Microbiological and Plant Engineering Aspects of Phosphate Biological Removal

A. Converti*, M. Zilli, R. Ghigliazza, and C. Sommariva**
Dipartimento of Chemical and Process Engineering "G.B. Bonino",
University of Genoa, Via Opera Pia, 15 I-16145 Genova (Italy)
**Ansaldo Energia s.p.a., P.O. Box 5711,
26584 Abu Dhabi Branch, U.A.E.

Original scientific paper
Received: March 17, 1999
Accepted: September 10, 1999

Biological models and microbial populations involved in phosphate removal from wastewater are reviewed and compared. After a brief presentation of the main "side stream" and "full stream" processes nowadays employed for this purpose, bench-scale experimental data obtained with innovations of well-accepted schemes of this technology, are presented and discussed. In particular, the effect of alternating anaerobic and aerobic phases in the presence of sludge enriched with *Acinetobacter calcoaceticus* is investigated, either in CSTR or in sequencing batch reactor. Up to about 75 mg l^{-1}, the removal yield in CSTR ranged between 95 and 99%, with negligible dependence on the fact that aerobic or anaerobic conditions were tested, while, over this threshold, efficiency sharply fell after 10 days of continuous working, thus proving the inability of poly-P bacteria to face P overloads. Phosphate uptake in sequencing batch reactor was quicker and more effective at higher rather than at lower COD_0/X_0 values, thereby suggesting that the "Sequencing batch" process might allow satisfactory results only under conditions of excess substrate. Finally, preliminary results on phosphate removal by aerobic sludge enriched with *A. lwoffi*, without resorting to any preliminary anaerobic stage, are evaluated from the viewpoint of a possible application on real scale. *A. lwoffi* behaved as a poly-P bacterium without the necessity of resorting to intermediate anaerobiosis. Experiments in CSTR under aerobic conditions allowed phosphate removals ranging from 65 to 80%, depending on the sludge retention time.

Keywords:
Biological phosphate removal, wastewater treatment, activated sludge, *Acinetobacter sp.*

Introduction

Srinath et al., observing in 1959 that phosphate uptake was stimulated by aeration of activated sludge and inhibited by toxic substances[1], were the first to suspect the biological nature of phosphate removal in water works. Subsequently, it was demonstrated that phosphate is released under anaerobic conditions, whereas the following uptake under aerobic conditions is favoured by wastewater addition[2].

Already in 1966 *Harold*[3] thought that stress conditions rather than oxygen supply or redox potential could be at the basis of excess phosphate uptake by aerated sludge ("overplus accumulation" or "luxury uptake"). About ten years later *Fuhs and Chen* postulated that bacteria belonging to the genus *Acinetobacter*, capable to accumulate large amounts of both polyphosphate and poly(hydroxybutyrate) (PHB), could be the most important agents of aerobic polyphosphate accumulation[4], provided that a preliminary fermentative anaerobic phase would be able to supply them with volatile acids as carbon source[5,6].

Following the observation that substrate might be stored as PHB by aerobic poly-P organisms under anaerobic conditions utilising the polyphosphate energy, it has recently been developed the hypothesis that the main role of the anaerobic phase would be, besides supplying volatile acids, the creation of a selective pressure against the other heterotrophic bacteria[7].

The present knowledge on poly-P bacteria microbiology allows to propose the general metabolic models depicted in Figure 1. Under anaerobic conditions (Figure 1a), the poly-P bacteria enzymatically hydrolyse polyphosphate and release phosphate[8]; the energy produced, temporarily stored as ATP, is used to uptake volatile acids, which are thereby reduced to poly(hydroxyalkanoates) (PHA), utilising the reducing power from the oxidation of glycogen to PHA[9]. In the absence of substrate, but in the presence of electron acceptors like oxygen (aerobic conditions) or nitrates (anoxic conditions), PHA is metabolised not only to support the growth, but also to produce the energy required to restore both polyphosphate and glycogen pools (Figure 1b). Finally, if the carbon source (volatile acids) and an electron acceptor are both available (Figure 1c), substrate is preferably accumulated as PHA utilising the energy from polyphosphate hydrolysis, rather than used for the growth[10], thereby following the typical behaviour of microorganisms subject to substrate depletion regime[11].

*To whom the correspondence should be addressed.

