8th Euro Biotechnology Congress

OMICS International

SURFACE ACTIVITY OF SURFACTIN UNDER EXTREME CONDITIONS (pH, TEMPERATURE AND IONIC STRENGTH) – A PROSPECTION FOR MICROBIAL ENHANCED OIL RECOVERY

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Co-supervisior – Dr. Francisco F. C. Barros
Supervisior (Split PhD) – Dr. Paula Jauregi

UNICAMP

Frankfurt am Main-BRD, 2015

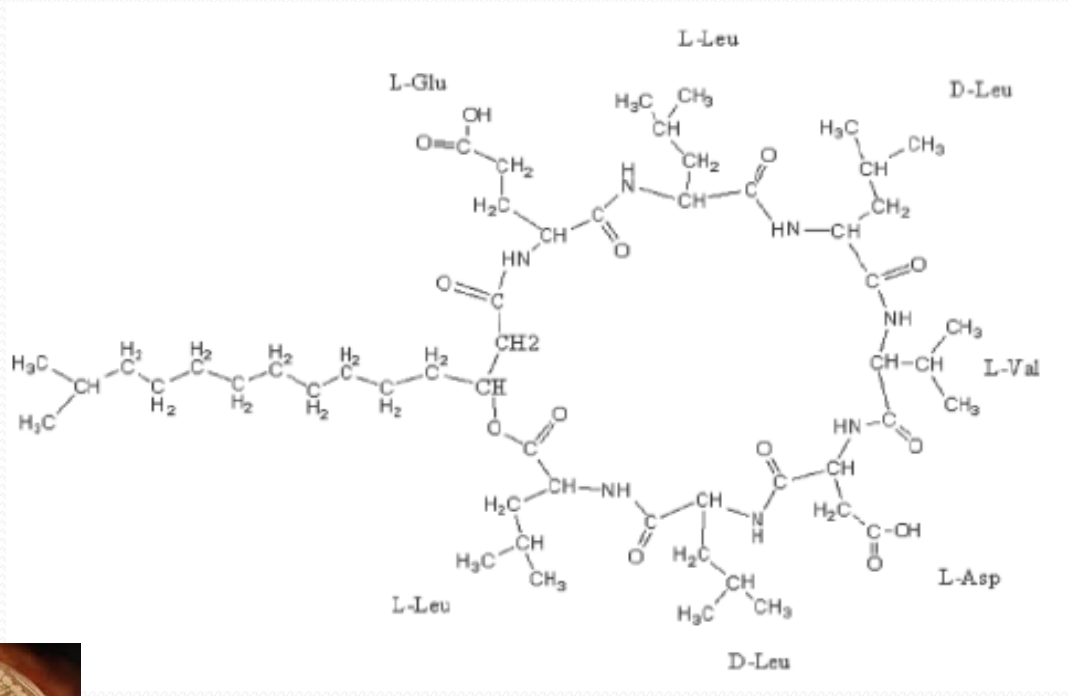
THE AIM OF THIS STUDY WAS…

- ✓ BIOTECHNOLOGICAL PRODUCTION
- ✓ PURIFICATION
- ✓ CHEMICAL STRUCTURE IDENTIFICATION
- ✓ APPLICATION - MEOR

SCREENING OF BIOSURFACTANT PRODUCERS

Bacillus subtilis LB5a

SCREENING OF BIOSURFACTANT PRODUCERS



SURFACTIN – lipopeptide

Bacillus subtilis LB5a

COMPOSITION OF CULTURE MEDIUM

Al (mg/L)	138-158	**Zn (mg/L)**	1.4-7.38
Total P (mg/L)	83.3-369	**Total N (g/L)**	1.72-2.67
K (mg/L)	895-3641	**NO_3- (mg/L)**	15.2-17.2
Ca (mg/L)	184-293	**NH_3 (mg/L)**	129-133
Mg (mg/L)	173-519.09	**COD (g O_2/L)**	45.36-60
S (mg/L)	38-154	**pH**	5.3-6,0
Fe (mg/L)	2.72-8.0	**Total sugar (g/L)**	33.78-58.18
Mn (mg/L)	1.5-3.46	**Reducing sugar (g/L)**	12.23-38
Cu (mg/L)	0.3-1.11	**Non-reducing sugar (g/L)**	20.1-23.3
B (mg/L)	3.0-5.0	**Total solids(g/L)**	60-62

PRODUCTION STRATEGIES

Batch fermentation	Feed-Batch fermentation	Continuous fermentation
Flask (250 mL) Top-bench bioreactor (7 L) Pilot scale (80 L)	Top-bench bioreactor (7 L)	Top-bench bioreactor (7 L)

Simultaneous production

diacetyl (2,3-butanedione), acetoin (3-hydroxy-2-butanone), and 2,3-butanediol,

Proteases, amylases and lipases

PRODUCTION

PURIFICATION

IDENTIFICATION

APPLICATION

PRODUCTION

PURIFICATION

IDENTIFICATION

APPLICATION

Bioresource Technology

Volume 97, Issue 2, January 2006, Pages 336–341

Production and properties of a surfactant obtained from *Bacillus subtilis* grown on cassava wastewater

