SRCAMB Collection Of Phosphate-Solubilizing Microorganisms As A Long-Term Bioresource

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State Research Center for Applied Microbiology & Biotechnology

- *Insulin production plant*
- *Nutrient media production*
- *Diagnostic media*
- *Biochip line*
- *Biopurification technique of oil-products*
- *R&D of cold plasma etc.*

SRCAMB is one of largest Russian research and production complexes, focused on biological and ecological safety, and capable to deal with all kinds of microbes including dangerous biological materials

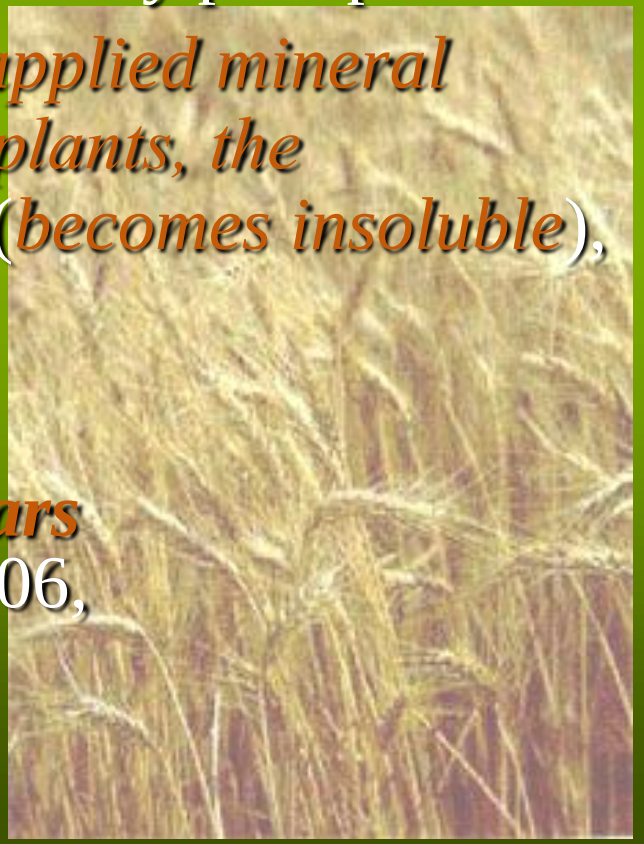
Microbial Phosphate Solubilization (PS) is Alternative Natural Process for P enriching

- ***First mention*** at 1948 (Pikovskaya). Returning interest in last years (regional strains search, mechanisms of activity, regional P source treating, genetics)
- What is the ***PSM*** group?
- ***SRCAMB scientists*** working more 10 years with ***biodiversity***, PS ***mechanism, formulation, application technologies***
- Our expedition search shows most active ***PSM are selecting*** from various niches deficient in nutrients. And many PSM display various useful properties