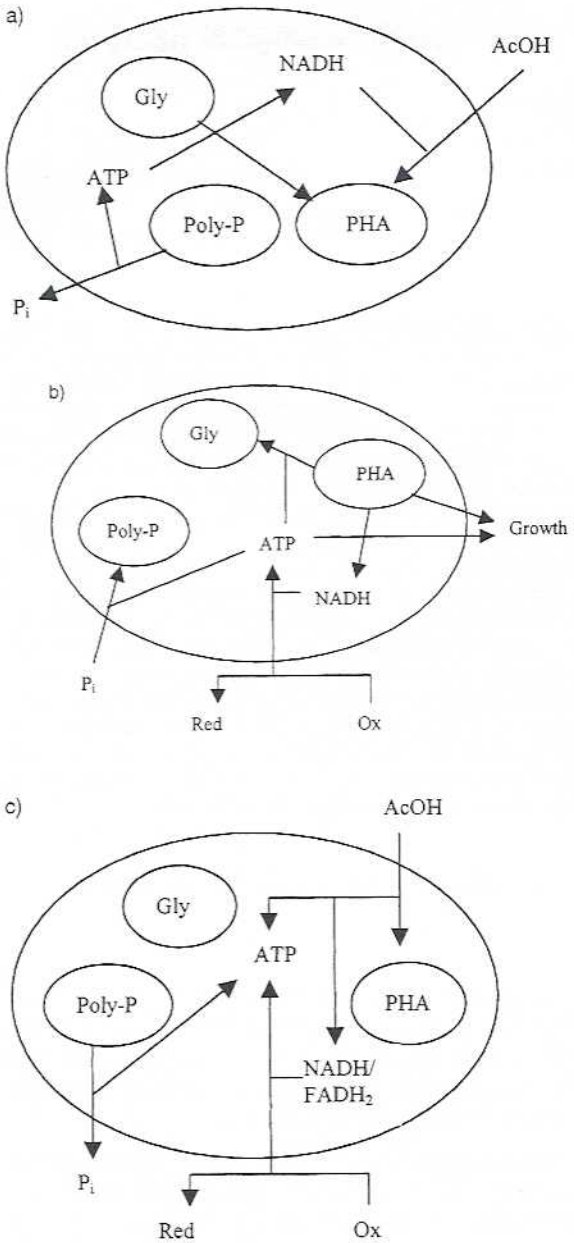

Fig. 1 – *Metabolic alternatives in poly-P bacteria: a) anaerobic conditions; b) aerobic or anoxic conditions in the absence of carbon source; c) aerobic or anoxic conditions in the presence of carbon source. Legend: AcOH = acetic acid; ATP = adenosine triphosphate; Gly = glycogen; $FADH_2$, NADH = reduced forms of coenzymes; Ox: electron acceptor like O_2 or NO_3^-; PHA = poly(hydroxyalkanoates); P_i = orthophosphate; poly-P = polyphosphate; Red: H_2O or N_2.*

BPR process configurations

The wide variety of BPR processes described in the literature, all deriving from the activated sludge process and implying remarkable cost savings with respect to chemical precipitation, can basically be grouped into two main categories: "full stream" processes, where the total wastewater flow rate is submitted to anaerobiosis, and "side stream" processes, where only a part of it is treated in this way.

Full stream processes for phosphate removal

The so-called "A/O process", nowadays widely used in the U.S.A., is the simplest type of full biological process for the sole phosphate removal, consisting in a series of anaerobic tanks followed by a series of aerobic tanks (Figure 2a). In the former, volatile acids are stored within the cells of poly-P bacteria in the reduced form of PHB, at the expense of polyphosphate hydrolysis and glycogen oxidation, with no need of external electron acceptors. In the latter, the substrate shortage allows only poly-P bacteria to grow at the expense of PHB and to accumulate polyphosphate, thus causing a net phosphate removal. Using a sludge age of 2–6 d, a hydraulic retention time of 1.0–3.5 h in the aerobic compartment and 0.5–1.5 h in the anaerobic one and a recycle ratio of 0.25–0.40, loads comparable with those of conventional activated sludge plants can be applied (0.2–0.7 kg_{BOD} kg_{MLSS}^{-1} d^{-1}). The most interesting installations of this scheme are those of Largo, Pontiac, and York River in the U.S.A. and those of Ruhleben and Marienfelde in Germany, which showed removal efficiencies ranging from 75 to 91%, according to the nitrate levels in the influent[12].

