

BACHELOR THESIS

Biomimetics of Extremophiles

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Abstract

Extremophiles are organisms capable of or dependent on living in extreme conditions which are considered toxic or deadly to other species. Their ability to thrive in such environments makes them interesting to study and promises a variety of coping mechanisms and unique traits which could be used in various fields. They could be especially promising in the field of bioremediation of waste that is problematic for the environment because of its longevity and toxicity, like non-decomposable plastic waste or even radioactive material.

This thesis starts with an overview on types of extremophiles and their methods of survival, and goes into detail on oil- and plastic degrading extremophiles. Their chemical and physiological mechanisms are expanded on, including reaction pathways for aliphatic, with open chained bonds, and aromatic, with one or more electron-unsaturated hexagonal rings, hydrocarbons, as well as the similarities and differences in bacterial, fungal and eucaryotic metabolisms. A few methods for biotechnological remediation of oil contamination are elaborated on.

The environmental impact of plastic waste and the comparatively small number of plastic degrading microorganisms is discussed. An overview of contributing factors in microbial plastic degradation is given, and recent discoveries of plastic degrading species prompts a closer look at the newly found biodegradation pathway of PET, the aromatic polymer polyethylene terephthalate, which is primarily used for manufacturing plastic bottles.

Finally, the surface erosion of Kevlar samples was imaged with atomic force microscopy after the application of a laccase mediator system, an enzymatic treatment to test the biodegradability of this polymer fiber. The laccase mediator system utilizes laccase, an enzyme which catalyzes redox reactions, and makes use of the broad range of substrates which laccase can reduce, by using them as mediators for the reactions.

This thesis is supposed to be the stepping stone to a biomimetic approach to bioremediation. This means that this information should be the basis to learn from the exceptional abilities of extremophiles, to be able to copy mechanisms and methods into innovative technology and get ideas for smart bioremediation solutions.

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1 Motivation

The state of our environment is critical. The influence of human civilization has impacted the earth and its nature severely, some say even beyond its natural recovery capabilities. This bachelor thesis is focused on only a small section of influencing factors caused by humanity, specifically the insufficiencies of our waste management systems and approaching shortage of natural resources. It aims to find methods in nature to reduce accumulated refuse and ways to aid the natural remediation capability, as well as looking for resource-friendly Waste-to-Value systems.

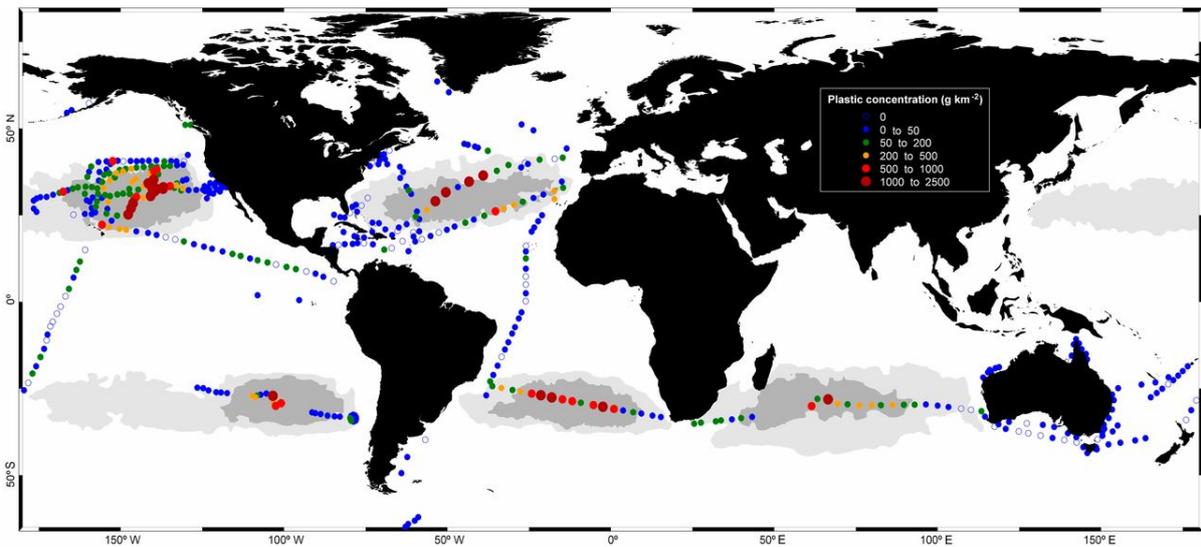


Figure 1: Concentrations of plastic debris in surface waters of the global ocean. Dark and light grey represent accumulation areas. Copyright Andrés Cózar et al. PNAS 2014, reused with permission [1].

The amount of waste which is dumped is still very high, even with rising effort for recycling and with more and more countries banning landfills as waste management option. Of 25.8 million (metric) tonnes of plastic refuse in official waste streams in 2014, 30.8 percent found their end in landfills in Europe, with a plastic materials production of 58 million tonnes in Europe, demand rising. World production is disproportionately higher: 322 million tonnes of plastic materials were produced worldwide, with China and the USA as the world's leading plastic producers [2]. This data still does not include how much (plastic) waste is dumped illegally, or how much of it enters the oceans and how. The fact is that plastic is chemically quite inert and polymers like PET take over 450 years to be broken down [3]. This means that the steady flow of plastic waste reaching the oceans will only increase with time, resulting in huge 'plastic islands' in accumulation areas, where the amount of floating plastic reaches up to 2500 g/km². In Fig. 1 the streams of plastic are shown, which follow numerical circulation models. Still, historical



Figure 2: Garbage heaps in the streets of Bangalore. Copyright belongs to <http://indians4socialchange.com>, Richa Gupta, 29 November 2015 [8]

data does not show a corresponding increase of plastic surface debris since the 1980s, which suggests that this is not the final destination for this floating refuse. The journey through the afterlife for plastic waste is still mostly unknown [1].

Waste dumping is not the only way to contaminate the environment with hazardous substances. The amount of crude oil finding its way into soil or the oceans is a significant concern. 668.000 tonnes of oil enter the environment every year by human exploit. Extraction of oil, including leakages from offshore mining platforms, atmospheric deposition and contaminated waste water, comprises 38.000 tonnes of oil input into the environment per year, transportation of petroleum another 150.000 tonnes per year. The source are pipeline and tank vessel spills, and operational discharges of cargo oil, with the atmospheric deposition of volatile organic compounds going along with this [4]. Big oil spills like the *Exxon Valdez* incident, where the tanker *Exxon Valdez* ran aground in 1989, emptying over 41.000 m³ of crude oil into the Gulf of Alaska [5], or the *Deepwater Horizon* oil spill in 2010, where a drilling rig explosion discharged 4.9 million barrels or 780.000 m³ of crude oil into the Gulf of Mexico, and killing eleven people [6, 7], are the incidents with the most media coverage and therefore have the most impact on the opinion of society towards environmental problems. Despite this, non-industrial consumption is the biggest contributor to oil pollution, and amounts to 480.000 tonnes per year [4].

With the steady rise of the IT sector and demand for electronic devices, as well as our current throw-away society, e-waste is becoming more difficult to separate and recycle

properly. This means that all metal components are dumped together with their plastic casings, sometimes with batteries or light bulbs filled with argon or other gases still inside, as well as with any heavy metal and other toxic components. That this could become a problem is well demonstrated by the Bangalore garbage crisis. Bangalore, sometimes called the 'IT capital of India', is the leading information technology exporter of India, which is why wealth and luxury have found entry to this city in the last decades. This is accompanied by consumerism, and this of course leads to a higher waste production. As this rise of wealth and prosperity has happened in a comparatively short period of time, government and management have not been able to handle the increase of waste. Garbage processing units can only process wet waste; mixed waste, like household refuse, can only be dumped in landfills. Official landfill sites have long reached their capacities, starting to close down one by one back in 2014, and having to send trucks of garbage away. With nowhere to go, these truckloads were discharged in the city itself, mostly on street corners and on the outskirts of the city, as seen in Fig. 2. Citizens living near processing sites have been protesting to close them down, going so far as blocking streets and going on strike, as this is affecting the quality of drinking water and fertile lands, but most of the people living in Bangalore seem to not care, refusing to segregate waste, as separating into wet and dry waste could severely better the situation [9, 10].

2 Introduction

2.1 What are extremophiles?

Extremophiles are microorganisms that live and thrive in extreme conditions. These conditions include temperature, redox potential, pH, salinity, hydrostatic pressure and nutrient concentration, which includes the availability of liquid water and light, as all but a very select few food chains are dependent on photosynthesis. Some extremophiles even live off of toxic nutrients, as they can decompose hydrocarbons, which are the major components of crude oil, or biodegrade different kinds of long-chained polymers, such as plastics.

Extremophile microorganisms are found in all domains of the ‘tree of life’: bacteria, archaea and eukarya, which include extremophilic fungi and plants. Of course, which conditions qualify as extreme is not universally defined, so ‘extreme’ in this case is characterized by the deviation to the standard physiological norm that we humans live at [11, 12].

