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Identification of surfactin lipopeptides isoforms produced by a newly isolated strain *Bacillus* sp. MI27 with potential use as main ingredients in detergent formulation: Application of mixture design for the formulation of a bio-based detergent

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ABSTRACT

The purification and characterization of lipopeptide biosurfactants produced by *Bacillus* sp. MI27 permits to identify two clusters of cyclic and linear surfactin isoforms. Aiming for a potential application in industrial biotechnology, we studied its potential washing capacity. The results show a good ability to wash oil stains, motor oil and mayonnaise evaluated by the determination of the % of stain removal and tissue bleaching indices after washing. Optimal washing capacities were unregistered for 300 and 400 mg/L at pH values ranging from 5 to 9 and temperatures ranging from 20 to 40°C. Using a 4-factor mixing design, we optimized a liquid formula composed only of BioS; silicate; carboxymethyl cellulose and calcium phosphate. The washing efficiency of the formula is of the order of 43.46% for petroleum and 49.10% for motor oil with a percentage reduction in surface tension of about 55.79% corresponding to 25.7 mN/m.

Key words: biosurfactant, detergent, formulation, mixture design, surfactin, washing capacity

HIGHLIGHTS

- Identification of cyclic and linear surfactin isoforms from *Bacillus* sp. MI27.
- Optimal washing capacities for 300–400 mg/L at pH values 5–9 and temperatures 20–40 °C.
- Mixture design for optimized liquid formula composed only of BioS, silicate, carboxymethyl cellulose and calcium phosphate.
- The washing efficiency of the formula is 43.46% for petroleum and 49.10% for motor oil.
- Surface tension value of 25.7 mN/m.

ABBREVIATIONS

MI27 BioS: Biosurfactant produced by *Bacillus* sp. MI27

INTRODUCTION

Detergents are mainly composed of surfactants that represent the active ingredient with a percentage ranging from 15 to 50% of all ingredients consisting of a set of chemicals called adjuvant (Mnif *et al.* 2023). Other compounds such as fragrances and optical brighteners are also added (Mnif *et al.* 2023). Notably, the surfactant activity has great importance on the properties and effectiveness of detergent formulations (Mnif *et al.* 2023). Having an amphiphilic character, surfactants tend to aggregate at the interface between two different phases (for example air/water, water/stain and stain/tissue) (Ranji *et al.* 2019; Cheng *et al.* 2020). There, the polar hydrophilic part is then directed inwards thus creating a discontinuity in the surface film (Ranji *et al.* 2019; Cheng *et al.* 2020). This causes a decrease in the surface tension (ST) of the solvent (Ranji *et al.* 2019; Cheng *et al.* 2020).

Generally, the main purpose of detergents is to clean the laundry, in other words, to remove all types of dirt. The secondary functions correspond to technical performance consisting of preserving or restoring the flexibility of linen, reviving colors, etc. Whereas, tertiary functions are called hedonic because they are associated with the pleasure that the consumer may experience during or after the use of the product (Ranji *et al.* 2019; Mnif *et al.* 2023). Moreover, the satisfaction of contributing to the respect of the environment by purchasing a product formulated from completely biodegradable ingredients and made from renewable natural resources is also needed. In fact, as petrochemical-derived surfactants cause great toxicity and damage to consumers and the environment (Giagnorio *et al.* 2017), we opt for the use of microbial-derived surfactants for the formulation of detergents (Ng *et al.* 2022; Sharma *et al.* 2022; Zahed *et al.* 2022).

Generally, BioS are characterized by a high structural diversity with amphiphilic character. They are subdivided into two parts; a polar hydrophilic moiety (peptides or proteins, mono or polysaccharides) and an apolar hydrophobic part composed of hydroxylated fatty acids (Mnif & Ghribi 2015a, 2015b). In addition, they are endowed by numerous functional properties including the power to reduce surface and interfacial tension, oil dispersion, emulsifying, foaming, solubilizing and wetting activities along with a low critical micelle concentration (Mnif & Ghribi 2015a, 2015b). These different capacities play an important role in what is called detergent power (Ranji *et al.* 2019; Cheng *et al.* 2020; Mnif *et al.* 2023). In addition, they are highly specific, biodegradable, non-toxic, biocompatible with the environment and less sensitive to biotopes of extreme temperatures, pH and salinity (Zahed *et al.* 2022; Mnif *et al.* 2023). As a result, BioS are classified as cleaning and bleaching products, whether domestic or industrial use (Mnif *et al.* 2023). Accordingly, the use of BioS for the production of household detergents, hygiene, beauty and well-being products could contribute to more than 56.8% of the global BioS market in 2018 (Mnif *et al.* 2023).

Lipopeptides BioS, constituted by a peptide moiety linked to a fatty acid chain, are among the most popular microbial surfactants with interesting properties (Carolin *et al.* 2021). In this aim, we propose in the present work to purify and identify lipopeptides BioS produced by a newly isolated strain *Bacillus* sp. MI27 (unpublished work). We apply an anionic exchange chromatography followed by high performance liquid chromatography coupled to a mass spectrometry (HPLC-MS). It is within this particular framework that the present work is situated; we tried to use MI27 BioS in the formulation of liquid detergent as a substitute for chemical surfactant. We opt initially to determine the washing ability of motor oil, petroleum and mayonnaise. After that, we formulate a new BioS-based liquid wash formula by applying a mixture design. The main constituents are MI27 BioS in combination with calcium phosphate (CP) and silicate as inorganic and organic complexants and carboxymethyl cellulose (CMC) as an emulsifying agent. The measurements of the ST reduction power, its motor oil washing capacity and oil washing capacity allow for the effectiveness of the optimized formula.

MATERIALS AND METHODS

Chemical product

Burned engine oil and diesel oil utilized in the present study were purchased from a local Mechanic's station in Sfax (Tunisia).

BioS production and extraction

The BioS-producing bacterial strain *Bacillus* sp. MI27 was streaked on an Lauria Bertani (LB) agar medium and incubated overnight at 37°C. Inoculum culture was prepared by dispersing one loop of cells in 50 ml LB medium (10 g/L peptone, 5.0 g/L yeast extract, 5.0 g/L NaCl). The mixture was then incubated at 37°C with shaking at 180 rpm for 18 h. For BioS production, 50 mL of sucrose-based medium was inoculated by an overnight inoculum at 4 %. The production media was composed of 20.0 g/L sucrose, 5.0 g/L yeast extract, 1.5 g/L KH_2PO_4, 0.1 g/L KCL, 0.5 g/L $MgSO_4$, 0.008 g/L $FeSO_4$, 0.05 g/L $CaCl_2$, 0.1 g/L KCl and traces elements (Cu, Mn, Zn, Br) and the pH was adjusted to 7.0 (Mnif *et al.* 2021a, 2021b). The culture medium was incubated for 24 h under agitation at 150 rpm followed by centrifugation at 10,000 rpm and 10 min to recuperate the supernatant and discard the cell pellet.

To recuperate the most BioS produced, we perform two cycles of acid precipitation–centrifugation–neutralization according to the protocol described by Mnif *et al.* (2021a, 2021b, 2022). Each time, the resulting BioS pellets were washed twice with acid distilled water (pH = 2) to eliminate impurities. They were then solubilized in distilled water and the pH was adjusted to 8.0 using 1 N NaOH to dissolve most of the lipopeptide

compounds. The obtained BioS extract was lyophilized, which served as the raw lipopeptide product for identifying the produced isoforms after purification, studying the washing capacity of the BioS and formulating the detergent.

