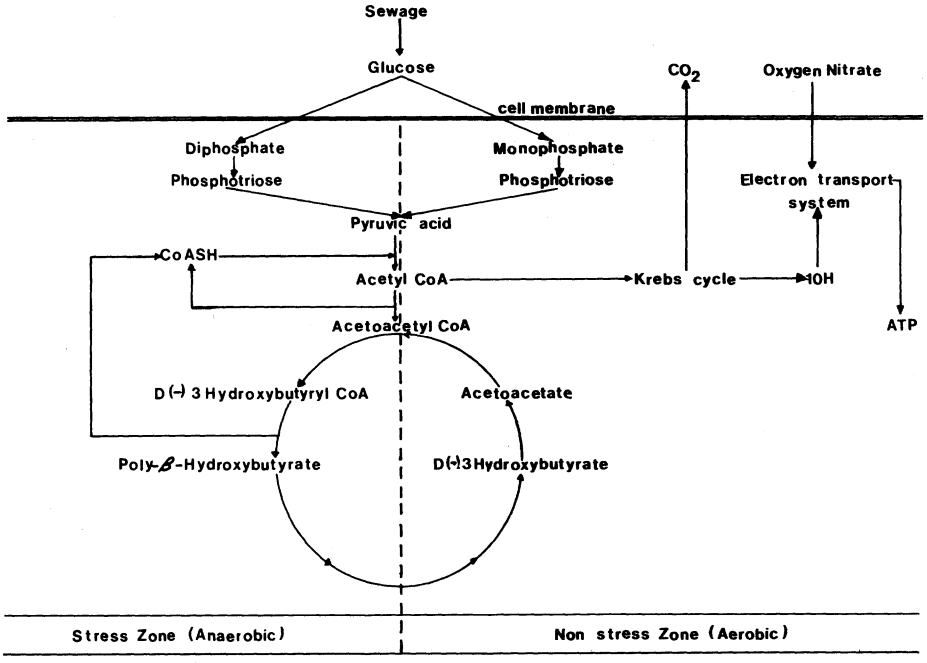


FIGURE 7. Simplified biochemical pathways showing the role of the hydroxybutyrate and Krebs cycles.

lular orthophosphate.[2, 17] Under certain conditions of stress this reservoir of high energy polymer may be called on to provide a limited amount of ATP for survival. Such conditions exist when aerobic bacteria are deprived of an oxygen supply, as in an anaerobic zone, where although phosphorus may be available, it cannot be converted to usable ATP because the intermediate Krebs cycle is inoperative as a result of the absence of a suitable electron acceptor. In these circumstances, poly P may be withdrawn from storage and converted by a reversed kinase reaction directly to ATP. Alternatively, and more likely, the stored poly P may be hydrolyzed to orthophosphate by the enzyme polyphosphatase for ultimate conversion to ATP.

FACTORS AFFECTING THE ACCUMULATION OF POLY-β-HYDROXYBUTYRATE

A surprisingly wide variety of bacteria can accumulate PHB, many species being found in activated sludge plants. PHB accumulates on the surface of the cell as lipids or as water-insoluble crystals contained as inclusions within the cell. *Hydrogenonomas eutropha* have been noted by Oedins and Schlegel [19] to accumulate up to 74% of its dry mass as PHB. Concentrations of 30 to 50% in other bacteria are not uncommon.[1]

Current experience with the modified activated sludge plants in Johannesburg suggests that it is only certain species of aerobic bacteria that accumulates PHB (Table III), thus supporting the conclusion arrived at by Senior *et al.*[20] that temporary deprivation of oxygen can result in the accumulation of PHB in certain bacteria.

At this point it is therefore expedient to examine in greater detail some of the enzymatic reactions that proceed in aerobic bacteria when they are exposed to stress conditions caused by anaerobiosis. Reference to Figure 7 will show that the extracellular substrate (or COD) can be metabolized up to the point of production of pyruvic acid under either aerobic or anaerobic conditions. This is further quantified in Equation 1.

$$\text{Wastewater (COD)} \rightarrow \underset{\text{Glucose}}{C_6H_{12}O_6} \rightarrow \underset{\text{Pyruvic acid}}{2CH_3COCOOH} + 4H^+ + 4e^- \quad (1)$$

Lawson,[21] working on a purified culture of *Acinetobacter,* (originally obtained from one of the Johannesburg activated sludge plants),

