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Chemical profile determination of biosurfactants produced by hydrocarbonoclastic bacteria

Supported on: 26/06/2024 **In front of the jury composed of:**

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Dedications

Not all letters can capture the right words. Not all words can fully express gratitude, love and respect. Thus, it is humbly that I dedicate this modest work

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ZAKIA

Abbreviations list

Asn: Asparagine

ATR: Total Attenuated Reflection

BS: Biosurfactants

BR: Brome

C/N: Carbon/Nitrogen

cg/cm3: Centigram per cubic centimeter

CIT: citrate

Cm: centimeter

CMC: Critical Micellar Concentration

CO_2: Carbon dioxide

CTAB: Hexadecyltrimethylammonium bromide

E24: The emulsification index

GC-MS: Gas Chromatography–Mass Spectrometry

GEL: Gelatin

Gln: Glutamine

Glu: Glutamic acid

GN: Nutrient Agar

H2S: Hydrogen sulphide

PAHs: Polycyclic aromatic hydrocarbons

He: the height of the emulsion layer

Ile: Isoleucine

IRTF-RTA: Fourier transform infrared spectroscopic analysis in attenuated total reflectance mode

KBr: potassium bromide

Leu: Leucine

LC: Length of hydrophobic chain

MDDS: Drug Delivery Systems

MG: Glucose Medium

mN/m: milli Newton per metre

MS: Mass Spectrometry

MSM: Mineral Environments

NRPS: Non ribosomal Peptide Synthetases

P/v: Weight / Volume

Rpm: Revolutions per minute

Ser: Serine

Thr: Threonine

TK: Temperature of Krafft

TS: Surface Tension

Val: Valin

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Introduction

Surfactants are widely used in several industrial domains such as agriculture, food processing, cosmetics, and medicines. The majority of these compounds are produced synthetically, which raises environmental concerns because of their toxic nature and the fact that they are not very biodegradable (Schramm et al., 2003 ; Makkar and Rockne, 2003).

The last few decades have seen significant progress in biotechnology, which has raised interest in biological surfactants. One of these surfactants' primary sources is microorganisms grown on media that dissolve in water or oil. They can be secreted into the culture medium or attached to the surface of the microorganisms (Gharaei-Fathabad, 2011). Some of their positive characteristics are high biodegradability, non-toxicity, and increased efficacy in harsh environments, including high salinity, pH, and temperatures (Makkar et al., 2011). They can also be made from regenerative resources (Dzięgilewska and Adamczek, 2013).

The variety of biosurfactant structures gives them a range of remarkable properties, including the capacity to create micelles and microemulsions between two immiscible phases and to lower the surface and interfacial tension of liquids (Yalaoui-Guellal, 2017). These properties make biosurfactants valuable tools for many sectors, including the environment, agri-food, pharmaceuticals, and cosmetics (Kee et al., 2021 ; Varjani et al., 2021).

Biosurfactant offer multiple advantages over synthetically produced surfactants (Pattanathu et al., 2008). For example, their action remains unchanged in the face of variations in temperature, and pH, while reducing their ecological footprint, and their action is often targeted and specific (Nitscke and Costa, 2007 ; Santos et al., 2016).

This work undertakes a detailed investigation of a biosurfactants's chemical and biological profiles. In this context, it comprises four chapters: Chapters 1 and 2 offer a detailed bibliographic review of biosurfactants covering categories, potential, particularities and fields of use. Chapter three describes in detail the experimental equipment and methodological protocols used to determine the chemical and biological profile of the biosurfactant treated. The final chapter provides an exhaustive analysis and nuanced interpretation of the results of the various analyses.

All in all, this work ends with a conclusion, highlighting the implications for future research in this field. It is followed by a list of references and then the appendices.

Part I: Literature review

Chapter I

I. General information on biosurfactants

I.1 Definition of biosurfactants

Biosurfactants are uniquely synthesized by a variety of microorganisms, such as bacteria, fungi, yeasts, and by some plants. They express diverse molecules with varied structures (Mulligan et al., 2001 ; Christofi and Ivshina, 2002). Their primary role is to reduce the interfacial and surface tensions in aqueous solutions and hydrocarbon mixtures, a property that sets them apart from other surfactants (Desai et al., 1997 ; Banat et al., 2000).

They can agglomerate at the interface of two immiscible surfaces, such as water and oil, and so lower both surface tensions simultaneously (Figure 01). The intricate process of biosurfactant production is heavily influenced by the environmental and cultivation requirements of the producing microorganisms, especially the carbon source, pH, nitrogen source, and temperature, adding to the complexity of their structure (Santos et al., 2016).

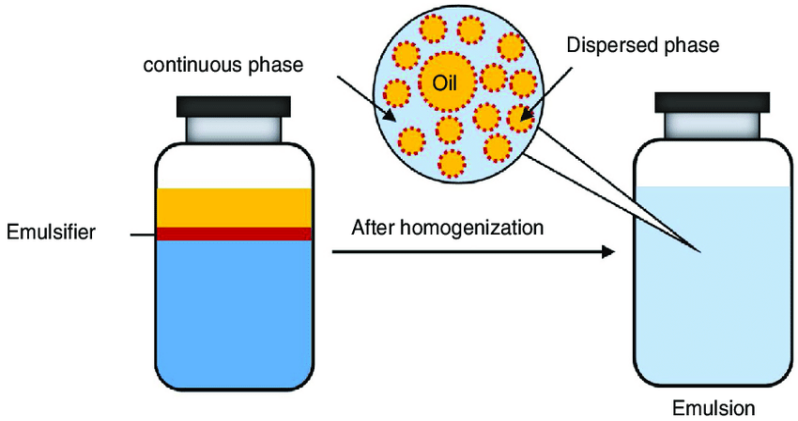

Figure 01 : Use of biosurfactants for emulsion emulsification (MeyIheuc et al., 2001).

I.2 Structure of biosurfactants

Biosurfactants comprise a polar hydrophilic portion and an apolar hydrophobic portion, as illustrated in (Figure 02). The interaction between the nature of the substrate, the kind of microorganism, and the production conditions employed determines the specific structure of each biosurfactant. There is a wide variety of functional groups in the hydrophilic water-soluble part, including amino acids, proteins, cyclic peptides, carboxylic acids, polysaccharides, and fatty alcohols (mono or di) (Dagbert et al., 2006 ; Sobrinho et al., 2013).

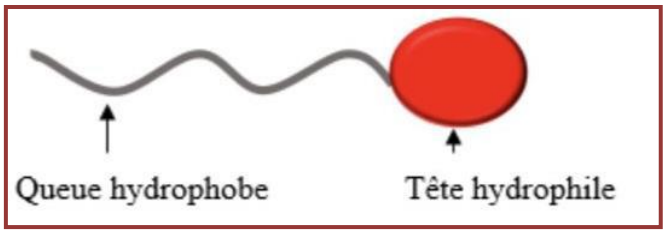

Figure 02 : Surfactant in monomer form (Santos et al., 2016).

However, in the hydrophobic part, there is a saturated or unsaturated aliphatic hydrocarbon chain, which gives these molecules their affinity for oils and insolubility in water, generally varying from C_8 to C_{22} and which can be linear or branched. It can also be built up by different types of fatty acids, like α-alkyl-β-hydroxy fatty acid, hydroxy fatty acid, and long-chain fatty acid (Dagbeert et al., 2006 ; Sobrinho et al., 2013). The hydrophilic nature of this part of the molecule facilitates the differentiation of the various kinds of chemical surfactants into 04 distinct categories (Banat et al., 2010 ; Zarasvand et Rai, 2014).

Cations, which carry a positive charge, are distinct from anionic surfactants which dissociate in aqueous solution, through the existence of one or more functional groups, the particles are transformed into ions with a negative charge. The absence of an ionic charge is a fundamental property of non-ionic surfactants, while amphiphiles (zwitterionics) have two hydrophilic groups, as illustrated in Figure 03 and listed in Table 01. Regarding the hydrophobic portion, the chemistry of surfactants is greatly influenced by three variables: aromaticity, the number of carbon atoms, and the degree of hydrocarbon chain branching (Banat et al., 2010).

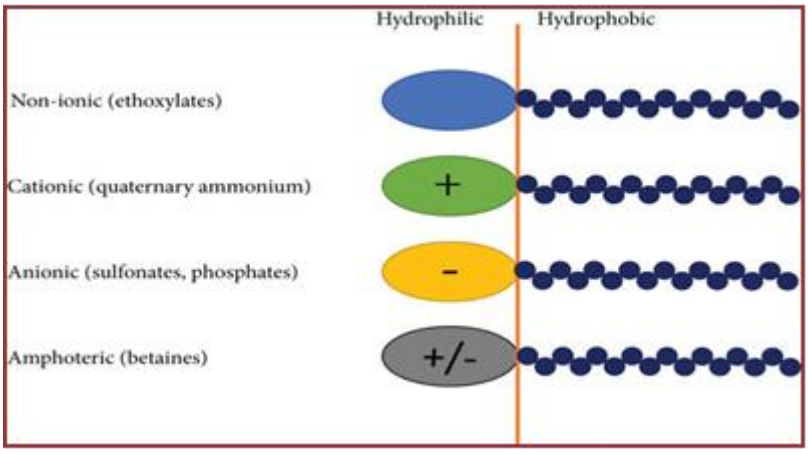

Figure 03 : Illustration of biosurfactant categories based on the polarity of their main functional group (Santos et al., 2016).

Table 01 : Overview of chemical surfactant diversity and their applications (Tabka, 2014).

Type	Process	Model	Use
Anionic	Dissociate in aqueous solution to generate negatively charged ions (anions).	SDS (Sodium Dodecyl Sulfate $NaSO_4(CH_2)_{11}CH_3$, sodium stearate, potassium laurate sodium dodecyl sulfate, sodium sulfosuccinate	Particularly used in detergents and cleaning products.
Cationic	Dissociate in aqueous solution to generate positively charged ions (cations).	CTAB (hexadecyltrimethylammonium bromide) $(CH_3)_3N(CH_2)_{15}CH_3Br$ Quaternary ammonium salts	Widely used in industrial and hospital environments for their powerful disinfectant action against micro-organisms.
Amphotere	Depending on the pH of the medium, these substances release either a positive or a negative ion.	N dodecyl-sulfobetaine	Exclusive formulations for pharmacies and cosmetics...
Non-Ionic	The molecule is electrically neutral, meaning it has no overall positive or negative charge.	Triton $C_8H_{15}C_6H_4(OC_2H_4)_9OH$ Polyglycol, esters d'acides gras, lécithine	These surfactants , appreciated for their mildness and low foaming power, are used in the textile, metalworking and cosmetics (body hygiene and beauty) industries.

I.3 Classification of biosurfactants

I.3.1 Based on molecular weight

I.3.1.1 Low molecular weight

Their ability to adjust to various interfaces is remarkable, making them effective at reducing stresses on the surface and between interfaces. As a result, they rapidly cover the new interface formed between oil and water during emulsification. This series includes a wide range of compounds, including fatty alcohols and fatty acids, as well as monoglycerides, lecithins and lysolecithins, glycolipids, and saponins (Healy et al., 1996).

I.3.1.2 High molecular weight or polymers

They are recognized as emulsifiers, offering optimal stabilization of oil-water emulsions without significantly altering surface tension, guaranteeing increased stability. They combine protein or lipopolysaccharide groups with polysaccharides (hydrocolloids) (Healy et al., 1996).

I.3.2 According to chemical structure

I.3.2.1 Glycolipids

Among the most well-known and valued biosurfactants are glycolipids. They arise from the reaction of carbohydrates with hydroxylated fatty acids or long-chain aliphatic acids corrected by an ether or ester. Notably, the most common glycolipids are sophorolipids, trehalolipids, and rhamnolipids (Shoeb et al., 2013).

a) Rhamnolipids

Extracellular glycolipids called rhamnolipids are made up of L-rhamnose and β-hydroxyalkanoic acid and they are generated by specific microbes like *Pseudomonas sp*., which includes *P. aeruginosa*, *P. fluorescens et P. putida*. According to (Banat et al., 2000) and (Cha et al., 2008), they are created when one or two β-hydroxydecanoic acid molecules fuse with one or two rhamnose sugar molecules. The hydroxyl (OH) group of β-hydroxydecanoic acid and a carbon atom at the reducing end of the rhamnose disaccharide form a covalent bond known as glycosidic bond, which is crucial to the structure of rhamnolipids. Another covalent link of the ester type is formed by the second acid's hydroxyl (OH) group (Karanth et al., 1999 ; Shoeb et al., 2013).

There are around 60 variants of rhamnolipids, but the most common are the four main types (Figure 04), designated Rh1 to Rh4 (Fessl, 2015).

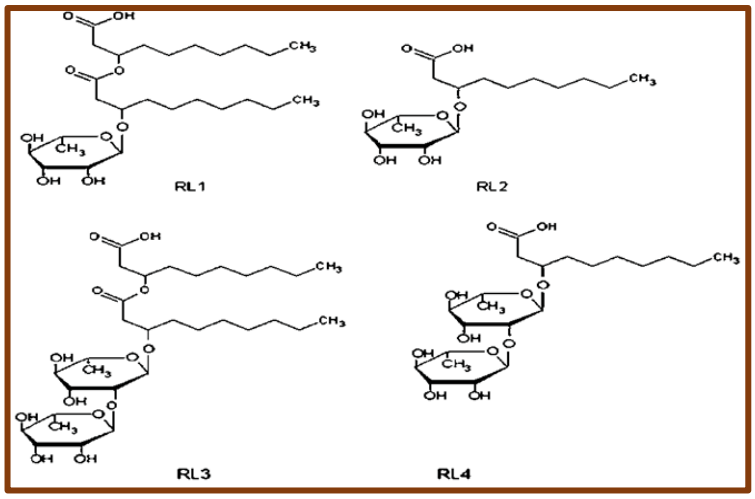

Figure 04 : Chemical structures of rhamnolipids produced by Pseudomonas (Irfan-Maqsood et Seddiq, 2014).

b) Trehalolipids

It has been possible to identify a large range of chemical structures for microbial trehalolipid biosurfactants.

Trehalose disaccharide is associated with t most *Mycobacterium*, *Nocardia and Corynebacterium* species and is connected to the C-6 and C-6' positions of mycolic acid. Branching at the α-position with the β-hydroxyls of fatty acids is a characteristic of mycolic acids. Trehalolipids produced by different microorganisms show significant structural variations, distinguished by the mycolic acid's composition, size, and unsaturation level in addition to its carbon atom count. The bacterial species *Rhodococcus erythropolis* produces trehalolipids, piqued scientists' curiosity of worldwide (Kaloorazi et Choobari, 2013).

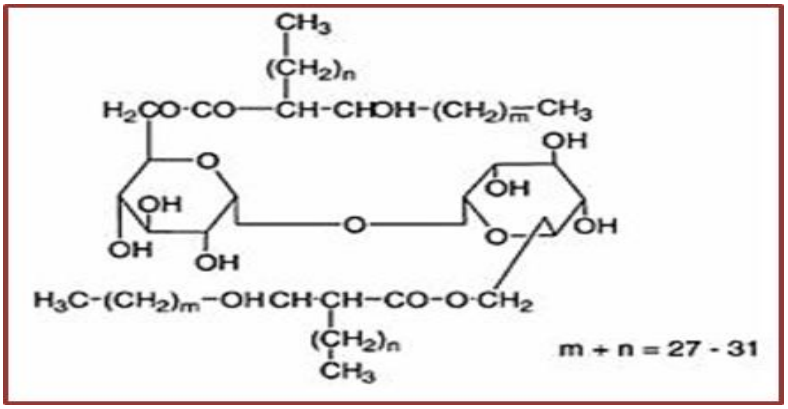

Figure 05 : Structure of a trehalolipid representative (Kedidi, 2014).

c) Sophorolipids

Yeasts produce glycolipids called sophorolipids, consisting of a dimeric carbohydrate and a long-chain hydroxylated fatty acid connected by a glycosidic bond (sophorose). The lactone form of sophorolipids, comprising 06 to 09 divergent hydrophobic lipids, is prized for its antibacterial, anti-inflammatory and immunoregulatory properties. These characteristics make them a preferred choice for medical and cosmetic applications, particularly because of their moisturizing and emulsifying properties. They fall into two main categories (Figure 06): acid sophorolipids (non-lactonic) and lactonic sophorolipids (Asmer et al., 1988 ; Gaur, 2019).

