Biowell Taken out after two years,2021

THE FATE OF BIODEGRADABLE GROUNDWATER MONITORING WELL

Master's thesis GEO4-6004 (30 ETC)

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Abstract

Accumulation of plastics worldwide has already aroused public concern due to its persistence in the natural environment, threatening environmental and human health in the coming decades. The utilisation of groundwater monitoring wells made of conventional plastic in groundwater and soil investigation projects aggravates plastic pollution in the subsurface. The development of biodegradable groundwater monitoring wells ('Biowell') provides an alternative. However, the biodegradation performance of a biowell placed in the soil has not been investigated in-depth and has led to the central research question: ***"What is the fate of the biodegradable monitoring well?"***

In the first step, external interviews with the producer, converter and researcher were held to provide background information. On the other hand, internal surveys were performed to gain deeper insight into the optimal conditions to utilise biowell. The results showed that the biowell is the best solution for short-term monitoring projects when sustainability is a critical request from clients and the government. However, deficiency of legislation and certification pose barriers to promote biowell on a large scale.

Besides, biodegradation performances of biowell in natural field conditions have not been investigated previously due to the deficiency of realistic cases. Two years ago, one biowell was installed underground in Utrecht. This used biowell is further chemically and physically analysed by visual inspection and Py GC-MS test. The results illustrated that biowell experienced slow-speed biodegradation under the natural field unsaturated condition.

Studies concentrating on the ultimate biodegradation under laboratory conditions are still scarce, which requires further investigation. Soil burial tests were designed under twelve different laboratory settings to investigate biodegradation performances. Results demonstrated that the biodegradation rate has a positive relationship with temperature while no obvious effects were noticed from soil humidity and bioaugmentation. The first-order kinetic model was further used to estimate the final biodegradation time, which anticipates that it would take approximately 3000 days and 3500 days under 20 ℃ and 40 ℃, respectively.

Briefly summarised, the biowell proves to be a more sustainable option in short-term soil & groundwater investigations providing appropriate implementation. Regarding the fate of biowell, it indicates that approximately 10 years is required to approach ultimate biodegradation in the natural soil environment.

Acknowledgements

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Furthermore, I would like to thank Marian Langevoort for finding me and providing me with this opportunity, during which I achieved a lot. Moreover, much gratitude to Carlo Bensaïah and ir. Tobias Praamstra from TAUW Netherland for all the patient guidance, encouragement and support during the research process. They always supplied me with detailed suggestions and constructive feedbacks to all questions throughout the entire period, no matter how big or trivial. They also encouraged me a lot to push me forward. Because of the corona, I didn't have much time to work with them in the office together, but the past five months are still one of the most unforgettable memories of my life in a foreign land. Also, I would like to thank Peter Lodder for his hospitability and support in helping me design experimental plans to conduct the whole tests step by step, leaving me with memories with warmth in Deventer.

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I hope you will enjoy reading the thesis.

Shiyue Yang
Utrecht, July 2021

Table of Content

List of Figures

Figures ***Pages***

List of Tables

Tables ***Pages***

List of Tables in Appendix

Tables ***Pages***

List of Equations

Equations ***Pages***

Abbreviations and Acronyms

AF	Acceleration Factor
EC	Electrical Conductivity
GHG	Green House Gas
NREU	Non-Renewable Energy Use
PHA	Polyhydroxyalkanoates
PHEE	Poly (hydroxyester-ether)
PLA	Polylactic Acid
PVC	Polyvinyl chloride
TPS	thermoplastic starch
WA	Water Adsorption
WHC	Water Holding Capacity
QPCR	Quantitative Polymerase Chain Reaction

Chapter 1. Introduction

1.1 Development of Biodegradable Plastics

Most conventional plastics are made from petroleum, which become exceptionally long molecules under heat and catalysts during the manufacturing process. Since those synthetic polymers cannot be found in nature, no organisms are able to decompose those plastics, leading to their persistence in the natural environment (Folino et al., 2020; Mooney, 2009). Despite improvements in technologies to recycle plastic wastes, the increasing population, which is anticipated to reach nine billion in 2050, will lead to a higher requirement for plastic products, eventually causing a massive amount of plastic waste to persist in the natural environment (Hughes, 2013). A large amount of plastic solid wastes will pose threats to human health and environmental pollution, such as groundwater contamination and sanitation issues (Al-Salem et al., 2017; Shafqat et al., 2020). Therefore, innovative and more environmentally-friendly plastic solutions have been explored worldwide (Bilo et al., 2018).

Biodegradable plastic has been recognized as an innovative alternative for conventional petroleum-based plastics owing to biodegradability. These biodegradable products are expected to eventually be mineralized into carbon dioxide, methane, water, inorganic compounds, or biomass, leaving no toxic residuals in the environment (Folino et al., 2020; Mooney, 2009; Standard, 2000). The production of starch-based plastics with biodegradability attracted much attention, considering the low price and availability of potato starch from the natural environment (Shogren et al., 1993). Potato starch-based plastics extracted from potato processing wastewater stepped into public attention approximately twenty years ago. As it is a waste-derived product, it will not compete for food production or land use. The replacement of conventional petroleum-based plastics with potato starch-based ones is also appealing from conserving limited petrochemical resources.

1.2 Biodegradable Groundwater Monitoring Wells in Soil Investigation

Biodegradable plastics have been utilised in different fields ranging from packing materials (e.g., trash bags, loose-fill foam, food containers, film wrapping, laminated paper), hygiene products (e.g., diaper back sheets and cotton swabs), consumer goods (fast-food tableware and containers) to other agricultural products such as mulch films and planters (Gadhave et al., 2018).

In soil investigation projects, monitoring wells are used to measure groundwater levels and water qualities. Short-term soil investigation projects merely require the groundwater monitoring well used for a short term (< 3 months; BRL K567). After successful soil investigation, monitoring wells are usually left in the soil, leading to plastics' long-term persistence in the natural environment. The groundwater monitoring well made of starch-based plastics might be a promising alternative for short-term soil investigation projects due to their biodegradability (Broeren et al., 2017; Folino et al., 2020).

1.3 Previous Research: Biowell Installed Two Years Ago

Due to the limited application of biowell, there lacks reliability test of biowell in reality. Two wells (one biowell and one PVC groundwater monitoring well) were set in Utrecht one meter away from each other (***Figure 1***) on 16th March 2019 by TAUW. Groundwater was taken from these two monitoring wells for field measurement and chemical analysis to compare water quality differences statistically.

a "Biobuis" b PVC groundwater monitoring well

Figure 1. Previous Project in Utrecht
((a) is the "Biobuis" and (b) is the regular groundwater monitoring well)

1.4 Knowledge Gaps

The promotion of biodegradable plastics still arouses concerns and controversy regarding their reliability, high cost and low mechanical strength (Emadian et al., 2017). Secondly, it is blended with other compatibilizers and additives to improve compatibility and mechanical properties, which may not necessarily biodegrade in the natural environment (Bátori et al., 2018).

Certification is merely valid under one specific given laboratory environment. Research concentrating on the biodegradation behaviour of starch-based plastics in the soil under different situations is scarce, which requires further investigation. Although the ultimate biodegradation time under a single circumstance can be achieved, complex natural environments may add uncertainty in the actual degradation behaviour under different environmental conditions, requiring further research and investigation towards this direction (Kjeldsen et al. 2019).

1.5 Research Questions and Aims

1.5.1 Research Questions and Objectives

Central Research Question:

"What is the fate of the biodegradable groundwater monitoring well in the soil?"

Sub Research Questions:

1. Which types of groundwater & soil investigation projects are most applicable to use biowell based on its characteristics and properties?
2. What are the changes occurring on the biowell, that are installed in situ for two years?
3. What is the rate and the extent of biodegradation of biowell in three months during lab conditions?

4. Can the biodegradation of the well be stimulated by temperature, soil humidity, bioaugmentation and larger surface areas?

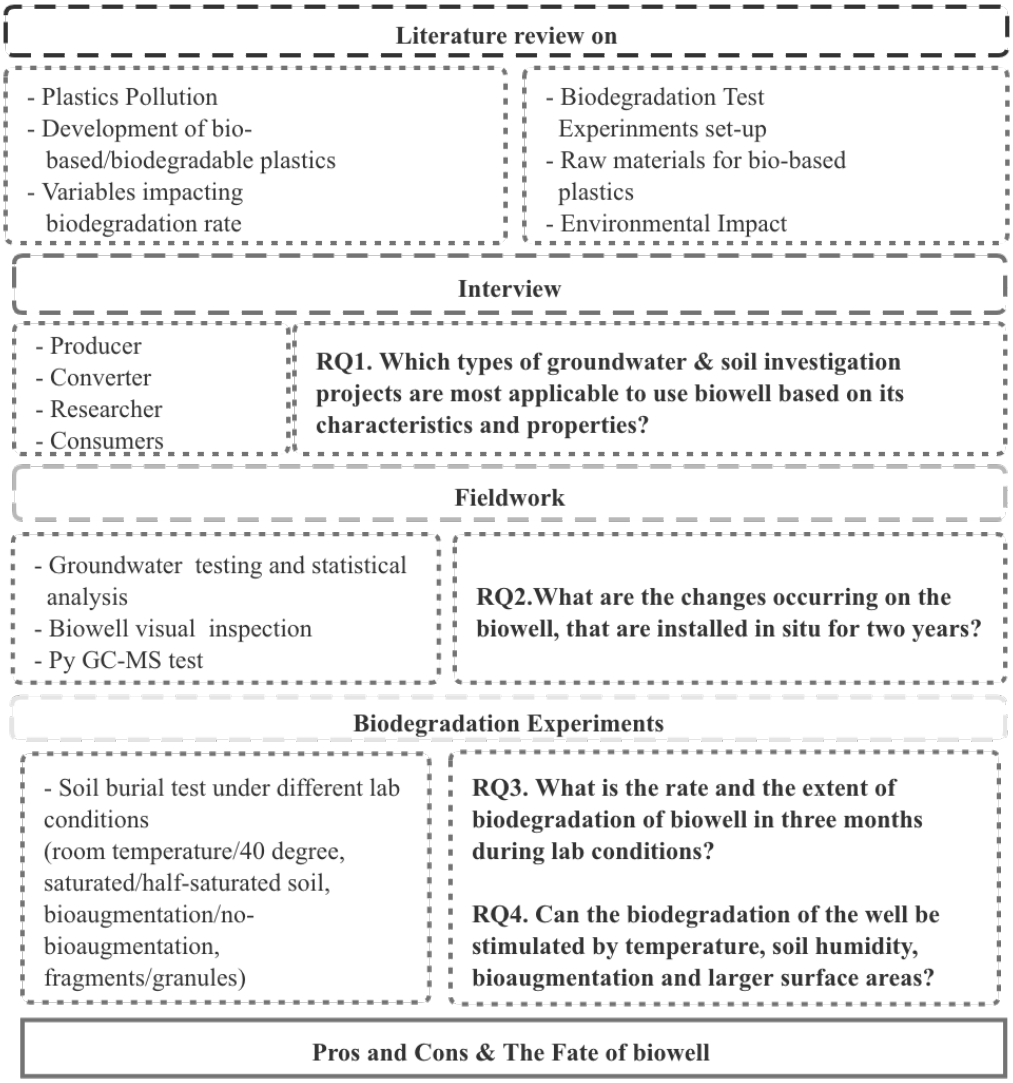

Figure 2. Research Framework

The research framework (***Figure 2***) demonstrates the procedures taken to answer research questions. The main research question was divided into four sub-research questions. The first phase consists of literature research and interviews regarding the actual compositions and people's attitudes towards promoting biodegradable monitoring well, aiming to determine the best conditions to use the biowell. Subsequently, the fieldwork phase was intended to examine the changes occurring on the biowell after two years' burial in the soil. Furthermore, the experiments played a vital role in understanding the actual biodegradation performances under different experimental conditions.

1.6 Outline of the Thesis

The thesis is structured as follows. Firstly, the introduction, objectives, and research questions are presented in **Chapter 1**. **Chapter 2** demonstrates the mechanisms of

biodegradation in the soil. **Chapter 3** presents a more specific description of the materials and methodologies used and research procedures to answer the research questions step by step. Results are demonstrated in **Chapter 4**, consisting of stakeholder analysis, groundwater monitoring results comparison, visual inspection of biowell, and biodegradation experiments outcomes. Subsequently, the discussion (**4.4.5**) and conclusion (**Chapter 6**) constitute the thesis's final parts. **4.4.5** mainly focuses on the results analysis and further explanation. **Chapter 6** is intended to provide the answers to the central research questions and the limitations and recommendations of this research.

Chapter 2. Theory: Aerobic Biodegradation Mechanisms

2.1 Biodegradation Process

Biodegradation is the terminology used in ecology. It is defined as the process in which microorganisms utilise biodegradable plastics as carbon and energy sources and produce inorganic substances (i.e., water and CO2) which is demonstrated in ***Figure 3***. (Bátori et al., 2018; Polman et al., 2021).

In the first step, the biodegradable materials are fragmented into small fractions under the combined function of microorganisms and abiotic factors (chemical and physical effects of humidity, temperature and sunlight) (Lucas et al., 2008). The first stage of biodegradation is called biodeterioration (Walsh, 2001).

The second step is called depolymerization. The solid and hydrophobic polymers are cut into dimers and monomers under the action of extracellular enzymes secreted by microorganisms in the soil (Bátori et al., 2018; Lucas et al., 2008).

The third step is called assimilation, in which transported molecules integrate the microbial metabolism. Energy, new biomass, storage vesicles and a quantity of primary and secondary metabolites are produced (Folino et al., 2020; Lucas et al., 2008). Subsequently, some metabolites (e.g., organic acids, aldehydes, terpenes, antibiotics, Etc.) might be excreted to the extracellular surroundings in the mineralisation stage. Other simple molecules as CO_2, H_2O and various salts produced from intracellular metabolites are released into the environment.

Research has been performed to investigate the biodegradability of single or combination of several types of pure bio-based/biodegradable plastics in natural settings, such as soil, compost, marine, and other aquatic environments (Folino et al., 2020). Soil and compost are mainly used due to a diversity of microorganisms inside, enabling higher biodegradation rates.

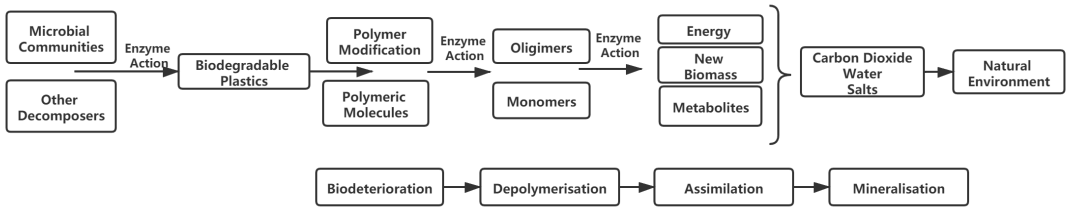

Figure 3. Biodegradation Process

Source: (Lucas et al., 2008)

2.2 Environmental Controls

Both internal and external aspects will influence biodegradation in the soil. With regards to the external environment, biodegradation depends on the selected soil types where it takes place. Differences in pH, soil water content, and microbial diversity also affect biodegradation rates. A soil with a humidity of 40%-60% proves optimal for aerobic biological processes (Folino et al., 2020). Considering internal aspects, the biodegradability of biodegradable plastics highly relies on chemical and physical properties (Emadian et al., 2017b). These properties include the surface feathers (hydrophobic or hydrophilic and surface area), the first-order structures (molecular

weight, molecular weight distribution, and chemical structures), and the higher-order structures (crystallinity, crystal structure, modulus of elasticity, glass transition temperature, and melting temperature) of polymers contained in the biodegradable plastics (Tokiwa et al., 2009).

Chapter 3. Research Methodologies

3.1 Materials

3.1.1 Biodegradable Groundwater Monitoring Well: "Biobuis"

The commercial biowell (***Figure 5***) used in this research from the brand "Biobuis" are certified as "OK Compost" and "OK biobased" by TÜV AUSTRIA Belgium, formerly known as Vinçotte (Šerá et al., 2020a). The development of ''Biobuis" is designed for projects which merely need (or is foreseen) to be sampled for a short period (<3 months; BRL K567). As expected, the biowell will break down under the effect of natural environments (i.e., temperature, water and microorganisms).

Solanyl C2201 from Rodenburg Biopolymers was used as the base material for the production of "Biobuis", which has a bio-based content of more than 67%. However, the concrete compositions could not be disclosed as a commercial secret. The potato starch used to manufacture Solanyl C2201 originated from the reclaimed potato processing industry (Yatigala et al., 2018). Several mechanical properties could be found on the website of Rodenburg Biopolymers, as shown in ***Figure 4*** & ***Table 1***. Solanyl C2201 appears to be a relatively stiff material with rather high brittleness (Zhou et al., 2016). Solanyl C2201 has a higher tensile yet a relatively lower strain at break strength than most C-grade Solanyl.

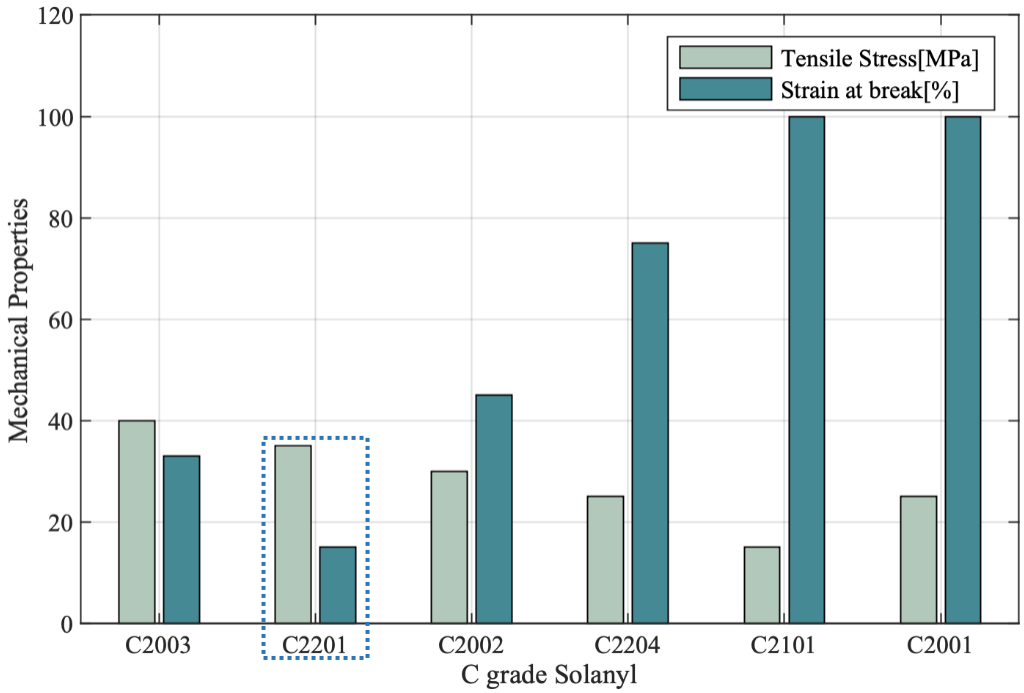

Figure 4. Mechanical Properties of C grade biopolymers
(***Sources:*** RODENBURG Biopolymer)

Figure 5. Biobuis

Table 1. Mechanical Properties of Solanyl C2201
(***Sources:*** RODENBURG Biopolymer)

Melting Temperature (℃)	Tensile Strength (MPa)	Strain at break (%)
140-150	37.5	15

3.2 Methods and Data Collection

The following sections discuss the research and data collection methods according to the guidance of the research framework
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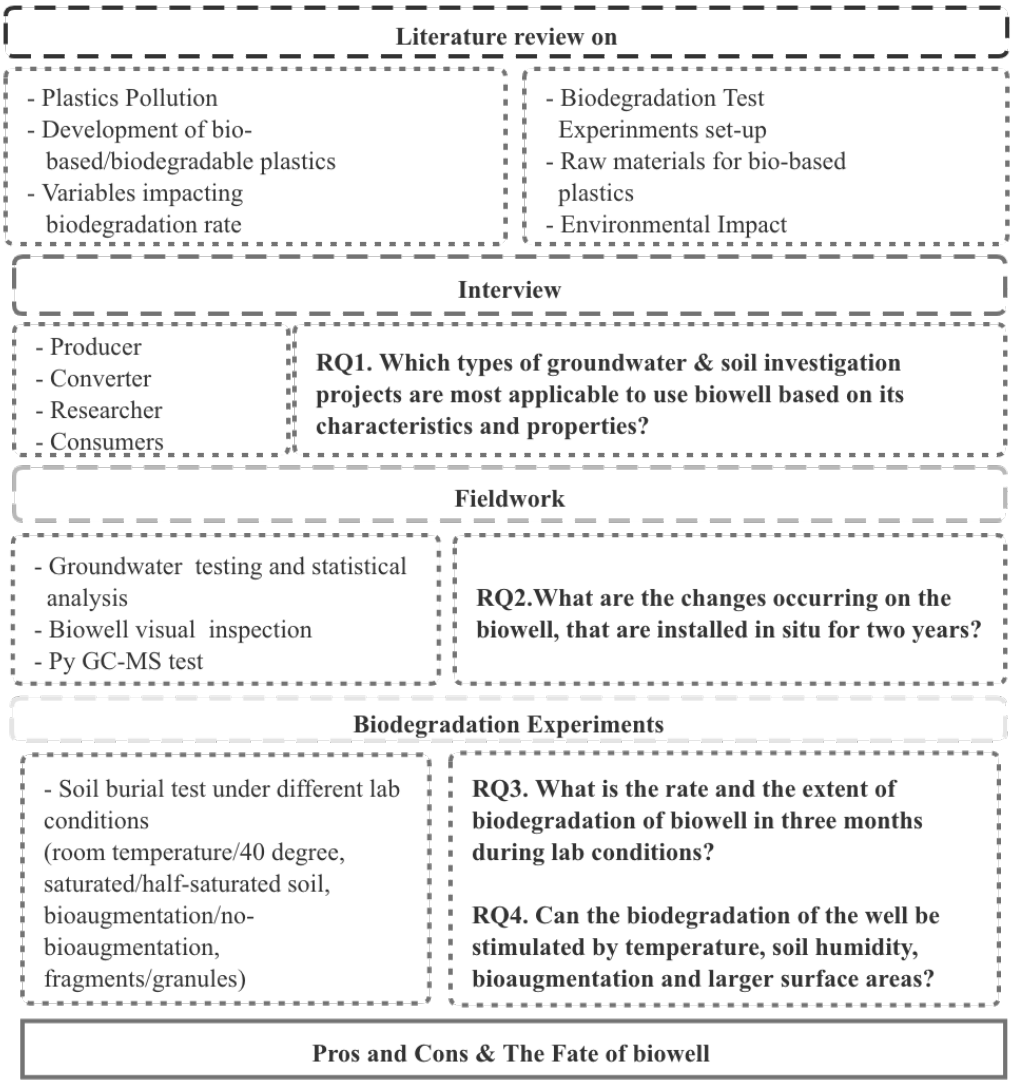


***Figure** 2*).

3.2.1 Literature Research

Literature research was done at the start of this project. The concepts and mechanisms of biodegradation of biodegradable plastics were identified from the literature review to illustrate basic concepts of biodegradable plastics. The literature was collected from the online catalogue. The main keywords used in this research were *"bioplastics," "biodegradation," "biodegradation in soil conditions".* Other literature was studied to determine the methodologies used in this project. Therefore, the search for information was through Scopus, Google scholar, and the online catalogue. To further narrow down the range of the search results, the function' most cited' and 'most recent' were used to ensure the literature review's quality. Apart from that, related files and reports from previous work were used through the internship position.