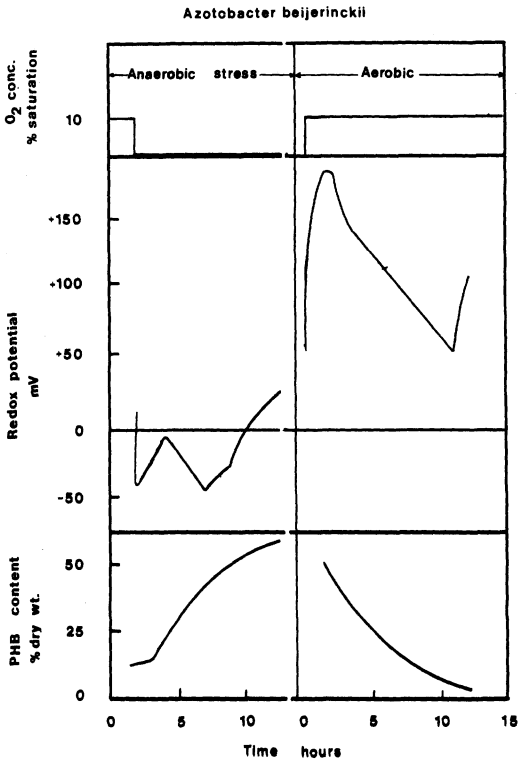


FIGURE 8. The effect of oxygen stress on the accumulation poly-β-hydroxybutyrate in a culture of *Azotobacter beijerinckii.*

has shown that substrate (COD) does in fact pass through the bacterial membrane under oxygen-limiting circumstances and without the need of any auxilliary energy source to accumulate as pyruvic acid.

Under normal aerobic conditions the hydrogen and electrons generated as Equation 1 would pass out of the enzymatic chain by the Krebs cycle and electron transport system as water. Under anaerobic conditions, the oxygen required for the operation of the latter transport system is not available, and unless alternative pathways are opened for the disposal of accumulated hydrogen and electrons, the reaction will cease at the pyruvate stage.

Dawes and Senior [1] have shown that certain bacteria do have the ability to convert these hydrogen atoms by acetyl coenzyme A to PHB, thus providing a "hydrogen sink" as a temporary alternative to the Krebs cycle (Figure 7). These reactions are of particular importance in relation to the Johannesburg plants as they indicate that aerobic bacteria can still remove COD even under severe oxygen-limitation conditions. Once aerobic conditions

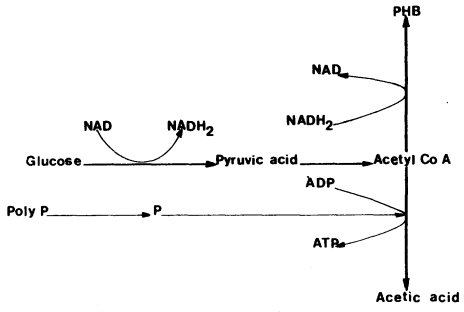


FIGURE 9. Postulated utilization of poly-β-hydroxybutyrate and polyphosphate as a survival mechanism in terms of stress.

are restored, the hydrogen ions stored temporarily as PHB pass back through acetyl coenzyme A as an intermediary and enter the normal Krebs cycle once again.

The above reactions have been very ably demonstrated by experiments conducted by Senior *et al.*[20] Using a chemostat they limited the supply of nitrogen, oxygen, and carbon in turn to a culture of *A. beijerinkii*. Their results are depicted in Figure 8 and indicate that only oxygen limitation produced an accumulation of PHB (up to 60% by mass). The intracellular concentration of the latter increased slowly with time. It will be noted that the initial drop in redox potential (ORP), because of the presence of hydrogen ions, increased to positive values as the PHB continued to accumulate. On restoration of aerobic conditions, the redox potential was raised to even higher values and then declined as the PHB was redirected through the Krebs cycle.

ROLE OF PHB AND POLY P IN THE SURVIVAL OF AEROBIC BACTERIA

Mention has already been made that the inclusion of quasi anaerobic areas into the Johannesburg activated sludge plants appears to have encouraged the prolific growth of a biota capable of storing PHB and poly P, and that the role played by these two substances obviously required further examination.

The formation of PHB occurs when aerobic bacteria find themselves under stress in an anaerobic environment. Under such circumstances ATP cannot be produced by the Krebs cycle, but Stanier *et al.*[3] have shown that the energy (ATP) necessary for survival can be supplied in terms of the following equation:

$$\text{Acetyl coenzyme A} + \text{ADP} + \text{P} \xrightarrow{+2\text{H}} \text{ATP} + \text{Acetic acid} + \text{CoAH} \quad (2)$$

Figure 9 shows that under anaerobic conditions, wastewater substrate can still be assimilated by aerobic bacteria capable of storing carbon and hydrogen as PHB. Acetyl coenzyme A is a necessary intermediate in such a reaction and is therefore available as one of the reactants required for Equation 2 to take place. Under stress conditions ADP will also be present as previously indicated. The remaining reactant required to satisfy Equation 2 is phosphorus, and it is postulated that this is made available under stress conditions from the polyphosphate pool.

