Grey biotechnology

purifying water with the help of **GMO**s (**G**enetically **M**odified **O**rganisms)

Elisa Coronado

Lisa Béglé

Touba Malik

Overview

Term Paper: Applications of Genetic Engineering and Biotechnology

1. Preface

For a long time, sewage treatment has been a purely mechanical and chemical process. Recently biotechnology came into play as well. However, the use of this method poses some challenges or maybe even threats to the natural environment.

We are intrigued by the biotechnological methods used in the field of water purification, because they are not a highly discussed, especially when genetically modified organisms (GMOs) are concerned. The applied engineering techniques do demand some skill and effort, but on the other hand the creation process and the resulting microorganisms are a fascinating subject. The process deals with one of our vital needs, i.e. clean water and it does have a promising future across the world. In this paper we will deal with the following questions:

* How has the method already been applied?
* What are the consequences of using genetically modified organisms in sewage-works and in the open environment?
* What's the potential of this method and what are possible future steps?
* Finally, how does this method contribute to solving the problem of water scarcity?

2. Introduction

The global problem of water pollution is becoming increasingly hard to control due to countless chemical factories and agricultural industries discharging their waste into water bodies and people throwing their trash into the sea. Some of the most problematic substances include chlorophenols, nitrophenols, BTEX (benzene, ethylbenzene, toluene and xylene), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls and organic solvents. Many of these substances are mutagenic and break down slowly which implies that they remain in the environment for a long period of time. These and other substances can accumulate in the food chain and have detrimental effects on our life quality.

Each year, approximately 13 billion dollars are spent worldwide to clean up the oceans. New methods and innovative ideas to counteract the problem are very much desired and grey biotechnology might be our saviour.

Grey biotechnology (or environmental biotechnology) is interested in the processing of drinking water, the clearing of sewage, the rehabilitation of contaminated soil, recycling and the purification of exhaust gases.

Since the 1980s, microorganisms are being used in the process of clearing contaminated water. Today, they are essential in sewage plants, industry and commerce. Biological cleaning systems are usually composed of a

mixture of bacteria, fungi and protozoa. They're able to catalyse the decomposition of organic and inorganic materials.

Microbes feed on fats, proteins, carbohydrates and nitrogen compounds. Under optimized conditions, they reproduce quickly and are thus able to decompose larger amounts of substances. The dangerous substances are often converted into less harmful ones.

The problem with these organisms is that they are not particularly efficient in normal conditions. The key to efficiency might be genetic engineering. There are multiple biotechnological approaches that enable us to overcome the limits of the naturally occurring microorganisms but the use of Genetically Manipulated Organisms (GMO) outside the lab is strictly regulated and is therefore mainly used in research alone.

Nevertheless, there are a couple of examples of genetic engineering of microorganisms worth to be mentioned:

In 1980, Ananda Chakrabarty modified a microorganism in a way that he almost got the patent for it. The so called "oil eater" was a naturally occurring bacterium that was given four plasmids that were naturally occurring as well. It was the combination of the four transferred plasmids in a single bacterial cell that was patent-worthy. The four plasmids allow the bacterium to degrade four components of crude oil which is very helpful, bearing in mind all the oil leak/spills that constantly occur.

Genetic engineering has also been used to modify the substrate specificity of a biphenyl dioxygenase enzyme involved in the degradation of polychlorinated biphenyl (PCB) in Pseudomonas sp. LB400 and Pseudomonas alcaligenes KF707. Different variants of the enzyme "biphenyl dioxygenase" were created through the combination of the substrate range of the enzyme from both organisms. The newly created variants were able to hydroxylate both double ortho- and double para-substituted PCBs.

Another example: Researchers at the Inter American University of Puerto Rico altered E. coli bacteria with genes that allowed the microorganisms to survive in mercury and even remove the mercury from waste sites. The genes that were used produce proteins called metallothionein and polyphosphate kinase that enable the bacterial cells to develop a resistance to mercury and to accumulate large amounts of the heavy metal within the organism. That way, the heavy metal is isolated. This is a very beneficial manipulation as mercury is very toxic and there are no naturally occurring organisms that possess the ability to bioremediate mercury.

However, in most cases it is solely the efficiency of the organism that needs to be increased through a few genetic adjustments.

