Technology Assessment for the usage of synthetic bacteria to reduce the amount of PET in marine environment (iGEM competition 2012)

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Contents

Abstract

Every year plastic industry produces 40 billion tons of PET. Unintentionally, a large amount of this PET finds its way into the ocean in consequence of inappropriate waste disposal or detachment of plastic in apparel in washing machines. Degradation of plastics on the beaches results due to surface embrittlement and micro cracking, yielding micro particles that are carried into water by wind or tidal action. In some marine test areas micro particles of PET already exceed the concentration of oceanic plankton. Combined with its property to bind toxins, these micro particles become a hazard for nature and it´s biological variability.

This year an interdisciplinary and self-organized team of students from the Technical University of Darmstadt are forcing this challenge at the International Genetically Engineered Machine Competition (iGEM). Their goal is to create a new bacterium which can transfer PET, normally not useable for organisms into components that can be metabolized. With assistance of techniques from synthetic biology they are building a biological machine, creating an organism from scratch.

The goal of the following manuscript was to develop a technology assessment based on a model by Wolfgang Bender. The used model first described the goals and intentions of the team before it draws two perspectives of what might happen in best and worst case scenario. In the second chapter the technology was described in detail so that possible risks and opportunities can be evaluated not only on a technical issue but even by looking at effects on society, human self-understanding and environment. For a final recommendation another model by Christoph Hubig was used. Different possibilities for the use of synthesized bacteria were evaluated for their option and bequest values.

The use of synthesized bacteria in industrial facilities seems to be most efficient and safety. The role of politics should be to strength research to develop profitable techniques to extract PET from sea water and set incentives for reducing the use of plastic products. If conditions are optimized, a responsible use of synthetic biology can help to clear the planet from human PET.

Introduction

A question maybe as old as mankind: “What is life?”. The discovery of the DNA as a kind of blueprint for all living organisms changed the way of thinking about life from something mystical, god given, to something based on matter which could therefore, be predictable and manipulable.The hope was, that if we understand the genetic code, we could understand principles of life and maybe one day be able to create life which has never occured before. In the last century new techniques have arisen, which made it possible to read and even write the genetic code. Automatic DNA analysis and gene synthesis became affordable and the completion of the human gene project lead to complete new possibilities and dreams. This progress has modified the researchers possibilities to not only manipulate single genes but even create new genomes. The foundation for a new field of biotechnology called synthetic biology was built. The results are comments like the following from Tom Knight of MIT: “The genetic code is 3.6 billion years old. It´s time for a rewrite”, which shows that mankind has not only the potential to create new forms of life in the future, it also has eventually the will to do so.

So what exactly is synthetic biology? Synthetic biology is a relatively new science. The term was first stated in 1912 in a work of Stepháne Leduc but was not used for a long time. In 1974 the geneticist Waclaw Szybalski picked up the term as you will see in the following citation of his: “Up to now we are working on the descriptive phase of molecular biology. […] But the real challenge will start when we enter the synthetic biology phase of research in our field. We will then devise new control elements and add these new modules to the existing genomes or built up wholly new genomes”. If we look at todays technical standard it seems that we are now very close to what Szybaski has predicted 40 years ago. Even if today there exists no real new synthesised organism, due to a daily increasing collection of standardized biological modules (called BioBricks), which can realize special functions in a cell and can easily be combined for difficult tasks, like metabolic pathways, it is only a question of time until scientists will be able to built new organisms.

Synthetic biology today is persuing three different goals.

The first goal is based on an engineering approach by which BioBricks can be combined in living organisms like components in machines. The annually competition iGEM requests students all around the world to built new BioBricks, usable for technical applications. Synthetic biology therefore focuses more on the technical aspects than the traditional biological focus on theory. As described in the beginning, understandig is replaced by

manipulation.
The second goal of synthetic biology is to create artificial biomolecules and with this leave the field of life principles. With a creation of new forms of biosystems a new spectrum of new features which do not exist in nature or even the creation of new aminoacids or DNA bases arises. This divergence from nature brings about an era change in biology.
The third goal is to create new genomes and maybe new organisms. Synthetic biology in this goal not only extends classical biotechnology and is not only an extreme genetechnic, due it is placed in contrast to existing technology because the spectum is much bigger. It is not only to manipulate parts of existing organisms, it is the creation of new ones."Although synthetic biology uses the same material and methods for DNA manipulation like conventional biotechnology and the difference may appear more quantitative then qualitative it can be argued, that the initially quanitative expansion of technical possibilities enables these goals which then lead due to their consequences and radicalism to a change also on a qualitative stage".It seems that synthetic biology leaves the traditional field of biology and its natural background. The question about what is life maybe will no longer be intersting for mankind and can be replaced by questions like: "What can I synthesize and how can I use life to reach my goals?" If life is just an artefact of engineering is it still be possible to talk about ethics? We think yes because the technical progress not only changes the way we think about organisms, it changes the way we are living. Social world can not be isolated from the world of technical science like Bruno Latour demanded.
Now it is time to think about descisions which change our future. This manuscript, a prospective technology assessment, is one trial to give an outlook what might happen if this new technology is used for in our example cleaning the planet from waste PET. We will compare different applications and give in the end a recommendation for future descisions. We know that we are restricted, because we can not look into the future and nobody knows to what consequences technical acting today really leads.

