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Combination Treatment of Biosurfactant and Copolymer Alkyl acrylate -Vinyl acetate to Improve the Cold Flow Properties of Waxy Gas

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Abstract

One promising bacterial strain (B3), recovered from petroleum-contaminated soil. The isolate was able to produce biosurfactant. Alkyl acrylate–vinyl acetate copolymer was prepared using varying lengths of the alkyl chain. In this study, the effect of copolymer additives along with biosurfactant on the crude oil wax crystallization method has been examined by solid point test and viscosity index measurement for gas oil. The FTIR spectrum analysis of the biosurfactant showed that it was a polymeric molecule. The application of only copolymer having a side-length of the chain of C_{13} and C_{22} reduced the solid point of gas oil by 3 and 9 ^{o}C respectively. Furthermore, the same copolymer along with biosurfactant decreased the solid point of gas oil by 15 and 21 ^{o}C, respectively. Many copolymer additives blended with biosurfactants, it is assumed that it might be used to slow the formation of wax crystals and shape shorter crystals. Because of this shift in crystal form, wax crystals' capacity to intergrow and interlock is substantially reduced. By increasing the length of the alkyl chain and mixing the copolymer and biosurfactant, the viscosity index of gas oil was raised. The blend P3 and P4 additives were also proved to a better viscosity index modifier at all concentrations in gas oil.

Keywords: Biosurfactant; Copolymer; point depressants; Viscosity index improvers; Wax precipitation

1. Introduction

Gas fuel is a petroleum product and generally consists of mixtures of hydrocarbon chains that vary from C_8 to C_{30}. Reduce wax crystal growth and create smaller crystals with a higher melting point. As the temperature drops, paraffin crystals form, generating a crystalline net that traps the remaining fuel in cage-like forms, causing cold flow issues [1]. The fluid flow characteristics of diesel fuel are weak in cold regions or at very low temperatures due to paraffin deposition in the fuel. A little volume of additives is known as pour-point depressants (PPDs). Most of these additives are considered to develop small shaped wax crystals by a number of different mechanisms, including nucleation, adsorption, co-crystallization, and an increase in wax solubility [2]. The traditional PPDs are mainly homo and co-polymers of different monomers [3]. The initial formation of the crystals is not altered by most PPDs, so they often do not influence the cloud point. Rather, the manufacture of PPDs according to different gas oil types has been examined using a general technique to illustrate the modification of wax crystals [4]. Several types of polymers have been produced, such as PPDs to impact the paraffin crystalline formation behavior [5]. Wax-alkylated naphthalenes, polymethacrylates, phenols, and styrene-ester copolymers are all examples of PPDs [6].

The viscosity index (VI) is a spot number that represents the fuel's resistance to viscosity transition with temperature.The chemical compounds that increase the viscosity index of fuel oils are known as viscosity index improvers. This addition changes the lubricant viscosity as the temperature varies. All commercially available essential VI improvers' are usually essential oil-soluble organic polymers. To be considered a good VI increasing additive, a chemical must not only have a beneficial effect on the viscosity index of new engine oil, but also maintain steady and gratify its VI improving the performance of an engine or other device in use. Furthermore, it is desirable that the VI boosting additive has a positive impact on the pour point of the fuel oil into which it is mixed, or at the very least that it has no negative impact on the pour point [7].

Treatment of waxy gas oil with pour point depressants is increasingly adopted by industry operators as an efficient, cost-effective method for

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managing flow assurance problems resulting from wax formation [8]. Pour point depressants interact with waxes, inducing changes in crystal morphology which culminate in increased oil flowability [9]. The majority of reported pour point depressants/flow improvers are high molecular weight polymeric compounds. High cost, well specificity, and eco-toxicity of conventional PPDs, are motivating factors for recent research aimed towards developing cheaper, eco-friendly, renewable alternatives through interaction of copolymer additives with biosurfactants.

The secondary metabolites and amphiphilic molecules consisting of both surface-active hydrophilic and hydrophobic moieties are biosurfactants [10]. These accumulate at the interface between two immiscible fluids, such as oil and water, to reduce surface and interfacial tensions [11]. Many microorganisms, including mould, yeast, and bacteria, such as *Pseudomonas, Klebsiella, Acinetobacter, Arthrobacter, Alcaligenes, Bacillus, Enterobacter,* and *Stenotrophomonas*, produce biosurfactants [12, 13]. Surface and interfacial tension between two molecular phases, such as liquid-liquid, solid-liquid, or liquid-gas, can be reduced by microbial cells [14, 15]. They have a method for altering the physical and chemical characteristics of a fluid, allowing them to deterge, lubricate, foam, and solubilize, as well as emulsify and reduce liquid viscosity [16]. As pour point depressants for crude oil, the fluids combine with any polymeric additive better than those of polymeric additives alone [17]. As a possible replacement for chemical surfactants, attention to biosurfactants has increased significantly today. Microbial surfactants have numerous properties, including temperature stability, pH, salinity stability, biodegradability, low toxicity and high foaming potential, which make them promising in many processing applications [18]. Biosurfactants are divided into five categories: phospholipids glycolipids, lipopeptides, neutral lipids, and polymeric substances [19, 20].

