Throughout the semester, we have focused on several aspects of water biology from a range of perspectives. In most cases, we have discussed interactions that take place in natural systems, although these interactions are often anthropogenically influenced. This presentation will focus on water biology in engineered systems and how biological interactions are often harnessed and optimized for applications to wastewater, drinking water, and groundwater remediation.

Overview

- Wastewater
  - Organic matter removal
  - Nutrient removal
- Contaminated groundwater
  - Chlorinated solvents
- Drinking water
  - 2-methylisoborneol
  - Membrane fouling

We will focus on wastewater, contaminated groundwater, and biological treatment of drinking water.

With regard to wastewater, I will discuss the use of biology for the removal of organic constituents and nutrients during secondary treatment.

For contaminated groundwater, I will focus on the removal of chlorinated solvents through the use of bioremediation, which may include natural processes, as well as induced processes.

And lastly, I will discuss some applications of biological treatment to drinking water. These applications include removal of 2-methylisoborneol and using biological means to pre-treat water prior to membrane filtration in order to reduce membrane fouling.

Wastewater Treatment

Wastewater Treatment

- Some constituents of concerns
  - Solid waste
  - Organic matter
  - Nutrients
  - Microorganisms
- Primary treatment
  - Settable solids
  - Physical/chemical treatment
- Secondary treatment
  - Dissolved/colloidal organic matter, nutrients
  - Biological treatment

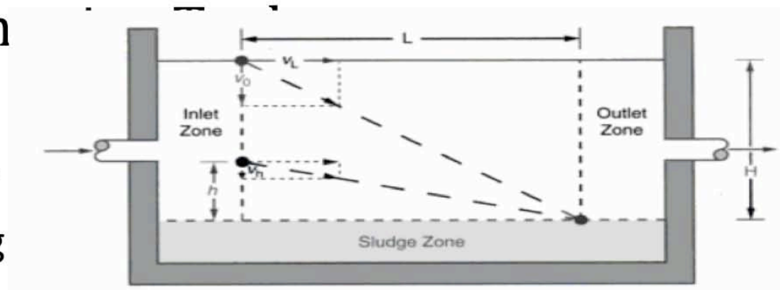
The generation of wastewater from human activities is inevitable. The characterization of this wastewater depends heavily on its source, thus also making its required treatment also source dependent. For example, the composition and treatment of domestic wastewater is likely to be very different than wastewater originating from industrial or agricultural uses.

With regard to domestic wastewater, there are several constituents of concern, the most obvious being solid waste, dissolved and colloidal organic matter, and nutrients. These constituents are of concern because wastewater is typically discharged into a receiving surface water body or reclaimed for reuse. Discharging or reusing wastewater with high concentrations of solid waste, organic matter, and nutrients would inevitably result in a wide range of pollution, oxygen depletion, eutrophic conditions, aesthetic issues, and gross violation of regulations. As a result, wastewater is sent to a centralized wastewater treatment plant or treated on-site prior to discharge or reuse. Conventional wastewater treatment can be separated into two segments: primary treatment and secondary treatment.

Primary Treatment

- Bar screens
- Grit chambers
- Sedimen

“Ideal” settling tank



Wastewater Treatment

Primary treatment is conducted prior to secondary treatment and it targets the solid waste portion of influent wastewater. Primary treatment is done through physical and chemical means, typically including bar screens, grit chambers, and quiescent settling, as well as preaeration and chemical coagulation if needed.

In this slide, a schematic of an “ideal settling tank” is shown (Vesilind, 2003). In an ideal settling tank, particles are removed from solution through gravitational settling. The time required for a particle to settle must be less than the time it takes the particle to travel horizontally to the end of the tank.

Primary treatment effluent then moves onto secondary treatment, where dissolved and colloidal constituents are targeted for transformation or removal by microorganisms. The microbial communities present during secondary treatment depend on the primary effluent quality and operating parameters.

