Modeling of Biosurfactant Production by *Lactobacillus* Strains

Lígia Rodrigues,[1*] Ana Moldes[2], José Teixeira[1] and Rosário Oliveira[1]

[1]Centro de Engenharia Biológica, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal, E-mail: lrmr@deb.uminho.pt*
[2]Department of Chemical Engineering, University of Vigo, Campus Ourense, As Lagoas s/n, 32004 Ourense, Spain.

ABSTRACT

Screening of biosurfactant-producing ability of three Lactobacillus strains was performed, being shown that, for all the tested strains, biosurfactant production is occurring mainly in the first 7 hours. All strains reduced 8 mN/m the surface tension of the fermentation broth at the end of fermentation and lysed blood agar with scores ranging between (++) corresponding to complete hemolysis with a diameter < 1 cm. Time courses of lactose, biomass and biosurfactant were modeled according to reported models, assuming product inhibition. Using optimized MRS broth as culture medium, the values estimated by the modeling of biosurfactant were P_{max} = 1.4 g of biosurfactant/L and r_p/X = 0.137g/L.h^{-1} for Lactobacillus casei CECT-5275, P_{max} = 1.5 g/L and r_p/X = 0.145g/L.h^{-1} for Lactobacillus rhamnosus CECT-288 and P_{max} = 1.5 g/L and r_p/X = 0.089 g/L.h^{-1} for Lactobacillus pentosus CECT-4023. Using whey as production medium, the values obtained for Lactobacillus rhamnosus CECT-288 were P_{max} = 1.3 g of biosurfactant/L and r_p/X = 0.078 g/L.h^{-1}. In conclusion, the results obtained showed that whey can be used as an alternative medium for biosurfactant production by Lactobacillus strains.

INTRODUCTION

Lactobacillus species are the most common members of indigenous microflora of the mammalian urogenital tract (Velraeds *et al.* 1996 (a)). *Lactobacillus* and *Streptococcus* species have been shown to be able to displace adhering uropathogenic *Enterococcus faecalis* from hydrophobic and hydrophilic substrata in a parallel-plate flow chamber, possibly through biosurfactant production (Velraeds *et al.* 1996 (b)). Velraeds and co-workers (1996(b)) concluded that certain strains of lactobacilli release protein-rich biosurfactants for which they proposed the name “surlactin”.

Biosurfactants are biological surface-active compounds released by microorganisms that can have some influence on interfaces. With regard to an anti-adhesive effect of biosurfactants, hypotheses have been forwarded in which adsorption of biosurfactants to a substratum surface alters the hydrophobicity of the surface and causes interference in microbial adhesion and desorption processes (Desai and Banat 1997). Biosurfactants have also been reported to have various degrees of antimicrobial activity (Banat *et al.* 2000). Several biosurfactants have strong antibacterial, antifungal and antiviral activity (Singh and Cameotra 2004). Interest in biosurfactants has increased considerably in recent years, as they are potential candidates for many commercial applications in the petroleum, pharmaceuticals, biomedical and food processing industries (Desai and Banat 1997). Dairy *Streptococcus thermophilus* strains, for example, can produce biosurfactants that cause their own desorption (Busscher *et al*. 1994) and Rodrigues *et al*. (2004 (a)) found that a biosurfactant obtained from *S. thermophilus* A showed a significant antimicrobial activity against *C. tropicalis* GB 9/9, being this strain a major contributor to the premature failure of voice prostheses. Additionally, the use of a biosurfactant from *Lactococcus lactis* 53 as antimicrobial agent and

its ability to inhibit adhesion of various microorganisms isolated from explanted voice prostheses has been demonstrated (Rodrigues *et al* 2004 (b)).

The aims of this study were to screen a number of *Lactobacillus* strains for biosurfactant production by blood agar method and surface tension determination, and to model the biosurfactant production as well as the time courses of lactose consumption and biomass growth. The relation between cellular growth and surface-activity of the biosurfactant in time (as a measure of its production) was determined for all the strains.

MATERIALS AND METHODS

Strains and culture conditions

The bacterial strains *Lactobacillus casei* CECT-5275, *Lactobacillus rhamnosus* CECT-288 and *Lactobacillus pentosus* CECT-4023 obtained from the Spanish Collection of Type Cultures (Valencia, Spain) were stored at –20ºC in MRS broth (deMan Rogosa Sharpe) containing 15% (v/v) glycerol solution until ready to use. From frozen stock, bacteria were streaked on MRS agar plates and incubated at the optimum temperature for each strain. Growth curves for the *Lactobacillus* strains were determined because biosurfactant production may be influenced by the growth phase of the organisms (Desai and Desai 1993). The bacterial strains were cultured in 100 ml optimized MRS broth (Rodrigues *et al.* 2005) and growth was measured by determining the optical density at 600 nm during different time intervals up to 72 h. The biomass concentrations (g dry weight l^{-1}) were determined using a calibration curve.

Blood Agar screening method

Each strain was streaked onto blood agar plates and incubated for 48 h at 37 ºC or 31ºC. The plates were visually inspected for zones of clearing around colonies, indicative of biosurfactant production. The diameter of the clear zones depends on the concentration of the biosurfactant and the zones of clearing were scored as follows: ‘-‘, no hemolysis; ‘+’, incomplete hemolysis; ‘++’, complete hemolysis with a diameter of lysis < 1 cm; ‘+++’, complete hemolysis with a diameter of lysis > 1 cm but < 3 cm; and ‘++++’, complete hemolysis with a diameter of lysis > 3 cm and green colonies.

