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ROCK PHOSPHATE SOLUBILIZATION BY SOIL BACTERIA

R. Ivanova, D. Bojinova, K. Nedialkova*

University of Chemical Technology and Metallurgy
8 Kl. Ohridski, 1756 Sofia, Bulgaria
E-mail: ralitsa_ivanova@abv.bg
**N. Poushkarov Institute of Soil Science,*
7 Shosse Bankya, 1080 Sofia

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ABSTRACT

The fact that the most of the bacteria isolated from the soil have the ability to dissolve rock phosphates both in the soil and in the culture medium is well known. The microorganisms produce low molecular mass organic acids, which attack the phosphate structure and transform phosphorus from non-utilizable to the utilizable for the plants form.

The objective of the present study is to establish the effect of the rock phosphate concentration in the liquid medium and its particle size. As well as the duration of the bioconversion on the solubilization of Tunisian phosphorite by Erwinia sp. *and* Azotobacter sp. *isolated from soil. It was established that the lower the concentration of the phosphate in the nutritive medium the greater is the conversion percentage of P_2O_5 into soluble forms. Also* Azotobacter sp. *have higher ability to dissolve Tunisian phosphorite than* Erwinia sp. *A maximum degree 96.7 % of P_2O_5 extraction has been obtained for the period of 12 days of incubation of* Azotobacter sp. *when in the medium was added 0.5 % w v^{-1} phosphate with particle size below 0.2 mm. Investigations with* Erwinia sp. *showed that a maximum extent of 76.6 % of the phosphate solubilization has been observed on the 6^{th} day of incubation at phosphorite concentration of 0.5 % w v^{-1}. It was observed a negative correlation between the degree of P_2O_5 extraction and the culture pH and a positive correlation between the titratable acidity and the quantity of phosphorus released.*

Keywords: Erwinia sp., Azotobacter sp., organic acids, phosphate-solubilizing microorganisms, phosphorus extraction, rock phosphate.

INTRODUCTION

It is well known that many microorganisms isolated from the soil are able to dissolve different kinds of rock phosphates in a liquid culture [1-4]. It is generally accepted that the major mechanism of the mineral phosphate solubilization is the action of organic acids synthesized by bacteria. Organic acids that solubilize phosphates are mainly: citric, lactic, gluconic, 2-ketogluconic, oxalic, tartaric, acetic, etc. [1, 3]. These organic acids are sources of biotical generated H^+ ion able to dissolve the mineral phosphate and to make it available for the plant [5]. Many results indicate that the phosphate solubilization is a consequence of the decrease of pH due to the production of organic acids. However, no correlation could be established between the acidic pH and the quantity of P_2O_5 liberated [1, 4].

The aim of this study is to establish the effect of the rock phosphate concentration in the liquid nutritive medium, the particle size of the phosphate and the time of bioconversion on the dissolution of Tunisian phosphorite by *Erwinia sp.* and *Azotobacter sp.* isolated from the soil.

Table 1. Chemical composition of Tunisian phosphorite with different particle size (% w w^{-1}).

Value of particle size (mm)	CaO	MgO	Al_2O_3	Fe_2O_3	K_2O	Na_2O	SiO_2	F	CO_2	H_2O	P_2O_5[a]	
											tot.	c.s.
below 0.2	48.9	0.2	<0.1	0.5	0.1	1.3	1.1	3.2	5.0	1.1	29.6	10.1
from 0.4 to 0.63	49.2	0.4	<0.1	0.3	0.1	1.4	1.0	3.5	4.8	1.2	31.4	9.9

[a] *$P_2O_{5\ tot.}$ – total content of P_2O_5; $P_2O_{5\ c.s.}$ - P_2O_5 soluble in 2 % citric acid*

EXPERIMENTAL

Tunisian phosphorite

The experiments have been carried out using two particle size of Tunisian phosphorite (TP) - below 0.2 mm and in the range from 0.4 to 0.63 mm. The chemical composition of TP is shown in Table 1. The P_2O_5 content was determined spectrophotometrically by vanado-molibdate yellow complex [6]. A complexometric method was used for CaO, Fe_2O_3 and Al_2O_3 determination, Atom Absorption Spectrophotometry (AAS) for magnesium, flame spectrophotometry for potassium and sodium, weight analysis for silicon, ion selective electrode method for fluorine and volumetric analysis for CO_2 as well.

