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USE OF BUTTER MILK AND POULTRY-TRANSFORMING WASTES FOR ENHANCED PRODUCTION OF *Bacillus subtilis* SPB1 BIOSURFACTANT IN SUBMERGED FERMENTATION

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ABSTRACT

Biosurfactants are valuable microbial amphiphilic molecules with effective surface-active and biological properties applicable to several industries and processes. Microorganisms synthesize them, especially during growth on water-immiscible substrates, providing an alternative to chemically prepared conventional surfactants. Microbial surfactants are not yet a sustainable alternative to chemically synthesized surfactants seeing their potentially high production charges. This study highlights the use of low-cost agro-industrial raw material for fermentative production of biosurfactants. The Box–Behnken Design and response surface methodology were employed to optimize the concentrations of the ratio butter milk /distilled water, poultry-transforming wastes and inoculum size for lipopeptide biosurfactant production by *B.subtilis* SPB1 in submerged fermentation.The best production yield was about 12.61 ± 0.7 g/L of crude lipopeptide biosurfactant. It can be obtained when using a ratio butter milk /distilled water of 1.5, poultry-transforming wastes of 23g/L and an inoculum size of 0.12. In comparison to the highest biosurfactant production yield reported for *Bacillus subtilis* SPB1, three fold increases were obtained.

Keywords: *Bacillus subtilis*, Biosurfactant, Optimization, Box–Behnken Design, Submerged fermentation

INTRODUCTION

Naturally occurring surface-active agents derived from microorganisms, are called biosurfactants **(Banat, 2000)**. Most microbial surfactants are complex molecules, comprising diverse structures that include lipopeptides, glycolipids, polysaccharide-protein complex, fatty acids and phospholipids. They have an amphiphilic structure with hydrophilic (amino acids, peptides, anionic or cationic, di- or polysaccharides) and hydrophobic (saturated or unsaturated fatty acid) moieties **(Desai and Banat, 1997)**. They possess very attractive properties such as their excellent surface activity, low toxicity, high biodegradability and high efficiency in extreme conditions such as pH, temperature and salinity **(Muthusamy *et al.*, 2008; Mnif *et al.*, 2012a)**. Biosurfactants are characterized by numerous biological activities. They can act as antimicrobial, anti-adhesive, anti-tumoral, antiviral and anti-inflammatory agents **(Rodrigues *et al.*, 2006)**. Also, it has been reported that biosurfactants exhibited a broad spectrum of action, including insecticidal activity against lepidopteran larvae **(Ghribi *et al.*, 2011a, b, 2012b; Mnif *et al.*, 2013)** and antimicrobial activity against microorganisms with multi-drug resistant profiles **(Ghribi *et al.*, 2012a)**. The potential applications of biosurfactants at pilot scales in industry include emulsification and foaming for food processing **(Mnif *et al.*, 2012c, 2013c)**, wetting and phase dispersion for cosmetics and textiles, or solubilization for agrochemicals **(Lin, 1996)**. In addition, biosurfactants can be used in environmental applications such as bioremediation, enhancing solubility of diesel and dispersion of oil spills **(Banat, 1995; Mnif *et al.*, 2012a)**. Even though interest in biosurfactants is increasing, these molecules do not compete economically with synthetic surfactants **(Gautam and Tyagi, 2006)**. To reduce production costs, scientists focus on enhancing the microbial production of surfactants **(Shih *et al.*, 2008)**. To improve yield production, many methods are possible like the optimization of media components, the development of economical engineering processes, the use of cost-free or cost-credit feedstock and even the strain improvement by mutagenesis or recombinant strains **(Mercade and Manresa, 1994; Nitschke *et al.*, 2004; Muthusamy *et al.*, 2008)**. 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. Peat hydrolysate **(Sheppard and Mulligan, 1987)**, lactic whey **(Koch *et al.*, 1988)**, olive oil mill effluent **(Mercade *et al.*, 1993)**, soybean curd residue **(Ohno *et al.*, 1995)**, molasses **(Makkar and Cameotra, 1997)**, potato process effluent **(Fox and Bala, 2000 ; Thompson *et al.* 2000)**, cassava waste water **(Nitschke and Pastore, 2006)** orange peels and soya bean **(Ghribi *et al.*, 2011b)**, tuna fish cooking residue and sesame peel flour **(Mnif *et al.*, 2012a)** are possible substrates for biosurfactant accumulation. Butter milk (a waste product from butter production) and poultry-transforming wastes flour are examples of agro industrial wastes or by-products easily available in Tunisia. The aim of this study was to develop a low-cost alternative medium for biosurfactant production by *B.subtilis* SPB1 based on these two unconventional substrates using a Box–Behnken Design.