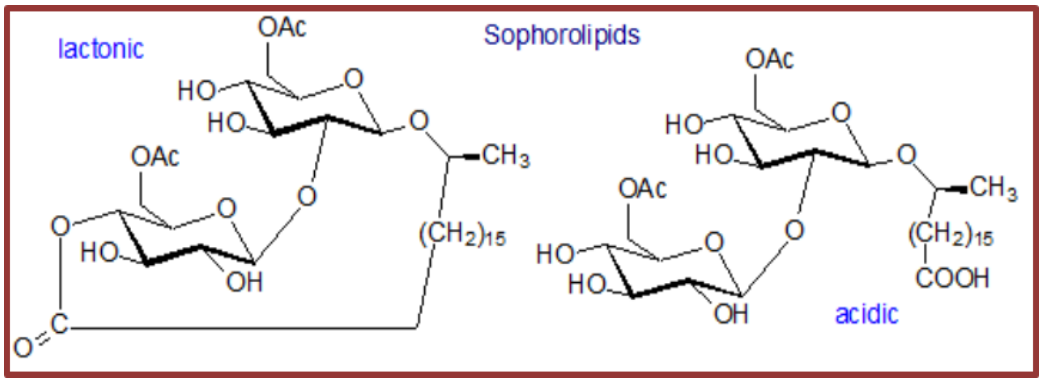

Figure 06 : Structures of the two types of sophorolipids (Ying et al., 2023)

I.3.2.2 Lipoproteins and lipopeptides

The cyclic lipopeptides synthesis gives rise to various compounds, containing lipopeptide antibiotics like polymyxins and decapeptide antibiotics like gramicidins. The lipid-to-polypeptide chain connection characterizes these compounds (Arima et al., 1968). Two examples of these lipopeptides are described below:

a) Surfactin

Bacillus subtilis ATCC-21332 produces surfactin, a biosurfactant that stands out for its remarkable properties. It can lower surface tension from 72 to 27.9 mN and has an extremely low critical micellar concentration of 0.017 g/l, making it one of the best-performing biosurfactants available. (Arima et al., 1968).

Seven amino acids and various 3-hydroxy fatty acids make up surfactin. Its central component is 3-hydroxyl-13-methylmyristine acid, which joins with a heptapeptide with the sequence Glu-Leu-Leu-Val-Asp-Leu-Leu (LLDLLDL configuration) to form a lactone ring

(Figure 07). (Cooper et al., 1987). This substance is created by a non-ribosomal enzyme called surfactin synthetase, which synthesizes peptides in a linear fashion (Chtioui, 2011). The amino acids L-Glu and L-Asp help give the molecule its amphiphilic character, and this gives it its surface-active characteristics (Cooper et al., 1987).

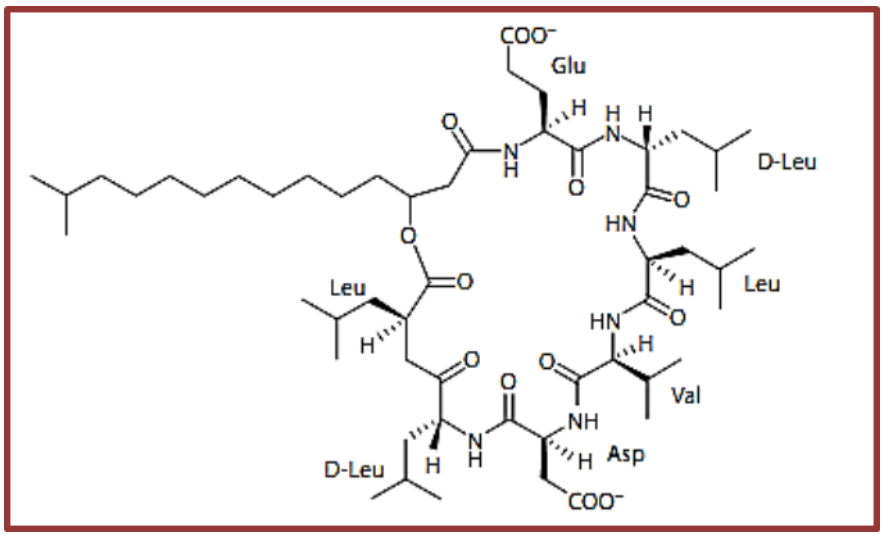

Figure 07 : Chemical structure of surfactin (Hausmann et al., 2014).

b) Lichenysin

The structure and physicochemical characteristics of Lichenysin, a biosurfactant derived from *B. licheniformis*, display characteristics similar to those of surfactin. The *B. licheniformis* BAS50 strain specifically produces *lichenysin A*, Characterized by a lengthy β-hydroxy fatty acid molecule (Yakimov et al., 1996). According to Arpita, the surfactants produced by *B. licheniformis* exhibit exceptional efficacy in decreasing the surface tension of water to 27 $mN.m^{-1}$ and the interfacial tension of water and n-hexadecane to 0.36 $mN.m^{-1}$ (Arpita, 2017). Lichenysin demonstrated superior efficacy as a cation chelator than surfactin (Grangemard et al., 2001).

c) Viscosines

The lipopeptides consist of a sequence of nine amino acids: L-Leu-D-Gln-D-Thr-D-Val-L-Leu-D-Ser-L-Ile. Viscosin is a C10-C12 hydroxydecanoyl residue to which they are connected (Janek et al., 2020). Viscosin is a product of bacteria present in marine and terrestrial environments such as *Pseudomonas sp*, using of non-ribosomal peptide synthetase enzymes (NRPS) (Geudens et al., 2017).

I.3.2.3 Fatty acids, phospholipids and neutral lipids

Micro-organisms, such as bacteria and yeasts, synthesize significant quantities of surfactants based on fatty acids and phospholipids when they grow on n-alkanes (Cooper et al., 1978 ; Shoeb et al., 2013). In the presence of n-alkanes, *Rhodococcus erythropolis* synthesize phosphatidylethanolamine, a substance with exceptional properties. The tension between water and hexadecane is below 1 mN/m. Additionally, it has an exceptionally low critical micelle concentration (CMC) of 30 mg/l (Shoeb et al., 2013).

I.3.2.4 Polymeric biosurfactants

Emulsan, synthesized by *Acinetobacter calcoaceticus*, is a representative example of polymeric biosurfactants, including manopoper, emulsan, liposan, and other polysaccharide-protein complexes among the most extensively studied. Liposan, synthesized by *Yarrowia lipolytica*, is a fascinating water-soluble emulsifier. Its composition, 83% carbohydrates and 17% proteins, is at the root of its complex structure. The fatty acids covalently bonded to the heteropolysaccharide skeleton give it exceptional emulsifying properties. (Shoeb et al., 2013).

I.3.2.5 Specific biosurfactants

Particulate biosurfactants can be categorized into extracellular vesicles and entire microbial cells. Extracellular membrane vesicles, by fragmenting, induce the formation of microemulsions from hydrocarbons, enhancing the uptake of these substances by microorganisms. Occasionally, the bacterial cell functions as a surfactant (Kappeli et Finnerty, 1979).

a) Vesicles

The extracellular vesicles generated by *Acinetobacter sp*. while growing on hexadecane were gathered and analyzed. These vesicles had a diameter ranging from 20 to 50 mm and a buoyant density of 1.158 g/cm3. They may potentially contribute to the assimilation of alkanes. They contain proteins, phospholipids, and lipopolysaccharides (Kappeli et Finnerty, 1979).

b) Whole microbial cells

Degradation capability of both hydrocarbon and non-hydrocarbon compounds is a striking example of the metabolic diversity of micro-organisms. Species such as cyanobacteria and certain pathogens illustrate this remarkable ability and are particularly sensitive to the

interfaces between water, hydrocarbons and air. In these specific situations, the microbial cell acts as a surfactant (Karanth et al., 1999).

I.4 Biosynthesis of biosurfactants

Biosurfactants (BS) are primarily produced by microbes in aerobic aquatic environments that contain one or more carbon sources, such as carbohydrates, oils, or hydrocarbons. Typically, these microorganisms consist of yeasts, bacteria, and fungi. Soils contaminated with hydrophobic substances such as polycyclic aromatic hydrocarbons (PAHs) often contain the bacteria used to produce BS. These bacteria are then extracted from their natural environment and cultivated in the laboratory. This facilitates testing to select the most effective carbon source and improve cultivation conditions to boost the production rate. However, the resulting BS is a mixture of different molecules (Dyke et al., 1993).

Although many species generate biosurfactants, their regulation remains largely unknown, except for strains of *P. aeruginosa* and *B. subtilis*, which are the most sought-after bacteria (Banat et al., 2000). The constituents of biosurfactants are in contact with bacterial membranes and degrade in the same way in the environment. (Thangamani et Shreve, 1994).

Reducing production costs is essential to ensure the efficient biosurfactant use and production. This reduce can be limited by exploiting affordable growth substrates. On the other hand, it is beneficial to use waste and agricultural products in order to limit costs and reduce the quantities of waste to be processed from different industrial sources, such as used motor oil (Makkar et Cameotra, 2002).

Household and secondary waste can also be used as raw materials for their production, offering significant advantages for large-scale production processes, particularly in oil-related technologies. This approach to biosurfactant production is considered economically viable (Mulligan et al., 2014).

I.4.1 Microorganisms producing biosurfactants

Primarily, bacteria that thrive in aerobic environments are responsible for the production of biosurfactants. In most cases, these microorganisms include fungi, yeasts or bacteria. Biosurfactants come from various microbial sources (Mulligan et al., 2001 ; Christophe et Ivshina, 2002).

The bacteria used to make biosurfactants are usually taken from hydrocarbon-contaminated soils, separated from their natural environment and grown in the laboratory. Using this method, it is possible to carry out trials to identify the most efficient carbon source and to improve the culture conditions in order to achieve maximum production yield (Van Dyke et al., 1993).

I.4.1.1 Physiological role of biosurfactants in microorganisms

Biosurfactants play an essential role in physiology by facilitating the proliferation of bacteria on substrates that are not soluble in water. They work by lowering surface tension at the end of the growth phase, facilitating substrate absorption and metabolism. However, the molecular mechanisms responsible for the absorption and metabolism of these substrates are not always elucidated and remain partially explained (Fakruddin, 2012).

I.4.2. Parameters influencing biosurfactant production

Biosurfactant production, the type and quantity of biosurfactants produced, is influenced by the content of the medium, including the source of carbon and other nutrients as well as culture parameters such as temperature, agitation, and pH.

I.4.2.1 Impact of the carbon source

The carbon source is essential for the production of biosurfactants, either by stimulating their production or decreasing the amount generated. One example is the production of rhamnolipids, which involves water-soluble carbon sources such as glycerol, glucose, mannitol, or ethanol. They seem to have a lower effectiveness level when used on insoluble substrates, such as n-alkanes or olive oil. Bacteria possess the capacity to proliferate on hydrophobic materials (Desai et Banat, 1997 ; Cameotra et Makkar, 1998).

I.4.2.2 Impact of nitrogen

According to a number of studies, rhamnolipids are formed when there is too much carbon in the environment or when the amount of available nitrogen is limited. The bacteria that produce them can use different forms of nitrogen depending on the situation (Lang et Wullbrandt, 1999). To optimize production performance, it is crucial to achieve an optimum C/N ratio, with nitrogen deficiency in particular inducing stress that stimulates the production of biosurfactants. (Gabet, 2004).

I.4.2.3. Impact of pH

The pH of the culture medium for a *P. aeruginosa* strain must be between 6.0 and 6.5. Biosurfactant production by *Ps. aeruginosa* is sensitive to pH variations and collapses rapidly when the pH deviates from the optimum range. In addition, other strains, such as *Norcardia corynbacteroides,* are not affected by pH levels between 6.5 and 8.0. (Arino et al., 1996).

I.4.2.4 Impact of mineral elements and ions in the growing medium

Limiting the availability of minerals and ions also affects the synthesis of biosurfactants in terms of both quantity and quality. For example, reducing iron and phosphate consumption increases the production of rhamnolipids. (Urai et al., 2006 ; Cameotra et al., 2010). Limiting metal ions, phosphate and sulfate chloride in the culture medium stimulates trehalolipid synthesis.

Iron and manganese in the culture medium enhance the production of biosurfactants by *B. subtilis* bacteria **(**Déziel et al., 1996 ; Laamari et al., 2010**)** and *Rhodococcus.sp* **(**Ruiz-Garcea et al., 2005). It seems that when the concentration of magnesium, calcium, potassium, sodium ions or trace elements is limited, this leads to an increase in the production of these elements (Abu-Ruwaida et al., 1991).

I.4.2.5 Influence of oxygen

Depending on the conditions, oxygen can stimulate or inhibit the cellular activity and growth of microorganisms (Gabet, 2004). Oxygen is crucial in controlling the production of biosurfactants by influencing the cellular functions and growth of microorganisms. Analysis of the synergistic effects on *Lactobacillus pentosus* revealed that these factors are closely linked and influence each other's impact. Oxygenation has a significant influence on lipopeptide production by *Bacillus subtilis* C9, with a three-fold increase in yield under conditions of low oxygenation (Kosaric and Sukan, 2000).

I.4.2.6 Impact of agitation speed

During the manufacture of biosurfactants, culture media undergo a stirring process. Increasing the speed of agitation of culture media leads to an increase in the mortality rate of bacteria, resulting in a decrease in their yield. Yeasts, as producer organisms, are distinguished

from bacteria by their positive response to increased agitation, which favors their productivity (Desai and Banat, 1997).

I.5 Mechanisms ofaction of biosurfactants

The use of biosurfactants in remediation has a number of advantages. According to West and Harwell, unlike cationic surfactants, which attach strongly to soils, molecules of biological origin, which are mainly non-ionic or anionic, have little affinity for soils and are therefore less absorbed. The critical micellar concentration (CMC) of biosurfactants is often lower than that of chemical surfactants, which means that they can act effectively at lower doses. A major advantage of using biosurfactants is their low toxicity and biodegradability, which simplify their handling and minimize their impact on the environment (West and Harwell, 1992). According to several authors, these agents work by mobilizing and solubilizing contaminants.

I.5.1 Mobilization

Biosurfactants possess the capability to decrease interfacial tensions between NAPLs (non-aqueous liquid phase) and water at their surface is essential for their mobilization (MC Cray et al., 2001). Mobilization involves two key phases: displacement and dispersion. The reduction in interfacial tensions between the NAPLs and the porous medium encourages the release of NAPL droplets, which constitutes the displacement phenomenon. The NAPLs are distributed in the aqueous phase in the form of fine emulsions, a phenomenon known as dispersion. (BAI et al., 1997).

I.5.2 Solubilization

Solubilization is the preferred method for treating contaminated soils, based on the formation of micelles. This process takes advantage of the unique ability of micellar solutions to solubilize hydrophobic contaminants that are usually insoluble in water (Gabet, 2004). Before reaching the critical micellar concentration (CMC), the monomers present in the solution generally have little impact on most hydrocarbons. (Pennel et al., 1993).

Above the CMC, hydrophobic hydrocarbons become incorporated into the micelles, considerably increasing their apparent solubility (Gabet, 2004). Reaching the actual critical micellar concentration (CMC) in the aqueous phase is essential to stimulate micelle formation. To achieve this, it is essential to add an adequate quantity of biosurfactants. The impact of biosurfactant concentration on contaminant sorption is complex. At low concentrations,

sorption predominates, while at high concentrations, solubilization becomes the main process (Van Dyke et al., 1993 ; Bai et al., 1998).

