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A new bacterial-derived biosurfactant for biotechnological applications in the oil industry: production, optimization, biosurfactant functional and physicochemical characterization

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ABSTRACT

Aiming the potential application of lipopeptide biosurfactant (BioS) in bioremediation, we studied its production by a novel, isolated strain of *Bacillus* sp. MI27. Using the experimental design methodology, a novel medium composed of 2% sucrose, 0.27% Na2HPO4, 0.2% ammonium sulfate, 0.02% NaCl, 0.02% $CaCl_2$, 0.02% $MgSO_4$, 0.001% $MnSO_4$, 0.06% KH_2PO_4, 0.005% FeSO4 and 0.005% $ZnSO_4$ was optimized. With this composition, strain MI27 produces 1.4 g/L BioS with maximum surface tension (ST) reduction of 23 mN/m and a dispersion diameter of around 10 cm. Emulsifying and foaming activities have been also confirmed. The critical micelle concentration (CMC) value was about 120 mg/L with corresponding ST of 23 mN/m. The dispersion and emulsification index (EI) were about 12 cm and 45% at 1,000 mg/L respectively. Moreover, the foaming capacity, stable over 1 h of incubation, was about 80% at 1,000 mg/L. Additionally, we studied the effect of different pH, temperature and salinities on MI27 BioS activity and stability. Obtained results showed interesting surface activities at extreme physicochemical conditions, especially at acidic and alkaline pH values, high and low temperatures and higher salinities. All these characteristics enable the possible application of BioS in water treatment biotechnology under diverse environmental conditions.

Key words: activity, biosurfactant, emulsification, foaming, oil dispersing, optimization, stability

HIGHLIGHTS

- Experimental methodology to optimize lipopeptide production by *Bacillus* sp. MI27.
- Optimized medium composed only of sucrose, ammonium sulfate and mineral elements.
- The lipopeptide had a CMC value of 120 mg/L with a surface tension of 23 mN/m. It disperses and emulsifies oil to about 12 cm and 45% with a foaming capacity of about 80%.
- The lipopeptide showed surface activity and stability at extreme physicochemical conditions.

ABBREVIATIONS

BioS	biosurfactant
ST	surface tension
ODA	oil dispersion activity
CMC	critical micelle concentration
CCD	central composite design

INTRODUCTION

Water pollution causes great damage to the environment and ecological equilibrium. Petroleum products consisting of aliphatic or aromatic hydrocarbons are the most polluting compounds. They have received special attention in recent years because of their toxic, carcinogenic and mutagenic characteristics (Sharma *et al.* 2022; Zahed *et al.* 2022). In pursuit of this aim, numerous physicochemical, biological and combined treatment methods were developed to combat hydrocarbon pollution. Biological decontamination or bioremediation uses the action of microorganisms (bacteria or fungi) to detoxify organic contaminants by complete or partial

biodegradation preferentially *in situ*, and has gained great attention due to the low implementation cost, simplicity and respect for the environment (Sharma *et al.* 2022; Zahed *et al.* 2022). Nevertheless, the low solubility and bioavailability of petroleum-derived products in water and soil, respectively, limits their biological treatments (Sharma *et al.* 2022; Zahed *et al.* 2022). In fact, they can form a stable oil layer on the surface of the water, tend to agglomerate in water, and adsorb strongly in soil, making them less accessible to attack by microorganisms and difficult to assimilate (Sharma *et al.* 2022). Therefore, increasing the dissolution of hydrophobic compounds in water and their desorption rates from soil particles are of great interest to enhance their depollution, especially by biodegradation. Furthermore, due to increasing exploitation, production, transportation and storage, oil spreads over a large area on the sea surface, provoking considerable detriment to plants and animals disturbing therefore all the ecological equilibrium (Mnif *et al.* 2017a). The use of synthetic dispersants, specified as a material that decreases the cohesive attraction between similar particles, is an effective means that facilitates the mechanical recovery of oil spills (Mnif *et al.* 2017a; Ng *et al.* 2022)

As known, surfactants defined as amphiphilic compounds with a hydrophilic head and a hydrophobic tail have the ability to reduce the surface tension (ST) of water along with oil dispersing, emulsification and foaming activities (Shaban *et al.* 2020). They are exploited, among other applications as detergents, emulsifiers, foaming and dispersants agents (Shaban *et al.* 2020). Most of the surfactants currently used are produced by chemical synthesis and are derived from petrochemical compounds (Shaban *et al.* 2020). Therefore, they are toxic and non-biodegradable (Shaban *et al.* 2020). However, interest in microbial surfactants called biosurfactants (BioS) has increased significantly over the past decade (Mnif & Ghribi 2015a, 2015b, 2015c; Fenibo *et al.* 2019). These BioS are very diverse and quickly biodegradable (Mnif & Ghribi 2015a, 2015b, 2015c; Fenibo *et al.* 2019). They are well characterized by their great properties to be active under extreme temperature, pH and salt conditions in addition to their stabilities (Mnif & Ghribi 2015a, 2015b, 2015c).

Owing these functional activities and physicochemical properties, BioS are applied in many fields, including the food and chemical industry, pharmaceutics and biomedicine, environment and bioremediation (Carolin *et al.* 2021; Karlapudi *et al.* 2018; Nikolova & Gutierrez 2021; Sakthipriya *et al.* 2021). They have a great interest in their use as an improver of hydrophobic compounds' solubility in water, mobility from soil particles and as an oil dispersant agent to combat oil spills, enhancer of oil biodegradation (Mnif *et al.* 2013a, 2013b, 2014, 2015a, 2017b). Thus, permitting their involvement in the water treatment process.

Basically, BioS are produced by multiple varieties of microbial strains during their growth on water-immiscible compounds (Mnif & Ghribi 2015a, 2015b, 2015c; Fenibo *et al.* 2019). However, their low yield and high-cost production limit their application. The enhancement of the yield of production and the optimization of economic bioprocesses has become of great interest to biotechnologists. It can be achieved by strain improvement by classical or directed mutagenesis, by using recombinant strains and by optimizing the nutritional requirements and physicochemical conditions of fermentation (Mnif & Ghribi 2015b, 2015c). In fact, numerous nutritional parameters can affect BioS production, such as carbon and nitrogen sources as well as mineral elements (Beltran-Gracia *et al.* 2017; Nurfarahin *et al.* 2018; Singh *et al.* 2019). To know, a broad variety of hydrophilic carbon sources, mainly carbohydrates and sugars (Zhang *et al.* 2016; Ghazala *et al.* 2017; Hmidet *et al.* 2017; Phulpoto *et al.* 2020) and hydrophobic carbon sources, mainly hydrocarbons and vegetable oils (Ndlovu *et al.* 2017; Ohadi *et al.* 2017; Patowary *et al.* 2017) can be exploited to produce BioS. These different carbon sources can be used separately or as mixed substrates (Sarubbo *et al.* 2016; Joy *et al.* 2017). However, glucose rests as the primary source for BioS production, as widely recognized (Eswari *et al.* 2016). However, in addition to glucose, sucrose can be used as an interesting sugar for lipopeptide production (Liu *et al.* 2012; Singh *et al.* 2014). Additionally, nitrogen sources can influence greatly BioS production. In pursuit of this aim, various organic or inorganic nitrogen sources can be employed. Well-known organic nitrogen sources like yeast extract, tryptone and glutamic acid can support higher production yields (Eswari *et al.* 2016; Beltran-Gracia *et al.* 2017). In addition, other organic nitrogen sources, namely peptone, casein acid as well as soybean flour have been used (Beltran-Gracia *et al.* 2017). Moreover, ammonium nitrate, ammonium sulfate, sodium nitrate, urea and glutamic sodium were assayed as inorganic nitrogen sources (Beltran-Gracia *et al.* 2017; Moshtagh *et al.* 2019; Phulpoto *et al.* 2020). On the other hand, previous research work denoted the important effect of trace elements, namely magnesium, manganese, iron, copper and nickel on BioS production (Lin *et al.* 2007; Beltran-Gracia *et al.* 2017; Bartal *et al.* 2018). Additionally, physicochemical factors like the initial pH of the culture medium, the incubation temperature as well as the rate of agitation have a great effect on lipopeptide production (Beltran-Gracia *et al.* 2017; Moshtagh *et al.* 2019; Phulpoto *et al.* 2020). For the agitation speed, moderate values in the order of 150–200 rpm can

support maximal BioS production (Mnif & Ghribi 2015b, 2015c). For the temperature, lipopeptides production can be accomplished between 25 and 45 °C (Mnif & Ghribi 2015b, 2015c). For the pH, a neutral value is the most favorable for an optimal BioS production (Mnif & Ghribi 2015b, 2015c).

