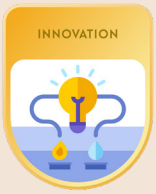
Remediation of Eutrophication Through a Phosphorus-Absorbing System



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In 2016, approximately sixty—five percent of freshwater and coastal water bodies in and around the United States were severely damaged by eutrophication. This phenomenon occurs naturally in surface water as organic matter from aquatic organisms accumulates. However, this process can easily be accelerated by the runoff of fertilizers into water, resulting in abnormally high nitrogen and phosphorus levels and, thus, overabundant algal and bacterial growth. The respiration of high populations of algae and bacteria deprives the surrounding air and water of oxygen and lowers the pH of the water as carbon dioxide levels in the water increase. These environmental consequences cause the deaths of aquatic organisms in the local ecosystem and impose a negative impact on neighboring ecosystems and human health.

Current remediations for eutrophication are mostly chemical based, such as applying ultrasonic irradiation, chemical algicides, or cyanophages. Our goal is to remediate eutrophication in an eco-friendly way by using genetically engineered *Escherichia coli* to absorb excess phosphorus from water bodies suffering from anthropogenic eutrophication. The system will incorporate regulatory genes to ensure environmental safety and will be contained in a semi-permeable buoy, ultimately creating an easily retrievable contraption to capture and remove phosphorus without the introduction of toxic chemicals.

Keywords: Eutrophication, *Escherichia coli*, phosphorus, eco-friendly, environment, remediation

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Background

Normally, eutrophication occurs naturally as surface water ages over thousands of years, creating a reserve of organic matter and minerals that can support large populations of algae. However, soil erosion and anthropogenic influences such as the use of synthetic fertilizers have rapidly deteriorated water quality in both saltwater and freshwater sources on Earth. Phosphorus attaches to soil particles, allowing runoff from agricultural areas to travel through soil and groundwater and contaminate local bodies of water. Being an important limiting nutrient of aquatic plants due to its insolubility in water, sudden spikes in phosphorus levels can result in the rapid saturation of surface water with algae, a dangerous and destructive phenome-

non known as an algal bloom—one of the key signals of eutrophication.

While long-term eutrophication can usually be prevented if total phosphorus levels are below 0.5 ppm and 0.05 ppm, levels of 0.08 to 0.10 ppm phosphate may trigger deadly algal blooms (Kotoski 1997). Therefore, excessive amounts of nutrients introduced into the water from agricultural runoff containing nitrogen- or phosphorus-based fertilizers and soil erosion can severely disturb the balance in the local ecosystem. A multitude of problems may arise: uncontrollable algal blooms that block sunlight, overgrowths of toxic cyanobacteria and other bacteria that feed on dead algae and deplete oxygen, and turbid water that is unsafe to drink from and unappealing for recreational use (Cornell University 2000). As a result, eutrophication harms and kills the marine and terrestrial organisms in the local aquatic ecosystem and neighboring ecosystems, posing a serious threat to their health and biodiversity.

Eutrophication also poses a threat to human populations. For instance, toxic cyanobacterial and algal blooms can cause gastrointestinal illnesses, infections, and respiratory problems, as in the case at Lake Okeechobee, Florida where contaminated coastal water resulted in a health state of emergency announcement. Eutrophication has resulted in reduced recreational usage of water bodies, reduced waterfront property values, damage to endangered species recovery programs and contamination of potable water in many major nations. In the United States alone, remediations for freshwater eutrophication have cost as high as $2.2 billion (Dodds et al 2008). The most common solutions to this problem as of now are to spray the affected water with algicides such as endothall, an acute toxin and irritant (National Center for Biotechnology Information 2017), and methabenzthiazuron, which is an environmental hazard and is toxic enough to inflict long term damage to aquatic ecosystems (National Center for Biotechnology Information 2018). While effective, these methods introduce extremely harmful organisms to the environment and humans (Thakur 2018). Thus, our system seeks to provide an eco-friendly alternative to remove phosphorus from affected water bodies using genetically engineered *E. coli*, reducing algae and bacteria populations.

Systems Level

This system is designed to extract phosphorus from water and store it to reduce the effects of eutrophication and allow for affected ecosystems to recover from eutrophication using genetically modified *E. coli* (strain K12). Buoys will be designed and constructed, containing the *E. coli* to prevent the *E. coli* from being released into the wild, allowing the phosphorus and bacteria to be removed from the ecosystem in the buoy by hand. The effectiveness of this system will be tested in the future: see more details in the "Future Experimentation" section.

Device Level

The system is designed to absorb phosphorus from the water and store it in bacteria cells. We plan to use the bacteria *Escherichia Coli* as our chassis for the system because *E. coli* is found naturally in the environment and serves as an organic and controlled alternative to harmful chemicals. *E. coli* optimally functions from 4–45º C and can survive in a pH of up to 2.6, but it functions optimally at a pH of around 7. Therefore, *E. coli* can withstand the fluctuation of water temperatures due to seasonal changes and geographic location, and its functionality aligns well with the pH of freshwater lakes, which is 6.5–8.5 (Doyle et al. 2006). Despite this, tests still need to be conducted to determine the optimal temperature for the system.

