Safety

Safety in our lab work and in handling the mealworms *Tenebrio molitor* was a very important aspect of our project. From the very beginning, it was clear that only two team members were allowed in the lab. Therefore, we were following our Universities SARS-CoV-2 safety instructions. The two team members were also given a safety instruction regarding the measurements taken in the SARS-CoV-2 situation.

The two team members also had a safety training for the lab facilities before start working in the laboratory. This included the topics:

- Lab access and rules
- Responsible individuals
- Differences between biosafety levels
- Biosafety equipment
- Good microbial technique
- Disinfection and sterilization
- Emergency procedures
- Transport rules
- Physical biosafety
- Personnel biosafety
- Dual- use and experiments of concern
- Data biosecurity
- Chemicals, fire and electrical safety

Additionally to the safety training, the team members were always working with lab coats, safety goggles, masks and gloves, especially when handling the mealworms. An instructor was present and supervising the experiments.

Dr. Frank Bengelsdorf from the Institute of Microbiology and Biotechnology of Ulm University is our safety guide and overviewed the whole process of handling the mealworms, i.e. he controlled the way the mealworms are stored. Additionally, we are trained by experienced instructors who are familiar with the experimental procedures and the handling of invertebrates. This includes the insect husbandry, safely killing of the mealworms, and gut preparation.

For more information, please view our Safety Form.

Handling of organisms:

Each experiment with the mealworms was performed in a laboratory at biosafety level 2, in order to handle risk of the mealworms infesting the lab. In our project we used *Escherichia coli* [pMTL83151], *E. coli* [pJIR750_ac2t2], *E. coli* [pJIR750_ac3t3] and *E. coli* XL1 blue MRF`, as well as the mealworm *T. molitor.*

To ensure that environment was not exposed to possibly harmful organisms, every sample containing a genetically manipulated organism was collected in a separate garbage and autoclaved. Work with bacteria was carried out under sterile working conditions.

In order to minimize health risks when working with the mealworms, we were only handling the insects with dusk masks, gloves, and laboratory coats. In addition, for the duration of the project, the university has provided us with an S2 laboratory in which the mealworms were housed. We purchased equipment suitable for the keeping of insects (scales, containers, hygrometers, etc.) that was cleaned and disinfected before and after use. In this way, we could minimize the risk of an allergic reaction for our team members and all other laboratory staff.

In addition, the university and the laboratory are adequately equipped with hand washers and hand sanitizers. Every employee was of course informed that mealworms were in the laboratory for the duration of our project. Only one person from our team was working with the insects. This person is not aware of having any allergic reactions.

For more information, please view our Check- In form.

Ethics

As we were working with the mealworm *Tenebrio molitor*, we needed to submit a Check- In form. From the very beginning, we had an open communication with the iGEM Safety and Security Committee. They made us aware of many aspects of handling the mealworms and encouraged us to perform an extensive research on the risks, ethics and the appropriate measurements. Therefore, we were researching in EU guidelines and were contacting Prof. Dr. Wolf at our university, who had worked with mealworms before. Prof. Dr. Wolf shared his experience with us and we learned how to properly handle and safely kill the mealworms after use.

Over the course of the experiment, the mealworms were kept under appropriate living conditions.

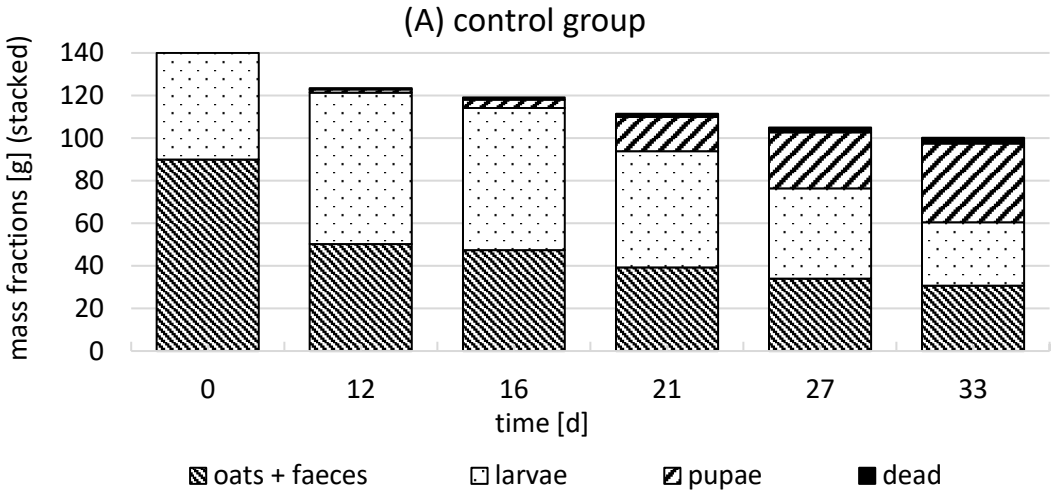
Pupations and escaped mealworms were killed by freezing them overnight in a -20 °C freezer.

Project description team UUlm

The inspiration for our project originated in a microbiology lecture by our PI, Prof. Dürre. We heard about tooth decay triggered by *Streptococcus mutans*. This bacterium deals with the hardly accessible apatite of the teeth by releasing acids that dissolve the substance. By forming holes and thereby increasing the surface area of the teeth, *S. mutans* can quickly destroy the chewing apparatus. As our future team leaders discussed the topic afterwards, they imagined a scenario in which a similar effect could be quite useful: plastic degradation. A great problem in that field is the high structural integrity of plastics and their small attackable surface area. If plastic eating bacteria could be equipped with the ability to excrete a solvent for the plastic, Katharina and Christoph thought, this could help them to degrade it faster. One plastic came to their minds that is especially prone to household solvents: polystyrene or PS for short. One of its solvents is acetone, which can be produced by bacteria such as *Clostridium acetobutylicum*. After a short research, they had found out where to look for PS degrading bacteria. As they searched for the topic online, they stumbled upon mealworms (the larvae of the beetle *Tenebrio molitor*) which are capable of PS degradation. Further research confirmed the thought that intestinal bacteria of the larvae were responsible for this ability. If these bacteria could be genetically modified to produce acetone, they may – just like *S. mutans* – be able to degrade their substrate better.

In 2020, the global pandemic of SARS-CoV-2 required everyone to comply with special safety precautions. One effect of these measurements was a very restricted and limited access to the laboratories at the university. Only initial pre-tests could be performed by the two students allowed in the lab. Nonetheless, important findings and experiences could already be gained. They will serve as a base for the continuation of the project in 2021. Namely one population dynamics study could be conducted on mealworms, the larvae of the beetle *Tenebrio molitor*. These arthropods possess the extraordinary natural ability of degrading PS with comparably high rates and serve as base of the project.

The population dynamics study was conducted to verify the polystyrene degrading ability of mealworms and learn about their handling. Two groups of mealworms were compared over the course of 36 days. One control group was fed with oatmeal and one test group was fed with a pure extruded polystyrene (EPS) diet. The compared parameters were the masses of five fractions: larvae, substrate (EPS or oatmeal), dead material, faeces, and pupations. The sections were divided by sieving and manual selection with tweezers. Fig. 1. shows the masses of the separated fractions.



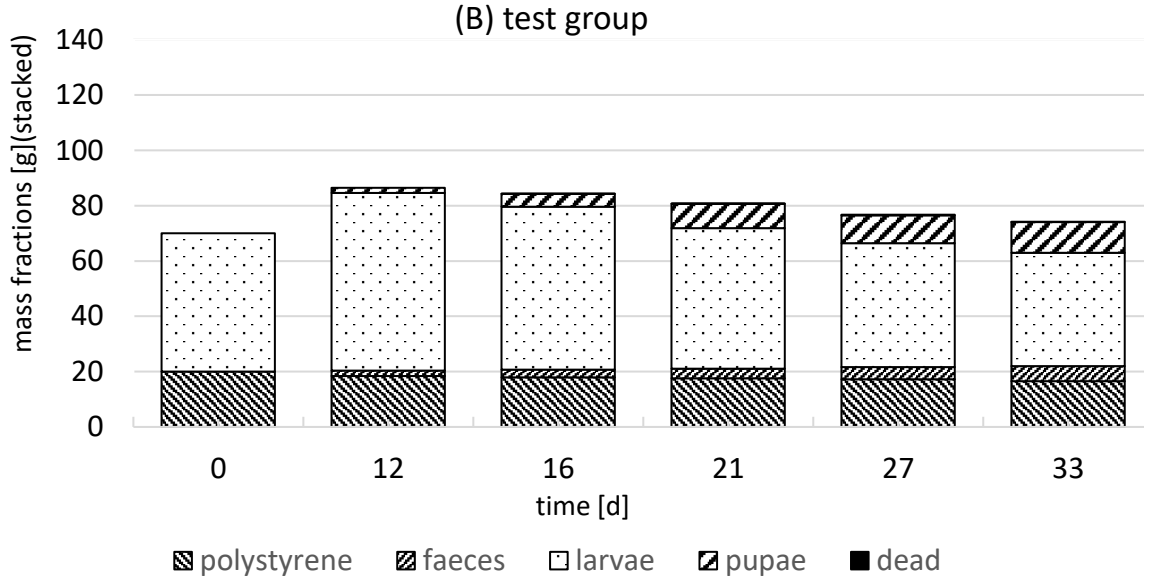
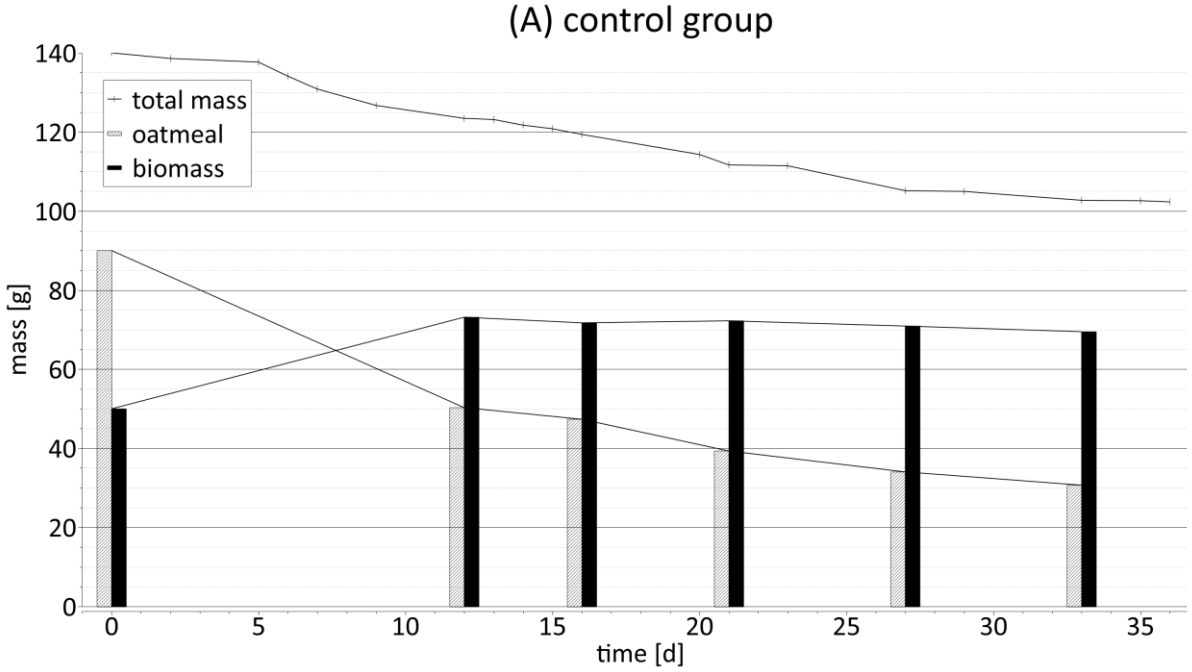


FIG. 1. *Population dynamics of* Tenebrio molitor *larvae. Control group (A) fed with oatmeal and test group (B) fed with extruded polystyrene. Mass fractions in stacked format: substrate (oats + faeces or extruded polystyrene), faeces, larvae of* T. molitor*, pupae of* T. molitor*, and dead mass including shed, parts of larvae or pupae and dead larvae or pupae.*

The total mass was measured by weighing the whole container and subtracting its empty mass. The total mass together with the substrate mass and summed up biomass (larvae, pupae, dead mass, and faeces) are depicted in Fig. 2.



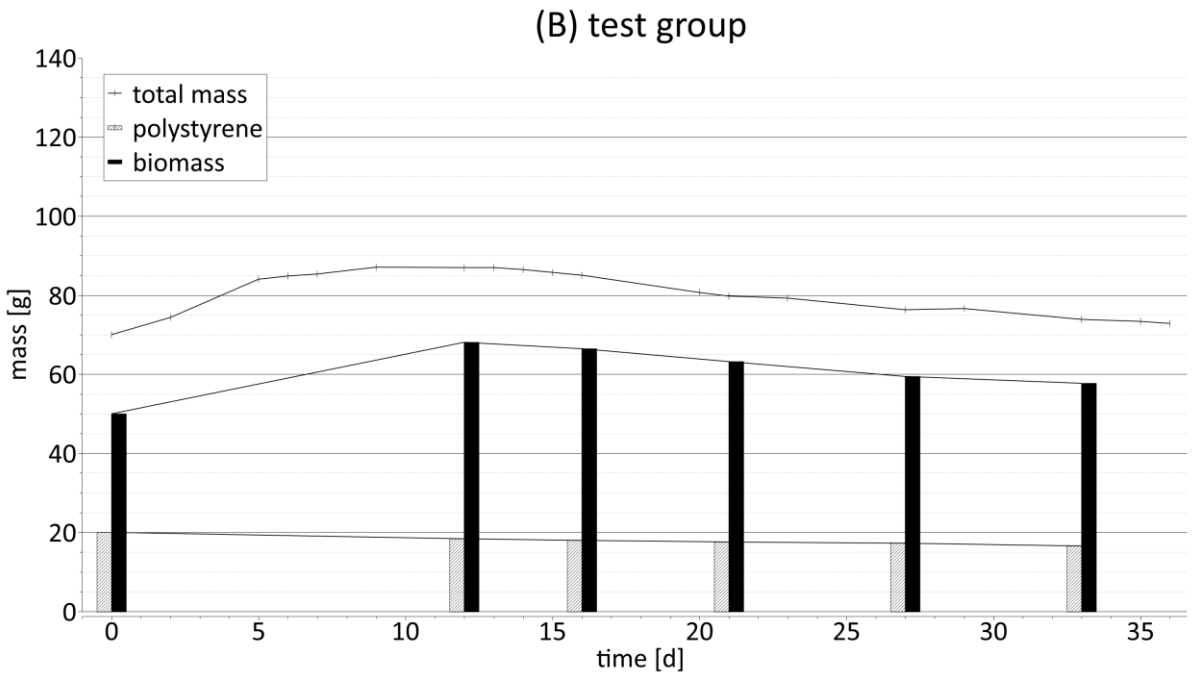


FIG. 2. *Population dynamics of* Tenebrio molitor *larvae. Control group (A) fed with oatmeal and test group (B) fed with extruded polystyrene. Total mass is determined by measuring the container and subtracting empty mass. Biomass as a sum of larvae, pupae, and dead material (A) and larvae, pupae, dead material, and feaces (B). Oatmeal includes feaces (A).*

From the resulting data, characteristic rates could be determined for the test and control groups. Therefore, a regression of linear area of the curves was performed to calculate the gradient. This gradient equals the respective change rates of the mass fractions. From this change rate, a relative change rate was determined by dividing the change rate through the initial mass of the used larvae, being 50 g for each group. The substrate change rate for the control group includes the change rate of the faeces, as they could not be physically divided and were measured together. Besides substrate change rates and larvae mass change rates, change rates for the total biomass were calculated by adding up the mass change rates of the larvae, the pupae and dead mass. These rates are stated in Tab. 1.

Tab. 1. *Characteristic change rates of control groups and test groups*

		Control group	Test group
Substrate change rate	[g/d]	-1,135	-0,082
Relative Substrate change rate	[1/d]	-0,023	-0,002
Larvae mass change rate	[g/d]	-2,03	-1,321
Relative Larvae mass change rate	[1/d]	-0,04	-0,026
Biomass change rate	[g/d]	-0,015	-0,709
Relative Biomass change rate	[1/d]	-0,0003	-0,014

In comparison to the relative substrate change rate of the oatmeal, the relative substrate change rate for the test group is about tenfold smaller. This small change rate indicates that even in the larvae, the degradation of PS is relatively low. Other research groups facing similar observations hypothesised that this is due to the disadvantageous unbalanced diet which only feeds the respective gut bacteria, but their energy yield is too small to provide enough calories for their

hosts.[1],[2] This hypothesis is encouraged by the overall loss in biomass. The mentioned research groups were able to compensate the effect by adding other, more accessible nutrients to the diet.[1], [2] For further comparison of the data, a starvation group of the mealworms would be helpful. Also, the residual undigested PS in the faeces and biomass in general was not measured, so the actual rate of degradation is probably even lower.

Parallel to the population dynamics study, acetone synthesis plasmids based on the work of our instructor M. Sc. Teresa Schoch were constructed. The aim is to enhance the ability of the gut bacteria by genetically modifying them to produce acetone. Acetone is a solvent for PS and weakens the polymer's intermolecular interactions. In theory, the presence of this chemical should therefore enlarge the attackable surface area for the bacteria and allow for a more efficient degradation.

The acetone synthesis plasmid carries the needed genes for the acetone production: encoding thiolase A (*thlA*), the butyrate-acetoacetate CoA transferase subunits A (*ctfA*) and B (*ctfB*), and the acetoacetate decarboxylase (*adc*). Acetone is produced from acetyl-CoA. The first step of this conversion is catalysed by thiolase A. Two molecules of acetyl-CoA are converted into one molecule of acetoacetyl-CoA. The transfer of coenzyme A (CoA) is achieved by the acetate/butyrate:acetoacetatyl-CoA transferase, which results in acetoacetate. The last step is carried out by the acetoacetate decarboxylase and is the elimination of CO_2. The product of this is acetone. The pathway is shown in Fig 3.

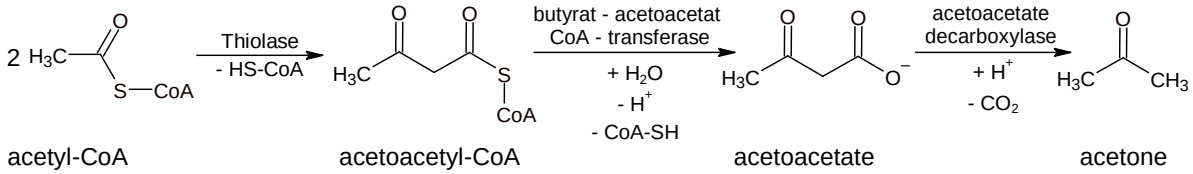


FIG. 3. *Acetone synthesis pathway. The synthesis of acetone starts with two molecules of acetoacetyl-CoA.*

The three genes mentioned above were obtained from two different plasmids (pJIR750_ac2t2 and pJIR750_ac3t3), which were already assembled by project instructor M. Sc. Teresa Schoch. These donor plasmids are variations of the gene constellations and gene origins on the same backbone. M. Sc. Teresa Schoch also designed the acetone synthesis plasmids for us, for which we are very grateful. For those designated plasmids, a pMTL83151 shuttle vector [19] was chosen as backbone. This backbone was originally obtained from Prof. Minton, University of Nottingham. It already contains resistance genes against chloramphenicol (*catP*), an origin of replication (*repH*), genes for replication in Gram-negative bacteria (*ColE1*) and genes enabling conjugation (*traJ*). The three genes obtained from the donor plasmids were combined with a *PthlA* promoter and inserted into the shuttle vector. The resulting plasmids are shown in Fig. 5.

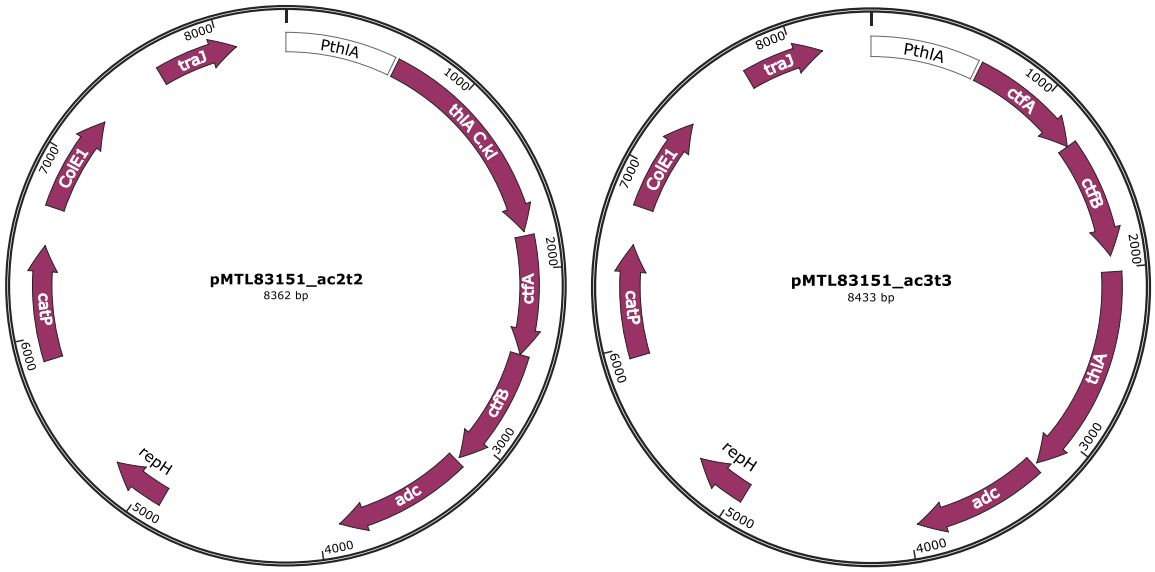


FIG. 4. *Acetone synthesis plasmid construction. Two plasmids with different gene constellations and gene origins. Genes assembled: PthlA promotor (*PthlA*), thiolase A (*thlA*), CoA transferase subunits A (*ctfA*) and B (*ctfB*), acetoacetate decarboxylase (*adc*), an origin of replication (*repH*), chloramphenicol (*catP*), genes for replication in Gram-negative bacteria (*ColE1*) and genes enabling conjugation (*traJ*).*

The plasmids were transformed into *E. coli* XL1 Blue MFR'. Unfortunately, sequencing the plasmids after isolating them from the transformed bacteria revealed that the targeted genes were not present. The experiment needs to be repeated to gain further information. Once the transformation succeeds, then the mutant strains can pass the acetone synthesis plasmid on to the intestinal bacteria. If they produce acetone, it can be examined if this has a positive impact on the PS degradation abilities. To learn more about the scientific work done and our findings, read **[Link: Engineering success]**. To learn about our future plans and the continuation of the project as well as all other parts of the project, please watch our **[Link: Team presentation video]**.

As a first participating team, the iGEM competition was especially hard to join for us under the special conditions of the year 2020. We still managed to participate and gain many valuable experiences. To give something back to the iGEM community, we decided to write a **[Link: beginner's guide]** for every team that wants to join in the following years. It is a summary of all the obstacles we were facing and figuring out ourselves the hard way. Hopefully, it will provide new teams the information they need and spare them the extensive research.

As the safety precautions were announced, we decided to focus more on other important aspects of our work. The **[Link: human practices]** are such an aspect. Besides the exchange with several experts in diverse fields that encounter plastics, our special focus was on the water cycle. Under the motto "it all comes back" we investigated the close loop from drinking to waste water and were invited to the Landeswasserversorgung Langenau, Germany that provides a whole region of Southern Germany with drinking water to discuss the impact of microplastics. Many other interviews and meetings are already planned. Besides exchanging with experts, we are eager to inform a broad public audience about the topic of our research. This is important because as mentioned earlier, plastics can be found in all our bodies. Besides spreading awareness and publishing what we are doing against plastic pollution, we also give tips on how everyone can reduce and avoid plastics in general. To spread this information, we maintain a series of social media channels:

[Links to all social media channels]

As we think that science and research are key to solving all of mankind's problems, including plastic pollution, we initiated a freely available series of fundamental life science info sheets called **[Link auf die website: iGEM explained]**. And as children are the scientists of tomorrow, we also published a series of free online video lectures on the same topic especially conceptualised for schools to be implemented into science classes called **[Link: iGEM at school]**.

Finally, to find out about how the plastic free future we dream about possibly looks like, read our **[Link: proposed implementation]** and watch our presentation video.

Team iGEM_UUlm

October 2020

References

[1] Yang S., Brandon A. M., Flanagan J. C. A., Yang J., Ning D., Cai S., Fan H., Wang Z., Ren J., Benbow E., Ren N., Waymouth R. M., Zhou J., Criddle C. S., Wu W. (2017). Biodegradation of polystyrene wastes in yellow mealworms (larvae of *Tenebrio molitor* linnaeus): Factors affecting biodegradation rates and the ability of polystyrene-fed larvae to complete their life cycle. Chemosphere, 191, 979-989, DOI: 10.1016/j.chemosphere.2017.10.117

[2] Weis M., Weis L. (2018). Tuning eines bioreaktors - Optimierung des styroporabbaus durch mehlkäferlarven. Jugend forscht

PROPOSED IMPLEMENTATION

This article offers a quick overview of possible implementations our project idea provides. The degradation of polystyrene into biomass and CO_2 with the help of *Tenebrio molitor* larvae is a promising option for improving plastic waste disposal at municipal waste management facilities. Our project idea could help to ensure that the public has access to a more environmentally friendly method of polystyrene disposal.

However, some prerequisites must be considered. Our concept is based on larvae which harbor genetically modified acetone-producing bacteria in their gut. Thus, the release or further use of larvae or beetles containing genetically modified organisms (GMOs) needs to be either legally permitted or prevented by physical barriers. A certain bureaucratic effort is needed to implement the approach at respective sites because the legal frameworks with respect to GMOs are different around the world. In addition, rather large installations are required to grow the larvae on polystyrene because of its low specific density.

The advantage of this biological polystyrene degradation compared to combustion (or final storage) is carbon fixation into biomass, instead of CO_2 (or microplastic) released into the environment. A further prerequisite before regionwide implementation at municipal waste management facilities would be legal framework to ensure considerable high prices for CO_2 emissions and plastic waste disposal.

An even simpler option could be accomplished through the isolation of the gut bacteria. Those are easier to handle and more scalable then arthropods. For their cultivation, fermenters could be used. This would enable fine control over the population and the degradation efficiency. Also, the CO_2 emissions could be further reduced, as bacteria are less dependent on complete oxidation of the carbon source in comparison to the insects. Furthermore, the produced acetone could be separated from the process, as it would not get used up. This chemical could be sold for various purposes and therefore would even allow for an economical profit.