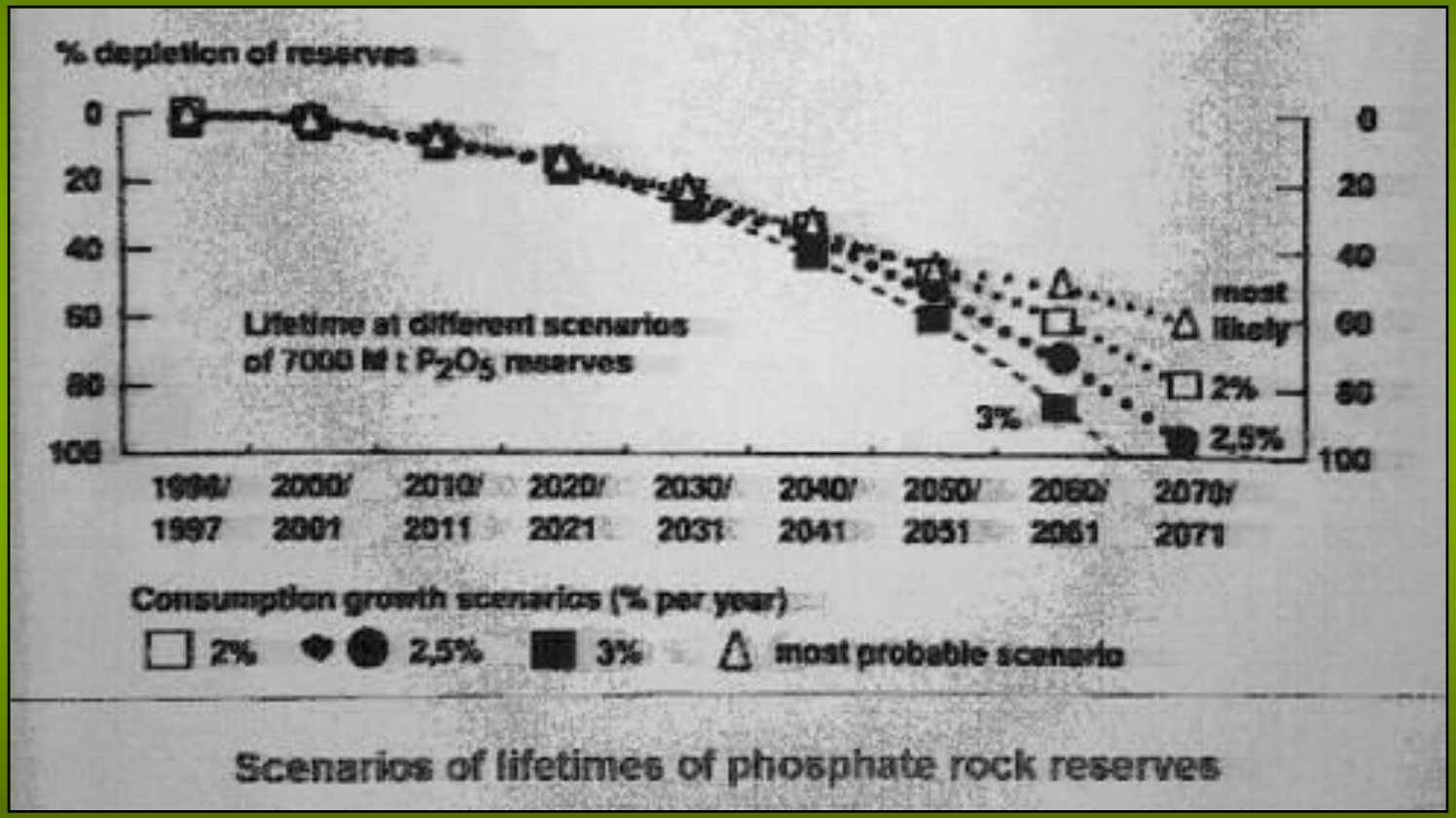
PS halozones around colonies

Challenges of P Industry

- *Phosphorus (P) deficiency in soils* is one of the most critical problems in agriculture. P mineral fertilizer used to correct for deficiencies is limited by availability of mineral phosphate ores and P in soil readily precipitates
- It's well known that ***only 12-25% of applied mineral fertilizer's phosphorous achieves the plants, the remainder*** of the P disappears in soil (*becomes insoluble*), groundwaters
- Recent *estimates of world phosphate reserves indicate only about **50-70 years** supply* for future use (Kirkby et al, 2006, IFS proceedings)
- *P is one of **non - renewable sources***



Forecast for Phosphate Rock Reserves



Scenarios of lifetimes of phosphate rock reserves

Cambridge, Kirkby et al., Dec. 13-15, 2006, 14th IFS annual meeting

Dramatic Impact on Environment of Phosphate Ore Mine and its Enrichment

P Mining

Wastes production

- 85-93% of mined phosphorus is lost during mining/treating/application **!!!**
- Hundreds of mln tones of low-grade ores and wastes

The Landscape View at Far North

PSM Collection at SRCAMB

- SRCAMB PSM collection ***includes more 700 cultures and is one of world largest now.*** Various genera: *Enterobacter*, *Bacillus*, *Azotobacter*, *Acinetobacter*, *Yarrowia*, *Pseudomonas* and others. ***Strict conditions technique*** for strain selection allowed to choose active, stable at environment and growing here
- Russia have ***unique variability of regions and climates*** (from arctic to subtropic) to screen for PS active isolates. Variable ecological niches (caves, minings, old mountains, reserves etc). ***It allows to use PSM for variable agricultural/soil needs and climate conditions***
- Most isolates are from ***expedition samples. 15 long and short term (7 years) expeditions*** to search PSM were carried out

