LOOKING BEYOND BIOSAFETY

an essay about the mechanisms to develop an ethical project

Prepared by the iGEM Team Bonn 2016

125 liters of water to make a kilogram of sheet paper, but it seems likely that this is the value for producing paper alone, and excludes the water required to grow the tree itself. *Water Content of Things, Pacific Institute, accessed March 13, 2011.*

Americans discard 4 million tons of office paper every year – enough to build a 12-foot high wall of paper from New York to California. *American Forest & Paper Association, 2004.*

When paper rots or is composted it emits methane gas which is 25 times more toxic than CO2. *International Institute for Environment and Development (IIED), founded in 1971, was commissioned by the World Business Council for Sustainable Development to do the study. "A Changing Future for Paper: A summary of the study "Towards a Sustainable Paper Cycle" Recycled paper requires.*

64% less energy than making paper from virgin wood pulp. *Energy Educators of Ontario, 1993.*

The pulp and paper industry is the third largest industrial buyer of elemental chlorine. *Printers National Environmental Assistance Centre, Fact Sheet by Todd MacFadden, and Michael P. Vogel, Ed.D. June, 1996*

1. INTRODUCTION: Biosecurity, much more than a matter of law.

> "Human Practices is the study of how your work affects the world, and how the world affects your work."
>
> — Peter Carr, Director of Judging

In developed societies the use of large amounts of paper- and board-based products is an everyday reality. The pulp for paper-making can be produced from virgin fiber by chemical or mechanical means or by the repulping of paper for recycling. In Europe, more than 50% of the fibers used by the paper industry come from recycled paper. Recycling paper involves removal of some contaminants prior to use and deinking, dependant upon the quality of and the requirements for the end product. [1]

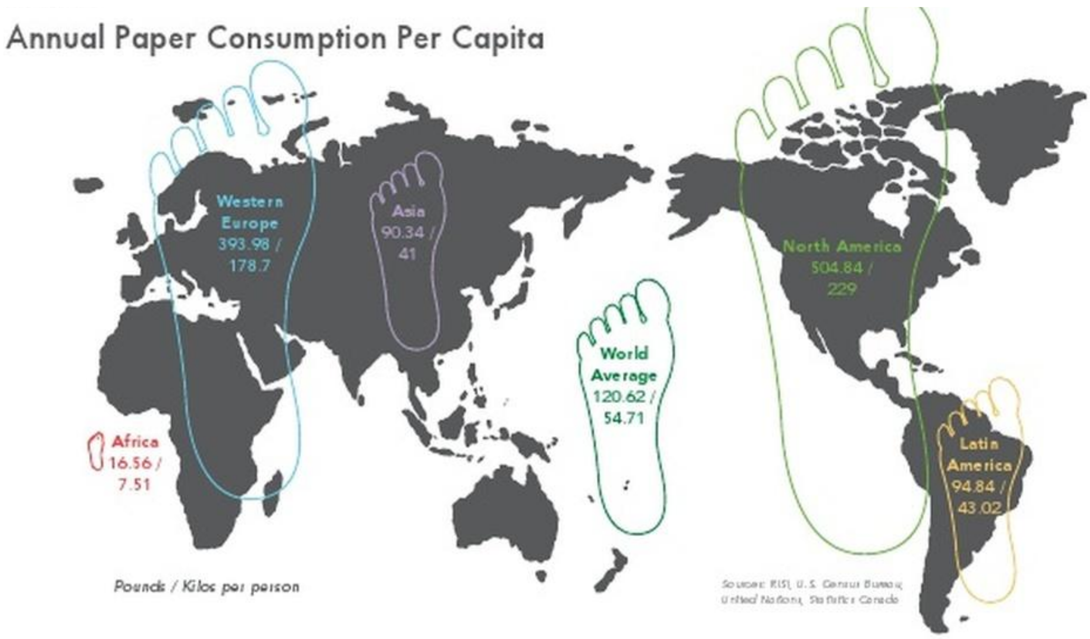

Figure 1. Annual Paper consumption per capita around the world. [2]

It seems undoubtedly a success that half of the paper production is not linked to deforestation, however, the repulping also needs chemicals which consume high amounts of energy. During this production, hazardous byproducts are also being released into the environment. Our proposal: using a standard enzymatic deinking system to eliminate the use of these harmful chemicals. Enzymes have been shown to be efficient in deinking paper [3], but have not yet found wide application in industry due to their costly production and purification. Basis of our proposal is a cheap and easy to use system, to continuously produce enzymes. Our approach relies on the use of synthetic biology to produce enzymes in the microorganism Bacillus subtilis.