Biosurfactant production and Surface-activity determination

The bacterial strains were cultured in 100 ml optimized MRS broth and grown for 72 h, in ambient air, at 31ºC for *L. pentosus* and 37ºC for the other *Lactobacillus* strains. For extracellular biosurfactant determination, at different time intervals samples were taken to assay the surface activity of the media broth.

For intracellular biosurfactant determination, at the end of the experiments (72 h) cells were harvested by centrifugation (10,000 × *g*, 5 min, 10ºC), washed twice in demineralized water, and resuspended in 20 ml of phosphate-buffered saline (PBS: 10 mM KH_2PO_4/K_2HPO_4 and 150 mM NaCl with pH adjusted to 7.0) (Velraeds *et al.* 1996 (a)). The bacteria were left at room temperature up to 24 h with gentle stirring for biosurfactant release. During extraction process, samples were taken at different time intervals, bacteria were removed by centrifugation and the remaining supernatant was tested for surface activity.

For all strains the biosurfactant production was also assayed growing the strain in whey. Commercial whey was prepared as follows: after adjusting the pH to 4.5 with 5N HCl, it was heated at 121ºC for 15 min to denature the proteins. The precipitates were removed by centrifugation at 4ºC and 8,000 × g for 10 minutes. The supernatants were adjusted to pH 6.3, sterilized at 121ºC for 15 minutes and used as culture media. The supernatant contained 56 g/L of lactose and lactose concentrations were determined by high performance chromatography (Agilent, model 1100, Palo Alto, CA) using ION-300 column (Transgenomic Inc., San Jose, CA) with refractive index detector. The mobile phase was 0.01 N H_2SO_4 with a flow rate of 0.4 ml min^{-1}.

The surface tension of the culture broth samples, of the PBS extraction samples and also of the whey fermentations was measured by the Ring method using a KRUSS Tensiometer equipped with a 1.9 cm De Noüy platinum ring at room temperature. The biosurfactant concentrations (g/L) were determined for each *Lactobacillus* strain using a calibration curve. The calibration curve was calculated for a commercial biosurfactant produced by several *Bacilli* (surfactin) using different concentrations of biosurfactant solution, below the critical micelle concentration, with known surface tension, as in this concentration range the decrease of surface tension is linear and it is possible to establish a relationship between the biosurfactant concentration and the surface tension (Kim *et al.* 2000).

Lactose consumption and biosurfactant production - Fitting of Data

The commercial software Solver of Microsoft Excel 2002 was used to fit the experimental data to the proposed models by nonlinear regression using the least-squares method. Biosurfactant production was mathematically modeled following the equation proposed by Mercier *et al.* (Mercier *et al.* 1992) for lactic acid production

$$P = \frac{P_0 P_{\max} e^{P_r t}}{P_{\max} - P_0 + P_0 e^{P_r t}} \quad (1)$$

where t is time, P is biosurfactant concentration, P_{max} is maximum concentration of biosurfactant, and P_r is the ratio between the initial volumetric rate of product formation (r_p) and the initial product concentration P_0.

From the series of experimental data biosurfactant concentration/time, the model parameters P_0, P_{max}, and P_r can be calculated for each *Lactobacillus* strain growing in optimized MRS broth or on whey.

Also biomass production was mathematically modeled and can be interpreted by equation (2)

$$X = \frac{X_0 X_{\max} e^{\mu_{\max} t}}{X_{\max} - X_0 + X_0 e^{\mu_{\max} t}} \quad (2)$$

where *t* is time, *X* is biomass concentration, X_{max} is maximum concentration of biomass, and μ_{max} is the ratio between the initial volumetric rate of biomass formation and the initial biomass concentration X_0. The model parameters X_0, X_{max}, and μ_{max} can be calculated from the series of experimental data biomass concentration/time.
Lactose consumption by the *Lactobacillus* strains can be interpreted by the equation (3)

$$S = S_0 - \frac{1}{Y_{P/S}}(P - P_0) - \frac{1}{Y_{X/S}}(X - X_0) \quad (3)$$

where $Y_{P/S}$ and $Y_{X/S}$ are the product yield for biosurfactant and biomass respectively, P and P_0 are the final and initial biosurfactant concentrations (g/L), X and X_0 are the final and initial biomass concentrations (g/L), and finally S_0 is the initial lactose concentration (g/L). The model parameters $Y_{P/S}$, $Y_{X/S}$ and S_0 were calculated for each *Lactobacillus* strain from the series of experimental data lactose concentration/time and the equations (1) and (2).

RESULTS

Blood Agar screening method

All the tested strains showed zones of clearing in the blood agar with scores ranging between (++) corresponding to complete hemolysis with a diameter < 1 cm. Also, these strains allowed for a surface tension reduction of 8 mN/m when compared with the control optimized MRS broth (55 mN/m) consistent with their ability to produce biosurfactants.

Biosurfactant production

Figure 1

Growth curves were obtained for the three *Lactobacillus* strains in order to establish the relation between cell growth and surface-activity of the biosurfactant in time as can be seen in Figure 1. For all the strains the biosurfactant production is occurring mainly in the first 7 hours, where substrate consumption and cell growth is still very low. As the decreases in the surface tension exceeded 8 mN/m (Busscher *et al.* 1994), all three strains were found to produce biosurfactants. The surface tension decreases were compared with the surface tension of optimized MRS broth (control) to correct for lower initial surface tensions values as a result of the medium ingredients that can have surface-active characteristics themselves.