The genus*Stenotrophomonas*is a Gram-negative bacterium that makes a large environmental role in the cycle of elements in nature [21]. The use of this species in bioremediation of soil pollution in the presence of biosurfactant was the goal of some researchers [12, 22].

The current study's goal was to isolate and identify a possible biosurfactant-producing bacterial strain from oil-contaminated soil. The synthesis, characterization, and performance evaluation of a number of polymer flexibility are the subject of the research. Two copolymers–one P1 (NAFOL 20+A acrylate-vinyl acetate) and another P2 (NAFOL 1822C acrylate - Vinyl acetate) were prepared. The prepared copolymers, their blending with produced biosurfactant and the effects of the prepared compounds were studied in the method of wax crystallization of gas oil.

2. Experimental

2.1. Isolation of bacteria that produce biosurfactants

The Egyptian petroleum research institute (EPRI) supThe Egyptian petroleum research institute (EPRI) supplied the oil-contaminated soil for isolating the biosurfactant-producing bacteria. The soil sample was collected in a plastic bag and delivered for isolation and screening of various bacterial strains producing biosurfactants to the laboratory. One gram of soil was mixed with ten ml of distillate water and incubated for two h at 30 ^{o}C.Biosurfactant production was carried out in a minimal salt medium (MSM) containing (g/L): $MgSO_4 \cdot 7H_2O$, 0.5; KH_2PO_4, 0.5; KCl, 0.1; NH_4NO_3, 4.0; K_2HPO_4, 1.0; NaCl, 2.0; $CaCl_2$, 0.01 and $FeSO_4 \cdot 7H_2O$, 0.01. With the solution of NaOH and HCl, the pH was modified to 7.0 using 2% glucose as a substrate. The 250 ml conical flasks were inoculated with 100 ml of MSM broth. The inoculated sample flasks were then incubated for 72 h at 150 rpm in a shaking incubator at 30 ± 2 °C during agitation. The media without inoculum was taken as a control sample at constant culture conditions [21]. The bacterial colonies formed on the plates were selected for further study.

For screening the production of biosurfactants, each bacterial isolate was inoculated into the nutrient media and shaken for 18-24 h at 150 rpm at 30 ^{o}C. Five ml of each bacterial culture was added separately to 100 ml of MSM, supported with 2 % glucose as a sole carbon source. In a shaking incubator at 30 ^{o}C for 72 h, the inoculated flasks were agitated. Cultural supernatants were separated by centrifugation of different inoculated flasks at 10,000 xg for 15 min after the incubation time. A drop displacement test, surface tension, and emulsification assay were performed to screen biosurfactant-producing bacterial isolates [23]. The highest biosurfactant-producing bacterial isolate was selected for further identification.

2.2. The surface activity of the produced biosurfactant

2.2.1. A drop oil displacement test

In this procedure, a 15 cm petri dish was filled with 40 ml of distilled water. After that, 2-3 ml of crude oil was dropped. After the stable oil film was formed, 500 µl of the cell-free supernatant was put to the middle of the film center. The diameter of the transparent regions on the oil's surface was calculated.

2.2.2. Surface tension

A ring tensiometer (Krüsstensiometer K6) was used to evaluate the surface tension of the biosurfactant formed by various bacterial isolates using a broth supernatant solution (20 ml) at 30 ^{o}C [24, 25].

2.2.3. Emulsification index

For the emulsification assay, the various bacterial isolates were inoculated in MSM broth for 72 h. The supernatants of the bacterial culture were harvested at 10,000 xg for 15 minutes by centrifugation. A test tube was filled with four ml of cultured supernatant and 6 ml of kerosene. The prepared mixture was stirred at an extremely high speed at room temperature for 2 min and left for 24 h at 30 ^{o}C and the emulsification index percentages were estimated by the following Cooper and Goldenberg equation [26]: (E_{24}) = 100 (height of the emulsion layer/total height).

2.3. Genetic identification of the most promising bacterial isolate

Sigma Scientific Services Co., Egypt, used 16s rRNA to genomically identify the biosurfactant-producing bacterial isolate. The bacterial cell was incubated for 24 h at 30 ^{o}C supplied with 2 % glucose as a sole carbon source on MSM agar media. The Gene Jet genomic DNA purification Kit (Thermo) (Sigma Scientific Services Co., Egypt) protocol was used to extract DNA for 16S rRNA gene amplification. The 16S rRNA genes were amplified using a polymerase chain reaction (PCR) with (5'-AGA GTT TGA TCC TGG CTCAG-3') and (5'-GGT TAC CTT ACG ACT T-3') as global bacterial forward and reverse primers, respectively. The PCR Purification Kit GeneJET^{TM}demands that PCR be cleaned for the manufacture of PCR. The completed PCR mixture was applied to 45 µl of binding buffer. After that, the solution was totally transported from step 1 to the GeneJET^{TM} cleaning column. The mixture was then centrifuged for 30-60s, at > 12000 xg before being released. A 100 l wash buffer was supplied to the GeneJET^{TM} cleaning column, centrifuged for 30-60 seconds, and the flow-through was ignored as the purification column was placed back into the aggregation channel. The mix was centrifuged for a further minute on the empty GeneJET^{TM} purification column to remove any remaining wash buffer. A clean 1.5 ml micro-centrifuge tube was used to transfer the purification column. A 25 µl elution buffer was then supplied to the center of the column membrane, and the column was centrifuged for 1 min before being discarded and the purified DNA stored at -20 ^{o}C. After purification of the PCR products, the DNA sequence of the positive clone was subjected to a similar search, BLAST on the NCBI website (http://www.ncbi.nlm.nih.gov) and deposited with Gen Bank. As references, several relevant 16S rRNA gene sequences having validly exported names were chosen from the Gen-Bank.

