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DECOMPOSITION OF PHOSPHORUS-CONTAINING COMPOUNDS IN AQUEOUS AND POLYSACCHARIDE SOLUTIONS OF ORGANIC ACIDS

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The purpose was to study the patterns of dissolution (solubilization) of phosphorus-containing minerals in aqueous and polysaccharide solutions of organic acids in order to model the mechanism of mineral destruction by soil bacteria synthesizing organic acids and exopolysaccharides.

Methods. Model, laboratory-analytical, microbiological, statistical.

Results. The destructive effect of organic acids on minerals is manifested both in aqueous and polysaccharide solutions. The introduction of bacterial polysaccharide into an aqueous solution of acids increases the decomposition of phosphorus-containing minerals by 1.34–4.96 times. The influence of the chemical structure of acid molecules on the intensity of mineral decomposition is mainly manifested in the presence of bacterial polysaccharide, while in an aqueous solution the effectiveness of acid action depends on the nature of the mineral being destroyed. To the maximum degree, polysaccharide increases the destruction of minerals in a solution of citric acid: molten magnesium phosphate —2.55 times, thermophosphate — 2.11 times, phosphate flour 4.96 times. Decomposition of phosphorus compounds in solutions of ascorbic and oxalic acids enhances bacterial polysaccharide to a lesser extent than in citric acid solution.

Modeling the destruction of phosphorus-containing minerals under non-sterile conditions (soil conditions) made it possible to establish that organic acids under non-sterile conditions are subject to consumption by soil microbiota, especially ascorbic and citric acids, and to a lesser extent — succinic. Aqueous solutions of organic acids after 18 hours of incubation in non-sterile conditions lose their leaching activity by 1.06–12.1 times. The introduction of a polysaccharide into aqueous solutions of acids makes it possible to avoid their rapid consumption by microorganisms, because of which the efficiency of mineral leaching under non-sterile conditions decreases by only 5–20% compared to sterile ones.

Conclusions. The introduction of a bacterial polysaccharide into a solution of organic acids enables the latter to be transferred to a sorbed state, as a result of which their susceptibility to consumption by microorganisms is sharply reduced. Thus, polysaccharide-forming bacteria not only destroy minerals more intensively than microorganisms synthesizing only low-molecular-weight metabolites, but also synthesize a more stable and long-term functioning leaching complex in the soil.

Key words: phosphorus; bacteria; organic acid; polysaccharide; modeling; dissolution; mechanism.

Phosphorus (P) is one of the five essential plant nutrients. Phosphate fertilizers have played an important role in agriculture since the beginning of the 20th. By 2051, global demand for phosphate fertilizers is expected to increase by 86% on arable land, especially in developing regions. However, phosphate rocks are a limited resource, and high quality phosphate rocks can be depleted in 300–400 years [1]. Most of the soluble phosphate applied to the soil as a chemical fertilizer is immobilized shortly after application and becomes unavailable to plants, resulting in an excess of fixed phosphorus in some arable soils. Microorganisms play a significant role in the natural P-cycle [2–4]. Insoluble forms of P and phosphorus in the composition of natural minerals can be converted into soluble P by soil phosphate mobilizing bacteria [2, 5, 6]. Greenhouse and field experiments have shown that application of phosphate mobilizing microorganism preparations promote plant growth in phosphorus deficient soils, indicating their potential as new biofertilizers to address food security issues, especially given the limited use of chemical fertilizers. However, it was shown that the effectiveness of the effect of phosphate mobilizing bacteria on plant growth varies under field conditions. Several biotic and abiotic factors can influence the activity and functions of phosphate mobilizing bacteria, as well as their effect on plant growth [2, 5]. Understanding the mechanisms of destruction (solubilization) of phosphate-containing minerals by microorganisms contributes to the development of effective strategies for using their potential to increase plant productivity, which was the goal of our research. Soil bacteria solubilize mineral phosphates mainly through the secretion of various organic acids, such as citric, gluconic, lactic, malic, oxalic, propionic, and succinic acids, which differ in the number of carboxy groups, hydroxy groups, and carbon double bonds [2, 5]. However, the role of organic acids in the processes of phosphorus mobilization is still denied by some authors. The authors base their conclusions as on the fact that there is a whole series of acid-forming bacteria that are incapable of dissolving natural phosphates, and on the basis of the fact that between the methods no acidification and decomposition of iron and aluminum phosphates no matter how significant the correlation [7].

