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PROTEOGENOMIC TOOLS FOR STUDYING MICROBIAL DEGRADATION OF POLYMERS AND ADDITIVES

BY
JAN STRUCKMANN POULSEN

DISSERTATION SUBMITTED 2023

AALBORG UNIVERSITY
DENMARK

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PREFACE

This dissertation is submitted in partial fulfilment of the requirements for obtaining the degree of Doctor of Philosophy (PhD). The thesis describes the outcomes of the PhD project "Proteogenomic tools for studying microbial degradation of polymers and additives", which was carried out from 1st of September 2021 to 31st of August 2023, at the Department of Chemistry and Bioscience at Aalborg University, under the supervision of Professor Jeppe Lund Nielsen. The results presented here were obtained as part of a work package under the "UPLIFT project" and the "IKEBA" project.

The primary focus of this PhD project was the development of an innovative screening platform consisting of a collection of approaches to identify novel enzymes and organisms with improved depolymerisation activities to any type of recalcitrant substrate – Both regarding resource recovery and degradation of micropollutants.

The thesis consists of an introduction that summarises the background and literature relevant to the PhD project, and is supported by 5 scientific papers, which are included in this thesis.

I hereby declare that this is my original work.

Jan Struckmann Poulsen

Aalborg, August 2023

ENGLISH SUMMARY

During the Carboniferous Age, the introduction of lignin led to a massive environmental imbalance, which persisted for 50 million years. Today, the demand for energy is rising, and sustainable and environmentally friendly energy sources are needed. Anaerobic digestion of agro-industrial waste streams is a practical and adaptable method for producing biogas. However, studies on how to exploit lignocellulosic biomass in the anaerobic digestion process are limited. As for the plastic pollution problem, plastic production started in the 1940s and 1950s, and today, plastics are essential in many sectors, especially in packaging. Plastic waste is a serious problem, as less than 20% of post-consumer plastic waste in developed countries is recycled, and most plastic ends up in landfills or leaks into the environment. Biodegradable polymers, such as poly(butylene adipate-co-terephthalate) (PBAT), and biobased plastics have gained interest in solving the environmental problems associated with conventional plastics made from fossil raw materials.

The objective of this project was to enhance the knowledge of the key degraders of different polymers and additives, with the intention of facilitating the recovery of resources in the future, as well as reducing the impact of pollutants. Key microorganisms were identified by means of amplicon sequencing, genome sequencing, and metagenome sequencing. Moreover, their metabolic and microbial physiology were explored through proteogenomic approaches for *in situ* studies and by retrieving and annotating metagenome-assembled genomes (MAGs).

The first two studies of the thesis describe a survey of anaerobic digestion (AD) systems processing maize silage and wheat straw, respectively. Each study was subject to both amplicon sequencing and protein-stable isotope probing to capture the microbial communities associated with these AD substrate types and to identify active degraders. In the first study, active thermophilic cellulose-degrading microorganisms were identified, these included members of the *Firmicutes* and *Candidatus* Bipolaricaulota, using a high-throughput linking of activity to identity. The second study observed a long-term adapted microbiome for degradation of wheat straw, and after the microbiome had adapted, key degraders were identified. Key degraders were shown to directly degrade the cellulose were *Defluviitoga tunisiensis*, *Syntrophothermus lipocalidus*, and *Pelobacter carbinolicus*.

This project has also been looking into the degradation of plastic polymers. The third study tracked the labelled carbon assimilation to identify key degraders of the monomers of PBAT. The direct metabolisers were affiliated to the genera *Bacteroides*, *Ichthyobacterium*, and *Methanosarcina*. An extracted HQ-MAG classified as *Anaerolinea thermophila*, contains enzymes putatively responsible for degrading butanediol. This study provides better insight into the identity of PBAT-degrading organisms and potential degradation processes that could be of interest for further study.

To gain deeper insight into a single organism's microbial physiology, analysis of differential protein expression was used in the fourth study. More specifically the physiological changes of *Ideonella sakaiensis* was studied during degradation of PET, to understand the degradation mechanism better. In continuation of studying the degradation of PET, a co-metabolic degradation of tetrabromobisphenol A (TBBPA), a flame retardant often used as an additive in PET, was investigated in the fifth study. For the first time, a labelled compound was used in concentrations of $\mu g \cdot L^{-1}$ as part of a co-metabolism. The utilization of a culture-independent protein-SIP method on biomass adjusted to grow on concentrations close to its environmental occurrence enabled the identification of active organisms involved in TBBPA degradation. The findings of this study have important implications for biotreatment technologies in engineered systems.

In conclusion, the combined results of this thesis highlight the usefulness of microbial studies in engineered systems to obtain a better understanding of the microbiology behind. On top of this the use of proteogenomics for obtaining insight into the functional role of the vast group of unculturable microorganisms.

DANSK RESUME

Under karbonalderen førte introduktionen af lignin til en massiv miljømæssig ubalance, som varede i 50 millioner år. I dag er efterspørgslen på energi stigende, og der er brug for bæredygtige og miljøvenlige energikilder. Anaerob udrådning af agroindustrielt spildevand er en praktisk og tilpasningsdygtig metode til at producere biogas. Imidlertid er undersøgelser af, hvordan man udnytter lignocellulosebiomasse i anaerob udrådningss-processen begrænsede. Hvad angår problemet med plastforurening, startede plastproduktionen i 1940'erne og 1950'erne, og i dag er plast essentielt i mange sektorer, især som emballage. Plastaffald er et alvorligt problem, da mindre end 20 % af plastikaffald fra forbrugere i udviklede lande genanvendes, og det meste plastik ender på lossepladser eller i miljøet. Biologisk nedbrydelige polymerer, såsom poly(butylenadipat-co-terephthalat) (PBAT) og biobaseret plast har fået interesse i forbindelse med at løse de miljøproblemer, der er forbundet med konventionel plast fremstillet af fossile råmaterialer.

Formålet med dette projekt var at øge kendskabet til de vigtigste nedbrydere af forskellige polymerer og additiver med den hensigt at lette genvindingen af ressourcer i fremtiden, samt at reducere påvirkningen af forurenende stoffer. Nøglemikroorganismer blev identificeret ved hjælp af amplikon-sekventering, genom-sekventering og metagenom-sekventering. Desuden blev deres metaboliske og mikrobielle fysiologi udforsket gennem proteogenomiske tilgange til *in situ* undersøgelser og ved at hente og annotere metagenom-samlede genomer (MAGs).

Afhandlingens to første studier beskriver undersøgelse af anaerob udrådnings-systemer, der behandler henholdsvis majs-ensilage og hvedehalm. Hver undersøgelse var genstand for både amplikon-sekventering og protein-stabil isotop-probing (protein-SIP) for at beskrive det mikrobielle samfund, der er forbundet med disse anaerob udrådnings-substrattyper og for at identificere aktive nedbrydere. I det første studie blev aktive termofile cellulose-nedbrydende mikroorganismer identificeret, disse omfattede medlemmer af *Firmicutes* og *Candidatus* Bipolaricaulota. Det andet studie observerede et langsigtet tilpasset mikrobiom til nedbrydning af hvedehalm, og efter at mikrobiomet havde tilpasset sig, blev centrale nedbrydere identificeret. Organismer, der blev vist direkte at nedbryde cellulosen, var *Defluviitoga tunisiensis*, *Syntrophothermus lipocalidus* og *Pelobacter carbinolicus*.

Dette projekt har også undersøgt nedbrydningen af plastpolymerer. Det tredje studie sporede den mærkede kulstofassimilering for at identificere de vigtigste nedbrydere af PBAT-monomere. De direkte metabolisatorer var tilknyttet slægterne *Bacteroides*, *Ichthyobacterium* og *Methanosarcina*. Et ekstraheret HQ-MAG klassificeret som *Anaerolinea thermophila*, indeholdt enzymer, der formodes at være ansvarlige for at nedbryde butandiol. Dette studie giver bedre indsigt i identiteten af PBAT-nedbrydende organismer og potentielle nedbrydningsprocesser, der kunne være af interesse for yderligere undersøgelse.

