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Low-cost fermentative medium for biosurfactant production by probiotic bacteria

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Abstract

Potential use of alternative fermentative medium for biosurfactant production by *Lactococcus lactis* 53 and *Streptococcus thermophilus* A was studied. Suitable models were established to describe the response of the experiments pertaining to glucose, lactose or sucrose consumption, cell growth and biosurfactant production. Synthetic media MRS and M17 broth were used as control experiments. When the synthetic media were replaced by cheaper alternative media, as cheese whey and molasses, fermentations were carried out effectively with high yields and productivities of biosurfactant. An increase about 1.2–1.5 times in the mass of produced biosurfactant per gram cell dry weight and 60–80% medium preparation costs reduction were achieved, for both strains.

In conclusion, the results obtained showed that supplemented cheese whey and molasses media can be used as a relatively inexpensive and economical alternative to synthetic media for biosurfactant production by probiotic bacteria, thus an attractive alternative as many of the potential applications for biosurfactants depend on whether they can be produced economically.

Keywords: Biosurfactant; Probiotic bacteria; Low-cost fermentative medium

1. Introduction

The 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 [1]. The biosurfactants have several advantages over chemical surfactants including lower toxicity and higher biodegradability, and effectiveness at extreme temperatures or pH values [2,3]. In spite of the advantages, fermentation must be cost competitive with chemical synthesis, and many of the potential applications that have been considered for biosurfactants depend on whether they can be produced economically. Fermentation medium can represent almost 30% of the cost for a microbial fermentation [4–6]. Complex media commonly employed for growth of lactic acid bacteria are not economically attractive due to their high amount of expensive nutrients such as yeast extract, peptone and salts [5,7,8]. Nevertheless, much effort in process optimization and at the engineering and biological levels has been done, and for some applications biosurfactants can be produced from several inexpensive waste substrates, thereby decreasing their production cost [6,9–14].

Biosurfactant production by probiotic strains, *Lactococcus lactis* 53 and *Streptococcus thermophilus* A, using conventional synthetic media and its applications was reported previously [15–17]. Rodrigues et al. [18] optimized the medium components by response surface optimization for the production of biosurfactants by probiotic bacteria and concluded that it was possible to determine optimal operating conditions to obtain a higher cellular growth, thus a higher biosurfactant production yield. Moreover, the authors suggested that since both bacterial strains studied shown higher amounts of biosurfactant produced with the optimized medium, it would be possible to develop strategies for biosurfactant production from whey. In another study [19] suitable kinetic models were established for several *Lactobacillus* strains biosurfactant producers using whey as an alternative medium. A great variety of alternative raw materials is currently available as nutrients for industrial fermentations, namely various agricultural and industrial by-products and waste materials. A good substrate for biosurfactant production is whey, as it is composed of high levels of lactose, protein, organic acids and vitamins. Whey is a waste product from cheese production

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Nomenclature

F value	F test statistical parameter
P	biosurfactant concentration (g/L)
P_{max}	maximum concentration of biosurfactant (g/L)
P_r	ratio between initial volumetric rate of biosurfactant formation (r_p) and initial biosurfactant concentration P_0 (h^{-1})
P_0	initial biosurfactant concentration (g/L)
r_p	initial volumetric rate of biosurfactant production (g/(L h))
r^2	correlation coefficient
S	substrate (glucose, lactose or sucrose) concentration (g/L)
S_0	initial substrate (glucose or lactose) concentration (g/L)
X	biomass concentration (g/L)
X_{max}	maximum concentration of biomass (g/L)
X_0	initial biomass concentration (g/L)
$Y_{P/S}$	yield of biosurfactant production per substrate consumption (g/g)
$Y_{P/X}$	yield of biosurfactant production per biomass growth (mg/g)
$Y_{X/S}$	yield of biomass growth per substrate consumption (g/g)

Greek letter

μ_{max}	maximum specific growth rate (ratio between initial volumetric rate of biomass formation (r_p) and initial biomass concentration X_0 (h^{-1}))

that represents a major pollution problem for countries depending on dairy economics and is normally used as animal feed. Sophorolipids production using whey was reported by Otto et al. [11]. On the other hand, molasses is also an interesting alternative. Molasses is a by-product of the sugar cane industry and it has many applications because of its low price compared to other sources of sugar, and the presence of several other compounds besides sucrose. These include minerals, organic compounds and vitamins, which are valuable for the fermentation process [20,21].