2.4. Extraction of the produced biosurfactant

The chosen bacterial strain was grown for 72 h in a biosurfactant production medium (MSM), then centrifuged at 10,000 xg for 15 min at 30 ^{o}C. The cell-free supernatant was acidified to pH 2 with 6N HCl and then kept at 4 °C overnight for deposition. The precipitate was collected by centrifugation at 10,000 rpm for 15 min at 5 ^{o}C to obtain the biosurfactant. It dissolves in deionized water (pH 8.0) to a maximum pH 7. Using chloroform: methanol (2:1), the biosurfactant was extracted [27]. In order to extract the solvent, the organic phase was then transferred to a round-bottom flask connected to a rotary evaporator, yielding a crude biosurfactant [22].

2.5. FTIR analysis for the characterization of biosurfactant

The chemical structure and components of the recovered biosurfactant were determined using FTIR spectroscopic analysis. The sample was made by homogeneous distribution of 1 mg of biosurfactant sample from potassium bromide pellets captured using the built-in plotter on the NicoletIS-10 FTIR spectrometer Infrared (IR) absorption spectrum. The IR spectra were obtained with a resolution of 1cm^{-1} in the range of 500-4000 cm^{-1}[28].

2.6. Determination of the biosurfactant activity

The following techniques were used to describe the performance of the obtained biosurfactant: emulsification power (E_{24}), surface tension (ST), and antimicrobial activity. The activity of emulsification (E_{24}) and surface tension have been discussed above.

2.6.1. Antibacterial activity

The antibacterial activity of the synthesized biosurfactant was tested using the agar diffusion method against Gram negative and positive bacteria, including *Pseudomonas aeruginosa* ATCC 10145, *Escherichia coli* ATCC 23282, *Bacillus subtilis* ATCC 6633, and *Staphylococcus aureus* ATCC 35556. The antifungal action of the produced biosurfactant has also been investigated through agar diffusion technique toward *Aspergillusniger*, IMI-31276, *Candida albicans*, IMRU-3669. The microbial strains used for the above tests were procured from the biotechnology lab, Egyptian Petroleum Research Institute, Cairo, Egypt. For standard drugs for antifungal and antibacterial resistance, Tobramycin and Mycostatin were used. In 5 mg / ml distilled water, all compounds were solubilized. For the antibacterial assay medium, the Luria-Bertani (LB) medium was used and the Wickerham media was used as an antifungal assay. The media were then inoculated with various

microorganisms and poured onto plates and sterilized and cooled to 40 ^{o}C. In all the petri plates, a hole with a diameter of 6 to 8 mm was created using a sterile cork borer.Afterward, the bottom of the hole was sealed with the respective molten media. Into the hole, 0.1 ml of biosurfactant was added. The plates were incubated at 30 ^{o}C for 24 h for bacterial and 25 ^{o}C for 3-5 days for fungal strains. On the studied microbes, the diameter of the inhibitory zone caused by the biosurfactant was assessed [29].

2.7. Materials for preparing of additives

Table 1 shows the standard analysis. The NAFOL 20 + A and NAFOL 1822 C linear saturated long-chain alcohol combinations were purchased from the Condeu Chemical Company in Germany. Vinyl acetate was obtained from Aldrich and, prior to use, purified under reduced strain. Hydroquinone, toluene, p-toluene sulphonic acid (PTSA), dibenzoyl peroxide, and other basic substances were employed as laboratory reagents. The cold flow efficiency of the above created copolymers and when blended with biosurfactant as cold flow improvers was determined for waxy gas oil supplied from waxy western desert crude oil in Cairo, Egypt. The urea adduction method was used to estimate the n-paraffin content of the gas oil composition [30]. Gas-liquid chromatographic (GLC) analysis was performed on the gas-liquid oil samples and their respective n-paraffin content. In this research (Table 2) discusses the physical characteristics of gas oil. The carbon number distribution of n-paraffin and average carbon number of the gas oil sample was measured [31].

2.8. Preparation of additives

2.8.1. Preparation of monomers

The molar ratio of 1.2:1 reaction of acrylic acid and different n-alkyl alcohols (NAFOL 20 + A, n = 13, NAFOL1822C, n = 22) was prepared using toluene as a solvent, PTSA as a catalyst, and hydroquinone as an inhibitor, and water was azeotropically purified using Dean-Stark equipment [4]. After the reaction at 120 ^{o}C for 6 h, the reaction products were washed with a 5% sodium hydroxide (NaOH) solution and then with excess distilled water. The final product contained a bright yellow or milky white latex substance.

