

Valorization of Oil Waste for Biodetergent Production Using *Serratia Marcescens* N2 and Gamma Irradiation Assisted Biorecovery

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Research Article

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Abstract

The aim of the present work is to valorize previously used frying oil and use it as biodegradable. *Serratia marcescens* N2 valorized 20% used oil and 8% cell concentration, the biosurfactant produced was a negatively charged lipopeptide with surface tension of 26.8 mN/m. Gamma radiation was used to obtain the higher yield of the biosurfactant by exposing the cells after growth under optimal conditions to low dose gamma radiation. The results showed that the use of radiation led to an increase in the amount of biosurfactant, and the biorecovery took place in a shorter time than usual. The chemical or functional form of the substance did not change at doses of 500 and 1000 gray, while there was a change in production and chemical and functional form at the dose of 2000 gray. The produced biosurfactant was used before and after irradiation to wash oil soiled cloths, the results showed 87% removal at 60°C under stirring conditions. Skin irritation tests performed on experimental mice showed that the surfactant does not cause any inflammation or red spots. Optical images of cloth patches showed no effect on fabric threads post washing the oil soiled cloth patches with biosurfactant. This study proved that 1) previously used oil can be bioconverted into biosurfactant and 2) the use of low doses gamma radiation results in an increase in biosurfactant yield by creating holes in the bacterial cell wall, which helps to recover more quantities of the biosurfactant without change in its chemical or functional form.

Introduction:

Biocircular and green (BCG) economy has been integrated in the realization of the 17 sustainable development goals (SDGs). Sustainability oriented society would entail the integration of multidisciplinary stakeholders who all work to pave the way for such a transformation to meet social, economic and ecological goals (D'Amato & Korhonen, 2021). The basic concept of BCG economy is to utilize waste as a new resource to obtain value added products, thus addressing sustainability (Arancon, Lin, Chan, Kwan, & Luque, 2013). One of the problematic wastes that is used as a new resource is the oil waste generated from frying foods. Pre-used frying oil result from the fast food industry, they are produced in huge amounts and the conventional methods of disposal as direct disposal in sewage system (Ganesh & Lin, 2009). This can lead to other problems such as clogging the drains due to the viscosity of pre-used oil and change in temperature during the disposal process can increase the clogging process. On the other hand, this problem has led to research concerned focusing on oil utilization and transformation into value added products. Oil cooking waste (OCW) is commonly recycled into other value added products such as soap, a very simple transformation that is attracting the attention of households and start-up companies in Egypt. Biosurfactant production using cooking oil waste as the sole carbon source is another attractive solution (Md Badrul Hisham, Ibrahim, Ramli, & Abd-Aziz, 2019).

Biosurfactants are prominent amphiphilic compounds that consist of hydrophilic and hydrophobic moieties. The hydrophobic part consists of a fatty acid or fatty alcohol chain with atoms ranging from C8 to C18. The hydrophilic component can be a carbohydrate or a protein. The diverse structural variations imply a variety of physicochemical properties (Kubicki et al., 2019). Biosurfactants are perceived as the next generation multifunctional biomolecules that can be used for an array of applications such as

medicine, industry, cosmetics and bioremediation. Yet, biosurfactant production face limitations that entail low microbial productivity and high cost for down streaming process (Fenibo, Ijoma, Selvarajan, & Chikere, 2019). Therefore, applying strategies for bioprocess upscale can be foreseen as a counteract approach to those limitations. The use of waste as low cost carbon source and computational tools for media selection and genetic engineering are among the methods for optimizing the biosurfactant production (Abbasi et al., 2013; Invally, Sancheti, & Ju, 2019; Jimoh, Senbadejo, Adeleke, & Lin, 2021). In addition to that, finding solutions to the down streaming process is imperative to the commercial production of biosurfactant. Reverse aqueous extraction method and calcium precipitation were used as novel approach to polish biosurfactant production (Invally et al., 2019). But apart from using the optimal media components for production, the amount released is dependent on the biosurfactant mobility from within the cell to the surrounding medium via the cell membrane. From this stand point; gamma radiation can be considered an effective tool for release of biosurfactant from bacterial cells. Gamma radiation was previously used to permeabilize the cell wall of gram positive bacteria to help release biomolecules to the media (Selim et al., 2020).

Therefore, the aim of the present study is to valorize pre-used frying oil in the production of biosurfactant, apply factorial design to reach optimal productivity and use gamma radiation to assist in biosurfactant down streaming process. Structural and functional changes post gamma radiation use will be assessed in terms of its application as a biode detergent.

Materials And Methods

Screening of different *Serratia marcescens* strains for biosurfactant production

Biosurfactant production screening was carried out in 250 mL Erlenmeyer flasks with 100 mL of production medium containing 1% peptone and 6% clean Mix frying oil with media were adjusted to pH 7.0 and autoclaved at 121 °C for 15 min. Then, they were inoculated at 2% inoculum size and incubated for 13 days at 28 °C, under orbital agitation (150 rpm)

Inoculum preparation of 5 *Serratia marcescens* strains (MN2 (KX601268), MN3(KX601278), MN4 (KX601721), MN5(KX601170) , N2 Bioproject ID PRJNA525074, Biosample ID SAMN11041520, and WGS accession SPSG00000000 Loop from plate in 10 ml Luria Bertani broth for 1 hour OD₆₀₀ adjustment then 2 ml from inoculum into flask. Surface tension of the collected cell-free metabolic cultures were obtained by centrifugation at 12,000×g for 20 min, and membrane filtration of culture media 0.22mm. Analyses were performed at 25 °C in a Kruss tensiometer (K20Kruss GmbH, Germany) using the Du Nouy ring method with Milli-Q water with surface tension of 72mN/m was used to calibrate the tensiometer (Araújo et al., 2019). Oil spreading techniques was performed as follows: 50 ml distilled water to petri dish followed by 100ml of vegetable oil to surface of water then 10ml of cell free biosurfactant was added slowly and detected in light after 30 s (Ghasemi, Moosavi-Nasab, Setoodeh, Mesbahi, & Yousefi, 2019).