I.6 Potential of biosurfactants

Characterizing biosurfactants is crucial for assessing the performance of producer micro-organisms and guiding their selection. Biosurfactants have a number of advantages over chemical surfactants (Pattanathu et al., 2008). Biosurfactants have several advantages, including:

I.6.1 Temperature and pH tolerance

Several biosurfactants are capable of being utilized under high temperatures and within a pH range of 2 to 12 (Santos et al., 2016). Lichenysin, produced by *B. licheniformis*, is an example of an effective biosurfactant that remains unchanged even when exposed to temperatures as high as 50°C and pH levels ranging from 4.5 to 9 (McInerney et al., 1990). *Arthrobacter protophormiae* has produced another biosurfactant that has shown resistance to heat (30-100°C) and stability over a wide pH range (2-12) (Singh and Cameotra, 2004).

I.6.2 Biodegradability

Biosurfactants degrade up to 99.96% and leave no harmful residues (Mohan et al., 2006). There are few studies regarding the biodegradation of biosurfactants. Nevertheless, the existing data indicate that biosurfactants have a higher susceptibility to degradation compared to their chemical equivalents. Additionally, a study conducted by (Mohan et al., 2006) demonstrated that rhamnolipids can be biodegraded in both aerobic and anaerobic environments. (Cappello et al., 2011), discovered that a biosurfactant made of exopolysaccharide can be easily broken down by specific bacterial strains in the marine environment.

Biodegradability experiments conducted on five biological surfactants produced by two *Bacillus sp, Flavobacterium sp, Dietzia maris*, and *Arthrobacter oxydans* revealed a clear correlation between the effectiveness of their degradation and the specific bacteria employed (Lima et al., 2011). There are therefore few recent studies evaluating the biodegradability of biosurfactants.

I.6.3 Low toxicity

While data on the toxicity of microbial surfactants is limited in the literature, they are generally considered to have low toxicity, making them suitable for use in sectors such as pharmaceuticals, cosmetics, food and the environment. Note that the toxicity of surfactants is related to their structure rather than their origin (Ferradji, 2014). The hydrophobicity of the surfactant's carbon chain is generally associated with toxicity: the longer the chain, the higher the hydrophobicity, and therefore the more toxic the surfactant (Eddouaouda, 2012).

Biosurfactants have been tested for their toxicity on marine organisms. The saltwater shrimp *Corophium volutator* showed increased sensitivity to synthetic surfactants compared to glycolipid-like biosurfactants (Lang and Wagner, 1993). After treatment with biosurfactants, the growth rate of *Acinetobacter calcoaceticus, Serratia marinorubra* and *Photobacterium phosphoreum* increases, while it decreases for flagellates (Poremba and Gunkel, 1991).

I.6.4 Specificity

Biosurfactants are complex organic compounds that have particular functional groups and frequently have a specific action. (Nitscke and Costa, 2007). This would be particularly suitable for cleaning specific pollutants, de-emulsifying industrial emulsions and even for specific applications in the cosmetics, pharmaceutical and food sectors (Gakpe et al., 2007).

I.6.5 Biocompatibility

When a biosurfactant is referred to as biocompatible, it signifies that it is easily accepted by living organisms and does not modify their biological activities upon interaction. These characteristic paves the way for its use in various fields such as cosmetics, pharmaceuticals and the food industry as functional additives (Santos et al., 2016).

Chapter II

II. Biosurfactants applications

II.1 Properties of biosurfactants

II.1.1 Biological properties

Glycolipids and lipopeptides, which are biosurfactants, may have specific antibiotic properties (table 02). It is possible that their antibiotic action is linked to their ability to solubilise certain components of the cell envelope (Bernheimer and Aviged, 1970).

Table 02 : Biological properties of biosurfactants

Biosurfactant	Properties	Reference
Surfactin **Irutin** **Viscosine**	Antimicrobial	(Besson et al., 1978) (Lesinger et Maragraff, 1979)
Gramicidines **Polymixins**	Antibiotics	(Marahiel et al., 1979) (Suauki et al., 1965)
Empedopeptine	Inhibits many aerobic and anaerobic gram(+) bacteria in vitro and in vivo.	(Sugawara et al., 1984)
Lichenysin A	Antibacterial	(Yakimov et al., 1995)
Lipid sophorosis	Special antibiotics with gram bacteria (+)	(Hommelet al., 1987)
Ramnose lipide (R1/R2)	Inhibits the growth of *B sublitis* and *staphilococcus epi*	(Lang et al., 1989)
Emulsane	Specific phage receptor	(Pines et Gutinck, 1981)

II.1.2 Physical and chemical properties of biosurfactants

Biosurfactants can have different characteristics, such as the ability to wet, foam, solubilize, disperse, emulate or demulsify. Depending on their surfactant activity, charge and chemical composition, the physicochemical characteristics of biosurfactants differ (Briant et Tenebre, 1989).

II.1.2.1 Decrease in surface tension

Liquid-gas interfaces have a surface tension that surfactants can reduce (Kroschwitz, 1994 ; Pugh, 1996). This tension is due to the anisotropic distribution of attractive forces experienced by the molecules of a liquid at its surface in contact with air, where each molecule is subject to attractive forces from other molecules, the result of which is zero. On the other

hand, a molecule at the surface feels a net attractive force directed towards the interior of the liquid, because the attractive forces exerted by the air molecules are very weak, so the contact surface between air and water is reduced. Surface tension results in an increase in the energy of the system when a molecule is displaced from the volume towards the surface, thus reducing the number of its first neighbors. Surface tension is a measure of the cohesive energy at an interface. The molecules of a liquid attract and interact with each other. These interactions between the molecules of the liquid are balanced by attractive forces acting in all directions (Schwartz and Perry, 1977 ; Puissieu and Suiller, 1983).

The force at the surface of a liquid is due to the attraction between the molecules that oppose the rupture of this surface. It uses the language Dyne.cm-1 (=mN.m-1). The surface tension of water is considerably reduced by biosurfactants, even in very dilute solutions. For example, pure water has a surface tension of 62.80 mN/m at 20°C, but in the presence of a biosurfactant it can drop to around 30 mN/m (Holmberg, 2001).

II.1.2.2 Decrease in interfacial voltage

Interfacial tension is the force required to break the surface between two liquids that do not mix. Biosurfactant molecules cluster at concentrations above CMC to form micelles, bilayers and vesicles. This micelles formation allows biosurfactants to reduce interfacial tension (Neindre, 1993 ; Pacwa-Płociniczak et al., 2011).

Interfacial tension is a molecular phenomenon that results from the energy difference between molecules at a fluid interface and their bulk counterparts. It can also be appropriately described as a measure of the energy required to create an interface unit between two solid liquids (Rotenberg et al., 1983).

Interfacial tension refers to the minimum amount of "reversible" work required to transport molecules (linked to each other by cohesion forces) from the core of the material or another phase to its surface. This offers the possibility of increasing the area of an increment (or increment) or modifying it. Energy is measured in joules per square meter (J/m2) (Andrieu and Müller, 2005 ; Béranger and Mazille, 2005). Water has an interfacial tension of 50.81 mN/m with alkane (n-octane) at 20°C, while in the presence of a biosurfactant it decreases to less than 1 mN/m (Holmberg et al., 2002).

II.1.2.3 Critical Micellar Concentration (CMC)

CMC is defined as the concentration in solution of a surface agent beyond which a fraction of the molecules dispersed in the solution tends to group to form micelles (Pore, 1992).

When micelles are formed from the hydrophobic parts, which cannot form hydrogen bonds in the aqueous phase, the free energy of the system increases considerably. By isolating the hydrophobic part from the water by adsorption on organic matrices or by forming micelles, this energy can be reduced (Haigaigh, 1996). At the center of the micelles, the hydrophobic parts are concentrated, while the hydrophilic parts remain in contact with the water (Figure 08).

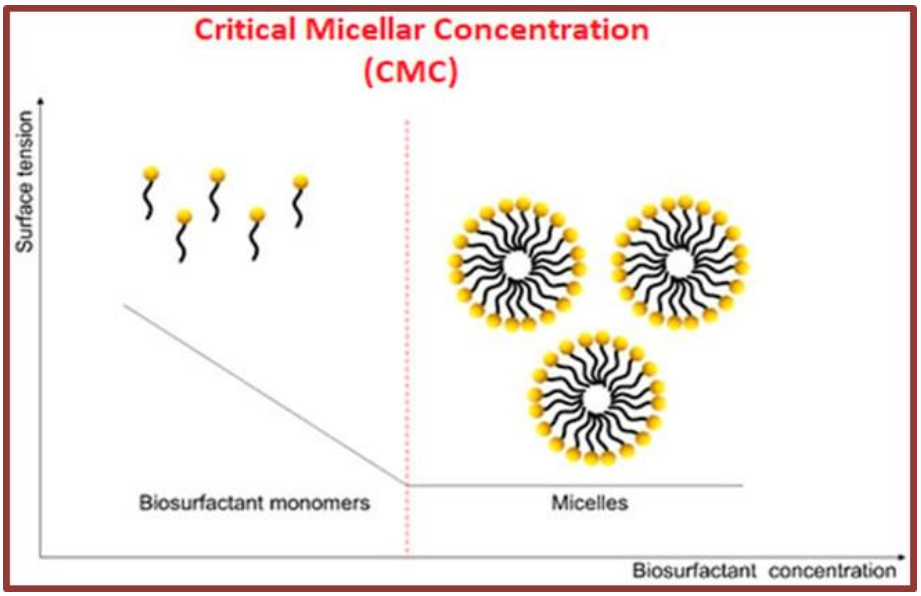

Figure 08 : Consequences of surfactant concentration on surface tension and micelles formation (Lee et al., 1995).

The CMC can also be defined as the concentration at which the surface tension is minimal (around 30 $mN.m^{-1}$ in an aqueous solution). Despite the almost identical minimum surface tension for many biosurfactants, the CMC differs according to their structure. CMC levels for biosurfactants fluctuate between 1 and 200 mg (Abalos et al., 2001).

Depending on its structure, the temperature of the solution and the presence of electrolytes or organic compounds, the CMC of a biosurfactant can fluctuate. (Edwards et al., 1991). The CMC of ionic biosurfactants is more influenced by electrolytes. An essential element is the fluctuation in the size of the hydrophobic region, and in general, the CMC decreases as the surfactant becomes more hydrophobic (Haigh, 1996).

II.1.2.4 Structure of biosurfactants and parameters influencing their micellization

Biosurfactant micelles come in a variety of microstructures, such as spheres, globules or cylinders. However, the most common are spherical and irregular vesicles, tubular bilayers and lamellar structures (Champion et al., 1995).

The internal volume of micelles varies according to their shape and the aggregation number, i.e. the number of monomers that make up the micelle. In general, the formation of a micelle requires between 50 and 100 monomers (Christofi et Ivshina, 2002).

The hydrophobic chain has a length of Lc and a volume of VH and an area of the hydrophilic part (ah). The biosurfactant also plays a role in the structure of the micelle. The shape of the micelle can be calculated using a geometric method using the ratio VH/(ah*Lc). When this proportion is less than 1/3, the micelle takes a spherical shape. By increasing the number of aggregations, the interior volume of the micelle increases. The morphology of the aggregates changes according to pH, biosurfactant concentration, temperature and ionic strength (Bai et al., 1998 ; Champion et al., 1995).

II.1.2.5 Solubility of biosurfactants as a function of temperature

The solubility versus temperature curve for ionic biosurfactants is irregular: above a specific temperature Tk, known as the Krafft temperature, solubility increases rapidly. The CMC curve as a function of temperature has also been included in this graph. (Shinoda et Fontell, 1995).

Solubility is low below the Krafft point, as it can only be attributed to monomers in solution. After reaching saturation of these monomers (at CMC), the biosurfactant pours out as a hydrated solid. As the temperature rises above Tk, solubility increases rapidly due to the formation of micelles, as the activity of the micelles remains constant. (Shinoda et Fontell, 1995). Micelles contribute to increased solubility, the biosurfactant may be present in the form of micelles or monomers.Nonionic biosurfactants do not have a Krafft temperature, but are characterized by a temperature known as a trouble point (Gabet, 2004).

II.2 Applications of biosurfactants

The popularity of biosurfactants lies in their non-toxicity, their ability to degrade and their use in extreme environments (Banat et al., 2000 ; Desai et Banat, 1997). This is why they are employed in various sectors.

II.2.1 Medical

Various biosurfactants are used in different fields, such as antimicrobial (antimalarial, antifungal and antiviral) and immunomodulatory activity. Adhesive and anti-adhesive compounds are utilized as adjuvants for antigens in the domains of vaccinations and gene therapy, serving a crucial function (Rodrigues et al., 2006 ; Varjani et al., 2020).

In the medical field, the incorporation of biosurfactants has both advantages and disadvantages. Although biosurfactants, such as rhamnolipids, have antimicrobial and anti-tumor properties, their production is associated with the pathogenicity of certain microorganisms, including *Pseudomonas*, even in plants. It is therefore essential to exercise great caution when using these biosurfactants because of the potential risk of allergic reactions and Epidermal irritation resulting from particular biosurfactants derived from pathogens found in cosmetic or medicinal items (Haque et Hassan., 2020).

II.2.2 In the pharmaceutical industry

In the pharmaceutical field, biosurfactants have been used to simplify the fusion of hydrophobic formulations, which has encouraged their use in drug distribution and in mixing and formulation processes. (Naughton et al., 2019 ; Ohadi et al., 2020).

Several researchers have investigated the properties and uses of biosurfactants as micro-emulsions in drug delivery systems (MDDS). Their study highlighted the importance of an in-depth study of the variety of biosurfactants in order to improve their use in drug delivery systems (Ohadi et al., 2020).

II.2.3 In cosmetics

Biosurfactants are widely used in the cosmetics and healthcare industries as cleaning, emulsifying and stabilizing agents. They are ideally suited to many essential products such as

shampoos, toothpaste, soaps, body creams, lotions and moisturizers Due to their minimal toxicity and strong biodegradability (Yea et al., 2019 ; Vecino et al., 2017).

Biosurfactants are frequently used as emulsifiers to stabilize emulsions in various cosmetic formulations. The effectiveness of these moisturizing creams for dry and anti-aging skin has been proven by studies (Vecino et al., 2017).

Similarly, the primary issue associated with cosmetic products s is skin irritation resulting from the application of chemical surfactants. These can be replaced by biosurfactants, which have powerful antimicrobial activity. Biosurfactants, which are influenced by the growth and hygrometry properties of plants, influence the metabolism of dermal fibroblasts and prevent the production of free radicals, which could cause skin damage (Kashif et al., 2022).

Vitamin C plays a crucial role in human health, as it is involved in collagen production and skin protection, but it degrades rapidly and the cosmetics and pharmaceutical industries use dehydrated vitamin C in their formulations to prevent it from degrading, which requires rehydration prior to use, which is a tedious procedure (Rincón-Fontán et al., 2020).

The majority of beauty products containing vitamin C are intended to add a touch of youth to the skin, but they are extremely unstable and are often combined with antioxidants. Research has demonstrated that the utilization of biosurfactants enhances the stability of vitamin C in water-based solutions (Chambial, 2013).

II.2.4 In food industry

Food formulation ingredients and substances with non-adhesive properties have been utilized to regulate fat accumulation, which improves the texture and shelf life of food products (Muthusamy et al., 2008).

II.2.5 In agriculture

Biological surfactants act as mobilizing agents to improve the solubility of chemical compounds that are potentially hazardous to health, such as PAHs. They enable microbes to adhere to contaminated soil particles. They are also used to make clay soils more sensitive to water in order to maintain their level of nutrition. Some biosurfactants are also used in biological control of plant diseases because of their antifungal properties. (Makkar et Rockne, 2003).

II.2.6 In environment

Biosurfactants possess amphiphilic characteristics that enable them to form stable micelles, making hydrocarbons and crude oil more readily available for biodegradation. This helps to improve soil quality and water management. In addition, biosurfactants have the ability to affect cloud precipitation by altering the surface tension of water droplets, and are also considered key players in the degradation of xenobiotics in benthic organisms (Markande et al., 2021).

