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Production of Microbial Biosurfactant Using Waste Frying Oil as a Low-Cost Substrate

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ABSTRACT

Biosurfactants are surface-active molecules made by bacteria, yeasts and fungi, and their cost of production remains the main obstacle to wider use. Because the growth substrate is often cited as a large share of that cost, waste frying oil, a lipid-rich residue that kitchens and food processors must dispose of anyway, has attracted attention as a cheap carbon source. This narrative review asks how far the published literature supports that idea. It draws on work about the chemistry of used frying oil, the main producer organisms and product classes, fermentation and recovery practice, and the assays used to detect and compare biosurfactants, and it reports no new experimental data. Used oil is a workable but variable substrate: it is rich in fatty acids, yet its composition depends on the oil, the frying history and the pretreatment. Rhamnolipid production by *Pseudomonas* species is the best documented route, although the opportunistic pathogenicity of the usual producers complicates scale-up. Yields, assays and units differ so widely between reports that direct comparison is rarely safe, and few papers count the cost of collecting, cleaning and processing the oil. The review concludes that waste frying oil is a plausible low-cost substrate, that the size of any saving is not established, and it lists the reporting items that would let studies be compared fairly.

Keywords: Biosurfactant; Waste frying oil; Rhamnolipid; Low-cost substrate; Fermentation; Narrative review

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Article Class: Narrative Review

1. Introduction

Bacteria, yeasts and some fungi release amphiphilic molecules that lower surface and interfacial tension, help the producer reach hydrophobic food and, in some cases, protect it from competitors (1, 2).

Collectively these molecules are called biosurfactants, and they have drawn interest as milder, more biodegradable alternatives to petrochemical surfactants in cleaning, oil recovery, soil remediation, food and cosmetic formulation (3, 4, 5, 6,



7, 8, 9).

Interest has not turned into widespread use, and the reason given most often is cost. Fermentation, recovery and purification are expensive compared with synthetic chemistry, and the raw materials for growth are repeatedly named as a large part of the bill (10, 11). Searching for cheap, abundant substrates has therefore become a field of its own, covering agricultural residues, industrial by-products and used oils (12, 13).

Used frying oil is an obvious candidate. Restaurants, snack makers and households generate it in large quantities, it has to be disposed of or reprocessed, and it is rich in the fatty acids that many producer organisms like to eat (14, 15). But cheap is not the same as easy, because heat alters the oil and its composition varies from one source to the next. This review looks at what the published literature says about producing microbial biosurfactant from waste frying oil, and how firmly it says it. The article reports no new experimental data and no local measurements of any kind. The aim is to separate what has been shown from what is routinely repeated.

2. Literature Review

2.1 Biosurfactants and Their Producers

Biosurfactants are usually grouped by chemistry. Low-molecular-mass products, mainly glycolipids and lipopeptides, are strong surface-tension reducers, while high-molecular-mass polymers tend to be better at stabilising emulsions (16, 17, 18). Table 1 lists the classes met most often in the production literature. Rhamnolipids from *Pseudomonas aeruginosa* are the best-studied glycolipids, with biosynthesis, diversity and regulation reviewed at length (19, 20, 21). Mono- and di-rhamnolipid forms with different chain lengths are often made together.

2.2 The Cost Problem

Several reviews make the same point from different angles. A biosurfactant that works well in the laboratory is not necessarily one that can be sold at a competitive price, because the price depends on yield, on how the product is recovered, and on what the producer was fed (10, 11, 26). Downstream processing is often an afterthought in laboratory studies, yet foaming, dilution and solvent or acid steps make it a substantial share of the effort (10, 27).

Table 1. Principal biosurfactant classes and typical producers.

Class	Typical producers	Notable features
Rhamnolipids	<i>Pseudomonas aeruginosa</i> and relatives (19, 21)	Glycolipids, strong surface activity, regulated by nitrogen supply (20)
Surfactin and other lipopeptides	<i>Bacillus subtilis</i> and relatives (22, 23)	Cyclic peptides with a fatty acid tail (24)
Sophorolipids and other yeast glycolipids	<i>Candida</i> and related yeasts (17)	Often made from oils and sugars together (25)
Polymeric bioemulsifiers	<i>Acinetobacter</i> and others (16)	High molecular mass, good emulsion stabilisers

Note: Qualitative summary; classes overlap and many strains make mixtures of congeners.

