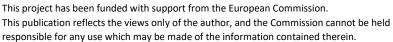


Erasmus+ project "biotechnology in our life" 2015 - 2018 VG-SPS-NW-15-36-013568

#### Disclaimer:





# THE TEAM:

Koidula Gymnasium (Pärnu, EST)

Coornhert Lyceum (Haarlem, NLD)

IES JOSE DE RIBERA (Xativa, ESP)

Liceo Scientifico Statale Galilei Galileo (Verona, ITA)

St Neots Learning Partnership (St. Neots, GBR)

Öffentlich-stiftisches Gymnasium Bethel (Bielefeld, GER)

Univ. Bielefeld teutolab-biotechnology (Bielefeld, GER)

"Biotechnology in our life" is a project (August 2015 till 2018) that strives for a compound structure between schools in Europe with science, companies, and politics.

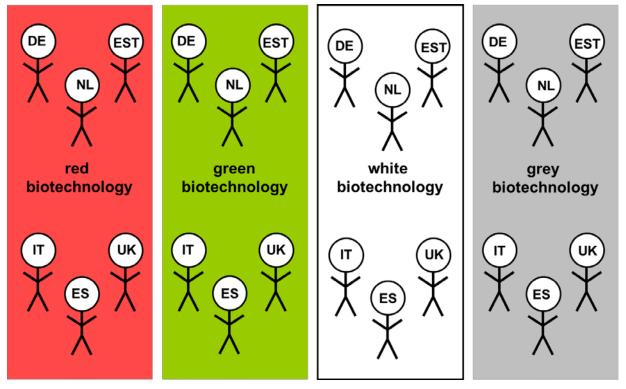
Biotechnology, as the "use of biological processes for technical applications" has both, a long history and an increasing prominence. For many years, living systems for technical appliances (brewing of beer, sewage clarification) have been used. The enormous potential of biotechnology has been recognized for only a few decades.

Regularly, new implementations and products are brought to the market. The awareness that, by transferring genetic material, interesting products can be "manufactured" in qualified production cells (e.g. human insulin, produced inside of bacteria), offers further possibilities. These genetic methods are equally admired as disregarded by the society.

The project aims at learning and experiencing the relevance of biotechnology in daily-life and to explore the potential of this research field for the future. The internationality (Germany, The Netherlands, Spain, Italy, United Kingdom and Estonia) of this project provides the chance to compare the nations' different experiences and bioethics perspectives.



24 international students aged between 15 and 18 participate the project and work in four international teams with four different focusses:



Each international team built three pairs. Each pair chose a topic they were interested in, made research and described their results in form of a poster, a presentation and an arcticle. The international students mainly worked virtually exchanging intermediate results on a special internet platform (eTwinning).

In addition the students meet at three **project meetings** in which they performed experiments, visited biotechnological companies and discussed their work and exchange their experiences. At the third meeting an exhibition took place.

1st meeting	independant/national team work transnational team work (virtual)	2nd meeting	independant/national team work transnational team work (virtual)	3rd meeting
01.10.2016 – 06.10.2016		04.02.2016 – 09.02.2016		25.03.2016 – 30.03.2016
Bielefeld Germany		St. Neots UK		Xativa Spain

This brochure provides an insight of the second year of this project.

In the **first part** of the brochure the respective host students report about the **transnational meetings**.

The articles in the **second part** of this brochure show the outcomings of the **students` work.** 

# Part I

Wastewater treatment plant

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Öffentlich-stiftisches Gymnasium Bethel, Bielefeld, Germany

Report from: Norman, Felix, Jakob and Nina

# Sunday, 02.10.2017

The first transnational meeting of the Erasmus+ Biotechnology-project 2016/17 has been in Bielefeld.



After the arrivals of the guest students on Saturday, the 1st of October 2016, we got to know each other better since we had to solve some "mysteries" on a rally through Bielefeld.





## Monday, 03.10.2017

Monday, we - the German group - held a presentation about genetic engineering and biotechnological basics.





After a lunch break, we had a lab course, named "What is in the sausage?" in which we learned how to use some basic biotechnological procedures like the PCR-method.

# Tuesday, 04.10.2017

On Tuesday, we left Bielefeld at 8 am to visit the Science Centre of "EVONIK Industries" and the "Chemiepark" in Marl. We were there for about 4 hours.





During this time, we were introduced to the youngster-project of "EVONIK"'s laboratories, which are bigger than the one we worked with in Bielefeld in the "CeBiTec". All together you can say that we had a interesting and informative day in Marl.

## Wednesday, 05.10.2017

On Wednesday, we had two presentations, one by Dr. Jan Mussgnug from University Bielefeld about "Biotechnology with Microalgae" and one about "Fermentation Technology" held by Prof. Frank Gudermann from the University of Applied Sciences in Bielefeld.

Furthermore, we started working in our transnational teams.

Finaly, we had a tour through the CeBiTec building, especially the labs.





## Thursday, 06.10.2017

The last day, Thursday, we showed our guests the FvB School buildings and told them a bit about our school system.

After lunch, we worked on our projects in the sub teams we formed the day before. Then we presented what we already did and what we wanted to achieve until the next meeting in February 2017, when we will see each other again in St. Neots in the United Kingdom.

After the great 5 days it has been time to say goodbye to our exchanges, but we all are looking forward to seeing each other again in St. Neots.

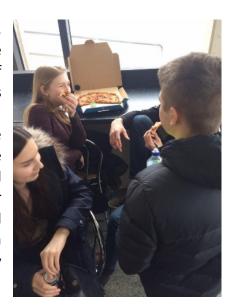


St. Neots Learning Partnership

Report from: Katy, Scott, Joe and Emily

The 2<sup>nd</sup> week of the Erasmus Project was held in St Neots, England from 4<sup>th</sup> February to 10<sup>th</sup> February. All of the English hosts were very excited to have the privilege of hosting everyone and especially excited to see our friends again!

This week was a tough week as our posters needed to be completed ahead of our trip to Xàtiva, however we, as the hosts, wanted to make sure the evenings were relaxed and fun and to make sure everyone got the most out of their stay in England. Some nights were spent in pubs and restaurants as well as trips to our local bowling alley and a trampoline park. Even trips to London were arranged by host families to ensure the best time!





On **Saturday 4**<sup>th</sup> **February**, our eagerly awaited visitors arrived and soon settled into their host families houses ready for a busy day on Sunday!

Our week began on Sunday 5<sup>th</sup> with coffee and biscuits, followed by a short introduction from Dr Miller to welcome everyone to our school, Longsands Academy. After catching up with everyone and reuniting with our groups, we were all set to work on our posters. Lunch came around very quickly and everybody's hard work was soon rewarded with A LOT of pizza! Following lunch, Dr Miller gave a presentation about the company 'Amgen' to us in order to prepare us for what was to follow on Monday. It was very interesting and gave us a deep insight into what we would be working on together with Amgen on Monday.

After a long hard Sunday at school, we were given a tour of St Neots by our very own, Dr Miller, the tour was a bit hectic but didn't fail to deliver everyone a slice of St Neots' history (even the hosts learnt something!).



On **Monday 6<sup>th</sup>**, we stayed at Longsands Academy to take part in a practical study on behalf of our project's industry partner called Amgen. We took part in the Amgen Biotech

experience using genetic engineering processes used to make insulin. As well as using gel electrophoresis and having the chance to look at how our own DNA is made up. Everyone was very intrigued to see the detailed results and it was a truly enriching activity. Everyone thoroughly enjoyed the experience and weren't at all phased by the complexity of such a subject



despite it being in, for many of the group, their second language. Once school had finished we took a coach to Cambridge University where we heard presentations from two PhD students who spoke about their individual studies that included looking at cancer developments within the University itself.

The next day (**Tuesday**, **7**<sup>th</sup>) we spent the morning at Ernulf Academy (our partner school in the town) where we took part in an Environmental Monitoring workshop which involved looking at how our water is contaminated by different sources from businesses and factories and concluding our groups ideas within posters. Every group got thoroughly involved and we had some very interesting discussions.







We then made a trip to Bury St Edmunds for the afternoon. Students were allowed to explore the city and, of course, pick up a coffee or something to eat! After an hour, we made our way to the city's famous Green King Brewery where we were given a guided tour, right from the learning about the fermentation tanks on the ground floor, to the roof top viewing area over the entire pipeline of the brewery as well as across the city. And what's a guided tour without some free samples? However, to the disappointment of some students over 18, we weren't given samples of beer, instead raw hops, one of the main ingredients of beer (I don't think many of us would recommend trying it!).

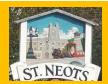
Wednesday 8<sup>th</sup> was a busy day, like any other, which started with us gathering in the library for а speech by representative from a company who look at medical drug development, potential drug molecules and the process of testing drugs. We were then given a couple of hours to finish our posters...the pressure on! Luckily everyone completed their posters and we even had time to spare for a



game of charades! But, trying to act out Gel electrophoresis is as hard as it sounds! During the afternoon, we had a tour around Cambridge and saw some of the university grounds! To the teachers delight, we also visited the Cavendish Laboratory, where the structure of DNA was determined by Watson and Crick.

The final day (**Thursday**, **9**<sup>th</sup>) was a very emotional and pungent day... A prompt arrival from everybody at school meant that we were soon on our way to Anglian Water in Leighton Linslade. Anglian Water is a large water company who supply water and water recycling services to just over 6 million homes and businesses, right from the Humber in Hull to the Thames Estuary and from Buckinghamshire to Lowestoft on the East Coast of England. This sites purpose is to filter the waste from our toilets (hence the pungent smell!) until it was clean enough to be put back into our local river systems. Despite the smell, it was very interesting to discover the complexity behind something you never really think about!

Once we had returned from Leighton Linslade it was time to say a BIG thank you to everyone who participated in our week in St Neots and our goodbyes to the students from Italy, Spain and The Netherlands.



On Friday, the German students departed in the morning, followed by the Estonian students in the afternoon. All farewells were very emotional however it was more of a 'see you soon' as it wouldn't be long until everyone would reunite in Xàtiva, Spain for the final week of the project together!

On behalf of all involved, I would like to dedicate this report to the memory of Dr Sarah Miller whose enthusiasm and commitment towards the Erasmus Project enabled us to be part of such an amazing opportunity.

