# The effects of pH and biodegradation on polymer-based granules

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Master thesis

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# Abstract

The use of biopolymers instead of traditional fossil fuel based and non-biodegradable polymers is seen as a positive step forward towards a more environmentally friendly future. This project investigates a granule product that is used in a dishwashing system with the aim of gaining knowledge about how it is affected by the storage in between uses and its biodegradability. The product is based on a known biodegradable polymer which is partially biobased. Experimental tests were performed to explore the effect of different pH values, in the pH range of foods, to give insight into how the granules could optimally be stored in between uses. Furthermore, biodegradation screenings were performed to investigate disposal options for the granules after they have been used. The results showed that a neutral pH value, 7, is preferred over a low pH, 3, during storage to minimize negative effects on the granules. Results from the biodegradation tests showed that the product is more degradable under aerobic conditions (in aerated soil and compost) than under anaerobic conditions (in anaerobic sludge). This could be further explored when looking into disposal options for the granules, but the relatively slow rate of degradation may be an obstacle.

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# 1. Introduction and objectives

Pollution and littering of plastic products and their waste is a problem when trying to move the world in a more environmentally friendly direction. In regards to polymer production and disposal the use of fossil fuels, emission of greenhouse gases and plastic waste accumulation are threats to the wellbeing of the planet. Although the use of oil and gas in the production of polymer materials account for a minor percentage of the total oil and gas use, which was 4-6% in Europe during 2017, the fact still remains that the amount of proven fossil fuel reserves are decreasing [1], [2]. It could be argued that these reserves only should be used when absolutely necessary and no other options are available.

The disposal of plastic waste can also lead to negative effects on the planet, ranging from situations such as greenhouse gas emissions from controlled incineration to uncontrolled littering of plastic bags in the natural environment [3], [4]. Accumulation of plastic waste in landfills and the natural environment is a widespread occurrence and it has been estimated that 79% of plastic waste, from all mass-produced plastics ever made, has ended up in such locations [5]. In Europe, the use of landfills for disposal of collected plastic waste is decreasing, but 24.9% of the 29.1 metric tons of collected post-consumer plastic waste in Europe during 2019 still ended up in landfills [6]. If the above mentioned problems are allowed to continue they can possibly lead to problems such as negative effects on wildlife and biodiversity, depletion of fossil fuel reserves and increased global temperature [2], [7]. To reduce such negative impacts from the polymer sector the implementation of biopolymers can be a positive step forward.

Biopolymer is a commonly used term, and can have various meanings. A biopolymer can be based on biological sources (biobased), degradable in biologically active environments (biodegradable) or both. Instead of using fossil fuels as a base for polymer synthesis, biobased polymers are completely or partially based on renewable resources such as cellulose from plants, chitosan from crustacean waste or lactic acid from bacterial fermentation [8], [9]. Biodegradable polymers can be degraded by living organisms which produce extracellular enzymes and/or chemicals in a biologically active environment, although this biotic degradation is often accompanied by abiotic degradation factors such as UV-light, heat and water [10]. Some examples of common biopolymers are polylactic acid (PLA), polyhydroxyalkanoates (PHAs), polycaprolactone (PCL) and poly(butylene adipate-co-terephthalate) (PBAT) which are used in multiple different sectors including packaging, agriculture, textiles and coatings. The granules in this study are based on PBAT, a aromatic-aliphatic copolyester, which will be further discussed in the background section.

If a fossil fuel based and non-biodegradable polymer is exchanged to a polymer which is based on renewable resources and can be subjected to biotic degradation it is considered to be part of a positive step towards a more environmentally friendly future, due to its lower stress

on the resources of our planet and incorporation back into the environment instead of leading to pollution and greenhouse gas emissions.

## 1.1 Granuldisk AB, their washing system and PowerGranules Bio

Granuldisk uses polymer-based granules in dishwashing systems for larger kitchenwares, which results in a system that requires less energy, water and chemicals than regular dish washers during their use. The granules are used in the systems to sputter the kitchenware and knock away remaining parts of foods. A new granule product, PowerGranule Bio, is a polyester granule based on PBAT together with $CaCO_3$ as a filler and some undisclosed minor ingredients such as a coloring additive and a thermal stabilizer. Furthermore, the PBAT component of the granule is partially biobased, meaning that parts of the polymer are derived from biological sources.

During the lifetime of a granule, from manufacturing to disposal after use, it is subjected to several different environments. A schematic overview of the granules movement can be seen in Figure 1. After they have been manufactured they are subjected to the atmospheric air and varying temperatures during transportation and storage. Following the step of manufacturing, they are packaged and sent to customers where they are used in their dishwashing systems. During use they are subjected to cycles of water combined with detergent followed by water combined with rinse aid, and the granules should optimally be able to withstand several thousands of minutes of use in the system before being replaced.

During use, handling and extraction of granules some of them might be lost from the cycle ending up either on the kitchen floor, in grease separators or in the sewage systems connected to the machine. After use, the granules are extracted into bins where they are rinsed by the user. The bins are then placed inside or outside of the dishwasher until the next time of use. It is believed that remaining parts of foods can have an effect on this environment, especially the pH, which might lead to negative effects for the granules. The storage time can vary from user to user and might sometimes be for a longer period, such as when granules are stored during vacations.

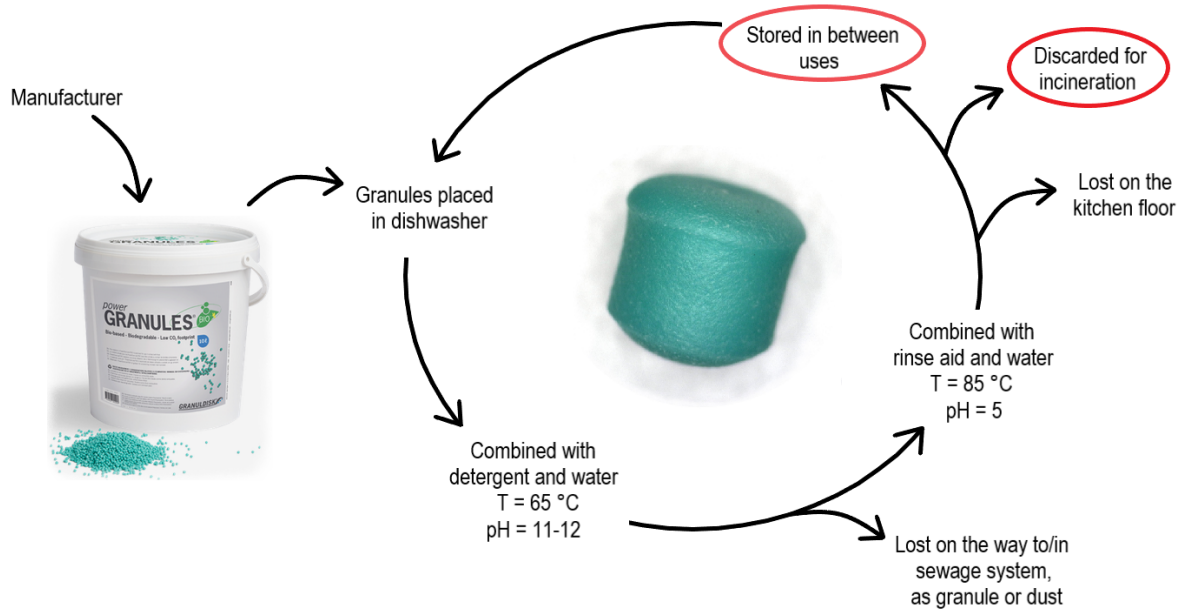


*Figure 1. A schematic of the movement of granules during its lifetime. The red circles are indicating the areas of interest for this thesis, namely the storage in between uses and the disposal of the granules after use.*

While many areas of interest can be found, this thesis focuses on the disposal of the granules after use and the effect of pH in the storage between uses, as indicated by the red circles in Figure 1.

Degradation of the product and changes to its surface during use limits its lifetime and functionality. It is believed that different pH values, originating from different kinds of foods, during the storage in between uses might affect the granules due to its effect on hydrolysis of PBAT in the product. It is also desirable to find signs of biodegradation if the granules are placed in compost, soil or anaerobic sludge to indicate if the granules might be biodegradable in these environments and therefore ultimately could be disposed of in another way than for incineration.

As mentioned previously the granule is partially biobased and should optimally be biodegradable at a fast rate after use, when placed in a biologically active environment. However, the granules also need to have the correct properties and be stable enough during a reasonably long time period for them to be practical for use in the system. This trade off, between biodegradability after use and properties/functionality during use, is often an obstacle within the design and choice of biodegradable polymers.

## 1.2 Objectives

1. Investigate the effect of pH on mass, density and surface when granules are exposed to aqueous conditions with varying pH which should correspond to the pH of different foods. This is done to investigate storage options for the granules in between uses.

2. Investigate the effects of biologically active environments on mass, density and surface when granules are exposed to biodegradation screenings based on International Organization for Standardization (ISO) standards in environments including compost, soil and anaerobic sludge. This is done to investigate the possibility of disposing the used granules in another way than for incineration.

# 2. Background

## 2.1 The granule

PowerGranules Bio is a polymer product in the form of approximately 3 mm sized granules that are based on PBAT with $CaCO_3$ as a filler, together with the addition of undisclosed minor additives (e.g. coloring agent). In this study two different batches (Batch 1 and Batch 2) of granules were tested in the pH-tests, while only granules from Batch 2 were used in the biodegradation tests. Both of these batches are a part of the development process to find an optimal recipe for the granules. The difference between the two batches is the addition of a thermal stabilizer together with a slightly lower density in the second batch (Batch 2), which results in more stable granules that were kept more intact during use in the dishwashing systems. To that point, the surfaces of granules from Batch 1 became much more uneven and flakey during use. Figure 3 shows images of unused, medium used and highly used granules from both Batch 1 and Batch 2, where the medium used and highly used granules have been used in a dishwashing system for approximately 4000 and 8000 minutes respectively.

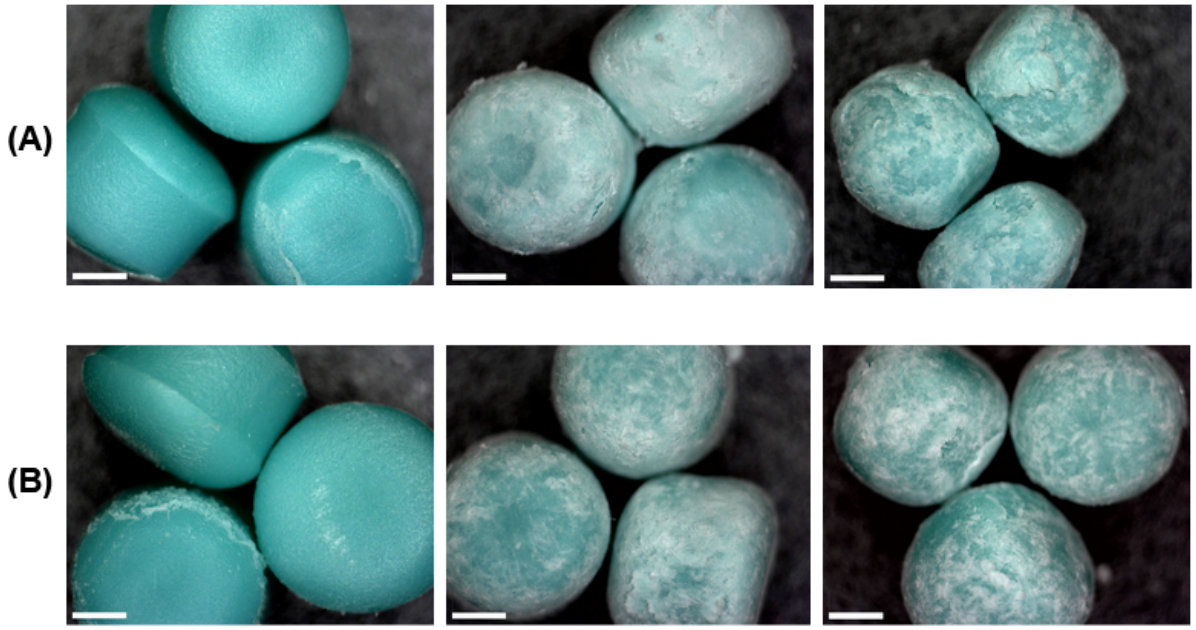


*Figure 3. The image shows samples of granules before any testing. Row (A) shows unused, medium used and highly used (from left to right) granules from Batch 1 and row (B) shows unused, medium used and highly used granules from Batch 2. White scale bars in the bottom left corners represent 1 mm.*

The filler, $CaCO_3$, is a widely used and common filler in plastic products, which can improve mechanical properties such as impact strength and stiffness of the plastic [30]. $CaCO_3$ has a lower solubility in solutions with high pH when compared to solution with low pH, due to the fact that it can react with hydrogen in solutions to form calcium ions and carbonate ions, hydrogen carbonate and/or carbonic acid, which are in equilibrium with each other [31], [32], [33]. Furthermore, this system is in balance with $CO_2$ and $H_2O$ in the solution, together with gaseous $CO_2$ in the surrounding atmosphere in the equilibrium

$$CO_2\,(g) \leftrightarrows CO_2\,(aq) + H_2O \leftrightarrows H_2CO_3 \leftrightarrows H^+ + HCO_3^- \leftrightarrows 2H^+ + CO_3^{2-}$$

This equilibrium is also recognized as the ocean carbonate system which contributes to the pH value of surface seawater at approximately 8.1 [34]. This indicates that polymer products containing $CaCO_3$ may be more likely to be affected by low pH solutions due to its higher solubility. Furthermore, $CaCO_3$ can form amorphous calcium carbonate which has incorporated water in its structure [35].

