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Optimization of the slaughterhouse water treatment rate by a new *Marinobacter carbonoclasticus* SF and its biosurfactant

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Abstract: The aim of this study consists of the production of a bio-surfactant from a new bacterial strain, *Marinobacter hydrocarbono clasticus* SF (96.76 % similarity) isolated from soil contaminated by hydrocarbons in Hassi-Messaoud (Southern Algeria) to treat liquid effluent from slaughterhouse water. The characteristics of organic matter biodegradation tests were discussed. Despite the high pollutant load and the unfavorable physicochemical composition of the effluent, the specific growth rate of the isolated strain after 10 days of incubation in the range of 0–30 g L^{-1} of NaCl was at neutral pH 7.4 and temperature of 45 °C. The best bio-surfactant production yield was obtained after 72 h of incubation and under the optimal production conditions such as diesel as carbon source, ammonium chloride as nitrogen source, and a C/N ratio of 5. The bio-surfactant produced is of glycolipid type with a low critical micellar concentration (*CMC*), good emulsifying power, and chemical and functional stability. Significant pollutant removal efficiency was obtained using the bacterial strain (up to 82 %) and the bio-surfactant (up to 96 %). Several anions, such as nitrates, phosphates, ammonium, and suspended solids, were measured.

Keywords: effluent; isolate; characterization; glycolipid; depollution.

INTRODUCTION

Water is a staple of most major feed processing companies. This water loaded with organic matter is discharged into the environment. It therefore becomes a major source of pollution for the receiving environment.

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To reduce the risk of contamination, wastewater must be treated by physical, chemical and biological methods. Slaughterhouses produce wastewater from slaughterhouse washing operations containing blood, triperia-tubular washing water and digestive contents, barn washing water, yard and truck washing water, as well as water from various devices and installations.

These effluents have a reddish appearance and are heavily loaded with fragments of meat, fat, excrement, stomach contents, trimming debris, blood clots, pieces of horns and hooves, scum, faeces, and straw. The volume of wastewater is linked to the number of slaughtered animals and varies according to the size of the slaughterhouse, the mode of operation and especially the type of slaughtered animals. The main problem of slaughterhouse effluent is the treatment of fats and organic matter that are present in large quantities.[1] Several authors were interested in aerobic[2] and anaerobic[3] processes for the characterization and the treatment of this type of wastewater.

Unlike other developed countries, Algeria provides little information on the quality of slaughterhouse wastewater, its characterization, and its treatment.[4,5] The treatment of this effluent is strongly recommended because it can be responsible for an irreversible ecological imbalance as well as the eutrophication of the waters of the receiving environment.

Microorganisms can remove hydrophobic organic compounds (HOCs) considered as contaminants because they harm the environment and human health.[5] Thus, the biodegradation of HOCs by hydrocarbonoclaste bacteria requires specific cellular functions to allow their assimilation and transport into the cytoplasm where they are metabolized.[6]

This type of bacteria, such as the genus *Marinobacter*, has been studied with the aim to characterize the biosurfactants and their properties that they excrete.[5] Biosurfactants are one of the main products of the bioeconomy because of their diverse economic activities,[7] low toxicity and useful physicochemical properties, they are promising for the environment bioremediation activities, including oil spill clean-up, removal of heavy metal contaminants and wastewater treatment.[8]

Among biosurfactants, rhamnolipids and other natural biosurfactants that can replace conventional surfactants could play a crucial role in the decontamination of contaminated water and soil.[9] It should be noted that there is a strong worldwide demand for surfactants in the various fields mentioned above.

Slaughterhouse treatment using the original bacterial strain *Marinobacter carbonoclasticus* and the biosurfactant produced from this strain were considered as alternatives to physicochemical methods used in wastewater treatment because synthetic surfactants are threatened with extinction due to the depletion of fossil sources. In this study, a bacterial strain *M. carbonoclasticus* SF was isolated from oil-contaminated soil in southern Algeria and identified by 16S rRNA sequencing.

