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Microbial cultivation in polymer vessels: Examples of stirred and static systems for the production of biostimulants and biocontrol agents used in agriculture and forestry

Oskars Grigs, Oskars Kurpnieks, Ilvars Sutris

Latvian State Institute of Wood Chemistry, Dzerbenes st. 27, Riga, LATVIA

* Correspondence: oskars.grigs@kki.lv

Objective Biotechnology equipment for microorganism cultivation often requires substantial capital investment. However, microbiological products used in agriculture and forestry - such as biostimulants, biocontrol agents, composting aids, and stump decomposition agents - must be affordably priced to compete with conventional fertilizers, fungicides, and stump treatment methods. The production of such products can be facilitated by technologies that require lower capital investment. This can be achieved by using relatively inexpensive polymer-based cultivation tanks.

Polymers (PP-R and HDPE) vs. glass and stainless steel Polymer materials like PP-R and HDPE are more affordable and easier to mold than glass or steel. Although they offer lower heat and chemical resistance, they are suitable for many low- to medium-sterility cultivation needs, providing a cost-effective solution for non-food, non-cosmetic, and non-health product manufacturing sectors.

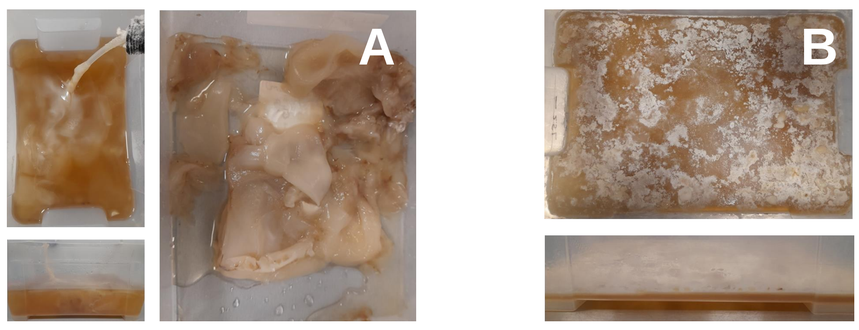
Material comparison for cultivation equipment

	Polypropylene (PP-R)	**High Density Poly Ethylene (HDPE)**	**Glass (borosilicate)**	**Stainless steel**
Thermal treatment (possible)	Can be steam-sterilized at 121 °C ($T_{cristalization}$ = 102-110 °C)	≤ 60 °C (deformations occur at ~65 °C)	> 200 °C	> 200 °C
Surface roughness	Ra =0.3-4 µm [DOI: 10.1163/016942410X511042]	Ra =0.3-4 µm [DOI: 10.1163/016942410X511042]	Ra = 0.010–0.020 µm [DOI: 10.1016/j.surfcoat.2006.03.034]	Ra=0.05-0.17 µm [DOI: 10.1021/acsomega.8b00769ACS Omega2018, 3, 6456−6464]
Contact angle	~95° [DOI: 10.1163/016942410X511042]	~96° [DOI: 10.1163/016942410X511042]	~33–50° [DOI: 10.1016/j.apsusc.2005.01.041]	~70–80° [DOI: 10.1016/j.surfcoat.2015.07.062]
Chemical sterilization (30–60 min)	H_2O_2 3–6%, NaOH 0.5–1%, NaOCl 0.5–1%, HCl ≤ 0.1% (limited effectiveness)	H_2O_2 3–6%, NaOCl 0.5–1%, NaOH 0.2% (≥30 min, limited ffectiveness)	H_2O_2 3–6%, NaOH 0.5–1%, NaOCl 0.5–1%, HCl 0.4–4%, H_2SO_4 5–10%	H_2O_2 3–6%, NaOH 1–2%, NaOCl 0.5–1%
	Thermaly and chemicaly suitable material with a hydrophobic and non-rough surface properties	Thermaly and chemicaly less suitable material with a hydrophobic and non-rough surface properties	Thermaly and chemicaly suitable material with a hydrophylic non-rough surface properties	Thermaly and chemicaly suitable material with a hydrophobic and non-rough surface properties

(1) *Phlebiopsis gigantea* cultivation in PP-R boxes

Phlebiopsis gigantea (PG) is a mycelial fungus used in plant protection (e.g., Rotstop) to control the spread of root rot caused by *Heterobasidion* spp. in conifer trees. PG also accelerates the biological decomposition of tree stumps.

The broth and the 11 L PP-R box were sterilized separately in an autoclave at 121 °C for 30 minutes. Depending on the process conditions, fungal mycelium (A) and spore production (B) can be achieved.