Biotechnology Research International
Volume 2013 (2013), Article ID 103960, 9 pages
http://dx.doi.org/10.1155/2013/103960

Research Article

Production of Enzymes from Agroindustrial Wastes by Biosurfactant-Producing Strains of *Bacillus subtilis*

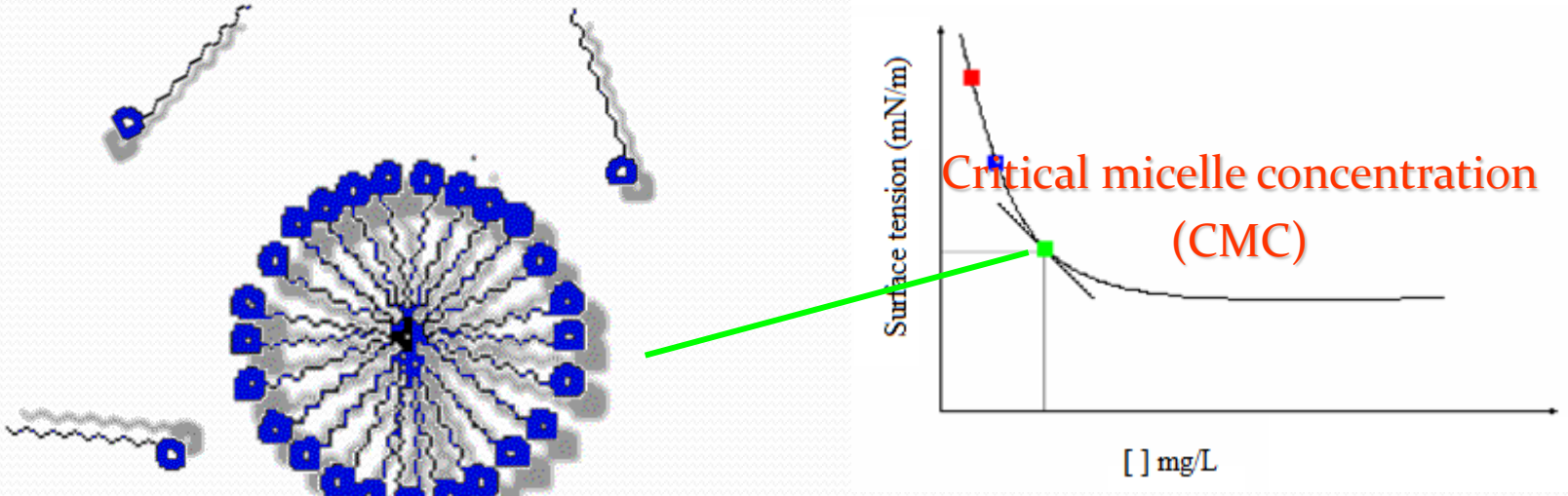
Francisco Fábio Cavalcante Barros, Ana Paula Resende Simiqueli, Cristiano José de Andrade, and Gláucia Maria Pastore

Department of Food Science, Faculty of Food Engineering, University of Campinas, P.O. Box 6121, 13083-862 Campinas, SP, Brazil