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The parameters influencing the growth of the isolate were determined. This review aims to discuss the most significant factors that influence and enhance microbial growth and the activity of this strain to treat slaughterhouse water as well as its power to produce biosurfactants. The impact of different parameters, such as pH, temperature and salinity, on the stability and emulsifying activity of the produced biosurfactant were investigated. Quantitative methods allowed the use of the isolated bacterial strain and the biosurfactant to be confirmed for the treatment of slaughterhouse water by measuring the chemical oxygen demand (*COD*), the biological oxygen demand over 5 days (BOD_5), as well as the rate of phosphates, nitrates, ammonium and suspended solids.

EXPERIMENTAL

Sampling of slaughterhouse water

The sample was taken at the exit of the slaughterhouse in Medea (Algeria), placed in inert polyethylene bottles, and stored at 4 °C. The analyzes were performed on the same day.

Isolation, growth conditions and identification of bacteria

Soil samples contaminated with hydrocarbons were taken in the region of Hassi-Messaoud (southern Algeria), transferred directly into sterile bottles, and stored in the dark at 4 °C until their use. The samples were pre-treated to determine pH, salinity, and electrical conductivity. Approximately 10 g of sediment, diluted 10 times with sterile water, was subjected to mechanical agitation for 24 h, then centrifuged at 4500 rpm for 20 min and filtered through a millipore filter (0.45 μm).[10] The pH was measured using a Metler Toledo pH meter comprising a selective electrode in contact with the solution. The conductivity was measured using a Consort C831 type conductivity meter (multi-parameter analyzer). The salinity (*S*) of the solid sample was obtained by comparing the result of the conductivity (σ) to the conductivity (κ) of a 32.43 g L^{-1} KCl solution according to:[11]

$$S = \frac{35\sigma}{\kappa} \tag{1}$$

where *S* is the salinity of the sample; σ is the conductivity of the sample in mS cm^{-1}; κ is the conductivity of a 32.43 g L^{-1} KCl solution.

The successive dilution method was used for the isolation. Thus, two grams of preserved sample were placed in 50 mL of basic enrichment culture medium containing 1 vol. % crude oil, then incubated at 37 °C at 180 rpm (shaker rotary, GFL3005, Germany). Transplanting was performed several times under the same conditions.

The SF bacterial strain used to produce the biosurfactant was maintained on nutrient agar. Crude oil was used as the sole carbon source in a minimum culture medium to enrich the strain for 14 h at 37 °C.[12] The optimal conditions for growth of the isolated bacterial strain (pH, temperature and salinity) were determined by measuring the specific growth rate (V_{max}) according to:

$$V_{max} = \frac{\log DO - \log(DO\ \tau_0)}{\tau_i} \tag{2}$$

where τ_0 is the initial incubation and τ_i the incubation time (h).

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Analysis of the 16S rRNA gene sequence as well as physiological and biochemical characteristics allowed the SF strain to be identified. The isolated strain underwent phylogenetic analyses by DNA sequencing corresponding to the 16S subunit of 16S ribosomal RNA (16S rRNA). For this, several steps were necessary.[13] The extraction of the genomic DNA (centrifugation, incubation and finally the resulting pellet containing the DNA was suspended in 20 µl of sterile water then stored at –20 °C) allowed genotypic identification of the isolated strain. Electrophoresis, quantification of genomic DNA extracted on agarose gel, and polymerase chain reaction (PCR) amplification of the 16S rRNA gene were performed using two universal primers, one forward (Fal125: AGAGTTTGATCCTGGCTCAG) extended from base position 8 to 27, and the other reverse (Ral126:

AAGGAGGTGATCCAAGCC, extended from base position 1.541 to 1.525, designed from the conserved zones within the rRNA operon of *Escherichia coli*.[14] An automatic sequencer automated 3100 genetic analyzer (Applied Biosystems) from the Biotechnology Centre of Sfax (CBS, Tunisia) for the analysis of electrophoresis and the quantification of the PCR product, Restriction analysis of the amplified ribosomal DNA, their purification and the 16S ribosomal DNA sequence. The sequence results were imported into BioEdit v5.0.9 and the complete or partial sequence of the fragment was reconstructed. Searching BLAST (blast, non-redundant) with the complete sequence, *via* GenBank,[13] identified the strain studied by comparison with the closest strains.