Biosurfactants (rhamnolipids, sophorolipids, surfactins) derived from micro-organisms such as *Pseudomonas, Bacillus* and *Candida* have been studied to solve oil spill problems. These biodegradable surfactants alter the surface tension of surfaces and have an impact on the influence of aerosols on rain. They are also used to improve bioremediation and are seen as a contemporary approach in nano-biotechnology for soil and water treatment (Markande et al., 2021).

II.2.7 In the oil industry

Biosurfactants have the ability to dissolve xenobiotic substances such as hydrocarbons, facilitating their degradation in the natural environment. They also have the ability to associate heavy metals such as zinc, lead, cadmium and copper, making them easier to remove from contaminated areas (Mulligan, 2005).

Despite their use in various fields, biosurfactants are increasingly being taken into account in contaminated site remediation strategies (Ron and Rosenberg, 2014). The use of biosurfactants for assisted remediation offers numerous benefits. In fact, biosurfactants increase the surface area of poorly soluble hydrocarbon compounds by reducing interfacial and surface tensions, which leads to an improvement in the bioavailability and mobility of disruptors. (Mahanty et al., 2006). Thus, by adding biosurfactants to a contaminated site, biodegradation can be improved by solubilizing, mobilizing or emulsifying metals (Ron et Rosenberg, 2014). Oil wells generally extract between a third and a half of the oil initially present in the reservoir using oil extraction methods (Santos et al.2016), the oil residues that remain in the small pores of oil reservoirs account for around 50 to 65% of the oil recovered. Capillary forces and interfacial tension between the hydrocarbon and the aqueous phase retain these residues. It is necessary to reduce the interfacial tension to release these trapped hydrocarbons, which can be achieved by using surfactants (Austad and Taugbol, 1995).

Through the use of heat, surfactants, microbial techniques and gas injection, much of the retained oil is recovered during oil recovery operations (Santos et al., 2016). The tension between oil and water and the capillary forces that prevent the passage of oil through rock pores have been reduced by biologically derived surfactants (Pacwa-Płociniczak et al., 2011). Biosurfactants create a stable barrier between oil and water, capturing the desorbed oil and allowing its easy removal by injection into the oil well (Pacwa-Płociniczak et al., 2011).

Part II:

Experimental procedures

Chapter III

III. Materials and methods

This study focuses on the production of biosurfactants by pure bacteria selected during previous research on the biodegradation of crude oil. These hydrocarbonoclastic bacteria, isolated from the sediments of the Soummam wadi, will be analyzed to characterize the biological and chemical properties of their biosurfactants. Biosurfactants (BS) were produced in the microbiology laboratory of the Akli Mohand Oulhadj faculty in Bouira, and their properties were analyzed in the laboratories of the Centre de Recherche et de Development (CRD) in Boumerdes.

SONATRACH, the flagship of the African oil industry since its creation on 31 December 1963, masters the entire hydrocarbon value chain, from exploration to marketing. Its own research laboratories, the Research and Development Centre (CRD), enable it to meet the technological challenges linked to the exploitation and processing of oil and gas resources, thus guaranteeing rigorous management of these national resources.

III.1. Materials

The strain studied was extracted from contaminated sediments of the wadi Soummam and stored on sloping nutrient agars.

Appendix 01 summarizes the equipment, equipment, glassware and reagents, while Appendix 02 presents the different culture media used in this study.

III.2. Methods

III.2.1. Revivification of the strain

The bacterial strain, which was stored in slanted tubes of nutrient agar, was revived using a solid-liquid plating technique in tubes containing nutrient broth. Subsequently, the tubes were placed in an incubator set at a temperature of 30°C for a duration of 24 hours. Petri dishes containing nutrient agar (NA) were then subjected to liquid-solid plating. The plates were placed in a controlled environment at a temperature of 30°C for a duration of 24 hours. NA medium enables the strain to be activated at origin, so that it can then be transferred to other cultural environments (Fonseca et al., 2007).

III.2.2. Verification of the purity of the strain

The nutrient agar streaking method was used to assess the purity of the bacterial strain. Using a Pasteur pipette, a few bacterial colonies were picked from the previously revived strain, then these colonies were plated with tight streaks on the surface of the nutrient agar, and the plates were incubated at 37°C for 24 hours, repeated several times until identical colonies were obtained on the same plate (Igbonekwu et al., 2014).

III.2.3. Preparation of the medium for the manufacture of biosurfactants

MSMG medium was prepared using a 500 ml Erlenmeyer flask containing 250 ml MSM base medium (Appendix 2). 5 g of glucose (2% as carbon source) was added to shake, filter and adjust its pH to 7.2±0.2, and then sterilized by autoclaving at 121.3°C for 21 minutes (Yalaoui-Guellal et al., 2018).

III.2.4. Biosurfactant production

A Batch type of submerged fermentation was used to produce biosurfactants, using a minimum mineral medium enriched with glucose (MSMG or MG). Faced with a Bunsen burner flame, colonies with similar morphologies were removed from Petri dishes containing the strain, then transferred to test tubes containing 9 ml of sterile physiological water, then vigorously vortexed until the mixture was homogeneous.

The fermentation process involved adding 1 ml of strain solution to a 500 ml Erlenmeyer flask containing 250 ml of MSM base medium. The flask was then placed in an incubator at a temperature of 30°C and a speed of 150 rpm for a duration of 7 days. After incubation, samples were centrifuged at a speed of 5000 rpm per minute for 30 minutes at a temperature of 4°C. Thanks to this centrifugation, the samples were divided into two different phases. The biosurfactants are present in a supernatant and the bacterial biomass is represented by a pellet (Yalaoui-Guellal et al., 2020).

III.2.4.1. Emulation test (E24)

This method involves combining the biosurfactant solution or culture supernatant with a hydrophobic substrate such as petroleum. The presence of biosurfactants is revealed by the formation of an emulsified layer (Walter et al., 2010).

In a test tube, equivalent amounts of supernatant and oil are mixed and stirred at the maximum vortex speed for five minutes.

After resting the tubes for 24 hours at room temperature, the emulsification index E24 is determined using the following formula:

$$E24 = (He / Ht) * 100$$

The coefficient E24 corresponds to the emulsion index after 24 hours, where *He* corresponds to the vertical dimension of the emulsion layer and *Ht* corresponds to the overall vertical dimension of the liquid (Emtiazi et al., 2009). The emulsions created by the isolates were compared with those created by a solution of synthetic surfactant, sodium dodecyl sulfate (SDS), in distilled water (Yalaoui-Guellal et al., 2018).

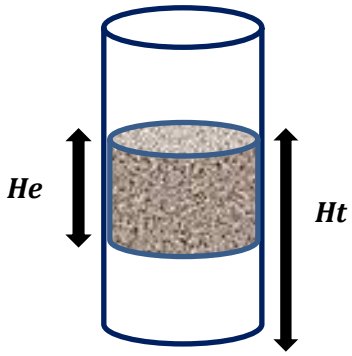

Figure 09 : Measurement of the emulsification index.

III.2.4.2. Biosurfactant extraction

Biosurfactants were obtained from the supernatants using the acid precipitation technique. The liquid portion (supernatant) was made acidic by altering its pH to a range of 2 to 2.5 using hydrochloric acid (HCl 6 N). It was then kept at a temperature of 4°C overnight to allow precipitation of the biosurfactants. The precipitate was then recovered by centrifuging at 5000 rpm for 30 minutes. After centrifugation, the precipitates were separated and placed in

glass Petri dishes, the initial weight (P0) of which had been measured beforehand (Figure 10). The precipitate was then frozen at -20°C and freeze-dried (Cazals, 2020).

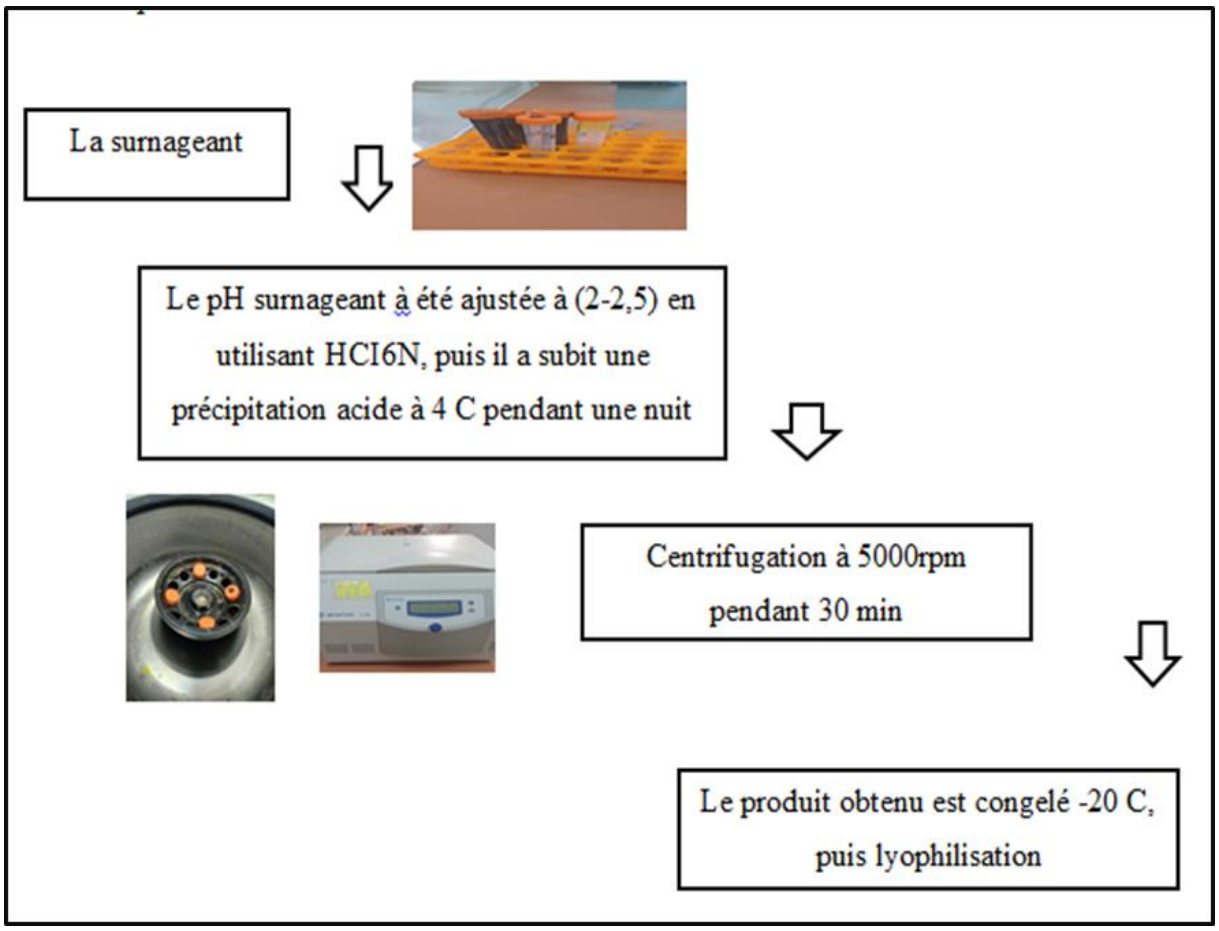

Figure 10 : Biosurfactant extraction (Original photos).

After the freeze-drying process, the following formula was applied to calculate the weight of the dry raw biosurfactants:

$$\mathbf{P_{BS}} = \frac{Pl - P0}{V}$$

PBS corresponds to the dry weight of the biosurfactants, expressed in mg/ml, Pl corresponds to the weight of the plates after freeze-drying, P0 corresponds to the initial weight of the empty plates, and V corresponds to the volume of debris (Yalaoui-Guellal et al., 2020).

The biosurfactants are now converted into a dry powder and carefully transported to pre-labeled Eppendorf tubes for preservation from any exposure to moisture.

III.2.5. Fourier transform infrared spectroscopy Analysis

Infrared absorption spectroscopy is a sensitive and reliable spectroscopic method, generally used in biology, based on the adsorption of infrared radiation by the material being analysed. It makes it possible to directly observe the characteristic frequencies of the modes of vibration of a molecule irradiated by an electromagnetic wave with a frequency in the infrared range, with a wavelength of between 2.5 μm and 25 μm (Hellal, 2014).

Scientists have used Fourier transform infrared (FTIR) spectroscopy to detect and examine the functional groups and chemical bonds found in the physiologically active portion of the biosurfactant, allowing its chemical nature to be determined (Das et al., 2008).

a. FTIR-RTA spectroscopy of biosurfactants without KBr

Infrared spectroscopy equipment analysis without KBr is carried out. A small quantity of powder is placed on the surface of the diamond and then pressed down using a support. Before analyzing the sample, it is essential to remove white elements such as CO_2 and H_2O from the apparatus (Gennet and Ley, 2004).

The instrument (figure 11) is adjusted to produce a vibration of 50 Hertz to improve peak resolution, then as soon as the spectrum is obtained, ATR (attenuated total reflection) corrections are applied to improve the sharpness of the peaks, and if CO_2 and H_2O are present, the ‘reduction’ option is chosen to obtain the final spectrum in a range of 400 to 4000 cm^{-1} (Gennet and Ley, 2004).

Figure 11 : Fourier Transform Infrared Spectrophotometer (FT/IR-4200) (Original photo).

b. FTIR-RTA spectroscopy of biosurfactants with KBr

The spectrometer was utilized to conduct FTIR analysis using KBr as a medium. The sample used for analysis consisted of biosurfactants dispersed in KBr pellets (Figure 12), which were subsequently freeze-dried with the active fraction. To prepare the sample, a 0.2 mg portion of biosurfactants was mixed with 90 mg of KBr to create a finely powdered mixture. This powder was then compressed using a hydraulic press to obtain a translucent pellet, which was subjected to analysis using the FTIR system (IRaffinity-1, SHIMADZU). The spectrum is acquired within the wavelength range of 400 to 4000 cm^{-1}(Yalaoui-Guellal, 2017).

The FTIR approach is utilized to identify the spectrum and compare it with a reference biosurfactant (surfactin) in order to verify the chemical nature of the biosurfactant extract (Das et al., 2008).

Figure 12 : Fourier Transform Infrared Spectrophotometer (Original photo).

III.2.6. Measurement of the surface tension

The easiest and most suitable screening technique to detect microbes that produce biosurfactants is to directly measure the surface tension of the culture substrate (Lin, 1996).

The surface tension of dilutions of biosurfactant is measured using the hanging drop technique (Dupeyrat, 1989). This technique (figure 13) involves forming a drop of biosurfactant solution at the end of a small-diameter needle (Kahnetics, internal Ø: 0.483 mm; external Ø: 0.711 mm). By analyzing the shape of the drop before it breaks, the surface tension can be measured using the FTA200 goniometer (First Ten Angstroms, VA, USA).

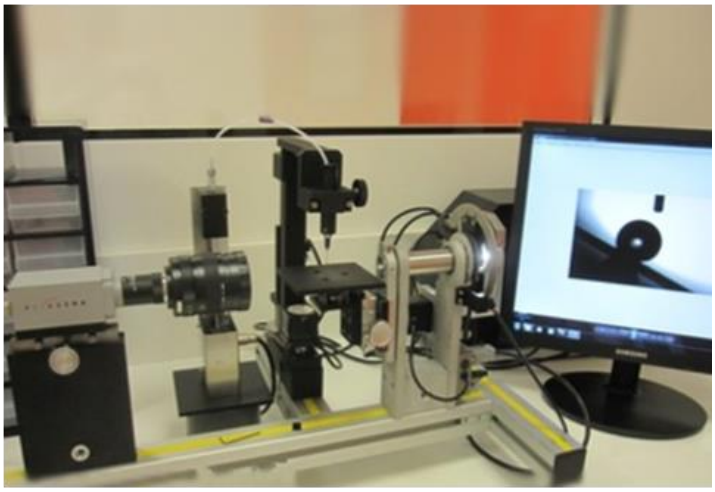

Figure 13 : Device for measuring surface tension (Original photo).

