# Phosphate recovery and fertilizer production from wastewater using iron-reducing bacteria

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## Abstract

**Introduction.** Food production is based on the use of fertilizers, in particular those containing phosphorus. Recovery of phosphorus from wastewater can prevent eutrophication of water bodies, meanwhile the removed phosphate can be used as a fertilizer.

**Materials and methods**. Wastewater containing phosphorous, iron ore, iron reducing bacteria are the main components of the proposed technology for phosphorous recovery. Microscopic observations were done using laser scanning confocal microscopy. Obtained fertilizer was tested for plant growth.

**Results and discussion**. An effective biological method for removal and recovery of phosphate from phosphate containing liquid wastes using precipitation of phosphate by microbially-produced ferrous ions was proposed. Performance of this biotechnology on the example of reject water (liquid fraction of anaerobic sludge) from municipal wastewater treatment plant was shown. This biotechnology is based on the application of iron ore treated by iron-reducing bacteria *Stenotrophomonas maltophilia* strain BK. Dissolved ferrous are produced from solid iron ore using iron-reducing bacteria and precipitate phosphate present in wastewater.

Phosphate-iron precipitate was assessed as a phosphorus fertilizer by addition of it to sandy soil poor with nutrients using for cultivation of three plants, namely *Lycopersicum esculantum* L. (tomato), *Casicum annuum* L. (pepper) and *Ipomoea aquatica* (water spinach). Addition of phosphorous precipitate to soil increased dry weight of stems and leaves of test plants by up to 5 times in comparison with control.

**Conclusion.** The biotechnology of anaerobic removal and recovery of phosphate from reject water of municipal wastewater treatment plants using iron-reducing bacteria *Stenotrophomonas maltophilia* strain BK and iron ore as a source of ferric has been developed. Produced phosphate – iron precipitate could be used as a phosphorus fertilizer.

## Introduction

This is due to the fact that a large part of soil phosphorus is adsorbed onto mineral particles or is present in form of slightly soluble salts and also is removed from soil because of water erosion (Alewell et al., 2020; Günther et al., 2018). Thus, not much soil phosphorus can be used by the plants for their growth. Phosphorus for agriculture is obtained from phosphate rock, which is a non-renewable resource and, according to preliminary calculations, its world reserves can be depleted in 50–100 years (Cordell et al., 2009). Phosphorus has even been called a "disappearing nutrient" (Gilbert, 2009). Therefore, recovery of phosphorus from various wastes in order to use it later as a fertilizer is a significant contribution to the problem of phosphorus shortage for agriculture.

From the other site, concentration of total phosphorus above 0.5 mg/l in freshwater systems causes their eutrophication. Growth and decay of phototrophic biomass lead to decrease of dissolved oxygen concentration in natural water that kills aquatic life and decreases quality of drinking water. According to European legislation, the concentration of phosphorus in purified municipal wastewater (discharged effluent from Municipal Wastewater Treatment Plant, MWWTP) should be below 0.5–1.0 mg/l (Sunner, 2017). According to the standards of United States Environmental Protection Agency (US EPA) the content of total phosphorus in streams that discharge directly into water systems should be less than 0.05 mg/l (US EPA, 1986). Meanwhile, concentration of phosphorus in municipal wastewater varied from 5 to 20 mg/l and only 30% of this concentration is removed by secondary treatment (Cieślik and Konieczka, 2017).

Concentration of total phosphorus in reject water (liquid fraction of anaerobic sludge) in MWWTP is in the range from 50 to 130 mg/l (Pitman, 1999). So, phosphorus, which is present in reject water, is from 10 to 50% of total phosphorus load to the aeration tank (Van Loosdrecht and Salem, 2006). Removal of phosphate from reject water can significantly decrease the phosphorus load on the aeration tanks of MWWTP, and helps to prevent eutrophication of natural water bodies. Besides that, removed phosphate probably can be used as a fertilizer in agriculture.