3. Description of engineering technique

Some microbes are naturally equipped with features that are used for the bioremediation of polluted water. However, they cannot be controlled and aren't very efficient. That's why we rely on genetic engineering. The creation of these genetically modified organisms (GMO) is a five-step process:

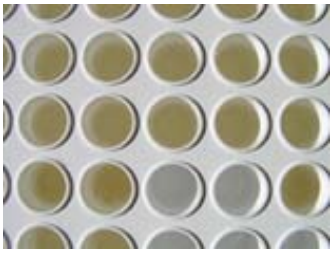
1. Cutting out the genes of interest
2. Recombination of the genes with the transfer vector
3. Transfer of the vector to the bacterial cell
4. Multiplication of the cell
5. Selection of the accomplished GMO

Three categories of useful genes can be inserted into the organism:

- degradative genes, such as the biphenyl dioxygenase, which encodes proteins required for the degradation of pollutants

- survival genes that allow the GMO not to die, despite the lack of a particular substances on which the GMO is dependent

- reporter genes (e.g. lux) that are able to apprise on pollutants



Samples of bioreporter contained water with different rates of arsenic concentration. The more intensify the colour yellow of the sample is, the more arsenic infested the water is.

After the gene of interest for the target organism is identified, it must be isolated. Its DNA information can be obtained from the cDNA or gDNA libraries. Many identical copies will be done on the stretch of DNA using the polymerase chain reaction (PCR) in order to amplify it. The gene will then be inserted into a transfer vector e.g. plasmid or bacterial conjugation with the help of restriction enzymes and ligases. The assembling of the recombinant vector with the chosen bacteria follows, which is done usually using complex methods. After transformation, the resulted organism will start to reproduce creating more of its kind. Afterwards the accomplished GMO can be separated from miscarriages that have not been modified. One method is screening with DNA probes that stick to the supposedly

transplanted gene. Another is to release antibiotics or herbicides into the vector, leading the GMO to resist, but not the failed ones.

The following paragraph will show an example of a method. A fair number of bacteria in nature are equipped with resistance mechanisms, which allows the organisms not to be poisoned by arsenic compounds. The same goes for the Escherichia coli plasmid R773, which is used for arsenic bioreporter assays.

Three proteins are involved:

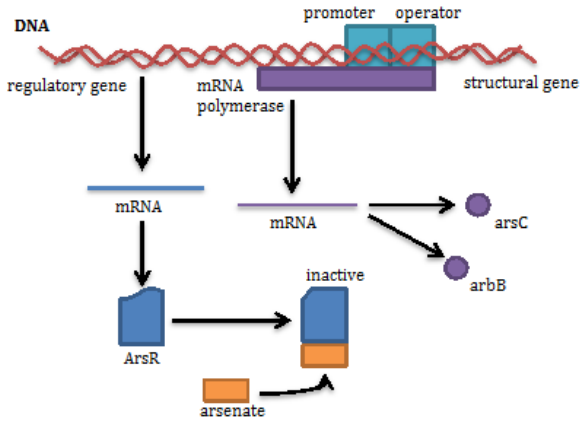
- an arsenate reductase (arsC) that converts arsenate to arsenite

- a membrane protein complex (arbB) that removes arsenite from the interior of the cell to the outside

- the arsenic sensing protein ArsR which has a regulatory function

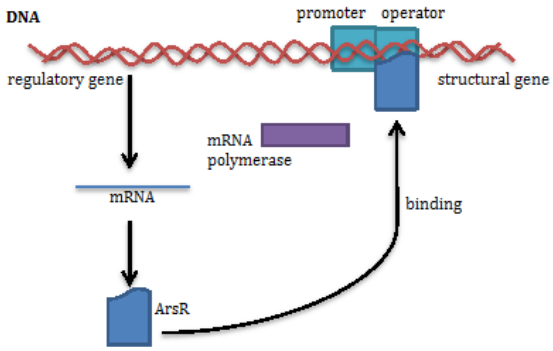
The ArsR is constantly produced by a regulatory gene. In the absence of arsenic ions, ArsR binds to a specific site on the bacterial DNA called an operator. It thereby prevents the synthesis of the arsenate reductase and the arsenite pump. If an arsenic ion enters the cell, it acts as an effector and interacts with the ArsR placing it in an inactive state. As a consequence, ArsR no longer represses the formation of the two other proteins which

In arsenic contaminated water :



can now reduce and expel the arsenic compounds.

In arsenic-free water :



The ArsR is used for the production of reporter genes of the arsenic biosensor. By genetic engineering the gene coding for the ArsR protein (the DNA binding site for ArsR) and a reporter gene coding for an easily detectable protein (like luciferase, beta-galactosidase or green fluorescent protein) are coupled. The reporter gene created is inserted into the host bacteria Escherichia coli DH5a which finalises the genetic modification. The purpose of the whole process was for the modified bacterial cells to continue to trigger alerts in the presence of arsenate or arsenite while no longer activating the resistance mechanisms.

4. Documentation

Interview with Jan Roelof van der Meer

J.R. Van der Meer is a professor at the University of Lausanne, where he teaches Biology and works at the van der Meer lab that focuses on Environmental and Evolutional Microbiology. In 2010 he was a recipient of the Erwin Schrödinger price for the development of arsenic bioreporter assays.