2.2 Classification of extremophiles

2.2.1 Hyperthermophiles - high temperatures

The first class of extremophiles discussed here are those that thrive at high temperatures, in expert circles called Hyperthermophiles. The term comes from ancient greek consisting of three parts: the prefix *hyper-* meaning *over, above* or *excessive*; *thermo-* meaning *heat*, like in popular words such as thermometer; and the suffix *-phile*, which means *loving* or *friendly*, indicating that these microorganisms have a predilection for extreme heat, or even a dependence on it, having an optimal growth temperature between 80°C and 110°C [13]. Hyperthermophiles can be found in geysers, hot springs near volcanoes or the ocean floor. Many of them are not only quite tolerant about their surrounding temperature, but have also had to adapt to other extreme conditions which come with it. For instance, many hot springs lie in solfatara fields, which are openings in the earth’s crust that emit sulfurous gases. The hyperthermophiles living there have also developed the amazing ability to survive in this environment by needing sulfur to live [12]. Other examples of hyperthermophiles are found at the bottom of the ocean, at sites called ‘black smokers’. These hot springs are about 3000 meters below the ocean surface with the hydrostatic pressure of about $3 \cdot 10^7$ Pa or 30 MPa, which raising the boiling point of water to above 400°C. Apart from that, the ejected hot sea water contains several kinds of chemical compounds, most of them heavy metal sulfides, which some inhabitants of these areas thrive off of [11].

2.2.2 Psychrophiles - low temperatures

This class of extremophiles can survive and even live at very low temperatures, as indicated by their name: the prefix *psychro-* means *cold*, and as we have already learned *-phile* means *loving*: cold loving microorganisms. In general there are three types: those that survive being deep frozen, for short or long periods of time, while their metabolism and reproduction systems cease to function, but can resume normal activity after being defrosted; those that function normally at such temperatures, and those that live at temperatures very close to the freezing point of water, by breaking the usual misconception that low cell yields and slow growth rates are symptoms of low incubation temperatures [14]. The major problem with surviving at subzero temperatures is the mechanical damage which is caused by the formation of large ice crystals in the cells. To avoid this problem, many psychrophiles have developed antifreeze agents which lower the freezing point of water, similar to antifreeze agents that are added to the screen wash of cars. Others have developed metabolic functions which prevent the formation of large crystals, and therefore prevent mechanical damage and can function normally after defrosting [11].

2.2.3 Halophiles - high salt concentration

Halophiles are salt loving organisms, their name including the prefix *halo-*, which means *salt* or *sea*. These creatures can survive in conditions with very high salt concentrations, from sea levels up to more than 20 volume percent of salt content in the medium.

Every cell needs a cell membrane to keep the important organelles inside, to keep the water balance and to do other very important tasks for the cell's survival. In this case the water balance is of interest, which is maintained by osmosis. This is a physical process which is based on diffusion, where two reservoirs are separated by a semipermeable membrane, which lets the solvent permeate from one side of the membrane to the other, but not any other particles or molecules, like ions. This means that water is transported through the membrane until the ion concentration on both sides is equal and balanced. This entails that if a cell is swimming in an environment with high salt concentration, osmosis causes the cell to dehydrate, as all water inside the cell is ejected through the membrane in the attempt to equalize the concentration. The cell dries out and dies. The upper salinity tolerance level is correspondent with the balance between the energetic cost of osmotic adaption and the amount of energy generated by each metabolic process in the cells [15].

Halophilic extremophiles are therefore very interesting creatures, as they have developed different techniques to survive in environments with high salt concentration. Some specimen, like bacteria and algae, negate the external osmotic pressure by making small organic molecules that fill the inside of the cell. Archaea on the other hand cancel out the imbalance in concentration by allowing and producing an even higher concentration on the inside of the cell. This however brings its own problems with it, as the salt will compete with the cell's proteins and other biomolecules for the available water, which would normally result in an accumulation of insoluble and useless lumps of biomass.

Halophilic archaea have adapted to this by developing acidic amino acids with lots of side chains that hold a negative charge each. They wrap around proteins in layers and massively increase the potential to bind water to the protein surface [11].

2.2.4 Acidophiles and alkalophiles - low and high pH levels

Acidophilic microorganisms are tolerant of very low pH values, which means very sour environments, like acids. Alkalophiles on the other hand like living in bases: high pH environments, their name derived originally from arabic meaning *the calcined ashes*. The pH value of a medium is defined by its concentration of dissociate hydroxide OH^- ions and their corresponding hydrogen H^+ ions (which is an approximation of the hydronium ion H_3O^+ concentration), specifically as the negative exponent of the hydroxide ion concentration: e.g. one liter of water has a hydroxide ion concentration of 10^{-7} OH^- ions and therefore has a pH value of 7, which corresponds to an equilibrium of OH^- ions and H^+ ions and is therefore called neutral pH.

The pH value of a cell's internal contents is very important for it to properly function and for its activity, and therefore the pH of the cell's environment is a very important influencing factor, as osmosis is again working to balance concentrations inside and outside the cell membranes and can easily tilt the cell out of its pH equilibrium into a non-active state. Most cell functions are performed by macromolecules, like proteins or nucleic acids. Proteins consist of chains of amino acids by bonding their carboxyl group ending COO^- to the amino group ending NH_3^+ of the next amino acid, so every macromolecule has a positive and a negative chain end [16]. These free ends react as either an acid or a base end to the macromolecule, and changes to the pH of its environment would effect them by binding a hydroxide or a hydrogen ion, thereby changing the charge state of the molecule and affecting the structure, functionality and reactivity [11].

Acidophiles Acidophiles are microorganisms that live optimally at pH levels of 3 or lower, a commonly known example is *Lactobacillus acidophilus*, which finds its home in curdled milk. They have to maintain a neutral inner pH value by forming a pH gradient inside the cell membrane, and have therefore developed a few techniques which still have not all been understood. The pH gradient in the membrane is induced by the positive potential inside, which is the exact reverse to non-acidophilic microorganisms, generated by potassium ions Na^+ . These positively charged ions inhibit the influx of hydrogen ions (protons) by a chemiosmotic barrier. The potassium ions needed for this process are transported by a high number of cation transporters in acidophiles.

The pH difference between inside and outside is the driving force behind the proton motive force PMF of these microorganisms, which is the generation of the cell's energy carrier, adenosine triphosphate ATP, by the movement of ions along their electrochemical gradient. This originates from the oxidation of NADH and facilitates an electron transport chain which functions as a proton pump. This produces an influx of protons inside the cell, which would rapidly dissipate the pH gradient. Therefore acidophiles use additional

methods to remove protons from inside the cell, for instance by pumping them out with secondary transporters, and having developed a nearly impermeable cell membrane and a reduced pore size in the membrane channels, as well as an efficient cytoplasmic buffering [17].

Alkalophiles Alkalophiles have their growth optimum at pH levels above 10. They also have to maintain a neutral inner pH level, but their mechanisms are not as convoluted as those of acidophiles, while still being similar. Alkalophiles have a well established cation exchange system, importing protons into the cell in exchange for potassium ions. They are utilizing membrane proteins as transporters, their numbers significantly higher than in non-extremophiles, as well as enzymes for proton capture and retention. Alkalophiles have developed changes in their cell surface layers which increases cytoplasmic proton retention, and additionally they have increased their metabolic acid production. Unlike their acidophilic counterparts, their pH difference between inside and outside is positive, same as the mesophilic membrane potential, which is favorable for their proton motive force and therefore for their energy production [18].

2.2.5 Piezophiles - high pressure

Extremophiles that have the ability to live under high pressures are called piezophiles, with *piezo-* meaning *to be squeezed or pressed* in ancient greek. This term has only recently been established, before they were known as barophiles, coming from *baro-*, which means *weight* or *heavy* in ancient greek.

The first piezophiles were found by the deep sea researcher A. Certes, who speculated that the lack of organic material on the ocean floor was due to degradation by microorganisms, and found evidence of this by incubating sterile sea water with samples collected by the deep sea vessels *Talisman* and *Travailleur* from up to 5100 meters below the sea level in 1884 [19]. The next big step in this field happened decades later by the barobiologist C. E. ZoBell in 1946, who was the first to publish reproducible data on piezophiles and their physiological behavior, because he was the first to implement the recent chemical discoveries in the field of thermodynamics by maintaining constant temperatures while working with varying pressures [20].

