microbiology research

MDPI

Article

Screening of Microorganisms from Wastes and Identification of the Optimal Substrate for Biosurfactant Production

Liliya Biktasheva *, Alexander Gordeev, Anastasia Kirichenko, Polina Kuryntseva and Svetlana Selivanovskaya

Institute of Environmental Sciences, Kazan Federal University, 18 Kremlyovskaya Street, 420008 Kazan, Russia; drgor@mail.ru (A.G.); anastasiyakirichenko696@gmail.com (A.K.); polinazwerewa@yandex.ru (P.K.); svetlana.selivanovskaya@kpfu.ru (S.S.)

* Correspondence: biktasheval@mail.ru; Tel.: +7-98-7007-4536

Abstract: The production of biosurfactants from organic wastes has received significant attention due to its potential cost savings. This study involved the isolation of biosurfactant-producing microorganisms from waste sources. The surfactant properties of the 37 studied isolates were assessed by reducing surface tension and their emulsifying properties, determined by the emulsification index E24. We assessed the ability of these isolated strains to produce biosurfactants using various waste substrates, namely potato peelings, waste cooking oil and sunflower cake. Our results showed that sunflower cake exhibited better growth and biosurfactant production for most of the strains studied. This highlights that sunflower cake is a potentially effective and economical substrate for the production of biosurfactants. The most effective strains allowing to achieve an emulsification index above 50% and reduce surface tension below 40 mN m^{-1} were *Enterobacter* sp. 2pp, strain 2wfo, *Peribacillus* sp. 1mo, *Sphingomonas* sp. 2mo, *Ochrobactrum* sp. 5mo, *Shouchella* sp. 6mo, *Bacillus* sp. 1os, *Bacillus* sp. 2os. Among these strains, both previously known strains as biosurfactant producers and previously unknown strains were found. Thus, we found that among representatives of the genus *Sphingomonas* there are effective producers of biosurfactants. The highest yield of biosurfactant on a medium with glycerol and glucose was shown by the *Bacillus* sp. 2os strain of 0.501 and 0.636 g L^{-1}, respectively.

Keywords: biosurfactant; organic wastes; surface tension; emulsification indices

Citation: Biktasheva, L.; Gordeev, A.; Kirichenko, A.; Kuryntseva, P.; Selivanovskaya, S. Screening of Microorganisms from Wastes and Identification of the Optimal Substrate for Biosurfactant Production. *Microbiol. Res.* **2024**, *15*, 152–163. https://doi.org/10.3390/microbiolres15010010

Academic Editor: Salam A. Ibrahim

Received: 23 November 2023
Revised: 2 January 2024
Accepted: 4 January 2024
Published: 6 January 2024

1. Introduction

Rising environmental concerns have propelled a quest for novel, eco-friendly methodologies across diverse domains. One such area of interest involves the investigation of substituting synthetic surfactants with biologically derived alternatives [1,2]. These biologically derived surfactants, termed biosurfactants, are currently under exploration for their potential applications in agriculture, the bioremediation of oil-contaminated soils, and enhanced oil recovery.

However, despite the burgeoning interest in biosurfactants, their practical applications remain restricted due to the high production costs associated with them. Potential resolutions to this challenge encompass the development and scaling of technological processes and reduction in the expenses tied to raw materials utilized. An emerging strategy involves the use of organic waste as a primary raw material, presenting a promising avenue to significantly diminish the cost of biosurfactants while simultaneously fostering the integration of waste materials into recycling cycles [3–5].

Organic wastes from food and agricultural industries, industrial byproducts such as wastewater, raw glycerol, and waste generated from meat production, as well as oil-contaminated soil, are all considered viable substrates for biosurfactant production. These waste materials are rich in nutrients, encompassing a wide array of sources such as sugarcane molasses, crop cakes, banana, orange, and potato peels, waste frying oils, coconut

oils, rapeseed oils, moringa and cassava residues, distillery waste, effluents from food and vegetable production, coffee wastewater, among others [6]

The selection of the substrate for biosurfactant production is pivotal as various carbon sources exert differing effects on product yield. For instance, Aparna et al. (2012) discovered that *Pseudomonas* sp. 2B yielded varying amounts of rhamnolipid, producing 4.14, 4.38, 3.24, and 4.09 g L^{-1} when cultivated on glycerol, coconut oil cake, orange peel, and whey, respectively [7]. Similarly, Moussa (2014) observed that the rhamnolipid yield of *Pseudomonas aeruginosa* TMN was 2.9 ± 0.02 g L^{-1} on a glucose substrate and 1.35 ± 0.01 g L^{-1} on glycerol, while sucrose resulted in a notably lower yield of 0.91 g L^{-1} [8].

The metabolic pathways and resultant homologues of biosurfactants display significant variability contingent upon the strain used, as well as the substrate employed [9–12]. Ndlovu et al. (2017) studied surfactin analogues obtained from *Bacillus amyloliquefasciens* ST34, noting the dominance of surfactins C13–C15 across samples. Similarly, *P. aeruginosa* ST5 generated six rhamnolipid congeners, with Rha–C10–C10 being the most abundant [13]. Mouafo et al. (2017) highlighted the ability of three *Lactobacillus* strains to produce biosurfactants with higher lipid content on a glycerol substrate compared to sugarcane molasses [12]. This difference was attributed to the mechanism of glycerol consumption primarily directed toward the lipolytic pathway and gluconeogenesis, allowing for the production of fatty acids and sugars [14].

Moreover, the properties of resulting surfactants are contingent upon the substrate used. Surfactant biomolecules, classified into surfactants and emulsifiers, exhibit distinct roles: surfactants reduce surface tension while emulsifiers partake in the formation and stabilization of emulsions [15]. Some biomolecules, however, possess both surfactant and emulsifying properties. Distinguishing these properties necessitates the application of diverse evaluation methodologies [16]. Biosurfactants are identified through methods assessing the reduction in surface and interfacial tension, while bioemulsifiers form stable emulsions without significant alterations in surface/interfacial tension across phases. For instance, Stoimenova (2014) reported the ability of an indigenous strain of industrial wastewater *Pseudomonas fluorescens* to produce glycolipid biosurfactants in a medium containing hexadecane, mineral oil, vegetable oil, and glycerol, with the highest emulsifying capability observed in the vegetable oil medium [17].

The influence of substrate on biosurfactant biosynthesis highlights the need for careful substrate selection. The use of organic wastes as substrates for the synthesis of biosurfactants has not been sufficiently studied, since only a small number of wastes have been studied as substrates. In particular, the study has not previously been carried out on the isolation of microorganisms and the build-up of biosurfactants on different types of organic municipal and agricultural wastes. In the present study, isolates were obtained and tested for their ability to synthesize biosurfactants on various organic wastes. Subsequently, the most effective waste-microorganism pair was selected, giving the highest biosurfactant productivity.

