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Phosphate removal using aerobic bacterial consortium and pure cultures isolated from activated sludge

Leyla Benammar[a], *Taha Menasria*[b,*], *Ammar Ayachi*[c], *Messaoud Benounis*[d]

[a] *Department of Natural and Life Sciences, Faculty of Sciences, University of Batna, Batna 05000, Algeria*
[b] *Department of Natural and Life Sciences, Faculty of Exact Sciences and Natural and Life Sciences, University of Tebessa, Tebessa 12002, Algeria*
[c] *Veterinary and Agricultural Sciences Institute, Department of Veterinary Sciences, University of Batna, Batna 05000, Algeria*
[d] *Department of Chemical Engineering, Faculty of Sciences and Technology, University of Khenchela, Khenchela 40004, Algeria*

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ABSTRACT

In the present study, an attempt is made to evaluate the kinetics of biological phosphate removal using a bacterial consortium of activated sludge, as well as screening for dominant polyphosphates accumulating bacteria. The results showed an efficient phosphate uptake ($P < 0.001$) of the consortium, with rates related to the initial concentration of both phosphate and carbon sources. Short chain volatile fatty acids presented the suitable substrates for enhanced biological phosphorus removal, of which maximum yield reached 99.23% and 78.51% in basal salt medium supplemented with 0.5% of sodium acetate and lactate respectively. Fifteen phosphate-accumulating bacteria were isolated from the activated sludge and only four isolates were selected and characterized as *Pseudomonas aeruginosa* AS1, *Moraxella lacunata* AS2, *Acinetobater junii* AS3 and *Alcaligenes denitrificans* AS4. The highest efficiency of phosphate uptake using pure culture was achieved with *Ac. junii* AS3 (83.36) followed by *P. aeruginosa* AS1 (81.78%), *Al. denitrificans* AS4 (76.72%), and *M. lacunata* AS2 with 50.6%.

1. Introduction

Phosphorus is one of the most important constituents of living organisms, which is involved in major physiological processes. In the environment, it occurs naturally in some igneous and sedimentary rocks, natural water, wastewater and sludge (Lévêque, 1996; Stednick, 1991; Pourriot and Meybeck, 1995). However, phosphorus can be considered as a pollutant, when excess content occurs in aquatic environments, which is mainly due to agricultural use of fertilizers, domestic sewage and industrial effluents (Giupponi et al., 1999; Krishnaswamy et al., 2009). In fact, high concentrations of phosphates in raw sewage affect the quality of receiving water resources, contributing to eutrophication and cyanobacterial blooms, which may induce health problems under specific environmental conditions (Jochimsen et al., 1998; Lacaze, 1996; Mcmeekin, 2009; Mino, 2000; Ploug, 2008; Wiegand and Pflugmacher, 2005). Therefore, a control of phosphate level in the waste has become an important key to improve the quality of aquatic ecosystems.

Phosphate removal can be performed by physic-chemical precipitation, biological treatment, or a combination of both processes (De Hass et al., 2000; Mino et al., 1998; Rybicki, 1997; Sathasivan, 2008). However, the significant costs associated

* Corresponding author. Tel.: +213 794180145.
E-mail address: tahamenasria@hotmail.com (T. Menasria).
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with chemical reagents addition, as well as the increased of chemical sludge production have limited the use of physic-chemical treatment on a large scale (Laridi, 2006). Therefore, alternative biological processes have been explored. This seems to be most competitive and best suited for phosphate removal from wastewater (van Loosdrecht et al., 1997).

The microbes have been widely examined in the context of environmental biotechnology as a means for pollutant removal. Currently, phosphates are biologically removed by absorption of dissolved orthophosphates, polyphosphates and organic phosphates using living microorganisms, such as bacteria, yeast, protozoa, microalgae, fungi and macrophytes (Akpor et al., 2007; de-Bashan and Bashan, 2004; Melasniemi and Hernesmaa, 2000; Shi et al., 2007). Thus, pollutant removal from wastewater, using activated sludge, is one of the most important biotechnological processes in a wastewater treatment plant (Han et al., 2012). Using the conventional methods, a variety of bacteria which have been isolated from activated sludge are able to accumulate polyphosphate, although *Acinetobacter* spp. are often considered to be typical representatives of poly-P cultivable bacteria and to play a role in enhanced biological phosphorus removal (EBPR) (Tandoi et al., 1998). However, recent studies using molecular-based methods showed that the phosphate accumulating organisms (PAO) population is much more diverse and instead of *Acinetobacter*, β-proteobacteria, α-proteobacteria and actinobacteria, were predominant in EBPR communities (Fang et al., 2002).