MATERIAL AND METHODS

Microorganism

The strain used in the present work was *Bacillus subtilis* SPB1 (HQ392822). It was isolated in our laboratory from a Tunisian soil contaminated by hydrocarbons, as reported by **Ghribi *et al.* (2012a);** *B. subtilis* SPB1 strain was streaked on a nutrient agar slant and incubated at 37°C. After 24 h, one loop of cells was dispensed in 3 ml of LB medium and incubated overnight at 37°C. Aliquots (0.2 ml) were used to inoculate 250 ml Erlenmeyer flasks containing 50 ml LB medium and incubated in a rotatory shaker at 200 rpm and 37°C (±0.5) during 4, 11, 14, 23, 24, 31 h in accordance with the purpose. Three ml of the obtained culture were used to inoculate the production medium. *Bacillus subtilis* SPB1 was shown to produce a highly effective biosurfactant that belongs to the class of lipopeptides. It was selected on the basis of the high haemolytic and emulsification activities of its biosurfactant which could reduce surface tension of the water from 70mNm^{-1} to 34 mNm^{-1} **(Ghribi *et al.*, 2011a)**.

Inoculum and culture conditions

The inoculum was prepared in 250 ml Erlenmeyer flasks containing 50 ml of LB medium and shaking at 150 rpm, overnight at 37°C (**Ghribi *et al.*, 2011a, b)**. It was used to inoculate the production medium composed only of butter milk,

poultry wastes flour and distilled water at the proportions given in Table 1. The production of biosurfactant was carried out in 250 ml Erlenmeyer flasks containing 50 ml of the medium and shaking at 150 rpm for 48 h at 37°C **(Ghribi *et al.*, 2011b, Mnif *et al.*, 2012a)**. Then, the material was used for extraction of lipopeptide and analysis.

Table 1 Box-Behnken design matrix of three independent factors along with experimental and predicted responses

Run	U_1: Butter Milk / distilled water	U_2: Poultry-Transforming Wastes Flour (g/L)	U_3 : Inoculum Size (OD_{600nm})	Y: Biosurfactant production (g/L)	
				Observed	Predicted
1	-1 (0.5)	-1 (15)	0 (0.2)	8.310	9.165
2	1 (1.5)	-1 (15)	0 (0.2)	11.940	11.893
3	-1 (0.5)	1 (45)	0 (0.2)	6.860	6.908
4	1 (1.5)	1 (45)	0 (0.2)	12.600	11.745
5	-1 (0.5)	0 (30)	-1 (0.1)	6.900	6.454
6	1 (1.5)	0 (30)	-1 (0.1)	11.540	11.996
7	-1 (0.5)	0 (30)	1 (0.3)	7.260	6.804
8	1 (1.5)	0 (30)	1 (0.3)	8.380	8.826
9	0 (1)	-1 (15)	-1 (0.1)	10.820	10.411
10	0 (1)	1 (45)	-1 (0.1)	8.050	8.449
11	0 (1)	-1 (15)	1 (0.3)	8.640	8.241
12	0 (1)	1 (45)	1 (0.3)	7.390	7.799
13	0 (1)	0 (30)	0 (0.2)	7.650	7.650
14	0 (1)	0 (30)	0 (0.2)	8.170	7.650
15	0 (1)	0 (30)	0 (0.2)	7.590	7.650
16	0 (1)	0 (30)	0 (0.2)	7.450	7.650
17	0 (1)	0 (30)	0 (0.2)	7.390	7.650

