

P-078 Effect of selected incubation parameters on the efficiency of bacteria immobilization on pumice stone

Kuźmicz U.^{1#} and Bartkowiak A.^{1*}

¹ C. Bioimmob & Innov Pack Mat, West Pomeranian Univ Technol Szczecin - Poland

* Supervisor # Contact email urszula.kuzmicz@zut.edu.pl



INTRODUCTION AND OBJECTIVES

Rigid, inorganic carriers seem to be an interesting alternative to the traditionally used hydrogel systems such as alginate/calcium beads or microcapsules. Their high mechanical strength enables the use of immobilization in a continuous fermentation processes. One of the main challenges of immobilization on solid carriers is to achieve high adhesion of microorganisms on their porous surface. Bacterial cells adsorption may be carried out on several porous materials, such as ceramics, sintered glass, resins (El-Naggar et al., 2006). Pumice is a type of extrusive volcanic rock, which has numerous open spaces and vesicles. Due to its high porosity can be used as a carrier for bacterial immobilization (Kaşıkara Pazarlıoğlu et al., 2005; Gungormusler et al., 2011).

The aim of this study was to evaluate the influence of temperature and shaking on the efficiency of bacterial immobilization on pumice stone.

MATERIALS AND METHODS

Bacterium: In studies of effect of temperature and shaking on the efficiency of immobilization *Lactobacillus casei* (collection of Poznań University of Life Sciences) was used.

Carrier for immobilization: Pumice stone, fraction 1-4 mm (Sigma- Aldrich).

Medium composition was as follows, [g/dm³]: enzymatic casein hydrolyzate - 10; yeast extract - 4; meat extract - 8; Tween 80 - 1; CH₃COONa - 5; MgSO₄ - 0,2;- K₂HPO₄ - 2; MnSO₄ - 0,05; C₆H₁₂O₆ - 20, ammonium citrate - 2.

Pumice stones (Fig. 1) were sterilized in autoclave at 121 °C; 2,2 bar for about 15 minutes; then they were added (3cm³- approx. 0,9g) to the culture medium (30ml) with bacteria (1,67x10⁵ CFU/ml). Afterwards each sample was incubated for 72 hours in four variants: 28°C with shaking (250rpm), 28°C without shaking, 37°C with shaking (250rpm) and 37°C without shaking.

To determine the number of immobilized cells after 24h, 48h and 72h each carrier was taken from media culture, then suspended in distilled water and homogenized (Ivankovic et al., 2010) in laboratory blender (Bag-Mixer). From each suspension serial dilutions were made. Cell concentration was expressed as colony-forming units (CFU) per ml and determined by making serial decimal dilutions and plating on MRS broth (Biocorp, Poland) with agar (Merck, Germany). CFU were counted after 24

hours plate incubation at 37 °C. Before homogenization serial dilutions from culture medium with pumice were performed as a control.

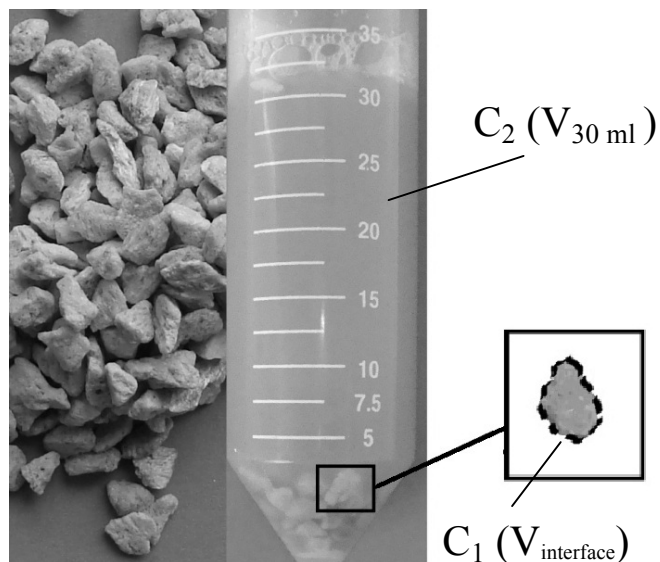


Figure 1: Pumice stone incubated with culture medium with bacteria

RESULTS AND DISCUSSION

Bacterial adhesion is a very complex process, which is affected by many factors (Merrit et al., 2000).

Bacterial adhesion has direct influence on the effectiveness of fermentation processes. It is important to obtain a high concentration (C₁ – Fig. 1) of microorganisms on the surface “interface” of the porous carriers. However, immobilization process conditions are dependent on the specific process, in which one would like to use immobilized cells.

The study has shown, that temperature is a very important factor in the process of immobilization *Lactobacillus casei*. If the process runs at 37° C, it enables the adhesion of a large number of bacterial cells on the surface of the porous carrier in a short time (Fig. 2). However after more than 24 hours, the number of immobilized cells significantly decreases (Fig. 2).