Chapter One: Problem description and goal definition

After describing the background of synthetic biology in the introduction, this chapter will be used as introduction for the evaluated project. Since the prospective technology assessment modell of Wolfgang Bender is used, two ethic modells which can be used for the reader as orientation in the following manuscript will be discussed in this chapter. It is no question that there is no happy medium and some day a descision has to be made. Either for the use of synthetically ingeneered organisms with all possibilities and consequences or against it with all negative effects which might emerge. In the next subchapter the future purpose of the new organism will be described.

Problem description

Every year plastic industry produces 40 billion tons of polyethylenterephtalate (PET). Unintentionally, a large amount of this PET finds it´s way into the ocean in consequence of inappropriate waste disposal or detachment of plastic in apparel in washing machines. Degradation of plastics on the beaches results due to surface embrittlement and micro cracking, yielding micro particles that are carried into water by wind or tidal action. Comparing to macro particles like plastic bottles, which can easily be removed by mechanical procedures, micro particles are very small (<5mm) so that they cannot be seen by the human eye. Their extraction is hard to handle and appropriate filtering would be very expensive. In some marine test areas micro particles of PET already exceed the concentration of oceanic plankton. Combined with it´s property to bind toxins, these micro particles become a hazard for nature and it´s biological variability.

Animals with gills and filter feeder integrate this particles in their organism and earlier estimations assume that 44% (Rios (2007)) of sea bird feed their offsprings with this not nutrient mass. The offsprings starve with filled stomachs (Andrady (2011)). It has been shown that PET enrichs in the global food chain and therefore also threatens the health of terrestrial beings. A threat wich rises every day new microparticles emerge and no arrangements are made to reduce the concentration of these particles. If this trend continues and it looks like it does, areas where the amount of plastic particles exceed the amount of plancton will no longer be exeptional, it will become normal. Consequences of this trend should not be underestimated.

Stupidly it is not only rubbish wich is careless thrown away indeed PET is a feedstock obtain

from crude oil. A rational consequence would be to extract this feedstock from where it causes massive damage and reuses it again. Until now there exist no efficient an economic procedures for extraction of PET from the oceans.
This year an interdisciplinary and self-organized team of students from the Technical University of Darmstadt is forcing this challenge at the International Genetically Engineered Machine Competition (iGEM). Their goal is to create a new bacterium which can transfer PET, normally not useable for organisms into components that can be metabolized. With assistance of techniques form synthetic biology they are building a biological machine, creating an organism from scratch. The new organisms should be used to convert the components of PET to endproduct which can be used for chemical or pharmaceutical industries. For this challange new mechanisms are integrated in the bacterium which will be descibed in detail in chapter two. A predictable kind of biology should be used to avoid the increase of PET in food chain, reuse feedstock and in the same step clean nature from human rubbish. The goal is intelligible but are this also the used methods? If the students create such an microorganism and want to free it into nature, they have to make it competetive to natural microorganisms otherwise it will not survive.
Goal of this manuscript is to investigate prospectivelly possibilies and consequences of this creation. In this technology assessment a model by Wolfgang Bender (IANUS) will be used wich focus on aspect of conservation and development. These aspects can be used for descision making. In a quite fictive view of what might happen in the future an utopia which emphasis positive possibilitys is pitted against a dystopia in which the focus lies on negative side effects.

Development or Ernst Blochs principle of hope

The thinking of youth is the most desirable thinking for Ernst Bloch in philosophy (Horster (1987, page 14). Preserving this kind of thinking is the only way mankind can actively create his future. Unbridled without any conventions or rules daydreaming about the future becomes colorful and unlimited, a cockaigne of possibilities. Some might say that this kind of thinking is quite unrealistic but haven´t they also said the same to Jules Verne when he dreamt about diving boots or flying to the moon? Ernst Bloch´s opinion is that a society needs people with visions, people who with their fresh ideas promote development and extend culture. The philosophy of Hegel (one of Bloch´s paragons) describes the development from the abstract to the concrete. Bloch following Hegel creates the idea of the concrete utopia, a possibility becoming reality. Bloch´s demand of being realistic by doing the impossible (Horster (1987, page 15) can be used as plea for thinking without any fears and doubts and with this create a surplus of ideas to built up new possibilities which then can someday be realities. Incentive for daydreams and developing utopia are deficiencies in actual human life which prevent the procedure of people to find themselves. In this case the deficiency lies in a great uncertainly concerning future mankind life. Will mankind die from its own rubbish? If we look at global pollution and the continuous enrichment of PET, question arise whether human can still trust in consuming foodstuffs or will there be health effects at the long range?