The aim of this study was to develop a low-cost alternative medium for biosurfactant production by *L. lactis* 53 and *S. thermophilus* A. Molasses and cheese whey were evaluated as alternative media and compared with the conventional synthetic medium. The yields of biosurfactant production for both strains were determined for all tested media. Additionally, the time courses of biosurfactant production, glucose, sucrose or lactose consumption and biomass growth were modelled.

2. Materials and Methods

2.1. Microorganisms and inoculums

The strains used in this work were *L. lactis* 53 and *S. thermophilus* A obtained from Nutricia (The Netherlands) and NIZO (The Netherlands), respectively. The bacterial strains *L. lactis* 53 and *S. thermophilus* A were stored at −20 °C in conventional synthetic MRS or M17 broth (OXOID, Basingstoke, England), respectively. From frozen stock, bacteria were streaked on MRS or M17 agar plates and incubated at 37 °C for further culturing. To prepare subcultures, the respective medium was inoculated with a colony from the plate and incubated overnight under the same conditions.

2.2. Fermentation experiments

To test biosurfactant production using alternative fermentation media, batch fermentations were carried out using the compositions described in Table 1. The conventional synthetic medium was prepared according to the supplier instructions (OXOID, Basingstoke, England). Appropriate dilutions were made in order to adjust lactose or sucrose initial concentrations of the medium. A 1-L bioreactor fitted with agitation control, as well as temperature and pH measurement and control were used. The temperature was maintained at 37 °C, the pH at 6.7 by automatic addition of a potassium hydroxide solution, and the agitation speed was set at 150 rpm. The total working volume was 0.5 L.

2.3. Cheese whey preparation

Commercial whey supplied by Sigma–Aldrich contained 65% (w/w) lactose and 11% (w/w) protein and was prepared as follows: after adjusting the pH to 4.5 with 5N HCl, it was heated at 121 °C for 15 min to denature the proteins. The precipitates were removed by centrifugation at 4 °C and 8000 × *g* for 10 min. The supernatants were adjusted to pH 6.7, sterilized at 121 °C for 15 min and used as culture media. The supernatant contained approximately 50 g/L of lactose. Yeast extract and peptone were added in suitable concentrations according to Table 1. In previ-

Table 1
Medium compositions used in the fermentation experiments for both tested strains

Medium	
L. lactis 53	
A	MRS broth
B	W (50 g/L lactose content) + 3 g/L YE + 5 g/L PEP
C	W (50 g/L lactose content) + 3 g/L YE + 10 g/L PEP
D	W (50 g/L lactose content) + 5.8 g/L YE + 44.8 g/L PEP
E	M (20 g/L sucrose content) + 3 g/L YE + 5 g/L PEP
F	M (20 g/L sucrose content) + 2.3 g/L YE + 18 g/L PEP
S. thermophilus A	
G	M17 broth
H	W (50 g/L lactose content) + 3 g/L YE + 5 g/L PEP
I	W (50 g/L lactose content) + 3 g/L YE + 10 g/L PEP
J	W (50 g/L lactose content) + 22 g/L YE + 43.8 g/L PEP + 231.6 g/L NGP
L	M (20 g/L sucrose content) + 3 g/L YE + 5 g/L PEP
M	M (20 g/L sucrose content) + 8.8 g/L YE + 17.5 g/L PEP + 92.6 g/L NGP

W, whey; YE, yeast extract; PEP, peptone; M, molasses; NGP, sodium glycerophosphate.

ous published results [18] peptone and sodium glycerophosphate were found to be significant factors for biosurfactant production by *L. lactis* 53 and *S. thermophilus* A, respectively. Thus, proportions of yeast extract, peptone and sodium glycerophosphate used in media D, F, J and M were defined according to this previous study.