Biosurfactant purification by anionic exchange chromatography and reversed phase HPLC

Having an anionic character, the raw lipopeptide extract was administered to a low-pressure chromatography column packed with Q Sefinose Resin (Bio Basic). The column was primarily equilibrated in 50 mM Tris-HCl and pH 8. For the elution, separate fractions were harvested every 5 min accompanied by the measure of the optical density at 280 nm (Bechard *et al.* 1998). The elution was done by a NaCl gradient ranging from 0.1 to 0.8 M in 50 mM Tris-HCl buffer (pH = 8). The flow rate is 1.0 ml/min.

The different fractions were subjected to the evaluation of the oil dispersing activity (ODA) according to the protocol described by Mnif *et al.* (2021a, 2021b). It was determined by the measurement of the area of dispersion of crude oil on the surface of water engendered by the surface active compounds. Active fractions were grouped and subjected to acid precipitation–centrifugation to collect the purified BioS pellets that were dissolved separately in alkaline ultrapure water (pH = 8.0). The retention of surface active compounds was verified by ODA measurement. After that, they were lyophilized to be fractionated by HPLC and identified by HPLC-MS.

HPLC-MS analysis

To identify the lipopeptides isoforms, the lyophilized BioS was re-suspended in a mixture of acetonitrile and water (75:25) at a concentration of 20 mg/mL, vortexed, sonicated for 10 min and centrifuged at 6,000 rpm for an additional 10 min. Two liquid phases and a precipitate were formed (an upper, colorless phase consisting of acetonitrile and a lower brownish phase containing a brown precipitate). The upper phase revealed the presence of peptide compounds detected at 220 nm by UV and MS, both in the HPLC and HPLC-MS, while the lower phase produced very poor signals.

The electro-spray ionization mass spectra (ESI-MS) were performed with a single quadrupole detector coupled to an HPLC equipped with a Phenomenex C18 reverse phase (0.46 × 25 cm) column packed with octadecylsiloxane of 5 μm diameter particle. Elution was carried out at a flow rate of 1 ml/min using linear gradients of 0.1% CHOOH/H_2O (A) and 0.1% CHOOH/ACN (B) (the gradient method was as follows: 0–2 min, 50% B; 2–15 50–100% B; 15–30 min, keeping 100% B, injection volume 20 μl). Liquid chromatography–mass spectrometry full scan positive and negative mode was conducted in the range from 100 *m/z* to 1,800 Dalton.

Determination of washing capacity

The washing capacity was determined as described by Bouassida *et al.* (2018). Small pieces of white fabric, of size 5 × 5 cm each, are soiled separately with engine oil, oil or mayonnaise and kept in this condition overnight at 30 °C. Subsequently, their precise weight was recorded before washing. The washing procedure was performed in an Erlenmeyer filled with 60 ml of tap water supplemented either with the MI27 crude BioS at various concentrations ranging from 100 to 500 mg/L; or a detergent formula diluted 100 times; or a commercial detergent also diluted 100 times. After washing, the tissues were rinsed twice with 100 ml of distilled water and dried overnight at 30 °C. Moreover, in order to observe the effect of certain physicochemical parameters on washing capacity, washing tests were carried out at different pH levels (5, 7 and 9), temperatures (20, 30 and 40 °C) and stirring times (20, 30 and 40 min). Afterwards, the percentage of removal of each spot was calculated using the following formula (Equation (1)) (Bouassida *et al.* 2018):

$$\text{Washing capacity } (\%) = \frac{(\text{Weight of fabric before washing and drying} - \text{Weight of fabric after washing and drying})}{(\text{Weight of fabric before washing and drying} - \text{Weight of clean fabric})} * 100 \quad (1)$$

After washing and drying, the tissues contaminated with motor oil, petroleum and mayonnaise, the whiteness indices were measured using a spectrophotometer (Color Eye 7000A, Gretag Macbeth).

Use of M27 biosurfactants for detergent formulation: elaboration of a liquid washing machine formula by a mixture design

The 4-factor mixture design conception

In order to optimize a new formula of automatic washing machine based on the MI27 BioS, we adopted a mixing plan with four factors to optimize the amount of the active ingredient (BioS, X1), the amount of sodium silicate ($Na_2Si_2O_5$, X2), the amount of CMC (X3) and the amount of calcium phosphate ($CaPO_4$, X4).

The matrix described by the Nemrodw software (LAPREI) contains 13 experiments with 5 different levels for MI27 BioS, of which these different levels are between 0.1 and 0.2. We used BioS levels less than or equal to 0.2 in order to minimize its quantity given its fairly high production cost. However, 7 different levels between 0 and 1 are assigned for the other 3 factors. Each experiment consisted of a formula whose sum of the different constituents is equal to 1 g corresponding to 1%. However, liquid lye is prepared at a concentration of 20%. Thus, the matrix of experiments in coded and real variables representing the composition of each formula is listed in Table 1. The coded variables indicate the composition of 1 g of ingredients and the actual variables indicate the percentage of the different ingredients to prepare 100 ml of liquid lye. During the washing tests, the detergent was diluted 100 times in the washing bath. Washing tests were realized with 60 ml tap water in which 600 µl liquid lye was dissolved. Tap water was used to simulate washing conditions with the automatic machine. The washing test takes 30 min under a shaking of 700 rpm. After that, the washing capacity is determined as described above.

Table 1 | Experimental mixture design in coded and real values

	X1: MI27 BioS		X2: Silicate		X3: CMC		X4: Calcium phosphate	
N EXP	Coded values	Real values (%)	Coded values	Real values (%)	Coded values	Real values (%)	Coded values	Real values (%)
1	0.1000	2.000	0.3000	6.000	0.3000	6.000	0.3000	6.000
2	0.1500	3.000	0.2833	5.666	0.2833	5.666	0.2833	5.666
3	0.2000	4.000	0.2667	5.334	0.2667	5.334	0.2667	5.334
4	0.1730	3.460	0.0000	0.000	0.4135	8.270	0.4135	8.270
5	0.1365	2.730	0.4500	9.000	0.2068	4.136	0.2068	4.136
6	0.1000	2.000	0.9000	18.000	0.0000	0.000	0.0000	0.000
7	0.1730	3.460	0.4135	8.270	0.0000	0.000	0.4135	8.270
8	0.1365	2.730	0.2068	4.136	0.4500	9.000	0.2068	4.136
9	0.1000	2.000	0.0000	0.000	0.9000	18.000	0.0000	0.000
10	0.1730	3.460	0.4135	8.270	0.4135	8.270	0.0000	0.000
11	0.1365	2.730	0.2068	4.136	0.2068	4.136	0.4500	9.000
12	0.10000	2.000	0.0000	0.000	0.0000	0.000	0.9000	18.000
13	0.1500	3.000	0.2833	5.666	0.2833	5.666	0.2833	5.666

Statistical method

The analysis of the results of the experimental plans is carried out by the Nemrodw software package version 2017 (LPRAI, Marseille, France). The various charts of the biochemical characterization of the MI27 BioS and the study of its washing capacity are carried out with the Excel software package version 2007.

RESULTS AND DISCUSSION

Purification and identification of MI27 bioS

Aiming at the identification of the lipopeptide compounds derived from *Bacillus* sp. MI27, a crude extract was prepared and purified by anionic exchange chromatography. An HPLC coupled with an ESI-MS permitted to determine the molecular weight of the constituting fractions. Results analyses demonstrated the acquirement of two clusters of peaks, the first at m/z values between 994 and 1,036 Da and the second at m/z values between 1,012 and 1,054 Da specified to Families A and B, respectively (Table 2).