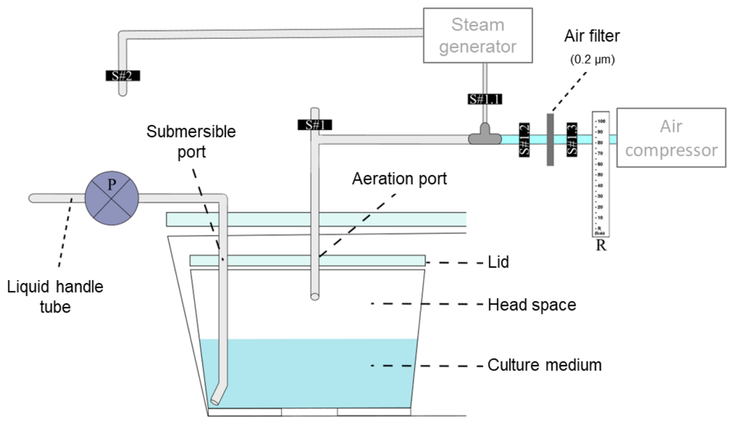


Filamentous fungi submerged cultivation: A – aerated process for mycelium production, 0.66 g(dry)/L ; B – passively aerated process for spore production (thiner broth level), (6±0.7)*10^6 spores/ml.

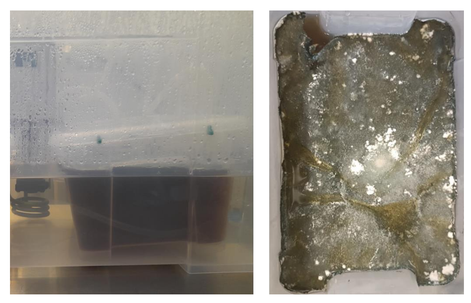
(2) *Trichoderma viride* cultivation in a PP-R out-of-autoclave steam-sterilized box

Trichoderma spp. is an effective plant biostimulant and fungicide. When cultivated on the surface of a liquid medium, it is possible to obtain both its spores and the broth containing bioactive compounds secreted by *Trichoderma* with antifungal activity. The feasibility of this cultivation method has been demonstrated using out-of-autoclave sterilization of the 5 L container with heated (~100 °C) water steam.

The cultivation box contains two ports used for steam inlet, condensate removal, medium addition, inoculation, and aeration. The culture medium was sterilized in an autoclave at 121 °C for 30 minutes. After 13 days of cultivation, a spore yield of 1.9×10^7 spores/mL was achieved, along with antifungal activity against *Cladosporium* spp., with potential activity against other plant pathogens as well.



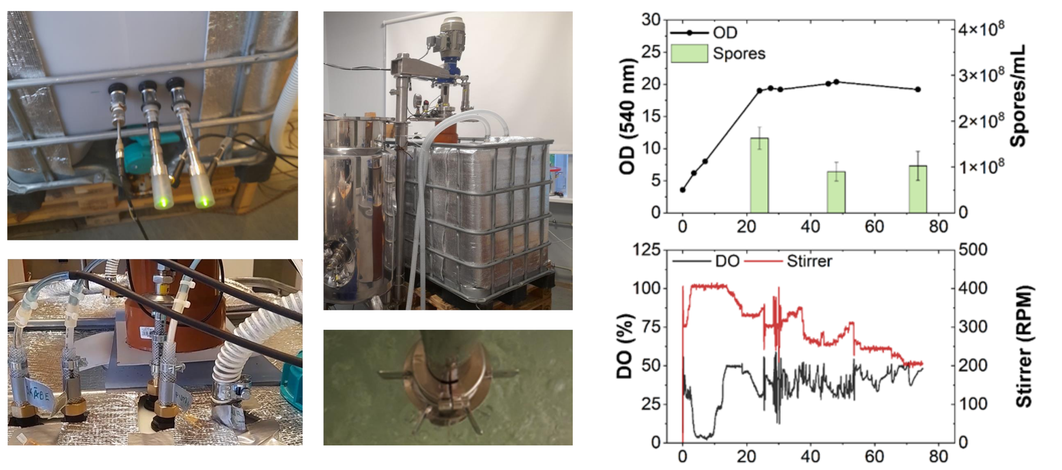
Pricipal diagram of the cultivation system



14th day culture

(3) *Bacillus subtilis* (Bs) cultivation in a HDPE rectangular container

Bacillus subtilis (Bs) is an endospore-forming bacterium used in the production of plant biostimulants and biological control agents. Due to its relatively low sterility requirements, Bs cultivation is well suited in an HDPE container-type bioreactor.



Prior to the process, the container was chemically washed and disinfected for 30 minutes using a 6% H_2O_2 aqueous solution. After disinfection, the hydrogen peroxide was neutralized with Na_2SO_3 and then rinsed with sterile water. The culture medium was heat-treated (~100 °C) in a separate container. Using this method, a Bs preparation with an endospore concentration of 1×10^8 per 1 mL was obtained.

Further work and collaboration opportunities

- ☼ The research group continues developing a 600 L HDPE bioreactor. Follow the progress ➡ https://kki.lv/zinatniska-darbiba/projekti/Subt4Potato
- ☼ The group has received funding for a project aimed at developing static 3-level (3x 50-60 L) out-of- autoclave steam-sterilisable tray system for filamentous fungi and their secondary metabolite containing preparation obtainment in a liquid surface cultivation procesess (Project No. 1.1.1.3/1/24/A/155, 10.2025.-09.2028.)

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