2.4. Molasses preparation

Molasses, by-product of the sugar cane industry, supplied by RAR (Porto, Portugal), contained 45% (w/v) sucrose, 20% (w/v) fructose and 10% (w/v) glucose. Molasses was diluted to a concentration of 20 g/L sucrose and supplemented with yeast extract and peptone as described in Table 1. The pH of the medium was adjusted to 6.7 prior to autoclaving (15 min at 121 °C).

2.5. Bacterial growth determination

Bacterial growth was measured by determining the optical density at 600 nm during different time intervals up to 30 h. The biomass concentrations (g dry weight/L) were determined using a calibration curve. The calibration curve was calculated for each strain using dilutions of a biomass suspension with known optical density. A fixed volume of the dilutions was filtered (0.22 μm) and left to dry at 105 °C for 24 h. All the filters were weighed before filtration and after drying. Thus, a relationship between biomass concentration (g/L) and optical density (600 nm) can be determined for each strain.

2.6. Sugar analysis

Sugar concentrations were determined by high performance liquid chromatography (Agilent, model 1100, Palo Alto, CA) using ION-300 column (Transgenomic Inc., San Jose, CA) with refractive index detector. The mobile phase was 0.01N H_2SO_4 at a flow rate of 0.4 mL/min.

2.7. Surface activity determination

The surface activity of biosurfactants produced by the bacterial strains was determined by measuring the surface tension of the broth samples by the Ring method [22] using a KRUSS Tensiometer equipped with a 1.9 cm De Noüy platinum ring at room temperature. To increase the accuracy an average of triplicates was used for this study.

2.8. Evaluation of biosurfactant concentration

The biosurfactant concentrations (g/L) were determined for each strain using a calibration curve (surface tension (mN/m) = −8.6465 concentration (g/L) + 76.984, $r^2 = 0.9729$). The calibration curve was calculated for a commercial biosurfactant produced by several *Bacilli* (surfactin—lowers the surface tension of water to 27 mN/m at 5.0×10^{-4} M [23]) using different concentrations of biosurfactant solution, below the critical micelle concentration, with known surface tension. In this biosurfactant 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 [22,24]. Nevertheless, to estimate biosurfactant concentration it was necessary sometimes to dilute the culture broth under the critical micelle concentration.

Surfactin was used as a standard just like for example albumin is used as a standard in protein quantification assays, since it is one of the best studied biosurfactants and presents proteinaceous characteristics as the biosurfactants produced [25], thus providing a suitable method for estimating the biosurfactant concentration.

2.9. Sugar consumption, biosurfactant production and biomass growth—fitting of data

Experimental data were fitted to proposed models using commercial software (solver of Microsoft Excel 2002) by nonlinear regression using the least-squares method. Biosurfactant production was mathematically modelled following the equation proposed by Mercier et al. [26] for lactic acid production:

$$\frac{dP}{dt} = P_r P \left(1 - \frac{P}{P_{max}}\right) \quad (1)$$

where t is the time (h), P the biosurfactant concentration (g/L), P_{max} the maximum concentration of biosurfactant (g/L), and P_r is the ratio between the initial volumetric rate of product formation (r_p) and the initial product concentration P_0 (g/L). Eq. (1) can be directly solved to give Eq. (2):

$$P = \frac{P_0 P_{max} e^{P_r t}}{P_{max} - P_0 + P_0 e^{P_r t}} \quad (2)$$

From the series of experimental data biosurfactant concentration/time, the model parameters P_0, P_{max}, and P_r can be calculated for each strain growing in the several tested fermentation medium.

Also biomass production was mathematically modelled and can be interpreted by Eq. (3):

$$X = \frac{X_0 X_{max} e^{\mu_{max} t}}{X_{max} - X_0 + X_0 e^{\mu_{max} t}} \quad (3)$$

where t is the time (h), X the biomass concentration (g/L), X_{max} the maximum concentration of biomass (g/L), and μ_{max} (h^{-1}) is the ratio between the initial volumetric rate of biomass formation and the initial biomass concentration X_0 (g/L). The model parameters X_0, X_{max}, and μ_{max} can be calculated from the series of experimental data biomass concentration/time.