III.2.7. Characterization by GC-MS

The most accurate method for detecting and measuring high molecular weight lipopeptides and biosurfactants is gas chromatography combined with mass spectrometry (GC/MS). It is, therefore, essential to analyze the lipid fraction of biosurfactants in order to elucidate their detailed structure, in particular to identify fatty acid structures. The hydrolytic cleavage of the link between the carbohydrate or peptide/protein part of the biosurfactants and their lipid parts is carried out by GC/MS, which allows the derivatization of the fatty acid chains resulting in fatty acid methyl esters (FAME) and convert them into trimethylsilyl derivatives (TMS) (Smyth et al., 2010).

Different columns were used for GC/MS analysis, such as the Hp 5Ms capillary column (length 30 m, internal diameter 0.25 mm, film thickness 0.25μm). GC-MS analyses can be performed with different columns (Yakimov et al., 1995).

The protocol of the GC/MS technique is as follows (Vater et al., 2002) :

- 5 mg of purified biosurfactant is dissolved in a 0.95 ml solution of methanol and 0.05 ml of sulphuric acid in a sealed tube. Heat it to 90°C for 15 hours, then evaporate the solvents and recover the residue with 1 ml of hexane.

- After that, wash with 1 ml of water and evaporate the hexane to obtain an extract containing the FAME.

- The experimental parameters employed are as follows: the injector temperature is set to 250°C, the oven program begins at 50°C and is maintained for 1 minute, then the temperature is increased at a rate of 25°C per minute until it reaches 175°C, after which it is increased at a rate of 4°C per minute until it reaches 230°C, and finally held at that temperature for 5 minutes.

- The characteristics of mass spectrometry include an electronic energy of 70 eV, a scanning range from 50 to 650 Da, and an injection volume of 1 μL. Additionally, the retention periods of fatty acid methyl esters increase with both chain length and degree of separation (Figure 14).

Figure 14 : Gas chromatography coupled with GC-MS mass spectrometry (Original photo).

Chapter IV

IV. Results and discussion

IV.1. Revivification and control of strain purity

The characteristic colonies of the strain can be observed after their recovery and incubation in the Na medium. The strain is pure thanks to the homogeneity of the characteristics of the Petri dish colonies.

IV.2. Emulsification test (E24)

After a full day, the biosurfactant was evaluated for its ability to form emulsions using the emulsification method (Cooper et Goldenberg, 1987).

According to the results of the emulsification test, it was observed that in the MSMG medium, the average value of E24 was 58.88 ± 1.478%, this result is better than obtained by the standards chemical surfactant SDS (50,23± 1.13%). It seems that this observation indicates that biosurfactants manufactured in the MSMG medium have the ability to emulsify biphasic solutions. (hydrophobic-hydrophilic). The emulsifying capacity of bacterial strains is an essential part of the biodegradation of crude oil in water. Thanks to this ability, the bioavailability of the oil is improved, resulting in an increase in the rate of biodegradation. Thus, these bacteria can be essential to eliminate sites polluted by hydrophobic substances such as hydrocarbons (Bredholt et al., 1998 ; Quek et al., 2006 ; Mnif et al., 2011).

The studied strain yielded a significant yield of freeze-dried biosurfactants, which was 5.15 ± 0.12 g/L after extraction by acid precipitation (HCl 6N) and after freeze-drying. However, this is a fairly significant amount compared to that observed by several authors (Kuyukina et Ivshina, 2010 ; Maalej et al., 2016 ; Yalaoui-Guellal et al., 2021).

IV.3. FTIR-RTA spectroscopy analysis

The analysis of the FTIR absorption spectra with and without KBr allowed use to identify all the absorption bands corresponding to the different chemical groups present in the biosurfactant, and thus to predict the constituents concerned.

Figure 15 represents the spectrum of the biosurfactant obtained by infrared spectroscopy with KBr between 400 and 4000 cm^{-1}.

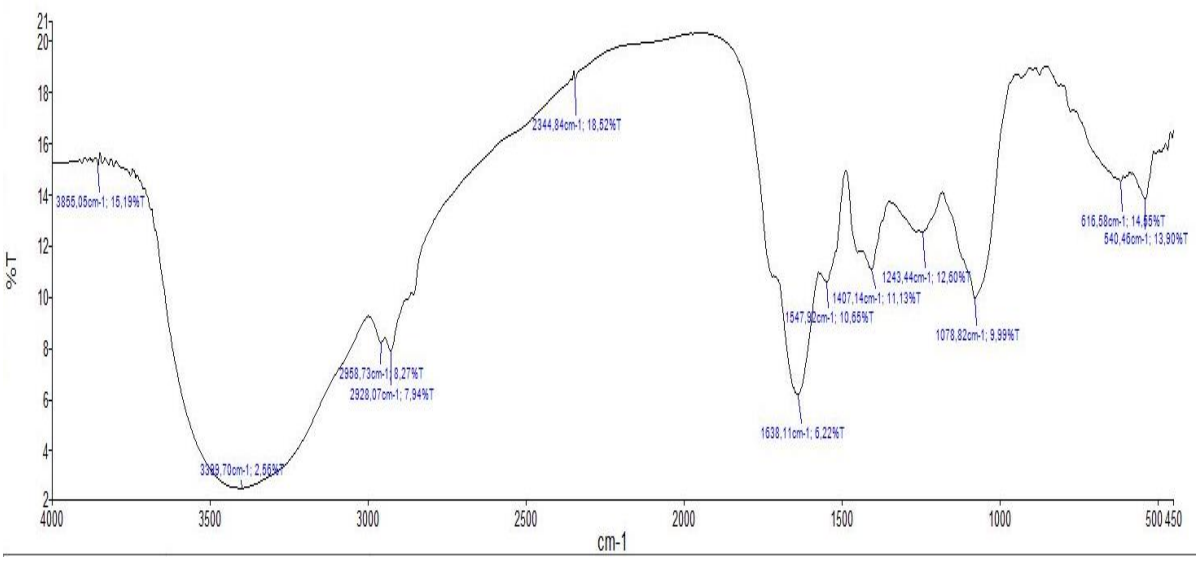

Figure 15 : Biosurfactant spectrum obtained by FTIR with KBr.

The results obtained in the graphic representation of the biosurfactant spectrum indicate the following characteristic bands (Gaffney et al., 2002 ; Collet, 2016) :

- The observation of three specific peaks at 3389 cm^{-1}, 3855 cm^{-1} and 1407 cm^{-1} in the spectrum confirms the presence of a hydroxyl group (OH) linked to alcohol or phenol structures.

- The presence of CH_2, CH_3 and CH groups in alkanes influences the peak position at 2958 cm^{-1} observed in the spectrum.

- The observation of a specific band at 1638 cm-1 in the spectrum confirms the presence of a carbonyl group (C=O) in carboxylic acid molecules or their derivatives.

- Infrared spectrum analysis reveals a characteristic peak at 1243 cm^{-1}, which is a distinctive signature of the presence of C-O bonds in alcohol or phenol structures.

- A specific peak at 1078 cm^{-1} confirms the presence of O-C groups in carboxylic acid molecules or their derivatives.

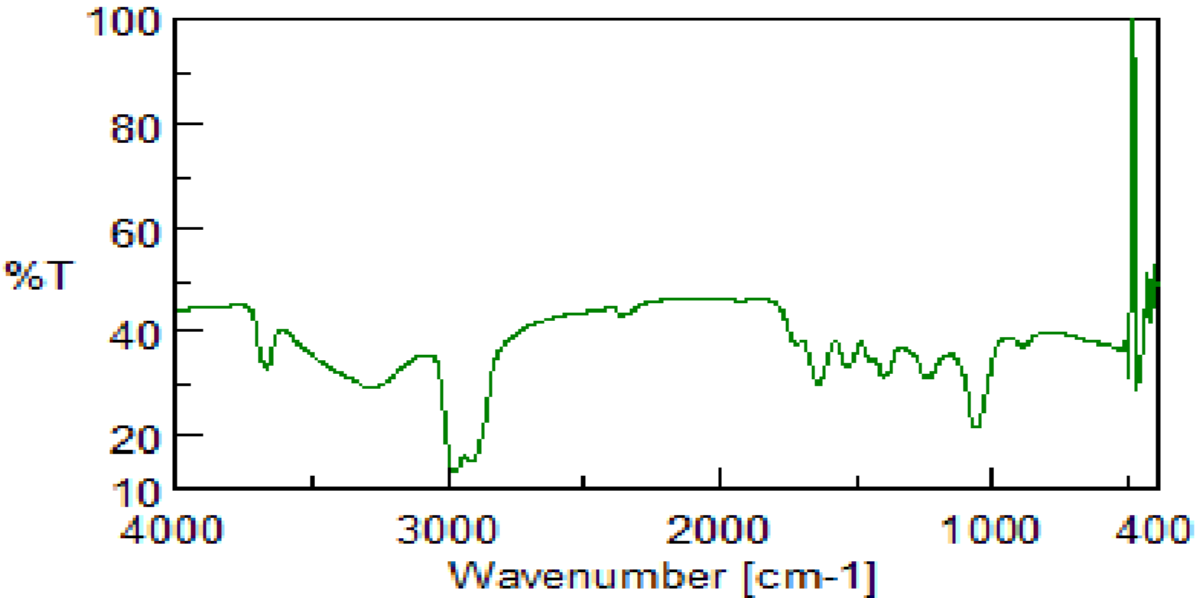

Figure 16 : FTIR spectrum without KBr of the biosurfactant studied.

Figure 16 shows the results obtained from the biosurfactant analyzed by Infrared spectroscopy without KBr between 400 and 4000 cm^{-1}.

The analysis of these results indicates (Gaffney et al., 2002 ; Collet, 2016) :

- Three peaks at 3669 cm^{-1}, 3320 cm^{-1} and 1401 cm^{-1} correspond to elongation vibrations of O-H bonds in alcohols and phenols.

- The presence of two absorption bands at 2987 cm^{-1} and 2910 cm^{-1} in the infrared spectrum confirms the presence of the CH_2, CH_3 and CH groups characteristic of alkanes.

- A vibration at 1646 cm^{-1} indicates the presence of a carbonyl group (C=O) of carboxylic acid and its derivatives.

- The elongation peak displayed at 1245 cm^{-1} is assigned to C-O bonds in alcohols and phenols.

- The observation of a specific peak at 1068 cm^{-1} in the IR spectrum indicates the presence of O-C groups in carboxylic acid molecules or their derivatives.

- The observation of a 900 cm^{-1} band in the infrared (IR) spectrum confirms the presence of a vinyl group (-$CH=CH_2$) in the analyzed molecule.

The comparison of the FTIR spectra of the two cases, with and without KBr, highlights similarities in the position of the peaks, indicating the presence of the same functional groups. However, peak intensity varies, suggesting a quantitative rather than qualitative difference in sample composition. It should be noted that peaks characteristic of a vinyl grouping are present in the spectrum without KBr, but absent in the spectrum with KBr.

These results are consistent with the conclusions of other work on biosurfactants where they observed that most biosurfactants systematically have a carboxylic or ester group in their structure (Banat, 1995 ; Ron et Rosenberg, 2002).

The use of FTIR spectroscopy to study biosurfactants revealed the simultaneous presence of aliphatic chains and peptide groups in these molecules. These results indicate the probable presence of lipopeptide biosurfactants, whose structure is comparable to that of surfactin and fengycin (Pereira et al., 2013 ; Liu et al., 2016).

IV.4. Evaluate the surface tension

On the surface of a liquid, molecules experience a lower force of attraction than those inside, which creates an imbalance and a tendency to regroup. This force of contraction, called surface tension, opposes any increase in the surface of the liquid (Holmberg, 2001). The analysis of the effect of surfactants on the surface tension of pure water allowed to determine a value of about 70 mN/m. Then, the surface tension decreases exponentially, reaching a minimum level of about 30 mN/m at a 2 mg/ml surfactant concentration.

Biosurfactants have the ability to significantly reduce the surface tension of water, bringing it into a range of values ranging from 25 to 30 mN/m (Rahman et al., 2002 ; Haba et al., 2003).

IV.5. Characterization by GC-MS

The methyl esters of the lipid part are analyzed in gas chromatography, which highlights four main peaks. According to the GC-MS analysis, it is observed that the lipid part is mainly composed of fatty acids as shown in Table 04. Identification was done using the Wiley, NBS and NIST databases.

Table 03 : Exploration of fatty acid composition of lipid parts of biosurfactants

Peak num	**1**	**2**	**3**	**4**
Retention time	17.24	16.72	14.98	17.23
Methyl ester	Acid (Z)-octadec-9-enoic	Acid (9Z,12Z)-octadeca -9,12-dienoic	Acid hexadecanoic	Acid (E)-octadec-9-enoic
Molecular mass (g/mol)	296	294	270	296

Figures 17, 18, 19 and 20 show the mass spectra obtained for the four methyl esters analyzed.

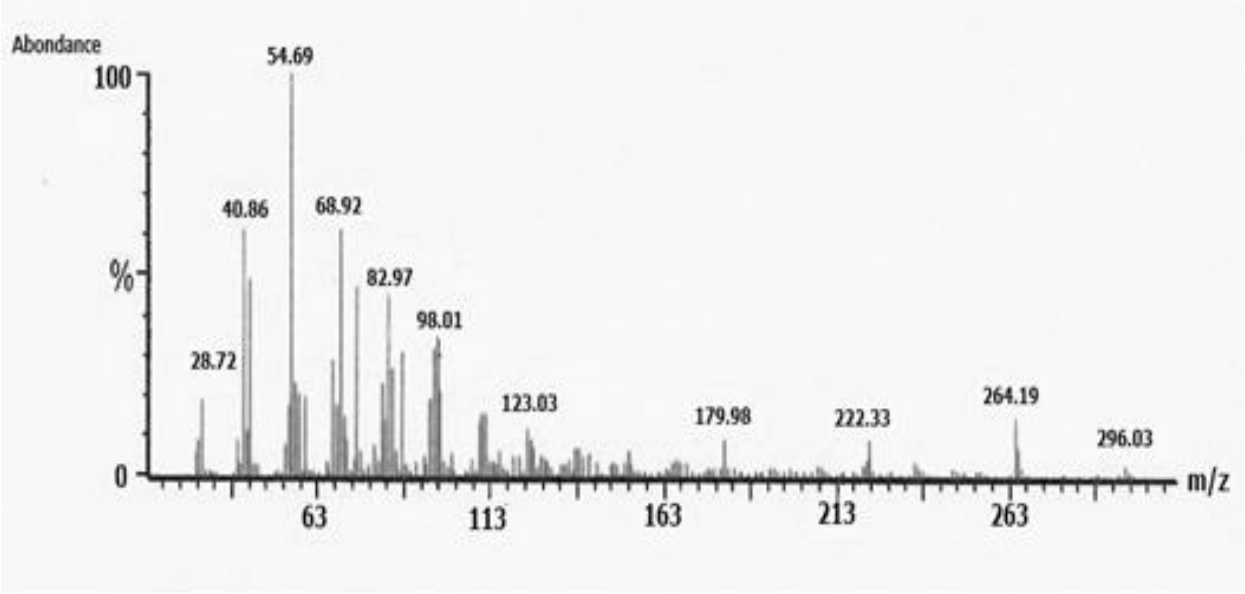

Figure 17 : Graphical representation of the fragmentation of methyl ester derived from acid (Z)-octadec9-enoic.