The experimental planning methodology can be applied to optimize nutritional and physicochemical conditions permitting a best BioS production (Mnif & Ghribi 2015a; Bertrand *et al.* 2018; Fenibo *et al.* 2019; Moshtagh *et al.* 2019; Singh *et al.* 2019; Phulpoto *et al.* 2020). In pursuit of this aim, we are interested in the present work to produce a new BioS with diverse functional properties derived from a new *Bacillus* sp. strain. It was isolated from a hydrocarbon-contaminated biotope in the region of Djerba in Tunisia and screened for its ability to reduce the ST of the culture medium along with its hemolytic activity. Microscopic observation and biochemical characterization by API 50 CH (Biomérieux; API Reference Guide) showed that the strain is Gram-positive isolate belonging to the *Bacillus* genera. As characterized in our previous work, *Bacillus* sp. MI27 produced lipopeptide isoforms belonging to the cyclic and linear surfactin families. The *m/z* values of the cyclic homologs of surfactin were about 994; 1,008; 1,022 and 1,036 Da. For the linear homologs, the *m/z* values were about 1,012; 1,026; 1,040 and 1,056 Da. Having the objective to be applied in environmental and industrial fields, we proposed a functional characterization of the produced BioS, including the ST-reducing power, the determination of the critical micelle concentration (CMC), the oil dispersing activity (ODA) and the emulsification and foaming capacities. In order to increase the production yield, we followed the experimental design methodology. Additionally, we studied the effect of various physicochemical conditions on the BioS surface activity and stability.

MATERIALS AND METHODS

Chemical product

The burned motor oil that served to determine the ODA was obtained from a local mechanic's station from Sfax, Tunisia.

Optimization of biosurfactant production under submerged fermentation

Submerged fermentation for BioS production

The new BioS-producing strain *Bacillus* sp. MI27 was exploited in the present study. The inoculum was prepared in an LB liquid medium as described by (Mnif *et al.* 2021a, 2021b, 2021c). It was incubated overnight at 37 °C under agitation of 180 rpm. During the optimization study of BioS production, 4% (v/v) inoculums were passed into 250-mL shake flasks containing 50 mL of the respective sucrose-based medium (pH = 7.0). The medium composition was indicated in the optimization study part according to the experimental design. The pH was adjusted by 1 N NaOH. The prepared culture medium was incubated at 37 °C under shaking at 150 rpm for 24 h.

Design of the experiments

The experimental planning methodology was selected to optimize BioS production by *Bacillus* sp. MI27 on a sucrose-based medium under submerged fermentation. The optimization study was divided into two steps: screening of the most significant compounds by a Plackett and Burman design and their optimization by a central composite design (CCD).

Identification of the most important nutrient components: Plackett and Burman design

In order to screen the most important medium compounds, a Plackett–Burman design was followed. This is a fractional plan that permits the exploration of up to '*N*-1' variables with *N* experiments. It supposes the absence of interactions between the different media components. During the present study, 11 parameters were nominated to evaluate their effect on BioS production, including sucrose, yeast extract, ammonium sulfate, Na_2HPO_4, NaH_2PO_4, $MgSO_4$, NaCl, $MnSO_4$, $FeSO_4$, $ZnSO_4$ and $CaCl_2$. Table 1 presents the described media components and their corresponding higher and lower levels by a total of 12 experiments of the Hadamard matrix (run N°1–12). Two coded levels (−1 and +1) were attributed for each variable. The production of BioS was managed by ST, the ODA measurements and the quantification of the BioS yield (g/L).

CCD to optimize the screened components: response surface methodology analyses

In the second part of the optimization study, a CCD was adopted. They were conducted to predict an empirical model of the process, find out the optimum values of the most important factors and enhance BioS production. The Nemrod-W Version 2007 software (LPRAI, Marseille, France) was used to generate the design, including 16

Table 1 | Plackett–Burman experimental design for 11 variables and the obtained results for BioS production

Exp.	U_1	U_2	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	ST reduction (%)	Oil dispersion activity (cm)	BioS yield (g/L)
1	1 (3)	1 (0.5)	− 1 (0.2)	1 (0.2)	1 (0.1)	1 (0.1)	−1 (0.02)	−1 (0.001)	−1 (0.001)	1 (0.01)	−1 (0.001)	58.16	5.00	0.620
2	−1 (1)	1 (0.5)	1 (0.2)	−1 (0.05)	1 (0.1)	1 (0.1)	1 (0.1)	−1 (0.001)	−1 (0.001)	−1 (0.001)	1 (0.01)	57.50	6.00	0.960
3	1 (3)	−1 (0.1)	1 (0.2)	1 (0.2)	−1 (0.02)	1 (0.1)	1 (0.1)	1 (0.01)	−1 (0.001)	−1 (0.001)	−1 (0.001)	45.00	1.00	0.084
4	−1 (1)	1 (0.5)	−1 (0.05)	1 (0.2)	1 (0.1)	−1 (0.02)	1 (0.1)	1 (0.01)	1 (0.01)	−1 (0.001)	−1 (0.001)	55.66	4.00	0.550
5	−1 (1)	−1 (0.1)	1 (0.2)	−1 (0.05)	1 (0.1)	1 (0.1)	−1 (0.02)	1 (0.01)	1 (0.01)	1 (0.01)	−1 (0.001)	45.00	2.00	0.064
6	−1 (1)	−1 (0.1)	−1 (0.05)	1 (0.2)	−1 (0.02)	1 (0.1)	1 (0.1)	−1 (0.001)	1 (0.01)	1 (0.01)	1 (0.01)	50.66	4.00	0. 52
7	1 (3)	−1 (0.1)	−1 (0.05)	−1 (0.05)	1 (0.1)	−1 (0.02)	1 (0.1)	1 (0.01)	−1 (0.001)	1 (0.01)	1 (0.01)	48.33	3.00	0.395
8	1 (3)	1 (0.5)	−1 (0.05)	−1 (0.05)	− 1 (0.02)	1 (0.1)	−1 (0.02)	1 (0.01)	1 (0.01)	−1 (0.001)	1 (0.01)	53.33	3.00	0.550
9	1 (3)	1 (0.5)	1 (0.2)	−1 (0.05)	−1 (0.02)	−1 (0.02)	1 (0.1)	−1 (0.001)	1 (0.01)	1 (0.01)	−1 (0.001)	53.66	3.00	0.580
10	−1 (1)	1 (0.5)	1 (0.2)	1 (0.2)	−1 (0.02)	−1 (0.02)	−1 (0.02)	1 (0.01)	−1 (0.001)	1 (0.01)	1 (0.01)	56.00	9.00	1.040
11	1 (3)	−1 (0.1)	1 (0.2)	1 (0.2)	1 (0.1)	−1 (0.02)	−1 (0.02)	−1 (0.001)	1 (0.01)	−1 (0.001)	1 (0.01)	53.33	3.00	0.440
12	−1 (1)	−1 (0.1)	−1 (0.05)	−1 (0.05)	−1 (0.02)	−1 (0.02)	−1 (0.02)	−1 (0.001)	−1 (0.001)	−1 (0.001)	−1 (0.001)	45.00	2.00	0.280

experiments, namely 8 experiments of the full factorial design experiments (2^3 = runs No. 1–8), 4 axial points (runs No. 9–12) and 4 replicates in the domain center (runs No. 13–16) to estimate the variability of the experimental results (Table 2). Three coded levels (−1, 0 and +1) were assessed for each variable. The response values (Y) denoted in each trial were the mean values of two repetitions. The BioS production was managed by the ST, the ODA and the BioS yield (g/L) measurements.

Table 2 | Three variables CCD along with the coded and real values of the different variables and the experimental and predicted responses

				Y_1: % of ST decrease		Y_2: oil dispersion (cm)		Y_3: BioS yield (g/L)	
Exp. No.	X_1: YE (%)	X_2: Na_2HPO_4 (%)	X_3: $CaCl_2$ (%)	Exp. response	Pred. response	Exp. response	Pred. response	Exp. response	Pred. response
1	0.50 (−1)	0.25 (−1)	0.020 (−1)	60.00	61.789	10.50	10.570	1.50	1.542
2	1.00 (+1)	0.25 (−1)	0.020 (−1)	59.13	58.608	8.00	8.449	1.10	1.075
3	0.50 (−1)	0.55 (+1)	0.020 (−1)	60.00	59.014	9.00	9.127	1.25	1.285
4	1.00 (+1)	0.55 (+1)	0.020 (−1)	59.65	61.013	8.00	8.006	1.20	1.217
5	0.50 (−1)	0.25 (−1)	0.050 (+1)	58.62	56.920	7.00	6.828	0.85	0.791
6	1.00 (+1)	0.25 (−1)	0.050 (+1)	48.27	48.919	5.50	5.207	0.70	0.623
7	0.50 (−1)	0.55 (+1)	0.050 (+1)	60.51	60.695	12.00	11.385	1.40	1.383
8	1.00 (+1)	0.55 (+1)	0.050 (+1)	60.00	57.874	11.00	10.765	1.70	1.615
9	0.33 (−1.6818)	0.40 (0)	0.035 (0)	60.68	60.941	9.00	9.271	1.20	1.179
10	1.17 (+1.6818)	0.40 (0)	0.035 (0)	55.68	55.895	7.00	6.9649	0.90	0.981
11	0.75 (0)	0.15 (−1.6818)	0.035 (0)	56.55	56.260	8.00	7.888	0.96	1.011
12	0.75 (0)	0.65 (+1.6818)	0.035 (0)	60.69	61.457	11.00	11.347	1.62	1.629
13	0.75 (0)	0.40 (0)	0.010 (−1.6818)	60.00	58.860	9.00	8.532	1.30	1.239
14	0.75 (0)	0.40 (0)	0.060 (+1.6818)	50.51	52.126	7.00	7.703	0.82	0.941
15	0.75 (0)	0.40 (0)	0.035 (0)	50.00	49.115	6.00	5.740	0.71	0.700
16	0.75 (0)	0.40 (0)	0.035 (0)	49,31	49.115	5.50	5.740	0.70	0.700
17	0.75 (0)	0.40 (0)	0.035 (0)	48.96	49.115	6.00	5.740	0.70	0.700
18	0.75 (0)	0.40 (0)	0.035 (0)	48.27	49.115	5.0	5.740	0.70	0.700