Sugar consumption can be interpreted by Eq. (4):

$$S = S_0 - \frac{1}{Y_{P/S}}(P - P_0) - \frac{1}{Y_{X/S}}(X - X_0) \quad (4)$$

where $Y_{P/S}$ (g/g) and $Y_{X/S}$ (g/g) 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 glucose, lactose or sucrose concentration (g/L). The model parameters $Y_{P/S}$, $Y_{X/S}$ and S_0 (g/L) were calculated for each strain

Table 2
Results obtained by regression of glucose, lactose or sucrose, biomass and biosurfactant concentration data in several fermentation medium for *L. lactis* 53[a]

Medium	Sugar consumption						Biomass					Biosurfactant production				
	S_0 (g/L)	$Y_{P/S}$ (g/g)	$Y_{X/S}$ (g/g)	$Y_{P/X}$ (mg/g)	r^2	F value	X_0 (g/L)	X_{max} (g/L)	μ_{max} (h^{-1})	r^2	F value	P_0 (g/L)	P_{max} (g/L)	P_r (h^{-1})	r^2	F value
A	29.0	0.05	0.30	163	0.958	45[c]	0.068	4.244	0.405	0.996	461[f]	0.030	0.693	0.640	0.983	116[e]
B	55.0	0.04	0.22	200	0.936	29[c]	0.064	5.963	0.315	0.997	766[g]	0.259	1.054	0.222	0.948	37[c]
C	52.0	0.04	0.22	159	0.940	31[c]	0.062	6.023	0.196	0.921	23[c]	0.097	0.919	0.419	0.979	92[d]
D	57.0	0.06	0.23	240	0.961	49[c]	0.036	5.680	0.372	0.984	119[e]	0.154	1.379	0.429	0.977	83[d]
E	26.3	0.07	0.36	197	0.997	632[g]	0.579	5.992	0.103	0.944	34[c]	0.053	1.041	0.338	0.958	46[c]
F	35.0	0.12	0.43	281	0.901	18[b]	0.064	5.990	0.202	0.959	46[c]	0.116	1.735	0.294	0.917	22[c]

[a] Parameters defined in the nomenclature table.
[b] SL > 90%.
[c] SL > 95%.
[d] SL > 97.5%.
[e] SL > 99%.
[f] SL > 99.5%.
[g] SL > 99.9%.

from the series of experimental data glucose, lactose or sucrose concentration/time and Eqs. (2) and (3).

The mathematical model proposed by Mercier et al. [26] was chosen because it fairly describes biomass growth, substrate consumption and product accumulation kinetic pattern, and is reasonable to predict that this mathematical model will adjust the biosurfactant production results with statistical significance of the parameters determined.

3. Results

3.1. Biosurfactant production using conventional synthetic medium

Fermentation control runs were carried out using the conventional synthetic medium MRS or M17 broth (A and G as defined in Table 1) for *L. lactis* 53 and *S. thermophilus* A, respectively. Experimental data were fitted to proposed models by nonlinear regression using the least-squares method. Tables 2 and 3 show the kinetic and regression parameters as well as the biosurfactant production yields. Both experiments show a kinetic pattern fairly described by the mathematical models with $r^2 > 0.952$, 0.996 and 0.983 for glucose or lactose consumption, biomass growth and biosurfactant production, respectively. It can be noted that *S. thermophilus* A presents a higher P_{max} (0.8 g of biosurfactant/L) compared to *L. lactis* 53 (0.7 g of biosurfactant/L). Regarding the $Y_{P/S}$ both strains present the same value (0.05 g/g). The $Y_{P/X}$ values listed in Tables 2 and 3 reflect the amount of biosurfactant produced (mg) per amount of dry cells (g). The $Y_{P/X}$ values obtained for both strains growing in control medium was 163 and 116 mg/g for *L. lactis* 53 and *S. thermophilus* A, respectively.