As with all products, safety is a major concern and additionally in biotechnological applications the special aspect of biosafety needs to be considered. Biosafety refers to the need to protect human health and the environment from the possible adverse effects of the products of modern biotechnology. [4] It is also described as security against the inadvertent, inappropriate, or intentional malevolent use of biological agents or biotechnology. This includes the development, production, stockpiling, or use of biological weapons as well as outbreaks of newly emergent and epidemic diseases. [5]

Biotechnology started in the seventies with the modification of the genome of *Escherichia coli* to produce different useful substances, such as insulin. The second phase was introduced with the design and development of modified genomes, associated with the production of new drugs, production of biofuels and genetically modified foods. Today we face plans to synthesize complete genomes or to even create entire new species. [6] Talking about such a new and powerful discipline raises many doubts and questions about possible unintended consequences. Aside from the real dangers which may arise from the abuse of these methods, a lot of fear originates from the unknown and the term biotechnology often remains negatively connotated. The laws imposed by European and International committees are intended to monitor the use of this technology, to ensure a benefit for the people. This plays an essential role in the concept of biosecurity.

When creating, developing and marketing a project, a good idea is not enough, the aims have to be clearly defined from the beginning as well. Factors such as cost, ethical implementation, safety and public reception all need to be carefully considered. For the success of our project, we need to ensure that we offer a safe product that poses no risk to human health or to the environment, but that is still economically profitable. For this we must examine and monitor the quality of the project and its economic viability while carefully considering all aspects of biosafety and implementing them into the design of our project. This also entails looking closely at the laws and legislations in place that must be adhered to for practical implementation of our project. However this is not enough, the success of our project is also fundamentally based on our product being bought by our consumers. A current obstacle here is the negative view of biotechnology still held by large parts of the general public, our client base. Thus it is also necessary to monitor the reaction of the scientific laymen to our designs and implement necessary changes to ensure a positive reception. Education of the public about both biotechnology in general and more specifically our implementation of the technology is equally important and was a central aspect of our human practice work.

A focus of iGEM is to facilitate the widespread implementation of biotechnological approaches in industry and to better the general understanding of the benefits of biotechnology. Aim of this essay is to discuss biosafety and public reception in regard to improving our project design and to summarize our finding for the future reference of other iGEM teams.

2. BIOSAFETY AND ITS TOOLS

Biosecurity should be understood as a doctrine aimed at achieving attitudes and behaviors that reduce the risk for both human health and the environmental. The framework of a risk reduction strategy is designed around following main points: establishing accident prevention measures and protecting the whole community and the environment from agents that are potentially harmful.

2.1. BIOSAFETY LEGISLATION

In the last fifteen years, institutions like the European Union or international committees have developed a series of regulations and regulator treaties for the young discipline of synthetic biology. As a European team, our project falls under the legislation used by the European Union and the international committees. The relevant legislations concerning the work with genetically modified micro-organisms that we must adhere to are listed and summarized below.

> Directive 2009/41 on the contained use of genetically modified micro-organisms.
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> "Any activity in which micro-organisms are genetically modified or in which genetically modified micro-organisms are cultured, stored, transported, destroyed, disposed of or used in any other way and for which physical, chemical or biological barriers, or any combination of such barriers, are used to limit their contact with, and to provide a high level of protection for, humans and the environment."

> Directive 2001/18 / EC on the deliberate release into the environment of organisms genetically modified.
>
> "In accordance with the precautionary principle, this Directive is to approximate the laws, regulations and administrative provisions of the Member States and to protect human health and the environment when:
> Deliberate release into the environment of genetically modified organisms for any other purpose than for marketing in the Community. Or genetically modified organisms as products or in products marketed in the Community."

> Cartagena Protocol
>
> "The Cartagena Protocol aims to ensure that the transboundary movement of Living Modified Organisms resulting from modern biotechnology is done under safe conditions for the conservation of biodiversity and human health. This movement must be preceded by an advance informed agreement to ensure that countries have the necessary information to make decisions regarding the

acceptance of imports of such organisms into their territory."