3.2.2 Interviews with Stakeholders

3.2.2.1 External Semi-Structured Interviews

The semi-structured interviews were conducted to gain a deep insight into the raw materials and production process (e.g., water and energy assumption) of the biowell with the producer and the converter. The converter refers to the company who manufactures the raw materials into final products (Broeren et al., 2017). Another interview was conducted with the research from Utrecht University whose team collaborated with the producer (Rodenburg Biopolymer) to do a cradle-to-factory gate Life Cycle Assessment for general grades of starch-based plastics (Broeren et al., 2017). External interviews aimed to reveal the environmental impact of the biowell.

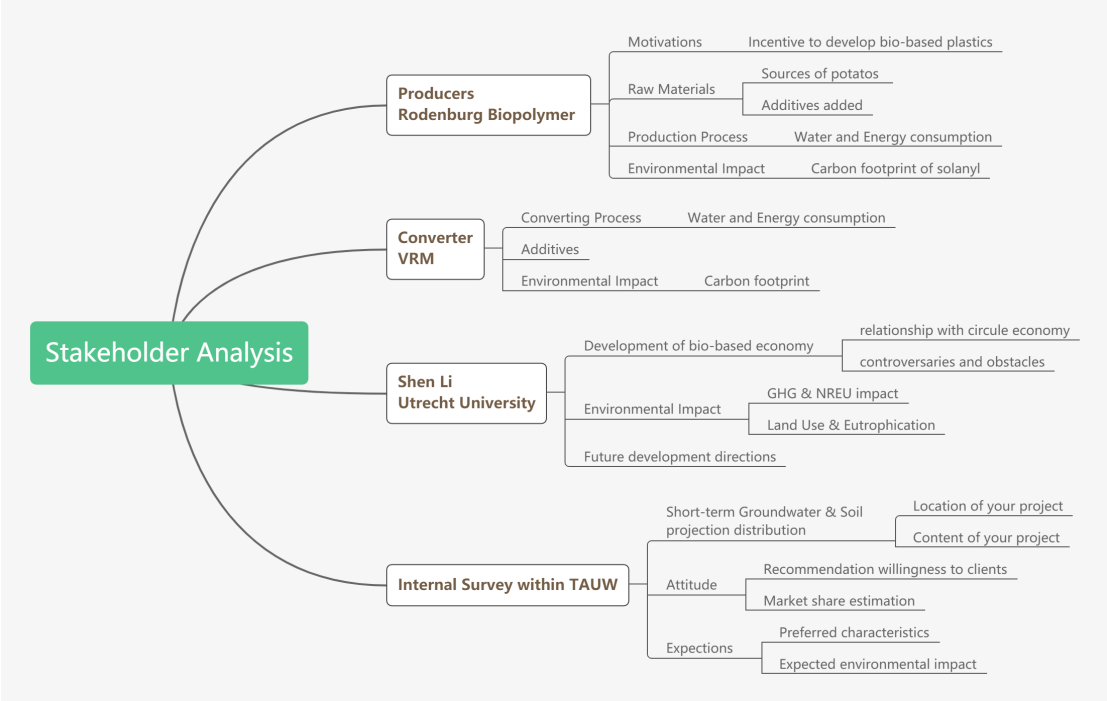


Figure 6. Approach for Stakeholder Analysis

These interviews began with a brief introduction regarding this research, and the objective of the interview was described. Apart from that, permission for the audio-recording of these interviews was asked from those interviewees. Interviewees were kept anonymous and with their numbers. In the results chapters, these numbers are

utilised to represent the corresponding expert.

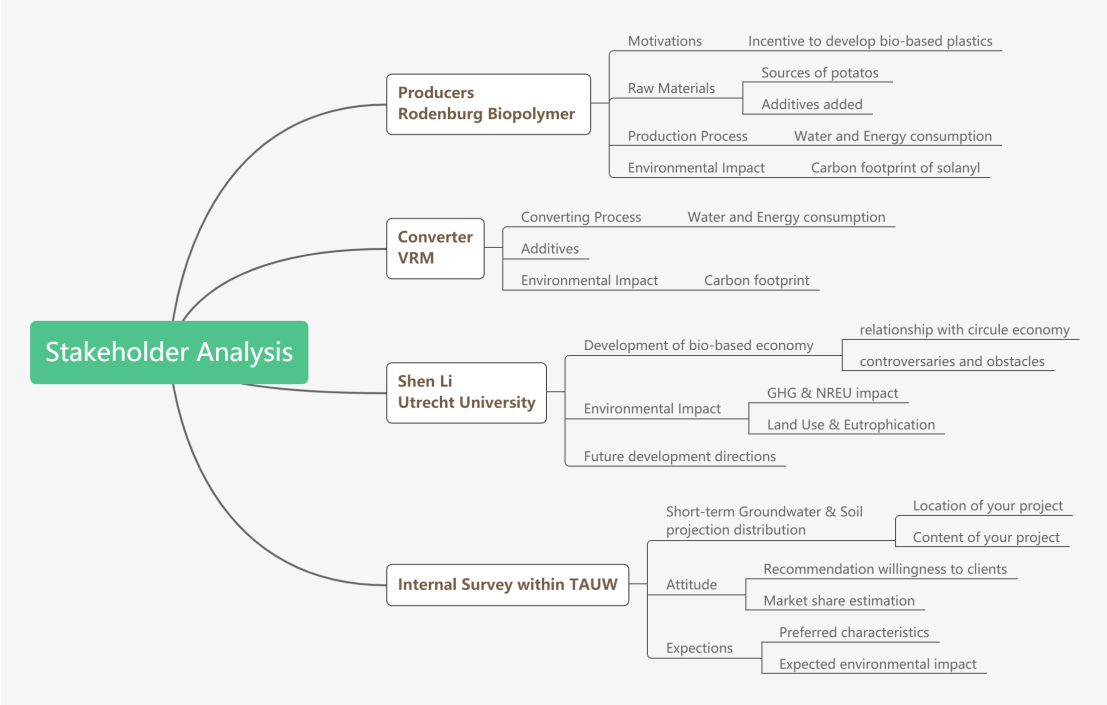


Figure 6 demonstrates the interview framework (see ***Appendix I*** for the interview guide)

Table 2. Overview of the Interviewees

Name	Organization (s)	Expertise	Function(s)	Date
A 1	Rodenburg Biopolymers	Biotechnology/Bio-based plastics	Producer	2021.05.17
A 2	Van Reekum Materials BV	Production of plastic pipes and screens	Converter	2021.03.09
A 3	Van Reekum Materials BV	Production of plastic pipes and screens	Converter	2021.03.09
A 4	Utrecht University	bio-based economy	Researcher	2021.03.22

3.2.2.2 Internal Questionnaire Survey: Attitudes towards Application of Biowell

The objective of internal surveys was to determine experts' attitudes in various fields within TAUW towards applying biowell into functional projects, thus determining the optimal conditions where the biowell could be employed in the future. At the beginning of the questionnaire, the background information of the biowell (e.g., raw materials, mechanical properties and price) was introduced. To fulfil the objective of this part, the following main questions were set out in the form of a questionnaire within TAUW and respondents were listed in ***Appendix II*** (McColl et al., 1998).

- Different types of short-term groundwater and soil investigation projects
- Utilization of biowell in practical projects
- Willingness to recommend biowell to clients and reasons
- Market share estimation of biowell in the future

Answers to each question were entered into a spreadsheet and grouped into different categories to present results for each question.

3.2.3 Field Investigations

Two years ago, the biowell and the PVC groundwater monitoring well were installed at *X: 135,639 Y: 453,058* on Avenue of Australia 5, Utrecht, one meter away from each other. To adequately evaluate the potential impact of the biodegradation process on the groundwater qualities, groundwater samples were collected from these two wells at the one-month interval. The groundwater samples were taken out on 15th March 2019, 19th April 2019, 25th April, 17th May 2019, 14th June 2019, 12th July, 16th August, 13th September, 13th December 2019, 10th March 2020 and 23rd March 2021, constituting the database of groundwater qualities for the entire year.

A suite of physicochemical parameters of groundwater samples taken from both wells were measured according to NEN 5740 (Groundwater Depth, pH, electrical conductivity (EC), Turbidity, Heavy metals (Ba, Cd, Co, Cu, Hg, Pb, Mo, Ni, Zn), aromatic hydrocarbons (Benzene, Toluene, Ethylbenzene, Xylene and Naphthalene), Volatile Hydrocarbons and Total Petroleum Hydrocarbon) (Charles et al., 2020). A two-tailed student *t*-test was employed to verify whether significant differences exist between measuring results from two different wells with Microsoft Excel (Charles, 2020; Ezenwaji, 2019). The entire groundwater monitoring results were placed in ***Appendix III.***

Secondly, the biowell and the PVC regular monitoring well were retrieved after two years' soil burial, and A visual inspection was conducted to characterise biodegradation performances based on all degradation indicators listed in ***Table 5*** (Kjeldsen et al., 2019; Ruggero et al., 2019).

3.2.4 Pyrolysis Gas Chromatography-Mass spectrometry (Py-GC-MS)

Py-GC/MS was performed to retrieve information about the polymer matrix of biowell (Westphal et al., 2001). It is an advantageous method to analyse the microstructures of natural and synthetic macromolecular substances (Llana-Ruíz-Cabello et al., 2016; Westphal et al., 2001). This test was facilitated at Utrecht University to distinguish changes of chemical compositions of the biowell after burial under natural field conditions for two years (Westphal et al., 2001).

Pyrolysis was carried out in Helium carrier gas on a Horizon Instruments Curie-Point pyrolysis device. Samples (typically 1–2 mg) were pressed onto Ni/Fe Curie point wires and subsequently heated for 5 s at 590°C. The pyrolysis unit was directly connected to a Carlo Erba GC8060 gas chromatography through a splitless injector set at 280°C, and the products were separated by a fused silica column (Varian, 25 m, 0.32 mm i.d.) coated with CP-Sil5 (film thickness 0.40 μm). The GC oven was initially kept at 40°C for 1 min, then heated at a rate of 7°C/min to 320°C and maintained at that temperature for 15 min. The column was coupled to a Fisons MD800 mass spectrometer (mass range m/z 45-650, ionization energy 70 eV, cycle time 0.7 s). Identification of the compounds was carried out from their mass spectra using a NIST library and/or by interpretation of the spectra, by their retention times and/or by comparison with data from the literature. Each day, prior to analysis of

samples, a standard (ball-milled oak root, Quercus robur L) is run in order to check pyrolysis, chromatography and mass spectrometry based on an array of compounds present, including polysaccharides, proteins, guaiacyl-lignin, syringyl-lignin, tannins, suberin, and triterpenoids. Each of these compounds has distinct features upon Py-GC-MS, thus allowing possible problems with the system to be traced. In case of maintenance or when too much sample was pyrolyzed (based, e.g., on peak overload or high intensity), a blank is run (either only GC-MS running or running a pre-extracted Curie-point wire).

Two duplicates were taken from the upper and lower part of the weathered biowell to provide a more accurate and all-round analysis towards biodegradation. For each component, the absolute chromatographic peak area was regarded as linear with its quantity, and the relative amount (peak area %) is linear with its content. Therefore, for each identified component, its peak area value obtained from the original biowell and different positions from the used one could be compared to reveal the changing of its yields. Moreover, the relative content (%) value could be compared to illustrate the changes of its relative content among the detected products (Lu et al., 2011).

3.2.5 Aerobic Soil Burial Test: Biodegradability Verification

The aerobic soil burial test is one of the most commonly utilised methods to determine biodegradation of biodegradable materials owing to its simplicity and feasibility. Besides, this method is similar to field conditions (Tai et al., 2019). Aerobic experiments were not conducted within this research because of the difficulty to construct a closed system without existing experimental equipment. What's more, samples could not be taken out for visual observation, or the closed system would be destroyed. Biodegradation and visual observation results could be found in ***Appendix V***.

3.2.5.1 Materials

3.2.5.1.1 Preparation of Biowell Samples

Biowell samples were tested in two forms with different surface areas (fragments and granules)(Lott et al., 2021). Biowell testing fragment samples were obtained by cutting the original material into small pieces as 1 cm x 1 cm x 0.5 cm (the wall thickness) and used to assess the biodegradability in soil burial test. Each portion weighed approximately 0.3500 g on average. To create granules samples, equal fragments of about 0.3500 g were ground to more diminutive size, equally distributed and placed in mesh bags consisting of around 0.3500 g for each specimen.

Mesh bags with 160 μm opening size were selected to hold the granules. A larger mesh size was abandoned as it might cause the non-biodegradable granules to fall out of the mesh bags after biodegradation, leading to measurement errors. The small mesh size allows meso- and microorganisms to enter the bags. Each sample was placed two cm apart and were recovered for further analysis at a two-week interval (Sintim et al., 2020).

3.2.5.1.2 Preparation of Soil Environment:

The biodegradation environment was sandy soil collected from the surface layer of

fields in the Veluwe (Šerá et al., 2020a). The soil was sieved over a 2.5 mm sieve to remove obvious plant material, stones, and other inert materials. The sampling site and the sampling date were recorded before the starting of the soil test. The procedure was based on (ISO 17556, 2020). The soil was stored in a sealed container under 4 ± 2 ℃ (ISO 17556, 2020). An overview of the entire experimental procedures is listed in ***Figure 7***.

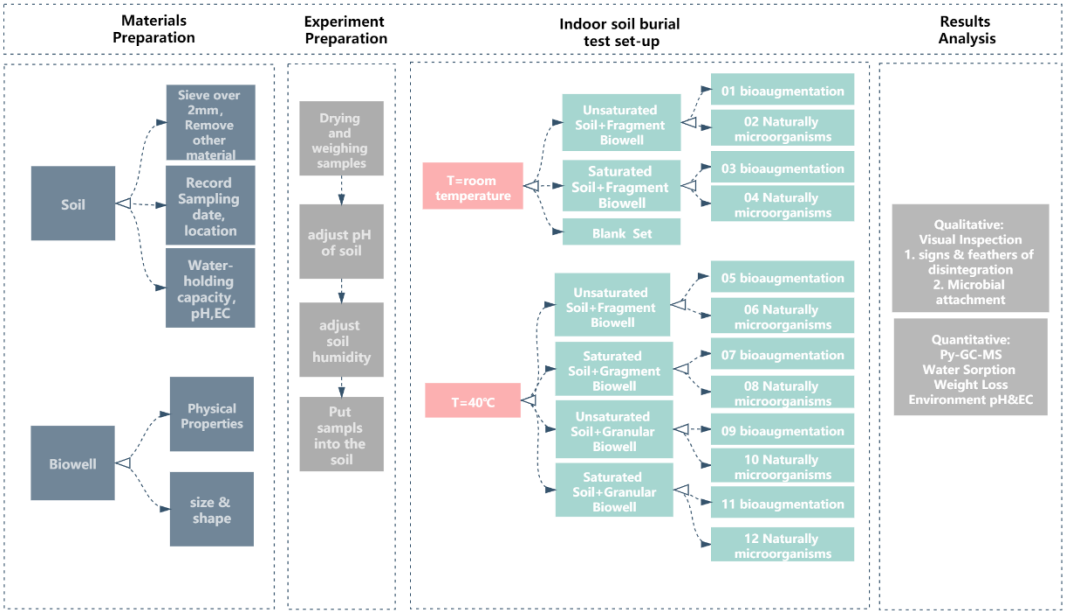


Figure 7. Experiment Procedures

3.2.5.2 Laboratory Testing Parameters and Procedures

3.2.5.2.1 Measurement of Soil and Biowell Characteristics Before the Test

Basic knowledge towards the soil characteristics and biowell plays an essential role in full interpretation of the results of the research. Several parameters of both the soil and the biowell samples were tested before the biodegradation test based on several standards (***Table 3***).

Table 3. **Measurement Methods**

Objective	Parameters	Methods	Objective	References
Soil	Total water-holding capacity (WHC)	a) Dry the soil sample around 105℃ and prepare the soil sample for around 25.00 g. b) Take a funnel and place it on a funnel stand. Put a filter paper in it. Fold the filter paper and moisten it with water. Put weighed soil in the funnel. Put a graduated cylinder below this funnel. Measure 100 mL of water and pour it slowly into the funnel. c) Wait for 10-15 minutes. Measure the water collected in the cylinder. Repeat the process with two more samples and calculate the water holding capacity of soil with the equation below. $$\boldsymbol{WHC = \frac{V_0 - V_i}{M_{soil}}}$$ ***Equation 1.*** Water Holding Capacity Where V_0 is the volume of water added and V_i is the volume of water running down. M_{soil} refers to the initial mass of soil added in the beginning.	The WHC was tested to calculate the water to be added to each soil environment to reach half-saturated/saturated conditions.	(ISO 11274, 2019)
	pH & EC of the soil	a) Take a representative test portion of at least 5 ml from the laboratory sample using the spoon in the bottle, five times its volume of water, potassium chloride solution, or calcium chloride. b) Calibration of the pH-meter/EC meter with buffer solution. c) Measure the pH/EC while being stirred.	The original pH & EC were measured as the original setting to determine the initial soil status and keep it within reasonable range.	(ISO 10390, 2005)
Biowell	Total Solid (TS)	Dry at 105℃ to constant weight and then weigh it to obtain TS.	The TS value represents reveals the water content within biowell, which is used to calculate actual biodegradation level later.	(ISO 10694, 1995)
	Volatile solids (VS)	Subtract the residues of a known amount of test materials after incineration at about 550℃ from the total dry content of the same sample.	VS means the combustible property of TS, one important indication for organic matter and biodegradability.	(ISO 10694, 1995)

3.2.5.2.2 Aerobic Soil Burial Tests

3.2.5.2.2.1 Variables Used in Soil Burial Tests

1) Temperature

The temperature was identified as an essential factor affecting biodegradation in the natural environment (Pischedda et al., 2019). For the accelerating purpose, the higher temperature at $40℃ \pm 2℃$ was used to investigate the effect of temperature on the rate of biodegradation and ultimate biodegradation levels after three months (Šerá et al., 2020a).

2) Soil moisture content

Soil's water content was adjusted to a suitable value for the test material by adding an appropriate amount of water to the soil based on testing results of WHC. The soil's optimum water content is usually between 40% and 60% of the total water-holding capacity (Pischedda et al., 2019). Two different soil sets were used, one reaching its water-holding capacity as the saturated soil, with 50% moisture water content as half-saturated soil.

3) Bioaugmentation

Bioaugmentation is commonly utilised to improve the capacity of contaminated soil to remove pollution with extra microorganisms (El Fantroussi & Agathos, 2005). It is also considered one promising technology to accelerate the biodegradation of compostable plastics (Castro-Aguirre et al., 2018). A mixture of commercially available bacteria was added to the bioaugmentation setting to investigate the impact of bioaugmentation (Castro-Aguirre et al., 2018).

4) Surface area

It has been proved that the biodegradation rate of biodegradable plastics in the soil is relevant to the available surface areas. Limited surface areas make it possible to anticipate biodegradation rates due to different reaction surface areas (Chinaglia et al., 2018). Four settings where biowell were made into granules were built to inquire into the effects of available surface area on biodegradation rate.

3.2.5.2.2.2 Experimental Settings in Soil Burial Tests

1) Common Experimental Procedures

A set of twelve equal round buckets with a volume of three litter were used as reactors. Each bucket was filled with approximately 2200 grams of soil. Biowell fragments were placed on the same layer of soil at a depth of five cm from the top surface. Two duplicates of each fragment were prepared to assess reproducibility. After the buckets were filled, they were covered with lids with holes on the top to prevent quick evaporation and ensure air circulation. The buckets were weighed weekly, and deionized water was added inside to retain the initial weight (Barbale et al., 2021). The total test duration was 84 days (Šerá et al., 2020a).

2) Experimental Settings under Different Conditions

In this research, twelve different reactors were separated into three groups. The first group (01-04) were placed under room temperature (~20 ℃), and the second (05-08) and third (09-12) settings were placed under 40 ℃. Besides, biowell granules were used in the third setting. Reactors with odd numbers were filled with saturated sandy soil, while those with even numbers were filled with half-saturated soil. Moreover, within each setting, the latter two were added with additional bacteria, and the other two were not. Combinations of different variables consisted of the twelve biodegradation environments, which are depicted in ***Figure 8***. And the sampling dates are listed in ***Table 4***.

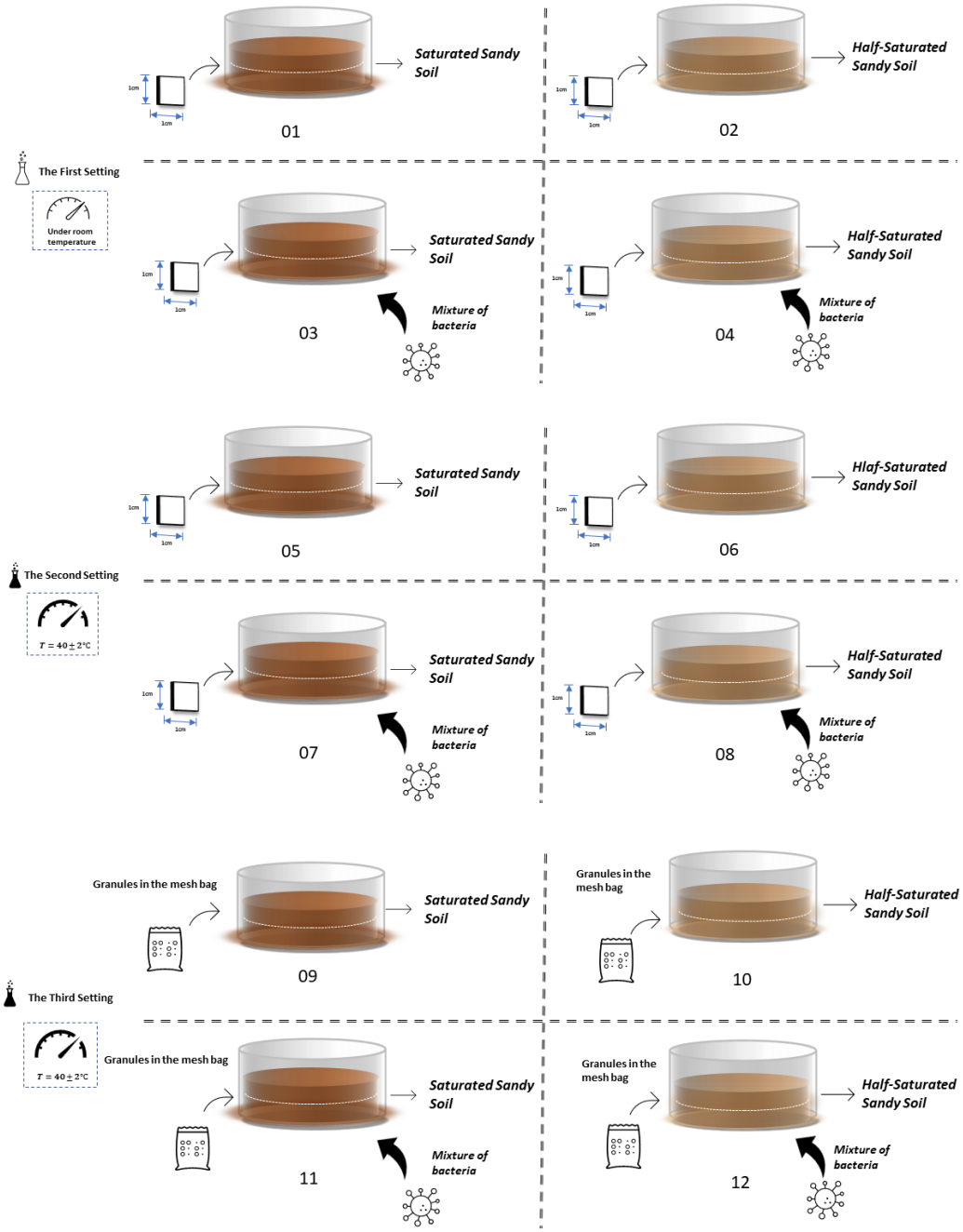


Figure 8. Experimental Set-ups

3) Sampling Intervals

Table 4. Sampling Intervals and Numbers

	Date / Number	April 26th & 29th	May 4th & 6th	May 18th & 20th	June 1st & 3rd	June 15th & 17th	June 29th & July 1st
I	01		01-01	01-02	01-03	01-04	01-05
	02		02-01	01-02	01-03	01-04	01-05
	03		03-01	03-02	03-03	03-04	03-05
	04		04-01	04-02	04-03	04-04	04-05
II	05		05-01	05-02	05-04	05-04	05-05
	06		06-01	06-02	06-03	06-04	06-05
	07		07-01	07-02	07-03	07-04	07-05
	08		08-01	08-02	08-03	08-04	08-05
III	09	09-01	09-02	09-03	09-04	09-05	09-06
	10	10-02	10-01	10-03	10-04	10-05	10-06
	11	11-01	11-02	11-03	11-04	11-05	11-06
	12	12-01	12-02	12-03	12-04	12-05	12-06
Note	Two duplicates were prepared for each sample to assess reproducibility, so 128 samples in total were taken. On April 26th only granules were collected for analysis.						