Microorganisms

Two Gram-negative bacteria – *Erwinia sp.* and *Azotobacter sp.*, designated below as bacteria A and B, were used. Bacteria were isolated from agricultural soils on glucose-asparagine agar medium with freshly precipitated calcium phosphate. Before setting the experiments cell suspensions for inoculation were prepared. Bacteria were incubated in a rotary water bath shaker for 30 h at 25°C. The amount of the viable bacterial cells was checked by plate counts technique. Final concentration of suspension was 10^9 cfu ml^{-1}.

Experimental methods

The bioconversion of TP was achieved through submerged incubation of the microorganisms in a liquid nutritive medium containing (g dm^{-3}): glucose 20; asparagines 1; K_2SO_4 0.2; $MgSO_4.7H_2O$ 0.2; $K_2HPO_4.3H_2O$ 0.5 and 0.02 % v v^{-1} maize extract. The nutritive medium was sterilized for 20 min at 110°C and 0.05 MPa. In 250 ml Erlenmeyer flask TP was added, so that the TP concentration was 0.5, 1 and 2 % w v^{-1}, respectively. The flasks were covered with cotton stopper and aluminum foil and sterilized for 3 h at 140°C. After cooling to room temperature 100 ml of nutritive medium and 2 ml of the bacterial suspension of bacteria A and B were put to each flask. The flasks were incubated in a shaking water bath at 25 ± 1°C for 15 days. At every 3 days (3rd, 6th, 9th, 12th and 15th day) the content of the flasks was centrifuged and pH, glucose concentration [7], titratable acidity through titration with 0.1 N NaOH and content of water-soluble P_2O_5 ($P_{2}O_{5\ w.s.}$) in this first filtrate (C_1) were determined. The precipitate (biomass and remaining mineral mass) was processed for 2 h at room temperature stirring with 80 ml 2 % citric acid. After filtration, the content of citrate-soluble P_2O_5 ($P_{2}O_{5\ c.s.}$) in the second filtrate (C_2) was determined.

On the basis of the results obtained the extent of extraction of P_2O_5 from phosphorite to the water-soluble (α_1) and the citrate-soluble (α_2) as well as the total extent of P_2O_5 extraction (α) were determined as follows:

$$\alpha_1 = \frac{P_2O_{5\,w.s.}}{P_2O_{5\,tot.}} 100\ ,(\%) \qquad (1)$$

$$\alpha_2 = \frac{P_2O_{5\,c.s.}}{P_2O_{5\,tot.}} 100\ ,(\%) \qquad (2)$$

$$\alpha = \alpha_1 + \alpha_2\ ,(\%) \qquad (3)$$

where $P_{2}O_{5\ w.s.}$ – P_2O_5 content in the first filtrate (g); $P_{2}O_{5\ c.s.}$ – P_2O_5 content in the second filtrate (g); $P_{2}O_{5\ tot.}$ – total content of P_2O_5 in the system (g).

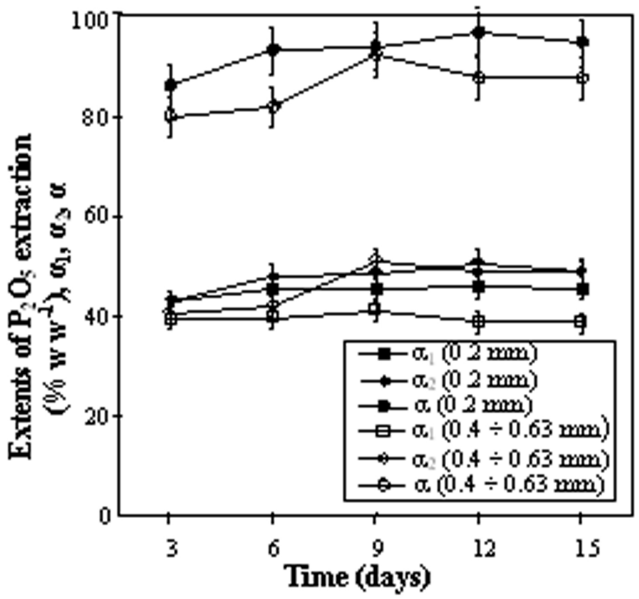

Fig. 1. Change in the extents of P_2O_5 extraction (α_1, α_2 and α) by bacteria B in nutritive medium containing 0.5 % w v^{-1} TP.