Production of biosurfactant

The bacterial strain isolated from the soil, identified previously, was kept in Petri dishes containing nutrient agar, recultivated monthly and stored at 4 °C. The culture medium used for production of the biosurfactant was incubated in Luria-Bertani broth consisting of 10 g of peptone: 5 g of yeast extract and 5 g of NaCl at pH 7. After 6 h incubation, a volume of gas oil at 1 vol. % was added followed by incubation for 72 h, shaking at 200 rpm and at a temperature of 45 °C. After centrifugation at 6000 rpm for 20 min, the biomass was separated from the supernatant containing the biosurfactant.

Optimization of operating parameters for biosurfactant production

Selection of carbon source and concentration. The type of carbon source used in bioprocesses is of paramount importance, it greatly influences the production yield, the quality, and the quantity of the biosurfactants produced.[15] In the present study, four carbon sources (1 vol. % were used to select the optimal source and to ensure maximum biosurfactant production: vegetable oil, olive oil, crude oil and hexadecane (Merck, Darmstadt). All substrates were sterilized using a 0.22 µm filter. The concentration of the optimized carbon source was determined by varying the concentration within a range of 0.5, 1, 2 and 3 %. The reduction in surface tension was measured with a tensiometer (TSD132389, Gibertini, Italy), which was also used with the emulsification index as a tool for selecting the carbon source and its concentration for optimal biosurfactant production.[15]

Selection of nitrogen source and concentration. Besides the carbon source, bacteria need nitrogenous substances to synthesize their proteins. Different sources of nitrogen (2 % g L^{-1}) were added to the culture medium: NH_4Cl, $NaNO_3$, NH_4NO_3 and urea. After optimization, its concentration was determined in the range 1, 2, 3 and 4 %.

Optimization of the C/N ratio. Several C/N ratios of 5, 10, 30, 40 and 50 were studied by varying the concentration of the carbon source while keeping the concentration of the nitrogen source constant.

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Kinetic study of the production in a free cell. A culture was prepared with the optimal synthesis conditions determined with the aim to study the kinetics of biosurfactant production by following the variations of the surface tension and of the emulsification index for a period ranging from 0 to 120 h.

Biosurfactant separation and purification. After optimal incubation of the production culture medium, the separation of the biosurfactant was performed by three-volume acetone in an ice water bath, kept for 24 h at 4 °C and followed by centrifugation at 200 rpm. The product was then dissolved in distilled water and recovered several times in acetone. The obtained crude biosurfactant was considered to be a partially purified.

Characterization of the biosurfactant. The characterization of the obtained biosurfactant was performed by first determining the surface tension of the biosurfactant and the emulsification number E_{24}. The emulsification index is defined as the ratio between the length of the total phase of the mixture (supernatant-hydrocarbon/oil) and the emulsified phase in which equal volumes of supernatant and hydrocarbon or oil (4 ml) were placed in a test tube and mixed with a vortex (VTX 400, France) at the maximum speed for 2min.

Analysis by FTIR was performed to determine the functional groups present in the biosurfactant product and to allow a preliminary structural characterization. A quantity of the precipitate obtained after drying the biosurfactant was dissolved in potassium bromide (KBr) (1/100 ratio), then the cell containing this mixture was placed in the light path that had a wavenumber between 500 and 4000 cm^{-1}.[16]

The critical micellar concentration (*CMC*) is an important parameter. The *CMC* corresponds to the lowest value of the surface tension of the biosurfactant. Above this concentration, no effect was observed on the surface activity. The *CMC* value was determined by plotting the surface tension against the concentration of biosurfactant in the solution.[17] In a test tube, a quantity of the separated precipitate was dissolved in distilled water. The mixture was manually stirred for 30 s and left to stand for 5 min. The emulsifying power of the biosurfactant was determined by mixing equal volumes of the supernatant and the hydrocarbon/oil (kerosene, heptane and olive oil), then stirred for 2 min at maximum speed and left to stand for 24 h. The interfacial tensions were again measured.