At the same time, it seems to us that this issue can be resolved in modal experiments with the use of solutions of organic acids, which give the possibility to evaluate the ability of individual organs Czech acids to the dissolution of phosphorus containing minerals. The introduction of polysaccharide into aqueous solutions of organic acids in a concentration of 0.5–1 0 g/l will allow to model the cultural liquid of those soil microorganism, which are synthesized at the same time organic acids and polysaccharides.

Materials and Methods

In the experiment, 0.1 M aqueous and polysaccharide solutions of ascorbic, oxalic, citric and succinic acids were used. Sterilization of acid solutions was carried out by filtration using cellulose acetate membrane filters MCA020013H with a pore diameter of 0.2 μm. Bacillus mucilaginosus C-3 polysaccharide was used as a bacterial polysaccharide. It is an anionic heteropolysaccharide, containing in its composition glucose — 10.6%, galactose — 4.0%, mannose — 11.9%, fukose — 0.5%, unidentified sugar — 0.5% (of dry weight).

We used the bacterial strain *B. mucilaginosus C-3*, obtained from the collection of the Institute of Mineral Resources of Mingeo of Ukraine. Bacteria were stored at room temperature on agar mineral medium with glucose, g/l: glucose — 15.4, $MgSO_4$ $7H_2O$ — 0.7, Na_2HPO_4 2 H_2O — 2.0, KNO_3 — 1.0, $FeCl_3$ $6H_2O$ — 0.05. The cultivation of *B.mucilaginosus* was carried out in a periodic process, which was carried out in 750 ml bah by volume with 100 ml of medium on a shaker at 240 rpm. The temperature of the cultivation was 30 °C, cultivation time was 18–24h. The sowing material was introduced in the amount of 5% of the medium volume. The polysaccharide preparation was obtained as follows: the culture liquid (18–24 h) was diluted 2–3 times with distilled water and centrifuged to separate the cells at 40000g for 40 minutes. The supernatant was drained and polysaccharide was precipitated from it with three volumes of ethanol. Precipitated EPS was separated from the supernatant by centrifugation at 6000 rpm for 10 min. The ethanol precipitate was dissolved in distilled water and dialyzed against tap water for one day, against distilled water for two days. The resulting polysaccharide was freeze-dried.

The lyophilized polysaccharide preparation was added to acid solutions at a concentration of 0.5 g/L. To carry out the process of polysaccharide macromolecules swelling and sorption of organic acid molecules on them, the samples were placed for 3 hours in a refrigerator at + 5 °C. For sterile variants of the experiment, the samples of minerals were sterilized at 1.5 atm for 1 hour by autoclaving and aseptically added to acid solutions. For non-sterile variants of the experiment, the samples of non-sterile mineral and 100 μl of water extract from meadow chernozem in a dilution of 10^5 were introduced into test tubes after 3 hours. The test tubes were incubated at 28 °C on a shaker with 160 rpm. The incubation time was 18 hours. The amount of polysaccharide in the solutions at the end of the experiment was controlled by viscosity (with the help of a VZh-1 viscometer) and by the weight method with preliminary precipitation of 3 times the ethanol volume.

The concentration of phosphorus in solutions was determined by the Murphy-Reilly method, after preliminarily precipitating the polysaccharide with three volumes of ethanol [8]. Measurement of the optical density of the solutions of the probes on FEK 56-2 and KFK-2 (light filter No. 8, λ = 590 nm, cuvette No. 30). The statistical significance of the obtained differences between the experimental variants was assessed by the value of the error of the mean result, calculated using the Statistica 10 program.

Results and Discussion

In aqueous solutions, oxalic acid has the greatest dissolving effect on the studied phosphorus compounds, followed by citric and ascorbic acids (Tables 1–3). Succinic acid realizes its dissolving effect depending on the properties of the minerals subjected to destruction: when dissolving fused magnesium phosphate and phosphate rock — at the level of citric acid, when dissolving thermophosphate — to a lesser extent than ascorbic acid.