3.2. Biosurfactant production using cheese whey

Fermentations were carried out using whey supplemented with yeast extract and peptone as culture broth for both studied strains. Different sets of medium composition in yeast extract and peptone were evaluated. Figs. 1A and 2A show the experimental data as well as the predicted values calculated by Eqs. (2)–(4) using the regression parameters listed in Tables 2 and 3 for *L. lactis* 53 and *S. thermophilus* A growing in medium D and J (as defined in Table 1), respectively. For both strains growing in all the tested cheese whey medium (B–D, H–J as defined in Table 1), the experiments show a kinetic pattern reasonably described by the mathematical model with $r^2 > 0.936$, 0.921 and 0.913 for lactose consumption, biomass growth and biosurfactant production, respectively. P_{max} values achieved with all cheese whey medium were higher than the observed for the control experiments. P_{max} values between 0.9 and 1.4 g of biosurfactant/L were obtained for both strains. Regarding the P_r the values obtained were between 0.22 and 0.429 h^{-1} for *L. lactis* 53, and between 0.078 and 0.725 h^{-1} for *S. thermophilus* A. Moreover, the $Y_{P/S}$ values obtained were similar for both strains and between 0.04 and 0.06 g/g, and X_{max} between 5.2 and 6.1 g/L with a μ_{max} between 0.196 and 0.447 h^{-1}.

Comparing the kinetic parameters obtained with the cheese whey medium experiments and control, it was possible to notice

Table 3
Results obtained by regression of glucose, lactose or sucrose, biomass and biosurfactant concentration data in several fermentation medium for *S. thermophilus* A[a]

Medium	Sugar consumption						Biomass					Biosurfactant production				
	S_0 (g/L)	$Y_{P/S}$ (g/g)	$Y_{X/S}$ (g/g)	$Y_{P/X}$ (mg/g)	r^2	F value	X_0 (g/L)	X_{max} (g/L)	μ_{max} (h^{-1})	r^2	F value	P_0 (g/L)	P_{max} (g/L)	P_r (h^{-1})	r^2	F value
G	35.0	0.05	0.41	116	0.952	40[b]	0.103	7.065	0.341	0.997	565[f]	0.060	0.828	0.862	0.988	171[d]
H	54.0	0.04	0.20	176	0.942	24[b]	0.255	5.246	0.201	0.929	26[b]	0.338	1.110	0.078	0.913	21[b]
I	52.4	0.04	0.26	155	0.991	227[e]	0.130	5.785	0.290	0.995	388[e]	0.040	0.914	0.725	0.990	206[e]
J	45.0	0.06	0.27	222	0.973	71[c]	0.035	6.079	0.447	0.968	61[c]	0.658	1.366	0.359	0.980	99[c]
L	20.0	0.08	0.49	164	0.989	177[d]	0.410	5.173	0.152	0.997	665[f]	0.031	1.017	0.152	0.986	140[d]
M	26.0	0.13	0.48	272	0.975	77[b]	1.099	5.9407	0.084	0.991	212[e]	0.119	1.401	0.257	0.982	107[c]

[a] Parameters defined in the nomenclature table.
[b] SL > 95%.
[c] SL > 97.5%.
[d] SL > 99%.
[e] SL > 99.5%.
[f] SL > 99.9%.