Table 2 | Assignment of the structure of Surfactin lipopeptides produced by *Bacillus* sp. MI27 by LC-ESI-MS/MS in this study

Family	Assignation	ESI + mode	ESI-mode	Lipopeptide molecular weight	Possible sequence
Family A Surfactin	C12/C13 Surfactin	995.1 $[M + H^+]$ 1,017.0 $[M + Na^+]$	993.4 $[M-H^+]$	994 [M]	 Xxx = Leu/Ile or Val $n = 9$ (Val +2Leu/Ile) or 10 (2Val + Leu/Ile)
	C13/C14 Surfactin	1,009.0 $[M + H^+]$ 1,031.1 $[M + Na^+]$	1,007.3 $[M-H^+]$	1,008 [M]	$n = 10$ (Val +2Leu/Ile) or 11 (2Val + Leu/Ile)
	C14/C15 Surfactin	1,023.1 $[M + H^+]$ 1,045.1 $[M + Na^+]$	1,021.3 $[M-H^+]$	1,022 [M]	$n = 11$ (Val +2Leu/Ile) or 12 (2Val + Leu/Ile)
	C15/C16 Surfactin	1,037.1 $[M + H^+]$ 1,059.1 $[M + Na^+]$	1,035.3 $[M-H^+]$	1,036 [M]	$n = 12$ (Val +2Leu/Ile) or 13 (2Val + Leu/Ile)
Family B Linear homologues of Surfactin	Linear C12/C13 Surfactin	1,013.1 $[M + H^+]$ 1,035.1 $[M + Na^+]$	1,011.1 $[M-H^+]$	1,012 [M]	 Xxx = Leu/Ile or Val $n = 9$ (Val +2Leu/Ile) or 10 (2Val + Leu/Ile)
	Linear C13/C14 Surfactin	1,027.0 $[M + H^+]$ 1,049.0 $[M + Na^+]$	1,025.3 $[M-H^+]$	1,026 [M]	$n = 10$ (Val +2Leu/Ile) or 11 (2Val + Leu/Ile)
	Linear C14/15 Surfactin	1,041.1 $[M + H^+]$ 1,063.1 $[M + Na^+]$	1,039.3 $[M-H^+]$	1,040 [M]	$n = 11$ (Val +2Leu/Ile) or 12 (2Val + Leu/Ile)
	Linear C15/16 Surfactin	1,055.0 $[M + H^+]$ 1,077.1 $[M + Na^+]$	1,053.3 $[M-H^+]$	1,054 [M]	$n = 12$ (Val +2Leu/Ile) or 13 (2Val + Leu/Ile)

According to previous research work reporting the identification of *Bacillus* sp. derived lipopeptides, each family could be attributed to different lipopeptide isoforms having probably the same amino acid sequence with differences in the length of the fatty acid chain. They correspond to the widely recognized isoforms of cyclic and linear surfactin compounds. The full scan LC-ESI-MS chromatogram for surfactins showed the main peaks from 11.5 to 17 min consisting of cyclic and linear isoforms of surfactin with fatty acid ranging from C12 to C16. Different variants of cyclic isoforms were identified as molecules $[M + H]^+$ *m/z* 995, 1,009, 1,023 and 1,037 Da (Figure 1) and $[M + Na]^+$ *m/z* 1,017, 1,031, 1,045 and 1,059 Da corresponding to C12/C13 surfactin, C13/C14 surfactin, C14/C15 surfactin and C15/C16 surfactin, respectively. They correspond to Family A. The isoforms with the same molecular weight having differences in the number of methylenes (CH_2) of the respective fatty acid maybe have differences in the nature of the composition of the amino acid sequence containing (Val + 2 Leu/Ileu) for the C12 surfactin or (2 Val + Leu/Ileu) for the C13 surfactin. This could explain the difference of 14 Da corresponding to the difference between the molecular weight of valine and leucine/ileucine. Also, the multiple 14 Da ($-CH_2$) difference can be assumed to different homologues with the same amino acid sequence but different in the length of the β-OH fatty acids. To conclude, we identified 8 surfactin isoforms with fatty acid ranging from C12 to C16 linked to amino acid sequence of Glu^1-Xxx^2-Leu^3-Xxx^4-Asp^5-Leu^6-Xxx^7 with potential variability in the Xxx amino acids of (Val + 2 Leu/Ile) or (2 Val + Leu/Ile) in positions 2, 4 and 7 as presented in Table 2.

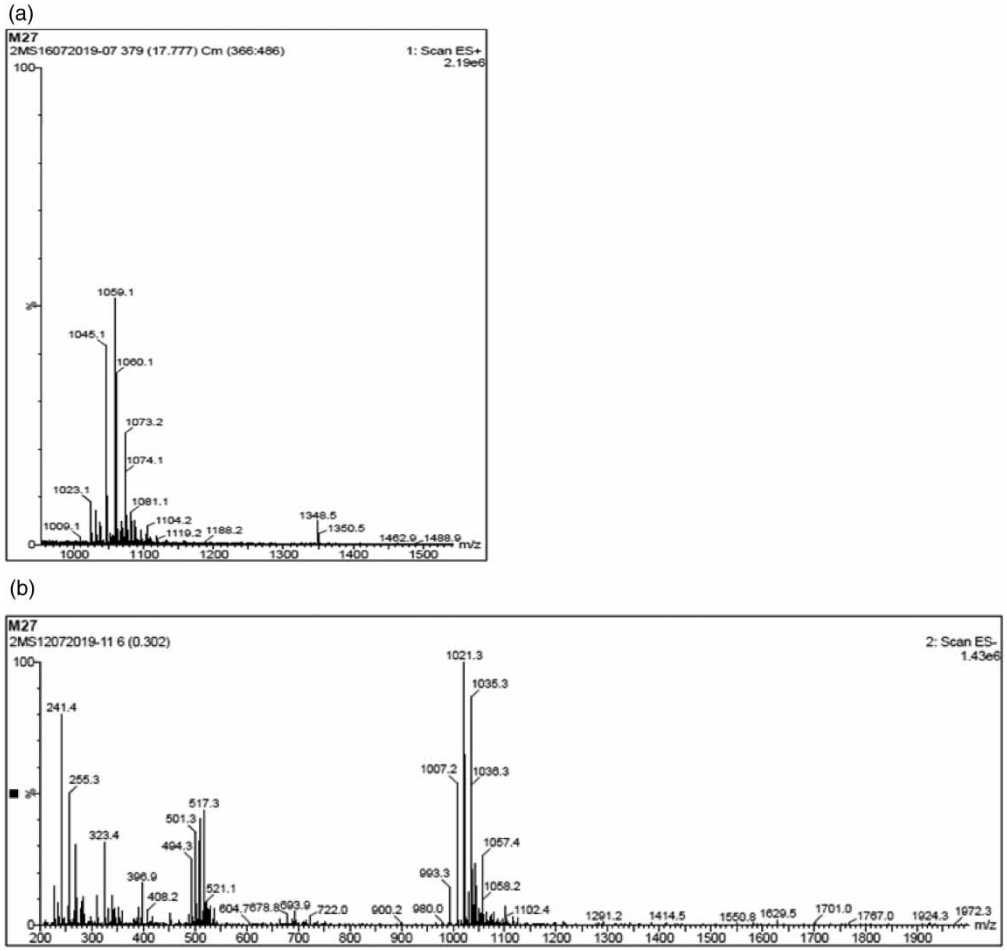

Figure 1 | Mass spectroscopy (LC/MSD-TOF) spectra of Surfactin Isoforms identified in the positive mode [M + H +] (a) and negative mode [M − H +] (b).