Biosurfactant extraction with PBS - fiting of data

Reduction of surface tension during the PBS extraction of cells in stationary phase were fitted to proposed models using commercial software (Table Curve Windows v1.11). For all the *Lactobacillus* strains an exponential fit was possible according with the following equation:

$$y = a + b \times e^{(-\frac{x}{c})} \quad (4)$$

where y is surface tension (mN/m) and x is the extraction time (h). The equation parameters obtained were very similar for all the tested strains, with a = 52 ± 0.6; b = 11 ± 1.5; c = 1 ± 0.32 and r^2 = 0.999. The surface tension values decreased along the 24 h extraction procedure with PBS and it was found that all the *Lactobacillus* strains released intracellular biosurfactants allowing for a surface tension reduction of 18 mN/m when compared with the control PBS (72 mN/m).

Fermentations in optimized MRS broth

Fermentation runs were carried out using the optimized MRS broth for all *Lactobacillus* strains and Figure 1 shows the experimental data as well as the predicted values calculated by equations 2, 3 and 4 using the regression parameters listed in Table 1. All experiments show a kinetic pattern fairly described by the mathematical models with r^2 > 0.951, 0.932 and 0.929 for lactose consumption, biomass and biosurfactant production, respectively. It can be noted that both *L. rhamnosus* and *L. pentosus* present the highest P_{max} (1.5 g of biosurfactant/L).

Table 1

Regarding the $Y_{P/S}$ all the *Lactobacillus* strains present the same value of 0.04 g/g which means that all the three strains showed a similar behavior concerning biosurfactant production. The r_p/X values listed in Table 1 reflect the activity of the microorganisms concerning biosurfactant production. It can be seen that *L. rhamnosus* presents the highest r_p/X value (0.145 g/L.h^{-1}) followed by *L. casei* and *L. pentosus*.

Fermentations in whey

Figure 2

Table 2

The lowest value of surface tension was achieved in the stationary phase (45 mN/m) and the reduction in the surface tension was between 11 and 12 mN/m when compared to the surface tension of whey broth (control). Figure 2 shows the experimental data as well as the predicted values calculated by equations 2, 3 and 4 using the regression parameters listed in Table 2. All experiments show a kinetic pattern fairly described by the mathematical models with $r^2 >$ 0.858, 0.941 and 0.990 for lactose consumption, biomass and biosurfactant production, respectively. The r^2 value obtained for lactose consumption was not so good (r^2 = 0.858) and this could be explained by the fact that not all the lactose was consumed during the cell growth. Similar P_{max} (1.3 to 1.4 g of biosurfactant/L), P_r (0.327 to 0.461 h^{-1}) and r_p/X (0.078 to 0.093 g/L.h^{-1}) values were achieved for all strains. Regarding $Y_{P/S}$ the values obtained were between 0.13 and 0.23 g/g, being *L. rhamnosus* the best producing strain with a X_{max} = 1.5 g/L and a μmax of 0.05 h^{-1}. From Figure 2 it was observed for all strains that they did not grow very well maybe because not all its nutritional requirements were full field, although similar concentrations of biosurfactant were achieved if compared to those obtained with optimized MRS medium. Comparing the kinetic parameters obtained with the two tested medium, it was possible to notice that a lower μmax (approximately 10 times less than with synthetic medium) was obtained with whey medium, as well as a lower X_{max} (approximately 4 times less than with synthetic medium). Also, very high residual sugar content was left on the end of the fermentation probably due to no pH control and also a small biomass growth.

DISCUSSION & CONCLUSIONS

The lactic acid bacteria *L. casei* CECT-5275, *L. rhamnosus* CECT-288 and *L. pentosus* CECT-4023 were found to be biosurfactant-producing strains. Depending upon the nature of the biosurfactant and the producing microorganisms, several patterns of biosurfactant production by fermentation are possible (Desai and Desai 1993). In our study, the biosurfactant production is occurring mainly in the first hours (7 h) where cell growth is and substrate consumption is very low. However, for all strains the biosurfactant production continues during all 72 hours of fermentation but at a very slow production rate. This slow production rate can be a consequence of product inhibition and pH reduction. The pH reduction results of simultaneous production of lactic acid that changes drastically the media conditions and can be responsible for the biosurfactant production inhibition. The lowest values of surface tension were obtained in the end of fermentation, therefore, our present observation that biosurfactant release by the selected lactobacilli strains is maximal for cells in the stationary cells is in accordance with the general notion on this point in the literature (Velraeds *et al.* 1996 (a), (b); Desai and Desai 1993).

In this study three *Lactobacillus* strains were screened for biosurfactant production by surface tension determination, and the biosurfactant production as well as the time courses of lactose consumption and biomass growth modeled. The approach used in this study allowed the determination of the fermentation parameters as well as a way to predict the biosurfactant extraction with PBS. From the PBS extraction results it was found that all the *Lactobacillus* strains tested allowed for a surface tension reduction of 18 mN/m, thus good biosurfactant-producing strains. The effectiveness of a surfactant is determined by its ability to lower the surface tension. For example, a good surfactant can lower the surface tension of water (air-water interface) from 72 mN/m to 35 mN/m (Mulligan and Gibbs 1993).