Substrates analysis

Butter milk was obtained from a dairy products factory (AGROMED SA, Sfax, Tunisia). Poultry-transforming wastes (bones, intestines, meat) obtained from a company specialized in farming, slaughtering, transforming and distributing poultry (CHAHIA, Sfax, Tunisia), were dried and thinly crushed. Total carbohydrates (sugars) were estimated using the phenol-sulfuric assay after total acid hydrolysis **(Daniels *et al.*, 1994; Dubois *et al.*, 1956; Israilides *et al.*, 1978)**. Protein Content was evaluated by the kjeldahl method according to **Pearson (1970)**. Lipid contents were determined gravimetrically after Soxhlet extraction using hexane as solvent **(AOAC, 1984)**. Dry matter was determined by oven drying at 105°C to constant weight **(AOAC, 1990)** and ash content was determined by combustion of the sample in a muffle furnace at 550°C for 12 h **(Bryant and McClements, 2000)**. Mineral content was determined by atomic absorption spectrophotometry **(Hernàndez *et al.*, 2005).**

Determination of the production yield

To determine the production yield, we adopt the protocol described by **Ghribi *et al.* (2011b)**. At the end of the cultivation, the culture was centrifuged at 10 000 rpm and 4°C for 20 min to remove bacterial cells. The supernatant-free cell was acidified using 6 N HCl at pH 2 and incubated overnight at 4°C. The precipitates were then collected by centrifugation at 10 000 rpm at 4°C for 20 min, washed three times with acid water (pH= 2) to collect the crude lipopeptide preparation followed by desiccation at 105°C for 24 h to determine the dry weight. Culture without inoculation was used as a negative control to take account of possible contributions of lipids and proteins from substrates. The negative control was included in each experiment and each cultural condition. Crude biosurfactant weight was calculated as the result of subtracting the grey white pellet weight obtained with the negative control from that measured with the culture containing the biosurfactant-producing strain. The values presented are the average of the results of three determinations of two separate experiments for each cultural condition.

Optimization of Biosurfactant Production Using Box–Bhenken Design

A Box–Bhenken design for three independent variables was adopted. It was generated using Nemrod-W version 2007 software **(LPRAI, Marseille, France)**. The three independent test variables chosen for the statistical experimental design are as follows: ratio of the liquid substrates: butter milk /distilled water (X_1), poultry-transforming wastes flour (X_2, g/L) and inoculum size (X_3, final OD_{600}). The range and the levels of the factors, which were varied according to the experimental design, in coded and real values, are given in Table 1.

Data analysis, modeling and response surface methodology

To analyze the experimental design data and determine the optimum conditions for enhanced biosurfactant production by *B. subtilis* SPB1, Response Surface Methodology (RSM) was used. In order to be correlated to the independent variables, the response variable was fitted by a second-order model that is represented below:

$$Y= b_0+ b_1 * X_1+ b_2 * X_2+ b_3 * X_3+ b_{1\text{-}1} * (X_1* X_1) + b_{2\text{-}2} * (X_2* X_2) + b_{3\text{-}3} * (X_3* X_3) + b_{1\text{-}2} * (X_1* X_2) + b_{1\text{-}3} * (X_1* X_3) + b_{2\text{-}3} * (X_2* X_3)$$