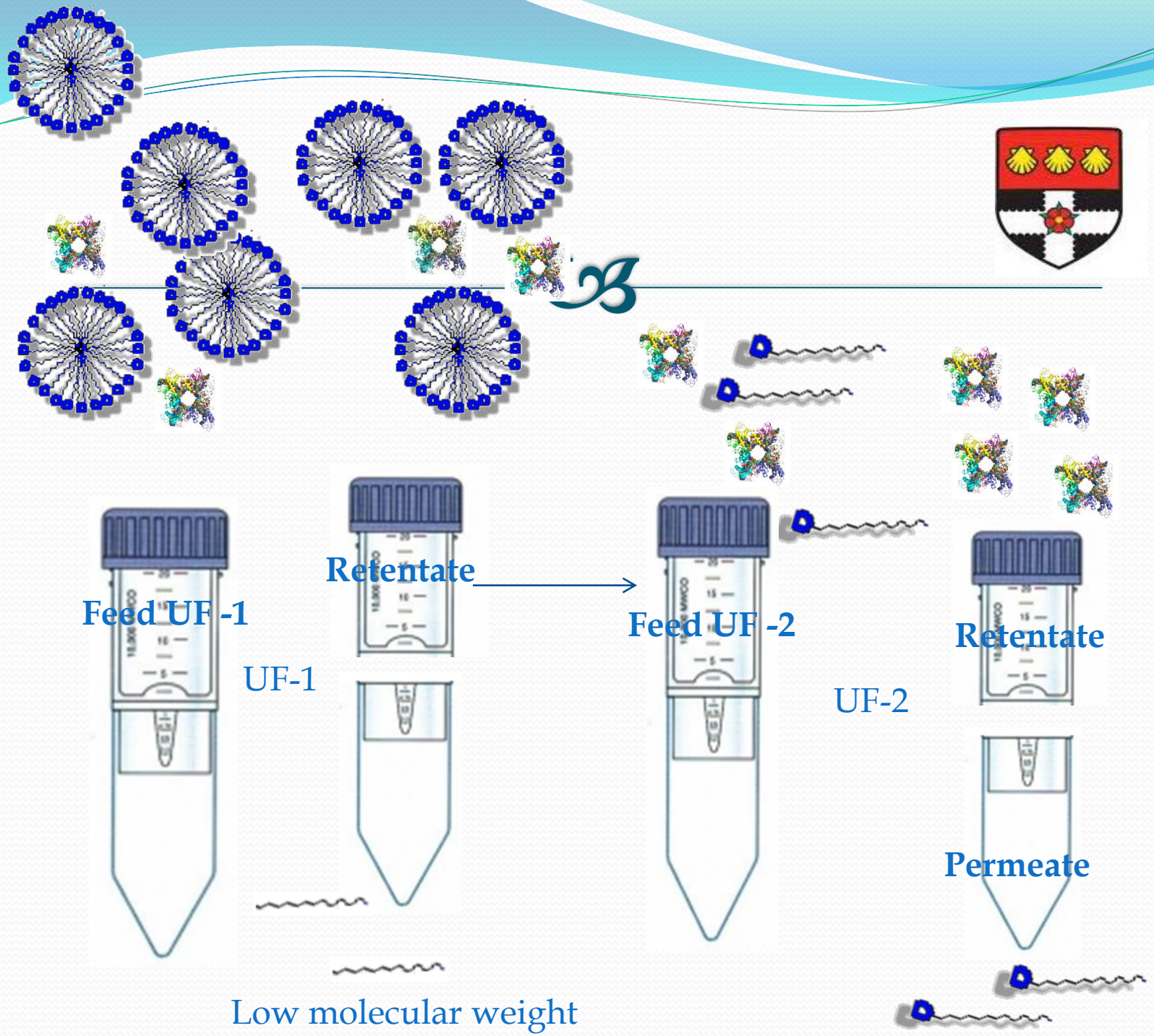
Francisco Fábio Cavalcante Barros, Mario Cezar Rodrigues Mano, Juliano Lemos Bicas, Ana Paula Dionísio, Cedenir Pereira Quadros, Mariana Uenojo, Iramaia Angélica Neri, Gláucia Maria Pastore

PURIFICATION...BUT HOW TO DO IT?

Ultrafiltration in two steps of surfactin produced by *Bacillus subtilis* LB5a using cassava wastewater as substrate



- Surfactin - CMC ≈10 mg/L
- Rhamnolipids- CMC ≈20 a 40 mg/L
- Synthetic surfactants - CMC from 590 to 2000 mg/L


Feed UF -1
Retentate
UF-1
Feed UF -2
Retentate
UF-2
Permeate
Low molecular weight

