MaCuMBA

Marine Microorganisms: Cultivation Methods for Improving their Biotechnological Applications

Project number: 311957
Start of the project (duration): August 1st, 2012 (48 months)

Collaborative Project
Seventh Framework Programme
Cooperation, KBBE

Deliverable D3.4

Deliverable D3.4: Immobilization of cells from extreme environments

Organisation name of lead contractor: UBO-LM2E (partner 3)

Due date of deliverable: M12
Actual submission date: M18

Revision: V.1

Project co-funded by the European Commission within the Seventh Framework Programme (2007-2013)	
Dissemination Level	
PU Public	
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	**X**

List of reviewers

Issue	Date	Implemented by
v.1		

Indicate any document related to this deliverable (report, website, ppt etc) and give file name

** Please attach deliverable documents and any additional material if needed. Presentation during the macuMBA General Assembly in Roscoff, September 2013*

Roscoff_LM2E_WP3.pdf

Summary

Write a short informative summary of your Deliverable (2 pages maximum), which should include the following elements:

Objective(s): of the deliverable D3.4
In the frame of the WP3 project, the LM2E is developing cell immobilization in order to improve existing free-cell continuous culture facilities. Microorganism immobilization should increase population stability while better mimicking the attached life-style for the culturing of deep-sea hydrothermal microbial communities. The objective of this deliverable is the development of a protocol dedicated to the immobilization of thermophilic marine microorganisms.

Rationale: describe the approach/methodology you chose to reach the objective(s)

The cultivation of thermophilic microorganisms in a gas-lift bioreactor is currently performed at LM2E, but the immobilization of thermophilic marine microorganisms has never been done. The immobilization parameters were chosen in order to preserve a maximum of cell viability, while allowing good microbial growth and high mechanical stability of the beads that will have to be maintained during several weeks of continuous culture in extreme conditions (high temperature, presence of sulphur and eventual pH variations). We chose to encapsulate the cells using an emulsification technique that is a gentle process feasible in the strictly anaerobic and high temperature conditions necessary for the maintaining of viability of the targeted microorganisms. The polymers mixture (gellan and xanthan) was chosen to be heat stable, to prevent water syneresis, to be not toxic for marine microorganisms and to sustain good microbial growth, while harbouring a high mechanical stability. Cell immobilization in a polymer matrix is based on a dispersion process in a two phase system composed of a polymer solution and oil under agitation, which allows the production of beads (1-2 mm diameter) recovered by sieving. The development of the immobilization for marine thermophiles required a large update because of the extreme conditions. The addition of salt in particular, necessary for the maintaining of cell viability, was a real challenge knowing the strong impact of the ionic strength on gel polymerisation. Moreover, the ATP luminometric method had to be adapted in order to measure cell viability and growth of encapsulated cells (bacteria and archaea).

Results: a short description of the results or decisions taken for this deliverable. If there is any delay, please explain why and mention if it affects any other deliverable or related deliverable.

The best immobilization conditions, allowing the production of a maximum of beads of good mechanical strength, consist in the use of a mixture of two polymers, gellan (2.5%) and xanthan (0.25%) with 12g/L of NaCl at an emulsion temperature of 80°C with a stirring speed of 250 rpm. Different salt concentrations were tested in order to allow beads formation while preserving cells from an osmotic stress. Rheological tests were carried out on 2 types of polymers (gellan or gellan/xanthan mixture) at different salt concentrations in order to choose the best conditions for the improvement of beads resistance during cell growth and stirring within the bioreactor. Beads resistance (beads size, shape and weight) in different growth conditions (temperatures ranging from 50 to 100°C, pH from 4 to 8, sulphur concentrations from 1 to 5 g/L and salt concentrations from 5 to

80 g/l) during 5 weeks of incubation were tested with a factorial experiment. The more detrimental incubation conditions were induced with high temperature (100°C) and acid pH (4). Incubation may then be performed between 50 and 90°C, pH of 5.4 to 8. A mixture of thermophile microorganisms (*Thermococcus kodakarensis* KOD1, *Marinitoga hydrogenitolerans* AT1271, and *Thermosipho* spp. MV1063 and AT1272) was immobilized in beads in order to check their survival and growth capacity during a preliminary batch experiment in Ravot modified medium at 60°C. The survival (*ca* 30%) of microorganisms after encapsulation together with the growth of thermophile microorganisms in gel beads suggest that this technique could be used for high temperature microbial community cultivation in a gas-lift bioreactor. Complementary studies are under investigation in the laboratory to prove the feasibility of the system during continuous culture experimentations.

Partner(s) involved in Deliverable production: UBO-LM2E partner 3

Protocol of immobilization of marine thermophile microorganisms

(The detailed protocol will be the subject of a publication)

Manufacture of beads under anaerobic condition

1. Transfer the autoclaved polymer solution (xanthan 0.25% and gellan 2.5%) and oil in the anaerobic chamber
2. Add 50 mL of the saline solution (12g/L NaCl) in the polymer solution and 2% of the inoculum under agitation
3. Pour the polymer solution in oil
4. Stir for 10 minutes in order to obtain beads of 1 to 2 mm diameters
5. Cool the emulsion up to 30°C under stirring
6. Stop the emulsion, remove the oil in excess and put the rest (including the magnetic bar) in a sterile beaker (2 L).
7. Add the hardening solution (0.1 M $CaCl_2$) to fill the beaker up to 1.4 L and shake for 30 minutes.