In the presence of *B.mucilaginosus* polysaccharide, the action effectiveness of the studied acids is identical in relation to all three connections: oxalic > citric > ascorbic > amber. and does not depend on the structural features of decomposed phosphorus fertilizers. Consequently, the minerals decomposition intensity in polysaccharide solutions of organic acids are defined, first of all, by the chemical structure of the acid molecule lots and does not depend on the structural features of the destructurable phosphorus fertilizers. As can be seen from the data presented in Table 3, the most effective bactericidal polysaccharide strengthens the decomposition of phosphate flour: the phosphorus removal in the presence of a polysaccharide exceeds the destruction of phosphate flour in an aqueous solution of ascorbic acid by 4.3 times, citric acid by 4.96, oxalic acid by 4.1, and succinic acid by 1.94 times.

In the maximum degree, polysakharid increases the destruction of minerals in the created citric acid: fused magnesium phosphate — 2.55 times, thermal phosphate — 2.11 times for, phosphate flour —

Table 1

Removal (solibilization) of phosphorus from fused magnesium phosphate in 0.01 M solutions of organic acids in the presence of *B. mucilaginosus* polysaccharide (2) and without it (1)

Organic acid Sterile conditions		Phosphorus removal (solibilization), P_2O_5 mg/l	
		Non-sterile conditions	
Ascorbic acid	1	100.0 + 8.14	30.3 + 2.88
	2	211.3 + 17.5	175.5 + 18.2
Lemon acid	1	124.2 + 10.6	39.4 + 5.11
	2	316.7 + 21.1	301.7 + 21.9
Oxalic acid	1	142.5 + 13.8	50.5 + 5.17
	2	213.1 + 15.9	218.4 + 20.5
Succinic acid	1	128.2 + 11.8	75.8 + 6.88
	2	190.4 + 18.0	192.2 + 20.1

Table 2

Removal (solibilization) of phosphorus from thermophosphate in 0.01 M solutions of organic acids in the presence of *B. mucilaginosus* polysaccharide (2) and without it (1)

Organic acid Sterile conditions		Phosphorus removal (solibilization), P_2O_5 mg/l	
		Non-sterile conditions	
Ascorbic acid	1	130.0 + 8.14	10.7 + 2.88
	2	175.2 + 17.5	165.5 + 18.2
Lemon acid	1	147.2 + 10.6	19.4 + 5.11
	2	310.7 + 21.1	223.8 + 21.9
Oxalic acid	1	188.5 + 13.8	34.5 + 5.17
	2	367.5 + 15.9	332.9 + 20.5
Succinic acid	1	99.4 + 11.8	85.6 + 6.88

Table 3

Removal (solibilization) of phosphorus from phosphate rock in 0.01M solutions organic acids in the presence of *B. mucilaginosus* polysaccharide (2) and without it (1)

Organic acid Sterile conditions		Phosphorus removal (solibilization), P_2O_5 mg/l	
		Non-sterile conditions	
Ascorbic acid	1	26.8 + 1.14	15.1 + 0.65
	2	115.4 + 17.5	120.5 + 8.24
Lemon acid	1	37.5 + 2.62	17.6 + 1.15
	2	185.9 + 12.5	185.6 + 11.4
Oxalic acid	1	55.2 + 1.84	50.5 + 5.07
	2	226.5 + 14.9	224.3 + 20.5
Succinic acid	1	42.6 + 2.18	40.3 + 2.88

4.96 times. Bacterial polysaccharide enhances decomposition of phosphorus compounds in solutions of ascorbic and oxalic acids to a lesser extent than in a solution of citric: fused magnesium phosphate by 1.50 times, phosphate rock — 4.31 and 4.1 times, respectively. However, during temophosphate decomposition, the opposite pattern is observed — in oxalic acid solution, the polysaccharide enhances the mineral decomposition by 1.95 times, and in ascorbic acid solution — by 1.34 times. To a minimal extent, the polysaccharide enhances the decomposition of the studied phosphorus-containing compounds in succinic acid solution.