Secondary Treatment

- Goal
- Oxidation-reduction conditions
  - Aerobic
  - Anoxic
  - Anaerobic

Wastewater Treatment

In secondary treatment, the overall goal is to establish and maintain a high population of microorganisms that are capable of converting biodegradable, organic wastewater constituents and certain inorganic fractions into new cell masses and byproducts. These new cell masses and byproducts are preferred over the original constituents because they can be subsequently removed from the system by air stripping, settling, or other physical means. The microbial communities that are active during secondary treatment, and thus the contaminants that are removed by these communities, depend on the operating parameters of the water treatment plant. More specifically, the results of secondary biological treatment are heavily influenced by the oxidation-reduction conditions and the hydraulic design.

Aerobic Conditions

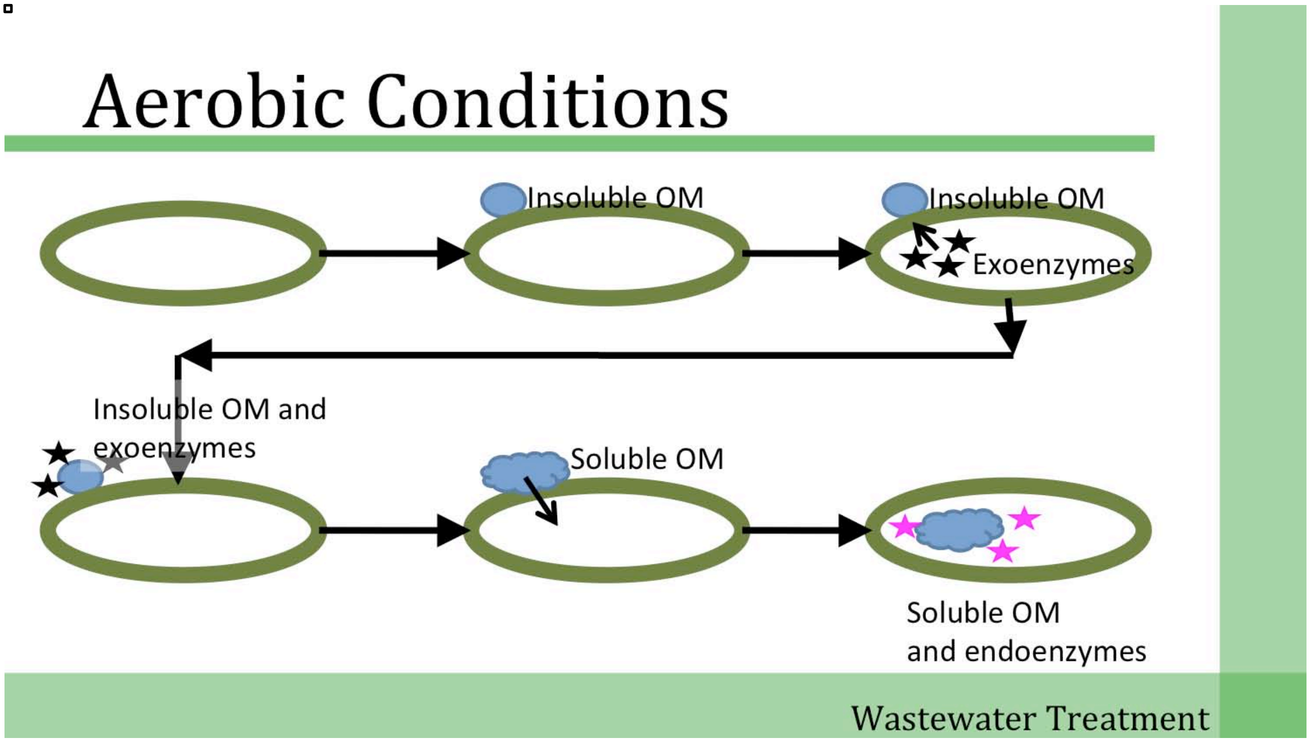
- Purpose:
  - Conversion of organic matter
- Microorganisms:
  - Mostly bacteria and protozoa
- Electron acceptor:
  - Dissolved oxygen
- Example:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

Wastewater Treatment

Under aerobic conditions, dissolved oxygen is available and it is therefore the primary electron acceptor. In conventional wastewater treatment in the United States, biological treatment is most often carried out under these conditions using mechanical aeration or bubbling techniques.