2. Materials and Methods

2.1. Waste Sampling

To isolate microorganisms, the following organic wastes were selected: waste from grease traps and oil traps of a water utility (Chelnyvodokanal LLC, Naberezhnye Chelny, Russia), potato peelings and used frying oil (catering restaurants, Kazan, Russia), rapeseed and sunflower cake (JSC Kazan Oil Extraction Plant, Kazan, Russia), oil-contaminated soil; soil contaminated with motor oil (car service areas, Kazan, Russia).

2.2. Isolation of Microorganisms from Wastes

Isolates of microorganisms from wastes were cultivated using a minimal medium (g L^{-1}): $NaNO_3$ 2.0, KH_2PO_4 0.5, K_2HPO_4 1.0, $MgSO_4$ $7H_2O$ 0.5, KCl 0.1, $FeSO_4$ $7H_2O$ 0.01. The pH was adjusted to 7.0 with 1 N HCl/NaOH. The investigated wastes were added as the sole carbon source to the autoclaved medium. Various carbon sources were separately

introduced into the medium. The initial waste materials, such as potato peelings, rapeseed and sunflower cake, oil-contaminated soil, soil contaminated with motor oils, and waste from water utility grease traps and oily sludge, as well as waste frying oil, were included in the mineral medium at a volume of 2% (v/v) [18].

Bacterial strains were inoculated in 250 mL flasks containing 100 mL of medium and incubated at 120 rpm at 28 °C for 72 h. Following incubation, the strains were subjected to the limiting dilution method and subsequently sown on a solid MPA medium. Individual isolates of microorganisms were obtained by evaluating the morphological characteristics of colonies on a solid medium in a Petri dish and through microscopic analysis utilizing an Axio Lab A1 light microscope (Carl Zeiss, Jena, Germany).

2.3. Assessment of Biosurfactant Production Ability

The assessment of emulsifying ability was conducted using the E24 method [19]. All measurements were carried out in triplicate. This procedure involved combining an equal volume of cell-free strain culture supernatant with crude oil. The cell-free supernatant was obtained via centrifugation of the liquid cell culture (10 min, 5000 rpm). The resulting mixture was vortexed and allowed to stand for 24 h at room temperature. Subsequently, the height of the emulsified column was measured, and E24 was calculated using the formula:

$$\mathrm{E24} = \frac{emulsion\ layer\ height}{total\ height\ of\ the\ liquid\ column\ in\ the\ test\ tube} \times 100\% \quad (1)$$

The cell-free strain culture supernatant was analyzed for the reduction in water surface tension (ST) using the Du Nouy ring method with a K20 tensiometer (KRUSS, Hamburg, Germany) at room temperature [20].

2.4. Determination of Strain Species

The total genomic DNA of the isolates was extracted utilizing a K-Sorb reagent kit for DNA extraction on microcolumns (SINTHOL Company, Moscow, Russia). Subsequently, the nucleotide sequence of the samples was determined through the Sanger sequencing method employing an ABI 3730 DNA Analyzer (Life Technologies, Carlsbad, CA, USA). Genomic libraries were constructed using general bacterial primers 27f-1492r [21]. The sequences obtained for each strain were matched with the sequences from the database using the BLAST NCBI. The sequences were aligned using MEGA 10.0 software.

2.5. Cultivation of Isolates on Different Waste Types

The chosen isolates were cultured on four distinct substrates: pure glycerol, potato peelings, waste frying oil, and sunflower cake. For the preparation of potato peelings and sunflower cake, both were dried at 55 °C for 4 days, crushed into fine powder, and subsequently, a 10% (w/v) solution of the powder in distilled water was autoclaved. The solution was filtered through gauze to obtain a clear filtrate, which was then added sterilely at a 4% (v/v) concentration to the previously described mineral medium. Waste frying oil and glycerol were directly added to the medium at a 2% (v/v) concentration before autoclaving. Biosurfactant producers were grown in 500 mL flasks containing 200 mL of medium at 120 rpm at 28 °C for 72 h.

2.6. Extraction of Biosurfactants

The cell culture was centrifuged at 5000 rpm for 10 min at room temperature to obtain cell-free supernatant. Extraction of biosurfactants was carried out using the acid precipitation method—bringing the cell-free supernatant to pH = 2 using 5 N HCl. The acidified supernatant was kept at 4 °C overnight and then centrifuged at 4 °C for 40 min at 3700 rpm. The biosurfactant precipitate was dissolved in a mixture of methanol and chloroform in a ratio of 2:1 and filtered using a 0.22 μm filter (Sartorius, Gottingen, Germany). The biosurfactant was obtained by evaporating the solvent on a rotary evaporator (IKA, Staufen im Breisgau, Germany) and evaluated gravimetrically.

2.7. Statistical Analysis

The error bars depicted in the figures indicate the standard error of means derived from the replicates. To evaluate the data within groups of values (characterizing each substrate type and individual strain), weights were assigned, and the weighted arithmetic means were computed. The statistical analysis was carried out using Statistica 10.0 software (StatSoft Inc., Tulsa, OK, USA). Graphs were generated using Microsoft Excel 2016 MSO (Microsoft, Redmond, WA, USA).

3. Results and Discussion

3.1. Isolation of Biosurfactant-Producing Microorganisms from Waste

Microorganisms capable of biosurfactant synthesis were isolated from various categories of wastes, including agricultural sources (potato peelings, rapeseed, and sunflower cake), industrial sources (oil-contaminated soil and soil contaminated with motor oils), and municipal wastes (waste from grease traps and water utility oily sludge, waste frying oil).

A total of 37 strains were isolated from these waste materials, and their genera were identified through Sanger sequencing (Table 1).

The investigation revealed a significant variance in the number of strains isolated from different waste sources. The largest number of strains was retrieved from oil-contaminated soils, while the smallest count originated from rapeseed cake. Among the total isolated strains, 33 were identified at the genus level, and four strains remained unidentified.

Table 1. Microorganisms isolated from waste.