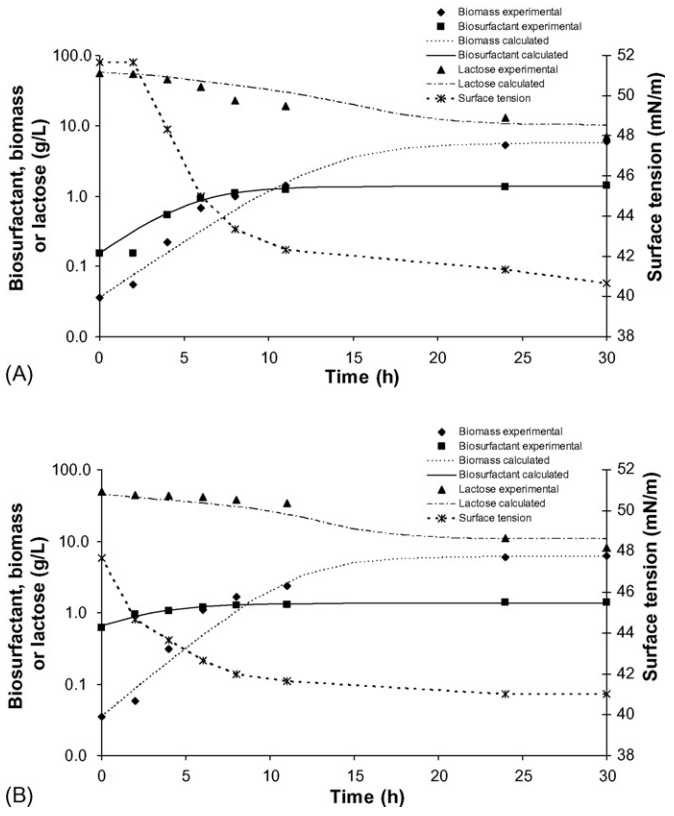

Fig. 1. Representation of the surface tension variation (- * -), experimental data and calculated time courses of biomass (♦, - - -), lactose (▲, - · -), and biosurfactant concentrations (■, —) during fermentations carried out with medium D (whey (50 g/L lactose content) + 5.8 g/L yeast extract + 44.8 g/L peptone) or medium J (whey (50 g/L lactose content) + 22 g/L yeast extract + 43.8 g/L peptone + 231.6 g/L sodium glycerophosphate) for (A) *L. lactis* 53 or (B) *S. thermophilus* A, respectively. Results represent the average of three independent experiments.

that higher $Y_{P/X}$ values were obtained. A mass of produced biosurfactant (mg/g cell dry weight) 1.5 times higher compared to MRS control medium was obtained for *L. lactis* 53 growing in medium D (as defined in Table 1). Similarly, for *S. thermophilus* A growing in medium J (as defined in Table 1) it was achieved an increase 1.9 times in the $Y_{P/X}$ values.

3.3. Biosurfactant production using molasses

In another set of experiments, fermentations were carried out using molasses supplemented with yeast extract and peptone as culture broth for both studied strains. Also, two different set of medium composition in yeast extract and peptone were evaluated. Figs. 1B and 2B show the experimental data and predicted values for *L. lactis* 53 and *S. thermophilus* A growing in medium F and M (as defined in Table 1), respectively. For both strains growing in all the tested molasses media (E, F, L, M as defined in Table 1), the mathematical model describes realistically the experimental data with $r^2 > 0.901$, 0.944 and 0.917 for sucrose consumption, biomass growth and biosurfactant production, respectively. P_{max} values between 1.0 and 1.7 g of biosurfactant/L and P_r values between 0.152 and 0.338 h^{-1} were obtained for both strains. Additionally, the $Y_{P/S}$ values obtained

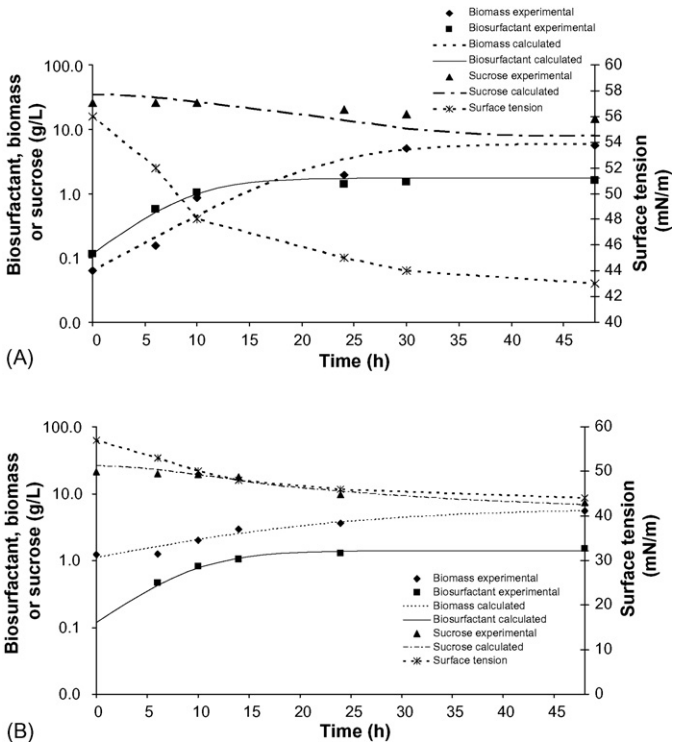

Fig. 2. Representation of the surface tension variation (- * -), experimental data and calculated time courses of biomass (♦, - - -), sucrose (▲, · · ·) and biosurfactant concentrations (■, —) during fermentations carried out with medium F (molasses (20 g/L sucrose content) + 2.3 g/L yeast extract + 18 g/L peptone) or medium M (molasses (20 g/L sucrose content) + 8.8 g/L yeast extract + 17.5 g/L peptone + 92.6 g/L sodium glycerophosphate) for (A) *L. lactis* 53 or (B) *S. thermophilus* A, respectively. Results represent the average of three independent experiments.