E. coli also replicates itself every 20 minutes under ideal growth conditions, which allows for an abundance of bacteria to form quickly. The genetically engineered *E. coli*, as well as a "substance V", a nutrient for the bacteria to live and grow, will be contained in buoys (go to the "Safety" section for more information on "substance V"). This is beneficial to the system as a large amount of phosphorus can be removed with the placement of a few bacteria in the buoys. Thus, *E. coli* seems to serve as an effective chassis due to the extensive studies and success rates of transformations executed on the bacterium.

Parts Level

Our system design consists of three sequences: one begins with the standard constitutive promoter BBa_J23100, the second is regulated by the inducible promoter P_{PHO89}, and the last is controlled by an artificial repressible operon. See a map of the system in Figure 1.

The first sequence starts with the constitutive promoter and codes for two proteins: the antitoxin CcdA, which is a component of the kill switch, and the gene ppk, which codes for the protein polyphosphate kinase. Polyphosphate kinase will be used to absorb phosphorus by catalyzing the reaction between ATP and phosphorus to form a stored long-chain polyphosphate. This sequence is on by default, consistently producing proteins to absorb phosphorus until cell death.

The second sequence consists of the inducible promoter P_{PHO89} followed by the ccdB gene. The ccdB gene codes for the toxin protein CcdB, the counterpart of CcdA in our kill switch. Originally from the yeast species *P. pastoris*,

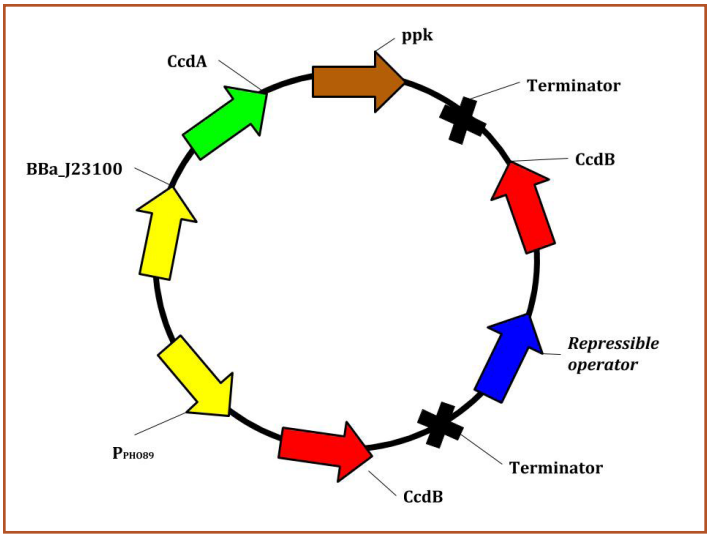

Figure 1. *Map of the plasmid containing the system, indicating the direction of gene transcription and the three promoters: BBa_J23100 is a constitutive promoter that induces the transcription of ccdA and ppk, P_{PHO89} is an inducible operator responsible for producing CcdB, and the repressible operator normally blocked by the presence of substance V controls the production of CcdB as well.*

the inducible promoter P_{PHO89} is switched off by default and is turned on when the phosphate level in the water is lower than a certain threshold level. In other words, when the remediation process is complete, the cells will terminate, preventing excess phosphorus absorption. The natural levels of phosphorus in freshwater aquatic systems are generally less than 0.03 mg/L. Since the default threshold level of P_{PHO89} is as high as 0.02 g/L (Ahn et al 2009), we will need to reengineer the promoter to reset that threshold, ideally at around 0.03 mg/L.

The third sequence is constituted of an artificial repressible operon controlling a ccdB gene. This too is a safety component.The repressible operon is normally repressed by an unknown, artificially synthesized substance V (discussed in more detail in the "Safety" section), but in the absence of substance V, ccdB expression is turned on, killing the bacteria. We plan to design and build an artificial repressible operon that will be compatible with the synthesized substance V we choose.

Safety

In order for a kill switch to be environmentally safe, it needs to be lethal for the cell to undergo apoptosis yet stable enough in its environmental surroundings to not pose a risk to surrounding ecosystems. Keeping these criteria in mind, we will implement the CcdA/CcdB Type II Toxin-antitoxin system as our kill switch.

