Microbial biopolymers and surfactants

Dad: we learned at school that microbes can be used to clean up fatty wastes. When we wash up, we use detergents to remove fats from the dishes. Do microbes also use detergents?

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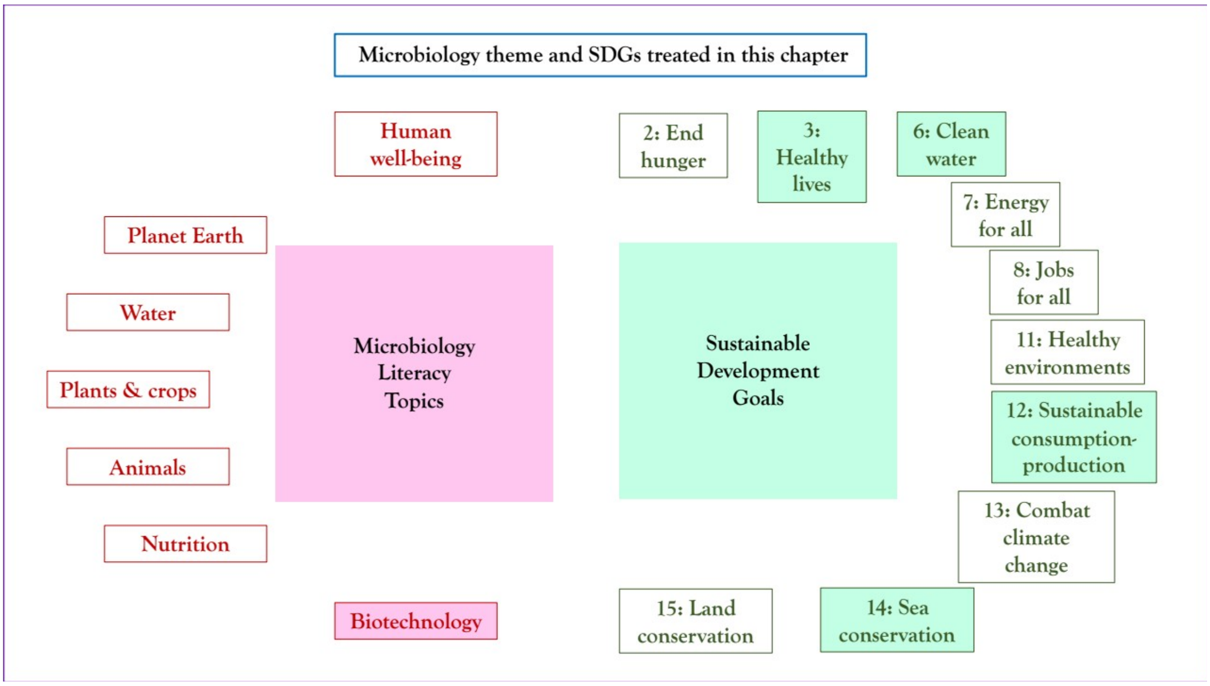
Storyline

"Oil and water don't mix!" We all know this truism, but we need to force them to mix sometimes, for example, every time we wash up after a nice meal, using clean water to remove oily remains of food on the dishes. To make them mix, we add washing-up liquid, which contains a detergent or surfactant. Microbes also need to force water and oil/fats to mix, e.g. when they degrade oil spills in the sea and use them as food. To do this, they produce biosurfactants and bioemulsifiers, both of which break up the oil into tiny droplets, thereby increasing the oil surface and hence the amount accessible for degradation: they increase its "bioavailability". Biosurfactants and bioemulsifiers play key roles in the biology and ecology of many microbes. However, they are not only important to microbes but also to us. They are used in the making of many foods and beverages, and in almost every sector of modern industry today (in textiles, construction, personal care products, medicine, etc.).