Cheap substrates are one answer, and the literature on them is large. Strains have been grown on cassava wastewater (28), potato substrates (29), cheaper sources for a glycolipid-producing *Bacillus* (30), refinery soapstock (31) and other low-cost raw materials for rhamnolipids (32).

2.3 What Frying Does to Oil

Oil that has been used for frying is no longer the oil that went into the pan. Heat, water and air drive hydrolysis, oxidation and polymerisation, leaving free fatty



acids, oxidised and polar compounds and larger polymeric material alongside the original triacylglycerols (14). Table 2 sets out which of these features matter for a fermentation. Free fatty acids are easier for cells to take up than intact triglycerides, but oxidised material can inhibit growth.

Table 2. Features of used frying oil that matter to a fermentation.

Feature	Why it matters	Practical consequence
Free fatty acids	Formed by hydrolysis during frying (14)	Easier uptake, but can inhibit growth
Oxidation products	Accumulate with heating and reuse (14)	Possible toxicity to the producer
Polar and polymeric material	Raises viscosity and polarity	Poorer dispersion in the medium
Food residues and water	Carried over from cooking	Filtering and settling needed
Batch variability	Different oils, different histories	Results hard to repeat

Note: Qualitative; the size of each effect depends on the oil, the frying history and the pretreatment.

Which effect dominates depends on the oil, how long it was heated, what was fried in it and whether it was filtered. Production studies rarely quantify this, so a sweeping statement that waste frying oil supports good yields is hard to defend. This variability is one reason the same oil can look excellent in one laboratory and disappointing in the next. The practical competition for the oil is also real: used cooking oil is a feedstock for biodiesel, and where that market is active the nominal price of the oil may not be near zero (33).

2.4 *Pseudomonas* and Rhamnolipids from Frying Oil

Work in the early 2000s screened *Pseudomonas aeruginosa* strains for rhamnolipid production from waste frying oils and selected a strain for further study (15), and the rhamnolipids it made were

later characterised for their physicochemical and antimicrobial properties (34). Other groups have reused waste frying oil with different strains (35), and crude products from other *P. aeruginosa* isolates have been described structurally (36).

These reports are encouraging, but they come with a built-in complication. *P. aeruginosa* is an opportunistic human pathogen, and an industrial process built on it raises safety and regulatory questions that a non-pathogenic host would not (27, 37), and a product made from an oil of uncertain history would need further testing before use in food (38). The evidence for safer hosts on waste frying oil specifically is much thinner.

3. Materials and Methods

3.1 Scope and Question

The review asks one question in two parts: does the published literature support waste frying oil as a practical carbon source for microbial biosurfactant production, and how well do reports allow the results to be compared? The question was broken into what a production study must state: the oil and its treatment, the organism and its safety, the medium and conditions, product recovery, and how production was measured. None of these questions is exotic, but published studies often leave them unanswered.

3.2 Literature Assembly

The review draws on peer-reviewed work in four areas: the chemistry of heated frying oil; producer organisms and product classes; fermentation, recovery and substrate studies; and methods of detection and measurement. Sources were chosen for relevance and clarity of method, and only work published up to 2013 is cited.

We did not run a formal systematic search and do not claim one. This is a narrative



synthesis, and the choice of sources reflects the authors' judgement. The review is therefore a better guide to the questions a production study should answer than to the size of any yield, and it avoids quoting figures that could not be checked against the primary reports.

3.3 Appraisal Framework

To judge how far a production report can be compared with another, we looked for the items in Table 3. They were drawn from the process and methods literature (10, 20, 39) and from the safety discussion around rhamnolipid producers (37).

Table 3. Items worth reporting so that biosurfactant studies can be compared.

Item	What to state	Why it matters
Substrate	Oil type, frying history, pretreatment	Composition drives outcome (14)
Strain	Identity, source, safety class	Pathogenicity and scale-up (37)
Medium and conditions	Nitrogen source, pH, aeration, time	Production is regulated (20)
Assay	Method, controls, replicates	Methods disagree (39, 40)
Yield	Basis and units, crude or purified	Crude mass is not product
Recovery	Method and losses	A large share of cost (10)

Note: Items are those implied by the cited methodological and process literature; no study is singled out.