If the process runs at 28°C, it needs more time to obtain the high concentration of bacteria on the surface of the support, but the efficiency of immobilization is then the highest (Fig. 2).

Within 24 hours no significant difference was noted in the concentration of bacterial cells immobilized on pum-

ice between the samples that were incubated with shaking and the samples incubated without shaking at both temperature (Fig. 2). The significant differences in the amount of immobilized bacterial cells were observed after 48 hours of the study. It has shown that shaking does not affect positively the adhesion of microorganisms (Fig. 2, 4). Within 24 hours no significant difference was noted in the concentration of bacterial cells in culture medium with pumice (before homogenization) between the samples that were incubated with and without shaking at both temperatures (Fig. 3). However after 48 hours concentration of bacteria in culture medium with pumice, incubated with shaking was higher than concentration of bacteria in culture medium incubated without shaking (Fig. 3).

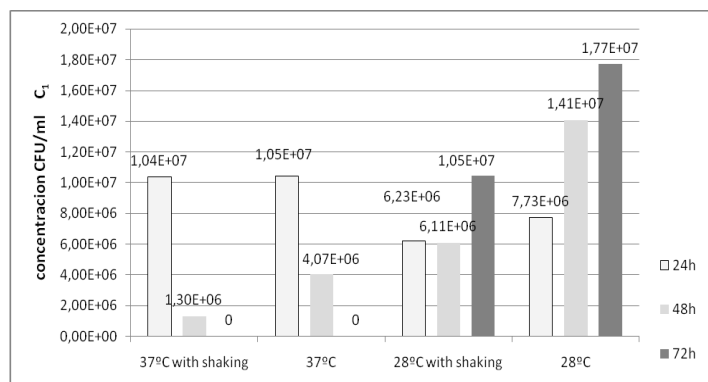


Figure 2: Concentration CFU on a porous surface of carrier within 3 days at incubation (temperature 37°C, 28°C, *L.casei*)

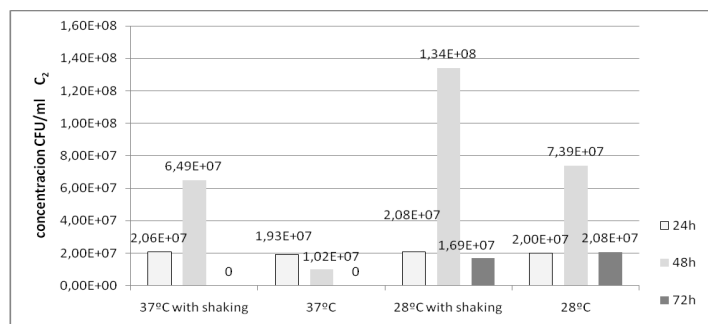


Figure 3: Concentration CFU in culture medium with carrier within 3 days at incubation (temperature 37°C, 28°C, *L.casei*)

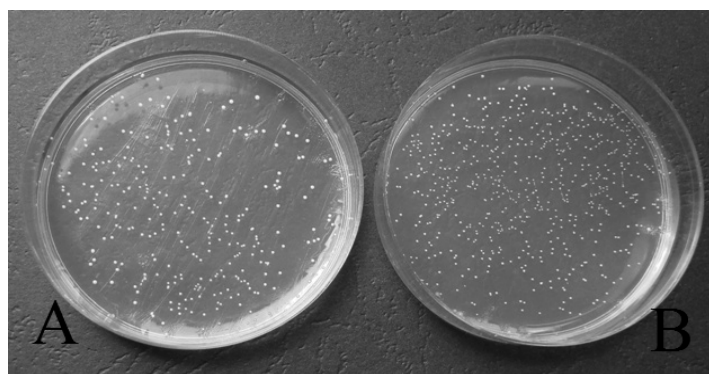


Figure 4: *Lactobacillus casei* on petri dishes after 48 hours at incubation at 28°C with shaking (A) and without shaking (B)

CONCLUSIONS

These preliminary experiments confirmed, that bacterial adhesion is a process, which is affected by many factors including temperature and shaking.

Incubation at temperature 37°C increases the number of bacteria on the porous surface of the carrier in a shorter time than at 28°C, however amount of bacteria is highly reduced after 48 hours. The incubation at lower temperature increases their lifespan and enables their further multiplication and thus affects the greater efficiency of the process of immobilization on the surface of the porous support. However, this process requires more time.

The results obtained in this work confirmed, that the bacteria adhesion on the porous carrier in samples, that were incubated with shaking is lower than adhesion in samples that were incubated without shaking. It has also confirmed, that adhesion of bacterial cells on the surface of pumice is weak and therefore this simply procedure of immobilization needs further modifications.

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