Linear or non-ester-cyclized isoforms of Surfactin with molecular weights of 1,012, 1,026, 1,040 and 1,054 Da (Family B) corresponding to C12; C13, C14, C15 and C16 Surfactin were identified as largely reported in recent studies (Wang *et al.* 2004; Pathak *et al.* 2014; Ma *et al.* 2016). Moreover, different variants of linear isoforms of

Surfactin were identified as molecules $[M + H]^+$ *m/z* 1,013, 1,027, 1,041 and 1,055 Da and $[M + Na]^+$ *m/z* 1,035, 1,049, 1,063 and 1,077 Da (Figure 2) corresponding to C12/C13 Surfactin, C13/C14 Surfactin, C14/C15 Surfactin and C15/C16 Surfactin, respectively. We assigned those *m/z* peaks to precursor analogues that are 18 Da bigger than the corresponding cyclic Surfactins. As we know, the relative molecular mass of the H_2O molecules is 18. Hence, we can conclude they were linear Surfactins based on their chromatographic behavior and fragmentation pattern. They correspond to Family B. Also, as described previously, they are also found as the different Surfactin homologues with multiple differences of 14 Da ($-CH_2$) corresponding to the difference in the length of the fatty acid tails that can vary from C12 to C16 (represented as the number of carbon atom) with the probable same amino acid sequence. Correspondingly, 14 Da could be attributed to the difference between the molecular weight of Val and Leu/Ile for two consecutive homologues.

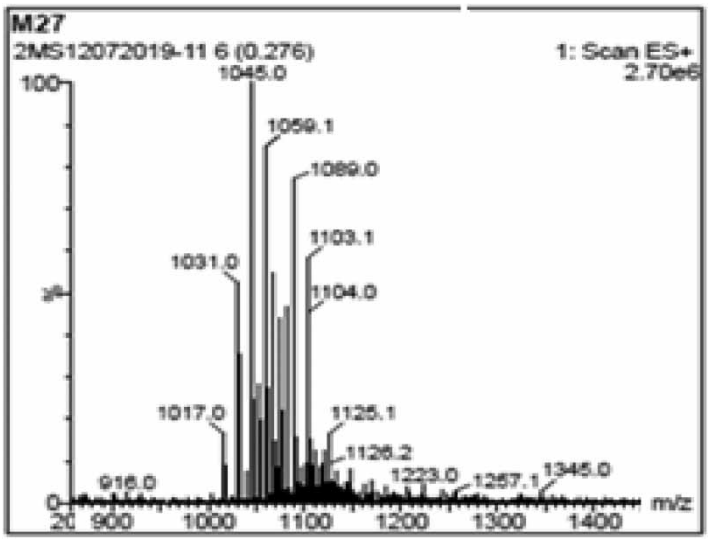

Figure 2 | Mass spectroscopy (LC/MSD-TOF) spectra of linear Surfactin Isoforms identified in the positive mode $[M + Na^+]$.

Figures 1 and 2 represent the mass spectroscopy (Liquid Chromatography/Mass Spectrometry Quadripole-TOF) spectrum of cyclic and linear Surfactin identified in the positive mode as $[M + H^+]$ and $[M + Na^+]$, respectively. In summary, Family A corresponds to cyclic isoforms of Surfactin with molecular weights of 994, 1,008, 1,022 and 1,036 Da (Ben Ayed *et al.* 2014; Pathak *et al.* 2014; Yang *et al.* 2015; Mnif *et al.* 2016; Jemil *et al.* 2017; Kecskeméti *et al.* 2018; Hentati *et al.* 2019; Lin *et al.* 2020) and Family B corresponds to linear analogues of Surfactin or non-ester-cyclized compounds with molecular weights of 1,012, 1,026, 1,040 and 1,054 Da corresponding to the (M + 18) of *m/z* 994; 1,008; 1,022 and 1,036 Da, respectively (Wang *et al.* 2004; Pathak *et al.* 2014; Ma *et al.* 2016). All these results with the possible sequences are presented in Table 2.

Structurally, lipopeptides consist of a hydrophobic fatty acid chain linked to a short hydrophilic linear or cyclic amino acids chain via ester or amide bonds or both. As described by Mnif & Ghribi (2015a) and Ali *et al.* (2022), the cyclic structure of this molecule was attributed to the lactone bridge between the β-hydroxyl function of the acid and the carboxyl-terminal function of the peptide moiety. Regarding the nature and the type of the fatty acid chain, as well as the number and the configuration of the amino acids in the peptide moiety, we distinguish different families among the lipopeptides class to know Surfactin, Iturin and Fengycin isoforms (Mnif & Ghribi 2015a, 2015b; Ali *et al.* 2022). Surfactin, identified in the current study, the most known lipopeptide isoforms, consists of a cyclic lipoheptapeptide, containing seven residues of D- and L-amino acids linked to one residue of a β-hydroxy fatty acid. Owing to the length of the lipid moiety, Surfactin can have six isoforms or nine isoforms (Mnif & Ghribi 2015a; Ali *et al.* 2022). When the length of the fatty acid chain ranges from C11 to C19, the molecular weight of Surfactins could vary from 980 to 1,078 Da (Bartal *et al.* 2018; Ali *et al.* 2022). For the peptide moiety, Surfactin has the common following structural traits: a heptapeptide with a chiral sequence LLDLLDL with a D-Leu in positions 3 and 6 and an L-Asp in position 5. The amino acid in positions 2, 4 and 7, they belong to Val, Leu and Ile (Mnif & Ghribi 2015a; Ali *et al.* 2022). However, we differentiate four isomers namely Surfactin, Lichenysin, Esperin and Pumilacidin among the Surfactin family that vary on the seventh amino acid (Mnif & Ghribi

2015a; Ali *et al.* 2022). Additionally, linear non-ester-cyclized isoforms were largely identified as described here and numerous previous work (Wang *et al.* 2004; Pathak *et al.* 2014; Ma *et al.* 2016; Théatre *et al.* 2021).

Study of the washing capacity of MI27 bioS

Generally, surfactants ensure the detachment of the dirt and its suspension in the laundry bath. The choice of surfactant depends on a large number of factors (washing temperature, type of textile, pH of washing water, washing time, etc.), but the essential parameters for achieving optimal detergency are the solubility of the surfactant and its CMC value. In general, BioS are more effective than chemical surfactants and their CMC are about 10 to 40 times lower. Thus, they can be very effective at very low concentrations. In this aim, we tried to assay the potential application of *Bacillus* sp. MI27-derived surfactant as a basis compound for detergent formula. It has the ability to decrease the ST of water to about 23 mN/m at a CMC value of 120 mg/L. Moreover, belonging to previous studies realized in our laboratory, MI27 BioS was able to disperse and emulsify oils to about 12 cm and 45% respectively at a concentration of 1,000 mg/L. It has a foaming capacity of about 80% at 1,000 mg/L stable over 1 h of incubation. Additionally, the MI27 BioS was active and stable under different physicochemical conditions of pH, temperature and salinity. All these properties are of great interest for potential use as a detergent ingredient.

In order to use the MI27 BioS in a detergent formula, we proposed to see its ability to wash oil, engine oil and mayonnaise. The washing capacity was monitored by the evaluation of the percentage of stain removal and the whiteness index. Increasing concentration of MI27 BioS ranging from 100 to 500 mg/L was assayed. The washing was carried out for 30 min at a temperature of 25°C using tap water. At the same time, sodium dodecyl sulfate (SDS) washes were performed using the same concentrations. The results presented in Figure 3 show an increase in washing capacity with an increase in BioS concentration, with an optimum of 300 mg/L for oil washing (36.51%) and 400 mg/L for mayonnaise washing (40.54%) and engine oil (45.86%). Above these concentrations, the washing capacity decreases slightly. This could be attributed to BioS aggregation, making it less effective at destabilizing and degreasing the tissue. Similar gaits are observed in washing with the chemical surfactant SDS. It also shows that the MI27 BioS shows a better washing capacity than the SDS only in the case of engine oil. These results are similar to those found by Bafghi & Fazaelipoor (2012), whose oil removal can reach almost 40% with increased rhamnolipid concentration under the same working conditions (pH = 7, time = 30 min temperature = 25 °C).