2.8.2. Copolymerization of n-Alkyl acrylate with vinyl acetate and blend with the produced biosurfactant

Copolymerization of n-alkyl acrylates with vinyl acetate was performed in a 100 ml four-necked round-bottom flask provided with a mechanical agitator, a condenser, a temperature controller, and a nitrogen-controlled inlet valve. In dry toluene, under the nitrogen atmosphere, the dibenzoyl peroxide (1 % w/w) was used as the initiator and N-alkyl monoester acrylate and vinyl acetate were added in molar ratios of (0.50: 0.50). After the reaction was completed, toluene was distilled at reduced pressure by keeping it under a vacuum for 2-3 h at 80 ^{o}C [32]. The copolymers were precipitated in the methanol overabundance and then removed. The copolymers were precipitated in overabundance methanol and then removed. The copolymers were purified by dissolution in toluene, then precipitation with methanol, filtration, and then dried under a vacuum at 40 ^{o}C. The prepared copolymers were designated as (P1 and P2). Then the blend P1 with biosurfactant designated (P3) and the blend (P2) with biosurfactant designated (P4).

2.9. Evaluation of the prepared copolymers as solidification point (SP) for gas oil

The solidification point SP is the temperature at which the fluidity of the diesel sample is lost [33]. The pour point (PP) is defined as the lowest temperature at which the test sample moves under specified experimental conditions as defined by ASTM D97, 2012. In general, the PP of gas oil is 1–3 °C above the SP or at the SP. In this study, the CFPP and SP were estimated using the GB/T510 method. To eliminate any inconsistencies, the SP determination was confirmed three times, and the average value is taken to represent the final value.

2.10. Estimation of the synthesized compounds as viscosity index improvers for gas oil/paraffin oil

Viscosity index (VI) created additives were tested as viscosity index improvers of gas oil. The kinematic viscosity of the oil containing the synthesized compounds was measured at 40°C and 100°C. The effect of the concentration of the synthesized additive on VI was investigated using different concentrations of a viscosity index test (VI) according to ASTM D2270-87 of 250, 500, 1000, 2000, and 3000 ppm.

Table 1. Typical analysis of linear long chain alcohol blends (NAFOL)

Long chain alcohol blend		
	NAFOL 20+A	NAFOL 1822C
Alcohol composition (wt. %)	C_{16-OH} + C_{18-OH} 18.0 C_{20-OH} 17.0 C_{22-OH} 17.5 C_{24-OH} 5.5 C_{26-OH} 4.5 C_{28-OH} + C_{30-OH} 3.0	C_{16-OH} 0.5 C_{18-OH} 5.0 C_{20-OH} 17.5 C_{22-OH} 76.5 C_{24-OH} 1.5
Average carbon number (calculated)	C_{av} =12.9~ 13	C_{av} = 21.4~21
Density (g/cm^3) approx.	at 80°C = 0.804	at 80°C = 0.803
Solidification point (°C) approx.	54- 58	66
Flash point (°C) approx.	207	205
Ester No. (mg KOH/g)	10.0	0.3
Acid No. (mg KOH/g)	0.1	0.06
Iodine No. (mg I/100 mg)	20.0	0.6
Water (Wt. %)	0.1	0.1

Table 2. Physical properties of waxy gas oil sample

Properties	Methods	Gas oil
Specific gravity at 60/60°F	IP 160/87	0.8568
Kinematics viscosity at 40°C (cSt)	IP 71/80	3.8
Cloud point (cp), °C	IP 219/82	24
Solid point (Sp), °C	GB/T510-2018	19
Sulfur content (wt %)	IP 266/87	0.22
Flash point, °C	IP 34/82 (87)	124
Total paraffins content (wt %)	Urea adduct	20.76
N-paraffin (wt %)	GLC	20.06
Iso-paraffins (wt %)	GLC	0.69

3. Results and Discussion

3.1. Isolation and screening of biosurfactant-producing bacteria

The bacterial population of oil-polluted soil showed a count of 2.8 * 10^8 CFU/g soil from the Egyptian petroleum research institute. Seven separate colonies of bacteria from oil-contaminated soil have been detected. The screening of bacterial isolates for producing biosurfactants and selecting potential biosurfactant producers were determined. The data of biosurfactant screening characteristics are listed in (Table 3).

After the collection of culture supernatants, the highest drop displacement test and emulsification index at 45 mm and 94 % respectively, and the lowest surface tension at 39 mN/m were detected by bacterial isolate (B3). Morikawa et al. [34] illustrated that the test for oil displacement is directly attributed to the solution's biosurfactant compound. The measurement of surface activity of the cultural supernatant shows a reduction in surface tension. There was a direct relationship discovered between drop collapse, oil displacement tests, and surface tension assays [35]. Likewise, this direct correlation was reported by Bodour and Miller-Maier [36] Therefore, the surface activity of the biosurfactant*Stenotrophomonasrhizophila* was established by the oil displacement method.The oil displacement test has various features in-demand for a small volume of sample. They are easy to accomplish and do not need the use of special equipment [37]. Rismani et al. [38] notified that the efficiency of biosurfactants has been measured as the area of the well-defined oil displacement zone. The drop collapsing test is not as susceptible to detect low levels of output of biosurfactants as the oil displacement test [39].The emulsification index is an indirect correlation between the oil displacement test and the surface tension used to screen biosurfactant production. The emulsification index > 30 % indicates the high activity of microbial strain for the production of biosurfactants [35]. Here, from the results, the bacterial isolate (B3) was selected for an upcoming study which attributed to this isolate gave high potential for production after 72 h.