3.2.5.2.3 Analysis of Experiment Results: Biodegradation Index

Two international standards ISO 17556, (2012) and ASTM D5988, (2018) provided standard criteria to assess the biodegradation of plastics within the soil environment (Briassoulis et al., 2020). Weight loss and CO_2 production amount were two frequently used indices to determine biodegradability in laboratory conditions (Folino et al., 2020). The weight-loss method is more feasible and applicable in the research due to the deficiency of existing respirometer equipment (Wu, 2014). Besides, other several indicators of biodegradation were further utilised here to analyse the fate of biodegradable monitoring well in the soil.

a) Water Sorption

Two samples were removed from the soil at the two-week intervals for each sampling time, carefully cleansed three times with deionized water, superficially dried with tissue paper, and weighed. Water uptake (%WS) was quantified by the following equation (Wu, 2014):

$$Water\ Sorption(\%) = \frac{W_h - W_d}{W_d} \cdot 100$$

Equation 2. Water Sorption Capacity

With:
W_d: the dry mass after biodegradation at time=t;
W_h : the humid mass.

Water adsorption capacity acts as an important indicator as low water sorption capacity delays the water intake and improves the product's stability. The results are calculated as the average of two replicates (Alvarez et al., 2006).

b) Weight Loss

$$Weight\ loss\ (\%)\ =\ \frac{M_b - M_d}{M_b} \cdot 100$$

Equation 3. Weight Loss Ratio

With:
weight loss (%): the percentage of weight loss after biodegradation;
M_b: the weight of the sample before biodegradation;
M_d: the weight of the piece after biodegradation.

The weight for each sample was measured and recorded before the experiments, after being dried under 105 ℃ and then cooled to room temperature in a desiccator (Wu, 2014).

c) Data Analysis

The degradation kinetics of biowell samples in all twelve different soil environments was described by a linear first-order model, which was verified as one optimal method to fit ultimate biodegradation kinetics (Barbale et al., 2021; Zhang et al., 1999). Regression was done by the following mathematical expression (Salunkhe et al., 2014):

$$M_t = M_0 \cdot \exp\left(-kt\right)$$

$$Biodegradation\ (\%) = exp\ (-kt)$$

Equation 4. First-order Mathematical Expression

With:
M_t: the mass (g) for biowell samples at time t (days);
M_0 is the initial mass (g) of biowell samples at time $t = 0\ days$
k: the degradation rate constant for biowell samples per day (day^{-1}).

The half-life time, which indicates that the mass of biowell samples is reduced to half of the initial weight, was calculated through the first-order model (Barbale et al., 2021).

d) pH & EC measuring

The digital pH-meter and EC-meter were used to measure soil's pH and EC value at a 2-week interval to maintain the soil pH and EC within approximately the same range (Badia et al., 2017; Sarasa et al., 2009).

e) Visual Observation

During the entire experiment period, the visual assessment was carried out for each recovered biowell piece under the microscope. Only one sample of the virgin biowell was observed under the microscope and photographed as the reference. The observed qualitative parameters are listed in ***Table 5*** and were systematically used for visual observation as described by (EN 14045, 2003). Visual results were recorded and photographed for each sampling time. The photographs reflecting each stage of

biodegradation could be found in ***Appendix V***.

Table 5 **.**Parameters for Visual Observation
(**Source**: EN 14045, 2003)

Period	Parameters
In the initial phase	Distribution of particle size Signs of microbial colonization (fungal hyphae, bacterial growth) should be described and photographed.
During the entire phase (Signs & Feathers of disintegration)	Roughness, porosities and cracks, structural deformation (destroyed surface, damage of the material, structure collapse, macroscopic deterioration for different times) Consistency, discolouring

f) Bacteria Counting

The Quantitative Polymerase Chain Reaction (QPCR) is a method to accurately detect and quantify different (groups) of organisms or genetic traits based on their DNA or RNA. Bacteria counting is intended to reveal the potential changes of bacteria amount before and after biodegradation after three months (Castillo et al., 2006; Kubista et al., 2006). Considering feasibility, simplicity and budget availability, several representative sample settings selected for the QPCR test are listed in ***Table 6***.

Table 6. Soil Samples Used for Bacteria Counting

In the initial stage	Original soil sample Soil sample with bio-augmentation
In the later stage	room temperature / no-bio-augmentation / half-saturated zone room temperature / no-bio-augmentation / saturated zone 40 degrees / bio-augmentation / half-saturated zone 40 degrees / bio-augmentation / saturated zone

Chapter 4. Results

4.1 Interview Results

This chapter is separated into two main sections. The first section deliberates the external interview results to gain more insight towards background information of "Biowell" itself. The second section focuses on the actual application of "Biowell" for soil and groundwater projects, which could provide a more comprehensive understanding of sustainable soil investigation approaches in the future. Results from interviews were designed to answer the first sub-research question.

4.1.1 External Interviews: Production of the Biowell

4.1.1.1 Raw Materials and the Production Process

The production of "Biowell" could be separated into two main stages. The first stage was the production of raw materials and the second one was the conversion of raw materials into final products. The Solanyl C2201, the raw material of "Biowell", is made from starch collected from the potato processing stream (*Rodenburg Biopolymers*). However, the concrete compositions cannot be disclosed as commercial secrets. The production process of starch-based plastics from Rodenburg biopolymer is demonstrated in ***Figure 9***.

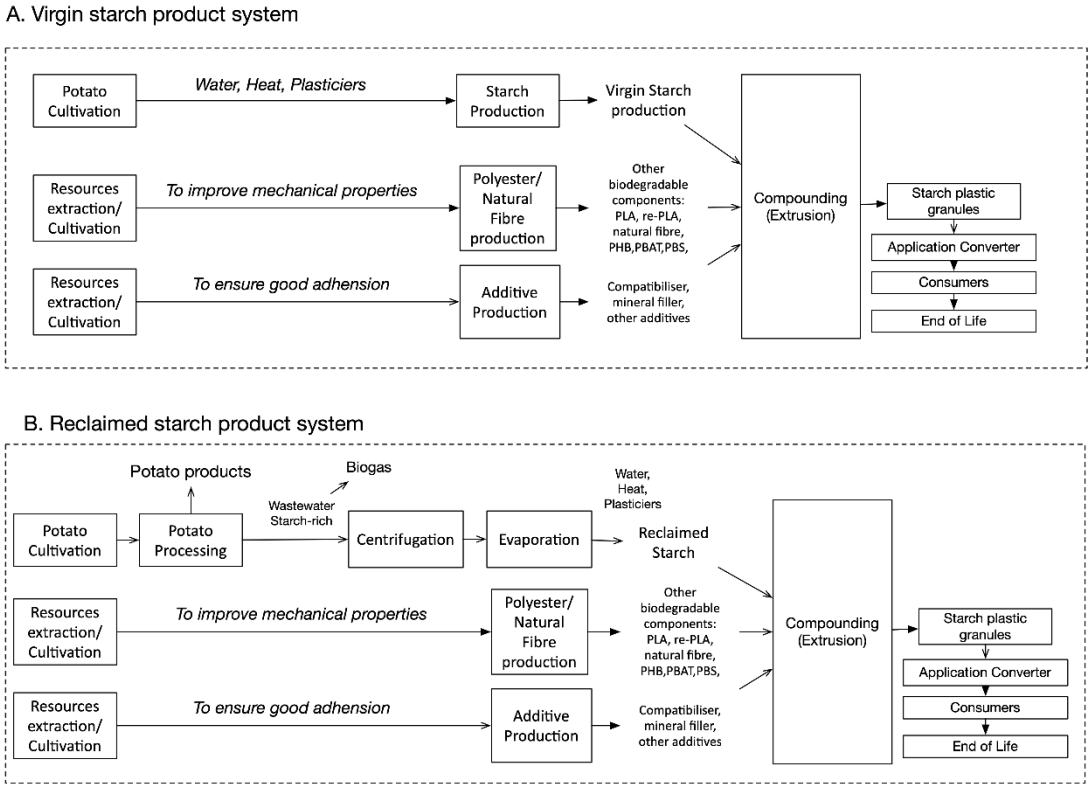


Figure 9. Production Process of starch-based plastics
(Broeren et al., 2017)

Starch could not be used directly in plastics. The introduction of water, heat and plasticizes broke off its structure to yield *thermoplastic starch (TPS)*, further proceeded in the following procedures, e.g., extrusion. It is usually compounded with

other polymers, especially aliphatic polyesters (PLA, re-PLA, PHB and natural fibres) to improve pure TPS's relatively poor mechanical properties. Natural fibres were introduced for many reasons: low price, fewer health hazards during the process and fair mechanical properties. Compatibilizer additives are introduced to ensure adequate adhesion between different components because there exists immiscibility between hydrophobic polyesters and hydrophilic starch/natural fibres (Broeren et al., 2017).

In the second stage, Solanyl C2201 was manufactured into the final "Biowell" product. The slot making was a challenge because of its relatively low melting temperature, making the production process slow and thus increasing the production budget.

4.1.1.2 Environmental Impact

Energy and Water Consumption - During the production process of Solanyl C2201, no more water was added to the compounding process compared with the conventional extrusion process. The only additional yet necessary step compared with the traditional production process was to dry the material, add energy consumption. The conversion process would not add extra water in a closed system and save approximately 60% of energy regarding the entire period.

Life cycle Assessment – Results from the interview with ***A 4*** confirmed that the environmental impacts of four selected starch-based plastics in their research are divergent from each other. Nevertheless, they all played a vital role in reducing the Green House Gas (GHG) emission and Non-renewable Energy Use (NREU), especially for reclaimed starch retrieved from the potato processing wastewater streams. Nevertheless, the production of starch-based plastics brought about a higher level of eutrophication and agricultural land use, as the negative aspects of the environmental impacts.

4.1.2 Internal Interview results: Attitudes towards application of Biowell

Of the thirty questionnaires sent out, twenty-two replies (73.4%) were received. Results of the internal survey were presented from ***Table 7***-***Table 10***.

Short-term investigation projects - Respondents working in different countries gave detailed explanations of those short-term groundwater and soil investigation projects they specialise in (***Table 7***). Short-term soil investigations are performed in the public domain and industrial sites as preparation for monitoring and planning. Moreover, short-term soil investigations are performed to gain insight into Health & Safety measures for utility companies (maintenance/work in subsurface on sewers, electricity cables, gas pipes, phone cables, etc). These projects may be one optimal opportunity to implement biowell.

Table 7. Short-term Groundwater and Soil Investigation Projects

Locations	Projects
TAUW Netherland	1. Preparations of infrastructural projects 2. Preparations for Transactions of locations 3. Industrial Sites Investigations 4. For the contract of Governments & Public Services companies 5. For the sake of government/construction/environment permit
TAUW France	Industrials and real estates related projects
TAUW Germany	Phase II in Due Diligence projects, detailed investigation programs for remediation planning, compliance monitoring and success monitoring during and after remediation projects
TAUW Italy	Phase II in Due Diligence projects in preliminary investigations for development of sites
TAUW Iberia	1. Phase II in Due Diligence projects for a site buying-selling process to assess the possible environmental liabilities 2. Industrial sites (e.g., Petrol station investigation)

Utilisation of biowell in practical projects - Firstly, all respondents have heard about "biodegradable groundwater monitoring well", but only 13.6% of them have used biowell in reality, which accounts for a small percentage. It was noticeable that biowell has been utilised in the contract with the Gemeente Groningen.

Table 8. Use of biowell in Practice

Have you ever heard about biodegradable groundwater monitoring well?	Have you ever used "biowell" in your groundwater and soil investigation projects?	Percentages
Yes (100%)	Yes	13.6%
	No	90.5%
No (0%)	Yes	0.0%
	No	0.0%

Willingness to Recommend Biowell to Clients and Reasons - Half of the respondents showed a strong willingness to recommend biowell to their clients in groundwater and soil investigation projects, considering its positive environmental impact and sustainability demand from clients nowadays. Another important reason mentioned several times was that the Dutch industry was making efforts to find sustainable methods in the industry. Moreover, one respondent also showed concern about consequences resulted from placing PVC-wells throughout the country. Biowell might relieve plastics accumulation in the future. However, half of the respondents' results were doubtful to recommend biowell mainly for two reasons. Firstly, there existed high risks that the monitoring well might be re-used in the future, which was difficult to anticipate. The lack of official authorization currently also led to their uncertainty towards this question.

Table 9. Willingness to Recommend Biowells to Clients and Responding Reasons

Answers	Reasons	Percentages
Yes (50.0%)	1. Biowell maybe the ideal solution for short-term projects.	9.1 %
	2. Biowell could meet sustainability demand from multinational clients.	13.6 %
	3. It is strange to put plastics all over the country without disposal afterwards.	4.6 %
	4. Governments are looking for methods to promote sustainability strategy.	9.1 %
	5. No reasoning.	22.7 %
No (0.0%)		
Maybe (50.0%)	1. Biowell may not be applicable for industrial sites due to contamination.	9.1 %
	2. It is hard to predict re-monitoring, so it will come with high risk.	4.6 %
	3. It is hard to predict long-term use in advance.	22.7 %
	4. Official approval by the authorization is needed.	4.6 %

Market share Estimation in the future – It is difficult for 45.5% of respondents to reasonably estimate biowell's market share. Lack of extra benefits in the industry and forced authorization were the most commonly mentioned reasons. They also thought that the market shares still depended on a handful of other elements, e.g., price, the importance of sustainability in contract and the actual percentage of short-term projects.

Fear and worries towards plastics pollution in the natural environment were the main driver for those who gave a higher estimation of biowell market share in the long term. 27.3% of respondents gave an estimation of 10% ~20% due to the low percentage of the short-term project in their past experiences.

Table 10. Market Share Estimation and Reasons

Market share estimation	Reasons	Percentages
95~100% (18.2 %)	1. Assuming that PVC/HDPE is forbidden by legislation in the future.	4.6%
	2. With the aim to avoid plastic pollution.	4.6%
	3. No reasoning	9.1%
60% (4.6 %)	1. No reasoning	9.1%
10~20% (27.3%)	1. Long-term monitoring is needed for most of the remediation projects	13.6%
	2. No reasoning	13.6%
no idea (45.5 %)	1. Financial driver: Extra value is needed for industry.	13.6%
	2. Need authorization promotion.	9.1%
	3. Shorting monitoring projects accounts for a small proportion.	4.6%
	4. Interesting for public domain	4.6%
	5. Depends on whether sustainability is important in the contract	4.6%
	6. No reasoning	4.6%

*Some respondents gave more than one answer.

4.2 Fieldwork Investigation: Biodegradation under natural conditions

4.2.1 Visual Inspection of the Biowell after Two Years of Burial

During the extraction process of the biowell **(Pb1F)** and the regular PVC groundwater monitoring well **(Pb2F)**, the Pb2F could be taken out easily with steady tensile

strength, and there did not exist biofilm formation on its surface compared with an unused one, as shown in ***Figure 10*** (a). By contrast, it took a longer time to extract the biowell as it has already attached to the soil much more closely. It was necessary to extract it with cautious attention because of its vulnerability. Therefore, merely approximately upper 60 cm of the biowell (above the groundwater level) has been pulled out successfully, as shown in ***Figure 11*** (a).

The visual inspection conducted from the extracted biowell demonstrated a few distinctive phenomena. With the biowell acting as the carbon source, microorganisms have colonised onto the surface of biowell. The obvious stratification of microbe presence could be distinguished from different colours on the upper and lower part of the biowell, respectively, in ***Figure 11*** (b) and (c).

Figure 10. The PVC Monitoring Well Taken Out After Two Years
((a) the upper one is the used PVC monitoring well and the lower one is a new one;
(b) the upper one is used biowell and the lower one is used PVC monitoring well;
(c) the upper one is used PVC monitoring well and the lower one is used biowell)

Figure 11. Biowell Taken Out After Two Years
((a) the upper one is a new biowell and the lower one is the used one;
(b) the upper part of used biowell;
(c) the lower part of used biowell)

In the upper part shown in ***Figure 11*** *(b)*, intensive black spots covered around the biowell, and the surface evolved to be rough. Moreover, one sizeable crack occurred in the upper part representing the structural deformation (***Figure 12***). Most fracture surfaces were still smooth and demonstrated no indication of discolouring. Concerning the lower part (***Figure 11*** (c)), there was no apparent alteration of structural disintegration on the biowell itself. However, it is noteworthy that different types of microorganisms have colonised the biowell surface.

a b c

Figure 12. Cracks on the Upper Part of Used Biowell

Apart from the underground environment, the biodegradation phenomenon was also witnessed from those biowells stored in the warehouse yet exposed to the air (***Figure 13***). A thin layer of water film was scented out on one small component of Biowells. Several black spots were also attached to the surface of the biowell in sections exposed to the air. A dense microbe community has already been distributed on the inner and outer surface of the bottom component in ***Figure 13*** (c).

a b c

Figure 13. Biowells in Stock

4.2.2 Microscope Observation

The weathered biowell was collected and photographed under a microscope to visualize the structural changes after two years of soil burial. Regarding biowells stocked in the warehouse and exposed to the air, the specimen exhibited a relatively smooth surface with fungi multiplied (***Figure 14***).

a b c

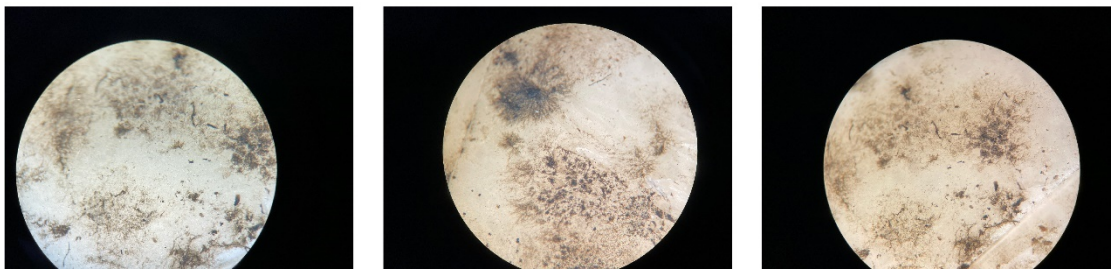


Figure 14. Microscope photographs of Biowell in stock

After two years, some evidence of erosion and cracks could be observed in detail under the microscope. Biodegradation caused heterogeneous damages on the surface of the biowell (***Figure 15***). Cracks and breaks were observed both in the outer and inner surfaces of the used biowell, companied by the surface erosion and distinct discolouring (***Figure 15***. (b)). The brittleness of the used biowell also increased as it could be destroyed without much effort.

Regarding the lower part of the biowell (***Figure 15*** (c) & (d)), which was placed deeper into the soil, no apparent structural fragmentation was appeared compared with the upper part under a microscope, retaining its original general structural properties. In contrast, small cracks could be observed under the microscope.

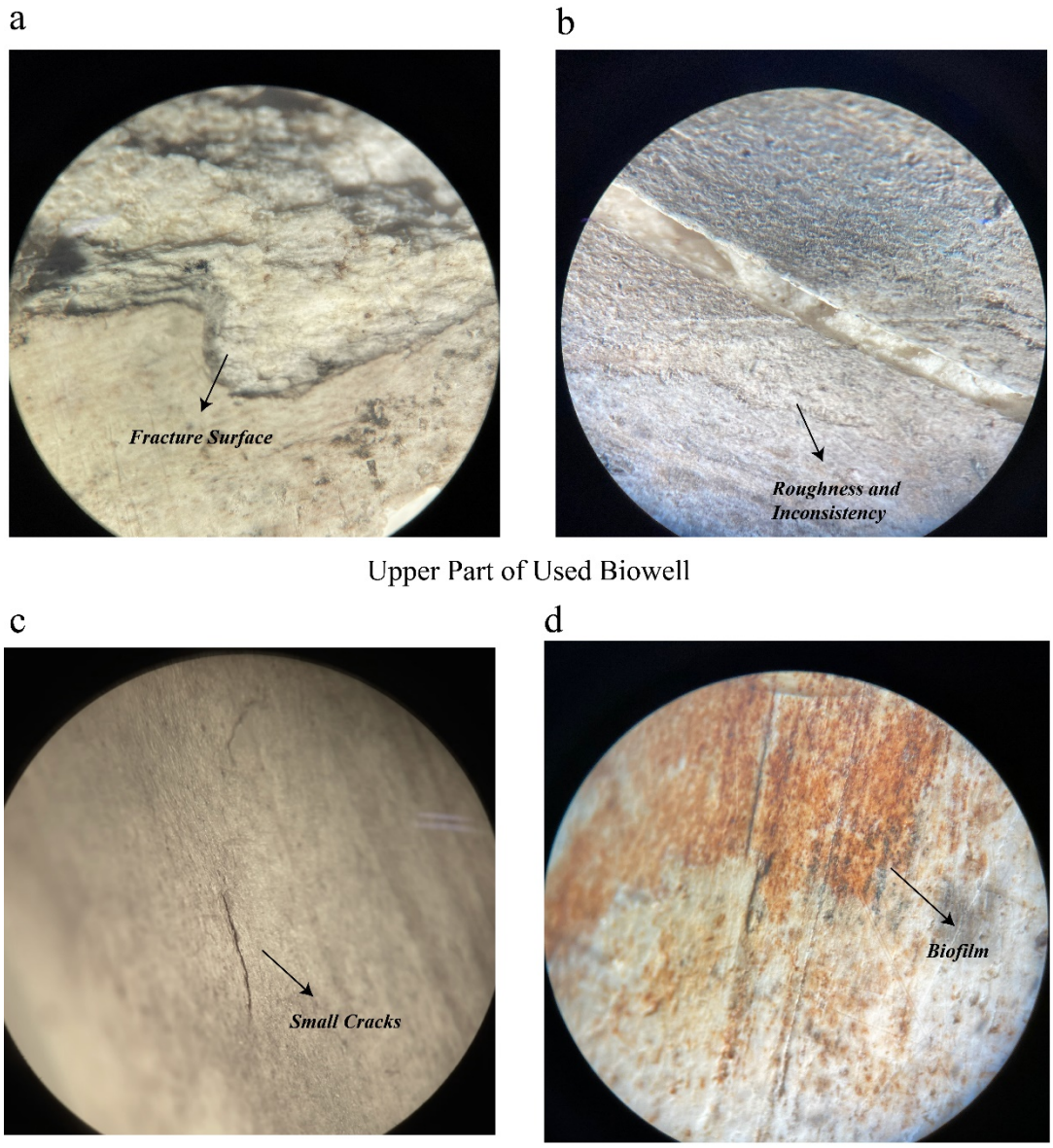


Figure 15. Microscope photographs of used biowell
((a) & (b) upper part, (c) & (d) lower part)

4.2.3 Field Testing & Groundwater Monitoring Data from 2019-2021

4.2.3.1 Field Measurements Data from 2019-2021

The study assessed the groundwater qualities from the biodegradable monitoring well (**Pb 1F**) and the regular PVC monitoring well (**Pb 2F**) within a total of eleven samples. Data that were the same between the two settings were not examined by *t*-test. The testing results were subjected to the two-tailed Student's *t* test at 95% confidence level (***Table 11***). Regarding field test results, groundwater depth, pH, EC,

and turbidity measured showed no significant deviation as *the p*-value of these four parameters lies within the range from 0.1386 to 0.7776 (*p*-value>0.05). The mean concentration of Arsenic (Ar) illustrated a significant difference (p-value=0.0357<0.05) between Pb 1F and Pb 2F. The concentration of Mercury (Hg), Cobalt (Co) and Barium (Ba) were higher in results from Pb1F than those in Pb 2F, yet without significant differences statistically. The mean concentration of Nickel (Ni) was higher detected in Pb2F than in Pb1F. However, the *t*-test results showed no noticeable variation (*p*-value=0.3478<0.05).

