3- Environmental Applications of Marine Biotechnology

Protecting and restoring the marine environment has become an increasingly high international priority, and marine biotechnology can play many roles. We have chosen to discuss six environmental application areas of marine biotechnology.

1. Waste processing and treatment
2. Monitoring of general ocean pollution
3. Bioremediation
   - Bioremediation of spilled oil
   - Bioremediation of dredged sediments
4. Environmental restoration and preservation
5. Marine biotechnology and global change
6. Biofouling (to be discussed in the next chapter)

1. Waste Processing and Treatment

Many coastal communities either do not treat their sewage or use only primary screening to remove solids before discharging it into the sea. Only some 10% of the total global production of waste is treated, and the remainder can place a severe burden on natural self-purification processes, particularly in the marine environment. The sediments that receive such sewage often become anoxic, so there is little marine life, and biodegradation, which is often most rapid under aerobic conditions, is slowed. Natural-cleansing processes are thus inhibited and, once problems occur, they tend to be self-amplifying so that the problem impinges on an ever greater area. Modern biotechnology has much to offer the waste-treatment industry that produces effluents that enter marine ecosystems.

Recent work has focused on better removal of nitrogenous and phosphorus nutrients, which stimulate algal growth in outfall areas. Removing nitrates and phosphates prevents eutrophication in coastal waters that receive sewage effluent.

   - The use of bacteria to accelerate the formation of vivianite \([\text{Fe}_3(\text{PO}_4).8\text{H}_2\text{O}]\) crystal aggregates in sediments. The method could be applied to aquatic systems that have received large amounts of phosphate from waste-treatment effluents.

![Optical micrograph of extracellular crystals of vivianite, produced by cells of *Magnetospirillum magnetotacticum* in cultures containing high concentrations of Fe(III) buffered with phosphate.](image)

**Figure** Optical micrograph of extracellular crystals of vivianite, produced by cells of *Magnetospirillum magnetotacticum* in cultures containing high concentrations of Fe(III) buffered with phosphate.
• Some waste-treatment systems that were designed to remove only organic compounds from wastewater aerobically could be modified to include **anaerobic zones** for removal of inorganic chemicals, such as nitrates. Under anaerobic conditions, microorganisms can transform nitrate to molecular nitrogen, thereby decreasing the nitrate in wastewater that might otherwise cause eutrophication of the receiving bodies of water.

• The use of microorganisms that can **immobilize phosphates** during wastewater treatment.

• The use of **genetically modified microorganisms** that can biodegrade compounds found in industrial plant wastewater, such as hydrocarbons and chlorinated solvents.

2. Monitoring of General Ocean Pollution

Modern biotechnology is providing numerous and powerful new ways to define the extent of marine pollution caused by coastal runoff and deliberate inputs. Understanding the extent of the effect of ocean pollution is clearly an **important determinant of whether remediation is needed, or will be needed in the future.**

Examples include:

• The use of common bivalves, such as blue mussels and oysters, to monitor chemical contaminants in coastal waters.

<table>
<thead>
<tr>
<th>Bivalves are potential organisms to monitor ocean pollution</th>
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<tr>
<td>Shellfish such as oysters and mussels are feed by drawing large volume of water through a membrane and trapping food i.e. filter feeders. The mussel <em>Mytilus edulis</em> for example can filter 100 L of water a day. Thus, by virtue of their feeding habit, they act as <strong>natural filters</strong> concentrating and accumulating various materials from the environment. Accordingly; these organisms may be of value as <strong>biological monitors</strong> for the presence of pollutants in sea water.</td>
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<td>It is the unique combination of different features which characterizes mussels as ideal biomonitors. The most important characteristics are:</td>
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<td>1. It is distributed in all marine ecosystems all over the world.</td>
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<td>2. It is a dominating species in many coastal areas.</td>
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<td>3. It is sessile as adults. Therefore, represent the contamination of their habitat ideally.</td>
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<td>4. It is relatively large and therefore easy to sample and handle.</td>
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<td>5. Its biology, physiology and way of life are well established. Consequently, biological effects of environmental stress in general and of contaminant exposure in particular are measurable at various levels of biological organization (from molecules to communities).</td>
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<td>6. It tolerates fairly high concentrations of contaminants in its tissues.</td>
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<td>7. It is consumed by humans, so its content of pollutants is relevant for considerations concerning food safety.</td>
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<td>8. Mussels can easily be translocated from clean to polluted locations.</td>
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• Enzymatic techniques are widely used to monitor the exposure of marine species to pollutants. e.g., the cytochrome P450- dependent monooxygenases is widely used as a **biomarker** of exposure to halogenated aromatic hydrocarbons (HAHs) and polynuclear aromatic hydrocarbons (PAHs).
Cytochromes P450 as biomarker (a detoxification markers)
The first step in detoxification of a pollutant that has entered living cells, is its hydrolysis, reduction or oxidation. The main enzymes that are associated with these actions are the class of the cytochrome P450 monooxygenases. Cytochromes P450 are able to perform a large range of chemical modifications on any foreign chemical molecule that has entered the cell (xenobiotic molecule): hydroxylation, epoxidation, deamination, oxidative and reductive dehalogenation, dealkylation,... A typical reaction is displayed below, with RH the (organic) molecule undergoing hydroxylation.