Most piezophiles do not need permanent high pressures to survive, some of them, for instance some hyperthermophiles from black smoker sites which live at up to 500 bar at 5000 meters below sea level, even prefer atmospheric pressure [11]. Yayanos was the first to isolate obligate piezophiles, which need high pressure to survive and high pressure is therefore obligatory for them, at the Mariana Trench at 10,500 meters below sea level and a pressure of 1050 bar [21], opening the pathway to more research on piezophiles. A handful of publications followed, researching life at the bottom of the Mariana Trench, which resulted in a number of isolated high pressure adapted bacteria and the discovery of their high pressure regulated system for gene expression. This system includes pressure regulated promoters and operons which encode the expression

of enzymes with increased activity at high pressures, making them very interesting for biotechnological use in bioreactor systems. Another interesting discovery is the correlation between piezophily and hyperthermophily: a number of enzymes were discovered which exhibits increased activity at high temperatures of up to 130°C while under high pressure, not only in hyperthermophiles, but also in mesophilic microorganisms such as *Escherichia coli*. This suggests that the origin of life developed at high pressures and then later had to adapt to moderate conditions [22].

2.2.6 Xerophiles - dryness

All living creatures need water to survive. Xerophiles are organisms that survive with minimal water in their immediate environment, their name also deriving from the ancient greek prefix *xero-* which literally means *dry*.

There are almost as many xerophiles as there are metabolic functions which allow such a tolerance of (almost) complete lack of water. The plant with the most impressive application of such a tolerance is *Welwitschia mirabilis*, which lives in the Namib desert with the total rainfall of 10 mm per year and the ability to reuse nutrients like carbon, nitrogen and potassium from dying ends of their leaves [11]. There are a number of xerophilic fungi which are of concern for human health, like *Aspergillus ochraceus*, which like to infest dry packaged foods like cereal and dried fruit [23]; and lichens, which are symbiotic organisms between fungi and algae or cyanobacteria. There are a number of bacteria that form drought-resistant endospores, which are inactive copies of the original cell incapable of metabolisms but is significantly more resistant to all kinds of stress [11].

Deinococcus radiodurans is a very interesting example of a xenophile, as it was first discovered for its remarkable resistance to radioactive and ultraviolet radiation. As it turns out, *D. radiodurans*' radioactivity resistance is only a side effect of its drought resistance. It has an extremely efficient mechanism that repairs damaged DNA, repairing cell damage caused by oxygen in the dry air and radiation damage in the same way [24].

2.2.7 Living on toxic nutrients

Organisms that use food sources which would be toxic for most other species also fall under the category of extremophiles. Fortunately, for every compound that is produced naturally in the environment, there seem to develop organisms which can utilize them in one way or another. Compounds exclusively produced by human technology are few and far between, but these especially carry a high risk of polluting the environment long term. The most amazing thing however is that some microorganisms have been found that degrade such new compounds, showing the adaptability of evolution. The following is a short overview of toxic or pollutant substances and their devourers.

Oil Crude oil and petroleum eating microorganisms use the chemically inert hydrocarbons as their sole carbon source. They have developed different metabolic pathways to

break them down to compounds suitable for the conventional carbon metabolism, by evolving new enzymes capable of hydrocarbon degradation.

Plastics Microbial polymer degradation is much more complex, because apart from proteins and DNA, most are manufactured artificially and only few have evolved naturally, giving microorganisms a chance to develop a polymer degrading metabolism, and additionally were constructed to be chemically inert and very durable. Abiotic degradation and microbial depolymerization reduce size and mass of a polymer and produces short chained products like monomers, dimers or oligomers. This happens extracellularly by secreting enzymes which either hydrolyze or oxidoreduce the polymer chains. These molecules are small enough to pass into the cell and be included in its metabolism. If all reaction products can be metabolized, only CO₂ and water remain and are discharged into the environment again. Only the conclusion of this last step completes a biodeterioration process feasible for bioremediation, otherwise reaction products stay in the environment, which may be just as difficult to remove, just as or even more toxic than the original polymer itself [25, 26].

Dioxins Dioxins are polyaromatic compounds with chlorine, bromine or fluorine ends and a dibenzodioxin or dibenzofuran base structure, which are two benzole rings linked by one or two oxygen atoms, respectively. Dioxins are highly carcinogenic and toxic and three metabolic pathways have been characterized. Two of them follow the same pathways as in oil degrading microorganisms: oxidative degradation with polyaromatic dioxygenases by aerobic microorganisms, see Reaction 2 on page 15, or fungal oxidation with cytochrome P-450, see Reaction 3 on page 17. The third reaction pathway is an anaerobic reductive dehalogenation [27].

Heavy metals Heavy metal bioremediation can be divided into two basic categories: biosorption and phytoremediation. Biosorption is the process of microbial cells extracting metal ions via non-metabolic pathways, by extracellular passive binding to a non-living biomass. The mechanisms involved are complex sequences of reactions, such as adsorption, desorption, complexation, ion exchange, coordination and precipitation [28, 29]. They are often specific to the biomass-solute pair, which limits the option to generalize these processes. These reactions usually involve binding the metals to the bacterial cell walls, either extracellularly (as for example with thorium in *Rhizopus arrhizus*) or intracellularly (like uranium in the same microorganism, *R. arrhizus*) [29]. The selection of a suitable kind of biomass for a specified biosorption application, as well as the decision to use living or non-living biomass depends on various considerations. The binding capacity of the biomass and the selectivity for the specified metal, contact solution toxicity, growth conditions for the cells and requirements to the mechanical strength of the sorbant medium are all to be considered. As these reactions are all basic chemical reactions and in basis are reversible, the biosorption process is as well, even though they are more difficult

in their complexity and one has to understand the complete biomass-solute pair specific sequence of the biosorption process [29]. This enables a neat biomimetic approach to resource management, implementing the biosorption of valuable metals from industrial waste waters and utilizing it to synthesize nanomaterials [28]. This involves the reduction of metal- or metal oxide ions to elemental nanoparticles. Most such syntheses then bind the metal nanoparticles extracellularly to the microbial biomass. One such example is the reduction of silver nitrate to elemental silver by the white rot fungus *Phaenerochaete chrysosporium*, another example is the synthesis of gold nanoparticles by *Aspergillus oryzae* var. *viridis* or *Penicillium rugulosum*.

Phytoremediation works by utilizing metal accumulating plants to remove, transfer or stabilize heavy metals from soils or wastewater, even with low contamination concentrations. It employs the growing and harvesting of metal accumulating plants and then treating the resulting biomass to recover the metal [28]. The advantage of this remediation method is that it induces minimal site destruction and destabilization. There are three plant categories viable for the use in phytoremediation: metal excluders, which are metal-tolerant plants that restrict the entry of potentially toxic heavy metals into most of their bodies, but still contain a high concentration in their roots. This is also called phytostabilization, which is the reduction of bioavailability and immobilization of contaminants in the rhizosphere of the plant and therefore reducing the risk to the environment. Metal indicators are plants which actively accumulate metals in their above-ground plant tissue and therefore reflect the metal concentration in the soil. Hyperaccumulators are plants capable of accumulating specific metals and reach considerable levels of this heavy metal in their upper plant biomass [30].

Nuclear waste As there are known bacterial species capable of surviving high radioactive activities, it follows naturally that there might be bioremediation applications for nuclear waste. Indeed, the microbial colonization of radioactive environments has recently been studied, as well as the mechanisms of uranium-VI reduction by sulfate reducing bacteria such as *Desulfovibrio* species. There seems to be a common metabolic pathway for metal and sulfur reduction mediated by cytochrome c_3 in combination with organic electron donors [31].

3 Bioremediation with extremophiles

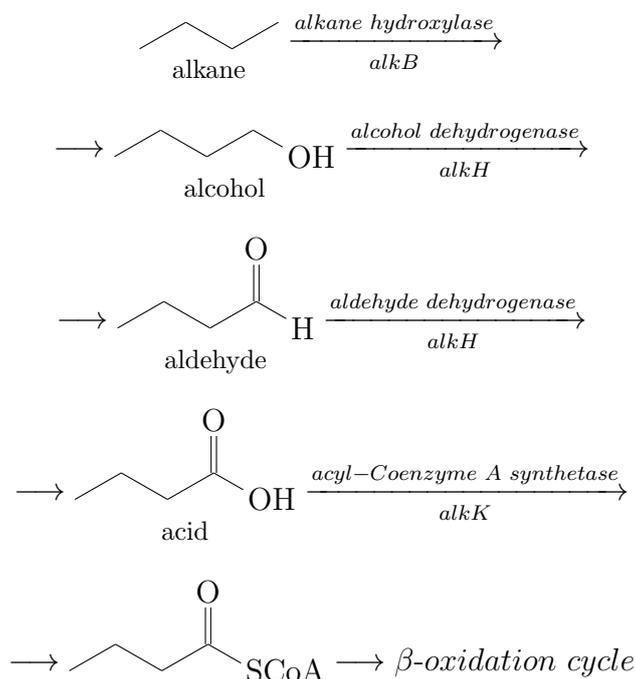
This thesis focuses on identifying extremophiles which are of use in refuse management. There are different possibilities where the capabilities of extremophiles could be of use, not only in the bioremediation of soil and water by decomposing crude oil or fossil-fuel-based plastics, but also by bioleaching soil contaminated with heavy metals or electronic waste, by recovering valuable metals. The ultimate goal of this research is not the biotechnological exploitation of extremophiles, but the biomimetic transfer of strategies to this end.