What fascinates you about this field?

The combination of trying to design a synthetic genetic construct in a simple bacterium that works as optimally as possible, and the idea that it might be applied somewhere to improve the quality of our environment.

How long did it take until you were first able to successfully modify the E. coli?

The procedure for the construction is very simple and a student can do it within a week. What takes longer, however, is the actual design of the synthetic genetic construct. We made dozens and dozens, and tried a lot of different things, in order to understand and model the system. Our first construct was made in 2003 – some of the more improved versions were published last year. Some further versions we are still working on, but they don't work very well for now!

What do you think the future will bring for genetically modified organisms?

Hard to say. Their construction becomes easier and easier, and this kind of constructions will also be permitted outside research labs (like in do-it-yourself-biology centers). On the other hand, there is still a lot of opposition against application of GMOs, and I don't see this changing (in Europe and CH) very rapidly. Our initial dream of having simple devices to detect environmental pollutants will therefore likely stay in the lab for a while.

There is now a similar hype in genetically modifying eukaryotic organisms. Quite a different discussion, about GMO food, medicine…

Is it possible to develop organisms that can detect substances other than arsenic?

Definitively. We have quite a range of different other bacteria, detecting other heavy metals, organic compounds, pesticides, toxicity…

5. Discussion

A lot of people associate bacteria with unsanitary conditions and see them as a threat to our well-being. But that's far from the truth. There are lot of examples of beneficial bacteria. Some of them are able to decompose dead plants and animals into their elements, cause fermentation -which is crucial in producing wine and vinegar-, enable cows to digest plants, etc.

Another useful trait of them is that they're very easy to genetically modify. We can take advantage of this trait and further improve their already existing capabilities to bioremediate harmful substances. This can also be done with other microorganisms.

In the following, we will be considering the advantages as well as the disadvantages of genetically manipulating microorganisms with regards to the purification of water systems.

Advantages

Low costs

Considering other types of remedial approaches, bioremediation (with and without GMOs) is relatively cheap.

High efficiency

Water scarcity is a big problem today -especially in less developed countries- and it is predicted that by 2025, 3 billion people worldwide will have no access to drinking water. It is our responsibility to prevent that from happening in the best way be possibly can since we humans have contributed a lot to this problem through the contamination of huge amounts of water each year. Since water is a renewable resource it is possible to clear it.

As mentioned before, there are microorganisms that are capable of degrading various organic and inorganic materials, but the problem is that they're very slow at it.

With the help of genetic engineering it is possible to significantly accelerate the process of degradation and thus eliminate a lot more waste.

The table below shows examples of a couple of engineered bacteria capable of expressing metal remediating molecules.

No.	Metal	Bacterial Species	Gene Expressed	Removal Efficiency
1	Arsenic	E.coli	Metalloregulatory protein ArsR	100.
2	Chromium(VI)	Methylococcus capsulatus	CrR protein	100.
3	Mercury	E.coli	Hg2+ Transporter	96.

4	Nickel	P.fluorescens	Phytochelatin synthase (PCS)	80.

Resistance to environmental conditions

It's possible to increase the resistance of organisms to different kinds of environmental factors like temperature, pH balance, etc.

As a result, a single type of microorganisms can be used in multiple different regions.

Detection of contaminants

Microorganisms plants can be modified to imitate bioluminescence which facilitate the detection of pollutants and toxins.

Creating of new jobs

Like any other technology, genetic engineering requires skilled workforce. New ways to manipulate genes (for example with new equipment) are waiting to be discovered. Researchers that study the biological degradation pathways in detail are of great importance as well as they provide the foundation improving the assets of microorganisms that are involved in the process of degradation.

The creation of new jobs improves the economy and raises the standard of living.

Disadvantages

Ineffectiveness

In an open environment the conditions can differ vastly from those in the lab. This can have a negative impact on the effectiveness of the organisms.

Risk of horizontal transfer of genetic material

It is possible that the genetically modified organisms will cross with non-modified organisms and create offspring. This could be harmful to the environment and negatively influence the ecosystem. The most common way to prevent horizontal transfer of genes is to induce a "suicide gene" into the organism. When there is no pollutant left, that gene causes holes to form in the cell wall and the organism dies.

Instability of plasmids

The plasmids in some cells are not stable and can easily be lost, thus rendering the organism useless. To ensure that the plasmids stay, further steps have to be taken, which costs time and money.

Toxic metabolites 

Metabolites are small molecules that form from natural processes, for example composting, and although there are none in the lab, they may occur in an open environment. Some metabolites are toxic for the genetically modified organisms and can cause a decrease in population, thus also leading to a decrease in efficiency.