Where X_1, X_2 and X_3 are the coded factors studied (Table 1); b_0 intercept, b_1, b_2 and b_3 linear coefficients; $b_{1\text{–}1}$, $b_{2\text{–}2}$ and $b_{3\text{–}3}$ squared coefficients; $b_{1\text{–}2}$, $b_{1\text{–}3}$ and $b_{2\text{–}3}$ interaction coefficients. The model coefficients were estimated using multi-linear regression, and their significance was determined by applying Student's t test. The Fisher's F test was applied to check statistical significance of the model **(Mnif *et al.*, 2013b)**. The quality of the fit of the polynomial model equation was expressed by the coefficient of determination R^2. ''Nemrod-W'' software **(Nemrod-W by LPRAI Marseilles, France) (Mathieu *et al.*, 2000)** was used for regression and graphical analysis. To describe the individual and cumulative effects of the variables as well as the possible correlations that existed between them, the two-dimensional graphical representations of the system behavior, called the iso-response contour plans were plotted. The optimum levels of the defined parameters were obtained by solving the regression equation and also by the analysis of the response surface contour plots **(Mnif *et al.*, 2013b)**.

RESULTS

Characterization of the substrates

The chemical composition of poultry-transforming wastes flour and butter milk is shown in Table 2. It was clear that the two by-products have relatively high sugar, protein, fat and ash contents. Therefore, poultry-transforming wastes flour and butter milk could be considered as potential sources of carbon and nitrogen. These findings suggested that the mixture of these two substrates might be a suitable medium to produce SPB1 lipopeptide biosurfactant in submerged culture.

Table 2 Chemical composition of Butter Milk and Poultry-Transforming Wastes Flour

Component	% Dry matter	
	Butter Milk	**Poultry-Transforming Wastes Flour**
Water	91.23± 3.54	5.83±0.43
Protein	3.68±0.7	36.39±1.94
Fat	0.81±0.14	16.63±2.13
Sugar	5.82±0.13	3.31±0.23
Ash	2.43±0.67	37.75±0.73
	mg/dl	
Ca	117±3.12	153.39±13.27
Mg	13±0.69	9.62±0.86
P	91±8.29	101.53±10.72
K	162±6.74	182±20.23
Na	107±4.67	91.57±8.24
Fe	0.07±0.001	0.1±0.02
Zn	0.56±0.04	0.82±0.06
Co	0.012±0.01	0.07±0.002
Mn	0.003±0.0001	0.006±0.0002

All the given values are means of three determinations ± standard deviation**.**

Experimental Design Study: Data Analysis and Modeling

In order to predict the levels of the liquid substrates ratio, poultry-transforming wastes flour and inoculum size, experimental planification methodology was adopted. After these experiments had been performed, the experimental and predicted values for biosurfactant production yields were determined (Table 1). A second-order polynomial quadratic model was generated by the multiple regression analysis using Nemrod-W software. It was designed to correlate the independent variables and to explain the behavior of the system in the design space **(Mnif *et al.*, 2013b)**. Thus, the following regression equation presented below shows the relative biosurfactant production as a function of the test variables in coded units:

$$\mathbf{Y=}\ 7.650+\ 1.891^{*}\ \mathbf{X_1}+\ -0.601^{*}\ \mathbf{X_2}+\ -0.705^{*}\ \mathbf{X_3}+\ 1.036^{*}\ \mathbf{(X_1*\ X_1)}\ +\ 1.241^{*}\ \mathbf{(X_2*\ X_2)}\ +-0.166\ ^{*}\ \mathbf{(X_3*\ X_3)}\ +\ 0.528^{*}\ \mathbf{(X_1*\ X_2)}\ +\ -0.880^{*}\ \mathbf{(X_1*X_3)}\ +\ 0.380^{*}\ \mathbf{(X_2*\ X_3)}$$

The goodness-of-fit of the regression model can be ascertained by applying the Fischer *F*-test (Akhnazarova and Kafarov, 1982). The values of correlation coefficient, Model F and Model P>F, were found to be 0.94, 60.4338, and <0.0001, respectively, which implies that the model is significant (Table 3). The value of the adjusted determination coefficient (Adj. R^2=0.863) was also very high to advocate for a high significance and a very good fit of the model. A higher value of correlation coefficient, R (0.94), justifies an excellent correlation between experimental and predicted values of biosurfactant production (Olivera *et al*., 2004). At the same time, a relatively lower value of the coefficient of variation (CV=10.92 %) indicates a better precision and reliability of the experiments carried out (Box and Wilson, 1951).