15 Long-Term and Short-Term Expeditions. Work during 7 yrs.










Climates variability:

- *Sub-arctic*
- *Temperate European*
- *Wet subtropic*
- *Dry subtropic*
- *Monsoon*
- *Alpine*
- *Maritime*

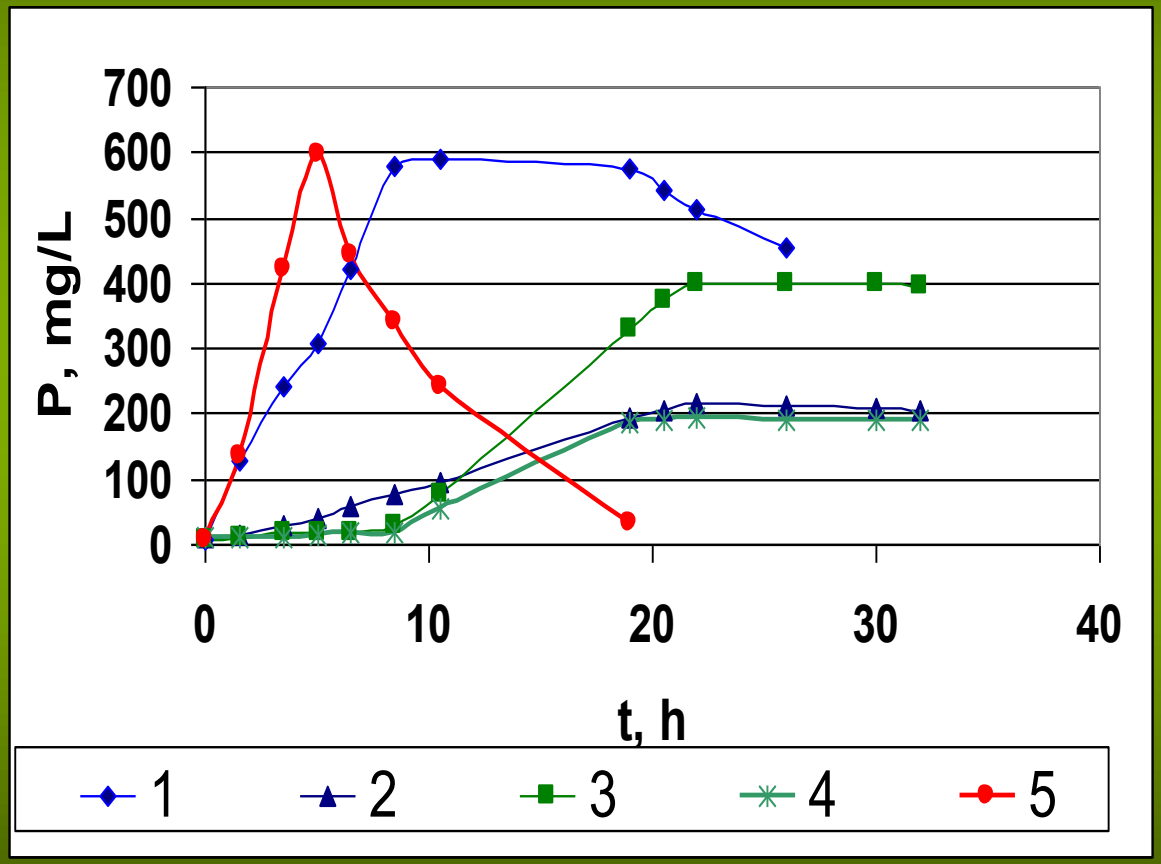
Sampling variability:

- *Ore deposits*
- *Reserves*
- *Volcanoes incl. mud*
- *Estuaries*
- *Salt lakes and reservoirs*
- *Canyons*
- *Mining plants*
- *Cliffs and mountains*
- *Relict caves*
- *Islands*