were similar for both strains and between 0.07 and 0.13 g/g, and X_{max} between 5.2 and 6.0 g/L with a μ_{max} between 0.08 and 0.202 h^{-1}.

The higher $Y_{P/X}$ values were obtained for both strains compared whether to control or cheese whey medium experiments. A mass of produced biosurfactant (mg/g cell dry weight) 1.7 times higher compared to MRS control medium was obtained for *L. lactis* 53 growing in medium F (as defined in Table 1). Similarly, for *S. thermophilus* A growing in medium M (as defined in Table 1) it was achieved an increase 2.3 times in the $Y_{P/X}$ values.

4. Discussion

In this study we focused on the potential use of alternative fermentative medium formulations for biosurfactant production. For *L. lactis* 53 and *S. thermophilus* A, suitable models were established to describe the response of the experiments pertaining to glucose, lactose or sucrose 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 modelling procedure allowed a better characterization of the biosurfactant production by the determination of the fermentation parameters and it was observed a reasonable fitting with a significance level over 90%.

The success of biosurfactant production depends on the development of cheaper processes and the use of low-cost raw materials, which account for 10–30% of the overall cost [6]. A great variety of agro-industrial wastes have been studied as potential substrates for biosurfactant production. Starch-rich wastes from potato-processing industries were successfully used for surfactant production [10]; molasses from sugar industry were assessed for biosurfactant production by *Bacillus* strains [20]; distillery and whey wastes were found to produce better results than conventional medium for rhamnolipid production [27,28]. Another good substrate for biosurfactant production is lactic whey, Daniel et al. [9] achieved production of high concentrations of sophorolipids from *Candida bombicola* ATCC 22214 and *Cryptococcus curvatus* ATCC 20509, using a two-stage fed batch process.

Whey is produced in large amounts by the cheese industry and is a huge waste disposal problem [29], being estimated a worldwide annual amount of about 4×10^7 tonnes. Cultivation of microorganisms on cheese whey has been proposed as an alternative to reduce waste disposal problem since it can reduce 90–95% of its biochemical oxygen demand (BOD), resulting in high-added value bio-ingredients for food industry. Several studies have been reported on the use of cheese whey for lactic acid production [30–35]. Also, cheese whey was used for the production of dextran and fructose by *Leuconostoc mesenteroides* NRRL B512 (f) [36]; production of ethanol [37] and for the production of yeast extract by *Kluyveromyces marxianus* [29]. Hinted by a previous work [18] and the fact that probiotic bacteria, especially *L. lactis* 53 and *S. thermophilus* A, have been used for the production of biosurfactants [15–17,19,38–40], three different sets of medium conditions using cheese whey were tested to see their potentials for biosurfactant fermentation. In the present study it was achieved an increase about 1.5–1.9 times in the mass of produced biosurfactant per gram cell dry weight, for *L. lactis* 53 and *S. thermophilus* A, respectively. From the different proportions of yeast extract, peptone and sodium glycerophosphate supplemented to cheese whey it was possible to observe that the best results were achieved with the medium D (50 g/L lactose content, supplemented with 5.8 g/L yeast extract and 44.8 g/L peptone) for *L. lactis* 53, and with the medium J (50 g/L lactose content, supplemented with 22 g/L yeast extract, 43.8 g/L peptone and 231.6 g/L sodium glycerophosphate) for *S. thermophilus* A; which is in accordance with previous published results [18] where peptone and sodium glycerophosphate were found to be significant factors for biosurfactant production by *L. lactis* 53 and *S. thermophilus* A, respectively. Table 4 presents the costs of the ingredients used in the formulation of the fermentation medium, as well as the costs of the medium used in this study. Moreover, the presented information allowed the evaluation of the most economical medium formulations. Despite a higher biosurfactant production yield was achieved with medium D (50 g/L lactose content, supplemented with 5.8 g/L yeast extract and 44.8 g/L peptone) for *L. lactis* 53, an increase of 40% in the medium preparation costs comparing with the synthetic MRS medium was estimated due to the high amounts of peptone supplemented; thus a compromise situation must be established to obtain higher biosurfactant production