The Microbiology and Societal Context

The microbiology: biosurfactants; bioemulsifiers; bioavailability of hydrophobic substrates; biodegradability and low toxicity versus synthetic petrochemical-derived counterparts: enhancing bioremediation processes. *Societal context*: growing preferences for "natural" and "bio" versus "synthetic" and "chemical", especially in food; sustainability: renewable versus fossil hydrocarbon chemical feedstocks;



Microbial biopolymers and surfactants: the Microbiology

1. ***Microbial surfactants.*** Surfactants are surface-active substances: you can see this when you add a drop of washing-up liquid to a washing-up bowl containing a greasy pan. The oil on the surface of the water moves away from the point where you add the drop of washing-up liquid because of its surface activity. Surface-active substances have both 'water-loving' (i.e. hydrophilic) and 'oil-loving' (i.e. hydrophobic) chemical groups. This allows surfactants to interact simultaneously with both aqueous and non-aqueous substances, and thereby permit water and water-soluble substances to mix with oil and oil-soluble substances – common examples are soaps and detergents which are largely surfactants that permit the removal of oily substances from your hands, pots, pans and other surfaces.

Every sector of modern industry today uses surfactants in one form or another, such as in the making of clothes and other textiles, as ingredients in foods and beverages, in the ink of pens and printers, and the list is endless. An important issue with many of the surfactants that are used today is that they are synthetic, which means they are not natural; rather, they are produced in industry using petroleum as the base material. There are problems with this because when these synthetic surfactants enter the environment, they can cause harm to natural ecosystems as many of these compounds can be toxic to living cells, especially when released into the environment in large quantities. There is also concern that synthetic surfactants, when used as ingredients in foods and beverages, may not be harmless to humans.

On the other hand, surfactants produced by biological sources (i.e. bio-surfactants), often by microbes or plants, are generally much less toxic and, as such, less problematic than those synthesized using petroleum. There is, therefore, significant interest in discovering new types of bio-surfactants for industrial and biotechnological applications. Bio-surfactants from microbes have gained increasing interest in recent years based on their associated lower levels of toxicity, higher degradability, and increased consumer demand for natural alternatives to synthetic surfactants. In recent years, the marine environment has become recognised as a rich and relatively untapped source for discovering microbial bio-surfactants. From an ecology point of view, these polymers serve important functions in marine environments, where they may be involved in microbial adhesion to solid surfaces and biofilm formation, the emulsification of petrochemicals (crude oil, diesel and other hydrocarbon pollutants) to enhance biodegradation (discussed below), and other functions. The global market revenues generated by bio-surfactants exceeded USD 1.5 billion in 2019 and is projected to grow at over 5.5% between 2020 and 2026.

2. ***Microbial polymers.*** Polymers are large molecules built up from many smaller units – building blocks – called monomers, strung together by chemical bonds, such as the familiar plastics, also made from petroleum.

Biopolymers are polymers produced by living things (especially microbes and plants) and are of particular interest for biotechnological and industrial applications. This is because they can exhibit a number of advantages for many commercial processes, such as possessing a large surface area on which multiple reactive groups may be expressed, the ability to confer texturizing and stabilizing properties to process and product formulations (e.g. the smooth texture of yoghurts on the palette), and the capacity to exhibit tensile strength and resistance to shear.