Table 11. The Student's *t*-test Results

Field Measurement							
Parameter	**Well**	**Mean**	**SD**	**t***	**t**	***P*-value**	**Decision**
Groundwater Depth (m)	Biowell	1.27	0.18	-0.32	-2.10 to +2.10	0.7509	Accepted H_0
	PVC	1.30	0.18				
pH	Biowell	6.82	0.31	-1.59	-2.18 to 2.18	0.1386	Accepted H_0
	PVC	7.05	0.20				
Electrical Conductivity *μS/cm*	Biowell	1089.40	644.50	0.29	-2.13 to 2.13	0.7776	Accepted H_0
	PVC	1020.10	406.64				
Turbidity (NTU)	Biowell	63.67	138.61	-1.14	-2.36 to +2.36	0.2934	Accepted H_0
Chemical Analysis							
Nickel (Ni) $\mu g/L$	Biowell	3.20	0.47	-0.99	-2.23 to +2.23	0.3478	Accepted H_0
	PVC	4.28	3.58				
Mercury (Hg) $\mu g/L$	Biowell	0.06	0.05	1.00	-2.23 to +2.23	0.3409	Accepted H_0
	PVC	0.05	0.00				
Cobalt (Co) $\mu g/L$	Biowell	2.32	1.06	0.53	-2.14 to +2.14	0.6076	Accepted H_0
	PVC	2.14	0.45				
Barium (Ba) $\mu g/L$	Biowell	242.73	203.62	1.69	-2.20 to +2.20	0.1198	Accepted H_0
	PVC	136.18	49.40				
Arsenic (Ar) $\mu g/L$	Biowell	18.35	16.49	2.43	-2.23 to +2.23	0.0357	Rejected H_0
	PVC	6.20	2.08				
Mineral oil C21-C30 $\mu g/L$	Biowell	16.73	4.82	0.24	-2.09 to +2.09	0.8163	Accepted H_0
	PVC	16.27	4.22				
Mineral oil C16-C21 $\mu g/L$	Biowell	12.09	6.93	0.67	-2.18 to +2.18	0.5183	Accepted H_0
	PVC	10.64	2.11				

4.3 Py GC-MS Results: Identification of Chemical Changes

4.3.1 Total Peak Areas of Pyrolysis Volatiles

Figure 16 shows chromatograms of Py GC-MS analysis at 320℃ of unaged biowell and aged biowell after two years buried in the soil. Acetic acid, Hydroxyacetaldenhyde, 5,6-Dilhydropyran-2,5-dione, and DL-lactide were among the compounds identified.

According to ***Figure 17,*** among all four aged samples, the peak area of four identified components in upper-2 increased dramatically compared with the other three samples.

It is noticeable that a large quantity of DL-lactide was formed from the upper-2 sample, ranging from 3~6.5 times of yields from different samples. Regarding Upper-1, Lower-1, Lower-2, there appeared a lower quantity of each component than the original sample.

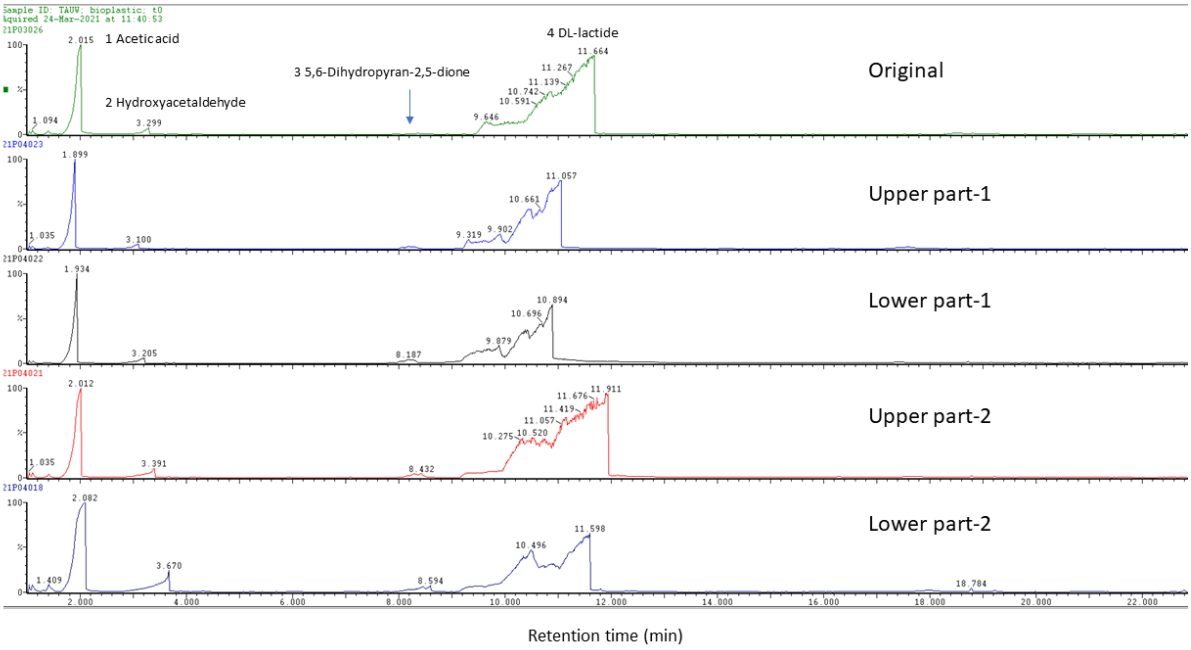


Figure 16. Chromatograms of Py GC-MS at 320℃
(a) Original biowell; (b) & (d) Aged Upper part biowell (c) & (e) Aged lower part biowell

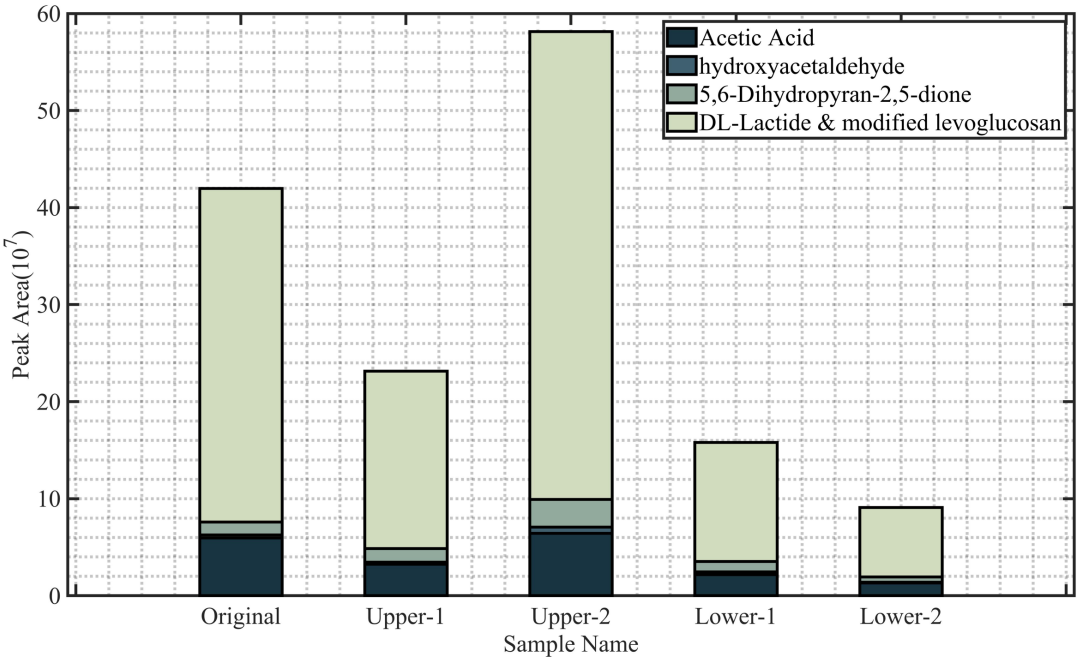


Figure 17. Peak Area of Different Identified Components

4.3.2 Relative Content of Identified Components

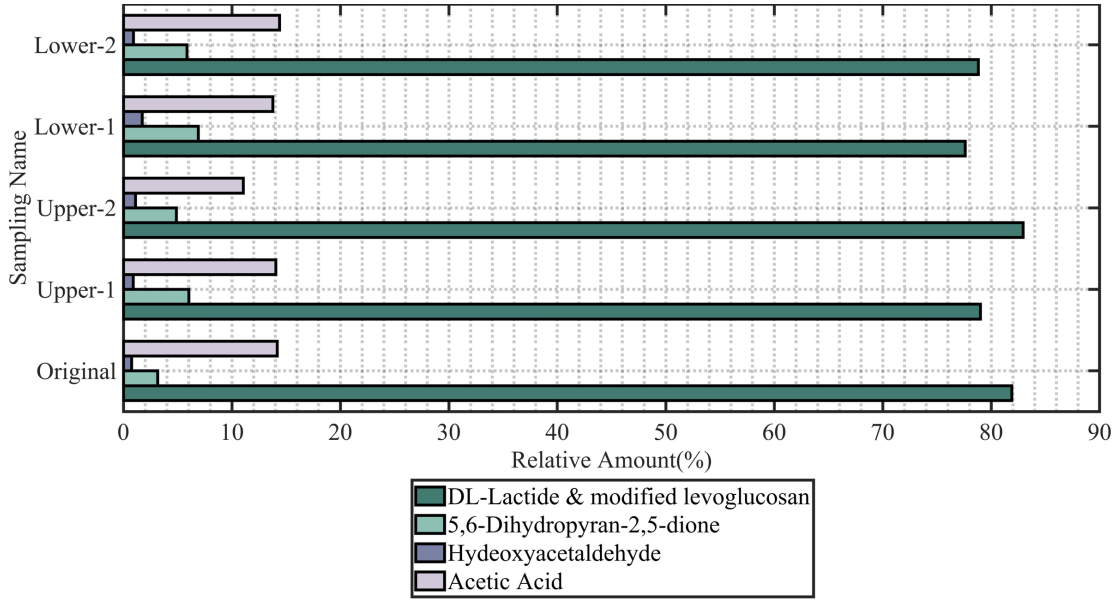


Figure 18. Relative Amount of Different Identified Components

The relative content of each component in the biowell compositions from virgin and aged biowell samples is demonstrated in ***Figure 18***. The relative content of each component remained approximately the same for all samples. With the relative content ranging from 77.60% to 82.94%, DL-lactide was always the dominant component before and after ageing for two years. Acetic acid, Hydroxyacetaldehyde, 5,6-Dihydropyran-2,5-dione accounted for 11.05% -14.40%, 0.75%-1.73%, 3.17%-6.90% respectively before and after two years of soil burial.

4.4 Soil Burial Test Results

The biodegradation data and photographs of the whole soil burial test are shown from ***Table A V 1*** to ***Table A V 12***. The biodegradation rate of each setting differs from the other, as seen from the biodegradation curves from ***Figure 19*** to ***Figure 22***. The biodegradation process was identified for all settings, deduced from the weight loss.

4.4.1 Basic Parameters

The measurement results for soil and the virgin biowell in the preparation stage are presented from ***Table 12*** to ***Table 13***.

Table 12. General Properties of the Biowell

Raw Material	Total Solids Ratio	Volatile Solids Ratio
Solanyl C2201	97.80%	95.45%

Table 13. General Soil Properties

Sampling date	Sampling location	Water holding capacity	Soil Moisture Content
2021.04.08	Nunspeet	49.23%	4.88%

4.4.2 Biodegradation Levels (%)

Weight loss were recorded and analysed for all biowell samples over the three months of the soil burial tests, thereby demonstrating the degradation process of these

samples. Four variables (i.e., temperature, soil water content, bioaugmentation and surface areas) illustrated different levels of accelerating effect on the biodegradation behaviour.

4.4.2.1 The Effect of Temperature

It could be observed from ***Figure 19*** & ***Table 14*** that biodegradation levels under 40 ℃ were higher (~5% difference) than that under room temperature with dashed lines lying over the solid lines. Deviation of biodegradation levels between room temperature and 40 ℃ also showed an enlarging tendency after eight weeks of soil burial test compared with that in the beginning period.

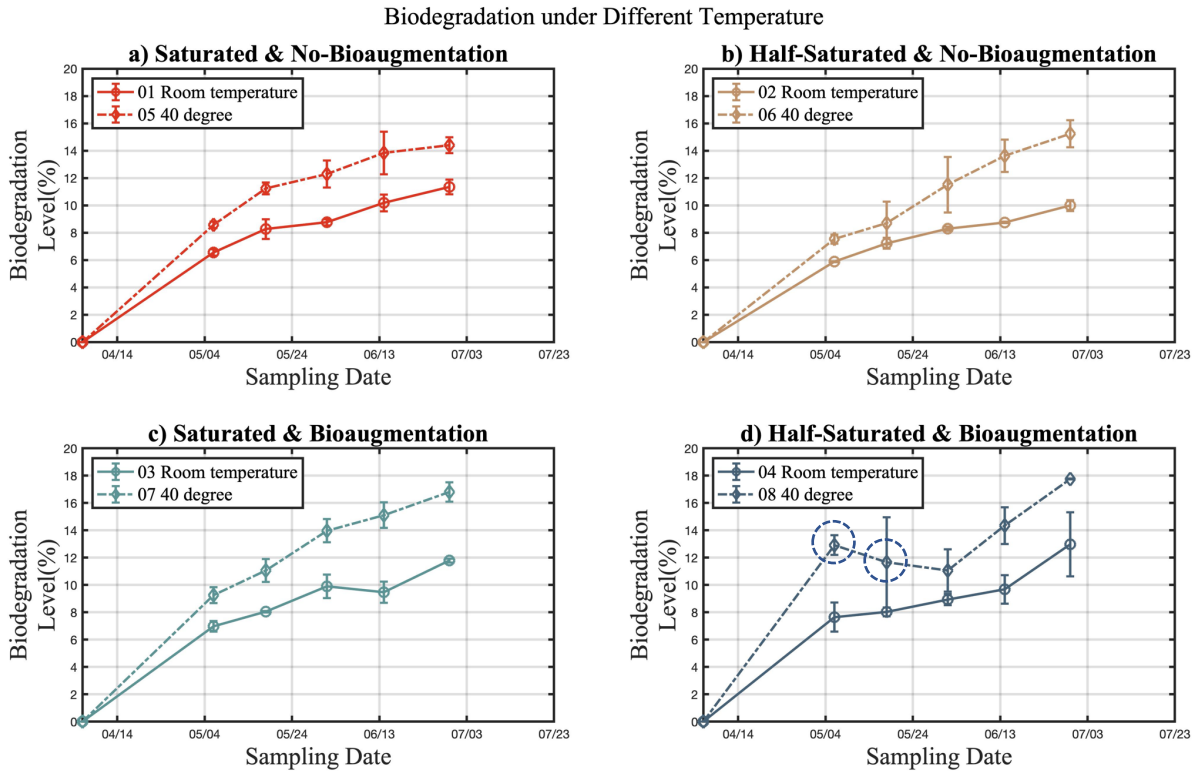


Figure 19. Weight Loss of Biowell under Different Temperature

Each value represents the mean of two replicates with error bars indicating standard deviation from the mean

Table 14. Final biodegradation levels under Different Temperature

	Saturated & No-bioaugmentation	Half-saturated & No-bioaugmentation	Saturated & Bioaugmentation	Half-saturated & Bioaugmentation
Room temperature	11.3494%	9.9885%	11.7805%	12.9701%
40 ℃	14.3989%	15.2415%	16.7994%	17.7537%
Absolute Differences	3.0495%	5.2530%	5.0189%	4.7836%

4.4.2.2 The Effect of Soil Water Content

The second comparison was intended to investigate the effect of soil humidity on biodegradation levels. Approximately 1% differences existed between biodegradation levels under saturated and half-saturated soil environments (***Figure 20 & Table 15***).

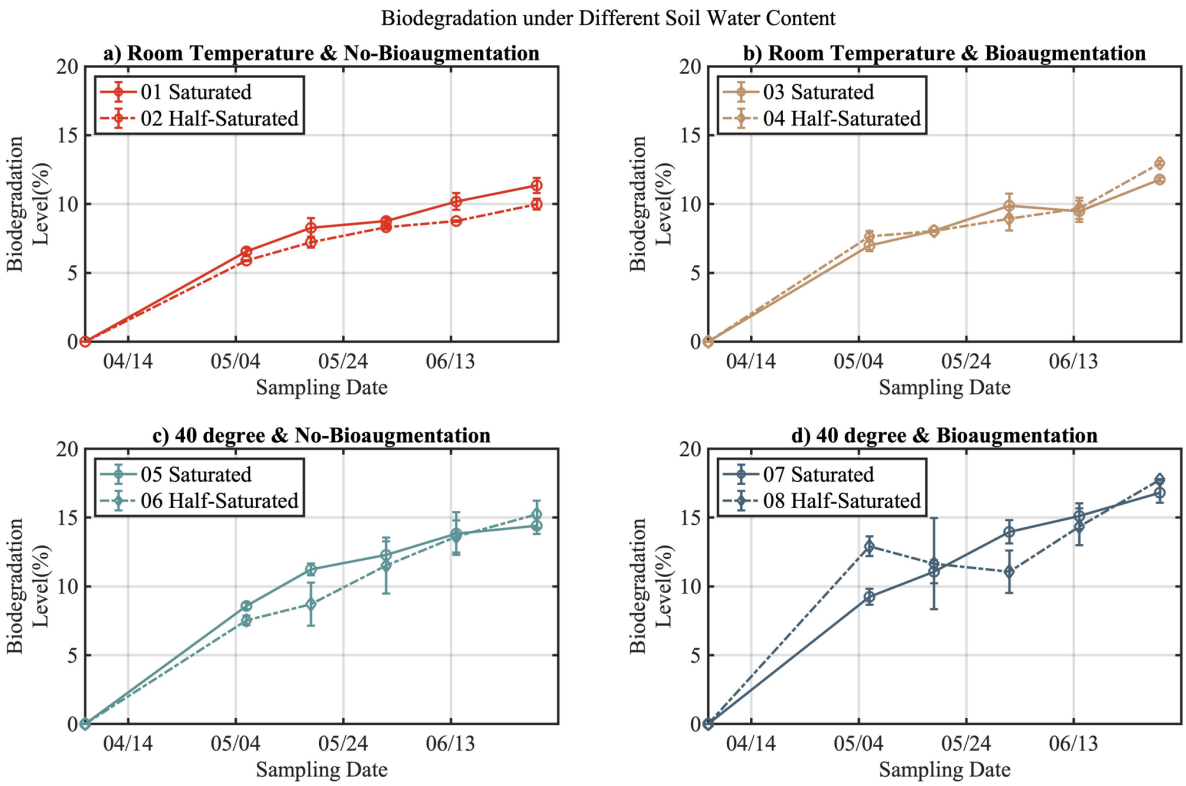


Figure 20. Weight Loss of Biowell under Different Soil Moisture

Each value represents the mean of two replicates with error bars indicating standard deviation from the mean

Table 15. Final Biodegradation Levels under Different Soil Moisture

	Room temperature & No-bioaugmentation	Room temperature & Bioaugmentation	40 degree & No-Bioaugmentation	40 degree & Bioaugmentation
Saturated Soil	11.3494%	11.7805%	14.3989%	16.7994%
Half-saturated soil	9.9885%	12.9701%	15.2415%	17.7537%
Absolute Differences	1.3609%	1.1896%	0.8426%	0.9543%

4.4.2.3 The Effect of Bioaugmentation

The third comparison was intended to reveal the effect of bioaugmentation on biodegradation levels. There existed almost no differences in biodegradation levels with and without bioaugmentation in ***Figure 21*** (a). For other three plots, a relatively larger deviation was remarked in the latter stage (***Figure 21*** (b), (c) & (d)). Absolute differences of biodegradation levels floated around 2% between settings with and without bioaugmentation (***Table 16***).

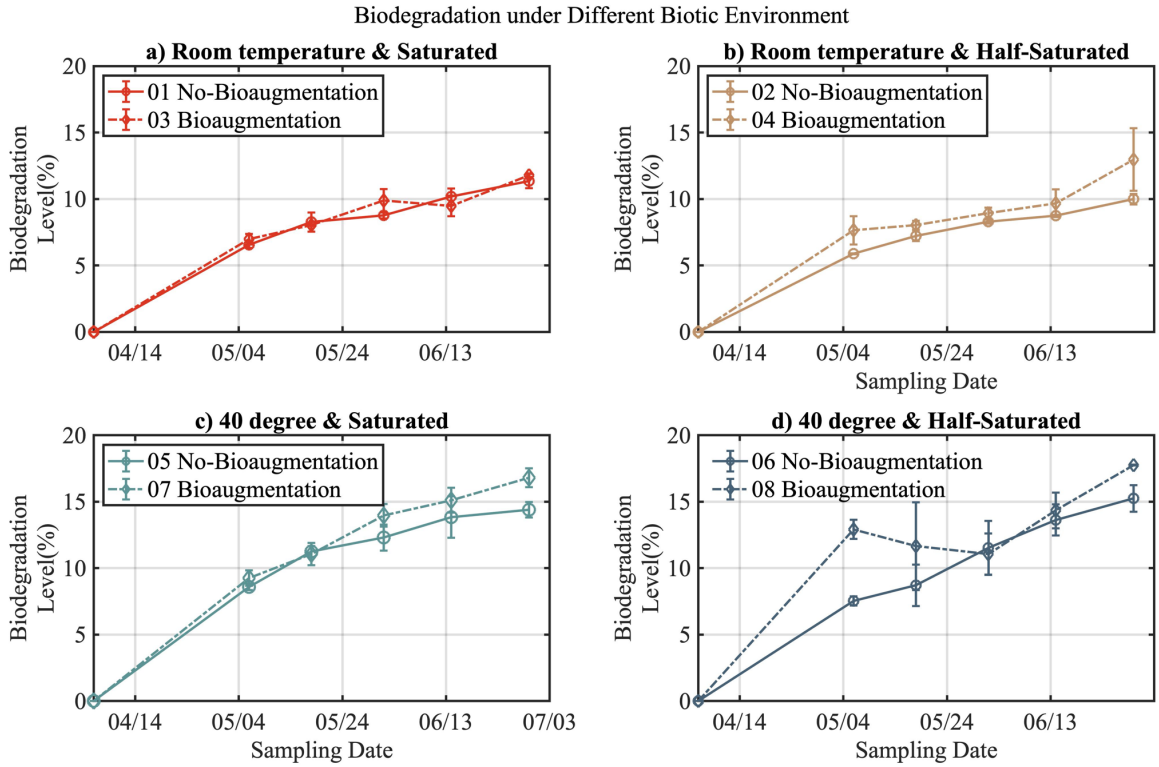


Figure 21. Weight Loss of Biowell under Different Biotic Conditions

Each value represents the mean of two replicates with error bars indicating standard deviation from the mean

Table 16. Final Biodegradation Levels under Different Biotic Environments

	Room temperature & Saturated	Room temperature & Half-saturated	40 degree & Saturated	40 degree & Half-Saturated
No-bioaugmentation	11.3494%	9.9885%	14.3989%	15.2415%
Bioaugmentation	11.7805%	12.9701%	16.7994%	17.7537%
Absolute Differences	0.4311%	2.9816%	2.4005%	2.5122%

4.4.2.4 The Effect of Surface Areas

The fourth comparison was made based on different surface areas (fragments and granules). The biodegradation data from granules fluctuated without a stable trend, and then it was difficult to establish a valid comparison between fragments and granules (***Figure 22*** **&** ***Table 17***).