PBAT is a linear random copolyester consisting of the two units butylene terephthalate (BT) and butylene adipate (BA), and is synthesized from the monomers butanediol, adipic acid and terephthalic acid through polycondensation [36]. Figure 4 shows the chemical structure of PBAT with its aromatic BT section (to the left) and aliphatic BA section (to the right). The polymer has a glass transition temperature around -30 °C and a melting temperature between 109-120 °C [37], [38].

It has been shown that the softer aliphatic sections (BA) are more susceptible to both biotic and abiotic degradation when compared to the more hydrophobic and stiffer aromatic sections (BT) [39], [40]. The combination of aromatic and aliphatic sections of the polymer contributes to the balance of mechanical properties from the BT sections and the degradable properties from the BA sections, making PBAT a good option for a biodegradable polymer.

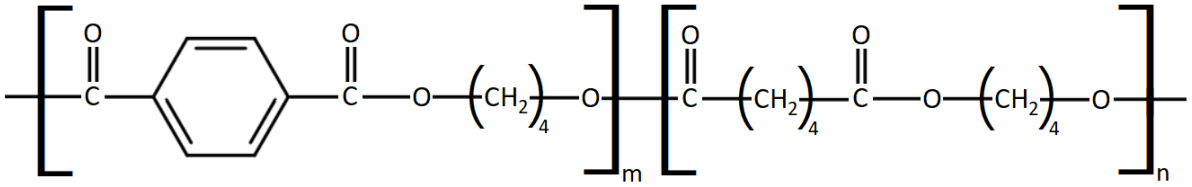

*Figure 4. Schematic picture of the chemical structure of PBAT, consisting of its BT (left) and BA (right) sections.*

Previously published research of biodegradability have shown that PBAT films are, to some extent, biodegradable under a range of different biological conditions such as in soil, compost, freshwater with sediment, and water with activated sludge [39], [41], [42]. Although biodegradation of PBAT has been found in various environments most reported results indicate that PBAT is more likely to biodegrade at a reasonably fast rate under aerobic conditions in soil and compost than in environments such as freshwater [43].

In soil and compost, where the application of PBAT based agricultural mulch films and the disposal of PBAT in compost are interesting, PBAT has been shown to be biodegradable in multiple studies [44], [45], [46]. Furthermore, Kijchavengkul et al. showed that PBAT is biodegradable at different rates in different kinds of compost, with manure compost showing the highest biodegradation rate when compared to food and yard compost [39].

Different kinds of more specific biotic involvement has also been shown to contribute to the biodegradation of PBAT, such as depolymerases from bacterium strain Roseateles depolymerans TB-87, bacterium Bacillus pumilus and fungal strain NKCM 1712 [47], [48], [49].

On the commercial market, some companies sell PBAT based products (e.g. Ecoflex© from BASF and ECOWORLD© from JINHUI) that have been certified according to standards such as the EN 13432 and/or ASTM D6400 which includes standardized tests to ensure that polymeric packaging products are safe to use in industrial composting. However, it should be noted that many PBAT based products are very thin, such as film blown plastic bags, and therefore have a large surface to mass ratio. A thicker polymer product such as granules might not pass these tests during the required time limits due to their thickness [50].

## 2.2 Polymer degradation

While many synthetic polymers are designed to withstand exposure to degradation factors with the goal of producing products with long lifetimes, some polymers are designed to be easily degraded in certain environments where the goal for the product is to be degraded into molecules that can be utilized by the surrounding environment [11]. As previously mentioned, PBAT has been designed to have a balance between the more easily degradable section and the less degradable, but stronger section. If used properly, this polymer can therefore be both functional during use and degraded at a desired rate after use.

Degradation of polymers can be the result of one or more different types of processes which can include both abiotic and biotic factors. Abiotic degradation factors include mechanical forces (e.g. strain and compression), light- (e.g. Norrish I and Norrish II reactions), thermal- and chemical (e.g. hydrolysis and oxidation) exposure which often work in combination with each other [12]. The effects of degradation can include changes in many types of properties such as strength, molecular weight, color and shape.

Biotic degradation of polymers occurs in a number of steps and processes ranging from surface deterioration to assimilation of monomers or short oligomers by microorganisms which can digest the molecules and produce biomass, water and gases [12]. Since polymers generally have very high molecular weight due to their long molecular chains they need to be broken down into smaller molecules before they are able to pass the cell walls of microorganisms, which is where the step of assimilation occurs [13].

One of the main chemical degradation factors for many polymers, especially aliphatic polyesters such as parts of PBAT, is the presence of water which can lead to hydrolysis. The presence of water is important during both abiotic and biotic degradation of polyesters due to the polymers susceptibility to nucleophilic attacks on the carbonyl carbon in the ester bonds.

### 2.2.1 Hydrolytic degradation of polyesters

Hydrolysis is an important chemical degradation mechanism for many polyesters, including PBAT. The rate of hydrolytic degradation depends on multiple factors including temperature, time, pH, type of chemical bonds in the polymer, copolymer composition, crystallinity and water uptake [14], [15]. Hydrolytic attacks on the ester carbonyl group are the main degradation mechanism, which results in chain scission of the polymer backbone and leads to the polymer breaking down into molecules with lower molecular weight. However, different values of pH in the surrounding environment can lead to different mechanisms responsible for the chain scission of the molecule.

Since the dish washing systems are used in larger kitchens which handle many types of different foods, it is important to consider what effect these foods might have on the pH in the storage environments for the granules since this may contribute to the rate of hydrolytic degradation. As reported by Bridges and Mattice, foods can have a wide range of pH with the most common values found in the range of 3-7 [16]. However, some exceptions were found of both lower and higher pH values, such as in the cases of lemon juice (pH 2-3) and egg whites (pH 8-9).

Figure 2 shows the general reaction mechanisms for hydrolysis of an ester bond under basic and acidic conditions. As can be seen in the figure, during base catalyzed hydrolysis a hydroxide ion performs a nucleophilic attack on the carbonyl carbon. This leads to the release of the ester oxygen containing part of the molecular chain, followed by protonation of the released oxygen creating an alcohol and a carboxylic acid as end products [17].

For hydrolysis in acidic environments the reaction starts with protonation of the carbonyl oxygen which leads to the carbonyl carbon being susceptible to a nucleophilic attack by the oxygen in a water molecule [17]. The ester oxygen containing part of the molecular chain is then protonated after which it leaves as an alcohol. Thereafter the carbonyl oxygen is deprotonated by a water molecule, resulting in a carboxylic acid as end product.

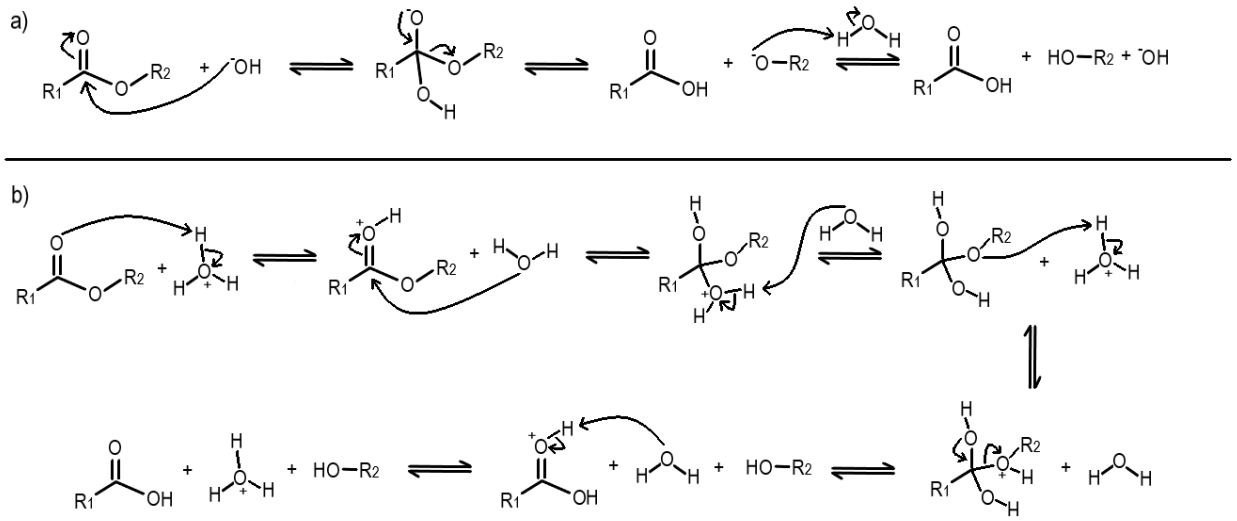


*Figure 2. General reaction mechanism for hydrolysis of an ester bond in a polyester under a) basic and b) acidic conditions. $R_1$ and $R_2$ correspond to the continued polymer molecule and the arrows in the figure indicate electron movement throughout the reactions. The figure has been adapted from reactions presented by Woodard et al. [17].*

### 2.2.2 Biotic degradation of polymers

Biotic degradation of polymers is often described in four different levels. These are biodeterioration, biofragmentation, assimilation and mineralization, and can account for the degradation of a polymer from high molecular weight molecules down to small molecules produced by microorganisms including biomass, water and gases.

During biodeterioration the surface of the polymer is subjected to degradation, usually by a combination of abiotic and biotic factors depending on the environment. The long polymer chains are cleaved into lower molecular weight products, which can then further be cleaved to oligomers and monomers through biofragmentation. Biofilms consisting of water, organic and inorganic particles, microorganisms colonizing the surface and a matrix consisting of the cells excretory products such as polysaccharides and proteins, are formed on the surface of the polymer [18]. The microorganisms present in the biofilms and the nearby environment can consist of a wide range of types of microorganisms such as fungi, bacteria, protozoa and algae.

Extracellular enzymes released by these microorganisms can adhere to the polymer surface leading to chain scission of the polymer, after which enzymes on the surfaces of the cells can further degrade the fragmented polymer sections into shorter molecules such as monomers, dimers and oligomers [19]. The enzymes can cleave the polymer chain through reactions with their active site where enzymatic hydrolysis or enzymatic oxidation of the polymer chain can occur, depending on the type of enzyme and polymer material [20]. For many polyesters, free radicals and enzymes such as esterases and lipases which are produced by microorganisms in the biofilm can catalyze the hydrolysis of the ester bonds in the polymer by lowering the activation energy needed for the hydrolysis reaction to occur [21], [22].

Some esterases have what is known as a catalytic triad located at their active sites. The catalytic triad includes serine, aspartic acid and histidine which can interact with each other and a water molecule to form free electrons on an oxygen in the serine molecule, which in turn can attack the carbonyl carbon through a nucleophilic reaction [23]. The reaction eventually leads to chain scission at the ester bond, creating an acid and an alcohol. In general this process can be called enzymatic depolymerization, which is also a term used to refer to certain enzymes that have been reported to be involved in degradation of specific polymers. Some examples of these are poly(3-hydroxybutyrate) (PHB) depolymerases which can depolymerize PHBs and PHA depolymerases which can depolymerize PHAs [24], [25].

Once the polymer has been fragmented into smaller molecules they can often be utilized as energy and carbon sources for microorganisms and their growth in the subsequent step of assimilation. For this to occur the polymer molecules need to be small enough to be transported into the cell, across the cell membrane, which generally requires the molecules to consist of less than 50 carbon atoms [26].

At this stage, the microorganisms can utilize the polymer fragments to produce biomass, adenosine triphosphate (ATP), minerals, salts, water and gases such as $CO_2$ and $CH_4$ [27]. If the products produced by the microorganisms are inorganic, such as inorganic salts and gases, the process is called mineralization.

Depending on the type of microorganism, and in which environmental conditions it can grow, the obtained polymer fragments can be broken down to useful molecules, e.g. for gaining energy via ATP production, in three different pathways called aerobic respiration, anaerobic respiration or fermentation [28]. One of the main difference between them is that oxygen is used as the final electron acceptor in aerobic respiration and other acceptors such as $NO_3^-$ and $SO_4^{2-}$ are used in anaerobic respiration, while the incomplete oxidation pathway of fermentation is used by some cells that do not have electron transport systems [29].