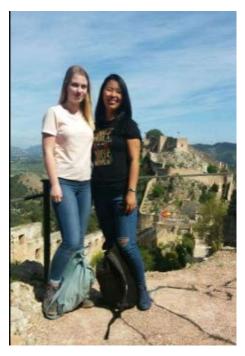
We'll remember these moments, with fondness in our hearts and smiles on our faces,

Katy, Scott, Joe, Emily

IES JOSE DE RIBERA, Xativa

Report from: Paula-Ming, Angela, Nadia and Mar

The third meeting took place in Xàtiva, Spain from Saturday 25<sup>th</sup> March to Friday 31<sup>st</sup> March. We were so excited about meeting again all our friends from last meeting (St. Neots) and also about becoming the host of the last meeting, so we took this as a responsibility because we wanted everyone to have the best time here in Spain while we were all learning, working, exchanging traditions, know-hows and also having a really good time.



The organization was also a little bit difficult because we wanted everyone to be happy with all the activities. Teachers organized all the activities that took place during the morning and sometimes the afternoon whereas hosts from Spain (us) organized activities for the evening and sometimes the night. We usually thought about light evenings like visiting parks, have a drink with everyone else and chill or even go out for dinner, although it was quite difficult to fit forty people in a café or a restaurant. In spite of that, according to the opinions some of the guests told us, they were really happy with these kind of activities; because in that way we could get to know each other so much better as we stayed together all the time.

First of all, the arrivals took place on Saturday 25<sup>th</sup>, luckily, everyone came and left the same day, so this Saturday we hung out, watched a football match and had dinner together and when we all met again, that feeling was such as one of the best ones ever.

Next day, despite of being Sunday, we all started working early in our presentations. Then, we had lunch and we went on a tour around Xàtiva. It was a really nice and sunny day, so we all enjoyed the Castle we visited which was located at the top of the mountain.

On Monday 27<sup>th</sup>, we went on a trip to the University of Valencia, where we did a laboratory practice called: "DNA Extraction". After that, at the Faculty of Physics, we attended a lecture by Prof. Dr. Joan Ferré about the safety of transgenic food.





Then, with a guide, we walked around the center of Valencia, visiting the most important monuments there such as the Cathedral or the City House. Next, we came back to Xàtiva and after hanging out together; we went home and had a rest.



On Tuesday, we had a lighter day, where we attended a bioinformatics workshop ("Bioinformatic analysis in detection and identification of plant viruses") lead by Dra. Ana Ruiz and Dr. Antonio Olmos. Then, after having a break, we continued working on our final presentations and we went home to do the "siesta", something guests really loved from Spanish culture. That afternoon we visited "El Palasiet" a garden grown symmetrically in town which also has a noble house and a specific area for animals. Then, as usual, we went to have a drink and we finally went home, because next day was going to be very exhausting.

One of the best days was Wednesday 29<sup>th</sup>, one of the most complete days where we first visited a Life sequencing company in Burjassot and then, we visited the City of Arts and Science which consist of different buildings about different matters. First we went to its aquarium called the Oceanographic where we saw lots of aquatic animals from all around the world. After lunch, we continued our visit through the Science Museum where there are several exhibitions and some of them consist of



practical experiments for the demonstration and the acquirement of the theorical explanation.

Afterwards, we went to the IMAX Cinema where we watched a film called "Mysteries of the Unseen World". When we came back to Xàtiva, we brought everyone to try the traditional Spanish hot chocolate with "Churros", which I think that was a complete success for our guests.



The last day before the departures was Thursday 30<sup>th</sup>, that day we finally presented our topics in the House of Culture in front of the people from the project and also some students of the hosting high school.

After presentations, Dr. Prof. José Miguel Mulet, a biotechnology researcher gave us the chance of asking him some controversial questions about GMOs and farming biotechnology. After the last official event everyone in the project went to have a Valencian paella cooked by the high school staff which was a complete success because some students ate "anxiously" large amounts of that typical dish. For ending in a good mood this outstanding story we lived together, that day we ended it having dinner out, dancing and singing everyone together, guests and hosts.



Finally, on the 31<sup>st</sup> March, all guests left during the morning giving an end to this wonderful experience which we recommend to everyone who has the chance, because that is where we all acquired skills about biotechnology mainly, but we also learnt English, sightseeing, about other cultures, how to have fun, how to respect, how to take care of each other, but the most important thing, we have learnt a little more, just a little, about life.

### What is biotechnology?

Biotechnology can be defined as the controlled and deliberate manipulation of biological systems for the efficient manufacture or processing of useful products.

Brewing and baking bread are examples of processes that fall within the concept of biotechnology. Traditional processes usually utilize the living organisms in their natural form while the more modern form of biotechnology will generally involve a more advanced modification of the DNA of biological system or organism.

One of the most important applications to biotechnology is personalized medicine, and this part of biotechnology, called biomedicine, also includes the development of new treatments to treat cancer.

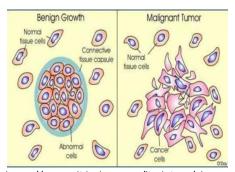


http://www.sterislifesciences.com/Market-Applications/Biotechnology.aspx

#### What is cancer?

Cancer is formed when body cells begin to divide without stopping and spread into surrounding tissue. This leads to abnormal, old and damaged cells surviving when they should be broken down by lysosomes, while new cells form unneccessarily and divide to form tumours.

Tumours can be solid masses of tissue or flow within the blood. They can also be either malignant or benign. Malignant is where the cancer cells on the edge of the tumour break off into distant sections in the body via the lymphatic and circulatory system to form new tumours. Benign tumours stay in one area, are large and can be removed because they dont grow back.

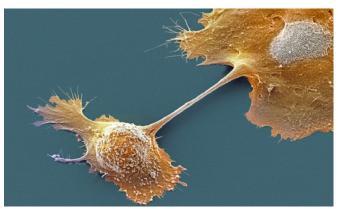


https://www.slideshare.net/liezlejoyg/characteristics-of-benign-and-malignant-neoplasms

In the image we can see the differences of benign tumours that stay in one area and malignant tumorus that can cause metastasis.

Cancer cells are less specialized than normal cells and can ignore signals telling them to stop dividing or begin apoptosis (programmed cell death). They can also influence their microenvironment to transform normal cells into blood vessels, in order to supply tumours with oxygen and nutrients for growth.

And here we have a picture where we can see pancreatic cancer cells:



http://www.mindsofmalady.com/2015/02/gene-pushing-normal-pancreas-cells-to.html

#### **History of cancer treatments**

Before the discovery of biotechnology, Cancer was treated with other methods, the most important two methods that are still used are:

**Chemotherapy**: has been used since the 1940s and 1950s. Is a type of treatment that includes a medication or combination of medications .The goal of chemo is to stop or slow the growth of cancer cells.

**Radiotherapy**: was first used in 1896. Radiotherapy uses high-energy rays to treat disease and orks by destroying cancer cells that cannot repair themselves after the treatment.

The most important issue of these treatments is that can also damage healthy cells and cause a lot of side effects.

## **Biotechnology and cancer**

Biotechnology has very important role in the development of antitumor mechanisms. Scientists are using vaccines against some molecules that are mutated in cancer cells.

The main way of treat cancer using biotechnology is a treatment called Immunotherapy.

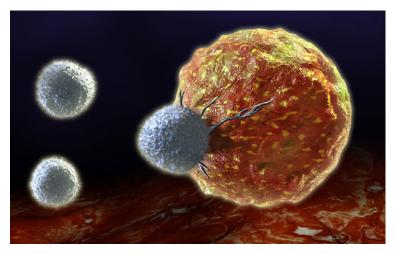
The best thing of this treatment in front of radiotherapy and chemotherapy are that we can attack cancer cells without attack the healthy cells of tumour's surroundings.

## **Immunotherapy**

Immunotherapy is treatment that uses certain parts of a person's immune system to fight diseases such as cancer. This can be done in a couple of ways:

- Stimulating your own immune system to work harder or smarter to attack cancer cells
- Giving you immune system components, such as man-made immune system proteins

Immunotherapy includes treatments that work in different ways. Some boost the body's immune system in a very general way. Others help train the immune system to attack cancer cells specifically.



https://scitechdaily.com/biologists-identify-a-new-approach-to-cancer-immunotherapy/

## **Gene therapy**

Before of explain what is immunotherapy, we need to know what are genes:

Genes are coded messages that tell cells how to make proteins. Proteins are the molecules that control the way cells behave. In this way our genes decide what we look like and how our body works. Genes are made of DNA and they are in the nucleus of the cell.

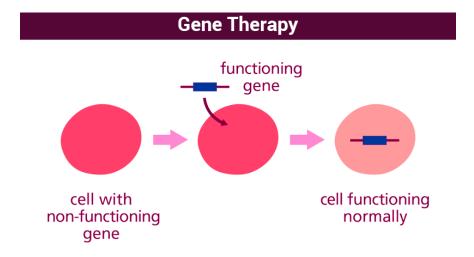
Cancer cells are different from normal cells. They have changes (called faults or mutations in several of their genes which make them divide too often and form a tumour.



http://www.sciencemag.org/news/2015/03/humans-may-harbor-more-100-genes-other-organisms

## And, what is gene therapy?

It's a method of immunotherapy. It can be defined as the insertions of genes into an individual's cells and tissues to treat cancer. A carrier, called vector must be used to deliver the gene to the patient's cells. The most common type of vectors are genetically modified viruses. Target cells become infected with the vector, and the the vector unloads the genetic material, containing the modified gene into the target cell.



http://byjus.com/biology/gene-therapy/

For decades' people with diabetes were meant to suffer from bad quality insulin from pigs. The use of this animal hormone was highly dangerous and caused many allergic and autoimmune reactions. This has changed since 1982 when the first bacteria were producing human insulin. But how can a bacterium produce a human hormone? How can we make them to? And why can they express human genes even if our mechanisms work completely different due to the difference between Eukaryotes and Prokaryotes. We want to answer these and other question with our work about artificial insulin.

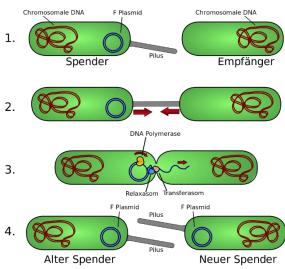
### Insulin, how it got discovered.

In the year 1890 Oscar Minkowski and Joseph von Mering took the pancreas from a dog. This dog went on to get diabetes, also known as sugar disease. This is how the existence of insulin got discovered. But it wasn't until 1921 that the first scientists successfully extracted and isolated the insulin from the pancreas. Later that year the first diabetes patient got treated with external insulin. Because it was now possible to treat people with insulin, people felt the need to produce insulin artificially, so without the help of animals like pigs, cows and horses. Now a day they produce the insulin using a bio-technology method called recombinant DNA technique. This technique works with bacteria. The gene that stands to produce insulin gets transferred into the DNA of bacteria. So, while the bacteria grow and multiply they start to produce insulin that later can be subtracted and used as a medicine with diabetes patients.