Table 4
Costs of the ingredients used in the formulation of fermentation medium

Ingredient	Cost (€/kg)	Medium	Cost (€/L)
Glucose	0.4	A	6.5
Lactose	0.4	B	2.6
Sucrose	0.4	C	3.6
Peptone	202.5	D	10.8
Yeast extract	52.9	E	1.2
Sodium glycerophosphate	32.4	F	3.8
Whey	27.88	G	6.5
Molasses	0.12	H	2.6
		I	3.6
		J	18.9
		L	1.2
		M	7.0

yields with lower medium preparation costs. With medium B (50 g/L lactose content, supplemented with 3 g/L yeast extract and 5 g/L peptone) the mass of produced biosurfactant per gram cell dry weight increased 1.2 times with an estimated 60% decrease in the medium preparation costs comparing with the synthetic MRS medium. Similar conclusions were established for *S. thermophilus* A, where the use of medium H (50 g/L lactose content, supplemented with 3 g/L yeast extract and 5 g/L peptone) resulted in a biosurfactant production yield 1.5 times higher with an estimated 60% reduction in the medium preparation costs comparing with the synthetic M17 medium.

A by-product of the sugar cane industry, molasses, represents an alternative for the biosurfactant production process as it is a relatively inexpensive raw material compared to other substrate sources, and it possesses other valuable compounds for the fermentation process. This alternative medium was also studied in the present work for biosurfactant production by probiotic bacteria. The biosurfactant production yields achieved with supplemented molasses medium were higher than the obtained whether with conventional or supplemented cheese whey medium. Although higher amounts of biosurfactant were produced with medium F (20 g/L sucrose content, supplemented with 2.3 g/L yeast extract and 18 g/L peptone) and M (20 g/L sucrose content, supplemented with 8.8 g/L yeast extract, 17.5 g/L peptone and 92.6 g/L sodium glycerophosphate) for *L. lactis* 53 and *S. thermophilus* A, respectively; resembling what was observed for cheese whey medium, a better compromise between good yields and low-costs is achievable with medium where peptone and yeast extract amounts are lower (20 g/L sucrose content, supplemented with 3 g/L yeast extract and 5 g/L peptone). Thus, an increase about 1.2–1.4 times in the mass of produced biosurfactant per gram cell dry weight and a 80% medium preparation costs reduction comparing with the synthetic MRS or M17 medium were achieved, for both strains.

Lactic acid bacteria ferment sugars via different pathways and are also capable of forming other products, e.g. flavours such as diacetyl and acetoin, bacteriocins or biosurfactants. The different carbon sources give varying amounts of by-products [5,41]. Hence, it can be speculated that the use of lactose or sucrose as carbon source instead of glucose induced the cells to use another metabolic pathway, and therefore the amount of mass of biosurfactant produced per gram cell dry weight varied. Lactic acid bacteria have already proven to be ideal hosts for metabolic engineering. The efficacy of metabolic engineering of lactic acid bacteria for the increased production of biosynthetic metabolites is yet to be demonstrated, but based on the results gathered in this study it seems to be an interesting approach for developing new strategies of biosurfactant production.

5. Conclusions

L. lactis 53 and *S. thermophilus* A showed a good performance for glucose or lactose to biosurfactant fermentation using the costly MRS or M17 broth, respectively, which includes among others yeast extract and peptone. When the conventional synthetic media were replaced by cheaper alternative media, as cheese whey heat precipitated and molasses, in all cases fermentations were carried out effectively with high yields and productivities of biosurfactant. The best results, even higher than those obtained with the conventional synthetic media, were obtained using supplemented molasses, thus it can be used as an alternative economical medium for biosurfactant production.

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