Dr. W.-D. Jülich

Rhamnolipids (strain JRV), microalgae, mineral composites: Roots for the development of coating materials for crop production

A presentation by PURMIN GmbH

Department of Pharmaceutical Biology

Natural products from the fields of

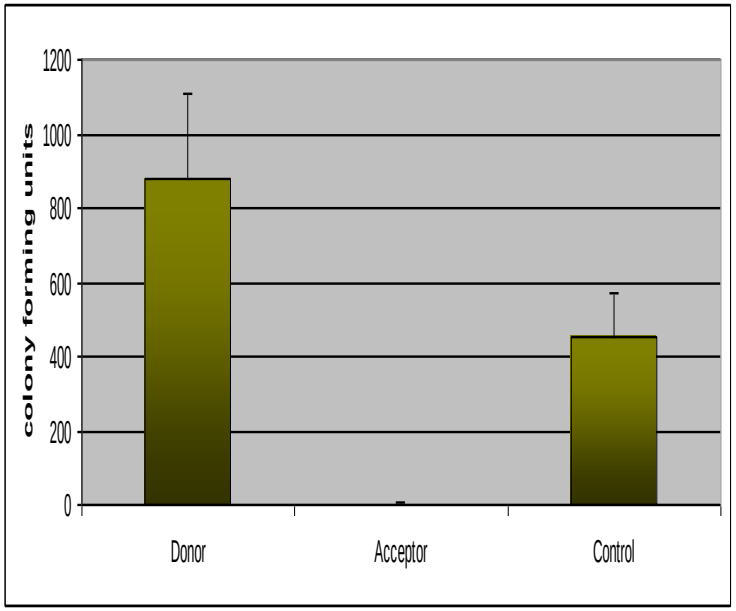
Organisms used ethnomedically

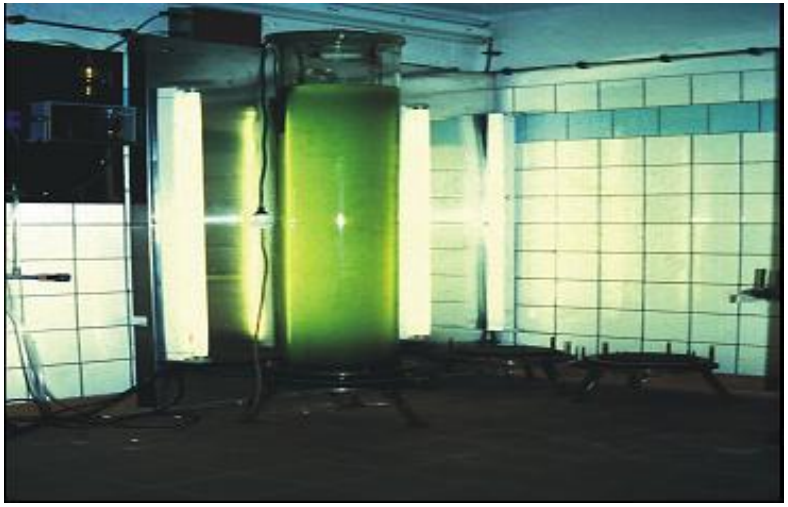
Aquatic organisms

Available for processing

Good analytics

Interesting experimental models for testing biological effects





Because Microalgae Bio 33 is already being used commercially, large-scale industrial production is available

Source: Lukowski G, Lindequist U, Mundt, S, Kramer, A, Jülich WD: Inhibition of Dermal MRSA Colonization by Microalgal Micro- and Nanoparticles, Skin Pharmacol Physiol, 2008; 21: -105

Results of antimycotic testing of Bio 33 extracts

(1.5 mg/paper-disc, specification of the mean values of the inhibition zone diameters)

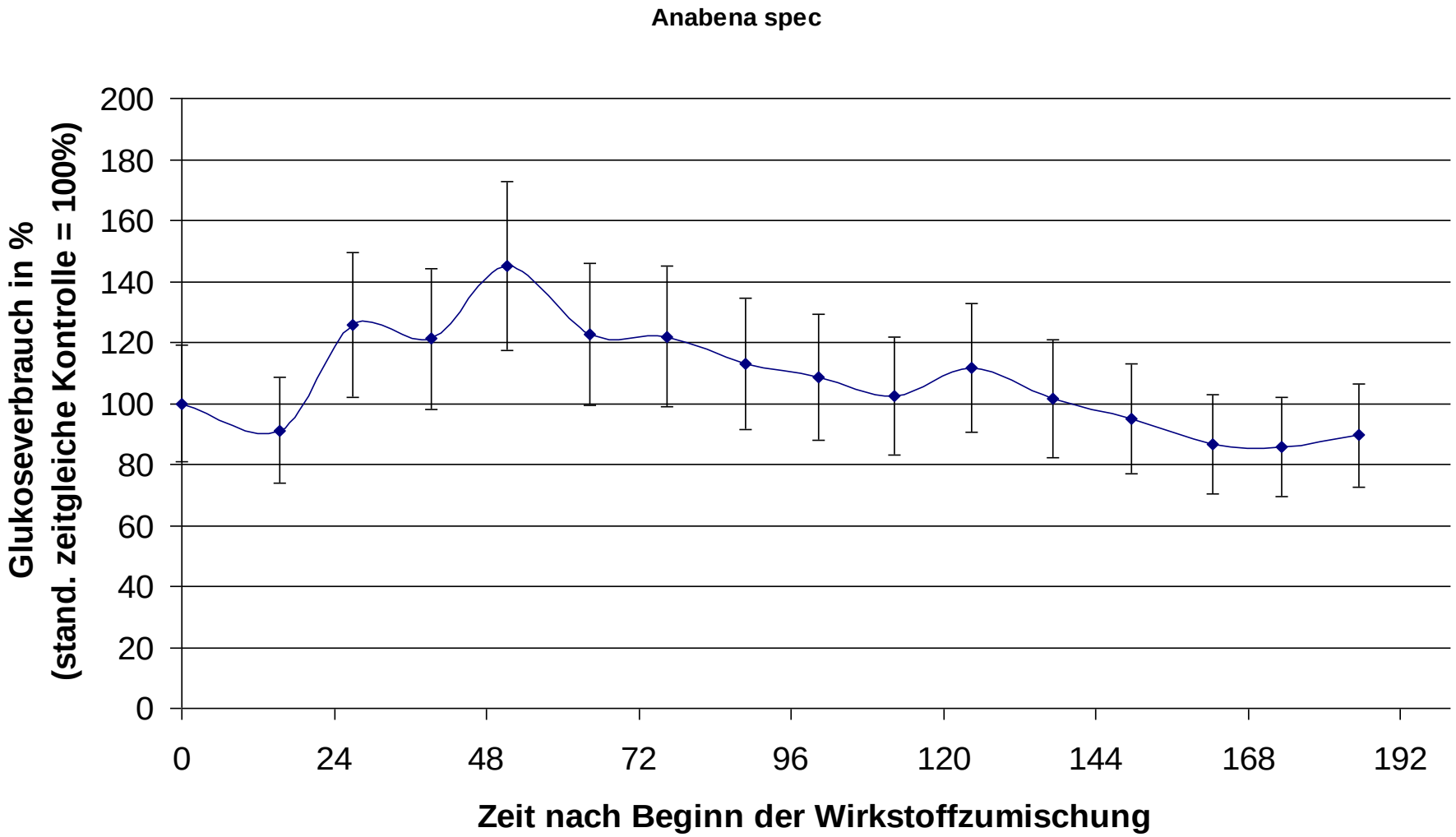
	C. albicans	*C. krusei*	*A. fumigatus*	*Mucor sp.*	*M. gypseum*
Incubation time/temperature	24 hrs/36°C	24 hrs/36°C	48 hrs/36°C	48 hrs/30°C	7 days/25°C
Substance no.	HHD/mm	HHD/mm	HHD/mm	HHD/mm	HHD/mm
Bio 33 Enriched extract	**21** **23**	**21** **20**	**26** **30**	**32** **26**	**N/a** **38**
EtOAc extract	**8**	**17**	**0**	**15**	**N/a**
Nystatin verif.	**23** **24**	**22** **19**	**21** **24**	**25** **25**	**N/a** **20**