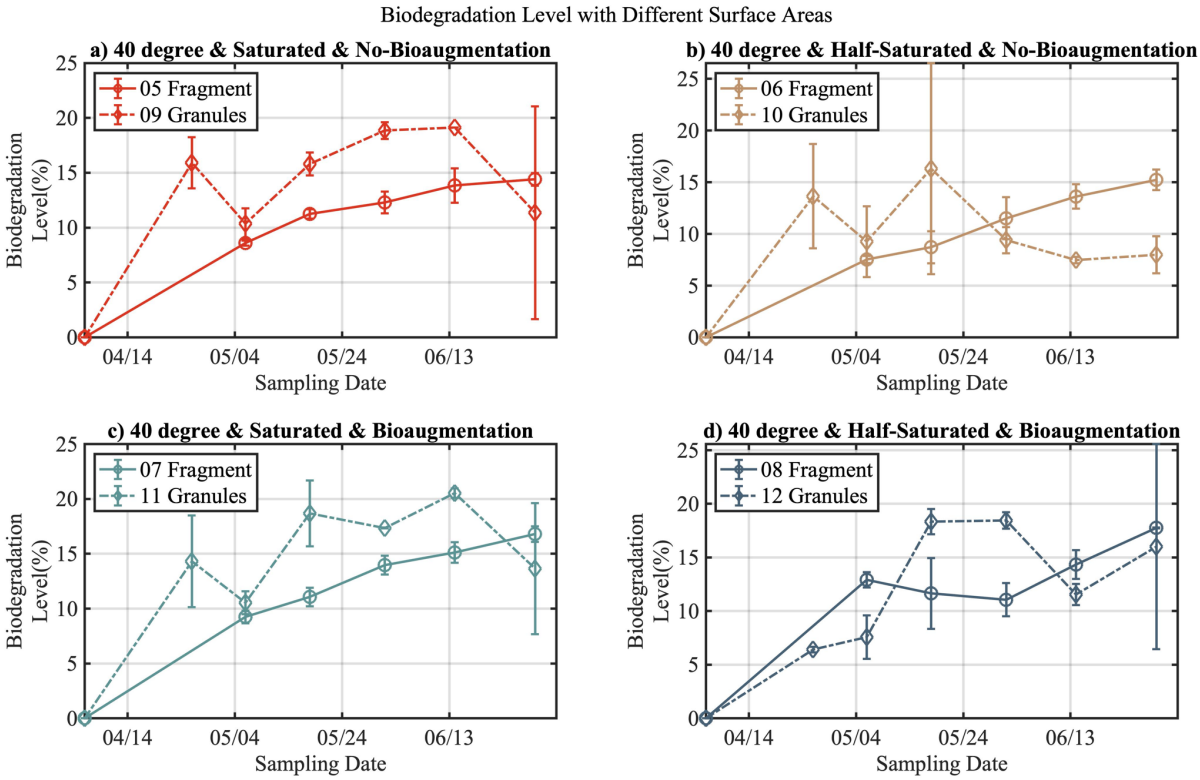


Figure 22. Weight Loss of Biowell with Different Surface Areas

Each value represents the mean of two replicates with error bars indicating standard deviation from the mean

Table 17. Final Biodegradation Levels with Different Surface Areas

40 degrees	Saturated & No-Bioaugmentation	Half-saturated & No-bioaugmentation	Saturated & Bioaugmentation	Half-Saturated & Bioaugmentation
Fragments	14.3989%	15.2415%	16.7994%	17.7537%
Granules	11.3576%	7.9832%	13.6428%	16.0056%
Differences	5.2808%	6.1577%	5.4205%	2.8007%

4.4.3 Estimation of Biowell Degradation in Soil

Data obtained from experiments were fitted with the first-order kinetic model (***Figure 23***). The kinetic parameters are summarised in ***Table 18***. As shown in **错误!未找到引用源。**, the first-order rate constant (k) is in the order of [unsaturated/with-bioaugmentation (04)]> [saturated /with-bioaugmentation (03)]> [saturated/no-bioaugmentation (01)]> [unsaturated/no-bioaugmentation (02)] under room temperature.

While under 40 ℃, the first-order rate constant (k) is in the order [unsaturated/with-bioaugmentation (08)]> [saturated /with-bioaugmentation (07)]> [saturated/no-bioaugmentation (05)]> [unsaturated/no-bioaugmentation (06)]. Extrapolation of this model could provide a rough estimation of the ultimate biodegradation time in the soil, approximately 3000 days and 3500 days under room temperature and 40 ℃, respectively.

Table 18. First-order Kinetic Parameters to Predict Ultimate Biodegradation

Setting Number	Biodegradation Rate $k(\%\ day^{-1})$	Estimated Half-Life Time $t_{1/2}$(days)	Biodegradation level after twelve weeks (%)
01	0.001612	429.99	11.3494%
02	0.001422	487.45	9.9885%
03	0.001646	421.11	11.7805%
04	0.001695	408.84	12.9701%
05	0.002192	316.22	14.3989%
06	0.002103	329.60	15.2415%
07	0.002453	282.57	16.7994%
08	0.002454	282.46	17.7537%

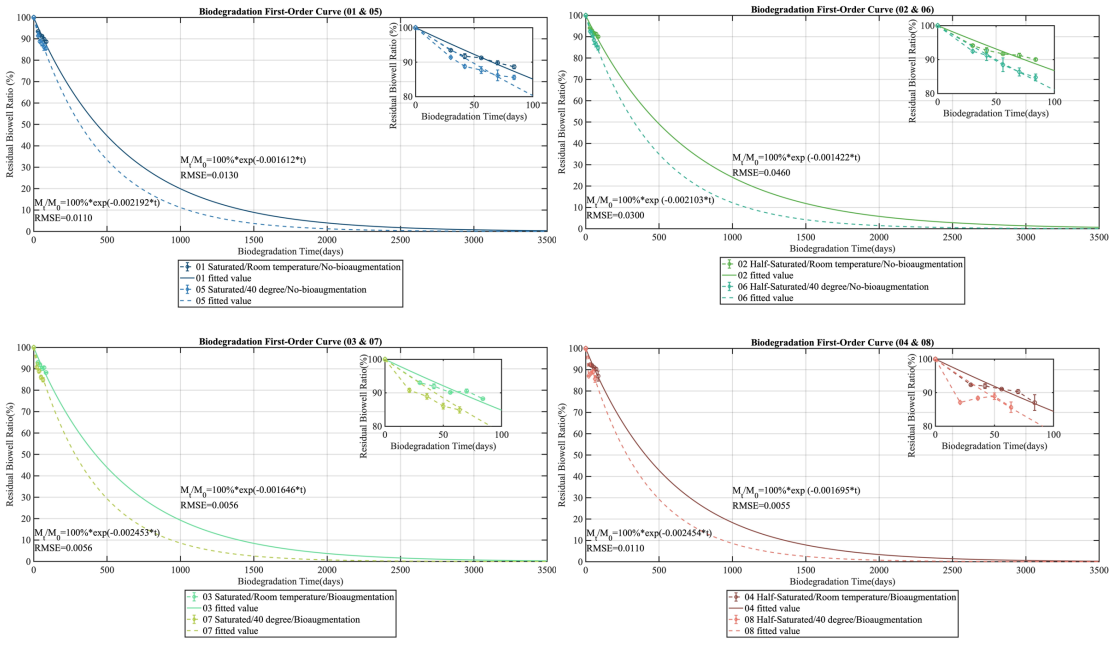


Figure 23. First-order Kinetics Model to Anticipate the Ultimate Biodegradation Time

4.4.4 Disintegration of Biowell Samples under Different Settings

Photographs from visual observations of each biowell sample are presented from ***Table A V 1*** to ***Table A V 12*** in **Appendix V**. A summarise of visual characteristics transformations are described in ***Table 19-Table 22.*** Structural disintegrations and microorganism's colonisation were observed on the sample surfaces during the three-month soil burial test.

On 26th April, only granules contained in mesh bags were taken out for the first

analysis after two weeks of burial. After washing, no visual differences (***Table A V 9*** **(a)-*Table A V 12* (a)**) were observed under the microscope. In the second sampling round, both fragments and granules samples were recovered for analysis. Shallow cracks could be observed on the inner surfaces of several pieces (Photographs of ***Table A V 1*****(a),** ***Table A V 3*** **(a)** and ***Table A V 4*** **(a**)). White spots and surface erosion began to appear from the edge of the biowell fragment (Photographs of ***Table A V 5*** **(a)**). Small cracks were witnessed in the third sampling time on more fragments (Photographs of ***Table A V 1*** **(b),** ***Table A V 2*** **(b),** ***Table A V 3*** **(b),** ***Table A V 4*** **(b)** and ***Table A V 7*** **(b)**). The breaks and cracks formed as a circle in the inner surfaces of those samples. On June 1st (after eight weeks), damages and delineation were observed to appear on the outer surfaces of several samples. Whereas, for the 06 setting, no apparent physical changes were noticed after eight weeks (***Table A V 6*** (c)). Different colours of microbial colonisation were observed on the surface of the 08 setting after eight weeks (***Table A V 8*** **(c)**). Some discolouring and whitish increments were observed on granules' surfaces, which also appeared on fragments. Compared with the third sampling round, cracks have become deeper and had an enlarging tendency (***Table A V 1*** **(d)-*Table A V 4* (d)**). White spots and dense erosion covered all over the inner surfaces. It was noticeable that different colours of biofilm demonstrated an extending tendency on the surface of the samples of setting 08 after ten weeks (***Table A V 8 (d)***). After twelve weeks, multi-layers of cracks occurred on the inner surfaces of setting 01-04, extending from the centre to the outer edge **(*Table A V 1* (e)-*Table A V 4* (e))**.

Table 19. Visual Observation for the First Time

The first sampling (2021.04.26)		
09	Granules	Granules clearly visible, no apparent differences
10		Granules clearly visible, no apparent differences
11		Granules clearly visible, roughness, discolouring
12		Granules clearly visible, no apparent differences

Table 20. Visual Observation for the Second, Third Time

The second sampling (2021.05.06)				***The third sampling (2021.05.18)***
Fragments	01	Cracks visible on the inner surface		Cracks visible on the inner surface and discolouring
	02	No apparent changes		Cracks visible on the inner surface
	03	Cracks visible on the inner surface and pink spots		Small cracks visible
	04	Discolouring		Cracks visible on the inner surface and discolouring
	05	Discolouring		Discolouring and surface erosion
	06	No apparent changes		Discolouring and surface erosion
	07	Discolouring		Deep cracks visible and soil attached into it
	08	Small cracks visible		Cracks visible on the inner surface
Granules	09	Granules clearly visible		Granules clearly visible
	10	Granules clearly visible		Granules clearly visible
	11	Discolouring		Cracks visible
	12	Granules clearly visible		Cracks visible on the inner surface

Table 21. Visual Observation for the Fourth and Fifth Time

		The fourth sampling (2021.06.01)		***The fifth sampling (2021.06.15)***
Fragments	01	Cracks visible on the inner surface		Circle-formed cracks visible on the inner surface; slight cracks on the outer surface
	02	Cracks visible on the inner, outer surface		Surface erosion; Circle-formed cracks visible in the inner surfaces
	03	Cracks visible on the inner, outer surface; discolouring		Circle-formed cracks visible on the inner surface; Small cracks on the outer surface
	04	Cracks visible on the inner outer surface; discolouring		Circle-formed cracks visible on the inner surface
	05	Pink spots; roughness on the inner, outer surface		Pinkish and whitish spots on the inner surface
	06	Slight cracks on the inner, outer surface		Black spots on the inner surface
	07	Roughness; whitish spots		Whitish spots
	08	With pink, black spots		Weathered and rough surfaces; black spots
Granules	09	Discolouring; soil attachment		Soil particles attached to the cracks
	10	Discolouring; roughness		Cracks and roughness on the surfaces
	11	Roughness; discolouring		Cracks on the surface
	12	Soil attachment; discolouring		Cracks on the surface

Table 22.Visual Observation for the Sixth Time

The sixth sampling (2021.06.29)					
Fragments	01	Cracks both in the inner and outer surface	**Granules**	09	Soil attachment, small cracks
	02	Cracks, erosion and surface roughness		10	Indication of disintegration
	03	Inner surface: multi layers of cracks		11	Cracks occurred on almost each granule
	04	multi layers of cracks, both in the central and side areas		12	Mycelium (probably) attached on the granules surface, serious erosion on the surface
	05	Erosion and roughness			
	06	Roughness, whitish spots colonization			
	07	High density of whitish spots			
	08	Severe discolouring and erosion, small cracks			

4.4.5 Water Adsorption

Water absorption (WA) was deduced from weight differences before and after drying. During the soil burial test period, the WA demonstrated an increasing tendency for the four samples under 20 ℃, from 22% to approximately 29%. The same trend was also observed from the other four samples under 40 ℃ with higher water adsorption values, starting from 33% to 42% on average.

4.4.6 Bacteria Counting

Table 23. Soil Samples Descriptions

Sample Code	Sample Name	Sampling Date	Sample type
01	Original Soil	26th April, 2021	Soil
02	Soil with Bioaugmentation	26th April, 2021	Soil
03	Room Temperature/No-Bioaugmentation/ Half-Saturated	29th June, 2021	Soil
04	Room Temperature/No-Bioaugmentation/Saturated	29th June, 2021	Soil
05	40 ℃/No bioaugmentation/Half-Saturated	29th June, 2021	Soil
06	40 ℃/Bioaugmentation/Saturated	29th June, 2021	Soil

Table 24. Bacteria Counting Results

Sampling Code	01 $(cells/g)$	02 $(cells/g)$	03 $(cells/g)$	04 $(cells/g)$	05 $(cells/g)$	06 $(cells/g)$
Sample Specific Detection Limit	$\mathbf{4.0 \times 10^3}$	$\mathbf{3.0 \times 10^3}$	$\mathbf{6.8 \times 10^3}$	$\mathbf{2.9 \times 10^3}$	$\mathbf{2.7 \times 10^3}$	$\mathbf{2.5 \times 10^3}$
Total Bacteria	$\mathbf{7.2 \times 10^8}$	$\mathbf{2.0 \times 10^9}$	$\mathbf{2.3 \times 10^{10}}$	$\mathbf{6.5 \times 10^9}$	$\mathbf{2.0 \times 10^{10}}$	$\mathbf{5.4 \times 10^9}$

*The detection limits are based on factors such as volume of samples, volumes used during analysis and efficiency of extraction. Therefore, the detection limit varies from sample based on internal control. Internal controls are used to ensure the quality of the Q-PCR reactions. Negative and positive controls are performed using pure cultures or by sequencing PCR products.

Basic soil information and bacteria counting results are demonstrated in ***Table 23*** and ***Table 24***, respectively. The Q-PCR results demonstrated that the original soil had contained a relatively high concentration of bacteria. Bioaugmentation increased the total bacteria amount in the original soil before experiments.

An increase in bacteria amount was observed in all soil samples after three months of experiments. Under room temperature, bacteria amount of half-saturated (03) was two orders of magnitude higher than the original soil (01), while that of saturated soil (04) was one order of magnitude higher. By contrast, under 40 ℃, bacteria merely increased by ten times in the half-saturated zone (05) and 2.7 times in saturated soil (06) than the original soil with bioaugmentation (02).

Chapter 5. Discussion

This research aimed to figure out the fate of the biowell in the subsurface and investigate the pros and cons of biowell. Firstly, interviews were conducted both internally and externally to provide robust evidence to arrange biowell appropriately in the future. Secondly, the biodegradation behaviour of biowell in different environments was tested with the aerobic soil burial test approach.

5.1 Feasibility to Implement Biowell in Practical Projects

The internal surveys showed that half of the respondents are willing to recommend biowell to clients whilst the other half still held uncertain attitudes (***Table 9***). It could be observed that lack of related knowledge towards biowell properties and mandatory provisions was the main barrier to promote it. Another worry might come from the unpredictability of re-monitoring in reality, under which circumstances biowell might bring about higher risk. At the same time, the pursuit of sustainability was the most vital element that respondents took into consideration. Thus, before implementing biowell into realistic projects, it is vital to take clients' and projects' demand and authorization into consideration to avoid high risk. The balance should be obtained between sustainable development and high-quality projects. A template to recommend biowell in the offer was suggested to provide a more environmentally-friendly opportunity for clients, promoting sustainability within soil & groundwater investigations.

The study conducted by Rujnić-Sokele & Pilipović (2017) confirmed that biodegradable plastics had been utilised in numerous short-time applications, where sustainability is an advantageous crucial characteristic. A comprehensive study by Ghosh & Jones (2021) presented the barriers and desired application state of biodegradable plastics. Their study illustrated that the trade-off between performance and degradability is expected be optimised and taken into consideration. Sudesh & Iwata (2008) also stated that biodegradable plastics might be superior regarding sustainability provided with the judicious and contented application.

5.2 Biodegradation under Natural Field Conditions

5.2.1 Indications from Visual Inspection

Visual inspection results demonstrated that slight structural transformations of biowell were observed, mainly due to its thickness. It is also noticeable that biowell became brittle after two years of burial as it could be broken by hand effortlessly. Besides, microbial colonisation was observed on the surface, with colour stratification on the upper and lower part of the used biowell (***Figure 11***).

Similar surface erosion and structural deformation were also observed on the biodegradable plastic films in the outdoor biodegradation experiments conducted by Amin et al. (2019). The biodegradation test done by Mumtaz et al. (2010) was also accompanied by structural disintegration and microbial colonisation characterises during biodegradation in natural soil conditions. Whereas, after 22 months of burial in their study, disintegrated tiny pieces of biodegradable plastics films were found attached with soil particles closely, demonstrating near-ultimate disintegration.

Rudnik & Briassoulis (2011) investigated that material thickness plays a vital role in influencing biodegradation rate, which could explain the low-speed biodegradation of biowell after 24 months of soil burial. Moreover, the results might also be attributed to the lack of favourable environmental conditions (temperature, microbial abundance, Etc.).

5.2.2 Interpretation of Groundwater Monitoring Data

Except for Arsenic (Ar) concentration, there existed no significant variance in other physical-chemical parameters between Pb1F and Pb2F statistically from the groundwater monitoring data (***Table 11***). Considering that Pb1F lay one meter away from the Pb2F, groundwater flow might lead to differences in metal ion concentration, resulting in a significant difference in Ar concentration. Briefly summarised, the results from the direct comparison of groundwater monitoring data indicate that the biowell is reliable to replace conventional groundwater monitoring well with regards to the physicochemical parameters in the short term.

5.2.3 Identification of Monomer and Small Oligomers

Py GC-MS test proved as an efficient means to estimate the degree of degradation (Westphal et al., 2001). This method helped gain insight into changes in chemical compositions after two years of outdoor soil burial. In this research, only noticeable changes in relative content and absolute composition of DL-latitude were detected (***Figure 17*** **&** ***Figure 18***). However, no significant changes were confirmed for the other three identified components, and no generated chemical compounds were identified.

Gallet et al. (2000) found similar results in the early period of his research when characterising matrix changes of biodegradable plastics exposed to the outdoor soil environment. However, lactic acid appeared due to hydrolysis between the 12th and 24th month of his research in the later stage. Lactic acid was not identified in aged biowell after two years of outdoor soil burial in this research. Lactic acid might probably be removed by assimilation while produced by hydrolysis at the same time (Gallet et al., 2000). Temperature and pyrolysis time also affect the number of chemicals that could be identified (Lu et al., 2011).

5.3 Differences of biodegradation rates under controlled conditions

5.3.1 Environmental Effects on the Biodegradation

Temperature - Temperature is regarded as one of the most significant environmental parameters affecting the biodegradation rate (Ho et al., 1999; Pischedda et al., 2019). Weight loss results from soil burial tests showed that degradation was higher under 40 ℃ than room temperature (***Figure 19***), because higher temperatures promote the soil microbial processes (Ho et al., 1999; Pischedda et al., 2019). The accelerating factor (AF) was defined as the ratio of the biodegradation rate at 40 ℃ and room temperature (***Table 25***). The AF values obtained were relatively consistent for the accelerating effect of higher temperature. Some previous studies also figured out that higher temperatures accelerated biodegradation rates (Lott et al., 2021; Šerá et al., 2020a). Analogous AF (~1.4) for PLA (Polylactic Acid, another biodegradation plastics made of corn starch) of the biodegradation rate at 37 ℃ and 25 ℃ was also

obtained by Šerá et al. (2020b). A greater extent of biodegradation under higher temperatures is also related to increased water absorption values (Wu, 2012).

Table 25. Biodegradation Rate and Acceleration Factors (Temperature)

Under Room Temperature	Biodegradation Rate k ($\% \, day^{-1}$)	Under 40 degrees	Biodegradation Rate k($\% \, day^{-1}$)	AF
01 (saturated/no-bioaugmentation)	0.001612	05 (saturated/no bioaugmentation)	0.002192	1.36
02 (half-saturated/no-bioaugmentation)	0.001422	06 (half-saturated/no-bioaugmentation)	0.002103	1.48
03 (saturated/bioaugmentation)	0.001646	07 (saturated/bioaugmentation)	0.002453	1.49
04 (half-saturated/bioaugmentation)	0.001695	08 (half-saturated/bioaugmentation)	0.002454	1.45

Soil Moisture - Contrary to the hypothesised association, no apparent differences were observed from biodegradation under saturated and half-saturated soil environments (***Figure 20***). The AF was defined as the ratio of the biodegradation rate under saturated and half-saturated soil (***Table 26***). The results were contradictory to the research done by Ho et al. (1999), who revealed that there was a positive correlation between the humidity and biodegradation rates. The results might be explained that the surface of the biowell is not sensitive to moisture. Zoungranan et al. (2020) figured out that soil moisture higher than 15% might cause excessive dilution, promoting the mobility of microorganisms. The movement of microorganisms might make them difficult to remain attached closely to biodegradable plastics.

Table 26. Biodegradation Rate and Acceleration Factors (Soil Moisture)

Saturated Zone	Biodegradation Rate k ($\% \, day^{-1}$)	Half-saturated Zone	Biodegradation Rate k ($\% \, day^{-1}$)	AF
01 (room temperature/no-bioaugmentation)	0.001612	02 (room temperature/no-bioaugmentation)	0.001422	1.13
03 (room temperature/bioaugmentation)	0.001646	04 (room temperature/bioaugmentation)	0.001695	0.97
05 (40 degree/no-bioaugmentation)	0.002192	06 (40 degree/no-bioaugmentation)	0.002103	1.04
07 (40 degree/half-bioaugmentation)	0.002453	08 (40 degree/half-bioaugmentation)	0.002454	1.00

Bioaugmentation - The AF was defined as the ratio of the biodegradation rate without and with bioaugmentation. Under the room temperature and saturated soil, bioaugmentation did not exert a positive effect on the biodegradation rate apparently (In the second raw of AF=1.02, ***Table 27***). In comparison, bioaugmentation exerted a slightly accelerating effect on biodegradation in the unsaturated zone (AF=1.19 under room temperature and AF=1.16 under 40 ℃, ***Table 27***).