Also the "Rotanox process", realized by *Best* et al. for phosphate removal in the existing wastewater treatment plant of Basingstocke (UK), is derived from the conventional activated sludge process. A restricted anaerobic contact zone was set up in the anoxic tank for nitrogen removal with the aim of ensuring, at high loads, the necessary anaerobic conditions. Removal efficiencies of 41, 76, and 95% were observed for phosphate, nitrogen, and BOD removals, respectively[12].

The "Sequencing-batch process" simply consists of successive batch treatment operations, each including the following phases: a) wastewater feeding of a reactor containing the sludge, b) unaerated (anaerobic) mixing, c) aerated (aerobic) mixing, d) sludge settling and e) effluent discharge[13]. No less than two parallel reactors working with alternate phases are obviously necessary to face the dead time between successive batch runs. Phosphate, nitrogen, and BOD removal efficiencies of 86, 25, and 95% were obtained in the pilot plant of Culver, using a total cycle time of nearly 5 h and loads of 0.16–0.42 kg_{BOD} kg_{MLSS}^{-1} d^{-1} [12].

The "Alternate process", which is mainly used in Denmark, consists of an adaptation to phosphate removal of existing plants for nitrification/denitrification[14]. The plant works cyclically in order to allow alternate aerobic/anaerobic conditions in two interconnected tanks, with a ratio of respective terms ranging from 0.5 to 5.0. The former reactor, which is fed with a mixed liquor rich

in nitrates, acts sequentially as anoxic and anaerobic tank. After this sequence, it is aerated for 30 min to favour the uptake of phosphate by *Acinetobacter sp.*, while the latter is fed under anoxic conditions. Residence time of 2 h for the anaerobic phase and at least of 0.5 h in the aerobic one and quite low loads (0.04–0.12 $kg_{BOD}\ kg_{MLSS}^{-1}\ d^{-1}$) are consistent with phosphate removals sometimes higher than 90%. The upgraded wastewater from both reactors is finally settled.

Side-stream processes

In these processes, the recycle of only a part of the mixed liquor into the so-called "stripper" has the aim to increase the residence time of the anaerobic phase with respect to full-stream processes, so as to enhance phosphate release and to ensure a more efficient overplus accumulation in the aerobic tank. In case the polluted wastewater is characterised by a very low COD/phosphate ratio or when using very long sludge ages to support denitrification, biomass growth is insufficient to sustain polyphosphate accumulation, and thus chemical precipitation is needed (combined biological/chemical processes).

In the "Phostrip process"[15], which is the only "side-stream" application on real scale, a part of the settled sludge coming from the aerobic tank, corresponding to 20–50% of the overall flow rate, is recycled into the anaerobic stripper where phosphate release is stimulated by acetate or wastewater addition (Figure 2b). A sludge retention time in the stripper of 5–20 h and a hydraulic residence time in the aerobic tank of 1–10 h are necessary to ensure satisfactory results. After sludge separation, phosphate present in the supernatant is removed in a separate reactor by precipitation at pH 9.0–9.5 with lime[16], whose consumption (100–300 mg l^{-1}) depends on the alkalinity.

All the main real scale applications were done in the U.S.A.[17] and consisted in adaptations of pre-existing activated sludge or nitrification plants treating influent flow rates varying from 3,400 to 114,000 $m^3\ d^{-1}$ (Seneca Falls, Landscale, Adrian, Savage, Southtowns, and Reno-Sparks). Removal efficiency ranged from 74 to 91%, depending on the stripper and the chemical reactor performances.

Combined removal of phosphate and nitrogen

Although these systems, all derived from the "Bardenpho" scheme proposed by *Barnard* in 1973[18], are out of the purposes of this study, it is necessary to remember, at least, that the adverse effect of nitrate on biological removal of phosphate[19] suggested that denitrification competes with this process for the same substrate, however it requires longer sludge ages.

The simplest scheme for the combined phosphate and nitrogen removal is the so-called "Phoredox process", which consists of a pre-denitrification reactor followed by an aerobic tank and preceded by an anaerobic reactor for phosphate release (Figure 2c). Phosphate, nitrogen, and BOD removals higher than 90% were reported at sludge ages of 22–25 d for a 23,000 $m^3\ d^{-1}$ flow rate[20].