Insulin is a hormone made by the cells in the pancreas. It gets produced when there is a high blood sugar level due to the metabolic process of degrading carbohydrates into sugar and it basically enables the cells to take up the sugar so they can create energy.

This procedure can be disturbed by several diseases. These diseases may create the need of artificial insulin. Due to the years, the possibilities and ways to produce artificial insulin have changed. They went from animal insulin to insulin produced by bacteria. But how can we tell a bacterium to produce a human gene?

Our knowledge according this field of research is based on Mr. Lederberg who in 1946 discovered 1. that bacteria have a circle inside them which is called plasmid and carries specific genes as an additional bit of DNA which can be expressed by the bacterium. The idea behind this is that bacteria can exchange bits of DNA which could improve their ability to survive specific conditions. It is also possible that bacteria exchange plasmids which carry toxic genes which will, later, kill them. We can use this plasmid to put human genes into a bacterium. By using restriction enzymes, we cut these plasmids open and basically glue the targeted 4. gene in. But this process isn't as easy as it might sound like because human or eukaryotic genes in



general have one big difference to bacterial DNA. Eukaryotic genes include some sequences which aren't important for the actuell product. That is why Eukaryotic cut this bit out before they express the gene. The bacteria can't. That is why we can't take the original gene. We need to take a human mRNA which doesn't have this so-called introns in it anymore. Then we use an enzyme called **reverse transcriptase** to change the way the way the mRNA is written into a way the DNA is coded. This gives us a cDNA which then can be putted in to the plasmid by using special enzymes. Afterwards the

by Charlotte and Jakob

plasmid is ready we put in to the bacteria through holes in their membrane created by chemicals. Afterwards the bacteria close the holes itself and starts to express the new gene, sucked in through the holes. Only trouble is that you can't just take the targeted gene. You always need to add a gene which gives an advantage to the bacteria like an ampicillin resistance. Otherwise there is no point for the bacterium to take the plasmid up and to express the genes located on it. This process is needed if the natural process in your body doesn't work properly anymore. For example caused by Diabetes. A few symptoms of diabetes type 2 are: being exhausted all the time, unusual thirsty, red eyes and blurry vision. If you find yourself having these and other symptoms the doctor can take a blood sample to find out what's wrong. If the results are that you do indeed have diabetes type 2 the first thing you should try is working out more, and eating healthy. If this new lifestyle does not work for you, you get treated with insulin. The insulin gets injected into the patient with a needle. The insulin usually doesn't get injected into the blood of the patient but into the fat so the body takes it up more slowly. Dealing with diabetes type 2 can be a challenge. It's very important for somebody with sugar disease to eat healthy because this can keep the sugar levels in your blood low. It's also important to stay active and exercise. This can help you control your blood sugar levels.

## Sources;

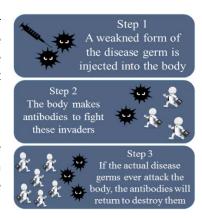
- "Bakterien vergiften sich von innen heraus" Max-Planck-Gesellschaft 22. März 2011 (seen 21.03.2017)
- https://www.drugwatch.com/diabetes/
- https://nl.wikipedia.org/wiki/Insuline
- https://www.vumc.nl/afdelingen/diabetescentrum/InfoDM/InvloedDagelijks/

#### What are vaccines?

A vaccine is a biological preparation used to produce or improve immunity against a particular disease. By inoculating killed or weakened disease-causing microorganisms (or crucial fragments, products or derivatives) the production of antibodies is stimulated. If and when the immune system encounters the disease-causing microorganism, it then itself prevents the disease from reacting rapidly and effectively.

#### How do vaccines work?

When the germs attack our body, they cause an infection; their surfaces are made up of molecules with a different and unique marker called **antigens**, so the immune system can recognize the microbes and fight against them. Macrophages attack and digest most parts of them, saving the antigens and carry them to the lymph nodes, where lymphocytes B produce protein substances called **antibodies** and highly specific cells that can fight the invading germs. The goal of most vaccines is to stimulate the antibodies response because it provides the protection against the specific disease. If a person later is exposed to that same pathogen again, the immune system will be able to produce the same type of antibodies.



Picture made by Susanna Gobbi

# How are vaccines produced?

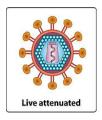
**Biotechnology** is used in three different ways in the development of vaccine:

- **1. Use of monoclonal antibodies for immunopurification of antigens:** this method is used to separate specific antigen from a mixture of very similar antigens. Once purified, the antigen is used for developing a vaccine against a pathogen. Individual interferons (which have the property of inhibiting viral infection and cell proliferation) have been purified using this technique.
- **2.** Use of cloned genes for the synthesis of antigens: hundreds of genes in eukaryotes have been cloned from genomic DNA or from cDNA. These clones genes included a number of genes for specific antigens and some have been used for the synthesis of antigens leading to the preparation of vaccines.
- **3. Synthetic peptides as vaccines:** vaccines can also be prepared through short synthetic peptide chains.

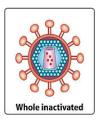
## **Types of vaccines**

All available vaccines can be divided into two main categories:

living: it uses live-attenuated organisms and they contain modified strains of a
pathogen (bacteria or viruses) that have been weakened but are able to
multiply within the body. These vaccines are able to induce a strong immune
response, however, there is a possibility they can revert to the virulent form at
any time.



 non-living vaccines: they are based on whole killed pathogens or components of them (subunit vaccines) as a capsule, partially purified toxins or polysaccharides.
 These vaccines are very efficacious and allowed the control and, in some cases,



the eradication of very important infectious diseases such as smallpox and polio, at least in industrialized countries.

Free pictures on: https://it.wikipedia.org/wiki/File:Various approaches for HIV vaccine development.jpg

## **Reverse vaccinology**

Reverse vaccinology is an innovative technique for the development of new vaccines through the sequencing of the genome of the pathogen.

This technique consists in:

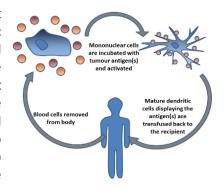
- 1. extracting entire bacteria genomes of chosen antigen and, thanks to the computer algorithms, we are able to locate a number of antigens more than the traditional approach;
- 2. Identifying the antigens.
- 3. On the basis of the DNA sequences, it is started the study on the biological role of each protein looking for those that could be used in the vaccine and which are easily recognized by the immune system.
- 4. This process leads to the identification of a few hundreds of interesting genes. Later these genes are rapidly cloned in order to produce the proteins they encode.
- 5. Proteins are evaluated for their ability to cause an immune response.
- 6. Isolate a dozen of antigens to be subjected to further analysis.

## **Experimental**

The availability of complete genome sequences in combination with novel advanced technologies, such as bioinformatics, microarrays and proteomics, have revolutionized the approach to vaccine development and provided a new impulse to microbial research.

These are a number of innovative vaccines in development and in use:

Dendritic cell vaccines— dendritic cell therapy is an immune therapy that harnesses the body's own immune system to fight cancer. The dendritic cell itself is an immune cell whose role is the recognition, processing and presentation of foreign antigens to the T-cells in the effector arm of the immune system. Although dendritic cells are potent cells, they are not usually present in an adequate quantity to allow for a potent immune response. Dendritic cell therapy thus involves the harvesting of blood cells (monocytes) from a patient and processing them in the laboratory to produce dendritic cells which are then given back to a patient in order to allow massive dendritic cell participation in optimally activating the immune system.

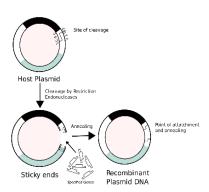


Free picture on <a href="https://commons.wikimedia.org/wiki/File:Dendritic\_cell\_therapy.png">https://commons.wikimedia.org/wiki/File:Dendritic\_cell\_therapy.png</a>

**Recombinant Vector** – they rely on the capacity of one or multiple defined antigens to induce immunity against the pathogen, when administered in the presence of adjuvants or when expressed by plasmids or harmless bacterial/viral vectors.

Several genes from different etiologic agents have been cloned, expressed and purified to be tested as vaccines. There are a variety of expression systems for antigenic protein components in which the DNA encoding the antigenic determinant can be inserted and expressed. However, several factors must be taken into account before selecting the system for antigen expression because they can interfere in the efficacy of production of recombinant antigens as vaccines.





**DNA vaccination** – an alternative, experimental approach to vaccination called DNA vaccination, created from an infectious agent's DNA, is under development. The proposed mechanism is the insertion of viral or bacterial DNA into human or animal cells. Some cells of the immune system that recognize the proteins expressed will attack these proteins and cells expressing them. One potential advantage of DNA vaccines is that they are very easy to produce and store but DNA vaccination is still experimental and is not approved for human use.

Plants as bioreactors for vaccine production – Transgenic plants have been identified as promising expression systems for vaccine production. Complex plants such as tobacco, potato, tomato and banana can have genes inserted that cause them to produce vaccines usable for humans. Bananas have been developed in order to produce a human vaccine against Hepatitis B.

## **Biography**

http://www.vita.it/it/article/2016/07/20/la-crisi-dei-vaccini-e-figlia-dellincrinarsi-del-rapporto-tra-scienza-/140222/http://www.biotechnology4u.com/biotechnology\_medicine\_development\_vaccines\_immunity.html

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http://www.caister.com/cimb/v/v6/17.pdf

http://www.vaccini.net/articolo/la-reverse-vaccinology-la-nuova-frontiera-per-lo-sviluppo-dei-vaccini#

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3854212/

#### **DEFINITION**

The starch potato Amflora owned by the BASF is a genetically modified organism in order to inhibit the production of one of the two starches naturally occurring in this kind of potato. These two starches are the amylopectin and the amylose. The amylopectin's main function is to jellify due to the process of starch retrogradation where while the amylose's one is to give resistance to the object which is applied to. These are the main reasons why Amflora has several industrial applications for

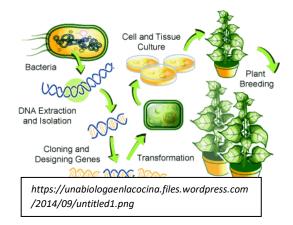
instance papermaking, textile industry or adhesive industry; where amylopectin, the jellifying substance, is needed. The aim of the development of Amflora is to modify the potato DNA in order to obtain the maximum amount of amylopectin (about a 99%), which is the most useful component of the potato. t also contains an antibiotic resistance gene.



