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Screening of Bacteria Isolated from Activated Sludges for Phosphate Removal from Wastewater

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ABSTRACT

The objective of the current study is to isolate phosphate removing microbes from activated sludge for wastewater remediation in the area of Khenchela (Eastern Algeria) . Phosphate rate was determined using colorimetric method and batch tests were developed to evaluate the biomass composition of the sludge. Four efficient pure strains isolated from activated sludge samples and identified as *Acinetobacter junii*, *Pseudomonas aeruginosa*, *Moraxella lacunata*, *Alcaligenes denitrificans*. Our results show that applying of mixed bacterial culture containing mostly isolated strains for bioremediation purpose can be used successfully for the elimination of phosphate from activated sludge of wastewater treatment plants.
KEY WORDS: phosphate, batch culture, activated sludge, phosphate reduction, wastewater.

INTRODUCTION

Phosphorus is one of the major components of all living cells. The control of the amount of phosphates in water or wastewater became an important key to improving the quality of our ecosystems. At high concentrations, the phosphorus in ecosystems affects the quality of freshwater resources and the practice of reusing wastewaters [1].

The method most commonly used for disposal of wastewater phosphates is the physico-chemical phosphate removal in which metal salts (iron, aluminum, or calcium) were used to precipitate phosphate. However, for his significant costs associated with additional chemical reagents and a significant sludge production have limited its widespread use [2, 3].

So, biological processes appear now to be the most competitive and best adapted to the treatment of phosphate present in wastewater. Phosphate can be efficiently removed from wastewaters by the activated sludge process which incorporates alternating anaerobic and aerobic periods [4, 5]. In their work [6] evaluate the feasibility of citric acid as external carbon source for biological phosphorus removal. A successful enhanced biological phosphorus removal was studied in both anaerobic-aerobic sequencing batch reactor to induce growth of phosphate accumulating organism and anaerobic-anoxic [7].

The purpose of our work is to study the kinetics of phosphate removal by mixed bacterial culture of activated sludge taken from the wastewater treatment plant of Khenchela (Eastern Algeria) and isolate pure bacterial cultures involved in this process.

MATERIALS AND METHODS

Sampling and composition medium

The activated sludge samples were taken from the aeration tanks of wastewater treatment plant of Khenchela which mainly receives domestic wastewater. Samples were collected in sterilized glass bottles and transported on ice to laboratory for analysis.

The synthetic medium used in this study contains the minimum of nutrients indispensable for phosphate removal bacteria growth [8]. The composition of medium diluted in distilled water was summarized in table 1.

Table 1: Composition of the synthetic medium [8].

Components	Concentration ($g.L^{-1}$)
$CH_3COONa.3H_2O$	0.4
K_2HPO_4	0.049
KH_2PO_4	0.028
NH_4Cl	0.107
$MgSO_4.7H_2O$	0.180
$CaCl_2.2H_2O$	0.0003
*Trace solution	0.3 $mL.L^{-1}$

* 10$g.L^{-1}$ EDTA, 1.54 $g.L^{-1}FeSO_4.7H_2O$, 0.15 $g.L^{-1}$ H_3BO_3, 0.03 $g.L^{-1}$ $CuSO_4.5H_2O$, 0.12 $g.L^{-1}$ $MnCl_2.4H_2O$, 0.18 $g.L^{-1}KI$, 0.06 $g.L^{-1}Na_2.MoO_4.2H_2O$, 0.12 $g.L^{-1}$ $ZnSO_4.7H_2O$, 0.15 $g.L^{-1}CoCl_2.6H_2O$.

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Phosphate removal by mixed culture

Inoculum preparation

Activated sludge samples were treated before inoculation to fermentors. First, the samples were diluted in 0.9% sterile physiological salt solution with stirring for 2 min by vortex mixer [9] and the suspended activated sludge was centrifuged at 300 rpm for 2 min. Then, the decanted supernatant was enriched in 100mLof prepared medium and incubated with shaking during 24 h at 30°C. After, the culture was centrifuged at high speed (10000 rpm) for 15 min and the pellets were washed in a physiological salt solution. Finally, the suspended pellet was added to the reactors.

Culture conditions

Mixed culture was inoculated to 500 mL fermentors contained 300 mL of medium supplemented with different concentrations of sodium acetate as carbon source and K_2HPO_4/KH_2PO_4 as phosphorus source. The fermentors were operated in batch mode and incubated under two alternating phases and aerobically in stirred bain-marie (GFL1083) for 2h, then aerobically at 30°C for 4 h in horizontal stirred operating at 150 mpm. Experiments were performed in triplicata.

Sampling and Analytical methods

To estimate phosphate concentration, liquid samples were withdrawn aseptically from each fermentor through a sampling port using sterile syringe, at regular time intervals. Orthophosphate is the most dominant in wastewater and its concentration was measured colorimetrically using the method of ascorbic acid [10].

Isolation of bacteria

During phosphate reduction by mixed culture, isolation was carried out from Fermentors inoculated with activated sludge and aliquot of culture were streaked onto minimal agar medium. Then, the inoculated plates were incubated at 30°C for 24 h to 72 h. Isolated Bacteria with distinct colony morphologies were selected and sub-cultured onto the same medium until achievement of growing in pure cultures. The isolated bacteria were grown in nutrient broth, stored in 10 % (v/v) glycerol at -20 °C and used as stock cultures in subsequent analysis.