Satti et al. (2018) found that bioaugmentation of soil could be successfully implemented to degrade biodegradable plastics waste in the soil with targeted isolated

microorganisms. However, due to time and research scope limits, no specific microorganisms aimed at promoting biowell biodegradation were isolated and used in this study, which might have a more profound effect on promoting biodegradation.

Table 27.Biodegradation Rate and Acceleration Factors (Bioaugmentation)

No Bioaugmentation	Biodegradation Rate $k\ (\%\ day^{-1})$	With Bio-Augmentation	Biodegradation Rate $k\ (\%\ day^{-1})$	AF
01 (Room temperature/Saturated)	0.001612	03 (Room temperature/Saturated)	0.001646	1.02
02 (Room temperature/Half-saturated)	0.001422	04 (Room temperature/Half-saturated)	0.001695	1.19
05 (40 degree/Saturated)	0.002192	07 (40 degree/Saturated)	0.002453	1.12
06 (40 degree/Half-Saturated)	0.002103	08 (40 degree/Half-Saturated)	0.002454	1.16

Abnormal Data - The first two biodegradation level values for setting 08 (fragments/40 ℃/unsaturated/bioaugmentation) were higher than that in the latter period, which was out of hypothesis (data points circled in ***Figure 19***). These abnormal data could be explained by the inhomogeneous distribution of microbial communities in the soil, which was also found by Boyandin et al. (2013).

5.3.2 Internal Effects on Biodegradation

Biodegradation rates for granules fluctuated during the three-month soil burial test and showed no uniform tendency (***Figure 22***). Firstly, measurement error might be caused by soil particles attached into some cracks on the surface of biowell granules, making it difficult to clean them thoroughly (see photographs after eight weeks in ***Table A V 9 - Table A V 12***). The soil particles attachment was apparently observed under the microscope. Secondly, it was difficult to separate tiny soil particles entering the mesh bags from some small biowell granules, which are likely to result in measurement errors. It seems no valid relationship between biodegradation rate and surface area could be drawn due to inaccurate weight loss results. Whilst interpreting this data, this should be taken into account. Based on these results, data showed no clear conclusion concerning biodegradation and surface area. However, visual results of granules provided different perspectives from fragments which is discussed in the next paragraph.

The study done by Chinaglia et al. (2018) has demonstrated that the available surface areas are correlated with the biodegradation rate positively. An enhanced degradation rate might be caused by increased depolymerization and fragmentations, enlarging reaction surface areas (Sintim et al., 2020).

5.3.3 Interpretation of Visual Results

Visual inspection results also qualitatively reveal potential deformation and disintegration features under different environmental settings, respectively (***Table 19-Table 22***). Surface erosion and structural deformations were regarded as representative indicators for biodegradation (Dutta et al., 2010; Tai et al., 2019; Weng

et al., 2010, 2013). Multi-layer of cracks gradually extended from the centre to the edge parts of the biowell fragments, both in the inner and outer surface for samples under room temperature. By contrast, discolouring and microbial colonisation appeared on surfaces of samples under 40 ℃. Mumtaz et al. (2010) found similar results when investigating the visual results of biodegradable plastics in soil using optical microscopy. They demonstrated that surface erosion indicated oxidation of the samples, accompanied by biofilm formation on its surface. Surface erosion could be used to indicated biodegradation owing to the contact and ingress of humidity (Tai et al., 2019).

An interesting phenomenon was captured under the microscope after twelve weeks of granules under 40 ℃/half-saturated/bioaugmentation settings. The potential web of mycelium grew on granules' surfaces (***Figure 24***), which indicated that fungi might promote biodegradation likewise. Mycelia growth of *P. stratus* was also observed on the surface of biodegradable plastics after 90 days from the study conducted by Maria Rodrigues da Luz et al.(2020). Their study addressed the performance of plastics biodegradation by Fungi. Lopez-Llorca & Colom Valiente (1993) confirmed soil fungi colonisation onto the starch-plastic film after placement in unsterile soil. No fungal appressoria was found on PHA films buried in the same soil conditions for a similar period. In this study, mycelia colonisation was not detected on surfaces of all samples after 12 weeks of burial as well. St.Leger et al. (1989) reported that polymer characteristics might play a significant role in fungal colonisation. Other factors such as nutrient concentration or surface hydrophobicity also influence fungal appressorium formation.

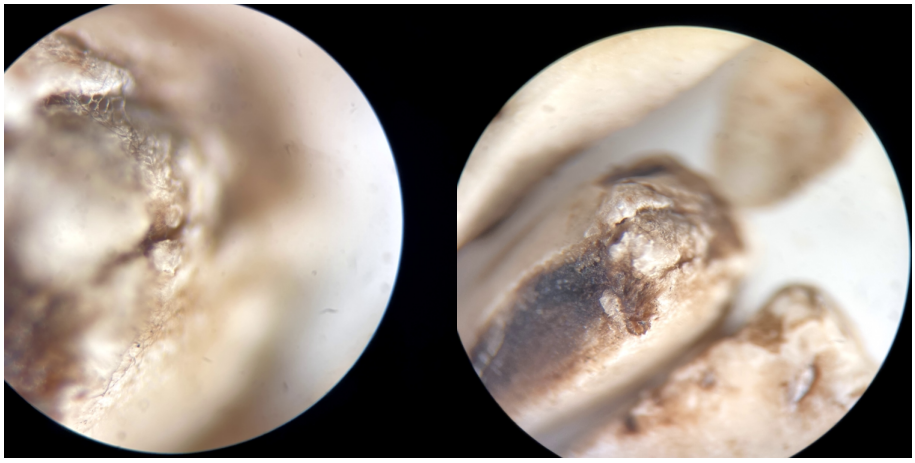


Figure 24. Mycelial Growth on the Surface of Biowell Granules

5.3.4 Biodegradation Time Estimation

According to ***Table 18***, it is estimated to take approximately 3000 (8.2 years) and 3500 (9.6 years) days for ultimate biodegradation under room temperature and 40 ℃. Several long-term studies were performed to estimate the ultimate biodegradation time of different types of biodegradable plastics.

Several previous studies addressed the 100% biodegradation time of different biodegradable plastics in soil conditions, mainly in mulch and film forms. Degradation studies conducted by Barragán et al. (2016) showed that MB mulch films

(Mater-Bi, another biodegradable plastic) buried under conditioned soil at 25 ℃ in an environmental chamber reached final biodegradation after 200 days of burial. It was also reported by Briassoulis et al. (2020) that the plateau phases of biodegradable plastics films with $25\ \mu m$ in thickness were approached at approximately 360 days of burial in clay-loam soil at 25 ℃. Moreover, Sintim et al. (2020) quantified the biodegradation time of plastic mulch films in agricultural soils using the second-polynomial function to extrapolate the experimental data. Their study demonstrated that it would take around 48-74 months for biodegradable plastic mulch films to attain 100% degradation in Mount Vernon.

One study done by Rudnik & Briassoulis, (2011) found that the thickness of biodegradable was related to biodegradation extent, which might justify the lower biodegradation rate of biowell compared with other studies. A possible explanation is that it is difficult for extracellular depolymerises to penetrate into the smooth and dense surfaces considering that biowell is 0.5 cm in thickness (Boyandin et al., 2013). However, the biodegradation rate is expected to increase in the later stage with the enlargement of reaction surface areas due to disintegration and fragmentation (Sintim et al., 2020). Combining the natural soil conditions, biowell thickness, and accelerating effect of disintegration, it implies that approximately 10 years is needed to approach 100% biodegradation for biowell.

5.3.5 Interpretation of Bacteria Amount

The microbial communities is a crucial evaluation parameter during the biodegradation process (Šerá et al., 2020a). Analysis of total bacteria amounts demonstrated that their concentrations were one or two orders of magnitude higher than that in the original soil after three months of soil burial test under room temperature. Meanwhile, total counts of bacteria were 2.7~10 times higher than that of the original soil with bioaugmentation under 40 ℃ (***Table 24***). An increase of similar magnitude of bacteria growth was also found by Balandin et al. (2013) after six months of natural soil burial of biodegradable plastics at 25 ℃.

Higher bacteria increasing were observed from half-saturated soil than saturated soil, which might be attributed to easier availability to oxygen (Llana-Ruíz-Cabello et al., 2016). Moreover, higher bacterial abundance were observed of soil under room temperature, which is in line with the findings of Sintim et al. (2020). Their study stated that temperature played a significant role to promote microorganisms' activities. They illustrated that bacterial abundances did not change in Knoxville (a humid tropical climate in Tennessee) while increasing in Mount Vernon (a cool Mediterranean climate in Washington). However, a greater extent of biodegradation of biodegradable plastic mulches was observed in the soil in Knoxville than in Mount Vernon, which might represent an increased capacity for biodegradation.

Chapter 6. Conclusions

This research aimed to determine the pros and cons of biowells in future groundwater & soil investigation projects and investigate the fate of biowells in the subsurface. The main research question was: ***"What is the fate of the biodegradable groundwater monitoring well in the soil?"*** This chapter covers the conclusions of this research and addresses the main research question. Furthermore, limitations of this study are presented, ending with recommendations for future research.

6.1 Favourable Conditions to Implement Biowell

Interviews were set out to figure out the compositions and mechanical properties of the biowell to identify the most appropriate occasions to implement biowell. The biowell is considered the favourable solution for short-term projects (e.g., projects in the public domain and Phase II in Due Diligence projects) due to its advantage of biodegradability and sustainability. Still, it lacks applicability in long-term projects considering risks of contamination and re-monitoring (e.g., remediation projects). The promotion of biowell still depends on forced authorization, extra benefits and significance of sustainability demand from clients. It could be concluded that implementation of biowell in a limited percentage, concentrating on short-term investigations and sustainability-orientated projects, might obtain the balance of high-quality projects and sustainability pursuit.

6.2 Biodegradation under Natural Soil Conditions

Py GC-MS test and visual inspection results of the used biowell gained insight into biodegradation performance under natural soil conditions. No newly-generated chemical compounds were detected after two years of burial. However, the absolute quantity of identified compounds differed after two years of burial, indicating the chemical transformations brought about from biodegradation. Combined with results from visual inspection, it demonstrated that biodegradation of biowell took place at a relative low speed under natural soil conditions, which retains at 10 ℃ of groundwater.

6.3 Biodegradation under Laboratory Soil Conditions

It was found that biowell samples in fragment and granules form could be biodegraded under laboratory conditions. The degree of biodegradation could reach 17.75% and 12.97% under room temperature and 40 ℃, respectively after twelve weeks under experimental conditions. In different environmental conditions, the biodegradation phenomenon demonstrated various characteristics. Cracks were observed under room temperatures, while surface erosion were noticed more frequently under 40 ℃. Biodegradation could be simulated via higher temperature by acceleration factor of approximately 1.4. Bacteria amounts in the soil under both room temperature and 40 ℃ increased than the original soil. Lower augmentation of bacteria amount under 40 ℃ might be caused by the increased biodegradability capacity of microorganisms activities.

Considering that laboratory studies were conducted under favourable conditions, higher temperatures under actual occasions might be unrealistic. So, it takes a longer

time to biodegrade under natural field conditions.

6.4 Overall Conclusion

Overall, the continuous biodegradability of biowell under a handful of laboratory settings was verified. Slowly-proceeding biodegradation behaviour under natural soil conditions was also observed and confirmed. Extrapolation with linear first-order kinetic modelling indicate that it might take approximately ten years for biowell to approach complete biodegradation under the natural soil conditions. To conclude, the research set out to investigate the fate of biodegradable groundwater monitoring well for better strategy determination. To further develop strategies of arranging biowell, results showed that it is necessary to reveal more background information with stakeholders and take projects demand (sustainability and re-monitoring) into account because the biowell is a feasible alternative in short-term monitoring projects, whereas in other projects will lose its applicability.

6.5 Limitations and Recommendations

It is essential to acknowledge the limitations of this research. First, background information of biowell production primarily came from stakeholder interviews rather than official documents support, resulting in the incompleteness and inaccuracy of information about raw materials. Furthermore, due to time limits and research scope, a long-term soil burial test could not be designed and conducted. The number of replicates had to be reduced considering feasibility. However, more replicates and a more extended period are necessary to provide more valid conclusions. For each variable (temperature, soil moisture, bioaugmentation and surface areas), merely two scenarios were utilised, adding difficulty to comprehensively evaluating their effects on biodegradation. Furthermore, it is beyond the scope of this study to investigate physical and chemical transformations on a molecular scale due to the deficiency of experimental methods.

An extended scope of respondents is also recommended to gain insight to people's attitude towards developing bio-based techniques in the future. To fully understand the biodegradation kinetics and ultimate biodegradation time, a long-term (> 2 years) soil burial test is recommended to determine the environmental persistence in the soil more accurately. Moreover, other analytical methods are also commonly recommended and used to monitor biodegradation of biodegradable plastics, such as Infrared (IR) Spectroscopy, Scanning Electron Microscope (SEC), Nuclear Magnetic Resonance (NRM), to better characterise different biodegradation behaviour.

References:

Al-Salem, S. M., Antelava, A., Constantinou, A., Manos, G., & Dutta, A. (2017). A review on thermal and catalytic pyrolysis of plastic solid waste (PSW). *Journal of Environmental Management*, *197*, 177–198. https://doi.org/10.1016/j.jenvman.2017.03.084

Alvarez, V. A., Ruseckaite, R. A., & Vázquez, A. (2006). Degradation of sisal fibre/Mater Bi-Y biocomposites buried in soil. *Polymer Degradation and Stability*, *91*(12), 3156–3162. https://doi.org/10.1016/j.polymdegradstab.2006.07.011

Amin, Md. R., Chowdhury, M. A., & Kowser, Md. A. (2019). Characterization and performance analysis of composite bioplastics synthesized using titanium dioxide nanoparticles with corn starch. *Heliyon*, *5*(8), e02009. https://doi.org/10.1016/j.heliyon.2019.e02009

ASTM D5988. (2018). *Test Method for Determining Aerobic Biodegradation of Plastic Materials in Soil*. ASTM International. https://doi.org/10.1520/D5988-18

Badia, J. D., Strömberg, E., Kittikorn, T., Ek, M., Karlsson, S., & Ribes-Greus, A. (2017). Relevant factors for the eco-design of polylactide/sisal biocomposites to control biodegradation in soil in an end-of-life scenario. *Polymer Degradation and Stability*, *143*, 9–19. https://doi.org/10.1016/j.polymdegradstab.2017.06.004

Barbale, M., Chinaglia, S., Gazzilli, A., Pischedda, A., Pognani, M., Tosin, M., & Degli-Innocenti, F. (2021). Hazard profiling of compostable shopping bags. Towards an ecological risk assessment of littering. *Polymer Degradation and Stability*, *188*, 109592. https://doi.org/10.1016/j.polymdegradstab.2021.109592

Barragán, D. H., Pelacho, A. M., & Martin-Closas, Ll. (2016). Degradation of agricultural biodegradable plastics in the soil under laboratory conditions. *Soil Research*, *54*(2), 216. https://doi.org/10.1071/SR15034

Bátori, V., Åkesson, D., Zamani, A., Taherzadeh, M. J., & Sárvári Horváth, I. (2018). Anaerobic degradation of bioplastics: A review. *Waste Management*, *80*, 406–413. https://doi.org/10.1016/j.wasman.2018.09.040

Bilo, F., Pandini, S., Sartore, L., Depero, L. E., Gargiulo, G., Bonassi, A., Federici, S., & Bontempi, E. (2018). A sustainable bioplastic obtained from rice straw. *Journal of Cleaner Production*, *200*, 357–368. https://doi.org/10.1016/j.jclepro.2018.07.252

Boyandin, A. N., Prudnikova, S. V., Karpov, V. A., Ivonin, V. N., Đỗ, N. L., Nguyễn, T. H., Lê, T. M. H., Filichev, N. L., Levin, A. L., Filipenko, M. L., Volova, T. G., & Gitelson, I. I. (2013). Microbial degradation of polyhydroxyalkanoates in tropical soils. *International Biodeterioration & Biodegradation*, *83*, 77–84. https://doi.org/10.1016/j.ibiod.2013.04.014

Briassoulis, D., Mistriotis, A., Mortier, N., & Tosin, M. (2020). A horizontal test method for biodegradation in soil of bio-based and conventional plastics and lubricants. *Journal of Cleaner Production*, *242*, 118392. https://doi.org/10.1016/j.jclepro.2019.118392

Broeren, M. L. M., Kuling, L., Worrell, E., & Shen, L. (2017). Environmental impact

assessment of six starch plastics focusing on wastewater-derived starch and additives. *Resources, Conservation and Recycling*, *127*, 246–255. https://doi.org/10.1016/j.resconrec.2017.09.001

Castillo, M., Martinorue, S., Manzanilla, E., Badiola, I., Martin, M., & Gasa, J. (2006). Quantification of total bacteria, enterobacteria and lactobacilli populations in pig digesta by real-time PCR. *Veterinary Microbiology*, *114*(1–2), 165–170. https://doi.org/10.1016/j.vetmic.2005.11.055

Castro-Aguirre, E., Auras, R., Selke, S., Rubino, M., & Marsh, T. (2018). Enhancing the biodegradation rate of poly(lactic acid) films and PLA bio-nanocomposites in simulated composting through bioaugmentation. *Polymer Degradation and Stability*, *154*, 46–54. https://doi.org/10.1016/j.polymdegradstab.2018.05.017

Charles, J. F. (2020). *Evaluation of the impact of flood on groundwater quality in hand-dug wells in Monrovia, Liberia*. 8.

Charles, J. F., Ngumbu, R. S., Toe, J. T., & Sangodoyin, A. Y. (2020). Evaluation of the impact of flood on groundwater quality in hand-dug wells in Monrovia, Liberia. *International Journal of Energy and Water Resources*, *4*(2), 181–188. https://doi.org/10.1007/s42108-020-00060-x

Chinaglia, S., Tosin, M., & Degli-Innocenti, F. (2018). Biodegradation rate of biodegradable plastics at molecular level. *Polymer Degradation and Stability*, *147*, 237–244. https://doi.org/10.1016/j.polymdegradstab.2017.12.011

Dutta, S., Karak, N., Saikia, J. P., & Konwar, B. K. (2010). Biodegradation of Epoxy and MF Modified Polyurethane Films Derived from a Sustainable Resource. *Journal of Polymers and the Environment*, *18*(3), 167–176. https://doi.org/10.1007/s10924-010-0161-8

El Fantroussi, S., & Agathos, S. N. (2005). Is bioaugmentation a feasible strategy for pollutant removal and site remediation? *Current Opinion in Microbiology*, *8*(3), 268–275. https://doi.org/10.1016/j.mib.2005.04.011

Emadian, S. M., Onay, T. T., & Demirel, B. (2017). Biodegradation of bioplastics in natural environments. *Waste Management*, *59*, 526–536. https://doi.org/10.1016/j.wasman.2016.10.006

EN 14045. (2003). *Packaging-Evaluation of the Disintegration of Packaging Materials in Practical Oriented Tests under Defined Composting Conditions.*

Ezenwaji, E. E. (2019). Spatial analysis of groundwater quality in Warri Urban, Nigeria. *Sustainable Water Resources Management*, 10.

Folino, A., Karageorgiou, A., Calabrò, P. S., & Komilis, D. (2020). Biodegradation of wasted bioplastics in natural and industrial environments: A review. *Sustainability (Switzerland)*, *12*(15), 1–37. https://doi.org/10.3390/su12156030

Gadhave, R. V., Das, A., Mahanwar, P. A., & Gadekar, P. T. (2018). *Starch Based Bio-Plastics: The Future of Sustainable Packaging*. https://doi.org/10.4236/ojpchem.2018.82003

Gallet, G., Lempiäinen, R., & Karlsson, S. (2000). Characterisation by solid phase microextraction–gas chromatography–mass spectrometry of matrix changes of poly(l-lactide) exposed to outdoor soil environment. *Polymer Degradation and Stability*, *71*(1), 147–151. https://doi.org/10.1016/S0141-3910(00)00165-8

Ghosh, K., & Jones, B. H. (2021). Roadmap to Biodegradable Plastics—Current State and Research Needs. *ACS Sustainable Chemistry & Engineering*, *9*(18), 6170–6187. https://doi.org/10.1021/acssuschemeng.1c00801

Ho, K.-L. G., Pometto, A. L., & Hinz, P. N. (1999). Effects of Temperature and Relative Humidity on Polylactic Acid Plastic Degradation. *Journal of Environmental Polymer Degradation*, *7*(2), 83–92. https://doi.org/10.1023/A:1021808317416

Hughes, R. (2013). On a European strategy on Plastic Waste in the Environment. *Journal of Chemical Information and Modeling*, *53*(9), 287.

ISO 10390, 2005. (2005). *Soil quality—Determination of pH*. https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/04/08/40879.html

ISO 10694. (1995). *Soil quality—Determination of organic and total carbon after dry combustion (elementary analysis)*. https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/01/87/18782.html

ISO 11274. (2019). *Soil quality—Determination of the water-retention characteristic—Laboratory methods*. https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/06/82/68256.html

ISO 17556. (2020). *INTERNATIONAL STANDARD Plastics—Determination of the ultimate aerobic biodegradability of plastic materials in soil by*. *2019*.

ISO 17556, 2012. (2012). *Plastics-Determination of the Ultimate Aerobic Biodegradability of Plastic Materials in Soil by Measuring the Oxygen Demand in a Respirometer or the Amount of Carbon Dioxide Evolved*. International Organization for Standardization Geneva.

Kjeldsen, A., Price, M., Lilley, C., Guzniczak, E., & Archer, I. (2019). A Review of Standards for Biodegradable Plastics with support from. *Industrial Biotechnology Innovation Centre IBioIC*, 28.

Kubista, M., Andrade, J. M., Bengtsson, M., Forootan, A., Jonák, J., Lind, K., Sindelka, R., Sjöback, R., Sjögreen, B., Strömbom, L., Ståhlberg, A., & Zoric, N. (2006). The real-time polymerase chain reaction. *Molecular Aspects of Medicine*, *27*(2–3), 95–125. https://doi.org/10.1016/j.mam.2005.12.007

Llana-Ruíz-Cabello, M., Pichardo, S., Jiménez-Morillo, N. T., Abad, P., Guillamón, E., González-Vila, F. J., Cameán, A. M., & González-Pérez, J. A. (2016). Characterisation of a bio-based packaging containing a natural additive from Allium spp. Using analytical pyrolysis and carbon stable isotopes. *Journal of Analytical and Applied Pyrolysis*, *120*, 334–340. https://doi.org/10.1016/j.jaap.2016.05.021

Lopez-Llorca, L. V., & Colom Valiente, M. F. (1993). Study of biodegradation of starch-

plastic films in soil using scanning electron microscopy. *Micron*, *24*(5), 457–463. https://doi.org/10.1016/0968-4328(93)90024-U

Lott, C., Eich, A., Makarow, D., Unger, B., van Eekert, M., Schuman, E., Reinach, M. S., Lasut, M. T., & Weber, M. (2021). Half-Life of Biodegradable Plastics in the Marine Environment Depends on Material, Habitat, and Climate Zone. *Frontiers in Marine Science*, *8*. https://doi.org/10.3389/fmars.2021.662074

Lu, Q., Yang, X., Dong, C., Zhang, Z., Zhang, X., & Zhu, X. (2011). Influence of pyrolysis temperature and time on the cellulose fast pyrolysis products: Analytical Py-GC/MS study. *Journal of Analytical and Applied Pyrolysis*, *92*(2), 430–438. https://doi.org/10.1016/j.jaap.2011.08.006

Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F., & Nava-Saucedo, J.-E. (2008). Polymer biodegradation: Mechanisms and estimation techniques – A review. *Chemosphere*, *73*(4), 429–442. https://doi.org/10.1016/j.chemosphere.2008.06.064

Maria Rodrigues da Luz, J., de Cássia Soares da Silva, M., Ferreira dos Santos, L., & Catarina Megumi Kasuya, M. (2020). Plastics Polymers Degradation by Fungi. In M. Blumenberg, M. Shaaban, & A. Elgaml (Eds.), *Microorganisms*. IntechOpen. https://doi.org/10.5772/intechopen.88608

McColl, A., Smith, H., White, P., & Field, J. (1998). *General practitioners' perceptions of the route to evidence based medicine: A questionnaire survey*. *316*, 7.