## 2.3 FTIR-ATR

Fourier Transform Infrared spectroscopy (FTIR) using Attenuated Total Reflection (ATR) is an analyzing technique that can be useful when identifying chemical bonds, and thereby identifying chemical substances, on the surface of solid samples. The system uses an infrared (IR) beam directed into an IR transparent crystal with high refractive index (e.g. Ge, Si or ZnS), that is in direct contact with the sample, after which the beam is reflected on their interface and then registered by an IR detector [51]. Figure 5 shows a schematic view of the setup for the IR beam, crystal and sample. When the beam is reflected at the interface of the crystal and the sample, a so-called evanescent electromagnetic wave is formed from which energy can be absorbed by molecules located at the surface of the sample, thereby decreasing the intensity of the reflected beam [51]. The difference in intensity between the incident and detected IR beams can then be assigned to absorbance by the vibrations of specific molecular bonds, depending on which wavelength (i.e. energy) was absorbed.

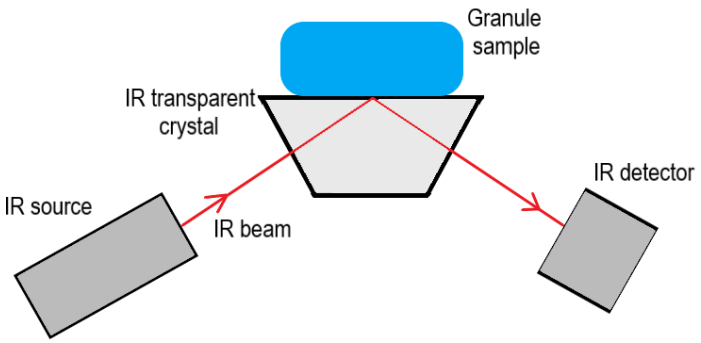


*Figure 5. A schematic view of an FTIR-ATR setup with incident IR beam, crystal, sample and IR detector. The figure is based on the image shown by Urbaniak-Domagala [51].*

The information from the IR beam that reaches the detector is collected and analyzed by software where the obtained information is translated, by Fourier transformation, to produce graphs showing spectras of absorbed wavelengths. These graphs are normally presented as absorption vs. 1/wavelength. For the purposes of this thesis, the main peaks of interest are a sharp peak at 850-900 $cm^{-1}$ and a broad peak at 1350-1500 $cm^{-1}$ which both corresponds to IR absorption by $CO_3$ vibrations together with a peak at around 1710 $cm^{-1}$ from C=O groups, a peak around 1260 $cm^{-1}$ from C-O in the ester linkage and a broad peak around 3350 $cm^{-1}$ for hydroxyl groups which relates to the degradation of PBAT [35], [39], [42]. A decrease in the absorption at these peaks indicates that degradation has occurred. The hydroxyl groups can, if an increase after tests is found, indicate degradation of the polymer but can also indicate the presence of amorphous calcium carbonate.

# 3. Materials and methods

As mentioned previously two different granule batches were used, which can be further divided into different types that have been used in the dishwashing systems for different amounts of time. A list of all types of samples used in the following tests can be seen in Table 1. Both batches and types were used in the pH tests while only two types from Batch 2 were used in the biodegradation tests. The approximately 3 mm sized granules contain PBAT, $CaCO_3$ and some undisclosed minor additives (e.g. coloring agent) and the difference between the two batches is the addition of a thermal stabilizer together with a slightly lower density in the second batch (Batch 2). All tests were run in triplicate, and any statistically significant differences were determined using t-tests with a significance level of $\alpha = 0.05$.

| Sample type | Minutes used | Batch number | Used in tests |
|---|---|---|---|
| Unused | 0 | Batch 1 | pH |
| Medium used | 3750 | Batch 1 | pH |
| Highly used | 7899 | Batch 1 | pH |
| Unused | 0 | Batch 2 | pH & biodegradation |
| Medium used | 4059 | Batch 2 | pH |
| Highly used | 8736 | Batch 2 | pH & biodegradation |

*Table 1. List of samples and their corresponding batches that were tested in the pH tests and in the biodegradation screenings.*

## 3.1 Biodegradation tests in compost, anaerobic sludge and soil

The following methods describe the screenings of biodegradability of the granule product, which are based on ISO standards for biodegradability in aerobic compost, soil and anaerobic sludge (ISO 14855-1, ISO 17556 and ISO 13975) under the corresponding environments. The standards were used to determine the preparation and composition of the mixtures of test material, compost, soil and anaerobic sludge together with the temperatures used during the tests and guidance for evaluation of disintegration. This evaluation of disintegration is performed by measuring the percentage of mass of the tested granules which can pass through a sieve with 2 mm seized holes after the tests are completed. For the biodegradation screenings two different types of granule samples, unused and highly used, from Batch 2 were tested (see Table 1). For all three biodegradation tests samples were taken out after three different time periods of 20, 47 and 60 days for mass, density, disintegration and surface evaluations.

### 3.1.1 Biodegradation test in compost

Compost-based soil (NSR, Sweden) was sieved using a sieve with a hole size of 2 mm to get rid of inert material such as larger pieces of wood. The percentage of dry matter in the compost was then determined to be 82.8 ± 0.7 % by drying a sample of the compost at 105 °C until constant weight was reached, which indicates that the moisture in the compost was removed. The pH of the compost was then measured to be 7.11 ± 0.09 by preparing a mixture of 1 part compost to 5 parts of deionized water and measuring the pH of the mixture using a pH meter (Mettler Toledo SevenGo Duo, USA). After this, 18 different 500 mL GL45 bottles were prepared with a mixture of 72.8 g compost, 5 g vermiculite (Nelson Garden, Sweden) and 45 mL of water each, giving the mixture a dry matter content of 53 %. Vermiculite was added to the mixture for compost structure and water retention purposes. 10 g of sample granules were then mixed into each bottle before they were placed in an incubator at 58 °C, to simulate commonly used composting conditions, together with two bottles of water to add moisture inside the incubator. 9 bottles received unused granules and the other 9 received highly used granules. During the incubation period, four of the bottles were weighed regularly to detect loss of water. Water was added to each bottle every 2-3 days to keep the dry matter content of the compost around 40-60 %. After the first weekend the water content had reduced significantly, after which 12 g of compost, 4 g of vermiculite and 15 mL of water was added to each bottle to help the mixture retain water content. An additional 2 g of vermiculite was added after 20 and 47 days for water retention purposes, due to the decrease in size of the original vermiculite particles that is believed to have occurred during stirring of the mixtures. Sample bottles were taken out and analyzed after 20, 47 and 60 days.

The content of the bottles was then sieved, using both a 2 mm and a 0.8 mm sieve in consecutive order, to locate the granules and evaluate the amount of disintegration. After this the granules were cleaned by washing with water and left at room temperature to dry until constant mass was obtained. The samples were then weighed and their density was measured. To evaluate loss of mass for the samples, the weight loss in percent ($w_{loss}$) was calculated according to

$$w_{loss} = \frac{w_{before} - w_{after}}{w_{before}} \times 100$$

where $w_{before}$ and $w_{after}$ is the measured sample weights before and after testing. The density measurements, both before and after tests, were performed using an analytical balance (Sartorius CP224S, Germany) and a 10:0.2 mL measuring cylinder. To do this, approximately 8 mL of granules were placed in the measuring cylinder and the weight of the granules ($m_{granules}$) was noted. 5 mL of water ($V_{water}$) was then added to the cylinder using a pipette after which the volume of the granules ($V_{granules}$) followed by their density ($\varrho_{granules}$) could be calculated using

$$\varrho_{granules} = \frac{m_{granules}}{V_{total} - V_{water}} = \frac{m_{granules}}{V_{granules}}$$

To evaluate surface changes of the samples, a Dino Light Edge Vision microscope (AnMo Electronics, Taiwan) was used together with DinoCapture 2.0 (AnMo Electronics, Taiwan) software to investigate the surfaces of the granules and possible changes during the tests. FTIR-ATR was then used on samples from the 47 day test samples to further investigate indications of surface changes.

### 3.1.2 Biodegradation test in anaerobic sludge

Anaerobic sludge (Va Syd Ellinge waste-water treatment plant, Sweden) was sieved using a sieve with a hole size of 2 mm to get rid of pieces of larger inert material after which the percentage of dry matter in the sludge was determined to be 2.9 ± 0.3 % by drying the sludge in 105 °C until constant mass was reached. The pH of the sludge was then measured to 7.57 ± 0.1 using a pH meter. A total of 18 different 500 mL GL45 glass bottles were then filled with sludge after which 4 g of sample granules were added to each bottle. 9 bottles received unused granules and the other 9 received highly used granules. A small gap of air was left at the top of the bottle to reduce the risk of spilling sludge during preparation and evaluation of the test. The bottles were then closed using lids with two holes (BPC Instruments, Sweden) connected via Tygon S3 gas tight tubing (BPC Instruments, Sweden) to check valves (BPC Instruments, Sweden). Check valves were used to release produced gases such as methane from the sludge out from the bottles while simultaneously keeping oxygen

away from the sludge. The bottles were then incubated in 35 °C, to simulate mesophilic anaerobic digestion conditions, for time periods of 20, 47 and 60 days. After the tests the samples were evaluated for disintegration and changes of their weight, density and surface according to the previously mentioned methods.

### 3.1.3 Biodegradation test in soil

Plantation soil (Plantagen, Sweden) was sieved using a sieve with a hole size of 2 mm to get rid of larger pieces of wood, rocks and other inert materials. The percentage of dry matter in the soil was then determined to be 46.7 ± 1.6 % by drying sieved soil in 105 °C until constant mass was reached. The pH of the soil was measured to 6.28 ± 0.14 by mixing 1 part soil with 5 parts of deionized water followed by measurement using a pH meter. When this was done, 18 different 500 mL GL45 glass bottles were filled with 100 g of soil followed by the addition of 4 g of sample granules. 9 bottles received unused granules and the other 9 received highly used granules. The bottles were then placed in room temperature (20.5 ± 1 °C) away from direct sunlight for a time period of 20, 47 and 60 days. During the experiments the soil was aerated by mixing once a week, together with the addition of water if the soil had evaporated moisture. After the tests the samples were evaluated for disintegration and changes of their weight, density and surface according to the previously mentioned methods.

## 3.2 pH tests

Unused, medium used and highly used granules from both batches (Batch 1 and Batch 2) were tested in solutions with four different pH values, 3.0, 5.0, 7.0 and 9.0. See Table 1 for a list of all samples. The solutions were prepared using deionized water with a pH of 5.8, conductivity of 0.73 µS/cm and a resistance of 1.22 MΩ*cm (Swatab, Sweden) together with HCl (VWR Chemicals, Sweden) for low-pH solutions and NaOH (Nitor, Sweden) for higher-pH solutions. The pH of the solutions were measured using a pH meter. Approximately 30 g samples of granules were then weighed using an analytical balance and three density measurements per sample were conducted. When this was done, the samples were put into 500 mL polypropylene bottles (VWR, Sweden) with 250 mL of their corresponding solution and placed in room temperature (20.5 ± 1 °C) for 20 days. The temperature was measured at several time points during the experiments to detect any larger changes. For the first batch of granules (Batch 1), the pH of the solutions were measured before and after the tests. For the second batch (Batch 2), the pH of the solutions were measured after 5, 10, 15 and 20 days. After 20 days, the samples were collected and dried at room temperature to remove moisture, until constant mass was obtained. The samples were then weighed and the density was measured. Samples in solutions with neutral pH (pH 7) were chosen as reference when comparing results to determine significant changes in weight losses. FTIR-ATR was then used to investigate surface changes on granules from Batch 2.

### 3.3 FTIR spectra

To look for further indications to surface changes, FTIR analysis was performed with attenuated total reflection using a Bruker Alpha II (Bruker, USA) together with OPUS software (Bruker, USA). Background scans were first measured for every sample, after which samples were scanned at room temperature in the scan range of 4000-400 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. The final results were averaged over 24 readings per sample. The data was normalized using the peak at 725 $cm^{-1}$ since the peak corresponds to =C-H bonds in benzene rings in the aromatic sections of PBAT [41]. This peak was chosen due to the fact that the aromatic sections of the polymer are less likely to be affected by degradation than other sections. FTIR-ATR was used on samples from the pH test of Batch 2 and on samples from 47 day biodegradation screenings. To determine significance of changes in the spectras before and after tests, areas under the peaks of interest mentioned in the background section were compared.