Optimization of production using factorial design

Serratia marcescens N2 was the strain with the lowest surface tension was used in the upcoming experiments, this strain was previously deposited at DDBJ/ENA/GenBank under Bioproject ID PRJNA525074, Biosample ID SAMN11041520, and WGS accession SPSG00000000 with the annotated genome of *S. marcescens* N2 deposited in the PATRIC database under genome number 615.1488 (Elkenawy et al 2021).. Biosurfactant production experimental design was done using Minitab 18 software (USA) for 2 factors, 3 levels. The design of the experiment is represented in Table S1. The cultivation was carried out in 250 mL Erlenmeyer flasks with 100 mL working volume. The production medium consisted of 1% peptone and carbon sources of 5, 10 and 20 % vol/vol of pre-used frying oil from local restaurant in Heliopolis area, Cairo. Cultivation media were adjusted to pH 7.0 and autoclaved at 121 °C for 15 min. Flasks were inoculated with inoculum sizes 2, 4 and 8% and incubated for 6 days at 28 °C, under orbital agitation (150 rpm). Surface tension was estimated as previously described and wet biosurfactant weight was calculated as the biosurfactant weight at the end of the production process in g/l.

Extraction and characterization of *S. marcescens*N2 biosurfactant

The biosurfactant produced by *S. marcescens* N2 after 6 days fermentation was isolated from cell-free metabolic liquid obtained by centrifuging (12,000×g for 20 min) the culture. The metabolic liquid was subjected to precipitation using conc. HCl to get pH 2.0 and kept at 4° C overnight. It was then centrifuged at 15,000×g for 15 min and the cell-free metabolic supernatant was collected and centrifuged at 5000×g for 15 min. The supernatant obtained was discarded and the crude biosurfactant was extracted three times with a chloroform-ethanol (2 : 1 v/v) mixture with vigorous shaking, The precipitate was collected, oven dried and used for analysis (Eraqi, Yassin, Ali, & Amin, 2016).

Fourier Transform Infrared Spectroscopy (FT-IR)

The identification of functional groups in the isolated biosurfactant was carried out using Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR). The obtained biosurfactant in its dry form was scanned within the range of 4000–400 cm⁻¹ using BRUKER VERTEX 70 device at NCRRT. The spectrum of the biosurfactant under study was compared to literature.

Emulsification Index

1ml of cell free metabolic liquid 1 ml used frying oil – paraffin oil control tween 80 and water vortex 2 min and leave on bench for 24 hrs measure height of emulsion layer divided by total height(Tripathi, Gaur, Dhiman, Gautam, & Manickam, 2020).

$$EI_{24} = \frac{\text{Height of emulsification layer (cm)}}{\text{Total height of the mixture (cm)}} \times 100$$

Zeta potential

The electrokinetic of potential zeta of biosurfactant aqueous solution was analyzed using PSSNICOMP Zeta Potential/Particle Sizer 380ZLS (PSS-NICOMP, Santa Barbara, CA, USA) with 2 mg of crude biosurfactant dissolved in 1mL of water (Araújo et al., 2019).

Biosurfactant identification of serrewettin gene

The WGS of *Serratia marcescens* N2 was used to identify the biosurfactant gene and the results were compared to other genes deposited in NCBI database repository. Data was represented as identity percentage.

Oil displacement activity (ODA) and Critical Micelle Concentration (CMC) of biosurfactant

ODA was performed by adding 10 µl of cell free broth on 100 µl of engine oil that was added to a petri dish containing 40 ml distilled water. The zone of displacement was recorded as positive. CMC of biosurfactant was determined using crude biosurfactant. Different concentrations (0.2-4 mg/mL) were prepared and surface tension was measured for each concentration. The value of CMC was obtained from graph (Araújo et al., 2019)

Gamma radiation for biosurfactant biorecovery and assessment of the structural and functional changes

At the end of cultivation period, the content was divided into four equal portions in clean sterile containers for gamma irradiation to test effect gamma radiation on biosurfactant biorecovery and yield. Irradiation process was carried out in Cobalt-60 (Co^{60}) 220 gamma cell, Canada Co. Ltd. located at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt at doses 500, 1000 and 2000 Gy. The dose rate was 1.119 kGy/ h at the time of experiment. At the end of the gamma irradiation process, the culture filtrate was collected and biosurfactant extracted as previously described. Surface tension, emulsification index, FTIR and Zeta potential were performed as previously described. Gravimetric analysis was performed by obtaining the dry weight for the biosurfactants after oven drying.

Oil valorization assessment

The whole content of 300 ml flask with 1% peptone, 20% oil content and 8% inoculum size cultivated for 13 days at 150 rpm and 28°C. The remaining oil content at the end of the experiment was measured to evaluate valorization of using the used frying oil into produced biosurfactant. The dry cell weight was measured to assess bacterial ability to valorize oil into biosurfactant. The consumed oil was divided by the initial oil to obtain the valorization percentage.