For at få dybere indsigt i en enkelt organismes mikrobielle fysiologi blev analyse af differential protein-ekspression brugt i det fjerde studie. Mere specifikt blev de fysiologiske ændringer af *Ideonella sakaiensis* undersøgt under nedbrydning af PET for bedre at forstå nedbrydningsmekanismen. I forlængelse af undersøgelsen af nedbrydningen af PET, blev en co-metabolisk nedbrydning af tetrabromobisphenol A (TBBPA), en brandhæmmer, der ofte bruges som tilsætningsstof i PET, undersøgt i det femte studie. For første gang blev en mærket forbindelse brugt i koncentrationer af $\mu g \cdot L^{-1}$ som en del af en co-metabolisme. Anvendelsen af en kulturuafhængig protein-SIP-metode på biomasse tilvænnet til at vokse på koncentrationer tæt på miljømæssige forekomst muliggjorde identifikation af aktive organismer involveret i TBBPA-nedbrydning. Resultaterne af denne undersøgelse har vigtige konsekvenser for biobehandlingsteknologier i konstruerede systemer.

Afslutningsvis fremhæver de kombinerede resultater af denne afhandling anvendelsen af mikrobielle undersøgelser i manipulerede systemer for at opnå en bedre forståelse af mikrobiologien bag. Oven i dette brugen af proteogenomiske tilgange til at opnå indsigt i den funktionelle rolle af den store gruppe af ukultiverbare mikroorganismer.

ACKNOWLEDGEMENTS

I am grateful for the numerous people who have helped along my journey here at Aalborg University and made possible in completing this study. First and foremost, I would like to express my deepest gratitude to my supervisor, Professor Jeppe Lund Nielsen, for giving me the opportunity to work on this project.

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Furthermore, I would like to extend my thanks to all participants in the UPLIFT project.

Lastly, I would like to thank my friends, family, and girlfriend for their support and for keeping me grounded during this project. To my parents, thank you for following me on this journey and helping me chase my dreams. To my friends, your support has been invaluable in completing this PhD project. To my girlfriend, thank you for the invaluable support and I know that together we can deal with everything.

Finally, thank you, reader of this thesis.

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OBJECTIVES OF THE PHD PROJECT

The overall objective of this PhD project was to utilise proteogenomics and bioinformatics in order to optimise biotechnologies for biofuel production and also demonstrate how proteogenomics can push us one step further in solving the plastic pollution by providing knowledge on the metabolism by key degraders.

The aim of the individual studies included in this PhD thesis were as follows:

Paper 1: Efficient utilization of lignocellulosic biomass for energy production is hindered by its recalcitrant nature. To enhance anaerobic digestion of plant-based feedstocks, it is essential to gain a deeper insight into the microorganisms capable of converting them. In this research, metagenome resolved protein stable isotope probing (protein-SIP) was employed to identify active thermophilic microorganisms that can break down cellulose from maize, in a full-scale anaerobic digester.

Paper 2: A long-term adapted microbiome for degradation of wheat straw was used to further elucidate which organisms is involved in the degradation of plant-based feedstocks. Provide the information regarding identity and function to enable the optimization of biotechnologies for biofuels production.

Paper 3: To track ^{13}C-labelled PBAT monomers in the sludge from an anaerobic digester of an waste water treatment plant in thermophilic conditions (55 °C). We examined three anaerobic digester reactors fed with fully ^{13}C-labelled adipic acid, butanediol, or terephthalate by retrieving simultaneous information on the identity of the microorganisms (metagenomics assemblies) and the activity from the ^{13}C-labelled proteins (Protein-SIP).

Paper 4: To study the physiological changes of *Ideonella sakaiensis* during degradation of polyethylene terephthalate. Determine the biochemical pathways and enzymes involved should lead to a better understanding of the degradation mechanisms.

Paper 5: Tetrabromobisphenol A (TBBPA), the most commonly utilized flame retardant globally, poses a threat to aquatic ecosystems. It is added to packaging plastics, including PET. Previous studies have suggested potential TBBPA degraders in anaerobic bioreactors, but the organisms that actively break down TBBPA under *in situ* conditions remain unidentified. To address this, a combination of protein-based stable isotope probing and metagenomics was employed to identify

and gain genomic insights into the TBBPA-degrading microorganisms.

Knowledge gathered by the project is to be applied towards obtaining a deeper understanding of the biodegradation of micropollutants, such as antibiotics/antifungals, microplastics, and additives to microplastics. This PhD project was part of a work package under the UPLIFT project and IKEBA project.

LIST OF SUPPORTING PAPERS

Paper 1: Poulsen JS, de Jonge N, Macêdo WV, Dalby FR, Feilberg A, Nielsen JL. Characterisation of cellulose-degrading organisms in an anaerobic digester. Bioresource Technology, 2022. DOI: 10.1016/j.biortech.2022.126933.

Paper 2: Poulsen JS, Macêdo WV, Bonde T, Nielsen JL. Energetically exploiting lignocellulose-rich residues in anaerobic digestion technologies: from bioreactors to proteogenomics. Submitted and under revision at Biotechnology for Biofuel and Bioproducts.

Paper 3: Poulsen JS[1], Santiso AT[1], Lema J, Echers SG, Wimmer R, Nielsen JL. Assessing labelled carbon assimilation from PBAT monomers during thermophilic anaerobic digestion. Submitted and under revision at Bioresource Technology.

[1]These authors have contributed equally to this study.

Paper 4: Poulsen JS, Nielsen JL. Proteomic characterisation of polyethylene terephthalate and monomers degradation by *Ideonella sakaiensis*. Journal of Proteomics, 2023. DOI: 10.1016/j.jprot.2023.104888.

Paper 5: Macêdo WV[1], **Poulsen JS**[1], Zaiat M, Nielsen JL. Proteogenomics identification of TBBPA degraders in anaerobic bioreactor. Environmental Pollution, 2022. DOI: 10.1016/j.envpol.2022.119786.

[1]These authors have contributed equally to this study.

Papers not included in this thesis:

Paper 6: Poulsen JS, Madsen AM, White JK, Nielsen JL. Physiological Responses of *Aspergillus niger* Challenged with Itraconazole. Antimicrobial Agents and Chemotherapy, 2021. DOI: 10.1128/AAC.02549-20.

Paper 7: White JK, Nielsen JL, **Poulsen JS**, Madsen AM. Antifungal Resistance in Isolates of *Aspergillus* from a Pig Farm. MDPI Atmosphere, 2021. DOI: 10.3390/atmos12070826.

Paper 8: de Jonge N, **Poulsen JS**, Vechi NT, Kofoed MVW, Nielsen JL. Wood-Ljungdahl pathway utilisation during in situ H_2 biomethanation. Science of the Total Environment, 2021. DOI: 10.1016/j.scitotenv.2021.151254.

Paper 9: Macêdo WV, **Poulsen JS**, Oliveira GHD, Nielsen JL, Zaiat M. Tetrabromobisphenol A (TBBPA) biodegradation in acidogenic systems: One step further on where and who. Science of the Total Environment, 2022. DOI: 10.1016/j.scitotenv.2021.152016.

Paper 10: Cydzik-Kwiatkowska A, de Jonge N, **Poulsen JS**, Nielsen JL. Unravelling gradient layers of microbial communities, proteins, and chemical structure in aerobic

granules. Science of the Total Environment, 2022. DOI: 10.1016/j.scitotenv.2022.154253.

Paper 11: Oliveira JMS, **Poulsen JS**, Foresti E, Nielsen JL. Microbial communities and metabolic pathways involved in reductive decolorization of an azo dye in a two-stage AD system. Chemosphere, 2023. DOI: 10.1016/j.chemosphere.2022.136731.