Engineering success

Introduction

For the years 2020 and 2021 we, the team UUlm, have dedicated our project to the biological degradation of polystyrene. Polystyrene, from here on referred to as PS, is a synthetic polymer with versatile applications in our modern society. Like most man-made polymers, PS degrades very slowly in nature. Even in environments with high microbial activity activated sludge the polymer is highly recalcitrant to biological decay.[1],[2],[3] However, one environment has been identified where the degradation rate of PS is comparably high: the guts of the larvae of the beetle *Tenebrio molitor.* [3],[4] Responsible for this capability of the larvae are several strains of intestinal bacteria which were able to degrade PS. [5] With a genetic modification to hese bacteria, we intend to improve their ability to degraded PS.

History of polystyrene degradation research

Early investigations into the biodegradability of PS date back to the 1970s. James E. Guillet *et al*. belong to the pioneers of plastics biodegradation research. In 1974 they used ^{14}C styrene monomers to produce a radioactively marked PS vinyl ketone copolymer. After mixing the polymer into soil, mud, sludge, etc., its concentration could be determined by the dose of radioactive irradiation from the decay of C^{14}. Based on this principle, the amount of non- and partially degraded polymer in the soil mixtures was measured, as well as the amount of $^{14}CO_2$ from the sealed environment which was collected by a CO_2 absorbent.[1] The process of measuring and understanding the degradation has been refined ever since. Already in 1979, Kaplan *et al*. compared the ability of fungi, invertebrates and microbes to degrade PS. Their study showed that of the tested organisms only fungi and microbes had limited abilities of degrading PS.[2] In 2015, Yang *et al*. investigated the larvae of *Tenebrio molitor* Linneaus, which offer an environment with comparably high PS degradation rates. They could show with ^{13}C labelled PS that it was indeed used metabolically. This realization stemmed from an increase in ^{13}C fatty acids. They also showed this ability of the worms to be dependent on bacteria in their intestinal biome by feeding them antibiotics.[3],[4]

Possible improvement of polystyrene degradation

Currently, the most promising basis for a more effective biological PS degradation lays in the identified intestinal bacteria from *T. molitor* larvae. Inspired by the principle of exoenzymes secreted by bacteria, a possible way of improving the degradation efficiency was reasoned. The macromolecules that make up the polymer form dense structures and leave a relatively small surface area that can be attacked by the bacteria. If this surface area could be increased, in theory the bacteria could degrade PS more efficiently. As the stability of the supramolecular assembly of PS is only due to van der Waals forces and π-π interactions[7], it can be strongly influenced by the solvent system.[8] Acetone, for example, can dissolve PS.[9] Coincidentally reliable methods of acetone production by bacteria have been shown.[10] Consequently the respective bacteria, genetically modified to produce acetone, could have a significant advantage in breaking down PS. As the bacteria themselves would produce the acetone, the process would not be dependent on the addition of any other adjuvants. As acetone is just a solvent and would theoretically not be used up,

it could be dissipated and used elsewhere. A two-bacteria-system would be conceivable and could avoid the possible obstacles of gene transfer into the wildtype intestinal bacteria. However, the stability and reliability of the bacterial culture could be assured by implementing the acetone production into the same bacteria which degrade the PS. As they would have evolutionary benefits over other bacteria when living on a pure PS substrate, they could be relatively resistant against invasion of other pathogenic bacteria.

Population dynamics study

To verify the PS degradation by *T. molitor* larvae and to assess the actual rate of degradation, a population dynamics study was conducted with groups of 50 g of larvae. Two test groups fed with 20 g of expanded PS (EPS) foam flakes (Ø 1 cm^3) only was compared to a control group fed with 90 g of oatmeal. The EPS foam was obtained as standard packaging material and the oatmeal was obtained as standard grocery product from a local supermarket.

All larvae were cultivated in a two-barrier containment. The inner barrier consisted of a small high-density polypropylene (HD PP) drawer cabinet from the hardware store. This plastic compound was chosen for two main aspects: mealworms have been reported to feed on a variety of plastics. The HD PP was chosen to offer a longer durability when being attacked by the larvae compared to non-high-density plastics.[20] Also, the inside of the drawer was checked for smooth surfaces. This measure ensured the lack of structural weak points that would be readily accessible by the larvae's mouthparts. The structural integrity of the drawer cabinet was checked during every measurement point. The second aspect was the material property offered by HD PP to be resistant against most household chemicals and solvents such as acetone. The outer barrier was put in place for any event of failure of the first barrier. This second barrier consisted of a fully closed acrylic glass cabinet. As this cabinet was completly sealed to prevent any outbreak of the larvae, the ventilation of the entire unit had to be done manually by opening the door of the outer container.

The four groups (two test groups, two control groups) were separated into four drawers with altering sequence (from top to bottom: control 1, test 1, control 2, test 2) to exclude possible local differences of external factors. As both substrates contain little to no water, a small dish (HD PP) filled with demineralized water was placed into each drawer and steadily refilled to increase the humidity. After the first 24 h of the experiment, the humidity was identified as problematic. The larvae gathered around the water bowl where the relative humidity was higher than in the rest of the drawer. After spraying the content of the drawers evenly with demineralized water, the larvae spread over the entire content of the drawers over the course of one hour. As the water shortage could harm the larvae and lower their productivity, a humidifier was designed to keep the humidity constantly at a high level. The humidifier was constructed as shown in Fig. 1 and placed inside the outer containment. The time switch was adjusted to power the unit every 3 h for a duration of five minutes. The drawers were provided with small holes on the upper side of the walls to ensure air circulation. Furthermore, humidity sensors were placed in each drawer to monitor the humidity.

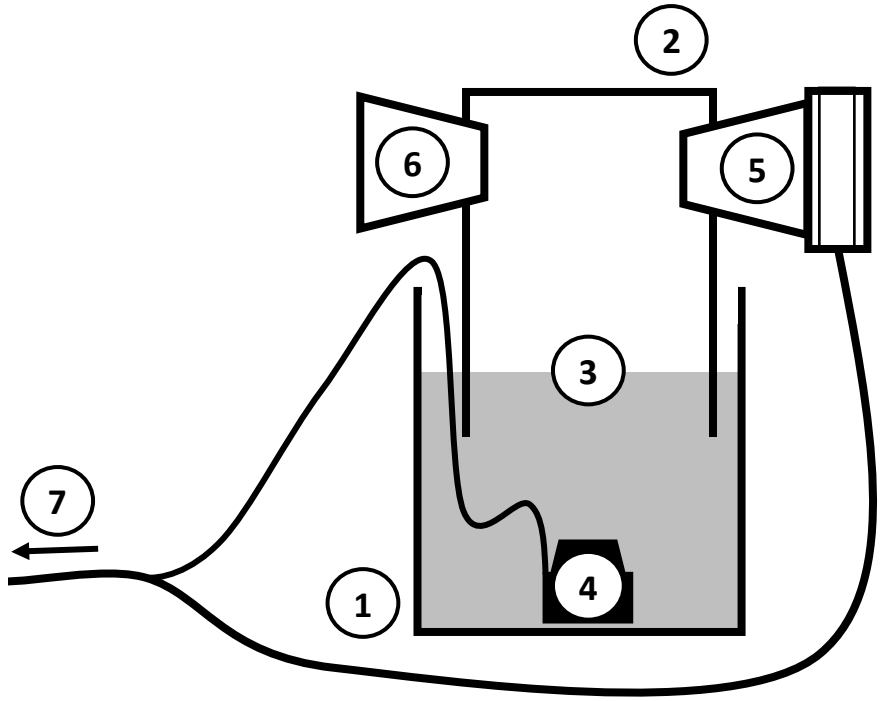
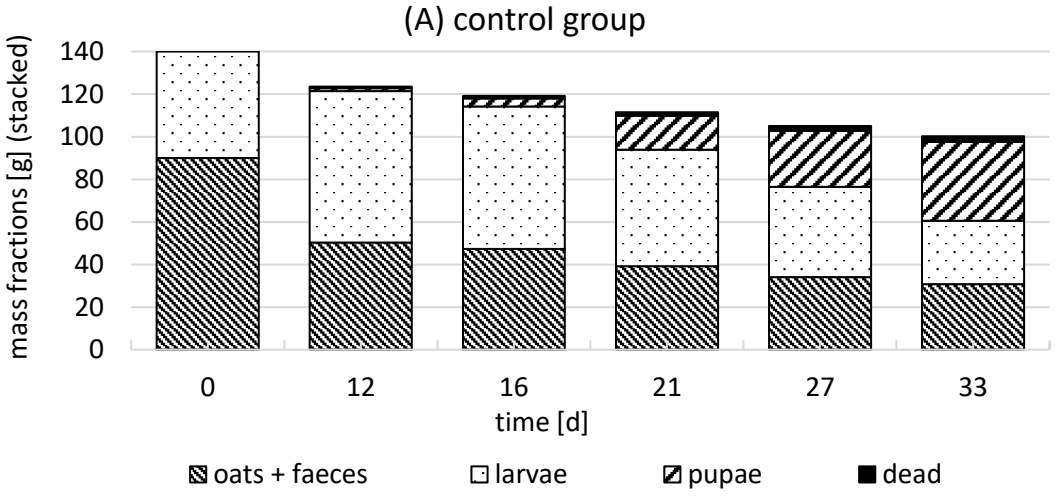


FIG. 1. *Air humidifier. Components: (1) outer beaker, (2) inner beaker, (3) water level, (4) ultrasonic "fogger" [power: 24 V * 1 A, fog production: 400 ml/h], (5) 24 V, 0.3 A fan and air inlet, (6) air outlet, (7) 24 V power supply with a time switch.*

After the installation of the humidifier on the fourth day of the experiment, an increase in humidity from approximately 30 % to 50 % relative humidity was displayed by the humidity sensors. Also, the larvae no longer gathered around the water bowls. The humidity remained at 50 ± 5 % during the rest of the experiment.

The relatively consistent humidity can be explained by the regular airing of the outer container. The humidifier itself was designed to be self-regulating: if the humidity is already high, the air becomes oversaturated more quickly and the formed fog is "heavier". This heavy fog deposits within the lower portion of the inner beaker and flows back into the outer beaker before it can reach the upper portion of the inner beaker and leaves through the outlet.



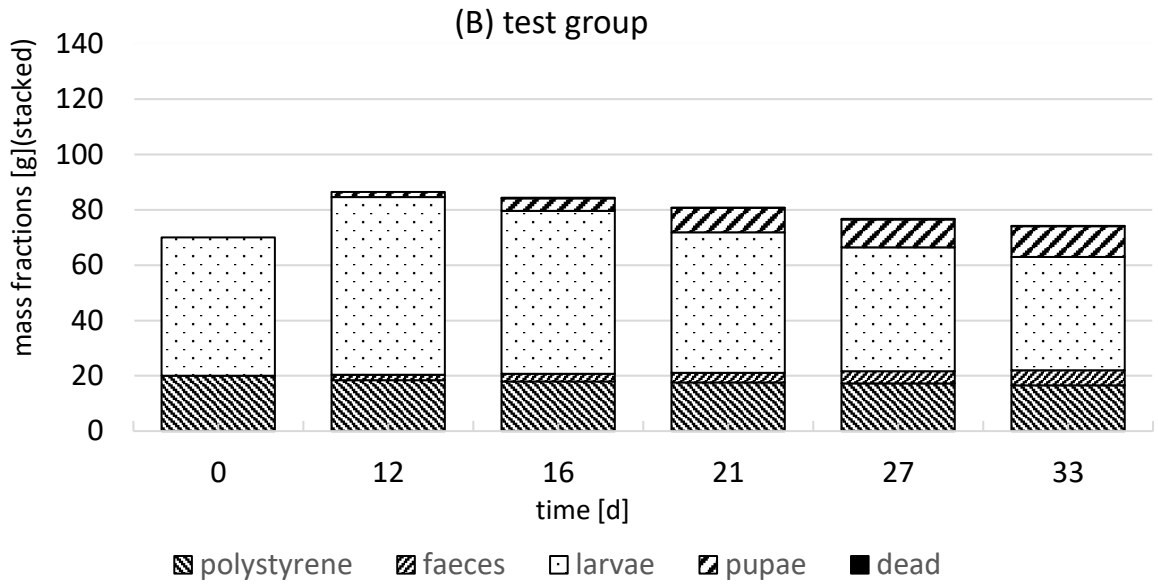


FIG. 2. *Population dynamics of* Tenebrio molitor *larvae. Control group (A) fed with oatmeal and test group (B) fed with extruded polystyrene. Mass fractions in stacked format: substrate (oats + faeces or extruded polystyrene), faeces, larvae of* T. molitor, *pupae of* T. molitor, *and dead mass including shed, parts of larvae or pupae and dead larvae or pupae.*

Over the course of 33 days, six measures were taken to determine the momentary composition of the drawers' contents. Therefore, sieves were used. The contents were separated into larvae, pupae, dead material (including shed, parts of larvae and pupae, dead larvae and pupae), faeces, and the substrate, being EPS or oatmeal. As the oatmeal disintegrated into particles of similar size as the faeces, they could not be separated and therefore are stated as one component. The masses of these fractions are depicted in Fig. 2. Besides the composition, the total mass of the drawers' contents was measured by weighing the drawer and subtracting its empty mass. These measurements together with a summed-up biomass (larvae, pupae, dead mass, faeces) and substrate mass (polystyrene or oatmeal, faeces) are shown in Fig. 3.

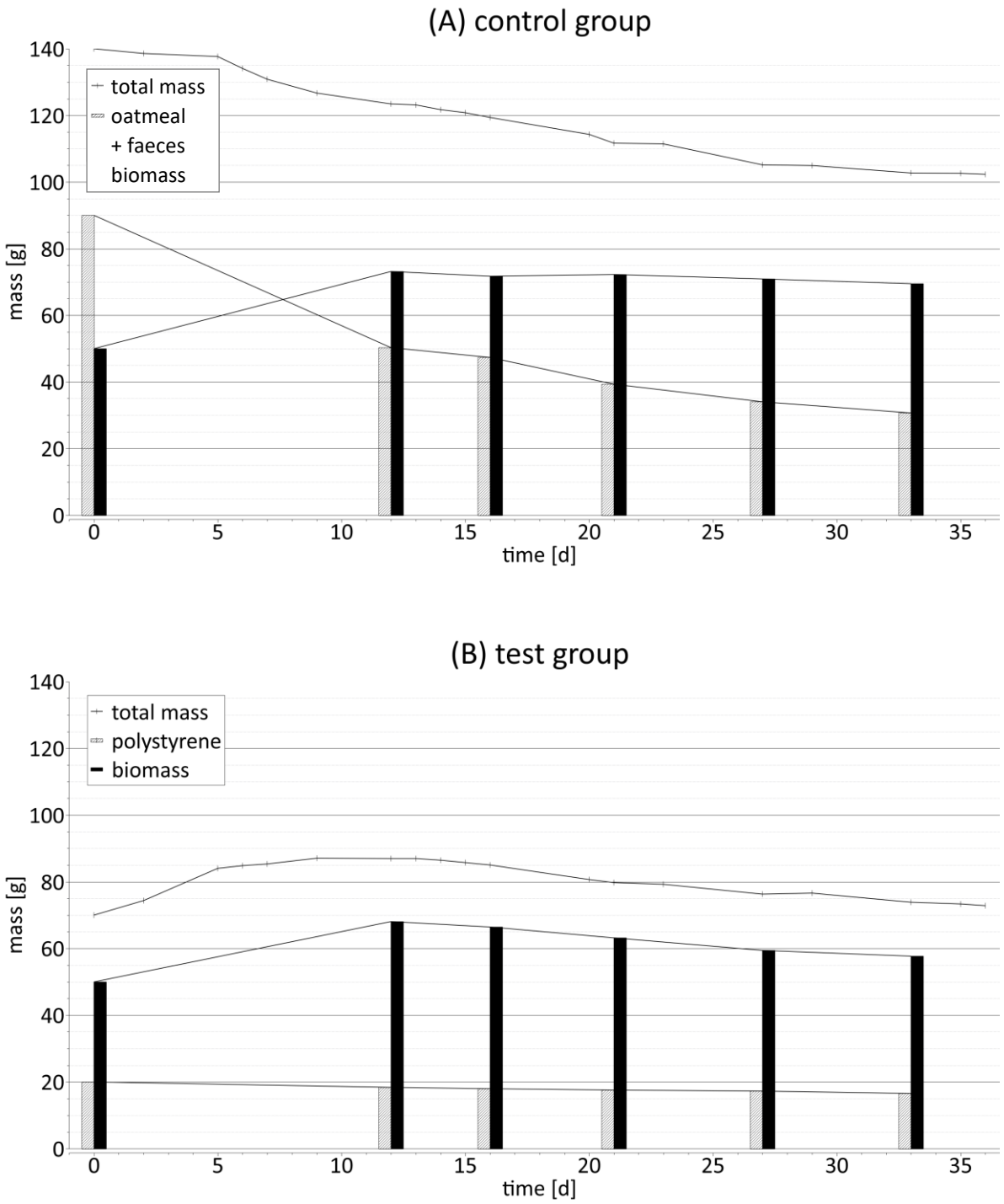


FIG. 3. *Population dynamics of* Tenebrio molitor *larvae. Control group (A) fed with oatmeal and test group (B) fed with extruded polystyrene. Total mass is determined by measuring the container and subtracting empty mass. Biomass as a sum of larvae, pupae, and dead material (A) and larvae, pupae, dead material, and faeces (B). Oatmeal includes faeces (A).*

From the resulting data, characteristic rates could be determined for the test and control groups. Therefore, a regression of linear area of the curves was performed to calculate the gradient. This gradient equals the respective change rates of the mass fractions. From this change rate, a relative change rate was determined by dividing the change rate through the initial mass of the used larvae, being 50 g for each group. The substrate change rate for the control group includes the change rate

of the faeces, as they could not be physically divided and were measured together. Besides substrate change rates and larvae mass change rates, change rates for the total biomass was calculated by adding up the mass change rates of the larvae, the pupae and dead mass. These rates are stated in Tab. 1.

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Biomass change rate	[g/d]	-0,015	-0,709
Relative Biomass change rate	[1/d]	-0,0003	-0,014

Acetone synthesis plasmid construction

To test if acetone has a positive impact on the degradation ability of mealworms (or rather their intestinal bacteria), certain modifications are planned. A recombinant *Escherichia coli* strain is to be created, containing an acetone synthesis plasmid. This plasmid bears the required genes for the acetone production: a thiolase A (*thlA*), an acetate/butyrate:acetoacetatyl-CoA transferase with subunits A (*ctfA*) and B (*ctfB*), and an acetoacetate decarboxylase (*adc*). Acetone is produced from acetyl-CoA.[10] The first step of this conversion is catalysed by thiolase A. Two molecules of acetyl-CoA are converted into one molecule of acetoacetyl-CoA. Next follows the transfer of CoA by the butyrate-acetoacetate CoA transferase, which results in acetoacetate. The last step is carried out by the acetoacetate decarboxylase and is the elimination of CO_2. The product of this is acetone. The pathway is shown in Fig 4.

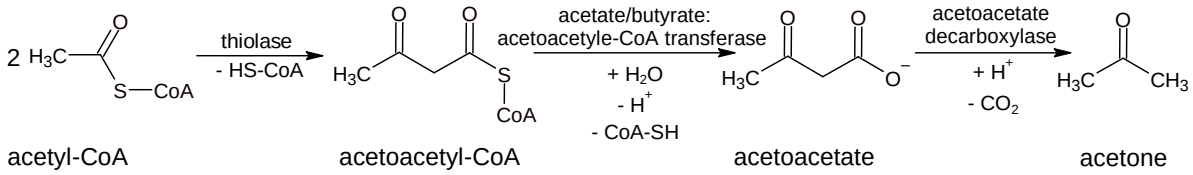

FIG. 4. *Acetone synthesis pathway. The synthesis of acetone starts with two molecules of acetoacetyl-CoA. CoA standing for coenzyme A.*

The three genes mentioned above were obtained from two different plasmids ([pJIR750_ac2t2] and [pJIR750_ac3t3]), which were already assembled by our project instructor M. Sc. Teresa Schoch. These donor plasmids are variations of the gene constellations and gene origins on the same backbone. M. Sc. Teresa Schoch also designed the acetone synthesis plasmids for us, which we are very grateful for. For those designated plasmids, a [pMTL83151] shuttle vector [19] was chosen as backbone. This backbone was originally obtained from Prof. Minton, University of Nottingham. It already contains resistance genes against chloramphenicol (*catP*), an origin of replication (*repH*), genes for replication in Gram-negative bacteria (*ColE1*), and genes enabling conjugation (*traJ*). The three genes obtained from the donor plasmids were combined with a *PthlA* promoter and inserted into the shuttle vector. The resulting plasmids are shown in Fig. 5. To confirm the insertion, an analytic digest was performed with the restriction enzymes *Sal*I and *EcoR*I. After amplifying them via PCR and running a gel electrophoresis, the expected bands on the gel indicated the successful

insertion. Via transformation, the two plasmids were transformed into *E. coli* XL1 Blue MFR'. After the transformed bacteria were plated on agar plates containing chloramphenicol and colonies were picked. A second analytical digest was performed similar too first one on plasmids isolated from the picked colonies , see notebook. The samples were sent to a commercial sequencing facility. After sequencing, the targeted genes could not be identified on the isolated plasmids. This experiment needs to be repeated. The source of failure has not been identified yet. One possible explanation is the partial destruction of the genes during the transformation.

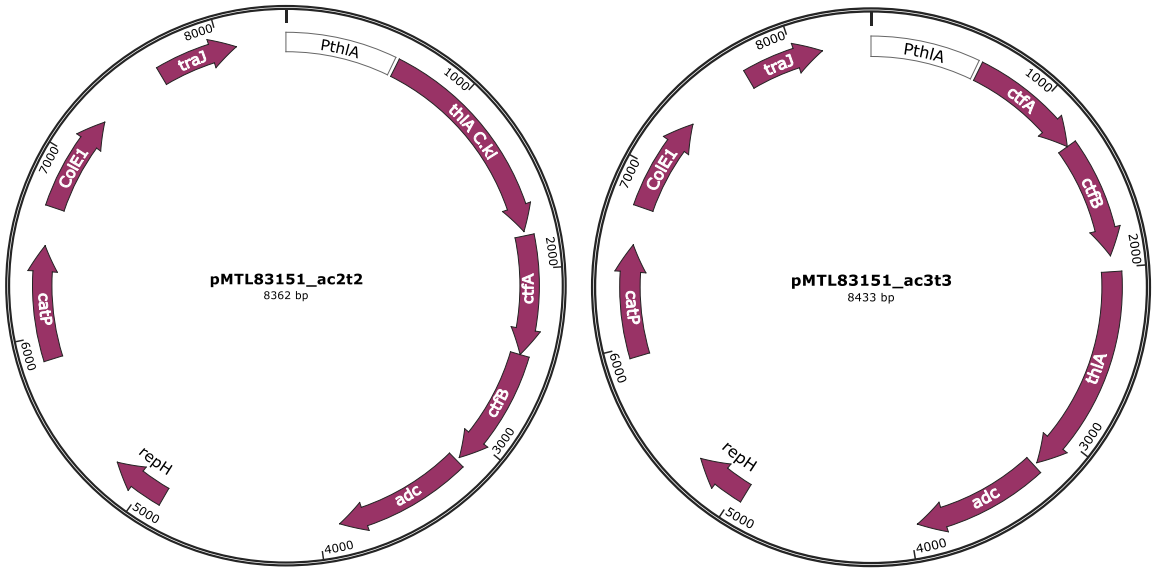

FIG. 5. *Acetone synthesis plasmid construction. Two plasmids with different gene constellations and gene origins. Genes assembled:* PthlA promotor (PthlA), thiolase A (thlA), CoA transferase subunits A (ctfA) and B (ctfB), acetoacetate decarboxylase (adc), *an origin of replication (*repH*), chloramphenicol resistance gene (*catP*), genes for replication in Gram-negative bacteria (*ColE1*) and genes enabling conjugation (*traJ*).*

Future plans

A possible next step is to isolate one or more bacterial strains with the ability to degrade polystyrene from the mealworm intestines. Tang *et al*. showed a very practical method for doing this.[5] To enrich the bacteria in the intestines, the mealworms were fed with polystyrene as a sole diet for 3 weeks. Then the guts were washed with basal medium. This suspension was plated on basal medium and incubated at 37 °C for 24 h under aerobic and anaerobic conditions. The microbes were transferred onto PS agar plates with yeast extract and incubated under the conditions stated above. The microbes were collected, and the pure colonies were preserved for the following experiments. To measure the degradation rate of polystyrene by these cultures a turbidity assay was used. [12] This is a qualitative and quantitative method because it can measure whether PS is degraded and how fast. An overnight culture was used and some additional medium containing a PS suspension was added. The resulting culture was incubated at 37 °C while simultaneously the PS concentration could be measured spectrophotometrically at 600 nm. To observe the degradation of PS, the measurement was repeated in defined intervals.

Also, the remaining PS concentration in larvae, pupae, dead material, and faeces needs to be measured. This is necessary to gain more information about the actual rate of PS degradation and for a better understanding of variations between differently treated larvae and the bacteria themselves. Besides the measurement of radioactively marked PS or isotopic distribution

measurements, either gel-permeation-chromatography (GPC) or turbidity systems were used by other research groups to determine these residues. As the analytical method needs to be reliable, easy, and safe to perform, lots of measurements, radioactively and isotopically labelled PS as well as GPC are unsuitable. The turbidity system is fairly convenient, but offers no exclusive sensitivity to PS. Therefore, the concentration is to be measured fluorometrically. PS fluoresces with a characteristic peak at 335 nm when dissolved in 1,2-dichloroethane and excited with ultraviolet light.[15] The reliability of this method with biological contaminants has to be tested.

Polystyrene degradation pathway

The ability to degrade synthetic polymers such as PS is most often shared by those bacteria which already degrade natural polymers such as lignin and cellulose. Especially lignin shows similarities regarding aromaticity. Both consist of aromatic rings linked with C-atoms and in the case of lignin with oxygen. The first step of degrading a polymer often is formation of monomers. This most certainly happens in the case of polystyrene too. How exactly the bacteria perform the cleavage of the PS polymer into monomeric units is not fully understood yet. Nakamiya *et al*. (1997) showed that the hydroquinone peroxidase from *Azotobacter beijerinckii* depolymerises PS in an artificial two-phase system.[15] This cleavage is depicted in Fig. 6.

