New binary polyelectrolyte systems for bacteria encapsulation.

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INTRODUCTION AND OBJECTIVES

Microencapsulation applied in biotechnology industry implies certain advantages: increases productivity, shorten time between each operation steps, simplified fermentation broth/ biomass separation and reduce amounts of byproducts that have a contact with the cells (Pourbafrani et al., 2007).

The main problem, that has to be overcome before final implementation of this technique on the large scale for example in bioconversion processes is the low mechanical stability, deterioration or even disintegration of typical hydrogel capsules such as binary alginate/Ca systems during use and storage caused by low molar mass ionic compounds present in the various mediums used in the biotechnology processes, namely phosphates, carbonates, monovalent metal cations and others.

In order to make encapsulation process as simple as possible and the obtained capsules much more stable than commonly use alginate/calcium complex system we looked for new pairs of polyelectrolytes that could meet the following needs and be use in a simple one step encapsulation protocol.

MATERIALS AND METHODS

In the screening experiments 13 polyanions and 24 polycations were applied, resulting in total number of over 300 reaction experiments. Based on preliminary capsule integrity study and secondary mechanical stability examinations one system with most enhanced mechanical resistance has been proposed. It consists of a polyanion solution having composition of 2% ι-carrageenan (PCI Worldwide Inc., Philippines) + 3% dextran (Biddle Sawyer Trade Co., Ltd., China) + 4% glucose and a polycation consisting of 1% poly(2-hydroxypropyl dimethylammoniumchloride) (PHPDMA) (LangfangBld Trade Co., China) + 4,6% glucose. Both solutions have osmolality of ~290 mOsmol/kg.

Microcapsules have been prepared by simply dropwise adding of sterile polyanion solution with bacteria *Citrobacter freundii* at a concentration of $1{,}6*10^7$ to sterile polycation solution. The reaction time was respectively 30 (C30) and 45 (C45) minutes. Then capsules were flushed and briefly washed in sterile demineralised water. After that they were placed in a bacterium medium used for bioconversion of glycerol to 1,3-propanediol. Medium contained: glycerol (50g/dm^3), K_2HPO_4 (48 g/ dm^3), KH_2PO_4 (12 g/dm^3), other salts such as: $(NH_4)_2SO_4$, $MgSO_4*7H_2O$, $CaCl_2*2H_2O$, $CoCl_2*6H_2O$, yeast extract, bactopeptone and bacteriological meat extract.

Concentration of 1,3-propanediol has been determined using HPLC method. In bioconversion process performed with encapsulated bacteria the same capsules were transfer 6 times to new mediums (roman numerals) and the whole process have been divide into 3 stages. First lasted for 72h, after which capsules were transfer to new medium. In second period capsules were moved three times to new medium and each time the process lasted for 24h. In the last stage capsules were used also three times but the process lasted for 10h. Each time before capsules were moved to new medium the bacteria concentration in old medium was determined using serial dilution technique to evaluate the protrusion of bacteria from microcapsules. Also after each stage concentration of bacteria inside the capsules was determined by transferring one capsule into 1mL of 0,9% NaCl, destroying capsule by pressing with nozzle, stirring and evaluating the bacteria concentration using serial dilutions technique. Each time two capsules were analyzed and the average have been calculated.

RESULTS AND DISCUSSION

The results of preliminary experiments indicated that polyanion- polycation surface complex formation resulted in most cases in precipitation (76.9%), either immediately or during the reaction process. For 14.4% capsules managed to maintained their integrity, but the membrane was to fragile and it was destroyed during removing from the polycation solution. While 8.3% complexes owned some minor mechanical strength, only one complex shown sufficient toughness to be consider as stable system. To avoid shrinking of such capsules during the reaction process a non-ionic polymer with high molecular mass, namely dextran, has been applied. Finally, to regulate osmolality and avoid osmotic shock glucose has been added to both solutions.

Bioimmobilisation of bacteria inside these novel microcapsules results in high bioconversion of glycerol into 1,3-propanediol during several medium change (figure 1).

The concentration of bacteria in case of encapsulated culture was much lower compare to free cell after 72h in first stage of process (figure 2).

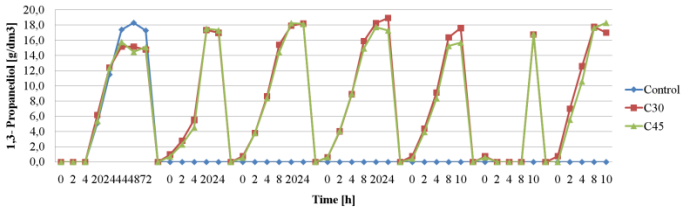

Figure 1. Concentration of 1,3-propanediol during the bioconversion process by encapsulated *Citrobacter freundii*.