Paper 12: Castronovo S, Helmholz L, Wolff, **Poulsen JS**, Nielsen JL, Ternes TA, Schmidt TC, Wick Arne. Protein Fraction and Shotgun Proteomics Analysis of Enriched Bacterial Cultures Shed New Light on the Enzymatically Catalyzed Degradation of Acesulfame. Water Research, 2023. DOI: 10.1016/j.watres.2022.119535.

Paper 13: Takman M, Cimbritz M, Paul C, Svahn O, Blomqvist S, **Poulsen JS**, Nielsen JL, Davidsson Å. Potential for water reuse with membrane bioreactor and granular activated carbon – a full-scale treatment plant operated over one year in Scania, Sweden. Submitted and under revision at Science of the Total Environment.

Paper 14: Poulsen JS, Nielsen CK, Pedersen NA, Wimmer R, Sondergaard TE, de Jonge N, Nielsen JL. Proteomic changes in methicillin-resistant *Staphylococcus aureus* exposed to cannabinoids. Submitted and under revision at Journal of Natural Products.

Paper 15: Deng Z, **Poulsen JS**, Nielsen JL, Weissbrodt DG, Lier JV, Spanjers H. Identification of protein-degraders in an anaerobic bioreactor by protein stable isotope probing combined with metagenomics. In preparation.

Paper 16: Dalby FR, Wilson H, **Poulsen JS**, Nielsen JL, Adamsen APS. Organic matter transformation rates and methanogenesis dynamics in pig slurry at ambient and low temperatures. Submitted and under revision at Environmental Quality.

Paper 17: Oliveira JMS, **Poulsen JS**, Foresti E, Nielsen JL. New insights into the mechanism of azo dye biodegradation by *L. lactis*. In preparation.

Paper 18: Macêdo WV, Harpøth RD, **Poulsen JS**, Nielsen JL, Fischer CH, Agneessens LM, Rickers CK, Vergeynst L. Resource recovery from hydrothermal liquefaction process water: toxicity reduction, bioenergy production, and microbial ecology. In preparation.

Paper 19: Sandeep R, Muscolino JF, Macêdo WV, Picullel M, Christensson M, **Poulsen JS**, Nielsen JL, Vergeynst L. Effect of Biofilm Architecture on the Activity and Community Composition of Phosphorus Accumulating Bacteria in a Moving Bed Biofilm Reactor. Submitted and under revision at Water Research.

Paper 20: Menanteau-Ledouble S, Löhn S, **Poulsen JS**, Hindersin S, Nielsen JL. The green algae *Haematococcus pluvialis* display inhibitory properties against the fish pathogen *Yersinia ruckeri*. In preparation.

INTRODUCTION

The natural environments of our planet have encountered numerous challenges over the past few centuries and are presently confronted with a range of issues resulting from human activities, including pollution, climate change, and depletion of resources. On top of this the awareness of such problematics has increased dramatically and therefore a need to act on them as well.

One of such problems is the production and use of polymers, both natural and synthetic polymers. The term "polymer" was coined by J. J. Berzelius, a chemist from Sweden, in 1833. It is derived from the Greek words "*poly*" meaning "many" and "*mer*" meaning "part", hence, "many parts". Collodion, a synthetic polymer, was one of the earliest types of polymers that was created by nitration of the natural polymer cellulose (1). Alexander Parkes discovered collodion in 1846, which was later commercialized under the name "celluloid". While celluloid dominated the polymer market for roughly three decades, its high flammability led to the development of more stable materials, such as plastics made from cellulose acetate (1).

1.1. THE CARBONIFEROUS AGE

During the carboniferous age, also known as the age of large plants, a new polymer was introduced into the world. With the introduction of this ‘new’ polymer, lignin, microbes’ ability/efficiency to degrade wood was decreased (2). This led to an enormous environmental imbalance, which persisted for the next 50 million years, resulting in the accumulation of dead wood. This reason is also used widely to explain the modern Industrial Revolution due to the greatest coal-forming interval in Earth’s history. The organic carbon burial plays an important role in Earth systems and influence the climate in regard to O_2 and CO_2 levels (2).

Natural organic polymers are essential components of living organisms, serving as structural materials, and participating in essential biological processes (3). Cellulose, lignin, and various resins are examples of polymers that constitute the solid parts of all plants (Figure 1). Cellulose, a polysaccharide composed of sugar molecules, and lignin, a complex three-dimensional network of polymers, are the two most abundant biopolymers on Earth (4).

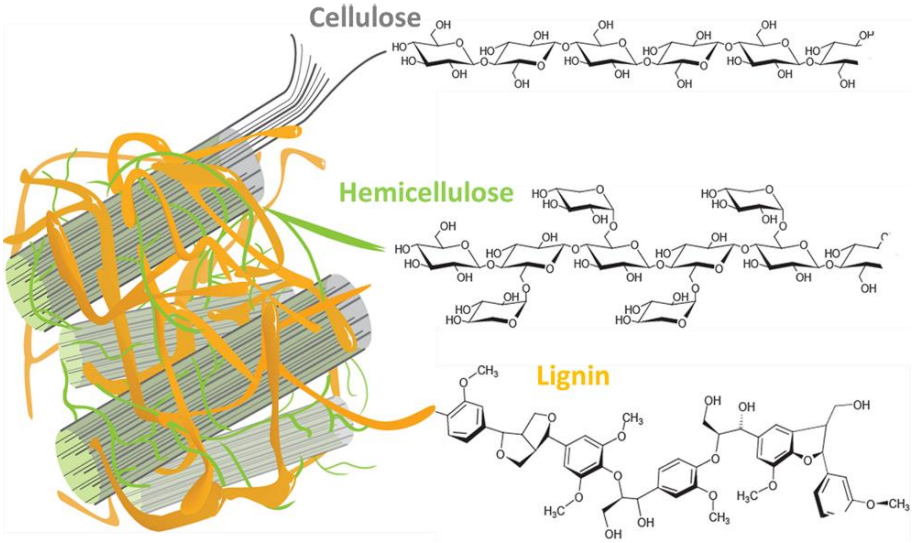

Figure 1: Some of the first polymers to be developed in nature (5).

Due to the presence of lignin, wood serves as a significant repository of organic carbon that is exceptionally resistant to decay. Lignin is a diverse polymer that imparts stability and firmness to wood, safeguards cellulose and hemicellulose from microbial degradation, and is the principal precursor to coal (6). Cellulose and lignin are the two most abundant organic compounds on Earth (Figure 1).

A significant amount of research has been dedicated to describing the complex process of wood degradation. Microorganisms have developed various enzymatic and non-enzymatic strategies to utilize the highly abundant biomass (7). Fungi and some bacterial species are responsible for the degradation of lignin. While the microbial breakdown of lignin has been extensively studied in fungi, it has not been thoroughly investigated in other organisms. Nevertheless, there are reports of bacteria that have the ability to decompose lignin. The exact number of fungal species that can break down lignin is unknown, but it has been reported that around 1600 species of wood-degrading fungi exist in North America (8). Lignocellulosic degrading bacteria represent mainly three phyla: *Proteobacteria*, *Actinobacteria*, and *Firmicutes* (4,7, **Paper 1**, **Paper 2**).

Due to the production of secondary metabolites and the use of extracellular enzymes, bacterial species of *Proteobacteria*, *Actinobacteria*, and *Firmicutes* are considered major decomposers of lignocelluloses. There are a number of *Actinomycetes* species, including *S. coelicolor*, *S. griseus*, and *S. psammoticus*, that produce three enzymes (laccase, lignin peroxidase, and manganese peroxidase) which are thought to be crucial in the biodegradation of lignin (9,10). Lignin peroxidases is capable of attacking lignin polymers by oxidizing different phenolic aromatic compounds and a variety of non-phenolic lignin model compounds (11).