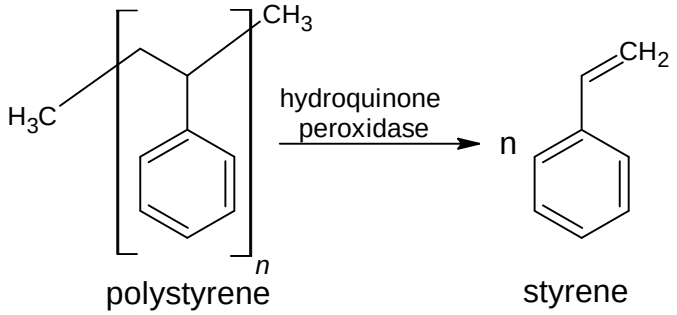

FIG. 6. *Suggested depolymerization step of PS with hydroquinone peroxidase.*[15]

For the degradation of styrene however, two pathways are largely known. One pathway leads from phenylacetic acid to acetyl-CoA and succinyl-CoA.[13] [14] This way is shown in Fig. 7. The second pathway proceeds via 3-vinylcatechol and ends with 2-vinylmuconate or with pyruvate and acetaldehyde.[13] This pathway is depicted in Fig. 8 and goes back to Mooney *et al*. (2006). [16]

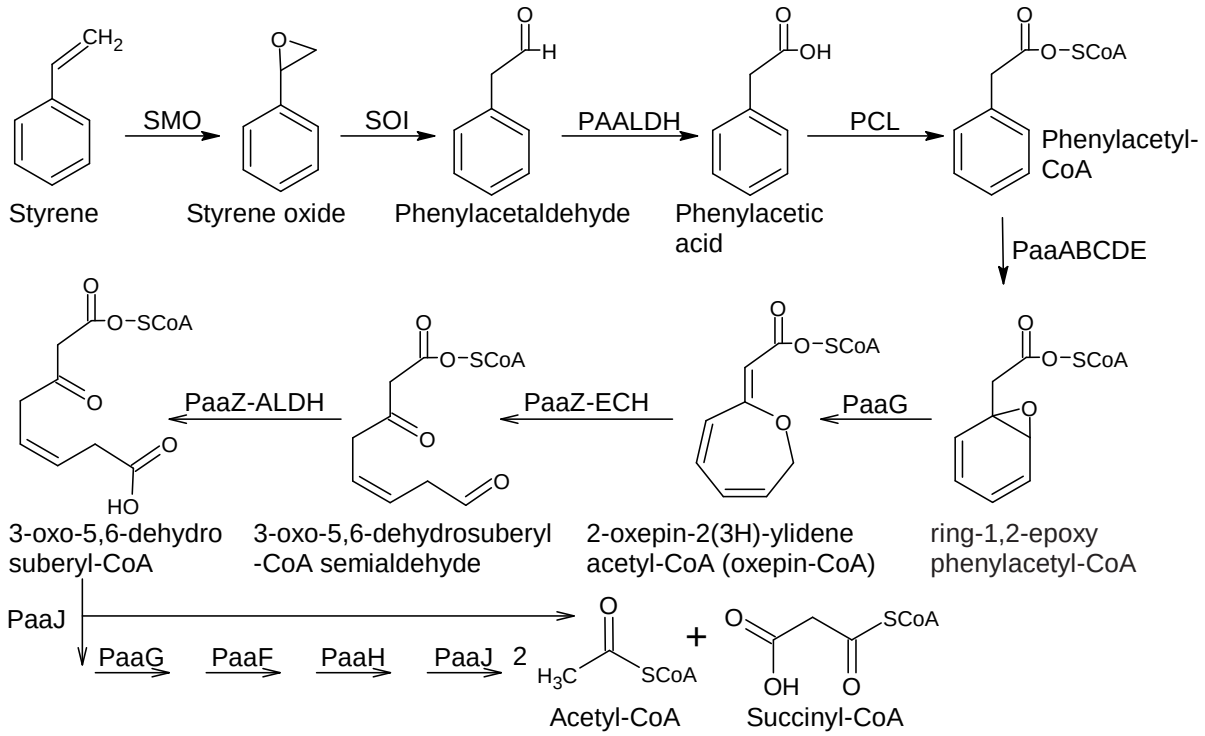

FIG. 7. *Suggested aerobic styrene degradation pathway by Teufel et al.[14] The enzymes shown are* styrene monooxygenase (SMO), styrene oxide isomerase (SOI), phenylacetaldehyde dehydrogenase (PAALDH), phenylacetate-CoA ligase (PCL), phenylacetyl-CoA 1,2-epoxidase (PaaABCDE), 2-(1,2-epoxy-1,2-dihydrophenyl)acetyl-CoA isomerase (PaaG), oxepin-CoA hydrolase (PaaZ-ECH), 3-oxo-5,6-dehydrosuberyl-CoA semialdehyde dehydrogenase (PaaZ-ALDH), 3-oxo-5,6-didehydrosuberyl-CoA thiolase (PaaJ), 2,3-dehydroadipyl-CoA hydratase (PaaF), 3-hydroxyacyl-CoA dehydrogenase (PaaH).

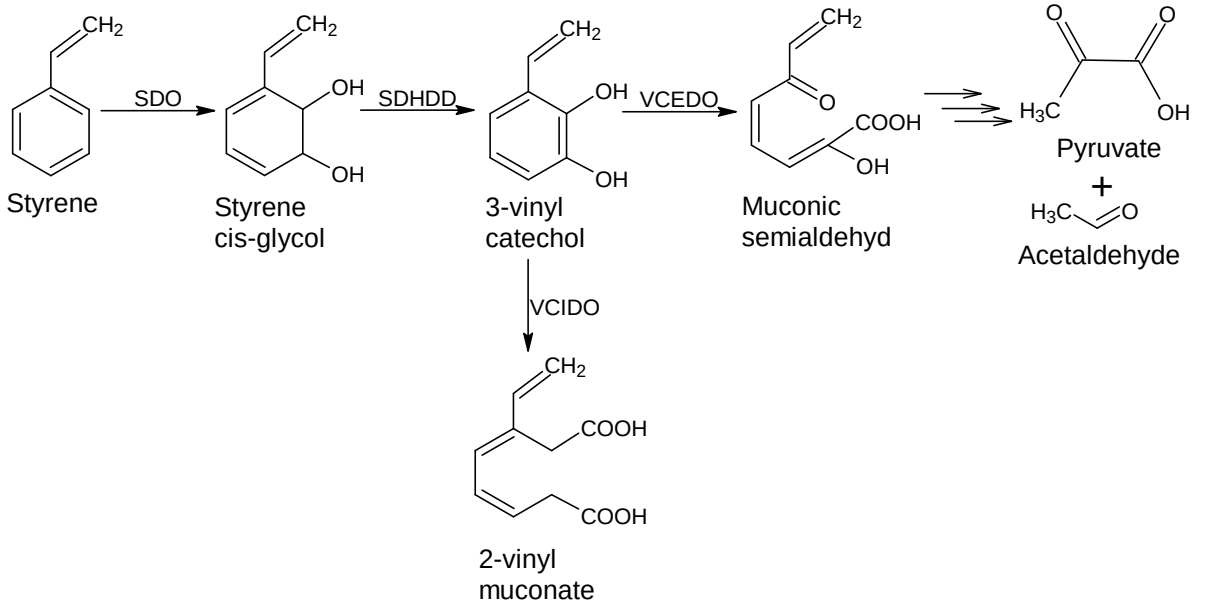

FIG. 8. *Suggested styrene degradation pathway by Mooney et al.[13] The enzymes shown are* styrene 2,3-dioxygenase (SDO), styrene 2,3-dihydrodiol dehydrogenase (SDHDD), 2,3-vinylcatechol extradiol dioxygenase (VCEDO) and 2,3-vinylcatechol intra-diol dioxygenase(VCIDO).

If the isolation of a bacterium from *T. molitor* fails, a second approach would be to build a bacterium that can degrade PS from scratch. In 2010, Teufel *et al*. showed that *E. coli* possesses the ability to degrade phenylacetic acid to acetyl-CoA and succinyl-CoA.[17] *Pseudomonas fluorescens* possesses the genes for the conversion of styrene over styrene oxide and phenylacetaldehyde to phenylacetic acid.[18] Thus, if it would be possible to transfer these genes from *P. fluorescens* to an *E. coli* strain, this *E. coli* would be capable of converting styrene into acetyl-CoA and succinyl-CoA. The only enzyme missing for the complete degradation of PS in this pathway would be one with the ability of depolymerising PS into styrene. *A. beijerinckii* posesses a hydroquinone peroxidase which can depolymerise PS.[15] With the respective gene transferred into *E. coli*, the resulting bacteria srain would theoretically have the ability of degrading PS completely.

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In the last few months, we managed to publish a few bits of our work.

In a [this video], we talk about how our project can contribute to the conservation of nature. This video was shot to participate in the Revive&Restore competition. Revive&Restore is an iGEM partner and in this year rewarded 12 teams with 5500 USD. In this video, we were able to collaborate with the Greenpeace Group Ulm/ Neu- Ulm and include their statement on the topic.

This video was published on our social media channels.

https://www.youtube.com/watch?v=LhA_aPgpQKk

We participated in the virtual German Meetup and had the opportunity to meet several other teams. For the Meetup, we prepared a [short video] about our project. This video was also published on our social media channels.

https://youtu.be/FEKCTqaHC50

Next to several other german teams, we had the chance to publish a short article in the journal BIOspektrum.

Our article is linked here: https://www.biospektrum.de/blatt/d_bs_pdf&_id=1765024 [1]

We were positively surprised about the interest from the public. After publishing the article, we received three responses to our project. One from students, who participated with a similar project in 2018 in a german student competition. They shared their results and experiences with us and were open to a discussion.

Another response came from a beekeepter, who observed the insects feeding on different plastics. He shared some pictures and information with us.

Lastly, another reader contacted us to let us know, that next to mealworms, other insects are capable of degrading polystyrene.

We are very thankful about the responses. Not only were people actually reading the article, it also led to a discussion amongst our team and different people, who shared the interest in our project.

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- **IGEM explained**

Since the beginning an integral part of our project was the education of our peers and raise more awareness for the variety of interesting topics in biology and especially synthetic biology. Therefore, we started our iGEM explained video series via our social media channels. We provided an overview about relevant lab methods, basic knowledge about topics such as immunology, virology, and the chemistry behind disinfectants, as well as specific information regarding our own iGEM project, that deals with mealworms. Through the variety of content that we provide as well as the media we chose we are sure that we were able to contribute to a better education regarding young potential scientists. Additionally, to check the acquired knowledge we provide tests on our website to review the most important facts. In the following figure you can get an idea of the iGEM explained series.

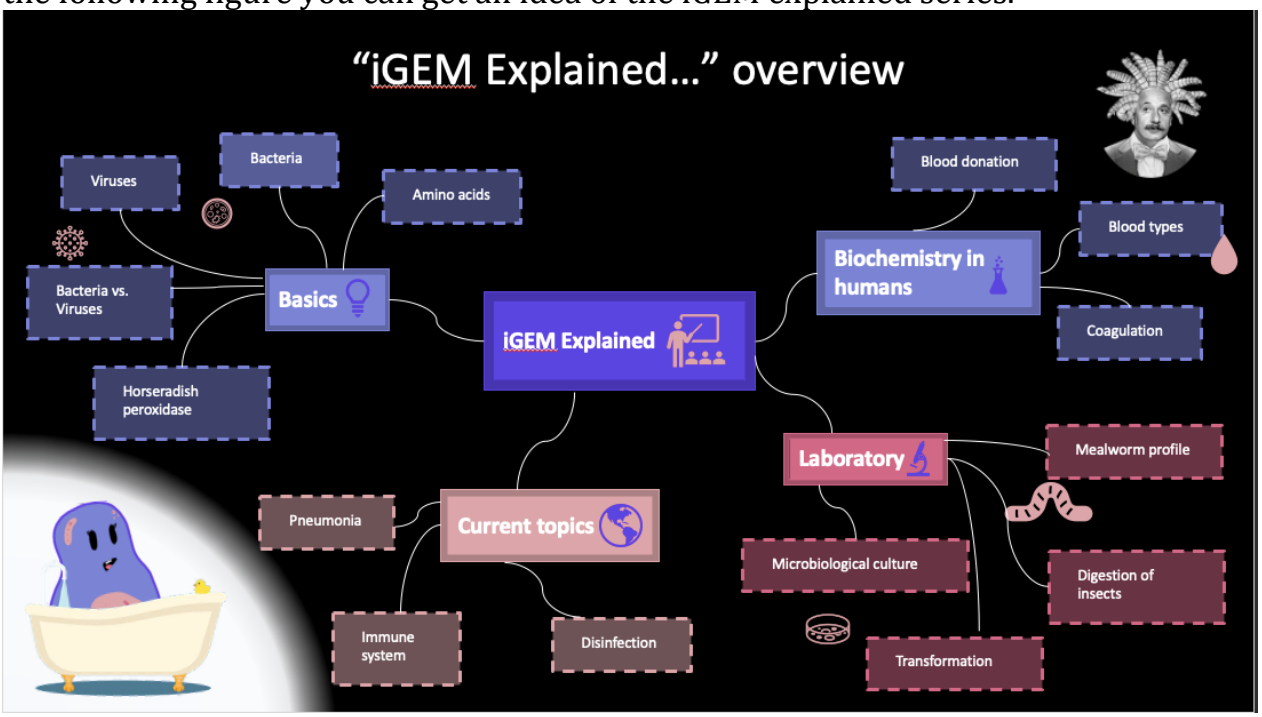

Figure 1: Overview on our iGEM explained topics

igem_uulm
Ulm

2/8

Gefällt 36 Mal

igem_uulm Today with our "iGEM Explained" we will focus on bacteria 🫦. For our research we will work with bacteria and genetically modify them... mehr

igem_uulm
Ulm

What are bacteria?

- Bacteria are microorganisms, i.e. living cells
- = Prokaryotes (gr.: before the nucleus)
  - Considered more primitive precursors of eukaryotes (gr.: with a nucleus), to which we humans and all higher organisms belong
- Bacteria is a word from old greek inferring „rod-like" as the first ones to be discovered looked like this
- They can grow to various sizes

Gefällt 36 Mal

igem_uulm Today with our "iGEM Explained" we will focus on bacteria 🫦. For our research we will work with bacteria and genetically modify them... mehr

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Ulm

What are they composed of?

- *Glycocalyx*: mucus layer for protection
- *Cell wall*: gives stability in various osmotic environments
- *Cell membrane*: allows transport, acts as a barrier
- *Pili*: adhesion, DNA Transfer
- *Flagellum*: allows propulsion
- *Lipid droplets*: act as nutrient storage
- *Ribosomes*: protein biosynthesis
- *DNA*: contains all crucial genetic information
- *Plasmids*: may contain genetic information useful but not essential to the bacterium, antibiotic resistance

Gefällt 36 Mal

igem_uulm Today with our "iGEM Explained" we will focus on bacteria 🫦. For our research we will work with bacteria and genetically modify them... mehr

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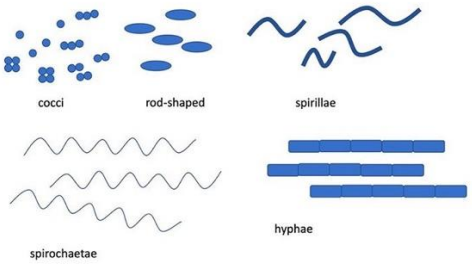
Ulm

How do they look like?



Gefällt 36 Mal

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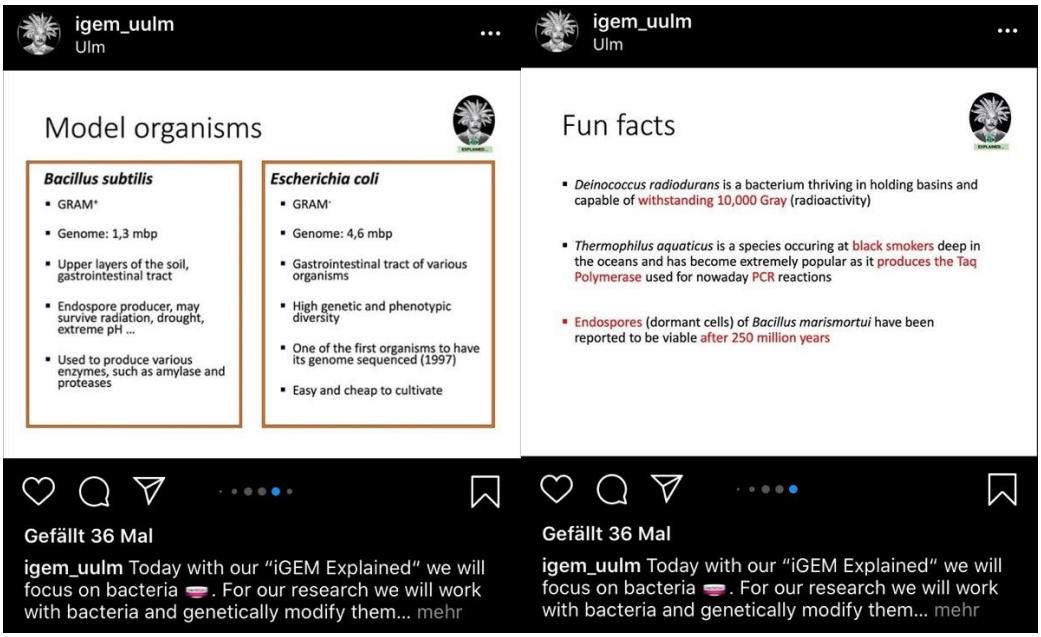
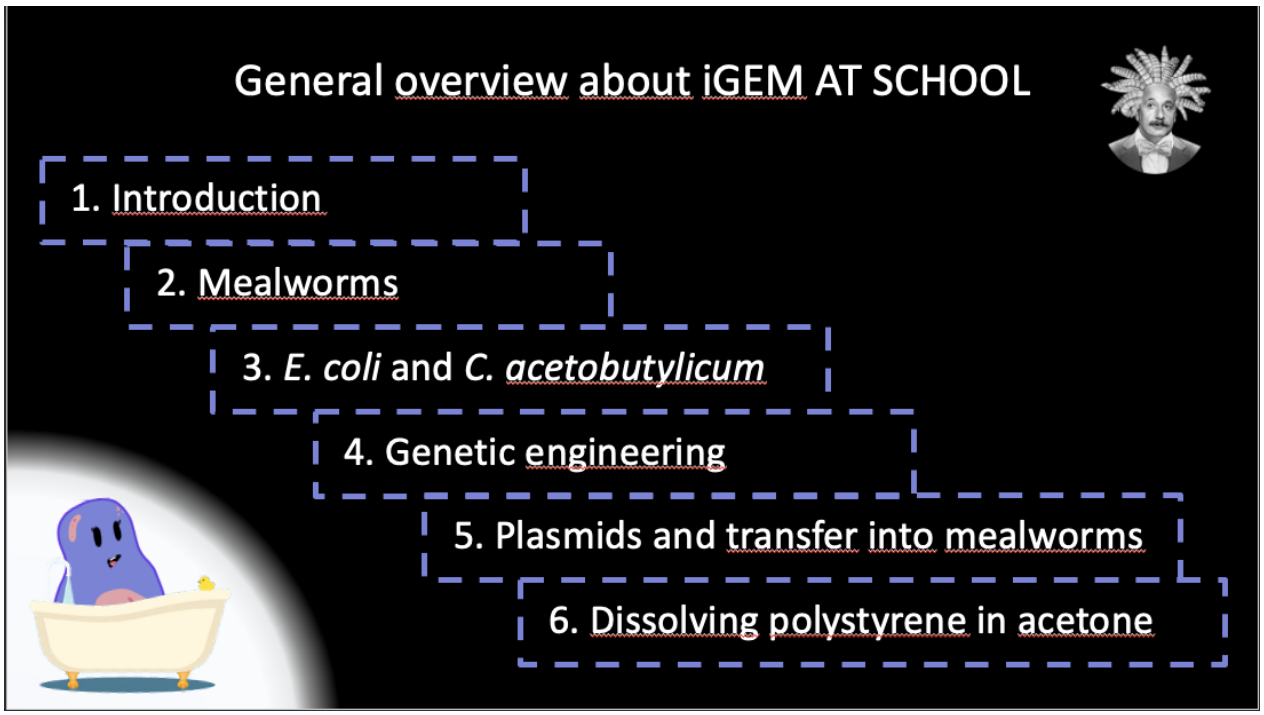


Figure 2: iGEM explained example from our instagram

- **IGEM at school**

Education is an important topic and due to the current SARS-CoV-2 situation schools and also the students are facing a hard time. Initially, we wanted to cooperate with schools in Ulm and hold lectures in class, which unfortunately wasn't possible due to COVID 19. Therefore, we designed an online lecture, which contains information about the iGEM contest, our specific topic, as well as the basic techniques in synthetic biology. Using this format, we wanted to make sure, that we are still able to reach students during the times of social distancing, and also we have the opportunity to reach a larger audience.
We recorded the lectures in multiple short videos, and also created a script for teachers with the links to the videos on our website, some tasks to review the learning process of the students and a feedback sheet so that we were able to gain knowledge on how to improve our contribution to the education process.

The videos are available on our website and on YouTube.



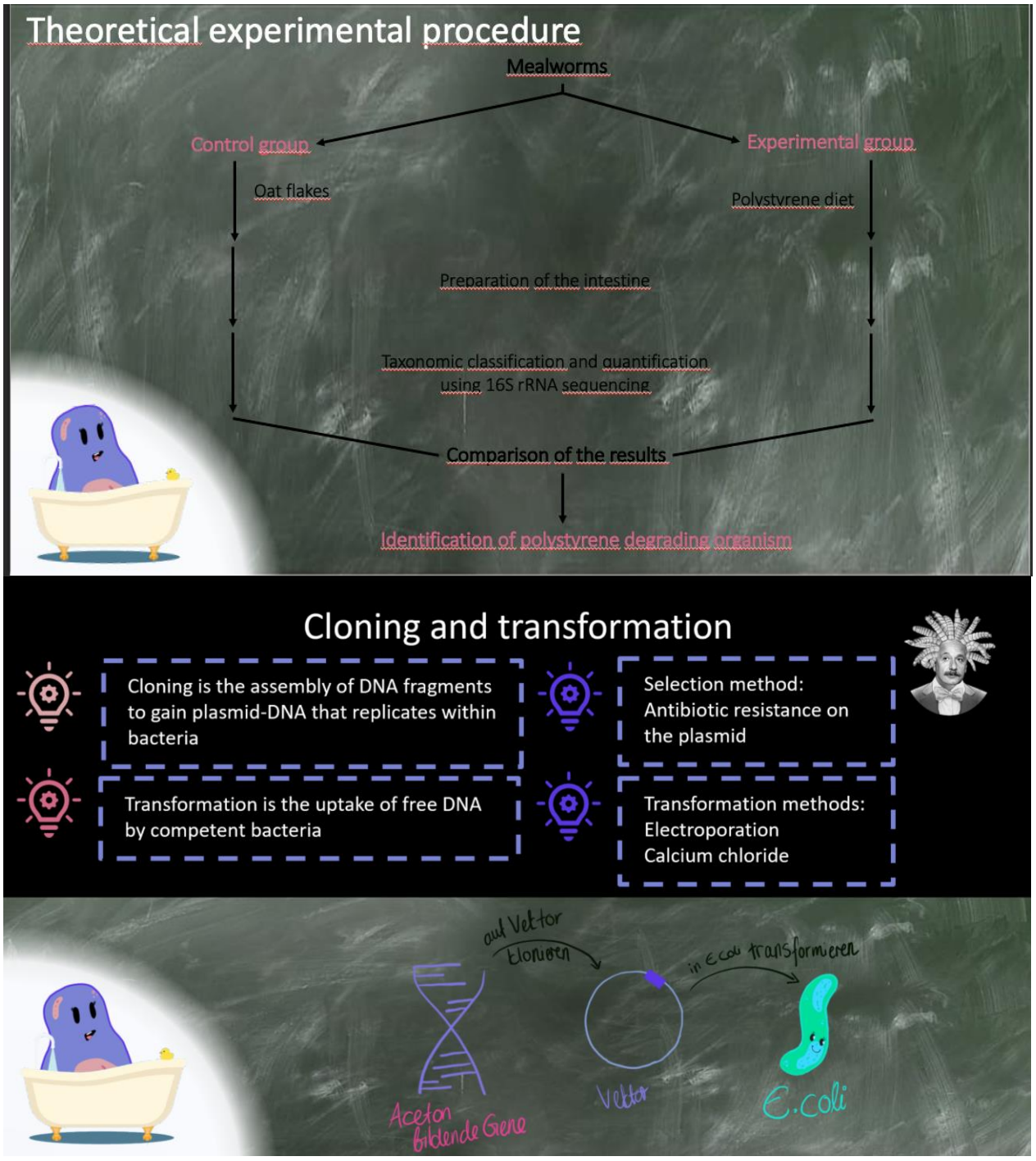


Figure 3: An overview about the topics on our iGEM at School lecture format. Detailed version is availabe on YouTube.

On our website there are lots of options to test your knowledge as well as playful options to gain more information about interesting microorganisms and their purpose.
We do have a bacteria quiz, where you can instantly check if your answers are correct, as well as a designed bacteria quartet that you can print out yourself and start a game with either family or friends, which is a great way to expand your

knowledge about various microorganisms and their properties.