3.1 Oil degrading extremophiles

Over 1.3 million liters of crude oil enters the environment every year [4]. In contrast to popular belief, the majority of contaminations does not happen through oil spills. More than half originates from natural leakage from the ocean floors, what stems from human impact originates from shipping, illegal dumping or deliberate discharge of processing sites. The fact that the oceans are not covered by the typical rainbow colored oil films is confirmation of the natural oil degrading capacity of our ecosystem, which is facilitated by hydrocarbon degrading microorganisms. This is very impressive, as more than 17.000 organic compounds have been identified in crude oil, which are not all equally susceptible to degradation [32]. The resistance to degradation is dependent on the stability of the chemical bonds of the hydrocarbon compound. As a rule, linear alkanes are more inclined to be metabolized than branched alkanes, then biodegradability decreases in order of molecular weight with polycyclic aromatic hydrocarbons as the most resistant to breakdown. Therefore we will regard the aromatic hydrocarbon metabolism separately from the aliphatic hydrocarbon metabolism in the following [33].

3.1.1 Aerobic aliphatic alkane biodegradation

One of the best characterized alkane degradation pathways is encoded by the OCT plasmid of *Pseudomonas putida*, described by the *alk* operon [34], which is a functioning unit of the DNA, encoding a specific enzyme or gene. The chemical pathway is as follows, with the necessary electrons for the alkane hydrolase being mediated by a monooxygenase (oxidizes molecular oxygen: $2\text{O}_2 + \text{NADH} \rightarrow \text{OH}^- + \text{H}_2\text{O} + \text{NAD}^+$) together with rubredoxin (low molecular weight Fe-containing protein in sulphur reducing organisms) and rubredoxin reductase [33]:



Reaction 1: Aliphatic alkane reaction pathway

There are other degradation pathways, for instance the genome of *Alcanivorax borkumensis* was documented in detail, with its complete gene set for alkane degradation, with the addition of encoding the production biosurfactants and exopolysaccharides with which they attach themselves to the oil-water interface [32]. This and other well-documented metabolism pathways all use similar sets of genes though, such as alkane hydroxylases, alcohol dehydrogenases and oxyreductases (whether mono- or dioxygenases) with variations in the intermediate products.

3.1.2 Aerobic polycyclic aromatic hydrocarbon (PAH) degradation

Polycyclic aromatic hydrocarbons are especially resistant to degradation, as the compounds are chemically very stable. The electrons of the benzene ring's double bonds are delocalized over the whole ring, which is because they are covalently π -bonded. This means two neighboring electron orbitals are overlapping and form a single hybrid orbital, meaning in the case of an aromatic ring the hybridization of all six C-orbitals. This makes breaking these bonds very difficult and causes the exceptionally low reactivity and facilitates a prolonged stay in the environment, even after all linear hydrocarbon compounds have already been degraded. They represent a severe health risk because of this, as most of PAHs are either toxic, mutagenic or carcinogenic [32].

There are a number of microorganisms that use PAHs as sole carbon and energy sources, and more are found continuously. The best studied communities are from the *Pseudomonas*

Bacteria The simplest PAH is naphthalene, an aromatic hydrocarbon with two rings. Its bacterial degradation is well described and characteristic for the metabolism of PAHs with more rings as well. First, two hydroxy groups are bonded to the aromatic ring, forming a catechol after oxidation, see Reaction 2. The aromatic ring of the catechol is then severed by a process called *meta*-cleavage of the ring. Using the example of Reaction 2, where the PAH has more than one aromatic ring, it is illustrated that the reaction can progress until all rings are cleaved and the product can be integrated into the central carbon metabolism of the organism. This works by splitting into pyruvate and an aromatic acetaldehyde, which reacts with a dehydrogenase to form a salicylate. This is then hydroxylized into another catechol which undergoes *meta*-cleavage again [33, 35].

This reaction chain is transcribed by the *nah* operon, which encodes the pathway from naphthalene to the salicylate, and the *sal* operon, which encodes the enzymes for salicylate oxidation and *meta*-cleavage. The regulator for both is a third operon, which is induced by the presence of salicylate in the organism [35].

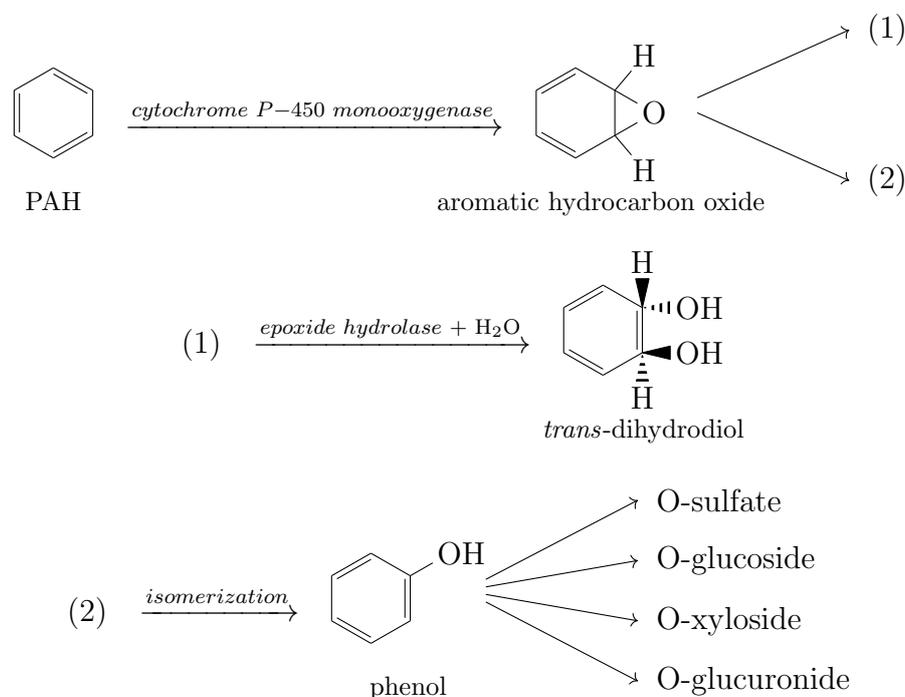
New genes for PAH degradation are isolated regularly and the diversity of these metabolic genes has become apparent. There are enzymes, like naphthalene dioxygenase, that catalyze a wide variety of reactions and therefore have the capability of degrading other PAHs as well. This relaxed initial enzyme specificity may be the reason for the broad PAH degrading capabilities in many strains.

Many strains of hydrocarbon degrading bacteria even encode more than one gene for similar enzymes, for instance by employing both monooxygenases and dioxygenases. They also often encode genes that do not directly participate in hydrocarbon degradation, but serve supplementary support functions. This can have synergistic effects on the degradation of various PAHs facilitated by different microorganisms by way of cometabolism. Different PAHs also have aiding or inhibitory effects on the degradation of other PAHs. The positive effect of the presence of another PAH is the increase of the total amount of degraded PAH of the original type, as some gene operons are induced by both PAHs or their intermediates. Inhibition of the degradation process is one way the presence of a different PAH can be detrimental, as some PAHs compete for the same enzymes. This sometimes even leads to total blockage of further metabolism, as enzymatic processes are inhibited by dead-end cometabolic products. Another reason for inhibition is the cytotoxicity of some cometabolic intermediates, which act harmful towards metabolic enzymes. It is therefore important to know the influence that specific PAHs have on specific microorganisms when comprising a suitable mixed organism culture for bioaugmentation so that detrimental effects can be avoided [33].

Fungi The PAH metabolism pathways of fungi are markedly different than bacterial pathways, as they do not use PAHs as their sole carbon and energy sources and therefore need an additional carbon source in the substrate. Two major pathways have been found, characterized by their respective enzymes facilitating the first attack on the PAH: intracellular cytochrome P-450 monooxygenase and extracellular lignin peroxidase, manganese peroxidase or laccase, which are all relatively non-specific towards PAHs. The

most notable representative of these fungi are *Cunninghamella elegans*, as they have been proven to metabolize a higher number of PAHs as other fungal species, including the five ringed benzo[a]pyrene. Other representatives are the white rot fungus *Phanaerochaete chrysosporium*, *Candida* sp. and *Penicillium* sp.

The cytochrome P-450 monooxygenase pathway, which is for instance the prevalent pathway of the white rot fungus, incorporates one oxygen atom into the aromatic ring forming an aromatic hydrocarbon oxide, which either undergoes an enzymatic hydration forming a *trans*-dihydrodiol, as opposed to the bacterial intermediate *cis*-dihydrodiol, or the arene oxide spontaneously isomerizes into a phenol without enzymatic influence. This then reacts with either sulfates, sugars such as glucose or xylose and their acids, like glucuronic acid, to stereochemically form large molecules at different torsion angles linked by an oxygen atom. This proves a regio- and stereoselectivity of the fungal PAH degradation pathways, which makes them very similar to mammalian metabolism.