The microbial communities found in aerobic systems are mostly aerobic bacteria and protozoa that oxidize organic matter for cellular activity and growth. Coupled with the oxidization of organic matter is the reduction of dissolved oxygen. For example, a bacterium may use glucose as food and oxygen as an electron acceptor, thus eventually producing carbon dioxide and water. In this case, the dissolved glucose would be eliminated and carbon dioxide could be removed through air stripping, assuming that dissolved oxygen is continuously supplied. In the presence of several different electron acceptors, microorganisms will continue to use dissolved oxygen until it is depleted; this is because electron acceptors are used based on the quantity of energy they supply.



The schematic shown in this slide was adapted from Gerardi, 2006. Aerobic bacteria are capable of oxidizing both soluble and insoluble organic matter through the use of exo- and endoenzymes. When insoluble organic matter adsorbs onto the bacterium, the bacterium produces exoenzymes that are exported out of the bacterium to the insoluble organic matter. The exoenzymes break the insoluble organic matter down until it is soluble and thus able to be taken up by the bacterium. Once absorbed by the bacterium, the soluble organic matter is degraded by endoenzymes.

Anoxic Conditions

- Purpose:
  - Nitrogen removal (denitrification)
- Microorganisms:
  - Facultative chemoheterotrophic bacteria
- Electron acceptor:
  - Nitrate
- Example:

$$2NO_3^- + 2H^+ \rightarrow N_2 + 2.5O_2 + H_2O$$

Wastewater Treatment

Under anoxic conditions, dissolved oxygen is absent and nitrate is available as the next most preferred electron acceptor. Nitrate may also be present under aerobic conditions, but dissolved oxygen would be preferentially used as an electron acceptor.

Microorganisms that are able to use nitrate as an electron acceptor continue to oxidize organic matter for cellular activity and growth; however, under these conditions, the focus is typically on denitrification instead of the conversion of organic matter. Denitrification is important because NO_3^- can eventually be removed as N_2 through air stripping, thus lowering the nutrient loading on the receiving surface water. Anoxic systems are also sometimes referred to as aerobic/anaerobic due to the fluctuating availability of nitrate and dissolved oxygen, as shown by the consumption of NO_3^- and production of O_2 in the equation above.

Anaerobic Conditions

- Purpose:
  - Sulfate removal, organic matter removal, methane production (fuel)
- Electron acceptors:
  - Sulfate and/or carbon dioxide
- Examples:

$CH_3COOH + SO_4^{2-} + 2H^+ \rightarrow H_2S + 2H_2O + 2CO_2$

$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$

Anaerobic conditions refer to a system in which both dissolved oxygen and nitrate are absent, thus forcing microorganisms to use other constituents as electron acceptors. Anaerobic conditions may be imposed on a wastewater system for organic matter removal, sulfate removal, or methane production because sulfate and carbon dioxide act as the electron acceptors and organic matter is still oxidized by microbial communities for cellular activity and growth. With regard to organic matter removal, the rate at which organic matter is oxidized will be less than that associated with aerobic conditions; however, costs associated with mechanical aeration and bubbling may be prohibitive and therefore worth the slower rate of oxidation.

In most cases, methane production is considered disadvantageous due to it being a greenhouse gas. In other cases, however, methane collection systems are put in place and methane production is encouraged. The collected methane can be used as a fuel. Similar set-ups can also be applied to landfills and composting facilities, in which operating parameters promote methane production through anaerobic digestion of organics.

Groundwater Remediation

Now, let's discuss the application of biological treatment to contaminated groundwater, using the example of chlorinated solvent removal through the use of bioremediation.