Wastes	Strains	The Closest-Related Strain	Identity, %	Identified as
potato peelings	1pp	*Enterobacter* sp. strain XN81	98.5	*Enterobacter* sp. 1pp
	2pp	*Enterobacter ludwigii* strain E8-13	97.6	*Enterobacter* sp. 2pp
	3pp	Bacterium strain BS0657	93.4	3pp
	4pp	Uncultured bacterium clone RBL10-19	94.9	4pp
	5pp	*Enterobacter ludwigii* strain 160-a blue	98.7	*Enterobacter* sp. 5pp
rapeseed cake	1rc	*Pantoea* sp. LL69	83.6	*Pantoea* sp. 1rc
grease trap	1gt	*Proteus mirabilis*, isolate AHI-2	99.5	*Proteus* sp. 1gt
	2gt	*Bacillus aerophilus* strain 0125	97.4	*Bacillus* sp. 2gt
oily sludge	1s	*Citrobacter freundii* strain E51	99.4	*Citrobacter* sp. 1s
	2s	*Sphingomonas echinoides* strain B18	94.7	*Sphingomonas* sp. 2s
waste frying oil	1wfo	*Sphingomonas* sp. strain FKP374	97.5	*Sphingomonas* sp. 1wfo
	2wfo	Uncultured *Sphingomonas* sp., clone: LR564B-24	89.8	2wfo
	3wfo	*Pseudomonas stutzeri* DSM 10701	99.6	*Pseudomonas* sp. 3wfo
	4wfo	Uncultured *Sphingomonas* sp., clone: LR564B-24	95.4	*Sphingomonas* sp. 4wfo
	5wfo	*Sphingomonas echinoides* strain KCOM 3301 (=JS364)	91.6	*Sphingomonas* sp. 5wfo
sunflower cake	1sc	*Sphingomonas* sp. strain FKP374	94.6	*Sphingomonas* sp. 1sc
	2sc	*Sphingomonas* sp. Hc_01N 16S	90.4	*Sphingomonas* sp. 2sc
	3sc	Bacterium strain BLEC3	89.5	3sc
	4sc	Uncultured *Sphingomonas* sp., clone: LR564B-24	87.4	*Sphingomonas* sp. 4sc
	5sc	*Sphingomonas* sp. PP-2 16S	83.9	*Sphingomonas* sp. 5sc
soil contaminated with motor oil	1mo	*Peribacillus frigoritolerans* strain TG15	94.6	*Peribacillus* sp. 1mo
	2mo	*Sphingomonas echinoides* strain MERYL5-24	94.1	*Sphingomonas* sp. 2mo
	3mo	*Nocardiopsis* sp. XLI-8	93.9	*Nocardiopsis* sp. 3mo
	4mo	*Bacillus gibsonii* strain S-2	95.0	*Bacillus* sp. 4mo
	5mo	*Ochrobactrum* sp. strain S2n90	81.2	*Ochrobactrum* sp. 5mo
	6mo	*Shouchella gibsonii* strain LMITABS00983	95.0	*Shouchella* sp. 6mo
	7mo	*Sphingomonas* sp. strain MEREH12	83.6	*Sphingomonas* sp. 7mo

Table 1. *Cont.*

Wastes	Strains	The Closest-Related Strain	Identity, %	Identified as
oil contaminated soil	1os	*Bacillus tequilensis* strain RS53	99.6	*Bacillus* sp. 1os
	2os	*Bacillus toyonensis* strain FORT 102	99.1	*Bacillus* sp. 2*os*
	3os	*Bacillus amyloliquefaciens* strain Sihong_838_1	81.7	*Bacillus* sp. 3os
	4os	*Staphylococcus* sp. strain FKR3-1	90.8	*Staphylococcus* sp. 4os
	5os	*Bacillus cereus* strain 2-2 16S	76.6	*Bacillus* sp. 5os
	6os	*Sphingomonas* sp. strain SA4_1	83.6	*Sphingomonas* sp. 6os
	7os	*Sphingomonas* sp. strain BWLP17	87.6	*Sphingomonas* sp. 7os
	8os	*Sphingomonas* sp. strain MERYL1-1	87.3	*Sphingomonas* sp. 8os
	9os	Uncultured *Sphingomonas* sp., clone: LR564B-24	83.6	*Sphingomonas* sp. 9os
	10os	Uncultured bacterium, clone SIP12-RT-12	86.8	10os

The dominant genus observed among these isolates was *Sphingomonas* sp., with ten strains, followed by six strains attributed to the genus *Bacillus* sp. Notably, five strains were isolated from potato peelings, predominantly affiliated with the genus *Enterobacter* sp., a common genus found in organic wastes [22]. The prevalence of *Sphingomonas* sp. in natural environments, such as soils, is noteworthy as they demonstrate the ability to degrade hydrocarbons [23].

The majority of the strains from oil-contaminated soils (10 strains) were divided between *Sphingomonas* and *Bacillus*, with each genus accounting for four strains. Conversely, the minimum number of strains emerged from rapeseed cake, predominantly featuring *Pantoea* sp. 1rc. The *Pantoea* genus encompasses a diverse array of bacteria isolated from various environments and is commonly found in the rhizosphere of rapeseed [24].

3.2. Evaluation of Isolates for Their Surface Tension Reduction Abilities

The isolates were assessed for their emulsifying and water surface tension reduction abilities, primarily on a conventional medium containing glycerol (Table 2). Research indicates that a crucial criterion for substantial surface-active properties is the reduction of surface tension to around 40 mN m^{-1} [25]. Consequently, among the isolates tested, six strains demonstrated notable surface tension reduction capabilities. Notable strains encompassed 3sc, *Sphingomonas* sp. 5sc (derived from sunflower cake), *Peribacillus* sp. 1mo, *Sphingomonas* sp. 2mo, *Ochrobactrum* sp. 5mo (isolated from soil contaminated with motor oil), as well as *Bacillus* sp. 5os (from oil-contaminated soil). Among these, there are well-recognized biosurfactant producers, such as representatives of the *Peribacillus* genus (previously known as *Brevibacterium*) [26], reducing surface activity to levels of 25.9–27.6 mN m^{-1} [27,28]. Additionally, the *Ochrobactrum* genus, isolated from motor oil-contaminated soil, managed to reduce the surface tension of the growth medium from 70 to 30.8 mN m^{-1} [29]. An intriguing aspect of this study is that the three members of the genus Sphingomonas we isolated exhibited significant emulsifying activity. In particular, strain 2mo, identified as *Sphingomonas* sp., showed a remarkable surface activity of 39.7 mN m^{-1}. Whereas previously, other studies noted that most *Sphingomonas* strains do not have the ability to produce biosurfactants [30].

The subsequent phase involved selecting more cost-effective substrates to augment biosurfactant production. To this end, three substrate types—potato peelings, used frying oil, and sunflower cake—were chosen. The efficacy of all isolated strains on these selected waste materials was evaluated by measuring the degree of surface tension reduction (Table 2). These particular wastes were identified as the most high-volume waste sources in the city of Kazan, Republic of Tatarstan, Russia.

Table 2. Surface tension on culture media with different carbon substrates.