Aspects of SRCAMB PS strains selection

- *Halozones forming (typical approach) isn't obligatory property of PSM. Moreover, about 1/3 strains was active in PS in liquid media but not formed HZ at agar with TCP. But are strong solubilizers*
- *Being selected in strict conditions PSMs displays high PS efficacy (PSE). Mineral media help to select more stable and cheaper strains*
- *Several PSM are participate in rock granite erosion*
- *Being selected from many unique econiches (deficient in nutrients, strong conditions etc) many PSMs have competitive mechanisms could be applied*

Dynamics of phosphate releasing from $Ca_3(PO_4)_2$ by different microorganisms



1- *Pseudomonas fluorescence* P469;

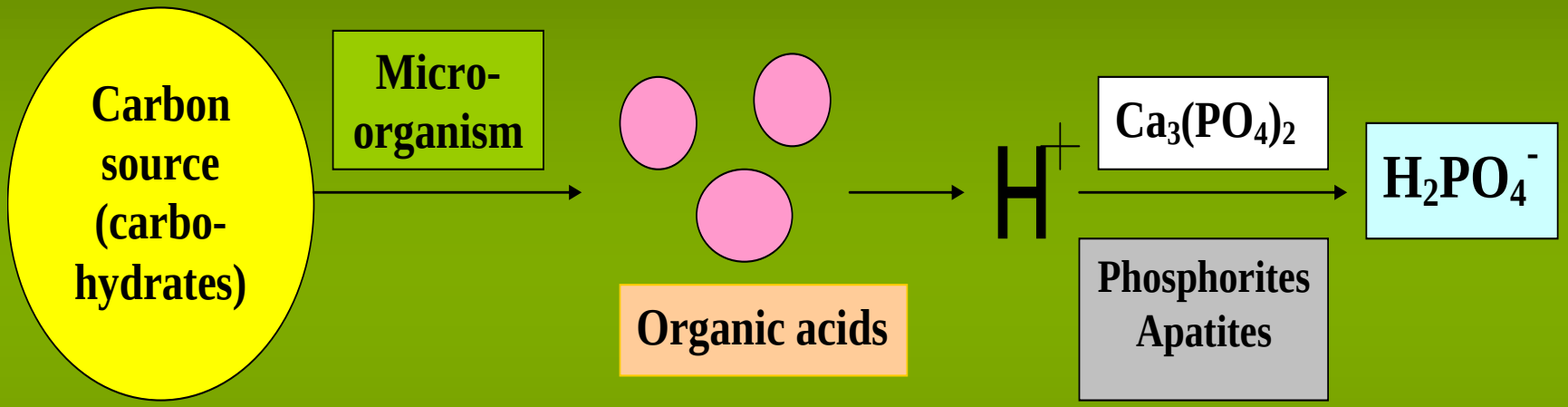
2 –*Bacillus subtilis* IPM 215;

3 - *Trichoderma longibrahiatum* 29;

4 –*T. asperellum* 16- GJS 03-35;

5 - *Burkholderia cepacia* E37

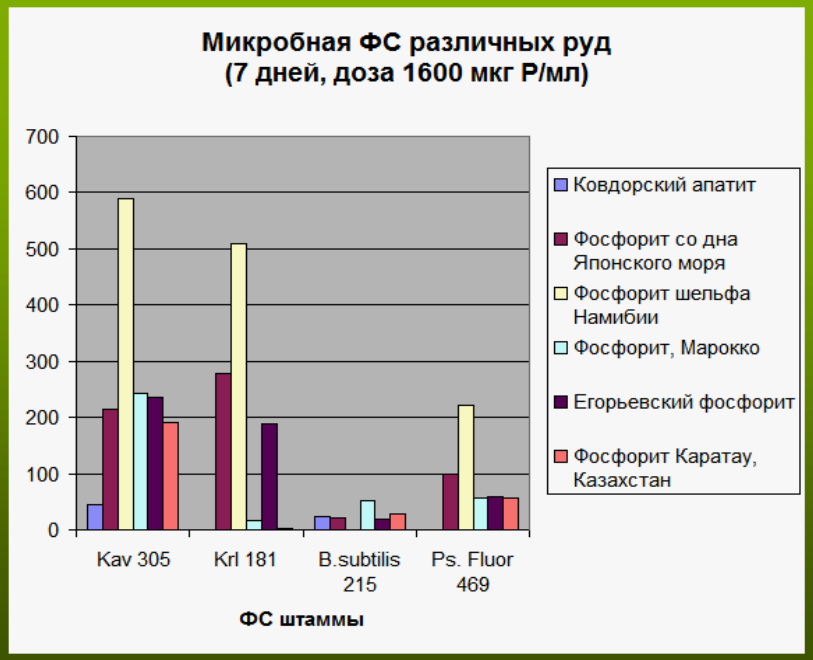
Most common mechanism of PS from mineral raw material by microbes