Source: S.Mundt, W.-D. Jülich, G. Lukowski, U. Lindequist: Microalgae with locally applicable, antifungal and/or antimycotic action, which simultaneously stimulate the metabolism and the immune system of vertebrate animals, as well as the preparations that are produced from them. PCT/EP 2010/059402

Request by Dr. Taubenrauch
Julius Kühn Institut, Quedlinburg

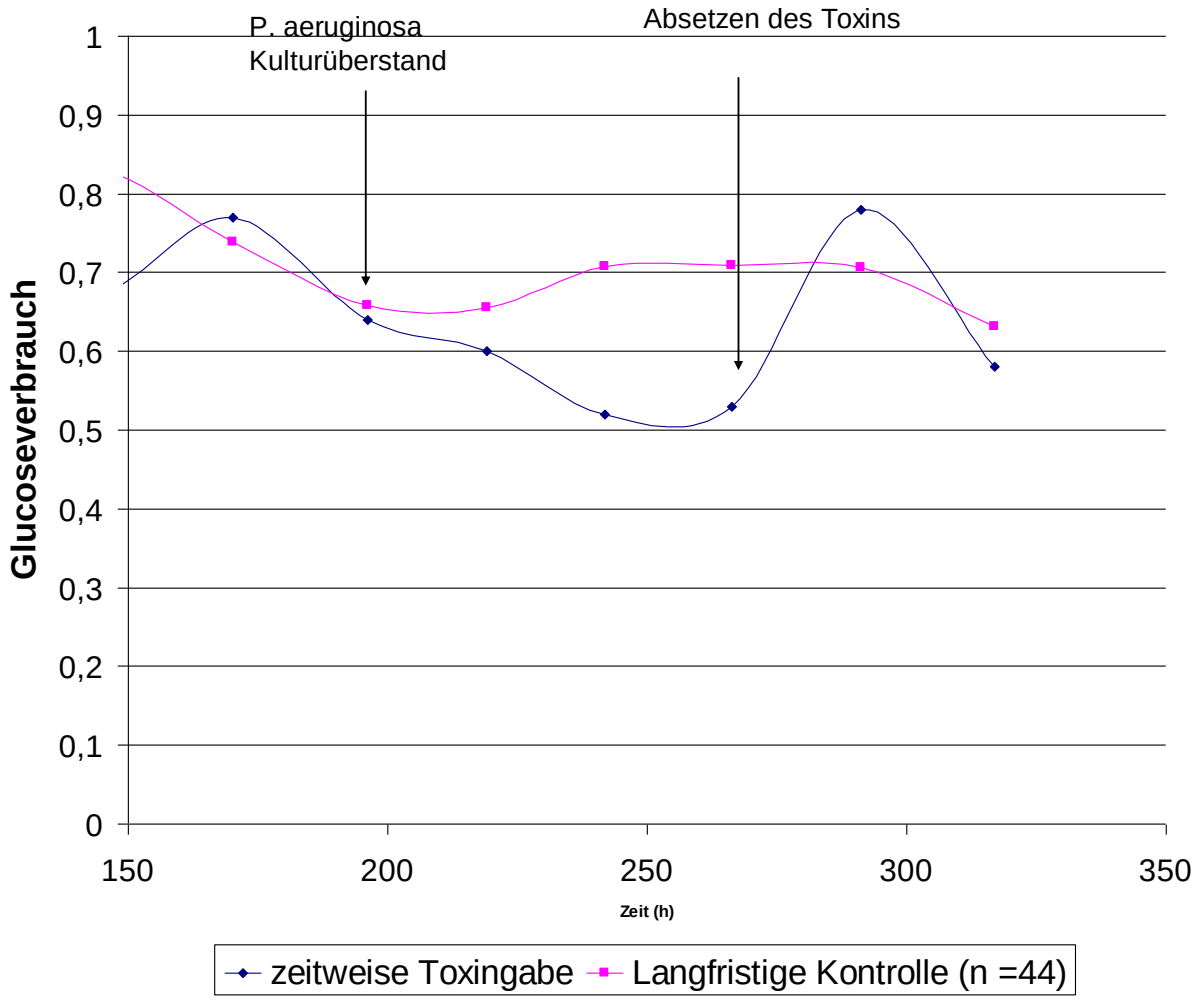
- Avoid seed-borne fungal infections in medicinal fennel
- In progress: Review of the antifungal effect of microalgae