The toxin CcdB not only can kill the cells quickly and effectively, it also further eliminates risks of naive organisms picking up stray engineered DNA floating in the environment by damaging DNA replication before introducing cell apoptosis, making it an ideal component of the kill switch. In the cells, CcdB targets the GyrA subunit of DNA gyrase and stops the transient double strand in the DNA strand, which decreases DNA synthesis and results in cell death. CcdB is produced in large quantities either when the phosphorus level in water has been lowered enough to be safe or when the bacteria escape the buoy container. Both situations are described in more detail later in this section. The antitoxin CcdA, on the other hand, binds onto CcdB to form a CcdA-CcdB complex, repressing its toxicity by physically blocking the activity of CcdB and removing the CcdB proteins from the DNA gyrase (Stirling et al 2017). In our system, the gene ccdA is set to be expressed at a constitutive low level to repress any leaky expression of CcdB. However, when one of the conditions mentioned above comes true and triggers the ccdB gene to produce a large amount of toxin, CcdB will be in excess of CcdA and de-repression occurs. The excess CcdB will then kill the cells, carrying out the task of a kill switch.

The CcdA/CcdB Type II Toxin-antitoxin system is implemented twice with different triggering conditions in our system. One of the two sequences contains the antitoxin and a phosphorus absorbing gene, which constantly produces proteins until the cell dies. The other involves an artificial repressible operon that operates on an artificially synthesized substance V. We are still researching for an ideal substance to use, but V will be a synthesized substance that does not exist in nature. A detailed explanation of the mechanism on the gene level is included in the "Parts Level" section.

We will supply V when cultivating the bacteria in the lab and place V in the buoys during remediation, but V will not be found anywhere else. Hence, in the event of some *E. coli* escaping from our buoy containers, these individuals will quickly die off without the presence of V, imposing no threat to the balance of the local ecosystem. The ideal substance of V is still in question so refer to "Future Experimentation" for more details.

Another mechanism we plan to implement to minimize potential environmental damage is to adhere the genetically engineered *E. coli* to a specific material. Although more research is required to identify an ideal adhesive material, our hope is to create a buoy that will prevent the *E. coli* from escaping the buoy and contaminating the water. The components of this are to include either a material that *E. coli* naturally adheres to or a gene that will serve to enhance the adhesion of *E. coli* to a material. We will try to minimize the impact of trapping the *E.coli* in a small container on the cells' survivability and metabolic activities by making the buoy permeable to water and ideally most or all of the bacterial nutrients

and waste substances, making the environment inside the buoy as open as possible.

Discussions

This system will alleviate the effects of eutrophication, which profoundly interfere with the surrounding environment. Eutrophication commonly causes a buildup of algae blooms in the water due to the abundance of phosphorus. The phosphorus absorbing system will reduce the phosphorus levels in the water and thus inhibiting the growth of algae. As the algal blooms decrease, oxygen levels will increase as the oxygen consumption levels decrease and the aquatic animals and plants will flourish once more.

The *E. coli* will be contained within a semi-permeable buoy by encoding genes that allow for better adhesion to synthetic or natural materials. Using said adhesion, the system can be contained to ensure safety and allow for controlled tests. A challenge that arose during development was lack of nearby freshwaters affected by eutrophication. Availability of such water would have provided better insight into the nature of eutrophicated waters, as opposed to researching the properties.

Future Experimentation

Having completed most of the research, the next step is to build the system. However, there are still various parts of the system that require further research. P_{PHO89}, an inducible promoter, turns on when the phosphate level in the water is less than a specific threshold value which has been identified as 0.02 g/L. As previously mentioned, the normal phosphate level of freshwater bodies is 0.03 g/L, so P_{PHO89} will need to be re-engineered. This can be done by adjusting the strength of the ribosome binding sites (RBS), changing the position of certain operators in the system, or by adding new operators into the system as a while.

Algal blooms form as a byproduct of eutrophication and causes the water to become basic. We plan to administer our system into waters already affected by eutrophication, thus it would be added to high pH environments. *E. coli* can survive in a pH range from about 4.4–9.7 thus we anticipate that the system working as algae generally makes the pH rise to about 8.2–8.7. We will find more about the system's efficiency to remove phosphorus upon experimentation.

Upon further research, we need to identify one synthesized substance that works the best with our purposes of substance V. The repressible promoter associated with substance V is also a component which we have to design on our own. The final implementation of the operon will vary based on the identity of V, and extensive testing will be needed to ensure its functionality.

Both promoters in the system control the gene coding for protein ccdB, which is part of the kill switch. However, all parts containing and/or expressing the ccdB protein have been called back, so research into another alternative for a toxin will be necessary.

Once built, the system will need to be tested and the results quantified to determine its effectiveness. More specifically, the experiments conducted will help determine the system's rate of phosphorus absorption, as well as its optimal temperature and pH. We will do so by taking a water sample with eutrophication from either a private property with permission or a nearby public park, namely the Acton Arboretum located in Acton, MA, USA. Should we choose to collect the water samples from the Acton Arboretum, we have verification from Natural Resources and Conservation to obtain a permit for testing in a controlled environment that can model environments that are affected by eutrophication.

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