Fatty acid utilisation by sludge from full-scale nutrient removal plants, with special reference to the role of nitrate*

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Abstract

The response of phosphate accumulating activated sludge, when exposed to various organic compounds, under anaerobic and anoxic conditions respectively, is described. Batch experiments were used to study the interactions between phosphate release, substrate utilisation and the concomitant concentration changes of ions such as calcium, magnesium, sulphate and potassium in sludge derived from four full-scale plants. The results obtained support the hypothesis that phosphate release occurs simultaneously with phosphate uptake under anoxic conditions. It is shown that substrate utilisation is partitioned between the requirements associated with phosphate release and with denitrification respectively, and that the relative amounts depend on the nature of the substrate and the origin of the sludge. In situations where these substrate consuming reactions occur in parallel, up to 100% of propionate is expended on phosphate release. The corresponding value for acetate may be as low as 33% while with compounds such as lactate the substrate is utilised almost exclusively for denitrification rather than phosphate release.

Introduction

The inhibition of biological phosphate removal by the ingress of nitrate into the anaerobic stage of nutrient removal plants has been widely observed. Among the researchers who have reported on this phenomenon are Menar and Jenkins (1969), Barnard (1976), Venter *et al.* (1978), Osborn and Nicholls (1978), Rensink (1981), Fukase *et al.* (1982) and Wentzel *et al.* (1985). Iwema and Meunier (1985) advanced two possible explanations for this phenomenon. On the one hand the presence of nitrate causes the redox potential to be so high that it either precludes phosphate release under anaerobic stress conditions or inhibits organic acid formation, thus depriving the phosphate removing bacteria of their preferred substrate. Alternatively, the easily biodegradable short-chain organic acids are consumed by denitrifying bacteria, leaving practically nothing for utilisation by phosphate removing bacteria.

Gerber *et al.* (1987) reported that the release of accumulated phosphate is governed by the nature of the substrate. Compounds such as acetate and propionate induced phosphate release not only under anaerobiosis but also under anoxic and aerobic conditions. On the other hand, with compounds such as ethanol and glucose, phosphate release occurred only after the onset of anaerobiosis, while substrates such as butyrate and lactate displayed variable behaviour which seemed related to the origin of the sludge.

The time course of phosphate concentration changes following contact between nitrified mixed liquor and short-chain fatty acids was shown to proceed in three segments, depending on the nature of the substrate and the amount of nitrate in the mixture. In the presence of substrate, which is capable of inducing phosphate release under anoxic conditions, an initial phase of rapid net release occurred. After complete substrate utilisation but with nitrate still present, a period of net phosphate uptake was observed. Ultimately, after complete denitrification, a secondary phase characterised by relatively slow phosphate release occurred (Gerber *et al.*, 1987).

These authors suggested that phosphate release and phosphate uptake occur simultaneously upon admixture of phosphate-laden sludge and specific substrates under anoxic conditions.

This paper is concerned with the response of activated sludge, derived from different nutrient removal plants, when exposed to selected substrates under anoxic and anaerobic batch conditions respectively. The primary objective of the study was to determine whether the consumption of selected organic substrate for denitrification purposes and for the initial phase of phosphate release, respectively occurred in parallel and, if so, to quantify the relative amounts being diverted into these processes. The experimental approach chosen for this study was designed to render an approximation of the sequence of events commonly occurring in practice, namely the anoxic/anaerobic period immediately following contact between nitrate containing return sludge and influent substrate at the head of a plant, and the subsequent aerobic phase.

Materials and methods

Batch tests

Batch experiments were conducted using sludge from four full-scale biological nutrient removal plants situated in the vicinity of Pretoria and Johannesburg respectively. The Baviaanspoort Treatment Works, situated east of Pretoria, is a three-stage modified Bardenpho process (16 Mℓ/d), as is the 13 Mℓ/d module of the Daspoort Sewage Works, near central Pretoria. The Goudkoppies plant and Northern Sewage Works, both of which are situated in Johannesburg, are five-stage modified processes each with a treatment capacity of 150 Mℓ/d. The Baviaanspoort Treatment Works receives industrial as well as domestic effluent while influent to the Daspoort Sewage Works consists mainly of domestic sewage and light industrial effluent. Effluent reaching the Goudkoppies plant includes discharge from a yeast factory while Northern Sewage Works was operated in a mode that, amongst others, involved *in situ* generation and elutriation of fermentation end products in the primary clarifiers.