PRODUCTION

PURIFICATION

IDENTIFICATION

APPLICATION

< Uso exclusivo do INPI >

INPI marca/cia

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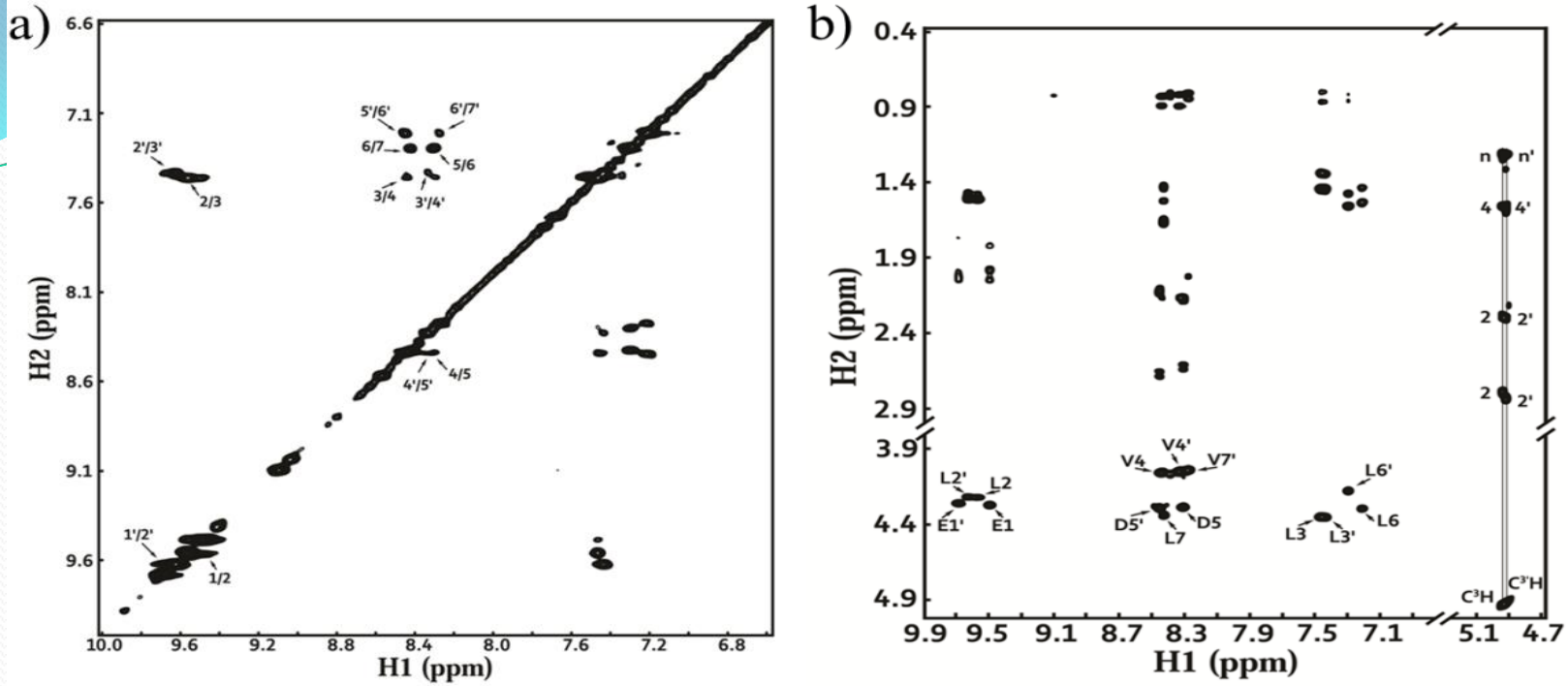
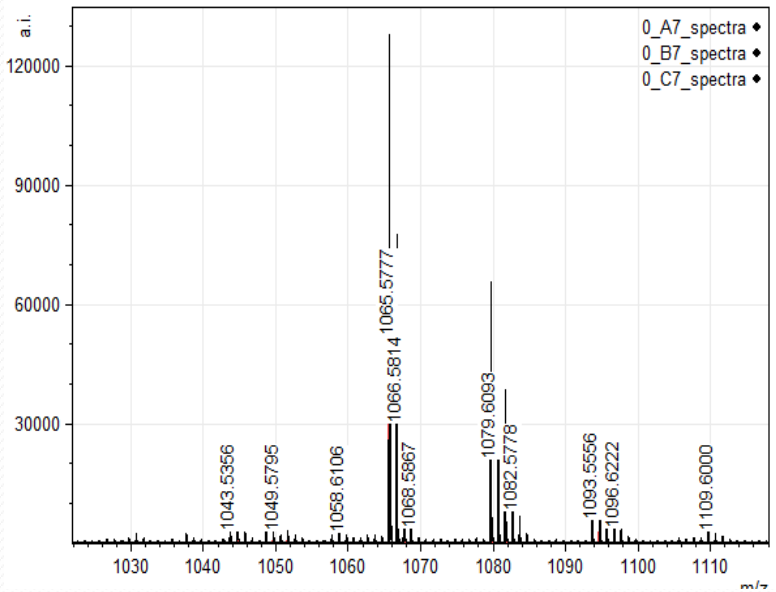
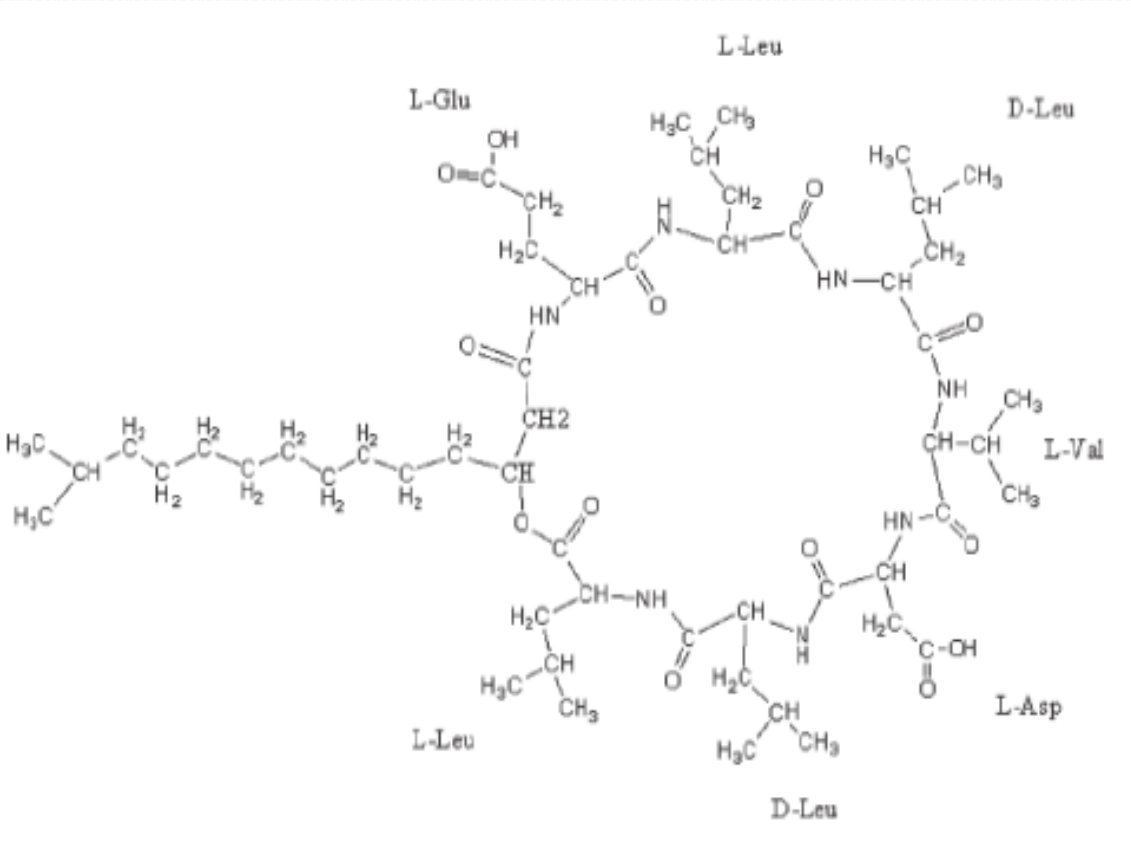
DIRPA	Tipo de Documento: Formulário	DIRPA	Página: 1/3
Título do Documento: Depósito de Pedido de Patente		Código: FQ001	Versão: 01
		Procedimento: DIRPA-PQ006	