Even in today's ecological systems consequences of phthalates (parts of PET) can be observed. On the basis of their structure, which is similar to those of the female sex hormone estrogen, phthalates bind to human and animal hormone receptors and act there as endocrine disruptors. Estrogens are not only known as sex hormones, they also regulate the growth of muscles and bones, play a big role in the evolution of the hormone and nervous system (Gilbert (2003, page 740). If this fragile system is disrupted by endocrine substances like phthalates, men can become sterile, women show a higher risk for breast cancer and a lot of other diseases like neurodegenerative ones or malformations in development are associated with phthalates. Although these facts are not mystery worldwide there are hardly any arrangements to change this situation and reduce the amount of PET in environment. This causes a huge deficiency for human beings. How can this deficiency be reduced or even eliminated? Bloch postulates for a new future technique which can be used to eliminate human deficiencies, increase freedom, justice and peace and leads to a better relation between human and nature. A true utopia in a way Bloch favorites can only be realized, if science is not orientated on profit goals but on human needs (Bloch, dPH, page 771). The inventor of this new technique should forget that we live in a world which is based on hyper production and profit maximization. He should better turn to new concepts which enable global crisis management borne by interest of the community. In this case it would mean to deal with the problems which arise by the use of PET. Let´s start dreaming:

Take the case that mankind has understand that micro particles of PET are a huge threat for life on earth and that there is no way to just stay in this situation. Primarily small interest groups try to convince other people that it is time for a change. After a while more and more interest groups start to communicate and build a global network. They mobilize other people to vote in their countries that this problem is one for their national political agendas. While public pressure is growing, politicians in the international community are forced to work together to find solutions for the PET problem and ignore the claims of the powerful lobbyist.

One outcome of the first meeting is that PET will be substituted by renewable resources but this does not solve the actual problem. In a second meeting the international community evaluates plans to use synthetically built organisms to help reducing the amount of PET. The bacterium which was engineered by students of the Technical University of Darmstadt for the iGEM contest 2012, after the contest used in industrial facilities, fulfills the most expectations of the politicians. They decide to modulate the organisms so that it could be also used in sea water. After a few positive tests in sea water tanks, the new organisms is set free to nature and starts degrading PET and modulates the subparts into amino acids. These amino acids can now be used by marine bacteria and algae for nutrition. After a few years all PET in the oceans is eliminated and due to the fact that no new PET is produced, the danger disappears. The strong decision from the international community was free of economic and profit goals. This builds up a new social climate of sustainable development and sanity. Now mankind is able to release itself from environmental pollution with new technologies. But can this problem be solved so easily? Is this kind of utopia realistic? What would Jonas say to this kind of solution?

Conservation or Hans Jonas principle of responsibility

To contrast Bloch´s idea of utopia we are now looking at Hans Jonas´ principle of responsibility which on the first view seems to be a dystopia. Due to its negative outcome dystopia includes the destruction of a belief in progress. But is Jonas´ ethic of the future really a dystopia? This will be examined in the next section.

Hans Jonas book the principle of responsibility was first printed in 1979. It includes answers for new ethical questions arising in the technical civilization. His goal was not to create a closed system of normative ethics or bioethics or even replace earlier ethics; his goal was to complement older ethics in respect of special problems which arise through acting of technological society. Reason for his book had probably been an at this time actual crisis in industrialized countries which leads to political and social problems like fear of an atomic war, exhaustion of global resources and the threat of an ecological collapse. Let´s first have a look at the former ethics which were dominated by the German philosopher Immanuel Kant. His ethics was based on evolution of acting in a nearer space and time. This means, that the people whose behavior is to judge are connected in common present. There was not animal or even nature ethics. Jonas stated now that the ethics of Kant is not useful for new challenges. The ethic is only useful for actual consequences but not for consequences emerging for future generations or nun human beings. Therefore Kant´s ethic cannot justify conservation of human existence. Confronting Kant´s category imperative stands Jonas ecological imperative. The basic of this imperative is that you should act in that way that the consequences of your acting are confirmative with humans living permanently on earth. This claim cannot be seen as a universal principle like Kant´s category imperative cause it only fits for special conditions. Not every action can be a danger for human life on earth.

In a first step we will now look at the philosophy of Hans Jonas. What does responsibility mean for him? Responsibility is a moral term. It means that actions are made responsible, that duties are fulfilled, that rights of others are respected and that the good will be saved and enhanced. New technologies which act on a global level force mankind to act responsible. The questions here are what is for what and to what extend is someone responsible for something. Often Jonas ethic of responsibility is identified as ethic of conservation but this statement is wrong. Jonas ethic is part of a transcendental ontology and his scope is the bottom of being. Therefore Jonas ethic has two sides, one is the subjective one and deals with responsibility for the Being, the second, objective one looks on the being as thing and for this thing somebody has to take responsibility. Or in other words axiology is becoming ontology. One trial to build a categorical duty is to preserve nature. The goal is to set objective validly over subjective judgment.