Preparation of the sludge consisted of collecting approximately 20ℓ of mixed liquor from the aerobic stage of the particular

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plant being studied, just before it enters the clarifier, and adding a sodium nitrate solution to an effective concentration of 20 mg/ℓ (as N) to prevent phosphate release during transportation. Filtered samples taken at the time of collection and after arrival were analysed to verify that phosphate had not passed to the liquid phase. The mixed liquor was subsequently aerated overnight (total period approximately 15 h) to ensure complete utilisation of unmetabolised substrate. Approximately 2ℓ of mixed liquor from the end of the anaerobic stage was collected simultaneously to determine phosphate uptake rates under anoxic conditions. This container was filled completely and kept closed overnight to maintain anaerobic conditions.

The sludges were separated from the supernatant by centrifugation at 5 000g for 3 min. Both pellets were collected by washing with small aliquots of a tap water medium to which had been added orthophosphoric acid, ammonium chloride and sodium bicarbonate to target concentrations of 10 mg/ℓ each for phosphate (as P) and ammonia (as N), and a total alkalinity of 200 mg/ℓ (as $CaCO_3$). The anaerobic mixed liquor was treated similarly to the aerobic sludge except that 40 mg/ℓ (as P) of phosphate and 10 mg/ℓ (as N) of sodium nitrate were added. Following screening through a sieve with 1,5mm square openings to remove coarse inorganic particles, the concentrated sludge mass was suspended in 18ℓ of the tap water medium, which was then flushed for about 15 min with nitrogen gas introduced through porous glass diffusers. Test volumes consisting of portions of this suspension were then dispensed and introduced into stoppered, magnetically stirred reactors. These had a total capacity of 2,5ℓ and were equipped with a sampling port and facilities for maintaining a continuously renewed nitrogen blanket above the liquid medium. The desired quantities of the organic substances being studied were dispensed from stock solutions, then adjusted to a standard volume of 75mℓ using distilled water, and introduced to the reactor at time zero. No substrate was added to the sludge derived from the anaerobic stage. Each substrate was introduced into two separate reactors, to one of which sodium nitrate had been added to achieve a concentration of 10mg/ℓ (as N). The other reactor was intended to function as a strictly anaerobic system but as a result of nitrate in the tap water supply this was never fully achieved from time instant zero.

The anoxic/anaerobic phase was maintained for 22 to 24 h. The concentration of ammonia, nitrate, orthophosphate and the organic substances involved was determined at discrete time intervals, using filtered samples. After completion of the experiment, mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (VSS) values were determined for each reactor. pH values were measured periodically but not controlled.

Organic compounds

The substrates selected for comparison included members of the group of compounds invariably capable of inducing phosphate release under anoxic conditions namely acetate, propionate and formate, and members of the class of compounds found to achieve this sometimes, depending on the origin of the sludge, namely butyrate and lactate. Where organic acids or their salts were used in the preparation of stock solutions these were adjusted before use to pH values in the range 6,5 to 7,5. A fixed quantity of each compound, equivalent to a theoretical COD of approximately 200 mg/ℓ, was added to each reactor.

Analytical methods

Orthophosphate, nitrate/nitrite, ammonia and sulphate were determined colorimetrically using auto-analytical equipment while calcium, magnesium and potassium were determined by atomic absorption spectrophotometry. The compounds acetate, propionate, formate, lactate and butyrate were determined at 214nm using a high performance liquid chromatograph with a variable wavelength detector. A Biorad fast acid column at an operating temperature of 65°C with an aqueous sulphuric acid solution (0,02 M) as mobile phase was employed for this purpose.

Results and discussion

Fig. 1 illustrates temporal phosphate release and substrate utilisation patterns observed for batch tests on mixed liquor from each of the four full-scale plants examined in this study. The corresponding relationships between phosphate and residual substrate concentration are given in Fig. 2. Initial phosphate release rates under anaerobic and anoxic conditions respectively, are summarised in Table 1 as a function of substrate and sludge origin. The amount of phosphate released per unit of substrate consumed is summarised in Table 2 while Table 3 contains substrate utilisation rates from which the relative amounts consumed as a result of phosphate release and of denitrification, when both occur simultaneously, may be computed. Finally the exchange ratios between phosphate and various ions are summarised in Table 4.