Application of biosurfactant as washing detergent

Washing experiment

Pieces of dry cotton cloth were cut into 2x2 cm pieces and each piece was stained with 0.3mL used frying oil. The pieces were left for 15 days to stabilize the stain as a challenge for stain removal, and their precise weights were recorded before staining, after staining and after washing.

The stained cotton cloths were washed using biosurfactant released from cells after culture exposure to 500, 1000 and 2000 Gy. The results were compared to non-irradiated biosurfactant producing culture at 1 g/litre tap water under both static and stirring in water bath at 50rpm and 30°C and 60 °C for 1 hour. After washing, the pieces were rinsed in water for half an hour, and dried at ambient temperature to a constant weight. The removal percentage of each stain was calculated using the precise weights of the pieces before and after washing (Khaje Bafghi & Fazaelipour, 2012; Tripathi et al., 2020).

$$\text{Detergency (\%)} = \frac{C - B}{A - B} \times 100$$

where *A* is the weight of a soiled cloth, *B* is the weight of a white cloth, and *C* is the weight of a soiled cloth after washing (Fei et al., 2020)

Optical microscopy

Optical images of clean, soiled and soiled clot patches washed with 500 Gy assisted biosurfactant at 60°C under string conditions were captured using AX10 Zeiss light microscope coupled with Axiocam 105 color (Germany) at NCRRT. Images representing the fabric threading were captured at 25X and 100X magnification.

Skin irritation test

Acute dermal irritation test was performed Co. based on OECD/OCDE404 method after the approval of Research Ethics Committee at the National research Center or Research and Technology (REC-NCRRT) with serial number 47 A/21, the result was expressed in terms of primary irritation index (PII). Irritation scores for erythema, eschar and edema formation at 1, 24, 48 and 72 hr after patch removal were summed up and divided by the number of observations, to obtain the individual PII. For the calculation of PII, all individual PII's were summed up and divided by the number of animals used during the test. The detailed information on experimental procedures of each test was described in (OECD, 2015)

Results

Screening of different *Serratia marcescens* strains for biosurfactant production

The results in Table 1 shows that although the 5 pigmented strains produced biosurfactant, yet their surface tension varied ranging from 29.8 to 38.9 mN/m with strain N2 with the highest production

Optimization of production using *Serratia marcescens* N2 using factorial design

A 2 factors 3 levels full factorial design was used to test *Serratia marcescens* N2 valorization of pre-used frying oil into produced biosurfactant and the possibility of increasing the amount of used oil to maximize valorization. Results obtained in Fig. 1a&1b show that 20% carbon source and 8% inoculum size resulted in the lowest surface tension (26.8 mN/m) and highest biosurfactant wet weight (4.34 g).

Characterization of *Serratia marcescens* N2 biosurfactant

Genome annotation for this bacterium shows the presence of serrawettin synthetase gene that is responsible for Serrawettin production. Table 2 represents the identity (%) of serrawettin synthetase produced by *Serratia marcescens* N2 as compared to other genes present in public database. Critical micelle concentration (CMC) of the produced biosurfactant was ca. 2 mg/ml (Fig. 2a). The biosurfactant was analyzed through ATR-FTIR (Fig. 2b) for determining the functional groups in their backbone. The biosurfactants showed broad and sharp peaks in the region of 3310–1657 cm^{-1} represent NH stretching and C-O-N indicating the presence of peptides, 2922 and 2666 cm^{-1} represent CH_2 and CH_3 groups. A strong peak at 1742 cm^{-1} suggested the presence of a carbonyl group attached to electron-withdrawing groups. The bands at 714 and 607 cm^{-1} corresponds to aromatic monosubstituted ring. Zeta potential of the biosurfactant was -22 mV, El_{24} was 90% and surface tension was dropped from 72 mN/m (standard ST for water) to 25.7 mN/m (Table 3).

Oil valorization

The oil valorization was calculated to be about 83.3% after 2 weeks. . Fig. 3 shows the valorization of oil after 1 h and 6 days as compared to zero time, the figures show speed valorization after 1 h and 6 days as seen from the emulsified layers in pictures Fig. 3b and c as compared to the picture at zero time (Fig. 3a).

Effect of gamma radiation as a tool for biosurfactant release from *Serratia marcescens* N2 cells on some biosurfactant structure and characteristics

To avoid the multiple steps, low dose gamma radiation was used as a single step, the cell cultures were exposed different doses of gamma radiation and the yield was calculated as 9.4, 16.5, 19.2 and 13.9 g/l for 0, 500, 1000 and 2000 Gy exposed cell cultures. The results in Fig. 4a shows that emulsification index, surface tension and zeta potential were almost the same and no evident functional changes while FTIR spectrum showed changes in broadness, sharpness and intensity of peaks at 3282, 1635, 1049 and 578 cm^{-1} for 2000 Gy irradiation assisted biosurfactant recovery (Fig. 4b).

Biodetergency and skin irritation test

The results shown in Fig. 5 represent crude biosurfactant that was used to clean fabric soiled with oil. The results show that the highest detergency of 87.5% was obtained after 500 Gy radiation assisted biorecovery this was followed by 62.5% for 1000 Gy and 57% for 2000 Gy radiation assisted biorecovery as compared to 50% for non-irradiated biosurfactant when the fabric was washed at 60°C under stirring

conditions. Images in fig. 6 shows that washing the soiled cloth patch with 500 Gy assisted biosurfactant at 60°C under stirring conditions did not affect the general threading of the fabric. Fig. 7 shows that all tested mice showed no redness or spots

Discussion

As an initial step for biosurfactant production, screening of different *Serratia marcescens* strains was performed using clean frying oil. Strain N2 with the highest production was previously recognized as prodigiosin hyper producing strain in a previous study (N. M. Elkenawy, Yassin, Elhifnawy, & Amin, 2017). Variation between *Serratia marcescens* isolates could be intrinsic within every strain capable of biosurfactant production in a variable extent. The initial screening was done using clean frying oil to produce biosurfactant in a minimal challenging condition to assess their production capability avoiding the complex composition of the pre-used frying oil that might be a challenge for bacterial production.