Statistical analysis and modeling

In order to check for errors and the significance of each parameter, an analysis of variance (ANOVA) was done. BioS production yield was taken as Y. The multiple regression analysis served to analyze the obtained data in order to acquire an empirical model that could relate the independent variables to the measured response. Thus, the following quadratic equation could explain the behavior of the system:

$$Y = b_0 + b_1{*}X_1 + b_2{*}X_2 + b_3X_3 + b_{1-1}{*}X_1^2 + b_{2-2}{*}X_2^2 + b_{3-3}{*}X_3^2 + b_{1-2}{*}X_1{*}X_2 + b_{1-3}{*}X_1{*}X_3 + b_{2-3}{*}X_2{*}X_3$$

where X_1, X_2 and X_3 are the coded factors studied; b_0 is the intercept; b_1, b_2 and b_3 are the linear coefficients, $b_{1\text{-}1}$, $b_{2\text{-}2}$ and $b_{3\text{-}3}$ are the squared coefficients and $b_{1\text{-}2}$, $b_{1\text{-}3}$ and $b_{2\text{-}3}$ are the interaction coefficients.

The multi-linear regression method served to estimate the model coefficients using the statistical software package (Nemrod-W by LPRAI Marseilles, France) as described in our previous works (Mnif *et al.* 2021b). The

statistical significance of the model was evaluated on the basis of *F*-test with unequal variance ($p < 0.05$). The response surface graphs, which represent the system behavior, were plotted after the conduction of the regression analyses on the experimental data. The isoresponse contour plot qualified as a two-dimensional graphical representation that depicted the individual and cumulative effects of the parameters. Moreover, they are used to forecast the potential correlations that could occur between the different variables.

Biosurfactant recovery

During the production experiments, a crude BioS extract was prepared according to the protocol described by Mnif *et al.* (2021a, 2021b). It consists of three consecutive cycles of acid precipitation–dissolution. The final BioS pellet was collected by centrifugation at 8,000 rpm/4 °C for 20 min, washed twice with acid distilled water (pH = 2) to eliminate any impurities and dissolved in alkaline-distilled water (pH = 8.0). After that, the concentration of the final dissolved extract was determined by the gravimetric method in order to quantify BioS production during the optimization study (Mnif *et al.* 2013a, 2013b). However, to characterize the produced BioS, the final extract was lyophilized and served as a crude lipopeptide preparation to evaluate the ST, the ODA, as well as the emulsification and foaming capacities.

Functional characterization of *Bacillus* sp. MI27-derived lipopeptide

ST evaluation and quantification of the CMC

The ST of the crude BioS was measured by a model Tensiometer Sigma 700, according to the Du-Noüy ring method. Except for special indications, experiments were realized at room temperature. To determine the CMC, we evaluated ST in the function of increasing BioS concentration. When an abrupt decrease in the ST was recorded, we denoted the CMC value (Mnif *et al.* 2021a, 2021b). It corresponds to the concentration at which BioS agglomerates into micelles.

Determination of the oil dispersion activity

The oil displacement test served to evaluate the ODA using burned motor oil. Assays were realized in a Petri dish with a diameter of 15 cm with 40 mL of distilled water. 100 µL of burned motor oil was dropped onto the surface of the water followed by the addition of 50 µL of the crude BioS. Hence, a clear zone of dispersion appeared and its diameter that corresponds to the ODA is recorded (Mnif *et al.* 2021a, 2021b).

Determination of the emulsification index

The emulsification assay was realized according to Mnif *et al.* (2021a, 2021b). For this, 1 mL of each BioS was mixed with 1 mL of motor oil in a 10 mL glass tube, vortexed for 2 min and allowed to settle for 24 h at room temperature. To quantify the emulsifying activity, we measure the quotient of emulsion-layer height (cm) by the total height after 24 h. The experiment was realized in triplicate and the Emulsification Index (EI-24%) was calculated according to Equation (1).

$$\text{EI} - 24\ (\%) = \frac{\text{Height of emulsion formed}}{\text{Total height}} \times 100 \tag{1}$$

Assay of the foaming activity

In a test tube, 10 mL of distilled water was mixed with different concentrations of MI27 BioS. The solutions were agitated correctly via an Ultra-Turrax (T18 basic, Germany) for 1 min at speed 3 and then placed on the laboratory bench. After resting, we measured the height of the foam above the water in centimeters (Bouassida *et al.* 2018). Foaming activity was calculated in accordance with the following equation (Equation (2)). The foaming stability was measured by the height of the foam after different incubation times for 1 h.

$$\text{Foaming activity}\ (\%) = \frac{\text{Height of the foam above the water}}{\text{Total height of the foam and water}} \times 100 \tag{2}$$

Effects of physicochemical factors on BioS surface activity and stability

Aiming to wide spread the application of MI27 lipopeptide BioS, we propose to study its surface activity and stability at different physicochemical factors by the measurement of the ST-reducing power (at the CMC

value = 120 mg/L) and the ODA as well as the emulsification and foaming capacities at 1,000 mg/L. Regarding the effect of temperature, surface activity was determined at increasing values ranging from 10 to 80 °C and after pre-incubation of the crude extract at the same range of temperature by evaluating only the ST and the ODA. For the pH and the salinity, the four activities were evaluated. The pH activity and stability were checked at different values ranging from 2.0 to 10.0 using glycine–HCl buffer (pH 2.0–3.0), acetate buffer (pH 4.0–5.0), phosphate buffer (pH 6.0–8.0) and glycine–NaOH buffer (pH 9.0–10.0) (all at a final concentration of 20 mM) (Mnif *et al.* 2013a; 2021a, 2021b). Different NaCl solutions at concentrations of 0, 0.5, 1, 1.5, 2, 3, 4 and 5% (weight/volume) were studied for the effect of salt on the surface activity and stability. Activity at standard conditions corresponding to pH = 7, ambient temperature, without salt addition and without pre-incubation served to determine the Residual Activity (RA) according to the present formula (Equation (3)) (Mnif *et al.* 2021a, 2021b). Each condition was performed in triplicates and average values were recorded.

$$\text{Residual Activity (\%)} = \frac{\text{Recorded activity at Standard Conditions}}{\text{Recorded activity after pH, tempearture and salts treatment}} * 100 \quad (3)$$

RESULTS AND DISCUSSION

Biosurfactant production optimization

Plackett–Burman experimental design for the screening of factors

The production of BioS by strain MI27 was evaluated by measuring the ST and the ODA in the supernatant and by the determination of the quantity produced by the gravimetric method. The experimental values of the three studied responses are presented in Table 1. The obtained results correspond to the average of three independent tests with two replicates for each test. For the percentage of reduction of ST, the results are between 45 and 58.16%; for the diameters of dispersion, they are between 1 and 9 cm; for the quantities produced, the results are between 0.064 and 1.04 g/L. All these results show a wide variability of the responses with a good correlation between them for each experiment studied, suggesting the right choice of the different parameters.

The effects of the different factors on the response studied are reflected in the Pareto diagrams presented in Figure 1. The results show that the amount of yeast extract significantly improves the different responses studied, followed by the amount of $CaCl_2$ and the amount of Na_2HPO_4 (in descending order). The various coefficients (from b_1 to b_{11}) are calculated using the least-square method. Thus, in order to improve the BioS production, we proposed to optimize these three factors while maintaining the amounts of sucrose at their average level, which is equal to 20 g/L ammonium sulfate, NaCl, $MgSO_4$ and $MnSO_4$ at their lower levels equal to 0.2, 0.02, 0.02 and 0.001%, respectively; and NaH_2PO_4, $FeSO_4$ and $ZnSO_4$ at their mean levels equal to 0.06, 0.005 and 0.005%, respectively.

Central composite design

The operating conditions of the 18 experiments of the three-variable composite plane are described by the lines of the experimental plan (in real variables) represented in Table 2. For each experiment, we determined the ST reduction, the diameter of the ODA and the amount of BioS produced. The presented values correspond to the means of three separate experiments with two replicates for each experiment. The estimated values of the coefficients and their standard deviations are given in Table 3. As the values of the multiple linear correlation factors R^2 are equal to 0.953 for the ST decrease, 0.973 for the ODA and 0.975 for the BioS yield, we conclude that the overall quality of the regression is considered very good. The values of the correlation coefficient, which are very close to 1, show that there is a good correlation between the experimental and theoretical results for the different responses recorded.