The surface tension values were calculated at different temperatures (20, 50, 70 and 120 °C), pH (2 to 11) and salinity (5 to 100 %) by incubation of the supernatant in the culture medium for 20 min.

Slaughterhouse water treatment

Characterization of the sample. The pH hydrogen potential was determined using WTWProfiLine pH 3310 pH meter. The measurement was performed with moderate stirring. The conductivity expressed in millisiemens per centimeter was measured using a Hanna Instruments HI8633 portable conductivity meter. Suspended solids (SS), refers to particles with a size between 10 nm and 1 µm, determined by vacuum filtration. They were determined by filtration through glass fibres using a vacuum filtration apparatus.[17]

The *COD* was determined according to the method described by Rodier,[17] under very precise operating conditions, Organic materials contained in the water were oxidized by an excess of potassium dichromate in an acidic medium and in the presence of silver sulphate and mercury sulphate. The BOD_5 reflects the amount of biodegradable effluent.

The amount of oxygen consumed was determined by incubating a volume of effluent for 5 days at 20 °C in the dark. The trace elements contained in the dosed effluent, such as nitrates, phosphates and ammonium, were determined using a typical spectrophotometer (DR/ /4000 V spectrophotometer, 230 Vac, Hach, USA) and glass cells with a capacity of 25 mL;

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detection in the visible range 300–1000 nm. Analysis of the compounds was realized after complexation with an added reagent that develops a colour. The nitrate assay was performed at 415 nm, ammonium at 655 nm, and phosphates at 880 nm.[18]

Treatment with biosurfactant and isolated strain. The slaughterhouse water was treated with 0.1 g of the obtained biosurfactant dissolved in 5 ml of distilled water, introduce into 500 mL of waste (slaughterhouse), incubated at 45 °C and at a speed of 150 rpm. Sampling and analysis were performed every 24 h for 7 days. In addition, water treatment was by the isolated strain. The microorganism used was the isolated aerobic bacterial strain *Marinobacter hydrocarbonoclasticus*. An amount of bacterial species was placed in LB medium and incubated on an orbital shaker at 45 °C and 150 rpm for 48 h. 3 % of the medium were put into 500 mL of polluted waste water, incubated at 45 °C and 150 rpm. A sample was taken every 24 h for measurement and analysis.

RESULTS AND DISCUSSION

Phylogenetic analysis

Molecular identification of the biosurfactant producing isolate was performed by sequencing the 16S rRNA gene and comparing to sequences from the NCBI 16S rRNA database with the 16S rRNA sequences of the isolate and the closely related strain. A rooted phylogenetic tree was constructed that is illustrated in Fig. S-1 of the Supplementary material to this paper. Analysis of the 16S rRNA gene sequence as well as physiological and biochemical characteristics allowed the strain SF to be identified as an *Marinobacter hydrocarbonoclasticus* species. Strain SF exhibits 99.76 % similarity using BLASTn with the type of strain of *M. hydrocarbonoclasticus*, ATCC 27132T (GenBank accession No. AB021372). The data were made available to ENA in Europe and to the ADN database in Japan.[19]

The accession number of the isolated strain SF is KX959991. A wide variety of *Marinobacter* species were described. *Marinobacter* sp. strains were collected from various locations around the world, exclusively from marine or halophilic habitats, with diverse physiochemical characteristics including seawater, sediments, marine hot-water springs, sea ice, deep seafloor, saline soil and offshore oil-producing well. Consistent with their habitat, most *Marinobacter* strains are moderate halophiles. Similarly, two new strains, SL014B61AT and SL014B11A, were isolated from an oil-polluted saline soil from Gudao in the coastal Shengli oil field, located in eastern of China. These two strains belonged to *Marinobacter* species.[20] Yi-Jing Luo *et al.*[21] were able to isolate *Marinobacter shengliensis* sp. from saline soil contaminated with oil, the isolates were found to grow at 10–40 °C (optimum 35 °C), pH 6.0–9.0 (optimal pH 8.0), and NaCl concentrations of 0.5–18.0 % (optimum 3.0–6.0 % NaCl). The specific growth rate was calculated according to Eq. (2). According to Fig. S-2a of the Supplementary material, it was observed that after 10 days of incubation, the SF strain grew over a pH range between 6 and 10.5, with a growth optimum at pH 7.4. Additionally, it is able to