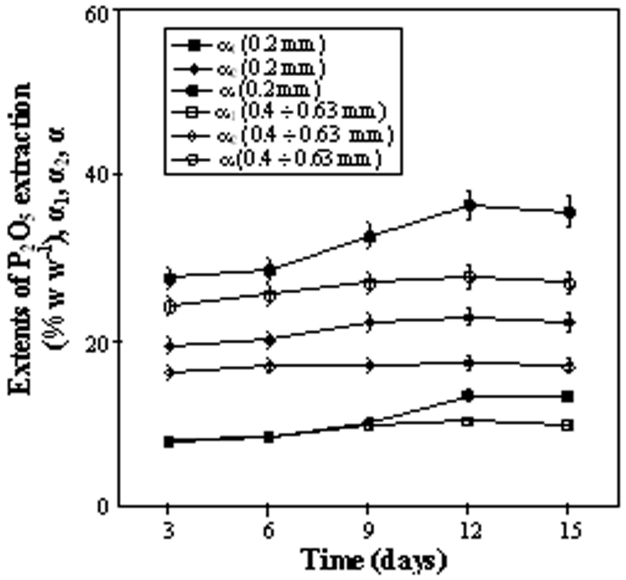

Fig. 3. Change in the extents of P_2O_5 extraction (α_1, α_2 and α) by bacteria B in nutritive medium containing 2 % w v^{-1} TP.

RESULTS AND DISCUSSION

Fig. 1 presents the results of the investigations with bacteria B at the lowest TP concentration (0.5 % w v^{-1}) in the nutritive medium using both phosphate fractions. The lower the TP fraction the higher the degrees of P_2O_5 extraction were obtained. α achieves a maximum of 96.7 % on the 12th day for the lower particle size. It reaches 92.2 % on the 6th day of incubation for the higher particle size, respectively.

At TP concentration 1 % w v^{-1} and particle size from 0.4 to 0.63 mm the total degree reaches 55.3 % on the 9th day (Fig. 2).

When the concentration of TP is increasing in the medium (2 % w v^{-1}) the extent of P_2O_5 extraction in

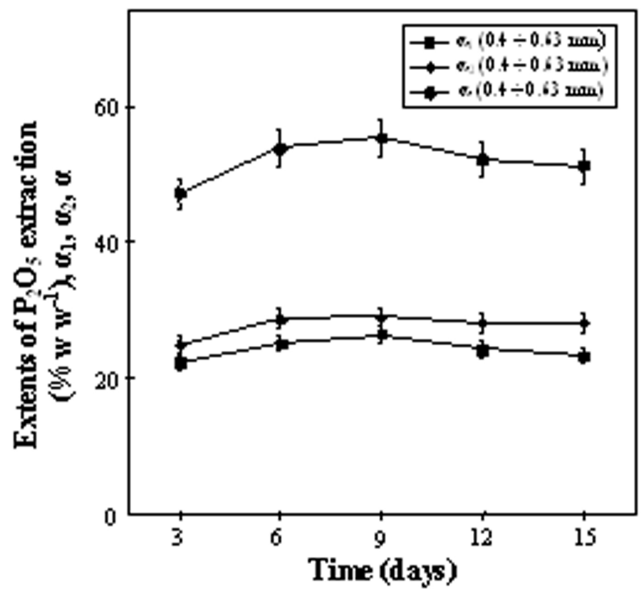

Fig. 2. Change in the extents of P_2O_5 extraction (α_1, α_2 and α) by bacteria B in nutritive medium containing 1 % w v^{-1} TP (particle size 0.4 - 0.63 mm).

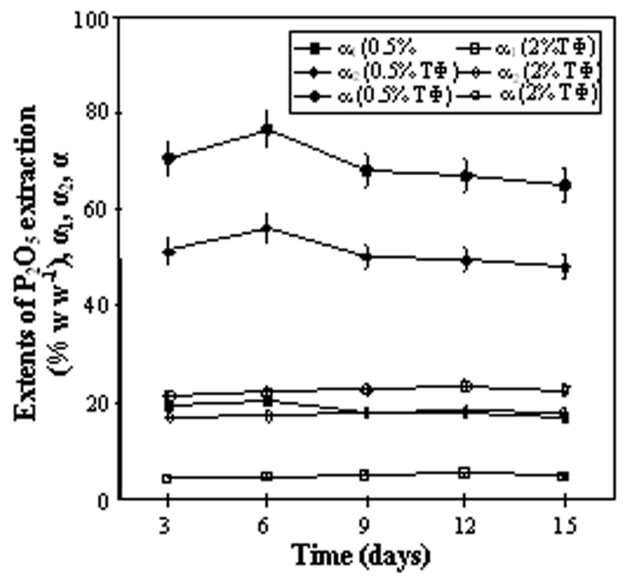

Fig. 4. Change in the extents of P_2O_5 extraction (α_1, α_2 and α) by bacteria A in nutritive medium containing different TP concentrations (particle size below 0.2 mm).