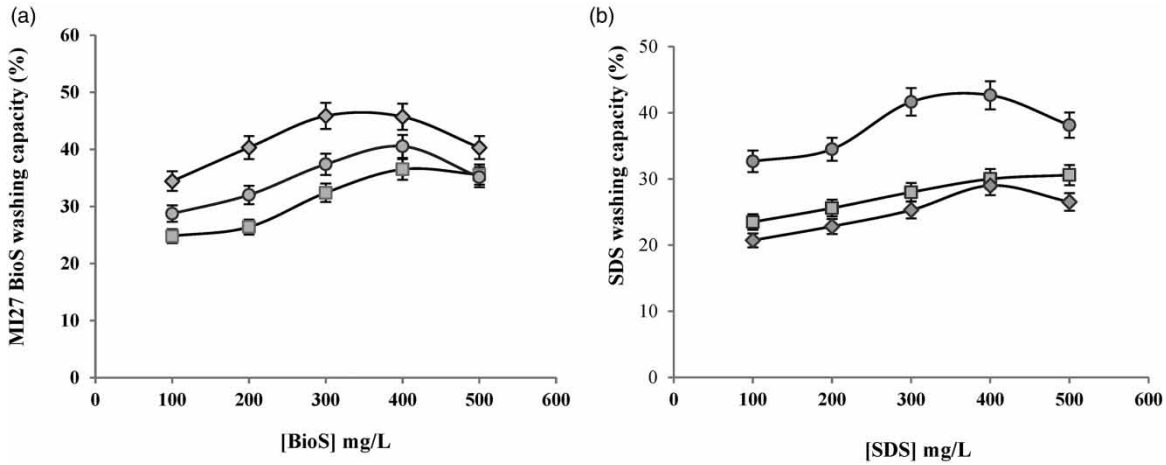

Figure 3 | Effect of different MI27 BioS and SDS concentration on the washing efficiencies of petroleum, motor oil and mayonnaise stains; (a) MI27 BioS and (b) SDS; —□—: petroleum; —◇—: motor oil and —○—: mayonnaise.

Regarding the tissue whiteness, as observed in Figure 4, there is a significant increase with an increase in BioS concentration up to an optimum of 400 mg/L for motor oil (67.84%), petroleum (83.14%) and mayonnaise (85.84%). Compared to SDS, the MI27 BioS shows better efficiency as a bleaching agent. The whiteness qualifies the cleanliness of the fabrics and constitutes a criterion of choice of the detergents; it is associated with the

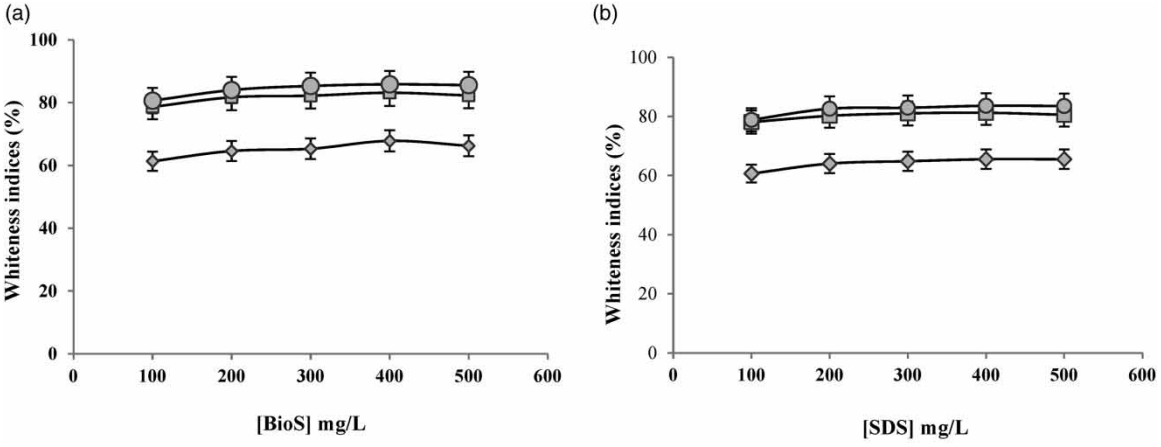

Figure 4 | Effect of different MI27 BioS and SDS concentration on the whiteness indices of the washed piece; (a) MI27 BioS and (b) SDS; —□—: petroleum; —◇—: motor oil and —○—: mayonnaise.

elimination of the colored impurities of the fabric. Basically, with contamination, the whiteness of tissues decreases due to associations of impurities to tissue fibers. All the results found are in favor of using the MI27 BioS as a chemical surfactant substitute in detergent formulas.

Optimization of a liquid washing machine biosurfactant-based formula

Experimentation plan and obtained results

Detergents are complex mixtures of numerous active ingredients containing up to 25 different compounds. The main ingredients of all washing machines are surfactants, adjuvants and fillers in addition to a few auxiliary additives such as enzymes, dispersants and bleaching agents (Gaubert *et al.* 2016). In this context, we will try to optimize a formula of liquid detergents based on MI27 BioS (X1) and three adjuvants; CMC (X2), sodium silicate (X3) and calcium phosphate ($CaPO_4$) (X4). Coded and real values of the experimental design are presented in Table 1.

Table 3 exposed the obtained results that are the mean values of three independent tests. It can be seen that the oil washing capacities vary from 29.24 to 55.13% and those of engine oil vary from 17.45 to 48.58%. For the capacity of ST reduction, a wide variability ranging from 24.5 to 26.8 mN/m was observed. All the results obtained suggest the correct choice of the various factors and their assigned levels. A first-order mathematical

Table 3 | Obtained results (experimental and predicted response)

	Y2 = Motor oil washing capacity (%)		Y1 = Oil washing capacity (%)		Y3 = Surface tension decrease (%)	
N° Exp	Exp response	Predicted response	Exp response	Predicted response	Exp response	Predicted response
1	40.85	33.85	42.00	40.23	56.89	56.042
2	42.38	41.00	42.38	37.23	56.37	56.524
3	48.30	48.14	38.83	34.24	56.72	57.022
4	47.64	50.21	39.39	38.74	57.24	56.263
5	35.73	35.58	29.41	36.35	57.75	56.692
6	17.45	20.95	37.00	33.95	56.89	57.110
7	36.72	37.45	29.24	30.14	57.06	57.543
8	33.93	43.09	34.00	41.41	55.68	55.939
9	48.58	48.72	55.13	52.67	53.79	54.325
10	47.800	45.190	32.000	38.691	56.03	56.455
11	39.820	38.545	37.630	36.385	56.89	56.579
12	30.470	31.895	31.530	34.069	56.03	56.692
13	46.000	41.000	42.630	37.232	56.37	56.524

model translating the dependence of each 'Y' response studied, according to the four coded variables was elaborated. It is written as follows:

$$Y = b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4$$

The values of the coefficients are calculated by the multiple linear regression method. The estimated values of the coefficients and their standard deviations and meanings are shown in Table 4. The values of the multi-linear correlation factors R^2 were 0.804 for engine oil wash capacity, 0.578 for oil and 0.622 for the power of ST reduction, indicating that the overall quality of the regression is considered very good. The estimation and statistics of the coefficients show that the quantities of silicate, CMC and $CaPO_4$ significantly affect the oil's washing capacity. However, the BioS content does not show any significant effect. For engine oil washing, it is highly dependent on the MI27 BioS concentration, CMC and $CaPO_4$. However, the amount of silicate in the mixture has no positive effect. The mathematical models of the two responses studied controlling the formulations of liquid lye are written as follows:

Table 4 | ANOVA analysis

	Sum of squares			Degree of freedom	Means squares			Ratio			Signification		
	Y1	Y2	Y3		Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
Regression	346.3289	778.1144	7.0909	3	115.4430	259.3715	2.3636	4.107	12.303		4.31*	0.155**	2.65*
Residual	252.9817	189.7383	4.2916	9	28.1091	21.0820	0.4757			4.968			
Total	599.3106	967.8527	11.3725	12									

Asterisks indicate significance at the level of 99.9% (**) and 99% (*).

$$Y1 = -13.675X_1 + 39.247X_2 + 60.043X_3 + 39.374X_4$$

$$Y2 = 162.466X_1 + 5.229X_2 + 36.085X_3 + 17.388X_4$$

$$Y3 = 64.808X_1 + 56.255X_2 + 53.160X_3 + 55.791X_4$$

With Y1, Y2 and Y3 refer respectively to the three responses recorded: the oil washing capacity, the engine oil washing capacity and the percentage decrease in ST in the washing bath. The values of the coefficients are calculated by the multiple linear regression method.

The analysis of variance (ANOVA) depicted in Table 5 permits verification of the validity of the three models described above. Obtained results indicated that the total sum of the squares of the average deviations estimated with 12 degrees of freedom is divided into two sums of squares. They are estimated with 3 and 9 degrees of freedom and are due respectively to regression and residual variation. Meanwhile, ANOVA proved significance of the regression for the two responses studied and the non-significance of the lack of validity.

Search for optimal conditions: study of isoresponse curves (two-dimensional graphic study)

Aiming to comprehend and define the effect of the described variables on the oil and engine oil washing capacity and on the percentage of ST decrease in the washing bath; the predicted model is presented in isoresponse curves (Figures 5–7). These isoresponse curves are plotted in the area of an equilateral triangle based on the simultaneous variation of the X1 components, X2 and X3 in the composition of the formulation ranging from 0 to 1 for each component while fixing the component X4 to 0.1. This will allow determining the optimal quantities of these factors giving maximum washing capacity with a good decrease in ST. The isoresponse curves are shown in Figure 5 for motor oil wash capacity, Figure 6 for oil wash capacity and Figure 7 for the percentage of ST decrease. The study of isoresponse curves of engine oil washing capacity shows that it is possible to work with a liquid washing machine formula consisting of 0.15 MI27 BioS; 0.15 silicate; 0.6 CMC and 0.1 $CaPO_4$ to obtain washing capacities of the order of 43.46% ($\pm$13.32) for petroleum and of the order of 49.10% ($\pm$11.54) for motor oil with a decrease in ST of the order of 55.79% ($\pm$1.7).

Table 5 | Statistical estimation of coefficients

	Coefficient			Ecart-type		t. experimental		Signification %	
Noun	**Y1**	**Y2**	**F. Inflation**	**Y1**	**Y2**	**Y1**	**Y2**	**Y1**	**Y2**
b_1	−13.675	162.466	14.83	39.244	33.986	0.35	4.78	73.6	0.100**
b_2	39.247	5.229	3.85	7.834	6.785	5.01	0.77	0.0729***	46.1
b_3	60.043	36.085	3.85	7.834	6.785	7.66	5.32	<0.01***	0.0482***
b_4	39.374	17.388	3.85	7.834	6.785	5.03	2.56	0.0713***	3.00*

	Coefficient		Ecart-Type	t. experimental	Signification %
Noun	**Y3**	**F. Inflation**	**Y3**	**Y3**	**Y3**
b_1	64.808	14.83	5.105	12.69	<0.01***
b_2	56.255	3.85	1.019	55.19	<0.01***
b_3	53.160	3.85	1.019	52.16	<0.01***
b_4	55.791	3.85	1.019	54.74	<0.01***

Y1 = Motor oil washing capacity.
Y2 = Oil washing capacity.
Y3 = Surface tension decrease.
Asterisks indicate significance at the level of 99.99% (***); 99.9% (**) and 99% (*).

However, the study of the oil washing capacity presented in Figure 6 gives a similar formula consisting of 0.15 MI27 BioS; 0.15 silicate; 0.59 CMC and 0.11 $CaPO_4$ to obtain washing capacities of the order of 42.13% (±13.08) for petroleum and 48.22% (±11.23) for motor oil with a decrease in ST of about 55.7% (±1.7).

Also, the isoresponse curves representing the change in the % of ST decrease as a function of the BioS concentration, silicate and CMC quantities confirm the results obtained previously (Figure 7).

To conclude, to have better washing capacities, we can establish a liquid lye formula composed of 0.15 MI27 BioS; 0.15 silicate; 0.6 CMC and 0.1 $CaPO_4$ in coded variables. In real variables, our formula will be prepared with 3% MI27 BioS; 3% silicate; 12% CMC and 2% CP. This new washing machine formula shows good washing efficiency with a good reduction in ST of the order of 25.7 mN/m. This facilitates the removal of contaminants. The optimized conditions were confirmed in 4 separate experiments, the mean values of which correspond to 52% for oil and 57% for engine oil. An average ST of 24 mN/m was found in the washing bath. Emulsifying (EI-24%) and foaming activities were confirmed. Therefore, the application of the experimental design methodology through a formulation design resulted in a new liquid washing formula based on a bacterial-derived BioS produced by a newly isolated strain. This chemical surfactant-free formula is ecological, biodegradable and non-toxic, thus making it possible to protect the environment.

Comparison of the MI27 elaborated formula with different detergents

In order to compare the efficiency of the MI27 formula, we carried out tests of oil washing, engine oil and mayonnaise using the MI27 formula; MI27 BioS alone, the SDS chemical surfactant and a commercial powder detergent "Ariel" generally used for washing laundry.

The results presented in Figure 8 show that the optimized formula based on MI27 BioS was more efficient than the BioS alone and the commercial detergent. In fact, the percentage of removal of stains by MI27 BioS, SDS and Ariel were only 35, 32 and 40% for petroleum stain, 28, 29 and 30% for engine oil stain and 46, 40 and 50% for mayonnaise stain. On the other hand, washing using the MI27 formula was able to remove about 52, 57 and 64% of the different stains, respectively. These results suggest the efficacy of the MI27 formula and the efficacy of MI27 BioS as substituent of chemical surfactants in detergent formulations. However, there is considerable research to prove the efficiency of cleaning BioS in comparison with chemical surfactants and commercial detergents.