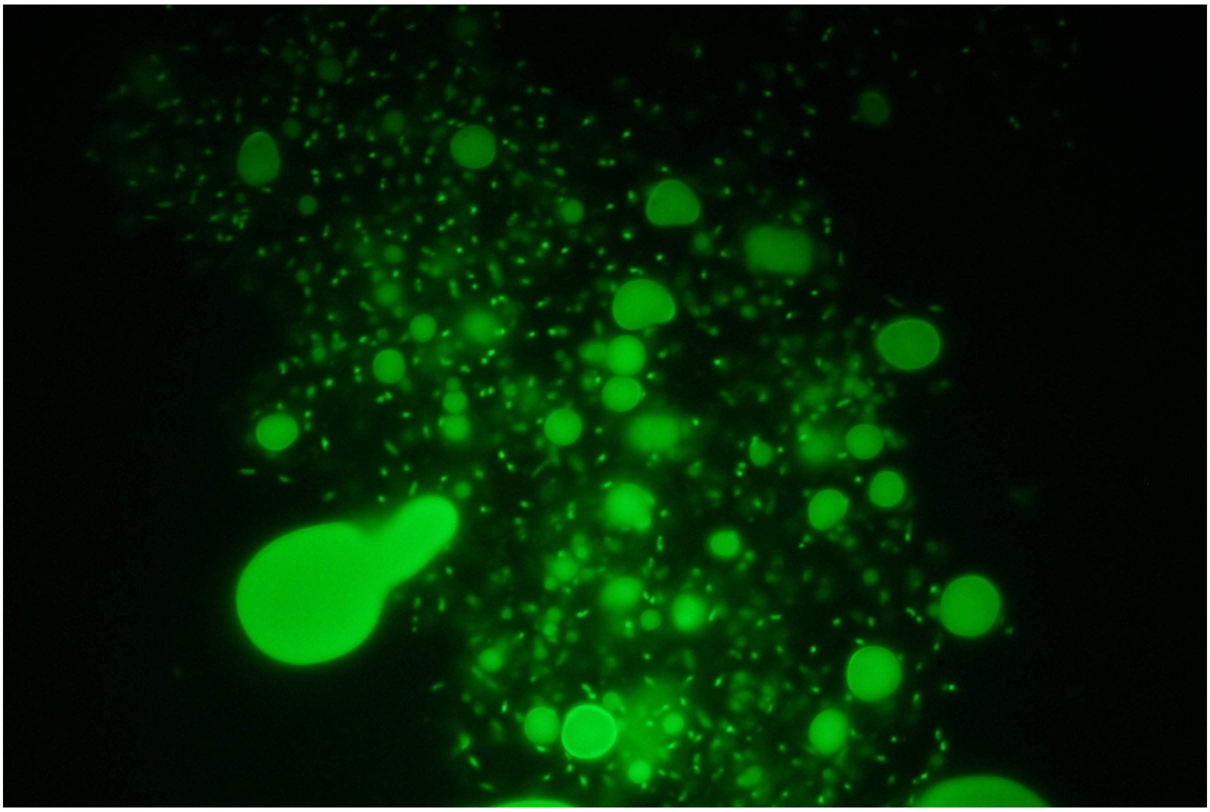
Biopolymers are largely composed of polysaccharides, but may also contain proteins and/or lipids. Like surfactants, some polymers may also be surface-active and, as such, be able to interact with both aqueous and non-aqueous substances. In this case, polymers are commonly referred to as emulsifiers (bio-emulsifiers if derived from biological sources) and are widely used to produce emulsions of water (or water-soluble) substances with oil (or oil-soluble substances). Typical examples of this are salad dressings which use emulsifiers to create emulsions of a food oil (e.g. olive oil) with vinegar (the aqueous phase). Some polymers are polyanionic – i.e. they have many negative charges on their surfaces – which allows them to bind positively-charged metals, thus having potential for use in the remediation and clean-up of environmental sites contaminated with toxic metals.

As is the case for bio-surfactants, microorganisms from the marine environment are recognized as a good source for discovering new types of biopolymers. In addition, microbially-derived polymers can exhibit enhanced performance and greater functional diversity than those produced synthetically using petrochemicals as the base material. One typical example of a microbial biopolymer is xanthan gum that is used widely in many applications, including in salad dressings that you buy at your local supermarket. Xanthan gum is a commercial hydrocolloid polymer produced by the bacterium *Xanthomonas campestris*. In addition to possessing surface-active properties, xanthan gum is also a viscosity builder (i.e. increases the viscosity of solutions), thus making it an important component of many healthcare products and food processing formulations; for e.g., it is used to improve the shelf life and texture of gluten-free foods, and you can find it as an ingredient in some salad dressings, ice creams, sauces and gravies. It has been widely used in the food industry as an authorised food additive due to its thickening, stabilising, emulsifying, antioxidant, and high viscosity properties. It has even been used to facilitate the extraction of crude oil from underground oil reservoirs.

3. ***Microbial surfactants in battling oil spills.*** Whether it be on land or in the sea, oil spills can be devastating, causing disease and death to many different types of living things and the destruction of whole ecosystems. Crude oil, and its petrochemical refined products (e.g. diesel, gasoline), are highly hydrophobic and, hence, very poorly soluble in water. When oil is spilled into the environment it will not mix with the water phase. At sea, it will float on the sea surface due to its lower density relative to water, and be seen as oil slicks. There are various species of microbes, mainly bacteria, that can use the hydrocarbons that comprise crude oil (and its refined products) as a food source. This is one of nature's most important and effective ways in cleaning up oil-contaminated sites. Some of these oil-eating bacteria can only feed on oil and a few related compounds, so are rather special. These bacteria are found in all seas and oceans around the world, in extremely low numbers; they are like sentinels, floating about in the water column or on the seabed until they come across oil, such as when an oil spill occurs, and then they start to munch on it. It's just another example that we should thank microbes for the wonderful and important work that they do; in this case in keeping our seas and oceans clean (see also the MicroStar ***Abo*** in the Microstars Gallery).