Mooney, B. P. (2009). The second green revolution? Production of plant-based biodegradable plastics. *Biochemical Journal*, *418*(2), 219–232. https://doi.org/10.1042/BJ20081769

Mumtaz, T., Khan, M. R., & Hassan, M. A. (2010). Study of environmental biodegradation of LDPE films in soil using optical and scanning electron microscopy. *Micron*, *41*(5), 430–438. https://doi.org/10.1016/j.micron.2010.02.008

Pischedda, A., Tosin, M., & Degli-Innocenti, F. (2019). Biodegradation of plastics in soil: The effect of temperature. *Polymer Degradation and Stability*, *170*, 109017. https://doi.org/10.1016/j.polymdegradstab.2019.109017

Polman, E. M. N., Gruter, G.-J. M., Parsons, J. R., & Tietema, A. (2021). Comparison of the aerobic biodegradation of biopolymers and the corresponding bioplastics: A review. *Science of The Total Environment*, *753*, 141953. https://doi.org/10.1016/j.scitotenv.2020.141953

Rudnik, E., & Briassoulis, D. (2011). Comparative Biodegradation in Soil Behaviour of two Biodegradable Polymers Based on Renewable Resources. *Journal of Polymers and the Environment*, *19*(1), 18–39. https://doi.org/10.1007/s10924-010-0243-7

Ruggero, F., Gori, R., & Lubello, C. (2019). Methodologies to assess biodegradation of bioplastics during aerobic composting and anaerobic digestion: A review. *Waste Management and Research*, *37*(10), 959–975. https://doi.org/10.1177/0734242X19854127

Rujnić-Sokele, M., & Pilipović, A. (2017). Challenges and opportunities of biodegradable plastics: A mini review. *Waste Management & Research: The Journal for a Sustainable*

Circular Economy, *35*(2), 132–140. https://doi.org/10.1177/0734242X16683272

Salunkhe, V. P., Sawant, I. S., Banerjee, K., Wadkar, P. N., Sawant, S. D., & Hingmire, S. A. (2014). Kinetics of degradation of carbendazim by B. subtilis strains: Possibility of in situ detoxification. *Environmental Monitoring and Assessment*, *186*(12), 8599–8610. https://doi.org/10.1007/s10661-014-4027-8

Sarasa, J., Gracia, J. M., & Javierre, C. (2009). Study of the biodisintegration of a bioplastic material waste. *Bioresource Technology*, *100*(15), 3764–3768. https://doi.org/10.1016/j.biortech.2008.11.049

Satti, S. M., Shah, A. A., Marsh, T. L., & Auras, R. (2018). Biodegradation of Poly(lactic acid) in Soil Microcosms at Ambient Temperature: Evaluation of Natural Attenuation, Bio-augmentation and Bio-stimulation. *Journal of Polymers and the Environment*, *26*(9), 3848–3857. https://doi.org/10.1007/s10924-018-1264-x

Šerá, J., Serbruyns, L., De Wilde, B., & Koutný, M. (2020a). Accelerated biodegradation testing of slowly degradable polyesters in soil. *Polymer Degradation and Stability*, *171*(2020). https://doi.org/10.1016/j.polymdegradstab.2019.109031

Šerá, J., Serbruyns, L., De Wilde, B., & Koutný, M. (2020b). Accelerated biodegradation testing of slowly degradable polyesters in soil. *Polymer Degradation and Stability*, *171*, 109031. https://doi.org/10.1016/j.polymdegradstab.2019.109031

Shafqat, A., Tahir, A., Mahmood, A., Tabinda, A. B., Yasar, A., & Pugazhendhi, A. (2020). A review on environmental significance carbon foot prints of starch based bio-plastic: A substitute of conventional plastics. *Biocatalysis and Agricultural Biotechnology*, *27*, 101540. https://doi.org/10.1016/j.bcab.2020.101540

Shogren, R. L., Fanta, G. F., & Doane, W. M. (1993). Development of Starch Based Plastics—A Reexamination of Selected Polymer Systems in Historical Perspective. *Starch - Stärke*, *45*(8), 276–280. https://doi.org/10.1002/star.19930450806

Sintim, H. Y., Bary, A. I., Hayes, D. G., Wadsworth, L. C., Anunciado, M. B., English, M. E., Bandopadhyay, S., Schaeffer, S. M., DeBruyn, J. M., Miles, C. A., Reganold, J. P., & Flury, M. (2020). In situ degradation of biodegradable plastic mulch films in compost and agricultural soils. *Science of The Total Environment*, *727*, 138668. https://doi.org/10.1016/j.scitotenv.2020.138668

Standard, E. N. (2000). 13432: 2000 Packaging—Requirements for Packaging Recoverable through Composting and Biodegradation—Test Scheme and Evaluation Criteria for the Final Acceptance of Packaging. *European Committee for Standardization: Brussel, Belgium*.

St.Leger, R. J., Butt, T. M., Goettel, M. S., Staples, R. C., & Roberts, D. W. (1989). Productionin vitro of appressoria by the entomopathogenic fungusMetarhizium anisopliae. *Experimental Mycology*, *13*(3), 274–288. https://doi.org/10.1016/0147-5975(89)90049-2

Sudesh, K., & Iwata, T. (2008). Sustainability of Biobased and Biodegradable Plastics. *CLEAN - Soil, Air, Water*, *36*(5–6), 433–442. https://doi.org/10.1002/clen.200700183

Tai, N. L., Adhikari, R., Shanks, R., & Adhikari, B. (2019). Aerobic biodegradation of starch–polyurethane flexible films under soil burial condition: Changes in physical structure and chemical composition. *International Biodeterioration & Biodegradation*, *145*, 104793. https://doi.org/10.1016/j.ibiod.2019.104793

Tokiwa, Y., Calabia, B. P., Ugwu, C. U., & Aiba, S. (2009). Biodegradability of Plastics. *International Journal of Molecular Sciences*, *10*(9), 3722–3742. https://doi.org/10.3390/ijms10093722

Walsh, J. H. (2001). Ecological considerations of biodeterioration. *International Biodeterioration & Biodegradation*, *48*(1), 16–25. https://doi.org/10.1016/S0964-8305(01)00063-4

Weng, Y.-X., Jin, Y.-J., Meng, Q.-Y., Wang, L., Zhang, M., & Wang, Y.-Z. (2013). Biodegradation behaviour of poly(butylene adipate-co-terephthalate) (PBAT), poly(lactic acid) (PLA), and their blend under soil conditions. *Polymer Testing*, *32*(5), 918–926. https://doi.org/10.1016/j.polymertesting.2013.05.001

Weng, Y.-X., Wang, Y., Wang, X.-L., & Wang, Y.-Z. (2010). Biodegradation behavior of PHBV films in a pilot-scale composting condition. *Polymer Testing*, *29*(5), 579–587. https://doi.org/10.1016/j.polymertesting.2010.04.002

Westphal, C., Perrot, C., & Karlsson, S. (2001). Py-GC/MS as a means to predict degree of degradation by giving microstructural changes modelled on LDPE and PLA. *Polymer Degradation and Stability*, *73*(2), 281–287. https://doi.org/10.1016/S0141-3910(01)00089-1

Wu, C.-S. (2012). Preparation, characterization, and biodegradability of renewable resource-based composites from recycled polylactide bioplastic and sisal fibers. *Journal of Applied Polymer Science*, *123*(1), 347–355. https://doi.org/10.1002/app.34223

Wu, C.-S. (2014). Preparation and Characterization of Polyhydroxyalkanoate Bioplastic-Based Green Renewable Composites from Rice Husk. *Journal of Polymers and the Environment*, *22*(3), 384–392. https://doi.org/10.1007/s10924-014-0662-y

Yatigala, N. S., Bajwa, D. S., & Bajwa, S. G. (2018). Compatibilization improves physico-mechanical properties of biodegradable biobased polymer composites. *Composites Part A: Applied Science and Manufacturing*, *107*, 315–325. https://doi.org/10.1016/j.compositesa.2018.01.011

Zhang, C., Valsaraj, K. T., Constant, W. D., & Roy, D. (1999). Aerobic biodegradation kinetics of four anionic and nonionic surfactants at sub- and supra-critical micelle concentrations (CMCs). *Water Research*, *33*(1), 115–124. https://doi.org/10.1016/S0043-1354(98)00170-5

Zhou, L., Zhao, G., & Jiang, W. (2016). Mechanical properties of biodegradable polylactide/poly(ether-block-amide)/thermoplastic starch blends: Effect of the crosslinking of starch. *Journal of Applied Polymer Science*, *133*(2). https://doi.org/10.1002/app.42297

Zoungranan, Y., Lynda, E., Dobi-Brice, K. K., Tchirioua, E., Bakary, C., & Yannick, D. D. (2020). Influence of natural factors on the biodegradation of simple and composite bioplastics

based on cassava starch and corn starch. *Journal of Environmental Chemical Engineering*, *8*(5), 104396. https://doi.org/10.1016/j.jece.2020.104396

Appendix I. Interview guide

Table A / 1. Interview Guide

Date:	
Location of interview:	
Interview:	
Organization:	
Function of interviewee:	

The objective of semi-structured interviews is to identify the pros and cons of the biodegradable groundwater monitoring well from producers, manufacturers, and consumers to reach the research objectives from one more social mood. Different types of questions are asked, which are dependent on the expertise of the interviewees. Questions are listed below, and approval was requested from interviewees for recording the interview content. These recordings are used for transcribing the results of the interviews and for further analysis.

1. **Introduction Questions:**
   - 1.1. Could you give a brief self-introduction and your research/working content please?
   - 1.2. What is your initiative interest in bio-based industry?
   - 1.3. (For producers and manufactures) What is your motivation in developing this kind of bio-based product?
2. **Presence of conditions**
   - 2.1. Could you provide a general oversight on the manufacturing cycle from crop growth to final distribution?
   - 2.2. What are the additives (mentals, resins, plasticizers or other natural raw materials) to the biobuis? How will they end up in the natural environment?
   - 2.3. Will the additives exert influence to the biodegradation process?
   - 2.4. What is the processing technology and production process of starch and the final product?
   - 2.5. Will it add water and energy requirement compared with conventional plastics production process?
   - 2.6. What are the physical parameters of this material?
   - 2.7. Will it take longer time for the materials to biodegrade for low temperature and high humidity conditions? Did you do tests on this yourself?
   - 2.8. Do you have a carbon footprint of the production of Solanyl?
3. **Presence of conditions**
   - 3.1. Where do TAUW always conduct short-term groundwater & soil research?
   - 3.2. Would you like to recommend biowell instead of conventional groundwater monitoring well to your clients?
   - 3.3. What is your attitude about using biodegradable monitoring well in your project?
   - 3.4. What are your expectations towards characteristics/properties of biowell when used in practical projects?

Appendix II. List of respondents

Table A // 1. Overview of the Interviewees from TAUW

Name	Business Unit	Function(s)	Location
TAUW Netherlands			
Elroy Houthuijzen	MIA public & private	TM/PL	Amsterdam
Alianne Bouma	MIA public	TM/PL	Assen
Marloes Cruijsen	MIA public & private	PL	Deventer
Micha van den Booger	BUI private	PL/TM	Deventer
Monica Martens	BUI private	PC	Deventer
Stefan Kasemier	MIA public & private	PA	Deventer
Wouter Hageman	BUI private	PL	Deventer
Joost Bakx	BUI private	TM/PL	Deventer/Rotterdam
Willem Hulsen	MIA public & private	PL	Eindhoven
Yacintha Mulder	MIA public & private	TM/PL	Rotterdam
Rob Rensen	BUI public & private	TM/PL	Rotterdam
Joost Pierik	MIA public & private	PL	Utrecht
Sanne Smouter	MIA public & private	PA	Utrecht
TAUW Belgium			
Herwig de Wilde	Soil exploration and remediation	PM	Lokeren
TAUW France			
Aurélie Houth	Industrials and real estate	EXP	Paris
TAUW Germany			
Patrick Jacob	Soil and Groundwater Investigation	EXP	Berlin
Matthias Sumann	Industrial Sites/soil & groundwater investigations and remediation	PM	Moers
Alfredo Perez de Mora	BU Site Development	PM	Munich
TAUW Iberia			
Anna Mestres	Industrial Sites/soil & groundwater investigations and remediation	Business manager	Barcelona
Ignacio Barco	Soil Manager	Senior Consultant	Madrid
TAUW Italy			
Luca Ballabio	Soil Department	PL	Milano
Andrea Piontkowsky	Soil Department	PL	Milano

EXP = expert; PA = project advisor; PC = project Coordinator; PL = project leader; PM = project manager; TM = team manager; MIA (Meten Inspectie en Advies), BULO (Business Unit Leefomgeving), BUI (Business Unit Industry)

Appendix III. Groundwater monitoring results from 2019-2021

Table A III 1. Groundwater Monitoring Data from Biowell (2019-2021)

Measure point		**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
Description		Pb 1 F (1,7-2,7)	Pb 1 F (1,7-2,7)	Pb 1 F (1,7-2,7)	Pb 1 F (1,7-2,7)	Pb 1 F (1,7-2,7)	Pb 1 F (1,7-2,7)	Pb 1 F (1,7-2,7)	Pb 1 F (1,7-2,7)	Pb 1 F (1,7-2,7)	Pb 1 F (1,7-2,7)
Range (cm-mv)		170-270	170-270	170-270	170-270	170-270	170-270	170-270	170-270	170-270	170-270
Date		20/3/15	26/4/19	01/5/19	27/5/19	26/6/19	17/7/19	24/9/19	18/12/19	17/3/20	29/3/21
METALS											
Arsenic (As)	ug/l	8,1	14	15	14	< 5	39	57	9	6,6	6,2
Barium (Ba)	µg/l	140	140	170	180	170	130	810	400	210	210
Cadmium (Cd)	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
Cobalt (Co)	ug/l	5,5	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Copper (Cu)	ug/l	< 2	< 2	< 2	< 2	< 2	< 2	3,4	< 2	< 2	< 2
Mercury (Hg)	ug/l	< 0,05	0,2	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05
Lead (Pb)	ug/l	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Molybdenum (Mo)	ug/l	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Nickel (Ni)	ug/l	4,2	< 3	< 3	< 3	< 3	< 3	4,1	< 3	< 3	< 3
Zinc (Zn)	ug/l	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
AROMATIC COMPOUNDS											
Benzene	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
Ethylbenzene	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
Toluene	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
Xylenen (som, 0.7 factor)	ug/l	< 0,21	< 0,21	< 0,21	< 0,21	< 0,21	< 0,21	< 0,21	< 0,21	< 0,21	< 0,21
Sum of Xylenes		**0,21	** 0,21	** 0,21	** 0,21	** 0,21	** 0,21	** 0,21	** 0,21	** 0,21	** 0,21

Styrene	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
som 16 aromatische oplosmiddelen (Bbk, 1-1-2008)	ug/l	** 0,77	** 0,77	**0,77	** 0,77	** 0,77	** 0,77	** 0,77	** 0,77	** 0,77	** 0,77
POLYCYCLIC AROMATIC HYDROCARBONS											
Naphthalene	ug/l	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02
CHLORINATED HYDROCARBONS											
Vinyl Chloride	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Dichloromethane	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
1,1-Dichloroethane	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
1,2-Dichloroethane	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
1,1-Dichloroethylene	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
1,2-Dichloorethenen (som, 0.7 factor)	ug/l	< 0,14	< 0,14	< 0,14	< 0,14	< 0,14	< 0,14	< 0,14	< 0,14	< 0,14	< 0,14
Sum of 1,2-Dichloroethenes			** 0,14	** 0,14	** 0,14	** 0,14	** 0,14	** 0,14	** 0,14	** 0,14	
Dichloorpropanen (0,7 som, 1,1+1,2+1,3)	ug/l	0,42	0,42	0,42	0,42	0,42	0,42	0,42	0,42	0,42	0,42
Dichloorpropaan		** 0,42	** 0,42	** 0,42	** 0,42	** 0,42	** 0,42	** 0,42	** 0,42	** 0,42	
Chloroform	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
1,1,1-Trichloroethane	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
1,1,2-Trichloroethane	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Trichloroethylene (tri)	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
Carbontetrachloride(tetra)	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1

Tetrachloroethylene (per)	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
OTHER COMPOUNDS											
Hydrocarbon FractioC10-C40	ug/l	< 50	< 50	< 50	< 50	75	< 50	< 50	< 50	< 50	< 50
Tribromomethane (bromoform)	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
Not in STI-list of the SPA											
--CKW (som)	ug/l	< 1,6	< 1,6	< 1,6	< 1,6	< 1,6	< 1,6	< 1,6	< 1,6	< 1,6	< 1,6
Total PAH 10 (Dutch Ministry)	DIMS LS	** 0	** 0	** 0	**0	**0	**0	**0	**0	**0	** 0
1,2-Dichloroethylene (cis)	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Hydrocarbon Fraction C10-C12	ug/l	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Hydrocarbon Fraction C12-C16	ug/l	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Orthoxylene	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
m,p-Xylene	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
1,2-Dichloroethylene (trans)	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
1,2-Dichloropropane	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
1,3-Dichloropropane	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
--aromaten (BTEX)	ug/l	< 0,9	< 0,9	< 0,9	< 0,9	< 0,9	< 0,9	< 0,9	< 0,9	< 0,9	< 0,9
Sum of Dichloroethenes		** 0,14									
1,1-Dichloorpropaan	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
Minerale olieC16-C21	ug/l	< 10	< 10	< 10	< 10	33	< 10	< 10	< 10	< 10	< 10
Minerale olieC21-C30	ug/l	< 15	< 15	< 15	< 15	31	< 15	< 15	< 15	18	< 15

Minerale olieC30-C35	ug/l	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	11
Minerale olieC35-C40	ug/l	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10

Table A III 2. Groundwater Monitoring Data from PVC Monitoring Well (2019-2021)

Measure point		2	2	2	2	2	2	2	2	2	2
Description		Pb 2 F (1,7-2,7)	Pb 2 F (1,7-2,7)	Pb 2 F (1,7-2,7)	Pb 2 F (1,7-2,7)	Pb 2 F (1,7-2,7)	Pb 2 F (1,7-2,7)	Pb 2 F (1,7-2,7)	Pb 2 F (1,7-2,7)	Pb 2 F (1,7-2,7)	Pb 2 F (1,7-2,7)
Range (cm-mv)		170-270	170-270	170-270	170-270	170-270	170-270	170-270	170-270	170-270	170-270
Date		20/3/15	26/4/19	01/5/19	27/5/19	26/6/19	17/7/19	24/9/19	18/12/19	17/3/20	29/3/21
METALS											
Arsenic (As)	ug/l	< 5	< 5	< 5	< 5	< 5	8,2	10	< 5	5,1	< 5
Barium (Ba)	µg/l	120	130	130	180	130	98	110	270	110	110
Cadmium (Cd)	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
Cobalt (Co)	ug/l	3,5	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Copper (Cu)	ug/l	< 2	< 2	< 2	< 2	< 2	< 2	< 2	4,8	< 2	< 2
Mercury (Hg)	ug/l	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05
Lead (Pb)	ug/l	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Molybdenum (Mo)	ug/l	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Nickel (Ni)	ug/l	3	3,8	4,3	< 3	< 3	< 3	15	< 3	< 3	< 3
Zinc (Zn)	ug/l	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
AROMATIC COMPOUNDS											
Benzene	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
Ethylbenzene	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
Toluene	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
Xylenen (som, 0.7 factor)	ug/l	< 0,21	< 0,21	< 0,21	< 0,21	< 0,21	< 0,21	< 0,21	< 0,21	< 0,21	< 0,21
Sum of Xylenes		** 0,21	** 0,21	** 0,21	** 0,21	** 0,21	** 0,21	** 0,21	** 0,21	** 0,21	
Styrene	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
som 16 aromatische	ug/l	** 0,77	** 0,77	** 0,77	** 0,77	** 0,77	** 0,77	** 0,77	** 0,77	** 0,77	** 0,77

oplosmiddelen (Bbk, 1-1-2008) (_US											
POLYCYCLIC AROMATIC HYDROCARBONS											
Naphthalene	ug/l	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02	< 0,02
CHLORINATED HYDROCARBONS											
Vinyl Chloride	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Dichloromethane	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
1,1-Dichloroethane	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
1,2-Dichloroethane	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
1,1-Dichloroethylene	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
1,2-Dichloorethenen (som, 0.7 factor)	ug/l	< 0,14	< 0,14	< 0,14	< 0,14	< 0,14	< 0,14	< 0,14	< 0,14	< 0,14	< 0,14
Sum of 1,2-Dichloroethenes			** 0,14	** 0,14	** 0,14	** 0,14	** 0,14	** 0,14	** 0,14	** 0,14	
Dichloorpropanen (0,7 som, 1,1+1,2+1,3)	ug/l	0,42	0,42	0,42	0,42	0,42	0,42	0,42	0,42	0,42	0,42
Dichloorpropaan		** 0,42	** 0,42	** 0,42	** 0,42	** 0,42	** 0,42	** 0,42	** 0,42	** 0,42	
Chloroform	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
1,1,1-Trichloroethane	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
1,1,2-Trichloroethane	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Trichloroethylene (tri)	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
Carbontetrachloride(tetra)	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Tetrachloroethylene (per)	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1

OTHER COMPOUNDS											
Hydrocarbon Fraction C10-C40	ug/l	50	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50
Tribromomethane (bromoform)	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
Not in STI-list of the SPA											
--CKW (som)	ug/l	< 1,6	< 1,6	< 1,6	< 1,6	< 1,6	< 1,6	< 1,6	< 1,6	< 1,6	< 1,6
Total PAH 10 (Dutch Ministry)	DIMSLS	** 0	** 0	** 0	** 0	** 0	** 0	** 0	** 0	** 0	** 0
1,2-Dichloroethylene(cis)	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Hydrocarbon Fraction C10-C12	ug/l	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Hydrocarbon Fraction C12-C16	ug/l	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Orthoxylene	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
m,p-Xylene	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
1,2-Dichloroethylene (trans)	ug/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
1,2-Dichloropropane	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
1,3-Dichloropropane	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
--aromaten (BTEX)	ug/l	< 0,9	< 0,9	< 0,9	< 0,9	< 0,9	< 0,9	< 0,9	< 0,9	< 0,9	< 0,9
Sum of Dichloroethenes		** 0,14									
1,1-Dichloorpropaan	ug/l	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2	< 0,2
Minerale olie C16-C21	ug/l	17	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Minerale olie C21-C30	ug/l	29	< 15	< 15	< 15	< 15	< 15	< 15	< 15	< 15	< 15

Minerale olie C30-C35	ug/l	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Minerale olie C35-C40	ug/l	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10

Appendix IV. Data for Py GC-MS

Table A IV 1. List of Products Formed during the Pyrolysis of Biowell Samples at 320 ℃

	Peak Area ($\times 10^7$)				
	Original	Upper-1	Upper-2	Lower-1	Lower-2
Acetic acid	5.9502468	3.249783	6.4244248	2.176614	1.3096889
Hydroxyacetaldehyde	0.3130038	0.208691	0.6538666	0.273249	0.0838809
5,6-Dihydropyran-2,5-dione	1.3286122	1.396766	2.841422	1.089773	0.5338528
DL-lactide	34.3801312	18.279052	48.220643	12.261450	7.1699976
SUM	41.971994	23.1342941	58.140356	15.801087	9.0974202
	Relative Amount				
Acetic acid	14.18%	14.05%	11.05%	13.78%	14.40%
Hydroxyacetaldehyde	0.75%	0.90%	1.12%	1.73%	0.92%
5,6-Dihydropyran-2,5-dione	3.17%	6.04%	4.89%	6.90%	5.87%
DL-lactide	81.91%	79.01%	82.94%	77.60%	78.81%