For all three *Lactobacillus* strains, suitable models were found to describe the response of the experiments pertaining to lactose consumption, cell growth and biosurfactant production. The models were validated by comparing the observed and predicted values, and a deviation of about 5% was found. The modeling procedure allowed a better characterization of the biosurfactant production by the determination of the parameters and it was observed a reasonable fitting with a significance level over 90%. Additionally, the blood agar method was used to screen biosurfactant-producing *Lactobacillus* strains. The hemolytic activity of biosurfactants was first discovered by Bernheimer and Avigad (1970) and blood agar lysis has been used to quantify surfactin (Moran *et al.* 2002), rhamnolipids (Johnson and Boese-Marrazzo 1980) and to screen for biosurfactant production by new isolates (Carrillo *et al.* 1996, Banat 1993). Carrillo *et al.* (1996) recommended the use of blood agar lysis as a primary method to screen for biosurfactant activity, however only 13.5% of the hemolytic strains lowered the surface tension of water below 40 mN/m. In addition, other microbial products such as virulence factors lyse blood agar and biosurfactants that are poorly diffusible may not lyse blood cells. Thus, as not all biosurfactants have a hemolytic activity and compounds other than biosurfactants may cause hemolysis it is not clear whether blood agar lysis should be used exclusively to screen for biosurfactant production and surface tension can then be used to confirm the results if required. Velraeds *et al*. (1996 (b)) screened 15 *Lactobacillus* isolates for biosurfactant production and found that *Lactobacillus acidophilus* RC14 and *Lactobacillus fermentum* B54 were strongly biosurfactant-producing strains. Additionally, they found that biosurfactant from several *Lactobacillus* strains inhibited the adhesion of uropathogenic *Enterococcus faecalis* strain to glass, despite the behaviour of all the strains tested was not the same. This indicates that it should not be expected that the products of different *Lactobacillus* strains would produce equivalent results for any given pathogen. Other biomedical applications of the biosurfactants were found in the literature, namely the use of biosurfactants obtained from *Lactococcus lactis* 53 and from *Streptococcus thermophilus* A to prevent the microbial colonization of silicone rubber voice prostheses (Rodrigues *et al.* 2004 (a), (b)). In our study, all three strains were found to produce biosurfactants after reaching the stationary growth phase and from all the above can be used for further investigation. The chemical composition of the crude biosurfactants produced by the lactobacilli strains tested was not analyzed and will be determined in further studies. However, Velraeds and co-workers (Velraeds *et al.* 1996 (b)) established a preliminary chemical structure for biosurfactants released by certain strains of lactobacilli and found that polysaccharide-containing groups occur in the biosurfactant mixture as the expense of proteinaceous groups. The authors concluded that the biosurfactants released by some lactobacilli strains are protein-rich and as they interfere with uropathogen adhesion, proposed the name “surlactin”.

Finally, the *Lactobacillus* strains were assayed for biosurfactant production using whey as the culture medium and comparing with the results obtained for the strains growing in optimized MRS medium it was possible to see that they did not grow well on whey medium probably due to some lack of nutrients, although similar biosurfactant concentrations were obtained, which means that with a culture medium optimization it could be possible to achieve higher biosurfactant concentrations.

In conclusion, a model could be established to follow the biosurfactant production at any fermentation time for all the tested strains with a significance level over 90%. The results obtained for *L. rhamnosus* CECT-288 showed that this is a good biosurfactant-producer strain and that whey can be used as an alternative medium for biosurfactant production.

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Table 1 Results obtained by regression of biosurfactant, biomass and lactose concentration data in optimized MRS broth fermentations [(a)].

***Lactobacillus* strains**	**Biosurfactant production**						**Biomass**					**Lactose consumption**				
	P_0 (g/L)	P_{max} (g/L)	P_r (h^{-1})	r_p / X (g/L.h^{-1})	r^2	F value	X_0 (g/L)	X_{max} (g/L)	μ_{max} (h^{-1})	r^2	F value	S_0 (g/L)	$Y_{P/S}$ (g/g)	$Y_{X/S}$ (g/g)	r^2	F value
L. casei	0.6	1.4	1.231	0.137	0.929	26 [(b)]	0.43	5.2	0.150	0.995	388 [(e)]	54.9	0.04	0.55	0.962	51 [(c)]
L. rhamnosus	0.6	1.5	1.139	0.145	0.976	81 [(d)]	0.12	4.6	0.299	0.932	27 [(b)]	53.8	0.04	0.55	0.956	44 [(c)]
L. pentosus	0.6	1.5	0.926	0.089	0.953	40 [(c)]	0.28	6.2	0.247	0.997	704 [(e)]	55.9	0.04	0.56	0.951	39 [(c)]

[(a)] P_0 = initial biosurfactant concentration (g/L); P_{max} = maximum concentration of biosurfactant (g/L); P_r = ratio between initial volumetric rate of biosurfactant formation (*rp*) and initial biosurfactant concentration P_0 (h^{-1}), r_p/X (g/L.h^{-1}) = represents the microorganisms activity; X_0 = initial biomass concentration (g/L); X_{max} = maximum concentration of biomass (g/L); μ_{max} = ratio between initial volumetric rate of biomass formation (*rp*) and initial biomass concentration X_0 (h^{-1}); S_0 = initial lactose concentration (g/L); $Y_{P/S}$ = product yield (g/g); $Y_{X/S}$ = biomass yield (g/g); r^2 = determination coefficient; F value = F test statistical parameter; [(a)], significance level >90%; [(b)], significance level >95%; [(c)], significance level >97.5%; [(d)], significance level >99%; [(e)], significance level >99.5%.