# 4. Results

### 4.1 Biodegradation tests

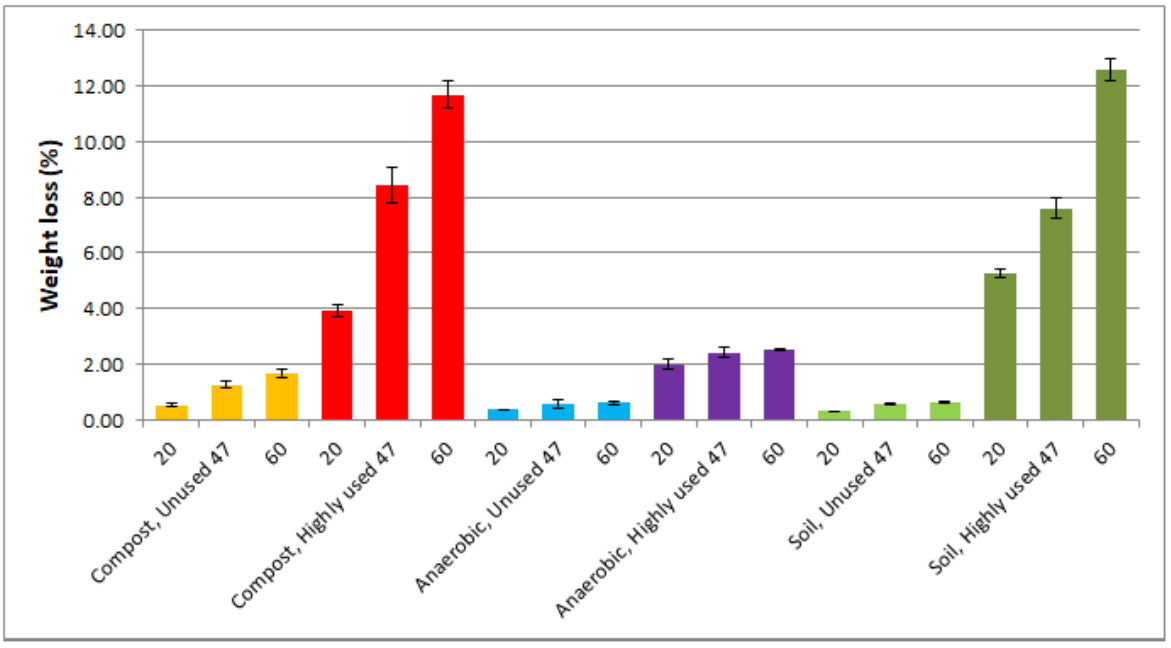


*Figure 6. The graph shows the measured weight loss of samples in all biodegradation tests after 20, 47 and 60 days of incubation time. The orange and red bars show weight loss results for the samples incubated in compost, the blue and purple bars show results for the samples in anaerobic sludge and the light green and dark green bars show the results for samples in soil. Error bars show standard deviation.*

The weight measurements from the biodegradation tests (Figure 6) after 20, 47 and 60 days showed that both the unused and highly used granules have lost more weight under aerobic conditions than under anaerobic conditions. Furthermore, it is clear that the highly used granules have lost more weight than the unused granules, possibly due to the fact that they have more surface area available.

After the first 20 days, the largest weight loss was seen in the samples with highly used granules that were placed in soil (5.28 ± 0.16 %), followed by the highly used granules in compost (3.94 ± 0.20 %) and the highly used granules in anaerobic sludge (1.99 ± 0.20 %). The samples of unused granules also showed a decrease in weight, although to a much lower degree. For these samples all environments resulted in a weight loss of <1 % after 20 days, with the samples in compost showing the largest weight loss (0.54 ± 0.05 %), followed by samples in anaerobic sludge (0.37 ± 0.01%) and soil (0.31 ± 0.01 %).

A similar trend could be seen after 47 days of incubation time where all environments gave an increased weight loss, compared to the 20 day samples, for both unused and highly used granules. For the highly used granules, compost samples showed the largest increase in weight loss to 8.44 ± 0.62 %, followed by the soil samples to 7.61 ± 0.35 % and the anaerobic sludge samples to 2.42 ± 0.18 %. The unused granules showed increased weight losses to 1.27 ± 0.14 % in compost, 0.57 ± 0.05 % in soil and 0.56 ± 0.13 % in anaerobic sludge after 47 days of incubation.

The trend of aerobic environments yielding the largest weight losses continued in the measurement after the full 60 days of incubation. The samples of highly used granules in soil showed the largest total weight loss (12.60 ± 0.41 %) and were closely followed by the samples of highly used granules in compost (11.70 ± 0.46 %). The highly used samples in anaerobic sludge reached a total weight loss of 2.50 ± 0.04 %. The weight loss of the unused granules also continued to increase, although at a slow rate. The total weight loss after 60 days of incubation for these were 1.66 ± 0.18 % for samples in compost, 0.63 ± 0.02 % for samples in soil and 0.60 ± 0.09 % for samples in anaerobic sludge.

The density measurements showed no significant difference for any of the biodegradation experiments after 20, 47 or 60 days when comparing the measured densities before and after the respective incubation periods. Density measurements can be seen in Appendix 1. Furthermore, 0% disintegration (i.e. percentage of sample mass that could pass through a sieve with 2 mm sized holes) was found for all samples after 20, 47 and 60 days.

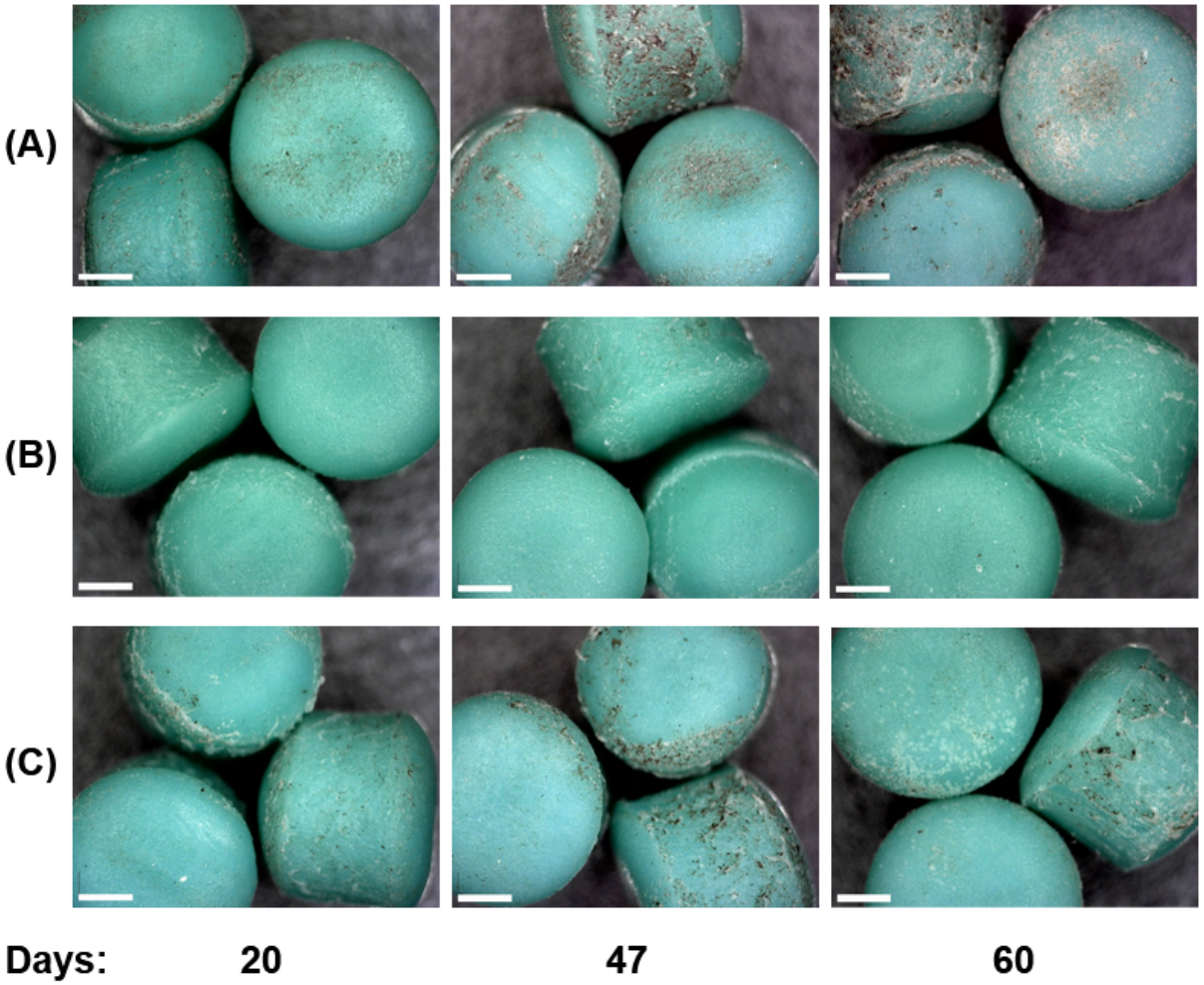


***Figure 7.*** *The figure shows images of different unused granules from samples retrieved from the biodegradation tests after 20, 47 and 60 days of incubation. Rows (A), (B) and (C) show granules from samples in compost, anaerobic sludge and soil respectively. White scale bars in the bottom left corners represent 1 mm.*

As can be seen in Figure 7 and Figure 9, the surfaces of both unused and highly used granules that were placed in aerobic conditions (soil and compost) were affected more by their environments than the granules that were placed in anaerobic sludge. It should be noted that the variance of surface structure between the individual samples, and granules within samples, makes it hard to compare minor details regarding surface structure in the images. Furthermore the structure of the surfaces led to remains of soil and compost being stuck on certain parts of the granules which were hard to clean off, as can be seen in the images.

In the images of samples that had an incubation time of 60 days, a whitening effect can be seen on the surface of some granules that were incubated in soil and compost, but not for samples in anaerobic sludge. This is believed to be due to surface degradation of PBAT for these samples. Figure 8 shows a closer look of this on an unused granule from incubation in compost. It shows that the whitening typically occurs around holes and uneven structures in the surface where remains of compost had accumulated.

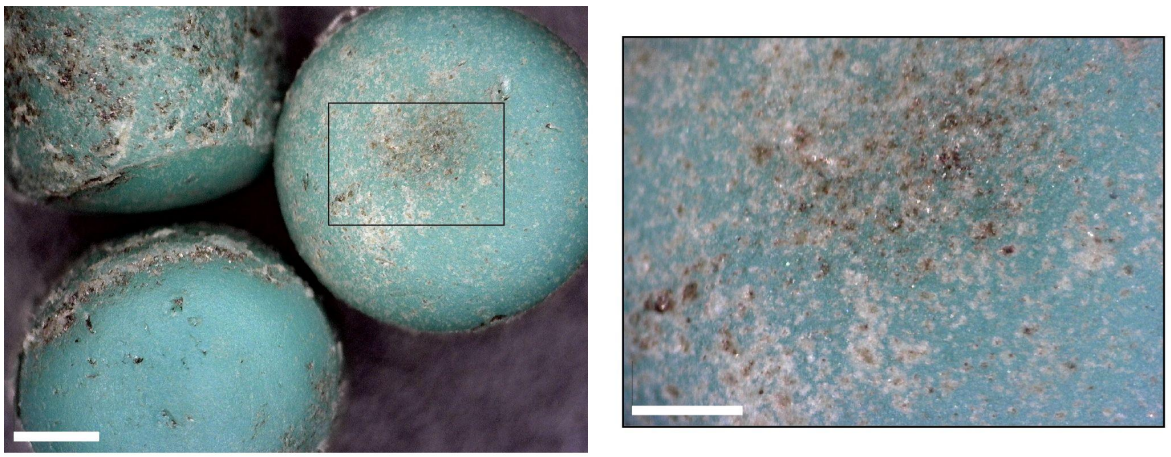

*Figure 8. The figure shows granules from unused samples that were incubated in compost for 60 days (left) and a zoomed in view (right) of the marked area showing signs of degradation on the surface of the granule. White scale bars in the bottom left corners represent 1 mm (left image) and 0.4 mm (right image).*