In this aim, numerous studies reported the use of lipopeptide BioS in detergent formulas. It knows a particular development. Several lipopeptide-based detergent formulations have been deferred. Bouassida *et al.* (2018) proved the successful use of *Bacillus subtilis* SPB1-derived lipopeptide in detergent formula. They formulated a new detergent based on sodium tripolyphosphate, sodium sulphate and a *B. subtilis*-derived lipopeptide that was effective in oil removal at pH = 7, a temperature of 65°C when stirring at 1,000 rpm during 60 min. The

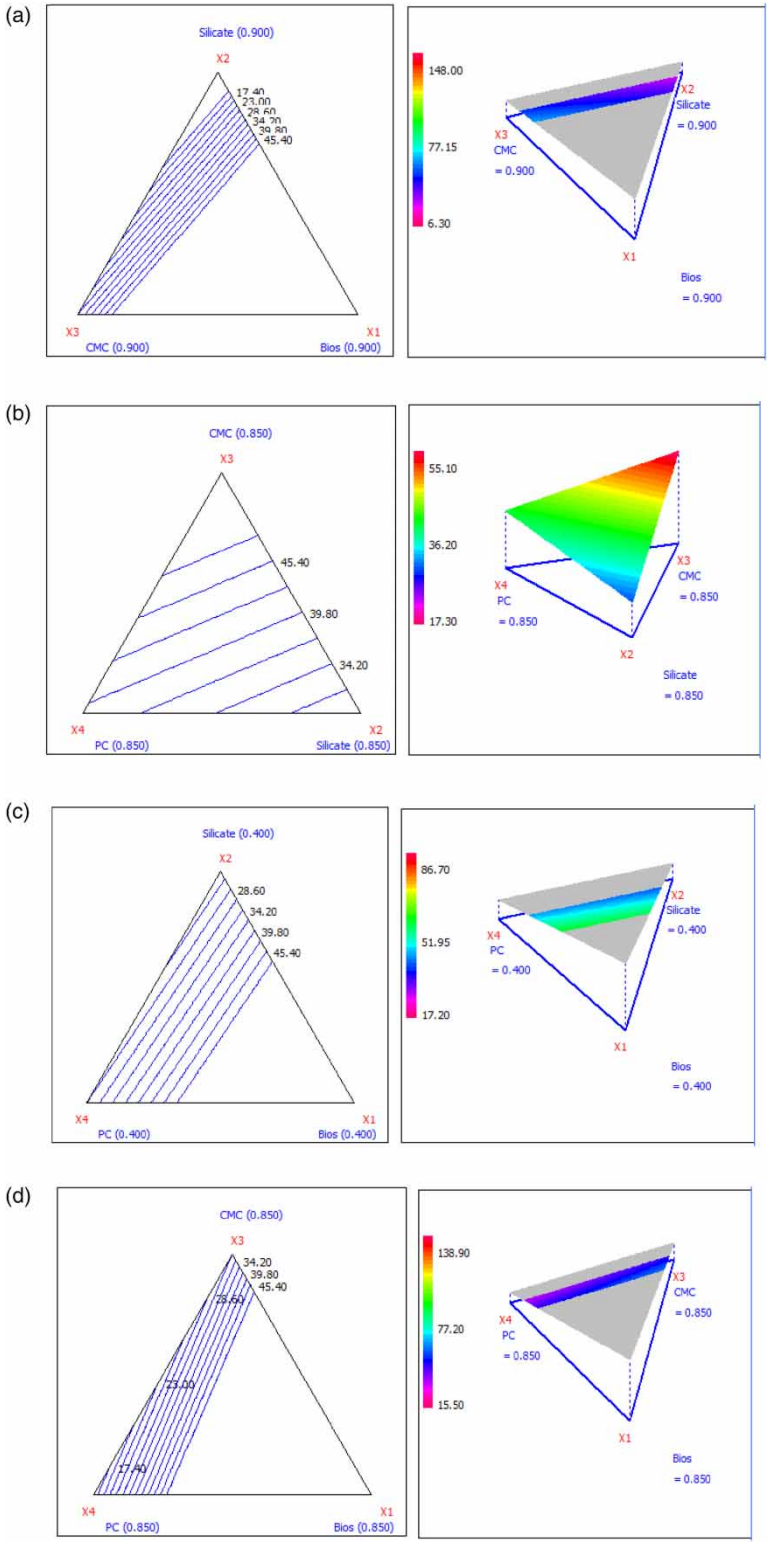

Figure 5 | Response area and isoresponse curves: variation of motor oil washing capacity as a function of: (a) MI27; silicate and CMC concentration, calcium phosphate = 0.1; (b) silicate, CMC and calcium phosphate contents, MI27 BioS = 0.15; (c) MI27 BioS, silicate and calcium phosphate contents, CMC = 0.6; (d) MI27 BioS, CMC and calcium phosphate. Contents, silicate = 0.15.

BioS-based formula demonstrated the best efficiency towards commercial detergent and a combined mixture of BioS for the removal of oil and tea stains with a bioscouring superior to 75% in terms of the stain removal against 60% than the commercial powders (Bouassida *et al.* 2018). Similar findings were reported by Mukherjee (2007) suggesting the detergent role of *B. subtilis* strain DM-04-derived lipopeptide that could enhance the elimination

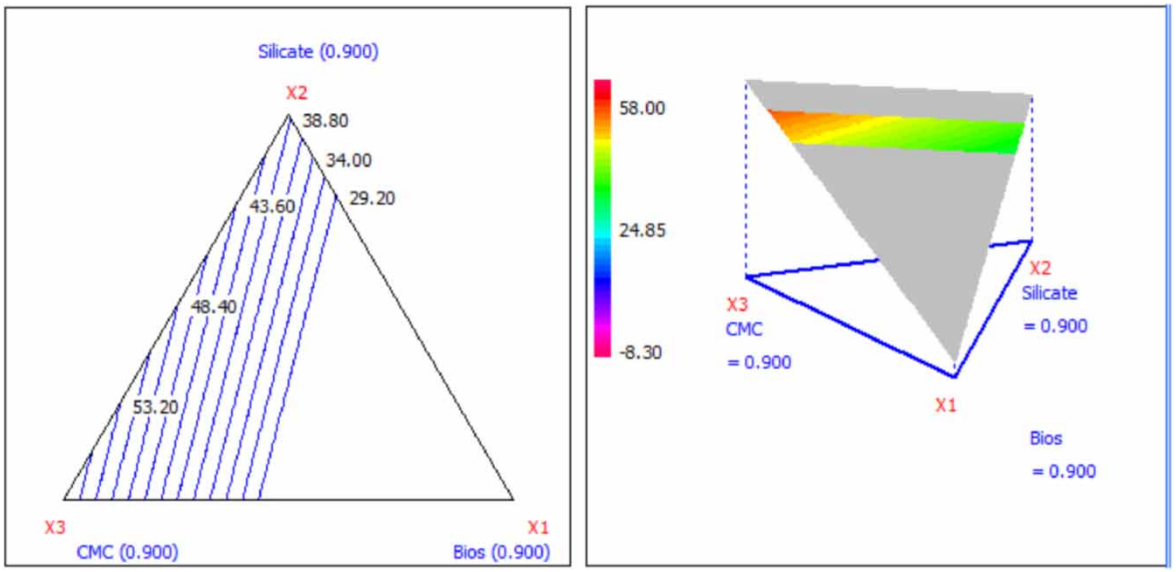

Figure 6 | Response area and isoresponse curves: variation in the oil washing capacity as a function of MI27 BioS, silicate and CMC contents; calcium phosphate = 0.1%.

