E-ISSN: 2664-6773
P-ISSN: 2664-6765
Impact Factor: RJIF 5.72
IJCBS 2025; 7(2): 102-105
www.chemicaljournal.org
Received: 15-06-2025
Accepted: 18-07-2025

Fatima Kareem Shandookh
Department of Biology, College of Science, University of Wasit, Ministry of Higher Education, Wasit, Iraq

Corresponding Author:
Fatima Kareem Shandookh
Department of Biology, College of Science, University of Wasit, Ministry of Higher Education, Wasit, Iraq

FTIR characterization of biosurfactants that produced from *Bacillus subtilis* isolated from wastewater samples contaminated with organic pollutants

Fatima Kareem Shandookh

DOI: https://www.doi.org/10.33545/26646765.2025.v7.i2b.158

Abstract
This study examined FTIR analysis of a biosurfactant made by *Bacillus subtilis*, by using wastewater samples contaminated with organic substances collected from the study area near the Al Ahdeb oil field is situated south of the Al Ahrar District in the Wasit Governorate/ Iraq. These water samples used for isolation of *Bacillus subtilis* and this bacterium identified according to biochemical tests such as starch hydrolysis, gelatin liquefaction, H_2S production, catalase test and acid and gas production. The results of biochemical tests confirmed that the bacterial species belongs to *Bacillus subtilis*.
Bacillus subtilis used for production of biosurfactant when used in decomposing of organic substances in water samples. FTIR spectroscopy of biosurfactant done by (400-4000) wavenumbers and confirmed that the biosurfactant contains a lipopeptide structure, protein residues and alkyl groups and including different chemical compounds appeared at various wavelengths.

Keywords: *Bacillus subtilis*, biosurfactant, ftir analysis, organic wastewater

1. Introduction

Although water covers around 70% of the Earth's surface, just 2.5% of it is freshwater suitable for terrestrial life, despite the fact that water is necessary for life. These days, living circumstances, industry need, and population growth are all contributing to the ongoing rise in water consumption. Nowadays, industrialized nations' societies understand the value of clean water, particularly in Western nations (Kristensen *et al.*, 2018) [14].
The extensive use of chemicals in daily life and unfettered access to chemicals have led to a rise in waste generation and a significant release of both common and novel organic compounds into the environment (Peteffi *et al.*,2019) [15]. Unchecked actions by humans and natural processes like dust deposition, evaporation, and bedrock weathering cause changes in the quality of water (Saud, 2014) [19].
Today, the most significant source of environmental pollution is linked to the irresponsible use of the planet's resources and excessive dependence on fossil fuels, which have led to major environmental issues such soil, surface water, and groundwater contamination (Erickson, 2014) [8].
Investigating microbial communities in aquatic environments has great potential to uncover novel and possibly useful chemical compounds with uses in environmental management and agriculture (Baker *et al.*, 2013) [3].
Major constituents of aquatic bacterial communities include species *like Bacillus subtilis* and *Bacillus cereus*, *Bacillus* species' ecological relevance and capacity for harsh circumstances are highlighted by their existence in these types of environments (Emon *et al.*, 2024) [7].
For instance, *Bacillus subtilis* is known for producing alkaline proteases and amazing biosurfactants, both of which are widely used in many different environmental management (Emon *et al.*, 2024) [7].
Biosurfactants are composed of a variety of compounds, including, glycolipids, phospholipids, fatty acids and lipoproteins. Lipopeptide biosurfactants are typically produced by *Bacillus* species (Gudiña *et al.*, 2013) [11].
FTIR spectroscopy has become a powerful tool for thoroughly examining changes in molecular structure because it can detect even the smallest changes in bond lengths and angles in large molecules (Garip *et al.*, 2009) [9].

Materials and Methods

Study Area Location

The Ahdab oil field is located in the southern part of Al-Ahalal District, Wasit Province, Iraq (Hassan, 2018) [12].

The discharge of organic pollutants into the Tigris River has surged due to the expansion of the Al-Ahdab oil field and industrial waste.

Bacillus subtilis isolated from wastewater samples collected from the study area.

The decomposition effect of *Bacillus subtilis* biosurfactant on organic substances evaluated using FTIR spectrum

Water Samples Collection

Collect one liter of each water sample into a sterile glass container and store on ice before transporting to the laboratory or storing in a refrigerator at 4 C°, to isolate bacteria resistant to organic pollutants (Gudiña *et al.*, 2013) [11].

