New frontiers in living cell encapsulation

DEPARTMENT OF AGRICULTURAL SCIENCE
UNIVERSITY DEGLI STUDI DI PERUGIA

UNIVERSITÀ DEGLI STUDI DI PERUGIA

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APPLICATION OF VIBRATING TECHNOLOGY FOR MICROENCAPSULATION OF BACTERIAL CELLS AND NISIN

8-9 MARZO 2013

Vibrating technology

Vibrating techonology is a new technique for the production of different types of microcapsules for application in biotechnological processes.

A) FEEDING SYSTEM:

The system is fed with a polymer-product mixture contained in a syringe or in a pressure bottle. The carrying capacity of the feeding mixture can be regulated by the PUMP function.

B) PULSATING CHAMBER AND NOZZLE:

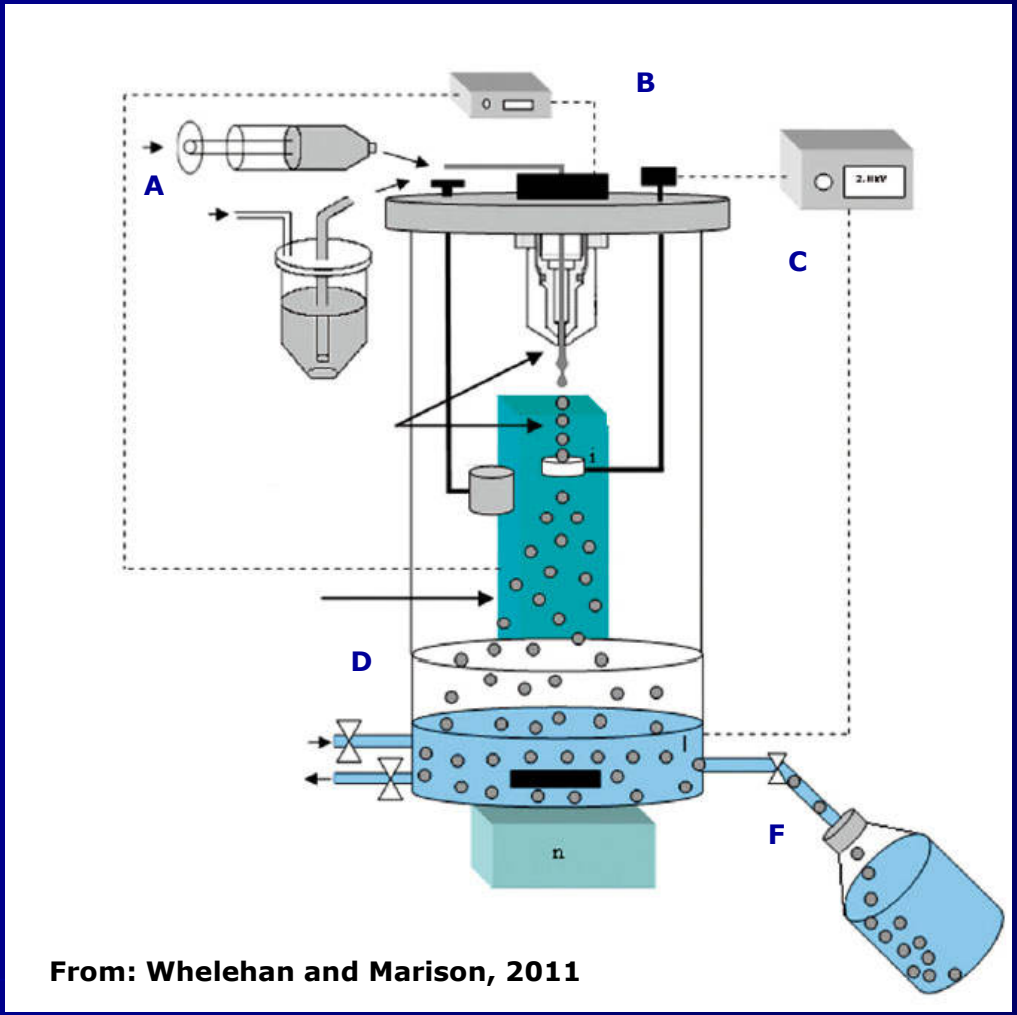
This mixture is pumped in a pulsating chamber where the vibration of a membrane breaks the mixture linear flow leading to the formation of droplets. Then, droplets are extruded through a nozzle. The number of the vibrations/sec. can be regulated by the FREQUENCY function.