Perfusion cell culture

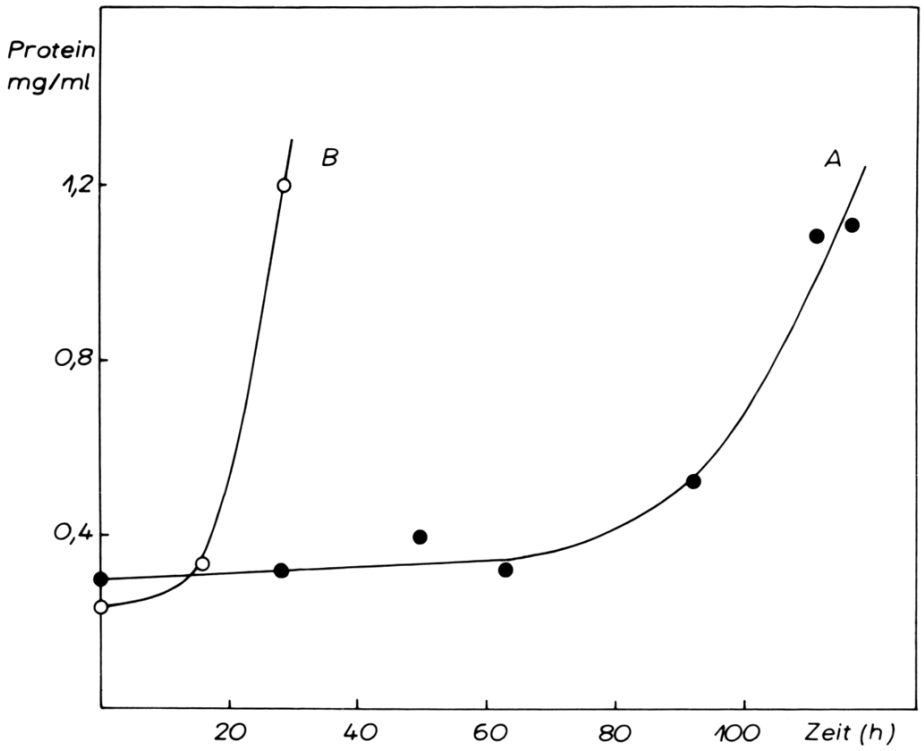


Source: J. Pörksen, W.-D. Jülich: Procedure and mechanism for establishing long-term biological effects in cells. PCT/EP2010/004002.

Reversible cellular damage is possible with the perfusion cell culture



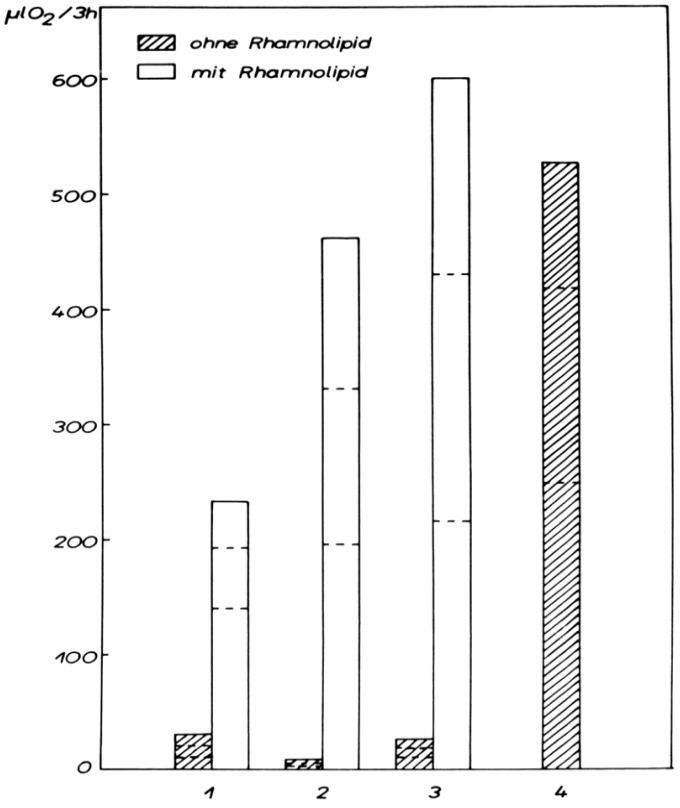
Rhamnolipids have one hydrophobic and one hydrophilic molecule moiety. This amphiphilic character reduces surface tension or interfacial tension. Improved emulsification is an important virulence factor for pseudomonas.