(10 ml) of sample was serially diluted and inoculated onto nutrient agar plates, incubated at 37°C for 24 hours. Colonies with different properties were then screened through repeated injections.

Bacillus subtilis isolated from wastewater contaminated with organic pollutants was diluted (10^{-1} to 10^{-7}) and inoculated onto nutrient agar (NA) plates and incubated at 30°C for 24 h. (Santos *et al.*, 2019) [17].

Biochemical properties of *Bacillus subtilis*

After isolation, cell morphology was examined using Gram staining under a light microscope, and B. subtilis biochemical properties were examined, including catalase test, starch hydrolysis, acid and gas production, gelatin liquefaction.

Finally, for the current study, isolates exhibiting rod-shaped, Gram-positive endospore formation were selected to break down organic substances (Sarode *et al.*, 2019) [17, 18].

Production and FTIR Characterization of Biosurfactants

- Conical flask contains 250 ml mineral salt medium consists of $MgSO_4$, NaCl, glucose and $(NH_4)_2SO_4$ and this medium contained wastewater sample injected with inoculum of *Bacillus subtilis* (8 x 10^9 cells) at 35 °C and 200 rpm for seven days.
- *Bacillus subtilis* culture was centrifuged to separate the biosurfactant, and the supernatant was precipitated by adding concentrated HCl overnight at 4°C until the final pH reached 2.0, thereby precipitating lipids and proteins (Santos *et al.*, 2019) [17].
- Bacterial cells were extracted from the liquid culture by centrifugation at 15,000 rpm for 15 minutes. After removing the supernatant, the bacterial pellet was washed twice with phosphate buffer. The material used for FTIR spectroscopy was washed again in phosphate buffer and stored at 20°C until freeze-dried (Garip *et al.*, 2009) [9].
- (2 mg) of partially purified biosurfactant was mixed with 150 mg of KBr and the mixture was compressed into tablets under dry conditions. The biosurfactant samples were then by using a Shimadzu spectrophotometer, biosurfactant analyzed by Fourier transform infrared spectroscopy (Aparna *et al.*, 2012). FT-IR spectra were measured in the frequency range of 400-4000 wavenumbers (Aparna *et al.*, 2012) [2].

Result and Discussion

Isolation and Identification of *Bacillus subtilis*

According to the strain's morphological test results, they are rod bacteria that generate spores and are Gram-positive.

Table (1) shows the primary biochemical features of the isolate under study.

Table 1: Biochemical properties of *Bacillus subtilis*

Biochemical properties	The result
Catalase test	+
Starch hydrolysis	+
Acid and gas production	+
Gelatin liquefaction	+
H2S production	+

Because of its extraordinary ability to produce a broad range of physiologically active compounds, *Bacillus subtilis* stands out as a bacterium of major industrial value. Numerous strains of this bacteria have been obtained by researchers from a variety of environmental sources, and each one exhibits distinct characteristics that underscore its potential uses in a range of industrial fields (Anand *et al.*, 2025) [1].

The water samples in the study area characterized by the high level of turbidity in water samples indicates that the water is not pure due to waste from oil fields and agricultural processes.

FTIR Analysis of Biosurfactants

- Except for optical isomers, compounds with various structures do not have the same FTIR spectra; as a result, distinct chemical fingerprints can be readily distinguished from other molecules' absorption patterns (Garip *et al.*, 2009) [9].
- FTIR spectroscopy was used to examine the functional groups of the biosurfactant generated by this isolate in order to further verify that the biosurfactants were of the glycolipid and lipopeptide types.
- *Bacillus subtilis* biosurfactants were found to possess substantial levels of alcohol and carboxylic acid when a preliminary evaluation of these substances was conducted. FTIR spectra shows that there are several absorption peaks,
- The figure (1) shows FTIR analysis of biosurfactants that produced from *Bacillus subtilis* isolated from wastewater samples.
- According to figure (1), alcohol's O-H bending is demonstrated by strong adsorption bands seen between 3878 and 3761 cm^{-1}.
- Around 3244 cm^{-1}, the FT-IR spectra of *Micrococcus luteus* exhibited the distinctive stretching vibration band of O-H (Yilmaz *et al.*, 2009) [21].
- (Rani *et al.*, 2022) [16] indicates the presence of hydroxyl group, is seen at 3217.67cm^{-1}
- Figure (1) displays the isolated biosurfactant's infrared spectrum. The N-H bending mostly shown by the band 3406 cm^{-1}. The $N-H_2$ bending concentrated in the band 3267 cm^{-1}. It proved that the structure contained protein residues.
- There was distinctive peak at 3300 cm−1, which results from the stretching vibrations of peptides N-H and O-H (Kong and Yu, 2007) [13].
- Yilmaz *et al.*, (2009) [21]. examined the separation and description of a biosurfactant that *Micrococcus luteus* (cocci) and *Burkholderia cepacian* (bacillus) produces. According to the FTIR spectrum, the biosurfactant contains a lipopeptide structure, protein residues and alkyl groups.