- *We have registered various organic acids during PS as lactic, acetic, citric, group of gluconic, maleic, oxalic and others stoichiometrically significant metabolites*
- *There are not exact description of full mechanism in PS by microorganisms*

Aspects of P Ores Availability

Khibiny's Apatite



- *Different P ores have extremely various bioavailability (more 10 times differ)*
- *For R&D* ***we have created P world ores Collection (>70 world ores)*** *from European mines, Asia, Far and Middle East, Africa, America, Oceania, shelfs*
- *To develop optimal fertilizer it should be cross-selection with microbes and P ore materials.*

Alternative for Chemical soluble P Fertilizers - Microbial P biofertilizers

- *Biological P fertilizers, based on the activity of phosphate solubilizing microorganisms (PSM), are capable of slowly releasing soluble P directly from insoluble phosphate ore materials,*
- *"green" direction of R&D*
- *Combining of alive cells, carbon source and P material in biogranule – prospective direction*

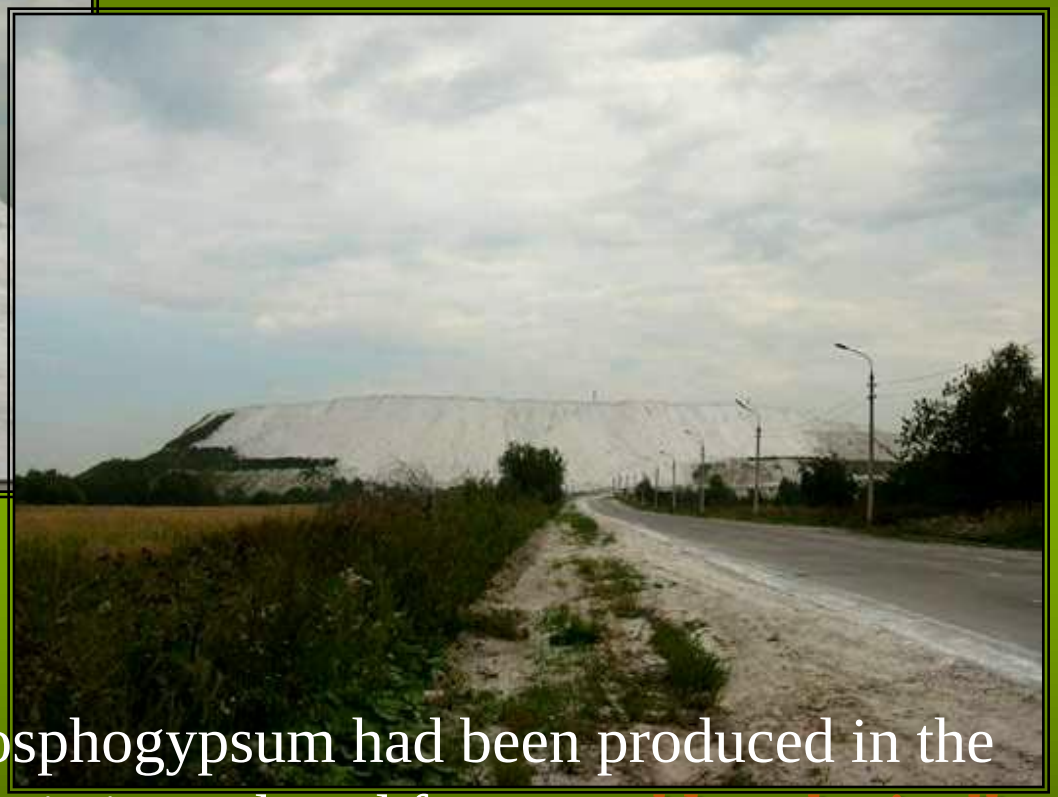
Plant testing of PSM preparations

Control Ore BF Sinergic BF

BF Control

- The effect of PSM biofertilizers (BF) on DW and plant P was 20.3-30.1% more controls
- Synergic microbial consortium makes additionally 13.3-21.2% over PSM effect (plus), so totally 33.6-51.3%