Growth of P. aeruginosa in alkane culture

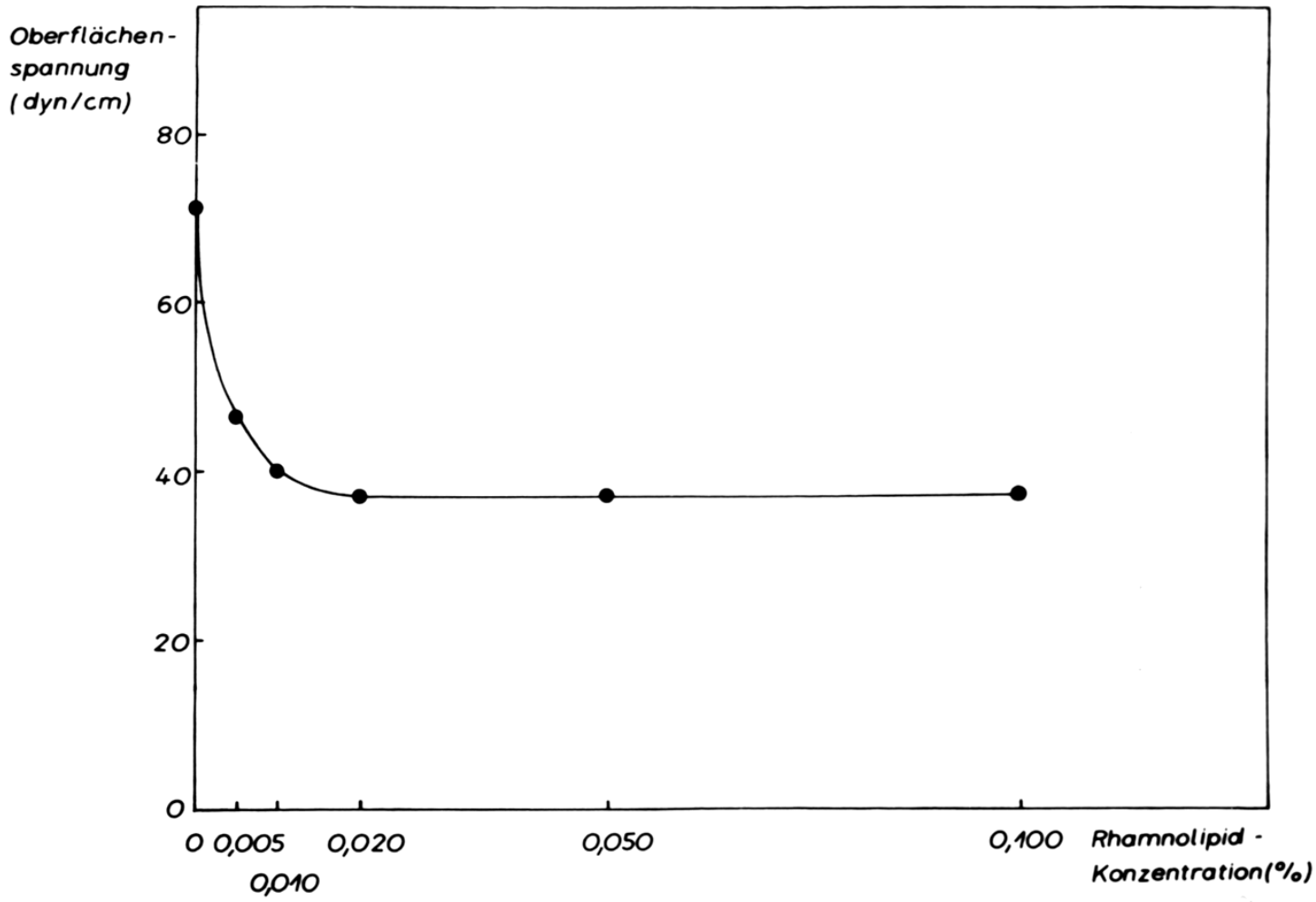
A mineral salt medium + 1 % dodecane

B addition of 0.025 % rhamnolipid

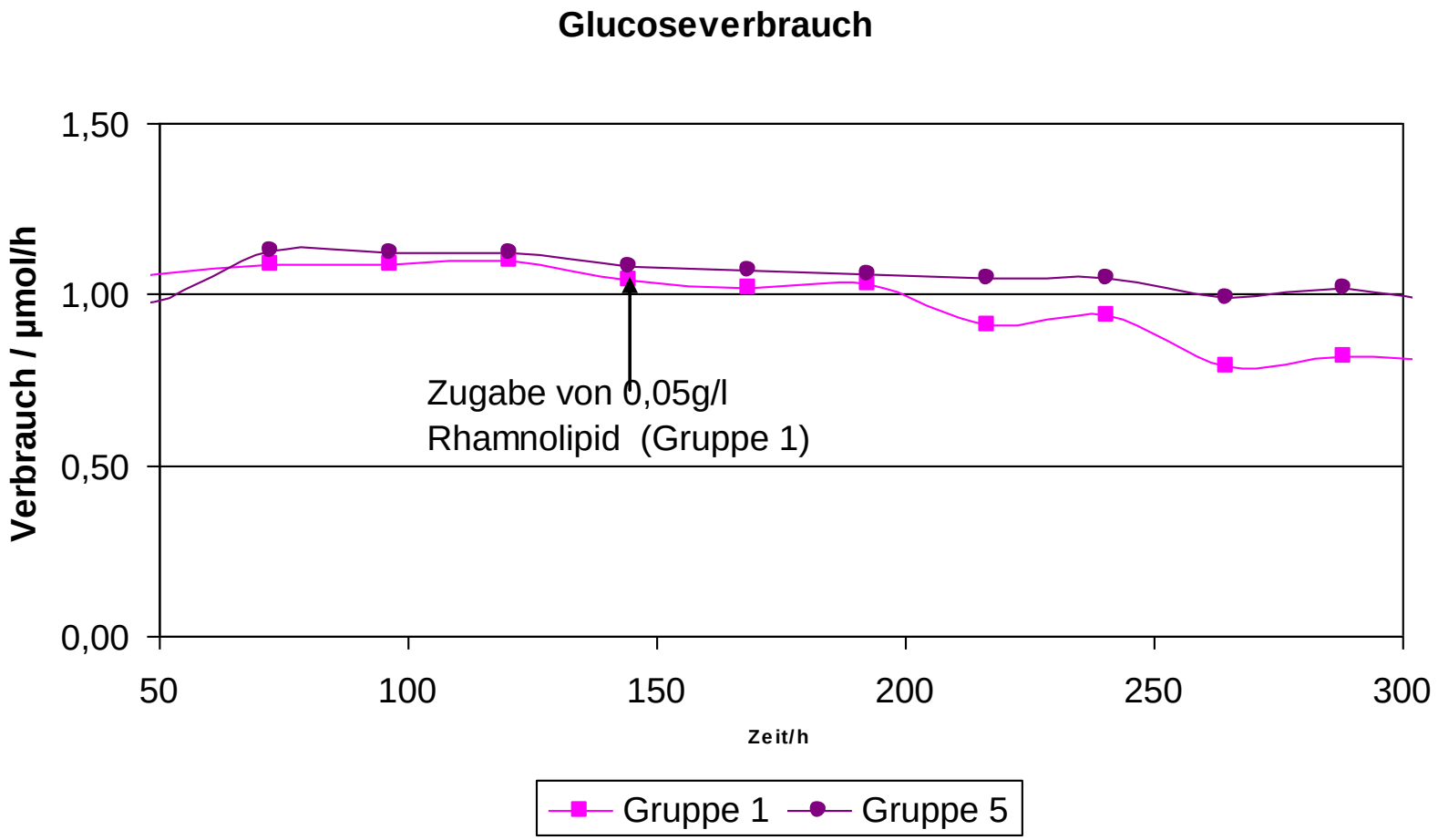


Oxygen consumption of resting cells after dodecane culture

Source: Institute of Microbiology of the University of Greifswald, Prof. Schauer



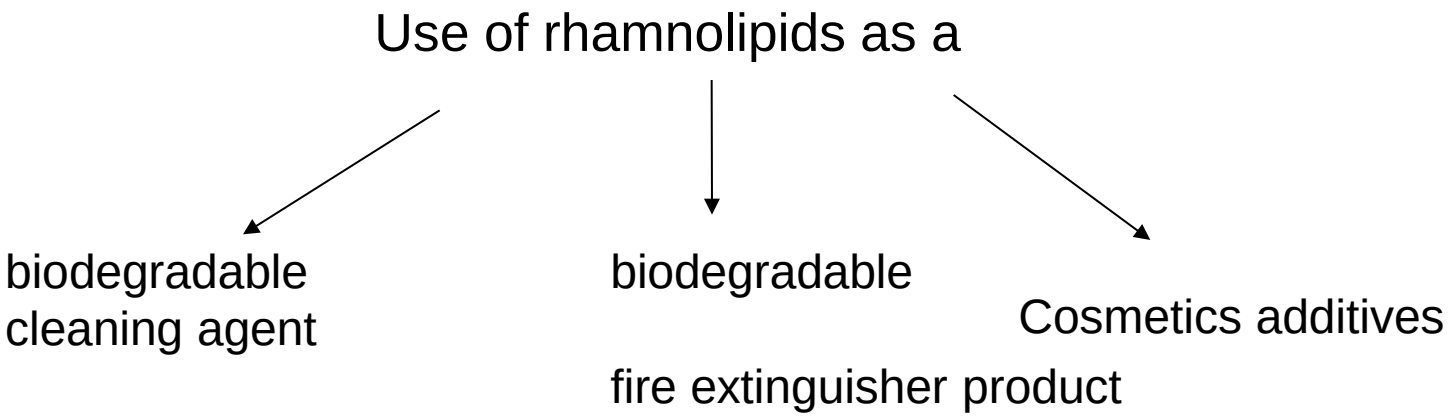
The reduction of surface tension is already reached by minimum concentrations. From a concentration of 0.005% (0.05 g/l), the surface tension remains constant.