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grow over a temperature range of 15 to 50 °C, with an optimum at 45 °C, as shown in Fig. S-2b.

The NaCl concentration for the growth of the SF strain was between 0 and 100 g L^{-1} with optimal growth between 0–30 g L^{-1}. No growth was observed at an NaCl concentration of 110 g L^{-1}, as shown in Fig. S-2c. Bacteria usually grow and live in an environment with a temperature range between 10 and 45 °C and in the pH range of 6–9.5.[22] The NaCl content is important for *M. hydrocarbonoclasticus*, because it can affect both the shape and flagellation of these cells.[23]

Production of biosurfactant

The production and characterization of a biosurfactant by the isolated strain SF by free cells was studied in batch cultures using the medium (LB) under conditions of temperature of 45 °C, pH 7.4, salinity of 30 % and a stirring speed of 200 rpm.

To date, the bacterial strain *M. carbonoclasticus* isolated from Algerian soil contaminated by hydrocarbons capable to produce biosurfactants has not been exploited. The strategy adopted was stepwise optimization and hence, by varying only one factor at a time, the kinetics of biosurfactant production were established. The optimum carbon source was gasoil at a concentration of 1 vol. %, a surface tension of 27.94 mN m^{-1} and an E_{24} of 75 % and a yield of 3.3 g L^{-1}.

There was also high induction of biosurfactant on all the hydrocarbons tested on diesel fuel (4.11 g L^{-1}).[24] In the present study, ammonium chloride was used as source of inorganic nitrogen (ST = 28.59 mN m^{-1} with an E_{24} of 68 %). SF species can grow on all four nitrogen sources, but ammonium chloride is the most efficient nitrogen source (2 %) compared to ammonium nitrate, sodium nitrate and urea.

The choice of inorganic source over organic nitrogen depends on the composition of the medium or the microbial strain.[25]

The synthesis kinetics were followed by a culture of the species *M. carbonoclasticus* SF in a medium containing diesel as the carbon source and NH_4Cl as the nitrogen source with different C:N mass ratios. The optimized C:N ratio 5:1 was achieved. This is the optimal condition for enhanced biosurfactant by *M. carbonoclasticus* SF. In a similar study conducted by Jimoh and Lin,[13] they found that a diesel fuel: $(NH_4)_2SO_4$ ratio of 3:1 resulted in the greatest production of biosurfactant.

The production of the biosurfactant started from the first hours of incubation, proven by the decrease in surface tension. This led to the use of a preculture that gives an appropriate and significant initial inoculum under optimal fermentation conditions favouring the production and a short lag phase.

After 72 h of fermentation, the production of the biosurfactant reached its maximum (ST = 27.94 mN m^{-1}) with a stable emulsion (E_{24} = 75 %). For 120

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hours, monitoring the pH showed the stability of the biosurfactant and its production that is associated with the increase in biomass, as shown in Fig. 1. It is a production associated with growth (secondary metabolism).