Breaking down lignin is accomplished by a complex process that involves multiple enzymes, including accessory enzymes, working together efficiently, and not solely by the four primary ligninolytic oxidases (11). It has been shown that a consortium of microorganisms could play an important role in the degradation of lignocellulosic biomass, since the possibility of all different enzymes being present is higher when a consortium of microorganisms is behind the degradation, compared to a single microbial strain (11). To advance application technologies, it's crucial to understand the functions and interactions of these enzyme combinations in lignin degradation through scientific research.

1.1.1. WASTE-TO-ENERGY TECHNOLOGIES

As the demand for energy continues to rise and fossil fuel reserves near depletion, the need for sustainable and environmentally friendly energy sources becomes more pressing. Anaerobic digestion (AD) of agro-industrial waste streams is a practical and adaptable method for producing biogas (12).

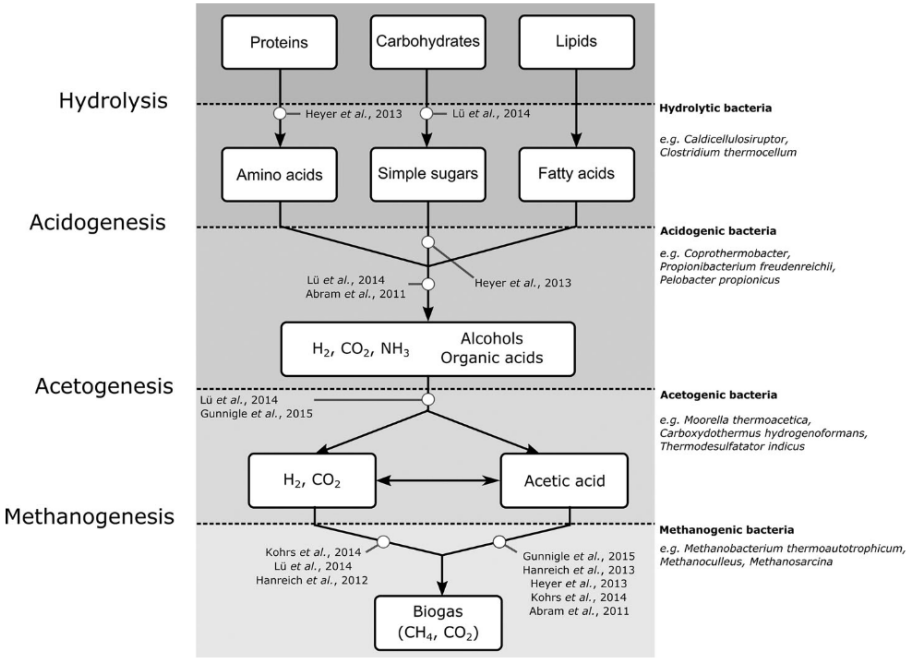

Figure 2: Schematic presentation of the major steps involved in the degradation of organic matter to biogas (13).

The AD process can be divided into four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 2), and different organisms with different abilities is involved in each phase of this process. During the initial stage, known as

hydrolysis, complex organic molecules such as carbohydrates, lipids, and proteins are broken down into their individual monomers, often extracellularly. Subsequently, in the second phase, referred to as acidogenesis, these monomers are transformed into volatile fatty acids (VFAs), H_2, CO_2, and NH_3. The next phase, acetogenesis, then transforms the VFAs into acetic acid, CO_2, and H_2, and final phase, the methanogenesis, convert the acetic acid and some of the H_2 into CH_4 and CO_2 (14).

A wide variety of feedstocks can be applied in the AD process, as long as they contain carbohydrates, proteins, and/or fats (12,14). However, the composition of the biogas and the methane yield is dependent on the type of feedstock, and the theoretical biogas yield varies with the digestibility of the feedstock and its content of carbohydrate, proteins, and fats (Table 1). The carbon-to-nitrogen (C/N) ratio is another commonly used measurement for the feedstock applied in AD. A general recommendation is that the C/N ratio should be between 20-30 and is usually achieved by co-digestion of feedstocks (15).

Table 1: Characteristics of major feedstocks used for anaerobic digestion (14).

Feedstock	***Main features***	***Biogas yield (m^3/kg VS)***	***Total solids (%)***	***C/N ratio***
Animal manure	Usually co-digested with bedding material (straw) or other biomass high in carbon. High buffer capacity. Complete source of nutrients and trace elements.	0.1-0.6	2-20	3-15
Food waste	Produced by hotels, restaurants, markets, and food processing companies. Requires size reduction. High variability in composition. Easily digestible, could generate inhibition by acidification.	0.3-0.8	5-50	15-35
Agricultural residues and energy crops	Abundantly in availability. Composed mostly of cellulose, hemicellulose, lignin, and/or starch. Highly recalcitrant: Needs pre-treatment to enhance degradability. Can be ensiled for storage.	0.2-0.5	20-80	40-150
Sewage sludge	By-product of wastewater treatment. High in solids and nutrients, but also potential for pathogens. Low digestibility, pre-treatment or co-digestion may improve it.	0.8-1.2	20-35	40-70

Not only the type of feedstock influences the efficiency and characteristic of the AD process, also the operational parameters influence the process. These parameters include feeding method, hydraulic retention time, organic loading rate, reactor temperature, etc. (14,16,17). Changes in any of these parameters not only influences the operational side of the AD, but also affect the microbial community in the AD.

The interest in implementation of lignocellulosic biomass in the AD process has increased over the years, due to its high energy potential, abundance, and to avoid competition with food and feed production (18). Therefore, investigations on how to exploit the lignocellulosic biomass has been in focus, however, so far most of the studies has investigated the settings and operational parameters of the bioreactors (19). Only recently studies have implemented metagenome-resolved protein stable isotope probing to extract multi-dimensional information of the AD process (20,21, **Paper 1**, **Paper 2**, **Paper 3**, **Paper 5**).

The macroscopic level of the AD process is well understood, but what is missing is the information regarding who is responsible for the specific bioconversion in the different stages (12). So far studies looking into enzymatic processes of the AD have only been able to provide information on either function or identity.

1.2. THE PLASTIC PROBLEM

Human history is defined periodically by ages, Stone, Bronze, and Iron Ages, and the modern age may arguably be defined as the Plastics Age (1), as plastic has become a prominent part of modern industry and society. With the invention of new polymer, different plastic polymers, a new environmental imbalance has been created, resulting in the accumulation of plastic waste in the environment. Immediate action is imperative for humanity, as it is not viable to wait another 50 million years for the evolutionary processes to reinstate equilibrium.

Plastic polymers have been invented for many different purposes, conserve food, technical innovations, safety innovations, energy conservations, etc. The production of plastic materials in an industrial scale started in the 1940s and 1950s (22), and in 2018 the annual production reached 359 million metric tons (23). There are five families of plastic that dominates the marked (accounts for approximately 75 % of the demand), these are polyethylene (including low density (LDPE), linear low density (LLDPE) and high density (HDPE)), polypropylene (PP), polyvinylchloride (PVC), polystyrene (solid PS and expandable EPS), and polyethylene terephthalate (PET) (24).

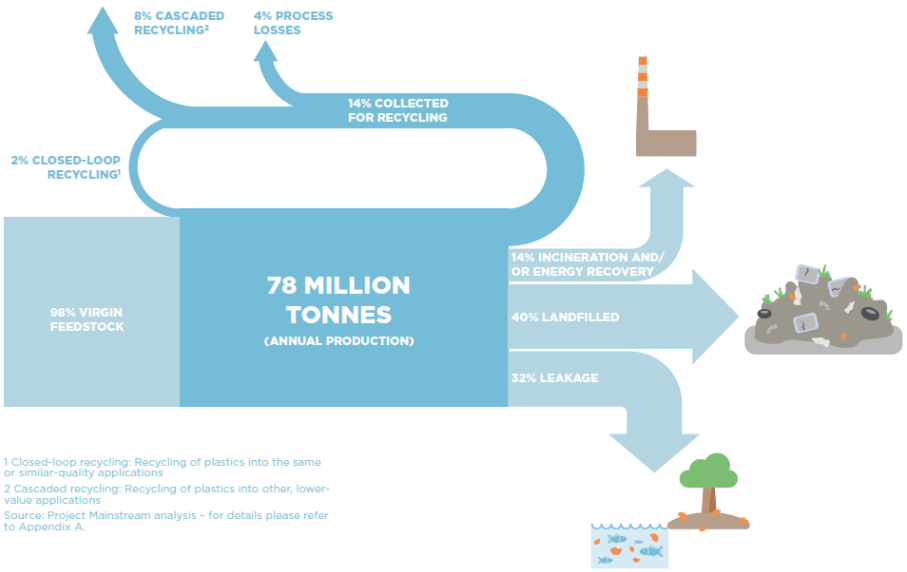

Figure 3: Global flows of plastic packaging materials in 2013 (25).