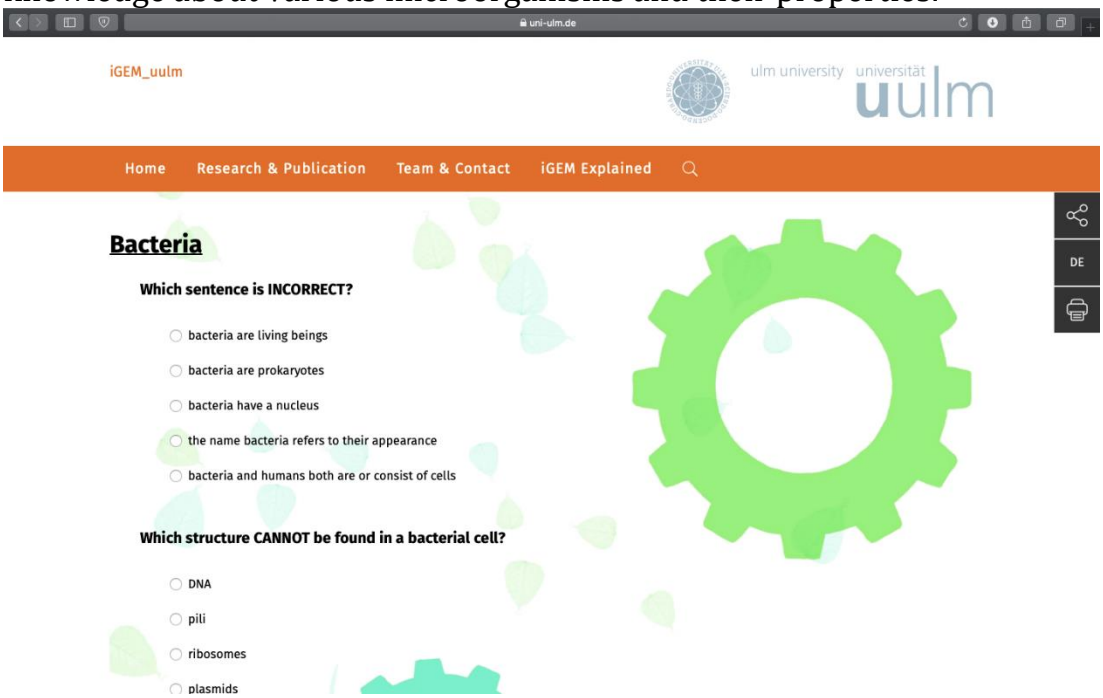


Figure 4: An example of the test questions available on our website

Figure 5: The iGEM explained is also on our website with a direct link to a quiz to review your knowledge

By offering material of different levels, we provided understandable content for people interested in science as well as professionals. Prior knowledge is not necessary to understand the provided content. With the variety of material, that we created, we are sure to offer fun ways to learn interesting things for every age.

iGEM social media

In our generation, social media are getting more and more important. During the lockdown in spring 2020 we experienced the chances of social media and video chatting to communicate within our team but also with other iGEM teams around the world. We think social media are a great way to educate and raise awareness for synthetic biology, since genetic engineering has become more controversial throughout society.

When we first started being active on social media, we choose to follow different strategies for different platforms. We compared aspects such as reach, number of followers, target group and defined goals for each platform. Our concept is shown in table 1 below.

Table 1: Comparison iGEM_UUlm social media accounts Facebook, YouTube, Instagram and Twitter under different aspects.

	Facebook	YouTube	Instagram	Twitter
Reach	Small and local reach	Small and local reach	International and larger reach	International and moderate reach
Typical follower/ target group	Family and friends Interested parties	German pupils/students Interested parties	Mixed Other iGEM teams International and local interested parties	Researcher Other iGEM teams Bioscience accounts
Age range	Especially over 30 years (75%)	Especially under 30 years (80%)	Especially under 30 years (90%)	Between 20 and 40 years
Our goal	Updates on our project	Educational input: “iGEM at school” Videos about research	International education: “iGEM Explained…” Insights into our work & research Communication with other teams Promotion within iGEM community Fun stuff	Updates on our project
Number of followers (October 2020)	22	19	314	61

After the first couple of weeks, we saw the biggest growth on our Instagram platform and hence decided to use it as our main platform within social media.

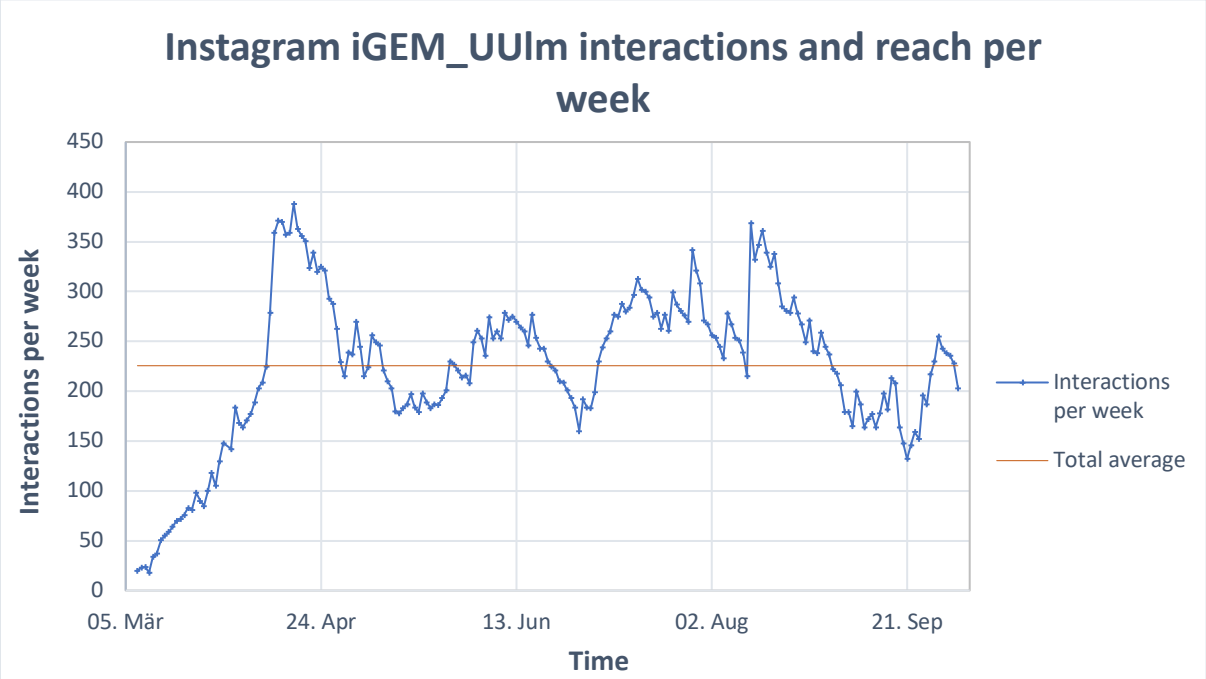
Instagram as the main platform

On Instagram, we want to share our project progress with followers and explain the theory of the research. This ensures that even people without a bioscientific background can keep up. We created the concept of "iGEM Explained…", where we outline biological topics illustrated with a short presentation including images and graphs. "iGEM Explained…" topics are explained from scratch and are suitable for non-professionals. However, even bioscience students can revise and fortify their knowledge with "iGEM Explained…". "iGEM Explained…" topics range from understanding the project idea and methods used to interesting and current topics concerning biochemistry in humans or SARS-CoV-2.

To achieve that people face up with science and build their own opinion about synthetic biology we provide them with basic knowledge to awaken their interest and break down complex processes into easy-understanding issues. To ensure consistency and a continuous learning process, every third post on our Instagram account is an "iGEM Explained…" post. Fun quizzes to control learning achievements take away the pressure one would find in a school or college. Spreading knowledge with Instagram is a casual way of learning new things without the need of a lecture.

Exchanging with other teams in the iGEM community is also very easy on social media. Talking with others about collaborations, research progress, feedback or challenges lifts the mood during lockdown and exam periods.

Since March 2020 we have documented our weekly interactions on Instagram. The correspondent graph is shown below.



Graph 1: Statistics of our Instagram account. Interactions and reach per week are plotted against the time.

On average we interacted with 225 accounts on Instagram weekly.

Educational videos and research information on YouTube
YouTube is the main platform where we share videos about the project design and our research background.

To support online teaching in German schools we produced the “iGEM at school” series. We explain biological methods like transformation and conjugation with the example of our research project. We aimed at middle and high school classes in the surroundings of Ulm. That is also the reason why the “iGEM at school” series is in German. All video presentations are available in our “iGEM at school” playlist on YouTube.

Project updates via Facebook and Twitter
We used Facebook and Twitter to keep our followers up-to-date on our projects. By giving basic information and sharing links to our posts on Instagram and videos we provided our audience with the latest updates. It is important for us to cover as much age groups and target groups as possible. Facebook enabled us to reach even older people, who might not be active on Instagram.

iGEM Collaborations

1. Instagram “This or That Challenge – Lab Edition“ by iGEM_UUlm

During the lockdown we created a template which can be used by other teams to connect and exchange on Instagram. With our “This or That Challenge” we tried to generate and keep anticipation for laboratory, even though the universities in many countries were closed. This situation was very frustrating for all of us. With the use of our collaboration we wanted to keep a piece of lab in our minds and hearts.
On the other hand, we aimed to entertain the iGEM community with “This or That Challenge- Lab Edition”, since the template and the possible answers can be quite stereotypical.
We are glad for the great response from many other iGEM teams and we want to thank you for joining us!

Joined teams:
igem_iisertirupati, India
igem_iisermohali, India
igem_iiserk, India
igem_bits, India
igem.thessaloniki, Greece
igemthessaly, Greece
igempatras, Greece
igemthrace, Greece
igemstockholm, Sweden
igem.kaiserslautern, Germany
igem.aachen, Germany

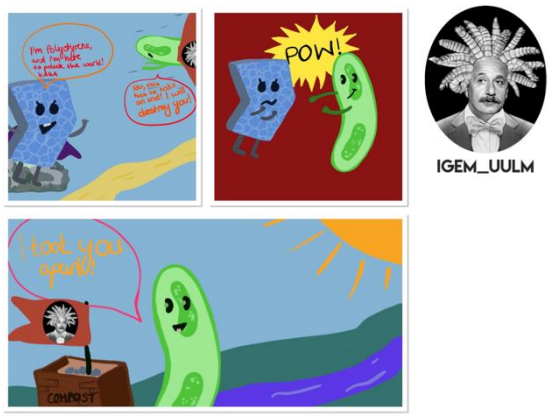
2. iGEM EXPLORE by iGEM UUlm

The aim of our collaboration was to connect with other teams, share scientifically interesting questions, and promote the iGEM contest and other teams.
The participants had to create a short and interesting video and/or a presentation about their research. These were uploaded to a cloud, which is accessible by all participating teams.
Each team then shared at least one post from another team on social media to promote that team.
A big thank you to all participating teams.

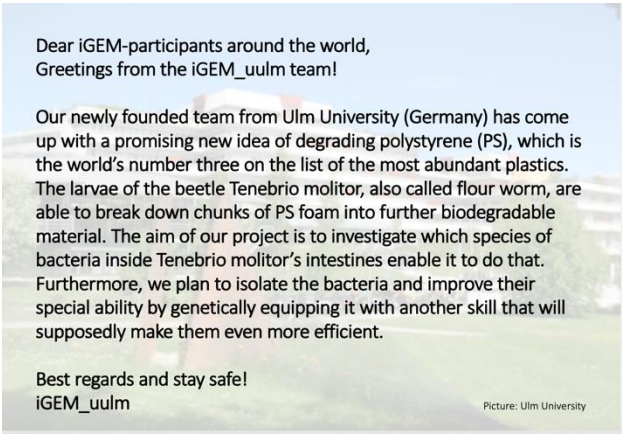
Joined teams:
igem_iiserp, India
igemrum, Puerto Rico
igemncku, Taiwan
igemhamburg, Germany
msp_igem, Netherlands

3. "Postcard project"– Team Duesseldorf

The iGEM Team Düsseldorf reached out to us offering us the chance to participate in their postcard challenge. Their idea was that every participating team would design an individual postcard either referring to the team's project or synthetic biology, as well as a short text about them. As many of the designed postcards as teams are participating in this collaboration should then be send to the iGEM Team Düsseldorf, who made sure that the sent in postcards were distributed to all participating teams to connect with each other. Of course, we did not want to miss this chance and submitted our designed and handcrafted postcards and waited for the postcards from all around the world. In the following you can take a look at our postcard design and the postcards we received from the other teams.



Postcard front side



Postcard back side

4. "Journal initiative"- Team msp_igem, Netherlands

iGEM team MSP-Maastricht gave us and many other teams the opportunity to learn how to write an abstract and scientific paper with their collaboration. We are happy to be part of it. iGEM team MSP-Maastricht provided us with a master for the paper with information what to write in which part, for example in an "abstract", in "results and findings" or in a "discussion". For the "journal initiative" our team wrote an abstract and a paper, as well as took part in their peer review sessions. After the peer review, we received feedback and helpful hints.
Unfortunately, we could not submit a final version of our paper and had to withdraw our participation. Nevertheless, the team from Maastrich was very interested in letting us take part in the collaboration. So, we wrote a report about the collaboration for the journal. We are very grateful for the consideration, the flexibility and the willingness to compromise.
All in all, the "journal initiative" was a great idea and exercise for us to learn and improve scientific writing skills. Thanks to iGEM team MSP-Maastricht for their excellent organisation and the creation of a platform!

5. collage of iGEM team´s logos -Team GW_DC, Washington

The team from Washington DC created a collage of all iGEM team's logos. With this collage they wanted to show that, despite the competition, we all have the same goal: trying to find solutions for serious problems to make the world a better place. We could identify with that directly and it was clear to take part.

6. youtube channel - MRIIRS_FARIDABAD, India

The team MRIIRS_FARIDABAD started a YouTube channel to share videos from different iGEM teams. Our team submitted a video about our project for this channel. It is important for us to spread awareness about synthetic biology and to show everyone what is possible by using synthetic biology.

2020

A year full of crises, uncertainties, fears and worries. Natural disasters, political challenges and of course the global SARS-CoV-2 pandemic kept the world in suspense this year. The pandemic continues to pose a major social, medical and economic challenge in many regions of the world.
As a team that is participating in the iGEM competition for the very first time, the starting conditions were somewhat different compared to previous years. We had to adapt our project to the current situation because we were unable to complete our laboratory work. Thus, we decided to use human practices as an opportunity to study the global issue of plastic waste and how we currently cope with it. The following article documents the progress and efforts we have made within the iGEM framework.

Before the world's population was busy coping with the SARS-CoV-2 pandemic, multiple environmental problems became increasingly popular at international scale. In particular, younger people began to demonstrate regularly for nature conservation and the protection of the environment. Humanity has been more than harsh with our planet in recent decades, with plastic waste in the environment being only one of the concerns. Global plastic production reached more than 6,300 metric tons in 2015, starting with 2 million tons in 1950[1]. The expiry time of plastics is long and they often end up in the environment mechanically crushed into microplastics. A significant part of plastics ends up in the oceans[2], where the plastic wastes become life-threatening traps for marine life [3],[4].
This makes the improvement of waste management systems across the world critical to reducing plastic pollution. This is where our approach in the iGEM competition comes into play. It is already known from several studies that some living beings are able to degrade different types of plastics. In our work we focused on the example of larvae of the beetle *Tenebrio molitor* that can break down polystyrene and use it as carbon source![5]

In our research we soon realized that the reduction of plastic waste can be far more beneficial for the environment than any kind of waste disposal. Even with recycling and reusing materials, the fastest and most effective solution to the plastic waste problem would be to stop plastics production at all. However, this is highly unrealistic. As much negative aspects plastics have, they also offer enormous benefits for our society. We began to understand, that we would have to base our project on various pillars to explore as many aspects of the topic as possible.

Seven main pillars are the base of our Human Practices approach (Figure 1).

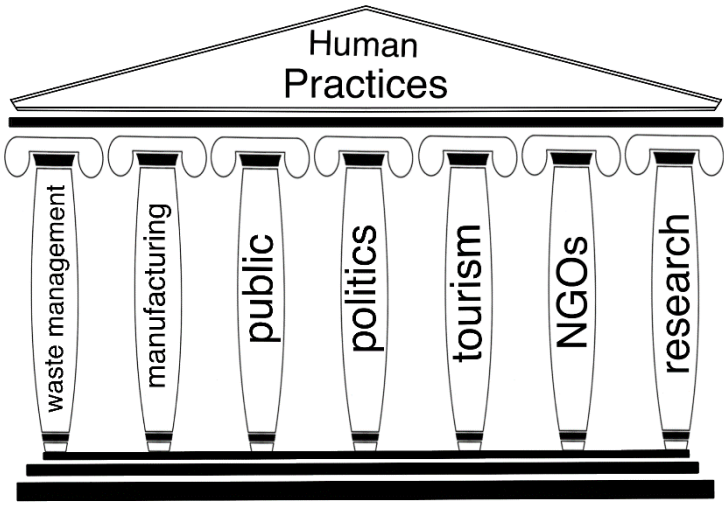


Figure 1. Seven pillars as base for our human practice

In February and March of 2020, our focus was on contacting as many stakeholders as possible.

Waste management

We have contacted several local waste management facilities and local recycling companies to learn more about the standard procedures of disposing plastics. We were able to get some insights about plastics waste management systems in Germany.

The company GEBR. BRAIG[6] (founded in 1966) offers a wide range of services, including municipal and commercial waste management.
The special waste/environmental consultant Alexander Eisele answered several of our questions. His point of view was, that separation of different wastes in Germany works very well and specific plastic wastes can be recycled quite successfully. Mixed plastics on the other hand are more difficult to recycle.

But even with our efforts in Germany to separate the waste before disposal, recyclable plastic can be sold and exported. The Greenpeace report: "A recycling Myth, Malaysia and the broken global recycling system" shows how European and international waste ends up in South East Asia and is often not processed there but burned.[7]

With the help of the stakeholders that we have contacted, we came to the conclusion that the waste management system is an area in which our approach can be implemented. For a closer look into this topic, please view Proposed Implementation. But we will come back to this as soon as our project is accessible to a wider audience. We also want to thoroughly research the decomposition of mixed plastics and focus more on this for our

project design next year.

Plastics manufacturing

We also tried to contact PlasticsEurope[8] to discuss plastic waste disposal, recycling, and export. Unfortunately, none of our inquiries were answered.

Public awareness

To integrate the public, we contacted local cities to learn more about their effort, regarding waste management, public awareness, and prevention of waste. We also planned to visit several schools, but due to the SARS-CoV-2 pandemic we instead prepared several short online lessons with respect to how synthetic biology can help to degrade polystyrene. To see the outcome of this topic, please visit our Education project.
We also created several social media channels, where we constantly uploaded different topics related to our project.

As part of our research, we visited the unpackaged store „Klare Kante“[9] in Ulm and had the chance to interview one of the owners André Wieland regarding the impact each and every person can make in their every- day life to reduce packaging waste. This gave us a better insight into what can be done in the future to raise awareness in society.

The newly opened store „Klare Kante“ focuses on unpackaged everyday items and tries to motivate people to buy and live a sustainable and plastic-free life.

In this years participation, we had the chance to publish an article[10] about our project in a journal. We documented our approach and our general project idea in BIOspektrum[11], our article was published in September 2020.
Since then, we received two feedback e-mails from people showing interest in our project. We used this platform to educate the readers and to reach a more scientific audience.

Politics

When we initially investigated the topic, we came across a lot of information on the website of the German Federal Environment Agency (Umweltbundesamt[12]). We got in touch with them but unfortunately we were not able to build up a collaboration yet.

Tourism

Plastics and waste in general entering the ecosystem is a problem linked with tourism. Overcrowded places and pollution in touristic areas lead to a great amount of plastic waste. To get a better understanding about the struggles and possible solutions, we were trying to contact hotels, bus- and train companies. As the consequences of the pandemic impacted the tourism industry heavily, we could not build a collaboration.

Non-governmental organisations

To learn more about the motivation and the difficulties regarding the much-needed changes in our society, we contacted several NGOs. We managed to arrange a skype call with our local GREENPEACE group in Ulm/ Neu- Ulm [13], where we had the chance to presented and discussed our project.

The Greenpeace group Ulm/Neu- Ulm pointed out several issues, where we could further improve the implementation of our project. This led to us to consider, for example, a more sustainable approach to the mealworms. Thanks to their input, we fed the not used mealworms to chickens! We also were able to contribute to their newsletter by writing an article about our project, that was not published yet.

Research

The LAKE CONSTANCE FOUNDATION[14] is a project-based foundation in Radolfzell, Germany. The foundation was founded in 1994 and since then has been working towards a more sustainable and responsible approach to Lake Constance and its surrounding areas. We have contacted the Lake Constance Foundation to learn more about a current project on nature and lake conservation.

The BLUE LAKES Project focuses on preventing and reducing the entry of microplastics into lakes. Therefore, the project wants to achieve a dialogue between water conservation experts as well as raise public awareness. The subjects of investigation of the project are Lake Constance and several other lakes in Italy over a period of four years. Furthermore, this project is a collaboration between several Italian partners and the Global Nature Fund[15] and is funded by the European Union. Based on these exchanges and the gathering of up to date information, the project aims to develop solutions for the increasingly important problem of pollution in lakes caused by microplastics.

We were able to interview Marion Hammerl, the managing director and program manager of the Blue Lakes Project. We had the opportunity to learn about their approaches, project management, research and difficulties they experienced during the pandemic.
We gained an insight into how important it is to spread awareness and public relations with respect to the plastic waste problem. To reduce microplastics in lakes and in general, a fundamental change in each and everybody's lifestyle and in the dealing with plastics is needed.

The consequences of the pandemic included difficulties in contacting the experts of specific fields. Many of them were working in home office and were not available for our inquiries or had to deal with problems related to the SARS-CoV-2 pandemic. Our initial plan for the Human Practices was to develop the mini video series "iGEM under water" to thoroughly educate the public an summarize all our findings in an entertaining and interesting way. We had already prepared and written nine short episodes that dealt with different perspectives on plastics in our society, based on our seven pillars. Over the months, however, we realized that our content was not enough to fill all the episodes of the series. So we decided to postpone the filming and production of the content until next year and to focus on microplastics in water this year.

Landeswasserversorgung

To get some information about the water supply system in Germany, we had a facility and laboratory tour of the Langenau drinking water treatment plant (Landeswasserversorgung[16]), as well as a detailed conversation with the press spokesman Bernhard Röhrle. Mr. Röhrle answered all our questions about drinking water treatment and water purity in Germany. He explained the water sources of the Swabian Alb, where the city of our university is located, and showed us the different steps involved in water purification. During the facility tour, we saw the treatment plant operating in action. We also had the opportunity to discuss the microplastic content in drinking water and in the water of the ecosystem. For our miniseries, we recorded the interview and the tour of the facility, unfortunately we were not able to publish video material on social media yet, but we´re looking forward to creating new content for our channels.

Figure 2. Katharina Werner and Sophie Ostwald listening to Bernhard Röhrle at the ferric chloride water purification system.

Due to the SARS-CoV-2 pandemic, only three of our team members had the chance to attend the facility tour. Of course, a precautionary distance of 1.5 m was always maintained.

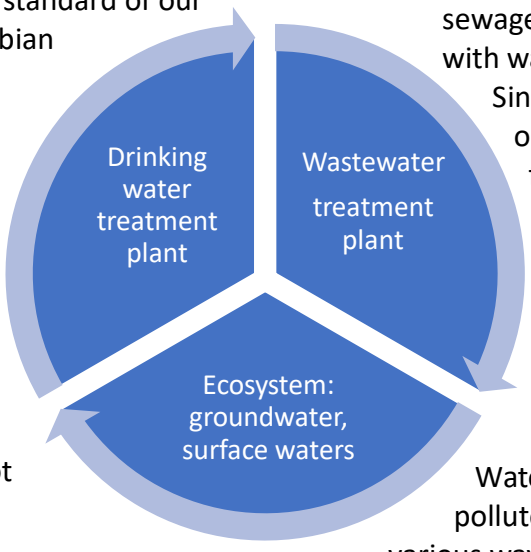
Bernhard Röhrle, press spokesman of “Landeswasserversorgung Langenau”, explaining us the water purification step with activated charcoal.

All our researches and conversations with the Landeswasserversorgung and the Blue Lakes Project lead to the development of the following water cycle.

The water cycle „It all comes back"

The result of our interview with the Landeswasserversorgung is that right now, the detectable share of microplastics in the drinking water is not at all that had to be concerned. The quality standard of our drinking water on the Swabian Alb is very high.

However, it is worth to invest time and effort to develop solutions and decomposition systems as early as possible, so microplastics in the closed loop water systems will not become a problem in the future.



Wastewater treatment is a large part of the water cycle. Today, up to 96% of the wastewater produced is recycled[17]. If the rain cannot seep away, it is fed into the sewage treatment plant, together with wastewater from households. Since not all particles get out of the wastewater through filtration, the water also goes through biological and chemical cleaning steps.

Water in the environment can be polluted with microplastics in various ways. In our conversation with the Blue Lakes Project, it became clear to us that microplastics can originate from cosmetic products, outdoor equipment, tire abrasion and other applications. The input into the ecosystem can be minimized by minor changes of our lifestyle, therefore raising awareness in the public and initiating a dialogue is the best solution to address this problem. Through the interview with Marion Hammerl from the blue lakes project, we came to the conclusion that one goal of our project must be to raise public awareness.

This year, we could contact few experts to learn more about the different perspectives on the topic. We were also able to prepare some possible collaborations for our iGEM participation next year.
As a grand finale of the project we will then produce the miniseries "iGEM under water".

Many thanks to all who helped us to improve and develop our project!

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Available at: www.biospektrum.de/blatt/d_bs_pdf&_id=1765024

[17] Federal Ministry for the Environment, Nature Conservation and Nuclear Safety: www.bmu.de/themen/wasser-abfall-boden/binnengewaesser/abwasser/

Get in contact:

[6] GEBR.BRAIG: www.braig-ehingen.de/

[8] PLASTICSEUROPE: www.plasticseurope.org/de

[9] KLARE KANTE: www.klarekanteunverpackt.de/

[11] BIOspektrum: www.biospektrum.de/

[12] UMWELTBUNDESAMT: www.umweltbundesamt.de/

[13] GREENPEACE Ulm/Neu- Ulm: www.greenpeace.de/gruppen/ulm
GREENPEACE Germany: www.ulm.greenpeace.de/

[14] LAKE CONSTANCE FOUNDATION: www.bodensee-stiftung.org/

[15] GLOBAL NATURE FUND: www.globalnature.org/de/home

[16] LANDESWASSERVERSORGUNG Langenau: www.lw-online.de/

How to iGEM?

A beginner's guide for participating at iGEM

Welcome to the iGEM beginner's guide. This guide is designed to make it as simple as possible for new teams to join the iGEM competition for the very first time. If you are an already experienced team you may still learn something from this guide. As we, the team from Ulm, Germany, participated for the first time in 2020 we wished this guide would have already existed back then to help us. So, we decided to make this our contribution to iGEM and all future teams. Cheers!

Proclaimer

This document is supposed to help you, finding your way through all steps of a successful participation at the iGEM competition. For this purpose, it contains many tips and information, we gained during our first year at the competition. This information is not universally applicable to every situation your team might experience. As everything you will experience will at least slightly differ from our own experiences, you should never take anything for granted and always take multiple sources into account when making important decisions. The judging criteria of the competition are very flexible for example, so we try to stay as general as possible, regarding information about those. Especially questions concerning legal matters are highly complex, dependent on your country, time, and many other factors. Therefore, we take no responsibility for any information provided in this document.

We do our best to make this document useful for everybody. So, if you find mistakes, don't agree with something, or if you experienced something different, please contact us at igem@uni-ulm.de. We highly appreciate your help!

Inhalt

How to use this document?