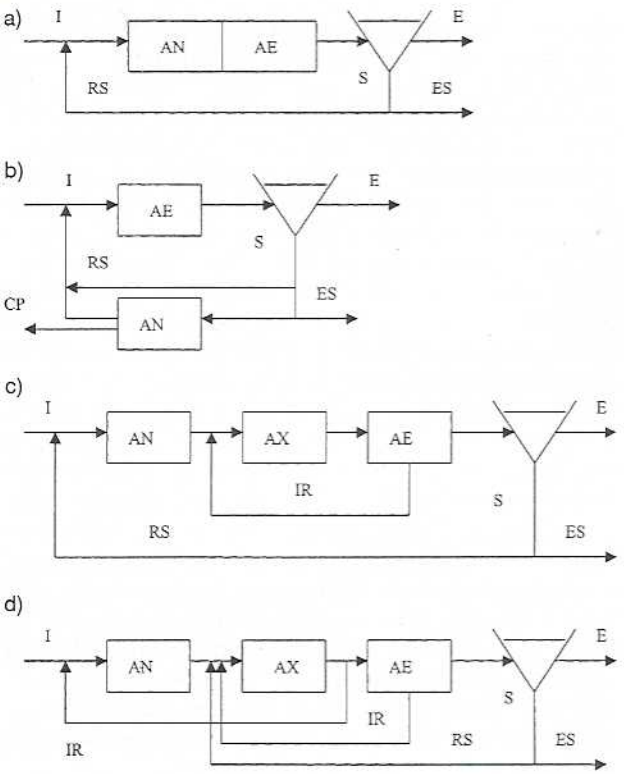

Fig. 2 – *Flow sheets of the main BPR processes: a) "A/O process"; b) "Phostrip process"; c) "Phoredox process" with nitrification/denitrification; d) "UCT process". Legend: AE = aerobic tank; AN = anaerobic tank; AX = anoxic tank; CP = chemical precipitation; E = effluent; ES = excess sludge; I = influent; IR = internal recirculation; RS = return sludge; S = settler.*

A modification of the last scheme has recently been set up in the University of Cape Town ("UCT process") to prevent the negative effect of high nitrate levels on phosphate release in the anaerobic tank[21]; it consists of partially recycling into the anoxic denitrification stage, both settled aerobic sludge rich in nitrates and mixed liquor from the aerobic compartment, as well as of transferring the denitrified sludge into the anaerobic reactor (Figure 2d).

Materials and methods

The different bench-scale apparatuses used for biological phosphate removal were previously described in detail[13,22,23]. Batch tests were carried out at 20 ± 0.1 °C and pH 6.5 ± 0.1 in 200 ml-Erlenmeyer flasks containing 5 g l^{-1} of activated sludge enriched with *Acinetobacter calcoa-*

ceticus. Continuous tests were performed under the same conditions using a synthetic sewage. When the "A/O process" was selected, a 3.2 l-CSTR and a 4.0 l-rectangular vessel, respectively used as anaerobic and aerobic compartments, were connected with a 1.2 l-glass cylindrical settler, so as to ensure a residence time of 6 h in both compartments and of 2.5 h in the settler with a sludge recycle ratio of 0.1. The "Alternate process" was simulated at the same residence times alternating anaerobiosis and aerobiosis of short duration in a single 6.0 l-CSTR, so as to ensure an overall cycle term of 24 h. The same reactor, regulated at working volume of 2.0 l, was used as aerated vessel or as "Sequencing batch" reactor; in the latter case, anaerobic phases of 6 h were followed by aerobic phases of 18 h during each cycle.

Batch and continuous tests in the "A/O process" and "Alternate process" were done, using a synthetic sewage containing, besides salts, 257 mg l^{-1} acetic acid, 40 mg l^{-1} sodium propionate, 40 mg l^{-1} glucose, 100 mg l^{-1} peptone, 20 mg l^{-1} yeast extract, and 44–440 mg l^{-1} KH_2PO_4, corresponding to P concentrations of 10–100 mg l^{-1}. Sometimes glucose was used as sole C source for comparisons.

Standard methods[24] were used to determine COD, phosphate, and suspended solids concentrations.