Aarhus Convention

"The Aarhus Convention on Access to Information, Public Participation in Decision-making and Access to Justice in Environmental Matters. The United Nations Economic Commission for Europe (UNECE) recognizes public rights on access to information, public participation and access to justice in the processes of government decision-making in matters affecting the average local, national or transboundary environment."

Convention on the Prohibition of Biological Weapons

"The Convention on the Prohibition of Biological Weapons (BWC) is to prohibit and prevent biological agents can be used as weapons of mass destruction against humans, animals or plants."

3. THE THREE PILLARS: PRECAUTIONARY PRINCIPLE, PRINCIPLE OF NONMALEFICENCE AND SOCIO-CULTURAL IMPACT

Within the EU, particular importance is placed on the ethical conduct when working with genetically modified organisms. When evaluating the ethics of work with genetically modified organisms three main principals are analysed: the precautionary principle, the principle of nonmaleficence and the socio-cultural impact. These three principles are the key aspects of all biosafety legislation (refer to 2.1) and must be fulfilled when genetically modified organisms are deliberately released or marketed as products or product components. In the following we are going to analyse our project design in regard to these three principles.

3.1. PRECAUTIONARY PRINCIPLE

The precautionary principle states that when a project may lead to morally unacceptable harm, actions shall be taken to avoid or diminish it before the begin of the project. [8] Its very name tells us what it is, although it would be clearer with the popular phrase "better safe than sorry". Every aspect of precaution implies that the proposal is safe, reliable and marketable and that safety measures are in place that account for different unusual circumstances.

In our project we work with the bacteria *Bacillus subtilis*, which naturally secretes proteins and enzymes. Our goal is to utilize this attribute for our enzyme production and to create a "cell factory". Instead of lysating bacteria, our project would allow for the continuous production of enzymes in a liquid culture that is easily isolated from the bacteria and can be sterilized either via filtration or gamma irradiation. Bacillus subtilis is classified as an S1 organism which is nonpathogenic and does not cause disease in healthy humans. It is generally regarded as safe (GRAS) by the American Food and Drug Administration and is widely used in laboratories all over the world, e.g. probiotics in pharmacies or fungicide in agriculture.

B. subtilis is not a human pathogen, but has on several occasions been isolated from human infections present in immunocompromised individuals. Overall, B.

subtilis has a low degree of virulence. [9] The possibility of human infection is not non-existent but it is low in the industrial setting where exposure to the bacterium is under controlled conditions. The use of B. subtilis in an industrial setting should not pose an unreasonable risk to human health.

Not only do we work with an organism type 1, meaning low probability of disease transmission, moreover there is no need to release it to the environment. Our process would take place in an enclosed factory setting under controlled conditions. All materials that come into contact with the bacteria would be sterilized thus preventing the unintentional release of the genetically modified microorganism into the environment.

A possible problem would be the import of this technology into other countries. GMOs can not be imported into countries that do not comply with its provision [7]. Since paper recycling is a global industry it would be important to be able to implement this new technology world-wide for the strongest environmental benefit.

A case-by-case environmental risk assessment should always be carried out prior to use. It should also take into account potential cumulative long-term effects associated with the interaction with other GMOs and the environment.[7] In the case of an unintentional release, the risks for the environment would probably be minimal since B. subtilis naturally occurs in the environment and the genetic modifications do not give the strain an evolutionary advantage beyond that which other antibiotic resistant strains have.

In general our project complies with the precautionary principle. Our project is safe in regard to human health and the unintentional release of genetically modified microorganisms and poses minimal risk.

3.2. PRINCIPLE OF NONMALEFICENCE

Besides adhering to general biosafety laws, it is also important to establish a clear principle of nonmaleficence. This ethical principle is basis for regulating human activity in such a way as to avoid harm. [10] To analyse our project based on this principle we will compare it to standard recycling procedures currently implemented to work out the benefits and potential risks our approach poses.