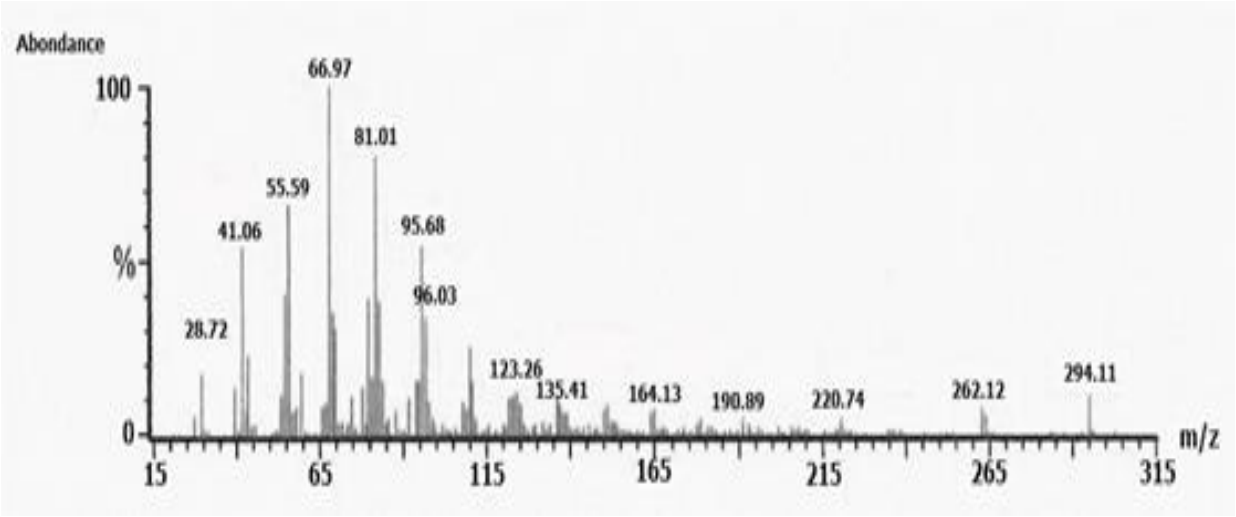

Figure 18 : Graphical representation of the fragmentation of acid-derived methyl ester (9Z,12Z)-octadeca-9,12-dienoic.

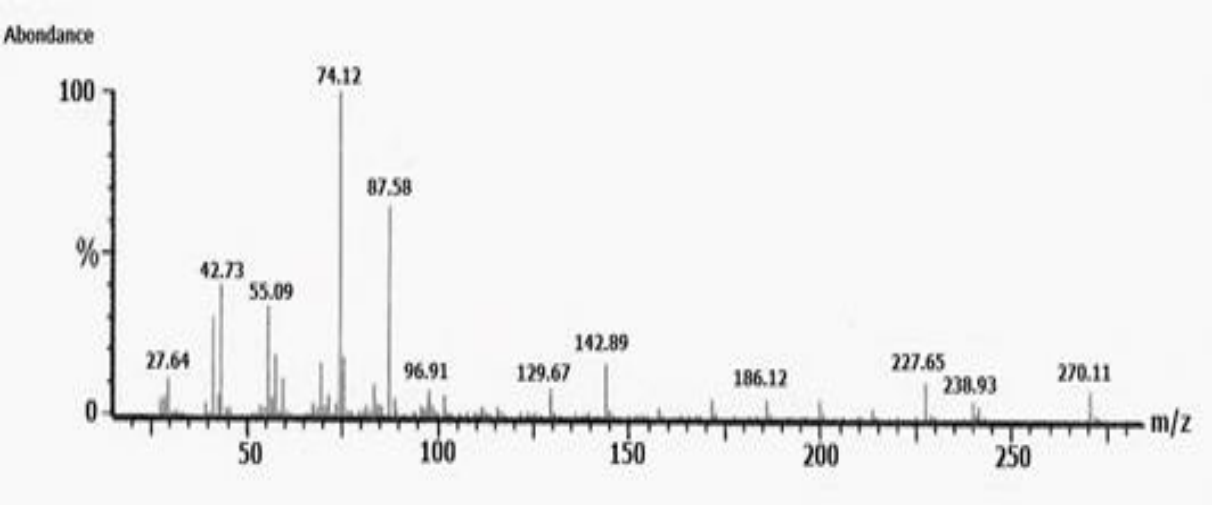

Figure 19 : Graphic mass representation of methyl ester of hexadecanoic acid.

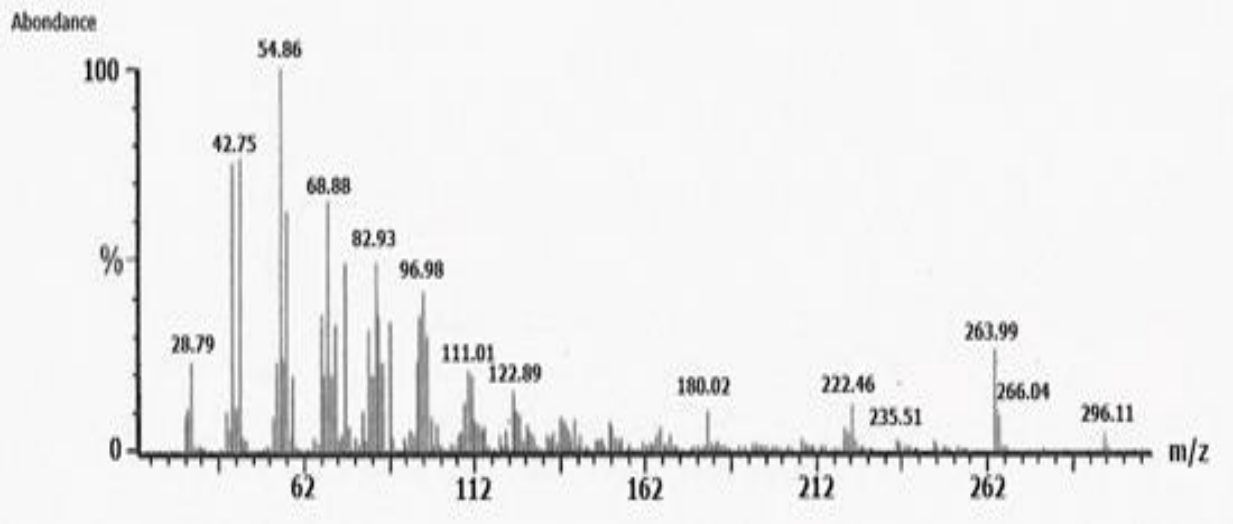

Figure 20 : Graphic mass representation of methyl ester of acid (E)-octadec-9-enoic.

There are the M+ fragments, which are the molecular ions of each peak in Table 04, as well as the [M-31]+ fragments, which are characteristic of methyl esters, in all the spectra shown in Figures 17, 18, 19, 20 as well as the fragments at m/z 74 which would be the result of a rearrangement McLafferty.

Each C-C bond is cleaved to form alkyl ions and an oxygen-containing ion has the formula $C_nH_{2n-1}O_2^+$. Table 04 shows the four compounds that have mass spectra that clearly indicate that they are fatty acid methyl esters. Thus, the lipid part is mainly composed of fatty acids in C16 (saturated) and C18 (mono and di-unsaturated). On prend l'exemple de l'hexadécanoate de méthyle

- Molecular ion M+ is at m/z 270
- The acylium ion $[CH_3\text{-}(CH_2)_{14}\text{-}C \equiv O]^+$ is at m/z 239.
- The ion from a McLafferty rearrangement is obtained at m/z 74
- Fragments at m/z 29, 43, 57, 87, 143 and 227 are also present.

According to Table 04 and comparing with the composition of the biosurfactant produced by the isolate of the strain *Natrinema*, which is composed of a benzene nucleus and a mixture of acids (palmitic acid 256,42g/mol, stearic acid 284,47g/mol and linoleic acid 280,44g/mol), the results are indicated, it is found that each biosurfactant whatever the producing strain has a different composition (Kiemla et al., 2016 ; Ariech, 2018).

The four compounds in Table 04 have mass spectra that clearly indicate that they are fatty acid methyl esters. Thus, the lipid part is mainly composed of fatty acids in C16 (saturated) and C18 (mono and di-unsaturated).

Conclusion

Biosurfactants are compounds with surfactant properties that are produced by microorganisms as byproducts of their metabolism. In this work, the objective is to study the physicochemical characteristics of biosurfactants produced from hydrocarbonoclast bacteria by performing different tests.

The first part of the study focused on a general bibliographic synthesis of the classification of biosurfactants, their potential and characteristics allow use to deepen our understanding of their nature, their structure, in particular surface and interfacial tension, and their various fields of application.

The second part focuses on the production of biosurfactants. Thus their physicochemical profile using different techniques such as surface tension, emulsification index, Fourier transform infrared spectroscopy and gas chromatography mass-spectrometry.

The results obtained highlighted the properties of biosurfactants obtained by hydrocarbonoclast strain:

- The studied strain gave a significant yield of freeze-dried biosurfactants, which was valued at 5, 15 g/L.

- An ability to emulsify hydrophobic-hydrophilic biphasic solutions with a value of E24 which was 53.88±4.479%.

- The lyophilizates of biosurfactants have a lower surface tension (30 mN/m) than that of water (70 mN/m), indicating their ability to decrease the surface tension.

- The results of Fourier's infrared transform spectroscopy revealed that biosurfactants are lipopeptide types, like surfactin and fengycin.

- Characterization by CG-MS shows that the lipid part is composed of fatty acids.

Following the results obtained, it is possible to complete this study by using more in-depth methods, such as:

- Molecular analysis of the strain analyzed using 16S RNA sequencing and assessment of the biological profile of its biosurfactants produced;

Production of biosurfactants at the semi-pilot scale by mastering all the factors influencing this production by optimization software;

- Atomic Absorption Spectrometry (AAS) analysis assesses the presence of metallic elements (alkaline, alkaline-earthy, transition metals) and metalloids in a sample.

- The combination of tandem mass spectrometry and liquid chromatography (LC-MS/MS) analysis allows the identification and quantification of the components of a mixture from the chloroform-methanol column chromatographic fraction.

-Analysis by mass spectrometry type MALDI-TOF for biochemical and molecular identification.

References

A

Abalos, A., Pinazo, A., Infante, M. R., Casals, M., Garcia, F., & Manresa, A. (2001). Physicochemical and antimicrobial properties of new rhamnolipids produced by *Pseudomonas aeruginosa* AT10 from soybean oil refinery wastes. *Langmuir*, 17(5), 1367-1371.

Abu-Ruwaida, A.S., Banat, I .M., Haditirto, S. & Khamis, A. (1991) Nutritional requirements and growth characteristics of a biosurfactant-producing *Rhodococcus* bacterium. *World. J. Microbiol. Biotechnol. 7: 53-61.*

Adesra, A., Srivastava, V. K., & Varjani, S. (2021). Valorization of dairy wastes: integrative approaches for value added products. *indian Journal of Microbiology*, 61(3), 270-278.

Andrieu, S., & Müller, P. (2005). Les surfaces solides: concepts et méthodes.

Arima, K., Kakinuma, A., & Tamura, G. (1968). Surfactin, a crystalline peptidelipid surfactant produced by *Bacillus subtilis*: Isolation, characterization and its inhibition of fibrin clot formation. *Biochemical and biophysical research communications*, 31(3), 488-494.

Arino, S., Marchal, R., & Vandecasteele, J. P. (1996). Identification and production of a rhamnolipidic biosurfactant by a *Pseudomonas* species. *Applied Microbiology and Biotechnology*, 45, 162-168.

Asmer, H.-J., Lang, S., Wagner, F., & Wray, V. (1988). Microbial production, structure elucidation and bioconversion of sophorose lipids. *Journal of the American Oil Chemists' Society,* 65, 1460-1466.

Austad, T., & Taugbøl, K. (1995). Chemical flooding of oil reservoirs 2. Dissociative surfactant-polymer interaction with a negative effect on oil recovery. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 103(1-2), 73-81.

B

Bages-Estopa, S., White, D., Winterburn, J., Webb, C., & Martin, P. (2018). Production and separation of a trehalolipid biosurfactant. *Biochemical Engineering Journal*, 139, 85-94.

Bai, G., Brusseau, M. L., & Miller, R. M. (1997). Biosurfactant-enhanced removal of residual hydrocarbon from soil. *Journal of Contaminant Hydrology*, 25(1-2), 157-170.

Bai, G., Brusseau, M. L., & Miller, R. M. (1998). Influence of cation type, ionic strength, and pH on solubilization and mobilization of residual hydrocarbon by a biosurfactant. *Journal of Contaminant Hydrology,* 30(3-4), 265-279.

Banat, I. M. (1995). Biosurfactants production and possible uses in microbial enhanced oil recovery and oil pollution remediation: a review. *Bioresource technology*, 51(1), 112.

Banat, I. M., ., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M. G., Fracchia, L., ... & Marchant, R. (2010). Microbial biosurfactants production, applications and future potential. *Applied microbiology and biotechnology*, 87, 427-444.

Banat, I. M., Makkar, R. S., & Cameotra, S. S. (2000). Potential commercial applications of microbial surfactants. Applied Microbiology and Biotechnology, 53, 495-508.

BERANGER, G., & MAZILLE, H. (2005). Approche scientifique des surfaces. Caractérisation et propriétés. Techniques de l'ingénieur. Matériaux métalliques, (M1425).

Berheinmer, A.W., Avigad, L.S. 1970. Nature and properties of a cytological agent produced by *Bacillus subtilis*. J. *Gen. Microbiol*. 61:361-369.

Bessson, F., Peypoux, F., Michel, G.,Delcambe, L. 1978. Identification of antibiotics of irutin group in various strains of *bacillus subtilis*. *J. Antibiot. Tokyo*. 31:283-288.

Bredholt, H., Josefsen, K., Vatland, A., Bruheim, P., & Eimhjellen, K. (1998). Emulsification of crude oil by an alkane-oxidizing *Rhodococcus* species isolated from seawater. *Canadian journal of microbiology*, 44(4), 330-340.

Briant, J., & Ténèbre, L. (1989). Généralités sur les phénomènes de surface. Phénomènes d'interface œ Agents de surface œ Principes et modes d'action, J. Briant, Ed. Technip, Paris.1-57.

C

Cameotra, S. S., & Makkar, R. S. (1998). Synthesis of biosurfactants in extreme conditions. *Applied microbiology and Biotechnology*, 50, 520-529.

Cameotra, S. S., Makkar, R. S., Kaur, J., & Mehta, S. K. (2010). Synthesis of biosurfactants and their advantages to microorganisms and mankind. Biosurfactants, 261-280.

Cappello, S., Crisari, A., Denaro, R., Crescenzi, F., Porcelli, F., & Yakimov, M. M. (2011). Biodegradation of a bioemulsificant exopolysaccharide (EPS 2003) by marine bacteria. *Water, Air, & Soil Pollution*, 214, 645-652.

Cattoir, V., Denis, F., Martin, C., Ploy, M.-C., & Poyart, C. (2016). Bactériologie médicale: techniques usuelles: Elsevier Health Sciences.

Cazals, F. (2020). Combinaison du lavage par biosurfactant et de la biodégradation

Cha, M., Lee, N., Kim, M., Kim, M., & Lee, S. (2008). Heterologous production of *Pseudomonas aeruginosa* EMS1 biosurfactant in *Pseudomonas putida*. *Bioresource Technology*, 99(7), 2192-2199.

Chambial, S., Dwivedi, S., Shukla, K. K., John, P. J., & Sharma, P. (2013). Vitamin C in disease prevention and cure: an overview. *Indian journal of clinical biochemistry*, 28, 314-328.

Champion, J. T., Gilkey, J. C., Lamparski, H., Retterer, J., & Miller, R. M. (1995). Electron microscopy of rhamnolipid (biosurfactant) morphology: effects of pH, cadmium, and octadecane. *Journal of Colloid and Interface Science*, 170(2), 569-574.

Christofi, N., & Ivshina, I. B. (2002). Microbial surfactants and their use in field studies of soil remediation. *Journal of Applied Microbiology*, 93(6), 915-929.

Chtioui, O. (2011). Production sélective de lipopeptides par *Bacillus subtilis* en bioréacteur disques tournants (Doctoral dissertation, Lille 1).

Collet, T. (2016). La spectroscopie infrarouge. Photoniques (82), 39-42.

Cooper, D. G., & Goldenberg, B. G. (1987). Surface-active agents from two *Bacillus* species. *Applied and environmental microbiology*, 53(2), 224-229.