Phosphate release versus anoxic or anaerobic conditions

Gerber and co-workers (1987) reported on the capability of various organic substances to induce phosphate accumulation. Their conclusions are reinforced by the results of this study and the following aspects, in particular, deserve to be highlighted:

- Substances such as acetate and propionate are consistently capable of inducing phosphate release, irrespective of whether the environment is anaerobic or anoxic (Figs. (1a) to (1h)). Table 1 shows that the initial phosphate release rates under these conditions generally vary between 10 and 20mg P/g VSS.h.
- Under anaerobic conditions the release pattern with butyrate is similar to the responses observed with acetate and propionate. Tables 1 and 2 show that phosphate release is associated with substrate utilisation although at a slower rate than with acetate and propionate (about 2 to 5 mg P/g VSS.h).
- In contrast, with lactate addition three out of the four tests (Fig. 1 and Table 3) indicate that no lactate utilisation accompanied phosphate release under anaerobic conditions. The exception is Daspoort mixed liquor, in which lactate utilisation continued after phosphate release had ceased, indicating some other, as yet unexplained reason for the lactate utilisation.
- Enhanced phosphate accumulation is not limited to aerobic conditions but occurs under anoxic conditions as well (Figs. 1(u) to 1(x)). These anoxic uptake rates varied from about 3 to 5 mg P/ℓ.h for the plants under discussion compared to anaerobic release rates ranging from about 15 to 30 mg P/ℓ.h at similar VSS levels (Figs. 1(a) to 1(h)).

If it is assumed that the anoxic uptake occurs also in the presence of substrates such as acetate and propionate then the reduced mass of phosphate released under anoxic conditions when compared to anaerobic conditions (Figs. 1(a) to 1(h)) is in line with the proposition that phosphate uptake and release occur simultaneously under such conditions. However, the relatively slow anoxic uptake rates referred to above would make it difficult to distinguish between the uptake and release components solely

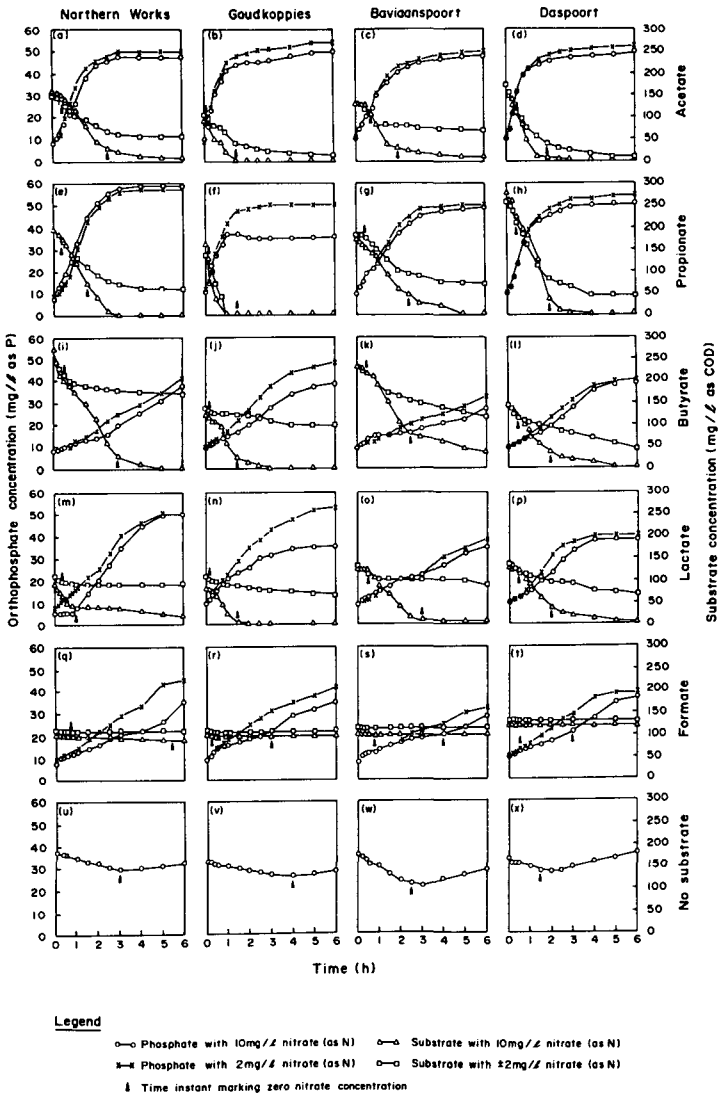

Figure 1
Time course of phosphate and substrate concentration under anoxic and anaerobic conditions.

on the basis of phosphate concentration profiles. In addition, phosphate release in the anoxic reactors terminated before that in the anaerobic batches, as a result of substrate utilisation by non-phosphate accumulating organisms for denitrification.