The current manufacturing process of pulp and paper is intensive in the consumption of electricity and steam, generating an expenditure of 18.41×10 ^ 9 KWh in 2014 alone [11]. For comparison, 60×10^9 kWh are used to power New York city and its suburbs for an entire year. Furthermore, the process involves the use of chemicals such as NaOH, Na2(SiO2)nO and H2O2 among others. Besides the chemicals themselves that need to be properly discarded, their production is also energy intensive and often leads to the accumulation of hazardous and toxic byproducts. In NaOH production for example Chloride, Mercury and Asbestos are released as byproducts. These substances have following harmful effects:

- Chloride increases the electrical conductivity of water and thus increases its corrosivity. In metal pipes, chloride reacts with metal ions to form soluble salts, thus increasing levels of metals in drinking-water. [12]

- Exposure to mercury, even in small amounts, can cause serious health problems and is dangerous for fetal development. According to World Health Organization (WHO), mercury is toxic to the nervous and immune, digestive systems, skin, lungs, kidneys and eyes.

- Asbestos intake can lead to asbestosis, a disease affecting the lung tissues, of which the number of deaths increased substantially from the 1960s to the 2000s and they are expected to continue occurring for decades. [13]

Employing enzymes instead of chemicals in the critical deinking step, drastically reduces the amount of these chemicals needed, as well as the toxic substances that are released into the environment. Furthermore, enzymatic deinking is carried out at lower temperatures than chemical deinking reducing the amount of energy necessary in this part of the process as well [14]. A comparison between the amounts of chemicals needed for enzymatic vs chemical deinking is made in table 1. The amount of chemicals saved through the use of enzymes is calculated. Since enzymes are completely natural and biodegradable substances their production has little to no impact on the environment.

So far enzymatic deinking shows a comparable if not even better deinking efficiency than conventional deinking methods, thus ensuring a comparable if not improved paper quality. [3]

Concerning the principle of nonmaleficence our project presents a great benefit for humans since the impact of paper recycling is dramatically reduced, while paper quality remains unaffected.

Chemical	**Conventional recycling**	**Enzymatic recycling**	**Amount saved**	**Amount saved annually (2013)**
Sodium Peroxide (NaOH)	26 kg/t recycled paper	16 kg/t enzymatically recycled paper	10 kg NaOH/ t paper	2 million t NaOH/ year
Sodium Silicate $Na_2(SiO_2)_nO$	10 kg/ t recycled paper	0 kg/ t enzymatically recycled paper	10 kg sodium silicate / t paper	2 million sodium silicate /year
Hydrogen Peroxide H2O2	25 kg/ t recycled paper	20 kg/ t enzymatically recycled paper	5 kg H2O2/ t paper	1 million t H2O2/ year

Table 1. kg of chemical per tonne of paper recycled in different deinking systems.

3.3. SOCIAL OPINION

Since 1998, in Europe, every citizen has the right to get access to environmental information. This can include information on the state of the environment, but also information on policies or measures taken. [15] Furthermore, people have the economical power and their opinion on a product will ultimately affect its success. As such it is extremely relevant that new applications are supported by the majority of the general public and they have a basic understanding of its function.

3.3.1. SYNTHETIC BIOLOGY SURVEY

As a part of our human practice a small-scale survey was launched to know more about the layman's comprehension of synthetic biology and whether a product based on enzymatic deinking would be bought. Our aim was to collect a large number of opinions across all levels of education. The survey was spread over various channels, Facebook attracting the most participants. The survey was available in four languages, which were English, German, Spanish and Vietnamese. To increase the number of participants, an incentive in the form of an Amazon gift certificate which was raffled under all participants was offered. The series of questions asked are presented below.

- Gender
- Age
- Educational status
- Do you know what synthetic biology is?
- From which sources (internet, television, books...) did you get your information about Synthetic Biology?
- Please shortly describe,in your own words, what Synthetic Biology is (only a few words).
- Do you think paper recycling is necessary?
- Do you think that paper recycling can be harmful to environment?
- Would you buy paper recycled with the help of Synthetic Biology?

Analysis of the results of the survey presented several issues that will need to be addressed for a proper implementation of our project.

3.2. RESULTS OF SURVEY

With a total number of 396 participants, meaningful results for every question were obtained. Consult them in detail here. In order to perform a social analysis, it was interesting to note the personal answers showing the ideological differences between groups. These are shown below by separating the participants by gender, age and educational level. The results also show a percentage of empty or incomplete questions.