Ferrous chloride and ferrous sulphate are conventionally used for chemical removal of phosphate from wastewater (Günther et al., 2018). The main disadvantage of this chemical precipitation of phosphate is the high cost of iron salts. Application of iron-reducing bacteria (IRB) for reduction of Fe(III), which is supplying to reject water as a cheap iron ore mining waste, produces dissolved Fe(II), which will precipitate phosphate. This technology can be a new method for phosphate removal and recovery (Ivanov et al., 2005; 2006; Korniienko et al., 2023; Stabnikov et al., 2004; Stabnikova et al., 2023; Tay et al., 2008). Altogether, obtained precipitate contains phosphorus, which is a non-renewable nutrient needed for plant growth.

The aim of the present study was to propose technological scheme for anaerobic removal and recovery of phosphate from reject water of MWWTP based on application of iron-reducing bacteria at the presence of iron ore and check the possibility to use of phosphate-iron precipitate as a fertilizer.

## Materials and methods

### Microscopic observations

The interaction of iron-reducing bacteria cells with $Fe(OH)_3$ particles was observed using a confocal laser scanning microscope Fluoview300 (Olympus, Japan). The combination of SYTO 9TM nucleic acid stain and propidium iodide was used according to the manufacturer's instructions for the LIVE/DEAD BacLightTM dye kit (Molecular Probes, Eugene, OR, USA). This kit is used to detect live and dead bacterial cells due to the ability of propidium iodide to selectively penetrate dead cells (Boulos et al., 1999). The green fluorescence of SYTO 9TM dye and the red fluorescence of propidium iodide were induced by a 10 mW argon laser at 488 nm, separated into two channels at 570 nm with a filter, and determined in channel 1 with a filter that transmitted light 1 with a filter that transmitted light from a wavelength of 580 nm to 640 nm.

**Microbiological analysis**

A 24-h direct plating method for fecal coliform enumeration at 37±1 °C for 21±3 h using Coliforms Chromogenic Agar was performed to assess the possibility of phosphorus-iron precipitate application as a fertilizer.

**Plant cultivation**

Tomatoes (*Lycopersicum esculantum* L.), chili peppers (*Casicum annuum* L.), and water spinach (*Ipomoea aquatica*) were selected to study the effect of phosphate-iron precipitate (PIP) obtained from wastewater on the growth of plants in ceramic pots (23 cm diameter, 18 cm high), each of which contained 4 kg of nutrient-poor soil with a moisture content of 14%. As controls, only soil without impurities (C1), soil with the addition of nitrogen fertilizer (C2), and soil with the addition of phosphate fertilizer (C3) were used. Ammonium sulfate was used as a nitrogen fertilizer in the amount of 0.46 g per kg of soil (0.098 g of nitrogen), which corresponded to the recommended rate of its use for nitrogen-poor soils (Roman et al., 1990). As a phosphate fertilizer, $KH_2PO_4$ was used, which was set in an amount corresponding to the amount of phosphorus in the experiment using obtained phosphate-iron precipitate. In the experiment, iron phosphate precipitate was added in an amount that corresponded to 0.0475 g P per 1 kg of soil and ammonium sulfate in the same amount as in C2. All chemicals were applied to the soil, thoroughly mixed and left for a week before making 10 seeds in each pot. After three weeks, 5 strong seedlings were left, which were grown for 8 weeks. At the end of cultivation, all plants were carefully removed from the soil and the roots were washed with distilled water. The length of the ground part of the plants and the root system was measured. The dry weight of the aerial part of the plant and roots was determined after drying at 60 °C for 48 hours.

**Statistics**

Experiments were carried out in triplicates. Statistical processing of the experimental results was carried out using special computer programs for personal computers.

Data on the effect of iron phosphate on plant growth were analysed by ANOVA and Tukey statistical procedures using the Statistical Package for the Social Sciences (SPSS) version 12.0 program (SPSS Inc, Chicago, Illinois).