Phosphate release and substrate utilisation

The results in Fig. 1 show that phosphate release is generally accompanied by steady utilisation of the substrate involved, a phenomenon which has already been firmly established. Also, the substrate utilisation rate under anoxic conditions exceeds that observed under anaerobiosis, a result which is likewise well-known from laboratory and full-scale experience. A notable exception is formate, the concentration of which remains practically constant even though some 30 mg/ℓ phosphate (as P) is released over the indicated 6h reaction period. This constancy of formate concentration in the bulk liquor holds for both anaerobic and anoxic conditions. This implies that both phosphate release and denitrification occurred without utilisation of formate, in line with results reported by Arvin and Kristensen (1985). Most likely this was due to the death of some cells with lysis of cell contents. However, nitrification during the subsequent aerobic phase proceeded as readily as with any of the other substrates (results not shown).

The apparently infinite phosphate release to COD consumption ratio observed for formate (Figs. 2(q) to 2(t)) is followed in reverse order of magnitude by values in the approximate range of 1 to 3 mg P/mg COD for lactate under anaerobic conditions (Table 2). This compares with values ranging from 0,1 to 0,6; 0,2 to 0,5 and 0,5 to 0,8 for butyrate, propionate and acetate respectively (Table 2).

A visual impression of the relationships between nitrate, phosphate and substrate may be gained by a study of Fig. 2. A horizontal displacement of the orthophosphate versus substrate concentration lines pertaining to anoxic and anaerobic conditions respectively would signify a significant effect of nitrate on the substrate utilisation rate. This situation is particularly well illustrated by the results pertaining to butyrate and lactate (Figs. 2(i) to 2(p)). However, one should not confuse such a displacement with the change in slope of the line occurring in situations where the rapid phosphate release phase has gone to completion before depletion of nitrate and/or substrate (Fig. 2(h)). With

TABLE 1
INITIAL PHOSPHATE RELEASE RATES UNDER ANAEROBIC AND ANOXIC CONDITIONS RESPECTIVELY, AS A FUNCTION OF SUBSTRATE AND SLUDGE ORIGIN (mg P/g VSS.h)

Substrate	Northern Works	Goudkoppies	Baviaanspoort	Daspoort
		Anaerobic conditions		
Acetate	14,4	18,1	7,9	18,0
Propionate	17,0	16,1	7,3	18,8
Butyrate	3,2	4,0	2,0	4,9
Lactate	6,1	6,4	2,8	7,7
Formate	4,6	3,4	2,0	5,3
		Anoxic conditions		
Acetate	13,8	18,0	7,3	18,4
Propionate	17,3	15,7	6,4	19,0
Butyrate	1,9	2,7	2,0	4,3
Lactate	0,0	3,9	2,5	5,4
Formate	2,7	1,9	1,7	2,8

TABLE 2
AMOUNT OF PHOSPHATE RELEASED PER UNIT OF SUBSTRATE CONSUMED UNDER ANAEROBIC AND ANOXIC CONDITIONS RESPECTIVELY, AS A FUNCTION OF ORGANIC COMPOUND AND SLUDGE ORIGIN (mg P/mg COD)

Substrate	Northern Works	Goudkoppies	Baviaanspoort	Daspoort
	Anaerobic conditions			
Acetate	0,62	0,81	0,68	0,50
Propionate	0,51	0,21	0,38	0,26
Butyrate	0,55	0,55	0,11	0,46
Lactate	1,46	1,26	2,93	1,07
	Anoxic conditions			
Acetate	0,33	0,36	0,24	0,20
Propionate	0,27	0,14	0,23	0,24
Butyrate	0,04	0,02	0,07	0,04
Lactate	0,00	0,11	0,09	0,14

acetate and propionate the amount of phosphate released per unit mass of substrate utilised varied from approximately 0,14 to 0,4 mg P/mg COD under anoxic conditions (Table 2). The corresponding values for lactate and butyrate varied from 0,0 to 0,15 mg P/mg COD. The generally lower values of the phosphate to COD exchange ratio under anoxic conditions as compared to anaerobic conditions is most likely due to additional substrate being used by the non-phosphate accumulating organisms for denitrification. Substrate utilisation by the phosphate accumulating organisms for denitrification cannot be ruled out, however, since the difference in phosphate release rates between the anoxic and anaerobic situations is small and difficult to detect from the time course of concentrations.