In Europe, the use of plastics is mostly dominated by packaging (38 %), but also in the health sector. Plastics are important in our society providing a range of benefits for human health and the environment (22). With such a diverse application of plastic, a just as diverse waste stream is being produced. Especially when talking about a product as plastic that has a short lifespan (~40 % of plastic products have a lifespan of less than 1 month) (26), resulting in serious environmental and management problems. Less than 20 % of post-consumer plastic waste in developed countries is recycled (23), and by far most of the plastic ends up in landfills (40 %) or leak into the environment (32 %) (Figure 3). With a waste stream of many different plastic polymers, with different types of additives and/or contaminants leads to substantial deterioration of the plastic properties during and after reprocessing (23). Today mechanical recycling is mostly applied, therefore, too often leads to a cascade recycling (downcycling) resulting in reduced quality and/or utility (Figure 3) (23,25).

In the field of plastic, the term "upcycling" refers to the practice of utilizing plastic waste, whether it be postindustrial or postconsumer, as a raw material for creating high-value products such as polymers, molecules, or materials (23). This method of upcycling is seen as complementary to chemical and mechanical recycling (Figure 4). The aim of upcycling is to convert waste plastic into products that have a greater perceived "value". However, in a sustainable plastics economy, the definition of "value" is not solely restricted to economic value, but also encompasses numerous external, personal, and sociological considerations (23,25). Therefore, addressing plastic waste problems necessitates a more holistic approach that is difficult to quantify.

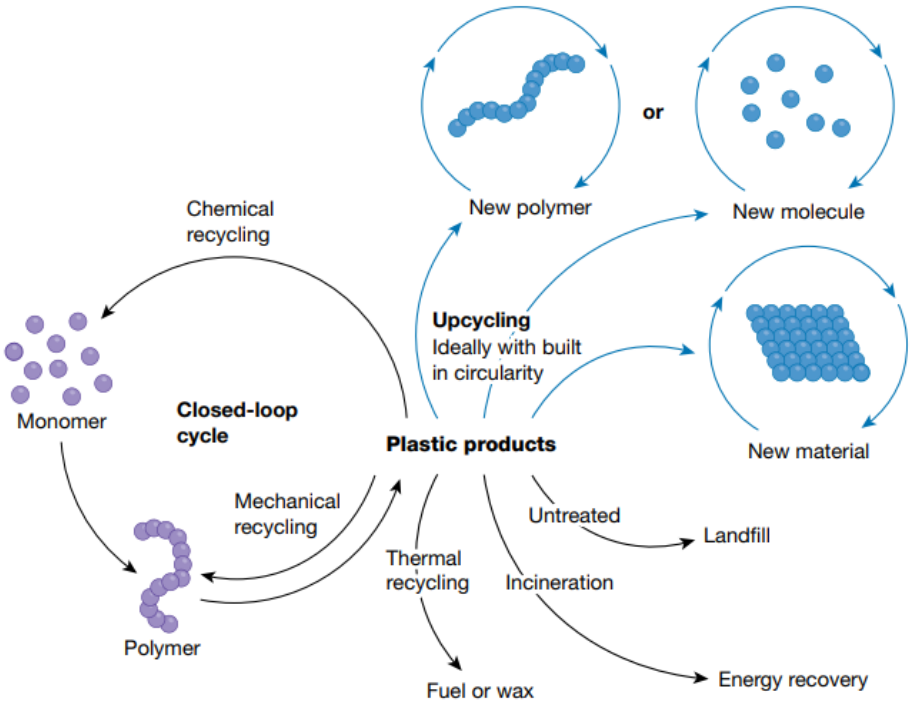

Figure 4: Upcycling of plastic and plastic waste. As one of the end-of-life options for plastics, upcycling is seen as a complementary approach to chemical and mechanical recycling. It involves using plastic waste to create new and valuable products such as polymers, molecules, or materials (23).

One approach is biological depolymerization technology, which can complement existing recycling practices by transforming persistent plastic waste into more readily recyclable and/or biodegradable polymers. The integration of biological depolymerization is expected to enhance the capacity to manage significant quantities of currently non-recyclable plastics.

1.2.1. PLASTIC POLYMERS

The term plastic is covering a very broad group of polymers with high molecular weight, and many of them are chemically synthetised from petroleum (27). In the case of petroleum-derived plastics, a waste-disposal problem arise since these materials often are not readily biodegradable and because of their resistance to microbial degradation they will accumulate in the environment (28). The breakdown of substances by living organisms or enzymes (biodegradation) occurs in a two-step process. First, polymers are broken down into smaller molecules through either abiotic

process such as oxidation, photodegradation, or hydrolysis, or biotic processes such as microbial degradation (28). The second step involves the assimilation of these polymer fragments by microorganisms and their mineralisation. The biodegradability of a substance is determined not only by its source but also by its chemical makeup and the environmental factors that promote degradation.

In order to solve the environmental problems associated with the conventional plastic types a growing interest is to design new biodegradable polymers. Besides the plastics made from fossil raw materials, biobased plastics has gained interest. These types of plastics are either fully or partially made from biological resources (29). But these are not necessarily biodegradable, which is why the entire life cycle of the plastic is important to examine. Therefore, plastics can be divided into fossil-based plastics and biobased plastics that can either be biodegradable or non-biodegradable. Biodegradable polymers derived from petroleum resources are synthetic polymers with hydrolysable backbones, such as ester, amide, and urethane, or could be polymers with carbon backbones, in which additives like antioxidants are added (28). The antioxidants will react when exposed to UV, inducing degradation of the polymer by photo-oxidation.

What influences the biodegradability is various factors, including polymer morphology, structure, chemical treatment, and molecular weight (30). The structure of biodegradable polymers is based on hydrolysable linkages that are exposed to degradation in the presence of microorganisms and hydrolytic enzymes. Not only the type of linkage in the structure influences the biodegradability. If the polymer is both hydrophobic and hydrophilic it is more prone to be degraded compared to polymers being only hydrophobic or hydrophilic (31). The morphology of the polymer also plays a role since the polymers' amorphous regions are more susceptible to enzymatic attack than crystalline regions, as the molecules in amorphous regions are farther apart, making them easier to degrade (30, **Paper 4**). Additionally, the enzymatic degradation of polymers is influenced by their melting temperature (Tm). Biodegradation of polymers decreases as the melting point of polymers increases, and the same can be said for the relation between the molecular weight and biodegradability (30). Increase in molecular weight will reduce the biodegradability.

Poly(butylene adipate-co-terephthalate) (PBAT)

One biodegradable plastic type of large importance in the market of mulch films is the poly(butylene adipate-co-terephthalate) (PBAT), an aliphatic-aromatic statistical co-polyester composed of adipic acid, 1,4-butanediol and terephthalic acid (Figure 5) (32). It is the second most produced biodegradable plastic after poly (lactic acid) (PLA) and approximately 9 % of all biodegradable plastic produced in 2022 (1.13 million tonnes) was PBAT (33). PBAT is marked as a fully biodegradable alternative to other conventional plastics, and consists of adipic acid, 1,4-butanediol, and purified terephthalic acid (34, **Paper 3**). Its global demand is mainly for shopping and garbage plastic bag production, followed by compostable food packaging (35).

