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# STERILITY PROBLEMS OF BATCH BIOTRANSFORMATION OF GLYCEROL TO DIHYDROXYACETONE USING IMMOBILIZED GLUCONOBACTER OXYDANS

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*Biotransformation of glycerol to dihydroxyacetone by the bacteria strain Gluconobacter oxydans immobilized in polyvinylalcohole gel has been studied. The sterility requirements necessary for successful course of reaction were examined. Suitable purity and treatment of reactor and all initial reaction components (water, glycerol, yeast extract, the air used for bacteria respiration and immobilized microorganisms) were found as necessary conditions against contamination.*

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## Introduction

At mild reaction conditions glycerol can be converted into commercially interesting product dihydroxyacetone by the bacteria strain *Gluconobacter oxydans* (G.o.) [1–4]. This biotransformation can be successfully carried out only when appropriate sterile requirements are fulfilled. This paper deals with these requirements. The main effects of contaminated reaction course are: low biomass concentration in poly(vinyl alcohol) capsules, uncontrolled excessive occurrence of parasitic microorganisms, zero production of dihydroxyacetone, increase in pH instead of its decrease and excessive foaming of the reaction mixture.

## Materials, Methods and Results

The biotransformation was carried out in a 1000 ml batch glass reactor equipped with 5 openings for air supply, measurement of dissolved oxygen, pH and temperature of the mixture, and addition of KOH aqueous solution. The initial reaction mixture consisted of aqueous solution of glycerol and yeast extract (which served as nitrogen and nutrient source for microorganisms) and of suspension of G.o. immobilized in the capsules from poly(vinyl alcohol) gel. The mixture was continuously saturated with oxygen from the air and kept at 25 °C. The pH was maintained at pH interval 4.8 – 5.1 by adding aqueous solution of 1.5 M KOH using a pH-stat.

The reaction was started by quick immixing of immobilized G.o. to the thermostated and with oxygen saturated aqueous solution of yeast extract and glycerol. The G.o. is a strictly aerobic microorganism which requires the presence of molecular oxygen for its activity. It was therefore essential to evaluate the requirements for purity of the air used for respiration. In addition, the requirements for sterility of the used water, glycerol, yeast extract, immobilized G.o. and internal surface of the whole apparatus had to be checked.

At first, several biotransformations without any sterilization steps were carried out for comparison with further experiments. Demineralized water without further treatment was used for preparing the glycerol and yeast extract solutions. Reaction apparatus was only washed with demineralized water and dried up with air. The air used for respiration was not treated. As early as 2 hours after the reaction start a huge amount of foam always appeared in the reactor and started to leave the reactor even against the overpressure of air (aprox. 20 – 30 torr) pumped into the reactor. At this state, the reactions were stopped and microbiological analysis was done. This analysis showed that the reaction mixture included (besides G.o.) multivarious mixture of microorganisms: genera *Rhodotorula*, *Micrococcus*, *Staphylococcus* and *Pseudomonas*. *Rhodotorula* were found already in the used demineralized water.

## Water Sterilization

At first the sterilization of water was tested by boiling for 30 minutes (excluded as unsufficient), then by distillation (great sterility improvement). As optimal was evaluated the water sterilization by means of the UV radiation of wavelength 254 nm for 120 minutes in a flow sterilizer (UV-Lampe, Warenimport&Handels GmbH, Austria).

## Yeast Extract and Glycerol Sterilization

The original freshly prepared aqueous concentrate of the solid yeast extract (Merck Fermtech, Austria) included genera *Micrococcus*, *Staphylococcus* and yeast genus *Rhodotorula*. *Rhodotorula* was easy destroyed at 100 °C (30 min), whereas *Micrococcus* and *Staphylococcus* are resistant to such conditions. Their destruction was carried out in an autoclave at 120 °C and overpressure 0.1 MPa for 20 min. The yeast concentrate adjusted this way already showed adequate biological purity for the studied bioreaction. The same sterilization procedure was used for glycerol (Lachema, Czech Republic).

## Sterile Conditions for Immobilized G.o.

The reserve of the original microorganisms G.o. immobilized in the pellets of poly(vinyl alcohol) (in the form of small disks of ca 5 mm diameter) were obtained from the firm MEGA a.s., The Czech Republic. The capsules were dispersed in the water solution of 50 % w/w glycerol . They were kept at 5 °C in a well closed glass bottle.

## Apparatus Sterilization

We initially tested its thermic sterilization. All parts of the apparatus were properly washed, rinsed with denatured ethanol and all openings were carefully packed with the tinfoil. So treated apparatus parts were exposed to 130 °C in a dryer. Four hours were enough for our purposes, but perfect sterility was reached only after 10 – 12 hours. However, even after that, contamination of the apparatus took sometimes place (probably during the spontaneous cooling of individual parts and/or assembling of the apparatus).

Therefore chemical sterilization was used. After washing and assemblage the apparatus was filled with 1 % aqueous solution of Persteril (Peroxides Sokolov, CZ) with peroxoacetic acid as the main sterilisation agent. This efficient

and volatile germicide is very advantageous because both its solution and its vapours sterilize. After only 20 – 30 minutes a perfect sterility of the whole apparatus was achieved. After that, Persteril was taken out with a sterile silicone hose and the whole apparatus was washed with sterile water treated by UV radiation. Such apparatus was ready for the start of the reaction.

### Air Sterilization

Because of potencial previous contamination it was not possible to use current laboratory air. As the most suitable sterile source of oxygen was found the air compressed in an air cylinder at 15 MPa. However, this way of $O_2$ saturation of the reaction mixture is very uneconomic considering the duration of experiments (24 – 48 h). Therefore we used as oxygen source the air from a compressor filtrated and sterilized by microfilter (Millex®GP, Millipore Co. Cork, Ireland) with 0.2 μm pores.

### Sterilization of Measuring Probe

The actual values of $O_2$ concentration, pH and temperature in the reaction mixture were measured by one complex probe ( WTW, Multi 340i, BRD). The part of this probe immersed in the reactor was carefully washed with denatured ethanol and then with demineralized water treated by UV radiation.

If all the above-mentioned sterility precautions were taken, every biotransformation of glycerol to dihydroxyacetone using immobilized bacteria G.o. proceeded without any sterility problems.

## Conclusion

The following optimal sterilizing conditions were determined for the studied bioconversion of glycerol to dihydroxyacetone by G.o. immobilized in the poly(vinyl alcohol) capsules:

1) Water used for every operation linked with the biotransformation has to be treated with UV radiation of 254 nm for 120 min.
2) Air from a compressor, required for saturating of the reaction mixture with oxygen, has to be treated by micro filter with 0.2 μm pores.
3) Glycerol and aqueous concentrate of yeast extract have to be sterilized in an autoclave at 120 °C and overpressure 0.1 MPa for 20 min.
4) The apparatus has to be sterilized before the reaction with 1 % aqueous solution of Persteril for 20 – 30 min.

5) The measuring probe has to be washed with denatured ethanol and subsequently with water treated by UV radiation before its immersion into the reactor.

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