The main result of Jonas philosophy is that, if there is an ethic decision needed future aspects have to be evaluated. An implementation of a new technique should only be allowed, if human survival is guaranteed. What can happen if we look at a worst case scenario? If bacteria are used for the degradation of PET in nature the consequences are not predictable. Even natural organisms can have massive effects on an ecosystem when they are inserted by coincidence. If bacteria start to mutate nobody knows what might happen and therefore there is no limit for worst case scenarios. Everything can be possible even if the risk might appear low. Human survival is not guaranteed if for example bacteria start do digest fatty acids like they did in experiments (see chapter two) or other bio substances.

A fictive vision into the future gives us foretell about new ways of techniques. But even so, to make a precise choice it takes first of all an understanding of the matter.
In this chapter the used tools will be discussed not only concerning the technical criteria, but also from an ethical point of view.

Technical criteria

a) Biological and biochemical basics

As already discussed in the previous chapter, the goal of the Darmstadt group is to create bacteria, which can cleave and utilize PET. In contrast to the utopia of Ernst Bloch the resulting initial product is Catechol and not amino acids. To achieve this goal the group has split up onto three wet labs and one simulating lab. On top the group has also taken some external groups onto the boot, who further support the process.
In the three wet labs it will be tried to bring genes for specific enzymes with biotechnological methods into the Chassis-bacteria and the activity of the corresponding enzymes will be tested. The Chassis-bacteria is so reduced in its gene pool that it could not survive in the environment. It completely lacks all pathogen factors and the ability to conjugate (gen exchange between bacteria). The three labs can be separated into following areas (1) degradation, (2) transport and (3) metabolism.

a1) Degradation

PET is a macromere from the group of polyester and is made of the individual substances Terepthalacid and Ethylenglykol. To recover these individual substances is the goal of the group degradation. Since PET is produced industrial, and as discussed in the previous chapter is inert for biological live forms, there is no enzyme found in the animal world that could cleave specific PET. Polyester on the other hand is frequent in Nature. One of them is Cutin, a part of plant cell walls. Molds attacking plants, like e.g. *Fusarium solani*, can degrade Cutin with an enzyme named Cutinase.
The isolated enzyme shows also a low activity on the degradation of PET. By the use of a targeted point mutation by the company Henkel, the Cutinase was altered in such a way that its enzyme activity was enhanced compared to the wild type enzyme. Biobricks, which were

coding for this altered enzyme, were bought by the scientists and built into *E.coli*. With the help of this additional membrane anchor, the enzyme could be expressed on top of the cell wall of the bacteria and be used for the degradation of PET. However, since the Cutinase degrades very unspecific and cleaves besides PET and Cutin also lipids, the cell wall of the bacteria was also degraded which leads to the death of the bacteria.

Evasive test in yeast showed due to different cell wall structure better results. Alternative to the Cutinase, another enzyme (Esterase) was tested. With this enzyme the degradation activity on PET could only be shown in theory until today. A realization in the desired targeted organism *Eschericha Coli* is therefore difficult, but eventually feasible with more time.

a2) Transport

In the transport group the challenge is to integrate a membrane channel that can transport the produced degradation product Terepthalacid inside the bacteria. Like this the substance is available to be metabolized by the organism. A trimer from *Comamonas testosterone KF* is used for that purpose. The different subunits of the transporter will be explored separately to be able to localize the desired function. The goal is to build a transport system that can work in a ph range that is optimal for E-coli.

a3) Metabolism

The goal of the group metabolism was to convert the imported Terephtalat to Catechol inside the bacteria. Catechol is as an important starting substance in the pharmaceutical and chemical industry. It serves for the generation of pesticides, colorants and medicines and can therefor used well economically. There are five enzymes needed for the conversion. The gene sequences of these enzymes will be incorporated into the genome of the bacteria. These are: TPHA1, TPHA1, TPHA3, TPHAB and AROY.

TPHA 1 and 3 together build an enzyme complex, which utilize the reaction from Terephtalat to a relatively unstable intermediate product. This is than further converted to Protocatechat by TPHAB.

Theoretically it would be feasible to convert the Protocatechat over a two-step enzyme reaction to Shikimiacid. Further steps would then produce amino acids. However, these steps are very complex since the chemical equilibrium tends to go in the other direction and end products would need to be directly skimmed or exported. Therefore, due to practical reasons, the aim is to convert Protocatechat to Catechol with the help of AROY.