The primary reason for the biodegradability of PBAT is the ester bonds, rather than the carbon-carbon chains found in non-biodegradable polymers like polyolefins. Previous studies have been able to prove the biodegradability of PBAT, however, what has been missing is information on who is the active degraders of this plastic (32). Hydrolases, lipases, and esterases has been confirmed able to release adipic acid, terephthalic acid, or butanediol from PBAT (Figure 5) (36) and afterwards metabolised by *Bacillus subtilis* and *B. licheniformis*, with strain-specific differences on their efficiency (37).

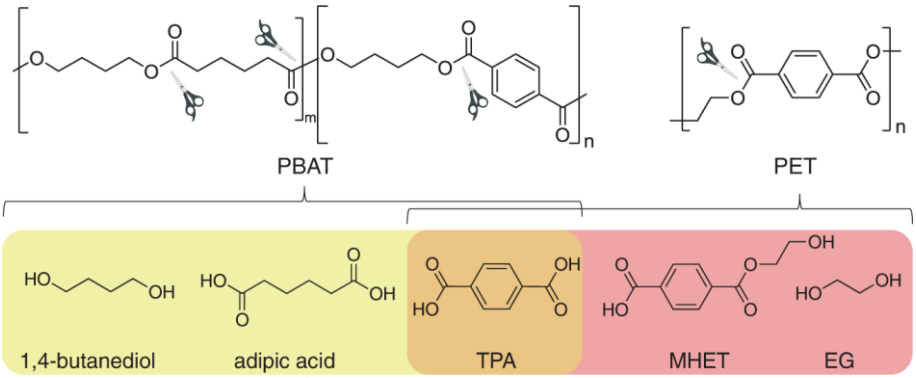

Figure 5: Chemical structure of poly(butylene adipate-co-terephthalate) (PBAT) and polyethylene terephthalate (PET) and the putative degradation products from ester hydrolysis. TPA: Terephthalic acid, MHET: Mono(2-hydroxyethyl) terephthalate, EG: Ethylene glycol (38).

Polyethylene terephthalate (PET)

Polyethylene terephthalate (PET) is a polymer which consist of multiple molecule units covalently bound together between an ethyl group and a single bonded oxygen of the ether-like side chain. The two monomeric units of PET are ethylene glycol and terephthalic acid (Figure 5) (39).

The biodegradation of low-crystalline PET has recently been discovered by an organism called *Ideonella sakaiensis* 201-F6 (40,41). This organism has previously been studied using genomics and transcriptomics, giving an indication of the specific activity of this organism when in presence of PET. PET hydrolase (PETase) and MHET hydrolase (MHETase) belongs to the group of cutinases, and is the two primary enzymes giving *I. sakaiensis* the ability to degrade PET (42, **Paper 4**). PETase breaks down PET into mono(2-hydroxyethyl) terephthalate (MHET), and MHETase further hydrolyses MHET into terephthalic acid and ethylene glycol (Figure 5) (38,42). However, to gain more information of how this organism degrade and metabolise PET a proteogenomic experiment was set up (**Paper 4**).

However, many organisms have the ability to degrade PET, including species of *Bacilllus*, *Apsergillus*, and *Spirulina* (43). Common for all of them is that they possess hydrolase-type enzymes, such as esterases, lipases, and cutinases, which can oxidise the PET ester bonds (43–45). Wild-type organisms capable of degrading PET have

been isolated mostly from soil (22.6 %), followed by aquatic habitats (12.4 %) and landfills (8 %), and the very first report on the topic of PET degradation was published in 2004 (43).

1.2.2. ADDITIVES

In plastic materials used in most products, additives play a distinct role in delivering and/or enhancing the functional properties of the plastic (22). Plasticizers, flame retardants, antioxidants, light and heat stabilizers, pigments, antistatic agents, and thermal stabilizers are some of the mostly used additives in different types of plastic when it comes to quantity and relevance of the environment (Table 2) (46,47).

Table 2: Brief description of commonly used plastic additives and the applied amount (Modified from 47).

Category/Type of additive	***Typical amount range (% w/w)***	***Substances***
Plasticisers	10-70	Short. medium and long chain chlorinated paraffins (SCCP/MCCP/LCCP); Diisoheptylphthalate (DIHP); Benzyl butyl phthalate (BBP); Bis (2-ethylhexyl)phthalate (DEHP); Bis(2-methoxyethyl) phthalate (DMEP); Dibutyl phthalate (DBP); Dipentyl phthalate (DPP); Di-(2-ethylhexyl) adipate (DEHA); Di-octyladipate (DOA); Diethyl phthalates (DEP); Diisobutylphthalate (DiBP); Tris(2 chloroethyl)phosphate (TCEP); Dicyclohexyl phthalate (DCHP); Butyl benzyl phthalate (BBP); Diheptyl adipate (DHA); Heptyl adipate (HAD); Heptyl octyl adipate (HOA).
Flame retardants	3-25	Short, medium, long chain chlorinated paraffins (SCCP/MCCP/LCCP); Boric acid; Brominated flame retardants (e.g., Polybrominated diphenyl ethers (PBDEs) and Tetrabromobisphenol A (TBBPA)); Phosphorous flame retardant (e.g. Tris(2-chloroethyl)phosphate (TCEP)).
Stabilisers, antioxidants, and UV stabilisers	0.05-3	Bisphenol A (BPA); Cadmium and Lead compounds; Nonylphenol compounds; Octylphenol; Butylated hydroxytoluene (BHT); 2- and 3-t-butyl-4 hydroxyanisole (BHA); Bisphenolics; Tris-nonyl-phenyl phosphate (TNPP); Tris(2,4-di-tert-butylphenyl) phosphite.
Pigments	0.001-10	Cobalt(II) diacetate; Cadmium compounds; Chromium compounds; Lead compounds.
Reinforcements	15-30	Glass fibers; Carbon fibers; Aramide fibers.

It is important to emphasize that additives are typically not chemically bound to the plastic polymer, except for reactive organic additives such as certain flame retardants which polymerise with the plastic molecules and become part of the polymer chain

(47). Some additives have a direct impact on the recyclability of the plastic. When talking about brominated flame retarded plastic recycling is also a problem, due to national or international regulations (48).

Flame retardants

Flame retardants is a term used to describe inorganic and/or organic substances which make different materials flame-proof (46). To meet the safety requirements, the use of flame retardants is a necessity in many cases, even though many of the flame retardants commercially used are classified as hazardous compounds.

One of the most widely applied flame retardants is the micropollutant Tetrabromobisphenol A (TBBPA) and is used as an additive to electric and electronic equipment, epoxy resins, and plastic products (PET, PBT, PET/PBT blends, polysulfone, etc.) to meet fire safety requirements (49). This micropollutant has already been detected in air, dust, sediment, biota, and water. Tetrabromobisphenol A (TBBPA) is a brominated flame retardant that is immunotoxic, neurotoxic, and may cause endocrine disruptions in organisms. Furthermore, the development of cancer in the uterus of rats, maternal transmission between fish generations, and genetic mutation in frogs have been associated with TBBPA exposure (50–54). Most studies investigating TBBPA bioconversion have been conducted using high concentrations of the chemical, which are much higher than its environmental occurrence, and have used pure or enriched cultures under laboratory conditions that are not representative of natural environments (55–57). Despite this limitation, these studies have provided valuable insights into the metabolic mechanisms of TBBPA bioconversion.

Upcoming meta-omics investigations on microbial communities that degrade TBBPA could provide new perspectives on its degradation under environmental relevant conditions. The current data suggest that TBBPA degradation is facilitated by varied and cooperative microbial communities using both co-metabolic and metabolic mechanisms that may occur simultaneously (49). Information regarding the biodegradation of TBBPA may lead to a better understanding of the ecological implications of other flame retardants and optimisation of the biotechnologies used to treat the waste, such as plastics, containing this type of micropollutants (49, **Paper 5**).