As presented in Table 4, ANOVA analyses permit to verify the validity of the model. The obtained results show that the total sum of the squares of the deviations from the mean evaluated with 17 degrees of freedom is divided into two sums of squares. The first, due to regression, is estimated with 9 degrees of freedom; the second, due to residual variation is estimated with 8 degrees of freedom. Otherwise, ANOVA permits us to conclude the significance of the regression (99.99%) for the ODA and the BioS yield. For the ST decrease, a significance threshold of 99.9% is observed. In addition, obtained results permit us to observe the adequacy of the adopted models to

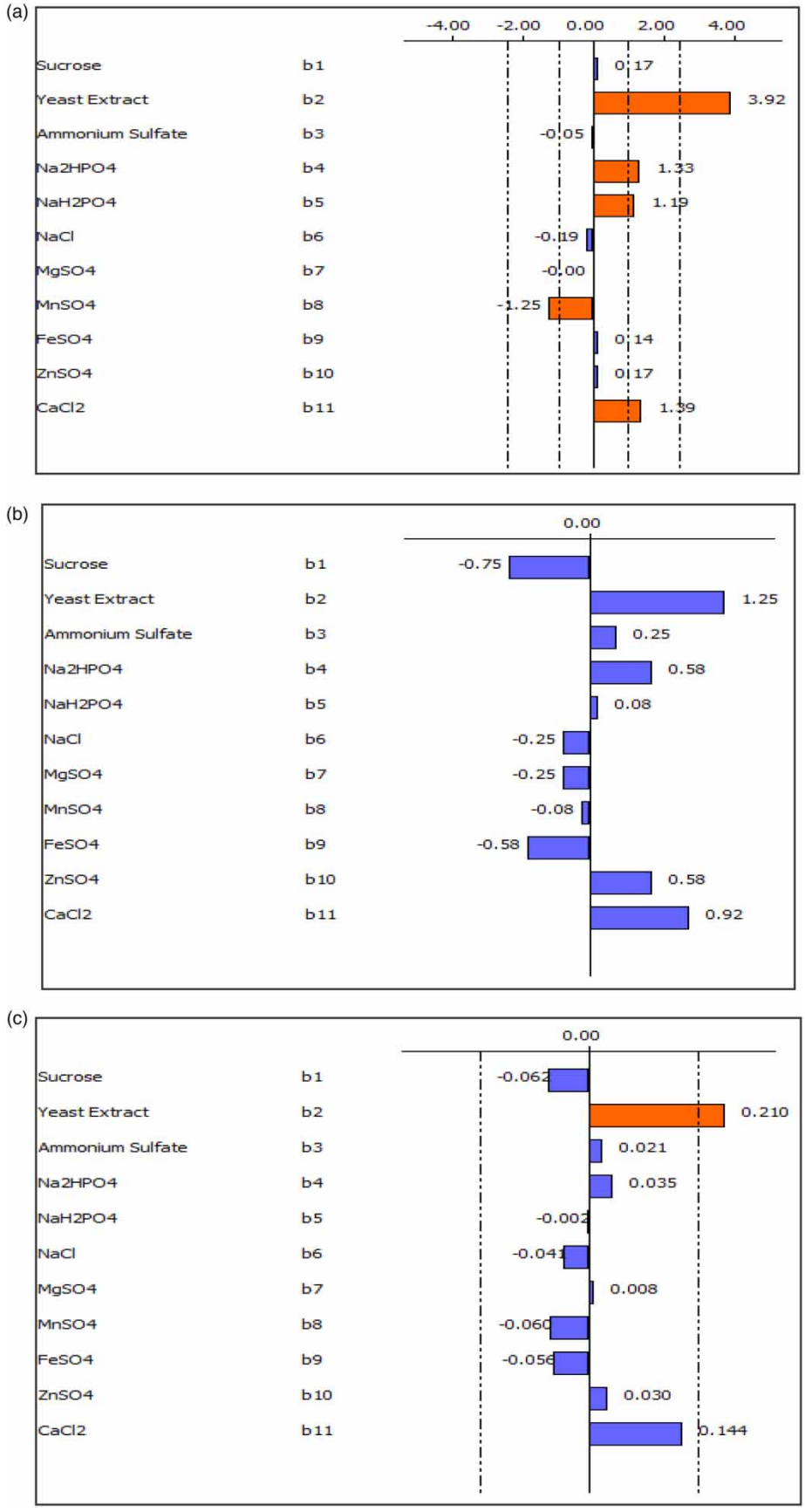

Figure 1 | Pareto design of the effect of the different nutritional parameters on BioS production: (a) Study of ST decrease response; (b) Study of the ODA response; and (c) Study of the BioS Yield response.

Table 3 | Estimated effect, regression coefficient and corresponding *t*- and *p*-values for BioS production in the CCD experiments

	Sum of squares			Degree of freedom	Mean squares			Rapport			Significations		
	Coefficients			F. Inflation	Ecart-types			t. expérimentales			Signification %		
Nom	Y_1	Y_2	Y_3		Y_1	Y_2	Y_3	Y_1	Y_2	Y_3	Y_1	Y_2	Y_3
b_0	49.115	5.740	0.700	1.00	0.798	0.246	0.002	61.57	23.32	280.039	< 0.01***	< 0.01***	< 0.01***
b_1	− 1.500	− 0.686	− 0.059	1.00	0.432	0.133	0.001	− 3.47	− 5.14	− 43.54	0.845**	0.0887***	< 0.01***
b_2	1.545	1.028	0.184	1.00	0.432	0.133	0.001	3.57	7.71	135.84	0.725**	< 0.01***	< 0.01***
b_3	− 2.002	− 0.246	− 0.088	1.00	0.432	0.133	0.001	− 4.63	− 1.85	− 65.34	0.169***	10.200	< 0.01***
$b_{1\text{-}1}$	3.289	0.841	0.134	1.00	0.449	0.139	0.001	7.32	6.06	95.56	< 0.01***	0.0302***	< 0.01***
$b_{2\text{-}2}$	3.445	1.371	0.214	1.00	0.449	0.139	0.001	7.67	9.89	155.92	< 0.01***	< 0.01***	< 0.01***
$b_{3\text{-}3}$	2.255	0.841	0.138	1.00	0.449	0.139	0.001	5.02	6.06	98.07	0.103**	0.0302***	< 0.01***
$b_{1\text{-}2}$	1.295	0.250	0.100	1.00	0.565	0.174	0.002	2.29	1.43	56.57	5.100	18.900	< 0.01***
$b_{1\text{-}3}$	− 1.205	0.125	0.075	1.00	0.565	0.174	0.002	− 2.13	0.72	42.43	6.500	49.410	< 0.01***
$b_{2\text{-}3}$	1.638	1.500	0.212	1.00	0.565	0.174	0.002	2.90	8.60	120.21	1.99*	< 0.01***	< 0.01***

Table 4 | ANOVA analysis for BioS production in the CCD

	Sum of squares			Degree of freedom	Mean squares			Rapport			Significations		
	Y_1	Y_2	Y_3		Y_1	Y_2	Y_3	Y_1	Y_2	Y_3	Y_1	Y_2	Y_3
Regression	418.0891	70.9995	18930	9	46.4543	7.8888	21.034	18.194	32.448	8413.499	0.0207***	<0.01***	<0.01***
Residual	20.4258	1.9450	491.24	8	2.5532	0.2431	6140.5						
Total	438.5148	72.9444	19422	17	49.0075	8.1319	6161.534						

predict the responses studied. Thus, the validated models of the three answers studied are written as follows:

$$Y1 = 49.115 - 1.500*X_1 + 1.545*X_2 - 2.002*X_3 + 3.289*X_1^2 + 3.445*X_2^2 + 2.255*X_3^2 + 1.295*X_1*X_2 - 1.205*X_1*X_3 + 1.638*X_2*X_3$$

$$Y2 = 5.740 - 0.686*X_1 + 1.028*X_2 - 0.246*X_3 + 0.841*X_1^2 + 1.371*X_2^2 + 0.841*X_3^2 + 0.250*X_1*X_2 + 0.125*X_1*X_3 + 1.500*X_2*X_3$$

$$Y3 = 0.700 - 0.059*X_1 + 0.18*X_2 - 0.088*X_3 + 0.134*X_1^2 + 0.219*X_2^2 + 0.138*X_3^2 + 0.100*X_1*X_2 + 0.075*X_1*X_3 + 0.212*X_2*X_3$$

with Y_1, Y_2 and Y_3 refer, respectively, to the three recorded responses, the decrease of the ST, the dispersion and the quantity produced. Combinations of factors (such as $X_1{}^*X_2$) represent an interaction between individual factors. Factors X_1^2, X_2^2 and X_3^2 represent the double effect of the different factors X_1, X_2 and X_3. The values of the coefficients are calculated by the multiple linear regression method.

Search of optimal conditions: study by response surface methodology

Aiming to appreciate the effect of the studied parameters on BioS production, the predicted model is presented in isoresponse curves and response surfaces as observed in Figure 2. The two-dimensional response surfaces are plotted to study the interaction between the compounds in the medium and to determine the optimal concentration of these compounds. The isoresponse curve reflecting the change in the ST decrease as a function of the amounts of yeast extract and Na_2HPO_4 is shown in Figure 2(a). The amount of $CaCl_2$ is fixed at its lower level, which is equal to 0.2%. An examination of this graph shows a strong interaction between the two factors studied. Indeed, to have an optimal decrease of the ST, it is necessary to prepare a medium containing 5 g/L (0.5%) yeast extract and 2.7 g/L (0.27%) Na_2HPO_4. Thus, under these conditions, we can achieve a production yield of about 1.47 g/L ($\pm$0.01) along with a dispersion diameter of the order of 10.15 cm ($\pm$1.43).