Table 3. Screening for the biosurfactants production from different bacterial isolates after 72 h

Bacterial isolate	A drop oil displacement test (mm)	Surface tension (mN/m) 30ºC	Emulsification index (E_{24})(%)
B1	35	43	50
B2	32	47	60
B3	45	39	94
B4	30	42	65
B5	25	44	60
B6	31	45	65
B7	35	43	55

3.2. Identification of the selected bacterial isolate and production of biosurfactant

The bacterial isolate (B3) was discovered to be a Gram-negative, rod-shaped, motile bacterium. The identity of the selected bacteria was determined by 16S rRNA gene sequence analysis to be *Stenotrophomonasrhizophila* strain e-p10 with a 96 % similarity. Ezebuiro et al. [40] stated that *Stenotrophomonas* sp. is a Gram-negative, rod-shaped, motile bacterium that is non-fermenting and is a mandatory aerobe strain. *Stenotrophomonas*sp. was first listed under the genus *Pseudomonas*; it was subsequently moved to the genus *Xanthomonas*, but the genus *Stenotrophomonas* was fully accepted and named in 1993.

3.3. Extraction and characterization of the produced biosurfactant

The production of biosurfactant is still deficient without effective and economical manners for the extraction of the product. The downstream costs of actual product performance are ~60-80 % of the overall cost of production. Traditional methods for extracting bioemulsifiers or biosurfactants from culture media include acid precipitation, solvent extraction, ammonium sulphate precipitation, centrifugation, and foam fractionation [41]. In the current research, the recovery of biosurfactant from supernatant broth culture by *Stenotrophomonasrhizophila* was done by solvent extraction and precipitation method [21, 42]. The yield of the biosurfactant was obtained from the cell-free culture at 1.6 g/l. The yield of the produced biosurfactant by *Stenotrophomonasrhizophila* AS42 at 0.63±0.05 was reported by Saisa-ard et al. [43]. In the current study, the procedure of recovering biosurfactant from culture media takes advantage of some other properties such as emulsification power and surface tension, which determine the efficiency of the produced biosurfactant. The purified biosurfactant of *Stenotrophomonasrhizophila*showed an emulsification index of 97 % and surface tension of 35 mN/m. These results agree with Hassen et al. [44] the author studied that the emulsification activity of the extracted biosurfactant of *Pseudomonas rhizophila* S211 is 90 %. Microbial surfactants have characteristics over their chemicals that are attributed to their strong physical and chemical characteristics, such as foaming, compliance with the environment, surface tension, emulsification capacity, and greater biodegradability. Also, it isused at high temperatures, concentrations of acidity and salt [45]. The biosurfactant extracted from cultural *Stenotrophomonasrhizophila* media was characterized by FTIR analysis (Figure 1). In their spectrum of biosurfactant shows a broad peak at 3365.86 cm^{-1} elucidating the OH group (free hydroxyl groups of rhamnose rings) due to partial esterification reaction. The strong absorption peak at 2929.36 cm^{-1} suggested the presence of the aliphatic chain (-CH) stretching bands confirmed the glycolipid type of the produced biosurfactant. The stretch, 1653.26 cm^{-1}, showed the –C [double bond, length as m-dash] O stretch. The absorption peaks at 1543.17 and 1454.37 cm^{-1} corresponded to the stretching model of the C-N bond. The presence of carbonyl functional group C-O was indicated by the peaks at 1454.37-1405.83 cm^{-1}, which were supported by the bond at

1243.86 cm^{-1}, which demonstrated C-O deformation vibration indicated the production of lipopeptidebiosurfactant [46]. According to Hassen et al. [44], the peaks observed in the range of 1.200-1000 cm^{-1} confirm the presence of bonds between carbon atoms and the hydroxyl groups discovered in the rhamnose units. The area between 720-557 cm^{-1} is due to the absorption of the bending vibration by aromatic C-H. According to the spectrum data, the produced biosurfactant is mainly a complex of lipopeptides with a tiny fraction of glycolipids that represents a polymeric molecule [43].