## Results and discussion

### Production of phosphate fertilizer

The main processes for removing phosphate from any phosphorus-containing liquid waste using iron-reducing bacteria are: (a) anoxic bacterial reduction of $Fe^{3+}$ from a cheap natural mineral such as iron ore and converting it into a soluble form $Fe^{2+}$; (b) precipitation of phosphate due the formation of an insoluble phosphate-iron precipitate:

$$4Fe_2O_3 + CH_3COO^- + 7H_2O \longrightarrow 8Fe^{2+} + 2HCO^- + 15OH^- \quad (1)$$

$$Fe^{2+} + HPO_4^- \longrightarrow FeHPO_4 \quad (2)$$

Ferrous phosphate could be further chemically or biologically oxidized by oxygen:

$$FeHPO_4 + HPO_4^{2-} + 0.5O_2 + H_2O \longrightarrow Fe_2(HPO_4)_3 + 2OH^- \quad (3)$$

The process of phosphorus recovery from phosphorus-containing wastewater, including reject water from municipal water treatment plants, using the reduction of iron from cheap iron ore sources by iron-reducing bacteria, has been studied and described in works (Ivanov et al., 2005; 2007; 2014; Stabnikov and Ivanov, 2004), as well as in a US patent (Tay J. H., Tay S. T. L., Ivanov V., Stabnikova O., Wang J.Y. US Patent 7 393452, Compositions and methods for the treatment of wastewater and other waste). The principal scheme of phosphate recovery and fertilizer production from reject water of the wastewater treatment plant using iron-reducing bacteria is shown in Fig. 1.

After mechanical treatment, wastewater enters the aeration tank A1. Wastewater after aerobic treatment in A1 is supplied by the pump P2 in the settling tank S3. A portion of activated sludge is returning to the aeration tank A1, but the excessive activated sludge is supplied by the pump P4 to the anaerobic reactor AR5. Treated wastewater after the aeration tank is discharged into aquatic systems. Biogas, which is produced during anaerobic digestion of activated sludge in the reactor AR5, can be used for heating and/or electricity generation. Anaerobic sludge after sedimentation in the settling tank S7 and dewatering is disposed of, for example as concrete from the mixture of cement and sewage sludge. Reject water with phosphate concentration from 100 to 200 mg/L is supplied to the reactor R8 for its precipitation by Fe(II). Part of the reject water is supplied for production of ferrous solution in the reactor R16. Settling of phosphate-iron precipitate (PIP) is going in the settling tank S9. Purified liquid from the settling tank S9 is returned to the aeration tank A1 for further treatment, but the precipitate in the form of suspension with the moisture content 90% is dewatered on the centrifuge C10 to the final moisture content 25%. PIP is drying on the belt dryer BD11 to the moisture content from 5 to10%. Hot air with a temperature 170°C is used as a heating agent. Final product enters the loading pocket LD12 and through screw conveyor SC13 is going for the packing to packing machine PM14.

Anaerobic cultivation of iron-reducing bacteria is going at temperature 28±2°C in the sealed reactor R15, which is filled by 90% medium (NM) with the following composition, g/l: $NaHCO_3$, 2.5; $NH_4Cl$, 1.5; $NaH_2PO_4$, 0.6; KCl, 0.1; yeasts extract 0.5; ferric citrate, $C_6H_8O_7Fe$, 5.0; ethanol, 1.0; tap water to 1 l; pH 7.0. Nitrogen gas is supplied into the reactor R14 to ensure anaerobic conditions. Inoculum (I) is preparing in the laboratory by anaerobic cultivation of IRB *Stenotrophomonas maltophilia* strain BK (Ivanov et al., 2005) on the solid medium (solidified reject water ) and then by the submerged cultivation in the liquid medium with the composition described above.