The reduction of surface tension by the rhamnolipid leads to a slight vitality reduction of the cells

Cooperation between the Ernst Moritz Arndt University of Greifswald and the Lviv Polytechnic National University (Ukraine)

The Ukrainian colleagues are dealing with the industrial production of rhamnolipids in large-scale fermenters.
They are doing so in cooperation with Biotensidon GmbH, Karlsruhe.



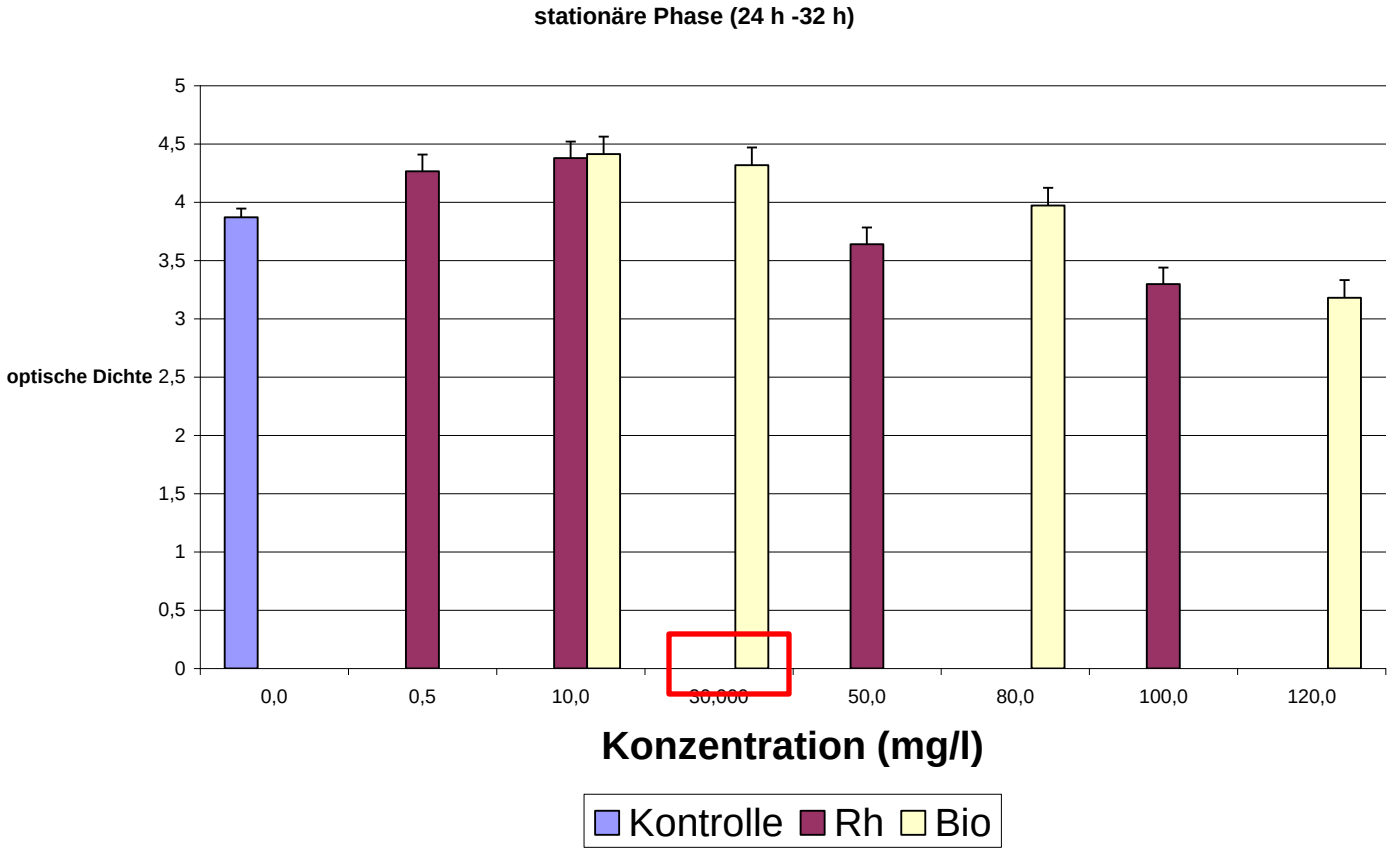
Cooperation between the Ernst Moritz Arndt University of Greifswald and the Lviv Polytechnic National University (Ukraine)

Dr. Karpenko's working group found that a rhamnolipid alginate complex, which is inexpensive to produce in a large-scale fermenter, has a favourable effect on plant growth

Surface-active compounds have effects on the entire ecosystem. With surfactants of the biosurfactant type, non-polar structural components can penetrate the lipid layer of the membranes of soil organisms. This could cause changes in the external load, conformation and permeability of the cell membranes of soil-dwelling organisms.

Source: Projects UKR 08/047 "Biosurfactants for the production of nanoparticles for use in the pharmaceutical industry and in improving environmental protection").

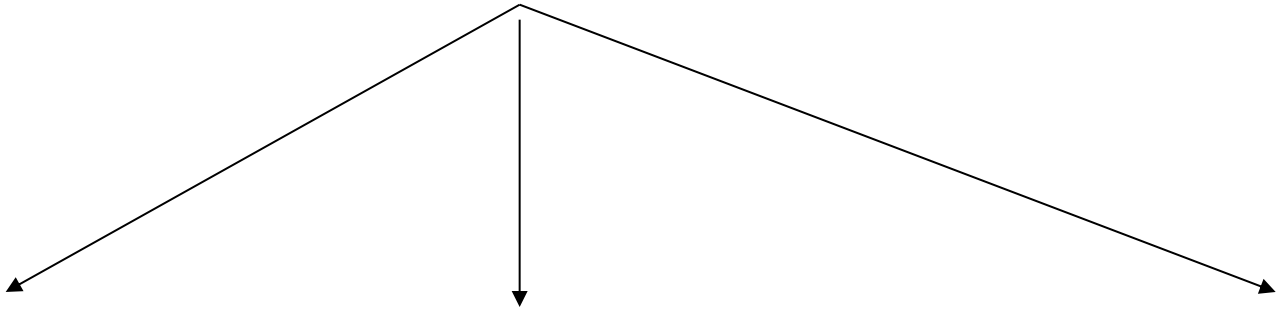
Determining an optimal concentration



Growth of *Saccharomyces cerevisiae* in the stationary phase (mean value of 5 measurements at 2-hour intervals) under the influence of biosurfactants (Rh) and rhamnolipid alginate complex (bio).