Usually, complex media is used to produce biosurfactants (Nehal and Singh 2021), but in the present study a simple media of peptone water was the base for biosurfactant production. Carbon source and inoculum size were chosen as two key factors that control *Serratia marcescens* N2 biosurfactant production. Although it was expected that the experimental design would reflect on the variation significance of the results it was not significant. However, the experimental design analysis showed significant interaction between Inoculum size and pre-used frying oil expressed as a direct relationship (Ghasemi et al., 2019) This result indicates that *Serratia marcescens* N2 has the ability to withstand and utilize a high content of pre-used frying oil and transform it to a biosurfactant which expresses the ability of *Serratia* to withstand the highest content of pre-used frying oil and transforming it to biosurfactant which could be either due to utilizing of oil as carbon source (Araújo et al., 2019) or as an inducer for biosurfactant production through the provoking biosurfactant biosynthetic cluster (Araújo et al., 2019). Balancing added nutrients and microbial growth is needed to reach a good biosurfactant production (Makkar & Cameotra, 1997). During this experiment, it was noted that the strain lost its pigment during the process which indicates that it interfered with prodigiosin production pathway resulting in shut down and directing the production to biosurfactant only. The production of secondary metabolites is highly dependent on cultivation conditions or environmental factors. *Serratia* metabolite production is regulated via quorum sensing (QS) system that influences cell-cell communication. Secondary active metabolites regulated by QS include biosurfactant and prodigiosin production (Su et al., 2016). The whole genome sequencing of *Serratia marcescens* N2 was performed in a previous study (Nora M Elkenawy, Youssef, Aziz, Amin, & Yassin, 2021) and the only biosurfactant identified within the genome was serrawettin synthetase, this enzyme is responsible for the synthesis of serrawettin. Serrawettin is a biosurfactant produced by *Serratia*, it can be present as Serrawettin W1 form which is a symmetric dilactone structure. The compound is composed of two serine residues, connected with two 3-hydroxydecanoic acids (Thies et al., 2014). Thus, according to the results of the IR spectra, *Serratia marcescens* N2 produces a cyclic lipopeptide, this is in accordance with literature describing biosurfactants with the same characteristics. It is known that *Serratia marcescens* N2 produces

serrawettin, this is a lipopeptide of low molecular weight (Viera et al 2021). Critical micelle concentration (CMC) of the produced biosurfactant value was low, a low CMC indicates that this compound can exert its effect at low concentrations(Kubicki et al., 2019).

Initial and consumed oil volumes were used to evaluate the oil valorization process by *Serratia marcescens* N2. In the present study, oil valorization reached its peak within 2 weeks, while oil consumption by *P. aeruginosa* PG1 was recorded to reach almost the same value after the fifth week of incubation with only 2% (v/v) crude oil added to MSM(Ganesh & Lin, 2009) This means that our result is very promising since the initial oil added was 20% (v/v) of the culture media, this means that *Serratia marcescens* N2 can valorize pre-used frying oil into another product and therefore reducing waste oil. Although gamma radiation was previously reported by some researchers to induce hyper producing bacterial mutants (El-Housseiny, Aboshanab, Aboulwafa, & Hassouna, 2019)yet in the current study, we used gamma radiation as a tool to enhance biorecovery and decrease the number of steps required for biosurfactant biorecovery. This is considered economic in the bioprocess of biosurfactant production since the steps involves acid precipitation overnight, refrigeration and centrifugation (Phulpoto et al., 2020), the yield obtained is dependent on the amount of biosurfactant released in the cultivation media. Our results indicate that *Serratia marcescens* N2 produces high yield of biosurfactant that increased with the exposure of cell cultures to gamma radiation, the highest yield was obtained at 1000 Gy. This result is higher than that reported by (Phulpoto et al., 2020) who obtained lower yields that were more than half what we obtained even after optimization of cultivation conditions. The use of gamma radiation did not incur any structural changes except in the peptide peak after exposing the cells to 2000 Gy, this change can be attributed to the effect of gamma radiation on the peptide fragment of the biosurfactant(Blanco et al., 2018)reported fragmentation as one of the effects exerted by ionizing radiation on proteins. This result confirms that exposure of cell culture to 1000 Gy can result in yield increase with no structural changes or functional changes. In a previous study, 1000 Gy was enough to induce permeability of *Bacillus* sp. producing biosurfactant(Selim et al., 2020) their results showed that this dose is enough to induce changes without compromising the bacterial viability. The obtained results confirm that low dose gamma radiation can be used for enhancing biorecovery without affecting biosurfactant structure and characteristics.