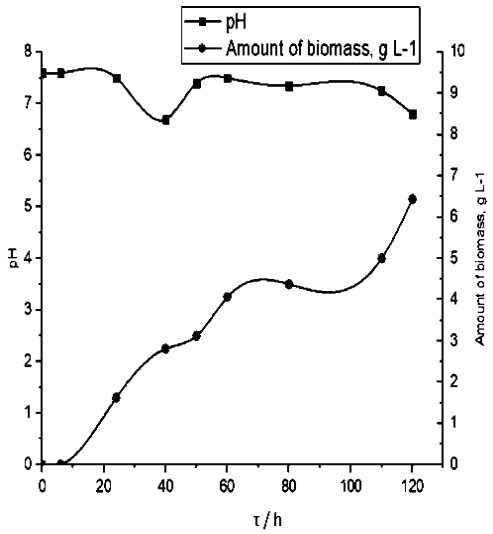

Fig. 1. Variation of pH and biomass with time.

Preliminary characterization of the biosurfactant

The results of a structural analysis of biosurfactant are shown in Fig. S-3 of the Supplementary material. A band greater than 3200 cm^{-1} was visible, indicating the presence of free hydroxyl groups of the rhamnose ring (-OH). Absorptions bands at around 2960 cm^{-1} are attributed to the symmetric C–H stretch of CH_2 and CH_3 groups of the aliphatic chains. Carbonyl ester groups are predicted by vibrations at 1100 cm^{-1}. The valence carbonyl band was found at 1685 cm^{-1}, corresponding to CO strain vibrations. The deformation vibration at 1450 cm^{-1} confirms the presence of alkyl groups. A similar study conducted by Ron and Rosenberg.[26] showed that almost all biosurfactants have an ester or carboxylic acid group in their structures and confirm the presence of bonds formed between carbon atoms and hydroxyl groups in the chemical structures of the glycosidic part. The biosurfactant obtained from the bacterial strain isolated from soil contaminated by hydrocarbons is initially a glycolipid. The determination of the biosurfactants *CMC* is crucial since there is no further modification in the surfactant activity above this concentration.[2] It is obvious from Fig. 2 that the surface tension decreased with increasing biosurfactants concentration until reaching 28.5 mN m^{-1} for a biosurfactant concentration greater than or equal to 850 mg L^{-1}.

Therefore, the critical micellar concentration (*CMC*) of the obtained product was 850 mg L^{-1}. Raddadi *et al.*[27] isolated 16 *Marinobacter* sp. such as strains

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from harbour sediments. They found *CMC* values greater than 900 mg L^{-1}. Comparable results were found by Zenati *et al.*[28] for *M. carbonoclasticus* SDK644 isolated from a marine environment. The *CMC* was 788 mg L^{-1}.

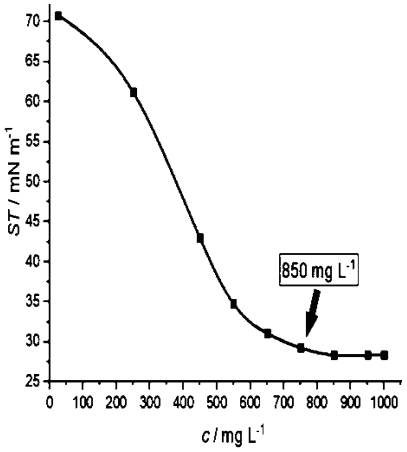

Fig. 2. Surface tension against the biosurfactant concentration.

The *CMC* is an index used to evaluate the surfactant activity and is the minimum concentration of biosurfactants necessary to reach the lowest values of surface tension, from which begins the formation of micellar aggregates. The interfacial tension of the mixture (supernatant/hydrocarbon or oil) showed a lowering capacity of the surface tension from 28 to 12 mN m^{-1}, The decrease in interfacial tension and the uniformity of the bubbles confirm the good emulsifying power of the biosurfactant on oils and hydrocarbons with good stability. The biosurfactant has a foaming power and foam stability was observed within 10 min. The biosurfactant produced was thermostable. Several studies showed that the glycollipids were unaffected by the rise in temperature (121 °C).[29] Moreover, the surfactant properties of the biosurfactant isolated from the SF strain remained stable in the pH range 2–11 and at a salinity of 5 to 100 mg L^{-1}. Hentati *et al.*[30] confirmed the stability of biosurfactants produced by a bacterial strain *Bacillus stratosphericus*, at different temperatures (4 to 121 °C) and pH (2.1–12) and salinity (0 to 150 %). A thermophilic and halotolerant strain of *Pseudominas aeruginosa*, isolated from petroleum contaminated soil, produced a biosurfactant when grown in media containing salinity in the range of 0–6 %.[31]

Slaughterhouse water treatment

Characterization of the sample. Some parameters which characterize the slaughterhouse waters were determined at pH 7.5 and a temperature of 11.5 °C. These parameters and their effluent water limit values are given in Table I.