4. Results

4.1 Waste Frying Oil as a Substrate

Waste frying oil is treated as a hydrophobic carbon source that cells convert to biomass and, under suitable conditions, to surface-active product. The route from kitchen to product runs through collection, removal of food particles and water, sometimes a heat or filtration step, sterilisation, fermentation, recovery and characterisation, as sketched in Figure 1.

Each step offers a point at which the result can change.

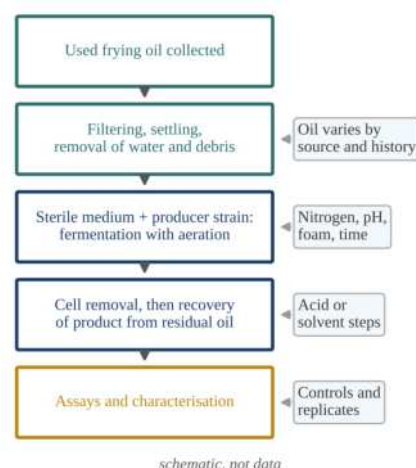


Figure 1. From used frying oil to a characterised biosurfactant (schematic, not data).

Oil is insoluble, so the medium has to be agitated and aerated well enough to disperse it, which is also what makes foam a nuisance. A substrate that arrives with contaminants may bring microbes or inhibitors with it, so pretreatment is part of the process design (11, 14). Small differences in medium or inoculum can look trivial on paper and still change a yield considerably.

4.2 Producer Organisms and Process Variables

Pseudomonas aeruginosa remains the best-documented producer of rhamnolipids from oils, including waste frying oil (15, 35). *Bacillus* strains make lipopeptides and glycolipids on many substrates (29, 30, 41), and yeasts have been grown on oils and sugars together (25). All depend on conditions. In rhamnolipid production, limiting nitrogen is widely reported to favour product formation, and the nitrogen source, iron, pH and temperature all matter (19, 20).

Whether product accumulates during growth or mainly after growth has slowed differs between organisms and conditions, as sketched in Figure 2. A culture sampled



early may look unproductive when product only appears later, and one harvested late may have lost product to degradation. A culture that has been given time to adapt to an oil behaves differently from one that has not.

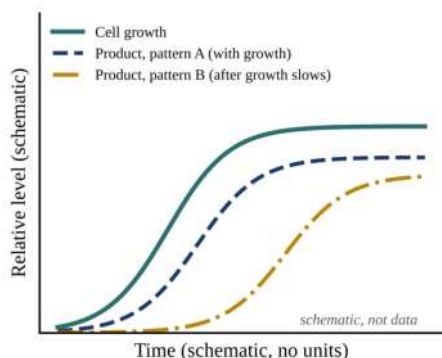


Figure 2. Conceptual time course of growth and product accumulation (schematic of two possible patterns, not data).

4.3 Recovery and Characterisation

Recovery usually begins with removing cells, then separating product from residual oil by acid precipitation or solvent extraction (10, 27). With oil substrates, residual oil is the main difficulty: it can be confused with product when mass is weighed, and it complicates any claim about yield. Crude extracts are often reported as if they were pure biosurfactant, which makes comparison unsafe.

4.4 Measuring and Comparing Production

No single assay answers every question. Drop collapse, oil spreading and emulsification tests are quick screens, surface tension and critical micelle concentration measure surface activity in a more quantitative way, and chemical analysis identifies the product. Figure 3 shows which kinds of question each assay can inform. A comparison of detection methods found blood agar lysis unreliable as a screen (40), and reviews stress that results from different assays are not interchangeable (39).

A further difficulty is how yield is expressed. Reports use grams per litre of crude extract, of purified product, of rhamnase equivalents or of emulsification activity, and comparing them as if they measured the same quantity is a mistake. Without a stated basis, a number in an abstract is hard to interpret. Read in this light, a failure to find activity in a single screening assay says little about the strain. An uninoculated oil control belongs in every report, since oils and their breakdown products can lower surface tension on their own. It is a habit worth keeping even when the answer seems obvious.