Appendix V. Original Microscope Observation and Data for Biodegradation

Table A V 1. Biodegradation of Biowell Fragments (01)

01 Room temperature/Saturated/No-bioaugmentation

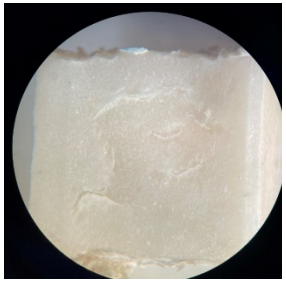
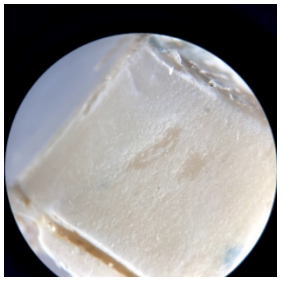
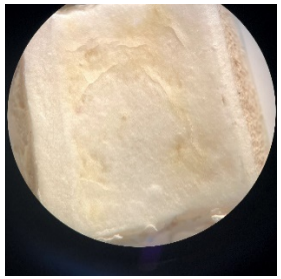
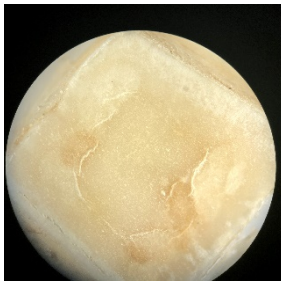
Time Period	**a) Four weeks**	**b) Six weeks**	**c) Eight weeks**	**d) Ten weeks**	**e) Twelve weeks**
Biowell Fragment					
Weight Loss	6.5487 %	8.2676 %	8.7594%	10.1869%	11.3493%

Table A V 2. Biodegradation of Biowell Fragments (02)

02 Room temperature/Half-saturated/No-bioaugmentation

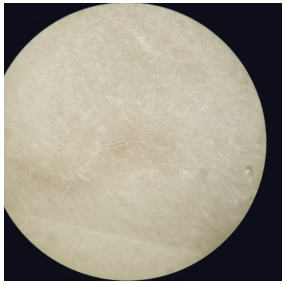
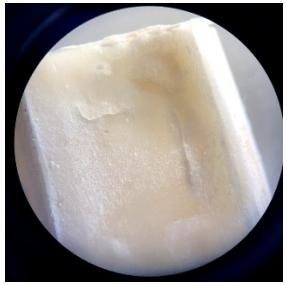
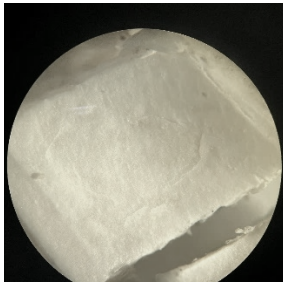
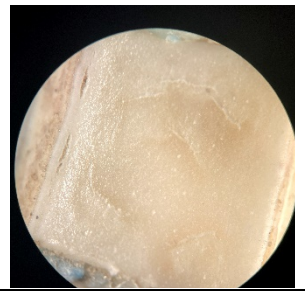
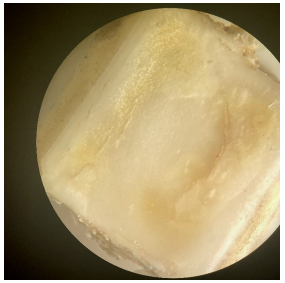
Time Period	**a) Four weeks**	**b) Six weeks**	**c) Eight weeks**	**d) Ten weeks**	**e) Twelve weeks**
Biowell Fragment					
Weight Loss	5.8809 %	7.2249 %	8.3051%	8.7570%	9.9885%

Table A V 3. Biodegradation of Biowell Fragments (03)

03 Room temperature/Saturated/Bioaugmentation

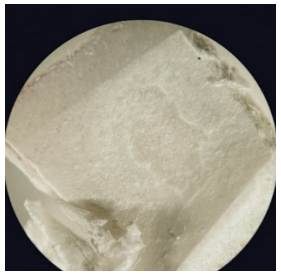
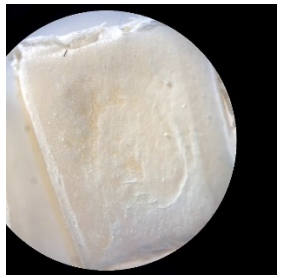
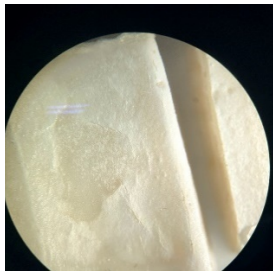
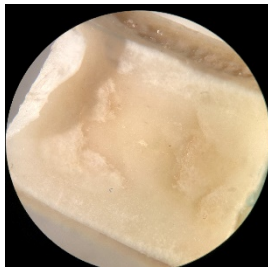
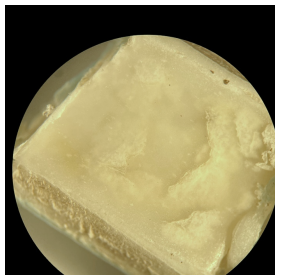
Time Period	**a) Four weeks**	**b) Six weeks**	**c) Eight weeks**	**d) Ten weeks**	**e) Twelve weeks**
Biowell Fragment					
Weight Loss	6.9770 %	8.0464 %	9.8879%	9.4747%	11.7805%

Table A V 4. Biodegradation of Biowell Fragments (04)

04 Room temperature/Half-saturated/Bioaugmentation

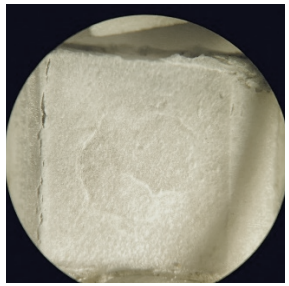
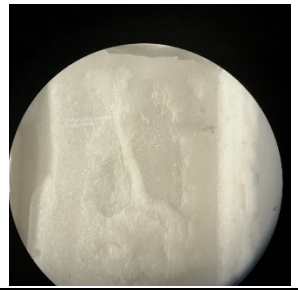
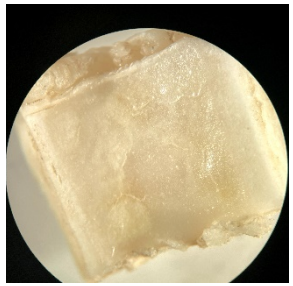
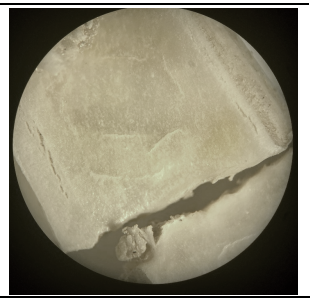
Time Period	**a) Four weeks**	**b) Six weeks**	**c) Eight weeks**	**d) Ten weeks**	**e) Twelve weeks**
Biowell Fragment					
Weight Loss	7.6480 %	8.0296 %	8.9321%	9.6806%	12.9701%

Table A V 5. Biodegradation of Biowell Fragments (05)

05 High Temperature/Saturated/No-Bioaugmentation

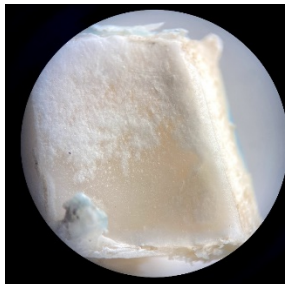
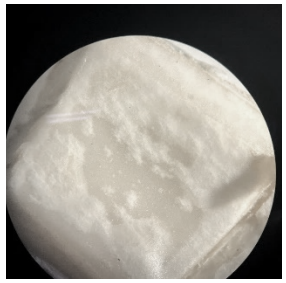
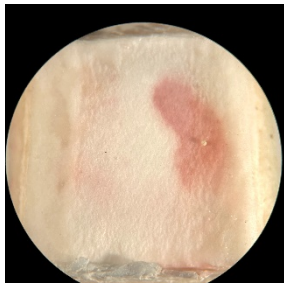
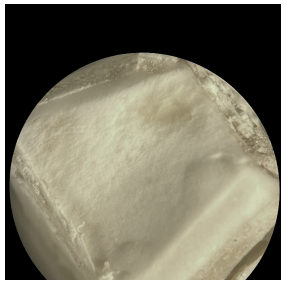
Time Period	**a) Four weeks**	**b) Six weeks**	**c) Eight weeks**	**d) Ten weeks**	**e) Twelve weeks**
Biowell Fragment					
Weight Loss	8.5834 %	11.2425 %	12.2945%	13.8366%	14.3989%

Table A V 6. Biodegradation of Biowell Fragments (06)

06 Room temperature/Half-saturated/No-Bioaugmentation

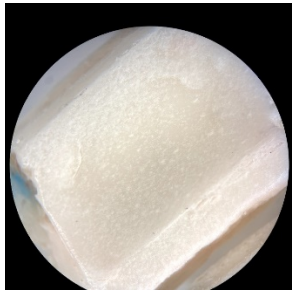
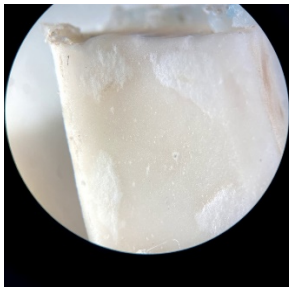
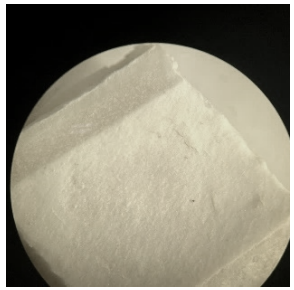
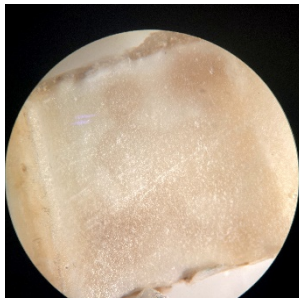
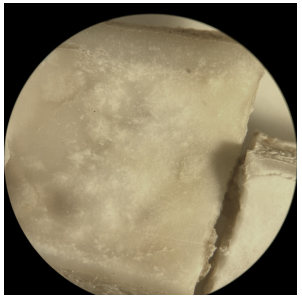
Time Period	**a) Four weeks**	**b) Six weeks**	**c) Eight weeks**	**d) Ten weeks**	**e) Twelve weeks**
Biowell Fragment					
Weight Loss	7.5370 %	8.7110%	11.5211%	13.6263%	15.2415%

Table A V 7. Biodegradation of Biowell Fragments (07)

07 High Temperature/Saturated/Bioaugmentation

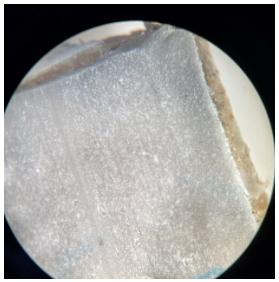
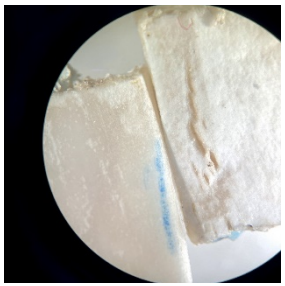
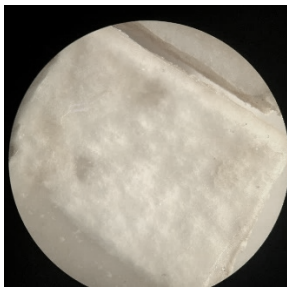
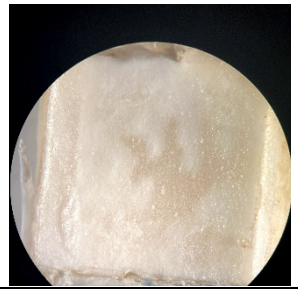
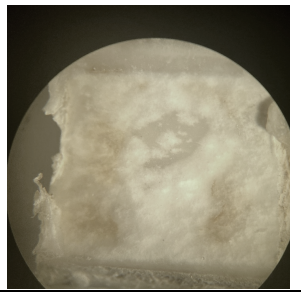
Time Period	**a) Four weeks**	**b) Six weeks**	**c) Eight weeks**	**d) Ten weeks**	**e) Twelve weeks**
Biowell Fragment					
Weight Loss	9.2468 %	11.0606 %	13.9687%	15.1105%	16.7994%

Table A V 8. Biodegradation of Biowell Fragments (08)

08 High temperature/Half-saturated/Bioaugmentation

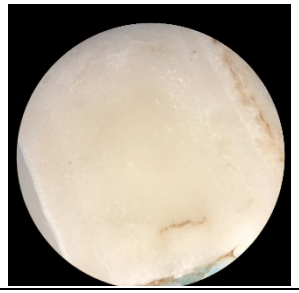
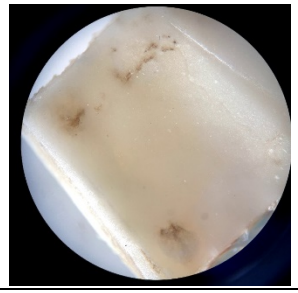
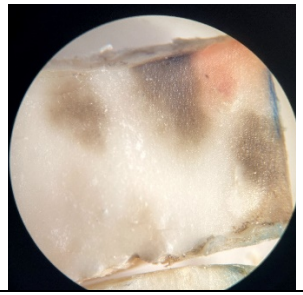
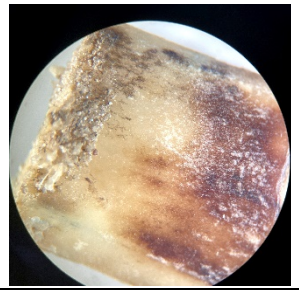
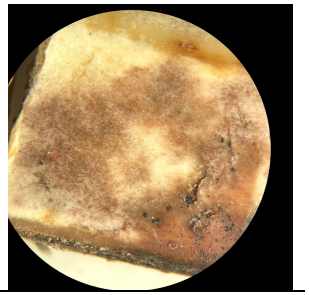
Time Period	**a) Four weeks**	**b) Six weeks**	**c) Eight weeks**	**d) Ten weeks**	**e) Twelve weeks**
Biowell Fragment					
Weight Loss	12.9124 %	11.6522 %	11.0608%	14.3399%	17.7537%

Table A V 9. Biodegradation of Biowell Granules (09)

09 High Temperature/Saturated/No-Bioaugmentation

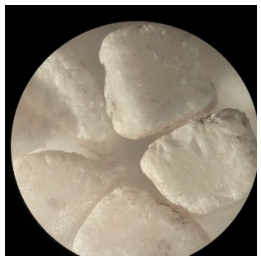
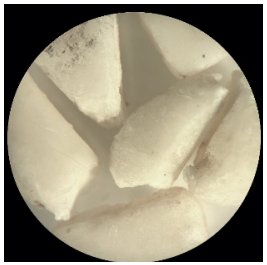
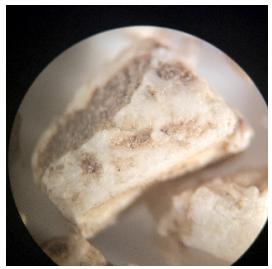
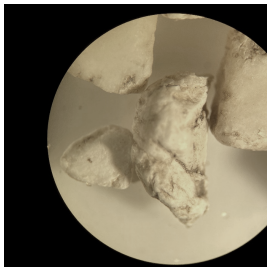
Time Period	a) Two weeks	b) Four weeks	c) Six weeks	d) Eight weeks	e) Ten weeks	f) Twelve weeks
Biowell Granules						
Weight Loss	15.9060 %	10.3598 %	15.8004 %	18.8447%	19.1174%	11.3576%

Table A V 10. Biodegradation of Biowell Granules (10)

10 High Temperature/Half-saturated/No-Bioaugmentation

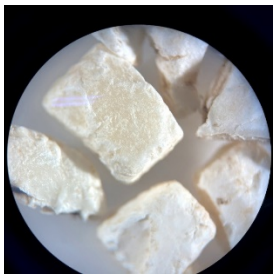
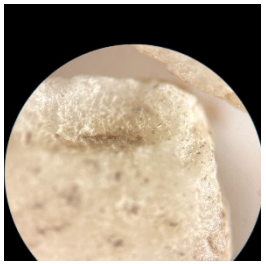
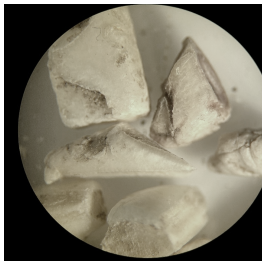
Time Period	a) Two weeks	b) Four weeks	c) Six weeks	d) Eight weeks	e) Ten weeks	f) Twelve weeks
Biowell Granules						
Weight Loss	13.6439 %	9.2589 %	16.3242 %	9.3930%	7.4686%	7.9832%

Table A V 11. Biodegradation of Biowell Granules (11)

11 High Temperature/Saturated/Bioaugmentation

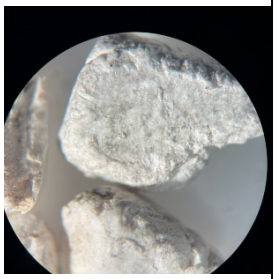
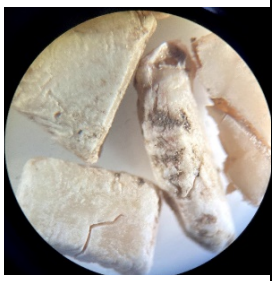
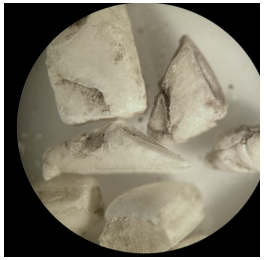
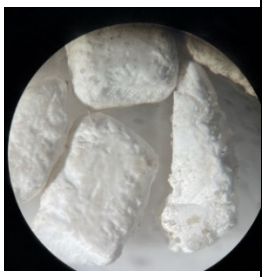
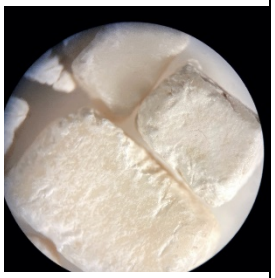
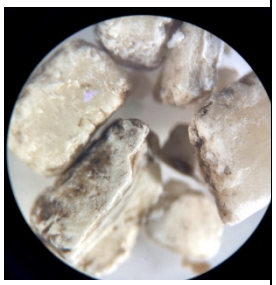
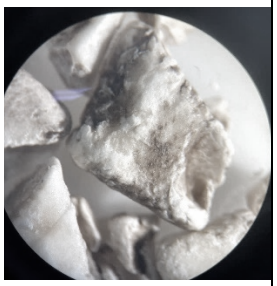
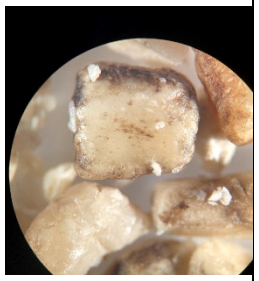
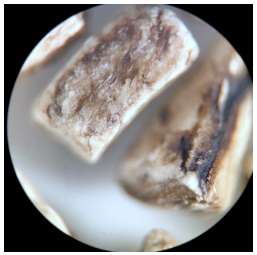
Time Period	a) Two weeks	b) Four weeks	c) Six weeks	d) Eight weeks	e) Ten weeks	f) Twelve weeks
Biowell Granules						
Weight Loss	14.3207 %	10.5429 %	18.6863 %	17.3544%	20.5310%	13.6428%

Table A V 12. Biodegradation of Biowell Granules (12)

12 High Temperature/Half-saturated/Bioaugmentation

Time Period	a) Two weeks	b) Four weeks	c) Six weeks	d) Eight weeks	e) Ten weeks	f) Twelve weeks
Biowell Granules						
Weight Loss	6.4158 %	7.5680 %	18.3460 %	18.4581%	11.5392%	16.0056%

Appendix VI. Acknowledgement in Chinese

致谢

2021 年 7 月 1 日，我在回 Utrecht 的火车上写下这段致谢。我想只有用母语，我才能最真诚而完整地表达我对过去五个月里所有经历的感谢。还记得 2020 年末的时候，因为想要在荷兰公司内拥有一份实习经历，并完成自己的毕业论文，我开始了漫长的找实习的过程。如果日子倒回两年前，我曾经是个坚定地做好打算要回到中国的人，我想念家里的一草一木。而如今，我想做一株蒲公英，即使漂泊不定居无定所，我却依然可以自在存活于这世间。对于一个国际生来说，想找到一份满意的实习，不太容易。因为疫情的原因，国际项目几乎处于停滞状态，没有一家公司愿意接收一个荷兰语几乎零水平的学生。可能也是缘分，我就这样接到了 Marian Langevoort 的电话，她告诉我，有一个很有趣的项目，你想不想了解一下看看。之后的日子里，一切都进展很顺利。因为疫情的原因，没法利用更多的时间前往公司与大家共事。本来以为会是一段平平淡淡就过去的日子。可我还是很清晰地记得每一次出现问题地时候，我总在犹豫不决要不要问出口的时候，Carlo Bensaïah 和 ir. Tobias Praamstra 总是给予我最详尽而耐心的解答，把我当作一个平等的人而不是处于学生阶段的后辈看待。永远给予我充分的肯定，支持，鼓励，引导，也总是在看到我进步之后指出之前所存在的不足。还有我每次孜孜不倦针对一些小问题执着发问的时候，Prof. dr. Stefan Dekker 总能以最快的速度帮我指点迷津，拨开迷雾，提供最可行却又准确的答案。

说过的无数句谢谢其实都不足以表达我对这过去五个月生活最诚挚的感谢。虽然可能，不再有机会可以相互合作或者共事，也有可能，我不会再停留在荷兰开启人生的下一个阶段，但这段日子里所有的收获与成长，都是我这两年生活里最值得回忆的东西。当以后回国的时候，我一定会骄傲地谈起，我在这里曾经有过五个月多么快乐而真挚的毕业生活。我会一直记得你们对我说的，you should be confident. 让我真诚地相信，不管我的下一站即将在哪里开启，我都会永远闪闪发光的行走着。

两年的时光转眼即逝，我还记得刚到 Utrecht Central 的第一天，因为航班延误又取消的原因，行李不知道丢在哪里，孑然一身来到一个陌生的城市，想起第一次爸妈送我到机场落泪的场景，想起很多个我独自一人在异国他乡走过的角落，想起那天 Peter Lodder 说，you are a tough person. 作为一个异乡人，留学生涯并不顺遂，刚刚来上课的时候甚至不知道要去哪里看课程要求，同学们都说着荷兰语，很多时候就默不作声度过一天又一天，求助无门的日子好像还历历在目。

所以我会越发感到自己的幸运，在过去的五个月里，我写下的每一字每一句都有人愿意帮我耐心查看，细心解答，给我最快的反馈，让我有动力和信心在这个研究里一步一步地走下去。今天我度过了在实验室地最后一天，我带了一些小礼物分给大家，Peter 说，我们留下一个中国结在实验室吧，这样以后大家来都知道，曾经有个中国女孩来过。我也会一直记得，曾经在欧亚大陆的另一端，书写过自己很重要的一部分人生。

其实不想写下最后的最后，因为还有无数话语不知如何抒发，不管是第一次来到 Deventer 这座城市的那天，Carlo 带我第一次参观实验室的日子，还是之后或飘雪或艳阳高照的荷兰天气，都成了我记忆里很重要的一部分。当然，还有这一路给过我鼓励，听过我在压力很大的时候抒发负面情绪，无条件支持我的父母，亲人，朋友。我们都将以更好的姿态迎接彼此。

在象牙塔中度过的六年终将结束，我看过南京的无数个日日夜夜，也在 Utrecht 这座小城里漂泊。终于要转变一个身份开启新的人生阶段，我也始终相信生活总会带来最美好的答案。