A major problem oil-eating bacteria face is that they cannot enter oil to eat it: they can only sit on the outside of a pool or drop of oil, so they can only access its surface. The rate-limiting factor for oil degradation is usually therefore the surface area of the oil:water interface. In order to increase the surface area of the oil interface, oil-eating bacteria produce bio-surfactants (akin to a soap or detergent). The bio-surfactants are released into the seawater where they can make direct contact with the oil. Consequently, this helps break up the oil into tiny droplets – akin to the formation of an emulsion – which massively increases the oil:water surface area, and hence the availability of oil to the bacteria; in other words, increasing the bioavailability of the oil to the bacteria. Bio-surfactants also increase the solubility of the hydrocarbons in the oil which the bacteria then feed on.

In the event of an oil spill at sea, synthetic chemical dispersants are often used as the first line of response, where they act like bio-surfactants in dispersing (or emulsifying) the oil and increasing the solubility of the oil's hydrocarbons for the oil-degrading bacteria to use as their food. Dispersants, however, are chemicals manufactured synthetically in a chemical plant. Therefore, because these chemicals are not natural, there is concern over their potential toxicity to life in the sea, for which there is some evidence. Scientists are therefore looking into developing new types of dispersants whose composition is largely, or entirely, made up of natural ingredients, such as those produced by oil-degrading bacteria and other microbes.



Surfactants produced by microbes during an oil spill. Biosurfactant-producing bacteria (small bright green dots) stained with acridine orange and viewed under the epifluorescence microscope. The biosurfactants that these bacteria produce has resulted in the emulsification of oil into small oil droplets (shown as green blobs).

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 3. Ensure healthy lives and promote well-being for all at all ages** (*improve health and consumer confidence on the ingredients in their foods, beverages and other products*). Although a significant fraction of the market demand for surface-active ingredients (surfactants and emulsifiers) is currently met by synthesis from petrochemicals, an important trend in the food and healthcare industries is the adoption of 'natural' ingredients with perceived benefits for the health of consumers. European legislation is pushing the market toward more natural and sustainable alternatives to chemically-synthesized surfactants and emulsifiers, driven to an extent by consumer awareness and a conceptual consideration for the environment. Driven partly by the fact that new EU directives have set a ban on the use of various commercial surfactants, there is a strong demand by the Food & Drink sector for replacing emulsifiers with the following E-numbers: E-431 to 436 Sorbates & Stearates, and the E-471 to 495 mono- and di-glycerides of fatty acids. These emulsifiers are synthetically derived or from animal sources and are thus not permitted for use on organic foods according to EU directive 2092/91 (49th Edition). Thus, there is a demand for new and natural alternatives to replace these emulsifiers.

In the cosmetic industry, an important issue about surfactants is their biological safety, specifically related to skin irritation. The most commonly used surfactants in this industry are non-ionic and anionic synthetic surfactants. Non-ionic ethylene oxide ethers are largely impure compounds and their safety has been questioned. Anionic sulfates and soap can elicit serious skin responses. Given also the poor bio-degradability of synthetic surfactants and emulsifiers, there is an urgent need to replace them with natural alternatives that are non-toxic, stable and environmentally friendly – qualities that are appealing and in demand by consumers. A number of natural plant-derived food emulsifiers (e.g. lecithin) used by food industries are increasingly being produced from genetically modified (GM) crops worldwide, particularly from GM soybean, which creates some limitations for food industries reluctant to use GM-based products, due to public perceptions or regulatory policies. The provision of natural compounds with the desired characteristics equal to such products traditionally available from animals/plants will add a lot of economic, social and environmental benefits. For this, microorganisms that produce bio-surfactants, bio-emulsifiers and polymers are commercially promising alternatives to these types of chemicals derived from petrochemicals, or even from plants and animals as these are often unsustainable sources. But despite the potential advantages of producing these chemicals from microorganisms, their commercial production is often limited because of typically low yields and high production costs, which is a major bottleneck that scientists and industry are trying to resolve.