Chlorinated Solvents

- Commercial and industrial applications
- Frequently released into the environment

Solvent	Amount released 1998-2001
Methylene chloride	33 million pounds
Perchloroethene	4 million pounds
1,1,1-trichloroethane	0.5 million pounds
Trichloroethene	11 million pounds

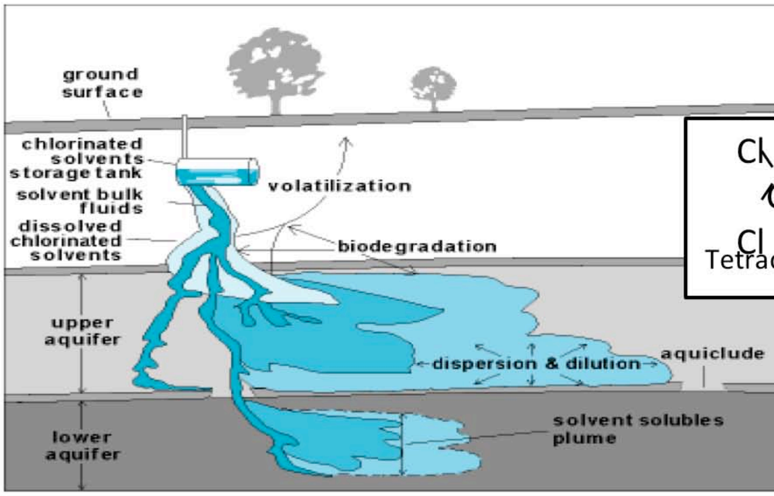
- Acute and chronic human health issues
  - Liver and kidney damage, reproductive failure, cancer
  - Low drinking water MCLs for some solvents

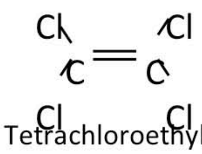
Groundwater Remediation

Chlorinated solvents are heavily used in commercial and industrial applications in the United States. Due to this widespread use, solvents are unfortunately frequently released and found in the environment. According to the U.S. Environmental Protection Agency Toxic Release Inventory, on- and off-site releases of solvents during 1998 to 2001 totaled the values shown in the table above. This is important to both human and environmental health due to the acute and chronic health issues associated with chlorinated solvents and our reliance on high quality groundwater.

When a solvent spill is known to occur, the responsible party is expected to address three aspects of the event: removal of the contaminant source, containment of the contaminant plume, and treatment of the contaminant plume.

Chlorinated Solvents





Tetrachloroethylene (PCE)

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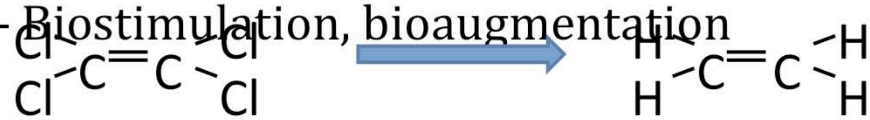
Groundwater Remediation

The figure shown here is a simplified depiction of chlorinated solvent movement after being released into soil and groundwater. Chlorinated solvents have a tendency for widespread groundwater contamination due to the following properties: high vapor pressure, high solubility, low organic phase partitioning, and low viscosity.

Chlorinated Solvents: Treatment

- Pump and treat methodologies
  - Costly and invasive
- Biological treatment
  - Natural attenuation
  - Biostimulation, bioaugmentation



Tetrachloroethylene Ethylene

Several methodologies can be used for treating chlorinated solvent plumes. In the past, "pump and treat" technologies were typically used in which the contaminated groundwater and soil were literally pumped out of the ground, treated, and then returned to the aquifer. As one might assume, pump and treat methodologies are intensive with regard to time, cost, and environmental invasiveness. As a result, biological means of treatment have gained popularity.

Natural attenuation involves plume management through natural processes, including adsorption of the solvent to soil particles, biodegradation, dilution, and dispersion. In order for natural attenuation to be accepted as a remedial solution, groundwater and soil data must be collected in order to predict the recalcitrance and movement of the solvents. If natural attenuation is chosen, modeling and long-term monitoring is required to ensure that biodegradation of the solvents occurs as predicted.

