Letter to the Editor with regard to the paper

CHANGES IN MORPHOLOGY AND PHOSPHATE-UPTAKE PATTERNS OF ACINETOBACTER CALCOACETICUS STRAINS

by EN Lawson and NE Tonhazy published in *Water SA* 6(3) 105—112, July 1980.

To those seeking to optimize plant control by attempting to elucidate the synergetic functioning of intracellular and extracellular phosphate removal mechanisms in activated sludge processes, the authors' meticulous work is of such importance and topical interest that it merits publication of certain additional data which may have been excluded from their published paper for the sake of brevity.

In particular, there is much interest and very little published quantitative data relating to the capacity of bacteria to contain phosphate intracellularly as an ordinary 'metabolic' requirement and as 'excess' stored in poly-phosphate form. For example, observed upper limits to the PO_4 contents of the total dry mass of living bacteria before and after 'luxury uptake', which are of interest to observers in this field, are not specifically stated in the paper.

Figures 3 and 4 seem to indicate that the maximum PO_4 'luxury uptake' recorded was 10% of the dry mass of living bacteria; but the percentage of PO_4 in the dry mass of living bacteria before the 'luxury uptake' occurred is not evident from Table 3.

It would be useful if the authors could confirm that PO_4 accounted for no more than 10% of the dry mass of living bacteria prior to 'luxury uptake', or state such other maximum percentage as they may have measured prior to 'luxury uptake'.

Publication of such additional data from the authors experiments or from literature available to them, might help to confirm or correct the impression that the maximum PO_4 content of living bacteria in activated sludge is unlikely to exceed about 20% their dry mass (which includes a maximum 'normal metabolic' PO_4 content of about 10% and a maximum 'luxury uptake' volutin content of about 10% of the total dry mass of living bacteria of which about 85% is volatile), thereby imposing a limitation on the phosphate removal capability of intracellular mechanisms in activated sludge treating municipal wastewaters without addition of chemicals. Such a limitation is not evident at Pinetown's Umhlatuzana activated sludge plant where wastewater total PO_4 concentrations in a 780 mg COD/ℓ wastewater are reduced from 36 mg PO_4/ℓ to near zero, by the apparent predominance of natural extracellular mechanisms of precipitation and polymer-binding of insoluble colloidal phosphate compounds in activated sludge.

On the basis of observation of the performance of the aforementioned plant, it has been postulated that release of PO_4 from intracellular poly-phosphate storage under anaerobic conditions, as mentioned by the authors, may play a significant role in temporarily enhancing extracellular PO_4 concentrations and consequently phosphate precipitation during a conditioning period which may be a necessary precedent to predominance of the natural extracellular mechanisms to which the best phosphate-removal performance of activated sludge may be attributable. In this context, if the authors measured the rates of release of phosphate under anaerobic conditions following the 'uptake' tests recorded by Figures 3 and 4, their observations and publication of the amounts and rates of PO_4 release per unit dry mass of organisms would be a useful addition to the record of their work.

With regard to extracellular precipitation, it would also be of interest to workers in this field if the authors could express their views on the possibility that a significant proportion of the PO_4 removal, attributed by them to luxury uptake and intracellular storage of poly-phosphate, might have been accounted for by phosphates extracellularly bound. For example, by precipitation and natural extracellular polymer-binding of sub-colloidal size (5 to 100 nm) insoluble phosphate compounds incorporating some of the cationic constituents of their 'phosphate-uptake medium'.

These comments and requests for more data are made in the belief that from the combined findings of intracellular luxury uptake research and extracellular precipitation and polymer-colloid-binding research, the understanding will come of phosphate removal mechanisms. This is essential for the optimization of full scale operation of activated sludge processes and more general circumvention of suggested limitations, such as 1 mg phosphorus removal capability per 100 mg COD application which may be effectively restrictive only in activated sludge processes so controlled that extra-cellular precipitation and binding mechanisms can play no more than an insignificant role by comparison with 'luxury uptake'. This belief springs from observations at Pinetown's Umhlatuzana plant, which indicate that the restrictions of the aforementioned kind may apply only when insufficient concentrations of relatively insoluble sub-colloidal phosphate compounds have been accumulated extracellularly in the activated sludge by natural polymer-binding.