Table 2 Results obtained by regression of biosurfactant, biomass and lactose concentration data in whey broth fermentations [(a)].

***Lactobacillus* strains**	**Biosurfactant production**						**Biomass**					**Lactose consumption**				
	P_0 (g/L)	P_{max} (g/L)	P_r (h^{-1})	r_p / X (g/L.h^{-1})	r^2	*F* value	X_0 (g/L)	X_{max} (g/L)	μ_{max} (h^{-1})	r^2	*F* value	S_0 (g/L)	$Y_{P/S}$ (g/g)	$Y_{X/S}$ (g/g)	r^2	*F* value
L. casei	0.2	1.4	0.461	0.091	1.000	1817316 [(e)]	0.18	1.2	0.036	0.969	63 [(c)]	54.1	0.23	0.17	0.858	12 [(a)]
L. rhamnosus	0.4	1.3	0.327	0.078	0.996	464 [(d)]	0.16	1.5	0.052	0.941	32 [(b)]	53.6	0.13	0.73	0.980	96 [(c)]
L. pentosus	0.4	1.4	0.353	0.093	0.990	195 [(c)]	0.19	1.5	0.049	0.959	46 [(b)]	52.8	0.22	0.22	0.955	43 [(b)]

[(a)] P_0 = initial biosurfactant concentration (g/L); P_{max} = maximum concentration of biosurfactant (g/L); P_r = ratio between initial volumetric rate of biosurfactant formation (*rp*) and initial biosurfactant concentration P_0 (h^{-1}); r_p/X (g/L.h^{-1}) = represents the microorganisms activity; X_0 = initial biomass concentration (g/L); X_{max} = maximum concentration of biomass (g/L); μ_{max} = ratio between initial volumetric rate of biomass formation (*rp*) and initial biomass concentration X_0 (h^{-1}); S_0 = initial lactose concentration (g/L); $Y_{P/S}$ = product yield (g/g); $Y_{X/S}$ = biomass yield (g/g); r^2 = determination coefficient; *F* value = *F* test statistical parameter; [(a)], significance level >90%; [(b)], significance level >95%; [(c)], significance level >97.5%, [(d)], significance level >99%, [(e)], significance level >99.9%.

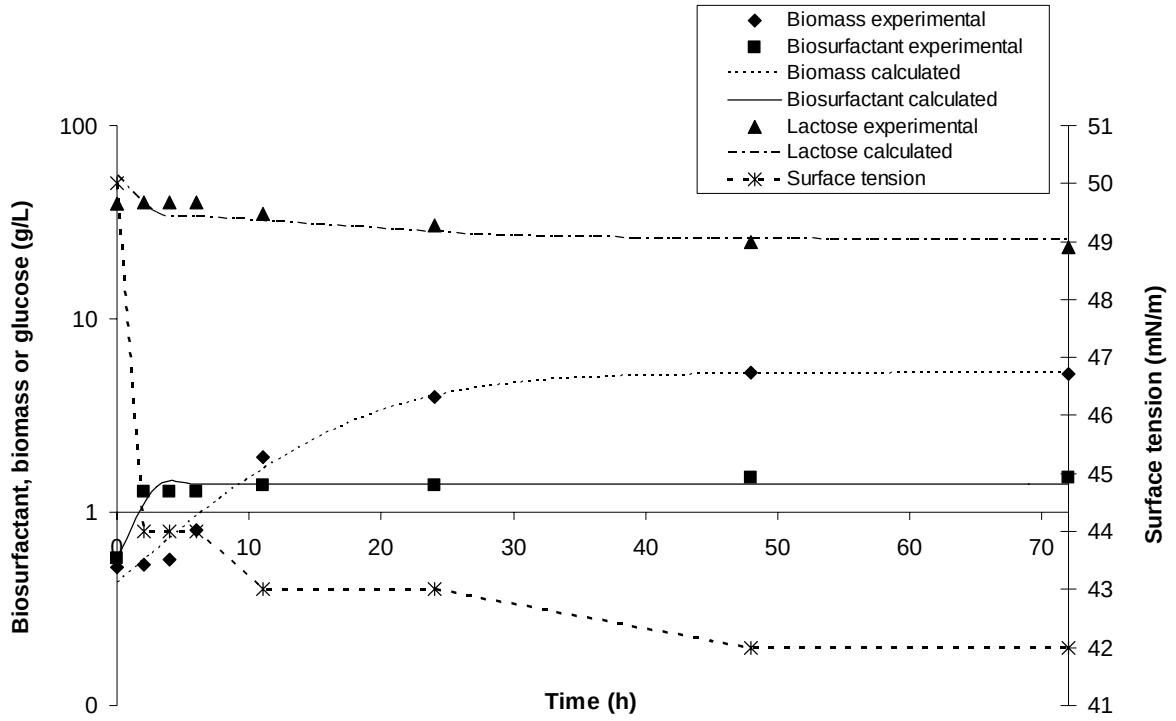
Captions

Fig. 1. Representation of the surface tension variation (--∗--), experimental data and calculated time courses of biomass (◆, -----), glucose (▲, — - —) and biosurfactant concentrations (■, ——) during fermentations carried out with optimized MRS broth using (A) *L. casei* CECT-5275, (B) *L. rhamnosus* CECT-288, (C) *L. pentosus* CECT-4023. Results represent the average of three independent experiments.