The detergency process occurs through the formation of micelles by surfactants that are able to form globules of impurities through a decrease in interface tension and with the help of electrostatic interactions between charges However; surface-active compounds cannot completely clean dirt from the surface without the presence of other additional compounds as support, namely builders, anti-redeposition, enzymes, and other additives(Helmy, Gustiani, & Mustikawati, 2020).stated that washing results that contain only 20% active ingredient in the form of biosurfactant are visually not good enough to clean stains (mild stain can be seen visually on the fabric). It is noteworthy to say that using the biosurfactant did not affect the fabric cloth threading as indicated by the captured images of clean, soiled and washed fabric cloths. Chemical surfactants were long ago reported to induce skin irritation(Wilhelm, Freitag, & Wolff, 1994), this is why the use of a biological based surfactant was sought to avoid any dermal effects. The biosurfactant under study was tested by applying it in a single dose to

the skin of experimental mice, untreated skin areas of the test animal served as the control. The degree of irritation/corrosion is read and scored at specified intervals and is further described in order to provide a complete evaluation of the. Our results indicate that all tested mice showed no redness or spots and this confirms that biosurfactant produced by *Serratia marcescens* N2 is safe to use on skin in addition to its efficiency as a biode detergent.

In conclusion, Biosurfactant production using pre-used oil is considered a step forward in achieving biocircular economy, it can be used as an alternative carbon source while at the same time reduce pre-used oil waste. *Serratia marcescens* N2 was able to produce an efficient biosurfactant with powerful surface tension reducing properties using 20% pre-used cooking oil. The biosurfactant was used as a biode detergent without causing any skin irritation. The use of gamma radiation is considered practical since it reduced the number of extraction steps and can be easily applied in down streaming process of biosurfactant production. Using low dose gamma radiation provided a single step for biorecovery, this can be introduced to production lines as an alternative to multiple step biorecovery. This work is to be continued for up-scale production of biosurfactant.

Declarations

Ethics approval and consent to participate

This work was approved by the Research Ethics Committee in the National Center for Radiation Research and Technology (REC-NCRRT), Egyptian Atomic Energy Authority, Cairo-Egypt, following the 3Rs principles for animal experimentation (Replace, Reduce and Refine). REC-NCRRT is organized and operated according to CIOMS and ICLAS International Guiding Principles for Biomedical Research Involving Animals 20212. Serial Number of the protocol 47/A/21.

Consent for publication

All authors agree to publish the following manuscript

Availability of data and materials

The obtained data will be available upon request.

Competing interests

The authors declare no competing interest

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Authors' contributions

Nora El Kenawy was responsible for conceptualization, experimental and data plotting, writing and editing. Ola M. Gomaa was responsible for conceptualization, data plotting, writing and final editing.

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Tables

Table 1. Screening of different *Serratia marcescens* strains for biosurfactant production, comparison was performed using surface tension

Strain	Surface tension (mN/m)
<i>Serratia marcescens</i> N2	29.8
<i>Serratia marcescens</i> MN2	36.6
<i>Serratia marcescens</i> MN3	33.3
<i>Serratia marcescens</i> MN4	38.9
<i>Serratia marcescens</i> MN5	33

Table 2. Identity percentage of serrawettin synthetase as compared to other genes in the public database

Gene from NCBI Library	% identity
<p><i>Serratia marcescens</i> swrW gene for putative serrawettin W1 synthetase, complete cds</p> <p>GenBank: AB193098.2</p> <p>GenBank Graphics</p> <p>>AB193098.2 <i>Serratia marcescens</i> swrW gene for putative serrawettin W1 synthetase, complete cds</p>	99%
<p><i>Serratia marcescens</i> strain N4-5 serrawettin W1 synthetase gene, partial cds</p> <p>GenBank: EF122074.1</p> <p>GenBank Graphics</p> <p>>EF122074.1 <i>Serratia marcescens</i> strain N4-5 serrawettin W1 synthetase gene, partial cds</p>	100%
<p><i>Serratia marcescens</i> strain ATCC 274 serrawettin W1 synthetase gene, partial cds</p> <p>GenBank: EF122077.1</p> <p>GenBank Graphics</p> <p>>EF122077.1 <i>Serratia marcescens</i> strain ATCC 274 serrawettin W1 synthetase gene, partial cds</p>	100%
<p><i>Serratia marcescens</i> strain A88copa13 serrawettin W2 gene, partial cds</p> <p>GenBank: JX667980.1</p> <p>GenBank Graphics</p> <p>>JX667980.1 <i>Serratia marcescens</i> strain A88copa13 serrawettin W2 gene, partial cds</p>	No match
<p><i>Serratia liquefaciens</i> serrawettin synthase (swrA) gene, partial cds</p> <p>GenBank: AF039572.1</p> <p>GenBank Graphics</p> <p>>AF039572.1 <i>Serratia liquefaciens</i> serrawettin synthase (swrA) gene, partial cds</p>	No match

Table 3. Characterization of biosurfactant

Feature	Surface tension (mN/m)	Oil spreading	El ₂₄ %	Zeta potential	CMC (mg/ml)
Value	25.7	+++	90	-22	2