Results and discussion

Batch tests

Batch tests at relatively low starting COD (257 mg l^{-1}) show, during the preliminary anaerobic phase, an increase in phosphate concentration in the medium as well as a COD reduction of about 200% and 70%, respectively, while the successive aerobic conditions are not able to ensure either satisfactory overplus accumulation or complete COD removal (Table 1). Although the anaerobic release seems to be inhibited by a starting COD increase up to 842 mg l^{-1}, aerobic phosphate uptake is notably improved exceeding 60%, while COD consumption keeps nearly the same.

Table 1 – *Results of batch phosphate removal tests*

	COD_0 = 257 mg l^{-1}			COD_0 = 842 mg l^{-1}		
Conditions		Anaerobic	Aerobic		Anaerobic	Aerobic
t/h	0	24	48	0	24	48
COD/mg l^{-1}	257.0	82.4	43.7	842	314	181
Y_{COD}/–	–	0.68	0.83	–	0.63	0.78
P/mg l^{-1}	4.8	15.3	4.0	9.2	15.2	3.6
[a]Y_P/–	–	–2.19	+0.17	–	–0.65	+0.61
SS/g l^{-1}	1.9	1.5	1.5	1.0	1.2	1.5

0 h = start of the anaerobic phase; 24 h = end of the anaerobic and start of the aerobic phases; 48 h = end of the aerobic phase.
[a]Negative values refer to phosphate release.

Additional batch tests were performed under microaerophilic conditions using sludge acclimated at 20 °C to check the influence of temperature changes (from 5 to 35 °C), like those taking place in open water works, on phosphate uptake. The results listed in Table 2 show that sludge is able to face quickly increases of 10–15 °C, with respect to the acclimation temperature (20 °C), ensuring removal yields higher than 60% within 70–140 h; on the other hand, decreases in temperature are responsible for intermediate phosphate release after about 50 h, which remarkably delays the achievements of both new acclimation and maximum final removal yield (about 60%). It should also be noticed that the greater the temperature fall, the stronger its negative effect, which means that the amount of phosphate released is higher.

Table 2 – *Effect of temperature changes on the sludge ability to retain polyphosphate.* P_0 = 36 mg l^{-1}

T/°C	5	15	25	30	35
P_f/mg l^{-1}	14	13	13	13	13
Y_P/–	0.60	0.62	0.64	0.63	0.62
P_{max}/mg l^{-1}	140	100	78	36	36
t_{max}/h	48	55	48	0	0

P_f = final phosphorus concentration in the medium; Y_P = yield of phosphorus uptake; P_{max} = maximum phosphorus concentration in the medium; t_{max} = time at which P_{max} is obtained.

Continuous tests according to the "A/O process"

During the start-up operation of the "A/O process", performed feeding a 10 mg_P l^{-1} synthetic sewage at residence time of 1.4 d, remarkable oscillations in phosphate removal yield (0.6–0.9) were observed, whereas COD consumption stabilised at a yield of about 85% within the first week. These preliminary tests showed that the best sludge acclimation is obtained in continuous rather than in batch mode and that the best COD of the feed is around 500 mg l^{-1}. In addition, the abundant presence in the sludge of *A. calcoaceticus*, *Pseudomonas paucimobilis*, and *Cryseomonas luteola*, that were likely responsible for the observed difficult sludge settling, has been demonstrated.

Since the above oscillations in phosphate removal were also observed after the achievement of pseudo-stationary conditions, it is likely that the physical separation of aerobic and anaerobic populations interferes with their normal interplay.

The results of these tests, carried out at residence times of 0.60 and 0.75 d in the anaerobic and aerobic compartments, respectively, well compare with those reported in the literature for similar systems: COD and phosphorus levels in the effluent were 30 mg l^{-1} and 1–2 mg l^{-1}, corresponding to respective average COD and phosphate removal yields of about 90% and 85%, while excess sludge production was 1.6 g_{SS} d^{-1}.

Continuous tests according to the "Alternate process"

The "Alternate process" start-up was performed submitting the sludge to alternate anaerobic/aerobic conditions at residence time of 1.43 d. This mode of operation strongly reduced the time necessary to reach steady-state conditions, eliminated the above oscillations, prevented the suspended solid decrease below a critical threshold, and allowed to achieve phosphate removal yields of about 90%, which means that the necessary interplay between anaerobic and aerobic populations is better established when they are simultaneously present. A progressive enrichment of sludge with poly-P bacteria was also detected.