Conducting an experiment on the minerals destruction in organic acids solutions in sterile conditions enables to find out in what way the structure of an acid molecule affects the degree of phosphate compounds decomposition. However, in soils, secondary microorganisms' metabolites experience the effects of the microbiota sides existing in soil, in particular, they can be consumed as sources of carbon and energy. Therefore, we delivered an experiment on the dissolution of minerals in non-sterile conditions I. As a result, it was found that the efficiency of minerals dissolution in aqueous solutions of organic acids under non-sterile conditions decreased compared to sterile conditions (Table 1–3). In particular, in the ascorbic acid and citric acids, the removal of phosphorus became minimal, especially when dissolving thermal phosphate and phosphate flour. This is probably related to the fact that citric and ascorbic acids to a greater extent are consumed by micro-organisms in comparison with oxalic and succinic acid [9]. In the smallest degree, succinic acid was consumed, therefore, the removal of phosphorus in its aqueous solution remained practically at the level of the experiment carried out under sterile conditions (Tables 1–3.). The consumption of organic acids by natural waters microbiota, soils is described in the literature [10,11], while it is noted that acids (acetic, alkaline, succinic) are consumed by soil micro flora with different intensities, and that the zinc content (leachable element) in percolate is closely correlated with the acids content. H.V. Hue [12] also notes that when conducting vegetation experiments, protocatechuic acid turned out to be the most effective in increasing the availability of phosphorus to plants as the most resistant to microbial destruction from the studied acids (acetic, malic acid, etc.).

The effectiveness of organic acids in aqueous solutions under non-sterile conditions decreased significantly after 18 hours of incubation. The solubilizing ability of acids decreased to the maximum when dissolving thermophosphate: ascorbic acid — 12.1 times, citric acid — 7.59, oxalic — 5.46, succinic — 1.16 times (Table 2). The dissolution of phosphate rock decreased minimally under non-sterile conditions: in ascorbic acid — 1.77 times, citric acid — 2.13, oxalic — 1.09, succinic — 1.06 times (Table 3). The removal of phosphorus from fused magnesium phosphate (Table 1) decreased under non-sterile conditions in a solution of ascorbic acid — 3.3 times, citric — 3.15, oxalic — 2.82, succinic — 1.69 times.

The effectiveness of the organic acids action in the presence of bakterial polysaccharide in non-sterile conditions turned out to be identical to that in sterile conditions (Table 1–3). The removal of phosphorus in polysaccharide solutions under non-sterile conditions did not decrease such significantly as in water. The reduction amount in phosphorus removal compared with sterile conditions was 5–20%. These data indicate that organic acids in the sorbed state on polysaccharides not only destroy minerals more effectively, but are also less accessible for consumption by soil microflora. The control of polysaccharide amount in solutions after the end of the experiment made it possible to draw a conclusion about its preservation for 18 hours by 95%. This is also confirmed by the data of E.I. Andreyuk et al.[13] about the fact that during the first 36 hours of cultivation bacteria do not consume in the same way as bacteria.

For exopolysaccharides, the function of extracellular enzymes action prolonging and antibiotics is known. [14]. Extracellular polysaccharides of some microorganisms are able to protect the producers exoenzymes from proteolytic degradation and other external influences. There is reason to believe that such an ability has a universal character. For example, the exopolysaccharide Arthrobacter citreus stimulated the activity of exoproteases Nocardia sp. [15, 16], and the extracellular glycan Mycobacterium lacticolum 121 doubled the thermal stability of the alkaline protein basics, derived from *Actinomyces* sp. [17]. The mechanism of the exglycans protektor action is apparently in the formation of a stable glycoprotein complex.

Preservation of exoenzymes activity is probably a special case of the universal protective function of bacterial polysaccharides. The second manifestation of it, as we believe, is protection from the consumption of low-molecular metabolites and ions absorbed by the polysaccharide during vegetative cell growth. Thanks to the synthesis of exopolysaccharide, microorganisms, as it were, create their own microenvironment around cells, ensuring more complete manifestation of trophic and functional capabilities.