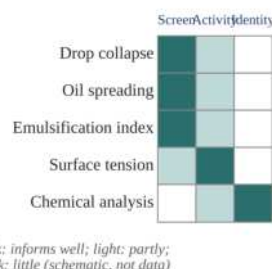


Figure 3. Which common assays inform which question (qualitative matrix, not data).

5. Discussion

5.1 Interpretation

The central finding of this review is a conditional one. Waste frying oil can serve as a carbon source for biosurfactant production, and the best-documented route is rhamnolipid production by *Pseudomonas*, but the strength of that conclusion is limited by variable oil, mixed assays and incomplete cost accounting. The oil is cheap to buy and expensive to standardise. That is a modest claim, but it is the one the literature will currently carry. The economic case is the weakest part of the literature. Papers often call waste oil low-cost without counting collection, transport, filtering and treatment, or the cost of recovering product from a medium that still contains oil (10, 11). A saving that



vanishes once collection, filtering and downstream work are priced is not one a factory would ever see.

5.2 Limitations

This review has the limits of any narrative synthesis. The sources were chosen by judgement, not by a documented search, so important work may be missing and the balance of what is cited reflects the authors' choices. We have deliberately not quoted yields from individual reports, because their bases and units differ. A reader who needs a number should consult the original studies and apply [Table 3](#). The point is not that any single report is poor, only that its numbers travel less far than its abstract implies. Reviews are more useful when they say where the evidence stops than when they smooth the edges.

5.3 Future Work

Three needs stand out. First, studies that characterise the oil used, including its frying history and treatment, and report the effect of that variation on the outcome. Second, comparable reporting of yield, with a stated basis, crude versus purified product, and consistent assays and controls. Third, honest economic analyses that include collection, pretreatment and recovery, and safety work on both producer strains and products, including non-pathogenic alternatives to the usual rhamnolipid producers ([27](#), [37](#)). Nothing here argues against valorising waste oil; it argues for measuring what happens when it is done. These cautions apply equally to any waste-derived substrate, not only to frying oil.

6. Conclusion

Waste frying oil is a plausible and widely studied substrate for microbial biosurfactant production, rich in fatty acids and available where food is fried. Reports of rhamnolipid production on such oil are credible, yet they rest on a variable

substrate, unharmonised assays and, with few exceptions, an incomplete account of cost. How much money the oil really saves is not established.

The practical message is short: describe the oil, justify the organism, state the basis of the yield, include the controls and count every cost.

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9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

T.S. conceived the review and wrote the sections on substrates and bioprocess design. S.S. wrote the sections on producer organisms and product classes. D.B. wrote the sections on measurement, comparison and microbiological safety. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No human participants or animals were involved and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

13. References

1. Ron, E. Z., & Rosenberg, E. (2001). Natural roles of biosurfactants. *Environmental Microbiology*, 3(4), 229–236. DOI: [10.1046/j.1462-2920.2001.00190.x](https://doi.org/10.1046/j.1462-2920.2001.00190.x)
2. Hommel, R. K. (1990). Formation and physiological role of biosurfactants produced by hydrocarbon-utilizing microorganisms.