1.3. A CONCENTRATION AND ACTIVITY INDEPENDENT HIGH-THROUGPUT METHOD

There are two primary categories of techniques employed to assess organisms' metabolic potential: Culture-dependent methods, involving the use of selective media to cultivate microorganisms of interest, aiming to obtain pure strains or well-defined consortia with the ability to decompose the specific substrate, and culture-independent

methods, which employ molecular biology and/or bioinformatics tools to analyse nucleotide or protein sequences (4).

Culture-independent methods generally consist of *in vitro* or *in silico* genetic analyses of gene sequences coding for 16S rRNA, or frequently encountered enzymes responsible for the process of interest (4). These methods avoid the bias caused by culture conditions and non-cultivable strains.

The genomic makeup of a microbial community not only determines its identity but also reflects its functional potential. However, it does not directly reveal what the community is actively doing at a particular time or under specific conditions (58). To fully understand what is determining the specific function of a specific organisms in the specific environment, additional multi-omics data types such as transcriptomics, proteomics, and metabolomics are needed.

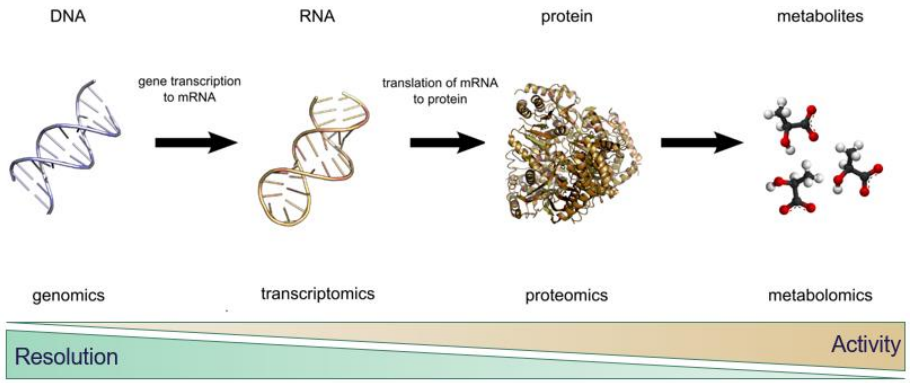

Figure 6: The "omics cascade". Biomolecules from DNA to metabolite holds information shifting from high resolution regarding the genotyping (identity) to a high level of information regarding the activity (phenotyping) (Modified from 58,59).

Different omics techniques target different biomolecules (Figure 6), therefore having different outputs, including different advantages and disadvantages (Table 3). Metagenomics and metatranscriptomics gives an indication of potential function, with the limitation that not all genes is being transcribed, and transcription of a gene does not necessarily lead to the synthesis of a protein (60). Quantification at protein level, using metaproteomics, can be used as the link between genomic identity of the microbial community and their specific activity, since proteins to some extent can be mapped to the original RNA sequence template. By metabolomics all metabolites from the microbial community are being analysed and identified. The identification and quantification of metabolites can give an understanding of all biological processes active in the system (59).

The advancement of sequencing technology is expected to further aid in the microbial profiling and understanding of metabolic pathways. Meanwhile, culture-dependent methods will provide an opportunity to experimentally confirm hypotheses generated

from omics research (61). Therefore, to enable a more comprehensive understanding of bacterial communities a complementary use of dependent and independent methods should be applied.

Table 3: Overview of advantages and disadvantages of different omics techniques. MS: Mass spectrometer, NMR: Nuclear magnetic resonance (Modified from 62).

Technology	*Advantages*	*Disadvantages/limitations*
Genomics	Central dogma of cellular function begins at the 'gene' level. All the information is present (introns, exons, promoter, intergenic, transcription start site, non-coding, etc.) Relatively easy lab handlings	Due to epigenetics, post-transcriptional and posttranslational changes, the final biological effect of your DNA is difficult to predict by performing only genome analyses
Transcriptomics	Analysis of the complete transcriptome. Relatively easy lab handlings.	Posttranslational modifications can influence the protein expression; therefore, the transcriptome should be regarded as an intermediate step.
Proteomics	Proteins are the major actors in cellular reactions. Proteomics allows the detection of changes in unexpected and unknown proteins.	Study of proteins is difficult due to the posttranslational modifications. Some proteins are difficult to separate.
Metabolomics	Metabolomics may reflect changes in cellular processes. MS fractioning allows for a more sensitive detection of metabolites than NMR. NMR is not destructive.	Relatively low numbers of metabolites (a few thousand), as can be measured. NMR is less sensitive than MS.

1.3.1. HOW CAN LABELLED SUBSTRATES ENABLE THE OPTIMIZATION OF BIOTECHNOLOGIES FOR RESOURCE RECOVERY AND MICROPOLLUTANT DEGRADATION?

A proteogenomics approach would be able to make the link between the genomic identity and the specific activity, and within this the protein-stable isotope probing (protein-SIP) is one of the promising approaches for the purpose.

Protein-SIP is based on the metabolic incorporation of heavy stable isotopes (usually ^{13}C or ^{15}N) into proteins and is a powerful tool for both qualitatively and quantitatively proteome studies (63). The assessment of the incorporation has proven to give valuable information regarding metabolic activity and protein turnover rates (63,64). Moreover, the approach, when used in time course experiments, also enables to follow

the carbon/nitrogen flux in the microbial consortia in order to identify food webs (**Paper 2, Paper 3**). Stable isotope labelled substrates have gained interest since the mass shift allows quantification of incorporation of the labelled substrate, giving an indirect measurement of the metabolic activity (63).

When comparing protein-SIP to other SIP approaches three advantages of protein-SIP are noteworthy (63,65). One being that the required incorporation of heavy isotope is minimal, only an additional 0.1 % ^{13}C is required, compared to DNA-SIP or RNA-SIP where a minimum of 50 or 10 % ^{13}C are required, respectively. Secondly, due to the two orders of magnitude, a dynamic range for quantification of isotope composition is possible. Finally, as mentioned before, by combining metagenomics with metaproteomics not only a high resolution of species and function is obtained, but it also enables the tracking of nutrient flux within the community.

The nutrient flux can be determined by looking into the shape of isotope pattern (Figure 7), in order to identify whether the incorporation of ^{13}C is due to a direct metabolism of the labelled substrate or if it is due to cross-feeding, where the incorporation of ^{13}C is by usage of labelled intermediates from the degradation process (65).

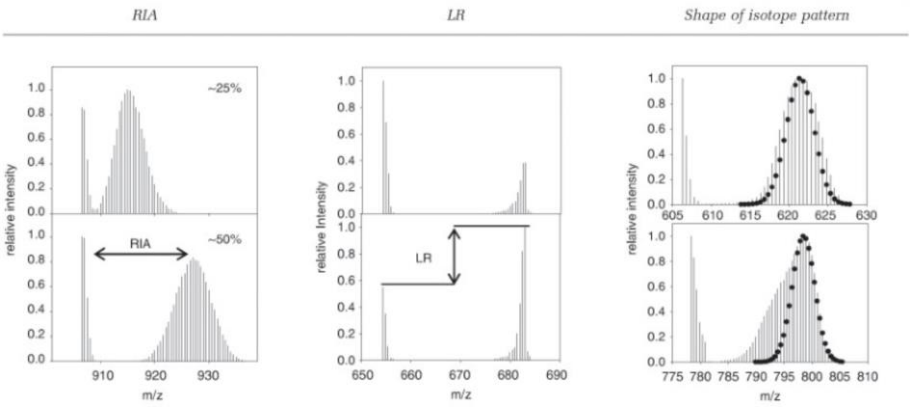

Figure 7: Features from the protein-SIP approach gives information regarding the relative isotopic abundance (RIA), labelling ratio (LR), and the shape of isotope pattern. The shape of the isotope pattern indicates whether a direct metabolization or cross-feeding has occurred. For direct metabolization the pattern will closely resemble a normal distribution, and cross-feeding will result in a tailed distribution due to the usage of labelled intermediates from degradation processes (65).

