List of frequently asked questions

1	**Could the use of microorganisms suppose a biological risk to society?**
	No, despite microorganisms are used in the project to degrade plastics and to create new bioplastics, these new bioplastics will be safe for the society as other bioplastics present in the market
2	**Is pre-treatment necessary to improve the transformation of plastics?**
	Yes, it is very important a correct pre-treatment to degraded bioplastics. Despite enzymes and microorganisms can directly attack to plastics their efficiency increases significatively when pre-treatments are done to increase the surface contact and to decrease the molecular weight of the target polymer.
3	**How will this project improve the life quality of citizens?**
	BioICEP project will contribute to improve the life quality of citizens in different aspects. The demand of bioplastics is increasing during the last years due to their environmentally friendly characteristics, however some of bioplastics are produced from food resources which can be a negative aspect for the citizens. Besides the plastic waste disposed in landfills is still very high and it is polluting different environmental compartments as soils and oceans. For this reason, this project will help to improve the life quality by one hand producing bioplastics and on the other hand the project will reduce the plastic waste.
4	**Where will the microorganisms for degrading plastics be isolated from?**
	A number of partners working on this project have access to biobanks of fungal and bacterial strains. They will screen these microbes for their ability to degrade plastic by incorporating the plastic into the microbial growing media and monitoring the growth of the microbes. As the plastic will be the only source of Carbon available for the microorganisms, they will only be able to grow if they can breakdown the plastic and release the Carbon for their own cell metabolism and growth. In addition to existing biobanks partners in the project will also isolate microbes from new sources such as plastic waste. Also, microorganisms with known plastic degrading abilities will be grown together as a consortium in BioICEP project so that mixed plastics can be treated simultaneously.
5	**Can enzymes produced by microorganisms be collected and used to breakdown plastics?**
	Microorganisms can be cultured on synthetic media in the laboratory which contains all the key nutrients for them to grow such as a carbon and nitrogen source. If they are grown on a plastic as its sole source of carbon, the enzyme they use to break down the plastic is excreted from the microorganism into the growing medium. After growth, the cells can be removed from the growth medium which will now contain the excreted enzyme. This enzyme can be

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	further concentrated in the growth medium and then used to treat waste plastic.
6	**Do microorganisms exist that can already degrade plastics?**
	Yes, bacteria and fungi have been identified that can degrade plastic by using an enzymatic approach. Microorganisms have been shown to produce enzymes which breakdown the plastics polyethylene terephthalate (PET) and polyurethane (PUR). However, the turnover rate of these enzymes is quite low. While microbes have been associated with the breakdown of polystyrene, polyamide, polyvinylchloride, polypropylene and polyethylene, no specific enzymes acting on these plastics have so far been discovered. These plastics comprise more than 80% of annual plastic production. This project aims to discover new enzymes from microbes which can degrade plastic. The pre-treatment of the plastic prior to exposure to microbes with plastic degrading enzymes will expose more points of attack for the enzymes and so should improve their performance.
7	**Are there already known plastic-degrading enzymes?**
	Yes, several microbial enzymes with the ability to break down plastics have been isolated and characterized. These enzymes are mostly able to hydrolyse biodegradable plastics. Some of them, however, are also able to degrade PET which is a petroleum-based recalcitrant plastic. These plastics are all polyesters. We aim to discover novel polyester-hydrolases from new sources with better characteristics than the known ones. Furthermore, our goal in BioICEP is to discover and/or engineer other enzymes with the ability to break down non-biodegradable petroleum-based plastics like polyethylene and polypropylene.
8	**What are the desired characteristics for a plastic-degrading enzyme?**
	An enzyme suitable to degrade plastics for industrial applications should have some specific characteristics. Such enzymes should have affinity for the target-material, meaning they should be able to be adsorbed on their surface. Thermostability has proven to be an important property since it is desired to perform degradation reactions at relatively high temperatures (~70 ˚C) for several hours in order to achieve high degradation yields.
9	**If the enzymes you discover do not have the desired characteristics, can you employ them for the degradation of plastics?**
	With the advancement of molecular biology, scientists have now the right tools to alter the properties of enzymes using directed mutagenesis techniques. This way, if for example our newly discovered enzymes do not have the right thermostability for the application, then we can enhance it by performing a directed evolution approach.
10	**Could the industrial upscaling in the BioICEP project pose a risk to society?**
	Since the process under development in the BioICEP project deals with plastic treatment and valorisation, the impact to society should be positive. The impact to society will be evaluated during the execution of the project.
11	**Could the industrial process developed in the BioICEP project have a negative impact in the environment? (i.e. more energy and chemicals needed to break down plastics)**
	During the upscaling of the BioICEP process careful evaluation of impact of the process and the process inputs/outputs (i.e. chemicals and wastes) to the environment will be taken into consideration. The negative impact to the environment will be minimized.