In order to confirm these optimal conditions, we have fixed the quantity of yeast extract at its optimal value, which is equal to 0.5%. Thus, an improved production yield of the order of 1.47 g/L with a dispersion diameter of the order of 10.10 cm and a ST decrease of the order of 60.68% can be achieved by working with 0.27% Na_2HPO_4 and 0.02% $CaCl_2$. Similar results were reached by setting the amount of Na_2HPO_4 at its optimum concentration of 0.27%. The corresponding isoresponse curves and surface plots are not presented. In order to see the variation of the second response studied, the dispersion diameter according to the factors studied, we fixed the amount of $CaCl_2$ to its maximal value, which is equal to 0.02% (Figure 2(b)). Analysis of the isoresponse curves representing the variation of the dispersion diameter as a function of the concentration of yeast extract and Na_2HPO_4 shows that it is possible to attain a diameter of the order of 10 cm with a 1.4 g/L BioS and a 60% ST reduction by working with 0.5% yeast extract and 0.27% Na_2HPO_2.

Additionally, in the study of the variation of the third response studied, the quantity produced by fixing the amount of $CaCl_2$ to its optimal value (0.02%) will allow obtaining the same optimal conditions found previously (Figure 2(c)). All the representations in Figures 2(a)–(c) confirm that the best production of BioS estimated at 1.41 g/L with a considerable ST decrease of the order of 60% (corresponding to a ST value of the order of 23.2 mN/m) and an ODA of about 10 cm can be obtained using 5 g/L yeast extract, 0.2 g/L $CaCl_2$ and 2.7 g/L Na_2HPO_4.

Generated optimum conditions were approved in four separate experiments, the mean of which corresponds to 1.4 g/L BioS, an average ST of 23 mN/m and an ODA of around 10 cm. Emulsifying (EI-24%) and foaming activities have been confirmed, suggesting a high production yield of BioS. Thus, the application of the experimental design methodology by a first screening plan of the most influential factors followed by a second CCD optimization plan allowed us to significantly improve the BioS production by the MI27 strain on sucrose-based medium. The composition of the culture medium is as follows: 2% sucrose, 0.27% Na_2HPO_4, 0.2% ammonium sulfate, 0.02% NaCl, 0.02% $CaCl_2$, 0.02% $MgSO_4$, 0.001% $MnSO_4$, 0.06% NaH_2PO_4, 0.005% $FeSO_4$ and 0.005% $ZnSO_4$.

In fact, the substitution of glucose by sucrose for the production of BioS allows a great economic gain. Also, adjusting the quantities of the various other components, including the nitrogen source and the mineral elements permits a significant decrease in the production cost with better quantities produced. The different nutritional parameters and their assigned levels strongly affect the BioS production yield and cost. Meanwhile, the

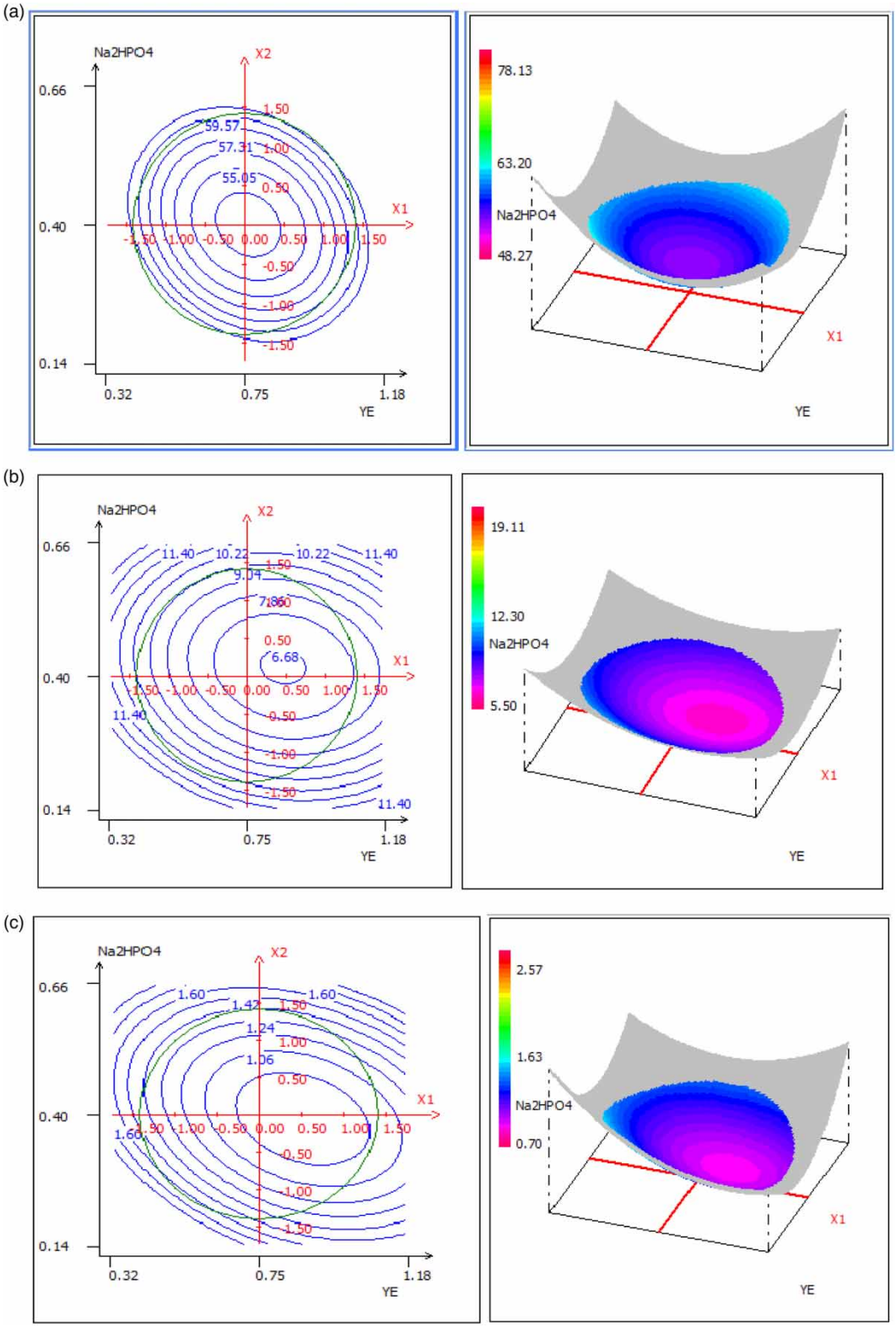

Figure 2 | Effect of the different nutritional parameters on the BioS production: response surface plot (left) and its contour plot (right) of interaction between yeast extract and Na_2HPO_4 with $CaCl_2$ concentration kept at 0.02 % evaluated by the measurement of the percentage of ST decrease (a); the Surface of oil dispersion (b) and the quantity of BioS produced (c).

applications of the experimental design methodology and response surfaces are experiencing a particular boom as fairly developed statistical tools to improve BioS production as they permit to adjust the media components (Bertrand *et al.* 2018). They also allow great economic gain and save time. Numerous factors can affect BioS

production, namely the nutritional parameters as well as the physicochemical parameters including pH, temperature, agitation and aeration (Bertrand *et al.* 2018). Previous studies have delayed the application of Plackett and Burman designs along with the response surface methodology to select the most influential factors on lipopeptide BioS production, especially surfactin isoforms (Haddad *et al.* 2014; Liu *et al.* 2014; Meena *et al.* 2020).

Functional characterization of the produced BioS

ST measurement and determination of the CMC value

An examination of the curve presented in Figure 3(a) showed the gradual decrease of the ST of water as a function of the increase in the BioS concentration. At a certain value called CMC, it is stable and maintains a constant level. From this point, the increased BioS concentration no longer leads to any reduction in the ST of water, indicating that CMC has been achieved. Thus, the BioS of MI27 lowers the ST of water to 23 mN/m with a CMC of the order of 120 mg/L. The ST evaluation is the most reliable technique for detecting, quantifying and characterizing BioS production. Indeed, BioS has the fundamental property of reducing the ST of water to a minimum reached their CMC. The reduction of this amount is attributed to the mono-molecular film formed between two non-miscible phases. CMC is the concentration at which BioS tends to be associated with micelles in solution and for which there is a sharp decrease in the ST (Pacwa-Plociniczak *et al.* 2011; Mnif & Ghribi 2015a). It is a physical magnitude that characterizes the surfactant potential of a compound and its effectiveness.

As largely recognized, each BioS was characterized by the ST and CMC values that evaluate its efficiency (Mnif & Ghribi 2015a). We denote an improvement in the surfactant's effectiveness as the values of ST and CMC decrease. By way of comparison, we note, for example, that the MI27-derived BioS is more effective than surfactin isoforms produced by *Bacillus subtilis* isolate LSFM-05 (de Faria *et al.* 2011), those produced by *B. subtilis* SL having a CMC value of 154 mg/L and a ST of 28.68 mN/m (Wu *et al.* 2022) and surfactin isoforms produced by *Bacillus nealsonii* S2MT (Phulpoto *et al.* 2020). In means of the ST reduction power, it is more efficient than surfactin isoforms produced by *B. subtilis* BS-37 displaying a CMC value of mg/L (Liu *et al.* 2014), *Bacillus amyloliquefaciens* MO13-derived surfactin isoforms displaying a CMC value of 36 mg/L and an ST of 27 mN/m (Moro *et al.* 2022), engineered surfactin isoforms produced by *B. subtilis* 168 having a CMC value of 38–36 mg/L with potential ST of 28 mN/m (Hu *et al.* 2021) and surfactin isoforms derived from *B. subtilis* #309 having a CMC value of 15 mg/L with potential ST of 28 mN/m (Janek *et al.* 2021).