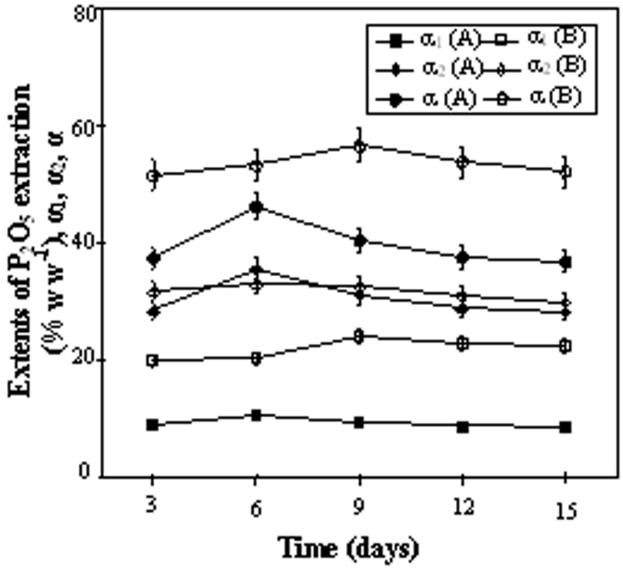

Fig. 5. Change in the extents of P_2O_5 extraction (α_1, α_2 and α) by bacteria A and B in nutritive medium containing 1 % w v^{-1} TP (particle size below 0.2 mm).

soluble forms (Fig. 3) is significantly lower compared with phosphate concentration of 0.5 % w v^{-1} (Fig. 1). The total degree of P_2O_5 extraction (α) reaches maximum on the 12th day and its value is 36.5 % for particle size below 0.2 mm. It is 27.8 % for fractions 0.4 - 0.63 mm, respectively.

Similar trend can be observed when bacteria A was used (Fig. 4). At a concentration of TP 0.5 % w v^{-1} the total degree reaches value 76.6 % on the 6th day and only 23.5 % for 2 % w v^{-1} concentration of TP on the 12th day, respectively.

As shown on Fig. 5 bacteria B has better solubilizing activity compared with bacteria A. When bacteria B was used the highest total degree of P_2O_5 extraction (α) is 56.5 % on 9th day, while with bacteria A reaches maximum of 46.2 % on the 6th day. The difference between bacteria A and bacteria B is essential considering the values of the extent of conversion of P_2O_5 to water-soluble form (α_1).

Table 2 represents the results from the culture pH, glucose concentration, titratable acidity, C_1 and C_2, as well as the total concentration of P_2O_5 ($C = C_1 + C_2$) for experiments with bacteria A for TP concentration 0.5, 1 and 2 % w v^{-1}. The initial pH of the nutritive medium is 7.4. As observed from the data, pH has values between 3.9 and 4.2 and fell considering to the lowest values on the 9th day.

Table 2. Results from the biochemical processing of Tunisian phosphorite (particle size below 0.2 mm and concentrations in the culture 0. 5, 1 and 2 % w v^{-1}) with bacteria A.

Day	pH	Glucose	Titratable acidity	P_2O_5		
				C_1	C_2	$C = C_1+C_2$
		g l^{-1}	μEq ml^{-1}	g l^{-1}		
0.5% TP						
3,0	3,9	13,0	5,2	0,3	0,8	1,0
6,0	3,9	9,7	7,4	0,3	0,8	1,1
9,0	3,9	9,4	5,6	0,3	0,7	1,0
12,0	3,9	9,0	4,0	0,3	0,7	1,0
15,0	3,9	9,0	4,2	0,3	0,7	1,0
1% TP						
3,0	3,8	13,0	5,0	0,3	0,8	1,1
6,0	3,8	9,5	6,6	0,3	1,1	1,4
9,0	3,8	9,5	5,4	0,3	0,9	1,2
12,0	4,2	9,0	5,2	0,3	0,9	1,1
15,0	4,2	8,7	4,6	0,3	0,8	1,1
2% TP						
3,0	3,8	12,2	7,4	0,3	1,0	1,3
6,0	3,8	11,7	8,6	0,3	1,0	1,3
9,0	3,7	11,3	10,2	0,3	1,0	1,3
12,0	3,8	10,1	10,6	0,3	1,1	1,4
15,0	3,9	9,9	10,6	0,3	1,1	1,3