Due to the lack of the enzyme called GBSS, the amylose that makes up a 20-30% of the potato starch is not produced. Therefore, almost only amylopectin is found in the Amflora composition. Amflora also contains an antibiotic resistance marker that was initially introduced to distinguish modified cells from non-modified, and that is of no use in the actual commercial plant, even if it is present and producing actively a protein that confers resistance to kanamycin, neomycin, gentamycin, gentemycin, paramomycin and framycetin, which are the main compounds of antibiotics.

### **BIOLOGICAL DATA**

The characteristics, appearance and abilities of a plant are determined by its genetic information. All of this genetic information is stored in the cell nucleus, on long molecules known as DNA (deoxyribonucleic acid). DNA itself is made up of different parts carrying information: the genes. Genes are ultimately responsible for all processes that take place in the plant, such as metabolic reactions, leaf shape and resistance to disease. One plant has approximately 50,000 genes.



Green genetic engineering is a field of biotechnology which is sometimes referred to as plant biotechnology. It is used in modern plant breeding. Genetic engineering allows us to identify individual genes, study their function and combine them in different ways. By doing this we can breed plants with certain characteristics.

Over the last 30 years biotechnologists have developed a range of techniques and methods for deactivating, removing or adding individual genes. One of these methods is the antisense strategy, which was used to develop the Amflora potato. This process involved deactivating the gene responsible for the formation of amylose. Researchers achieved this by copying the gene and inserting a mirror image of it back into the genetic information - hence the term 'antisense'. The copy attaches itself to the original gene, blocking it so it cannot produce any more amylose. Consequently, the potato will only produce the industrially valuable amylopectin.

## **DISADVANTAGES OF AMFLORA**

- Harmful health risks.
- Can cause antibiotic resistance.
- Cause health problems for animals eating the product.
- Some GM foods may contain allergens and toxins.
- Gene transfer may occur between organisms.
- Long term health concerns.
- Cross pollination of natural potatoes and Amflora will make a mixture.
- Change the natural environment. Harm the diversity of species.
- Against some religion—"Things should all be made by God".

#### **ADVANTAGES OF AMFLORA**

- The decrease of the cost of production, less money spent on pesticides and insecticides it actually saves money.
- Better for the environment: In Amflora production less energy, water and chemicals are used.
- Less chemical used to resist pests.
- They use less pesticides so it is better for farmers' health.
- High in nutrients: the protein packed potato.
- It makes paper and yarn glossier and stronger.
- Reduction of soil erosion. Better quality potatoes.

#### **HISTORY**

Originally registered on 5 August 1996, Amflora was developed by geneticist Lennart Erjefält and agronomist Jüri Känno of Svalöf Weibull AB. After the European Commission's approval of the potato, BASF announced it was going to produce Amflora seed starting in April 2010 in Germany's Western Pomerania (20 ha) and Sweden (80 ha). It also announced it was planting 150 ha in the Czech Republic "for commercial aims with an unnamed partner."

Due to lack of acceptance of GM crops in Europe, BASF Plant Science decided in January 2012 to stop its commercialization activities in Europe and would no longer sell Amflora there, but it would continue seeking regulatory approval for its products in the Americas and Asia.

In 2013, an EU court annulled the approval of BASF's Amflora, saying that the EU Commission broke rules when it approved the potato in 2010.

#### **LINCESING PROCEDURE**

Amflora could not be sold within the European Union without approval, and its licence could only be issued after voting at the Council of Ministers of the European Union with a 74 percent threshold of support. Two rounds of voting were held, first by experts in December 2006 and then by the agricultural ministers July 2007, but both failed to reach the 74 percent threshold. Although the voting was by secret ballot, the New York Times reported that Amflora was supported by the agricultural



in

https://es.pinterest.com/traceyking/gmo-food-say-no/

ministers of Germany and Belgium, and was opposed by the agricultural ministers of Italy, Ireland, and Austria, while the agricultural ministers of France and Bulgaria abstained from voting.

#### **AMFLORA AND THE EUROPEN UNION**

The General Court had annulled an EU Commission decision authorizing BASF's genetically modified (GM) potato 'Amflora' because the European Union's executive had departed from the rules governing the authorization procedures, the European Union's second-highest court announced on Friday.

The ruling came as EU members Hungary, France, Austria, Poland and Luxembourg challenged an EU Commission decision in 2010 that had cleared BASF's Amflora potato for industrial starch production and animal feed use.



The Commission gave its approval after the European Food Safety Authority (EFSA) said in a consolidated opinion in 2009 that it believed Amflora posed no threat to human health or the environment. However, the Commission failed to submit the EFSA report, which included dissenting opinions, to two advisory committees made up of representatives of EU member states.

In 2011, BASF already stopped selling its Amflora potatoes in the EU because the genetically modified (GM) crop was struggling to gain a market share amid widespread popular and political resistance. In 2012, the German chemical giant announced that it was moving its biotech headquarters to North Carolina and halting the commercialization of GM products for the European market.

#### **Cotton in our lives**

Cotton is a soft and fluffy fibre that grows in a boll, or protective case, around the seeds of the cotton plant. It is the world oldest commercial crop and is one of the most important fibres in the material industry.



https://pixabay.com/en/cotton-fruitopen-country-flower-1721144/



https://pixabay.com/en/toweltextile-fabric-cotton-color-1838210/

We use cotton every single day it is part of a lot of things that we use like towels or suites but we also use it in the production of soft toys. Cotton is grown all over the world. Cotton is vital for the survival of many low income countries in Central and West Asia and Africa however most of it is grown in America, China and India. However growing cotton is a very hard job, it usually involves hard working conditions in places such as India.

Cotton covers 2.5% of the world's cultivated land however it uses 16% of the world's pesticides, this is more than any other largely grown crop. The cotton plant requires warm weather and lots of water, it takes 2 liters of water to produce one t-shirt. Also farmers in India get a low price for their cotton due to it being very cheaply and more efficiently produced in America and China.



https://pixabay.com/en/co tton-field-agricultureharvest-2180566/

Cotton is also up against other problems along with the weather, these include weeds causing a negative effect on the successful growth of cotton. And they have pests such as Varmints and bollworm. These destroy the plants make the plants useless to the farmers.

A solution to this has been found through biotechnology and genetic engineering. The bacterium Bacillus thuringiensis naturally produces a chemical harmful to the bollworm. This gene in the bacillus thuringiensis which creates this chemical was extracted and inserted into the cotton seeds DNA as a transgene. This then causes the cotton (now called Bacillus thuringiensis cotton) to produce its own chemical to defend its self against the bollworm.

by Felix and Emma

This means less cotton plants are destroyed due to the pest, this means a higher yield of cotton for the farmers, giving more to sell.

In many cotton farming areas there are pests such as the lepidopteran larvae which also damages the cotton plant. Usually pesticides are used against this, however it is also killed by the Bacillus thuringiensis toxin now in the transgenic ocotton, and this reduces the need of pesticides used on the cotton, causing was pollution from the pesticides. Further more the cotton now has a permanent or genetic resistance to the bollworm which will not be affected by environmental factors.

However farmers from smaller and poorer countries cannot afford these expensive genetically modified seeds as they are much more costly than regular non genetic ay modified seeds. This is leaving a gap in their profits against countries such as America who can afford these seeds and reduce produce loss. Also the genetically modified cotton is only effective for up to 120 days (cotton takes at least 140 days to grow) after this the efficiency of the toxin production is greatly reduced. This still leaves the plant vunrable to pests near the end of growth.

These days lots of people are against GMO (Genetically Modified Organisms) but in some ways, GMO have always existed, and farmers have often relied on them: in fact, plants which were more suitable for cultivation (because they produced more and better fruits or maybe were less infected by pathogens) were selected and then cloned with a horticultural technique named as grafting. This technique consists in the positioning of a part of the plant, usually a branch, in a second plant, compatible with the first one. In this way, it is possible to clone plants that have a genetic modification which has some benefits for us. For example, the bananas used for consumption do



not have seeds because their set of chromosomes is 3n and this makes them sterile (they are a cross between 2n and 4n bananas, which are not sterile): so, all the bananas that are for sale in supermarkets could be the fruit of clones of the same banana tree! Furthermore, fruits like lemons or grapefruits are hybrids, they are a cross between other different fruit. Gregor Mendel, who discovered the basis of genetics by crossing different type of peas, was the first one to show that lots of things can be done with plants!

A non-sterile banana
Free picture on https://en.wikipedia.org/wiki/Banana

Plants have been the basis for medical treatments through much of human history. For example, it is known from "Ebers Papyrus", a medical papyrus of herbal knowledge, that Egyptians knew a lot about the use of plants in medicine; the Greeks and the Romans too used plants for medical purposes. Such traditional medicine is still widely practised today: in China, 50% of the total use of medicines is from herbal preparations. Nowadays, everyone knows that plants are effective because of their active ingredients: lots of them have been identified, and about a quarter of prescription drugs are still of plant origin<sup>1</sup>.

But if plants already have their own active substances, why should they not be modified to increase the amount of these substances in them, or maybe to create new ones that we need? Thanks to genetic engeneering, this has become possible. It has been discovered that plants have a very high potential: they can protect themselves from pathogens, they can be made richer in active ingredients or in nutritional values, vitamins for example, but they can also obtain characteristics (and applications) they did not have before. They can be used for the production of biofuels and for bioremediation, a technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site, and they can be used as bioreactors for the production of substances which can treat diseases.

Pharming is a combination of the words "pharmaceutical" and "farming": it refers to the insertion of genes that code for a pharmaceutical into an organism that does not have them in its genome. Pharming in plants is the procedure carried out in these organisms.

Genetic engeneering in fact allows us also to use plants to produce drugs, vaccines, hormones, cytokines, antibodies and other specific molecules. These pharmaceuticals are produced in specific parts of the plant, which can be seeds or chloroplasts mainly. But why should plants be modified in order to create these molecules and then harvest them, rather than make them in a lab, using bacteria or mammalian cells, as usually done before? Well, the use of genetically modified plants

to produce these molecules is cheaper, and it produces better molecules than the ones made by bacteria: in fact, proteins that are usually glycosylated in humans (glycosylation is a process that gives the protein its correct shape) are not glycosylated by bacteria, but they are in plants.