Table 3 Analysis of variance (ANOVA) for the selected quadratic model for biosurfactant production

Source of variation	Sum of Squares	Degree of freedom	Mean square	F-value	Significance
Regression	51.8885	9	5.7654	60.4338	**
Residual	3.3149	7	0.4736		
Lack of fit	2.9333	3	0.9778	10.2492	*
Pure error	0.3816	4	0.0954		
Total	55.2034	16			

** Significant at 0.01 level;
*Significant at 0.05 level.

The F value 60.4338 was large that implies the adequacy of the model, and the interaction between variables are also significant. To check to significance of the different factors, Student's t test was done. The student *t* distribution and the significance of each coefficient are given in Table 4. Results indicate that most of all factors influence biosurfactant production.

Table 4 Estimated effect, regression coefficient and corresponding t and p values for biosurfactant production

Noun	Coefficient	F.inflation	Ecart-type	t.exp	Signification (%)
$\mathbf{b_0}$	7.650		0.13813037	55.38	***
$\mathbf{b_1}$	1.891	1	0.10920165	17.32	***
$\mathbf{b_2}$	-0.601	1	0.10920165	-5.51	*
$\mathbf{b_3}$	-0.705	1	0.10920165	-6.46	*
$\mathbf{b_{1\text{-}1}}$	1.036	1.01	0.15052408	6.88	*
$\mathbf{b_{2\text{-}2}}$	1.241	1.01	0.15052408	8.25	*
$\mathbf{b_{3\text{-}3}}$	-0.166	1.01	0.15052408	-1.1	33.1
$\mathbf{b_{1\text{-}2}}$	0.528	1	0.15443445	3.42	#
$\mathbf{b_{1\text{-}3}}$	-0.880	1	0.15443445	5.7	*
$\mathbf{b_{2\text{-}3}}$	0.380	1	0.15443445	2.46	7.0

(***) Significant at the level 99.99%
(*) Significant at the level 99%
(#) Significant at the level 95%

3D Response Surface Plots Analysis and Optimum Validation

Response surface methodology was adopted to optimize economical medium component supporting maximum biosurfactant production yield. The response surface plots and their respective contour plots for the predicted response Y (biosurfactant production yield), based on the second-order model, provided information about the interaction between two parameters and allowed an easy interpretation of the results and prediction of the optimal values **(Mnif *et al.*, 2012b)**. The effect of the interaction between the three conditions used for biosurfactant production by *B. subtilis* SPB1 was investigated by plotting the response surface curves against any two independent variables while keeping the third independent variable at zero level. According to the Student's t test, the most of mutual interactions between the different factors are significant. Furthermore, ratio of liquid substrates (butter milk / distilled water) was highly significant and as the estimate coefficient was positive; an increase in this ratio may increase the production yield, while inoculum density and poultry transforming waste would affect negatively biosurfactant production since their estimate coefficients were negatives.

So, in order to determine the optimal conditions of the three operational parameters supporting a maximum biosurfactant production yield, the 3D response surface curve and their respective 2D contour plot representing the pair-wise interaction between poultry-transforming wastes flour level and the liquid substrates ratio (butter milk/distilled water) were then plotted as well as the interaction between the ratio butter milk/distilled water and inoculums size at constant poultry-transforming wastes flour level (Fig 1). It can be clear from Figure 1A that in spite of the poutry transforming waste concentration, the increase in the value of the ratio butter milk / distilled water induced a significant increase in biosurfactant production yield. Also, Figure 1B shows that iso-responses are near parallel to inoculum size axis. This suggests that an increase neither a decrease of the inoculum density did not affect significantly the production yield.

