Fish heads, fish heads: For biosurfactant production

Stacy Kish

- Biosurfactants are compounds produced by some bacteria that replicate the properties of petroleum-based surfactants.
- While many research studies have explored different bacteria grown on different medium, fish waste is proving to be a viable option to grow microbes for industrial-scale biosurfactant production.
- The Scottish start-up company, Eco Clean Team, has partnered with a researcher at the University of St. Andrews to develop a pilot project for scaling biosurfactant production with fish oil from the local aquaculture industry.
- While many hurdles remain, the biosurfactant industry and fish peptone industry are both on the upswing.

In 2010, Deep Water Horizon released over 130 million gallons (https://tinyurl.com/3brsh5u8) of oil, the equivalent to 200 Olympic-sized swimming pools, into the Gulf of Mexico. Twenty years earlier, the *Exxon Valdez* oil tanker ran aground in Alaska, releasing 11 million gallons of oil into Prince William Sound.

Oil spills harm marine plants and animals, and render seafood unsafe to eat. Environmental remediators apply different techniques, including skimming and burning, to remove oil pollution from the water's surface; however, synthetic dispersants created using petrochemicals, paradoxically, are the best means of clean-up. They reduce the opportunity for the contaminant to reach the shoreline by dispersing the oil and breaking it up into smaller droplets that are easier for microbes to consume. However, research shows synthetic surfactants are often toxic to marine organisms, changing their behavior, physiology, and reproduction patterns. These adverse effects raise concerns about which is worse for the environment—the dispersants applied to break up an oil slick or the oil itself.

Synthetic surfactants are a common class of molecules found in laundry and dishwashing detergents, among other household products. They are ubiquitous in our lives, yet they rarely garner the negative attention of an oil spill, because they are typically unseen. Although, in many instances synthetic surfactants are more pervasive and insidious to the environment.

"When people think about petroleum-based problems, they may think about the plastic trash pile floating in the middle of the Pacific Ocean, but other molecules, [like synthetic surfactants] that are not so readily evident, are also harming the planet," said Rick Ashby, research scientist with the United States Department of Agriculture, Agriculture Research Service. "We are looking at biosurfactants as a means to avoid ecological contamination by petroleum-based products. The challenge is to make them at low-enough cost so they are competitive and compelling for industry to adopt."

MICROBES TO THE RESCUE

During different stages of growth, many microorganisms produce secondary metabolites in the form of surfactants. In nature, these microbes enzymatically convert the long-chain carbon compounds in oils into a chemical consortium that includes biosurfactants. The compounds vary in molecule length and structure, including glycolipids, lipopeptides, fatty acids, polysaccharide–protein complexes, peptides, phospholipids, and neutral lipids. As with any surfactant, biosurfactants have a polar, hydrophilic head group and a hydrophobic tail, that in this case is typically composed of one or more long chain fatty acids (https://tinyurl.com/35tappu2).

"The benefit of biosurfactants is that they are biodegradable and renewable," said Ashby. "They will degrade into nontoxic materials in nature and can also be produced using renewable materials, like fats, oils, and plant biomass."

When compared to synthetic counterparts, biosurfactants have a similar chemical composition while being just as effective at breaking up oil and creating lather for personal care products. They function over a wide-range of temperature, pH, and salinity conditions. They interact with a diverse variety of functional groups, which is beneficial when cleaning heavy minerals during environmental remediation. When produced within a renewable framework, biosurfactants also have a smaller carbon footprint than their synthetic equivalents.

Common strains of bacteria and fungi that produce biosurfactants include, but are not limited to, *Pseudomonas aeruginosa*, *Starmerella bombicola, Bacillus subtilis,* and *Ustilago maydis*. Scientists still speculate on the reason microbes produce biosurfactants. Incorporating hydrophobic carbon sources into their cells may somehow aid in microbe survival. Biosurfactants are known to protect against gram positive bacteria, like *Staphylococcus*, *Streptococcus, Listeria*, and *Bacillus*.