- Biodegradation*, 1(2–3), 107–119. DOI: [10.1007/BF00117055](https://doi.org/10.1007/BF00117055)
3. Desai, J. D., & Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Reviews*, 61(1), 47–64. DOI: [10.1128/mmbr.61.1.47-64.1997](https://doi.org/10.1128/mmbr.61.1.47-64.1997)
 4. Banat, I. M., Makkar, R. S., & Cameotra, S. S. (2000). Potential commercial applications of microbial surfactants. *Applied Microbiology and Biotechnology*, 53(5), 495–508. DOI: [10.1007/s002530051648](https://doi.org/10.1007/s002530051648)
 5. Mulligan, C. N. (2005). Environmental applications for biosurfactants. *Environmental Pollution*, 133(2), 183–198. DOI: [10.1016/j.envpol.2004.06.009](https://doi.org/10.1016/j.envpol.2004.06.009)
 6. Mulligan, C. N., Yong, R. N., & Gibbs, B. F. (2001). Surfactant-enhanced remediation of contaminated soil: A review. *Engineering Geology*, 60(1–4), 371–380. DOI: [10.1016/S0013-7952\(00\)00117-4](https://doi.org/10.1016/S0013-7952(00)00117-4)
 7. Pacwa-Płociniczak, M., Płaza, G. A., Piotrowska-Seget, Z., & Cameotra, S. S. (2011). Environmental applications of biosurfactants: Recent advances. *International Journal of Molecular Sciences*, 12(1), 633–654. DOI: [10.3390/ijms12010633](https://doi.org/10.3390/ijms12010633)
 8. Singh, A., Van Hamme, J. D., & Ward, O. P. (2007). Surfactants in microbiology and biotechnology: Part 2. Application aspects. *Biotechnology Advances*, 25(1), 99–121. DOI: [10.1016/j.biotechadv.2006.10.004](https://doi.org/10.1016/j.biotechadv.2006.10.004)
 9. Cameotra, S. S., & Makkar, R. S. (2004). Recent applications of biosurfactants as biological and immunological molecules. *Current Opinion in Microbiology*, 7(3), 262–266. DOI: [10.1016/j.mib.2004.04.006](https://doi.org/10.1016/j.mib.2004.04.006)
 10. Mukherjee, S., Das, P., & Sen, R. (2006). Towards commercial production of microbial surfactants. *Trends in Biotechnology*, 24(11), 509–515. DOI: [10.1016/j.tibtech.2006.09.005](https://doi.org/10.1016/j.tibtech.2006.09.005)
 11. Marchant, R., & Banat, I. M. (2012). Microbial biosurfactants: Challenges and opportunities for future exploitation. *Trends in Biotechnology*, 30(11), 558–565. DOI: [10.1016/j.tibtech.2012.07.003](https://doi.org/10.1016/j.tibtech.2012.07.003)
 12. Makkar, R. S., & Cameotra, S. S. (2002). An update on the use of unconventional substrates for biosurfactant production and their new applications. *Applied Microbiology and Biotechnology*, 58(4), 428–434. DOI: [10.1007/s00253-001-0924-1](https://doi.org/10.1007/s00253-001-0924-1)
 13. Makkar, R. S., Cameotra, S. S., & Banat, I. M. (2011). Advances in utilization of renewable substrates for biosurfactant production. *AMB Express*, 1, 5. DOI: [10.1186/2191-0855-1-5](https://doi.org/10.1186/2191-0855-1-5)
 14. Choe, E., & Min, D. B. (2007). Chemistry of deep-fat frying oils. *Journal of Food Science*, 72(5), R77–R86. DOI: [10.1111/j.1750-3841.2007.00352.x](https://doi.org/10.1111/j.1750-3841.2007.00352.x)