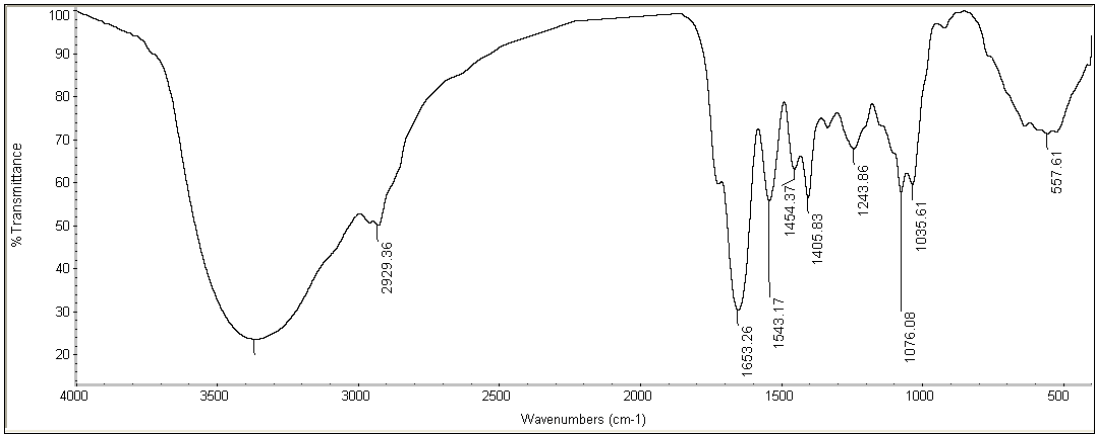

Figure 1. FTIR spectrum of the produced biosurfactant by *Stenotrophomonasrhizophila*

3.4. Antimicrobial activity of produced biosurfactant

Worldwide, due to the current problem of antibiotic resistance, surfactants produced from different microbial strains are currently being studied as a substitution for other potential antimicrobial agents [47]. Therefore, in this work, the antimicrobial potential of the produced biosurfactant (polymeric molecule biosurfactant) from*Stenotrophomonasrhizophila* was determined against the tested microbial strains, including Gram-positive and Gram-negative rods and cocci bacteria, yeast strain, *Candida albicans*, and *Aspergillusniger* as a fungi strain. After 3-5 days at 28 ^{o}C and 24 h at 30 ^{o}C for zone growth inhibition of the fungal strains and bacterial strains respectively were evaluated. Table (4) shows that 0.1 ml of biosurfactant has significant antibacterial activity against all bacterial species under examined (Gram-positive and negative bacterial strains) and fungi strains (fungi and yeast strains). Because this form of biosurfactant is a polymeric molecule that is characterized a combination of lipopeptides with a tiny fraction of glycolipids, it has a high activity. The results suggest that a glycolipid molecule with both hydrophobic and hydrophilic groups may be capable of inserting its fatty acid components into a cell membrane that causes major changes in the cell wall structure and composition that interrupt the cytoskeleton and plasma membrane components, enabling the membrane to remove the cytoplasmic components [48, 49].Iturin A, bacillomycin D, bacillomycin F, bacillomycin L, and bacillomycinLc are members of the lipopeptide family, which include β-hydroxy fatty acid that is thought to inhibit various species of fungi and bacteria [50].

Table 4. Antimicrobial test of produced biosurfactant by*Stenotrophomonasrhizophila* after 72 h

Component	Inhibition zone (in mm)					
	Bacteria				Fungi	
	Gram positive		Gram negative			
	Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Aspergillus niger***	***Candida albicans***
Produced biosurfactant	30	22	26	23	25	22
Tobramycin*	13	14	12	13	-	-
Mycostatin*	-	-	-	-	13	15

Tobramycin*: Control broad antibiotic against gram positive and negative bacteria species
Mycostatin**: Control antibiotic against fungi species

3.5. Chemical characterization of the prepared additives

The chemical structure of the produced monomers and copolymers was investigated further using infrared spectral analysis, which revealed similar patterns. A representative illustration is demonstrated for the monomers (NAFOL20 + A acrylate) and copolymers with vinyl acetate (P1) were verified by IR spectroscopy. The characteristic peaks showed that the long-chain alkyl (- (CH_2) n- and n = 13) absorption peak is 721 cm^{-1} as a repressor for the monomer at 1638 cm^{-1} (C = C stretch), 1722 cm^{-1} (C = O stretch), 1297, and 1161 cm^{-1} (-COC- stretch). The monomer failed to detect high absorption peaks at 3600-3200 cm^{-1}, demonstrating that the monomers did not include alcoholic hydroxyl groups (-OH) or acid hydroxyl groups (-COOH). The signature absorption peaks of $-CH_3-$ and $-CH_2-$, are observed in the FTIR spectra of P1, as are the high absorption peaks of C = O at 1727 cm^{-1} , and the vibration peak of -COC- stretching at 1241 and 1150 cm^{-1} 1226 and 1172 cm^{-1}. The long-chain alkyl (-(CH_2) n- and n =13) absorption peak is 696 cm^{-1} 720 cm^{-1}. In the copolymer with vinyl acetate, the characteristic C = C peak of (NAFOL20 + A acrylate) vanished completely. The characteristic C=C peak of (NAFOL20+A acrylate) vanished completely in the copolymer with vinyl acetate illustrated in (Figure 2). Therefore, the P1 copolymers were synthesized successfully. The average molecular weight and polydispersity of the produced copolymers were determined by gel permeation chromatographic analysis.