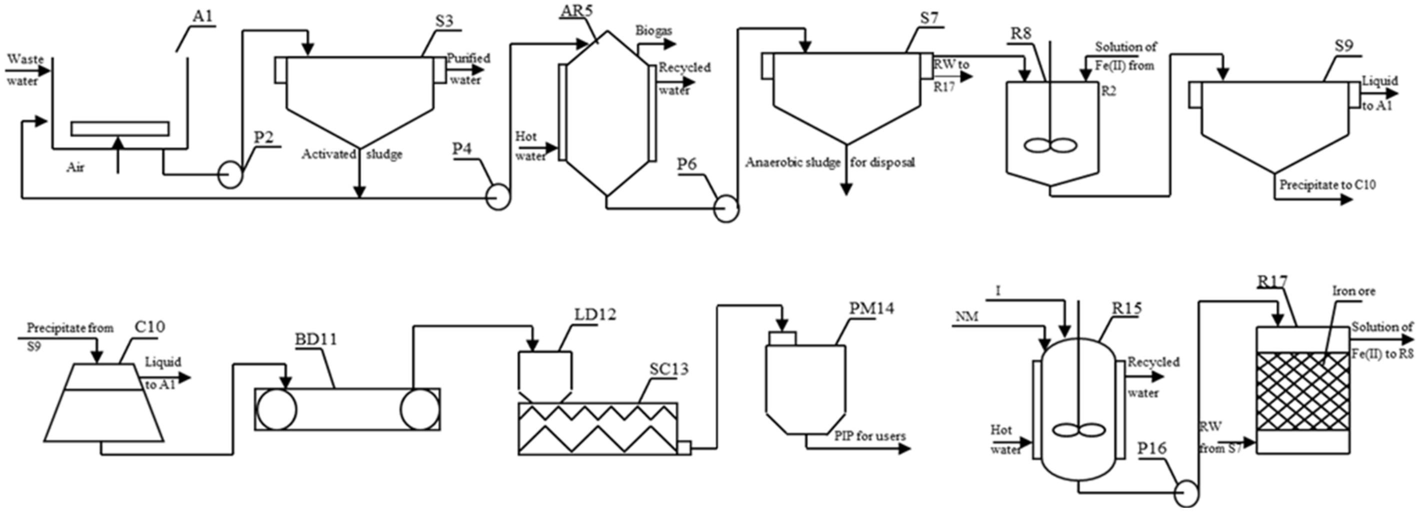


**Figure 1. The scheme of phosphate recovery and fertilizer production from reject water of wastewater treatment plant using iron-reducing bacteria:**
A1, aeration tank;
P2, P4, P6, P16, pumps;
S3, S7, S9, settling tanks;
AR5, anaerobic reactor;
R8, R14, R15, R17, reactors;
C10, centrifuge;
BD11, belt dryer;
LP12, loading pocket;
SC13, screw conveyor;
PM14, packing machine;
I, inoculum;
NM, nutrient medium.

*S. maltophilia* strain BK is Gram-negative, rod-shaped, non-spore forming bacteria, facultative aerobe. *S. maltophilia* strain BK is able to reduce Fe(III) using m-cresol, 2,4-dichlorphenol, p-phenylphenol and diphenylamine, a third by importance compound in the European Union list of priority pollutants, as a sole source of carbon under anaerobic conditions. This property of strain BK can be used in the anaerobic (anoxic) treatment of wastewater or reject water polluted with xenobiotics. Image of iron-reducing bacteria on iron hydroxide particles obtained using a confocal laser scanning microscope is shown in Fig. 2.