\[ \text{RH} + \text{NADPH} + \text{O}_2 + \text{H}^+ \rightarrow \text{ROH} + \text{NADP}^+ + \text{H}_2\text{O} \]

The consequence of this step is, that the xenobiotic molecule in general becomes less lipophilic, and, hence, more prone to be taken up into the watery elimination pathways of a cell.

- **Biosensors:** Many groups are working to explore the use of aquatic organisms as biosensors to detect low concentrations of pollutants and toxins in waterways. **Bioluminescent strains of bacteria** e.g. *Vibrio* can be used as biosensors. In response to changing environmental conditions, the intensity of light emitted by bacteria can change. Because of this ability, *Vibrio* has been used to detect pollutants such as organic chemicals and nitrogen-containing compounds in marine environments. **Another approach** is to fuse the **lux genes with a promoter for stress-related genes.** The promoters are activated when bacteria encounter such stress factors (such as pollutants). Samples are collected, added to cultures of the genetically engineered bacteria with the lux genes and the intensity of light emitted by the bacteria is measured.

3. Environmental Remediation
Not only are some marine organisms useful for detecting environmental pollutions, but many marine species are also believed to possess unique mechanisms for breaking down substances including toxic organic chemicals such as phenols and toluene, toxic metals and oil products found in harbors and adjacent to oil rigs.

- One of the earliest techniques used in marine remediation involved **increasing the quantity of shellfish in polluted areas.** Scientists introduced racks of shellfish into polluted waters to take advantage of the natural feeding method of bivalves such as clams, oysters, and mussels. Because these organisms strain the water, they act as a form of estuarine filters to remove wastes such as nitrogen compounds and organic chemicals. These chemicals, in turn, are absorbed in tissues of the shellfish. After periods of time, these shellfish can be harvested, thus removing wastes from the water.

- **Heavy metal contamination** of marine waters is the result of many industrial manufacturing processes. As a solution to this problem, scientists have isolated marine **bacteria that oxidize metals** such as iron, manganese, nickel, and cobalt. Some of these bacteria can also be used to **extract important metals** from low-grade ores. Additionally, some marine bacteria and single-cell algae express **metallothioneins**, a family of metal-binding proteins. These species thrive in water contaminated with cadmium and other heavy metals, where they filtrate
cadmium from the surrounding environment and then degrade toxic metals into harmless by-products. Scientists are looking at ways to use these organisms to extract, recover, and recycle important and expensive metals such as gold and silver from manufacturing processes.

- Many aquatic organisms produce substances that are valuable for degrading and processing a variety waste materials. By using aquatic organisms, it is possible to stimulate waste degradation in natural environments.

- The use of algae as natural filters Microbiologists group have experimented with growing nitrogen-metabolizing algae on large mats called scrubbers (see the next Figure) so they can used as natural filters. These scrubbers work like charcoal filters in an aquarium in that they bind nitrogenous wastes. Water contaminated with farm animal wastes is passed over the scrubbers, and the algae absorb and metabolize the wastes. These wastes provide nutrients for the algae, which grow into thick mats. The algae are periodically harvested by cutting them back, but they are allowed to re-grow like grass. Water cleaned in this way has been used for irrigation, and some of the harvested algae have even been used as livestock food.