- **Goal 6. Sustainable management of water and sanitation**. Both household wastewaters and some natural waters polluted by chemicals contain fatty/oily components whose microbial removal during treatment is facilitated by surfactants and emulsifiers produced by the participating microbes. Thus: biosurfactants and bioemulsifiers are central to both natural and engineered processes biological cleansing of waters.
- **Goal 12. Achieve environmentally sound management of chemicals and wastes**. Many of end-of-pipe treatments of chemicals that would otherwise pollute the environment, i.e. engineered treatments of industrial wastewaters prior to release into the environment, involve fatty/oily compounds whose biodegradation involves and indeed requires the participation of biosurfactants and bioemulsifiers produced by the microbes involved.
- **Goal 14. Reduce marine pollution** (*reduce the impacts of oil pollution and develop mitigation measures for treating oil spills*). Dispersants that are currently stockpiled around the world for use in the event of oil spills are considered non-toxic to most aquatic animals. However, recent studies have found that exposure to medium and high concentrations of certain dispersants has been shown to cause significant decrease of larval settlement and survival of two common coral species (*Porites asteroids* and *Montastraea faveolata*) and to deep-sea corals (*Leiopathes glaberrima*, *Paramuricea* type B3, and *Callogorgia delta*). Growing awareness in society regarding the environmental hazards associated with the use of chemical dispersants has led to increased interest towards the use of naturally-derived bio-surfactants which are commonly associated with low toxicity, high biodegradability, better environmental compatibility, and can sustainably sourced from microorganisms. The development of a new generation of dispersants that are as, or more, effective than commercial synthetic dispersants, cost efficient, and have minimal side effects when they come in contact with, or are ingested by, marine organisms and humans is a path that has gained traction since the Deepwater Horizon oil spill that occurred in 2010. One recurring major challenge with this is in

producing large enough quantities of the bio-surfactants from the microbes, considering that they are used in the hundreds of kilograms to treat large oil spills.

Pupil participation

1. Class discussion

a. Present to pupils images of some historic oil spills, such as of the Deepwater Horizon disaster that occurred in the Gulf of Mexico in April of 2010, or the Exxon Valdez spill that occurred in Prince William Sound, Alaska in March of 1989. Take a look at images showing the oil slicks and discuss the impact they caused to marine life, ecosystems and local economies (e.g. fishing, tourism).

b. Discuss the issues and challenges associated with developing surface-active substances (bio-surfactants/bio-emulsifiers and polymers) for commercialisation. Many different types of microorganisms produce these chemicals, and industry and consumers are keen to use them over those that are produced from petroleum. But why are so few that are produced from microbes in use today?

2. Exercises

a. Look inside your kitchen pantry, and also go to your local supermarket, and have a look at your favourite (and non-favourite) processed foods, such as ice cream, butter/margarine, salad dressings, chocolate bar, ready-made meals and others. Look at the ingredients section on the packaging and see if there are any surfactants/emulsifiers or polymers shown on the list – these are often shown as 'E' numbers which are typically derived from petrochemicals. But you may also find surfactants, emulsifiers and polymers derived from plants or microorganisms, such as lecithin (commonly from soy bean), gellan gum and xanthan gum (from bacteria), gum Arabic (from the Acacia tree), guar gum (from leguminous shrub plant), agar and carrageenan (from red seaweeds), and alginate (from brown seaweed). On some cosmetics and eye wash solutions, you might find hyaluronate (a polymer surfactant) which is produced by the group A streptococci bacteria.

The Evidence Base, Further Reading and Teaching Aids

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Glossary

Aqueous – water itself, or a substance able to dissolve in water.
Bio-emulsifiers – same as emulsifiers, but derived from biological sources.
Biogenic – derived from a biological source or living thing.
Bio-surfactants – same as surfactants, but derived from biological sources.
Emulsifiers – a group of chemicals that are generally of high molecular weight (large molecules) having both hydrophilic and hydrophobic groups and that emulsify (mix) aqueous (water soluble) and non-aqueous (non-water-soluble; e.g. oil) substances together.
Hydrophilic – water loving; able to dissolve or mix in water, or interact with water molecules.
Hydrophobic – water-hating, or oil-loving; able to dissolve or mix in oil, but not in water.
Non-aqueous – oil itself, or a substance unable to dissolve in water.
Surface-active substances – these are surfactants and emulsifiers that have both hydrophilic and hydrophobic groups.
Surfactants – a group of chemicals that are generally of low molecular weight (small molecules) having both hydrophilic and hydrophobic groups and that lower the tension between water and oil molecules.