After the achievement of steady state, a synthetic sewage containing 8–10 mg_P l^{-1} was continuously fed into the reactor, using a ratio of 0.6 between the terms of aerobic and anaerobic phases as well as an overall cycle time of 24 h. These tests allowed to check the performance of a system, like that under consideration, which is expected to allow remarkable cost savings, not only because of the elimination of the aerobic tank but also because of the reduced total aeration time. In addition, the use of a single vessel was also probably responsible for the observed peculiar absence of anaerobic phosphate release and of the practically constant phosphate uptake (about 90%), which did not seem to depend appreciably on the oxygen availability (Figure 3). This strange behaviour can be ascribed to the occurrence of a pseudo-steady state under which anaerobic and aerobic populations, which are simultaneously present, interact in such a way that phosphate is uptaken as soon as it is released.

The results of tests carried out using sewage containing increasing levels of phosphorus (Figure 4) show that, up to about 75 mg l^{-1}, the removal yield ranged between 95 and 99%, with negligible dependence on the fact that aerobic or anaerobic conditions were tested, while, over this threshold, efficiency sharply fell after 10 days of continuous working, thus proving the inability of poly-P bacteria to face P overloads. Comparable results were obtained using different single carbon sources (glucose, acetate, and propionate).

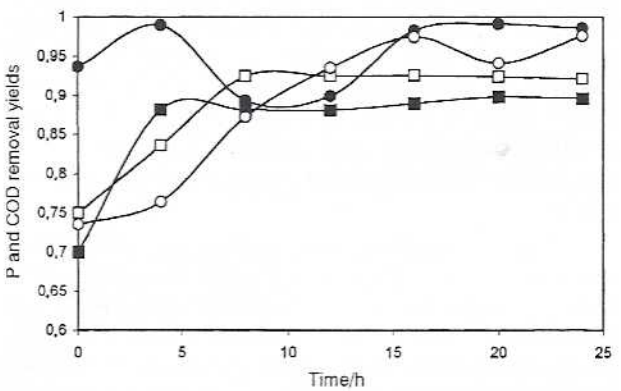

Fig. 3 – *Experimental results of continuous removals of phosphate and COD through the "Alternate process". P_i = 8–10 mg/l; COD_i = 150–445 mg/l. Aerobic conditions (●) Y_P; (■) Y_{COD}. Anaerobic conditions: (○) Y_P; (□) Y_{COD}.*

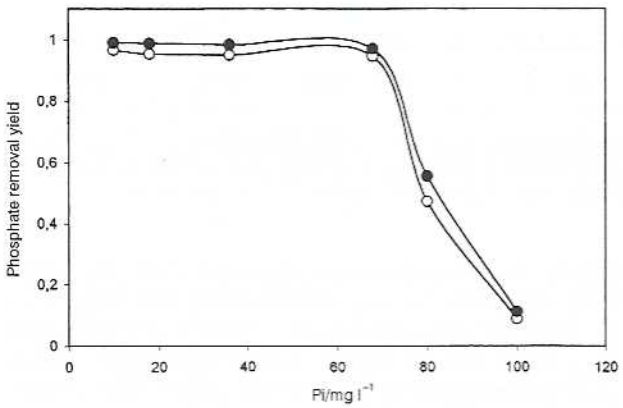

Fig. 4 – *Effect of the phosphorus concentration in the feed on the yield of phosphate removal through the "Alternate process", under steady state conditions. (●) Aerobic phase; (○) Anaerobic phase.*

Phosphate uptake tests according to the "Sequencing batch" scheme

"Sequencing batch" tests were carried out by alternating anaerobic and aerobic conditions of about 6 and 18 h, respectively. The ratio between starting COD and biomass concentrations was varied between 0.36 and 4.6 in order to evaluate the minimum threshold (COD_0/X_0 = 0.5) below which the peculiar advantages associable to low values of this parameter (stability of population composition, excellent removal yield, and poor sludge production) are completely vanished by inhibition of biomass growth to an extent unsuitable to sustain the process.