Obviously, the ecological role of microorganisms synthesizing organic acids in the dissolution of hard-to-reach phosphates is limited by the susceptibility of these acids to their consumption by soil microorganisms at a fairly high rate. These are microorganisms with sufficiently high speed. Microorganisms synthesizing exopolysaccharides simultaneously with organic acids represent not more active (2–5 times) higher complex, but less protective — molecular secondary metabodies from the rapidly consumed soil microbiota consequently the absorbed state is less accessible.

Conclusions

The effectiveness of the organic acids action in aqueous solutions depends on the chemical properties of their molecules and the structural features of the dissolved compounds. The greatest dissolving effect on the studied phosphorus compounds has an oxalic acid, then — citric and ascorbic acids

Bacterial polysaccharide accelerates the minerals decomposition compared to acids aqueous solutions, for example, phosphate flour: in a solution of ascorbic acid by 4.3 times, citric acid — 4.96, oxalic acid — 4.1, succinic acid — 1.94 times.

Under nonsterile conditions, the effectiveness of aqueous solutions of organic acids is significantly reduced. For 18 hours of incubation, the solubilizing ability of acids decreases to maximum when dissolving thermal phosphate: ascorbic by 12.1 times, citric — 7.59, oxalic — 5.46, succinic — 1.16 times.

The solubilizing ability of polysaccharide solutions of organic acids in non-sterile conditions decreases slightly — by 5–20%.

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ДЕСТРУКЦІЯ ФОСФОРОВМІСНИХ СПОЛУК У ВОДНИХ І ПОЛІСАХАРИДНИХ РОЗЧИНАХ ОРГАНІЧНИХ КИСЛОТ

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Мета: дослідження закономірностей розчинення (солюбилизації) фосфоровмісних мінералів у водних і полісахаридних розчинах органічних кислот з метою моделювання механізму деструкції мінералів ґрунтовими бактеріями, які синтезують органічні кислоти і екзополісахариди.

Методи: модельний, лабораторно-аналітичний, мікробіологічний, статистичний.

Результати: Розчинювальна дія органічних кислот на мінерали проявляється як у водному, так і полісахаридному розчині. Внесення бактеріального полісахариду у водний розчин кислот посилює розкладання фосфоровмісних мінералів у 1,34–4,96 раза. Вплив хімічної будови молекул кислот на інтенсивність розчинення мінералів проявляється, в основному, в присутності бактеріального полісахариду. У водному ж розчині ефективність дії кислот залежить від природи мінералу, що деструктується. В максимальному ступені полісахарид інтенсифікує розкладання мінералів у розчині лимонної кислоти: плавлений магнієвий фосфат – у 2,55 раза, термофосфат — в 2,11 раза, фосфоритне борошно — в 4,96 раза. Розкладання фосфорних сполук в розчинах аскорбінової та щавлевої кислот бактеріальний полісахарид інтенсифікує у меншому ступені, ніж у розчині лимонної кислоти.

Моделювання розкладання фосфоровмісних мінералів у нестерильних умовах (умови ґрунту) дозволило встановити, що органічні кислоти у нестерильних умовах споживаються ґрунтовою мікробіотою, особливо аскорбінова і лимонна, у меншому ступені — бурштинна кислота. Водні розчини органічних кислот через 18 годин інкубування у нестерильних умовах втрачають деструктувальну активність у 1,06–12,1 раза. Внесення полісахариду *Bacillus mucilaginosus* у водні розчини кислот дозволяє запобігти швидкому споживанню їх мікроорганізмами, в результаті чого ефективність розкладання мінералів у нестерильних умовах зменшується порівняно зі стерильними лише на 5–20%.

Висновки. Внесення бактеріального полісахариду до розчину органічних кислот дозволяє перевести їх у сорбований стан, у результаті чого схильність кислот до споживання мікроорганізмами різко зменшується. Таким чином, бактерії, що синтезують екзополісахариди, не тільки більш інтенсивно деструктують мінерали, ніж мікроорганізми, що синтезують тільки низькомолекулярні вторинні метаболіти, але й синтезують більш стійкий і довго функціонувальний у ґрунті комплекс метаболітів, що розкладають мінерали.

Ключові слова: фосфор; бактерії; органічна кислота; полісахарид; моделювання; розкладання, механізм.