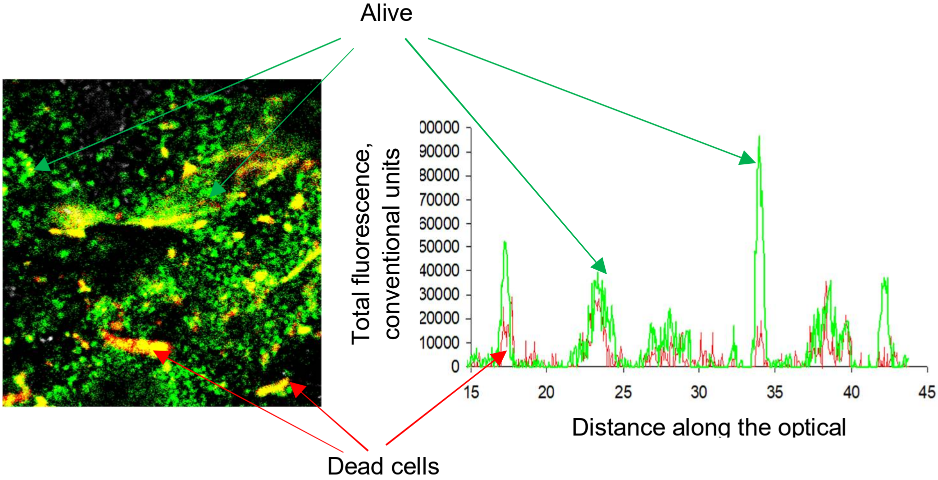


**Figure 2. Image of iron-reducing bacteria on iron hydroxide particles (image obtained using a confocal laser scanning microscope):**
a – cells were stained with nucleic acid dye SYTO 9TM (green fluorescence) and propidium iodide (PI), penetrating only into cells with a broken membrane.
Dead cells are yellow due to a combination of green and red fluorescence;
b – fluorescence indices of SYTO 9 (upper curve) and PI (lower curve) in the optical section of the image.

Suspension of IRB biomass is supplied to the reactor R15 for initiation of ferric reduction from iron ore particles with size less than 4.75 mm and the content of Fe(III) higher than 65%, which is periodically loaded in the reactor R15. Further reduction of Fe(III) is performed by IRB present in the reactor R16. Reject water is continuously supplied to the reactor R17 from the settling tank S7.

Reduction of iron from iron ore leads to production of Fe(II) solution, which is used for phosphate precipitation in the form of phosphate iron precipitate, PIP. PIP after dewatering and drying can be used as a phosphorus fertilizer in agriculture.

**Assessment of biological safety of use in the cultivation of agricultural plants**

An important aspect of the use of wastewater treatment products in agriculture is to check its safety, in particular, the presence of pathogenic microorganisms. The United States Standard for the use of anaerobically treated activated sludge (biological solid waste) includes allowable limits for faecal coliforms (US EPA, 1999). An important aspect of the use of wastewater treatment products in agriculture is to check its safety, in particular, the presence of pathogenic microorganisms. The United States Standard for the Use of Anaerobically Treated Activated Sludge (Biological Solid Waste) includes allowable limits for fecal coliforms. According to the content of faecal coliforms, biological solid waste is divided into two classes: class B with a faecal coliforms content of less than $2\times10^6$ cells per gram of dry matter and class A with a faecal coliforms content of less than $1\times10^3$ cells per gram of dry matter. Unlike class B biological solid waste, class A waste has virtually no restrictions regarding the place of application and the regarding the place of use and the nature of crops.

The concentration of faecal coliforms was $(4.0\pm0.6)x10^6$ cells/ml in suspensions of anaerobic sludge (activated sludge after anaerobic treatment) with a solids concentration of 5.5%; $(3.1\pm0.8)x10^1$ cells/ml in the liquid fraction of anaerobic sludge; $(1.8\pm0.2)x10^2$ cells per 1 g of dry matter in phosphorus-iron sediment with moisture content of 75%. The growth of colonies of faecal coliforms was not observed in the analysis of the dried phosphorus-iron sediment, which makes it possible to consider the possibility of its use as a fertilizer.

## Application of phosphate iron precipitate as fertilizer for plant cultivation

The chemico-physical characteristics of the sandy soil used in the experiment are given in Table 1.

**Table 1**

**Chemico-physical characteristics of soil**

| Parameters | Soil | Parameters | Soil |
|---|---|---|---|
| pH | 6.4±0.1 | N, % from dry matter | 0.29±0.02 |
| Organic substances, % from dry matter | 4.8±0.2 | P, % from dry matter | 0.00±0.00 |
| Density, g/cm$^3$ | 1.26±0.11 | K, % from dry matter | 0.16±0.01 |

Sandy loam soil poor by nutrients was used as control (C). Plants were grown in soil with addition only a nitrogen fertilizer (C1), in soil with addition only a phosphorus fertilizer (C2) and in soil with addition of ammonium sulphate as a nitrogen fertilizer and phosphate iron precipitate as a phosphorus fertilizer in ratio N: P = 2:1 (E). Quality of phosphate iron precipitate as the phosphorus fertilizer was tested using cultivation of three plants: *Lycopersicum esculantum L.* (tomato), *Casicum annuum L.* (pepper) and *Ipomoea aquatica* (water spinach). Results are shown in Table 2.