The values listed in Table 3 point out how the growth yield (Y_G) progressively decreases with decreasing the above ratio, because of the progressive cell activity reduction provoked by substrate shortage. At COD_0/X_0 = 0.36 the growth is practically stopped, which is consistent with the nearly negligible COD consumption rate nec-

Table 3 – *Effect of COD_0/X_0 ratio on the main kinetic and yield parameters of the "Sequencing batch" process*

COD_0/X_0 (g_{COD}/g_{SS})	Phase	Y_G –	Y_{COD} –	μ_{max} h^{-1}	r_{COD} $g_{COD}\ g_{SS}^{-1}\ h^{-1}$	r_P $mg_P\ g_{SS}^{-1}\ h^{-1}$
4.6	Anaerobic	0.78	0.40	0.117	0.270	–6.83
	Aerobic	0.50	0.38	0.022	0.086	+4.79
1.8	Anaerobic	0.48	0.41	0.083	0.121	–
	Aerobic	0.40	0.42	0.020	0.042	–
0.6	Anaerobic	0.19	0.42	0.033	0.042	–
	Aerobic	0.14	0.39	–	0.013	–
0.5	Anaerobic	0.14	0.38	0.030	0.029	–2.43
	Aerobic	0.01	0.43	0.018	0.013	+1.02
0.36	Anaerobic	0	0.25	0.000	0.015	–1.66
	Aerobic	0	0.31	0.000	0.006	+0.37

Y_G = yield of growth; Y_{COD} = yield of COD consumption; μ_{max} = maximum specific growth rate; r_{COD} = specific rate of COD consumption; r_P = specific rate of phosphate release (–) or uptake (+).

essary for maintenance requirements. In addition, contrary to any expectation, the values of Y_G and of the maximum specific growth rate (μ_{max}) are always higher under anaerobic rather than under aerobic conditions, which is probably due to an excess oxygen shock suffered by fermentative bacteria responsible for the production of substrates directly metabolisable by poly-P bacteria.

Finally, phosphate uptake is quicker and more effective at higher rather than at lower COD_0/X_0 values, thereby suggesting that the "Sequencing batch" process might allow satisfactory results only under conditions of excess substrate. At COD_0/X_0 = 0.36 phosphate is even released rather than uptaken.

Phosphate removal in aerated stirred tank reactor

It has been demonstrated in recent studies that when activated sludge enriched with *Acinetobacter calcoaceticus* var. *lwoffi*, commonly called *A. lwoffi*, is submitted to alternate anaerobic/aerobic conditions, phosphate uptake beyond metabolic needs and nearly complete COD removal take place during the aerobic phase, without any appreciable intermediate phosphate release during the anaerobic phase[25,26]. On the basis of the new findings on the role of the anaerobic phase, it appears, that contrary to what happens in other poly-P bacteria, *A. lwoffi* does not require this phase to compete favourably with the heterotrophic population.

Although, metabolic mechanisms in this strain are not yet clearly defined, its ability to remove phosphate under exclusive aerobic conditions has been checked by a series of aerobic tests whose main results are compared in Table 4 with those obtained under alternate anaerobic/aerobic conditions. While COD removal is nearly the same in both series, phosphorus uptake beyond metabolic needs (0.015 $g_P\ g_{SS}^{-1}$) is remarkably higher under exclusively aerobic conditions. This result together with the observation of very high P contents of cells (9–11%) prove the capacity of *A. lwoffi* to behave as a poly-P bacterium without the necessity of resorting to intermediate anaerobiosis as well as the possibility of using it to emphasise the removal ability of activated sludge. Successively, experiments in CSTR allowed phosphate removals ranging from 65 to 80%, depending on the sludge retention time.

Table 4 – *Comparison of the results obtained from batch tests of phosphate removal carried out under alternate anaerobic/aerobic and exclusively aerobic conditions. Carbon source: sodium acetate*

Conditions	Y_{COD}/–	Y_P/–	$\%_P/g_P\ 100\ g_{SS}^{-1}$	Y_N/–	$Y_{P/SS}/g_P\ g_{SS}^{-1}$
Alternate	0.90–0.95	0.55–0.65	9.0	0.55–0.60	0.051
Aerobic	0.94	0.74	11.0	–	0.096

Y_{COD} = yield of COD consumption; Y_P = yield of phosphate uptake; $\%_P$ = phosphorus content of cells; Y_N = yield of nitrogen removal; $Y_{P/SS}$ = yield of phosphorus uptaken per gram of biomass.

Conclusions

The results obtained in bench-scale apparatuses simulating innovations of well-accepted schemes for biological phosphate removal demonstrate that enriching activated sludge with poly-P bacteria is in general an optimal criterion to accelerate start-up of continuous operation. Although *A. calcoaceticus* allows better removal yields than *A. lwoffi*, it requires preliminary anaerobic conditions and intermediate release to emphasise the successive aerobic uptake of phosphate.

