



Scaling study for obtaining microbiological fertilizers and plant protection products in depth and surface cultivation processes, no. 1.1.1.1/19/A/150

Progress report for the 5th quarter period 01.04.-30.06.2021.

Trichoderma spp. extraction in the liquid surface cultivation process

A series of experiments were started, within the framework of which it is planned to evaluate the production of liquid *Trichoderma* preparation by realizing *Trichoderma* cultivation on the liquid surface under bioreactor conditions. Within the series of experiments, it is planned to test different (3-4) medium and aeration modes, maintaining the “liquid surface” / “liquid volume” ratio, which is close to the ratio in flask cultures. During the reporting period, 2 cultivation experiments were performed in two different mediums, testing two aeration modes for each medium. The effect of aeration on the thickness of the fungal layer formed on the surface of the liquid was observed. Samples were analyzed for antifungal activity, number of colony forming units or spores.

Trichoderma spp. extraction in the process of depth cultivation

A series of experiments has been started, in the framework of which it is planned to test the influence of *Trichoderma* and *Bacillus* co-cultivation in the flask process by shaking. A series of experiments will test the various pre-selected mediums. In the first experiment, anti-fungal activity was detected in one of the selected culture mediums. The first flask experiments were performed to evaluate the effect of surfactants Tween 80 and Tween 40 (0.2-2%) on *Trichoderma* biomass formation and fungal micromorphological properties. The obtained biomass - paste - was treated with several chemicals (hydrochloric acid, copper sulphate and starch) and their combinations, creating a total of 12 variants, and the preservation of fungal viability and antifungal activity against the phytopathogenic fungus *Fusarium graminearum* during storage at room temperature was evaluated. On this topic M.T. Dzierkale developed and excellently defended the course paper at the Department of Microbiology and Biotechnology of the University of Latvia. The results of research on the production of *Trichoderma* biomass in different depth cultivation conditions are presented at the “World Microbe Forum, 20-24 June, 2021. Online worldwide. An ASM & FEMS collaboration”.

Trichoderma spp. extraction in the surface (solid phase) cultivation process

Improvement of the principal scheme of the solid phase cultivation pilot plant and pilot plant design works have been performed, conformity assessments for the applied sensors and performance conformity assessment for the selected process have been performed. Prototype design solutions and their compliance with the experiments to be performed have been approved together with the manufacturer. During the following quarter, *Trichoderma spp.* solid-phase cultivation experiments on coffee bean bran, which is a by-product of the food industry (waste). In the future, it is planned to determine the physical parameters of coffee bean bran and their influence on cultivation processes in a static solid phase system with wheat bran.



Bacillus spp. extraction in the depth cultivation process

A series of experiments are performed in flasks to obtain the optimal medium composition, which is suitable for cultivation processes in laboratory and pilot-scale bioreactors. The medium uses industrial reagents such as bean, pea and soy flour, which are supplemented with various sources of sugar to increase productivity, such as molasses, food sugar and glucose. The combination of the medium composition with the highest yield and / or the best economic calculation is used for further experiments to obtain the optimal medium composition. The experimental results are analyzed by accumulating data on changes in optical density over time, the number of colony-forming units, incl. vegetative cells and spores, etc. methods.

A series of experiments were performed in a 5L laboratory-scale bioreactor according to Chen et al. (10.1007 / s00253-009-2162-x) and analyzed the dynamics of *B. subtilis* cell biomass and spore accumulation. In the 24th hour of the culture process, a high cell biomass result was achieved - $1.78 * 10^9$ CFU / ml and $1.34 * 10^9$ spores / ml. Three cultivation processes were also performed in a 100L pilot-scale bioreactor, testing the suitability of the respective optimal composition medium previously determined in flask experiments for industrial-scale cultivation processes. In the experiment with additional substrate feeding, the biomass concentration of $7.15 * 10^9$ CFU / ml was reached, but a relatively low fraction of cells formed spores - $4.95 * 10^8$ spores / ml (7%). The data obtained in the cultivation processes on the dynamics of cell biomass growth and spore formation, mixer speed range and aeration will be used in the implementation of further experiments.