Phosphogypsum as Life Reality

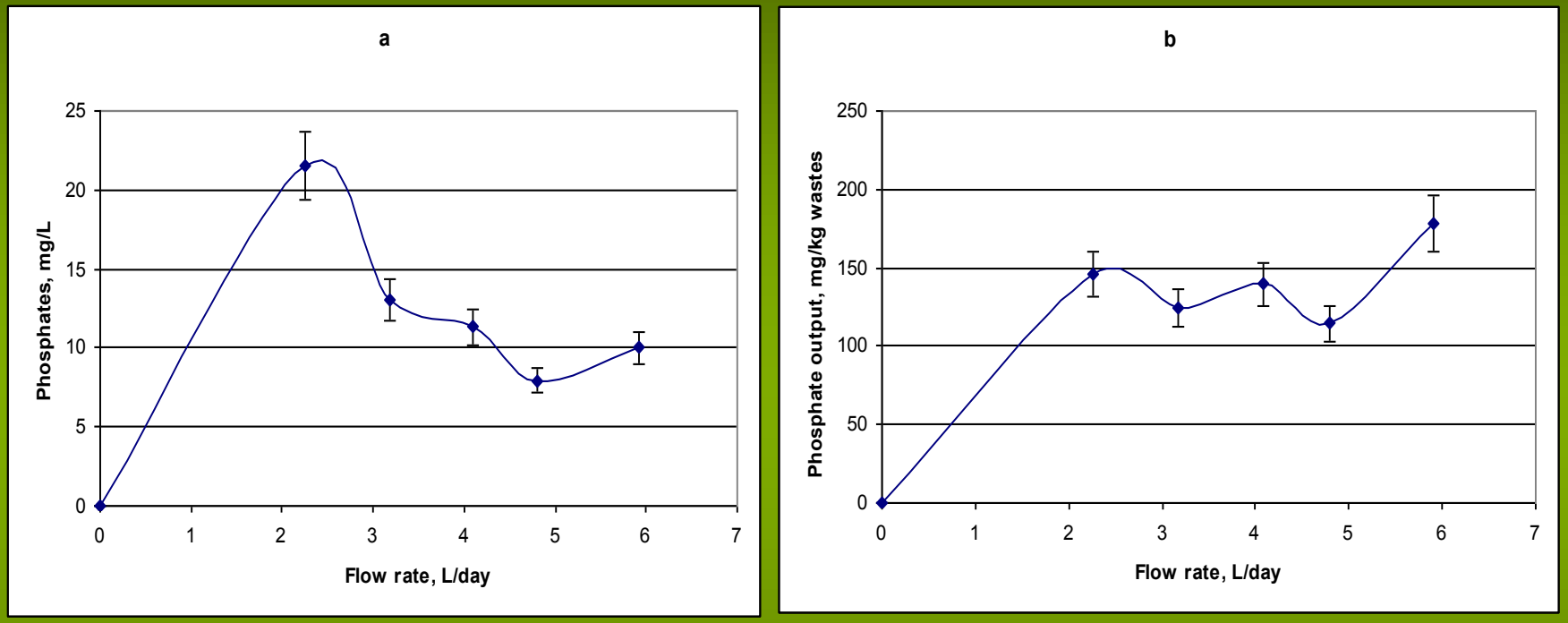


- Hundreds of mln tons of phosphogypsum had been produced in the world P industry. And in Russia it produced from ***world ecologically purest Khibiny apatite ores,*** and in case of remaining phosphates dismissal it could be secondary used for industry

We Have Shown Alternative – to Use PSM in P Extracting and Waste Treating

- *For poor P-containing ore wastes (hundreds of mln tons)*
- *For PhosphoGypsum (n*100 mln tons) and remaining P extracting*
- *For use of low-grade phosphorite mining*

Phosphates Release from P-containing Wastes of Sludge Fields



Treating of P-containing wastes of sludge fields: a – P outlet concentration, b – specific P output