Type of Wastes	Strains	ST, mN m^{-1}			
		Glycerol	Potato Peelings	Waste Frying Oil	Sunflower Cake
Potato peelings	*Enterobacter* sp. 1pp	58.05 ± 0.681	54.75 ± 0.415	63.28 ± 0.453	55.66± 0.269
	Enterobacter sp. 2pp	43.84 ± 0.206	56.73 ± 0.486	43.41± 0.136	51.96± 0.269
	3pp	52.86 ± 0.599	56.42 ± 0.442	47.99± 0.198	59.03± 0.234
	4pp	45.06 ± 0.198	55.99 ± 0.441	47.74± 0.187	46.53± 0.190
	Enterobacter sp. 5pp	49.58 ± 0.069	61.93 ± 0.305	53.96± 0.354	51.22± 0.56
rapeseed oil	*Pantoea* sp. 1rc	43.41 ± 0.263	53.81 ± 0.328	52.57± 0.512	49.8± 0.345
grease trap	*Proteus* sp. 1gt	65.14 ± 0.695	61.58 ± 0.269	48.37± 0.353	58.61± 0.61
	Bacillus sp. 2gt	65.95 ± 0.438	60.27 ± 0.361	56.25± 0.553	62.09± 0.655
oily sludge	*Citrobacter* sp. 1s	58.59 ± 1.419	52.52 ± 0.225	48.37± 0.335	53.36± 0.489
	Sphingomonas sp. 2s	66.53 ± 0.545	52.11 ± 0.315	56.25± 0.256	58.99± 0.605
waste frying oil	*Sphingomonas* sp. 1wfo	53.53 ± 0.433	47.33 ± 0.120	49.82± 0.487	50.45± 0.180
	Sphingomonas sp. 2wfo	54.27 ± 0.508	27.83 ± 0.033	47.87± 0.303	29.78± 0.03
	Pseudomonas sp. 2wfo	55.64 ± 0.488	49.70 ± 0.169	43.04± 0.233	58.81± 0.61
	Sphingomonas sp. 4wfo	60.34 ± 0.578	53.60 ± 0.598	54.13± 0.321	55.75± 0.614
	Sphingomonas sp. 5wfo	55.93 ± 0.596	57.91 ± 0.501	54.8± 0.567	58.32± 0.576
Sunflower cake	*Sphingomonas* sp. 1sc	69.4 ± 0.288	62.02 ± 1.073	63.56± 0.679	65.34± 0.678
	Sphingomonas sp. 2sc	69.29 ± 0.284	64.62 ± 0.761	66.45± 0.665	65.12± 0.608
	3sc	27.83 ± 0.034	59.09 ± 1.147	60.34± 0.64	65.73± 0.603
	Sphingomonas sp. 4sc	68.14 ± 0.351	63.89 ± 0.914	64.87± 0.677	65.99± 0.555
	Sphingomonas sp. 5sc	35.78 ± 0.352	67.87 ± 0.604	65.45± 0.501	63.39± 0.679
soil contaminated with motor oil	*Peribacillus* sp. 1mo	21.64± 0.018	45.76 ± 0.174	43.56± 0.344	57.57± 0.456
	Sphingomonas sp. 2mo	39.71± 0.03	50.34 ± 0.379	36.77± 0.09	28.32± 0.05
	Nocardiopsis sp. 3mo	48.61 ± 0.097	29.98 ± 0.098	45.34± 0.185	3.45± 0.025
	Bacillus sp. 4mo	47.9 ± 0.648	64.75 ± 0.654	45.1± 0.205	52.11± 0.432
	Ochrobactrum sp. 5mo	29.15 ± 0.077	50.85 ± 0.125	33.89± 0.1	34.49± 0.061
	Shouchella sp. 6mo	59.15 ± 0.389	29.15 ± 0.082	42.58± 0.186	3.14± 0.02
	Sphingomonas sp. 7mo	47.88 ± 0.355	55.95 ± 0.820	48.91± 0.307	53.98± 0.399
oil contaminated soil	*Bacillus* sp. 1os	53.45 ± 0.484	33.07 ± 0.480	45.13± 0.205	24.19± 0.056
	Bacillus sp. *2os*	60.35 ± 0.564	30.86 ± 0.257	32.56± 0.11	3.99± 0.01
	Bacillus sp. 3os	50.11 ± 0.494	31.02 ± 0.234	35.76± 0.101	29.17± 0.055
	Staphylococcus sp. 4os	45.76 ± 0.284	53.30 ± 0.384	44.55± 0.234	49.75± 0.155
	Bacillus sp. 5os	29.42 ± 0.042	28.8 ± 0.090	58.63± 0.566	28.66± 0.04
	Sphingomonas sp. 6os	63.43 ± 0.355	62.11 ± 0.588	61.23± 0.453	63.54± 0.666
	Sphingomonas sp. 7os	64.91 ± 0.566	43.37 ± 0.948	50.08± 0.334	51.1± 0.456
	Sphingomonas sp. 8os	59.78 ± 0.67	49.91 ± 0.142	53.87± 0.788	53.08± 0.489
	Sphingomonas sp. 9os	68.08 ± 0.561	45.12 ± 0.074	45.31± 0.305	47.55± 0.311
	10os	57.51 ± 0.475	50.34 ± 0.086	53.45± 0.398	55.67± 0.499

reduction of surface tension to 40 mN m^{-1} is highlighted in red.

An analysis of these waste sources for cultivating biosurfactant producers revealed certain trends (Table 2). Notably, sunflower cake emerged as the most effective substrate, facilitating significant biosurfactant production with noteworthy surface activity in nine strains spanning various genera: 2wfo, *Sphingomonas* sp. 2mo, *Nocardiopsis* sp. 3mo, *Ochrobactrum* sp. 5mo, *Shouchella* sp. 6mo, *Bacillus* sp. 1os, *Bacillus* sp. 2os, *Bacillus* sp. 3os, *Bacillus* sp. 5os.

Conversely, used frying oil was the least effective substrate for biosurfactant production, leading to an effective reduction in surface tension in only four strains of genus—*Sphingomonas* sp. 2mo, *Ochrobactrum* sp. 5mo, *Bacillus* sp. 2os, *Bacillus* sp. 3os.

Among representatives of the genus *Bacillus*, for example, the species *Bacillus toyonensis* is known, which was previously isolated from oil-contaminated areas and demonstrated the ability to reduce surface tension to 47 mN m^{-1} [31]. Similarly, Chaurasia et al. (2020) isolated *Bacillus tequilensis* LK5.4 from soybean, displaying the capacity to reduce the surface tension of the culture medium by up to 40% [32]. *Nocardiopsis* sp. B4, isolated from seawater, exhibited a surface tension decrease to 29 mN m^{-1} during cultivation, with an E24 emulsification index of 80% [3].

3.3. Evaluation of Emulsifying Properties of Isolates

In the subsequent stage, all isolates were examined for the presence of emulsifying properties in their produced metabolites. Emulsification is considered significant when the emulsification index exceeds 50% [33,34] (Table 3).

Table 3. E24 values in culture media with different carbon substrates.