3.6. Effect of the n-alkyl chain length of prepared additives and blend these additives with biosurfactant on solid point (SP) for gas oil

The SP of untreated gas oil is 18 °C. The depression of P1, P2, P3, and P4 of gas oil is determined in Table (5) and Figure (3). The ΔSP shows the reduction in SP of gas oil before and after additive treatment. Table (5) illustrates that not all PPDs can successfully increase gas oil fluidity at low temperatures. The carbon number of gas oil is closely related to the depressing effects of additions. When compared to P1, P2, P3, and P4, the P4 showed better depression. As more P4 was added, the general trend of ΔSP gradually elevated, then stabilized or decreased. The ΔSP reached a maximum of 21^{o}C when the P4 concentration was 3000 ppm. This study suggested that additives had a stronger and more noticeable depression impact than the length of the n-alkyl chain in PPDs close to the average gas oil carbon number [51]. In gas oil, however, the structural n-alkyl chain is easier to incorporate into and co-crystallize with n-alkanes. P1, P2, and P3, on the other hand, have a smaller depressive effect. The ΔSP of gas oil was generally 6 to 15°C after adding P3. While P2 lowered the ΔSP of gas oil by 0 to 9°C. The P1 only had a minor influence on the ΔSP of gas oil.

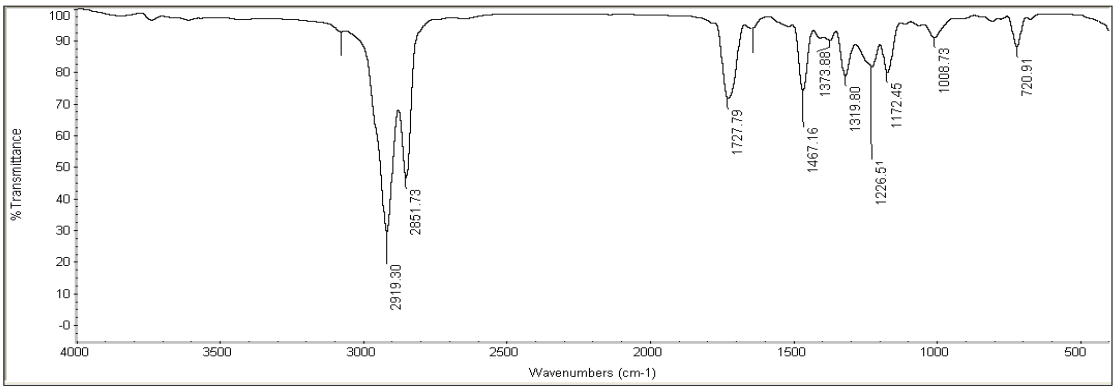

Figure2. FTIR spectrum copolymerization of n-alkyl acrylate with vinyl acetate

Table 5. Effect of type and concentration of the additive on the solid point for gas oil

Additives Designation	Mol. Wt	Polydispersity index	Additive concentration pm	SP, °C	Maximum change in solid point,°C	ΔSP, °C	Extend of solid point depression,°C
P1	43.111	1.70	0 250 500 1000 1500 2000 3000	18 18 18 15 15 15 18	15	0 0 0 3 3 3 0	3
P2	40.231	1.60	0 250 500 1000 1500 2000 3000	18 18 15 15 12 9 9	9	0 0 3 3 6 9 9	9
P3	38.853	1.42	0 250 500 1000 1500 2000 3000	18 12 12 9 9 3 3	3	0 6 6 9 9 15	15
P4	35.512	1.20	0 250 500 1000 1500 2000 3000	18 3 6 6 0 -3 -3	-3	0 15 12 12 18 21 21	21

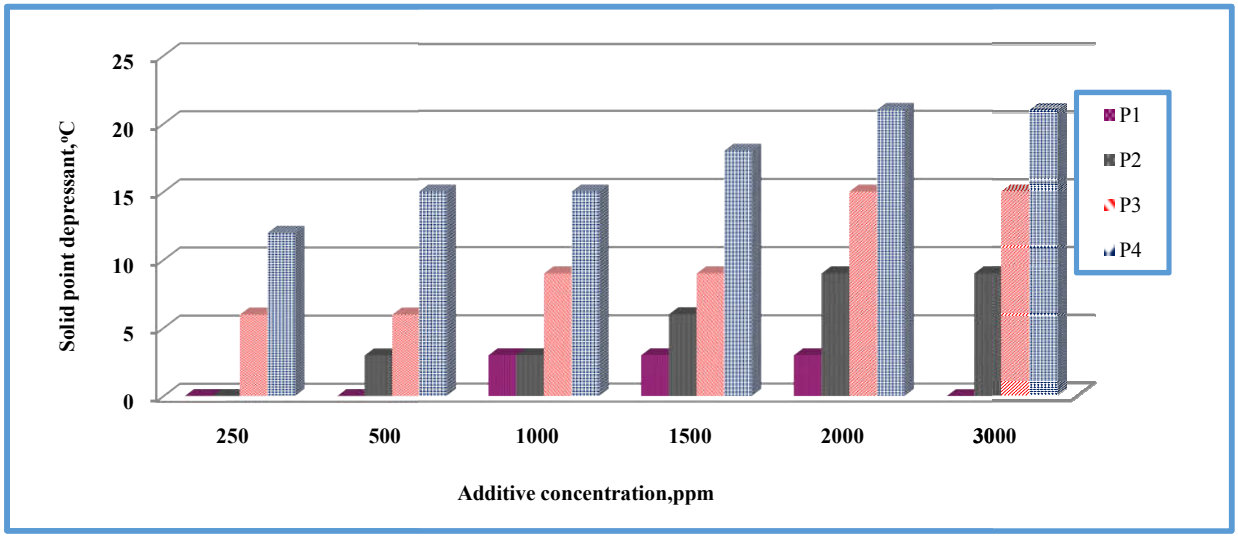

Figure 3. Effect concentration of additives (P1-P2) and their blend with bio surfactants (P3-P4) on solid point depressant for gas oil