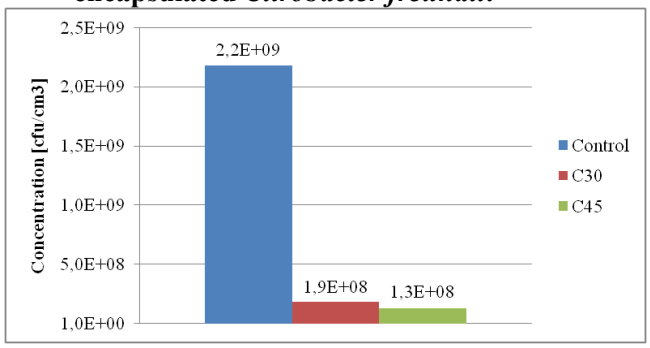

Figure 2. Concentration of bacteria in medium after 72h.

The lower concentration of bacteria for capsules obtained after 45 minutes (C45) could be the result of toxic effect of polycation.

In subsequent stages the protrusion of bacteria from the capsules was initially decreasing and then it was set at an approximately steady level (figure 3).

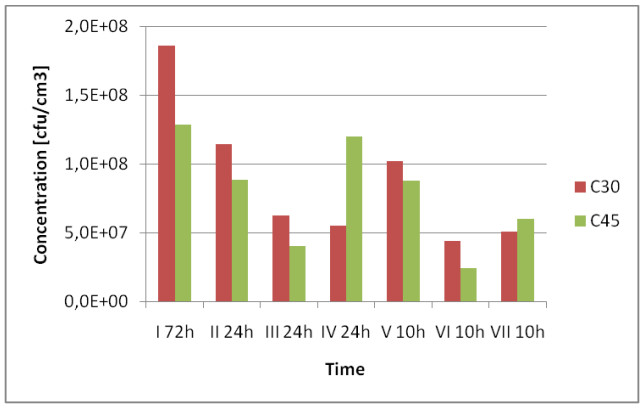

Figure 3. Protrusion of bacteria from capsules.

Concentration of bacteria inside capsules has increased and reached level of $2,4*10^8$ and $2,2*10^8$ per capsule for capsules of reaction time 30 and 45 minutes, respectively (figure 4).

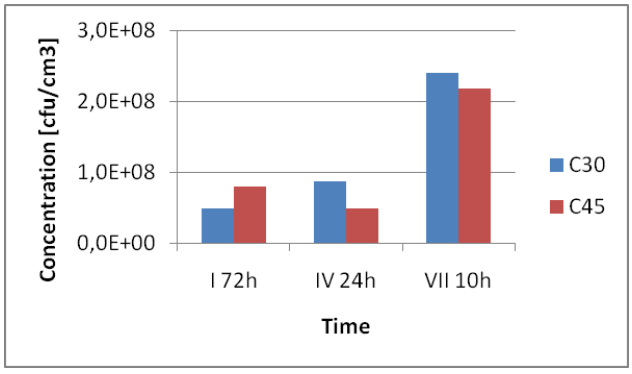

Figure 4. Concentrations of bacteria inside capsules in various periods (I, IV and VII).

After the preparation capsules had a dense membrane structure with a polymeric scaffold network inside (figure 5.)

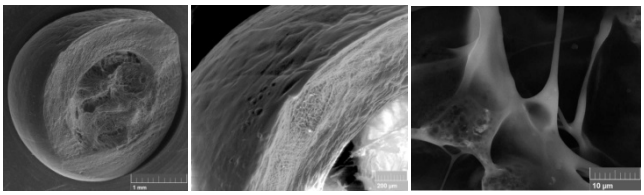

Figure 5. Structure of capsule after preparation.

At the end of bioconversion process (after stage VII) capsule inside became hollow, however their outer membranes remained compact (figure 6).

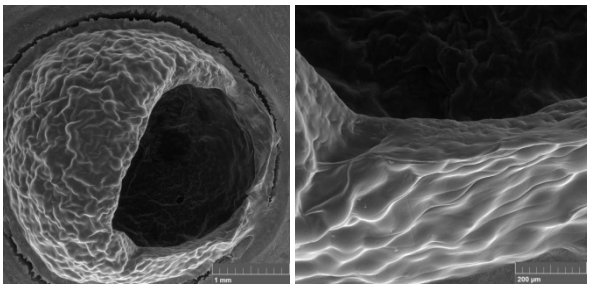

Figure 6. Structure of capsule at the end of bioconversion process.

For some of the capsules due to high gas production of *Citrobacter freundii* a breakage of capsule membrane could be observed which explains the presence of bacteria in outer culture medium.

CONCLUSIONS

We have presented novel binary polyelectrolyte microcapsule system, which can be obtained in a simple one step method as result of reaction between two oppositely charged polymers. This method could be applied in bioimmobilisation of bacteria to enhance the typical biotechnological processes. These capsules are much more stable in typical medium compare to standard alginate/calcium complex and can be utilized continuously at least several times.

Thus system will be further developed to overcome the breakage of some of the capsules due to the gas production of *Citrobacter freundii* bacteria.

REFERENCES

- Pourbafrani M. et al. (2007) *Protective Effect of Encapsulation in Fermentation of Limonene-contained Media and Orange Peel Hydrolyzate* Int. J. Mol. Sci. 8, 777-787.

ACKNOWLEDGEMENTS

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