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Indeed, the blood present in this type of effluent mainly contains protein complexes (fibrogen, coagulation protein, antibodies, *etc*.) that are active at neutral pH. The slaughterhouse waste is characterized by high alkalinity, high chemical oxygen demand, high volatile content, high ammonium, and phosphorus content.[33] The high BOD_5 value can be explained by the abundance of organic matter (rumen debris), by the concentration of this effluent and by the blood of slaughterhouse waste as well as the *COD* value. The COD/BOD_5 ratio of 1.13 is less than 3 in line with that of predominantly domestic urban wastewater.[32] Therefore, the wastewater from this urban discharge has a high organic load, and is easily biodegradable and hence biological treatment of slaughterhouse water is suitable. To characterize industrial pollution, the BOD_5/COD ratio must be considered, the latter being equal to 0.88, the polluted water is loaded with OM, unstable water with the risk of odour release.

TABLE I. Physicochemical parameters of slaughterhouse water and their limit values

Values	*COD* / mg L^{-1}	BOD_5 / mg L^{-1}	c_{SS} / mg L^{-1}
Limit[32]	800	250	200
In slaughterhouse waters	1920	1700	12.5

Treatment with biosurfactant and isolated strain

With isolated strain. *M. hydrocarbonoclasticus* exhibits significant enzymatic activities, which allow the bacteria to degrade hydrophobic substances in an aerobic environment.[34] Therefore, *M. hydrocarbonoclasticus* can be considered as a potential bioremediation agent. Good results were obtained using this strain for the treatment of polluted water. Pollution by organic matter contained in an effluent can be evaluated by the chemical oxygen demand (*COD*). According to Fig. S-4a of the Supplementary material, in the light of the results found, it was noticed a considerably remarkable decrease in *COD* from the first day with a purification efficiency of 80 % (*COD*) and 85 % (BOD_5), which explains the purifying power of the *Marinobacter* sp. used. At the end of the treatment, the obtained *COD* value is within the standards required for discharges. Likewise, for BOD_5, given the high biodegradability of the effluent to be treated. Rates of up to 81 % reduction in organic matter (OM) contained in the slaughterhouse water were obtained, which allow to conclude the effectiveness of the biological treatment method. The disadvantages of this method, it presents problems related to the reduction of the concentrations of activated sludge, the need for supervision by experts in the design of biological systems and the high cost of the elimination of large quantities of effluents from sludge during treatment. Gnowe *et al*.[35] in their study, used the biological treatment method for the treatment of slaughterhouse water. Organic matter reduction rates of up to 81 % were obtained. This percentage is demonstrated in Fig. S-4b. Moreover, as shown in

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Figs. S-4c and d, the presence of ammonium and MO in the medium facilitated the bioelimination of phosphates (dephosphatation), the suspended solids increased considerably with increasing quantity of nitrates and decreasing quantity of ammonium. This is due to the nitrification of the wastewater which consists in an oxidation of ammoniacal nitrogen into nitrites, which in turn oxidize and transform into nitrates.[36] After the second day (homogeneous sample), suspended solids increased markedly. This could be explained by the formation of aggregates during the treatment (concentration of pollution in the form of sludge) The reduction in phosphates and ammonium, with the formation of nitrates (nitrification) created a favourable environment for the biodegradation and reflect the good purifying capacity of the strain used. Wastewater temperature values of less than 30 °C were recorded, they are considered as the limit values for direct discharge into the receiving environment, The pH values varied slightly and remained around 7.5. When the pH was lower than 5 or higher than 8.5, the growth of microorganisms was directly affected. The electrical conductivity reflects the degree of overall mineralization, the measured values varied between 0.88 and 3.5 mS cm^{-1}. This increase can be explained by the formation of new ions during the oxidation of organic matter and by substantial variation of the mineralization.