Figures

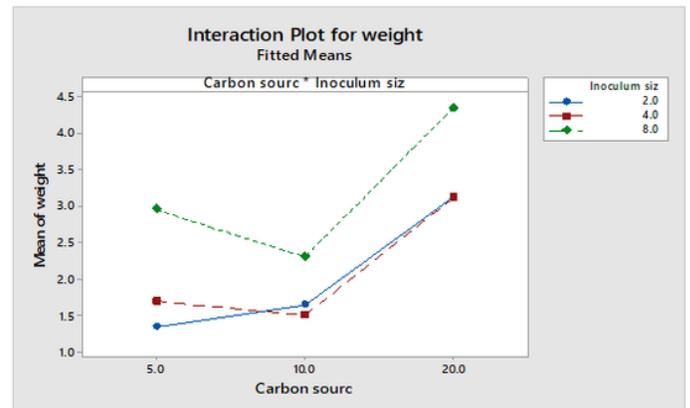
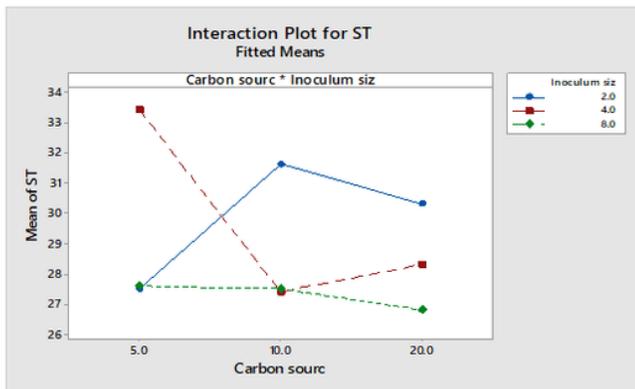
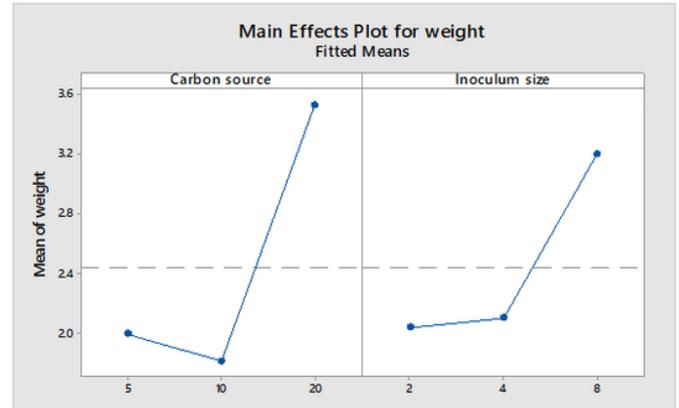
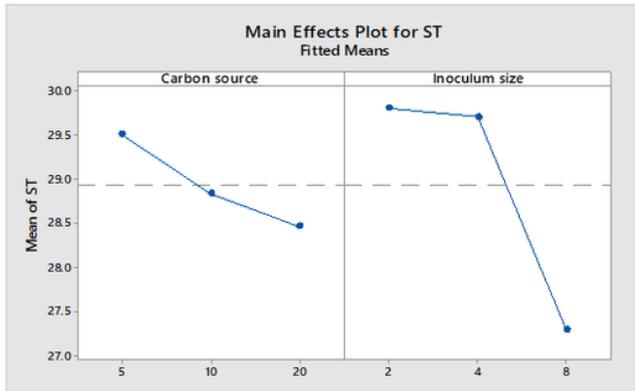


Fig. 1a

Fig. 1b

Figure 1

a: Main effects and interaction plots for surface tension of produced biosurfactant under conditions applied through factorial design. b: Main effects and interaction plots for surface tension and weight of produced biosurfactant under conditions applied through factorial design.

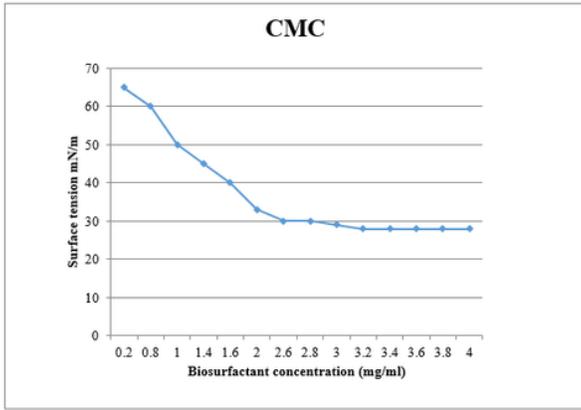


Fig. 2a

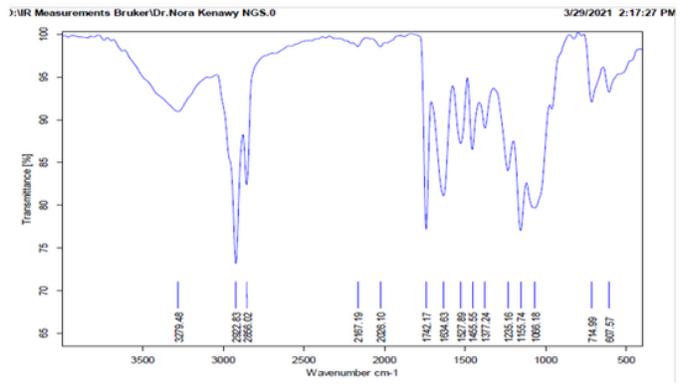


Fig. 2b

Figure 2

a: CMC of the crude isolated biosurfactant b: ATR-FTIR spectrum of *Serratia marcescens* N2 biosurfactant.