Recent results indicate that favourable operating conditions (such as those at Pinetown's Umhlatuzana plant where intracellular uptake and anaerobic release may predominate during a relatively brief conditioning period, prior to steady state predominance of extracellular phosphate removal mechanisms) may be more generally attainable in activated sludge plant of appropriate configuration and capable of producing effluent filtrate phosphate concentrations an order of magnitude lower than those observed at the pilot plant, referred to by the authors as the source of their bacteria, namely 3 to 7 mg P/ℓ orthophosphates as recorded in the tables of H.A. Nicholls' paper ("Kinetics of phosphorus transformations in aerobic and anaerobic environments" presented in Copenhagen 19—21 June 1978 at Post Conference Seminar PS1 following the 9th IAWPR Conference in Stockholm).

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EN Lawson replies as follows:

From your letter it appears that you consider that extra-cellular precipitation and binding of phosphate is as important, or even more important than intracellular uptake and that this may be enhanced by phosphate release by bacteria. This may very well be true in activated sludge. We have no evidence so far that extracellular binding plays an important role in phosphate removal in our system.

However, we do consider that because our system is much simpler and more controllable than activated sludge, it is possible to get answers to questions which are unanswerable with activated sludge. This is due to the inability to regulate in activated sludge other factors interplaying with those under investigation. If we can determine the behaviour of the bacteria towards various environmental factors and why they have this behaviour, then hopefully this can be extrapolated to activated sludge. But it is necessary to see the limitations of our system and we see them only too clearly.

You wished to have further quantitative data on the experiment described in our paper. In addition, amounts and rates of phosphate release under anaerobic conditions was requested. This can be provided by the results of a very similar experiment where phosphate release under anaerobiosis was also assessed. Materials and methods were as described and apart from minor differences the results were also very close to those presented in our paper.

Acinetobacter calcoaceticus strains cultured under conditions of decreased oxygen supply (air bubbled into the medium) increased in mass after 21,5 h (converted to dry weight) as follows:

Culture type I

Strain	SW1b	16 mg/100 mℓ
	SW5a	10 mg/100 mℓ
	SW8a	11 mg/100 mℓ
	SW9b	9 mg/100 mℓ

and in

Strain	SW1b	27,5	mg phosphate were taken up per 100 mg dry weight
	SW5a	36	
	SW8a	5	
	SW9b	42	

Thus, apart from SW8a, the bacteria removed phosphate in excess of the 20% referred to in your letter but the cells are of course abnormally large under these conditions of growth and have an increased fluid content. This was clear from a dry weight comparison with bacteria showing similar turbidities when suspended in liquid, but cultured in the presence of more oxygen.

In the case of growth under conditions of increased oxygen supply (the surface of the growth medium was continuously disturbed by rotation at 100 r/min) the dry weight of the cell mass obtained was as follows:

Culture type II

Strain	SW1b	84 mg/100 mℓ
	SW5a	76 mg/100 mℓ
	SW8a	77 mg/100 mℓ
	SW9b	88 mg/100 mℓ

These are the average values of 5 sets of 200 mℓ of culture

and in

Strain	SW1b	6	mg phosphate were taken up per 100 mg dry weight
	SW5a	6,6	
	SW8a	6,5	
	SW9b	5,7	

Now, it might not follow that the bacteria contained exactly the same amount of phosphate when placed into the non-growth medium (to assess luxury phosphate uptake) because we had found originally that sometimes "shock" release of phosphate was exhibited when bacteria were placed into another medium. Fortunately centrifugation (used for harvesting the cells) had little effect and we believe that under conditions of this experiment little or no phosphate was released by the cells. The reasons for this should become clear to you later when I refer again to shock release. Our experiments on shock release gave results which provided some insight on the upper limit of phosphate content of the bacteria and excretion of phosphate under anaerobic conditions.

Luxury phosphate uptake of the four strains after 6 h aeration under non-growth conditions was found to be:

Culture type I

Strain	SW2b	100 mg dry weight removed 26 mg phosphate
	SW5a	100 mg dry weight removed 10 mg phosphate
	SW8a	100 mg dry weight removed 11,5 mg phosphate
	SW9b	100 mg dry weight removed 13,5 mg phosphate

Culture type II

Strain	SW1b	100 mg dry weight removed 11 mg phosphate
	SW5a	100 mg dry weight removed 5 mg phosphate
	SW8a	100 mg dry weight removed 4 mg phosphate
	SW9b	100 mg dry weight removed 13 mg phosphate

Here clearly, as stated in our paper, the strains with greater nutritional versatility (SW1b and SW9b) showed an increased capacity for phosphate removal. It can also be seen that under conditions of oxygen deprivation during growth more phosphate is removed.