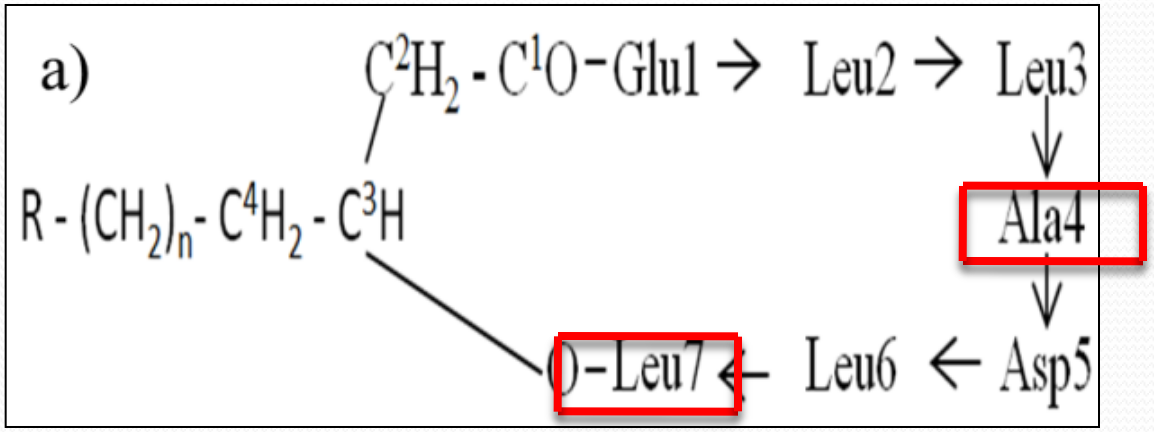


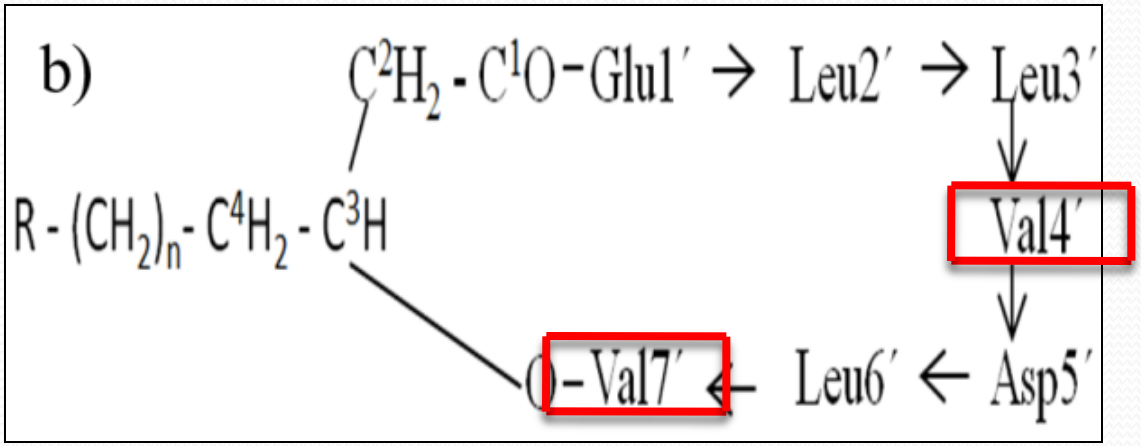
NMR



MALDI-TOFMS


L-Leu
L-Glu
D-Leu
L-Val
L-Asp
L-Leu
D-Leu


a)
C^2H_2 - C^1O - Glu1 → Leu2 → Leu3
R - $(CH_2)_n$ - C^4H_2 - C^3H
Ala4
O - Leu7 ← Leu6 ← Asp5