Fig. 2. Representation of the surface tension variation (--∗--), experimental data and calculated time courses of biomass (◆, -----), glucose (▲, — - —) and biosurfactant concentrations (■, ——) during fermentations carried out with whey broth using (A) *L. casei* CECT-5275, (B) *L. rhamnosus* CECT-288, (C) *L. pentosus* CECT-4023. Results represent the average of three independent experiments.

Fig. 1.

A)


Biomass experimental
Biosurfactant experimental
Biomass calculated
Biosurfactant calculated
Lactose experimental
Lactose calculated
Surface tension
Biosurfactant, biomass or glucose (g/L)
Surface tension (mN/m)
Time (h)
100
10
1
0
51
50
49
48
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43
42
41
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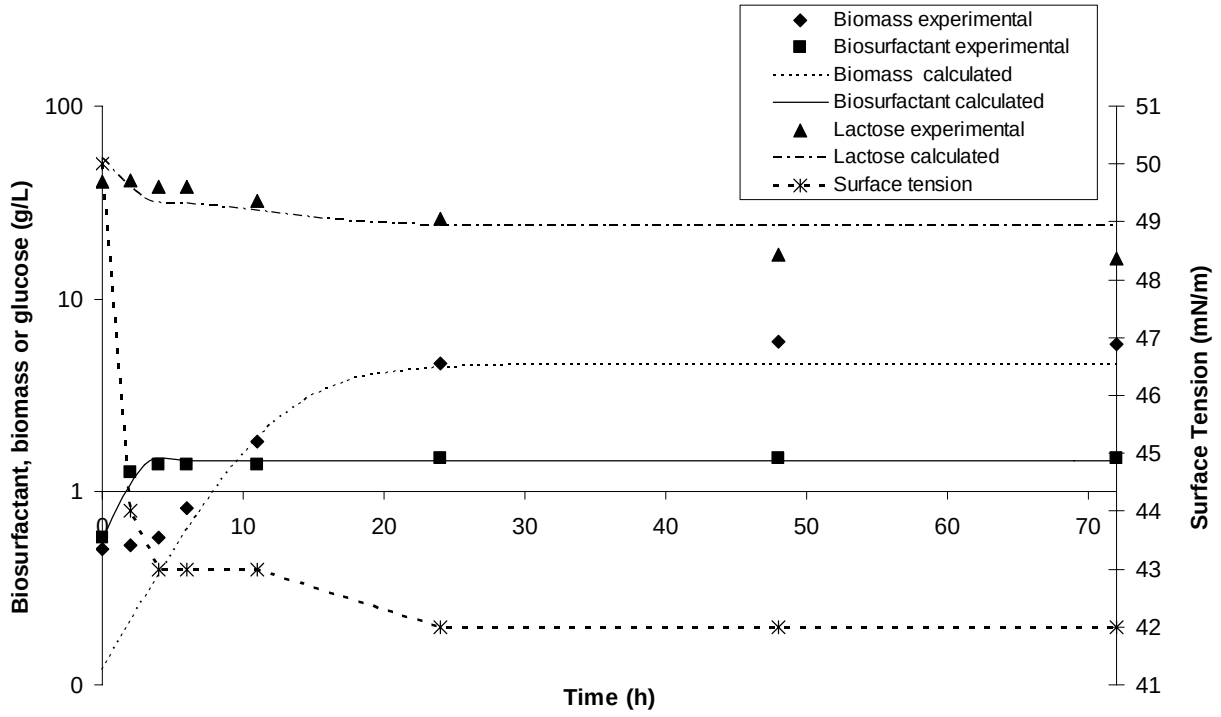
60
70

B)


Biomass experimental
Biosurfactant experimental
Biomass calculated
Biosurfactant calculated
Lactose experimental
Lactose calculated
Surface tension
Biosurfactant, biomass or glucose (g/L)
Surface Tension (mN/m)
Time (h)
100
10
1
0
51
50
49
48
47
46
45
44
43
42
41
10
20
30
40
50
60
70

C)