Type of Wastes	Strains	E24, %			
		Glycerol	Potato Peelings	Waste Frying Oil	Sunflower Cake
potato peelings	*Enterobacter* sp. 1pp	20 ± 1	7 ± 3	20 ± 5	15 ± 5
	Enterobacter sp. 2pp	50 ± 5	36 ± 5	35 ± 5	50 ± 3
	3pp	20 ± 2	29 ± 3	20 ± 2	20 ± 1
	4pp	50 ± 5	14 ± 2	20 ± 1	21 ± 5
rapeseed oil	*Enterobacter* sp. 5pp	30 ± 5	9 ± 1	10 ± 3	5 ± 3
	Pantoea sp. 1rc	50 ± 5	4 ± 0	4 ± 0	30 ± 3
grease trap	*Proteus* sp. 1gt	5 ± 0	21 ± 0	10 ± 1	10 ± 2
	Bacillus sp. 2gt	0 ± 0	7 ± 3	5 ± 0	5 ± 0
oily sludge	*Citrobacter* sp. 1s	5 ± 0	7 ± 4	5 ± 0	5 ± 0
	Sphingomonas sp. 2s	10 ± 2	7 ± 4	10 ± 1	10 ± 2
waste frying oil	*Sphingomonas* sp. 1wfo	0 ± 0	14 ± 5	7 ± 2	0 ± 0
	Sphingomonas sp. 2wfo	30 ± 2	29 ± 5	30 ± 2	7 ± 4
	Pseudomonas sp. 2wfo	20 ± 1	50 ± 5	30 ± 3	20 ± 2
	Sphingomonas sp. 4wfo	5 ± 0	14 ± 3	10 ± 1	5 ± 0
	Sphingomonas sp. 5wfo	5 ± 0	14 ± 2	10 ± 2	10 ± 3
sunflower cake	*Sphingomonas* sp. 1sc	20 ± 3	14 ± 2	14 ± 4	14 ± 5
	Sphingomonas sp. 2sc	30 ± 5	0 ± 0	5 ± 0	5 ± 0
	3sc	50 ± 5	14 ± 2	30 ± 2	21 ± 4
	Sphingomonas sp. 4sc	10 ± 2	14 ± 3	10 ± 1	14 ± 5
	Sphingomonas sp. 5sc	20 ± 1	14 ± 4	14 ± 2	29 ± 6
soil contaminated with motor oil	*Peribacillus* sp. 1mo	50 ± 3	14 ± 0	50 ± 3	50 ± 2
	Sphingomonas sp. 2mo	50 ± 2	14 ± 0	50 ± 2	71 ± 4
	Nocardiopsis sp. 3mo	30 ± 1	14 ± 1	7 ± 1	7 ± 2
	Bacillus sp. 4mo	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Ochrobactrum sp. 5mo	50 ± 5	14 ± 2	20 ± 2	21 ± 1
	Shouchella sp. 6mo	30 ± 5	29 ± 3	29 ± 2	7 ± 3
	Sphingomonas sp. 7mo	10 ± 2	50 ± 3	30 ± 1	20 ± 5
oil contaminated soil	*Bacillus* sp. 1os	50 ± 2	29 ± 5	50 ± 1	57 ± 5
	Bacillus sp. 2os	5 ± 0	50 ± 1	30 ± 4	7 ± 0
	Bacillus sp. 3os	20 ± 0	21 ± 5	20 ± 1	14 ± 2
	Staphylococcus sp. 4os	5 ± 0	14 ± 3	5 ± 0	5 ± 0
	Bacillus sp. 5os	20 ± 0	0 ± 0	10 ± 0	14 ± 3
	Sphingomonas sp. 6os	30 ± 2	14 ± 2	10 ± 0	10 ± 3
	Sphingomonas sp. 7os	10 ± 1	14 ± 1	10 ± 2	10 ± 4
	Sphingomonas sp. 8os	30 ± 3	14 ± 3	20 ± 3	20 ± 2
	Sphingomonas sp. 9os	20 ± 1	29 ± 5	20 ± 5	20 ± 1
	10os	20 ± 1	50 ± 2	20 ± 1	20 ± 4

E24 > 50% are highlighted in red.

On traditional glycerol-based sources, eight strains—identified as genus *Enterobacter* sp. 2pp, 4pp (from potato peelings), *Pantoea* sp. 1rc (from rapeseed cake), 3sc (from

sunflower cake), *Peribacillus* sp. 1mo, *Sphingomonas* sp. 2mo, *Ochrobactrum* sp. 5mo (from soil contaminated with motor oil), and *Bacillus* sp. 1os (from oil-contaminated soil)—demonstrated substantial emulsifying activity (over 50%).

Several of these genus have been previously identified for their emulsification capabilities. For instance, Rabiei (2013) noted that a consortium of *Enterobacter* sp. genus representatives could produce biosurfactants with an emulsification index exceeding 70%, thereby reducing the surface tension of the nutrient medium from 72 to 31 mN m^{-1} [35]. Additionally, Essghaier et al. (2023) demonstrated that endophytes of *Pantoea alhagi* species are capable of producing biosurfactants with an emulsifying activity of 82% [36]. The species representative of *B. tequilensis* LK5.4 exhibited a maximum emulsification index of 52% [32].

Upon assessing new waste substrates, it was observed that the selected waste materials yielded substances with comparatively lower emulsifying activity than glycerol. Specifically, while glycerol facilitated the synthesis of bioemulsifiers in 8 strains, potato peelings and cake showed 4, and used frying oil demonstrated this trait in 3 strains.

These results indicated that metabolites from strains exhibiting high surface activity may not always exhibit high emulsifying activity. However, metabolites of such strains as 4pp, *Peribacillus* sp. 1mo, *Sphingomonas* sp. 2mo, and *Ochrobactrum* sp. 5mo have both of these activities. It is known from the literature that many bacterial strains produce a diverse mixture of analogues and congeners of biosurfactants under the influence of a single carbon source present in the nutrient medium [37]. Moreover, studies show that surface tension values and emulsification indices may not be uniformly correlated for the same strains [16]. Microorganisms are capable of synthesizing various mixtures of heteropolysaccharides, lipopolysaccharides, lipoproteins and proteins, potentially indicating varying degrees of biosurfactant and bioemulsifying properties. It is known that substances with low molecular weight have mainly surface activity, while substances with high molecular weight are effective as emulsion stabilizers [38]. The combination of polysaccharides, fatty acids and protein components in bioemulsifiers allows achieving greater emulsifying potential [16]. Some studies note the influence of the substrate C/N ratio on surface and emulsifying activity, which is not detected at the same C/N values [39]. Notably, other studies also note the ability of microorganisms of the genera *Peribacillus* sp., *Sphingomonas* sp. and *Ochrobactrum* sp. to produce both biosurfactants and bioemulsifiers, while representatives of the genera *Enterobacter* sp. and *Pantoea* sp. produce substances that are mainly biosurfactants.

It's evident that different strains exhibited varied performance across different substrates. Notably, strains such as *Bacillus* sp. 2os, *Bacillus* sp. 3os и *Sphingomonas* sp. 2mo displayed activity across multiple substrates, whereas *Peribacillus* sp. 1mo solely showed effectiveness in the glycerol substrate. This variability might be attributed to the individual metabolic capabilities of each specific strain.

The process of identifying the most effective substrate involved the assignment of weight to each parameter (Supplement Table S1). Weight scores were designated to the lowest surface tension (ST) values and the highest E24 values, which were then used to calculate the weighted arithmetic mean (Supplement Table S2). Based on these assessments, it was determined that sunflower cake served as the most effective medium for cultivating microorganisms with surface-active properties. In contrast, glycerol displayed greater suitability for emulsifying properties. Sunflower cake not only supported the growth of microorganisms with emulsifying properties but also secured the second-highest position in the ranking. Considering our objective to select a cost-effective substrate for further investigations, sunflower cake was selected, while potato peelings were identified as the least effective substrate. The efficacy of sunflower cake as a substrate for biosurfactant production has been corroborated by numerous studies [40,41]. For instance, Ciurko et al. (2022) reported that sunflower cake enabled the enhancement of *Bacillus subtilis* surfactin at a concentration of 1.19 ± 0.03 g L^{-1} [39].