3.3.2. SOCIO ECONOMIC PROFILE OF PARTICIPANTS

The age of the population sample peaks at 18-24 (figure 2) with most participants being between the ages of 18 and 34. 53.4% of the participants are women compared to 31.06% men. The majority of participants has a high level of education (figure 3) and are active on social networks such as facebook. The also preferably make use of the Internet for research purposes. This bias may be due to the distribution method of the survey via electronic means.

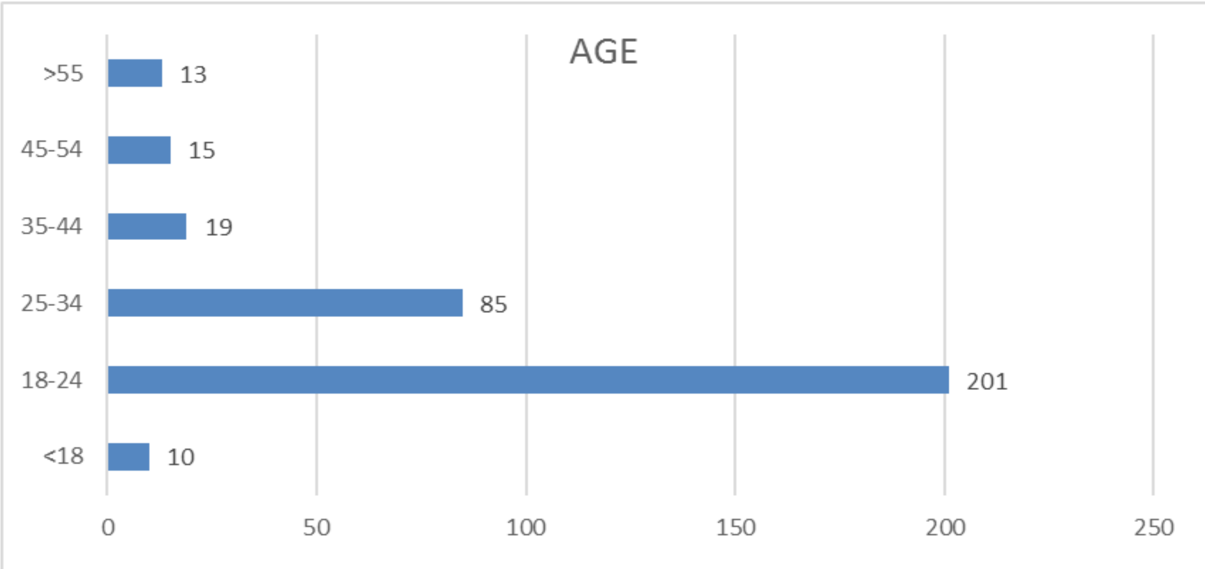

Figure 2. Age range of the surveyed

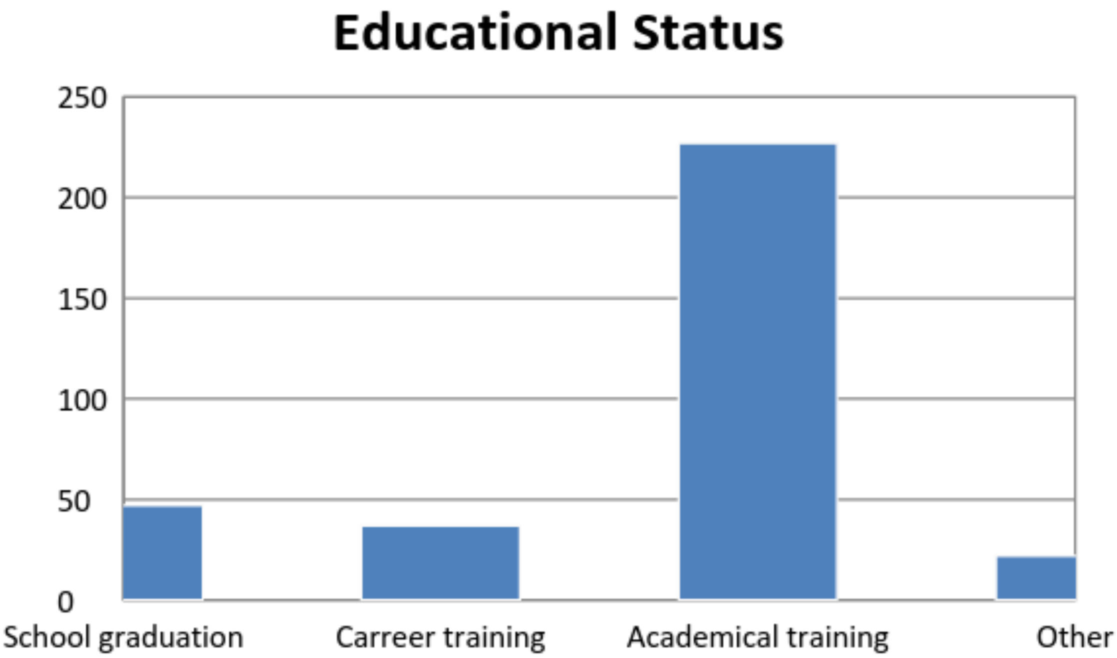

Figure 3. Educational Status of the Surveyed

3.3.3. KNOWLEDGE ABOUT SYNTHETIC BIOLOGY AND PAPER RECYCLING

30.56% of the participants stated that they understood the concept of synthetic biology, whereas 49.75% stated that they did not. Those who do, state they know the term mainly through the internet, but also from college and/or books. Interestingly, some of the responses to our request to briefly describe synthetic biology

Paper recycling was considered an important matter by 76.26% of our participants, whereas 2.78% considered it not important. When asked if conventional recycling could also be harmful to the environment the responses were divided between 48.99% yes and 30.05% no. Luckily for the future of our project, most participants would purchase our recycled paper. Only a small fraction of 4.55% were against it.

3.3.4 DISCUSSION OF SURVEY RESULTS

Analysis of the results of the survey presented several issues that will need to be addressed for a proper implementation of our project.

It is worrying that although most of the respondents are highly educated young people, the do not know what synthetic biology is. What information they do have mainly comes from the internet and is often very vague. Some of the participants had heard of synthetic biology in school, but there appears to be an apparent lack of general knowledge especially in the field of biology. This indicates a necessity to make information on synthetic biology more widely available also through non-technological means and to further educate the public on this important topic. Work in the classroom or at public events, competitions and workshops would be important. Other media such as radio, television or newspaper should also be considered. This way we might also reach other age groups than those questioned in our studied via the internet.

According to our data, the population is interested in recycling paper and they think it is an important area of development. It does not seem necessary to further promote this topic, but indicates that we can continue with the current strategy.