Optimal system requirements

This document is designed for the usage with a Microsoft Windows 10 running computer and to be opened with adobe acrobat reader. It will probably still work if you use another operating system and/or another pdf reader. However, you will get the best results, when using optimal system requirements. Software we will discuss is also always focused on Microsoft Windows 10, as this is the mostly used operating system. A free version of the recommended pdf reader adobe acrobat reader can be downloaded here: https://get.adobe.com/uk/reader/

Interactive links and table of contents

To make it easier to navigate through the document, we embedded interactive links in this document. For weblinks hold [Ctrl] and click the link to open it in your browser.

"Print section" function

Print section

Some chapters contain information which you might want to isolate from this document. We included the "Print section" function to allow you to do that conveniently. It enables you to either physically print the chapter or to virtually print it into a separate PDF file.

The great iGEM Checklist

To make it easier for you to see where you stand in the competition, here is a short checklist. These are some basic steps, that will make the start a little easier. This is not meant to be a list where you start out with 1. and check off one point after the other. The aspects are roughly organized in chronological order; however, many things need to be worked on simultaneously.

1. Find your topic
2. Find principle investigators, instructors and advisors
3. Assort a team
4. Get your schools approval/assistance
5. Open an account
6. Take care of the legal aspects of starting a scientific group (these can vary for every country)
7. Secure funding and financial support from sponsors
8. Register to iGEM
9. Start your lab work
10. Think about human resources
11. Join collaborations
12. Think about your contribution
13. Create BioBricks
14. Present your work

Important parts of the competition

To start off, here is a short overview of some of the more important aspects of iGEM. Of course, your research and lab work are the central point of your project. Apart from that, there are a lot of other parts to consider. These are all found in the medal criteria. Aim and apply for gold. That way if you do not meet all the criteria it is still possible to get a lower medal.

One of the most important medal criteria is human practices. Here you are supposed to show that your project is aimed to improve the world and that you are working together with the community.

As well as working with the community, you should also get into touch with other iGEM teams. Plan and organize collaborations well and contact teams early on.

A medal criterion that is in close relation to your lab work, is BioBricks. These are biological parts, e.g. DNA or proteins, that you create during the work in the laboratory. To find out more about adding BioBricks to the database go to "Creating parts".

Finding your topic

The first step of the competition is choosing the topic. It is important to take some things into consideration. Firstly, the project should not only aim to discover something new but mainly seek to improve or solve a relevant problem. It is helpful if the groundwork has already been laid and the basics have been studied. However, make sure that your project is original. It should leave a positive impact on the world, so aim for the stars but still stay grounded and consider the logistics. Keep the specifications from the iGEM Headquarters in mind and be aware of your limits regarding scientific work.

Team

Responsibilities

For a successful project it is important to have a good team. It should consist of members with a broad spectrum of expertise. You should think about whom you want on your team beforehand. That way you can target specific groups of people during recruiting. The makeup of your team depends mainly on your project topic, however there are a few members every team needs. The first people you should ask are your principle investigators, instructors, and advisors. Apart from these members you will also need people, who…

… work in the lab
… take care of funding and finances
… know about legal matters
… work human practices
… collaborate with other teams
… know their way around computers
… can create creative content (drawing, photoshop, edit videos, …)
… take care of social media

Recruitment

Once you know who you want on your team, you need to find out how to get them to join. The easiest way is to go into lectures and ask the people there. By selecting the lectures carefully, you can already choose what kind of people you are recruiting. For example, if your team is missing someone who can handle the software aspects of the competition, you can go into a lecture about computer science. Don't go unprepared! Put together a short presentation explaining iGEM and your project idea.

A great motivation for joining is the fact that participating in an international competition is an excellent reference on your resume, as well as a once in a lifetime chance to conduct scientific research as a student.

Team management

For a team to function well, the members need to stay connected and be able to communicate. Thanks to social media, messenger services and video calls this is not very difficult. However, it might help to have team meetings in person to discuss important matters. For those meetings to go smoothly, it is best to make an agenda beforehand and send it to the rest of the team. That way everybody can prepare for the meeting and it will be more efficient.

In our experience it is also helpful to divide your team into smaller work groups. These consist of two or three people depending on the task and have a group leader. The smaller groups can then report back on their progress to the whole team in meetings.

Organizing many people can be difficult. Fortunately, there are a lot of tools, that can help with that.

Excel

It has proven useful to have a table with the contact information of every team member (phone number, e-mail, …) and their part in the team (IT, lab, …). This way you have an overview over who is doing what and how to reach them. Watch out for data protection and be careful about handing out this information. (This is of course something that is different in every country see “Legal matters” for further information)

Online calendar

Even without a pandemic it is recommendable to have a team calendar. It allows for long term planning and gives a great overview over deadlines and dates. For example, you can use Microsoft Outlook calendar or similar programs.

Online surveys

For example, doodle surveys are helpful in finding dates for meetings and such.

Communication

As already mentioned, communication is key for a good work relationship. If meetings in person are not possible (for example during a pandemic), team meetings can be held by video conferences. Programs that can be used are Zoom, Skype or others. Another way to stay in contact is via e-mail or over messenger systems. Especially with messenger systems you want to be careful about security. Some are safer than others, so be mindful about what you share.

Data sharing

If you want to share files with your teammates there are a lot of ways to do that. The easiest way is to send the file via e-mail. This doesn’t work for larger files, since there is a limit to the size of a document that can be sent by e-mail. That is why larger files should be uploaded into a dropbox, cloud, etc. (Our team shares papers and other files over the “shared documents” option in our mailing list.)

Important websites

There are four important websites for the competition. They are all linked together but it is often easier to visit them directly.

Main page (igem.org)

https://igem.org/Main_Page

This page is very helpful to finding out what iGEM is and gives a short overview over the contest. It is great for getting to know iGEM. However, it does not show information on the current competition and is thus not very helpful regarding dates and deadlines. For these you need to visit the current page of the year.

Page of the current year ([year].igem.org)

e.g. https://2020.igem.org/Main_Page

This is the page where you can find all the relevant information for this year's competition. It includes a calendar with all the deadlines, as well as the judging forms and medal criteria for the respective year.

Team page

This page is very important for your team. From here you can get to your team's wiki and your submitted parts. It also shows information on your team. Every member has to create an account and sign up in order to be part of the competition. A guide to creating an account can be found here.

The team wiki shows all the work your team has accomplished and is an important part of the competition. Please note that there is a deadline for the wikis. The servers are often overloaded in the time before the wiki freeze, so be sure to upload your articles with time to spare.

To create your wiki, you use HTML. This is a "programming language" used to design websites. It is not very hard to learn and there are many tutorials. A few commands are enough to configurate the team wiki.

Creating parts (parts.igem.org)

http://parts.igem.org/Main_Page

Another important part of iGEM is creating biological parts and registering them on your team page. The name of a part is always built like this: BBa_[letter][team number][part number]

To keep an overview of your submitted parts it is best to compile all the information in a table. An example table can be found here.

A guide to submitting parts can be found here.

If you want to find out how to edit parts, click here.

Apart from submitting parts electronically, it is also possible to send them directly to the iGEM headquarters.

Legal matters

When setting up and organizing an iGEM team, there are some legal things that must be observed. It is advisable to open a bank account immediately after the establishment. Usually this is possible through the university. With a little luck, the university will provide you with an accompanying person who will support you in legal questions. Allow some time for this step.

Once the account has been opened, you can search for sponsors. The tax law of the respective country must be observed! Maybe you can get around this with donations in kind. You should also secure yourself legally against the sponsor with a contract. Here the services of both contractual partners must be precisely recorded. This serves to avoid misunderstandings.

Publishing

When publishing logos, images and videos, make sure that you have the license or that you are using license-free material. It is possible to apply a filter to your search engine for license-free pictures. However, this is not a guarantee, so inform yourself about the origin of your materials.

In the case of self-created material, all persons involved must agree to its publication. This is (at least in the EU) also valid for pictures of team members. Make sure you are legally safe in this regard.

When publishing a scientific text e.g. a paper, it is also very important to quote and reference correctly. The APA style referencing is recommended here. There are support programs for this, e.g. Microsoft Word.

Software

Software for editing images and videos, but also software that is required in the laboratory, are important tools for the project. There are many free programs out there, but payware usually has some advantages. In most cases it is possible to obtain the license from the university.

Some helpful software:

- plotting graphs:

  - Wolfram Mathematica
  - SciDavis (Freeware)

- drawing structural formulas:

  - ChemSketch (Freeware)

- DNA handling

  - CLC Workbench
  - Genome Compiler

- other software:

  - Microsoft Word
  - inDesing
  - Photoshop
  - Blender

CORONA- our experience with the pandemic

In the end we would like to share a few experiences we have gained over the last year, especially with regard to the global pandemic. If you are thinking about participating in iGEM for the first time during the outbreak of a global pandemic – well, there are better ideas. Participating in a global contest for the first time without experience is not easy at the best of times. Adding a global pandemic does not help.

Two of our main problems, apart from not being able to meet in person, were that we were not able to work in the lab and that finding sponsors was very difficult. The pandemic effected the economy which is why finding sponsors wasn't easy. It is common to get a lot of rejections from possible sponsors, however, being an unestablished team with no previous successes to show, we heard "no" quite often. It is important not to let that get you down, but instead to keep on trying.

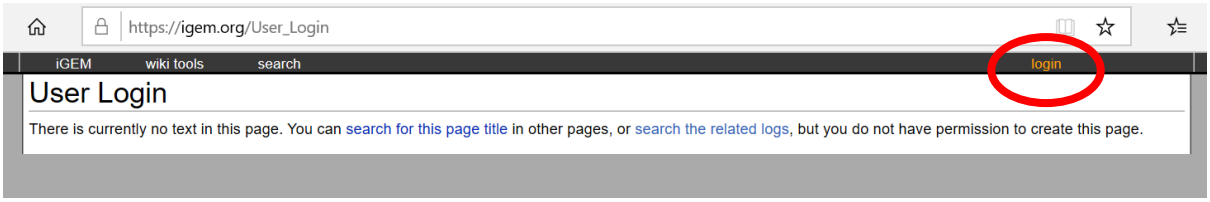
We also had the problem that getting an account took longer than we expected, especially with the University being in lockdown. That is why it took us quite a while to contact potential sponsors.

Our University went into lockdown in March 2020. There are still many regulations and working from home is very much encouraged. These circumstances caused us to shift our focus away from the lab work. We are glad that eventually it was possible for us to start our work there.

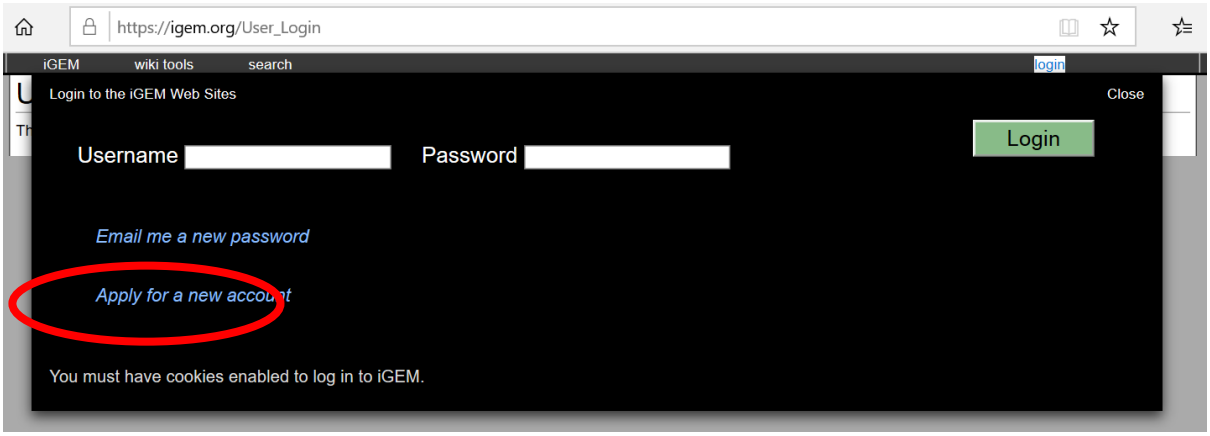
Of course, the most difficult part was that it was impossible for us to meet up in person. Instead we held a lot of video conferences and learned to be very patient with the internet.

All in all, it has been an interesting year and we had a lot of fun and learned many things. We are looking forward to participating again next year, after all the groundwork has now been laid and the starting difficulties have been overcome. We are hoping this guide will encourage and help other first-time teams and make their start a little easier.

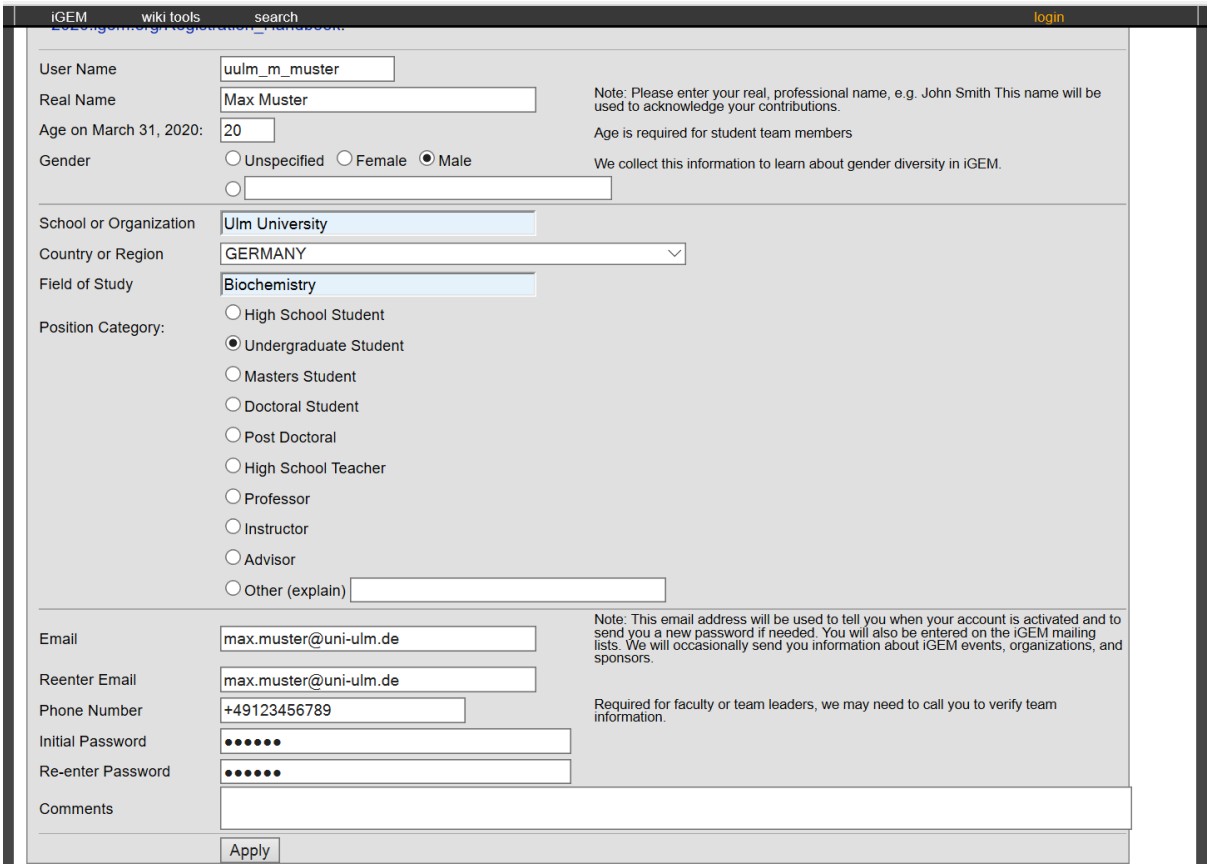
1. Go to https://igem.org/User_Login
2. Click on login.



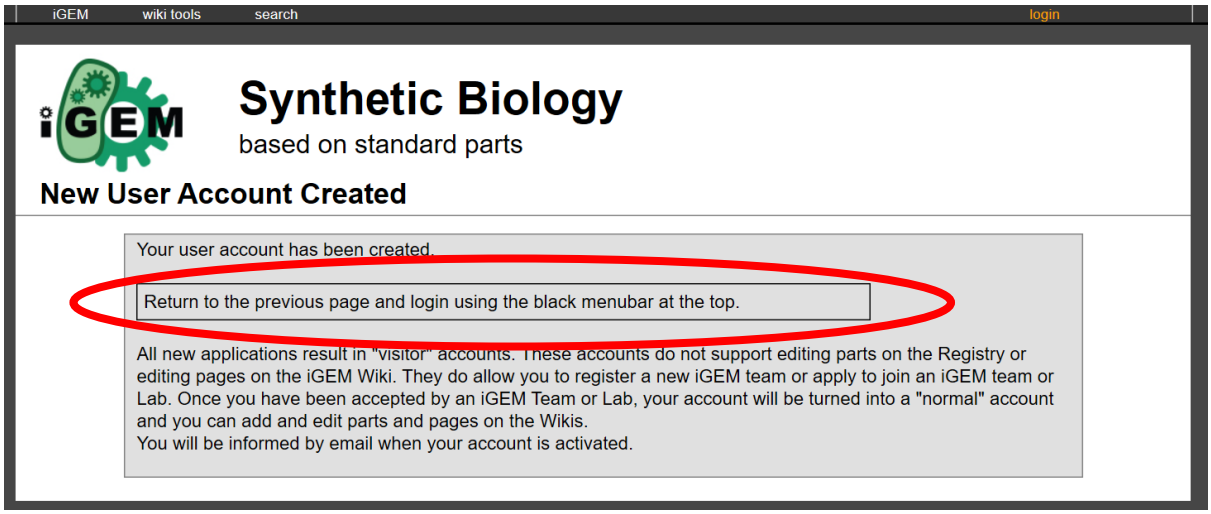
3. Click on „Apply for a new account".



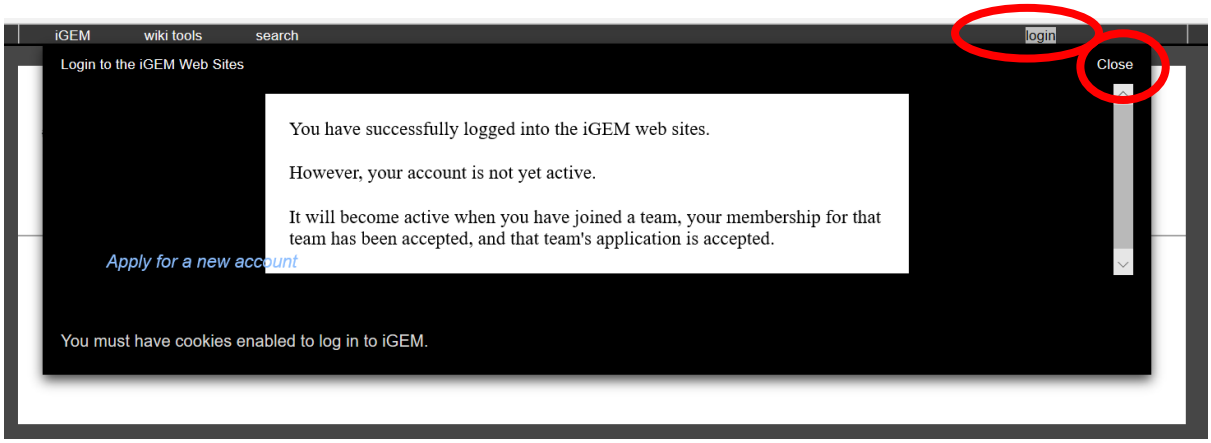
4. Enter your personal data and select a password. Click "Apply" to confirm.



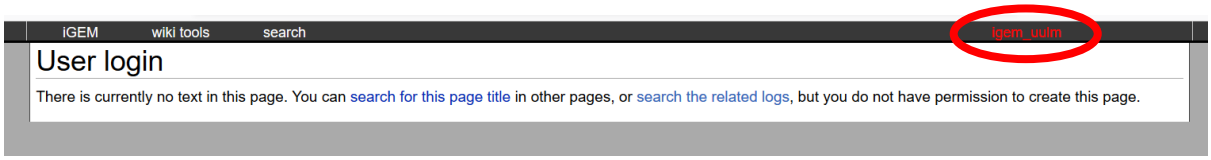
5. After successfully creating your account you will return to the login page.



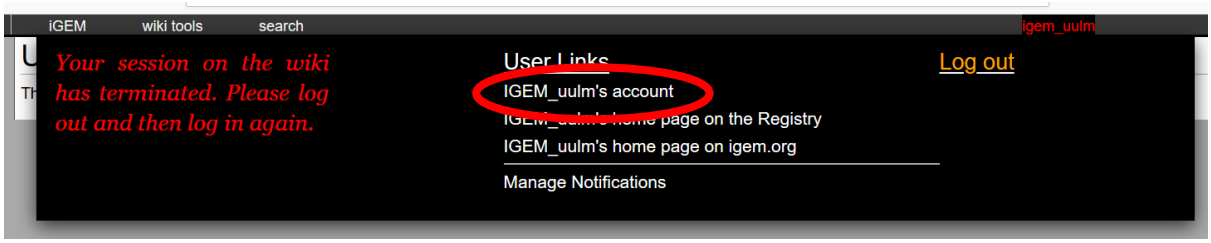
6. By pressing login again (step 2) you can now access your account using your chosen username and password.
7. Close the information box by clicking „Close“ on the top right.



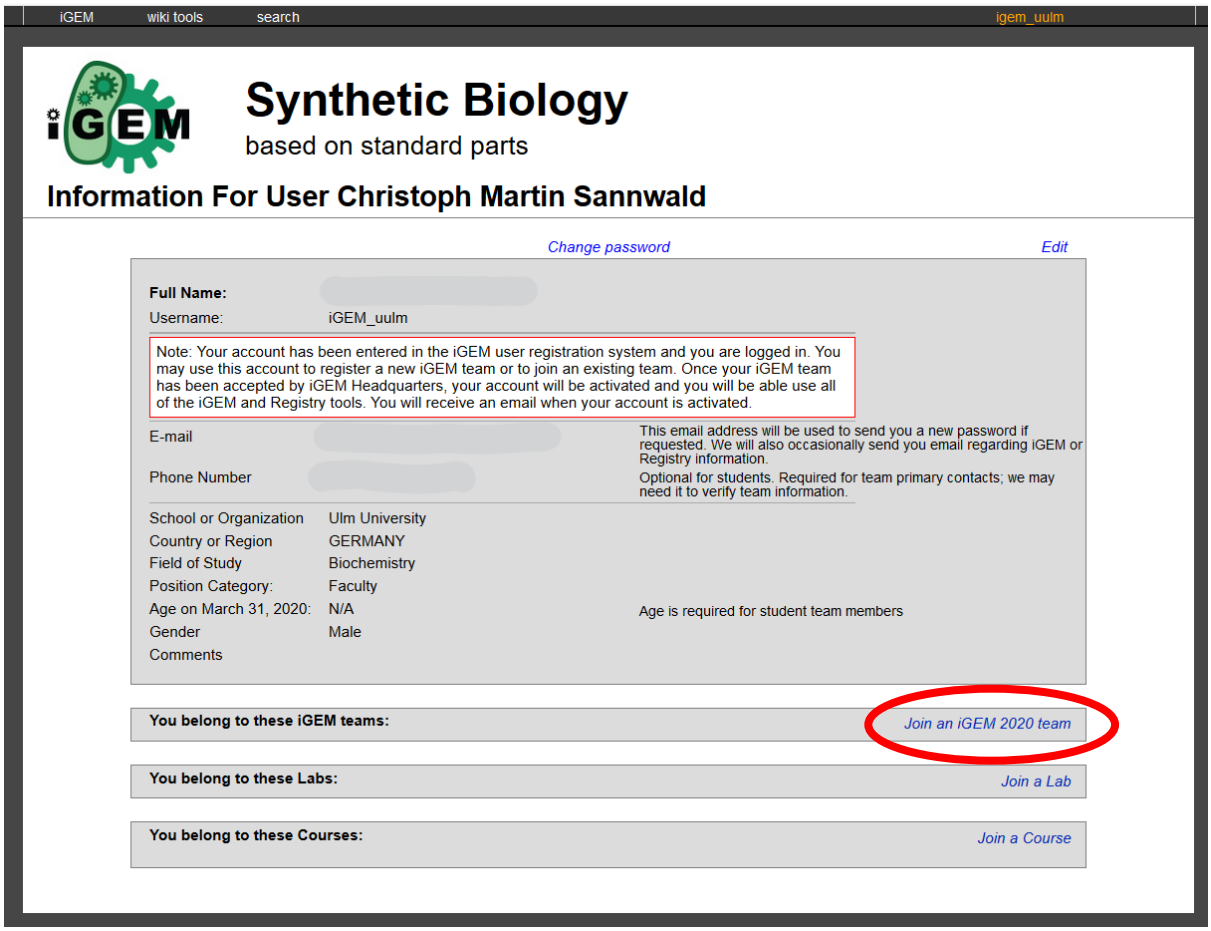
8. Move the cursor onto your chosen username in the top righthand corner.