 15. Haba, E., Espuny, M. J., Busquets, M., & Manresa, A. (2000). Screening and production of rhamnolipids by *Pseudomonas aeruginosa* 47T2 NCIB 40044 from waste frying oils. *Journal of Applied Microbiology*, 88(3), 379–387. DOI: [10.1046/j.1365-2672.2000.00961.x](https://doi.org/10.1046/j.1365-2672.2000.00961.x)
 16. Rosenberg, E., & Ron, E. Z. (1999). High- and low-molecular-mass microbial surfactants. *Applied Microbiology and Biotechnology*, 52(2), 154–162. DOI: [10.1007/s002530051502](https://doi.org/10.1007/s002530051502)
 17. Lang, S. (2002). Biological amphiphiles (microbial biosurfactants). *Current Opinion in Colloid & Interface Science*, 7(1–2), 12–20. DOI: [10.1016/S1359-0294\(02\)00007-9](https://doi.org/10.1016/S1359-0294(02)00007-9)
 18. Van Hamme, J. D., Singh, A., & Ward, O. P. (2006). Physiological aspects: Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. *Biotechnology Advances*, 24(6), 604–620. DOI: [10.1016/j.biotechadv.2006.08.001](https://doi.org/10.1016/j.biotechadv.2006.08.001)
 19. Maier, R. M., & Soberón-Chávez, G. (2000). *Pseudomonas aeruginosa* rhamnolipids: Biosynthesis and potential biotechnological applications. *Applied Microbiology and Biotechnology*, 54(5), 625–633. DOI: [10.1007/s002530000443](https://doi.org/10.1007/s002530000443)
 20. Soberón-Chávez, G., Lépine, F., & Déziel, E. (2005). Production of rhamnolipids by *Pseudomonas aeruginosa*. *Applied Microbiology and Biotechnology*, 68(6), 718–725. DOI: [10.1007/s00253-005-0150-3](https://doi.org/10.1007/s00253-005-0150-3)
 21. Abdel-Mawgoud, A. M., Lépine, F., & Déziel, E. (2010). Rhamnolipids: Diversity of structures, microbial origins and roles. *Applied Microbiology and Biotechnology*, 86(5), 1323–1336. DOI: [10.1007/s00253-010-2498-2](https://doi.org/10.1007/s00253-010-2498-2)
 22. Peypoux, F., Bonmatin, J. M., & Wallach, J. (1999). Recent trends in the biochemistry of surfactin. *Applied Microbiology and Biotechnology*, 51(5), 553–563. DOI: [10.1007/s002530051432](https://doi.org/10.1007/s002530051432)
 23. Cooper, D. G., & Goldenberg, B. G. (1987). Surface-active agents from two *Bacillus* species. *Applied and Environmental Microbiology*, 53(2), 224–229. DOI: [10.1128/aem.53.2.224-229.1987](https://doi.org/10.1128/aem.53.2.224-229.1987)
 24. Morikawa, M., Hirata, Y., & Imanaka, T. (2000). A study on the structure–function relationship of lipopeptide biosurfactants. *Biochimica et Biophysica Acta*, 1488(3), 211–218. DOI: [10.1016/S1388-1981\(00\)00124-4](https://doi.org/10.1016/S1388-1981(00)00124-4)
 25. Sarubbo, L. A., Farias, C. B. B., & Campos-Takaki, G. M. (2007). Co-utilization of canola oil and glucose on the production of a surfactant by *Candida lipolytica*. *Current Microbiology*, 54(1), 68–73. DOI: [10.1007/s00253-006-0012-4](https://doi.org/10.1007/s00253-006-0012-4)