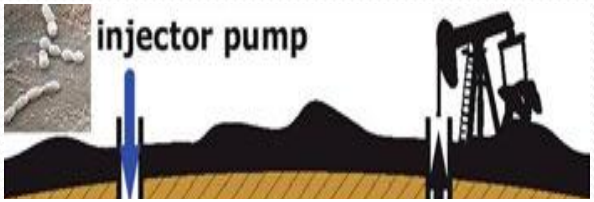

b)
C^2H_2 - C^1O - Glu1′ → Leu2′ → Leu3′
R - $(CH_2)_n$ - C^4H_2 - C^3H
Val4′
O - Val7′ ← Leu6′ ← Asp5′

POTENTIAL APPLICATIONS

- ✓Antimicrobial
- ✓Emulsifier
- ✓Therapeutic agents
- ✓Pesticide
- ✓Food
- ✓Cosmetic
- ✓Etc

✓MICROBIAL ENHANCE OIL RECOVERY

MEOR

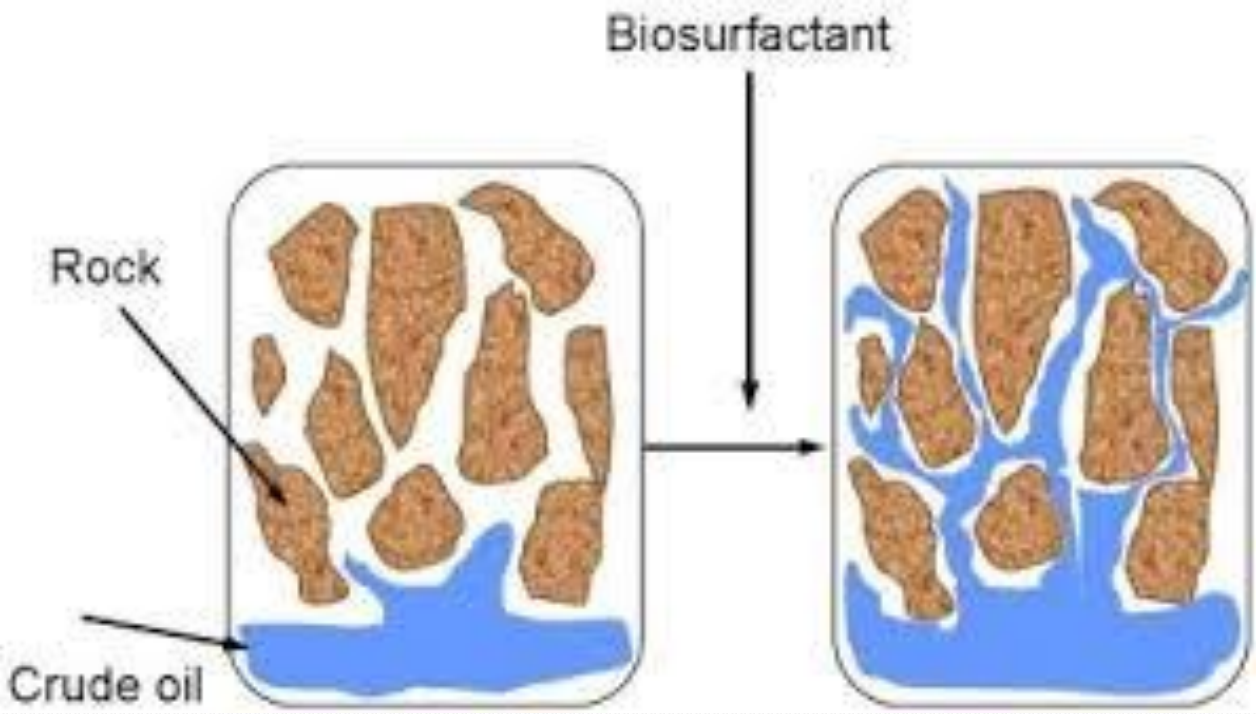


Thus, the aim of microbial Enhanced Oil Recovery technologies is mainly the remaining oil in the wells – after the primary and secondary techniques - which is ≈3 trillion barrels (Shibulal et al. 2014 - Scientific World Journal).

PRIMARY RECOVERY

SECONDARY RECOVERY

MEOR



MEOR

ELSEVIER

International Biodeterioration & Biodegradation

Volume 97, January–February 2015, Pages 188–194

Review

Progress in pilot testing of microbial-enhanced oil recovery in the Daqing oilfield of north China

Jian-Jun Le, Xiao-Lin Wu, Rui Wang, Ji-Yuan Zhang, Lu-Lu Bai, Zhao-Wei Hou

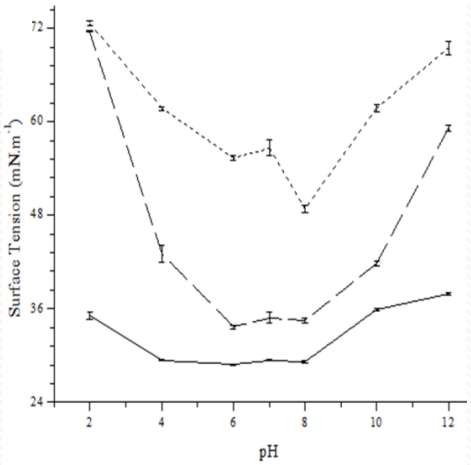
It was described that in the Daqing oilfield, the temperature ranged from 45 to 890C and the ionic strength ≈15g.L^{-1}, that is, the interaction of these parameters has to be consider.