C) MAGNETIC FIELD:

The application of a magnetic field generated between the nozzle and the electrod, leds to adroplet's surface charge. Electrostatic repulsion forces among droplets cause the dispersion of the beads. The intensity of the magnetic field can be regulated by the ELECTRODE function.

D-F) GELIFING SOLUTION:

Finally, beads are collected in an hardening solution, and microcapsules are subsequently recovered.



From: Whelehan and Marison, 2011

Microencapsulation of *Lactobacillus reuteri* DSM 17938 and nisin: the experimental plan

Microencapsulated products:

***Lactobacillus reuteri* DSM 17938**

having probiotic properties:

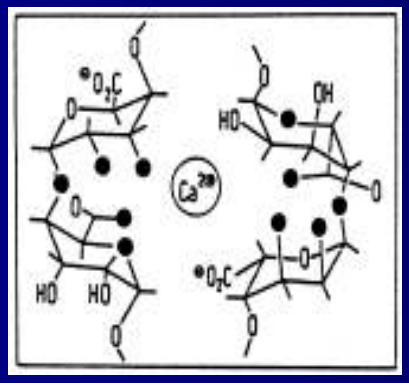
- stimulation of immune response;
- reduction of intestinal mucosa permeability;
- regulation of intestinal microbiota;
- prevention of bacterial colonization;
- production of antimicrobial substances.

Nisin

a bacteriocin produced by *Lactococcus lactis* subsp. *lactis* recognized as safe (GRAS) for food application by FDA. Bacteriocins are ribosomally synthesized by lactic acid bacteria; these compounds are antimicrobial peptides active against Gram+ bacteria.

Microencapsulating agent:

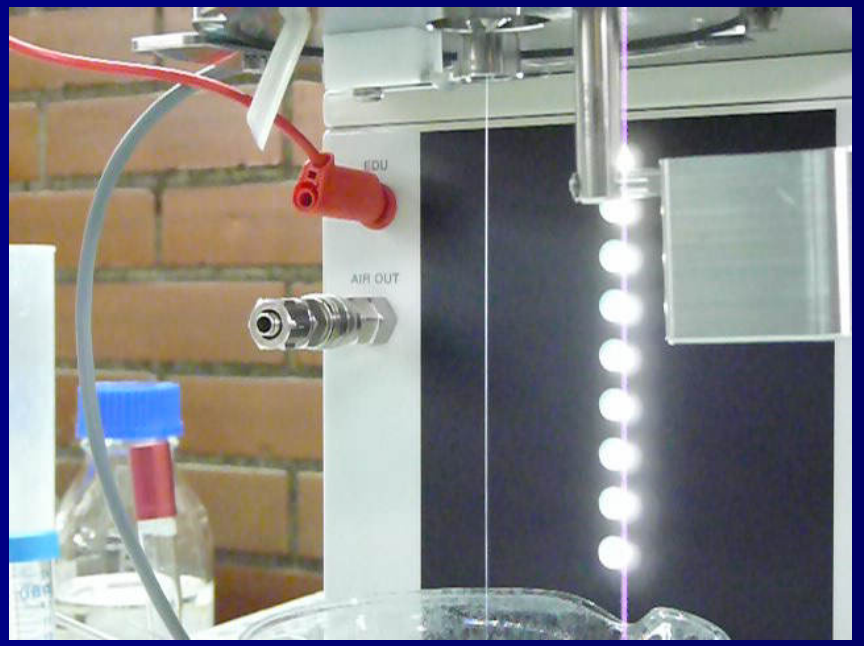
$CaCl_2$ → **Alginic acid**



Encapsulation parameters

Lactobacillus reuteri DSM 17938	
Parameter	**Value**
Encapsulated solution	*L. reuteri* in 2% alginate
Pump	2.91 ml/min
Frequency	1700 hz
Electrode	850 V
Hardening solution	0.5 M $CaCl_2$
Nozzle diameter	80 μ m