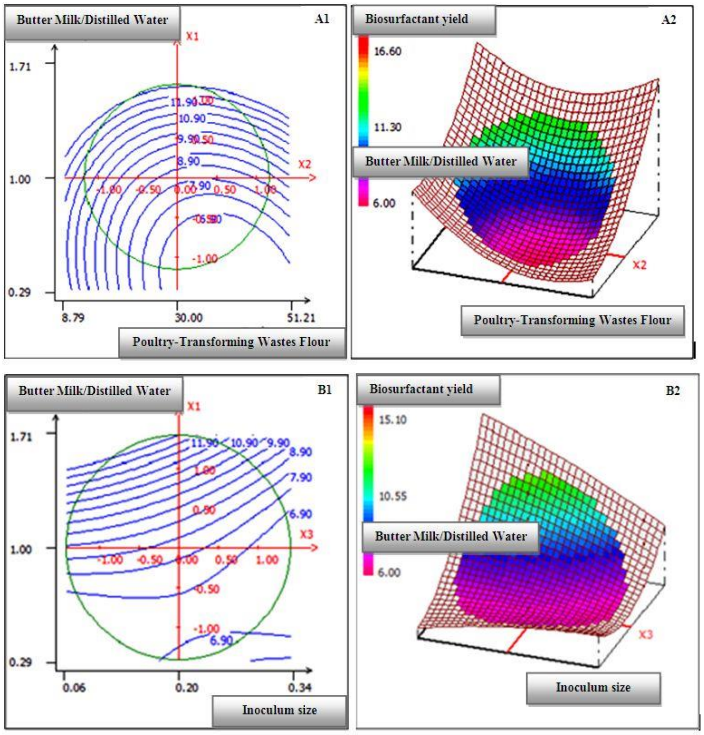

Figure 1 Contour Plots (A1) and three-dimensional response surface (A2) of the interaction between the ratio Butter Milk/Distilled Water and Poultry-Transforming Wastes Flour level at constant Inoculum Size. Contour Plots (B1) and three-dimensional response surface (B2) of the interaction between the ratio Butter Milk/Distilled Water and Inoculum Size at constant Poultry-Transforming Wastes Flour level.

In fact, 3D response surface curve represents generally the graphical representation of the regression equation **(Gu *et al.*, 2005)**, permit an easy prediction and interpretation of the results and are helpful in determining the optimal factors levels **(Ghribi *et al.*, 2011b)**. The effect of the interaction of various parameters on biosurfactant production by *B. subtilis* was investigated by plotting the response surface curves against any two independent variables while keeping the third independent variable at constant level. Therefore, in the first time we have fixed the inoculum size at its zero coded level OD_{600}= 0.2. So, the response was represented as function of the interaction between liquid substrates ratio and poultry-transforming wastes flour level. The optimal values for the two later variables were estimated to 30 g/l and 1.7, respectively (data not shown). When fixing poultry-transforming wastes flour at its zero coded level (30 g/L), the optimal biosurfactant production (12.4 g/L) was obtained with an inoculums size of 0.12 and butter milk/distilled water ratio of 1.5. Similarly, when fixing the inoculum size at its optimal value (0.12), the optimum predicted lipopeptide production was of about 12.4 (±0.71) g/L which could be obtained when operating with a liquid substrates ratio of 1.5 and a poultry-transforming wastes flour level of 23g/L. The corresponding experiment was carried out in four replicates and the average yield value was calculated. The biosurfactant production was 12.61 g/L while the predicted value was 12.4 (± 0.7) g/L. This production was, interestingly two fold much higher than that obtained when using the defined medium composed of basal salts, urea and glucose and even so much better than the production yield obtained under optimized conditions reported in our previous work **(Ghribi and Ellouze-Chaabouni, 2011c ; Mnif *et al.*, 2012 a, b)**.