Overall, there are some great advantages to using modified organisms for bioremediation. However, the open environment is very different from the lab, and it can take several years of research before the organisms can actually be used to clean water systems. In Switzerland, there are strict laws in place to ensure that no genetically engineered organisms can harm the ecosystem. Before any modified organism can be put into an open environment it has to be proven that it won't influence other organisms, doesn't have a negative impact on the environment and that it or its modified genes won't spread.

The future goal is to take the organisms out to the open field, where they can clean most of our water systems. This could also help with the water scarcity, because by using the genetically modified organisms a much larger quantity of water can be cleaned and because they're relatively cheap and easy to use, the water can also be cleaned directly in the areas where it's urgently needed.

As for the arsenic bioreporter assays, they are currently used in the Bengal basin, the place with the highest arsenic concentration in the groundwater in the world. There they help to analyse tap water, so that the 40 million people who live there can have access to safe drinking water.

6. Summary

We were initially interested in grey biotechnology because it seemed like an interesting topic that isn't talked about often enough. We wanted to know how big of an impact it has on our lives and that of other organisms and whether it might even be the solution to the water scarcity we have on our planet. During our research, we looked at some of the challenges the scientists face when working on genetically modified organisms and the threats they pose to the ecosystem.

Water pollution is a global problem that is becoming increasingly hard to control because trash often gets disposed in the sea and some of the substances it is composed of take very long to decompose. This is where grey biotechnology comes into play. It focuses on the preservation of safe drinking water and the cleaning of water systems. Since the 1980s microbes have been used to clean contaminated water, but they're not efficient enough. The efficiency can be increased by using modified organisms. For a gene to be implemented into an organism it has to go through a five-step process. An example for an organism that is used for bioremediation is the arsenic bioreporter assay. The bacterium E. coli is naturally resistant to arsenic. When a reporter gene is inserted in the bacterium, it triggers alerts in the presence of arsenic, showing that the water is contaminated and not safe to drink.

We also talked to J.R. Van deer Meer, one of the creators of the arsenic bioreporters, about genetically modified organisms and what the future has in store for them.

After looking at how grey biotechnology is used we discussed its advantages, for example the low costs of it, and the disadvantages as for example the risk of horizontal transfer of genes. We came to the conclusion that it has a lot of potential, but it's hard to implement in the open environment.

7. References

https://www.researchgate.net/publication/258979391_Bioremediation_and_Genetically_Modified_Organisms [accessed Apr 23 2018]

https://en.wikipedia.org/wiki/Bioremediation#cite_note-Menn_et_al_2001-17 [accessed Apr 27 2018]

https://www.unil.ch/arsenicsensors/home/menuinst/principle.html [accessed Apr 27 2018]

https://www.admin.ch/opc/de/classified-compilation/19996136/index.html [accessed Apr 27 2018]

https://www.biologie-seite.de/Biologie/Graue_Biotechnologie [accessed Apr 27 2018]

https://gentechniken.de/graue-gentechnik/ [accessed Apr 27 2018]

http://www.chemikinternational.com/pdf/2012/08_2012/chemik_8_2012_02.pdf [accessed Apr 27 2018]

https://de.m.wikipedia.org/wiki/Umweltbiotechnologie [accessed Apr 27 2018]

https://en.m.wikipedia.org/wiki/Ananda_Mohan_Chakrabarty [accessed Apr 27 2018]

https://www.google.ch/amp/s/www.popularmechanics.com/science/environment/amp7176/how-microbes-will-clean-up-our-messes/ [accessed Apr 27 2018]

http://www.oekosystem-erde.de/html/wasserzukunft.html [accessed Apr 27 2018]

https://www.projectguru.in/publications/genetic-engineering-bioremediation/ [accessed Apr 27 2018]

https://www.flickr.com/photos/usaid_images/8404847495 [accessed Apr 27 2018]

https://mysterytacklebox.com/blog/mtb-cleanup-water-pollution/ [accessed Apr 27 2018]

https://www.wur.nl/en/Expertise-Services/Chair-groups/Agrotechnology-and-Food-Sciences/Laboratory-of-Microbiology/Events/Microbiology-Centennial-Symposium/Keynote-speakers.htm [accessed Apr 27 2018]

Handout “genetic engineering”

Handout “the regulation of gene expression”

Pictures:

https://www.flickr.com/photos/usaid_images/8404847495

https://www.unil.ch/arsenicsensors/home/menuinst/background.html

https://www.unil.ch/arsenicsensors/home/menuinst/protocols.html

https://www.wur.nl/upload_mm/4/1/0/132509ec-0479-43aa-9d2e-74d6a623dce6_Jan%20Roelof2_41ce62da_180x180.jpg