In the present study, we investigated the kinetic of bio-phosphate removal by means of a bacterial consortium of activated sludge as well as optimization tests were conducted to study the relationship between initial concentrations of phosphate and carbon sources in the medium and efficiency of phosphate uptake. Furthermore, we have focused on isolation of dominant polyphosphate-accumulating bacteria, which were tested in order to compare their phosphate storage capacities.

2. Materials and methods

2.1. Activated sludge sampling

Activated sludge was taken from the aerobic zone of a local wastewater treatment plant in Khenchela city (North-east Algeria). Sludge samples were collected in sterile glass bottles and used within 24 h for later determination of phosphate removal capacity. Physico-chemical characteristics of the sludge are summarized in Table 1.

Table 1 – Physicochemical characteristics of activated sludge.

Parameters	Activated sludge
pH	6.46
Temperature (°C)	23.9
Conductivity (μS/cm)	476
Salinity (‰)	0.2
Turbidity (NTU)	11.05
Total dissolved solid (TDS)	230
Nitrate ($mg\,L^{-1}$)	0.56
Nitrite ($mg\,L^{-1}$)	0.27
Ammoniac ($mg\,L^{-1}$)	10.39
Orthophosphate ($mg\,L^{-1}$)	17.66

2.2. Enrichment of bacterial consortium

The growth media used to simulate the sewage for all the experiments consisted of basal acetate salt medium (BASM) as described by Saito et al. (2004). The basal medium contained (g/L): $CH_3COONa{\cdot}3H_2O$ (0.4 g), NH_4Cl (0.107 g), $MgSO_4{\cdot}7H_2O$ (0.180 g), $CaCl_2{\cdot}2H_2O$ (0.014 g), K_2HPO_4 (0.049 g), KH_2PO_4 (0.028 g) and trace element solution (0.3 mL/L). The trace element composition is: EDTA (10 g/L), $FeSO_4{\cdot}7H_2O$ (1.54 g/L), H_3BO_3 ($150\,mg\,L^{-1}$), $CuSO_4{\cdot}5H_2O$ ($30\,mg\,L^{-1}$), $MnCl_2{\cdot}4H_2O$ ($120\,mg\,L^{-1}$), KI ($180\,mg\,L^{-1}$), Na_2–$MoO_4{\cdot}2H_2O$ ($60\,mg\,L^{-1}$), $ZnSO_4{\cdot}7H_2O$ ($120\,mg\,L^{-1}$), $CoCl_2{\cdot}6H_2O$ ($150\,mg\,L^{-1}$). The pH of the medium was adjusted to 7 ± 0.1 using 1N HCl or 1N NaOH solutions, before autoclaving at 121 °C for 15 min. The enrichement of bacterial consortium was made according to Pauli and Kaitala, (1995). The activated sludge was mixed with 0.9% NaCL solution. This mixture was homogenized to disperse flocks and detach microorganisms by vortexing for 2 min to 5 min. Inoculation series were made from the homogenized mixture on BASM, incubated aerobically in flask on a rotary shaker at 30 °C for 2–3 days. The cultures were centrifuged (10,000 rpm), washed and used as activated consortium for batch cultures.

2.3. Batch experiments and optimization in mixed culture

In order to assess the phosphate uptake capacities of the activated sludge, a batch test was adopted under aerobic/anaerobic conditions. The batch culture was carried out in 500 mL lab-scale reactors supplied by 300 mL working volume of synthetic basal media, inoculated by suspending 2.5% of sludge inoculums. The inoculated flasks were incubated at 30 °C on a rotary shaker at 130 rpm. To increase the efficiency of the phosphate removal, the effect of different carbon sources (glucose, lactose, acetate and lactate) at 0.5% was assayed. Therefore, phosphate uptake optimization was carried out using different initial concentrations of phosphate and sodium acetate.

2.4. Phosphate and bacterial growth estimation

At regular intervals, samples were taken aseptically from reactors, in order to carry out the monitoring of bacterial growth, phosphate concentrations and pH changes. Cell growth was determined by measuring the optical density at 600 nm. Appropriate calibration curves were used to recalculate OD values to cell densities. The linear extrapolation method was used to calculate lag phases. To measure phosphate concentration, ascorbic acid standard method was used (Rodier et al., 2009). Samples were centrifuged at 7000 rpm for 15 min and the supernatants were diluted as required for the analyses. The formed blue complex was measured at 700 nm.

Phosphate uptake efficiency (%) was calculated using the equation: Phosphate removal efficiency = $[(C1–C2)/C1] \times 100$. Where C1 and C2 are the initial and final concentration of phosphorus, respectively.

2.5. Screening and identification of efficient phosphate removal bacteria

Isolation of dominant microorganisms was achieved from different reactors by streak inoculating biomass into solidified BASM. The plates were incubated at 30 °C for 3 to 7 days.