BioS are generally characterized by low CMCs with a better decrease in ST compared to chemical surfactants (Mnif *et al.* 2021a, 2021b). In this aim, similar results were approved for MI27-derived BioS. As mentioned in our previous work, we determined the CMC and the ST decrease (γCMC) values of four chemical surfactants. The values are about 200 mg/L and 32 mN/m for the Triton X-100, 31 mg/L and 35 mN/m for the CTAB and 794 mg/L and 35 mN/m for SDS (Mnif *et al.* 2021b). On the basis of these values, the concluded results proved the effectiveness of *Bacillus* sp. MI27-derived BioS toward the three surfactants in terms of the ST decrease. For the CMC value, MI27-derived BioS is less efficient than the CTAB. However, all the results were found to favor the efficacy of the BioS of MI27 and its high application potential. Similar results suggesting the successful use of *B. subtilis* SPB1 in comparison to chemical surfactants in the bioremediation of dyes and hydrocarbons contaminated soil and water were denoted by Mnif *et al.* (2015a, 2015b, 2015c, 2016, 2017a).

Study of oil dispersing activity: comparison with chemical surfactants

In addition to the ST's decreasing power, BioS are able to disperse the oil. As shown in Figure 3(b), a maximum dispersion of around 120 mm is obtained at concentrations greater than or equal to 1,000 mg/L. There is a gradual increase in the dispersal area with the increase in BioS concentration, which will stabilize at a certain level. Thus, our BioS showed a good ability to disperse burned motor oil. Recent studies reported the ODA of bacterial-derived surfactin isoforms, suggesting their utility in the environmental field for the treatment of oil contaminants (Liu *et al.* 2014; Phulpoto *et al.* 2020; Barale *et al.* 2022).

A comparison of the ODA with that of chemical surfactants shows that the BioS of MI27 is significantly more effective. Indeed, maximum dispersion diameters of the order of 8.8, 9.2, 8.8 and 8.7 cm were obtained, respectively, with 900 mg/L Dehydol, 1,000 mg/L Triton X100, 1,000 mg/L CTAB and 85 mg/L SDS (Mnif *et al.* 2021c). Some literature studies show the effectiveness of hydrocarbon dispersal from BioS by contribution to chemical surfactants such as SDS, Tween 80 and Trition X100 (Das & Chandran 2011; Silva *et al.* 2014). Thus, having better efficiency and considering the toxicity of chemical surfactants, the BioS of MI27 presents a healthier and ecological alternative for various environmental applications. Basically, the enormous usage of

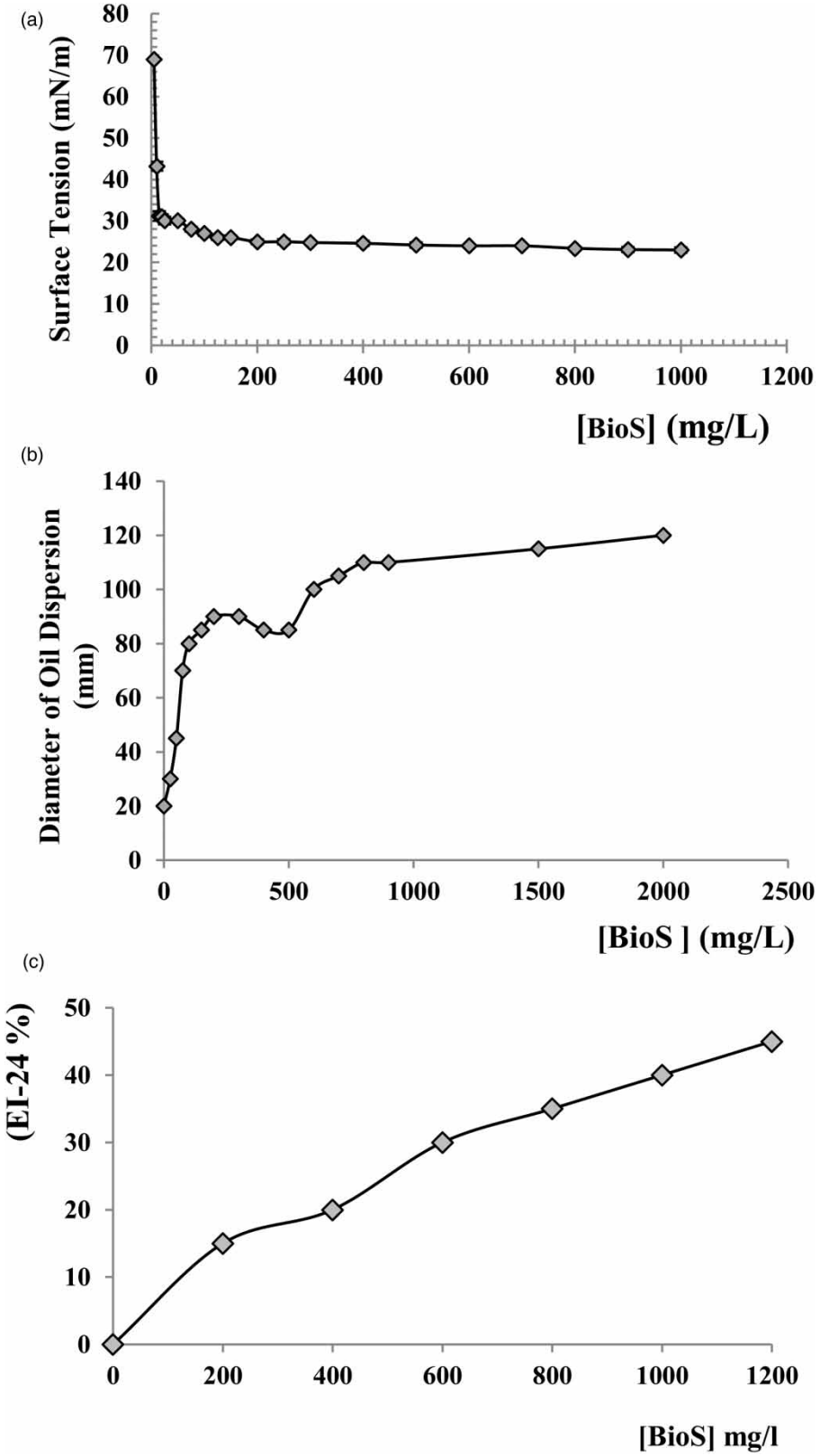

Figure 3 | Evolution of the ST (a), diameter of oil dispersion (b) and EI (c) as function of the MI27 BioS concentration.

petroleum-derived products imparts highly to water pollution provoking a major environmental risk to human health. The high hydrophobicity and solubility of hydrocarbons and vegetables inhibits their uptake and biodegradation by microorganisms. The bio-dispersion of these oils and hydrocarbons discharged to the surface of

the water facilitates their disposal by physical methods. The use of BioS as a bio-dispersant is a better alternative due to its lower toxicity and biodegradability.

Study of emulsification activity

As shown in Figure 3(c), the BioS of MI27 shows a maximum emulsification of around 45%, which is obtained at concentrations greater than or equal to 1,000 mg/L. In this aim, numerous bacterial-derived surfactin, exhibited emulsification activities of around 67.6% for those derived from *B. subtilis* isolate LSFM-05 (de Faria *et al.* 2011), around 98% as reported by Long *et al.* (2017), around 55% for surfactin-like BioS produced by novel strain *B. nealsonii* S2MT (Phulpoto *et al.* 2020), between 58 and 64% for *B. subtilis* #309 derived surfactin (Janek *et al.* 2021) and between 56, 67, 54 and 60% for *B. subtilis* LS-derived surfactin isoforms (Wu *et al.* 2022). This emulsification property makes BioS excellent candidates for the bioremediation of water and soil contaminated with oils and hydrocarbons (Maier & Soberon 2000).

Study of foaming activity

Measurements of the foaming activity of the BioS of MI27 and the stability of the foam for 60 min are observed in Figure 4. We note that the foaming activity of MI27 increases gradually with the increase in BioS concentration. Thus, the BioS of MI27 has the ability to form foam even at low concentrations. It is about 48% at 100 mg/L to touch 80% at 1,000 mg/L. This foaming power is slightly stable for 60 min with small gradual decreases every 15 min to lose about 50% of its foaming power after 60 min of incubation. In addition, BioS are characterized by interesting foaming activities, allowing them to be used as cleaning agents in detergent formulation for laundry as well as cosmetics and in the petroleum industry (Mnif & Ghribi 2015a, 2015b, 2015c). In this regard, various studies have mentioned the foaming activities of surfactin isoforms produced by *B. subtilis* (Razafindralambo *et al.* 1996).