DISCUSSION

Surfactants constitute an important class of industrial chemicals widely used in almost every sector of modern industry. At the moment, most of the commercially available surfactants are chemical surfactants, mainly petroleum-derived. The chance of biosurfactants replacing their chemical counterparts is mainly related with the cost, functionality and production capacity to meet the need of the intended application. It can be accepted a high production cost for a biosurfactant if it is a high value product and/or the production volumes are low, such as for medicines for example. However, for the most common biosurfactant applications, namely environmental ones, the high volumes required make high production costs unbearable. Therefore, research efforts must focus on the development of processes of biosurfactant production with reduced costs. Some of the factors that can influence the costs are the selected or engineered microorganisms; the developed process; the choice of the growth substrate; the process by-products and product recovery. Moreover, as discussed previously, to reduce the production costs it is desirable to use low-cost raw materials. This paper highlights the use of butter milk and poultry-transforming wastes for *B. subtilis* SPB1 biosurfactant production in submerged culture. It demonstrated also the effectiveness and feasibility of the use of statistical models to optimize culture medium components and conditions for enhanced biosurfactant production as it was previously confirmed by **Sen and Swaminathan (1997); Kiran *et al.* (2010); Ghribi *et al.* (2012a) and Mnif *et al.* (2013b)**. Therefore, a high degree of similarity was observed between the predicted and experimental values that reflected the accuracy and applicability of response surface methodology to optimize the process for enhanced SPB1 biosurfactant production. Here, it permitted to define optimum conditions supporting high biosurfactant production defined as inoculum size (0.12), liquid substrates ratio (milk butter/distilled water) (1.5) and poultry-transforming wastes flour level (23g/L). Also, response methodology allows an analysis of the individual, cumulative and interactive effects of these three parameters on biosurfactant production **(Mnif *et al.*, 2013b)**. According to Table 4, all the factors affect significantly the response; also, the interactions between the different factors are significant. Figure 1 shows that an increase in the liquid substrates' ratio enhances the biosurfactant production yield. A maximum biosurfactant production of 12.61 g/L was achieved. Validation experiments were also carried out to verify the adequacy and the accuracy of the model and results showed that the predicted value (12,4g/L ± 0.7) agreed with the experimental value (12,61g/L) well and more than six fold increase compared to the original medium was obtained **(Ghribi and Ellouze-Chaabouni, 2011c)**. Interestingly, the production yield obtained in this study is three fold higher than this reported in recent studies dealing with the optimization of *B. subtilis* SPB1 biosurfactant production conditions under submerged fermentation. Indeed, **Ghribi *et al.* (2011b)** showed that, the optimal biosurfactant production by *B. subtilis* SPB1 (4.45 g/L) was obtained when using orange peels (15.5 g/L), soya bean (10 g/L) and diluted sea water (30%). Similarly, **Mnif *et al.* (2012a)** reported SPB1 biosurfactant production of about 4.5 g/L with a medium composed of 33 g/L sesame peel flour mixed with diluted tuna fish cooking residue (40%). The results reported in this paper are unique and encouraging. Also, they give a basis for further study with large scale fermentation for *B. subtilis* SPB1 biosurfactant production using low-cost materials which contribute to the valorization of wastes and effluents obtained from local industry. This approach of bioconversion of residues to value added products can considerably reduce the production charges of biosurfactants as well as lead to a reasonable utilization of residues, which could contribute in the maintain of the ecological balance. Taking count of many reports and studies; efforts are concentrated on different processes of biosurfactant production to find an appropriate and economically practical method to make microbial surfactants competitive with chemical surfactants.

CONCLUSION

Bacillus subtilis SPB1 produces a biosurfactant that belongs to the class of lipopeptides having excellent emulsifying properties. The results reported in this paper indicate that *Bacillus subtilis* SPB1 can be cultivated under submerged fermentation (SmF) for the production of biosurfactant using agro-industrial residues. The use of butter milk and poultry-transforming wastes flour as low cost substrates is unique. The optimum conditions supporting high biosurfactant production yield (12.61 g/L) were defined as inoculum size (0.12), liquid substrates ratio (1.5) and poultry-transforming wastes flour level (23g/L). In comparison to original level and the optimized production yields described for *Bacillus subtilis* SPB1, six fold and three fold increases were obtained, respectively. Even though, much work should be done before its application in the field, the results presented in this paper are encouraging and of special economic interest for countries with abundance of agroindustrial residues and lower the production cost of metabolites.

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