A toxic limit concentration of 100 mg/l was determined in the luminescent bacteria test for the bio-complex.

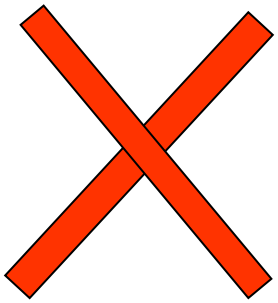
Opportunities to use the positive impact of biosurfactants on plant growth



Distribution of a biosurfactant solution

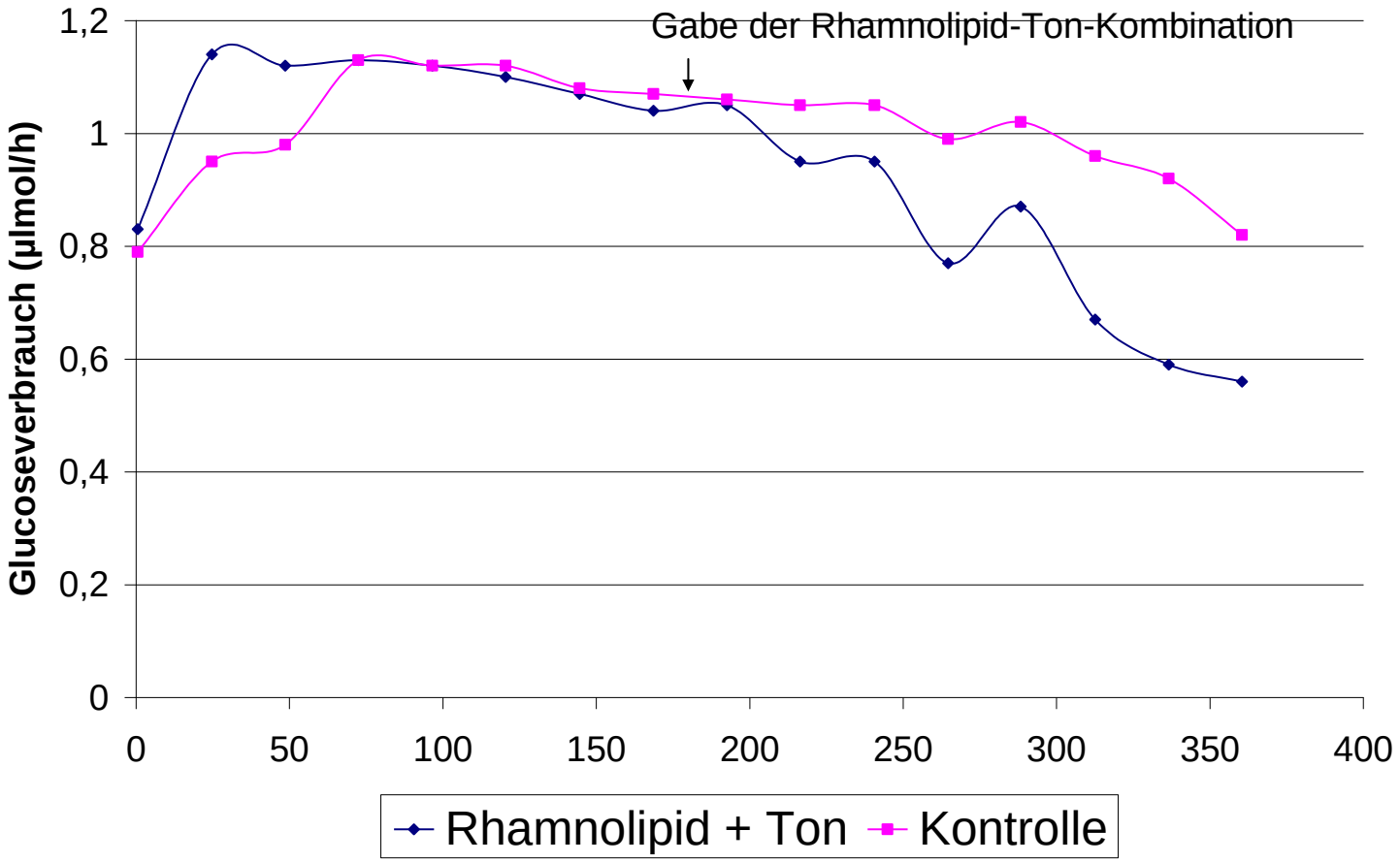
Binding to geopolymers

Coating of seeds



Soil improvers
in the form of granules

Pelleted seeds for
- cereal cultivation
- turf and lawns
- flower seeds
- forestry



A composite of biosurfactants and clay minerals reduces the glucose metabolism in perfusion cell cultures at a biosurfactant concentration of as little as 50 mg/l

Purmin GmbH

- Founded as a UG (Limited Liability Entrepreneurial Company) from the University of Greifswald by Dr. Schmidt and Dr. Jülich

- **Special expertise: Production of mineral composites**

➢ Hydrogels are initially produced from mixed layer clay minerals, which are used to disperse zeolites.

➢ Mixed layer clay minerals break open during dispersion along the intermediate layer areas as well as transversely to these layers.

➢ Zeolites and other geominerals are incorporated into this "house of cards" structure in order to stabilise the colloidal state

Mineral composites for application to skin

Reduction of itching by Lithoderm®Liquid

Produced by PURMIN GmbH

German sales: Medicaris GmbH, Buxtehude

Photos: M.Kummer

Decreased itching in
< 1 min. after application of
Lithoderm®Liquid

24 hours after the application of
Lithoderm®Liquid

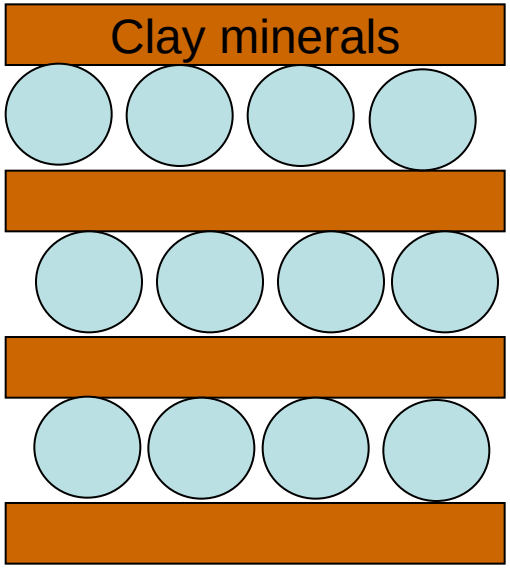
Result of the cooperation with Lviv:

Use of mineral composites

as carriers of the rhamnolipid alginate complex

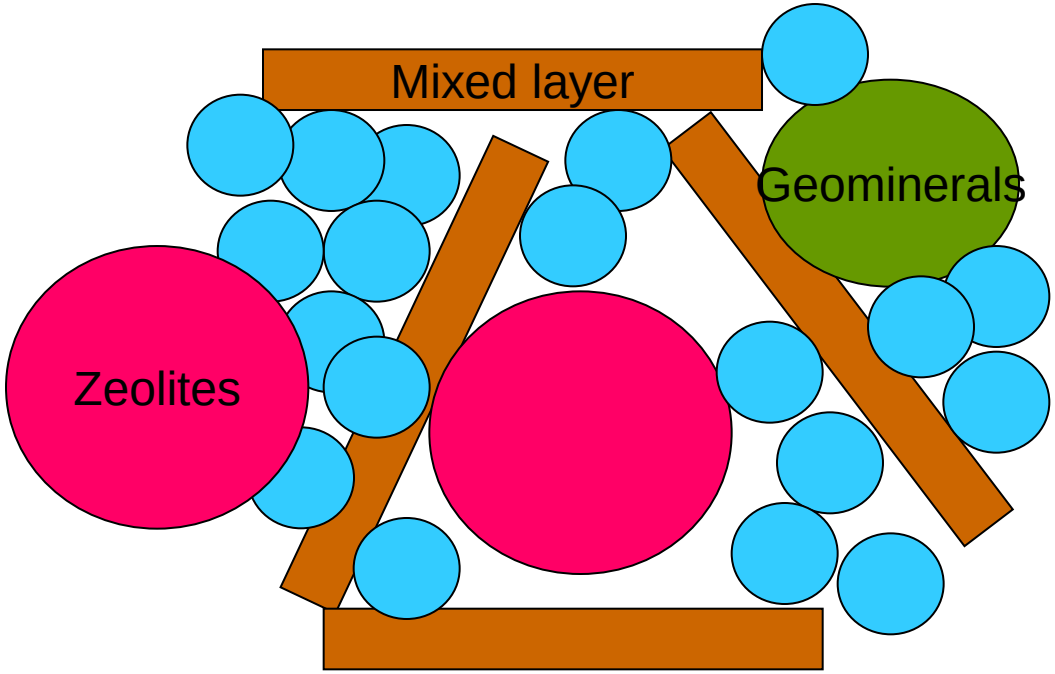
- The rhamnolipid alginate complex is incorporated into the structure of the mineral composite during production
- The mineral composite biosurfactant can be granulated in this form.
- The granules produced can absorb and store large quantities of water after injection into the ground.
- Water and biosurfactants are made available in a form that is not harmful to plants.
- The absorption of water and nutrients by the plant is also improved by the mineral composites during dry periods

Pure clay minerals



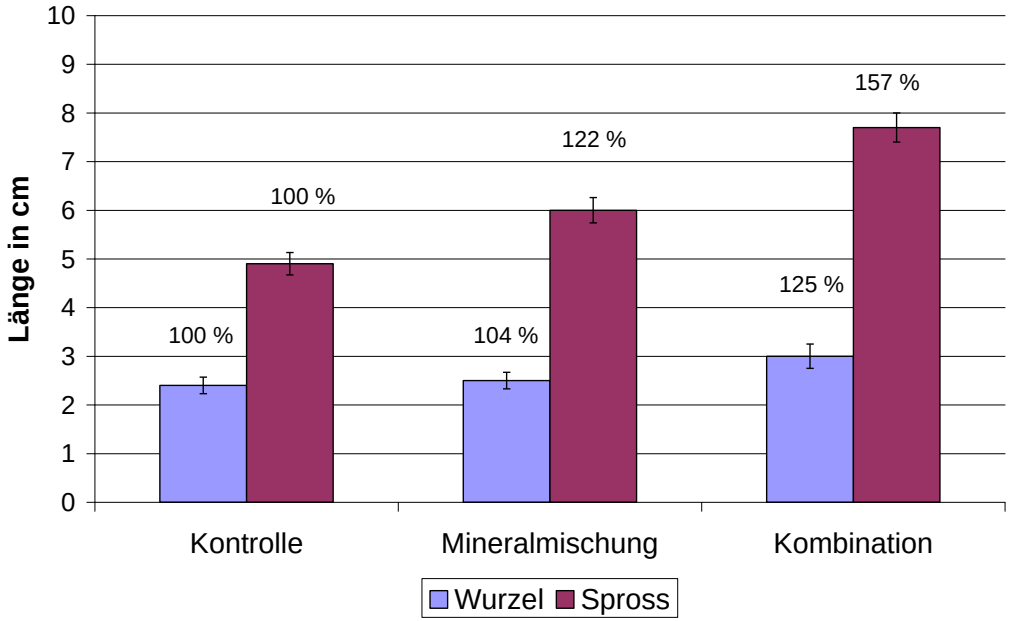
For soils with a high clay content, the particles stick together and form few interspaces. The water is bonded so strongly that it can only be delivered to the plant in small quantities. This means that deadheads can occur in clay soils although sufficient water is available. Ventilation is generally poor in clay.

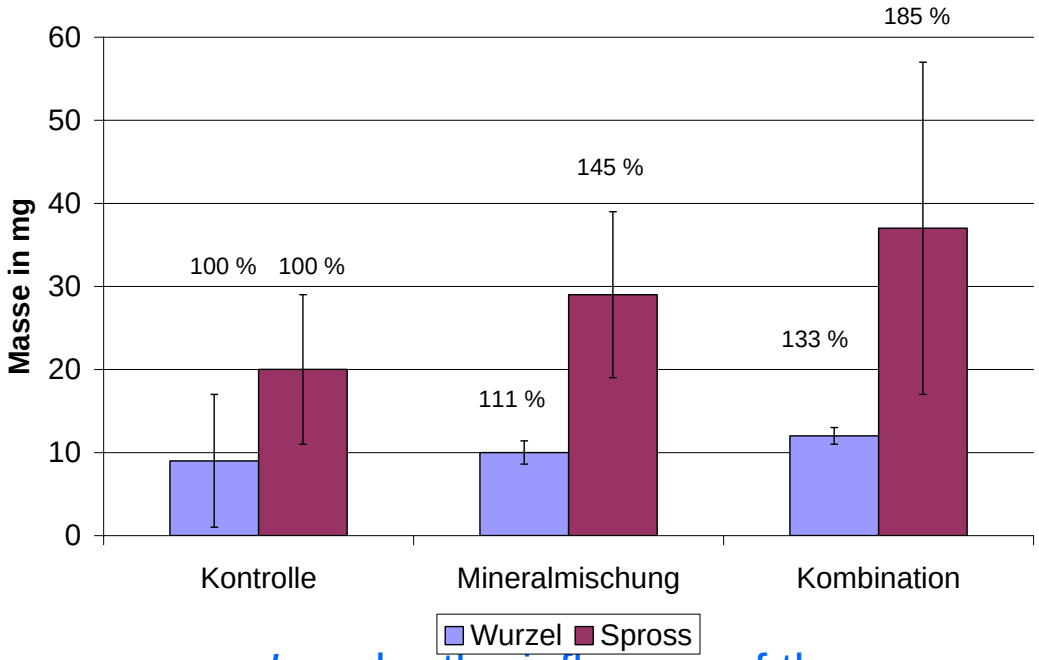
Mineral composite granules with stored water of which the surface tension is reduced by rhamnolipid alginates.