𝒟

Dagbert, C., Meylheuc, T., & Bellon-Fontaine, M. N. (2006). Corrosion behaviour of AISI 304 stainless steel in presence of a biosurfactant produced by *Pseudomonas fluorescens*. *Electrochimica Acta*, 51(24), 5221-5227.

Das, P., Mukherjee, S., & Sen, R. (2008). Antimicrobial potential of a lipopeptide biosurfactant derived from a marine *Bacillus circulans*. *Journal of Applied Microbiology*, 104(6), 1675-1684.

Derguine-Mecheri, L. (2019). Production et caractérisation des biosurfactants par des levures et des moisissures. Thèse de doctorat. Universite Mouloud Mammeri Tizi-Ouzou. Algerie.167 p.

Desai, J. D., & Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. *Microbiology and Molecular biology reviews*, 61(1), 47-64.

Déziel, É., Paquette, G., Villemur, R., Lepine, F., & Bisaillon, J. (1996). Biosurfactant production by a soil *Pseudomonas* strain growing on polycyclic aromatic hydrocarbons. *Applied and environmental microbiology*, 62(6), 1908-1912.

Dupeyrat, M. 1989. Méthode de mesure de la tension superficielle ou interfaciale. In Technip et Institut francais du Pétrole (ed.) Phénoménes d'interfaces agents de surface, Briant. 39-69.

Dyke, M. V., Couture, P., Brauer, M., Lee, H., & Trevors, J. T. (1993). *Pseudomonas aeruginosa* UG2 rhamnolipid biosurfactants: structural characterization and their use in removing hydrophobic compounds from soil. *Canadian Journal of Microbiology*, 39(11), 1071-1078. dynamisée, pour la dépollution des eaux souterraines de sites contaminés par des HAP. Université Paris-Est.

Dzięgielewska, E., & Adamczak, M. (2013). Evaluation of waste products in the synthesis of surfactants by yeasts. *Chemical Papers*, 67, 1113-1122.

E

Eddouaouda, K. (2012). Etude du potentiel biodegradatif de souches isolées du sol de Hassi Messaoud contamine par du pétrole

Edwards, D. A., Luthy, R. G., & Liu, Z. (1991). Solubilization of polycyclic aromatic hydrocarbons in micellar nonionic surfactant solutions. Environmental Science & Technology, 25(1), 127-133.

Emtiazi, G., Saleh, T., & Hassanshahian, M. (2009). The effect of bacterial glutathione S-transferase on morpholine degradation. *Biotechnology journal*, 4(2), 202-205.

F

Ferradji, F. Z. (2014). Etude des voies metaboliques de la biodegradabilite de l'hydrocarbure en vue d'une application d'un procede de biodepollution des eaux de mer (Doctoral dissertation, Blida).

Fessl S. biosurfactants- effective fat solvents made by bacteria. BIOPRO Baden-Wurttemberg Gmbh. 2015.

Fonseca, R., Silva, A., De França, F., Cardoso, V., & Sérvulo, E. (2007). Optimizing carbon/nitrogen ratio for biosurfactant production by a *Bacillus subtilis* strain. Paper presented at the Applied Biochemistry and Biotecnology: The Twenty-Eighth Symposium Proceedings of the Twenty-Eight Symposium on Biotechnology for Fuels and Chemicals Held April 30–May 3, 2006, in Nashville, Tennessee.

G

Gabet, S. (2004). Remobilisation d'Hydrocarbures Aromatiques Polycycliques (HAP) présents dans les sols contaminés à l'aide d'un tensioactif d'origine biologique (Doctoral dissertation, Limoges).

Gaffney, J. S., Marley, N. A., & Jones, D. E. (2002). Fourier transform infrared (FTIR) spectroscopy. Characterization of materials, 1-33.

GAKPE E., PATTANATHU K. S. M. R., et HATHA M. A.A., 2007 – Microbial Biosurfactants . *J.Mar. Atmos. Res*, 3(2): 1-17.

Gaur, V. K., Regar, R. K., Dhiman, N., Gautam, K., Srivastava, J. K., Patnaik, S., . . . Manickam, N. (2019). Biosynthesis and characterization of sophorolipid biosurfactant by Candida spp.: application as food emulsifier and antibacterial agent. *Bioresource technology*, 285, 121314.

Gennet, D., & Ley, C. (2004). Introduction à la spectroscopie Infrarouge (I.R.). Consulté le 07 mai 2024, à l'adresse https://culturesciences.chimie.ens.fr/thematiques/chimie-analytique/spectroscopies/introduction-a-la-spectroscopie-infrarouge-ir.

Geudens, N., Nasir, M. N., Crowet, J. M., Raaijmakers, J. M., Fehér, K., Coenye, T., ... & Deleu, M. (2017). Membrane interactions of natural cyclic lipodepsipeptides of the viscosin group. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1859(3), 331-339.

Gharaei-Fathabad, E. (2011). Biosurfactants in pharmaceutical industry: a mini-review. *American Journal of Drug Discovery and Development,* 1(1), 58-69.

Grangemard, I., Wallach, J., Maget-Dana, R., & Peypoux, F. (2001). Lichenysin: a more efficient cation chelator than surfactin. *Applied biochemistry and biotechnology*, 90, 199-210.

Guerra-Santos, L. H., Käppeli, O., & Fiechter, A. (1986). Dependence of *Pseudomonas aeruginosa* continous culture biosurfactant production on nutritional and environmental factors. *Applied Microbiology and Biotechnology*, 24, 443-448.

H

Haba, E., Pinazo, A., Jauregui, O., Espuny, M. J., Infante, M. R., & Manresa, A. (2003). Physicochemical characterization and antimicrobial properties of rhamnolipids produced by *Pseudomonas aeruginosa* 47T2 NCBIM 40044. *Biotechnology and bioengineering*, 81(3), 316-322.

Haigh, S. D. (1996). A review of the interaction of surfactants with organic contaminants in soil. *Science of the Total Environment*, 185(1-3), 161-170.

Haque, E., & Hassan, S. (2020). Physiochemical Characterization and Anti-colon Cancer Activity of Biosurfactant Produced from Marine *Pseudomonas sp*. *International Journal of Pharmaceutical Investigation, 10(2).*

Hausmann, R., & Syldatk, C. (2014). Types and classification of microbial surfactants. Biosurfactants: production and utilization-processes, technologies, and economics, 159, 1.

Healy, M. G., Devine, C. M., & Murphy, R. (1996). Microbial production of biosurfactants. *Resources, conservation and recycling*, 18(1-4), 41-57.

Holmberg, K. (2001). Natural surfactants. *Current Opinion in Colloid & Interface Science*, 6(2), 148-159.

Holmberg, K., Jönsson, B., Kronberg, B., & Lindman, B. (2002). Polymers in aqueous solution. Wiley-Blackwell.

Hommel, R., Stiiwer, O., Stuber, W., Haferburg, D., & Kleber, H. P. (1987). Production of water-soluble surface-active exolipids by *Torulopsis apicola*. *Applied microbiology and biotechnology*, 26, 199-205.

I

Igbonekwu, C. J., Osonwa, U. E., Umeyor, C. E., Ikegbunam, M. N., Uronnachi, E. M., & Esimone, C. O. (2014). Biosurfactant produced by novel halo-thermophilic bacterial strain from unconventional substrates. *Int J Pharmacogn*, 1(1), 73-81.

Irfan-Maqsood, M., & Seddiq-Sha0ms, M. (2014). Rhamnolipids: well-characterized glycolipids with potential broad applicability as biosurfactants. *Industrial biotechnology*, 10(4), 285-291.

J

Janek, T., Mirończuk, A. M., Rymowicz, W., & Dobrowolski, A. (2020). High-yield expression of extracellular lipase from *Yarrowia lipolytica* and its interactions with lipopeptide biosurfactants: a biophysical approach. *Archives of Biochemistry and Biophysics*, 689, 108475.

Johnson J.L., Phelips S.L., Cummins C.S., London J., Gasser F. (1980). Taxonomie of *Lactobacillus acidophillus* group, *international journal of systematic Bacteriology*, 30 :53-68.

K

Kaloorazi, N. A., & Choobari, M. F. S. (2013). Biosurfactants: Properties and applications. *J Biol Today's World,* 2, 235-41.

Käppeli, O., & Finnerty, W. R. (1979). Partition of alkane by an extracellular vesicle derived from hexadecane-grown *Acinetobacter*. *Journal of bacteriology*, 140(2), 707-712.

Karanth, N. G. K., Deo, P. G., & Veenanadig, N. K. (1999). Microbial production of biosurfactants and their importance. *Current Science*, 116-126.

Kashif, A., Rehman, R., Fuwad, A., Shahid, M. K., Dayarathne, H. N. P., Jamal, A., ... & Choi, Y. (2022). Current advances in the classification, production, properties and applications of microbial biosurfactants–A critical review. *Advances in Colloid and Interface science*, 306, 102718.

Kedidi A. Effets de la salinité sur la stabilité des biosurfactants produits par des souches bactériennes telluriques en présence du gasoil. Thèse MASTER. Université kasdi merbah Ouargla- faculté des sciences de la nature et de la vie département des sciences biologiques : spécialité microbiologie appliqués. 2014. p 68.

Kee, S. H., Chiongson, J. B. V., Saludes, J. P., Vigneswari, S., Ramakrishna, S., & Bhubalan, K. (2021). Bioconversion of agro-industry sourced biowaste into biomaterials via microbial factories–A viable domain of circular economy. *Environmental Pollution*, 271, 116311.

Kiemle, D. J., Silverstein, R. M., & Webster, F. X. (2016). Identification spectrométrique de composés organiques-3ème édition. De Boeck Superieur.

Kosaric, N., & Sukan, F. V. (2014). Biosurfactants: production and utilization—processes, technologies, and economics (Vol. 159): CRC press.

Kroschwitz, J.I. (1994) Encyclopedia of Chemical Technology 4th edition, John Wiley & Sons, New York.

L

Laamari, M. R., Benzakour, J., Berrekhis, F., Derja, A., & Villemin, D. (2010). Etude de l'inhibition de la corrosion du fer en milieu sulfurique 1 M par l'acide hexa méthylène diamine tétra méthyle phosphonique. Les technologies de laboratoire, 5(20).

Lang, S. (1993). Biological activities of biosurfactants. Biosufactants: production-properties applications, 48, 251-268.

Lang, S., & Wullbrandt, D. (1999). Rhamnose lipids–biosynthesis, microbial production and application potential. *Applied microbiology and biotechnology*, 51, 22-32.

Lang, S., Katsiwela, E., & Wagner, F. (1989). Antimicrobial effects of biosurfactants. *Lipid/Fett*, *91*(9), 363-366.

Lee, W., Bohra, N. C., Altschaeffl, A. G., & White, T. D. (1995). Resilient modulus of cohesive soils and the effect of freeze–thaw. *Canadian Geotechnical Journal*, *32*(4), 559-568.

Leisinger, T., & Margraff, R. (1979). Secondary metabolites of the fluorescent pseudomonads. *Microbiological Reviews*, 43(3), 422-442.

Lima, T. M., Procópio, L. C., Brandão, F. D., Carvalho, A. M., Tótola, M. R., & Borges, A. C. (2011). Biodegradability of bacterial surfactants. *Biodegradation*, 22, 585-592.

Lin, S. C. (1996). Biosurfactants: recent advances. Journal of Chemical Technology & Biotechnology: International Research in Process, *Environmental and Clean Technology*, 66(2), 109-120.

Liu, B., Liu, J., Ju, M., Li, X., & Yu, Q. (2016). Purification and characterization of biosurfactant produced by *Bacillus licheniformis* Y-1 and its application in remediation of petroleum contaminated soil. *Marine pollution bulletin*, 107(1), 4651.

M

Makkar, R. S., & Rockne, K. J. (2003). Comparison of synthetic surfactants and biosurfactants in enhancing biodegradation of polycyclic aromatic hydrocarbons. *Environmental Toxicology and Chemistry: An International Journal*, 22(10), 2280 2292.

Makkar, R. S., Cameotra, S. S., & Banat, I. M. (2011). Advances in utilization of renewable substrates for biosurfactant production. *AMB express*, 1, 1-19.

Makkar, R., & Cameotra, S. (2002). An update on the use of unconventional substrates for biosurfactant production and their new applications. *Applied microbiology and biotechnology*, 58, 428-434.

Marahiel, M. A., Danders, W., Krause, M., & Kleinkauf, H. (1979). Biological Role of Gramicidin S in Spore Functions: Studies on Gramicidin-S-Negative Mutants of *Bacillus brevis* ATCC9999. *European Journal of Biochemistry*, 99(1), 49-55.

Markande, A. R., Patel, D., & Varjani, S. (2021). A review on biosurfactants: properties, applications and current developments. *Bioresource Technology*, 330, 124963.

McCray, J. E., Bai, G., Maier, R. M., & Brusseau, M. L. (2001). Biosurfactant-enhanced solubilization of NAPL mixtures. *Journal of Contaminant Hydrology*, 48(1-2), 45-68.

McInerney, M. J., Javaheri, M., & Nagle Jr, D. P. (1990). Properties of the biosurfactant produced by *Bacillus licheniformis* strain JF-2. *Journal of industrial microbiology and biotechnology*, 5(2-3), 95-101.

Md, F. (2012). Biosurfactant: production and application. *J Pet Environ Biotechnol*, 3(4), 124.

Meylheuc, T., Van Oss, C. J., & Bellon-Fontaine, M. N. (2001). Adsorption of biosurfactant on solid surfaces and consequences regarding the bioadhesion of *Listeria monocytogenes* LO28. *Journal of Applied Microbiology*, 91(5), 822-832.

MM, Y., HL, F., & KN, T. (1996). Effect of heterogeneity of hydrophobic moieties on surface activity of *lichenysin A*, a lipopeptide biosurfactant from *Bacillus licheniformis* BAS50. *Biotechnology and applied biochemistry*, 23(1), 13-18.

Mnif, S., Chamkha, M., Labat, M., & Sayadi, S. (2011). Simultaneous hydrocarbon biodegradation and biosurfactant production by oilfield-selected bacteria. *Journal of Applied Microbiology*, 111(3), 525-536.

Mohan, P. K., Nakhla, G., & Yanful, E. K. (2006). Biodegradability of surfactants under aerobic, anoxic, and anaerobic conditions. *Journal of Environmental Engineering*, 132(2), 279-283.

Mulligan, C. N., Sharma, S. K., & Mudhoo, A. (2014). Biosurfactants. Research Trends and Applications. *CRC Press, Boca Raton*, 34.

Mulligan, C. N., Yong, R. N., & Gibbs, B. F. (2001). Surfactant-enhanced remediation of contaminated soil: a review. *Engineering geology*, 60(1-4), 371-380.

Muthusamy, K., Gopalakrishnan, S., Ravi, T. K., & Sivachidambaram, P. (2008). Biosurfactants: properties, commercial production and application. *Current science*, 736-747.

N

Naughton, P. J., Marchant, R., Naughton, V., & Banat, I. M. (2019). Microbial biosurfactants: current trends and applications in agricultural and biomedical industries. *Journal of applied microbiology*, 127(1), 12-28.

Neindre B.L., (1993).Tensions superficielles des composés organiques. Ed. Techniques Ingénieur.

Nitschke, M., & Costa, S. G. V. A. O. (2007). Biosurfactants in food industry. *Trends in Food Science & Technology*, 18(5), 252-259.

O

Ohadi, M., Shahravan, A., Dehghannoudeh, N., Eslaminejad, T., Banat, I. M., & Dehghannoudeh, G. (2020). Potential use of microbial surfactant in microemulsion drug delivery system: a systematic review. *Drug design, development and therapy*, 541-550.

P

Pacwa-Płociniczak, M., Płaza, G. A., Piotrowska-Seget, Z., & Cameotra, S. S. (2011). Environmental applications of biosurfactants: recent advances. *International journal of molecular sciences*, 12(1), 633-654.