The other ingredient of PET, Ethylenglykol, could be used as a food source for the bacteria if further genes are incorporated.

a3) Combination of elements

After all single biobricks had been integrated in the bacteria genome the next step was to combine the single biobricks to one unit. Until now this goal is not achieved because one biobrick was not compatible with e-coli and was therefore inserted in yeast. One chance to built one complete organism for degradation, transport and metabolism is that all groups switch to yeast. Like described earlier synthetic built bacteria are not able to survive in nature. If they should, they need pathogenic factors and a new receptor for the output transfer of synthesized amino acids. In the next stage possible application areas are discussed which might be realistic in the nearer future.

b) Technical area of application

b1) Utilization in a defecator

Concepts of how to apply the produced bacteria in defecators are currently researched together with students of environmental sciences at the Technical University.
The purification of drinking water concentrates on removing biological degradation products. Many chemical substances such as PET, drugs or hormones cannot be absorbed by most defecators and are found in rivers or even drinking water (Esperanza (2006)).
As outlined in discourse part 2, these substances can be harmful to health and interfere the fragile endocrine system of humans and animals.
The group in Darmstadt is working on the decomposition of PET while other groups who attend iGEM this year generate micro-organisms to degrade hormones, like the group from Bielefeld. The idea is to construct a defecator that uses different synthetically produced bacteria to filter the harmful substances from the water and thus reduce the burden of man and nature.
At the current level of the project you would construct the bacterium so it can absorb Terephtalat and process it to Catechol which would be excreted by a transporter. The fission of PET would be possible by prefixing a tank in which PET would be segregated by a Cutinase. The use of Hiq Cutinase, which has its optimum at 80° Celsius, would be more efficient. No new organism is needed at this point, the cleaned up enzyme will do.
The water containing the dissolved components would be carried to another tank containing the bacteria, after being cooled down to 40° Celsius. An outdoor basin would not be practical

because of the fragility of the bacteria, which does not survive variation of temperature. The discarded Catechol would crystallize and could easily be skimmed. Catechol is toxic, so only water free of Catechol may be recycled to the water circuit.

b2) Valorization of waste

Items made of PET can be recycled if they are properly disposed. But in every recycling process, the quality of the PET decreases. After several processes, it can only be used for example for clothes. After about five times of recycling, the PET cannot be used anymore and is burnt in waste incineration plants. Toxic gases may occur if the PET is of low purity. This material is available at a very low price and could be transformed to Catechol by means of the method explained above, which would lead to an immense increase in value. Instead of burning it, the PET would be brought to special plants; the extracted Catechol could be sold at a high price.
This procedure offers a great economic incentive and could lead to a better strategy of collecting PET, so less waste would be disposed in the environment. A closed plant would be simple to install and probably be approved with regulations easy to satisfy.

b3) Cleansing of the global oceans

The motive of the project, cleaning the world from PET-waste, stays utopian at the current state of the art. As mentioned before, the only possible model-organism that comes into question would be yeast, for it combines the ability to degrade, transport and metabolize. Yeasts do not occur in salt water, so a matching carrier-organism that is viable in salt water has to be found. Amino acids are supposed to be the metabolic products.
Upon questioning the team of students, 63 % come out against the settlement of an artificially created organism (27 % no opinion, 10 % agree). The possible risks as mixture with other micro-organisms, uncontrolled mutation, digestion of other even natural polyesters and uncontrolled spread are unpredictable. The cleansing of the global oceans is not possible this way.

c) Aspects of security

Even if the risk of unplanned genetic exchange with other bacteria is low, because the bacteria’s ability to conjugate has been removed, it can still not be fully eliminated. Consequences of mixture are unpredictable for the lack of empirical values. If the artificially designed bacterium is set out in the environment and if it comes to a genetic exchange, the now missing factors of pathogenicity may be built-in. This scenario is unlikely, but not impossible. Completely new, unknown bacteria strains may evolve.

For this reason, the bacteria may only be used in closed facilities. If used in a defecator, the water previously purified by the bacteria must be filtered or heated to avoid transport of the germs to the global water circuit. Usage in open basins must be excluded. For the security of populace, the bacteria should have certain features which prevent viability outside of determined laboratory conditions. It makes sense to convert the energy generating metabolism to ethylene glycol. In nature, this substance occurs in small quantities only, so the risk of unintended spread is minimized.

The accumulation of Catechol in the defecator holds another minor security risk. As mentioned before, it must be guaranteed that the water is completely cleaned of Catechol. This can be accomplished by heating the water to 80° Celsius. Catechol boils at this temperature and could be extracted by condensation.

In plants built for the extraction from waste, this risk is rated lower, still the Catechol must be removed properly from the nutrition medium.

d) Aspects of economy

It is often the aspects of economy, not sustainability, which determine whether technologies establish on the market or not. Considering the processing from PET waste to Catechol, it seems that both aspects are reconciled. An economic incentive is the immense accretion of non-recyclable PET to Catechol, so investors for plants with appropriate safety standards should easily be found. As opposed to defecators, here the input of PET and thus the expected profit is known, so a precise calculation is easy.

In economic use, the patents have to be regarded, for example those on the most effective Cutinase by the Henkel company. Otherwise, the requested enzyme might be developed by selective evolution and would be patent-free. It still has to be clarified whether the research unit can apply for a patent for the newly designed bacterium and thus preserve turnover rights. Anyhow, given the approval for use of the bacteria, the plant would be profitable. Compared to chemical methods, it can operate with milder requirements. The crude material as well as ingredients for the cultural medium is quite cheap. At this time, the bacteria can only degrade new, pure PET, but it is presumably only a matter of time until methods are developed to process old PET and filter additives like Bisphenol A.