- The CH*3* bonds are identified by the bands with centers at 2924 cm^{-1} and 2862 cm^{-1}. The distinctive stretching vibration band of COOH was present in the FTIR spectrum of *Bacillus subtilis* at about 1649 cm^{-1}.
- At this point the C-H stretching bands of CH_2 and CH_3 alkyl groups were detected at 1446 cm-1. It indicated that aliphatic chains were present. C-H stretching bands comprising CH_2 and CH_3 alkyl groups and -C=O ester groups, respectively, were seen in the 1411 cm^1 and 1271 cm^1 areas (Yilmaz *et al.*, 2009) [21].
- This result agreement with (Yalcin and Cavusoglu, 2010) [20] founded that the CH_2 bending is mostly shown by the band centered at 1450-1455 cm^{-1}.
- The peaks at around 1101 and 1037 cm−1 in the *Bacillus subtilis* spectra were associated with stretching vibrations of −C=O.
- It was discovered that the (C-O-C) stretching of polysaccharides is linked to the region between 1200 and 950 cm^{-1}. (Dean *et al.*, 2010) [5].
- Esters' C-O-C vibrations are due to the peaks at 1238 and 1118 cm^1. (Das, *et al.*,2008) [4].

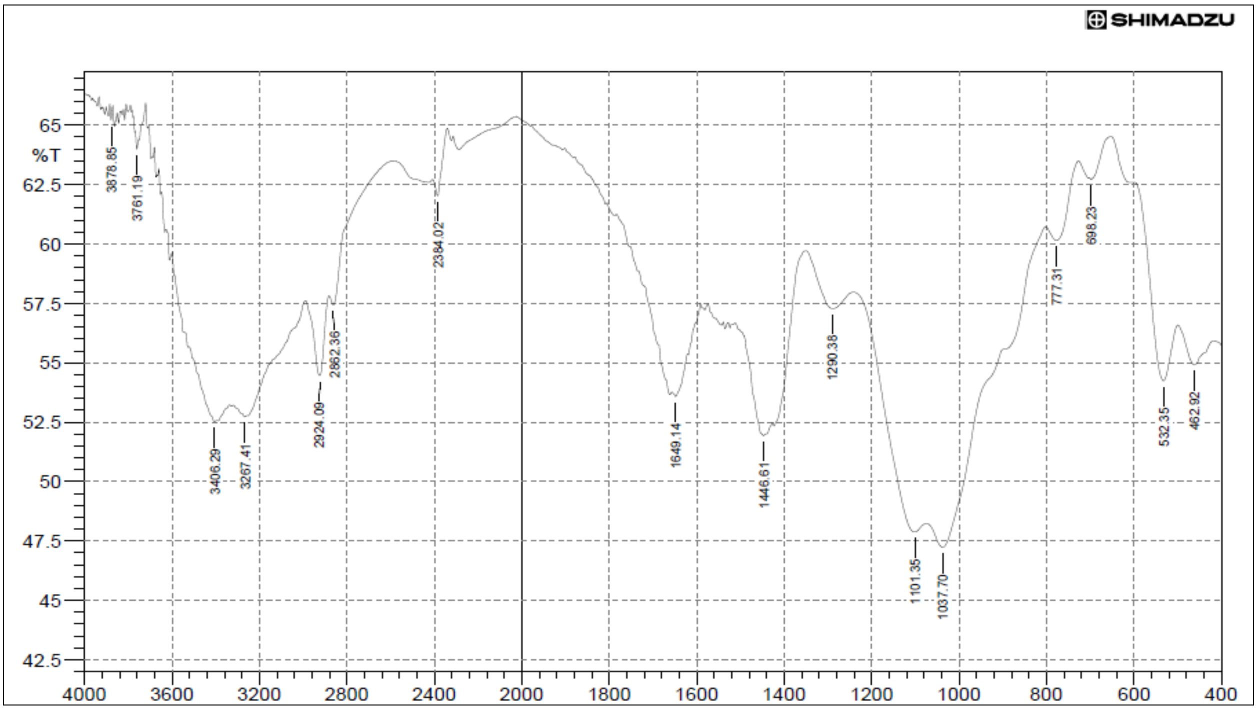

Fig 1: Shows FTIR analysis of biosurfactants that produced from *Bacillus subtilis* isolated from wastewater samples.