- Bioremediation of Spilled Oil
Crude oil has been a part of the natural marine environment for millennia; oil seeps add an estimated $90 \times 10^6$ gal/year. It is not surprising that hydrocarbon-degrading bacteria are found in all environments where they have been looked for. Today, total input of oil into the sea is perhaps ten times greater, with most of the additional oil coming from human’s activities including:

- Urban or municipal runoff.
- The extraction operation from the seabed,
- The transportation across the oceans,
- The refining in plants along the coast.
- Discharging oil by oil tankers while unloading.
- The illegally cleaning the tankers tanks at sea and emptying the water they use as ballast on their return trips to load oil.
- The blowouts of offshore rigs used in the extraction of oil.
- The sinking or collision of supertankers.
The major role of bioremediation in the marine environment has been in speeding the natural biodegradation of spilled oil. Bioremediation processes include:

1. Adding nitrogen and phosphorus to stimulate microbial degradation
   Oil is said to be almost completely biodegradable because, though very slowly, it is broken down, or decomposed, by bacteria. Unlike many other carbon sources, however, oil contains little nitrogen or other essential nutrients. In aerobic environments, the degradation of substantial amounts of oil, therefore, is typically limited by the supply of nitrogen and phosphorus. Hence, adding such nutrients is a promising approach to stimulating degradation. It is important to remember, however, that the growth of marine algae is also typically limited by the availability of nitrogenous nutrients, so care needs to be taken to stimulate the growth of oil-degrading microorganisms without stimulating algal growth.

2. The use of oleophilic nutrients
   Another approach to overcome the problem of water-soluble nutrient washout is to utilize oleophilic organic nutrients that are able to adhere to oil, providing and maintaining optimal nutrient concentrations at the oil-water interface where biodegradation occurs.

3. The use of slow-release fertilizer.

4. The most successful application of bioremediation
   to a marine oil spill has been attained by adding a combination of oleophilic and slow-release fertilizers to stimulate biodegradation by the indigenous microflora where it sped degradation substantially and had no adverse ecological effects, and the process took several years less than unassisted natural cleansing would have taken.

5. The use of genetically engineered organisms to degrade hydrocarbons.

Bioremediation of Dredged Sediments

Most harbors require periodic dredging to maintain safe water depths, but sediments in many harbors have been contaminated with a variety of pollutants that makes disposal of dredged material problematic. A major problem for a bioremediation approach is that the varied contaminants require different approaches for safe and effective degradation, and appropriate strategies for some contaminants might
increase the potential hazard from others. For example, dechlorination of polychlorinated biphenyls requires initial anaerobic reductive dehalogenation followed by aerobic degradation. Current hydrocarbon bioremediation technologies of contaminated sediments rely on aerobic degradation, but immobilization of potentially harmful metal ions requires anaerobic conditions. Nevertheless, bioremediation technologies potentially have much to offer in treating dredged material; perhaps the issue of handling such mixed contamination requires much more research, and modern molecular tools (e.g. engineered organisms) may have an important role to play once the fundamental microbiological processes are understood.

4. Environmental Restoration and Preservation

Modern biology is providing important insights into ways of improving, maintaining, or restoring the marine environment. Example include:

- The cultivation of macroalgae to prevent eutrophication.
- The use viruses which are capable of controlling the red or brown tide. Perhaps the virus can be used to keep these potentially toxic organisms under control, and it is likely that viruses can control blooms of other nuisance organisms.
- The use of biomarkers to monitor compliance with international and state laws e.g. to differentiate between wild-caught fish and farmed fish by analyzing their fatty acid composition to distinguish fish species and populations, which is vital for sound stock management. Fatty acid profiles also allow the detection of marine turtle oils in cosmetics and other preparations.
- A rather different aspect of the use of biotechnology in the pursuit of a cleaner environment is in pollution prevention. Examples of problems in the marine environment include oil from motor vessels, plastic trash along the world's shorelines, and contamination by antifouling paints. Fully biodegradable lubricating oils are now available for marine lubrication; fully biodegradable plastics for the marine market, made from bacterial polymers, are available to replace nonbiodegradable petrochemical plastics.

5. Marine Biotechnology and Global Change

The tremendous consumption of fossil fuels since the dawn of the industrial revolution has undoubtedly resulted in increasing level of greenhouse gases (GHGs) primarily CO₂ in the atmosphere. These gases act as a protective layer, causing the planet to be warmer (global warming) than it would otherwise be. The influence of this increase is changes in global climate with rise of sea levels and change in the amount and pattern of precipitation, probably including expansion of subtropical deserts. Warming is expected to be strongest in the Arctic and would be associated with continuing retreat of glaciers, permafrost and sea ice. Other likely effects include changes in the frequency and intensity of extreme weather events, species extinctions, and changes in agricultural yields. Warming and related changes will vary from region to region around the globe.
Political and public debate continues regarding global warming, and what actions to take in response.