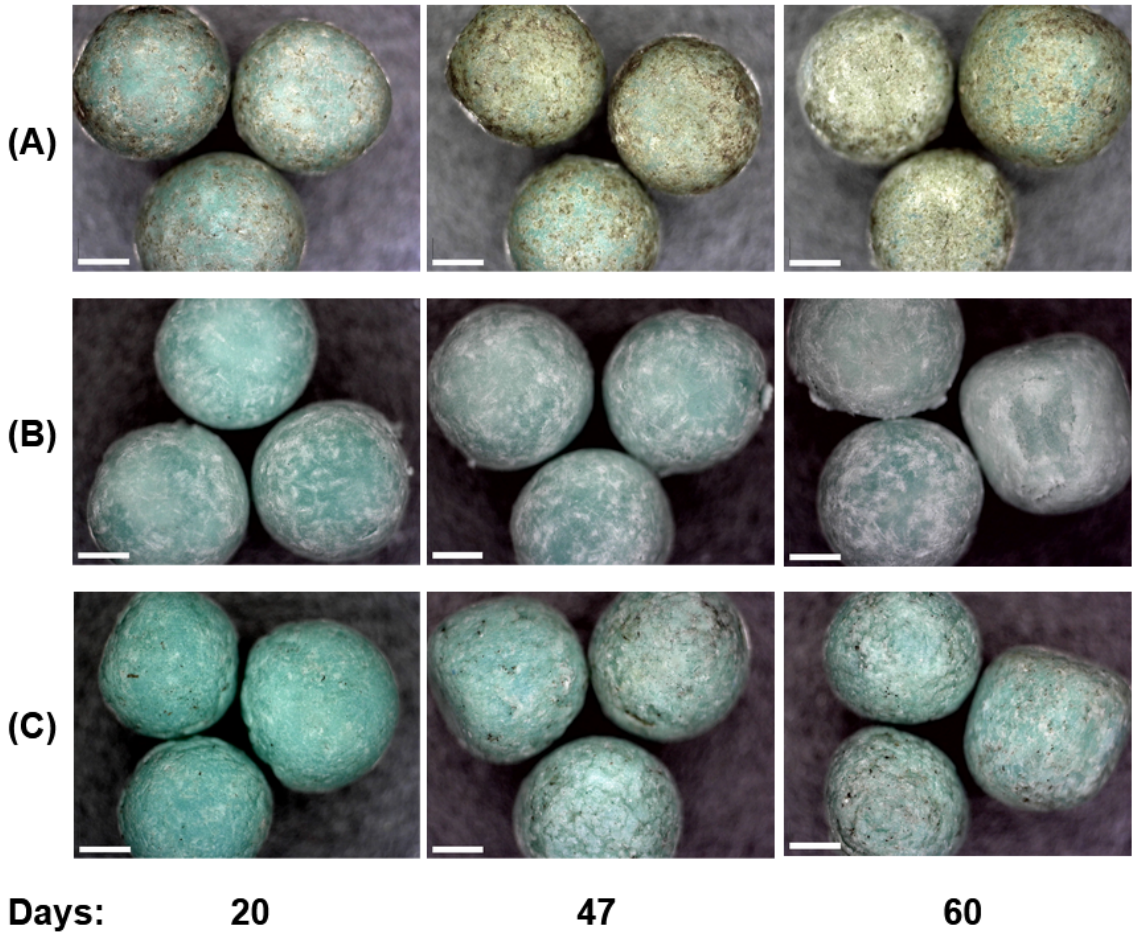


*Figure 9. The figure shows images of different granules from samples retrieved from the biodegradation tests after 20, 47 and 60 days of highly used granules. Rows (A), (B) and (C) show granules in compost, anaerobic sludge and soil respectively. White scale bars in the bottom left corners represent 1 mm.*

The graphs below (Figure 10, Figure 11 and Figure 12) show results from the FTIR-ATR analysis performed on granule samples that were incubated for 47 days in compost, anaerobic sludge and soil. Significant changes in absorption were found in peaks of interest at 850-900 $cm^{-1}$ and 1350-1500 $cm^{-1}$ (decreased $CaCO_3$) for unused granules in soil, 1710 $cm^{-1}$ (decreased C=O) for highly used granules in soil and compost, 1260 $cm^{-1}$ (decreased C-O in ester) for all highly used granules, and 3350 $cm^{-1}$ (increased O-H) for unused granules in anaerobic sludge and soil. However, it is believed that these results were strongly affected by the remains of organic matter from their environments during incubation. Large increases in peaks between 2800-3000 $cm^{-1}$, which corresponds to absorption by $CH_2$ and $CH_3$ bonds, together with a large variance between some samples are strong indicators that this is the case. Therefore these results should be regarded as inconclusive.

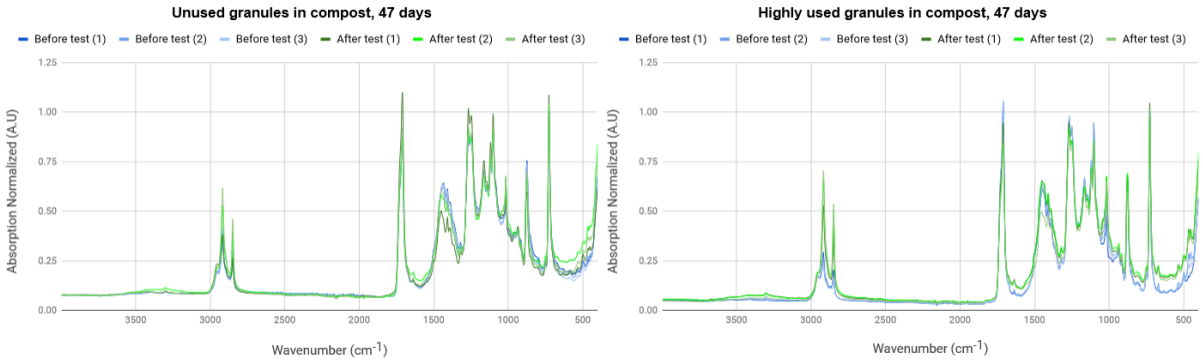


*Figure 10. FTIR-ATR results from biodegradation tests of unused and highly used granules from Batch 2 in compost after 47 days. Blue curves show results from granules that were not incubated and the green curves show results from tested granules.*

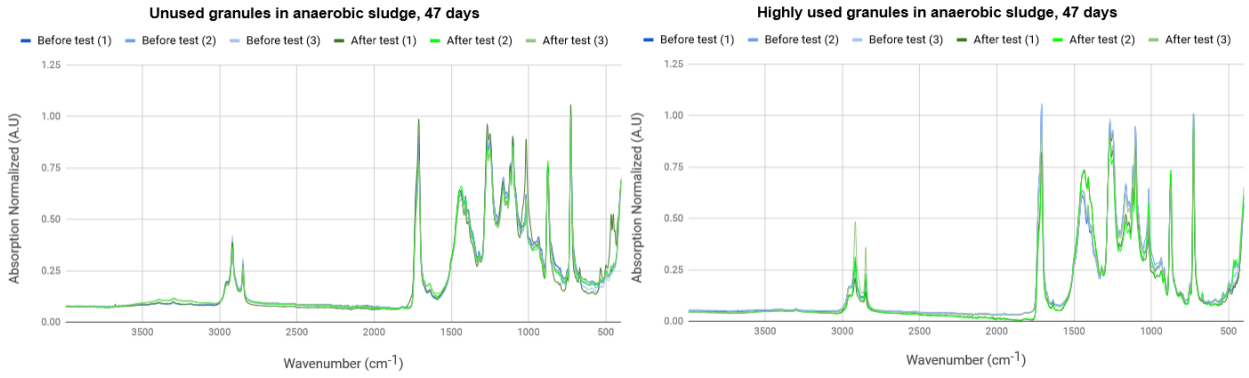


*Figure 11. FTIR-ATR results from biodegradation tests of unused and highly used granules from Batch 2 in anaerobic sludge after 47 days. Blue curves show results from granules that were not incubated and the green curves show results from tested granules.*

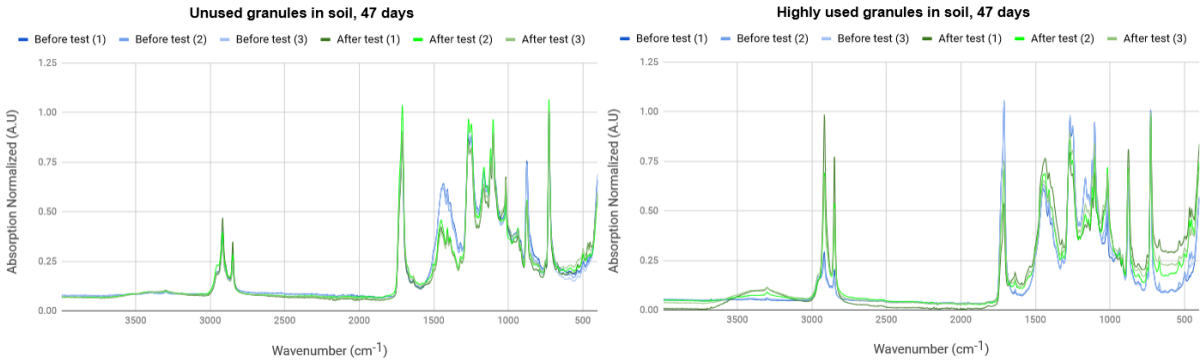


*Figure 12. FTIR-ATR results from biodegradation tests of unused and highly used granules from Batch 2 in soil after 47 days. Blue curves show results from granules that were not incubated and the green curves show results from tested granules.*

## 4.2 pH tests

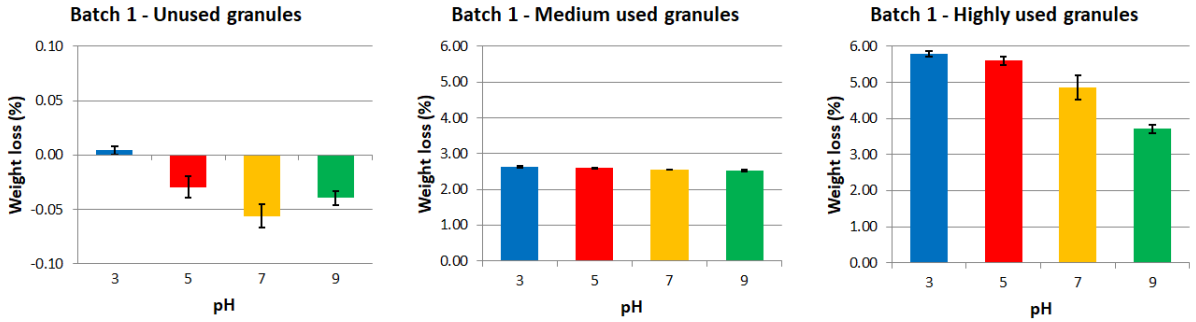


*Figure 13. The graphs show the results from the weight loss measurements of samples from pH tests with granules from Batch 1. The scale of the y-axis in the graph for unused granules (left) is varied to show the difference between samples. Error bars show standard deviation.*

The results from the weight loss measurements of the unused granules from Batch 1 (Figure 13) showed that the granules that were placed in solution with a pH of 3 had kept their weight (0.00 ± 0.00 % weight loss). For the other solutions, with pH 5, 7 and 9, an increase in total weight of the samples was measured (0.03 ± 0.01 %, 0.06 ± 0.01 % and 0.04 ± 0.01 % respectively). However, this increase is very small and it is believed that the results indicate that moisture has been incorporated into the granules of these samples.

The medium used granules from Batch 1 shows a weight loss of 2.64 ± 0.03 % for the granules in pH 3 solution, 2.60 ± 0.01 % for samples in pH 5 solution, 2.56 ± 0.01 % for samples in pH 7 solution and 2.54 ± 0.03 % for the samples in pH 9 solution.

The weight loss measurement of the highly used samples showed a further increase of weight loss in all samples when compared to the less used granules. Samples from pH 3 solution showed a weight loss of 5.80 ± 0.08 % followed by a weight loss of 5.60 ± 0.11 % for pH 5 samples, 4.86 ± 0.34 % for pH 7 solution and 3.72 ± 0.12 % for samples in pH 9 solution.

The measurements show a statistically significant difference in weight loss, when comparing results from samples that were placed in pH 3 solution with samples placed in pH 7 solution, for all types of granules from Batch 1. Furthermore, statistically significant weight losses were found between samples placed in pH 5 and pH 7 in tests of unused and medium used granules, but not for highly used granules. For samples in pH 9 solution a statistically significant weight loss, compared to samples in pH 7, was only found for highly used granules. However, it should be noted that even though a statistically significant difference was found in the weight loss measurements the difference is very small, especially for the unused and medium used granules.

After the tests, the pH of all solutions was measured in the range of 6.57 for pH 3 solutions to 7.95 for pH 9 solutions, which might indicate that $CaCO_3$ has reacted with the more acidic solutions and consumed hydrogen. Furthermore, no significant change in density was measured for any of the samples in pH tests of Batch 1. Microscopic images of the granules after testing did not show any distinct changes, and can be found in Appendix 2 together with the density measurements in Appendix 3.