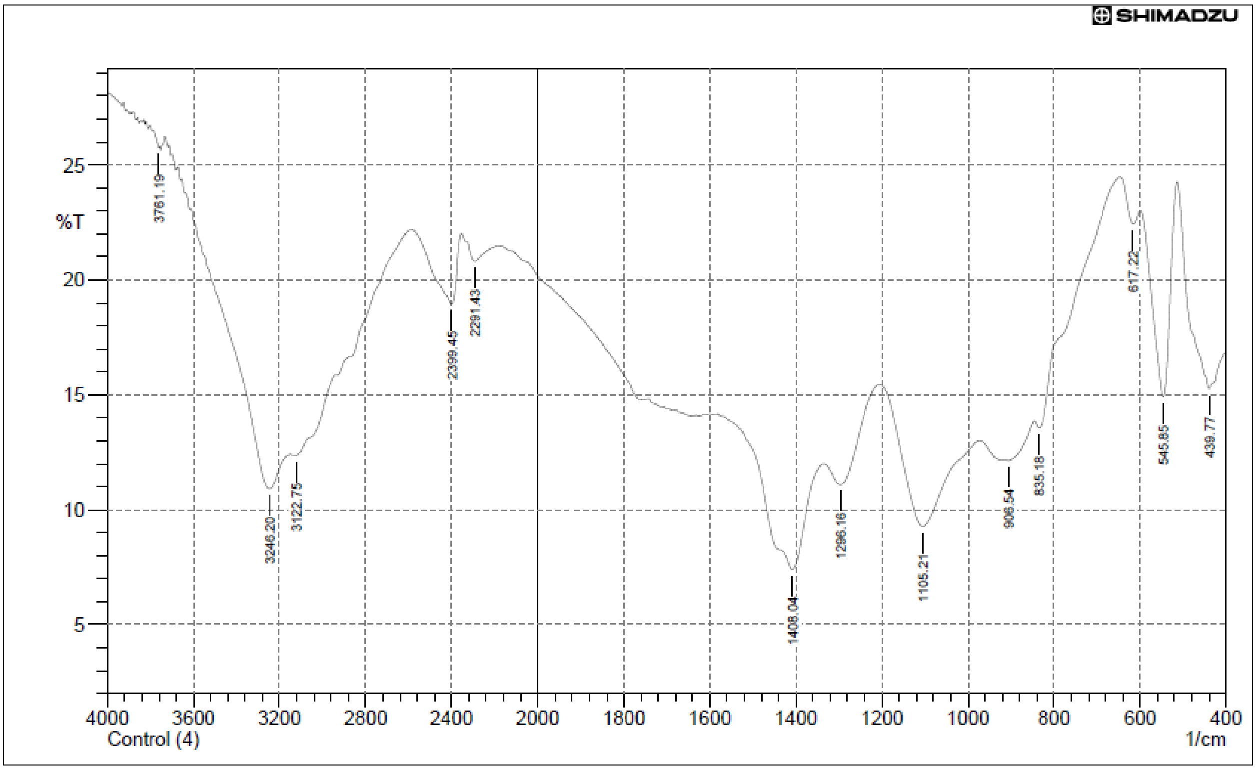

Fig 2: FTIR analysis of control sample

Fast and simultaneous characterization of many functional groups, including lipids, proteins, nucleic acids, and polysaccharides, is made possible by FTIR (Dogan *et al.*, 2007) [6].

Surface tension is decreased by the interaction of surfactants with phases of varying polarity, such as oil-water and air-water. The presence of microbial surfactants is one of the elements that can result in a decrease in surface tension (Yilmaz *et al.*, 2009) [21]. FT-IR spectra were used to confirm the functional groups of biosurfactants, indicating the potential existence of amino, carboxylic, hydroxyl, and carbonyl groups of bacterial isolates *Micrococcus luteus* (cocci) and *Burkholderia cepacian* (bacillus) (Yilmaz *et al.*,2009) [21].

Conclusion

The results indicate that biosurfactant obtained from *B. subtilis* has different chemical compounds appeared at various wavelengths, and the study reveals the value of FTIR spectroscopy in determining the bonds found in biosurfactant.