Reaction 3: Fungal reaction pathway

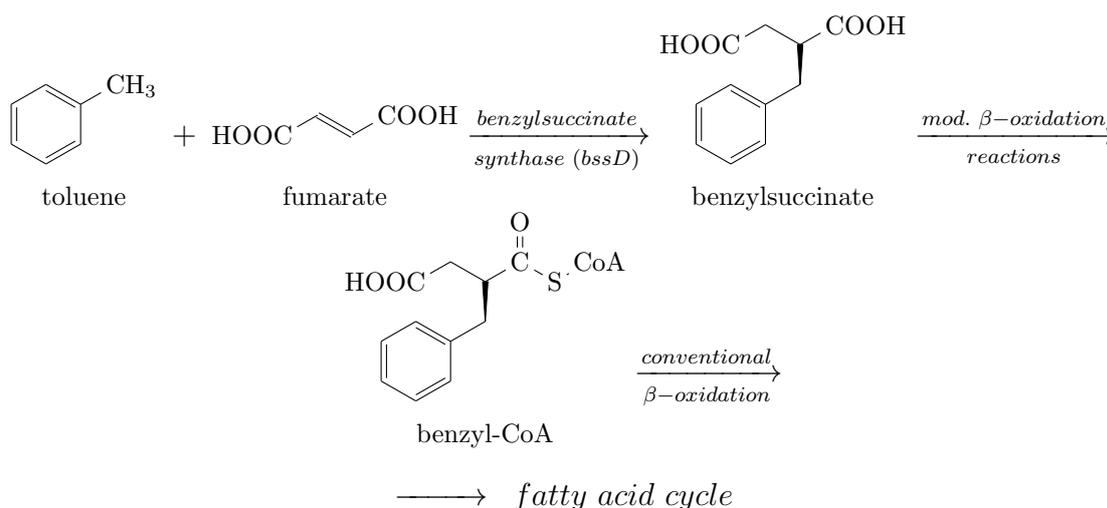
The other fungal pathway is facilitated by extracellular enzymes like lignin peroxidase, which oxidize PAHs with ionization energies lower than 7.6eV by a free radical attack, forming quinones, which are aromatic compounds with two double bonded oxygen atoms. These quinones can then undergo ring cleavage [35, 36].

Cyanobacteria and eucaryotic algae Some cyanobacteria and algae have been known to metabolize PAHs since the 1980s. They execute this decomposition by oxidizing them under photoautotrophic conditions, as is done by red, green and brown algae. Others, such as *Oscillatoria* sp. strain JCM or *Agmenellum quadruplicatum* use a similar pathway as fungi, forming aromatic hydrocarbon oxide intermediates which then isomerize [35].

3.1.3 Anaerobic metabolic pathways

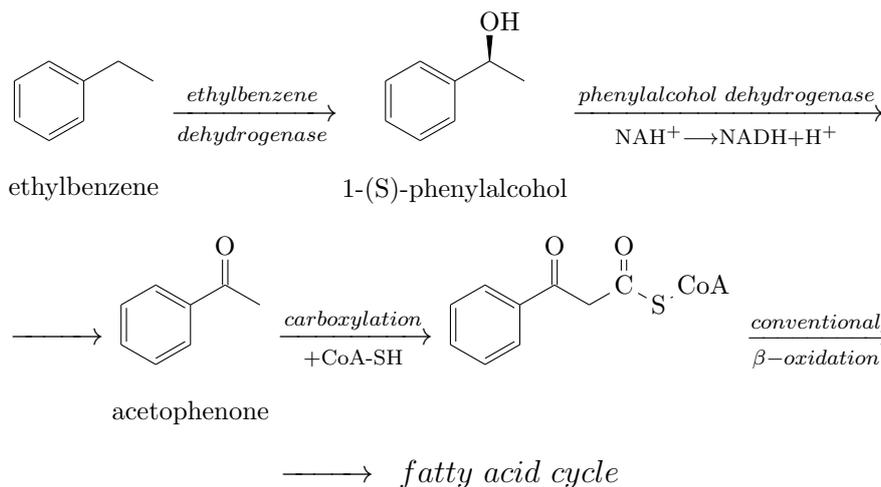
Anaerobic bioremediation is performed by anoxygenic photosynthetic bacteria, which perform bacterial photosynthesis under anaerobic conditions, or cultures of methanogenic bacteria, which are microorganisms that produce methane as a metabolic end product along with CO₂ and water under anoxic conditions, while no strains of fungi have been found to date. These bacteria are mostly sulfur-reducing or denitrifying bacteria, but there are Fe(III)-reducing species as well, for instance of the *Geobacter* family: *G. grbiciae* and *G. metallireducens*. A range of pathways has been found with the detection of new intermediate compounds suggesting even more possible pathways.

The first metabolic pathway, see Reaction 4, titled benzyl-CoA pathway, is employed by denitrifying bacteria degrading mostly monoaromatic compounds like toluene or xylene, examples are *Azoarcus* sp. strain T or some *Thauera aromatica* strains [33]. However for a few strains of sulfur-reducing bacteria a similar and comparable pathway has been detected, which also utilize it to degrade two ringed hydrocarbons, like naphthalene and its derivatives [37]. This reaction pathway starts with toluene reacting with either a fumarate (HOOC-CH=CH-COOH) or a succinate (HOOC-CH₂-CH₂-COOH) into a benzylsuccinate, then by modified β -oxidation reactions to benzyl-Coenzyme A and finally reaches the fatty acid metabolism by conventional β -oxidation.



Reaction 4: Anaerobic reaction pathway 1

The second pathway used by denitrifying bacteria, for instance *Azoarcus* sp. strain EbN1, is a novel reaction system, as it is utilizing the first known enzyme that oxidizes a non-activated hydrocarbon without molecular oxygen as a cosubstrate, e.g. bound by hydroxylation. Ethylbenzene is directly oxidized into a 1-(S)-phenylalcohol by an ethylbenzene dehydrogenase, which then is further oxidized by 1-(S)-phenylalcohol dehydrogenase with NAD^+ as the usual electron acceptor into acetophenone [38].

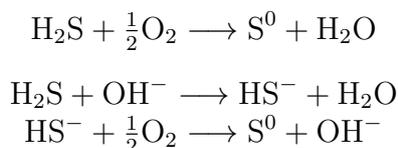


Reaction 5: Anaerobic reaction pathway 2

Pathways for PAHs with two or three rings are rarer, but can also be metabolized under anaerobic conditions. These reactions are activated by the carboxylation of the hydrocarbon by binding a $-\text{COOH}$ group and forming an acid.

3.1.4 Desulfurization

The treatment of liquid or gaseous waste streams to remove sulfur and sulfur compounds like H_2S is important for the environment, and finds its place in this thesis because oily waste streams often have a high sulfur concentration. Current chemical methods with all their disadvantages can be replaced by simple bioremediary techniques utilizing sulfur-reducing microorganisms by a process under the trade name Thiopaq O&G™ for the oil and gas industry, named by Paqell, the company which developed this process. This is a desulfurization process comprised of two steps: the waste gas streams are first washed with a liquid washing agent, which solves the sulfur compounds into the liquid phase, in which the bacterial conversion into elemental sulfur commences with the help of thiobacilli, for instance *Thiocalovibrio* or *Thioalcalobacteria* species [33].



Reaction 6: Thiopaq O&G process

3.1.5 Biostimulation

Biostimulation is the aiding of nature's own capacity of remediation by observing the limiting factors to microorganism activity and helping to overcome them in the case of an oil spill. Once oil enters the sea it distributes over the water's surface. It will spread and be transported by waves, which disperse it and enable emulsification. If the oil is washed up at shore it enters and settles in the sediments, which restricts availability to microorganisms. The oil film on the sea's surface is subject to evaporation, volatilizing lighter hydrocarbons that contaminate the atmosphere and reduce air quality. Strategies dealing with these phenomena and enhancing microbial bioremediation differ in complexity and technological requirements, from passive processes to implementing bioreactors and biofiltration systems, especially when dealing with contaminated soils [33, 39].

There are many factors that influence hydrocarbon biodegradability in the environment. One of these factors is the availability of supplements: oxygen and water, but also a sufficient supply of nitrogen and phosphorus, which would otherwise limit growth of hydrocarbon degrading microorganisms. Therefore the most basic form of biostimulation is the fertilization of oil spill sites, by trying to achieve an optimal ratio of P:N:C of 1:10:100 [40].