Factor	Transgenic plants	Plant cell cultures	Bacteria	Yeast	Mammalian cell culture	Transgenic animals
Production costs	Low	Medium	Low	Medium	High	High
Product quality	High	High	Low	Medium	High	High
Time effort	High	Medium	Low	Medium	High	High
Productivity	High	Medium	Medium	Medium	Medium	High
Contamination risk	No	No	Yes	No	Yes	Yes
Storage	RT	-20°C	-20°C	-20°C	N <sub>2</sub>	N <sub>2</sub>
RT = Room temperature	N2 = In liquid nitroge	n				

Pharming in plants compared to other types of pharming Adapted from Fischer et al. 2000; created by Michele Di Palma

Moreover, the cost of recombinant proteins made by plants is very low compared to other ways to obtain them (about 2-10% of the cost of recombinant proteins made in E. coli, 0.1% of the ones made in mammalian cells<sup>2</sup>).

But how can a plant be genetically modified? There are three main ways to do that. The first one, called agroinfiltration, uses a common bacteria which usually infects plants and makes them sick (called Agrobacterium Tumefaciens). This bacteria has a plasmid, called Ti (Tumor inducing), which is responsible for the transformation of normal cells into tumoral cells. A part of the DNA in the plasmid (called T-DNA) becomes part of the DNA of the cell and modifies it. The extremities of T-DNA are essential parts for its insertion into the plant cell, so we can remove what is in the middle of T-DNA, replace it with the DNA we want to replicate and connect it to the extremities with ligase enzyme. Now the bacterium is not dangerous anymore and it modifies the plant cell so that it has the desired gene. In this plasmid there is also a gene marker which makes plants resistant to an antibiotic: in this way plant cells which have been successfully modified can be identified by



putting them in a Petri dish with the chosen antibiotic. The ones which have not been modified will then be discarded.

The second way to modify a plant is called biolistic particle delivery system (or gene gun): this method consists in shooting dense particles of tungsten or gold coated with DNA into a plant tissue, but this way is less effective than agroinfiltration. The last way, called electroporation, is a technique by which an electrical field is applied to cells in order to increase the permeability of their cell membrane, allowing the DNA to be introduced into the cell.

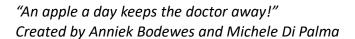
Agroinfiltration technique
Free picture on https://en.wikipedia.org/wiki/Agroinfiltration

The first antibiotic resistant plant was produced in 1982. Since then there have been lots of trials for the production of molecules in plants, for example both the cytochine Interleukin-10 (I-10, an immunosuppressor which "breaks" your immune system) and ZMapp, a pharmaceutical which can treat the Ebola virus disease, have been produced in a tobacco plant. Also, there have been trials for glucocerebrosidase, an enzyme that, if lacking, is responsible for Gaucher's disease, and many other trials for the production of hormones and proteins in plants.

But the most interesting topic regards edible vaccines. If a plant can be modified in order to produce a molecule in any part of the plant (in fact it is possible to choose the place where this

molecule should be produced: in the leaves, in the seeds, in the roots etc.), a vaccine could be produced in a fruit or a vegetable and people could then just eat it. This would be very fascinating, but there are some problems connected to it. First of all, there needs to be enough vaccine into this fruit or vegetable, and this is not as simple as it seems. It is difficult to reach amounts of the vaccine higher than 1% of total soluble proteins<sup>3</sup>. Furthermore, the vaccine has to pass through the digestive system without being degraded, and this one too is not an easy task.

Anyway, do not think this is impossible: the vaccine for hepatitis B has been produced in a tuber, and it is resistant to digestion!





Pharming in plants can save our lives, but it can also be very dangerous. In fact, the molecules produced, stored in the plant, can pollute the air, the soil and water, and they can also accumulate in non-target organisms: that is why genetically modified plants must be cultivated in greenhouses. If I-10, for example, got into a virus which can incorporate it in its DNA, this virus could become a "doomsday pathogen", killing all of us through the inhibition of our immune system. We must be careful.

The greater use of transgenic plants for the production of biopharmaceuticals would lead to a greater availability of these molecules all around the world where required, this is the reason why this technique should be studied in depth, in order to improve it.

Maybe one day a pharmaceutical that can prevent cancer will be found. And it would be great if we could just simply find it in our food, eat it and stay healthy.

<sup>&</sup>lt;sup>1</sup> Rainer Fischer & Neil Emans / Molecular farming of farmaceutical proteins, Transgenic Research 2000; 279-299

 $<sup>^{\</sup>rm 2}$  J.W. Larrick et al. / Biomolecular Engeneering 2000; 18: 87-94

<sup>&</sup>lt;sup>3</sup>Henry Daniell et al. / Trends in Plant Science 2001; 222

<sup>&</sup>quot;This project has been funded with support from the European Commission. This publication [communication] reflects the views only of the author, and the Commission cannot be held responsible for any use which may be made of the information contained therein."



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Claudio and me have done our project about biofuels, a specific area of biotechnology. First, we are going to explain some facts about biotechnology and then, we are going to focus in our project.

# What's biotechnology?

**Biotechnology** is the use of living systems and organisms to develop or make products. It's understood as the application of principles of science and engineering for the treatment of organic and inorganic materials by biological systems to produce goods and services.

# Benefits of biotechnology

The main advantages of biotechnology are:

- <u>Increase performance:</u> through genetically modified organisms, the performance of the crops augments.
- <u>Pesticides reduction:</u> genetically modified organisms to resist pests.
- <u>Improve nutrition:</u> can till in extreme conditions.



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# Risks of biotechnology

It has some dangers that can be classified in:

- Harm the health of humans and animals.
- Environmental consequences



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# Applications of biotechnology

It has some applications that are classified into:

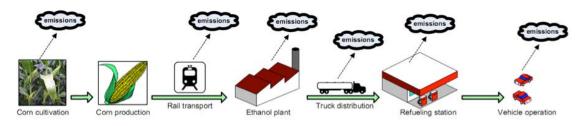
- Red biotechnology: is applied to medical processes. Some examples are the designing
  of organisms to produce antibiotics, and the engineering of genetic cures through
  genetic manipulation.
- <u>Green biotechnology:</u> is biotechnology applied to agricultural processes. An example would be the selection and domestication of plants via micropropagation. Another example is the designing of transgenic plants to grow under specific environments in the presence or absence of chemical.
- <u>Blue biotechnology:</u> is a term that has been used to describe the marine and aquatic application of biotechnology. It's use is relatively rare.
- <u>Grey biotechnology</u>: is the biotechnology of the environment. An example is the maintenance of biodiversity and preservation of species.
- Orange biotechnology: is a term to designate the educational biotechnology. It applies to the diffusion of the biotechnology and its formation.
- White biotechnology: is biotechnology applied to industrial processes. An example is
  the designing of an organism to produce a useful chemical or to use enzymes as
  industrial catalysts to either produce valuable chemicals or destroy polluting
  chemicals. Is the part of the biotechnology in that we will focus.

# White biotechnology: biofuels - an overview

A **biofuel** is a fuel produced through contemporary biological processes, such as agriculture and anaerobic digestion, rather than a fuel produced by geological processes such as those involved in the formation of fossil fuels, such as coal and petroleum, from prehistoric biological matter.

Renewable biofuels generally involve contemporary carbon fixation, such as those that occur in plants or microalgae through the process of photosynthesis. Other biofuels are made through the use or conversion of biomass. This biomass converted to convenient energy-containing substances in three different ways: thermal conversion, chemical conversion and biochemical conversion. This biomass conversion can result in fuel in solid, liquid or gas form. This new biomass can also be used directly for biofuels.

There are various social, economic, environmental and technical issues relating to biofuels production and use, which have been debated in the popular media and scientific journals. These include: the effect of moderating oil prices, the "food vs fuel" debate, poverty reduction potential, carbon emissions levels and nitrogen dioxide emissions.



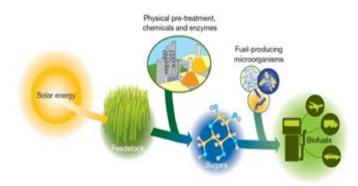
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# First generation of biofuels

The **first generation** or conventional biofuels are made from **sugar, starch** or **vegetable oil** that are contained in materials such as: sugar cane juice, corn kernels, beet juice... Also used as inputs are animals fats and waste oil from cooking food processing.

These types of biofuels are produced using conventional technology such as **fermentation** (for sugars and carbohydrates), **transesterification** (for oils and fats), and **anaerobic digestion** (for organic waste).

Through these processes can be obtained: **ethanol**, **biodiesel**, **biofuel gasoline**, **vegetable oil**, **biogas**, **solid biofuels**...



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# Second generation of biofuels

The **second generation** biofuels, also known as advanced biofuels, are fuels that can be manufactured from various types of **biomass**. Biomass is a wide-ranging term meaning any source of organic carbon that is renewed rapidly as part of the carbon cycle. Biomass is derived from plant materials, but can also include animal materials.

First generation biofuels are made from the sugars and vegetable oils found in arable crops, which can be easily extracted using conventional technology. In comparison, second generation biofuels are made from lignocellulosic biomass or woody crops, agricultural residues or waste. This makes it more difficult to extract the required fuel. A series of physical and chemical treatments might be required to convert lignocellulosic biomass to liquid fuels suitable for transportation.

Some of the second generation biofuels are still developing, such as **cellulose**, **ethanol**, **algae fuel**, **biohydrogen**...

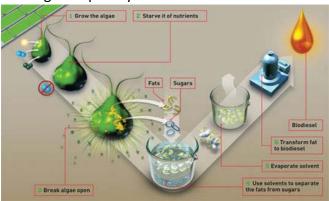


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# Third generation of biofuels

Beyond second-generation biofuels, scientists are also working with so-called **third-generation biofuels**, which are derived from **microalgae** and **cyanobacteria**.

Advantages to this process include the facts that microalgae is an aquaculture, that it reproduces rapidly, that it can grow in salt water, that it can generate biomass with a higher energy content than ethanol, and that the resulting biofuel can be used as a pure product in existing transport systems.