- [10.1007/s00284-006-0412-5](https://doi.org/10.1007/s00284-006-0412-5)
26. Banat, I. M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M. G., Fracchia, L., ... Marchant, R. (2010). Microbial biosurfactants production, applications and future potential. *Applied Microbiology and Biotechnology*, 87(2), 427–444. DOI: [10.1007/s00253-010-2589-0](https://doi.org/10.1007/s00253-010-2589-0)
 27. Henkel, M., Müller, M. M., Kügler, J. H., Lovaglio, R. B., Contiero, J., Sylatk, C., & Hausmann, R. (2012). Rhamnolipids as biosurfactants from renewable resources: Concepts for next-generation rhamnolipid production. *Process Biochemistry*, 47(8), 1207–1219. DOI: [10.1016/j.procbio.2012.04.018](https://doi.org/10.1016/j.procbio.2012.04.018)
 28. Nitschke, M., & Pastore, G. M. (2006). Production and properties of a surfactant obtained from *Bacillus subtilis* grown on cassava wastewater. *Bioresource Technology*, 97(2), 336–341. DOI: [10.1016/j.biortech.2005.02.044](https://doi.org/10.1016/j.biortech.2005.02.044)
 29. Fox, S. L., & Bala, G. A. (2000). Production of surfactant from *Bacillus subtilis* ATCC 21332 using potato substrates. *Bioresource Technology*, 75(3), 235–240. DOI: [10.1016/S0960-8524\(00\)00059-6](https://doi.org/10.1016/S0960-8524(00)00059-6)
 30. Thavasi, R., Jayalakshmi, S., Balasubramanian, T., & Banat, I. M. (2008). Production and characterization of a glycolipid biosurfactant from *Bacillus megaterium* using economically cheaper sources. *World Journal of Microbiology and Biotechnology*, 24(7), 917–925. DOI: [10.1007/s11274-007-9609-y](https://doi.org/10.1007/s11274-007-9609-y)
 31. Benincasa, M., Contiero, J., Manresa, M. A., & Moraes, I. O. (2002). Rhamnolipid production by *Pseudomonas aeruginosa* LBI growing on soapstock as the sole carbon source. *Journal of Food Engineering*, 54(4), 283–288. DOI: [10.1016/S0260-8774\(01\)00214-X](https://doi.org/10.1016/S0260-8774(01)00214-X)
 32. Rahman, K. S. M., Rahman, T. J., McClean, S., Marchant, R., & Banat, I. M. (2002). Rhamnolipid biosurfactant production by strains of *Pseudomonas aeruginosa* using low-cost raw materials. *Biotechnology Progress*, 18(6), 1277–1281. DOI: [10.1021/bp020071x](https://doi.org/10.1021/bp020071x)
 33. Math, M. C., Kumar, S. P., & Chetty, S. V. (2010). Technologies for biodiesel production from used cooking oil: A review. *Energy for Sustainable Development*, 14(4), 339–345. DOI: [10.1016/j.esd.2010.08.001](https://doi.org/10.1016/j.esd.2010.08.001)
 34. Haba, E., Pinazo, A., Jauregui, O., Espuny, M. J., Infante, M. R., & Manresa, A. (2003). Physicochemical characterization and antimicrobial properties of rhamnolipids produced by *Pseudomonas aeruginosa* 47T2 NCBIM 40044. *Biotechnology and Bioengineering*, 81(3), 316–322. DOI: [10.1002/bit.10474](https://doi.org/10.1002/bit.10474)
 35. Zhu, Y., Gan, J. J., Zhang, G. L., Yao, B., Zhu, W. J., & Meng, Q. (2007). Reuse of waste frying oil for production of rhamnolipids using *Pseudomonas aeruginosa* zju.u1M. *Journal of Zhejiang University Science A*, 8(9), 1514–1520. DOI: [10.1631/jzus.2007.A1514](https://doi.org/10.1631/jzus.2007.A1514)
 36. Pornsunthorntawe, O., Wongpanit, P., Chavadej, S., Abe, M., & Rujiravanit, R. (2008). Structural and physicochemical characterization of crude biosurfactant produced by *Pseudomonas aeruginosa* SP4 isolated from petroleum-contaminated soil. *Bioresource Technology*, 99(6), 1589–1595. DOI: [10.1016/j.biortech.2007.04.020](https://doi.org/10.1016/j.biortech.2007.04.020)
 37. Müller, M. M., Kügler, J. H., Henkel, M., Gerlitzki, M., Hörmann, B., Pöhnlein, M., ... Hausmann, R. (2012). Rhamnolipids: Next generation surfactants? *Journal of Biotechnology*, 162(4), 366–380. DOI: [10.1016/j.jbiotec.2012.05.022](https://doi.org/10.1016/j.jbiotec.2012.05.022)
 38. Nitschke, M., & Costa, S. G. V. A. O. (2007). Biosurfactants in food industry. *Trends in Food Science & Technology*, 18(5), 252–259. DOI: [10.1016/j.tifs.2007.01.002](https://doi.org/10.1016/j.tifs.2007.01.002)
 39. Satpute, S. K., Banpurkar, A. G., Dhakephalkar, P. K., Banat, I. M., & Chopade, B. A. (2010). Methods for investigating biosurfactants and bioemulsifiers: A review. *Critical Reviews in Biotechnology*, 30(2), 127–144. DOI: [10.3109/07388550903427272](https://doi.org/10.3109/07388550903427272)
 40. Youssef, N. H., Duncan, K. E., Nagle, D. P., Savage, K. N., Knapp, R. M., & McInerney, M. J. (2004). Comparison of methods to detect biosurfactant production by diverse microorganisms. *Journal of Microbiological Methods*, 56(3), 339–347. DOI: [10.1016/j.mimet.2003.11.001](https://doi.org/10.1016/j.mimet.2003.11.001)
 41. Joshi, S., Bharucha, C., & Desai, A. J. (2008). Production of biosurfactant and antifungal compound by fermented food isolate *Bacillus subtilis* 20B. *Bioresource Technology*, 99(11), 4603–4608. DOI: [10.1016/j.biortech.2007.07.030](https://doi.org/10.1016/j.biortech.2007.07.030)