A significant relationship was detected between solubilization, measured as soluble phosphate and titratable acidity in the culture medium (Table 2, Figs. 4 and 5). The correlation coefficients between soluble phosphate and titratable acidity are: 0.89 at 0.5 % w v^{-1} TP, 0.97 at 1 % w v^{-1} TP and 0.90 at 2 % w v^{-1} TP, respectively. Instead of the fact that the values of titratable acidity achieved higher value (10.6 μEq ml^{-1}) after 9 days of the bioconversion of TP (2 % w v^{-1}), the phosphorus concentration did not change significantly (Table 2).

The relationship between the soluble phosphate detected in the first and second filtrate expressed as P_2O_5 (C_1 and C_2) and their sum (C), pH and titratable acidity are shown in Tables 2, 3 and 4. Soluble phosphate levels (C_1, C_2 and C) were found to be nearly equivalent to the two values of the particle size of TP by phosphate concentration of 0.5 % w v^{-1} (Tables 3 and 4). Above this concentration, there was an increase in the amount of the soluble phosphate. When TP with particle size bellow 0.2 mm and concentration 2 % w v^{-1} was used a maximum value of soluble phosphate 2.2 g dm^{-3} up to the 12th day was observed. This maximum amount of phosphorite solubilized corresponded to the highest

Table 3. Results from the biochemical processing of Tunisian phosphorite (particle size below 0.2 mm and concentrations in the culture 0. 5, 1 and 2 % w v^{-1}) with bacteria B.

Day	pH	Glucose	Titratable acidity	P_2O_5		
				C_1	C_2	$C = C_1+C_2$
		g l^{-1}	μEq ml^{-1}	g l^{-1}		
0.5% TP						
3,0	3,7	15,4	7,4	0,6	0,6	1,3
6,0	3,7	7,7	9,4	0,7	0,7	1,4
9,0	3,6	7,1	10,2	0,7	0,7	1,4
12,0	3,6	6,5	11,0	0,7	0,8	1,4
15,0	3,6	6,2	10,4	0,7	0,7	1,4
1% TP						
3,0	3,7	16,2	6,8	0,6	0,9	1,5
6,0	3,7	7,7	7,8	0,6	1,0	1,6
9,0	3,5	7,4	12,2	0,7	1,0	1,7
12,0	3,7	7,0	11,2	0,7	0,9	1,6
15,0	3,7	6,9	11,2	0,7	0,9	1,5
2% TP						
3,0	3,7	15,0	8,2	0,5	1,2	1,6
6,0	3,8	8,2	10,6	0,5	1,2	1,7
9,0	3,4	7,7	14,4	0,6	1,3	1,9
12,0	3,4	7,2	16,4	0,8	1,4	2,2
15,0	3,4	6,4	17,0	0,8	1,3	2,1

Table 4. Results from the biochemical processing of Tunisian phosphorite (particle size 0.4-0.63 mm and concentrations in the culture 0.5 and 2 % w v^{-1}) with bacteria B.

Day	pH	Glucose	Titratable acidity	P_2O_5		
				C_1	C_2	$C = C_1+C_2$
		g l^{-1}	μEq ml^{-1}	g l^{-1}		
0.5% TP						
3,0	3,7	7,9	7,2	0,6	0,6	1,3
6,0	3,7	4,3	8,0	0,6	0,7	1,3
9,0	3,4	3,8	13,6	0,6	0,8	1,4
12,0	3,7	3,6	11,6	0,6	0,8	1,4
15,0	3,7	3,2	12,2	0,6	0,8	1,4
1% TP						
3,0	3,5	10,3	10,5	0,7	0,8	1,5
6,0	3,3	8,1	12,9	0,8	0,9	1,7
9,0	3,3	7,0	16,7	0,8	0,9	1,7
12,0	3,3	4,6	16,3	0,8	0,9	1,6
15,0	3,3	4,0	15,5	0,7	0,9	1,6
2% TP						
3,0	3,7	7,0	8,8	0,5	1,0	1,5
6,0	3,7	5,7	12,2	0,5	1,1	1,6
9,0	3,6	3,6	13,8	0,6	1,1	1,7
12,0	3,5	2,9	14,4	0,7	1,1	1,8
15,0	3,5	2,6	14,2	0,6	1,1	1,7

value of the organic acid produced. The sugar consumption and organic acid liberation are seen to be most active up to the 6th day. A partial neutralization of the acids with ions, liberated due to the decomposition of the phosphate, is the reason for maximum of organic acid production on the 12th and 9th day for the experiments with TP concentration of 0.5 and 1 % w v^{-1}.