With biosurfactant. The slaughterhouse water treatment was performed using a biosurfactant produced from the bacterial strain *M. carbonoclasticus* SF. The good results shown in Fig. S-5a of the Supplementary material confirmed the effectiveness of the use of the biosurfactant in the field of polluted water treatment.

As illustrated in Fig. S-5b, the obtained purification efficiency was 82 and 96 % for *COD* and BOD_5, respectively. A decrease in the concentration of phosphates and ammonium, an increase in nitrates and a large amount of suspended solids were observed. The study conducted by Al-Wahaib *et al.*[37] on different media to produce biosurfactants using strains of *Marinobacter* sp. found that biodegradation efficiencies were of 90, 84, 76 and 72 % for crude oil, diesel, C32 and C40, respectively. An excellent treatment of contaminants was found. This is due to the low CMC value and the good emulsifying power of the biosurfactant obtained.[37]

CONCLUSION

The strain isolated from Algerian soil polluted by hydrocarbons was a *Marinobacter carbonoclasticus* SF. The latter was able to grow on a minimum culture medium consisting of diesel as a carbon source (1 %), ammonium chloride as a nitrogen source (2 %), and a C/N ratio of 5 under optimal conditions of pH 7.4, a temperature of 45 °C, and salinity of 0–30 %. The isolated strain showed its ability to produce a glycolipid-type biosurfactant after 72 h of incubation, a surface tension of 27.94 mN m^{-1} was obtained and an emulsification number of

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75 %. The *CMC* of the biosurfactant was 850 mg L^{-1} and a yield of 3.3 g L^{-1}. The obtained biosurfactant was both thermally and chemically stable. Physico-chemical pollution parameters of the wastewater from the municipal slaughter-house of the city of Medea (Algeria) have values that relatively exceed the limit values for discharges into the receiving environment (Oued Lahrech) which represents a real risk that requires a thorough treatment. The water treatment trials resulted in significant depollution rates, whether by the isolated bacterial strain *M. hydrocarbonoclasticus* SF (more than 81 %) or by the biosurfactant produced by this strain (more than 96 %).

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11335, or from the corresponding author on request.

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ИЗВОД

ОПТИМИЗАЦИЈА ТРЕТМАНА ВОДЕ ИЗ КЛАНИЦЕ ПОМОЋУ НОВОГ *Marinobacter carbonoclasticus* SF И ЊЕГОВОГ БИО-СУРФАКТАНТА

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Циљ ове студије је производња био-сурфактанта из новог соја бактерија, *Marinobacter hydrocarbonoclasticus* SF (96,76 % сличности), изолованог из земљишта контаминираног угљоводоницима у Hassi-Messaoud (Јужни Алжир), за пречишћавање течних ефлуента из воде која долази из кланице. Размотрене су карактеристике тестова биоразградње органске материје. Упркос великом оптерећењу загађујућим материјама и неповољном физичко–хемијском саставу ефлуента, специфична брзина раста изолованог соја после 10 дана инкубације била је на неутралном pH 7,4 и температури од 45 °C, у опсегу од 0–30 g L^{-1} NaCl. Најбољи производни принос био-сурфактанта је постигнут након 72 h инкубације и под оптималним условима као што су дизел као извор угљеника, амонијум-хлорид као извор азота и однос C/N од 5. Произведени био-сурфактант гликолипидног типа са ниском критичном мицеларном концентрацијом (*CMC*), добрим емулгационим својствима и хемијском и функционалном стабилношћу. Значајна ефикасност уклањања загађивача постигнута је коришћењем бактеријског соја (до 82 %) и био-сурфактанта (до 96 %). Измерена је концентрација неколико јона, као што су нитрати, фосфати, амонијум, као и суспендоване чврсте супстанце.

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