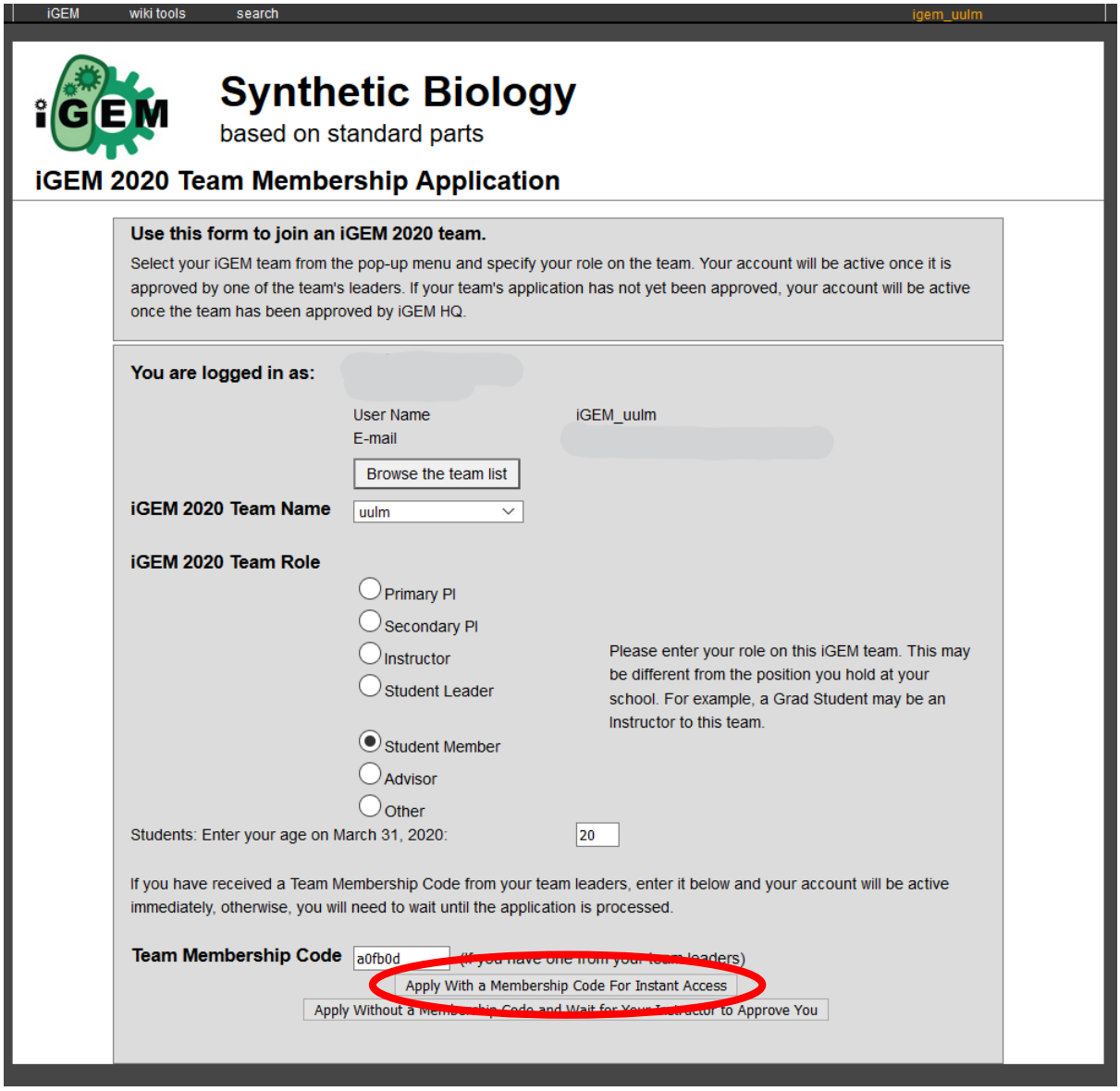
9. Open your account.



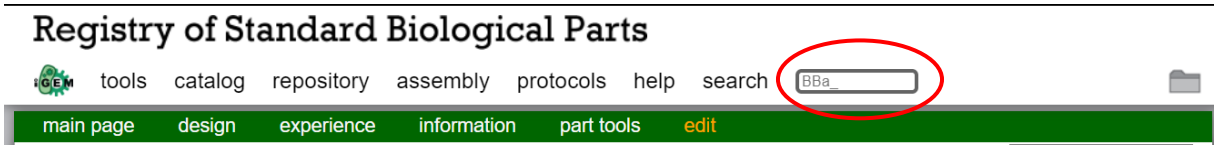
10. Enter the group over „Join an iGEM [year] team”



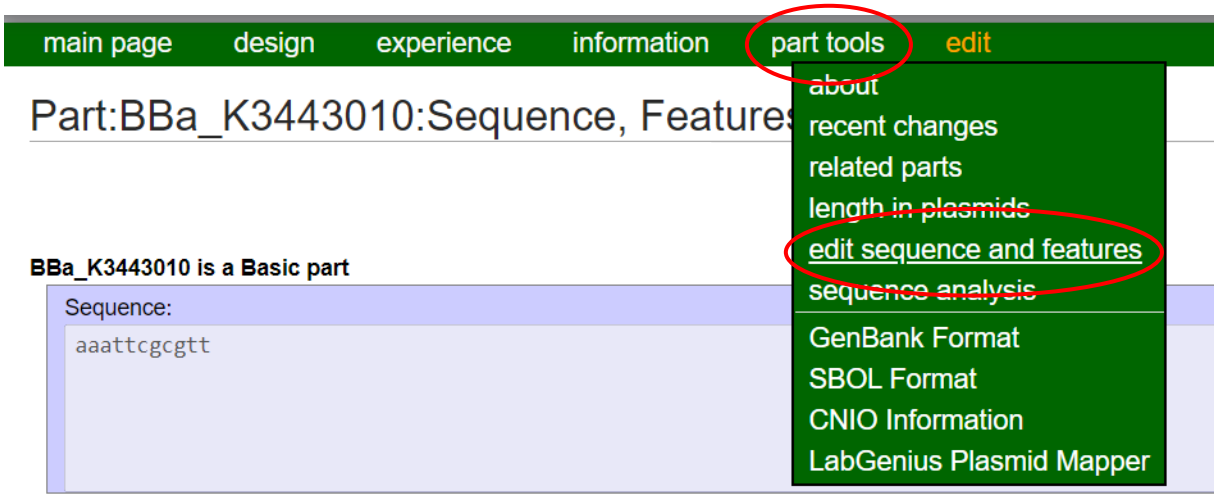
11. Choose your team name from the list.
12. Select your role in the team.
13. Confirm your age at the time of registration
14. Enter the membership code you have received from your team leader.
15. Confirm by clicking on the button for Instant Access.


iGEM
wiki tools
search
igem_uulm
Synthetic Biology
based on standard parts
iGEM 2020 Team Membership Application
Use this form to join an iGEM 2020 team.
Select your iGEM team from the pop-up menu and specify your role on the team. Your account will be active once it is approved by one of the team's leaders. If your team's application has not yet been approved, your account will be active once the team has been approved by iGEM HQ.
You are logged in as:
User Name
iGEM_uulm
E-mail
Browse the team list
iGEM 2020 Team Name
uulm
iGEM 2020 Team Role
Primary PI
Secondary PI
Instructor
Student Leader
Student Member
Advisor
Other
Please enter your role on this iGEM team. This may be different from the position you hold at your school. For example, a Grad Student may be an Instructor to this team.
Students: Enter your age on March 31, 2020:
20
If you have received a Team Membership Code from your team leaders, enter it below and your account will be active immediately, otherwise, you will need to wait until the application is processed.
Team Membership Code
a0fb0d
Apply With a Membership Code For Instant Access
Apply Without a

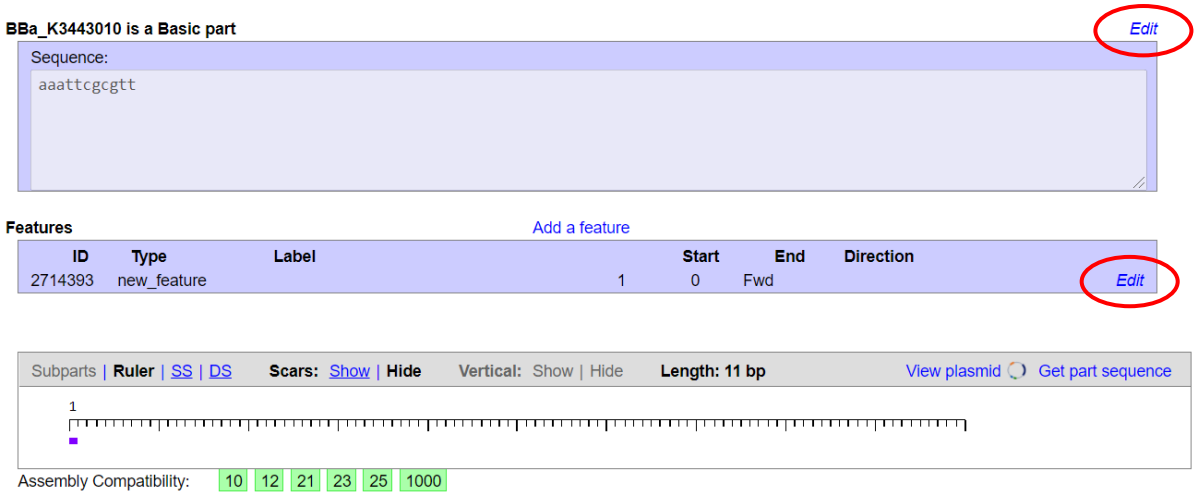
1. To edit a part or add features, find the part using the search function.



2. Move your cursor on “part tools”. Click on “edit sequence and features”.



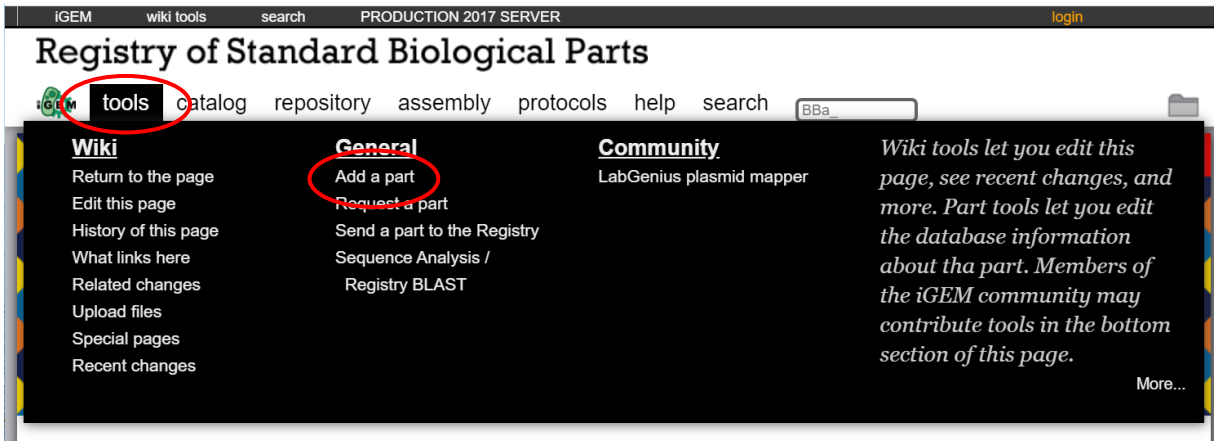
3. Now you can make changes to your part by clicking on “Edit”



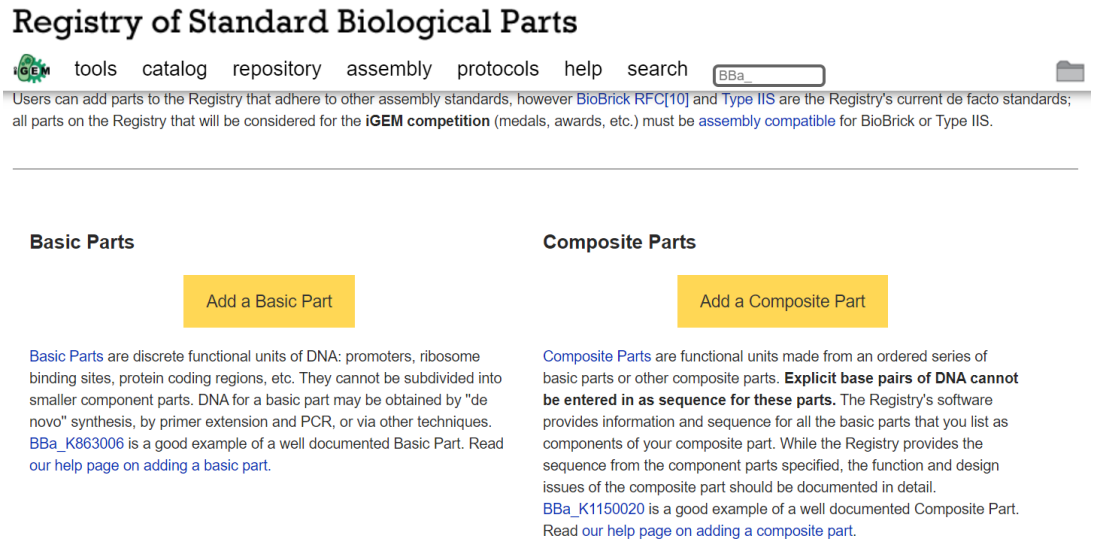
4. Don’t forget to save your changes by clicking on “Save”.

1. Go to http://parts.igem.org/Main_Page
2. Log into your account

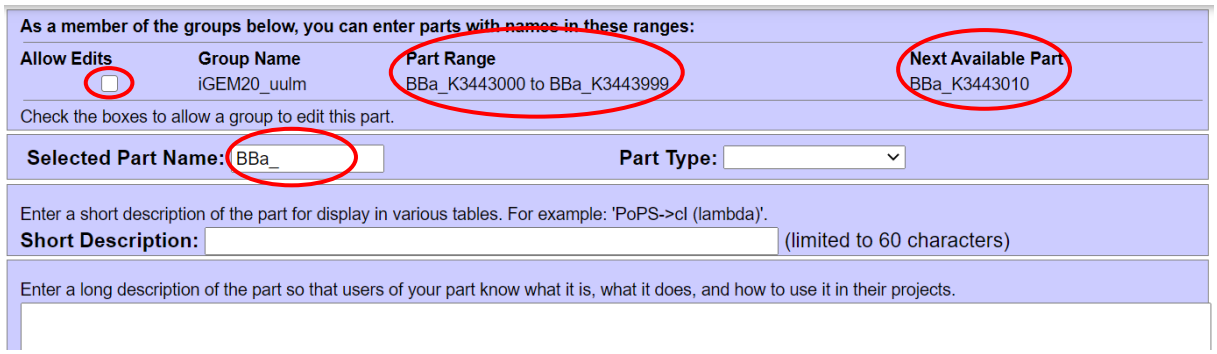
3. Click on “tools“
4. Click on “Add a part”



5. Select “Add a Basic Part” or “Add a Composite Part” depending on what kind of part you are adding.

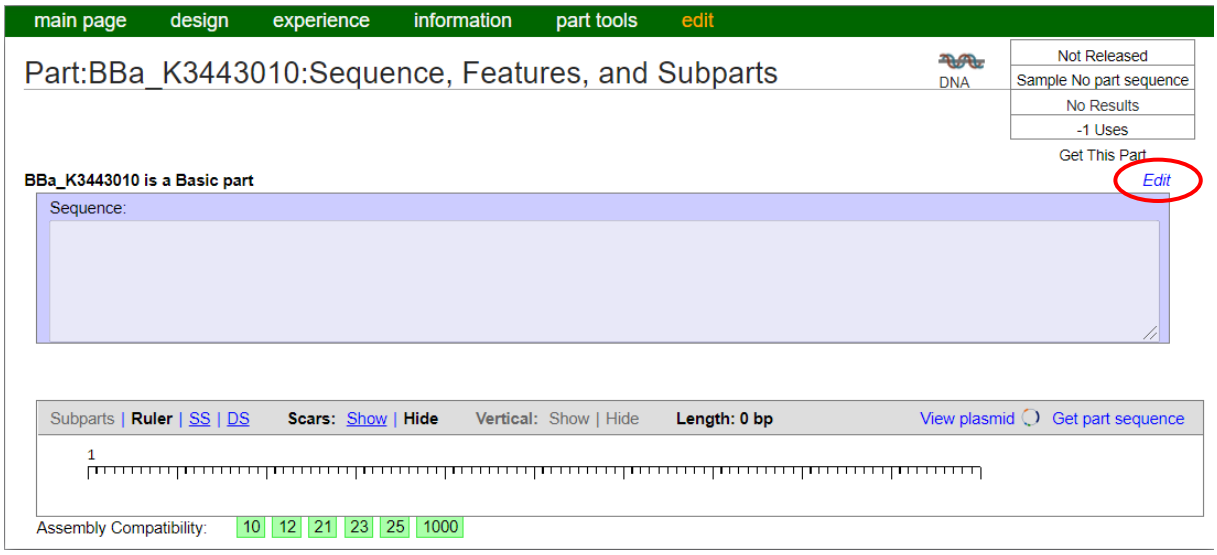


6. Choose the part name from your part range and enter in “Selected Part Name” box (it is easiest to use the next available part).
7. Check “Allow Edits”

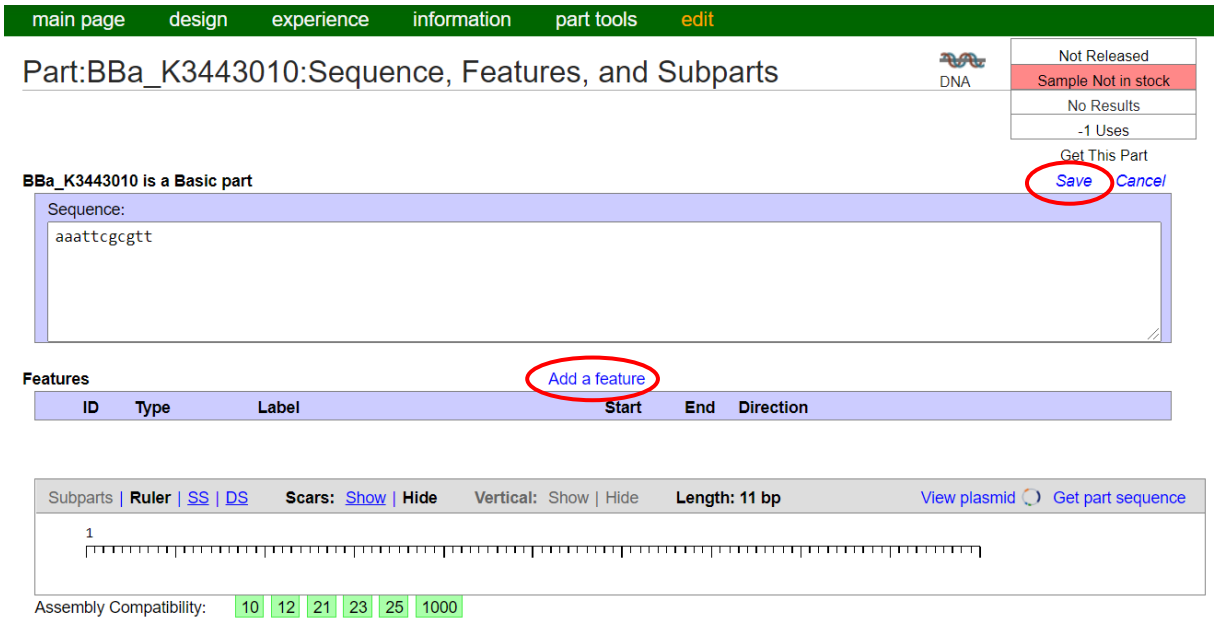


8. Fill out the rest of the form according to the description.
9. Confirm with “Go on to enter the sequence and add feature annotations”

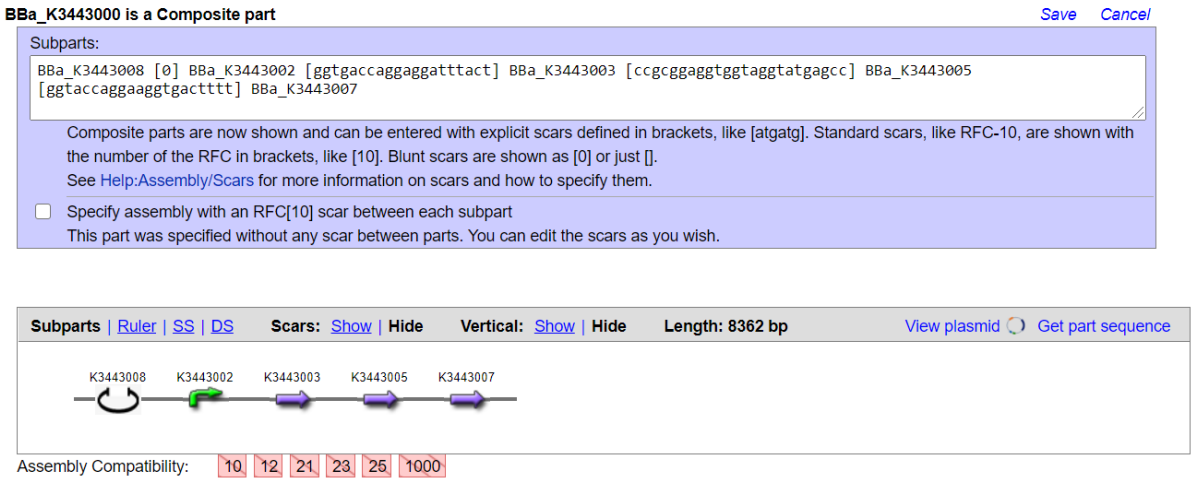
10. To enter sequence, click on edit.



11. Enter sequence and features and select “Save”.



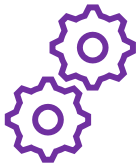
Note: the sequences of composite parts consist of the names of basic parts. Scars are written in brackets.



Attributions

This year, the UUlm team participated for the first time with a project in the iGEM competition. Our participation would not have been possible, without for the support, guidance, and encouragement of various people. We have been working on different aspects of this project for almost a year now, and during this time, we came across many helping hands we want to thank today. With this page, we want to acknowledge all the help and support we received. Thank you!

Student Team

	WetLab and mealworm maintenance, Team leaders	Katharina Werner Christoph Sannwald
	Education	Camilla Förster Jana Zäh Maike Buck
	Human Practices	Katharina Werner Sophie Ostwald
	Proposed Implementations	Hanna Maier
	Contribution	Annika Siewert Sophia Stöferle Christoph Sannwald
	Engineering Success	Christoph Sannwald Peter Bübl
	Collaborations	Sophia Stöferle Jana Zäh Maike Buck Camilla Förster Katharina Werner Christoph Sannwald

	Project Description	Dominik Frank
	Social Media	Jana Zäh
	Poster Design	Christoph Sannwald Katharina Werner
	Wiki design	Alexander Lodemann Christoph Sannwald Katharina Werner
	Art Design	Camilla Förster
	Bacteria quiz	Hanna Maier Peter Bübl Camilla Förster
	Video editing	Wolf- Hendrick Zillmann Christoph Sannwald
	Wiki editing Brainstorming Filming	Team effort

Supervisors

First Principal Investigator	Prof. Dr. Peter Dürre	Many thanks to Prof. Dürre for founding and managing the team with us! A participation without your support would not have been possible. Thank you for giving us a lab space and the funding to get started with iGEM – even in these extraordinary conditions this year. We are also especially thankful for letting us borrow the camera equipment to film our iGEM contents.
Secondary Principal Investigator	Prof. Dr. Nils Johnsson	Thank you to Prof. Johnsson for your support regarding our project! Thank you for taking the time and reviewing all our work.
Advisor	PhD. Frank Bengelsdorf	Many thanks to Frank for always taking the time to answer all our questions! Thank you for your countless support and the knowledge and ideas you shared with us. Thank you for your commitment and the help from day one, especially your input regarding iGEM at school and providing us with the necessary contacts.
Instructor	M. Sc. Teresa Schoch	Teresa – thank you for your patience with us! Thank you for teaching us in the lab and always providing us with information and valuable advices! Thank you for always being open with us and helping us to develop our project. This project would not have been possible without your input. Also, many thanks for letting us work with your plasmid designs!

Special Thanks To:

Annerose Frank Barone	Thank you Anne for your administrational support! Thank you for figuring out the payment procedure with us and for always answering all our questions.
Prof. Dr. Harald Wolf	Thank you Prof. Wolf for providing us with your expertise and valuable information about the handling of the mealworms.
Manuela Niessing	We want to thank Mrs. Niessing for your administrational help with our bank account. It took us a long time to find a solution for this

	issue, and we could not be more grateful for your support!
Frank Volz and Sandra Zell	A huge thank you for your supporting our team! You were always open for all our questions regarding the financial administration.
Karin Dengler- Wupperfeld Iris Steiner Brigitte Ehrler	For their support in the lab and for our project, we want to thank the laboratory technicians!
Ulmer Universitäts Gesellschaft (UUG)	Thank you to the UUG for funding our participation this year! We are very grateful for getting this opportunity and a big role in making this possible was the support of the UUG.
Ulm University	A huge thank you also goes to our *alma mater*. As well as the UUG, the Ulm University funded our participation this year. Thank you for your faith in us and giving us the chance to represent Ulm University in an international competition.
Oliver Heidu	Thank you for advising us in the beginning stages of our project.
Felix Rieg	Thank you for your help and advice with editing our wiki pages.

A Thank You to all the iGEM teams for collaborating with us

Team Duesseldorf	Postcard project
Team MRIIRS_FARIDABAD	YouTube video project
Team MSP- Maastricht	Journal Initiative
Team GW_DC	Team Logo collaboration
Team UUlm iGEM Explore	Participating Teams: igem_iiserp, India igemrum, Puerto Rico igemncku, Taiwan igemhamburg, Germany msp_igem, Netherlands
Team UUlm This or That Challenge – Lab Edition	Participating Teams: igem_iisertirupati, India igem_iisermohali, India igem_iiserk, India igem_bits, India igem.thessaloniki, Greece igemthessaly, Greece igempatras, Greece igemthrace, Greece igemstockholm, Sweden igem.kaiserslautern, Germany

	igem.aachen, Germany

Thank you for your expertise to help our Human Practices professionals!

In this years participation, we had the chance to interview several professionals to talk about different aspects of our project. For more information, please view our Human Practices article.

We do have permission from each professional to use the companies logos in our Wiki article.

Landeswasserversorgung	Bernhard Röhrle	Landeswasserversorgung Ulm
Bodensee Stiftung Lake Constance Foundation	Marion Hammerl	Lake Constance Foundation
GEBR. BRAIG Entsorgungsfachbetrieb	Alexander Eisele	GEBR. BRAIG
		Greenpeace Team Ulm/ Neu- Ulm
KLARE KANTE	Andre Wieland	Klare Kante

Thanks to the Journal BIOspektrum for giving us the chance to publish an article about our project.

Our Helping Hands

Next to all the people listed above, other helping hands were significantly responsible for our participation. After first thinking about a participation in the competition, we quickly realized, we needed some advice from experienced teams. We contacted the **iGEM Team HU Berlin** and **the iGEM Team LMU & TU Munich** to talk about their experiences, do´s and don´ts about getting us started and tips and tricks for a successful participation. We are very grateful for your help!

In the starting phase of our project, we were facing different organizational difficulties. **Gerold Brackenhofer** from Ulm University helped us to establish a foundation at the University, from where we could further build our team.

Prof. Dr. Olga Pollatos is our University´s Vice President for Education. In the beginning stages of our project, we had the chance to present our projects idea to her and to talk about establishing the iGEM UUlm team as a permanent enrichment for the University. Ulm University also partly funded our participation, for which we are very grateful.

Thanks to the **StudierendenVertretung**, the student representation of the Uni. Thanks to them, we are now an officially recognized University group.

Last but not least, a thank you to the **Kreismedienzentrum Ulm**. They gave us the opportunity to borrow camera and filming equipment, which we needed to film Human Practices.

Notebook

What are we doing – our idea!

We aim to develop a mechanism to break down polystyrene with the help of mealworms. Mealworms can feed on and digest styrofoam and use it as a carbon source to complete their life cycle. This is possible due to bacteria in their gut. These bacteria are necessary for the degradation of polystyrene. Our approach is based on the fact that acetone is a commonly known solvent for styrofoam. The acetone molecules intercalate in between the polymer strands and weaken the polystyrene stability. This gives the gut bacteria more surface to do their magic.