During the evaluation of the most effective strains, the individual weights for each value and their corresponding weighted arithmetic mean were analyzed. Remarkably,

the most effective strains, those with weight scores surpassing 20, included eight specific strains: *Enterobacter* sp. 2pp, strain 2wfo, *Peribacillus* sp. 1mo, *Sphingomonas* sp. 2mo, *Ochrobactrum* sp. 5mo, *Shouchella* sp. 6mo, *Bacillus* sp. 1os, *Bacillus* sp. 2os.

3.4. Biosurfactants Yield

The yield of biosurfactants for proficient strains in a medium based on sunflower cake was evaluated (Figure 1). Additionally, the yield of biosurfactants in a conventional glycerol-containing medium was also assessed for comparison.

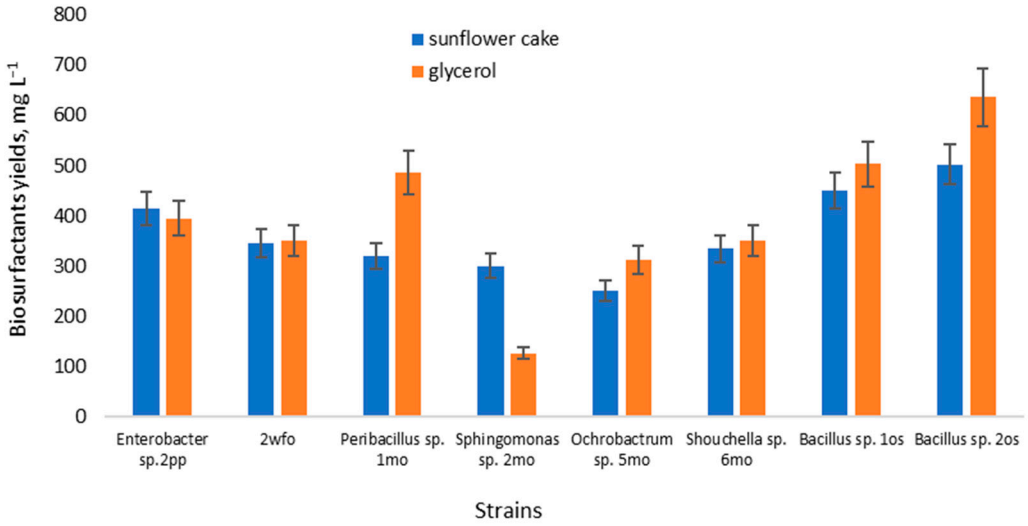

Figure 1. Biosurfactant yield from glycerol and sunflower cake medium.

In the glycerol-based medium, the biosurfactant yield ranged from 126–636 mg L^{-1}, while in the medium with sunflower cake, it was between 250–502 mg L^{-1}. Notably, the strain of *Sphingomonas* sp. 2mo exhibited the lowest yield in the glycerol-based medium, while a strain of the genus *Ochrobactrum* sp. 5mo showed the lowest yield in the sunflower cake medium. These yields align with existing literature data [8,42]. Rane et al. (2017) reported biosurfactant production in minimal media, noting 0.207 g L^{-1} when glucose served as the sole carbon source, and 0.241 g L^{-1} with molasses [18]. However, biosurfactant production wasn't feasible using whey as the carbon source. Additionally, Rane found that agro-wastes, bagasse, and orange peel extracts yielded 0.127 and 0.089 g L^{-1} of biosurfactant, respectively. Das (2018) discovered that *Pseudomonas azotoformans* AJ15 yielded rhamnolipids in the range of 0.6–0.76 g L^{-1} while cultivating on potato peelings ranging from 5–15 g L^{-1} [43]. Similar concentrations of sugarcane waste resulted in yields of 0.6–0.97 g L^{-1}.

Notably, the strain *Bacillus* sp. 2os achieved the highest yield in both glycerol and sunflower cake mediums. Other studies confirm the potential of this genus to attain biosurfactant yields within the range of 1–2.5 g L^{-1} [30]. However, these results indicate the need for further optimization of the nutrient medium. Despite the slightly superior yield observed based on glycerol, sunflower cake exhibited comparable results and proved to be more economically advantageous.

The manifestation of surface-active and emulsifying properties in biosurfactants of the same microorganisms on different substrates can be explained by the different hydrophilic-lipophilic balances of these substrates. The different hydrophilic-lipophilic balance of sunflower cake and glycerol ensures the synthesis of biosurfactants of different molecular weights with different properties [44].

4. Conclusions

The findings of this study revealed that strains isolated from various sources, including potato peelings, rapeseed and sunflower cake, oil-contaminated soil, soil contaminated

with motor oils, waste from water utility grease traps and oily sludge, as well as used frying oil, possess the capacity for biosurfactant production. From these isolates, 8 microorganisms were identified as displaying the greatest potential in biosurfactant synthesis with surfactant and emulsifying properties (*Enterobacter* sp. 2pp, strain 2wfo, *Peribacillus* sp. 1mo, *Sphingomonas* sp. 2mo, *Ochrobactrum* sp. 5mo, *Shouchella* sp. 6mo, *Bacillus* sp. 1os, *Bacillus* sp. 2os), which will be utilized in further investigations. In this study, for the first time, representatives of the genus *Sphingomonas* were isolated that have the ability to synthesize biosurfactants with significant emulsifying and surfactant properties. Based on the biosurfactant yields, the *Bacillus* sp. 2os strain demonstrated the highest biosurfactant production capability—0.501 and 0.636 g L^{-1} for glycerol and glucose, respectively. The study investigated the feasibility of utilizing three types of waste as carbon sources: potato peelings, rapeseed and sunflower cake, and waste frying oil. It was determined that among all substrates, sunflower cake exhibited the most promising potential for biosurfactant production. This substrate led to the most substantial reduction in surface tension and the highest emulsification index among the majority of the strains tested.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres15010010/s1, Table S1: Weight scores; Table S2: Weighted arithmetic means.

Author Contributions: Conceptualization, S.S.; methodology, L.B.; validation, A.G.; formal analysis, P.K.; investigation, L.B. and A.K.; data curation, S.S.; writing—original draft preparation, A.G. and L.B.; writing—review and editing, P.K.; visualization, A.K.; supervision, S.S.; funding acquisition, P.K. All authors have read and agreed to the published version of the manuscript.