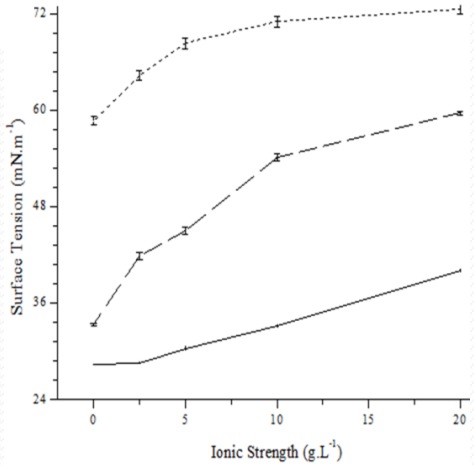
MEOR

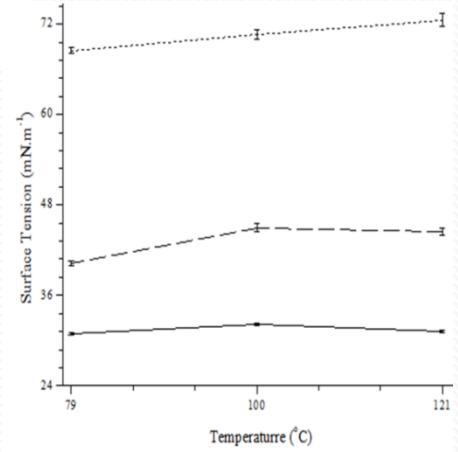
This study describes for the first time, the surface activity measurements of surfactin at extreme conditions of temperature, ionic strength and pH (one at a time) and its interaction.

