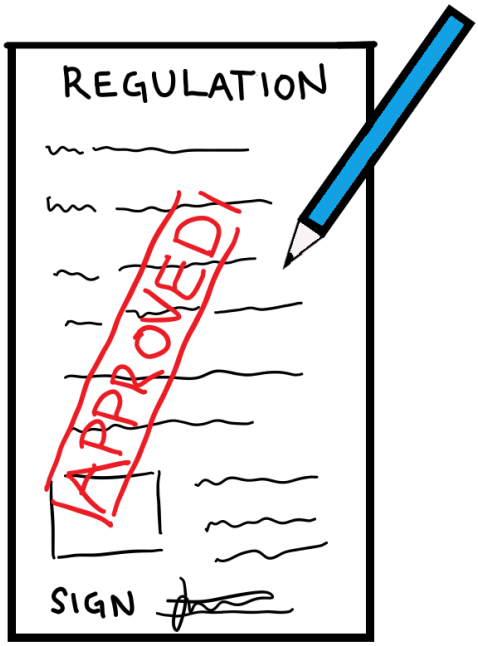
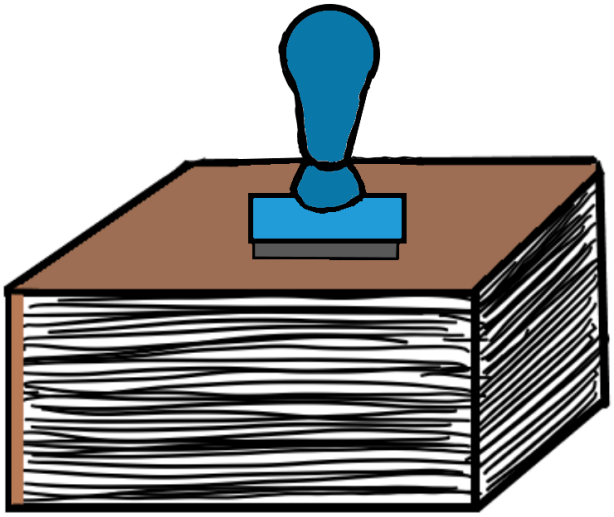
Pharmaceuticals in wastewater effluents

Biotic Blue, regulation compliance and proposed Work of conduct

iGEM Stockholm 2018





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The problem

Today, increased levels of pharmaceuticals found in waste water effluents, influents and rivers pose a threat to both aquatic marine life as well as for higher organisms and species including humans. Especially, antibiotics have been found in high levels in water effluents [1] that could lead to bacteria developing antibiotic resistance. Antibiotic resistance is an increasing problem and one of our greatest global health threats today [2] ultimately making bacterial infections that were once easy to treat untreatable.

Biotic Blue

Laccases are copper-containing oxidase enzymes, naturally found in white rot fungi [3]. These enzymes have high promiscuity, meaning that they can work on many different substrates. Laccases are of high interest to us humans today, due to their ability to modify a range of antibiotics, where sulfamethoxazole (SMX) is one of them [4]. Therefore, we in the iGEM Stockholm team of 2018 set on the task of making a mutant laccase that is more specific towards modifying SMX, to inactivate it and therefore reduce its potency to convey antibiotic resistance to non-pathogenic bacteria. To establish the reaction mechanism, we used quantum mechanics (QM). We then used quantum mechanics and molecular mechanics (QM/MM) to visualize the substrate-enzyme association. Through modeling, we came up with a new, mutated and optimized laccase. This mutated laccase is then immobilized on magnetic beads, to be used in an industrial setting to clean wastewater. This product is Biotic Blue, and it is here to help us clean the water from antibiotics that could worsen the situation of antibiotic resistance in water effluents.

Regulations currently enforced in Sweden

In order to implement Biotic Blue in Sweden, to fully comply with the specific regulations, it is necessary to analyze the Swedish directives and codes relevant to pharmaceuticals in wastewater. In our case, it is specifically important to look at regulations that could affect our product Biotic Blue, but also the importance of Biotic Blue on the market, and the

implementation on the market as a treatment solution in wastewater treatment plants (WWTPs).

The Swedish Environmental Code Ds 2000:61 is a major piece of legislation that promotes sustainable development by fundamental environmental rules. Especially, chapter 3 concerning the management of land and water areas is of interest for Biotic Blue. In chapter 3, section 3 it is mentioned that "water areas that are particularly vulnerable from an ecological point of view should be protected against measures that could potentially damage the natural environment" [5]. Section 5 is also touching upon the subject, stating that "water areas that are important for aquaculture should be protected against measures that could interfere with the culture" [5].