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#### Bioplastic

The official IUPAC definition of a bioplastic is: "a bio based polymer derived from the biomass or issued from monomers derived from the biomass and which, at some stage in its processing into finished products, can be shaped by flow." But bioplastic is more often defined as a plastic made from renewable biomass sources, such as vegetables fats and oils, corn starch, or microorganisms. Or plastics that are biodegradable. When a plastic material meets one or both of these requirements it is considered to be a bioplastic. As mentioned before some, but not all, bioplastics are biodegradable. This means that they can break down in either anaerobic or aerobic environments, depending on how they are produced. The term "bioplastics" can be misleading because it suggests that any polymer derived from biomass is environmentally friendly. This is not true, not all bioplastics are biodegradable and made from biomass. Some bioplastics can still be bad for the environment, not as bad as other more common plastics but some bioplastics are certainly not 100% environmentally friendly.

There are many different types of bioplastic, the most common ones at this moment are: Starch-based plastics, polylactic acid (PLA) and polyurethanes (PUR). Thermoplastic starch (starch-based plastic) currently represents the most widely used bioplastic, constituting about 50 percent of the bioplastics market. They are often blended with biodegradable polyesters to produce blends that are used for industrial applications and are also compostable. Or blends that have a lower carbon footprint than other plastics. Polylactic acid (PLA) is a transparent plastic produced from corn or dextrose. It can be processed using standard equipment that already exists for the production of some conventional plastics. PLA and PLA blends generally come in the form of granulates with various properties, and are used in the plastic processing industry for the production of plastic containers, cups and bottles. Polyurethanes (PUR) is a plastic made out of long chains of organic units. It is mostly used because of its many properties including elasticity, transparency, resistance to oil, grease and abrasion.

Later in this article the production process of these plastics will be explained in more detail.

	Biodegradable	Non-biodegradable
Bio based	PLA, starch blends	PET, PUR
Fossil based	PCL, PBAT	PVC,PP

The names of different plastics, the plastics in **bold** are considered bioplastics.

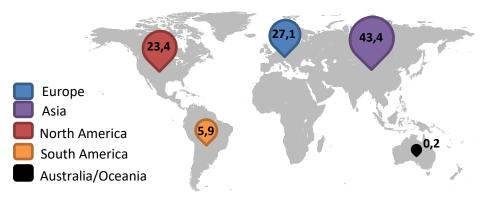
### The market

Bioplastics are used in an increasing number of markets, from packaging, catering products and consumer electronics, to textiles and a number of other segments. Packaging remains the largest fields of application for bioplastics with almost 40 percent (1.6 million tonnes) of the total bioplastics market in 2016. However, it is predicted that the use of bioplastics in other markets will increase. Customer goods and the automotive and transport sector are the most promising markets (beside packaging) for bioplastics in the future. Also the construction and building sector, where technical performance polymers will be used looks quite promising. Today, bioplastics are mainly used for disposable items such as containers, bags, straws and the bottling of drinks. A small portion of these bioplastics is also used for non-disposable applications like phone casings and plastic piping. Another interesting use of bioplastic is use in the medical sector. Medical implants made from PLA can dissolve in the human body witch can save patients from a second operation.

But the market for bioplastic at this moment is still very small. Currently, bioplastics represent about one per cent of the about 300 million tonnes of plastic produced annually. But as demand is rising and with more sophisticated materials, applications, and products emerging, the market is already growing by about 20 to 100 per cent per year. In the years 2000 to 2008 the market increased by 600%, and it is also predicted that the market will increase another 300% from 2014 to 2021. The main drivers of this growth are bio-based, non-biodegradable plastics, such as polyurethanes (PUR). Bioplastics that can biodegrade such as Starch-based plastics and polylactic acid (PLA) are also growing steadily from around 0,9 million tonnes in 2016 to almost 1,3 million tonnes in 2021.

Most of this growth takes place in Asia, with more than 40% of the global production capacities of bioplastics Asia is by far the biggest producer of bioplastics. Europe and North America are far behind with 27,1% (Europe) and 23,4% (North America). Asia will probably further expand its role as major production hub. It is predicted that in 2021, more than 45 percent of bioplastics will be produced in Asia. Around a quarter of the global production capacity will probably be located in Europe.

# Global production of bioplastics in 2016 (in %)



# The production of bioplastic

As mentioned before Starch-based plastics, polylactic acid (PLA) and polyurethanes (PUR) are the most commonly used bioplastics today. All of these plastics consist of long chains of polymers (a large <u>molecule</u>, or <u>macromolecule</u>, composed of many repeated subunits). Although the production process is similar, it is not exactly the same. The last part of the processes is almost the same for all three plastics, they differ from each other in the first part of the process.

Starch-based plastics can be formed by the same processes as current commercial plastics, giving it similar mechanical strength to some polyolefin plastics. First, extrusion of the starch takes place. This will then be pressed together in a vacuum and put into a reactor where it will be moulded into a plastic. Using a glycerol based modifier results in a totally sustainable and biodegradable material.

To make polylactic acid corn or other raw materials are fermented to produce lactic acid, which is then polymerized to make PLA. The lactic acid is put into a reactor and converted into a type of preplastic under high temperature and in a vacuum. This pre-plastic will then be broken down in to the building blocks of PLA. Out of these "building blocks" the bioplastic will be put together.

There are a lot of different versions of PUR, this is one way of making PUR. This process utilizes a spontaneous reaction between polyamines and cyclic carbonates to produce polyhydroxurethanes. This is than put in to a reactor under high temperature and in vacuum were it is moulded into the right plastic.

# Advantages of bioplastic

There are a lot of benefits of bioplastic, beside the fact that it is safer to use than normal plastic the main two benefits are: the environmental benefits and the fact that bioplastic is a sustainable and reliable source of plastic.

Almost everyone agrees that global warming is a real threat. That is why the European Union made the plan "Europe 2020". Europe 2020 contains concrete targets, including 20 percent lower greenhouse gas emissions compared to 1990, 20 percent increase in energy efficiency, and a total share of 20 percent of energy from renewables. The use of bioplastics can be an important step towards achieving these targets. Life cycle analyses show that bio based plastics enable a significant CO2 saving , up to carbon neutrality, compared to more common other plastics, depending on the feedstock, the product and the application.

Another very serious environmental problem is the Great Pacific garbage patch. This is the collection of plastic waste and litter in the north pacific ocean. The size of this patch is estimated to be between 700,000 square kilometres (270,000 sq mi) and more than 15,000,000 square kilometres (5,800,000 sq mi). A lot of sea mammals are at risk due to this patch. They get stuck in the plastic or they eat it. Also algae and plankton communities are threatened, if this goes on it could mean that the entire food web may change. Animals that feed on algae and plankton, such as fish and turtles, will have less food. If populations of those animals decrease, there will be less food for apex predators such as tuna, sharks, and whales. Eventually, seafood becomes less available and more expensive for people. This indicates that it is a very serious problem for humans as well. To make this all worse, plastic attracts toxic gasses witch is also bad for animals. This means that the problem is making itself worse. The use of bioplastics is not going to solve this problem but because most bioplastics are biodegradable they will also not make it any worse witch is a huge step in the right direction because with the use of normal plastics the patch is still growing very fast. During biodegradation microorganisms that are available in the environment convert materials into natural substances such as water, carbon dioxide, and compost without needing any artificial additives. The process of biodegradation depends on the surrounding environmental conditions, the material and the application.

The last benefit for bioplastics is the sustainable and reliable source of plastic. Because the prices of petrol (which is used by the production of normal plastics) are rising and the amount of petrol decreasing, a lot of producers are looking for a new way of making plastics. Bioplastic is an ideal option for this demand because it is a reliable and sustainable way of making plastic.

# The future of bioplastic

As mentioned before the market for bioplastics is growing really fast and is predicted to keep growing tremendously in the future. But these are not the only positive things to come for bioplastics in the future. There is still a lot of innovation possible for the production and use of bioplastics. There are a lot of studies hoping to find an even better way of producing these plastics and making new types of bioplastic.

Genetic modification of plants for example. Genetic modification (GM) is a very big challenge for the bioplastics industry. None of the currently available bioplastics require the use of GM crops, although GM corn is the standard feedstock. Looking further ahead, some of the second generation bioplastics manufacturing technologies under development employ the "plant factory" model, using genetically modified crops or genetically modified bacteria to optimise efficiency. This can lead to a self-sustainable, carbon neutral and cheap way of making plastics.

Besides optimising the efficiency of the production process there are also a lot of people who try to find new uses for bioplastics. As described before bioplastic is already being used in the medical sector, saving people from operations. This can drastically expand in the future. As mentioned before it is predicted that bioplastics will be used in a lot more markets than only packaging in the future.

# Conclusion

Taking everything into consideration we think that bioplastics are a very good alternative for normal petrol based plastics. We expect an enormous growth on the market of bioplastics in the next few years and we do believe that bioplastics have a lot of potential to get the world one step closer to a carbon neutral, self-sustainable and reliable society.

#### Sources

The following sites have been used to make this article:

- www.european-bioplastics.org
- www.IUPAC.org (international union of pure and applied chemistry)
- www.wikipedia.org
- www.wetenschap.infonu.nl
- www.nationalgeografic.org
- <u>www.WUR.nl</u> (Wageningen university and research)

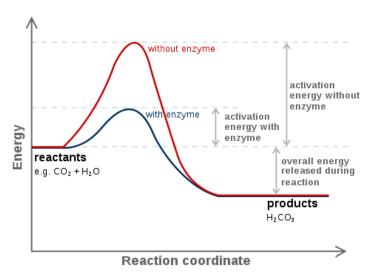
Throughout this project, we have been using our knowledge gained from excursions and lab work in both St Neots and Bielefeld to create a poster in which all of our information is collated into a friendly yet informative style. Our project title was 'who cleans our laundry?' – our initial thought was of course our parents(!) but there is a huge amount of chemistry behind the detergents your parents use. This article with reemphasise all of our key points from the poster and also expand on our findings.

# Who cleans our Laundry?

Firstly we will answer the main question itself, laundry detergents are the most popular and common substance used to clean our clothes. The detergent, combined with a temperature above room temperature, is the the perfect duo to increase the activities of the chemicals which increase the solubility of stains and therefore clean clothes more efficiently using enzymes for specific stains.

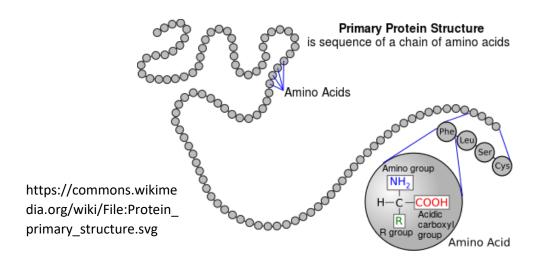
# What are enzymes?