Although we only had very limited time in the lab, we managed to start our research. We were able to start assembling our acetone synthesis plasmids, but could not finish in time. We also conducted a behavioral study with two groups of mealworms to learn more about their natural ability to digest polystyrene. This is what we achieved.

November 2019 – February 2020:

- literature research
- choosing a topic
- team finding
- connecting with experienced iGEM teams

March – April 2020: lockdown due to the Corona virus

- Brainstorming for new ideas which we could achieve from home
- preparing education and human practices
- contacting experts
- finances and organization

Time table for the acetone synthesis plasmid construction

Mon	Tue	Wed	Thur	Fri	Sat	Sun
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18 Cell seeding Preparing medium	19 Plasmid isolation Analytic digest Gelelectrophoresis PCR	20 Preparative digest Gelelectrophoresis PCR Preparing agar plates	21	22	23	24
25 Gelelectrophoresis DNA purification Gel (ac3t3) DNA purification PCR product (ac2t2) Infusion cloning	26 Transformation	27 Transformation	28 Picking colonies	29 Plasmid isolation Analytic digest Gelelektrophoresis	30	31

May

June

Mon	Tue	Wed	Thur	Fri	Sat	Sun
1	2 Picking colonies	3 Plasmid isolation Analytic digest Gelelectrophoresis Picking colonies	4 Plasmid isolation Analytic digest Gelelektrophoresis Prepare for sequencing	5	6	7
8	9	10	11	12	13	14
15	16	17	18	18	20	21
22	23	24	25	26	27	28
29	30					

Time table for the behavioral study of mealworms

May

Mon	Tue	Wed	Thur	Fri	Sat	Sun
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31

June

Mon	Tue	Wed	Thur	Fri	Sat	Sun
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	18	20	21
22	23	24	25	26	27	28
29	30					

July

Mon	Tue	Wed	Thur	Fri	Sat	Sun
		1	2	3	4	5
6	7	8	9	10	11	12

13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

Results

Acetone synthesis plasmids

When assembling the plasmids for acetone synthesis, we discovered that the lab we were working in already worked with the necessary BioBricks. We were able to use and simply reassemble the needed sequences on a suitable backbone. We also chose to assemble two different plasmids. The plasmids are on the same backbone, but they are different in gene order and origin of the genes.

Our works, as well as the work of many others, was heavily impacted by the COVID-19 pandemic. Unfortunately, we were not able to finish the experiments. We were having difficulties transforming the assembled plasmids into a competent *Escherichia coli* strand and didn't have the time to repeat the transformation.

The following results show our progress assembling the acetone synthesis plasmids.

Monday, May 18th:

1. LB media was prepared (see protocols)
2. *Escherichia coli* [pMTL83151] (backbone)
   [pJIR750_ac2t2]
   [pJIR750_ac3t3]

   were seeded in 2x 5ml media and incubated over night at 37°C while shaking.

Tuesday, May 19th:

1. LB media was autoclaved
2. Plasmid isolation of the three plasmids from the *E. coli* ([pMTL83151], [pJIR750_ac2t2], [pJIR750_ac3t3])
3. Analytic restriction digest, PCR
4. Gel electrophoresis to confirm the plasmid fragments after restriction (see protocols)

Procedure analytic digest

1. Prepared Mastermix for 6 tubes as follows:

Chemicals	Volume
FastDigest Green Buffer	14 µL
Restriction enzyme 1 *Eco*RI	7 µL
Restriction enzyme 2 *Sal*I	7 µL
H_2O	105 µL

2. Divided mastermix into 6 Eppendorf tubes and added 1 µL plasmid DNA per tube. Two tubes per plasmid ([pMTL83151], [pJIR750_ac2t2], [pJIR750_ac3t3])
3. Incubated at 37°C for 20 min; inactivated at 65 °C for 5 min

Plasmids	Enzymes	expected fragments
[pMTL83151]	*Eco*RI	1001 bp + 3475 bp
[pJIR750_ac3t3]	*Sal*I + *Eco*RI	3963 bp + 6535 bp
[pJIR750_ac2t2]	*Sal*I + *Eco*RI	3892 bp + 6535 bp

PCR

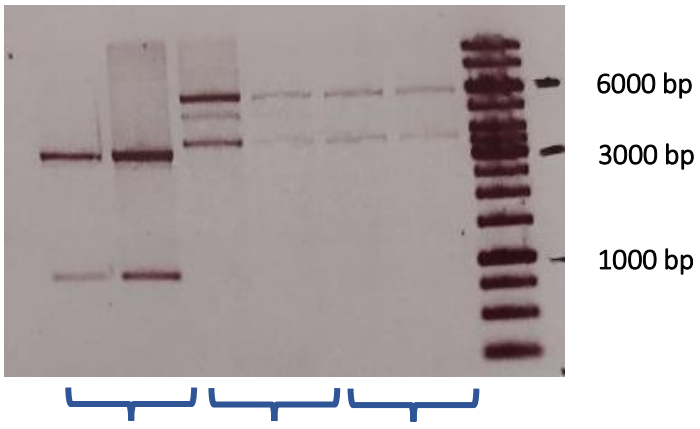
1. Prepare mastermix PCR for 5 tubes:

Chemicals	Volume
CloneAmp™ HiFi PCR Premix	75 µL
Primer forward	3 µL
Primer reverse	3 µL
H_2O	63 µL

2. Added 6 µL plasmid DNA, two tubes per plasmid ([pJIR750_ac2t2], [pJIR750_ac3t3]). End volume 25 µL.
3. Started thermocycler protocol (see protocols). Products are insert 1: ac2t2 and insert 2: ac3t3.

Gelelectrophoresis

1. Prepare agarose gel as described in protocols
2. Fill pockets with 6 µL plasmid fragments and one pocket with 4 µL Ladder



Results: [pMTL83151] has two bands at approximately 1000 bp and 3000 bp, expected fragment length 1001 bp + 3475 bp.

[pJIR750_ac3t3] has two bands at approximately 4000 bp and 6000 bp, expected fragment length 3963 bp + 6535 bp. There was also a third band for [pJIR750_ac2t2] at about 5000 bp. This was probably the non-digested plasmid.

[pJIR750_ac2t2] has two bands at approximately 4000 bp and 6000 bp, expected fragment length 3892 bp + 6535 bp.

➔ the fragments could be confirmed

Wednesday, May 20th:

1. Preparative digest of [pMTL83151] (see protocols)
2. Repeat gel electrophoresis with PCR products from yesterday
3. Preparing agar plates (1.5 % w/v) with chloramphenicol (end concentration 30 µg/mL))

Procedure preparative digest

1. Prepared mastermix for 4 tubes as follows:

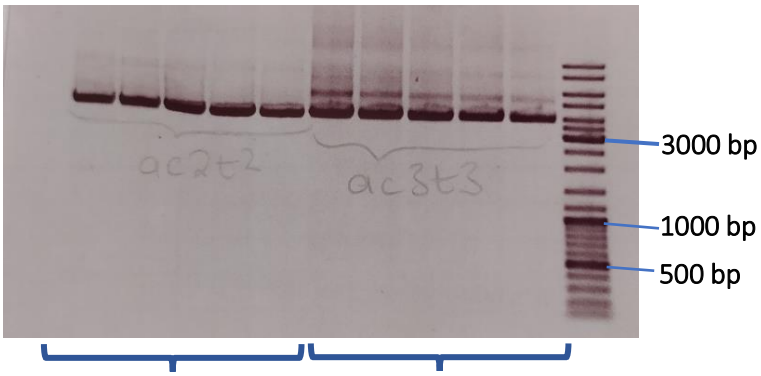
Chemicals	Volume
FastDigest Green Buffer	20 µL
Restriction enzyme 1 *Bam*HI	10 µL
Restriction enzyme 2 *Xba*I	10 µL
H_2O	140 µL

2. Divided mastermix into 4 Eppendorf tubes and added 5 µL plasmid DNA per tube. End volume 50 µL.
3. Incubated mixture at 37 °C for at least 3 h; inactivate at 6 °C for 5 min

Plasmid	Enzymes	expected fragments
[pMTL83151]	*Bam*HI + *Xba*I	140 bp + 4466 bp

Gelelectrophoresis:

1. Prepare gel as described in protocols
2. Fill pockets with mix in the pattern described in protocols:
    - 5 µL 1x TAE buffer
    - 2 µL LD
    - 1 µL PCR product from yesterday



Insert 1: ac2t2 Insert 2: ac3t3

Note: ac2t2 has one band at approximately 4000 bp, expected fragment length 3936 bp → DNA purification from PCR product

ac3t3 has two bands at approximately 4000 bp and 5000 bp, expected fragment length of this insert is at 4007 bp → DNA purification from gel → repeat gel electrophoresis with bigger comb and more PCR product

Monday, May 25th:

1. Repeated gel electrophoresis for insert ac3t3
2. DNA purification (see protocols)
3. Infusion Cloning (see protocols)

Gelelectrophoresis

1. Repeated gel electrophoresis for insert ac3t3, used bigger comb and filled with 25µl PCR product and 5 µL LD in the following pattern:

	Ladder	1	2	3	4	5	Ladder
Volume	4 µL	5 µL	30 µL	30 µL	30 µL	30 µL	4 µL

2. Cut the gel in sections 2-5 shortly over and under the bands.
3. Put sections 2 + 3 in one Eppendorf tube and 4 + 5 in another one for DNA purification

DNA purification

1. DNA purification from gel for two tubes ac3t3 (see protocols)
   → Two tubes with purified DNA
2. DNA purification of the PCR product (pMTL83151, ac2t2) (see protocols)
   → Two tubes with purified DNA

Infusion Cloning

1. Measure DNA concentration using a NanoDrop
2. Needed concentrations were calculated with the infusion calculator by TakaraBio Inc.

Vector: [pMTL83151]:
   a. 145.5 ng/µL
   b. 148.4 ng/µL

Infusion calculator, relation: 2
Length vector: 4466 bp
Length ac2t2: 3936 bp
Length ac3t3: 4007 bp

Insert: ac3t3:
   a. 125.8 ng/µL

→ Vector: 150 ng, Insert: 269 ng

b. 58 ng/µL

Insert: ac2t2:
a. 220.9 ng/µL
b. 305 ng/µL´

→ for insert ac2t2: Vector 1 µL from b and insert 2 µL from a
→ for insert ac3t3: Vector 1 µL from b and insert 1 µL from b

3. Prepared Infusion Cloning mixture:

	pJIR750_ac2t2	pJIR750_ac3t3
5x InfusionHD Enzyme Premix	2 µL	2 µL
vector	1 µL from b.	1 µL from b.
insert	1 µL from b.	2 µL from a.
water to 10 µL	6 µL	5 µL

Tuesday, May 26th:

1. Transformation [pMTL83151_ac2t2] and [pMTL83151_ac3t3] in *E. coli* XL1 blue MRF` (see protocols)

Wednesday, May 27th:

1. Repeat transformation of [pMTL83151_ac2t2] and [pMTL83151_ac3t3] in *E. coli* XL1 blue MRF`

Note: yesterdays plates overgrew, use two other plates this time. Chloramphenicol inactive?

Thursday, May 28th:

Notes: New Plates look good! Throw away old plates, chloramphenicol is obviously inactive. Prepare more chloramphenicol (conc 30 mg/mL): weigh 0.4 g into 13 ml EtOH (99 %)
Prepare more chloramphenicol agar plates

1. Pick colonies 1-6 (see protocols), 6 per plasmid

Friday, May 29th:

1. Plasmid isolation from picked colonies 1-6 for both plasmids
2. Analytic digest
3. Gelelectrophoresis

Analytic digest:

1. Prepared Mastermix for 12 tubes as follows:

Chemicals	Volume
FastDigest Green Buffer	28 µL
Restriction enzyme 1 *Xho*I	14 µL
Restriction enzyme 2 *BamH*I	14 µL
H_2O	210 µL

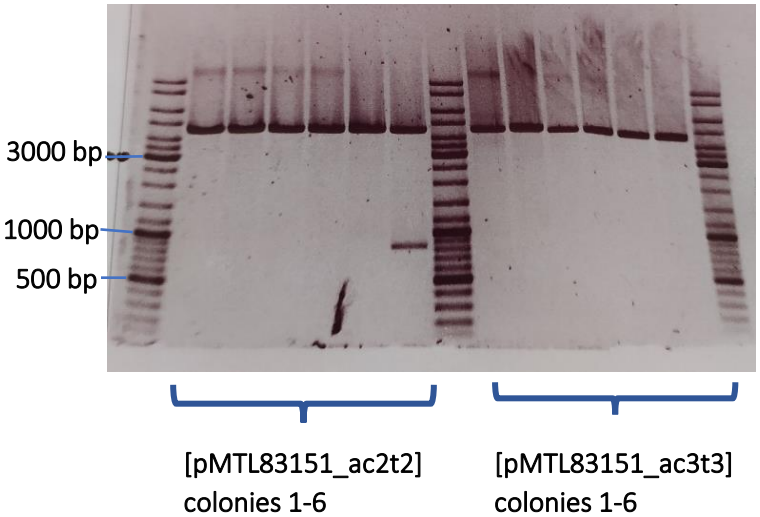
2. Divided mastermix into 12 Eppendorf tubes and added 1 µL plasmid DNA per tube. six tubes per plasmid ([pMTL83151_ac2t2], [pMTL83151_ac3t3]). End volume 20 µL per tube.
3. Incubated at 37 °C for 20 min and at 65 °C for 5 min.

Plasmids	Enzymes	expected fragments
[pMTL83151_ac3t3]	*Xho*I + *Bam*HI	617 bp + 3377 bp + 4439 bp
[pMTL83151_ac2t2]	*Xho*I + *Bam*HI	2413 bp + 1510 bp + 4439 bp

Gelelectrophoresis

1. Prepared gel as described in protocols
2. Filled pockets with 6 µL digest product and 4 µL Ladder in the following pattern:

Ladder	ac2t2 1	ac2t2 2	ac2t2 3	ac2t2 4	ac2t2 5	ac2t2 6	Ladder	ac3t3 1	ac3t3 2	ac3t3 3	ac3t3 4	ac3t3 5	ac3t3 6	Ladder



Results: A transformation of the acetone synthesis plasmids did not happen in any colony. None of the fragment showed expected lengths, just the backbone was transferred. Repeat picking colonies.

Tuesday, June 2nd:

1. Picking 6 more colonies (7-12) for plasmids [pMTL83151_ac2t2] and [pMTL83151_ac3t3]

Wednesday, June 3rd:

2. Plasmid isolation from picked colonies 7- 12 for plasmids [pMTL83151_ac2t2] and [pMTL83151_ac3t3]
3. Analytic digest
4. Gelelectrophoresis
5. Picking 6 more colonies (13-18) from [pMTL83151_ac2t2] (see protocols)

Analytic digest:

1. Prepared mastermix for 12 tubes as follows:

Chemicals	Volume
FastDigest Green Buffer 10,0 % (v/v)	28 µL
Restriction enzyme 1 *Xho*I	14 µL

Restriction enzyme 2 *BamH*I	14 µL
H_2O	210 µL

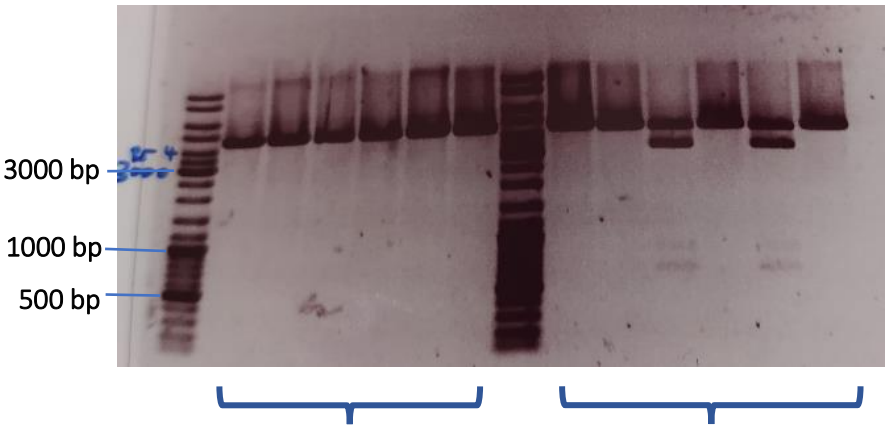
2. Divided mastermix into 12 Eppendorf tubes and added 1 µL plasmid DNA per tube. Six tubes per plasmid ([pMTL83151_ac2t2], [pMTL83151_ac3t3]). End volume 20 µL per tube.
3. Incubated at 37 °C for 20 min and at 65 °C for 5 min.

Plasmid	Enzyme	Fragments
[pMTL83151_ac3t3]	*Xho*I + *Bam*HI	617 bp + 3377 bp + 4439 bp
[pMTL83151_ac2t2]	*Xho*I + *Bam*HI	2413 bp + 1510 bp + 4439 bp

Gelelectrophoresis

1. Prepared gel as described in protocols
2. Filled pockets with 6 µL digest product and 4 µL Ladder in the following pattern:

Ladder	ac2t2 7	ac2t2 8	ac2t2 9	ac2t2 10	ac2t2 11	ac2t2 12	Ladder	ac3t3 7	ac3t3 8	ac3t3 9	ac3t3 10	ac3t3 11	ac3t3 12	Ladder



Results: A transformation of the acetone synthesis plasmid did not happen in any colony from plasmid [pMTL83151_ac2t2], ony the backbone was transferred. The transformation did happen for the acetone synthesis plasmid [pMTL83151_ac3t3] in the colonies 9 and 7. Here, the fragment lengths of 617 bp + 3377 bp + 4439 bp could be proven with the digest and gelelectrophoresis → prepare plasmids for sequencing! Repeat picking colonies for the plasmid [pMTL83151_ac2t2].

Thursday, June 4th:

1. Plasmid isolation from picked colonies 13-18 for [pMTL83151_ac2t2]
2. Analytic digest
3. Gelelektrophoresis
4. Prepare and send [pMTL83151_ac3t3] plasmids from colonies 9 and 11 to being sequenced

Analytic digest:

1. Prepared Mastermix for 6 tubes as follows:

Chemicals	Volume
FastDigest Green Buffer 10,0 % (v/v)	14 µL
Restriction enzyme 1 *Xho*I	7 µL
Restriction enzyme 2 *BamH*I	7 µL
H_2O	105 µL

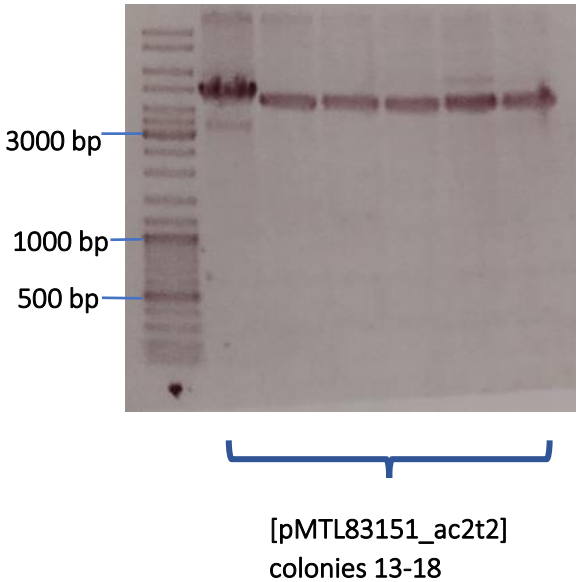
2. Divided Mastermix into 6 Eppendorf tubes and added 1 µL Plasmid DNA per tube. End volume 20 µL.
3. Incubated at 37 °C for 20 min and at 65 °C for 5 min.

Plasmid	Enzymes	expected fragments
[pMTL83151_ac2t2]	*Xho*I + *Bam*HI	2413 bp + 1510 bp + 4439 bp

Gelelectrophoresis

1. Prepared gel as described in protocols
2. Filled pockets with 6 µL digest product and 4 µL Ladder in the following pattern:

Ladder	ac2t2 13	ac2t2 14	ac2t2 15	ac2t2 16	ac2t2 17	ac2t2 18



[pMTL83151_ac2t2]
colonies 13-18

Results: A transformation did not happen in any colony for the acetone synthesis plasmid [pMTL83151_ac2t2]. None of the fragments showed the expected length. Only the backbone was transferred.

Behavioral study mealworms

Wednesday, May 13th:

1. Initial setup of the experiment
2. Weighing out initial masses

Setting up the experiment:

A drawer cabinet (polypropylene) inside an acrylic glass container was set to contain four groups of mealworms. Of the six drawer positions in the cabinet only the four middle ones were occupied by two control groups and two test groups in the following order:

Position in the cabinet	Drawer content
1 (top)	empty
2	Control group 1
3	Test group 1
4	Control group 2
5	Test group 2
6 (bottom)	empty

The alteration between test groups and control groups show relative possible local differences in external factors like temperature, light etc.

Weighing out initial masses:

The control group drawers were filled with 90.00 g of oatmeal from the grocery shop and 50.00 g of mealworms. The control group drawers were filled with 20.00 g of extruded polystyrene (EPS) from standard packaging material and 50.00 g of mealworms. Water bowl (polypropylene) was filled with 50 ml of demineralized water were placed in each drawer.

Thursday, May 14th:

1. Weighing total masses

Setting up the experiment:

See weighing protocol.

Friday, May 15th:

1. Weighing total masses

Monday, May 18th:

1. Weighing total masses
2. Installation of air humidifying unit

Installation of an air humidifying unit:

Water shortage was identified as a problem of the setup. A specially designed air humidifier was placed in the outer containment. For further details see [Link: Engineering success]. Additionally, humidity sensors were placed in the containers. The relative humidity increased from approx. 30% to approx. 50 % in each drawer in the course of 2 hours.

Tuesday, May 19th:

1. Weighing total masses

Wednesday, May 20th:

1. Weighing total masses

Friday, May 22nd:

1. Weighing total masses
2. Setup of new parallel test group

Setting up new parallel test group:

To test a more manageable test group only four pieces of EPS were placed in a new drawer:

Position in the cabinet	Drawer content
1 (top)	New parallel test group
2	Control group 1
3	Test group 1
4	Control group 2
5	Test group 2
6 (bottom)	empty

A water bowl filled with approx. 50 ml of demineralized water was placed in the drawer too.

Monday, May 25th:

1. Weighing total masses
2. Separating and weighing the fractions
3. Weighing fractions of parallel test group

Separating and weighing the fractions:

See weighing protocol.

Tuesday, May 26th:

1. Weighing total masses
2. Weighing fractions of parallel test group

Wednesday, May 27th:

1. Weighing total masses
2. Weighing fractions of parallel test group

Thursday, May 28th:

1. Weighing total masses
2. Weighing fractions of parallel test group

Wednesday, May 29th:

1. Weighing total masses
2. Separating and weighing the fractions
3. Weighing fractions of parallel test group

Tuesday, June 2nd:

1. Weighing total masses
2. Weighing fractions of parallel test group

Wednesday, June 3rd:

1. Weighing total masses
2. Separating and weighing the fractions
3. Weighing fractions of parallel test group

Thursday, June 4th:

1. Weighing total masses

2. Weighing fractions of parallel test group

Friday, June 5th:

1. Weighing total masses
2. Weighing fractions of parallel test group

Tuesday, June 9th:

1. Weighing total masses
2. Separating and weighing the fractions
3. Weighing fractions of parallel test group

Wednesday, June 10th:

1. Weighing total masses
2. Weighing fractions of parallel test group

Monday, June 15th:

1. Weighing total masses
2. Separating and weighing the fractions
3. Weighing fractions of parallel test group

Tuesday, June 16th:

1. Weighing total masses
2. Weighing fractions of parallel test group

Wednesday, June 17th:

1. Weighing total masses
2. Weighing fractions of parallel test group

Tuesday, June 23rd:

1. Weighing total masses
2. Separating and weighing the fractions
3. Weighing fractions of parallel test group
4. Finalize experiment

Finalize experiment:

All larvae frozen at -20 °C and substrates retained for further analysis. Drawers and cabinet cleaned and disinfected. See weighing protocol.