As long as non-recyclable PET is available, profitability is ensured. Possibly the price of PET

would increase, so eventually there will be an incentive to filter it from the sea.
To cleanse the global oceans on that account would be a way of reason through profit.

e) Aspects of legality

Apart from the patent rights mentioned before, approvals have to be obtained before a plant can be built. The utilization of genetically engineered micro-organisms in closed systems is regulated in the European directive 2009/41/EG. The directive ensures the safety of human health and environment. For this purpose, means of confinement and protection that prevent exposition are reviewed. Emergency guides are mandatory in case of accidents.
These requirements should easily be met when constructing a plant to degrade PET by using artificial bacteria, if the planning is done conscientious. The usage in a defecator is more difficult. Can this still be assumed a closed system?
Respective requests will be harder to achieve. An eventual release of the bacteria to the global oceans for the purification of the environment will most possibly be rejected, since the side effects cannot be evaluated. Another aspect is the question of property on the PET. If eventually it will be profitable to filter PET from the sea for use as a crude material to process industrial usable substances, it raises the question of who possesses the PET and if everyone could exploit it at their own discretion.
Within the territorial waters it would be the assignment of the states to issue options of exploitation. Beyond these zones this is more difficult. At this time, these questions are of no importance, since there are no practicable methods of filtering and the technique is not established yet. They have to be kept in mind though.

Ethical criteria

One important aspect of technology assessment is an ethical discussion about the used appliances, especially when biological or anthropological techniques are used. The evaluation of those in the prospective technology assessment of Bender is divided into four parts. Each part is evaluated for the compatibility and it´s possibilities of promotion. The four parts are:

- Human orientation
- Social orientation
- Environmental orientation
- Future orientation

a) Human orientation

Like described earlier, humankind is threatened by the consequences of PET enrichment. A reduction of the concentration of PET would lead to an improvement of living conditions for every human being indipentent of social and financial standard. A technology is human compatible when it reduces risks for health without leading to other grave consequences.
A production of toxic side products has to be averted. This is the case when Catechol is the end product. Therefore special filters have to be used to eliminate Catechol from water. The new synthesized organisms must not be harmful for humans especially when they find their way out into nature. This risk is reduced by lowering the viability of the bacteria like described before. A risk of gene transfer with other bacteria cannot be ruled out absolutely and therefore a small possibility exists that in a worst case new pathogen germs can develop. It would be a train wreck, if these pathogenic bacteria were also able to degrade fatty acids. Imagine such an organism in human gut. This fiction is one reason why a release of the bacteria into nature is not desirable. Consequences for humans and animals are not appreciable.
New possibilities arise when bacteria can be used low-risk and successful in sewage treatment. If the technique works with PET maybe it can also be used to eliminate other substances like hormones, medicaments or other toxins from drinking water. This means higher possibilities of human promotion.

b). Social orientation

What kind of social consequences will occur when a non natural synthesized bacteria is inserted into the ecosystem? First of all is there a difference between organisms which are engineered by synthetic biology and those manipulated with conventional biotechnology? Since 1977 human insulin is produced by bacteria (Porro (2011) and since thousands of years organisms are used for producing foodstuff like beer, cheese or wine. These organisms were cultivated over years to show the optimal results. „A concrete contentual distinction between synthetic biology and other existing fields in science and technique is not easy. One reason is that these fields are historically related“(Boldt (2008)). Compared to conventional biotechnology in which new enzymes are inserted into existing bacteria, in synthetic biology the goal is to easily manipulate even metabolic pathways and signal cascades via biobricks. In the actual case, where we look at the results of the team of the TU Darmstadt, a bacteria is engineered for the degradation and metabolization of PET.
It seems that engineered, planned organisms are manageable but there can be no 100% safety.

Mutations and adaptations are essential parts of what we call life and they do not disappear when new creatures are built (Saint-Ruf (2006)). In synthetic biology new combinations of biobricks are possible. First simulated in computer models there are no borders for creativity. By generating new possibilities on a creative and creationistic scope synthetic biology enriches society. Options seem to be limitless and visions known as science fiction become realistic. Mankind can now make the old dream real of being a creator of nature and will use this new option to optimize natural processes after their interests and goals. It is not so that man was not manipulating the world around him before but now it comes to a new dimension. The manipulator becomes the creator and diverges even more from his natural roots. One risk is that nature becomes artificial, using the term biofact already points in this direction. A global system in which man is just a small part is too complex to be understood in details. The state of not knowing does not prevent man from intervene in natural processes and so he is often confronted with unwanted results. Is nature really a playground for human creativity? Why can mankind demanding adaptations of nature to man? Where is an acceptable limit? Possible consequences of using synthetic biology can lead to an even additional alienation of nature and mankind with effects on human self-image. If nature is becoming plannable and predictable then also humans will become so. Cultural values like optimization and profitability will no longer used only for economic systems, it will now also be used for ecologic and biologic systems. Society only benefits when a moderate use of synthetic biology based on a respectful handling of nature is accomplished. The chance is there to create a new society where the term sustainability is rated with a higher value. A new powerful tool is available when synthetic bacteria can transform PET to Catechol. This tool can be used as alternative to the now used combustion strategy. PET what was no longer useable in the past is now becoming a popular feedstock with a higher price.