If biodegradation of the chlorinated solvent spill is deemed the best option, but indigenous communities are not expected to degrade the solvents naturally, biostimulation or bioaugmentation can be implemented. Biostimulation involves altering environmental conditions in order to encourage degradation of the chlorinated solvents, such as through the addition of nutrients, growth substrates, or oxygen. When natural attenuation and biostimulation do not seem promising, exogenous microbial communities are selected based on specific characteristics and introduced.

With regard to chlorinated solvents, the goal of biological treatment is anaerobic reductive dechlorination in which the end product is ethylene.

Bioaugmentation

- *In situ* treatment
- Examples:
  - *Burkholdia cepacia* ENV435
    - 78% reduction in total mass of TCE, DCE, and VC in treated area after 2 days
  - KB-1 culture (phylogenetic relatives of *Dehalococcoides ethenogenes)*
    - ~99% reduction in PCE, TCE, and DCE under reducing conditions after 200 days

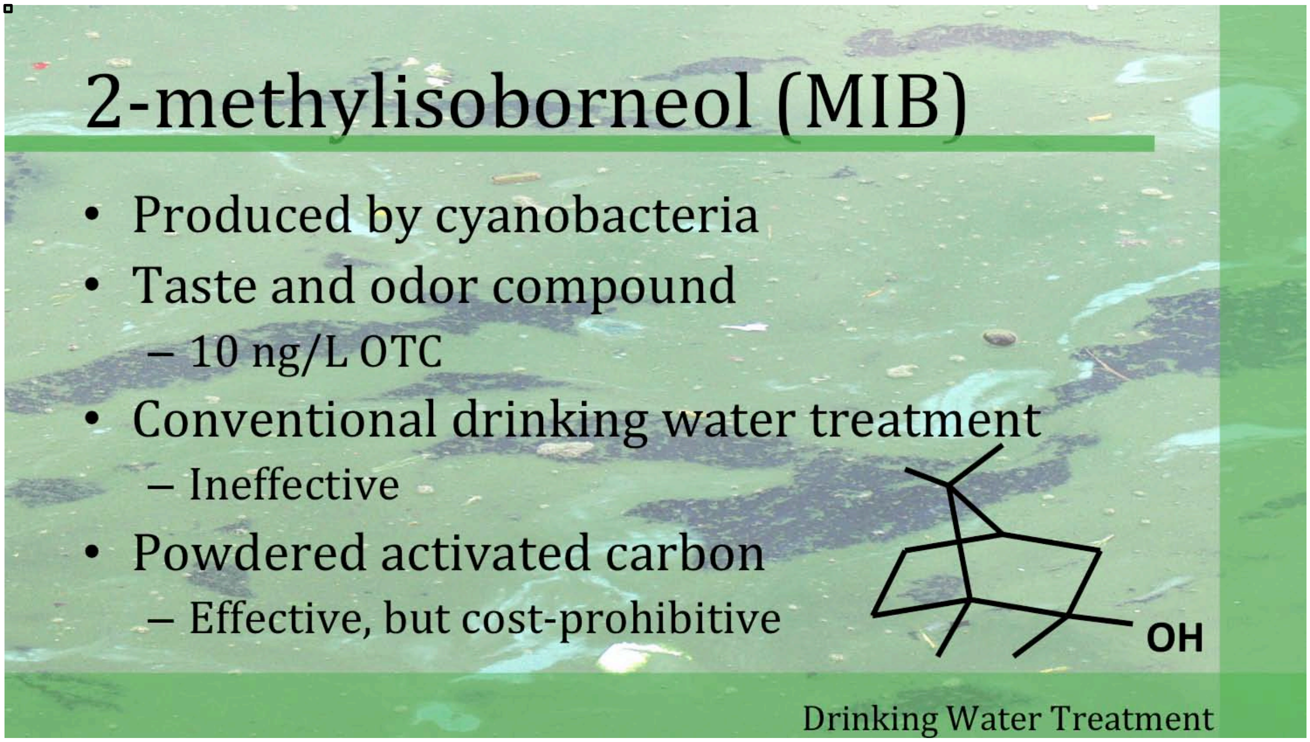
In one study, bioaugmentation was used for a test system located at an industrial facility in New Jersey that was contaminated with chlorinated solvents. A special strain of bacteria, *Burkholdia cepacia* ENV435, was chosen for bioaugmentation because it was shown to exhibit limited adhesion to aquifer solids, degrade chlorinated ethenes, and grow to high cell densities.