In addition, the Swedish Environmental Agency (EPA) (responsible for advising municipalities, county administrative boards and plant operators on sewage systems and treatment facilities designed for more than 200 people) [6], has in a very efficient way presented the two most important directives that are important in wastewater treatment. These directives are the Urban Waste Water Treatment Directive (91/271/EEC) and the EU Water Framework Directive (2000/60/EC), concerning urban wastewater treatment discharge from industrial and domestic sectors, as well as objectives to involve citizens in the work towards cleaner water in Europe [7][8]. These regulations are interesting from a sustainable point of view, since they are greatly intertwined with the sustainability development goals (SDGs). The SDGs are addressing global challenges that we are facing at the moment, in which goal 6 is clean water and sanitation [9].

In summary, there are regulations that are targeting health protection and environmentally hazardous activities, but they are still lacking the focus on pharmaceuticals.

Situation within the European region today

There are no regulations that are aimed at monitoring the levels of pharmaceuticals in wastewater in any countries except for Switzerland up to this moment (Personal

communication Christian Baresel 20 Jun 2018, expert at the Swedish Environmental Research Institute, study visit at Pharem Biotech Martin Ryen 25 Sept 2018, CEO of Pharem Biotech). Switzerland has also developed a strategy against the levels of micropollutants, defined as substances found at low concentrations in waters (nano- to microgram level per liter). This strategy is aiming at reducing the occurrence of micropollutants in Swiss waters, and many of the WWTPs that are active in Switzerland are required to implement an additional treating step aiming at reducing micropollutants, until 2040 [10].

The fact that Switzerland is the only country in the European region that is monitoring and working towards reducing micropollutants is problematic as mentioned previously. We are not aware of what kind of chemical mixtures that are found in the wastewater that is coming in to the WWTPs, and what is leaving the WWTPs. However, the lack of monitoring could be attributed to the lack of a standardised way of measuring the pharmaceuticals in the wastewater, which we have proposed a work of conduct for below.

Further defining the problem that we are faced with

By scrutinising the regulations and through communication, we have established that it is important that correct safety measures are taken into consideration to ensure that the ecosystem is kept intact, not being affected by man-made industries and personal use. However, we are failing this on several points. The first point is that we are not currently measuring the pharmaceuticals in wastewater on a regular basis. Especially interesting are the antibiotics, due to their shown damaging effect. Going hand in hand with this matter is the absence of enforced regulations aimed at actively monitor the levels of the antibiotics. Furthermore, there are no regulations that are enforced that are stating how the measurements will be performed. As of today, there is no standardised way of measuring pharmaceuticals and antibiotics (Personal communication C. Baresel 20 Jun 2018). It is as if the regulations that are present on the market already are invalidated (see chapter 3 and chapter 5 of The Swedish Environmental Code Ds 2000:61 on page 4), due to the lack of safety measures regarding dangerous compounds in water, that can have a deleterious effect on aquatic life.

Our proposition of work of conduct

Extensive research within different regulations that are enforced within the area of treatment of wastewater has been conducted, as well as discussions with professionals within the field. These discussions and reviews of current regimes have resulted in a suggestion of a suitable work of conduct that should be validated and performed at all municipal and private WWTPs in the whole of European region when it comes to the treatment of antibiotics.

Our proposed work of conduct for antibiotics in wastewater:

1. Measurement of pharmaceuticals should be performed on a monthly or bi-monthly basis
2. The antibiotics tested should be following the antibiotics on the watch list of pharmaceuticals in surface waters, published by The European Commission, provided by the Joint Research Centre (JRC)
3. The pharmaceutical testing should be based on the workflow of the international standard "Limit of Sulfamethoxazole in wastewater", developed by the Standard Student Society (SSS) from the International Standardization Organization (ISO)
4. Regulations that are working for the enzymatic treatment of pharmaceuticals should be enforced in municipal and private WWTPs, directed towards the most ecotoxic and potent antibiotics

We encourage that this work of conduct is followed, and encourage the use of Biotic Blue in the treatment flow, due to the ability for laccases to work on a wide arrange of antibiotics, where Biotic Blue has a higher specificity for antibiotics, acquired by modeling and engineering.

Concluding remarks

It is of importance that Biotic Blue complies to the regulations that are already enforced

on the global market, and that the correct safety measures are taken to consideration. Furthermore, it is essential to solve the absence of developed frameworks aimed at dealing with the emerging problem of antibiotics in wastewater. The solution to this issue is not found on national level, but on a regional level, and should be done in collaboration between nations that border the same water sources. This is a matter that should be addressed now and not later, due to the dangerous threat that antibiotics are posing on humans and other species in the ecosystem.

References

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