As pointed out above, formate is not utilised under either anaerobic or anoxic conditions. Lactate behaves similarly in that comparatively very small amounts are utilised during phosphate release. It is, however, readily utilised as a carbon source for denitrification and under anoxic conditions this constitutes the main destination of this substrate. With phosphate-laden sludge under anoxic conditions butyrate is also utilised mainly for denitrification purposes. With the exception of the Daspoort Sewage Treatment Plant, only about 20% of the butyrate utilised is associated with phosphate release under conditions where both phosphate release and denitrification can occur (Table 3). With acetate the amount associated with denitrification under such conditions constitutes between 50 and 67% of the total amount of substrate utilised. With propionate, on the other hand, only about 25 to 40% of the available amount is utilised for denitrification purposes and then only in the Johannesburg plants. In the Pretoria plants propionate was used virtually exclusively for effecting phosphate release even though denitrification could in principle also occur.

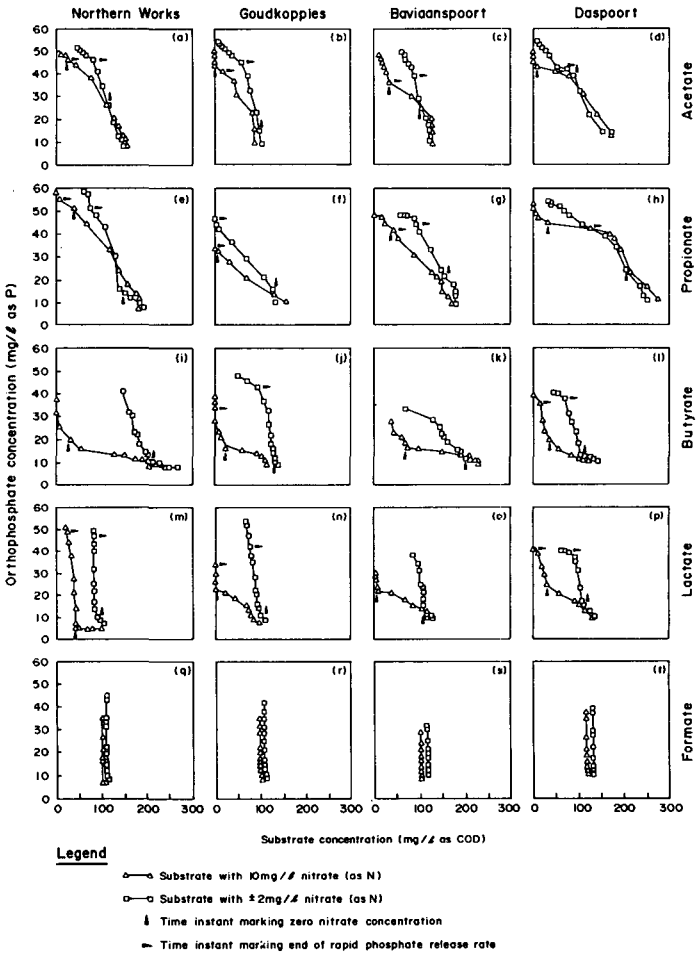

Figure 2
The relationship between phosphate release and organic substrate utilisation during anoxic and anaerobic conditions (Northern Works MLSS = 2 090 mg/ℓ, VSS = 1 830 mg/ℓ; Goudkoppies MLSS = 2 422 mg/ℓ, VSS = 2 018 mg/ℓ; Baviaanspoort MLSS = 2 754 mg/ℓ, VSS = 2 122 mg/ℓ; Daspoort MLSS = 2 552 mg/ℓ, VSS = 1 766 mg/ℓ).