Data were analysed using one-way ANOVA and Tukey's HSD tests by the SPSS statistical package. The length and dry weight of stems in the experiment was increased significantly: for pepper by 3.1 and 19; for tomato by 4.4 and 40; for water spinach by 2.1 and 5 times in comparison with controls, respectively (Figures 3 and 4).

The length and dry weight of the ground part of the plant (stem) increased when phosphate iron precipitate was added as a phosphorus fertilizer (E) to the soil containing nitrogen fertilizer (C1): pepper by 3.1 and 19; tomatoes by 4.4 and 40; water spinach by 2.1 and 5 times, respectively.

**Table 2**

**Influence of phosphate iron precipitate addition to soil on the plant growth**

| Parameters of plants | C | C1 (with N) | C2 (with P) | E |
|---|---|---|---|---|
| **Pepper** | | | | |
| Length of stem, cm | 6.0±0.4$^{a}$ | 6.1±0.3$^{a}$ | 5.9±0.,4$^{a}$ | 19.0±1.4$^{b}$ |
| Length of root, cm | 4.7±0.8$^{a}$ | 4.7±0.,7$^{a}$ | 4.6±0.8$^{a}$ | 12.6±1.5$^{b}$ |
| Dry weight of stem, g | 0.02±0.00$^{a}$ | 0.02±0.00$^{a}$ | 0.02±0.00$^{a}$ | 0.38±0.14$^{b}$ |
| Dry weight of root, g | 0.01±0.00$^{a}$ | 0.01±0.00$^{a}$ | 0.01±0.00$^{a}$ | 0.08±0.03$^{b}$ |
| **Tomato** | | | | |
| Length of stem, cm | 7.6±0.7$^{a}$ | 7.2±0.4$^{a}$ | 7.0±0.4$^{a}$ | 31.8±2.2$^{b}$ |
| Length of root, cm | 6.0±1.1$^{a}$ | 5.8±0.6$^{a}$ | 5.9±0.4$^{a}$ | 10.4±2.3$^{b}$ |
| Dry weight of stem, g | 0.04±0.0$^{a}$ | 0.04±0.0$^{a}$ | 0.04±0.0$^{a}$ | 1.59±0.37$^{b}$ |
| Dry weight of root, g | 0.05±0.0$^{a}$ | 0.04± 0.0$^{a}$ | 0.05±0.0$^{a}$ | 0.32±0.11$^{b}$ |
| **Water spinach** | | | | |
| Length of stem, cm | 19.2±0.5$^{a}$ | 16.2±0.8$^{b}$ | 11.6±1.3$^{b}$ | 33.6±3.7$^{b}$ |
| Length of root, cm | 15.1±2.0$^{b}$ | 10.4±1.,3$^{a}$ | 7.8±1.1$^{a}$ | 15.3±1.6$^{b}$ |
| Dry weight of stem, g | 0.32±0.03$^{a}$ | 0.23±0.01$^{a}$ | 0.16±0.02$^{a}$ | 1.17±0.27$^{b}$ |
| Dry weight of root, g | 0.15±0.03$^{ab}$ | 0.12±0.02$^{ab}$ | 0.10±0.02$^{a}$ | 0.18±0.03$^{b}$ |

Note: Values in the same line followed by the same letter are not statistically different at $P<0.05$ according to the least significant difference test (calculated using statistical procedures ANOVA and Tukey).