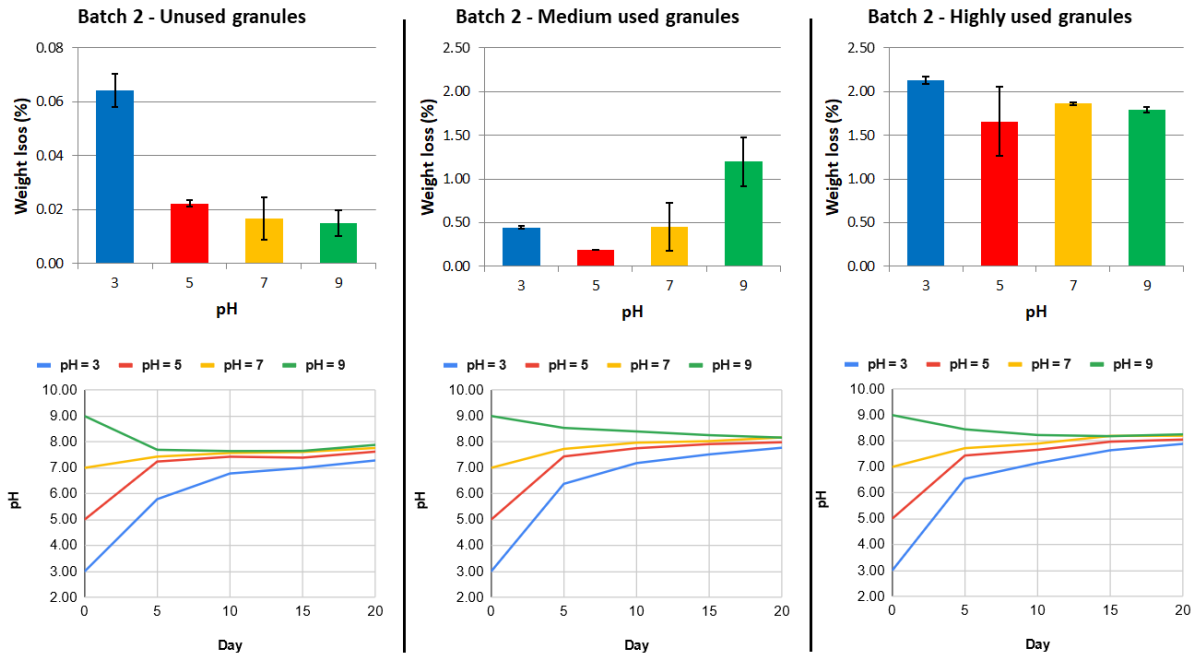


*Figure 14. The graphs show the results from weight loss measurements (after 20 days) and their corresponding pH measurements (mean values after 0, 5, 10, 15 and 20 days) for samples with granules from Batch 2. The scale of the y-axis in the graph for unused granules (top left) is varied to show the difference between samples. Error bars show standard deviation.*

The weight measurements from the pH tests of granules from Batch 2 (Figure 14) showed a slight decrease in weight for all samples in all tests. Compared to the samples from Batch 1 they showed a lower amount of weight loss, possibly due to their improved performance during use. The unused samples of granules showed the lowest weight loss at 0.06 ± 0.01 % for samples in pH 3 solution followed by 0.02 ± 0.00 % for samples in pH 5, 0.02 ± 0.01 % for samples in pH 7 and 0.01 ± 0.00 % for samples in pH 9 solution.

When looking at the weight loss results of the medium used granules, the samples that were placed in a solution of pH 9 showed the largest weight change which is not in line with the previous results. Here, a weight loss of 0.45 ± 0.01 % was found in the samples that were placed in pH 3 solution, 0.19 ± 0.00 % for samples in pH 5, 0.45 ± 0.27 % for samples in pH 7 and 1.20 ± 0.28 % for samples in pH 9 solution. To investigate these results further, the test of medium used granules from Batch 2 was repeated, although with a smaller amount of granules, 10 g of granules instead of 30 g, due to the lack of availability of these granules at the time of the repeated test. The results of this test can be seen in Appendix 4 and showed similar results to those of the previous tests, namely that the samples in pH 3 solution lost slightly more weight when compared to samples in solution with a pH of 5, 7 and 9.

The test of highly used granules from Batch 2 showed that the pH 3 solution had the largest effect of the weight of the samples. A weight loss of 2.13 ± 0.05 % was found for samples in

pH 3, 1.66 ± 0.40 % for samples in pH 5, 1.86 ± 0.01 % for samples in pH 7 and 1.79 ± 0.03 % for samples in pH 9 solution. It should be noted that one of the samples of highly used granules in pH 5 was accidentally spilled out and non-retrievable so the results for these samples are based on duplicate instead of triplicate tests.

A statistically significant weight loss was found in the measurements for samples in pH 3 solution, when comparing the weight loss of them to samples in pH 7 solution, for the tests of unused and highly used granules, but not for medium used samples. No such differences in weight loss were found for samples in pH 5 solution. For samples in pH 9 solution a statistically significant weight loss was found for the medium and highly used granules. Once again the difference is very small, especially for the unused and medium used granules.

The pH measurements during the tests of granules from Batch 2 are shown in the bottom row of Figure 14. It can be seen that the pH values in all solutions moves towards a pH value of approximately 8 in all tests. Furthermore, the density measurements for all samples in pH tests of granules from Batch 2 showed no significant change and imaging of the samples did not show any distinct changes of the surfaces, and can be seen in Appendix 5 and Appendix 6.

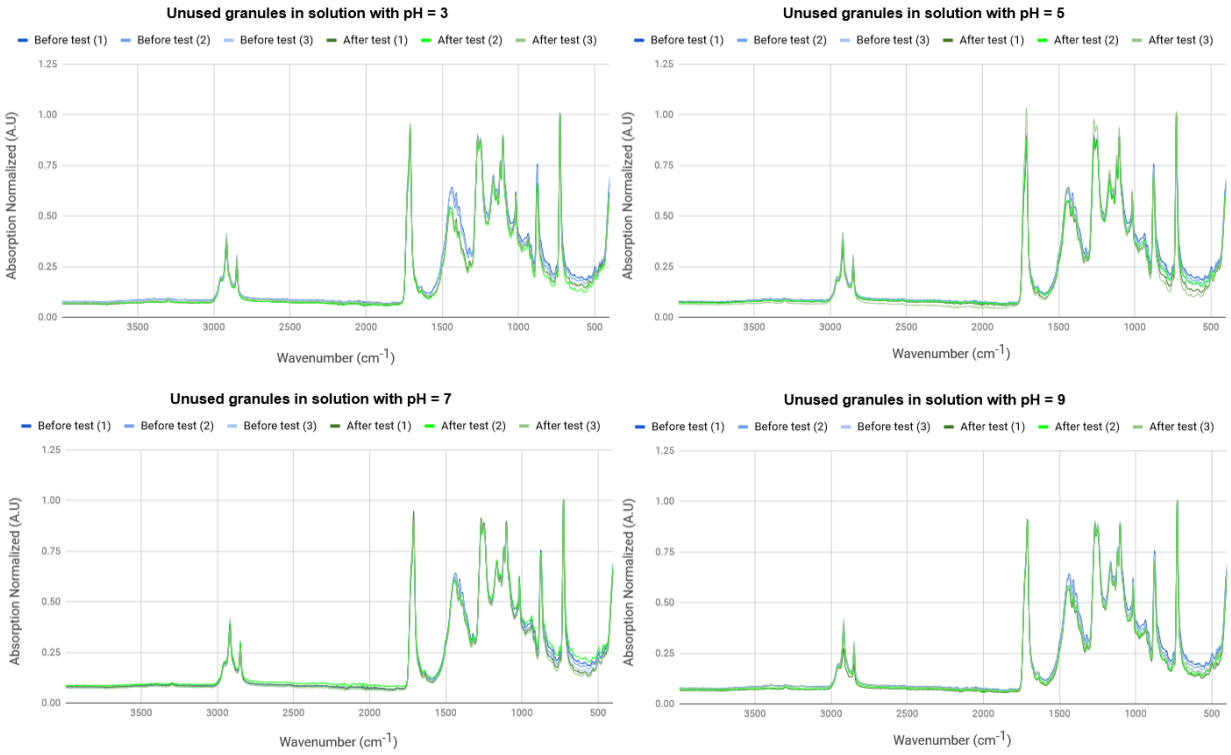


*Figure 15. FTIR-ATR results from pH-test of unused granules from Batch 2 in their corresponding solutions. Blue curves show results from granules that were not incubated and the green curves show results from tested granules.*

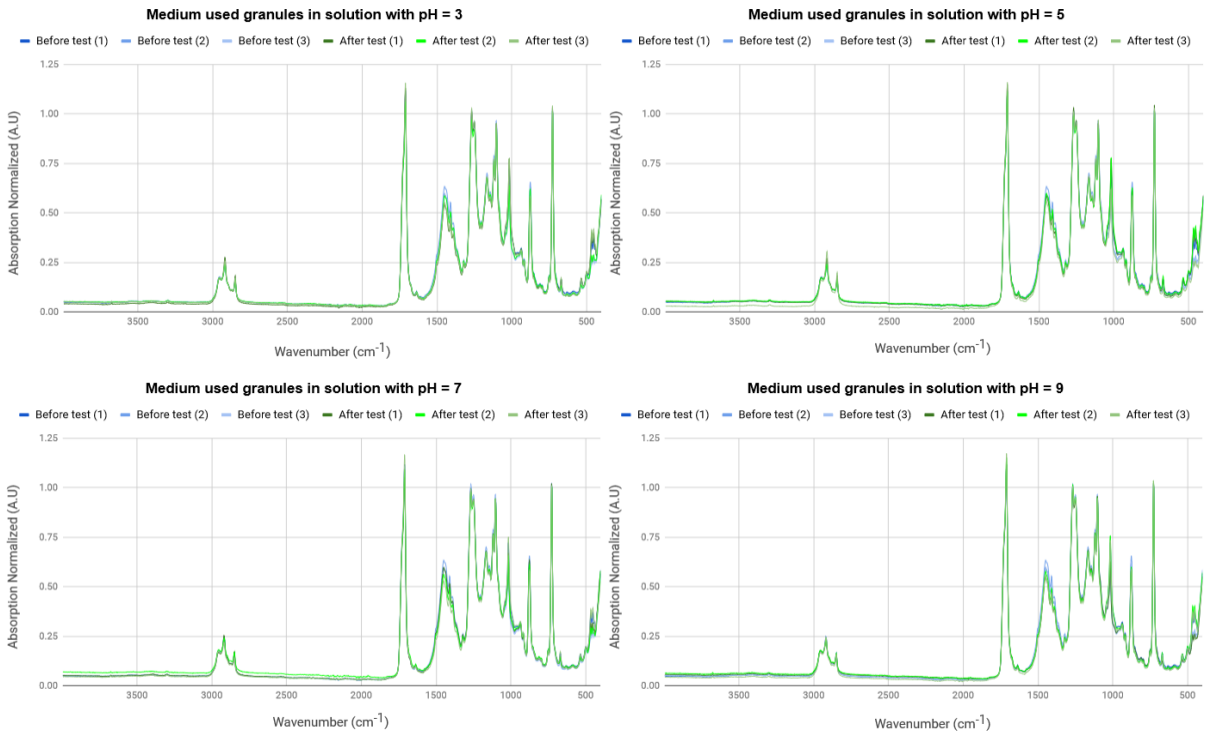


*Figure 16. FTIR-ATR results from pH-test of medium used granules from Batch 2 in their corresponding solutions. Blue curves show results from granules that were not incubated and the green curves show results from tested granules.*

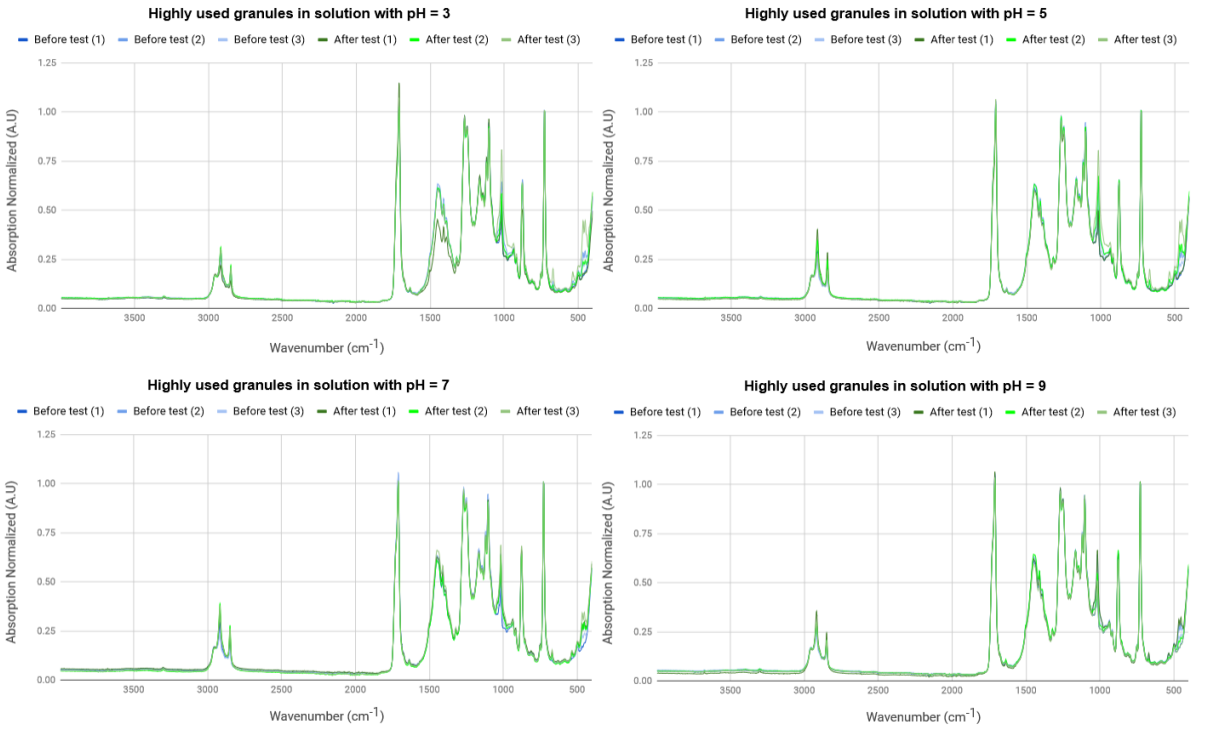


*Figure 17. FTIR-ATR results from pH-test of highly used granules from Batch 2 in their corresponding solutions. Blue curves show results from granules that were not incubated and the green curves show results from tested granules.*

The FTIR-ATR measurements of unused granule samples in pH tests from Batch 2 (Figure 15) showed a statistically significant decrease in absorption at the broad peak around 1350-1500 $cm^{-1}$, which indicates loss of $CaCO_3$, for all samples except those in pH 5 solution. For the sharp peak at 850-900 $cm^{-1}$, a significant decrease was found, which also indicates loss of $CaCO_3$, for all samples except those in pH 9 solution. However, in all cases except for the samples in pH 3 solution these changes were very small.