Enzymes are globular proteins that act as biological catalysts and speed up chemical reactions without being used up themselves which allows them to be reused and are therefore effective in small amounts. The enzymes work by lowering the activation energy of the reaction so less energy is needed for the reaction to take place.

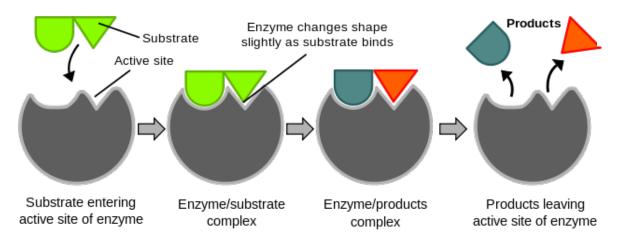


https://commons.wikimedia.org/wiki/File:Carbonic\_anhydrase\_reaction\_in\_tissue.svg

Enzymes are made from an amino acid sequence which is dependant on the gene sequence. They are very specific according to their 3D tertiary structure which is created by folding the secondary structure thousands of times and determines the function of the enzyme. A specific reigon of the enzyme is functional and allows substrates to bind to it, this is called the active site. The specific substrate molecule for the enzyme can fit inside the active site and is held by bonds which temporarily form betweeen certain amino acids. The substrate can then be converted into different moleules called products, via the enzyme.



Enzymes have one function only, and work like a key that fits in a lock (lock and key theory). Only when the right enzyme finds the right material it can work upon, a biochemical reaction occurs. The active site is the 'lock' and the substrate is the 'key' and only one key fits in a certain lock.



https://upload.wikimedia.org/wikipedia/commons/2/24/Induced fit diagram.svg

Enzymes are able to work at low temperature and moderate pH which overall means, given the right conditions, the enzyme can go on and on for as long as needed so this lowers costs.

# What are detergents?

Laundry detergent contains enzymes harvested from microorganisms such as bacteria. Biological detergents clean in the same way as non-biological ones with additional effects from the enzymes, whose purpose is to break down protein, starches and fat in dirt and stains on clothing, a biological detergent can contain  $\alpha$ -amylase, a cellulase, a protease and a lipase. Tests by the Consumers' Association in the UK published in their 'Which?' magazine showed that the performance of various makes of biological powders ranged from 58% to 81%, and non-biological powders scored from 41% to 70%.

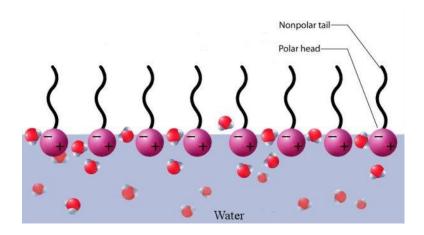
# **How do detergents work?**

The surfactants detergents contain are made of molecules that have two different ends. One end is strongly attracted to water (hydrophillic); the other is attracted to oily substances like grease (hydrophobic).

During the wash cycle, the surfactant mixes with water. The grease-loving ends of the surfactant molecules start to attach themselves to the dirt on your jeans. The tumbling motion breaks the dirt and grease into smaller, easier-to-remove pieces. During the rinse cycle, water molecules moving past attach themselves to the opposite, water-loving ends of the surfactant molecules. The water molecules pull the surfactant and dirt away from the jeans. During the final spin, the dirty water flushes away.

This is why detergents and water work better than either alone. Surfactants are even more efficient with the use of enzymes and specific qualities.

Modern detergents contain a variety of surfactants, so they work in different ways. For example, oxidizers use the oxygen molecule in water to produce a chemical reaction and whiten material.



https://commons.wikimedia.org/wiki/File:Surfactant.jpg

# **How are Enzymes made?**

The starting point for enzyme production is a vial of a selected strain of microorganisms which can produce large amounts of enzyme. They will be nurtured and fed with the optimum conditions to improve growth until they multiply many thousand times. Then the desired end-product is recovered from the fermentation broth and sold as a standardised product.

This is the standard way of producing enzymes however enzymes nowadays can be genetically modified to produce specific enzymes which would be most effective as a detergent.

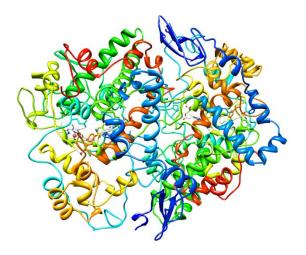
# **How are enzymes modified?**

Subtilisin is the most common enzyme which is genetically modified within a bacterium. Subtilisin is 274 amino acids long, and one of these, the methionine at position 222, lies right beside the active site of the enzyme, the reaction in the active site results in the breaking of a peptide bond in the backbone of the protein. Unfortunately, methionine is an amino acid that is very easily oxidized, and laundry detergents are often used in conjunction with bleach, which is a strong oxidizing agent, therefore the enzyme is usually inactivated.

To overcome this problem, genetic engineering techniques were used to isolate the gene for subtilisin, and the small part of the gene that codes for methionine 222 was replaced by chemically synthesized DNA fragments that coded for other amino acids. The experiment was done in such a way that nineteen new subtilisin genes were produced to replace the 222 position. Some resulted in inactive versions of the enzyme being produced but most were fully functional. So now it is possible to use laundry detergent and bleach at the same time and still remove protein-based stains which leads to more efficient cleaning altogether.

From this example, we can conclude that modifying enzymes allow people to have the certain characteristics, some of which will improve cleaning chemically and some will reduce the need for as much detergent, or the temperature in which we wash our clothes, therefore saving energy.

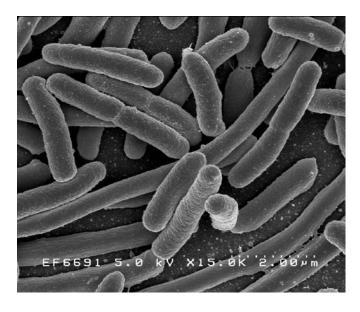
https://upload.wikimedia.org/wikipedia/commons/e/e6/Cyclooxygenase-2.png



#### **HOW MICROBES ARE USED TO CLEAN WASTE?**

Microbes are nature's ultimate garbage disposal, devouring the dead, decomposing and inert material that litters Earth's surface. The concept is called bioremediation, and it involves using organisms that either naturally love to eat contaminants or have been genetically altered to give them the taste for toxins. Scientists are designing or deploying microbes to purge sites of contaminants such as PCBs, oil, radioactive waste, gasoline and mercury, and new bioremediation research appears regularl

A widely used approach to bioremediation involves stimulating naturally occurring microbial communities, providing them with nutrients and other needs, to break down a contaminant. This is termed biostimulation. Biostimulation can be achieved through changes in pH, moisture, aeration, or additions of electron donors, electron acceptors or nutrients. Another bioremediation approach is termed bioaugmentation, where organisms selected for high degradation abilities are used to inoculate the contaminated site. These two approaches are not mutually exclusive- they can be used simultaneously.



Free picture from:

https://upload.wikimedia.org/wikipedia/commons/3/32/EscherichiaColi\_NIAID.jpg

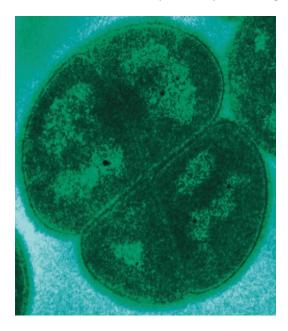
#### **DEINOCOCCUS RADIODURANS**

Deinococcus radiodurans is an extremophilic bacterium, one of the most radiation-resistant organisms known. It can survive cold, dehydration, vacuum, and acid, and is therefore known as a polyextremophile and has been listed as the world's toughest bacterium in The Guinness Book Of World Records.

D. radiodurans was discovered in 1956 by Arthur W. Anderson at the Oregon Agricultural Experiment Station in Corvallis, Oregon. Experiments were being performed to determine whether canned food could be sterilized using high doses of gamma radiation. A tin of meat was exposed to a dose of radiation that was thought to kill all known forms of life, but the meat subsequently spoiled, and D. radiodurans was isolated.

D. radiodurans is capable of withstanding an acute dose of 5,000 grays (Gy) of ionizing radiation with almost no loss of viability. A dose of 5,000 Gy is estimated to introduce several hundred double-strand breaks (DSBs) into the organism's DNA.

Several bacteria of comparable radioresistance are now known, including some species of the genus Chroococcidiopsis and some species of Rubrobacter. Deinococcus radiodurans also has a unique ability to repair damaged DNA. It isolates the damaged segments in a controlled area and repairs it. This bacteria can also repair many small fragments from an entire chromosome.



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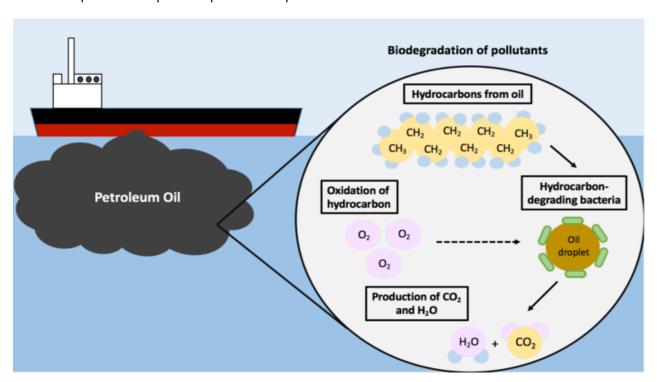
https://en.wikipedia.org/wiki/Deinococcus\_radiodurans#/media/File:Deinococcus\_radiodurans.jpg

#### **ALCANIVORAX BORKUMENSIS**

In our fossil-fuel age, oil spills remain a major problem. Petroleum oil is toxic for most life forms and pollution of the environment by oil causes major ecological problems. The total amount of oil lost to the environment through tanker incidents in 2016 was approximately 6,000 tonnes. Scientists developing cleanup strategies have looked to the microbes that thrive in the wake of such spills as one solution. Now, thanks to a detailed breakdown of one of the most effective of these oil-eaters, they are closer to having biologically based remedies for such environmental disasters.

Alcanivorax borkumensis is a marine bacterium that uses exclusively petroleum oil hydrocarbons as sources of carbon and energy. Its ubiquity, unusual physiology and demonstrated role in biodegradation show that it is globally important in the removal of hydrocarbons from polluted marine systems.

Vítor A. P. Martins dos Santos of the German Research Center for Biotechnology and his colleagues broke the marine organism's genome into more than 3 million base pairs and then pieced them together into a complete genetic map. That map contains several so-called islands that are unique to *A. borkumensis*, such as a set of genes that allow the organism to break down the alkanes in oil and use them as food. By sequencing the genome of this oil-eating microbe, the scientists hope to harness its power to help clean up future oil spills.