The occurrence of both PHB and poly P deposits in bacteria, found in activated sludge systems designed to induce some degree of stress to the biota, is therefore not a matter of chance but rather a dire necessity and a means of survival.

DISCUSSION

It is suggested that the presence of bacteria containing stored materials could confer a considerable degree of stability to an activated sludge plant, particularly in the event of a power failure. To illustrate this point, some sludge from the Johannesburg Olifantsvlei Works was stored without the addition of any further substrate for a period of 1 month at 4°C. Considerable release of phosphorus took

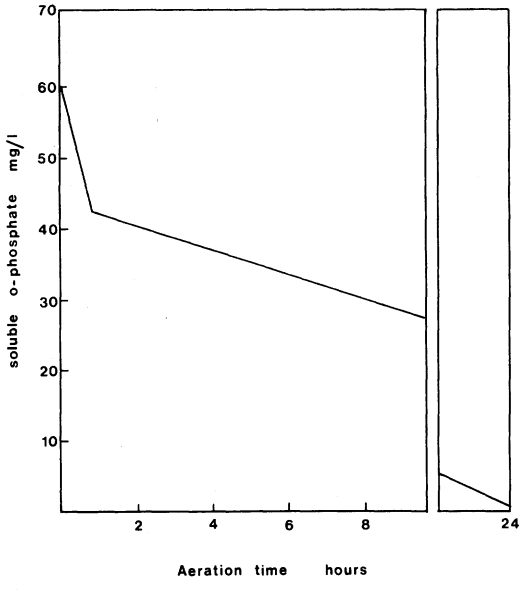


FIGURE 10. Uptake of phosphorus by activated sludge that has been stored under anaerobic conditions for 4 weeks then re-aerated for 24 hours.

FIGURE 11. The 150 000-m³/d Johannesburg Goudkoppies Works under construction in 1977. (1) coarse and fine screens, (2) primary sedimentation tanks, (3) balancing tank, (4) anaerobic zone, (5) primary anoxic zone, (6) primary aeration zone, (7) secondary anoxic zone, (8) secondary aeration zone, and (9) final clarifiers.

place during this period and microscopic examination revealed that considerable shrinkage of the poly P pool had taken place.

After 24 hours of aeration the bacteria appeared to completely recover; the phosphorus was once again taken into the bacterial cells, and the poly P storage facilities were noted to have been replenished. This sequence of events is recorded in Figure 10 from which it will be noted that the re-adsorption of phosphorus is biphasic—the more rapid rate being strongly indicative of the overplus mechanism.

The broad concepts presented thus far have been incorporated into the new 150 000-m³/d Johannesburg Goudkoppie Works(Figure 11), completed in May 1978. Costing $11.0 million at 1977 prices, this plant was designed to handle settled wastewater with a biochemical oxygen demand of 300 mg/l in an anaerobic/anoxic/aerobic/secondary anoxic/secondary aerobic zone configuration having retention times of 1, 2.3, 7.1, 2.3, and 1.3 hours, respectively, based on the incoming wastewater flow. Provision has been made for a maximum internal recycle rate from the primary aeration to the primary anoxic zone of 16:1, but more recent pilot-plant experiments indicate the possibility of ultimate use of only one quarter of this capacity. The recycle rate from the secondary clarifiers to the anaerobic zone is rated 1.6:1 on average dry-weather flow.

This plant was originally designed for denitrification only, but while under construction the advantage of including an anaerobic zone became increasingly apparent and the construction was accordingly modified. A further variation from the original design was to make it possible for sludge from the primary clarifiers to be added to the system to provide an additional operational facility to ensure good denitrification. Effluent quality from this plant is expected to comply with orthophosphate < 1 mg/l and total nitrogen as < 10 mg/l for 95% of the time.

Concurrently with the construction of Goudkoppie Works, a pilot plant was established at the Johannesburg Northern Works, where an almost exactly similar activated-sludge plant is under construction, scheduled to become operational in 1979. Some of the observations made on the pilot plant are pertinent to the current discussion, and are therefore briefly commented on in the following.

Effects of nitrate. Osborn and Nicholls [10] have suggested that nitrates in the final effluent from the plant should be $N \not> 2$ mg/l to ensure that nitrates returned with the underflow from the final clarifier do not seriously affect the performance of the initial oxygen limiting zone. This can be achieved provided COD is available in the feed to the plant.