References

1. Anand SS, Nair, BG, Sadasivan Nair S, & Gopalakrishna Pai, J. Proteases from marine endophyte, *Bacillus subtilis* ULB16: Unlocking the industrial potential of a marine-derived enzyme source. Biocatalysis and Agricultural Biotechnology, 2025;64:103503.
2. Aparna A, Srinikethan G, & Smitha H Production and characterization of biosurfactant produced by a novel Pseudomonas sp. 2B. Colloids and Surfaces B: Biointerfaces, 2012;95:23-29.
3. Baker S, Harini BP, Rakshith D, & Satish S. Marine microbes: invisible nanofactories. Journal of Pharmacy Research, 2013;6(3):383-388.
4. Das P, Mukherjee S, & Sen R. Antimicrobial potential of a lipopeptide biosurfactant derived from a marine Bacillus circulans. Journal of applied microbiology, 2008;104(6):1675-1684.
5. Dean AP, Sigee DC, Estrada B. and Pittman, Jk. Using FTIR spectroscopy for rapid determination of lipid accumulation in response to nitrogen limitation in freshwater microalgae. Bioresource Technology. 2010;(101):4499-4507.
6. Dogan A, Ergen K, Budak F, & Severcan F. Evaluation of disseminated candidiasis on an experimental animal model: a Fourier transform infrared study. Applied spectroscopy, 2007;61(2):199-203.
7. Emon TH, Hakim A, Chakraborthy D, & Azad AK. Enhanced production of dehairing alkaline protease from *Bacillus subtilis* mutant E29 by consolidated bioprocessing using response surface modeling. Biomass Conversion and Biorefinery, 2024;14(16):19501-19517.
8. Erickson J. Environmental geology: facing the challenges of our changing earth. Infobase Publishing. 2014
9. Garip S, Gozen AC, & Severcan F. Use of Fourier transform infrared spectroscopy for rapid comparative analysis of Bacillus and Micrococcus isolates. Food Chemistry, 2009;113(4):1301-1307.
10. Garip S, Gozen AC, & Severcan F. Use of Fourier transform infrared spectroscopy for rapid comparative analysis of Bacillus and Micrococcus isolates. Food Chemistry, 2009;113(4):1301-1307.
11. Gudiña EJ, Rangarajan V, Sen R, & Rodrigues LR. Potential therapeutic applications of biosurfactants. Trends in pharmacological sciences, 2013;34(12):667-675.
12. Hassan RAA. The Impact of Al Ahdeb Oil Field on The Environment Pollution in Al Ahrar District, Wasit Governorate, Iraq (Doctoral dissertation, M. Sc. Thesis. College of Science, University of Baghdad, Iraq); 2018.
13. Kong J, & Yu S. Fourier transform infrared spectroscopic analysis of protein secondary structures. Acta biochimica et biophysica Sinica, 2007;39(8):549-559.
14. Kristensen P, Whalley C, Zal FNN, & Christiansen T European waters assessment of status and pressures; 2018.
15. Peteffi GP, Fleck JD, Kael IM, Rosa DC, Antunes MV, & Linden R. Ecotoxicological risk assessment due to the presence of bisphenol A and caffeine in surface waters in the Sinos River Basin-Rio Grande do Sul-Brazil. Brazilian Journal of Biology, 2019;79:712-721.
16. Rani R, Kumar S, Munjal N, & Kamboj U. May). Characterization of ground water using spectroscopic techniques. In Journal of Physics: Conference Series IOP Publishing. 2022;2267(1): 012022).
17. Santos ECLD, Miranda DADR, Silva ALDS, & López AMQ. Biosurfactant Production by Bacillus strains isolated from sugar cane mill wastewaters. Brazilian Archives of Biology and Technology, 2019;62:e19170630.
18. Sarode CA, Bramhankar SB, Kakad SA, Labhasetwar AA, Bhure SS, Isokar SS, & Tathod DG. Biochemical and physiological characterizations of *Bacillus subtilis*. Int J Chem Stud, 2019;7(1):1957-1960.
19. Saud QJ. Effects of selected contaminants on the physical, chemical, and geotechnical properties of aquifer solid. University of Missouri-Kansas City; 2014.
20. Yalcin E, & Cavusoglu K. Structural analysis and antioxidant activity of a biosurfactant obtained from *Bacillus subtilis* RW-I. Turkish Journal of Biochemistry-Turk Biyokimya Dergisi, 2010;35(3):243-247.
21. Yilmaz F, Ergene A, Yalçin E, & Tan S. Production and characterization of biosurfactants produced by microorganisms isolated from milk factory wastewaters. Environmental technology, 2009;30(13):1397-1404.