As hydrocarbons tend to be hydrophobic, the addition of surface tension reducing chemicals can enhance the efficiency of biostimulation. Thankfully there are microorganisms that produce their own biosurfactants, which they synthesize in their cell membrane and then release extracellularly [33]. Biosurfactants with low molecular weight reduce the surface tension of oil so that it can separate into small oil droplets which form a water-oil emulsion, examples are glycolipids and lipopeptides. The large oil surface area gives microorganisms a larger area of contact to utilize, and the efficiency of biodegradation increases. High molecular weight biosurfactants are stabilizers of oil-in-water emulsions, most notably polysaccharides and lipoproteins [32, 33].

Bioremediation of soil and waste sludge When dealing with shoreline contaminations, soils and industrial oily waste sludges, the remediation options can be sorted by grade of human interference, which goes hand in hand with rate of degradation.

The least invasive method, just like with bioremediation at open sea, is biostimulation by fertilizing the soil with necessary nutrients for optimal growth of microorganisms. This is not very effective however, as nutrient accessibility is dependent on homogeneity of

the soil or sediments and its type, whether fine sand or coarse cobbles, and penetration depth of the contaminant. These simple bioremediation systems also do not factor in substrate saturation kinetics that dictate the decrease of degradation rates as contaminant concentrations fall. This is further worsened by insufficient diffusion in solid phase environments, and shows that passive bioremediation does not provide sufficient capacity for high volumes of oily wastes.

One very special example for non-invasive bioremediation is phytoremediation. This method uses plants and their rhizospheric microorganisms. Plant roots excrete necessary nitrogen sources, which supplements microbial hydrocarbon degradation [33, 41]. A severe disadvantage in phytoremediation is posed by the absorption of volatile organic carbons by the plants which are consequently released into the atmosphere.

To counteract the problem of homogeneity, one can aerate and plow the soil, in addition to fertilizing with necessary nutrients [5, 32, 33]. Studies have shown an increase of the natural degradation rate of two to seven times by fertilization and tilling [5], but there are a number of disadvantages. The process of plowing allows mixing of soil, but it also promotes gas transfer, which boosts volatilization of light toxic organic carbons into the atmosphere [33].

There are two possible implementations of this method: the first is the treatment of oily wastes by landfarming. A previously uncontaminated expanse of land is used to make a refinery and is therefore deliberately contaminated, which makes this area unusable for the foreseeable future [33]. The other possibility of implementation is by using this method on beaches which were contaminated by floating oil because of an oil spill. However, tilling is very disruptive to plant life, animals and the ecosystem of the beach and most often does more harm than good [32].

The most developed method for bioremediation of oily waste sludges is that of the bioreactor. Most rate limiting factors are eliminated by controlling influencing parameters like temperature, moisture, pH, aeration and mixing. Solid components are ground to smaller pieces and contact with the aqueous phase containing hydrocarbon degrading microorganisms is promoted, which results in a significant increase in degradation rates. This is further advantageous, as acceleration of biodegradation minimizes volatilization of lighter toxic organic carbons, and microbial degradation becomes the dominant disposal mechanism [5, 32, 33].

3.1.6 Bioaugmentation

Bioaugmentation is the process of adding specific microorganisms to the environment to facilitate bioremediation. This could potentially improve bioremediation techniques, as there are many options and traits specific to different microorganisms to choose from, as described above. There is already a lot of research into genetically engineering organisms with a broad metabolic skillset. However, research into this field is forbidden

in Austria, and the use of genetically engineered microorganisms has been a controversy regarding safety and ecological damage, and many countries, Austria among them, do not permit their use. But as mentioned, the variety of functions is great and the gene pool facilitating them even greater. The strain of maintaining all of these genes makes a potential engineered organism very vulnerable outside of a lab environment. Still, there is already the first ever patent on a living organism: an engineered *Pseudomonas* sp. capable of degrading two types of PAHs, camphor, salicylates and octane.

A genetically engineered organism is not the only way to facilitate biodegradation with the required diversity of metabolic pathways. It is even more flexible to use a mixed culture of microorganisms suitable to the substrate and can be adapted to any given situation individually [32, 33].

3.1.7 Biofiltration of volatile organic carbons (VOC)

Volatilization of light organic compounds is a severe problem for the environment and governments are increasing restrictions and requirements on waste disposals. VOCs are organic compounds with a boiling temperature of down to 0°C and their vapors are very hazardous to health and facilitate tropospheric ozone production.

Biofiltration is the biological oxidation of volatile organic compounds in a reactor where microorganisms are immobilized as a biofilm on a solid phase or in a liquid phase support material. The VOC vapors pass through the solid support phase, which should have a large surface area for microbial reaction, or are bubbled through the liquid support phase by a fine bubble diffuser.

For highest efficiency with acceptable reactor dimensions it is endeavored to optimize retention time in the support phase and microbial activity [33].

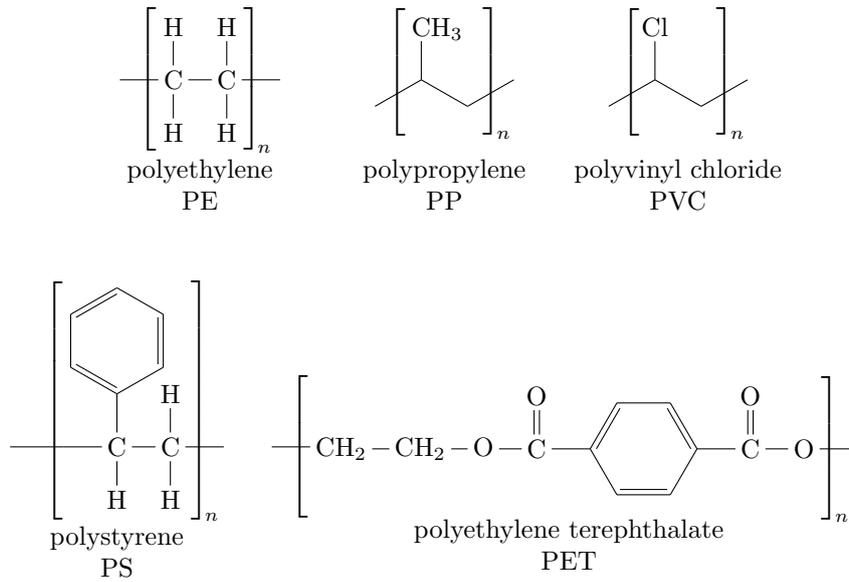


Figure 3: Most common polymers

3.2 Plastic and polymer degrading extremophiles

The increasing production of plastic goods and subsequent increase in plastic waste is becoming an environmental concern, with average time of natural degradation in landfills being around 450 years [3] and the necessity for closing landfills all over the world. Rising pressure is being put on governments, and growing land and water pollution calls for alternative plastic waste disposal methods like the development of biodegradable plastics and the biodegradation of existing plastic wastes. Unfortunately, bioremediation of plastics is not yet as advanced, because very few polymer degrading organisms have yet been found. As a rule one can say that for anything freely available in the environment there are things that eat it. The problem with plastic as a workable material is that it is a wholly man-made product, which has its origin in the middle of the nineteenth century in the rubber industry and advancement accelerated rapidly in the 1950s with the development of thermoplasts. This is a comparatively short period of time for the potential evolution of plastic eating organisms and is the reason why such a small number of organisms with this ability has been found to date.

The word plastic is an umbrella term for any material made out of a polymer or polymer blends. The simplest polymer is polyethylene (PE), seen in Fig. 3, it is a chain of only carbon atoms, with the two carbon atoms of the polymer backbone repeating *n* times and forming a long chain molecule. Polypropylene (PP), polyvinyl chloride (PVC) and polystyrene (PS) are similar to polyethylene in that their polymer backbone consists of a pair of carbon atoms as well, but they each have a different functional group bound to one of those carbon atoms. With dozens of standard polymers (polyethylene PE, polypropylene PP, polyvinyl chloride PVC, polystyrene PS and polyethylene terephthalate

PET being the most common, see Figure 3) and even more derivatives chemically adjusted to different kinds of prerequisites, there must be almost as many degradation methods as kinds of polymers, and different species for each one. But because plastic is used for its durability, stability and longevity, not many species are known to biodegrade them. Therefore environmental influences are integral to the biodeterioration process.

3.2.1 Environmental effects

Three factors influence the biodegradability of polymers: mechanical-, photo- and thermal degradation [42, 25]. The biodegradability of plastics is governed by different factors, like their polymer characteristics such as mobility, crystallinity, molecular weight and their functional groups and ligands [26]. The aforementioned factors all contribute to changing the polymer properties to conditions favorable for decomposition, as they all result in the deterioration of the material, affecting the polymer properties by either breaking them into smaller pieces or causing molecular fission.

Mechanical deterioration is due to compression, tension or shear forces and can be caused by any number of stress factors, like natural aging of materials or (over)load [25].

Photodeterioration is caused by high-energy radiation like ultraviolet light, which allows electrons to absorb energy and cause excitation and higher reactivity, which results in oxidation, producing free radicals, or in cleavage of covalent bonds and other chemical effects like hydrolysis. It is by far the most effective abiotic factor occurring in the environment, with hydrolysis as the most common initiator for degradation [25, 26].