Funding: The work has been performed with the financial support of the Russian Science Foundation, grant No. 23-24-00611.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Mohanty, S.S.; Koul, Y.; Varjani, S.; Pandey, A.; Ngo, H.H.; Chang, J.S.; Wong, J.W.C.; Bui, X.T. A Critical Review on Various Feedstocks as Sustainable Substrates for Biosurfactants Production: A Way towards Cleaner Production. *Microb. Cell Fact.* **2021**, *20*, 120. [CrossRef] [PubMed]
2. Galitskaya, P.; Karamova, K.; Biktasheva, L.; Galieva, G.; Gordeev, A.; Selivanovskaya, S. Lipopeptides Produced by Bacillus Mojavensis P1709 as an Efficient Tool to Maintain Postharvest Cherry Tomato Quality and Quantity. *Agriculture* **2022**, *12*, 609. [CrossRef]
3. Khopade, A.; Biao, R.; Liu, X.; Mahadik, K.; Zhang, L.; Kokare, C. Production and Stability Studies of the Biosurfactant Isolated from Marine *Nocardiopsis* sp. B4. *Desalination* **2012**, *285*, 198–204. [CrossRef]
4. Carolin, C.F.; Senthil Kumar, P.; Mohanakrishna, G.; Hemavathy, R.V.; Rangasamy, G.; M Aminabhavi, T. Sustainable Production of Biosurfactants via Valorisation of Industrial Wastes as Alternate Feedstocks. *Chemosphere* **2023**, *312*, 137326. [CrossRef] [PubMed]
5. Gaur, V.K.; Sharma, P.; Sirohi, R.; Varjani, S.; Taherzadeh, M.J.; Chang, J.-S.; Yong Ng, H.; Wong, J.W.C.; Kim, S.-H. Production of Biosurfactants from Agro-Industrial Waste and Waste Cooking Oil in a Circular Bioeconomy: An Overview. *Bioresour. Technol.* **2022**, *343*, 126059. [CrossRef] [PubMed]
6. Singh, P.; Patil, Y.; Rale, V. Biosurfactant Production: Emerging Trends and Promising Strategies. *J. Appl. Microbiol.* **2019**, *126*, 2–13. [CrossRef] [PubMed]
7. Aparna, A.; Srinikethan, G.; Smitha, H. Production and Characterization of Biosurfactant Produced by a Novel *Pseudomonas* sp. 2B. *Colloids Surf. B Biointerfaces* **2012**, *95*, 23–29. [CrossRef]
8. Moussa, T.A.A.; Mohamed, M.S.; Samak, N. Production and Characterization of Di-Rhamnolipid Produced by *Pseudomonas Aeruginosa* TMN. *Braz. J. Chem. Eng.* **2014**, *31*, 867–880. [CrossRef]

9. Bonmatin, J.-M.; Laprevote, O.; Peypoux, F. Diversity Among Microbial Cyclic Lipopeptides: Iturins and Surfactins. Activity-Structure Relationships to Design New Bioactive Agents. *Comb. Chem. High Throughput Screen.* **2012**, *6*, 541–556. [CrossRef]
10. Das, P.; Mukherjee, S.; Sen, R. Substrate Dependent Production of Extracellular Biosurfactant by a Marine Bacterium. *Bioresour. Technol.* **2009**, *100*, 1015–1019. [CrossRef]
11. Singh, A.K.; Rautela, R.; Cameotra, S.S. Substrate Dependent in Vitro Antifungal Activity of Bacillus Sp Strain AR2. *Microb. Cell Fact.* **2014**, *13*, 67. [CrossRef] [PubMed]
12. Mouafo, T.H.; Mbawala, A.; Ndjouenkeu, R. Effect of Different Carbon Sources on Biosurfactants' Production by Three Strains of *Lactobacillus* spp. *Biomed Res. Int.* **2018**, *2018*, 5034783. [CrossRef] [PubMed]
13. Ndlovu, T.; Rautenbach, M.; Khan, S.; Khan, W. Variants of Lipopeptides and Glycolipids Produced by *Bacillus Amyloliquefaciens* and *Pseudomonas Aeruginosa* Cultured in Different Carbon Substrates. *AMB Express* **2017**, *7*, 109. [CrossRef]
14. Santos, D.; Rufino, R.; Luna, J.; Santos, V.; Sarubbo, L. Biosurfactants: Multifunctional Biomolecules of the 21st Century. *Int. J. Mol. Sci.* **2016**, *17*, 401. [CrossRef] [PubMed]
15. Alizadeh-Sani, M.; Hamishehkar, H.; Khezerlou, A.; Azizi-Lalabadi, M.; Azadi, Y.; Nattagh-Eshtivani, E.; Fasihi, M.; Ghavami, A.; Aynehchi, A.; Ehsani, A. Bioemulsifiers Derived from Microorganisms: Applications in the Drug and Food Industry. *Adv. Pharm. Bull.* **2018**, *8*, 191–199. [CrossRef] [PubMed]
16. Uzoigwe, C.; Burgess, J.G.; Ennis, C.J.; Rahman, P.K.S.M. Bioemulsifiers Are Not Biosurfactants and Require Different Screening Approaches. *Front. Microbiol.* **2015**, *6*, 245. [CrossRef] [PubMed]
17. Stoimenova, E.; Vasileva-Tonkova, E.; Sotirova, A.; Galabova, D.; Lalchev, Z. Evaluation of Different Carbon Sources for Growth and Biosurfactant Production by Pseudomonas Fluorescens Isolated from Wastewaters. *Z. Fur Naturforschung Sect. C J. Biosci.* **2009**, *64*, 96–102. [CrossRef]
18. Rane, A.N.; Baikar, V.V.; Ravi Kumar, D.V.; Deopurkar, R.L. Agro-Industrial Wastes for Production of Biosurfactant by Bacillus Subtilis ANR 88 and Its Application in Synthesis of Silver and Gold Nanoparticles. *Front. Microbiol.* **2017**, *8*, 492. [CrossRef]
19. Cooper, D.G.; Goldenberg, B.G. Surface-Active Agents from Two Bacillus Species. *Appl. Environ. Microbiol.* **1987**, *53*, 224–229. [CrossRef]
20. Yin, H.; Qiang, J.; Jia, Y.; Ye, J.; Peng, H.; Qin, H.; Zhang, N.; He, B. Characteristics of Biosurfactant Produced by *Pseudomonas Aeruginosa* S6 Isolated from Oil-Containing Wastewater. *Process Biochem.* **2009**, *44*, 302–308. [CrossRef]
21. Ludwig, W. Nucleic Acid Techniques in Bacterial Systematics and Identification. *Int. J. Food Microbiol.* **2007**, *120*, 225–236. [CrossRef] [PubMed]
22. Waghmare, P.R.; Patil, S.M.; Jadhav, S.L.; Jeon, B.H.; Govindwar, S.P. Utilization of Agricultural Waste Biomass by Cellulolytic Isolate Enterobacter sp. SUK-Bio. *Agric. Nat. Resour.* **2018**, *52*, 399–406. [CrossRef]
23. Pei, X.H.; Zhan, X.H.; Wang, S.M.; Lin, Y.S.; Zhou, L.X. Effects of a Biosurfactant and a Synthetic Surfactant on Phenanthrene Degradation by a Sphingomonas Strain. *Pedosphere* **2010**, *20*, 771–779. [CrossRef]
24. Berg, G. Rhizobacteria of Oilseed Rape Antagonistic to Verticillium Dahliae Var. Longisporum STARK. *Z Pflanzenkr Pflanzenschutz* **1996**, *103*, 20–30.
25. Kalvandi, S.; Garousin, H.; Pourbabaee, A.A.; Farahbakhsh, M. The Release of Petroleum Hydrocarbons from a Saline-Sodic Soil by the New Biosurfactant-Producing Strain of *Bacillus* sp. *Sci. Rep.* **2022**, *12*, 19770. [CrossRef] [PubMed]
26. Montecillo, J.A.V.; Bae, H. Reclassification of Brevibacterium Frigoritolerans as Peribacillus Frigoritolerans Comb. Nov. Based on Phylogenomics and Multiple Molecular Synapomorphies. *Int. J. Syst. Evol. Microbiol.* **2022**, *72*, 005389. [CrossRef]
27. Khondee, N.; Ruamyat, N.; Luepromchai, E.; Sikhao, K.; Hawangchu, Y. Bioconversion of Lignocellulosic Wastes to Zwitterionic Biosurfactants by an Alkaliphilic Bacterium: Process Development and Product Characterization. *Biomass Bioenergy* **2022**, *165*, 106568. [CrossRef]
28. Manetsberger, J.; Caballero Gómez, N.; Soria-Rodríguez, C.; Benomar, N.; Abriouel, H. Simply Versatile: The Use of Peribacillus Simplex in Sustainable Agriculture. *Microorganisms* **2023**, *11*, 2540. [CrossRef]
29. Ibrahim, H.M.M. Characterization of Biosurfactants Produced by Novel Strains of Ochrobactrum Anthropi HM-1 and Citrobacter Freundii HM-2 from Used Engine Oil-Contaminated Soil. *Egypt. J. Pet.* **2018**, *27*, 21–29. [CrossRef]
30. Renard, P.; Canet, I.; Sancelme, M.; Wirgot, N.; Deguillaume, L.; Delort, A.M. Screening of Cloud Microorganisms Isolated at the Puy de Dôme (France) Station for the Production of Biosurfactants. *Atmo. Chem. Phys.* **2016**, *16*, 12347–12358. [CrossRef]
31. Darwesh, O.M.; Mahmoud, M.S.; Barakat, K.M.; Abuellil, A.; Ahmad, M.E. Improving the Bioremediation Technology of Contaminated Wastewater Using Biosurfactants Produced by Novel Bacillus Isolates. *Heliyon* **2021**, *7*, e08616. [CrossRef] [PubMed]
32. Chaurasia, L.K.; Tamang, B.; Tirwa, R.K.; Lepcha, P.L. Influence of Biosurfactant Producing Bacillus Tequilensis LK5.4 Isolate of Kinema, a Fermented Soybean, on Seed Germination and Growth of Maize (*Zea mays* L.). *3 Biotech* **2020**, *10*, 297. [CrossRef] [PubMed]
33. Rosenberg, E.; Rubinovitz, C.; Gottlieb, A.; Rosenhak, S.; Ron, E.Z. Production of Biodispersan by Acinetobacter Calcoaceticus A2. *Appl. Environ. Microbiol.* **1988**, *54*, 317–322. [CrossRef] [PubMed]
34. Mendes Lopes, E. Emulsification Properties of Bioemulsifiers Produced by Wild-Type and Mutant Bradyrhizobium Elkanii Strains. *J. Bioremediat. Biodegrad.* **2014**, *5*, 1000245. [CrossRef]