On December 12, 2015, 195 countries adopted the first global agreement addressing climate change called Paris Agreement. In this agreement the participating countries agreed to reduce emissions as part of the method for reducing greenhouse gas.

Obvious approaches to reduce greenhouse gas emissions include reducing the use of fossil fuels by

- improving energy efficiency or
- by using renewable energy sources and producing CO$_2$-neutral fuels,
- growing biomass to sequester atmospheric CO$_2$ out of the biosphere.

It is widely viewed, that marine biotechnology has the potential to lower global atmospheric CO$_2$, both by sequestering carbon and by producing CO$_2$-neutral fuels.

Carbon sequestration is a potential approach to reducing CO$_2$ accumulation in the atmosphere; it is capturing the carbon dioxide produced by burning fossil fuels and storing it safely away from the atmosphere.

- Capturing the carbon dioxide produced can be enhanced through stimulating marine photosynthesis by adding some limiting nutrient, so that CO$_2$ would be taken from the atmosphere. Perhaps the best-known suggestion is what is sometimes referred to as the ocean Geritol effect: The addition of extraordinarily small quantities of the common element iron to the upper ocean in certain life-poor ocean areas to stimulate a phytoplankton bloom. This is intended to enhance biological productivity, which can benefit the marine food chain and remove carbon dioxide from the atmosphere. Iron is a trace element necessary for photosynthesis in all plants and algae, however it is highly insoluble in sea water and is often the limiting nutrient for phytoplankton growth. Zinc and phosphate might be also used to stimulate algal growth and hence to reduce carbon dioxide levels in an effort to address climate change issues.

- Cell walls of microalgae contain highly cross-linked, macromolecular, paraffinic hydrocarbons that resist biological and chemical degradation. It is reasonable to view the macromolecular paraffinic hydrocarbons in algae as a carbon sink because they can be buried, thereby removing CO$_2$ from the carbon cycle.

- Ocean disposal of the biomass is another potential option. Transporting material, such as crop waste, out to sea and allowing it to sink into deep ocean storage has been proposed as a means of sequestration of carbon. Carbon that sinks below the marine thermocline (100–200 meters) is effectively removed from the atmosphere for hundreds of years. Since deep ocean currents take so long to resurface.

- Renewable fuels: Most discussion of renewable fuels focuses on land plants, but marine algae both macroalgae (e.g., kelp) and microalgae (microscopic) have also been considered. They could be grown in the sea in enclosures, or on land with otherwise-unexploited saline water in arid regions. Cultivated macroalgae are reported to have yields from 30 to 150 tonnes dry weight per hectare per year which is higher than average yields of sugarcane. The simplest way to use algal biomass would be to harvest it, dry it, and use it as a CO$_2$-neutral fuel. Such a process could provide electricity at half the cost of using coal.
Carbon Neutral Fuel
Burning algae releases the same amount of carbon dioxide that is absorbed whilst growing, creating a balance between carbon emitted and absorbed. Therefore burning algae as a fuel is considered to be carbon neutral.

- Catalytic gasification is another alternative. Converting algal biomass to methane by anaerobic digestion would be only slightly more complicated. The process can be modulated so that most of the biomass is converted to CO₂ and CH₄, typically with a large excess of methane. The CO₂ could be recycled back to algae, leaving a clean methane fuel. Marine algae are a particularly good feed for anaerobic digestion because they contain no lignin; land plants typically contain some 20% lignin (dry weight), which is quite resistant to anaerobic degradation. Kelp is perhaps the best source of biogenic methane yet found. An important caveat is that if anaerobic gasification were to be used on a large scale in a greenhouse-limited scenario, any leakage of methane would have to be minimized, because methane is a substantially more potent greenhouse gas than CO₂.

- Another simple approach would be to ferment the algal biomass e.g. kelp to produce acetone, butanol, and ethanol, the ABE fermentation.

- An approach that conceivably would yield a more valuable product would be to grow microalgae that produce oils; It is estimated that total microalgal productivity could be as high as 30 ton/acre (81 tonne/ha) if saturating concentrations of CO₂ were provided, perhaps from power station flue gases. If 50% of the product were oil, a reasonable goal, this would be the equivalent of more than 100 barrels of petroleum per acre per year. Current estimates are that liquid fuel could be produced from microalgae at a cost of 177 dollars per petroleum barrel equivalent, with realistic hopes to reduce it to 50 dollars.