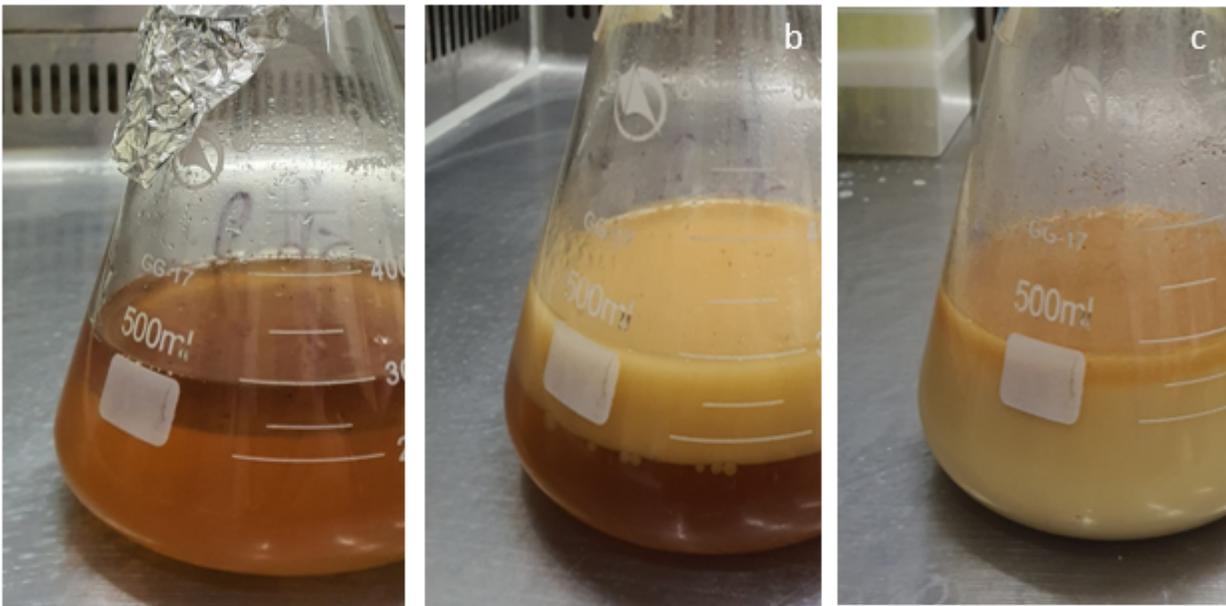


Fig. 3

Figure 3

Oil valorization steps for *Serratia marcescens* N2 at zero time incubation (a), 1h (b) and 6 days (c).

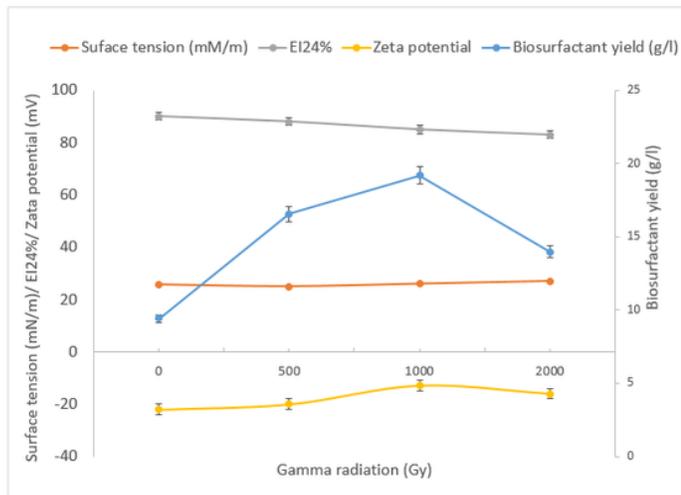


Fig. 4a

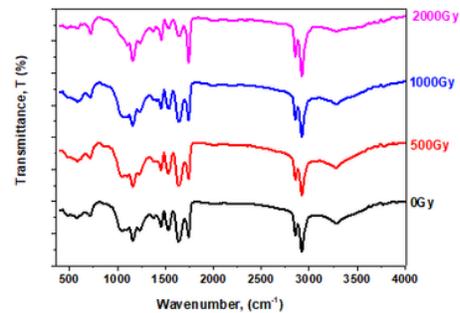


Fig. 4b

Figure 4

a: Biosurfactant yield, surface tension, EI24% and Zeta potential of biosurfactant recovery after exposing *Serratia marcescens* N2 cultures to gamma irradiation. b: ATR-FTIR spectrum of *Serratia marcescens* N2 biosurfactant structure after extraction using different gamma radiation doses.