Effect of COD. Martin and Marais,[22] Carberry and Tenney,[15] and others have claimed that phosphorus removal can be optimized at particular ratios of COD to phosphorus at a given sludge age. While such claims are un-

doubtedly true of the specific cases under investigation, they do not provide an understanding of the fundamental interactions caused by introducing a COD load at a specific point into a delicately balanced biological system. Observations on the Johannesburg pilot plant indicate that phosphate removal is not necessarily directly proportional to the mixed liquor suspended solids (MLSS) concentration, the growth of which is in turn dependent on DO and COD concentrations together with the liquid retention time and the sludge age. It appears to be more closely related to the conditioning received by the sludge during its passage through the anaerobic zone. A larger COD load will result in more active growth at the start of the aeration basin in a plug-flow system. This additional growth will in turn result in more ATP production that could give rise to greater kinase activity. Nesmeyanova [17] has shown that some of the stored poly P is utilized during this rapid growth phase. Therefore the greater the amount of this poly P that can be utilized, the greater will be the orthophosphate concentration required to replenish the poly P pool.

Effect of stress in an oxygen-limiting zone. Varying degrees of stress can be imposed on strict aerobes by altering the COD concentration entering the anaerobic zone or by changing the residence time in the zone. Thus the type of bacterial population present in the system can be varied by regulating the "stress" in the oxygen-limiting zone. The greater the stress, the greater the preponderance of a biota with survival characteristics, which are usually associated with phosphorus storage capabilities. As a corollary to this thesis, it must be accepted that the conventional interpretation of the term "sludge age" now needs to be reconsidered.

Effect of plug-flow. It has been noted previously that if "luxury" uptake of phosphorus is to occur, growth of the biomass must have virtually ceased so that competition for ATP is relieved for it to be converted by kinase to poly P for storage. This is less likely to happen in a completely mixed plant where cells are brought into frequent contact with the influent to the aeration basin.

In a denitrifying plant with an internal recycle, provided the winter temperature does not affect nitrification efficiency to a marked extent, the point of abstraction of the returned sludge should be immediately after the point of complete nitrification. Recycling from the end of the aeration basin appears to be contraindicated. In a nonnitrifying plug-flow plant, there might be some advantage in wasting sludge from near the influent end of the aeration basin.

Design of the new 150 000 m^3/d Johannesburg Bushkoppies Works, which is under construction, has been much influenced by the work described. Briefly, this will be a diffused air, extended-aeration plant in which about one-third of the flow will be roughly settled and the remaining unsettled wastewater added directly into an anaerobic/anoxic/aerobic basin configuration with the retention time in the anaerobic zone being about 2 hours, based on the flow of the untreated wastewater only. The aerated portion will operate with plug-flow at its lower end and will have provision to vary the point of abstraction of the internally recycled liquor. The rough sedimentation tanks will serve to accumulate untreated solids that will be released as required into the system via any of the three zones. In this way some degree of balancing will be achieved and the settled material can be fed to the plant when the main peak has passed or to the anaerobic zones to control both denitrification and the degree of stress applied to the biota.

CONCLUSION

The experiments described and the general experience gained in Johannesburg have led to the belief that the incorporation into an extended-aeration, activated sludge plant of an anaerobic zone with a retention time of about 2 hours (based on the incoming wastewater flow) has definite advantages in controlling the uptake of phosphorus by bacteria and in encouraging the storage of stable organic compounds such as PHB.

The stress applied to bacteria in this zone can be varied, particularly when a controlled source of untreated wastewater solids is available, and in this way the type of biological life present may be manipulated to favor those species capable of storing phosphorus for use in times of need. Such a process has the added advantage of producing effluents of similar quality to conventional plants but with a considerable savings in externally applied energy.

The lower oxygen levels prevailing in this type of plant are likely to lead to the production of slightly greater quantities of waste sludge that must be handled aerobically if further dewatering is required.

Subsequent to these experiments conducted in Johannesburg, it is encouraging to note that *in situ* stress zones have been successfully applied in existing plants in Pretoria and the Durban area. New plants with a specifically designed anaerobic area have also been installed in several towns including Meyerton, the new gasoline-from-coal plant at Secunda and at Salisbury and Umtali in Rhodesia.

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Authors. H. A. Nicholls is the Scientific Officer responsible for the laboratory and pilot plant facilities at the Northern Waste Water Purification Works, and D. W. Osborn is Chief Scientific Officer responsible for the Laboratory and Technical Services Branch of the City Health Department of Johannesburg.

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