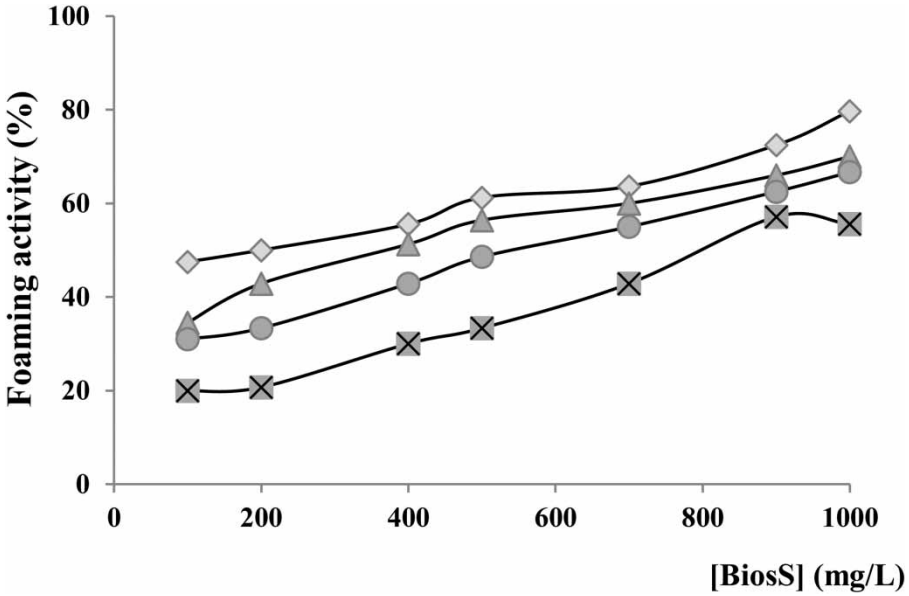

Figure 4 | Study of MI27 BioS foaming activity: increase of the foaming activity in function of BioS concentration and stability of the foam during 60 min; —◇— After 1 min ; —▲—: After 15 min ; —●—: After 30 min ; —✕—: After 60 min.

Effects of different physicochemical factors on MI27 BioS surface activity and stability

Owing to excellent surface activities, the lipopeptide derived from *Bacillus* sp. MI27 has potential use in the environmental field for the bioremediation of hydrocarbon-contaminated soil and water. Then, in order to enlarge its scope of application, we study its activity and stability at diverse and extreme physicochemical conditions. The abilities to decrease the ST, to disperse oil, to emulsify oil and to form foam were assessed at different pH values ranging from 2 to 10 and salt concentrations ranging from 0 to 5%. For the temperature,

we evaluated only the ST reduction and oil dispersion from 10 to 80 °C. The ST was assayed at the CMC value of 120 mg/L and the other activities at 1,000 mg/L.

Effect of temperature on MI27 BioS activity and stability

The ST decreasing power and the ODA of burned motor oil were evaluated at increasing temperatures (from 10 to 80 °C). Recorded results indicate that both activities reach their optimum values when the temperature is 30 °C. In addition, there has been a gradual decrease in activity, especially at high temperatures (Figure 5(a)). However, MI27-derived BioS depicted an interesting thermal activity, as it maintained more than 70% of its ODA and more than 50% of its ST decreasing ability at 80 °C.

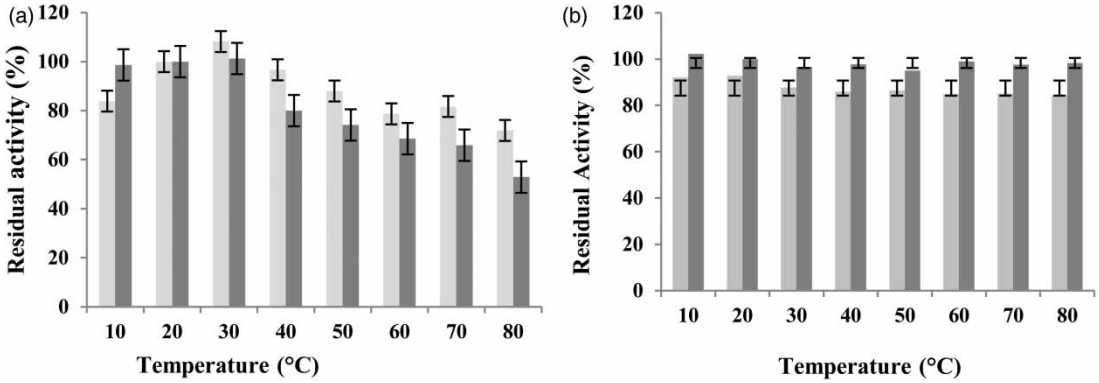

Figure 5 | Effect of temperature on MI27 BioS surface activity (a) and stability (b) evaluated by the measurement of ■: dispersant activity and ■: ST reduction.

When observing the impact of elevated temperature on MI27 BioS stability, we remark a perfect thermal-stability, especially for the power of ST reduction (Figure 5(b)). The lipopeptide maintains about 100% of its ST reduction capacity and more than 90% of its ODA in the mentioned temperature values. These results are of growing interest as the prospective application of BioS in numerous industrial processes necessitates thermal stabilities. Previous research works exhibited similar findings (Chen *et al.* 2017; Ghazala *et al.* 2017; Kiran *et al.* 2017; Martins *et al.* 2018; Feng *et al.* 2019; Purwasena *et al.* 2019; Zouari *et al.* 2019; Jimenez *et al.* 2021; Mnif *et al.* 2021a, 2021b; Umar *et al.* 2021; Wu *et al.* 2022).

Effect of pH on MI27 BioS activity and stability

The assay of the effect of pH on MI27-derived BioS behavior shows its activity over a broad range of pH. In fact, it maintains between 60 and 100% of its capacity to decrease the ST, from 40 to 100% of its ODA (Figure 6(Aa)), from 78 to 100% of its foaming power and from 50 to 100% of its emulsifying activity for pH ranges from 4 to 10 (Figure 6(Ab)). Nevertheless, a minor decrease in its activity is seen at the extremely acidic pH of 2 and 3. Recorded results are comparable to those documented by Mnif *et al.* (2013b), Feng *et al.* (2019); Phulpoto *et al.* (2020) and Mnif *et al.* (2021a, 2021b). The MI27-derived BioS retains 20 and 40% of its ODA, 35 and 55% of the ST-reducing power, 42% of its emulsifying activity and 65 and 85% of its foaming power for pH 2 and 3. Generally, pH is one of the main prominent environmental parameters affecting the performance of any BioS. It modifies the net load of the surfactant molecule and thus its orientation at the interface, explaining the attenuation of the activity of the majority of BioS at the strongly acidic pH. This can be assigned to the physicochemical properties of lipopeptide BioS, which precipitate at the acid pH thus losing their activity and are completely soluble at the neutral and alkaline pH thus keeping their different activities (Takashi *et al.* 2009).

The results presented in Figure 6 show that the MI27 lipopeptide preserves more than 80% of its ST-reducing capacity at pH levels differing from 4 to 10 (Figure 6(Ba)). However, a strong attenuation of its ODA is observed for acid pH 2, 3 and 4. For emulsifying and foaming activities, very good stability is observed for pH 3–10 (more than 70% of relative activities are retained) with maximum neutral pH activity (Figure 6(Bb)). The stability of MI27-derived BioS toward a wide pH range offers a good opportunity for use in various industrial processes. Similar results were published for lipopeptide BioS (Chen *et al.* 2017; Ghazala *et al.* 2017; Kiran *et al.* 2017;