3.7. Evaluation of the prepared compounds additives as viscosity index improvers for gas oil

The activity of polymeric compounds as viscosity index improvers is mostly dependent on the action of polymer molecules in the dispersed phase (gas oil). Table (6) and Figure (4) show the results of testing the produced compounds (P1-P4) as viscosity index improvers for gas oil. In this regard, the kinematic viscosity of untapped oil and oil containing various amounts of the investigated additives was evaluated

at 40 °C and 100 °C. Different concentrations of the manufactured additives (P1-P4) ranging from 0.00 ppm to 3000 ppm were utilized to investigate the influence of additive concentration on VI; data is reported in Table 6 and Figure (4). It was reported that when the concentration of the produced additives in the solution increases, the VI increases. This is evident by the fact that polymer solubility increases as temperature rises. As a result of the increased interaction between the polymer chain and the solvent molecule, the polymer molecules transform from a tight coil to an extended coil. This increase in volume raises the viscosity of the mixture, compensating for the oil's usual reduction in viscosity when temperature rises. An increase in the concentration of the polymer causes an increase in the overall volume of polymer micelles in the oil solution. As a result, a high polymer concentration of the same polymer should have a higher viscosity index than a low polymer concentration of the same polymer, for example, additive P4 at 250 ppm VI equals 5.32, whereas 3000 ppm VI equals 12.6 [52]. The effect of alkyl chain length on the efficiency of prepared additives as viscosity index improvers has been shown to increase as the length of the acrylate used in the alkyl chain increases. As shown in Table (6) and Figure (4), with P1 containing alkyl chain length C_{13} VI at 3000 ppm equal to 7.6 and P2 containing alkyl chain length C_{22} VI at 3000 ppm equal to 8.2 [53, 54].

4. Conclusions

In the current work, seven bacterial isolates were isolated from oil-contaminated soil. The bacterial isolate (B3) gave the highest biosurfactant production. Therefore, the bacterial isolate (B3) was selected for upcoming study. The 16S rRNA gene sequencing of the most efficient bacterial isolate (B3) identified it as *Stenotrophomonasrhizophila* strain e-p10. From the culture media of *S. rhizophila* the biosurfactant was extracted at 1.6 g/l. The evaluation and characterization by FTIR analysis of biosurfactants were determined. The ability of *S. rhizophila* to produce a polymeric molecule type of biosurfactant with high emulsifier characteristics has been proven. From the results of the antimicrobial activity, it can be concluded that, the polymeric molecule type of biosurfactant production capacity of *S. rhizophila* for emphasizes its potential bio-control activity. A variety of the copolymers of alkyl acrylate-vinyl acetate with different alkyl side-chain lengths have been prepared. A major factor influencing the effectiveness of copolymer in lowering the solid point of gas oil was the alkyl side-chain length and blending the copolymer with biosurfactants,

The side-chain lengths of C_{22} were ideal for gas oil in copolymer with biosurfactants, which decreased the solid points of the corresponding gas oils by 21 °C. The addition of the alkyl side-chain improved and accelerated wax solubility. A copolymer with a biosurfactant reduced the amount of wax and modified the mechanism of gas oil wax crystallization.As viscosity index enhancers, the copolymers prepared from C_{22} acrylate with biosurfactant additives are more effective than those prepared from alkyl C_{13} acrylate with biosurfactants.

5. Conflict of Interest

The authors declare that they have no conflict of interest.

6. Formatting of funding sources

No list funding sources

Table 6. Dependence of VI on concentration of additive with gas oil on viscosity index for gas oil

Conc., ppm	P1	P2	P3	P4
0	4.22	4.22	4.22	4.22
250	4.32	4.52	5.11	5.32
500	4.94	5.2	5.61	6.2
1000	5.1	5.9	6.9	8.9
2000	6.8	7.2	8.1	10.5
3000	7.6	8.2	9.8	12.6

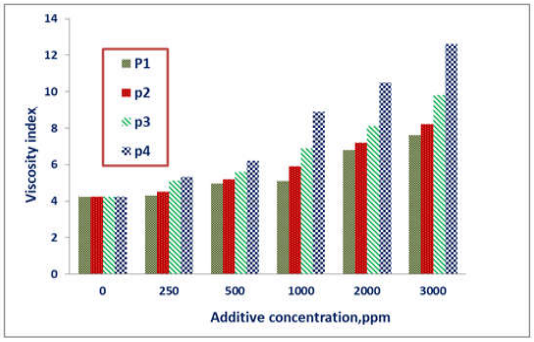

Figure 4. Effect of alkyl chain length of additives (P1-P2) and their blend with biosurfactants (P3-P4)

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