Pennell, K. D., Abriola, L. M., & Weber Jr, W. J. (1993). Surfactant-enhanced solubilization of residual dodecane in soil columns. 1. Experimental investigation. Environmental science & technology, 27(12), 2332-2340.

Pereira, J. F., Gudiña, E. J., Costa, R., Vitorino, R., Teixeira, J. A., Coutinho, J. A., & Rodrigues, L. R. (2013). Optimization and characterization of biosurfactant production by *Bacillus subtilis* isolates towards microbial enhanced oil recovery applications. *Fuel*, 111, 259-268.

Pines, O., & Gutnick, D. L. (1981). Relationship between phage resistance and emulsan production, interaction of phages with the cell-surface of *Acinetobacter calcoaceticus* RAG-1. *Archives of Microbiology*, 130, 129-133.

Poré, J. (1992). Émulsions, micro-émulsions, émulsions multiples: Les agents de surface. 2, Balance hydrophile. Éd. techniques des industries des corps gras.

Pugh, R. J. (1996). Foaming, foam films, antifoaming and defoaming. *Advances in colloid and interface science*, 64, 67-142.

Puissieu, F. & Suiller, M. (1983) Agents de surface et émulsions, technique et documentation Lavoisier.

Q

Quek, E., Ting, Y.-P., & Tan, H. M. (2006). *Rhodococcus* sp. F92 immobilized on polyurethane foam shows ability to degrade various petroleum products. *Bioresource technology*, 97(1), 32-38.

R

Rahman, K. S. M., Thahira-Rahman, J., Lakshmanaperumalsamy, P., & Banat, I. M. (2002). Towards efficient crude oil degradation by a mixed bacterial consortium. *Bioresource technology*, 85(3), 257-261.

Rahman, P. K., & Gakpe, E. (2008). Production, characterisation and applications of biosurfactants-Review. *Biotechnology*.

Rincón-Fontán, M., Rodríguez-López, L., Vecino, X., Cruz, J. M., & Moldes, A. B. (2020). Potential application of a multifunctional biosurfactant extract obtained from corn as stabilizing agent of vitamin C in cosmetic formulations. *Sustainable chemistry and pharmacy*, 16, 100248.

Rodrigues, L., Banat, I. M., Teixeira, J., & Oliveira, R. (2006). Biosurfactants: potential applications in medicine. *Journal of antimicrobial chemotherapy*, 57(4), 609-618.

Ron, E. Z., & Rosenberg, E. (2002). Biosurfactants and oil bioremediation. *Current opinion in biotechnology*, 13(3), 249-252.

Rotenberg, Y., Boruvka, L., & Neumann, A. (1983). Determination of surface tension and contact angle from the shapes of axisymmetric fluid interfaces. *Journal of colloid and interface science*, 93(1), 169-183.

Roy, A. (2017). Review on the biosurfactants: properties, types and its applications. *J. Fundam. Renew. Energy Appl*, 8(2).

Ruiz-Garcia, C., Bejar, V., Martinez-Checa, F., Llamas, I., & Quesada, E. (2005). *Bacillus velezensis sp*. nov., a surfactant-producing bacterium isolated from the river Vélez in

Málaga, southern Spain. *International Journal of Systematic and Evolutionary Microbiology*, 55(1), 191-195.

S

Santos, D. K. F., Rufino, R. D., Luna, J. M., Santos, V. A., & Sarubbo, L. A. (2016). Biosurfactants: multifunctional biomolecules of the 21st century. *International journal of molecular sciences*, 17(3), 401.

Sayad L., (2008). Qualité physicochimique et bactériologie des eaux de l'écosystème lacustre Lac des Oiseaux (wilaya de Taraf). Mémoire de Magister. Université Badji Mokhtar Annaba. Algérie. 125 p.

Schramm, L. L., Stasiuk, E. N., & Marangoni, D. G. (2003). 2 Surfactants and their applications. *Annual Reports Section" C"(Physical Chemistry*), 99, 3-48.

Schwartz, A. M., Perry, J. W., & Berch, J. (1966). Surface active agents and detergents.

Shinoda, K., & Fontell, K. (1995). Ionic surfactants capable of being used in hard water. *Advances in colloid and interface science*, 54, 55-72.

Shoeb, E., Akhlaq, F., Badar, U., Akhter, J., & Imtiaz, S. (2013). Classification and industrial applications of biosurfactants. *Academic Research International*, 4(3), 243.

Singh, P., & Cameotra, S. S. (2004). Potential applications of microbial surfactants in biomedical sciences. *TRENDS in Biotechnology*, 22(3), 142-146.

Smyth, T. J., Perfumo, A., Marchant, R., Banat, I. M., Chen, M., Thomas, R. K., . . . Parry, N. J. (2010). Directed microbial biosynthesis of deuterated biosurfactants and potential future application to other bioactive molecules. Applied microbiology and biotechnology, 87,1347-1354.

Sobrinho, H. B., Luna, J. M., Rufino, R. D., Porto, A. L. F., & Sarubbo, L. A. (2013). Biosurfactants: classification, properties and environmental applications. Recent developments in biotechnology, 11(14), 1-29.

Sugawara, K., Numata, K. I., Konishi, M., &Amp; Kawaguchi, H. (1984). Empedopeptin Bmy-28117), A New Depsipeptide Antibiotic Ii. Structure Determination. *The Journal of antibiotics*, 37(9), 958-964.

Suzuki, T., Hayashi, K., Fujikawa, K., & Tsukamoto, K. (1965). The chemical structure of polymyxin E: the identities of polymyxin E1 with colistin A and of polymyxin E2 with colistin B. *The Journal of Biochemistry*, 57(2), 226-227.

$\mathcal{T}$

Thairu, Y.,Nasir, I.A., Yahaya, U. 2014. Laboratory perspective of Gram staining and its significance in investigations of infectious diseases. *Sub-Saharan African Journal of Medicine*, 1(4), 168-174.

Thangamani, S., & Shreve, G. S. (1994). Effect of anionic biosurfactant on hexadecane partitioning in multiphase systems. *Environmental science & technology*, 28(12), 1993-2000.

$\mathcal{U}$

Urai, M., Aizawa, T., Anzai, H., Ogihara, J., Iwabuchi, N., Neilan, B., ... & Sunairi, M. (2006). Structural analysis of an extracellular polysaccharide produced by a benzene tolerant bacterium, *Rhodococcus sp*. 33. *Carbohydrate research*, *341*(5), 616-623.

$\mathcal{V}$

Varjani, S., Rakholiya, P., Ng, H. Y., Taherzadeh, M. J., Ngo, H. H., Chang, J.-S., . Bui, X.T. (2021). Bio-based rhamnolipids production and recovery from waste streams: Status and perspectives. *Bioresource technology*, 319, 124213.

Varjani, S., Upasani, V. N., & Pandey, A. (2020). Bioremediation of oily sludge polluted soil employing a novel strain of *Pseudomonas aeruginosa* and phytotoxicity of petroleum hydrocarbons for seed germination. Science of the Total Environment, 737, 139766.

Vater J., Kablitz B., Wilde C., Franke P., Mehta N. et Cameotra S.S. (2002). Matrix assisted laser desorption ionization time of flight mass spectrometry of lipopeptide biosurfactants in whole cells and culture filtrates of *Bacillus subtilis* C-1 isolated from petroleum sludge. Appl. Environ. Microbiol. 68: 6210-6219.

Vecino, X., Cruz, J. M., Moldes, A. B., & Rodrigues, L. R. (2017). Biosurfactants in cosmetic formulations: trends and challenges. *Critical reviews in biotechnology*, 37(7), 911 923.

$\mathcal{W}$

Walter, V., Syldatk, C., & Hausmann, R. (2010). Screening concepts for the isolation of biosurfactant producing microorganisms. Biosurfactants, 1-13.

W-Christie W. Rhamnolipids, Sophorolipids and Relates Glycolipids. website: The LipidWeb. [Consulté le 14 mars 2024]. Dispoible sur “http://www.lipidhome.co.uk/lipids/simple/rhamno/index.htm”.

West, C. C., & Harwell, J. H. (1992). Surfactants and subsurface remediation. *Environmental Science & Technology*, 26(12), 2324-2330.

Wilson, A.J. (1996) " Foams, Surfactant Sciences Series" Vol. 57, Ed. Marcel Dekker, New York.

Y

Yakimov, M. M., Timmis, K. N., Wray, V., & Fredrickson, H. L. (1995). Characterization of a new lipopeptide surfactant produced by thermotolerant and halotolerant subsurface *Bacillus licheniformis* BAS50. *Applied and environmental microbiology*, 61(5), 1706-1713.

Yalaoui-Guellal D. (2017). Production des biosurfactants par des souches hydrocarbonoclastes isolées des sédiments du bassin versant de la Soummam de Bejaia, Algérie. Thèse de doctorat : Microbiologie. Université A. Mira-Bejaia. 126p.

Yalaoui-Guellal, D., Brahmi, F., Touati, A., De Champs, C., Banat, I. M., & Madani, K. (2018). Production of Biosurfactants by Hydrocarbons degrading bacteria isolated from Soummam watershed Sediments of Bejaia in Algeria. *Environmental Progress & Sustainable Energy*, 37(1), 189-195.

Yalaoui-Guellal, D., Fella-Temzi, S., Djafri-Dib, S., Brahmi, F., Banat, I. M., & Madani, K. (2020). Biodegradation potential of crude petroleum by hydrocarbonoclastic bacteria isolated from Soummam wadi sediment and chemical-biological proprieties of their biosurfactants. *Journal of Petroleum Science and Engineering*, 184, 106554.

Yea, D., Jo, S., & Lim, J. (2019). Synthesis of eco-friendly nano-structured biosurfactants from vegetable oil sources and characterization of their interfacial properties for cosmetic applications. *MRS Advances*, 4, 377-384.

Ying, W., Chen, J., & Liu, X. (2023). A review on antimicrobial activity, Anti-Biofilm and synergistic effects of sophorolipids since their discovery. *Applied Biochemistry and Microbiology*, 59(5), 580–596.

Z

Zarasvand, K. A., & Rai, V. R. (2014). Microorganisms: induction and inhibition of corrosion in metals. *International Biodeterioration & Biodegradation*, 87, 66-74.

Appendices

Appendix 01: Equipment, materials, glassware and reagents used:

Equipment	Materials and glassware	Reagents
Hotplate stirrer	Beakers	HCl
Autoclave	Petri dishes	Distilled water
Etuve	Glass boxes	Ethanol
Analytical balance	100 ml specimens	Glucose
Bec bunsen	1000ml Erlenmeyer	Methanol
Incubator	250 ml bottles	Sulfuric acid
Centrifuge	Micropipettes	Hexane
PH meter	Pliers	
Refrigerator	Test tube holders	
Vortex	Absorbent paper	
Microscope	Aluminum foil	
Sifter	Transparent Cellophane Paper	
Spectrophotometer	Pipette pasteur	
Freeze-dryer	Tapered tube	
Blood pressure monitor	Test tube	
Infrared Spectrophotometer to Fourier transform	Spatula	
	Strainer	
	Eppendorf tubes	
	Funnels	
	Blades and lamella	
	Spectrophotometer cuvette	

Appendix 02: Composition of culture media used

Nutrient agar medium (g/l)	**Salt Mineral Medium (MSM)**
Nutrient Agar Powder..... 28 Distilled water ..……..….....1000	NaCl…….….30g KH_2PO_4……..……..... 2,0g NH_4NO_3……..…..……1,0g Na_2HPO_4…….....……3,0g $MgSO_4.7H_2O$….....0,7g Trace element solution ……..…1,0 ml Distilled water …….....1000 ml pH = 7,2 ± 0,2

Abstract: Surfactants are surface active agents synthesized chemically or biologically (biosurfactants). These molecules are mainly produced by micro-organisms that develop aerobically in a contaminated environment. Biosurfactant as an effective biological surfactant, it is less toxic and biodegradable, as well as it has the ability to reduce surface and interfacial tension. For this study, we examined data concerning the biosurfactant produced from hydrocarbonoclast bacteria, which is considered as a biological alternative to chemical surfactants due to its biotechnological properties, environmental and industrial. The optimization of biosurfactant production depends on environmental factors (pH regulation, temperature, oxygen, etc.). The measurement of biosurfactant production using a minimum mineral medium enriched with glucose (MG) is carried out by several methods such as the measurement of surface tension (about 30 mN/m) and emulsification index (58.88%) and 5.15 g/L of freeze dried biosurfactants. The identification is assembled for characterization using advanced analysis techniques such as Fourier transform infrared spectroscopy (FTIR), gas chromatography coupled with mass spectrometry (GC/MS).
Keywords: Biosurfactant, Emulsification test (E24), GC-MS, IRTF-RTA.

Résumé : Les surfactants sont des agents à activité de surface synthétisés chimiquement ou par voie biologique (biosurfactants). Ces molécules sont principalement produites par des micro-organismes qui se développent de manière aérobie dans un milieu contaminé. Le biosurfactant en tant qu'agent tensioactif biologique efficace, il est moins toxique et biodégradable, ainsi qu'il a la capacité de réduire la tension superficielle et interfaciale. Pour cette étude, nous avons examiné les données concernant le biosurfactant produit à partir des bactéries hydrocarbonoclastes, qui est envisagé comme une alternative biologique aux surfactants chimiques en raison de ses propriétés biotechnologiques, environnementales et industrielles. L'optimisation de la production des biosurfactants dépend des facteurs environnementaux (la régulation de pH, la température, l'oxygéne…). La mesure de la production des biosurfactants en utilisant un milieu minimum minéral enrichi avec le glucose (MG) est effectuée par plusieurs méthodes telles que la mesure de la tension de surface (environ 30 mN/m) et de l'indice d'émulsification (58,88%) et 5,15 g/L de biosurfactants lyophilisés. L'identification est assemblée pour la caractérisation en utilisant des techniques d'analyses avancées comme la technique spectroscopie infrarouge à transformée de Fourier (FTIR), la chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC/MS).
Mot clés : Biosurfactant, Test d'émulsification E24, GC-MS, IRTF-RTA

ملخص: المواد الخافضة للتوتر السطحي هي عوامل نشطة سطحية مصنعة كيميائياً أو بيولوجياً (المواد الخافضة للتوتر السطحي). تنتج هذه الجزيئات بشكل أساسي عن طريق الكائنات الحية الدقيقة التي تتطور هوائيًا في بيئة ملوثة. خافض للتوتر السطحي الحيوي كخافض للتوتر السطحي البيولوجي الفعال، فهو أقل سمية وقابل للتحلل، وكذلك لديه القدرة على تقليل التوتر السطحي والبيني. في هذه الدراسة، قمنا بفحص البيانات المتعلقة بالخافض للتوتر السطحي الحيوي المنتج من بكتيريا الهيدروكربونوكلاست، والتي تعتبر بديلاً بيولوجيًا لخافضات التوتر السطحي الكيميائية نظرًا لخصائصها التكنولوجية الحيوية والبيئية والصناعية. يعتمد تحسين إنتاج الخافضات السطحية الحيوية على العوامل البيئية (تنظيم درجة الحموضة ودرجة الحرارة والأكسجين وما إلى ذلك). يتم قياس إنتاج الخافضات السطحية الحيوية باستخدام الحد الأدنى من الوسط المعدني الغني بالجلوكوز بعدة طرق مثل قياس التوتر السطحي (حوالي 30 مللي نيوتن/متر) ومؤشر الاستحلاب (58.88٪) و 5.15 جم/لتر من المواد الخافضة للتوتر السطحي الحيوي المجففة بالتجميد. يتم تجميع التعريف للتوصيف باستخدام تقنيات التحليل المتقدمة مثل التحليل الطيفي للأشعة تحت الحمراء، وكروماتوغرافيا الغاز إلى جانب قياس الطيف الكتلي.
الكلمات المفتاحية: خافض التوتر السطحي الحيوي، اختبار الاستحلاب، وكروماتوغرافيا الغاز إلى جانب قياس الطيف الكتلي، التحليل الطيفي للأشعة تحت الحمراء.