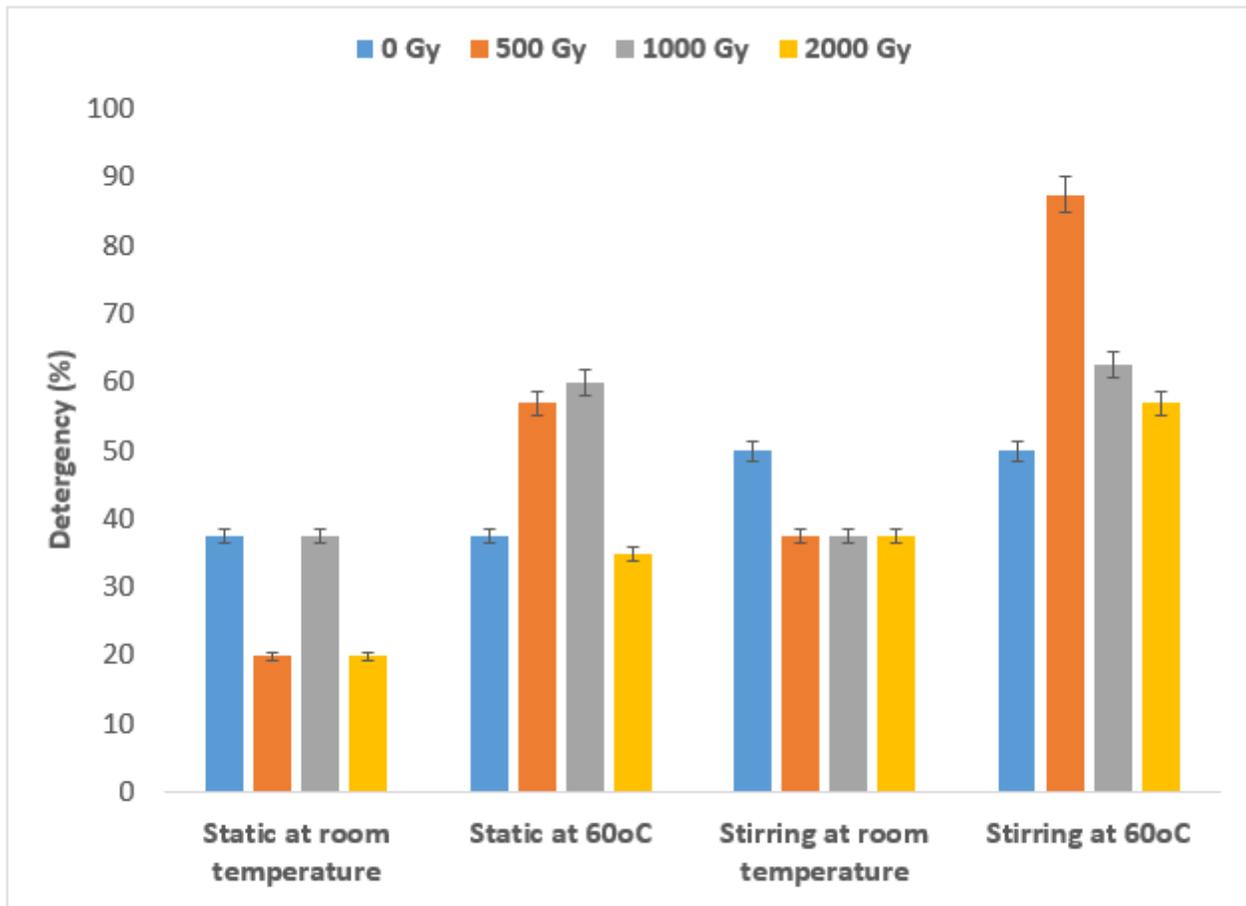


Fig. 5

Figure 5

Effect of using gamma irradiation on *Serratia marcescens* N2 biosurfactant detergency (%) under static and stirring conditions at room temperature and 60oC.

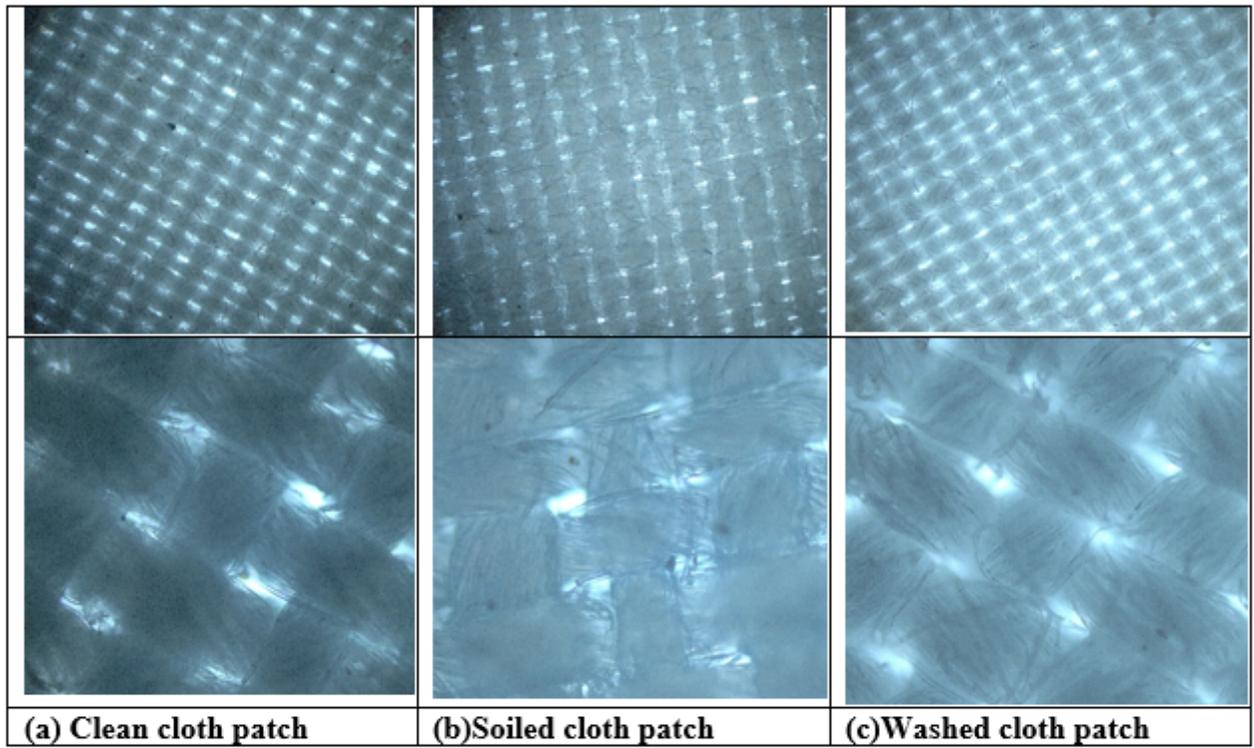


Fig. 6:

Figure 6

Optical images at magnification 25X (upper row) and 100X (lower row) representing clean cloth patch (a), soiled cloth patch (b) and soiled patch washed with *Serratia marcescens* N2 500 Gy assisted biosurfactant at 60oC and stirring conditions (c).



Fig. 7

Figure 7

Images representing the effect of *Serratia marcescens* N2 biosurfactant on skin of mice after 24 h application of cotton pad soaked with 1ml of biosurfactant (b) as compared to water soaked cotton pad (a)

Supplementary Files

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