Prior to bioaugmentation, the groundwater contained 1000-2500 µg/L of chlorinated ethenes (trichloroethylene (TCE), dichloroethylene (DCE), and vinyl chloride (VC)). Approximately 550 liters of the bacteria (~100 g/L) were injected with oxygen into a 4.6 m x 12 m plot. After 2 days, the groundwater chlorinated ethene concentration in the bioagumented plot was compared to that in another plot, equal in size, that had been injected with basal salts. In one trial, the total mass of TCE, DCE, and VC in the plot that had been treated with the specialized bacteria had been reduced by ~78%.

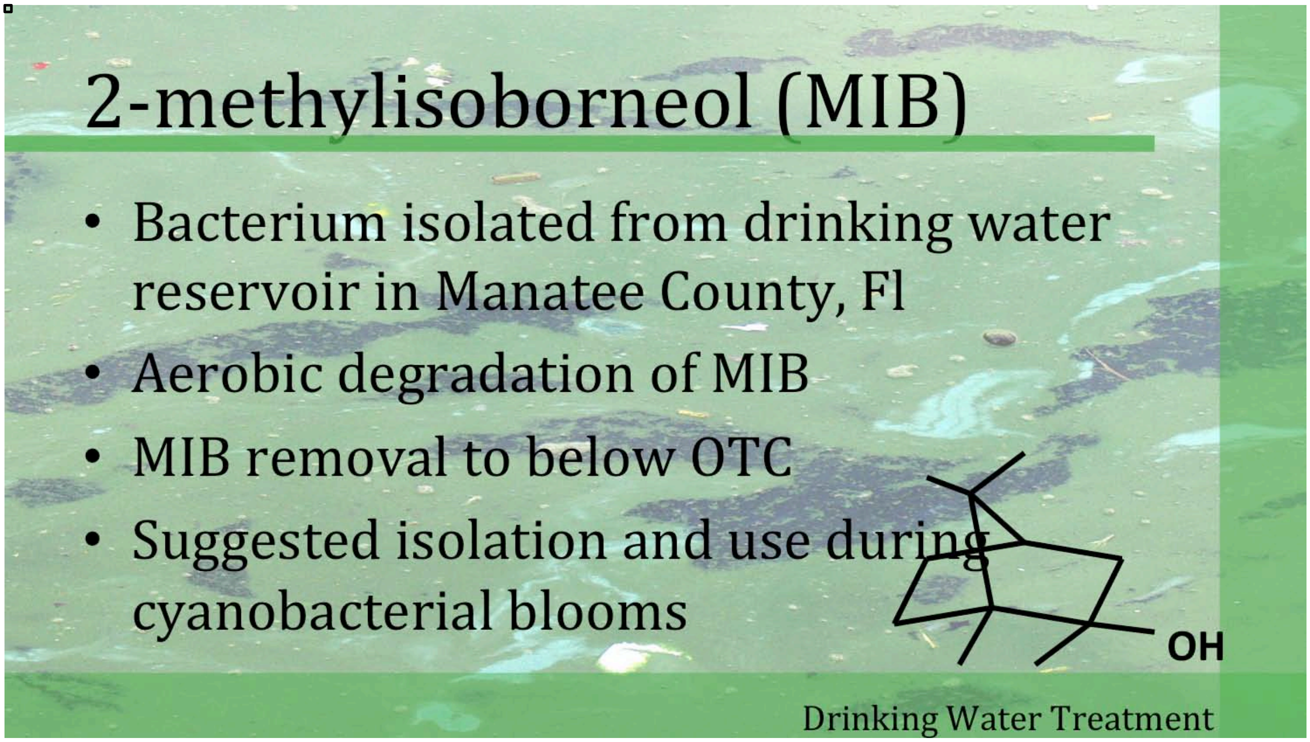
In another study, a pilot test area at Kelly Air Force Base was injected with a dechlorinating enrichment culture known as KB-1 after being amended with methanol and acetate to establish reducing conditions. After 200 days, chlorinated solvent concentrations had decreased from ~1 mg/L to ~5 µg/L and a corresponding increase in ethene was observed.