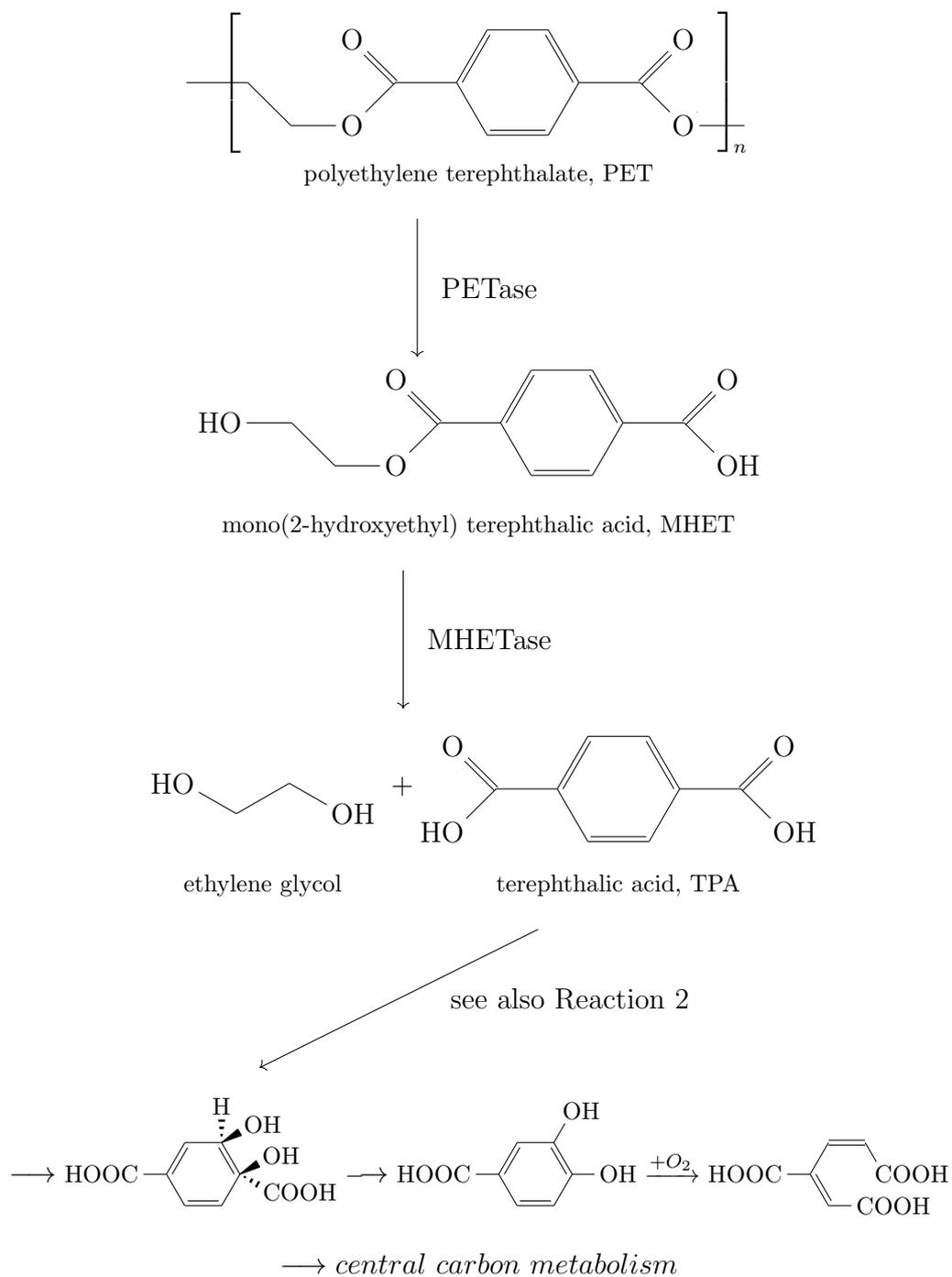
Thermal degradation is the molecular deterioration of polymers at the transition between solid and liquid state at their melting point. It can be accelerated or caused by infrared radiation, composting or human influence. Overheating modifies the organization of the molecular framework, and changes the crystalline or semi-crystalline structure, thereby altering properties like flexibility and chemical inactivity [25].

3.2.2 Microbial degradation

The biodegradation of polymers can be generalized by the following steps: first, the long-chained polymer structure is fragmented into smaller molecules, mostly by abiotic forces. Then these shorter molecules are gradually reduced in size and molecular weight to monomers, dimers or oligomers by depolymerization of microorganisms. These pieces are small enough to penetrate the cell wall and can move into the plasmic membrane to be integrated into the metabolism as a source of carbon and energy, in either the β -oxydation cycle or the tricarboxylic acid cycle, producing CO_2 , water and in the case of anaerobic degradation also methane [25, 26].

Some polymers have been found to be vulnerable to microbial degradation even with their chemical resistance to change. This is attributed to the production of specific enzymes, which can be categorized as intra- and extracellular depolymerases. These are either hydrolases or oxidoreductases, and include lipases, cutinases, esterases or amylases

[25, 26, 43].



Reaction 7: PET biodegradation

The chemical mechanisms of degradation can differ greatly depending on the polymer,

enzyme and organism, with more than one suggested chemical reaction possible. They all agree upon the incorporation of either water, in the form of hydrogen- and hydroxyl ions hydrolyzing ester bonds, or by incorporating one or two oxygen atoms in oxo-biodegradation, forming either an alcohol or a peroxide, similar to the aliphatic alkane reaction pathway of oil degrading microorganisms in Reaction 1. They are then reactive to chain cleavage, resulting in short organic acid molecules [25, 44]. By the example of polyethylene it is obvious that not all polymers have an easily available ester bond to hydrolyze. This constitutes the forming of said ester bonds to facilitate the breakdown by oxo-biodegradation [45].

Polyethylene however has been the focus of some very recent media coverage in 2017, which proves its biodegradation by caterpillars of the wax moth *Galleria mellonella* [46]. It was found that these caterpillars biodegrade polyethylene with the end product ethylene glycol. Apart from these wax moth larvae, the fungus *Penicillium simplicissimum* and the bacterium *Nocardia asteroides* have been known to biodegrade polyethylene [44, 46].

The biodegradation of polyethylene terephthalate, an aromatic polymer with two ester groups in the chain backbone, is quite of interest as well. Just a year ago in 2016 a bacterium called *Ideonella sakaiensis* was discovered by Yoshida and his research group in a Japanese plastic waste landfill, which prefers PET to aliphatic esters, as well as normal carbon sources such as glucose and cellulose [47]. It is assumed that this species has only recently evolved, given that PET has only been available as part of the natural food chain for a very short period of time. They have proposed a degradation pathway with a unique PET hydrolyzing enzyme, which they titled PETase, and another enzyme which is capable of hydrolyzing the major product of PET hydrolysis, see Reaction 7. Interestingly, following along this degradation pathway, the hydrolysis of PET leads into the same reaction pathway of PAH degrading bacteria, see Reaction 2. This is reasonable, as the benzene ring needs to be cleaved for a complete degradation, but also suggests that PAH degrading microorganisms could be promising in the field of plastic biodegradation as well.

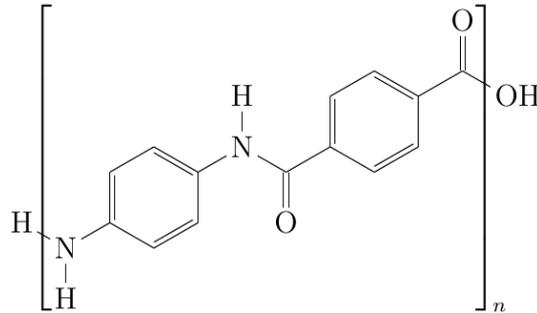


Figure 4: The polymeric structure of Kevlar.

3.2.3 Experimental work

As experimental illustration of the possibilities in plastic degradation, a cooperation with the research group of Professor Guebitz at the Institute of Environmental Biotechnology at the University of Natural Resources and Life Sciences Vienna was established. This has made it possible to gain access to Kevlar samples which were treated with a laccase mediator system (LMS). This system utilizes laccase, an enzyme of the oxidoreductases group with a broad range of substrates, including aromatic compounds, which catalyzes the reduction of molecular oxygen into water. The main function of laccase in nature is the polymerization and depolymerization of lignin, with the ability to oxidize hydroxyl groups linked to a benzene ring, before the cleavage of alkyl side chains or ring-cleavage of non-phenolic lignin structures [48].

Kevlar, the examined plastic sample material, is a large polymer from a class of highly heat resistant, strong synthetic fibres, called Aramid fibres. It features a high degree of orientation that makes it similar to fibres such as ultra-high molecular weight polyethylene, and exhibits good strength-to-weight proportionality. Its chemical structure is depicted in Fig. 4.