If the price of PET increases mankind will hopefully start to use alternative materials for packing. New materials should be bio-degradable. It is a pity that price and convenience are such important factors in the acting of humans. Today still more plastic bags are used than for example linen of paper bags. It is a task for politics to set possible incentives for a change in the right direction via a clever price strategy or directed prohibitions. Additionally funding should be given into investigative fields of research for finding appropriate materials. This would be a good strategy in the long way. Using these new nature conservating materials must not be a privilege for rich people. This should be fundamental right for each human being.

c) Environmental orientation

Aspects of environmental orientation have already been suggested earlier. Now these aspects should be intensified. A reduction of PET would not only lead to a higher quality in human health it would also increase environmental quality. Since a large amount of PET find its way to the oceans so that in some marine areas the concentration of PET exceeds the concentration of plankton, a reduction of PET would be in any case desirable. Degradation time for plastic is estimated with 450 years but there is no concrete knowledge. If we believe in this number then a use of PET degrading bacteria in industry or in a sewage treatment would not make the situation better. The amount of PET in the oceans would not be reduced. The only benefit is that it is not getting worse. The problem would not be solved. For lowering the concentration of PET in oceans we have to think of building organisms that degrades PET and can survive in marine environment. Experience gained in the use of green genetics (manipulation of plant genomes) show that consequences are not predictable. Results gained in labors cannot be used to set prognosis for field trials. Like already pointed, the complexity of global systems with its huge amount of influences and interactions is too high for humans to understand. There is no possibility to fulfill the claims of calculability or foreseeable consequences. Destruction of the whole ecological system is not excusable. Therefore an introduction into marine environment is not makeable. But what about the 450 years left? Can we just live with this situation without intervention? PET in the oceans has to be removed. In today's society the best mechanism to stimulate people to act are financial benefits. Politics should promote the research on useful and inexpensive techniques to filter sea water. Focus should here lie on the one hand on effectiveness and on the other hand on compatibility with marine organisms. Filtered PET could then be transferred into Catechol. It seems that there is a long way to clean environment from human rubbish but anyway a first step has to be done. There is no time for waiting any longer. Waiting will make the situation worse for nature and for humankind.

d) Future orientation

Future orientation is one of the important aspects in a prospective technology assessment. In this part the technique will be evaluated on its sustainability. Sustainability means as well that no other future technologies are inhibited as life of humans is not restricted inappropriate. Another criterion for sustainability is that situation is getting better by using the technology. Looking at possibilities which might arise in the future when using bacteria to degrade PET there can be seen an extension of the scope of action for man. New systems of recycling can emerge not only for minimizing damage but also to create additional economic attractions. Recycling can become a profitable part of industry. These new economic interests can be used to clean the world from human rubbish and save natural resources. The new principle from cradle to cradle can change industry in the way it adopts to natural cycles. The idea is to create a form of giving and taking considering the ecologic system. If there would be a possibility to clean the planet from PET then this chance should be used to create alternative, eco friendly techniques. Mankind can find it´s peace with nature like Ernst Bloch postulates.

In this chapter information gained in this manuscript is used to give a recommendation for ethic decisions like they have to be taken for example in politics. The two principles described in chapter one (Bloch and Jonas) are quite contrary and allow to distinguish only between two options. These two options are that technology should be used or not. Most decisions and their consequences are ambivalent and situation-dependent so that a binary model cannot be sufficient. Another model from Christoph Hubig deals with basic values which he thinks are essential for human beings (Becker (2008), page 128). In the model two basic values are distinguished. These are option and bequest values. Option values imply the variability of options humans can take. The more options remain after a new technique is implemented the better it is for the option values. Options also mean a prevention of factual constrains. A perfect example here is the use of atomic energy. Once praised as being a really efficient and eco friendly technique now the problems became visible. To deal with nuclear waste other techniques have to be developed to prevent mankind from harm; costs are no longer manageable. A good technique therefore allows for alternatives and implies little factual constrains.

Bequest values focus on the rights of individual persons. A new technique must not interfere with basic rights like building an own identity or having the possibility to educate oneself. The individual must always keep its possibilities to take own decisions. If a technique for example leads to the loss of human sanity let´s say by interfering with brain waves it is not compatible with bequest values. If there is a discrepancy between option and bequest values, bequest values should be preferred.

After giving a short introduction into Hubig´s model we will now use these to evaluate the use of synthesized bacteria under different conditions. Like described earlier in this text we will focus on four different possibilities. These possibilities are:

- no intervention
- Use of bacteria in marine environment
- Use of bacteria in sewage treatment
- Use of bacteria in industrial facility

Table 1 gives an overview over the option and bequest values for every possibility.