Previous studies have gathered these oil-munching organisms from contaminated water and soil environments. A 2021 study combined a lactonic sophorolipid biosurfactant, produced by *Starmerella bombicola,* with choline myristate and choline oleate ionic liquid surfactants to create a greener remediation product (https://doi.org/10.1016/j.envpol.2021.117119). The study examined two mixtures of different concentration of these compounds and found each to be thermodynamically stable and effective at dispersing crude oils. The maximum dispersion effectiveness was 78.23% for the 80:20 lactonic sophorolipid-choline myristate blend and 81.15% for the 70:30 lactonic sophorolipid-choline oleate blend. The high dispersion rates for these two mixtures are attributed to the hydrophobic tail and unsaturation of the additional surfactants, which improved the interactions between compounds in the mixture.

NATURALLY LESS TOXIC

A series of toxicity studies compared the effect of various biosurfactants and synthetic surfactants on marine organisms. Indicator species provide a way to evaluate how the different compounds affect the organism's physiological, nutritional, structural, and morphological characteristics.

A 2003 study in the journal *Marine Pollution Bulletin*, compared three synthetic surfactants (Corexit 9500, PES-61, and Triton X-100) with three biogenic surfactants (Bio-EM, Emulsan, and PES-51) to evaluate their toxicity on marine organisms and their effectiveness for dispersing oil (https://doi.org/10.1016/S0025-326X(03)00238-8). The study focused on two indicator species: *Mysidopsis bahia*, an epibenthic mysid shrimp living in estuarine waters spanning the Gulf of Mexico to Florida, and *Menidia beryllina*, an inland minnow living in waters from Mexico to Massachusetts. The study found that biosurfactants were intermediate to those of the synthetic surfactants in toxicity. *M. bahia* was generally more sensitive to the synthetic surfactants. The authors stress that application of the results requires balancing site-specific considerations of dilution, biodegradation, and exposure duration and depth when selecting a surfactant for remediation.

Almost two decades later, a 2020 study in the same journal obtained the biosurfactant, lipoprotein, from cultivated *Bacillus cereus* to evaluate the toxicity on two indicator species (https://doi.org/10.1016/j.marpolbul.2020.111357). The study also evaluated the hurdles to scaling-up production from the bench to industrial applications (fig.1). The toxicity portion of the study focused on the fish *Poecilia vivipara*, which is sensitive to potassium dichromate, sodium dodecyl sulfate, copper, and zinc. The second indicator species was the bivalve *Anomalocardia brasiliana,* which lives along the Brazilian coast. The results suggested that biosurfactants are safe and effective to remediate marine environments. The study also concluded that this biosurfactant was biocompatible for industrial-scale production, producing 4 g/L in only 48 h using a low-cost renewable raw material.