For the medium used granules in the pH tests (Figure 16), the FTIR-ATR results only showed a statistically significant decrease in absorption at the peaks around 1350-1500 $cm^{-1}$ and 850-900 $cm^{-1}$ for the samples that were placed in pH 9 solution. However, this decrease was very small. The measurements from the other samples showed similar spectras, but a larger variance was seen within those samples.

The FTIR-ATR measurements of the highly used granules in the pH tests (Figure 17) only showed a significant decrease in one peak for all of the samples, which was a decrease in the peak around 1710 $cm^{-1}$ for samples in pH 7 solution. This corresponds to absorption by C=O bonds. No other significant changes in absorption at the peaks of interest were found for the highly used granules.

# 5. Discussion

## 5.1 Biodegradation tests

All biodegradation tests were originally planned to be analyzed after 20, 40 and 60 days to give measurements with even increments of incubation time, but due to unforeseen events the 40 day tests were delayed and ended after 47 days. Nonetheless, the weight measurements from the biodegradation tests showed that the granules have lost more weight in aerobic conditions than under anaerobic conditions. This is consistent with the findings of previously published research regarding PBAT, although those are usually studies of thin films made from pure PBAT material.

The results also showed that there is a large difference between the weight loss of the unused and the highly used granules which is believed to be, at least partially, due to the fact that there is more surface area available for degradation of the highly used samples. This stems from the fact that the sample weights were the same for both unused and highly used samples but the highly used granules were slightly smaller and had a more uneven and torn surface, resulting in a higher number of granules used and more surface area available. Furthermore, it is possible that mechanical forces from repeated stirring of the mixtures, while aerating and

refilling water, may have affected the samples that were placed in compost and soil. Since the water in the anaerobic sludge did not need to be refilled in this way, they were not subjected to the same amount of mechanical forces during the incubation. If this occurred, it is believed to have had the largest effect in the beginning of the tests due to the fact that the potentially removed flakes on the surface of the granules would have been removed during the first few rounds of stirring resulting in an added weight loss at the 20 day measurements. Moreover, there is a possibility of granules being lost during sieving and inspection of the test mixtures, although this was done very carefully and is deemed not very likely.

During the incubation time, water was refilled in the mixtures of soil and compost where the moisture evaporated during testing. For the tests in soil this was done once per week while for the compost tests this was done every 2-3 days due to the higher incubation temperature. The water content of the compost environments were therefore not optimal during the entire period, which might have negatively affected the degradation for these samples. It is also good to note that this compost test was done at the high temperature of 58 °C which is a commonly used temperature in industrial composting facilities, and not in home composting which would occur at lower temperatures.

The results from FTIR-ATR measurements of samples from these tests are deemed to be inconclusive, due to the large variance and the likely presence of contaminations from remains of organic matter from the incubation environments even though they were carefully cleaned as much as possible. It is therefore concluded that FTIR-ATR is not an effective tool for analyzing these types of granules after incubation in these environments.

Although the results show weight loss of the samples in their respective biologically active environments, it can not be firmly concluded that biodegradation or assimilation of the polymer has occurred. It is however assumed that biodegradation has occurred due to the measured results. It would have been good to use blanks, such as granules in air or water, to compare the assumed biotic effects to abiotic effects from water and heat. For more signs of biodegradation to be seen other testing methods would give more clear indications, such as $CO_2$ production measurements used in the long-term studies stated in ISO standards for biodegradability. However, since there was a substantial weight loss, difference between the aerobic and anaerobic environments and the microscopic images showed signs of surface degradation around indentations where compost and soil had accumulated, the tests are believed to be adequate as screenings for biodegradation. It is therefore believed that the tested granules are biodegradable in the used aerobic environments of soil at room temperature and compost at 58 °C, but that total degradation of the granules will take a substantially longer time.

At the rate of weight loss observed in the measurements for soil and compost, it would take approximately 500 days for the highly used granules to lose 100 % of their weight. However, once the granules would lose a substantial amount of weight, their surface area would most likely increase and the rate of weight loss would therefore also increase. Even so, if the rate of weight loss does not increase very rapidly the granules will most likely not pass tests

required for certifications regarding disposal of the granules to environments such as industrial compost sites. For the granules to be able to achieve such certification they would need to show a disintegration of 90 % (only 10 % of the remaining granules being larger than 2 mm) after 3 months and a 90 % conversion of its organic material to $CO_2$ within 6 months. Furthermore, they would need to pass chemical, ecotoxicity and practical tests. Based on the results in this project, it is therefore believed that these granules would not be able to get a certification where such requirements are stated.

## 5.2 pH tests

The results from the pH tests showed that solutions with pH values of 3 had the largest effect on the samples of granules. There was a substantial difference in the weight losses between Batch 1 and Batch 2 which indicates that the granules from Batch 2 were more resistant to degradation by the solutions. The results most likely originate from the fact that the granules of Batch 2 were much more durable during use, and therefore were more intact when they were placed in the solutions. Furthermore, an interesting change in pH values of the solutions were seen, where the values of all solutions shift towards a pH value of around 8. A potential reason for the decreased pH values from 9 to around 8 is the hydrolysis of ester bonds in the polymer which end products, carboxylic acid and alcohol, can be deprotonated. The increase in pH of solutions with starting values of 3, 5 and 7 is believed to be due to uptake of hydrogen by carbonate ions which can form hydrogen carbonate and carbonic acid.

The pH test of medium used granules from Batch 2 showed results which did not follow the same trend as seen in the other tests, where samples in pH 3 solution lost the most amount of weight. However, as stated in the results, a repeated test was done to investigate this further which showed that the medium used granules placed in pH 3 solution did lose the most weight. Even though only one repetition of this test was performed it is believed to have been more accurate, since the results from the repeated test follow the same trend as seen in the other pH tests.

As mentioned previously, one of the triplicate runs of the highly used granules from Batch 2 was accidentally spilled out, resulting in a large variance for that measurement. Variance in sample measurements can also stem from individual variance in granules, depending on how much they were affected by their use in the dishwashing systems previous to testing. Furthermore, additional accidental loss of granules during and after testing is a possibility, although not likely due to the careful handling of the samples throughout the process.

The FTIR-ATR measurements of granule samples from Batch 2 showed more consistent results throughout the samples, compared to the FTIR-ATR measurements of the samples from the biodegradation tests, and are believed to be reliable measurements. Even though there were statistically significant changes in absorption for multiple samples, only the

unused granules from solutions with pH 3 showed a slight decrease in absorption of $CaCO_3$ after the tests. However, the measurements indicate similar results as most of the weight and pH measurements, which is that $CaCO_3$ in the granules is affected by the solutions with lower pH values.

### 5.2 Future aspects

While results from the biodegradation tests did not show that the granules degraded extremely rapidly, as one would optimally like, it would be interesting to perform studies during longer time periods. Such studies could give further information about the disposal opportunities of the granules. It is however believed that the trade-off between rapid degradation after use versus properties and durability during use is hard to achieve for these types of products. An interesting idea is to focus on the development and use of a fully biobased PBAT granule, instead of a partially biobased granule, if such product would have the same properties and durability as the now used granules have which it should since it has the same chemical structure.

Furthermore, since the pH tests showed that the solutions with a pH value of 3 had a larger effect on the granules than the solutions with higher pH values, the results lead to questions about how the granules would be affected by even higher pH values, beyond the pH values of foods. It would therefore be interesting to study the pH effects in a wider range.

## 6. Conclusions

The two main objectives in this project was to investigate the effect on polymer granules by the pH corresponding to that of foods, to give insights towards optimal storage in between uses, and to investigate the effects of biologically active environments to give insights into the biodegradability of the product and its possible disposal alternatives. The results of the study showed that the granules were affected the most by solutions with a pH value of 3, when compared to solutions with neutral pH, which indicates that the more neutral conditions are preferred during storage in between uses.

Furthermore, the results from the biodegradation tests showed that aerobic conditions (soil and compost) had a larger effect on the granules than anaerobic conditions (anaerobic sludge). Even though the product seems to be biodegradable in soil and compost the observed rate of degradation is relatively slow. More long-term studies, and possibly using other methods, would be needed to truly investigate the possibilities for a different disposal of the granules than today's disposal for incineration.

# 7. Acknowledgments

I extend my deep gratitude to everyone who assisted and gave support during the time this work was performed. A special thanks to supervisors Balazs Franko, Johanna Friman and Per Walter for their support, help and guidance throughout this project. Furthermore I would like to extend a large thank you to Lisa Mårtensson and Åsa Håkansson at Granuldisk for all the guidance, support and company in the lab. Last, but far from least, a gigantic thank you to friends and family who supported and kept me going throughout this process.

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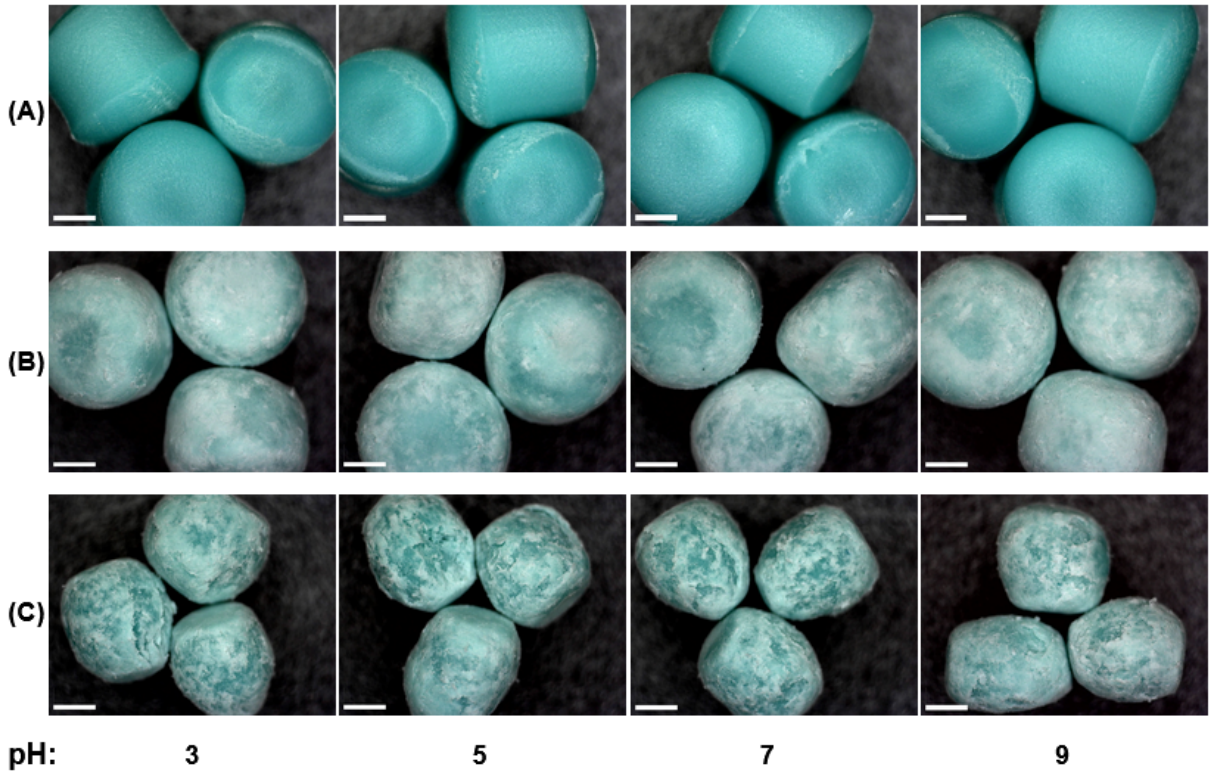
# Appendix