Drinking Water Treatment

Similar to wastewater treatment and groundwater remediation, biological treatment of drinking water can be used to target a wide range of contaminants. Today I will be focusing on the use of biology for the removal 2-methylisoborneol and as a pretreatment process prior to membrane filtration.



In addition to providing safe drinking water safe, it is important that water treatment facilities provide palatable drinking water to consumers. As a result, 2-methylisoborneol (MIB) has become a very well-known taste and odor compound. MIB is produced by cyanobacteria and it is well-known for two main reasons: 1) the associated odor threshold concentration (OTC) is very low at approximately 10 ng/L and 2) conventional water treatment typically results in poor removal of MIB. The addition of powdered activated carbon has proven to be effective for MIB removal; however, the required doses, disposal, and regeneration of powdered activated carbon make this treatment process economically infeasible. In response to the need for MIB treatment, biological treatment has gained popularity as an alternative treatment method.



In a study conducted by researchers at the University of Florida, a bacterium was isolated from a drinking water reservoir in Manatee Country. The bacterium was shown to effectively degrade MIB under aerobic conditions at high and low concentrations of MIB. Furthermore, this bacterium removed MIB to below its associated odor threshold concentration of 10 ng/L. The authors of the study suggest that this type of bacteria could be isolated and used during cyanobacteria blooms. The feasibility of this option is further supported by the fact that the bacterium can use MIB in addition to other carbon substrates, thus indicating that the bacterium is likely to survive and be present in the environment in the presence and absence of MIB.

The isolated bacterium was characterized as spore-forming, flagellated, and most closely related to *Bacillus fusiformis* and *Bacillus sphaericus*