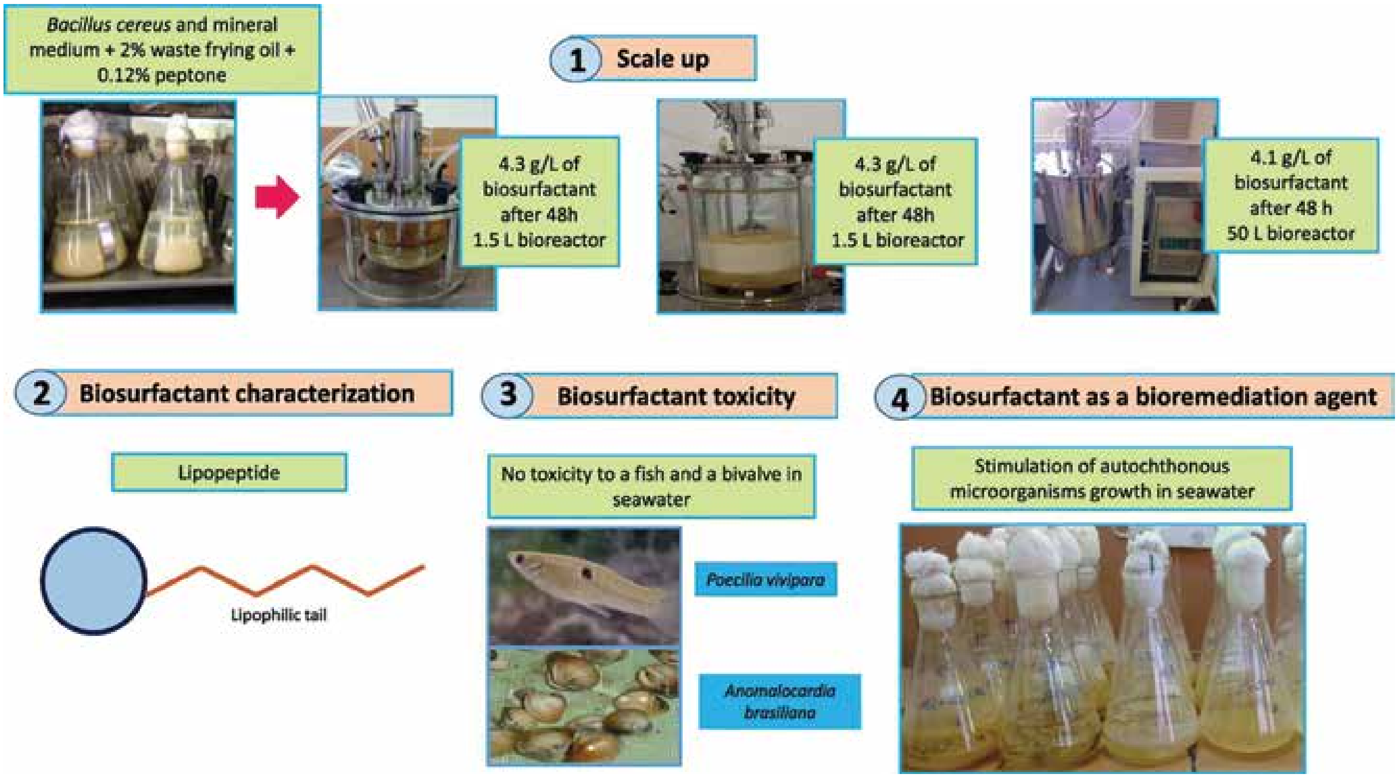

FIG. 1: Studies demonstrate the potential for industrial-scale production biosurfactants and their biocompatibility in the remediation of marine environments polluted by oil spills. Source: Duval, *et al., Marine Pollution Bulletin, 157*,111357, 2020.

WITH AQUACULTURE WASTE, SCALE-UP MAY BE POSSIBLE

Despite these advantages, scaling the production of biosurfactants remains cost prohibitive, and hindered by the availability of suitable oils to grow the microbes. To compete in the global market, low-cost production is essential.

Using industrial wastes as feedstocks for biosurfactant production reduces the impact of pollution on the environment and offers a nutrient-rich medium for growing microbes. When processing fish, up to 60% of the total weight of the product ends-up as waste and a troubling source of local environmental pollution. Fish waste consists of a slurry of fish heads, fish skin, fish bones, meat, and viscera. Microbes can transform this rich source of suspended solids, organic carbon, and nitrogen into surfactants. Several studies have evaluated a series of microbes grown on fish waste, compared to other feedstocks, to produce biosurfactants for industrial-scale applications.

Some early work investigated the use of glycolipids as biopesticides to thwart fungal infections and kill mosquito larvae. These compounds are now being explored as biosurfactants. In 2011, researchers grew the phytopathogenic smut fungus, *Ustilago maydis*, to produce glycolipids using soy- and fish-based oils. The study found fish oil with the addition of lipase, an enzyme to break down fats, produced the highest yield (16.8 g/L of biomass), especially after seven days of incubation (https://doi.org/10.5897/AJMR10.814).

Another study examined two strains of *Pseudomonas aeruginosa* (H1 and SY1) that were collected from soils surrounding olive oil and fish oil factories across Turkey (http://doi.org/10.1590/S1517-838246320140727). This microbe produces rhamnolipid biosurfactants. The study examined the potential of industrial production of rhamnolipid for bioremediation applications. The researchers grew the two environmental strains as well as a control strain (ATCC 9027) on two carbon feedstocks: kefir, a fermented milk drink, and fish meal. They found that the quality and quantity of biosurfactant produced was influenced by carbon and nitrogen substrates, the concentration of nutrients in the growth medium, culture conditions,—such as pH, temperature, and agitation—and culture dilution rate. The three microbial strains grown in the fish oil medium produced more rhamnolipid than those grown on the kefir medium. The results suggest that fish waste can be an important additive when exploring ways to ramp-up production of rhamnolipid biosurfactants.