| S. # | Density, before (g/ml) | Density, after (g/ml) | S. # | Density, before (g/ml) | Density, after (g/ml) | S. # | Density, before (g/ml) | Density, after (g/ml) |
|---|---|---|---|---|---|---|---|---|
| **#CU20.1** | 1.34 | 1.35 | **#AU20.1** | 1.34 | 1.34 | **#SU20.1** | 1.33 | 1.34 |
| **#CU20.2** | 1.35 | 1.34 | **#AU20.2** | 1.35 | 1.35 | **#SU20.2** | 1.35 | 1.34 |
| **#CU20.3** | 1.35 | 1.35 | **#AU20.3** | 1.35 | 1.34 | **#SU20.3** | 1.35 | 1.34 |
| **#CU40.1** | 1.35 | 1.34 | **#AU40.1** | 1.35 | 1.35 | **#SU40.1** | 1.34 | 1.35 |
| **#CU40.2** | 1.35 | 1.35 | **#AU40.2** | 1.34 | 1.35 | **#SU40.2** | 1.35 | 1.34 |
| **#CU40.3** | 1.33 | 1.34 | **#AU40.3** | 1.35 | 1.34 | **#SU40.3** | 1.34 | 1.34 |
| **#CU60.1** | 1.34 | 1.33 | **#AU60.1** | 1.35 | 1.35 | **#SU60.1** | 1.34 | 1.35 |
| **#CU60.2** | 1.35 | 1.35 | **#AU60.2** | 1.34 | 1.35 | **#SU60.2** | 1.35 | 1.33 |
| **#CU60.3** | 1.35 | 1.34 | **#AU60.3** | 1.34 | 1.34 | **#SU60.3** | 1.35 | 1.35 |
| **#CH20.1** | 1.34 | 1.35 | **#AH20.1** | 1.34 | 1.34 | **#SH20.1** | 1.34 | 1.33 |
| **#CH20.2** | 1.35 | 1.33 | **#AH20.2** | 1.35 | 1.35 | **#SH20.2** | 1.35 | 1.34 |
| **#CH20.3** | 1.35 | 1.35 | **#AH20.3** | 1.34 | 1.34 | **#SH20.3** | 1.35 | 1.34 |
| **#CH40.1** | 1.35 | 1.35 | **#AH40.1** | 1.34 | 1.35 | **#SH40.1** | 1.34 | 1.35 |
| **#CH40.2** | 1.34 | 1.34 | **#AH40.2** | 1.35 | 1.35 | **#SH40.2** | 1.34 | 1.33 |
| **#CH40.3** | 1.35 | 1.34 | **#AH40.3** | 1.35 | 1.34 | **#SH40.3** | 1.35 | 1.34 |
| **#CH60.1** | 1.34 | 1.35 | **#AH60.1** | 1.34 | 1.35 | **#SH60.1** | 1.35 | 1.34 |
| **#CH60.2** | 1.35 | 1.35 | **#AH60.2** | 1.34 | 1.33 | **#SH60.2** | 1.34 | 1.35 |
| **#CH60.3** | 1.34 | 1.33 | **#AH60.3** | 1.35 | 1.35 | **#SH60.3** | 1.34 | 1.34 |

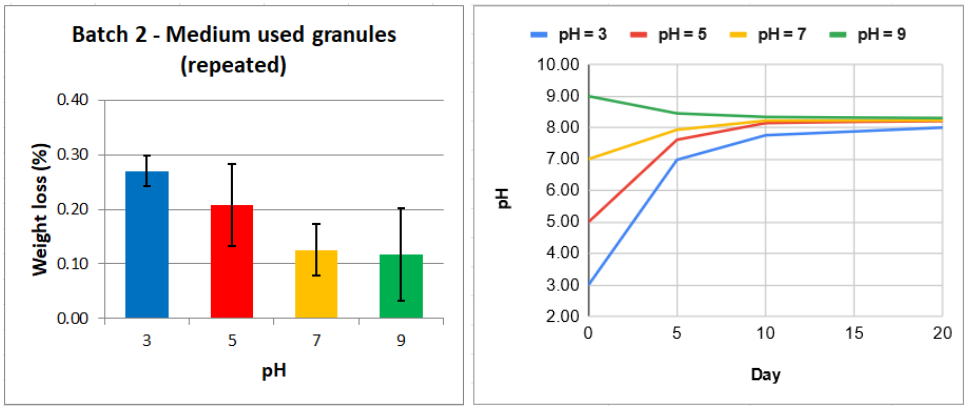
*Appendix 1. Table shows results from the density measurements for granule samples before and after biodegradation tests. Sample numbers (S. #) corresponds to Compost Unused (CU), Compost Highly used (CH), Anaerobic sludge Unused (AU), Anaerobic sludge Highly used (AH), Soil Unused (SU) and Soil Highly used (SH) granules. The numbers 20, 40 and 60 in the sample numbers denote the incubation time.*




*Appendix 2. Images of granules after pH-tests of Batch 1. Rows (A), (B) and (C) show test samples of unused, medium used and highly used granules respectively. White scale bars in the bottom left corners represent 1 mm.*

| S. # | pH | Density, before (g/ml) | Density, after (g/ml) | S. # | pH | Density, before (g/ml) | Density, after (g/ml) | S. # | pH | Density, before (g/ml) | Density, after (g/ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **#1.1** | 3 | 1.37 | 1.38 | **#5.1** | 3 | 1.38 | 1.37 | **#9.1** | 3 | 1.37 | 1.38 |
| **#1.2** | 3 | 1.38 | 1.37 | **#5.2** | 3 | 1.39 | 1.38 | **#9.2** | 3 | 1.36 | 1.37 |
| **#1.3** | 3 | 1.38 | 1.38 | **#5.3** | 3 | 1.38 | 1.38 | **#9.3** | 3 | 1.38 | 1.38 |
| **#2.1** | 5 | 1.38 | 1.38 | **#6.1** | 5 | 1.39 | 1.39 | **#10.1** | 5 | 1.38 | 1.37 |
| **#2.2** | 5 | 1.37 | 1.38 | **#6.2** | 5 | 1.39 | 1.40 | **#10.2** | 5 | 1.37 | 1.38 |
| **#2.3** | 5 | 1.38 | 1.36 | **#6.3** | 5 | 1.39 | 1.39 | **#10.3** | 5 | 1.37 | 1.38 |
| **#3.1** | 7 | 1.39 | 1.37 | **#7.1** | 7 | 1.38 | 1.40 | **#11.1** | 7 | 1.37 | 1.37 |
| **#3.2** | 7 | 1.38 | 1.36 | **#7.2** | 7 | 1.39 | 1.40 | **#11.2** | 7 | 1.37 | 1.38 |
| **#3.3** | 7 | 1.38 | 1.38 | **#7.3** | 7 | 1.39 | 1.39 | **#11.3** | 7 | 1.36 | 1.37 |
| **#4.1** | 9 | 1.39 | 1.38 | **#8.1** | 9 | 1.39 | 1.38 | **#12.1** | 9 | 1.37 | 1.38 |
| **#4.2** | 9 | 1.37 | 1.38 | **#8.2** | 9 | 1.39 | 1.40 | **#12.2** | 9 | 1.38 | 1.38 |
| **#4.3** | 9 | 1.39 | 1.37 | **#8.3** | 9 | 1.38 | 1.39 | **#12.3** | 9 | 1.36 | 1.37 |

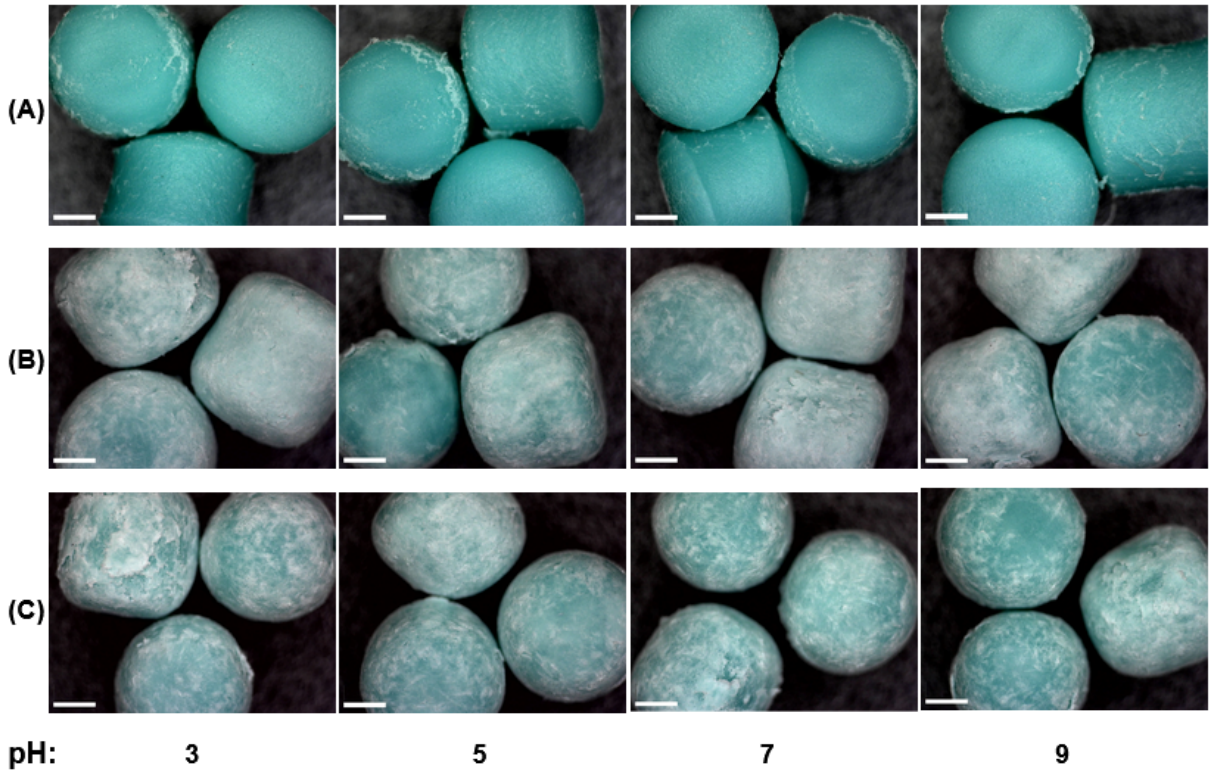
*Appendix 3. Table shows measured density for the granule samples before and after pH tests of granules from Batch 1. Sample numbers (S. #) 1-4, 5-8 and 9-12 correspond to unused, medium used and highly used granules respectively.*




*Appendix 4. Results from the repeated pH test of medium used granules from Batch 2, showing both the measured weight loss (left) and the pH measured in the solutions after 0, 5, 10, 15 and 20 days (right).*

| S. # | pH | Density, before (g/ml) | Density, after (g/ml) | S. # | pH | Density, before (g/ml) | Density, after (g/ml) | S. # | pH | Density, before (g/ml) | Density, after (g/ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **#13.1** | 3 | 1.34 | 1.35 | **#17.1** | 3 | 1.36 | 1.35 | **#21.1** | 3 | 1.35 | 1.35 |
| **#13.2** | 3 | 1.33 | 1.34 | **#17.2** | 3 | 1.36 | 1.36 | **#21.2** | 3 | 1.35 | 1.34 |
| **#13.3** | 3 | 1.33 | 1.34 | **#17.3** | 3 | 1.35 | 1.34 | **#21.3** | 3 | 1.35 | 1.35 |
| **#14.1** | 5 | 1.34 | 1.34 | **#18.1** | 5 | 1.35 | 1.34 | **#22.1** | 5 | 1.35 | 1.36 |
| **#14.2** | 5 | 1.34 | 1.33 | **#18.2** | 5 | 1.35 | 1.35 | **#22.2** | 5 | 1.35 | 1.34 |
| **#14.3** | 5 | 1.33 | 1.35 | **#18.3** | 5 | 1.36 | 1.35 | **#22.3** | 5 | 1.35 | 1.35 |
| **#15.1** | 7 | 1.34 | 1.33 | **#19.1** | 7 | 1.36 | 1.35 | **#23.1** | 7 | 1.34 | 1.35 |
| **#15.2** | 7 | 1.34 | 1.34 | **#19.2** | 7 | 1.35 | 1.36 | **#23.2** | 7 | 1.35 | 1.34 |
| **#15.3** | 7 | 1.33 | 1.35 | **#19.3** | 7 | 1.36 | 1.35 | **#23.3** | 7 | 1.35 | 1.34 |
| **#16.1** | 9 | 1.33 | 1.34 | **#20.1** | 9 | 1.35 | 1.34 | **#24.1** | 9 | 1.35 | 1.34 |
| **#16.2** | 9 | 1.33 | 1.35 | **#20.2** | 9 | 1.36 | 1.36 | **#24.2** | 9 | 1.34 | 1.35 |
| **#16.3** | 9 | 1.34 | 1.34 | **#20.3** | 9 | 1.35 | 1.34 | **#24.3** | 9 | 1.34 | 1.35 |

*Appendix 5. Table shows measured density for the granule samples before and after pH tests of granules from Batch 2. Sample numbers (S. #) 13-16, 17-20 and 21-24 correspond to unused, medium used and highly used granules respectively.*




*Appendix 6. Images of granules from pH-tests of Batch 2. Rows (A), (B) and (C) show test samples of unused, medium used and highly used granules respectively. White scale bars in the bottom left corners represent 1 mm.*