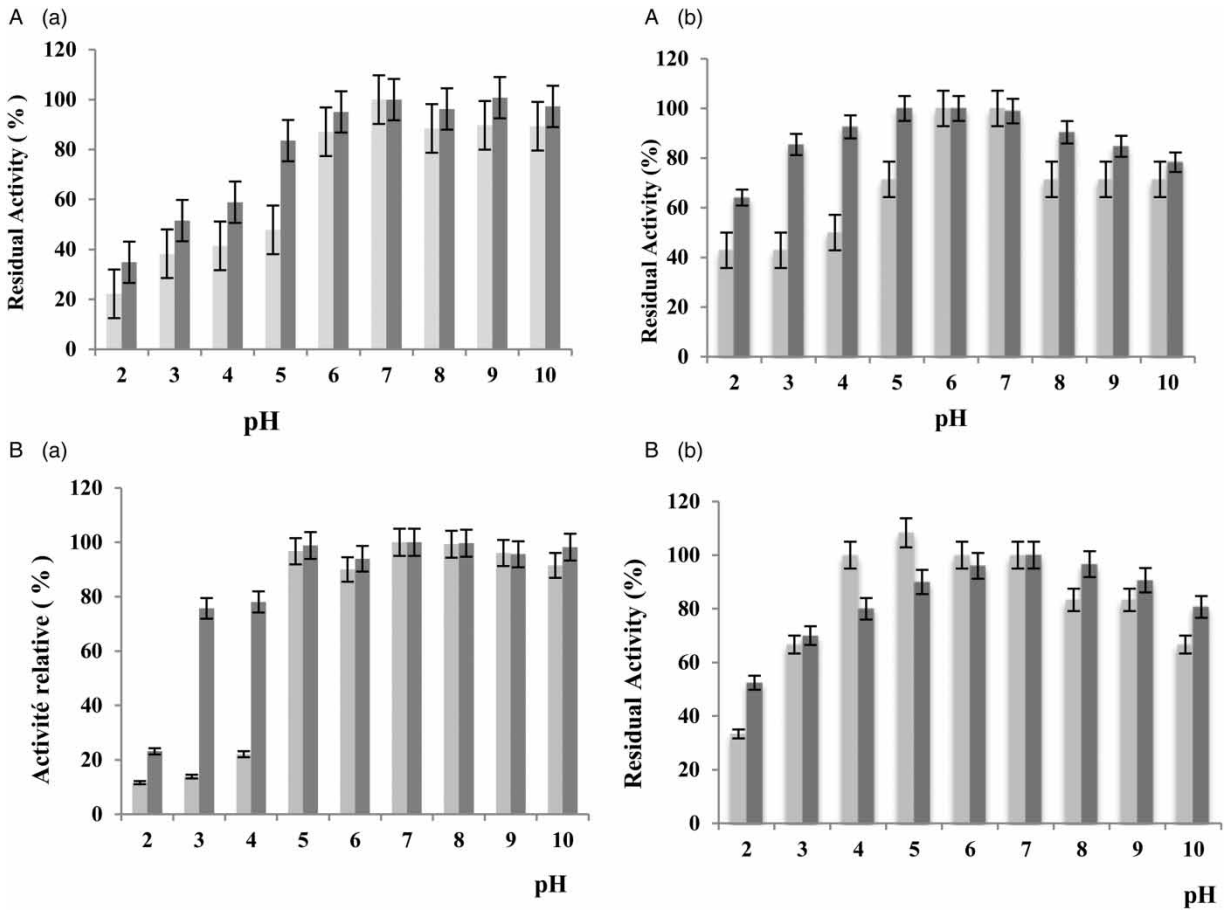

Figure 6 | Effect of pH on MI27 BioS surface activity (A) and stability (B) evaluated by the measurement of [: dispersant activity and : ST reduction] (a); [: EI-24% and : foaming activity] (b).

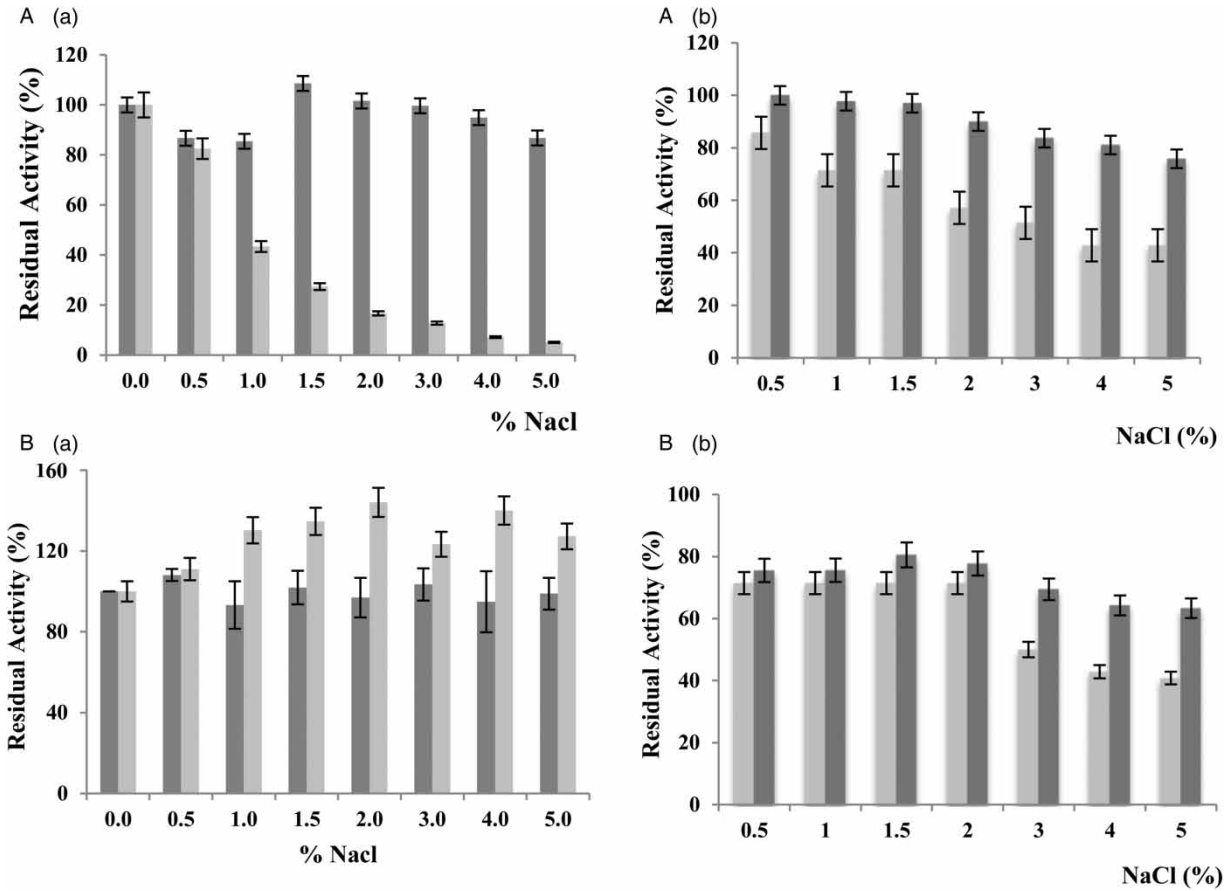

Figure 7 | Evaluation of the effect of NaCl on MI27 BioS surface activity (A) and stability (B) evaluated by the measurement of [: dispersant activity and : ST reduction] (a) and [: EI-24% and : foaming activity] (b).

Martins *et al.* 2018; Feng *et al.* 2019; Purwasena *et al.* 2019; Zouari *et al.* 2019; Jimenez *et al.* 2021; Mnif *et al.* 2021a, 2021b; Umar *et al.* 2021; Wu *et al.* 2022).

Effect of salinity on MI27 BioS activity and stability

Figure 7(a) shows that the addition of sodium chloride significantly increases the ST reduction of the MI27 BioS at NaCl concentrations of 1.5 and 2% with a maximum activation of 110% in the presence of 1.5% NaCl. So we can suppose the activation of MI27-derived lipopeptide BioS by the Na^+ cation. Additionally, it declared an emulsifying activity of interest for the different concentrations ranging from 0.5 to 5% with slight improvements. This activating effect of sodium chloride is similar to that of Huszcza & Burczyk (2003). A probable explanation for that is the modification of the molecular area of BioS by the effect to salt exposure (Thimon *et al.* 1992).

However, salinity inhibits the ODA of MI27-derived lipopeptide by losing more than 50% of its activity in the presence of concentrations greater than or equal to 1%. Additionally, we note a decrease of 14–58% of the MI27 emulsification power at NaCl concentrations ranging from 0.5 to 5%, respectively (Figure 7(Aa)). Nevertheless, MI27 lipopeptide has an interesting foaming power in the range of NaCl concentration studied (loses a maximum of 24% of its foaming activity to 5% NaCl) (Figure 7(Ab)). Numerous research works presented similar results (Mnif *et al.* 2013b, 2021a, 2021b; Pathak & Keharia 2014; Feng *et al.* 2019; Phulpoto *et al.* 2020). This good activity under saline conditions will allow the potential investigation of MI27 lipopeptide in the bioremediation of hydrocarbons contaminated seawater (Mnif *et al.* 2017a).

In addition to the study of MI27 BioS activity at different salt amounts, we explored its stability after pre-incubation in the presence of salts to mitigate seawater. Looking at Figure 7(Ba), we observe a perfect stability of the MI27 lipopeptide after measurement of the power of decrease of the ST (relative activities are around 100%) with activation of the ODA. As observed in Figure 7(Bb), for foaming power, there is a slight attenuation of activities at high NaCl concentrations of 3, 4 and 5%. Similar results are observed for emulsifying activity with higher attenuation than foaming power. These results go in favor of broadening its purview of use in different areas, especially for the enhanced oil recovery and biological remediation of sea water contamination(Chen *et al.* 2017; Ghazala *et al.* 2017; Kiran *et al.* 2017; Martins *et al.* 2018; Feng *et al.* 2019; Purwasena *et al.* 2019; Zouari *et al.* 2019; Jimenez *et al.* 2021; Mnif *et al.* 2021a, 2021b; Umar *et al.* 2021; Wu *et al.* 2022).

CONCLUSION

To conclude, experimental planning methodology was applied to maximize *Bacillus* sp. MI27 BioS production. The produced BioS illustrated interesting functional properties, especially its surface activity with potent emulsion-forming and foaming capacities. The crude lipopeptide preparation was characterized by great ST reduction, low CMC value along with emulsification, oil dispersing and foaming activities. The physicochemical characterization of the crude surfactin isoforms showed perfect activity and stability at extreme conditions of temperature, salinity and pH by means of the evaluation of their different functional properties. All these findings exhibited the prospective usage of MI27 lipopeptide preparation in industrial and environmental biotechnology.

ETHICAL APPROVAL

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

CONSENT TO PARTICIPATE

Informed consent was obtained from all individual participants included in the study.

CONSENT TO PUBLISH

All the authors gave the publisher the permission to publish the work in Water Practice and Technology.

AUTHORS' CONTRIBUTIONS

The first author of this paper M.E. and the second author M.B. elaborated the experimental parts of the present work. The fourth author D.G. and the fifth author I.M. participated in the elaboration of the experimental plan of the work and corrected this paper.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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