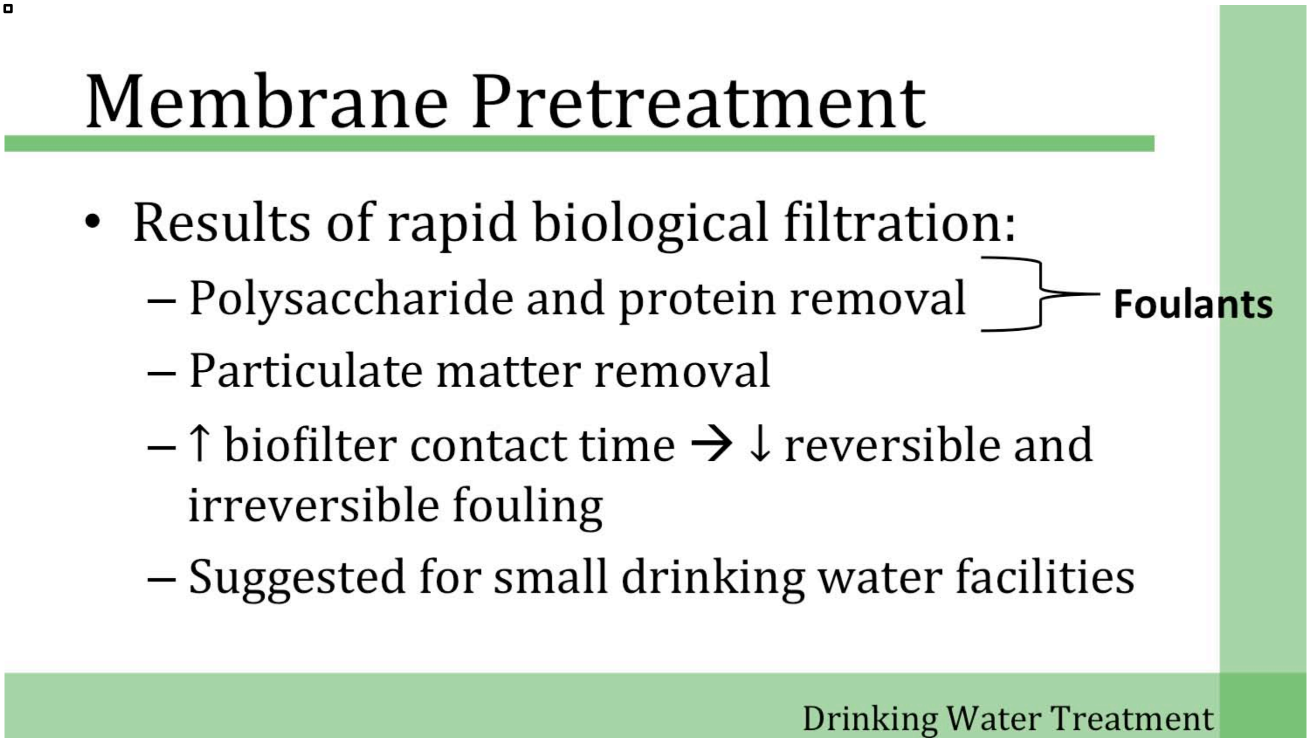
Low Pressure Membrane Filtration

- Microbial and turbidity removal
- Organic fouling
  - Soluble and colloidal OM
  - Reversible and irreversible
  - Flux decline
  - Reduced water quality
  - Increased operating costs
- Chemical free rapid biological filtration for pretreatment?

Drinking Water Treatment

In addition to contaminant removal, biological treatment can be used to enhance the performance of other treatment options. For example, low pressure membrane filtration is increasingly used for microbial and turbidity removal. However, membrane filtration can be a costly process to maintain if membrane fouling is not avoided. As a result, influent organic matter is of concern because organic fouling of membranes can result in both reversible and irreversible fouling, thus causing flux declines, reduced water quality, and increased operating costs.

In order to protect these costly and relatively sensitive membranes from fouling, influent water often undergoes pretreatment in order to remove some of the important foulants. In response to organic fouling, chemical free rapid biological filtration has been proposed as a pretreatment option.



In response to this proposal, one study investigated the effect of biological filtration of drinking prior to ultrafiltration with regard to membrane fouling. Results showed that biological filtration improved influent water quality with regard to polysaccharide, protein, and particulate removal. Additionally, a negative association was established between contact time in the biofilters and subsequent reversible and irreversible ultrafiltration membrane fouling. As a result, the researchers in this study suggest that biological filtration be considered by small drinking water facilities as a pretreatment option for low pressure membrane filtration.

Summary

- Wastewater treatment
  - Organic matter and nutrient removal
  - Ensure removal of microorganisms before discharge
- Groundwater remediation
  - Chlorinated solvents
  - Natural attenuation, biostimulation, bioaugmentation
  - Investigate long-term effects of new microbial population
- Drinking water
  - MIB and membrane pretreatment
  - Ensure removal microorganisms before chlorination and distribution

Understanding aquatic biology is incredibly important when studying natural and engineered systems. Over the course of the semester, we have discussed several aspects of water biology and today we have discussed how these natural interactions are often intentionally applied for remedial purposes. With regard to wastewater treatment, secondary treatment typically includes biological processes for the removal of organic matter and nutrients. During these processes, it is important to ensure that conditions within the system support the appropriate microbial communities and that microorganisms are thoroughly removed or inactivated before discharging or reusing the treated wastewater.

Secondly, groundwater contamination requires attention because chemicals that are heavily used within our society are often inadvertently released, thus posing harm to human and environmental health. Chlorinated solvents are a common issue with groundwater quality and studies have shown that bioremediation, including natural attention, biostimulation, and bioaugmentation, can oftentimes attenuate the issue. When using these biological methodologies, it is important to study the long-term effects in order to ensure that new microbial contaminants are not introduced.

Lastly, biological treatment can also be used for a wide range of applications in the drinking water industry. For example, biological treatment can be used to remove MIB, a common taste and odor compound, and membrane foulants in order to increase the palatability of water and reduce membrane fouling.


Questions?

References

- **Wastewater**
- **Groundwater**
- **Drinking water**

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