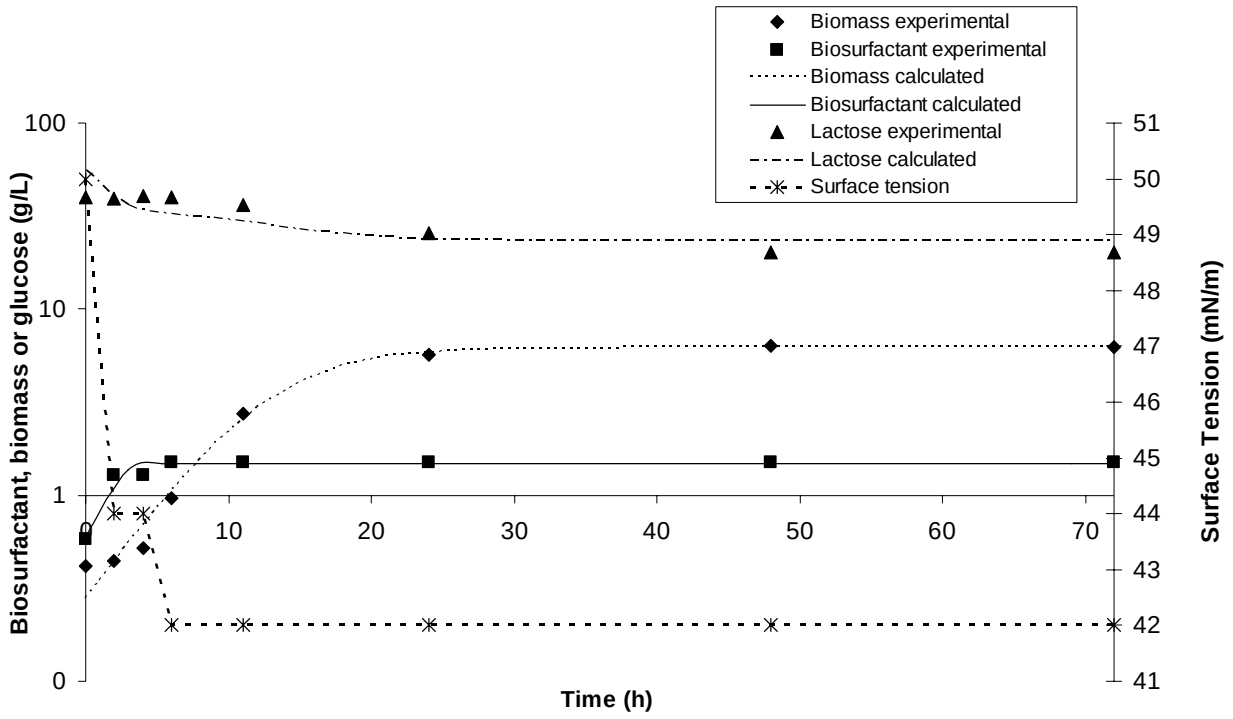
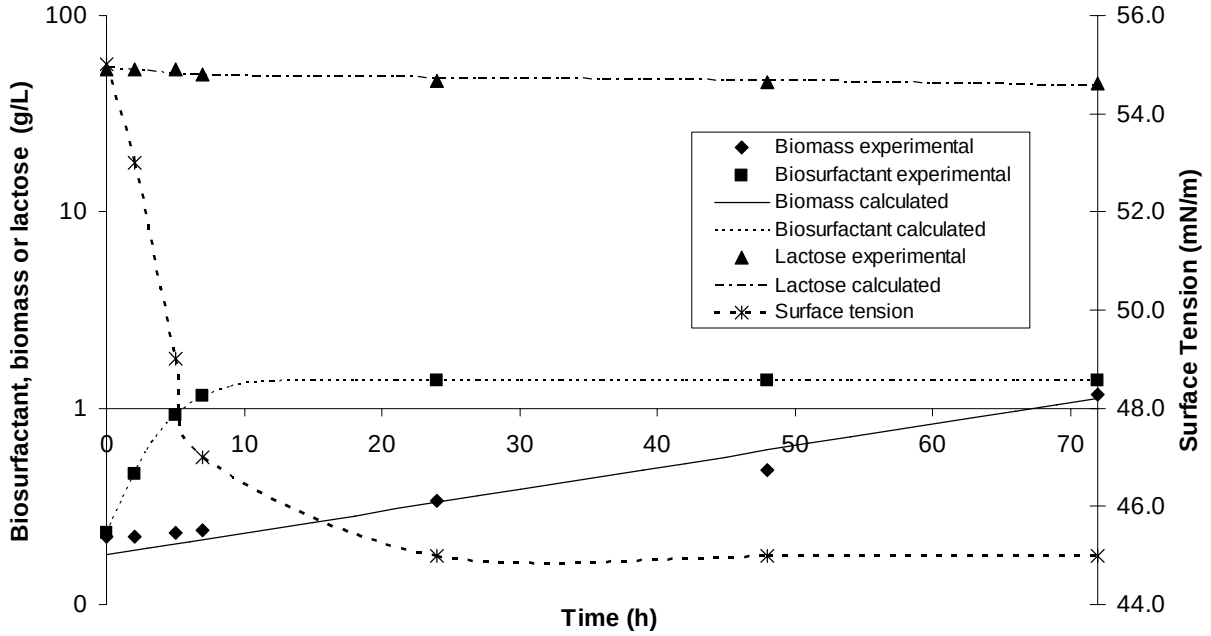
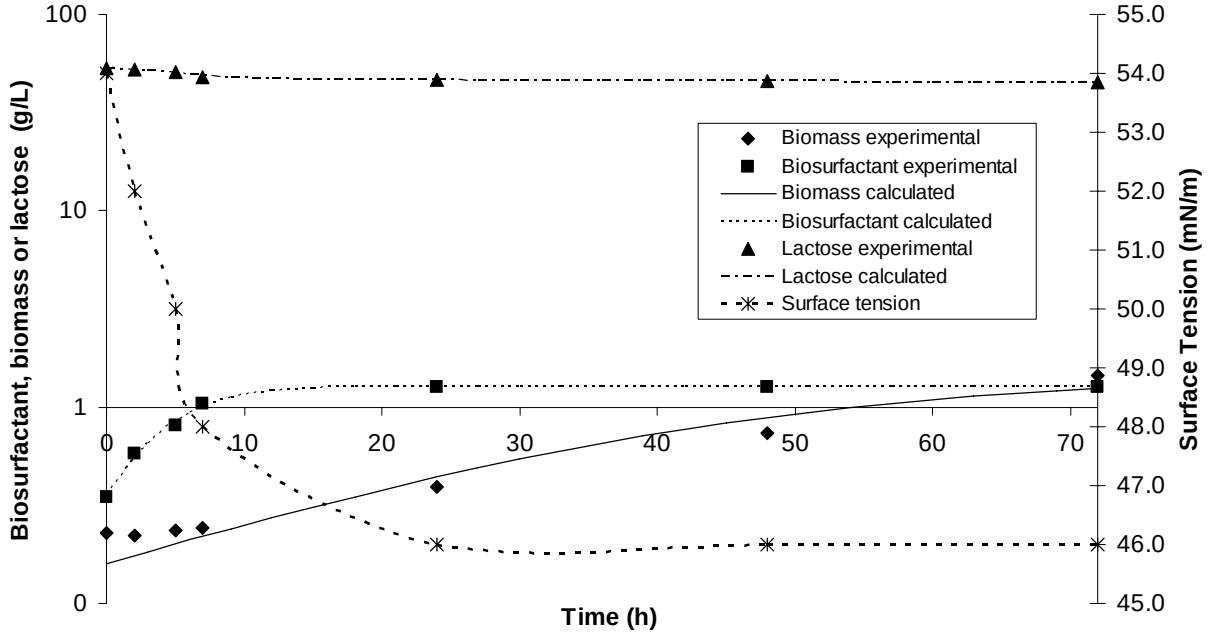