The mineral composite granules can absorb and store large quantities of water

Water and biosurfactants are made available in a form that is not harmful to plants.





Increase in length and mass of *Lolium perenne L* under the influence of the geominerals and of the combination of biosurfactants and geominerals

Cooperation with SUET Saat und Erntetechnik GmbH

Coating of seeds

- Plant seed is coated with a thin but adherent layer of mineral composites.
- After injection into the ground, the coating layer swells as a result of water absorption. This surrounds the seeds with a geopolymer biosurfactant water film.
- This biosurfactant water film contains dissolved nutrients.
- The thickness of this geopolymer biosurfactant water film is critical to conditions that are favourable to germination. Increased water content in the vicinity of the seed is optimal for germination, even during dry periods.

Advantages of the new technological solution

- The coated seeds bond with the ground quickly and intensively.
- The layer thickness can be increased significantly (10-40% of the grain weight)
- The optimal concentration of 20 mg of biosurfactant is reached in the vicinity of the germ, while the toxic effects on soil-dwelling organisms are safely eliminated.
- The structure-forming geopolymers used ensure that there is an adequate water supply without soil pores becoming clogged during the germination and initial growth phases.
- Special seed-dependent advantages: As an example, coating grass seed when planting lawns and afforestation seed in the forestry sector also prevents the seed from being blown away and or being destroyed by birds. The uniform distribution of the lawn is promoted and maintenance is reduced.
- Insecticides can be incorporated in a single step

First joint field trial at Gut Schmerwitz

Questions: Can any effect at all be expected at a coating/material biosurfactant concentration of 20 mg/kg?

Conditions still inadequate:

- Pelleting with only 10% of the grain weight
- Scheduling difficulties owing to long period of frost
- Seed verum/verification consequently not possible at the same location
- Verum not sown at the optimum time

Results from the field trial at Gut Schwerwitz

- Significantly shortened growing season

  prox. 20 – 25 days less

  Verification: Sown 14 days earlier, not yet ready for harvesting in early August
- Even growth over the entire area
- Tillering better than during verification
- Planting of stronger, longer grains according to subjective assessment by the agriculturalist

How can an optimal nutrient supply be achieved in the vicinity of the germ?

- The best solution is to use a biological compound fertilizer, which ensures the supply of phosphate and nitrogen.
- Animal meal is obtained in large quantities
- Only cat. III is currently authorised for use as a fertilizer
- The majority (cat. I and II) is currently being burned.
- In coated seed form, fully hygienically safe recovery is achieved.
- However, animal meal does not adhere to the seed.
- We achieved animal meal bonding to the biosurfactant geopolymer complex.
- We achieve a biosurfactant geopolymer animal meal complex of 10 - 40% of the grain weight.

Cooperation with Schaap GmbH - an animal meal manufacturer

Advantages of the combined application of soil improvement granules and coated seed

- Although the quantity of biosurfactants and geopolymers is limited by layer thickness during seed coating, the quantity is arbitrary during distribution in granule form and can be adapted to soil types, i.e. a larger quantity of granules would be applied to light sandy soils than to heavy clay soils.
- Coating supports germination and early growth.
- During the later growth stages, the storage of water and fertilizer is achieved through the injection of the fertilizer biosurfactant geopolymer complexes in granular form.
- Reversible swelling and absorption by the plants/soil in interspaces ensure good ventilation and the unobstructed inflow of nutrients, during both initial use and subsequent repeated use. This supports the soil ecosystem by improving optimum soil structure and will become a prerequisite for the existence of a newly created population of organisms that is both rich in species and has high numbers, promising high yields in the long term.

Statement by the Scientific Board of Agricultural Policy at the Federal Ministry of Food, Agriculture and Consumer Protection in 2012

- "The projected growth in demand of about 1.8% per year by 2050 cannot be countered by the mere continuation of current trends"
- "Future increases in crop production will need to be achieved primarily through higher yields per hectare"
- "In recent decades, the increased use of agricultural chemicals has contributed significantly to revenue growth," but is also "associated with negative environmental effects such as nitrate leaching, eutrophication and biodiversity loss"
- "In addition, raw materials that are important for the production of mineral fertilizers are becoming scarcer and more expensive"
- "The production of nitrogen fertilizers is energy intensive, meaning that fertilizer prices are closely correlated to the price of crude oil"
- "According to some experts, the more easily exploitable world phosphorus reserves could be depleted in the near future"

The new technology meets these demands exactly

- Soil fertility and water retention capacity will be improved in the long term,
- Better use will be made not only of scarce phosphate resources, but also of nitrogen fertilizers
- Leaching of nitrate into groundwater will be reduced and negative impacts on the ecosystem prevented
- Yields can be increased considerably

We therefore propose that a collaborative research project be applied for to enable you to contribute your expertise to the development of new technology

Thank you

To my Greifswald colleagues

Department of Pharmaceutical Biology, University of Greifswald:

Prof. U. Lindequist, Ms. PD S. Mundt

Institute of Microbiology of the University of Greifswald: Prof.F. Schauer, Dr. Schlüter

Purmin GmbH: Doz.D. D. Schmidt

Institute of Marine Biotechnogy: Dr. G. Lukowski

To the cooperation partners

Biotensidon GmbH: J.Joegel, R. Hartmann

National University Lviv Polytechnic (Ukraine):
Dr. A. Shulga, Dr. E. Karpenko, Dr. N. Shcheglova, Dr. R. Vildanova

analysio GmbH: J. Pörksen

SUET Saat und Erntetechnik GmbH: Dr. sc.agr. H. Lamprecht, Dr. J.Dau, Dr. D. Göbke,

Schaap GmbH: J. Schaap

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Thank you for attention!