Measurement data

TAB.1. *Total masses for control groups and test groups. Yellow highlighted data are measurements before and after the separation and weighing of the fractions. These mass differences were corrected for graphical analysis.*

date	number	Total mass of drawers with contents [g]			
		Control 1	Control 2	Test 1	Test 2
13/05/2020	0	391.35	391.32	320.91	323.78
15/05/2020	2	390.08	389.81	325.64	327.79
18/05/2020	5	390.57	387.49	334.21	338.43
19/05/2020	6	386.18	384.74	334.89	339.39
20/05/2020	7	382.73	381.73	335.41	340.04
22/05/2020	9	378.15	377.87	337.57	341.34

25/05/2020	12	374.88	374.73	337.16	341.45
25/05/2020	12	414.56	409.85	332.3	337.65
26/05/2020	13	414.06	409.71	332.16	337.84
27/05/2020	14	412.6	408.32	331.64	337.31
28/05/2020	15	411.57	407.45	331.05	336.53
29/05/2020	16	410.01	406.2	330.41	335.79
29/05/2020	16	409.35	407.13	330.39	332.19
02/06/2020	20	403.68	402.64	326.64	327.07
03/06/2020	21	401	400.12	325.76	326.08
03/06/2020	21	398.62	393.24	320.98	320.77
04/06/2020	22	397.8	393.68	319.88	320.88
05/06/2020	23	395.57	391.55	319.84	319.6
09/06/2020	27	391.39	387.44	317.58	317.25
09/06/2020	27	385.75	381.94	314.91	313.75
10/06/2020	28	384.92	382.31	313	316.28
15/06/2020	33	382.78	380.07	310.55	313.27
15/06/2020	33	370.76	371.74	309	310.87
16/06/2020	34	371.64	370.62	308.43	310.45
17/06/2020	35	371.22	370.35	308.02	309.96
18/06/2020	36	369.9	369.65	307.66	309.6

TAB. 2. *Masses of separated fractions of control groups*

date	nr.	Masses control group 1 [g]				Masses control group 2 [g]			
		oatmeal	larvae	pupae	dead	oatmeal	larvae	pupae	dead
13/05/2020	0	90.000	50.00	0	0	90.000	50.00	0	0
25/05/2020	12	48.937	73.15	0.799	0.647	51.651	69.12	1.942	0.694
29/05/2020	16	86.937	68.22	2.80	0.7	87.163	65.42	2.158	0.144
03/06/2020	21	80.863	56.41	12.072	0.308	82.984	52.85	12.175	0.787
09/06/2020	27		44.16	10.696	0.503	84.807	40.56	10.019	0.736
15/06/2020	33		29.41	10.033	0.198	86.629	30.22	11.192	0.706

TAB. 3. *Masses of separated fractions of test groups*

date	nr.	Masses test group 1 [g]					Masses test group 2 [g]				
		EPS	faeces	larvae	pupae	dead	EPS	faeces	larvae	pupae	dead
13/05/2020	0	20.00	0.000	50.00	0.000	0.000	20.00	0.000	50.00	0.000	0.000
25/05/2020	12	18.27	2.250	63.02	2.106	0.000	18.49	1.763	65.39	1.589	0.000
29/05/2020	16	18.16	0.709	57.71	2.620	0.358	17.79	0.677	60.30	2.735	0.000
03/06/2020	21	17.45	0.677	49.6	4.102	0.000	17.75	0.842	51.95	4.465	0.000
09/06/2020	27	17.35	0.866	46.7	0.1789	0.000	17.2	0.992	42.90	2.339	0.000
15/06/2020	33	16.8	1.003	41.27	0.682	0.000	16.33	1.012	40.74	1.383	0.000

TAB. 4. *Masses of EPS pieces of parallel test group*

date	Nr.	Masses of EPS pieces [g]			
		piece a	piece b	piece c	piece d

22/05/2020	0	0.471	0.694	0.705	0.723
26/05/2020	4	0.346	0.551	0.562	0.562
27/05/2020	5	0.321	0.545	0.555	0.542
28/05/2020	6	0.281	0.507	0.503	0.511
29/05/2020	7	0.263	0.489	0.483	0.493
02/06/2020	11	0.196	0.443	0.389	0.445
03/06/2020	12	0.187	0.431	0.371	0.434
04/06/2020	13	0.177	0.42	0.353	0.419
05/06/2020	14	0.167	0.404	0.339	0.408
09/06/2020	18	0.14	0.376	0.312	0.382
10/06/2020	19	0.136	0.371	0.303	0.372
15/06/2020	24	0.116	0.34	0.253	0.337
16/06/2020	25	0.13	0.322	0.238	0.323
17/06/2020	26	0.095	0.311	0.221	0.304
18/06/2020	27	0.086	0.298	0.204	0.284

TAB. 5. *Masses of separated fractions of parallel test group (masses of EPS pieces summed)*

date	Nr.	Masses [g]				
		EPS	faeces	larvae	pupae	dead
22/05/2020	0	2.593	0	50.02	0	0
26/05/2020	4	2.021	0.923	46.23	0	0
27/05/2020	5	1.963	1.116	45.60	0	0
28/05/2020	6	1.802	1.217	48.73	0	0
29/05/2020	7	1.728	1.347	48.16	0	0
02/06/2020	11	1.473	1.889	49.03	0.322	0
03/06/2020	12	1.423	2.125	47.72	0.586	0
04/06/2020	13	1.369	2.27	45.42	2.077	0
05/06/2020	14	1.318	2.423	43.10	3.544	0
09/06/2020	18	1.21	2.969	34.60	8.818	0
10/06/2020	19	1.182	3.118	33.01	9.863	0
15/06/2020	24	1.046	3.775	26.301	13.539	0
16/06/2020	25	1.013	3.905	25.13	14.227	0
17/06/2020	26	0.931	4.033	24.31	14.357	0
18/06/2020	27	0.872	4.148	23.68	14.658	0

Parts

part nr.	part	type	basic/composite
BBa_K3443000	ac2t2	plasmid	composite
BBa_K3443001	ac3t3	plasmid	composite
BBa_K3443002	PthlA	promotor	basic
BBa_K3443003	thlA (*C. kluyveri*)	Enzyme Gene	basic
BBa_K3443004	thlA (*C. scatologenes*)	Enzyme Gene	basic
BBa_K3443005	ctfA ctfB (*C. aceticum*)	Enzyme Gene	basic
BBa_K3443006	ctfA ctfB (*C. scatologenes*)	Enzyme Gene	basic
BBa_K3443007	adc	Enzyme Gene	basic
BBa_K3443008	[pMTL84151]	plasmid	basic

PCR

Introduction

This protocol for a Polymerization Chain Reaction (PCR) is used to amplify DNA fragments.

Materials

- CloneAmp™ HiFi PCR Premix by Takara Bio Inc.
- Primer (forward and reverse)
- Plastic tubes
- Thermocycler
- Table- top centrifuge
- Water
- Vortexer

Procedure

1. Prepare primers, dilute 1:10
2. Prepare mastermix for each plasmid (see Table 1). Each tube should contain 25 µL.

   Table 1: Mixed solution PCR for one tube

Chemicals	**Volume**
CloneAmp™ HiFi PCR Premix	12.5 µL
Primer forward	0.5 µL
Primer reverse	0.5 µL
water	to 25 µL
Total volume	**25 µL**

3. Add 1 µL DNA per tube.
4. Place tubes in thermocycler and start following protocol:

Step	**Temperature**	**Time**
1	98°C	10 min
2	98°C	10 s
3	55- 60°C	10 s
4	72°C	2:30 min
5	Go to step 2 and repeat 30 times	
6	72°C	10 min

5. Hold product on 4°C

Masterplate and picking colonies

Note: Work under sterile conditions while picking colonies!

Material:

- Agar plates (LB + chloramphenicol antibiotic (30 µg/mL)) with clones
- Agar plates (LB + chloramphenicol antibiotic (30 µg/mL)) not inoculated used as masterplate
- Autoclaved toothpicks
- Test tubes with 5 mL LB- media and chloramphenicol

Procedure:

1. Divide not inoculated agar plates into squares and label with one number per picked colony
2. Open the agar plates with colonies and pick a single colony with an autoclaved toothpick
3. Carefully scratch the respective square of the not inoculated agar plates. Place toothpick in a test tube. Repeat with 6 colonies.
4. Incubate the masterplate and inoculated test tubes with selected colonies at 37°C overnight use the liquid culture for plasmid isolation.

Plasmid isolation

This protocol is adapted from Zyppy Plasmid Miniprep Kit (ZYMO RESEARCH).

Materials:

- Zyppy Plasmid Miniprep Kit
- Table- top centrifuge
- Eppendorf tubes
- Demineralized water
- Bacterial overnight culture (*Escherichia coli*: 37 °C)

Procedure:

1. Harvest cells by centrifuging 2 ml of liquid culture at 13000 rpm for 1 min at room temperature
2. Repeat step 1 with the same tube
3. Resuspend pelleted bacteria in 600 µLH_2O
4. Add 100 µL 7xLysis Buffer, mix
5. Add 350 µL Neutralization Buffer, mix until yellow
6. Centrifuge mixture at 13000 rpm 5 min at room temperature
7. Transfer supernatant in tube with filter in collection tube
8. Centrifuge at 13000 rpm for 15 s at room temperature → discard collection tube
9. Add 200 µL Endo-Wash-Buffer to the column
10. Centrifuge 30 s 13,000 rpm at room temperature → discard collection tube
11. Add 400 µL Zyppy-Wash-Buffer
12. Centrifuge at 13000 rpm for 2 min at room temperature
13. Transfer column into a new tube, add 25 µL H_2O on the filter without touching
14. Incubate 2 min at room temperature
15. Centrifuge at 13000 rpm 1 min at room temperature to eluate the plasmid

Gelelectrophoresis

Material:

- 1x TAE buffer
- Agarose (0.8 % w/v)
- Gene Ruler LadderMix (Thermo Fischer)
- 6x Loading Dye (LD) (Thermo Fischer)
- PCR Product
- Gelelectrophoresis Station
- Ethidium bromide
- UV illuminator and camera

Procedure:

Gelelectrophoresis:

1. Cast gel in a gel camber and add appropriate comb
2. Wait until gel is fully polymerized
3. Fill chamber with 1x TAE buffer
4. Remove comb and load gel in the following pattern
5.

Pocket 1	**Pocket 2**	**Pocket 3**	**Pocket 1**	**Pocket 4**	**Pocket 5**	**Pocket 6**
4 µL Ladder Mix	Mix PCR Product colony 1	Mix PCR Product colony 2	Mix PCR Product colony 3	Mix PCR Product colony 4	Mix PCR Product colony 5	Mix PCR Product colony 6

   Fill pockets with Mix:
   - 5 µL 1x TAE buffer
   - 2 µL LD
   - 1 µL PCR product

6. Run gel at 120 V for 40 min
7. Stain gel in ethidium bromide solution for 5 min
8. Image the gel using an UV illuminator and take a picture

Sieving and weighing protocol

To get a better understanding of the subjects of research namely the larvae of the beetle *Tenebrio molitor* (mealworms), a population dynamics study was conducted. Therefore, different groups of mealworms were kept in drawers inside a special setup. Further details on the setup are described under **[Link: engineering success]**. The larvae were kept inside the drawers for 36 days. During this time, the drawers themselves including their contents were weighed. But also, the drawer's contents were weighed in different fractions. To separate the larvae into the different mass fractions, sieves were used. This protocol was followed to create the measuring data found in the **[Link: Notebook]**.

Safety note

During the hole process of sieving and weighing fine dust accumulates in the work area. This dust can cause allergic reactions and asthmatic symptoms in sensitive persons. Skin contact is to be avoided by wearing proper lab coats (covering the hole arm to wrist) and gloves and eye protection. To avoid inhalation of dust particles a mask needs to be used. Good ventilation of the work area needs to be ensured by regularly opening windows.

Sieving

The drawer's contents had to be separated into five fractions. Tab. 1. shows the five fractions for the control group and the test group.

Tab. 1. *Fractions of control group and the test group. EPS standing for extruded polystyrene.*

fraction	Control group	Test group
(1) substrate	(1a) EPS chunks approx. 20*20*20 mm^3	(1b) Oatmeal approx. 8*6*1 mm^3
(2) faeces	Particles < 1 mm^3	Particles < 1 mm^3
(3) larvae	Wormlike 30*4*4 mm^3	Wormlike 30*4*4 mm^3
(4) pupae	10*6*4 mm^3	10*6*4 mm^3
(5) Dead material	Shed or (parts of/dead) larvae/pupae	Shed or (parts of/dead) larvae/pupae

The separation was achieved by sieving. When testing out different screen sieves, their low efficiency against longish objects was observed, as they need to be oriented vertically to match their smallest site with the square pores. To improve this, "pet toilet shovels" were purchased from a local pet store. Those posses' rectangular slits with one large site of about 60 mm and a special geometry that helps the longish objects to orient parallel to the slits. The used sieves and the permeability against the five fractions stated in Tab. 1. is shown in Tab. 2.

Tab. 2. *Permeability of different fractions stated in Tab. 1. through different sieves*

sieve	permeable
#0 none	(1a), (1b), (2), (3), (4), (5)
#1 Pet toilet shovel large	(1b), (2), (3), [(4)], [(5)]
#2 Pet toilet shovel small	(1b), (2), [(3)]
#3 Screen sieve 1*1 mm^2	[(1b)], (2)

[]: semipermeable

Two achieve the best separation, the sieves stated in Tab. 2. were used in descending order. As some irregular small larvae, pupae or pieces of the dead mass sometimes permeated the sieve unintentionally (semipermeable), the screening material was manually processed with tweezers. A complete separation of faeces (2) and oatmeal (1b) could not be achieved (The larvae produced such fine abrasion of the oatmeal that it was indistinguishable by size from the faeces). The mass fractions were decanted into tared containers (mass written thereon) which were sealed before weighing. The empty drawers were rinsed several times with taped water until no debris were visible and rinsed and disinfected with 70 % ethanol. After the ethanol is dried, the containers were rinsed a last time with demineralized water and set to dry. The water bowls were emptied and cleaned the same way. The humidity sensors were wiped with a wet towel until they were clean and disinfected with 70 % ethanol. After drying, they were wiped with a wet towel (demin. water) and dried.

Weighing

The tared containers were placed on an analytical balance and when the reading was stable, the mass in grams with (if possible) three decimal places was noted. For weighing the extruded polystyrene, large containers were necessary (large specific density of EPS). Those are stronger influenced by air blasts and a stable reading of the balance was only possible for two decimal places. The slight movement of the larvae also allowed for only two decimal places.

Sample collection

Faeces and faeces mixed with oatmeal were permanently stored in containers for later analysis. Pupae (and larvae at the end of the experiment) were frozen at – 20 °C and stored at this temperature for later analysis.

Restoring the experimental setup

Oatmeal was weight out to fill the remaining material up to 90 g and added to the remaining oatmeal. The substrate was added to the containers and the larvae were added to the containers.

After weighing the total mass: All drawers were checked a last time for structural integrity and possible damages. Notes were carefully taken of all possible damages and those were repaired if possible (so far, no damage to the containers was observed). The dishes were filled with approx. 50 ml of demineralized water and placed in the drawers. The humidity sensors were added back into the drawers. All drawers were slid all the way in into the cabinet. The outer container was properly sealed.

Weighing the total mass

The drawers with content, but without water bowls were weight on an analytical balance. The stable reading in grams was noted with a precision of two decimal places.

Transformation

Introduction:

This protocol describes the transformation of DNA in competent *Escherichia coli* via heat shock.

Note: Work under sterile conditions.

Materials:

- Competent *E. coli* cells
- Plasmids
- Heatblock
- Eppendorf tubes
- Table- top centrifuge
- LB- media

Procedure:

1. Thaw *E. coli* on ice
2. Add 5 µL Infusion Product, gently mix, incubate 10 min on ice
3. Heatshock at 42°C for 1 min
4. Incubate 10 min on ice
5. Add 800 µL LB-Medium
6. Incubate under shaking conditions for 35 min at 37 °C
7. Centrifuge at 5000 rpm 3 min at room temperature
8. Discard 800 µL of the supernatant and resuspend cell pellet in the remaining medium
9. Transfer the solution onto agar plate and spread with clean Drigalski spatula
10. Incubate overnight at 37°C

Restriction Digest

Introduction:

Restriction digest is used to check if the plasmid shows the correct fragment length.

Note: Always handle enzymes and buffer on ice.

Material:

- FastDigest enzymes (Thermo Fischer)
- FastDigest Green Buffer (Thermo Fischer)
- Plasmid DNA

Procedure analytic digest

1. In a tube combine the following:

Chemicals	Volume
FastDigest Green Buffer	2 µL
Restriction enzyme	1 µL
Plasmid DNA	1 µL
H_2O	to 20 µL

2. Incubate at 37 °C for 20 min; inactivate at 65 °C for 5 min

Procedure preparative digest

1. In a tube combine the following:

Chemicals	Volume
FastDigest Green Buffer	5 µL
Restriction enzyme	2.5 µL
Plasmid DNA	1 µL
H_2O	to 50 µL

2. Incubate at 37 °C for at least 3 h; inactivate at 65 °C for 5 min

DNA Extraction

A. DNA Extraction from Gel

Introduction:

Zymoclean™ gel DNA Recovery Kit protocol was adapted.

Material:

- Gel with DNA of interest
- Zymoclean™ gel DNA Recovery Kit
- Eppendorf Tubes and tubes with filters
- water
- Table- top centrifuge

Procedure:

1. Weigh 2 ml Eppendorf tube (1g)
2. Excise the DNA fragment to be purified from the agarose gel using a clean tool and add it to the Eppendorf tube.
3. Weigh again to determine the weight of the gel slice
4. Add ADB buffer three times the volume of the gel slice
5. Melt gel at 55°C
6. Transfer into tube with filter
7. Centrifuge at 13000 rpm 1 min at room temperature, discard collection tube
8. Add 200 µL wash buffer and centrifuge at 13000 rpm 1 min at room temperature, discard collection tube
9. Add 200 µL wash buffer and centrifuge at 13000 rpm 2 min at room temperature, discard collection tube
10. Transfer tube with filter into clean Eppendorf tube (1,5 ml)
11. Add 10 µL H_2O to the filter without touching and incubate 1 min at room temperature
12. Centrifuge at 13000 rpm 1 min at room temperature
13. Repeat steps 11 and 12

B. DNA Extraction from PCR Product

Materials

- PCR product
- Wash buffer
- Binding buffer
- Table- top centrifuge
- Eppendorf tubes and tubes with filters
- water

Procedure

1. Add 200 µL binding buffer to PCR product, mix carefully
2. Transfer mixture into tube with filter
3. Centrifuge at 13000 rpm 1 min at room temperature, discard collection tube
4. Add 200 µL wash buffer to the filter
5. Centrifuge at 13000 rpm 1 min at room temperature, discard collection tube
6. Add 200 µL wash buffer to the filter
7. Centrifuge at 13000 rpm 2 min at room temperature, discard collection tube
8. Transfer tube with filter to clean Eppendorf tube (1,5 ml)
9. Add 10 µL H_2O to the filter without touching and incubate 1 min at room temperature
10. Centrifuge at 13000 rpm 1 min at room temperature
11. Repeat steps 9 and 10

DNA Purification

A. DNA Purification after gel electrophoresis

Introduction:

Zymoclean™ Gel DNA Recovery Kit protocol was adapted.

Material:

- Gel with DNA of interest
- Zymoclean™ Gel DNA Recovery Kit
- Eppendorf tubes
- Table- top centrifuge

Procedure:

1. Weigh 2 mL Eppendorf tube (1g)
2. Excise the DNA fragment to be purified from the agarose gel using a clean tool and put the gel fragment in the Eppendorf tube
3. Weigh again to determine the weight of the gel fragment
4. Add ADB buffer three times the volume of the gel fragment
5. Melt gel at 55 °C
6. Transfer the solution into the column
7. Centrifuge at 13000 rpm for 1 min at room temperature, discard collection tube
8. Add 200 µL wash buffer and centrifuge at 13000 rpm for 1 min at room temperature, discard collection tube
9. Add 200 µL wash buffer and centrifuge at 13000 rpm for 2 min at room temperature, discard collection tube
10. Transfer tube with filter into fresh Eppendorf tube (1.5 mL)
11. Add 10 µL H_2O to the filter without touching and incubate for 1 min at room temperature
12. Centrifuge at 13000 rpm for 1 min at room temperature
13. Repeat steps 11 and 12

B. DNA purification after PCR

Materials

- PCR Product
- Wash Buffer
- Binding buffer
- Table- top Centrifuge
- Eppendorf tubes

Procedure

1. Add 200 µL binding buffer to PCR product, mix carefully
2. Transfer mixture into the column
3. Centrifuge at 13000 rpm for 1 min at room temperature, discard collection tube
4. Add 200 µL Wash buffer and centrifuge at 13000 rpm for 1 min at room temperature, discard collection tube
5. Add 200 µL Wash buffer and centrifuge at 13000 rpm for 2 min at room temperature, discard collection tube
6. Transfer tube with filter to fresh Eppendorf tube (1.5 mL)
7. Add 10 µL H_2O to the filter without touching and incubate for 1 min at room temperature
8. Centrifuge at 13000 rpm for 1 min at room temperature
9. Repeat steps 7 and 8

Lysogeny broth Media

LB- media:

• NaCl	10	g
• Trypton	10	g
• Yeast extract	5	g
• H_2O	*ad* 1000	mL

Agar plates: add 1.5 % w/v agar

To fluid LB- media: add 10 µL chloramphenicol antibiotic (30 mg/mL stock concentration) per 5 mL LB- media

Ligation: Infusion Cloning

Introduction:

Infusion Cloning ligates PCR products (inserts) to a linearized vector and produces a new plasmid.

Note: keep enzymes on ice.

Material:

- 5x In- Fusion HD Enzyme Premix by Takara Bio Inc.
- Linearized vector
- Purified inserts
- demineralized water
- Eppendorf tubes

Preparation:

Measure DNA concentration with NanoDrop and calculate needed volumes.

Procedure:

1. Prepare the following mixture in an Eppendorf tube (1.5 ml) on ice:

Chemicals	**Volume**
5x In- Fusion HD Enzyme Premix	2 µL
Linearized vector	70 ng
Inserts	100 ng each insert
H_2O	to 10 µL

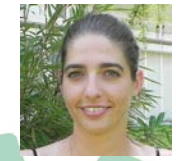
2. The total volume should be 10 µL. Incubate at 50°C for 20-35 min and proceed with transformation or store at -20 °C.

Where the magic happens

Our research takes place at Ulm University in Southern Germany. As Ulm is the birthplace of Albert Einstein, we chose the famous scientist in combination with our research subject T. molitor as our logo. Our lab space is located in the Institute for Microbiology and Biotechnology. Our instructors Dr. Frank Bengelsdorf and Teresa Schoch frequently support us. Both are very experienced in the fields of our research and therfore very auxiliary for our project.

On organisatory issues and questions about feasability and strategy we can count on our PIs, microbiologist Professor Dürre and Professor Johnsson, who is a geneticist.

Dr. Frank Bengelsdorf
Instructor



Teresa Schoch
Instructor

We are enthusiastic our project could finally come about and very grateful for the nice and competent assistance in everything we do. We would especially like to thank the four mentioned above, but also everyone else who was involved in the challenging process of making this project possible.

Thanks to all of you!

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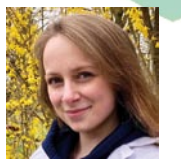

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IGEM_UULM

Degradation of Polystyrene

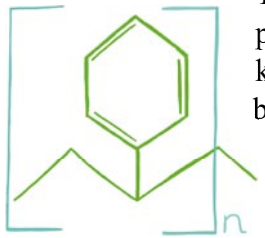
iGEM_uulm igem_uulm igem_uulm iGEMuulm

What is iGEM?

As a team of 14 students from Ulm University (Germany) we are participating in an international science contest called iGEM competition which is organized by the iGEM Foundation. The abbreviation iGEM stands for "international genetically engineered machine". The aim of the competition is to solve problems of humanity with the help of synthetic biology. The final competition takes place in Boston, USA, in form of the so-called Giant Jamboree, where all teams present the results of their research.

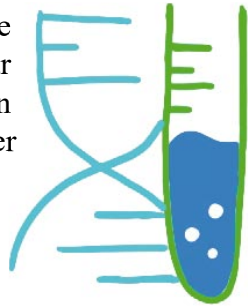
The aim of our research

Everyone has heard of the problems of plastic pollution. Especially in the oceans it causes severe damage to sea animals and the ecosystem as a whole.



The most common plastic is polyethylene terephthalate, better known as PET. It is found in plastic bottles and many other products of daily use. Methods of breaking down this type of plastic have already been developed by iGEM teams in the past. But is there a universal solution applicable to all the other types of plastic?

Unfortunately, no. Plastics are a very heterogeneous group of substances and their chemical structures differ a lot from each other, which is why one needs different approaches to attain their biological degradation. Our newly founded team has come up with a promising new idea of degrading polystyrene, abbreviated PS, which is the world's number three on the list of the most abundant plastics: the larvae of the beetle Tenebrio molitor, also called flour worm, are able to break down chunks of PS foam into further biodegradable material.



The aim of our project is to investigate which species of bacteria inside Tenebrio molitor's intestines enable it to do that. Furthermore, we plan to isolate the bacteria and improve their special ability by genetically equipping it with another skill that will supposedly make them even more efficient.

RESEARCH TOPIC

The pollution of our environment by plastics is increasing, one of the most abundant being polystyrene [1]. As previous research demonstrates, the larvae of *Tenebrio molitor* can break down polystyrene. Recent studies have shown that the gut bacteria of those larvae play a huge role in the degradation of polystyrene. They are able to metabolically use polystyrene for growth and break it down to CO_2 [2].

Moreover, acetone can be used to dissolve polystyrene [3]. In our project, we want to combine these two factors to improve the degradation of polystyrene with genetically modified bacteria in the gut of the mealworms. For our approach, we decided to use a plasmid encoding the enzymes responsible for acetone production, in oder to make the polystyrene more accessible for the degradation in the gut.

To produce acetone, the enzymes thiolase (ThlA), acetoacetyl- CoA: acetate/ butyrate- CoA transferase subunits A and B (CtfA/CtfB) and acetoacetate decarboxylase (Adc) are necessary (Fig. 1A).

Two acetone synthesis plasmids were constructed, harboring different gene constellations (Fig. 1B and 1C).

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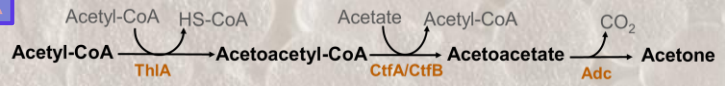
PLASMID CONSTRUCTION

FIGURE 1

A

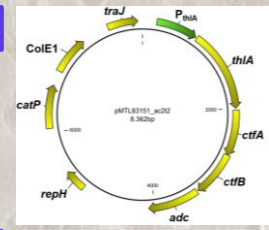


A: Pathway used for the acetone synthesis operon, consisting of thiolase (ThlA), acetoacetyl-CoA:acetate/butyrate-CoA transferase subunits A and B (CtfA/B), and acetoacetate decarboxylase (Adc)

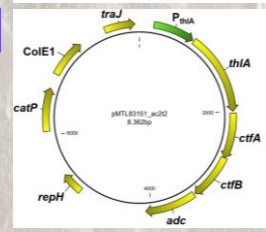
B: Plasmid pMTL83151_ac3t3 with and *ctfA/B* (CACET_c04240/CACET_c04250) from *C. aceticum*, *thlA* (CKL_3698) from *C. kluyveri*, and *adc* (CA_P0165) from *C. acetobutylicum*

C: Plasmid pMTL83151_ac3t3 with *thlA* and *ctfA/B* (EG59DRAFT_00772-00774) from *C. scatologenes*, and *adc* (CA_P0165) from *C. acetobutylicum*

B



C

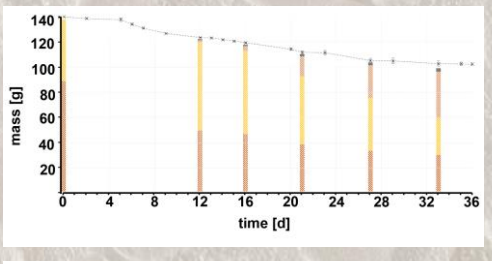


FIRST RESULTS

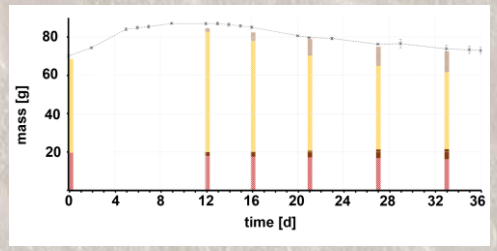
The first results show that the larvae can survive on both oatmeal and polystyrene diet (Fig. 2A and 2B). Fig. 2C illustrates the population of mealworms living only on polystyrene. The population in the control group is displayed in Fig. 2D. Although the experimental group solely lives on polystyrene, the mealworms complete their life cycle and show vital behavior.

FIGURE 2

A



B



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[1] Geyer *et al*., 2017, DOI: 10.1126/sciadv.1700782
[2] Brandon *et al*., 2018, DOI: 10.1021/acs.est.8b02301
[3] Cowie *et al*., 1983, DOI: 10.1126/sciadv.aaz7301

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