8% nisin solution	
Parameter	**Value**
Encapsulated solution	*Nisin* in 1.5% alginate
Pump	2.80 ml/min
Frequency	1700 hz
Electrode	950 V
Hardening solution	0.25 M $CaCl_2$
Nozzle diameter	80 μ m



In *vitro* test

Microencapsulated *Lactobacillus reuteri*

I) **Staining of *Lactobacillus reuteri* microcapsules with Bac-Light**

II) **Enumeration of microencapsulated *Lactobacillus reuteri before* and after alginase digestion**

III) **Heat treatment of *Lactobacillus reuteri before and after microencapsulation***

Work in progress…

In *vitro* test

Microencapsulated nisin

I) Staining of microencapsules containing nisin with isothiocyanate fluorescein (FITC)

II) Biological activity of free and microencapsulaed nisin against *Brochothrix thermosphacta* 7R1

III) Enzymatic digestion by protease and alginase of microcapsulated nisin

Work in progress...

Microencapsulation of *Lactobacillus reuteri* DSM 17938: results

I) Viable staining of microencapsulated cells:

All microcapsules appeared as spherical structure of aboute 150 μm in diameter; stained microcapsules mainly contain green-coloured cells (viable cells) and very few red-coloured cells (damaged cells). Furthermore, fluorescence microscopy images show that rarely free cells were observed.

II) Enumeration (UFC/ml)ml of microencapsulated cells before and after alginase treatment:

	Free cells	Microencapsulated cells	Microencapsulated cells treated with alginase
L. reuteri UFC/ml	5.5×10^9	5.5×10^7	5.5×10^8

III) Heat treatment of microencapsulated cells:

	Free cells	Free cells after heat treatment	Microencapsulated cells	Microencapsulated cells after heat treatment
L. reuteri UFC/ml	5.5×10^9	4.5×10^5	5.5×10^7	5.5×10^6

Work in progress...

Microencapsulation of *nisin:* results

I) Staining of microencapsulated nisin:

Microcapsules appeared as no perfect spherical structure but….. The size was of aboute 100 μm in diameter; fluorescence microscopy immages show that all nisin was contained in microcapsule structure and non microencapsulated nisin was detected.

II) Biological activity of free and microencapsulaed nisin against *Brochothrix thermosphacta* 7R1:

	Free nisin	Microencapsulated nisin
Antimicrobial Activity UI/ml	25600	12800
Diameter of inibition zone (cm)	2.7±0.16	1.48±0.13

III) Biological activity of nisin microencapsules against *Brochothrix thermosphacta* 7R1 after enzymatic digestion by protease and alginase:

	Microencapsulated nisin	Microencapsulated nisin after protease digestion	Microencapsulated nisin after alginase digestion
Antimicrobial Activity UI/ml	12800	6400	6400
Diameter of inibition zone (cm)	**1.48**±0.13	0.91±0.07	1.13±0.9

Work in progress…

Conclusions

Microencapsulation by emulsion

Antimicrobial activity of carvacrol was not revealed in any test: our data suggest that experimental plan applied in this work has not been able for carvacrol microincapsulation

Microencapsulation by spray drying

Results observed suggest that dilution in $CaCl_2$ is a limiting factor for microcapsules antimicrobial activity. After that, carvacrol proved to be a compound able to resist the spray drying process, thanks to its physical properties and to maintain its antimicrobial activity.

Thanks
for your attention