Concomitant phosphate and cation/anion release

Phosphate release was accompanied by a corresponding increase in potassium, magnesium and sulphate concentrations in all cases. For a particular sludge and a particular anion/cation, no significant differences were observed among the ratios of phosphate released to concomitant ion release as effected by the different substrates. Average values of the experimentally determined ratios are given in Table 4. The ratios pertaining to potassium varied between 0,38 to 0,64 mol K/mol P for the different plants. These values are slightly higher than the 0,41

TABLE 3
SUBSTRATE REMOVAL RATE UNDER ANAEROBIC AND ANOXIC CONDITIONS RESPECTIVELY, AS A FUNCTION OF ORGANIC COMPOUND AND SLUDGE ORIGIN (mg COD/g VSS.h)

Substrate	Northern Works	Goudkoppies	Baviaanspoort	Daspoort
		Anaerobic conditions		
Acetate	17	23	26	33
Propionate	32	80	24	55
Butyrate	7	4	8	12
Lactate	0	2	0	12
		Anoxic conditions		
Acetate	46	62	44	77
Propionate	68	139	31	55
Butyrate	36	34	39	35
Lactate	13	38	21	32
		Ascribed to denitrification		
Acetate	29	39	18	44
Propionate	36	59	7	0
Butyrate	29	30	31	23
Lactate	13	36	21	20

found by Arvin and Kristensen (1985) and 0,25 mol K/mol P found by Gerber *et al.* (1987). The average 0,34 mol K/mol P is similar to the values found by Miyamoto-Mills *et al.* (1983) and Arvin and Kristensen (1985).

The release ratios for sulphate varied between 0,44 and 0,53 mol SO_4/mol P. This aspect is a perplexing one, since a decrease in sulphate concentration would normally be expected under anaerobic conditions.

It is noteworthy that calcium concentrations remained constant throughout the anaerobic phase for 3 of the 4 sludges investigated, which agrees with the work of Gerber *et al.* (1987). The exception was Daspoort where calcium was removed from the medium during anaerobiosis at a ratio of 0,57 mol Ca/mol P. This calcium removal might be ascribed to calcium phosphate precipitation. It needs to be recorded, however, that the Daspoort Sewage Works had been modified to operate as a three-stage modified Bardenpho process only shortly prior to this study and this might be the cause of the deviant behaviour of the sludge.

Summary and conclusions

The results of this study allow the following conclusions to be drawn:

- Compounds such as acetate and propionate are consistently capable of inducing phosphate release from phosphate-laden sludge under anoxic and anaerobic conditions but with substances such as butyrate and lactate, release may be either retarded or inhibited until after the onset of anaerobiosis.
- Phosphate uptake occurs not only under aerobic but also under anoxic conditions and this suggests that simultaneous phosphate release and uptake can occur under anoxic conditions, a situation which might wrongfully be ascribed to suppression of phosphate release.
- Organisms which use nitrate as electron acceptor compete for substrate with phosphate accumulating organisms under anoxic conditions. In situations under which both denitrification and rapid phosphate release can occur, between 50 and 66% of available acetate is utilised for denitrification. Propionate is less available to the denitrifying organisms and the amount consumed for this purpose generally varies between 0 and 50%. With butyrate typically 80% of the available substrate is utilised for this purpose while lactate is utilised practically exclusively for denitrification.
- Formate does not directly support either phosphate release or denitrification although both these phenomena occur in the presence of this substrate.
- Phosphate release exhibits interaction patterns with inorganic ions such as magnesium, potassium and sulphate. The exchange ratio varies considerably from plant to plant but generally ranged from 0,27 to 0,64 mol ion/mol P. Calcium concentration generally remained constant during anaerobiosis except with Daspoort sludge in which case calcium was removed from solution during the anaerobic phase.

TABLE 4
CONCOMITANT UPTAKE AND RELEASE RATIOS BETWEEN PHOSPHATE AND VARIOUS OTHER INORGANIC IONS (mol ion/mol P)

Ion	Northern Works	Goudkoppies	Baviaanspoort	Daspoort
mol K/mol P	0,43	0,53	0,38	0,64
mol Mg/mol P	0,35	0,50	0,27	0,54
mol SO_4/mol P	0,44	0,52	0,45	0,53
mol Ca/mol P	0	0	0	0,57

- The microbiological competition for acetate, propionate and butyrate to achieve phosphate release and denitrification, respectively, implies that in situations where substrate is limiting a reduced amount of substrate will be available for phosphate release if nitrate is present. This will result in reduced phosphate release and hence reduced excess phosphate removal.

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