In 2021, Memorial University of Newfoundland, Canada, civil engineering professor Bing Chen collaborated on a project with Chinese researchers to study generating fish peptones from the enzymatic hydrolysis of tuna fish waste (https://doi.org/10.3390/catal11040456). Fish peptones are the protein decomposition product obtained from different marine fish species. They are used as a nitrogen source for bacteria (fig. 2). Within their fish slurry, the researchers grew the bacterium, *Bacillus subtilis* (ATCC® 21332™). Then, they added a small amount of manganese to ensure the microbes produced sufficient lipopeptide biosurfactant.

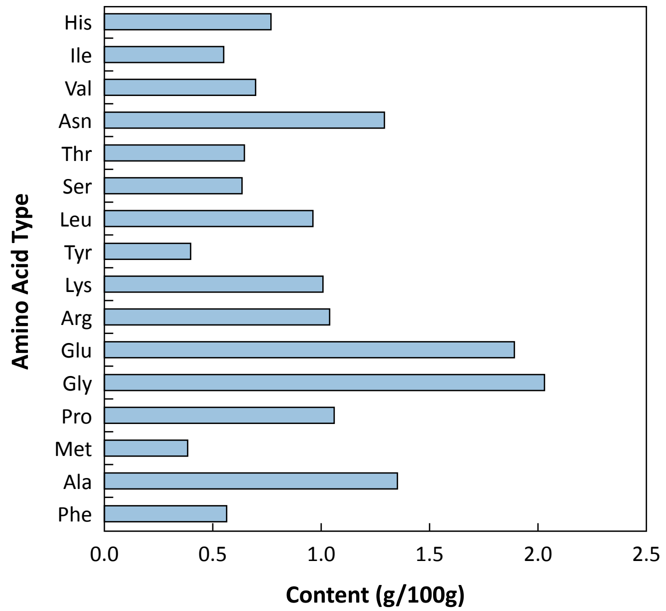

FIG. 2: Using the trichloroacetic acid (TCA) method, researchers achieved 44.2% enzymatic hydrolysis of fish waste. This is the amino acid composition in tuna waste-based peptone that resulted. Source: Hu, J. *et al., Catalysts,11*(4), 456, 2021.

The resulting biosurfactant reduced surface tension, as well as exhibited emulsifying, foaming, and biocatalytic activity. During the study, the highest biosurfactant production (274 mg/L) occurred between 24 to 36 hours of the fermentation process. The study authors are optimistic that it may be possible to scale-up surfactant production cost-effectively, noting that carbon and nitrogen supplements may be necessary to optimize the substrate (fig 3).

REAL WORLD INDUSTRIAL-SCALE FERMENTATION

Alfredo Bonaccorso, senior research fellow at the Institute of Behavioral and Neural Sciences at the University of St.

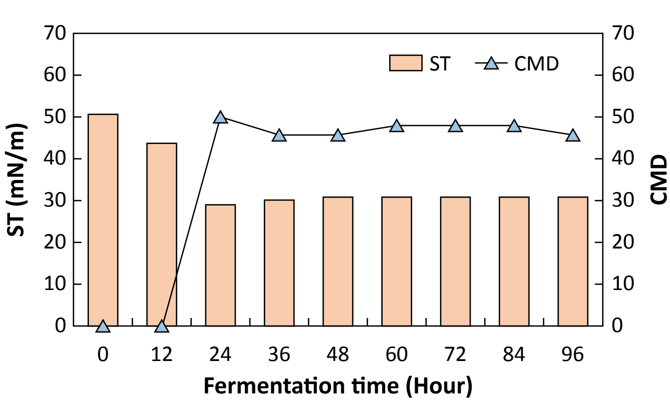

FIG. 3: The results from batch-scale experiments showing biosurfactant production between 12-24 hours, as indicated by a lower surface tension (ST) and a higher critical micelle dilution (CMD).
Source: Hu, J. *et al., Catalysts,11*(4), 456, 2021.