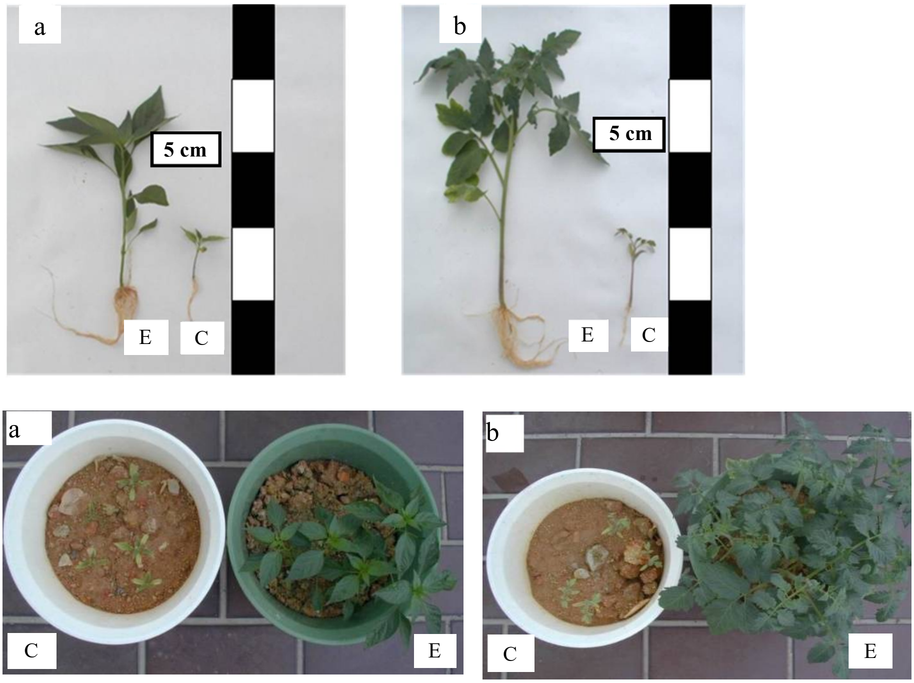


**Figure 3. Growth of pepper (a) and tomatoes (b) in control (C) and in the experiment with phosphate-iron precipitate (E).**

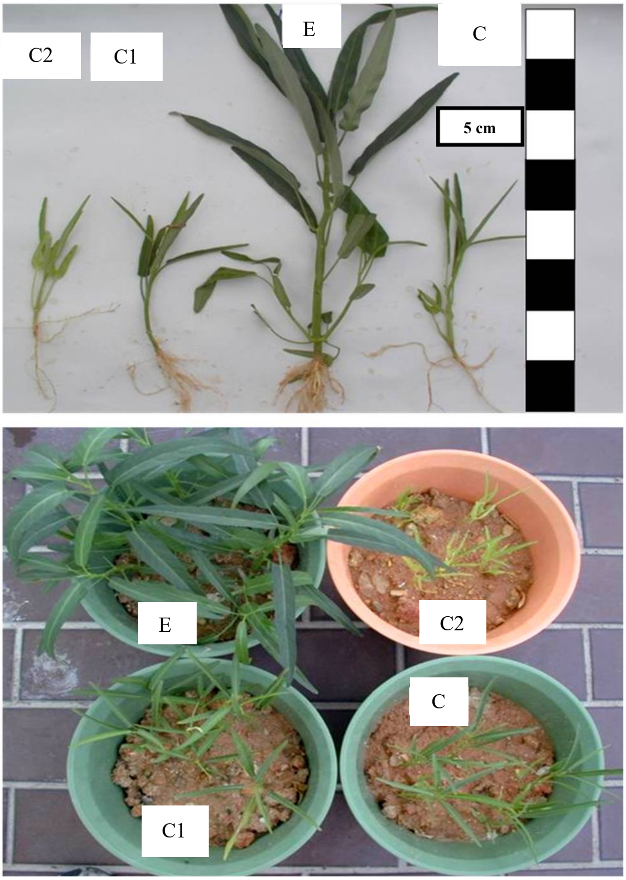


**Figure 4. Growth of water spinach in control (C), in control added with nitrogen fertilizer (C1), in control added with phosphorus fertilizer (C2) and in the experiment with phosphate iron precipitate (E).**

## Conclusions

The biotechnology of anaerobic removal and recovery of phosphate from reject water of municipal wastewater treatment plant using iron-reducing bacteria *Stenotrophomonas maltophilia* strain BK and iron ore mining waste as a source of ferric has been developed. Produced phosphate – iron precipitate could be used as a phosphorus fertilizer. Addition of phosphate iron precipitate in poor by nutrients soil increased the dry weight of stems and leaves of test plants by 4–5 times in comparison with control.

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