When analysing the peptides from the protein-SIP studies two other features is of great importance, the relative isotope abundance (RIA) and the labelling ratio (LR) (Figure 7). RIA gives information regarding the number of labelled atoms in a peptide, whereas the second feature, LR, gives the ratio of labelled to natural peptide, and in time series can be used to determine the protein turnover rates after addition of the labelled substrate (63, **Paper 1**, **Paper 2**, **Paper 3**, **Paper 5**).

One of the critical parts when setting up/running the protein-SIP experiment, is the selection of appropriate time points for sampling (64). As a rule of thumb sampling closely after the exposure with the labelled substrate is recommended since long exposures can lead to incorporation of labelled substrate by a cross-feeding mechanism (64,65).

Analysing metaproteomic data presents a range of computational obstacles, such as managing large databases, selecting the best search parameters, and utilizing suitable search algorithms (64). Additionally, to achieve a satisfactory level of quality and quantity in search hits, the results must undergo reliable FDR filtering. When studying environmental samples, the choice of database is another critical part of the protein-SIP workflow. Metagenome sequencing gives the possibility to mirror the sample and thereby making a database specific for the sample or a two-step database search method wherein matches derived from a primary search against a large database were used to create a smaller subset database (64,66,67). A target-decoy database, of the subset database, merged with a host specific database is used for the second search (66).

In general, proteomic techniques for analysing environmental samples face three main limitations (64). The first is the challenge of extracting metaproteomes, which is complicated by the presence of significant amounts of organic and occasionally inorganic background material in the samples, as well as limited biomass availability. The second limitation is the high complexity of the consortia being studied, and the third is the limited genetic coverage in databases. Nevertheless, protein-SIP has proven effective to increase coverage by identifying active members of a community in relation to a specific substrate (64, **Paper 1**, **Paper 2**, **Paper 3**, **Paper 5**).

1.3.2. ANALYSIS OF DIFFERENTIAL PROTEIN EXPRESSION

Once an organism has been isolated or a culture has been highly enriched the analysis of differential protein expression can be used as a powerful tool for studying the physiological state between two groups, with high level of resolution (68,69, Paper 4). Expression proteomics involves determining the absolute or relative quantities of proteins in a mixture. However, expression proteomics has a distinct advantage as it measures the mature protein, meaning the end product of the gene expression cascade, which is more closely linked to biological function than message levels (70). Another advantage is that expression proteomics can determine the levels of gene products in subcellular compartments and organelles, unlike transcriptomics.

Within the analysis of differential protein expression, one could say that both a classic and a new approach exist. The classic process being the use of two-dimensional gel electrophoresis, and the new approach is the LC-MS based techniques (shotgun proteomics) (69). If intact proteins and study of post translational mutation variants is of interest the two-dimensional gel electrophoresis method is preferred. However,

using this method will only give identification by MS/MS on the significantly regulated proteins (68), and even with presence of more than thousands of spots, only a few hundred proteins of high abundance can be determined (70). Furthermore, the methods suitability for highly hydrophobic or very large proteins is limited (68).

On the other hand, using shotgun proteomics gives identification and quantification of all detectable proteins in the samples and the method is less biased regarding chemicophysical protein properties (69,70). Comparing the knowledge provided by other high-throughput methods, the proteomic based strategy gives more complex and comprehensive knowledge on the studied situation (71).

CONCLUSION AND PERPSECTIVES

Over the course of the PhD project, detailed studies of the degradation of different polymers and additives were carried out. In Paper 1 and 2, the degradation of lignocellulosic biomass was investigated in AD systems treating maize silage and wheat straw. A step further on the optimization of biotechnologies for biofuels production has been achieved by providing information on the identity and function of organisms, from a long-term adapted microbiome only fed with wheat straw, directly degrading the cellulose, namely *Defluviitoga tunisiensis*, *Syntrophothermus lipocalidus*, and *Pelobacter carbinolicus* (**Paper 2**). These studies are some of the first to give a direct link between an identity and activity (**Paper 1 and 2**). Several studies have identified microbial communities that could potentially play a key role in the degradation of lignocellulosic biomass and cellulose, however, most of these studies are based on correlation between degradation rates of lignocellulosic straw and/or cellulose with changes in specific genes or enzymes involved in cellulose degradation (17,19).

In Paper 3 and 4, the biodegradation of plastic, PBAT and PET, were studied. Three genera (*Bacteroides*, *Ichthyobacterium*, and *Methanosarcina*) were identified as direct consumers of PBAT monomers (**Paper 3**). A recovered HQ-MAG was taxonomically classified as *Anaerolinea thermophila* and by looking into the genomic material of this HQ-MAG, the enzymes putatively responsible for degrading part of the PBAT were revealed. Paper 3 offers a more comprehensive understanding of the biodegradation of PBAT monomers in anaerobic digestion. This knowledge is pertinent in developing innovative technological solutions for a more efficient and intensive treatment of its residues.

By the use of proteomics new and deeper insight into the physiological changes during degradation of PET was revealed (**Paper 4**). Knowledge to the pathways used by *I. sakaiensis* during degradation of PET, and more specifically, which proteins/enzymes from *I. sakaiensis* are responsible for the complete mineralisation/degradation, can be used to stop the degradation at the monomer level and use these for resynthesizing new polymers (**Paper 4**). Blocking the expression of specific enzymes makes it possible to favour specific monomers of interest.

In Paper 5 the biodegradation of the flame retardant Tetrabromobisphenol a (TBBPA), often used as an additive in PET, was studied. This is the first study to combine protein-SIP and metagenomic data to identify and elucidate functional prediction of microorganisms actively involved in the cometabolic degradation of a micropollutant at concentrations close to what has been detected in the environment (**Paper 5**).

The results reported/discussed in Paper 1, 2, 3, and 5 represent the wide applicability of combining omics techniques as a screening methodology to link identity with specific activity of the community, as the same approach was applied in different environments and setups.

This work was motivated by the need of extending knowledge on how polymers and micropollutants are biodegraded in different settings (anaerobic and aerobic environments), including how to exploit different waste streams to either upcycle the waste or to recover energy from the waste. Insufficient knowledge of the microbial communities that participate in anaerobic digestion is a significant obstacle to improve the efficiency of AD technology and operational management both regarding pollutant degradation and resource recovery from recalcitrant substrates. State-of-the-art proteogenomics approaches have shown to provide the link between identity and function, both for single culture organisms and for mixed communities in a high-throughput manner. The protein-SIP approach has been proven to be highly applicable for active systems, recalcitrant substrates, and for pollutants in low concentrations (environmentally relevant concentrations).

The current PhD therefore clearly demonstrates that proteogenomics tool holds the capacity to link function with identity in complex ecosystems in a high-throughput manner.

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PAPER 1. CHARACTERISATION OF CELLULOSE-DEGRADING ORGANISMS IN AN ANAEROBIC DIGESTER

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PAPER 2. ENERGETICALLY EXPLOITING LIGNOCELLULOSE-RICH RESIDUES IN ANAEROBIC DIGESTION TECHNOLOGIES: FROM BIOREACTORS TO PROTEOGENOMICS

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PAPER 3. ASSESSING LABELLED CARBON ASSIMILATION FROM PBAT MONOMERS DURING THERMOPHILIC ANAEROBIC DIGESTION

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PAPER 4. PROTEOMIC CHARACTERISATION OF POLYETHYLENE TEREPHTHALATE AND MONOMERS DEGRADATION BY *IDEONELLA SAKAIENSIS*

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PAPER 5. PROTEOGENOMICS IDENTIFICATION OF TBBPA DEGRADERS IN ANAEROBIC BIOREACTOR

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