Bitterness blocker

While cleaning products may be the focus of industry today, Ashby believes that biosurfactants could one day be found in a host of other products. He explained that some glycolipid biosurfactants (sophorolipids) have been shown to block the perception of bitterness (https://doi.org/10.1002/jsde.12526). This characteristic could garner interest from the food industry for hygiene products, like mouthwash and toothpaste. Pharmaceutical companies could also benefit from this characteristic to remove the bitter taste or aftertaste of some medications.

Andrews, has partnered with a Scottish start-up company, Eco Clean Team, to overcome the challenges of scaling biosurfactant production by tapping into a local resource—fish oil waste from Scottish aquaculture. According to an article in *Fish Focus,* the Scottish aquaculture sector processed 192,000 tons of Atlantic salmon in 2020. This market is anticipated to grow, with the potential to net up to $4.45 billion by 2030 (https://tinyurl.com/4znzmprx).

Bonaccorso and the Eco Clean Team have not revealed details, but say they aim to identify new approaches to scale production of biosurfactants using fish waste, which offers a near continuous and homogenous supply of oil that does not fluctuate in price like fossil fuels. It also provides added value to the aquaculture sector by creating jobs, and reducing and reusing the waste stream.

Mark Hamilton, co-founder and director at Eco Clean Team, told *Fish Focus*, "We have already proved the feasibility in a previous study and hope that, by the end of this project, we will find ourselves closer to full-scale commercialization and seeing the surfactant used in a range of industrial and selected household products."

Bonaccorso says, he and his team have devised a more cost-effective, efficient, and eco-friendly process than traditional methods for producing biosurfactants. Bonaccorso anticipates it will be possible to create a pilot project in the next few years to begin the process of scaling production for industrial applications.

THE FUTURE OF THE BIOSURFACTANT MARKET

The ability to scale biosurfactants production will be critical for adoption by industry for a variety of applications. Fish waste may be the key to these future production goals. According to a March 2022 *Future Market Insights* article, the fish peptone market is on the rise (https://tinyurl.com/2x3rphd7). In the United States and Canada, fish peptones obtained from fish heads and fish livers are used as nitrogen and carbon sources for microbial growth and biosurfactant production. The new process to convert fish peptones has reduced the cost for lipopeptide production on an industrial scale.

The rise in fish peptones parallels the rise in the biosurfactant market. A January 2022 *Kingpin Market Research* article reports the biosurfactant industry is anticipated to grow at a steady rate due to consumer demand for bio-based, eco-friendly biosurfactants (https://tinyurl.com/2p87wre9). In 2021, the global market for microbial biosurfactants was valued at $19.41 million and is anticipated to reach $38 million by 2027 (https://tinyurl.com/fb9sfpjn). The report focuses on regions of primary biosurfactant production, including China, the United States, Europe, and Japan where the market is dominated by five companies — Evonik Industries AG, BASF SE, Ecover, Jeneil, and Givaudan — that hold more than 80% of the market share (https://tinyurl.com/2s3dbe7s).

Evonik Industries AG has developed a line of products under the REWOFERM® trademark. These products leverage two biosurfactants: sophorolipids and rhamnolipids. Compared to traditional surfactants used in laundry formulations, rhamnolipids allow excellent cleaning using a lower surfactant concentration, thereby reducing the overall carbon footprint of the formulation.

"It is ok for a new, green product to be more expensive than the conventional product on the market if you do not need to use as much of it in a formulated product," said Derek Dagostino, director Global Marketing Cleaning Solutions at Evonik Corporation. "It really depends on the market application and the formulation for customers to better understand the value that can be created for them by biosurfactants."

According to Dagostino, the rising consumer demand for green products motivates companies to formulate products that are safe, effective, and have lower volatile organic compounds, common to petroleum-based products. While the Evonik Industries AG currently uses a vegetable feedstock in the production of their two biosurfactant lines, they are constantly innovating and evaluating new feedstocks. Maybe, their next innovation will be fish waste.

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