MEOR

ONE A TIME





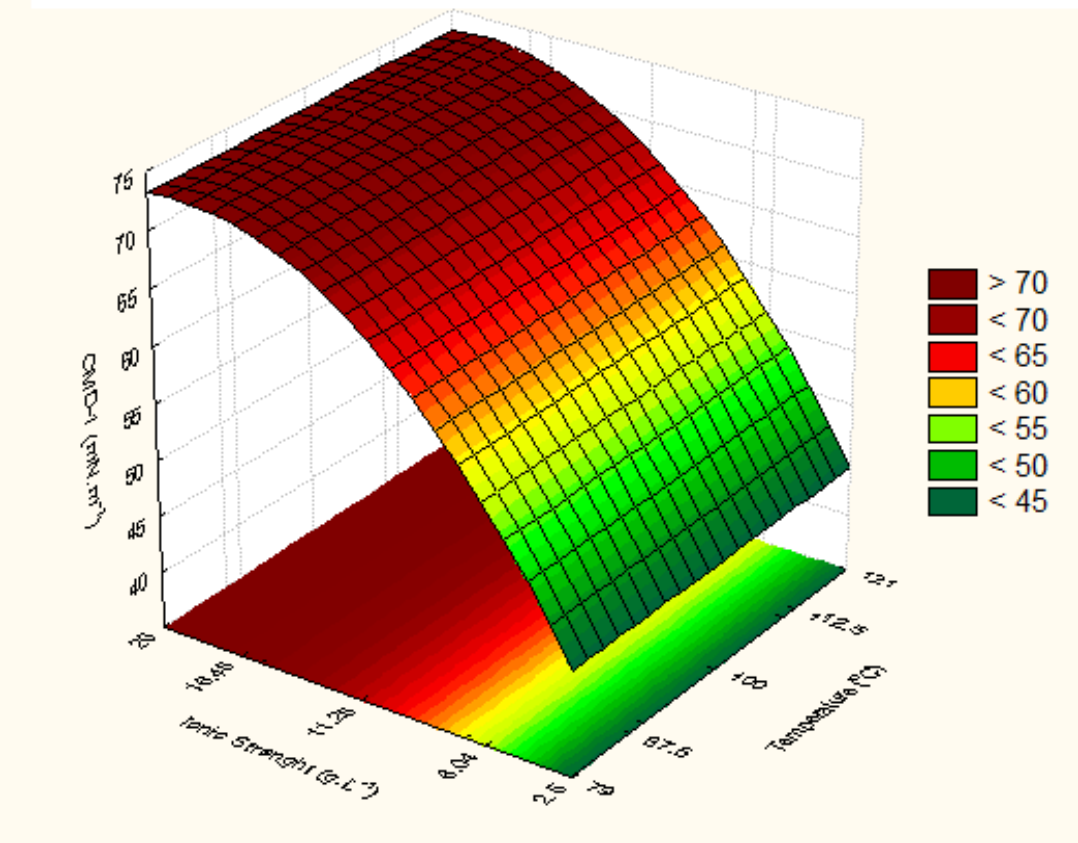


Temperature does not affect the surface activity of surfactin

Central composite design

Experiment	Coded levels			Experimental Levels			Produced surfactin	
	x_1	x_2	x_3	Temperature (°C)	pH	Ionicstrength (g.L^{-1})	ST (mN.m^{-1})	CMD-1 (mN.m^{-1})
1	-1	-1	-1	87.5	4.02	6.04	33.38	48.91
2	+1	-1	-1	112.5	4.02	6.04	37.53	62.44
3	-1	+1	-1	87.5	9.97	6.04	37.67	48.29
4	+1	+1	-1	112.5	9.97	6.04	35.02	51.23
5	-1	-1	+1	87.5	4.02	16.45	41.27	72.34
6	+1	-1	+1	112.5	4.02	16.45	43.95	72.54
7	-1	+1	+1	87.5	9.97	16.45	45.42	72.54
8	+1	+1	+1	112.5	9.97	16.45	43.15	72.71
9	-1.68	0	0	80	7	11.25	38.36	72.5
10	+1.68	0	0	121	7	11.25	46.65	60.95
11	0	-1.68	0	100	2	11.25	47.53	72.74
12	0	+1.68	0	100	12	11.25	40.94	63.6
13	0	0	-1.68	100	7	2.5	29.95	49.87
14	0	0	+1.68	100	7	20	42.07	72.21
15	0	0	0	100	7	11.25	38.71	71.99
16	0	0	0	100	7	11.25	37.36	70.28
17	0	0	0	100	7	11.25	37.76	67.36

MEOR


75
70
65
60
55
50
45
40
20
16.66
13.33
10
6.66
3.33
0
Ionic Strength (g.L-1)
79
87.6
100
112.5
125
Temperature (°C)
> 70
< 70
< 65
< 60
< 55
< 50
< 45

MEOR

The ANOVA of ST and CMD-1 indicates that parameters (temperature, pH and ionic strength) are statistically different (95% of confidence) [(Fcalregression 23.02; Ftab 3.74); (Fcal lack of fit 5.01; Ftab19.41)] - (r2 of 0.76). Thus, the CCD analysis of surfactin - CMD-1 – resulted the following equation:

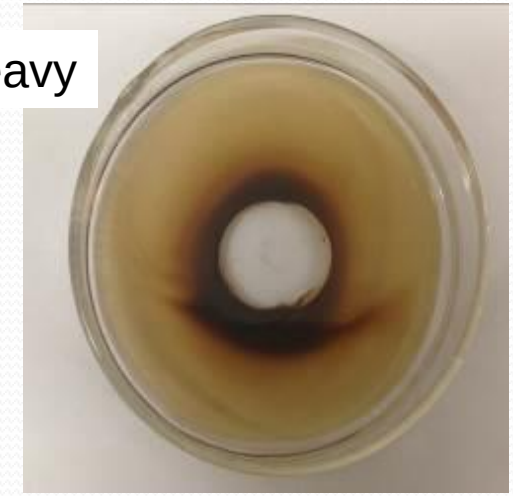
$$\text{Equation (1): } Y = 67.28 + 8.5x - 3.02x^2$$

Y is CMD-1 of surfactin, x ionic strength and x^2 ionic strength squared.

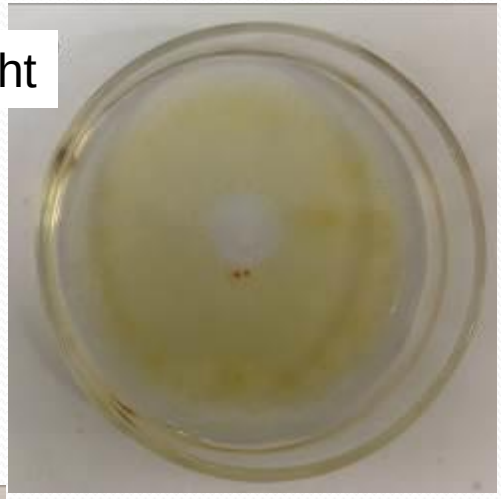
The ionic strength is the most significant parameters on the surface activity. It was observed that pH minimally influences the surface activity of surfactin. The same trend that temperature. The derivate of equation 1 with $Y' = 0$ (maximum– red area) indicates that at 18.58 $g.L^{-1}$ of brine is the threshold, when associate to extreme pH and temperature, in order to keep the surface activity.

MEOR

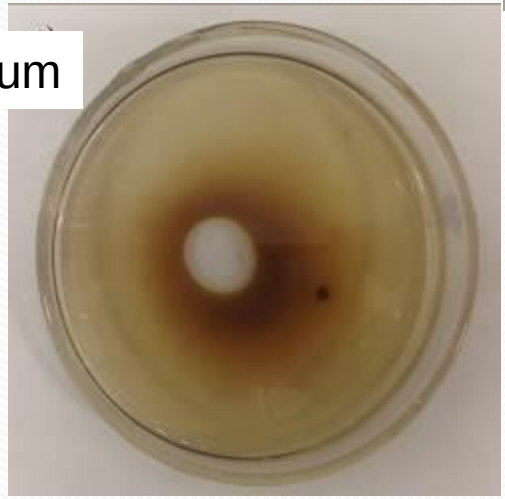
heavy



light



medium



CONCLUSION

✓The surfactin, that was produced by cassava wastewater as culture medium and purified by ultrafiltration, has remarkable surface activiy under extreme conditions and also great potential to be used for MEOR .

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Danke schön