- *At all conditions investigated, the essential phosphate release from phosphate-containing ores and wastes was observed*
- *Microbial treatment was estimated to remove all reminder P from phosphate containing materials during 1-2 warm seasons*

Many our PSM suppress greatly the developing of fungal soil burn pathogens

Fusarium treated wheat growth: Without PSM (left) and with (right) PSM Bacillus sp 26a

Content of mycotoxins (4-deoxynivalenol, DON) in wheat grain (infected by F. graminearum)

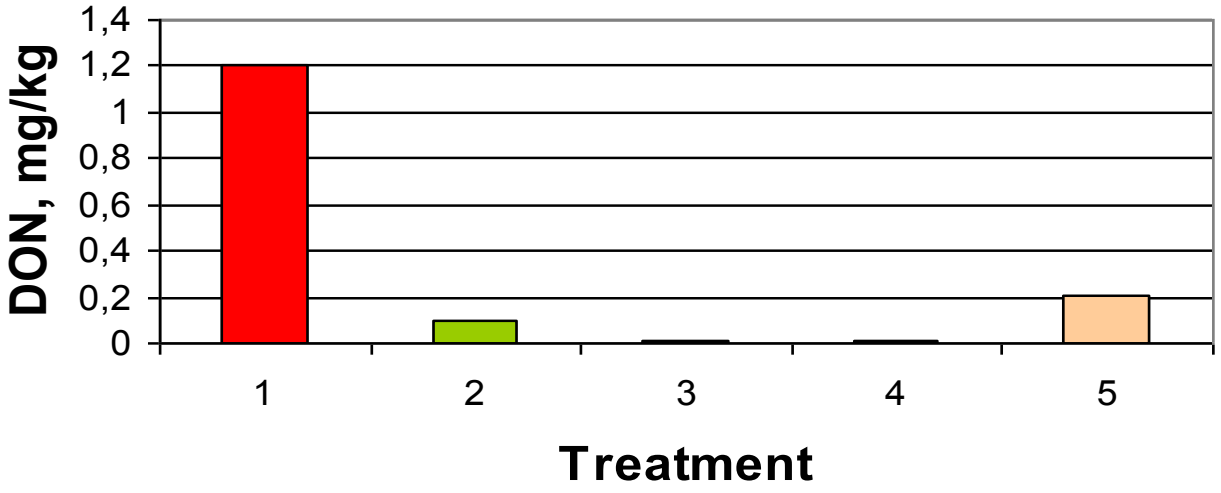
1- infected control

2-*Trichoderma* #16 (seed treatment)

3-*Trichoderma* #16 (seed treatment + spraying)

4 -Agat - chemical fungicide (seed treatment)

5- TMTD - chemical fungicide (seed treatment)



As Producers of Bioactive Substances SRCAMB PSM Collection Have Great Potential for Industrial Application

- *PSM usually produce stoicheometrically max quantity of* ***various organic acids*** *and it could be screened for* ***production of it***
- *From own and other research it could be conclude that many of PSM would be effective* ***producers of enzymes*** *(dehydrogenases, phytases etc.). So, PSM collection is very prospective for screening of enzymes producers*
- *Other possibility of PSM is variable metabolites helping in environmental competition, and we found such activity for several strains. And* ***new bactericides and fungicides*** *are very prospective by PSM collection screening*

As Producers of Bioactive Substances SRCAMB PSM Collection Have Great Potential for Industrial Application (continued)

- ***Soil bounded phosphorus*** *is long-term problem and one of the reason to screen and select PSM for the P mobilizing preparation or technique*
- *PSM usually produce large quantity of biopolymers and it could be screened for* ***production of biopolymers***
- *Biodiversity of PSM in various earth regions and numerous long-term direction of described applications above – are the subjects for world R&D groups in collaboration*

R&D on PS Processes Requires to Join Various Specialists

SRCAMB Team has it

IT'S NEED TO COMBINE:

- General & soil microbiologists
- Expeditioneers, field lab
- Chem. analysts
- Metabolites biochemists
- Fermentation scientists & technologists
- Drying and formulation scientists
- Phosphate ores geochemists
- Agroscientist
- Pot & field trials researcher
- Ecologic biotechnologist

Thanks for your attention!

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