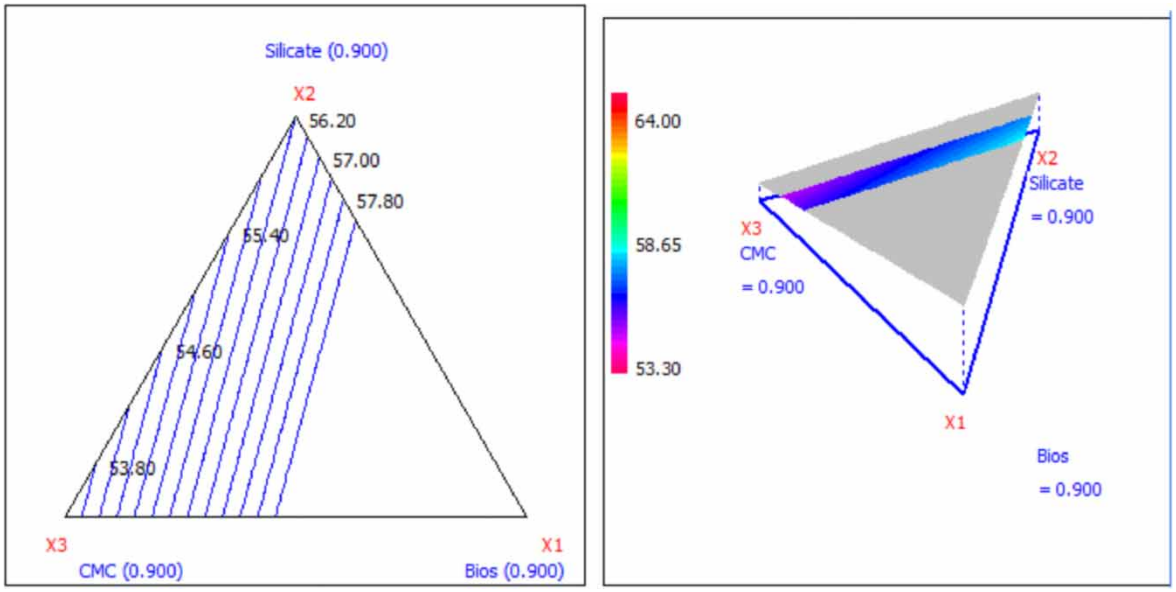

Figure 7 | Response area and isoresponse curves: variation in the percentage of ST decrease in the wash bath as a function of MI27 BioS, silicate and CMC contents; calcium phosphate = 0.1%.

of oily stain from fabric by 9 to 12%. Moreover, the mentioned BioS enhance the cleaning performance of commercial detergent. In addition, Fei *et al.* (2020) suggested the effective role of *B. subtilis* HSO121-derived Surfactin in a detergent formula.

The increase in washing capacity of the formulated BioS may be due to the activities of the different added ingredients, particularly CP, which reduces the hardness of tap water and CMC, which has emulsification capacity thus improving the elimination of hydrophobic compounds. In particular, this adjuvant can retain and stabilize the BioS molecule during storage so that it retains its optimal activity. The different ingredients tested in this formula meet the requirements of a good commercial detergent. Sodium silicate and CP are classified as building agents. A potential manufacturer must satisfy a large number of requirements, including

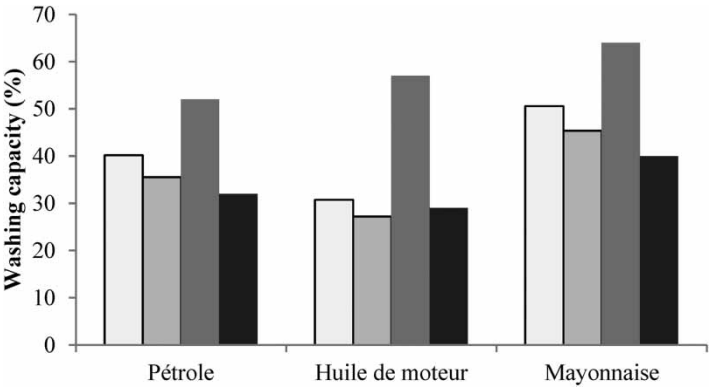

Figure 8 | Comparison between the effect of commercial detergent 'Ariel'; MI27 BioS; MI27 BioS-based formula and SDS for the removal of oil, motor oil and mayonnaise (Time = 30 min; stirring = 700 rpm and temperature = 25 °C); □: Ariel; ▨: MI27 BioS; ■: MI27 BioS-based formula; ■: SDS.

compatibility with bleach, oral non-toxicity and absence of adverse effects on the eyes, skin and other environmental and economic practices. Crystalline silicate in layers ($Na_2Si_2O_5$), an organic complex agent that combines great efficacy per unit mass with a high degree of multi-functionality was widely used in the past as an adjuvant in liquid detergent formulation (Yangxin *et al.* 2008).

These advantages over other adjuvant reside in its solubility in water and good ability to exchange magnesium and calcium ions thus effectively reducing the hardness of the wash water (Yangxin *et al.* 2008). Its high solubility assists very little to the sludge formation in wastewater treatment station and mitigates partially the alkalinity of aqueous liquor (Yangxin *et al.* 2008). In addition, it has a corrosive inhibiting activity and can be mixed with any other adjuvant, being used in formulations of liquid detergents and very compact. It is also low to non-toxic and biodegradable (Yangxin *et al.* 2008). All these benefits make it a good manufacturer for detergents.

Calcium phosphate ($CaPO_4$), an inorganic complexion agent, has a high capacity to chelate calcium and magnesium ions present in hard water, thereby improving the effectiveness of detergents. It also has low toxicity (Yangxin *et al.* 2008). Also, it facilitates the dissolution of detergents, maintains alkalinity during washing and prevents the re-collection of dirt on washed fabrics. In particular, it is used in the medical field for its antiseptic activity (Belouafa *et al.* 2006). However, its phosphate ion content poses environmental problems when discharged into the washing water. Phosphate ions cause eutrophisation problems and excessive algal blooms. But used at a very low dose (less than 4%), such as in the case of our MI27 formula; it does not present any environmental risks (Yangxin *et al.* 2008). CMC is a better additive for synthetic detergents. It is primarily used in detergent formulations for its emulsification power and protective colloidal properties (Palmer *et al.* 2011). In the washing process, it produces anions which can act as a repellent of dirt particles preventing their re-condition on the washed material. It also improves the viscosity, solubility and fluidity of detergent.

In particular, because it has negative charges when ionizing in water, it can play the role of chelating divalent ions, thereby reducing water hardness (Palmer *et al.* 2011). Having these interesting properties, it has been used in many fields including in the mining industry as a soap thinner and synthetic detergent, in the food industry for the manufacture of ice cream, in the textile industry for textile finishing and in the pharmaceutical industry as an adjuvant in drug formulas (Palmer *et al.* 2011). It is also biodegradable and non-toxic (Yangxin *et al.* 2008).

CONCLUSION

New lipopeptide isoforms were extracted from a *Bacillus* sp. MI27 strain. They were identified as linear and cyclic isoforms of Surfactin. The crude lipopeptide preparation was characterized by great ST reduction, a 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 salinity, temperature and pH. Different activities were studied including the ST-reducing power, the emulsification index, the foaming capacity and the washing ability of stains. All these characteristics advised the potential use of MI27 lipopeptide preparation in industrial and environmental biotechnology. In addition, having lower toxicity and greater biodegradability, BioS are considered safer and more ecological replacements to chemical

surfactants. On this basis, a new formula was prepared for potential use in detergency. Its interaction with the different ingredients of the washing machine can improve washing performance by effectively eliminating engine oil, oil and mayonnaise leading us to finalize that the potential use of these BioS as an additive for laundry detergent is very promising. To conclude, the described BioS can be of great interest in the chemical industry for detergent formulation and healthcare product.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper.

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AUTHORS' CONTRIBUTIONS

All authors directly participated in the planning, execution, or analysis of this study. All authors contributed in the realization of the work, read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare there is no conflict.

CONSENT TO PARTICIPATE

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

CONSENT TO PUBLISH

All the authors give the Publisher the permission to publish the Work in Water Practice and Technology.

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