Intervention	Usual recycling and combustion	Synthetic bacteria in marine environment	Synthetic bacteria in sewage treatment	Synthetic bacteria in industrial facility
Result	No degradation of PET	PET is converted to amino acids	PET is converted to Catechol	PET is converted to Catechol
System	No System	Open System	Semi-open System	Close System
Concentration of PET	Rises fast	Decreases	Rises slower than with no intervention	Rises fast (eventually decrease when extraction becomes attractive)
Option value	All options open Eventually force to act when situation is becomming worse Consequences of omission (+/-)	PET is degraded therefore no other options New side effects (uncontrolled gene exchange, global proliferation,..) force humans to act Crises management necessary (-)	Use of already existing facilities Eventual new technologies needed to prevent bacteria outburst New options when system can also be used for other substances End products must not be toxic or biohazardous (+/-)	Eventually elimination of other techniques due to high efficiency and profitability If outburst is prevented then following techniques are not needed No new infrastructure for transport or logistic needed. Infrastructure of combustion can be used Building of new facilities necessary (+)
Bequest value	Health threatening (neurodegenerative diseases and cancer) Danger for live of human and animals Reduction of food quality (-)	Possible improvement of live quality Eventually enormous consequences for human and animals High fear factor concerning possible side effects → Acceptance for technique low (+/-)	If used appropriately no hazard for humans and animals Acceptance for technique difficult, fear of bacteria outburst (+)	If used appropriately no hazard for humans and animals Acceptance for technique comparable to classical biotechnology (+)
Possibilities	None	New possibilities of designing biology Creation of new material cycles	Degradation from other substances (environmental toxins) New methods for PET extraction	Research for finding new techniques for PET extraction becomes attractive Degradation of other substances
Recommends	Not useful	Not useful, side effects are not predictable	Useful if bacteria stay in closed system (security standards)	Only useful if PET is extracted from marine environments

Table 1: Overview of different possibilities for the use of synthesized bacteria to degradate PET and first evaluation

Technique	Usual recycling and combustion	Marine environment	Sewage treatment	Industrial facility
Option value	+/-	-	+/-	+
Bequest value	-	+/-	+	+
Ranking	4.	3.	2.	1.

Evaluation of the different possibilities is not so simple. The only thing which is clear that there has to be a change and doing nothing is no possibility because the situation is getting worse every day with enormous consequences for marine and terrestrial beings.
When we look at the result it seems that setting bacteria free into marine environment is the only possibility where the situation can directly become better because PET is degraded. The consequences of this action are not predictable and even if the risk is low that bacteria might become harmful for mankind this scenario is non-waivable. Cause of this lack of knowledge it is not responsible to choose this possibility. The use in sewage treatment does not reduce the amount of PET in oceans but it slows the enrichment of PET in rivers. However a semi-open system cannot guarantee that there will be no outburst of bacteria. If this possibility is chosen, it has to be clear that high security standards have to be fulfilled.
The only technique which is rated with positive option and bequest values is the use of synthetic bacteria in an industrial facility. Since there is no direct reduction in marine PET concentration, this possibility is only useful in a combination with other techniques which can be used to extract PET from marine environments. Due to its low risks in bacteria outburst and the possibility to use already existing transport and logistics infrastructure this possibility seems makeable.
Like already discussed in previous chapters the option of reducing the amount of PET in marine environment should also lead to a behavioral change in the use of plastic. So a combination of converting PET to Catechol in industrial facilities, filtering PET from sea water and a behavioral change is recommended. Since human behavior is not always grounded on rationality there have to be certain kind of conditions to change this behavior. Two major motivations for humans to change behavior are the improvement of convenience or the reduction of costs. Some attraction can be set to trigger these motivations like increasing the costs of plastic. There is no reason why clothes are packed in plastic bags if these bags are only used by the consumer to transport these from the shop to his home. Paper bags would be enough here. What if salespersons have to pay penalty if they give away plastic bags? Small steps may change a lot here. Looking at the underlying problem a huge amount

of PET is used for package. A focus on regional products would help to prevent ecologic cost not only concerning plastic but also exhaust gases (transport). Why is PET used in clothes? Are there no natural alternatives? What about forcing producers of washing machines to include filters for extracting PET?

There are a lot of things which might be changed to reduce the amount of new PET in marine environments. Additional to these restrictions for commercial activities, politics should also give more funding for extraction methods (of PET from sea water) and basic material research. Materials from nature are often healthier and bio friendly then chemical synthesized ones. So why do not use them? Is it really true that politics can take no rational decision as long as there are economic interests and lobby work.

If synthetic biology can be used to help mankind to clean the planet why should we not use it responsible and sensitive? For politics this means that there has to be more focus on long range plans and sustainability. Decisions should be taken in order to improve life quality for everyone. Maybe it is time for a value change. Maybe it is time for synthetic biology?

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