Biomass experimental
Biosurfactant experimental
Biomass calculated
Biosurfactant calculated
Lactose experimental
Lactose calculated
Surface tension
Biosurfactant, biomass or glucose (g/L)
Surface Tension (mN/m)
Time (h)
100
10
1
0
51
50
49
48
47
46
45
44
43
42
41
0
10
20
30
40
50
60
70

Fig. 2.

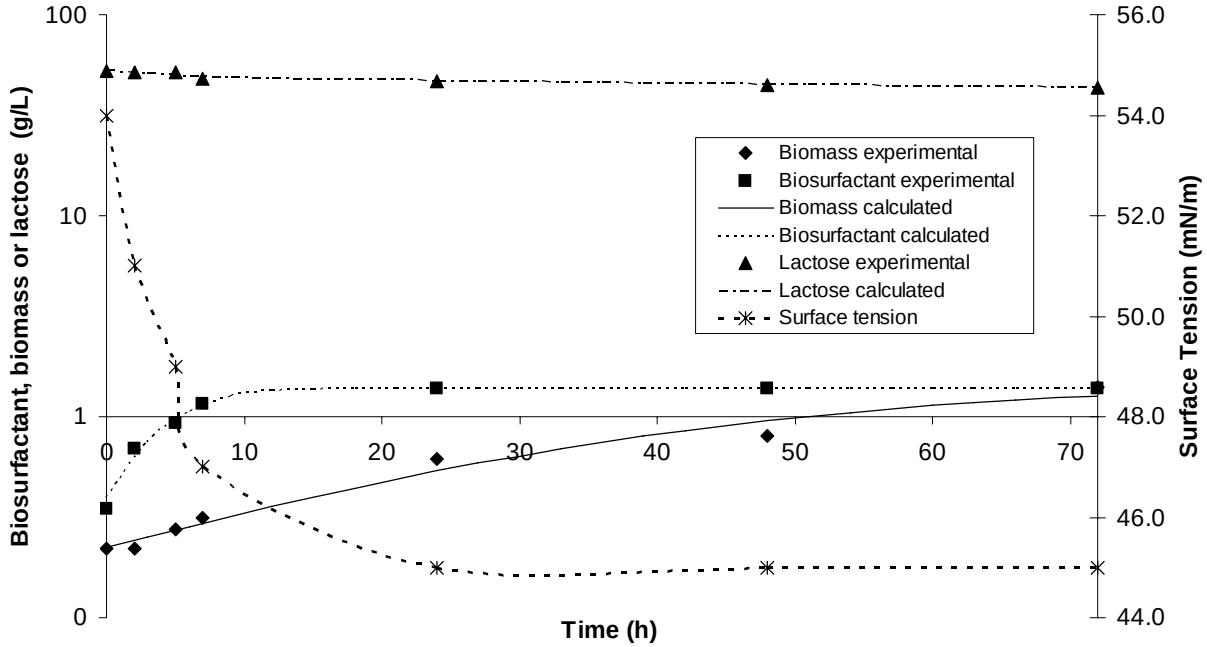
A)


Biosurfactant, biomass or lactose (g/L)
Surface Tension (mN/m)
Time (h)
Biomass experimental
Biosurfactant experimental
Biomass calculated
Biosurfactant calculated
Lactose experimental
Lactose calculated
Surface tension
100
10
1
0
56.0
54.0
52.0
50.0
48.0
46.0
44.0
0
10
20
30
40
50
60
70

B)


Biosurfactant, biomass or lactose (g/L)
Surface Tension (mN/m)
Time (h)
Biomass experimental
Biosurfactant experimental
Biomass calculated
Biosurfactant calculated
Lactose experimental
Lactose calculated
Surface tension
100
10
1
0
55.0
54.0
53.0
52.0
51.0
50.0
49.0
48.0
47.0
46.0
45.0
0
10
20
30
40
50
60
70

C)


100
10
1
0
56.0
54.0
52.0
50.0
48.0
46.0
44.0
0
10
20
30
40
50
60
70
Biosurfactant, biomass or lactose (g/L)
Surface Tension (mN/m)
Time (h)
Biomass experimental
Biosurfactant experimental
Biomass calculated
Biosurfactant calculated
Lactose experimental
Lactose calculated
Surface tension