The samples were prepared as (2 x 2) cm² sheets. Two samples were prepared, with two accompanying control samples. The first was incubated in a shaker at room temperature with a laccase mediator system, the second at 50°C with LMS and subsequently washed with succinic buffer, pH ~ 4.0; the two control samples were incubated in the same way with only succinic buffer. The buffer was made by solving succinic acid in Milli-Q™ ultra pure water with a concentration of 0.2M, and used to maintain the pH level. The samples were dried and then measured under the atomic force microscope (Model: MFP-3D, Asylum Research, Santa Barbara, CA, USA).

In Fig. 5 you can clearly see a relatively smooth surface of the control sample with heightened veins visible all across the surface, whereas in Fig. 6 the surface has become eroded, with small circular pits visible with a slightly darker coloring and the veins from the control sample clearly missing.

In Fig. 7 the surface of the control sample prepared at 50°C is relatively smooth as well, with a few larger ridges, as well as a few smaller hill-like structures at the top,

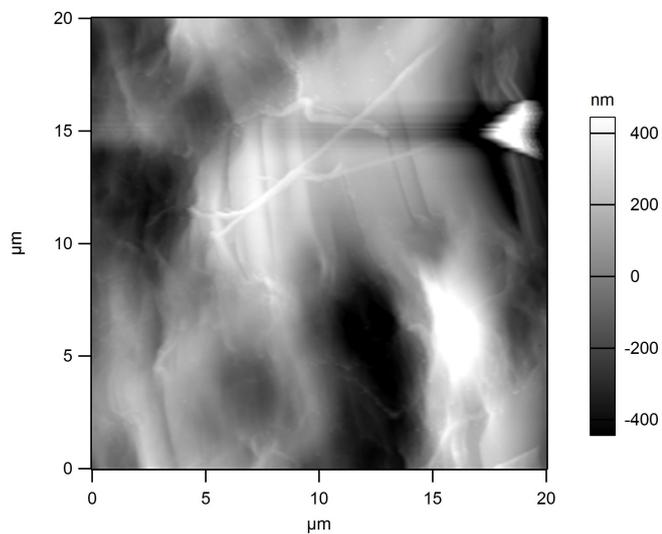


Figure 5: AFM image of Kevlar, control sample

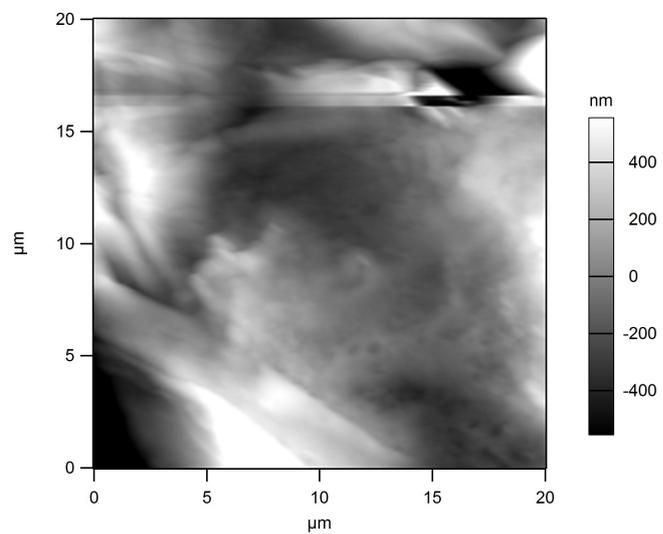


Figure 6: AFM image of Kevlar, treated with lac-case mediator system

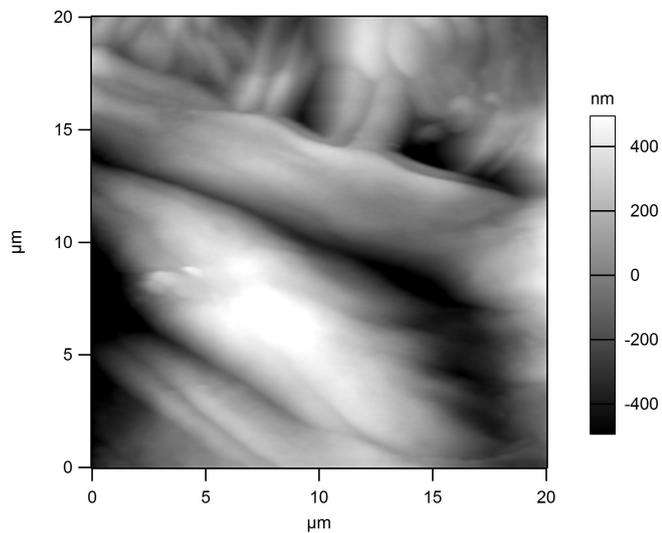


Figure 7: AFM image of Kevlar, treated at 50°C, control sample

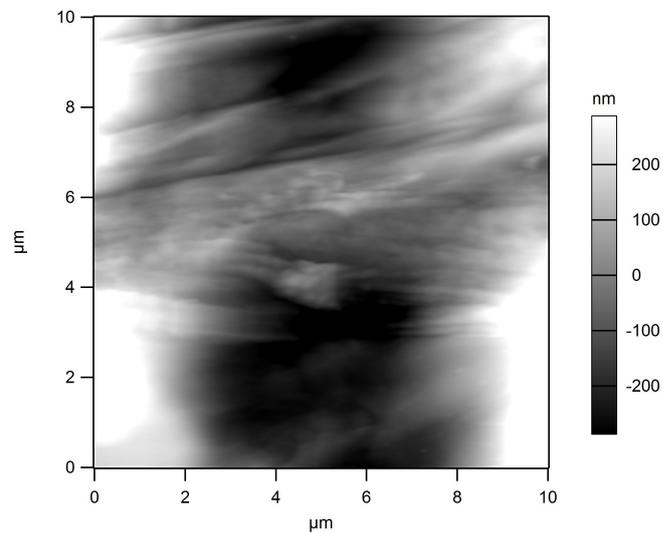


Figure 8: AFM image of Kevlar, treated with lac-case mediator system at 50°C

which also have a smooth surface. The LMS treated sample in Fig. 8 shows an uneven surface, especially in the middle parts of the picture, which could well be an area of surface erosion.

As a preliminary test to determine surface erosion of Kevlar samples by enzyme treatment of this type, the measured images point towards positive erosion of the surface, but are too inconclusive as sole testing procedures.

4 Summary and Outlook

The environmental impact of oil and plastic waste in oceans and landfills and the growing plastic demand and production was discussed. Problem zones like major oil catastrophes or the Bangalore garbage crisis, as well as the trend towards a throw-away society and inadequate waste management were debated.

An overview of extremophilic organisms was given, and their possible benefit to bioremediation by their ability to biodegrade and consume different toxic or otherwise dangerous materials was related. This includes hydrocarbons of crude oil and petroleum, plastics, dioxins, heavy metals and radioactive materials such as nuclear waste. This thesis put a special weight on the possibilities regarding the bioremediation of oil and plastic.

A broad overview was given on different metabolic pathways in extremophiles for the degradation of oil, detailing necessary enzymes and their corresponding organisms; in aerobic and anaerobic conditions; in different states, like emulsions or soil and waste sludges; for different kinds of hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs); and the differences in bacterial, fungal and eucaryotic metabolism is elaborated.

Expanding on the application of extremophiles in bioremediation, biostimulation and bioaugmentation are presented, where one is the aiding of natural growth by supplementing nutritional supplies, and the other is implanting foreign organisms to facilitate biodegradation, as well as the method of biofiltering volatile organic carbons (VOCs) with high evaporation rates to avert them from entering the atmosphere.

It was noted that plastic biodegradation is yet to become a bigger field of research, and therefore only a general overview was given on this subject. Recent research was discussed in the field of biodegradation of polyethylene terephthalate (PET) and polyethylene (PE) by newly found extremophile species, the bacterium *Ideonella sakaiensis* and the wax moth larvae *Galleria mellonella* respectively.

The experimental part of this thesis focuses on the surface erosion of Kevlar, a synthetic polymer fiber. The enzymatic treatment of the Kevlar samples was done by Sara Vecchiato and Lukas Skopek from the research group of Professor Guebitz at the Institute of Environmental Biotechnology at the University of Natural Resources and Life Sciences Vienna, using a laccase mediator system. They were imaged under the atomic force microscope (AFM) at our facility at the Technical University of Vienna.

The AFM measurements point towards positive surface erosion of the Kevlar samples, but should be verified by other testing procedures. Future experiments could include the preparation of a sample on a microscope slide with optical markers for referencing the position of the measurement. This would allow the imaging of the same sample before and after enzymatic treatment by measuring the control sample and subsequently treating it with LMS. Alternatively, a dynamic measurement in a flow-through fluid cell could be set up, imaging the sample with the AFM continuously, to record surface erosion over time.

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