Free picture on: https://commons.wikimedia.org/wiki/File:Biodegradation\_of\_Pollutants.png

#### **HOW SCIENTISTS PLAN TO CLEAN UP PLASTIC WASTE?**

Scientists have worked out the best way of removing the millions of tons of plastic waste floating in the oceans. About 8 million tons of plastic waste such as food packaging and plastic bottles are being washed into the oceans each year. PET a plastic resin and the most common type of polyester. Two monomers—modified ethylene glycol and purified terephthalic acid—are combined to form the polymer called polyethylene terephthalate.

Motivated by the accumulation of PET in the environment, Shosuke Yoshida of Keio University and colleagues searched for a type of bacteria that is capable of digesting the plastic polymer. The researchers collected 250 environmental samples, such as soil and sludge, from the yard of a PET bottle-recycling factory and analyzed many different species of bacteria that were growing within the samples.

One new bacterium, which they named *Ideonella sakaiensis* 201-F6, could nearly completely degrade a thin film of PET after six weeks, at a temperature of 30°C. The 201-F6 strain of bacteria uses just two enzymes to "eat" PET. The first enzyme (called a PETase) breaks down PET into a compound called MHET. The second enzyme (called a MHETase) further breaks down MET.

But *I. sakaiensis* 201-F6 is only effective on land-based plastic pollution. PET's compounds are denser in water, which means they more easily sink towards the bottom.

The discovery of *Ideonella sakaiensis* has potential importance for the recycling process of PET plastics. The bacterium can currently break down a thin film of PET in a little over six weeks, so it is thought that any prospective applications in mass recycling programs will have to be preceded by enhancement of its abilities through genetic modification.



Free picture on: http://maxpixel.freegreatpicture.com/Plastic-Bottles-Recycling-Bottles-115071

### **Used materials:**

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- https://link.springer.com/referenceworkentry/10.1007%2F978-3-540-77587-4 89
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#### **PLASTIC**

The word "plastic" refers to a series of (usually synthetic) polymers, made up of chains of carbon-based monomers. It is one of the most common and used materials in the world, since it is light, inexpensive, durable and resistant. The most common types of plastic are polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), polyethylene or polyethene (PE) and polyethylene terephthalate (PET).



free picture at https://upload.wikimedia.org/wikipedia/commons/e/e6/WasteFinalDeposited.jpg

#### **DISPOSAL OF PLASTIC WASTE**

There are different ways to dispose plastic waste: storing it in landfills is the least convenient, given that plastic requires an extremely long time to degrade into the environment, from 100 to 1000 years. Moreover, the substances composing waste are not reused in any way, causing a waste of materials and energy [1].

#### **RECYCLING**

New objects are produced from waste through recycling.

Thermosetting plastics can only be ground and used as aggregates for new products, e.g. concrete or asphalt; thermoplastic materials, instead, can be given a new shape when heated and moulded. The main advantage of this disposal method is the production of plastic from plastic itself, without

using petroleum.

## **WASTE-TO-ENERGY**

This method allows to obtain electricity from waste: in the majority of waste-to-energy plants, garbage is burnt in order to heat water, which steam is used to run turbines. A large part of the process consists in managing the remaining – both solid and volatile – substances, some of which are toxic. This represents the major disadvantage of waste-to-energy, whereas its main advantages are the reduction of the amount of waste that is landfilled and the possibility to use the energy produced from plastic.



Free picture at https://upload.wikimedia.org/wikipedia/commons/a/ad/Oxodegradable\_plastic-

Logo.jpg

#### **BIODEGRADABLE PLASTICS AND BIOPLASTICS**

Plastic polymers are very resistant to environmental factors; biodegradable plastics contain additives that make them less resistant to sunlight and oxydation.

Given that fungi and bacteria are capable of decomposing organic molecules, attempts are being made to produce plastic polymers (called "bioplastics") that are based on natural polymers, e.g. cellulose, starch, lignin etc., in order to make them more suitable for microbial degradation [3],[4].

The best feature of these materials is the possibility to compost: they degrade in non-harming substances in a few weeks time.

Nevertheless, these "eco-friendly" plastics also have some disadvantages, for example there are less resistant to mechanical stimulation, or some of them release methane – a powerful greenhouse gas – while degrading.

#### **BIOREMEDIATION**

One of the most recent ways to dispose plastic waste is bioremediation, which is the removal of polluting substances from an area through microorganisms.

While recycling and using bioplastics can be efficient methods to prevent waste from being landfilled, bioremediation can solve the problem of environments that are already polluted.

Microbial degradation can be studied in various ways, of which each one leads to different results and has its own pros and cons, e.g. the reproducibility of experiments or the reliability of results [5].

#### **BIOREMEDIATION: POLYURETHANE AND ENDOPHYTIC FUNGI**

In 2008 a group of researchers from Yale University tested the ability of various microorganisms to degrade polyurethane (PUR).

59 endophytic fungi were grown on solid PUR medium. In the first assay, 18 of these organisms produced a halo of clearance on the surface of the growth medium, therefore, they have been screened in other assays.

In the first one, all 18 fungi could degrade solid PUR medium in test tubes.

The five most active fungi were further examined in other assays.

These other experiments showed that *Pestalotiopsis microspora* (E2712A) and *Pestalotiopsis microspora* (E3317B) are the quickest to degrade PUR as the sole carbon source in liquid cultures and the only two that are able to grow in anaerobic conditions.

Afterwards, it was discovered that the activity is extracellular (since a cell-free filtrate can break down the polymer) and also induced by the exposure to PUR - because the fungi which have not been grown on the polymer cannot degrade it.

The enzyme responsible for degradation is probably a serine hydrolase, given that the activity is stopped by a serine-hydrolase inhibitor.

These organisms, especially *Pestalotiopsis microspora*, can be one of the solutions to the issue of plastic waste disposal. In the future, other organisms could be found to be capable of clearing polymers that are even more resistant and dangerous than polyurethane [6].

#### **BIOREMEDIATION: PET AND BACTERIA**

As with polyurethane, various studies have been carried out about microorganisms which can degrade polyethylene terephtalate.

In 2016, one of the most significant experiments was performed by Dr. Yoshida and his team, who screened 250 plastic samples to identify PET-degrading microorganisms. In this way they discovered a new species, *Ideonella sakaiensis*, which was able to degrade a plastic film of 0.31 mg/cm<sup>2</sup> per day.

Two enzymes are involved in the degradation activity: ISF6\_4831 produces mono(2-hydroxyethyl) terephtalic acid through hydrolysis. The second enzyme, ISF6\_0224, transforms mono(2-hydroxyethyl) terephtalic acid into terephtalic acid and ethylene glycol, two non-harming substances [7].

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There are certain characteristics that water must have to be able to be used for domestic and industrial purposes. This includes not containing pathogenic organisms and it must be free from toxic substances and organic matter. However water does not always possess these things at the source (Rivers, streams, lakes or wells.)

### Coliform

Coliform bacteria are a commonly used indicator of sanitary quality of foods and water. Coliforms can be found in the aquatic environment, in soil and on vegetation; they are universally always present in the digestive tracts in animals, including humans. While coliforms themselves are not normally causes of serious illness, they are easy to culture, and their presence is used to indicate that other pathogenic organisms of fecal origin may be present. Such pathogens include disease- causing bacteria, viruses, or protozoa and many multicellular parasites.

A well-known coliform is E coli. E coli is rod-shaped and is considered to be the best species of coliform that can give an indication of whether pathogenic organisms are present. During rainfalls, snow melts, or other types of precipitation, E. coli may be washed into creeks, rivers, streams, lakes, or groundwater. When these waters are used as sources of drinking water and the water is not treated or inadequately treated, E. coli may end up in the drinking water.



http://www.popsci.com/scientists-design-genome-for-upgraded-e-coli

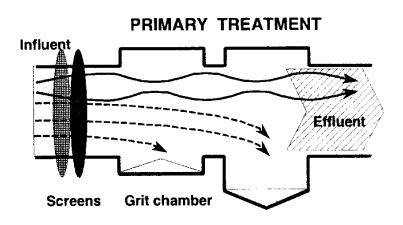
### Wastewater treatment process

Before treatment plants, wastewater would be drained into water ways. The volume of clean water in the systems would dilute the waste, and the microorganisms in the water would turn the sewage into new bacterial cells, carbon dioxide and other organic matter. However due to an increase in the human population, the demand for domestic use of water has increased, so the natural purification system has required industrial help.

Wastewater treatment is done in a series of steps that can have increasing effectiveness and complexity depending on the resources available. The normal sequence goes from primary, secondary, to tertiary treatment. The treatment of primary water can be different depending on the chemical and microbiology characteristics of the available water, and also the end properties of water that are required by the user.

## **Primary treatment**

As sewage enters a plant for treatment, it flows through a screen, which removes large floating objects that may block the pipes or cause damage to the equipment. For example cloth or sticks. After sewage has been screened, it will pass into a grit chamber. This is where sand and small stones settle to the bottom. However the sewage will still contain inorganic and organic matter, as well as suspended solids. A sedimentation tank will remove these minute particles by reducing the speed of flow so the suspended solid fall to the bottom of the tank and form primary biosolids (sludge). The biosolids that are removed from the tank and can be used as fertilizers, incinerated or put into landfills.



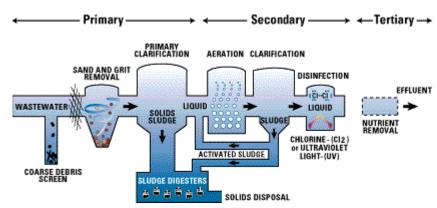
https://www3.epa.gov/npdes/pubs/bastre.pdf

Sedimentation tank

# Secondary treatment

Once the sewage has left the primary treatment stage it will be pumped into an aeration tank. This is where the sewage is mixed with air and sludge. The sludge has been mixed with bacteria. This mixing of materials will speed the process up because the bacteria will break down the organic matter that was still remnant in the wastewater into harmless by products. The sludge will now contain lots of extra useful bacteria and can be pumped back into the aeration tank to be mixed with new sewage. The treated waste water will flow into another sedimentation tank where the excess bacteria can be removed. For the secondary treatment to be completed, the water is then usually disinfected by killing pathogenic bacteria and odors with chlorine. 99% of bacterium can be killed via proper chlorination.

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