Notwithstanding the significant differences showed by these strains naturally present in activated sludge, obviously implying quite different operational modes, the satisfactory removal yields obtained by continuous operation (even > 90% in the former and 65–80% in the latter case) suggest that this bio-technique could already be mature to compete effectively with the traditional chemical precipitation, if only little addition and/or improvement would be made in the existing wastewater treatment plants.

References

1. *Srinath, E.G., Sastry, C.A., Pillai, S.C.*, Experientia **15** (1959) 339
2. *Levin, G.V., Shapiro, J.*, J. Wat. Pollut. Control Fed. **37** (1965) 810
3. *Harold, F.M.*, Bacteriol. Rev. **30** (1966) 772
4. *Fuhs, G.W., Chen, M.*, Microbiol. Ecol. **2** (1975) 119
5. *Barnard, J.L.*, Wat. Res. **9** (1975) 485
6. *Nicholls, H.A.*, Water S.A. **1** (1975) 121
7. *Maurer, M., Gujer, W., Hany, R., Bachmann, S.*, Wat. Res. **31** (1997) 907
8. *van Groenestijn, J.W., Bentvelsen, M.M.A., Deinema, M.H., Zehnder, A.J.B.*, Appl. Environ. Microbiol. **55** (1989) 219
9. *Smolders, G.J.F., van Loosdrecht, M.C.M., Heijnen, J.J.*, Wat. Sci. Technol. **31** (1994) 79
10. *Kuba, T., Wachtmeister, A., van Loosdrecht, M.C.M., Heijnen, J.J.*, Wat. Sci. Technol. **30** (1994) 263
11. *van Loosdrecht, M.C.M., Pot, M.A., Heijnen, J.J.*, Wat. Sci. Technol. **35** (1997) 41
12. *Andreottola, G., Canziani, R., Cossu, R.*, Rimozione Biologica dei Nutrienti dalle Acque, Istituto per l'Ambiente, Milan, 1990, p. 191
13. *Ghigliazza, R., Lodi, A., Converti, A., Nicolella, C., Rovatti, M.*, Bioproc. Eng. **14** (1996) 131
14. *Janssen, P.M.J., Rensink, J.H.*, In Biological Phosphate Removal from Wastewaters (R. Ramadori, ed.), Proc. IAWPRC Specialized Conference, September 1987, Rome, Italy. Pergamon Press, Oxford, 1987, p. 365
15. *Levin, G.V., Della Sala, U.*, In Biological Phosphate Removal from Wastewaters (R. Ramadori, ed.), Proc. IAWPRC Specialized Conference, September 1987, Rome, Italy. Pergamon Press, Oxford, 1987, p. 249
16. *Eggers, E., Dirkzwager, A.H., van den Honing, H.*, Wat. Sci. Technol. **24** (1991) 333
17. *U.S.E.P.A.*, Process Design Manual for Phosphorus Removal, Center for Environmental Research Information, Technology Transfer, Cincinnati, Ohio, U.S.A., 1987
18. *Barnard, J.L.*, Wat. Pollut. Control **72** (1973) 6
19. *Hascoet, M.C., Florentz, M.*, Water S.A. **11** (1985) 1
20. *Barnard, J.L., Stevens, G.M., Leslie, P.J.*, Wat. Sci. Technol. **17** (1985) 233
21. *van Loosdrecht, M.C.M., Hooijmans, C.M., Brdjanovic, D., Heijnen, J.J.*, Appl. Microb. Biotechnol. **48** (1997) 289
22. *Converti, A., Zilli, M., Poloniecki, R.H., Del Borghi, M., Ferraiolo, G.*, Wat. Res. **27** (1993) 791
23. *Converti, A., Rovatti, M., Del Borghi, M.*, Wat. Res. **29** (1995) 263
24. *APHA*, Standard Methods for the Examination of Water and Wastewater, 16th Ed., American Public Health Association, Washington, DC, 1985
25. *Ghigliazza, R., Lodi, A., Rovatti, M.*, Bioproc. Eng. **18** (1998) 207
26. *Ghigliazza, R., Lodi, A., Rovatti, M.*, Bioproc. Eng. **20** (1999) 257