This is a very positive point we noticed in our study since this shows the development of general awareness for environmental conservation.

An aspect where more information needs to made available to the public is the detrimental environmental effects of the current recycling strategies. Only 48.99% of the questioned participants were aware of the fact that paper recycling can also have negative effects. This indicates that people may not be informed about the entire process of recycling, specifically the deinking stage. It would be necessary to inform people about this process with its pros and cons and report on synthetic biology.

A very positive aspect presented in our survey is that the concept of using methods of synthetic biology to recycle paper was very well received by the questioned participants. This together with the fact that paper recycling is a subject of interest with the general public and is regarded as a topic of relevance makes our project both socially and economically viable. Nevertheless it remains important to educate the public about current technologies implemented in industry, synthetic biology and the benefits that alternative biotechnological approaches can bring with them.

Looking at our project in regards to social-cultural impact it seems that in combination with further efforts to educate the public about synthetic biology it will be well received and will have benefits for society.

4. CONCLUSIONS

We currently have a very high paper consumption and are accumulating waste from all aspects of society. Current methods for paper recycling are useful, but still need to be improved. Our project represents an economical alternative, with a low toxicity and risk, which not only saves huge amounts of electricity but at the same time gallons of water.

Analysing our project in regard to biosafety it fulfills all three aspects upon which biotechnological work is analysed in the EU. Considering all aspects of biosafety legislation our project poses little to no health risk and will be implemented in a controlled environment with the appropriate security measures. As such it complies with the precautionary principle. Second, it presents no possibility for harm as it involves saving energy and water as well as reduced toxicity while remaining economically viable and thus fulfills the principle of nonmaleficence. Finally, social opinion seems to support our project, although further work in public education needs to be done.

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