Bacterial identification

Morphology and biochemical characteristics of isolates

The cell morphology was determined microscopically after Neisser stain and Gram stain preparation. The biochemical characteristics were determined using tests including Catalase, Oxidase, Nitrate reductase, Tryptophane desaminase, Decarboxylase, Urease, Indole production, Carbohydrate fermentation and Mixed acid fermentations. Growth of strains on King A and King B media was tested too.

Identification of bacteria by API system

Pure bacterial strains were identified using API 20NE systems, according to the manufacturer's instructions (BioMérieux). The API strips were examined after 24 h and 48 h at 30 °C and the identifications were carried out by identification database APixl.

RESULTS AND DISCUSSION

Culture medium optimization

Optimization of culture medium was performed to find the optimal initial concentration of sodium acetate, the optimal initial concentration of phosphate and the appropriate carbon source. The values of the kinetic of optimization parameters are summarized in Table.2.

Table.2. Optimization of culture parameters

Parameters	Phosphate removal rate (%)	Final Phosphate concentration ($mg.L^{-1}$)
Initial concentration of sodium acetate		
1000 ppm	83.38	2.71
2000 ppm	93.08	1.16
3000 ppm	93.80	1.03
4000 ppm	96.19	0.64
5000 ppm	99.23	0.13
Initial concentration of phosphate		
0 ppm		
5 ppm	99.68	0.027
10 ppm	98.87	0.14
30 ppm	93.91	0.24
50 ppm	71.09	14.98
Carbon sources		
Acetate	99.23	0.13
Glucose	71.22	3.82
Lactose	61.05	6.8
Lactate	78.51	4.22

As shown in (Table.3), acetate provides the best rate of phosphate removal, 99.23 %, followed by lactate with a rate of 78.51 % and glucose with 71.22%, while lactose eliminate 61.05 % of phosphate. Our results are in agreement with the results obtained in the literature [11, 12, 13, 14].

Phosphate removal by mixed culture

For mixed culture, bacteria involved in the phosphate accumulation process present a latency phase of about 8 h. This short adaptation period could be the result of synergistic interrelation ships between co-cultured species [15]. In [16, 17] they reported that the synergistic inter actions between phosphate-solubilizing co-cultured species resulted in high phosphatase activity and enhanced available Phosphate in the soil [18]. After latency phase, phosphate accumulating starts with an accumulating rate of about 0.31 $mg.L^{-1}.h^{-1}$ during the first 50 h, exponential phase. Finally, the stationary phase was reached after 72 h of mixed culture treatment with a dephosphatation rate of about 92.87%.

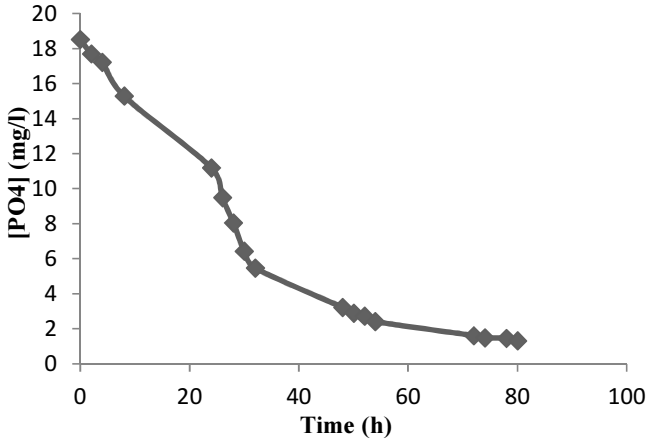

Fig 1: Kinetic of Phosphate removal using mixed bacterial culture.

Identification of isolates

Different types of bacterial culture were isolated from activated sludge using streak plate method. Four strains were selected on the basis of morphological and biochemical characteristics, bacterial culture was tentatively identified as *Acinetobacter junii*, *Pseudomonas aeruginosa*, *Moraxella lacunata*, *Alcaligenes denitrificans*. These results were confirmed using API 20NE system and the numerical profile is presented in Table 3.

Table 3: Identification of isolates using API 20NE system.

	S1	S2	S3	S4
Numerical profile	1001051	1354575	1010204	1000467
Strain Name	*Acinetobacter junii*	*Pseudomonas aeruginosa*	*Moraxella lacunata*	*Alcaligenes denitrificans*

Our results show the biodiversity of the activated sludge samples obtained from wastewater plants of Khenchela. Three of these strains which are *Acinetobacter junii, Pseudomonas aeruginosa, Moraxella lacunata* belong to the γ class of *Proteobacteria,* while *Alcaligenes denitrificans* belongs to *ß* class of *Proteobacteria* [19].

We also show that *Proteobacteria* are the most abundant with a rate of 87.5%. Similar results were obtained in wastewater by [20]. They found that the *Proteobacteria* are a major group of bacteria and represent more than 50 % of all the micro-organisms present in five wastewater treatment plants.

In this study, the most frequently isolated class is that of *Proteobacteria* with three species, which was comfirmed by several studies [21, 22, 23, 24, 25].

Conclusions

The objective of our work consists to isolate phosphate removing microbes from activated sludge for wastewater remediation in the area of Khenchela (Eastern Algeria). For this, four efficient pure strains isolated from activated sludge samples and identified as *Acinetobacter junii, Pseudomonas aeruginosa, Moraxella lacunata and Alcaligenes denitrificans*. Our results show that mixed bacterial culture containing mostly isolated strains can be used successfully for removing phosphate from activated sludge of wastewater treatment plants.

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