35. Rabiei, A.; Sharifinik, M.; Niazi, A.; Hashemi, A.; Ayatollahi, S. Core Flooding Tests to Investigate the Effects of IFT Reduction and Wettability Alteration on Oil Recovery during MEOR Process in an Iranian Oil Reservoir. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 5979–5991. [CrossRef] [PubMed]
36. Essghaier, B.; Mallat, N.; Khwaldia, K.; Mottola, F.; Rocco, L.; Hannachi, H. Production and Characterization of New Biosurfactants/Bioemulsifiers from Pantoea Alhagi and Their Antioxidant, Antimicrobial and Anti-Biofilm Potentiality Evaluations. *Molecules* **2023**, *28*, 1912. [CrossRef] [PubMed]
37. Sen, R. Response Surface Optimization of the Critical Media Components for the Production of Surfactin. *J. Chem. Technol. Biotechnol.* **1997**, *68*, 263–270. [CrossRef]
38. Jiménez-Peñalver, P.; Koh, A.; Gross, R.; Gea, T.; Font, X. Biosurfactants from Waste: Structures and Interfacial Properties of Sophorolipids Produced from a Residual Oil Cake. *J. Surfactants Deterg.* **2020**, *23*, 481–486. [CrossRef]
39. Ciurko, D.; Czyżnikowska, Ż.; Kancelista, A.; Łaba, W.; Janek, T. Sustainable Production of Biosurfactant from Agro-Industrial Oil Wastes by Bacillus Subtilis and Its Potential Application as Antioxidant and ACE Inhibitor. *Int. J. Mol. Sci.* **2022**, *23*, 10824. [CrossRef]
40. Umar, A.A.; Saaid, I.B.M.; Sulaimon, A.A.; Pilus, R.B.M. A Review of Petroleum Emulsions and Recent Progress on Water-in-Crude Oil Emulsions Stabilized by Natural Surfactants and Solids. *J. Pet. Sci. Eng.* **2018**, *165*, 673–690. [CrossRef]
41. Saimmai, A.; Rukadee, O.; Onlamool, T.; Sobhon, V.; Maneerat, S. Isolation and Functional Characterization of a Biosurfactant Produced by a New and Promising Strain of Oleomonas Sagaranensis AT18. *World J. Microbiol. Biotechnol.* **2012**, *28*, 2973–2986. [CrossRef] [PubMed]
42. Wu, J.Y.; Yeh, K.L.; Lu, W.B.; Lin, C.L.; Chang, J.S. Rhamnolipid Production with Indigenous *Pseudomonas Aeruginosa* EM1 Isolated from Oil-Contaminated Site. *Bioresour. Technol.* **2008**, *99*, 1157–1164. [CrossRef] [PubMed]
43. Das, A.J.; Kumar, R. Utilization of Agro-Industrial Waste for Biosurfactant Production under Submerged Fermentation and Its Application in Oil Recovery from Sand Matrix. *Bioresour. Technol.* **2018**, *260*, 233–240. [CrossRef] [PubMed]
44. Liepins, J.; Balina, K.; Soloha, R.; Berzina, I.; Lukasa, L.K.; Dace, E. Glycolipid Biosurfactant Production from Waste Cooking Oils by Yeast: Review of Substrates, Producers and Products. *Fermentation* **2021**, *7*, 136. [CrossRef]