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12	**Could the industrial process developed in the BioICEP project be technically and economically feasible?**
	The technical and economic feasibility of the process will be determined during the execution of the project.
13	**Will the plastics produced in BioICEP, from the decomposition of mixed plastic waste, be biodegradable?**
	Yes, BioICEP aims to produce biodegradable plastics from the decomposition of mixed plastic waste. Biodegradability of a bioplastic and time required depend on various factors, such as temperature and the amount of moisture present.
14	**What type of bioproducts can be made in BioICEP from the decomposition of mixed plastic waste?**
	Highly sought after bioproducts including: 1) biodegradable polymers, such as PHAs and nanocellulose, with applications in segments such as the food packaging industry; 2) rhamnolipids, as important bio-surfactant for the cosmetic and pharmaceutical industry. In addition, polymer compatibilizers to prepare polymer blends suitable for 3D printing.
15	**Will BioICEP help to tackle the plastic pollution in the oceans and other environments?**
	BioICEP will contribute to 12 out of the 17 UN Sustainable Development Goals SDGs ¨ which contain targets to improve waste and resource management directly and contain the highest proportions of targets aiming to alter waste and resource flows in our economy. BioICEP is designed to directly impact the global goals on: a) achievement of affordable and clean energy via very low overall energy consumption associated with the triple action depolymerisation and biosynthesis processes; b) clean water, sanitation and life below water, by alleviating the streaming of microplastics into water systems and life on land and; c) by the low carbon footprint biotransformation of enormous mixed plastic waste stockpiles into equivalent ubiquitous recalcitrant plastics replacements with biodegradable products.
16	**How can you intensify plastic degradation by enzymes?**
	With the advancements in protein engineering, enzymes with known sequences can be modified rationally and randomly via process called directed evolution to obtain improved variants. In addition, enzymes as biocatalysts can be improved by various stabilization techniques and mixture of enzyme can also be applied in order to achieve synergy. We aim to apply all these approaches on the enzymes reported to have the ability to depolymerise plastic materials, including PETases, cutinases and other.
17	**How sustainable are enzymes as biocatalysts?**
	To produce pure enzymes on industrial scale is still quite expensive process. However, their ecological footprint is lower in comparison to some metal-based catalysts. We are also exploring the possibility to use semi-purified enzyme preparations, to reuse stabilized enzymes and/or to use microbial-whole cells as biocatalysts.
18	**What is an end product of plastic degradation by enzymes?**
	It is expected that monomeric units of the plastic materials would be obtained at the end of the enzymatic depolymerisations. We are also looking into utilising that material as substrate for bacterial fermentations to produce virgin biopolymers such as PHA and nanocellulose, to add to the circularity of the plastics.

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One of #BioICEP 's objectives is to develop accelerated, high-efficiency biodegradation incorporating microorganism communities expressing at least three novel and improved enzymatic activities enabling the degradation of mixtures of plastics. #BioICEP is funded by #H2020
The second objective of the #BioICEP project is the sustainable degradation of at least 20% of mixed plastics. Do you want to know more about the project? https://bioicep.eu/index.php #BioICEP was made possible thanks to #H2020 funding @EU_H2020
The third objective of the #BioICEP project is to obtain high added value bioproducts, including equivalent bioplastics recovered from mixed plastic waste. Know more about it! https://bioicep.eu/index.php #BioICEP was made possible thanks to #H2020 funding @EU_H2020
Objective #4 of the #BioICEP project is to achieve a sustainable prototype system plan, paving the way to bring the developed solution to the market, fulfilling current needs, future expectations, and delivering a seamless bio-innovative route for a circular economy for plastics.

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