The results obtained showed that the solubilization of Tunisian phosphorite depends on: phosphate concentration in the nutritive medium, the particle size of TP, the decrease in pH, the acid production and the microbial isolate used.

The data indicate that the lower the quantity of phosphate applicable, the greater is the conversion percentage of P_2O_5 in soluble forms. The similar correlation has been found in [8]. According to Venkaterswarlu et al. [9] by increasing the phosphate concentration over 0.25 % the quantities of the released P are not significant in the conditions of rock phosphate solubilization with *Aspergillus niger*. The additional accumulation of soluble phosphate was changed insignificantly (0.2-0.5 g dm^{-3}) in the case when TP concentration was increased from 0.5 to 1 and 2 % w v^{-1}. These results are in conformity with those recorded by Venkateswarlu et al. [9] and Nahas et al. [10].

In the present study the maximum solubilization occurred with the particle size below 0.2 mm (Table 3) by equivalent conditions, confirming the observations of Ghosh and Banic [8]. Smaller particle size material is more susceptible to microbial attack than the larger sized ore. Decreasing the particle size, the surface area of the phosphate particles increases and hence better contact occurs, which aids maximum solubilization of the rock phosphate.

Many results indicate decrease in pH due to the formation of organic acids and support the view that the acids and products of microbial activity affect the solution of phosphate. A correlation was detected between culture pH and titratable acidity (r = -0.87 to 0.25) which is in accordance with other results [11, 12]. However, no correlation has been observed between the degree of solubilization and pH change [13, 14]. According to Kucey [1] the lack of relationship between pH drop and P solubilized may be due to the liming effect of the rock phosphate and the production of other metabolites by the microbes, or on the type of acid produced [15].

In the present study negative correlations between the quantities of phosphate solubilized and decrease in pH was observed. The values of the correlation coeffi-

cients are from –0.55 to –0.94. A maximum of the total P_2O_5 concentration, respectively a maximum of TP solubilization was achieved at the lowest values of the culture pH in accordance with the results obtained by Venkaterswarlu et al. [9] and Nahas [12].

Some authors indicate that no correlation has been observed between phosphate solubilization and titratable acidity [13]. Cerezine et al. [11] consider that even though the concentration of soluble phosphates was related to pH, it was not related to titratable acidity, which confirms that the solubilizing ability is not related to organic acid production but to the nature of the organic acid produced. The results showed a significant correlation between the titratable acidity and the quantity of the released P. The value of the correlation coefficient is about 0.9 in all the experiments. This is in accordance to the results obtained from Nahas et al. [10], Nahas [12] and Goldstein [16].

The variables that affect the mechanisms of rock phosphate solubilization have been discussed by different authors. Some of them considered that the organic acid produced by microorganisms have an important role in solubilization of inorganic phosphates [3, 9]. According to Goldstein [16] the bioprocess of rock phosphate ore involves much more processes than simple acid dissolution. He assumes that the bacteria form biofilms on the surface of the ore particles, which produce unique physicochemical conditions. That result in true biocatalytic events that greatly enhance the rate and efficiency of phosphorus bioleaching from the phosphates.

Obviously the bioconversion was performed in a complex heterogeneous system with simultaneously occurring biosynthetic and chemical reactions, which have different velocity depending on various effected parameters: continuously changeable concentration of macro- and microelements, organic acid concentration, diffusion velocity, etc. Phosphorus containing compounds and other ions (Ca^{2+}, Mg^{2+}, K^+, Na^+, Fe^{3+}, etc.) move to the liquid face medium after defined period of bioconversion. As a result of the phosphate solubilization and the high Ca^{2+} ions concentration it is most probably its bound part of the produced organic acids to form slow soluble salts. The effect is decreasing of the acids concentration in the culture medium, which will limit the process of phosphate decomposition.

CONCLUSIONS

It was established that all the investigated factors have a significant influence on the solubilization of Tunisian phosphorite. The most essential are the TP concentration and the duration of bioconversion. Further investigations can explain the mechanism of phosphate dissolution using microbial soil isolates on the conditions used in this study.

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