After aeration the bacteria were placed under anaerobic conditions, achieved by using BOD-type bottles, completely filled with the cell suspensions.

We had found from previous investigations that most phosphorus was released shortly after anaerobiosis was imposed and reached a maximum after 3,5 h. In this experiment 4 h of anaerobiosis resulted in phosphate release (again calculated to 100 mg dry weight of cells) as follows:

Culture type I

Strain	SW2b,	released 4 mg phosphate
	SW5a	released 3,5 mg phosphate
	SW8a	released 6,5 mg phosphate
	SW9b	released 4,5 mg phosphate

Culture type II

Strain	SW1b	released 4,5 mg phosphate
	SW5a	released 5 mg phosphate

SW8a released 4 mg phosphate
SW9b released 5 mg phosphate

It would thus appear that all strains, with the possible exception of strain SW8a, released similar amounts of phosphate under the anaerobic conditions used and that the method of culture employed to obtain the cells did not affect that. Interestingly also, a comparison of phosphate uptake and release in strains SW5a and SW8a tended to indicate that only phosphate accumulated under non-growth conditions was released.

Referring again to the shock excretion of phosphate mentioned earlier, we found that if the cells were grown in a medium which contained phosphate far in excess of that provided in the experiment just described then "shock release" occurred. The only exception was strain SW9b which never exhibited shock release. High amounts of phosphate were taken up but the bacteria became voided of phosphate when they met conditions of less phosphate in the environment. Cells exhibiting "shock release" also excreted greater amounts of phosphate under anaerobic conditions.

The following results illustrate this. Strain SW1b was used. Two types of media were employed for growth. One had a phosphate content of 60 mg/ℓ while the other had additional phosphate in the form of 6 g NaH_2PO_4 per litre. Unfortunately no analysis of phosphate uptake during growth was made, but results from other experiments indicate that phosphate uptake was only slightly higher than the amount released (if shock release occurred). After growth the cells were harvested and placed in a non-growth medium which had a phosphate content of 68 mg/ℓ.

Within 15 min after start of the experiment, to determine luxury uptake, cells grown with high phosphate had released 17,5 mg phosphate per 100 mg dry weight. After 1 h the bacteria had removed the same amount of phosphate as was originally released. Only an additional amount of phosphate uptake of 3 mg was found after the usual 6 h. In the case of the bacteria grown under conditions of low phosphate, luxury-uptake started immediately and 25 mg per 100 mg dry weight was removed in 5 h. More-or-less all the phosphate in the medium had disappeared in 6 h.

When put under anaerobiosis the bacteria which had exhibited shock excretion again released 17 mg phosphate per 100 mg dry weight. They (i.e. samples from the same batch) were also put under anaerobiosis with the addition of metabolic inhibitors (sodium iodoacetate, sodium malonate and sodium fluoride) and in all cases release dropped to approximately 4 mg per 100 mg dry weight. In contrast the culture obtained from growth with low phosphate only released 2 mg phosphate in the absence of inhibitors. Thus growth in the presence of excess phosphate resulted in a high phosphate excretion and most, if not all, was under metabolic control of the cells.

In conclusion it appears that the bacteria do have an upper limit of phosphate uptake which is less than 20% of their dry weight but this varies from strain to strain and can be affected by environmental conditions during growth, like concentration of oxygen (where it can be more than doubled under the right conditions, but to the detriment of growth and, probably, future viability) and the amount of phosphate present. Increased phosphate removal might also involve extracellular mechanisms of precipitation which cannot be assessed from our experimental data.

High amounts of phosphate release under anaerobic conditions reflect an excessive uptake of phosphate during growth, when oxygen is not limiting. If less phosphate is taken up during growth than the bacteria are capable of doing, then a high "luxury-uptake" is induced but no phosphate is released on anaerobiosis. The value of anaerobic release of phosphate may therefore reside in a rectification of a phosphate imbalance in the cell created during growth. If this is true, then if that imbalance is not present anaerobiosis has little value.

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GUIDE TO AUTHORS

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