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From waste to self-healing concrete: A proof-of-concept of a new application for polyhydroxyalkanoate

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ABSTRACT

Polyhydroxyalkanoate (PHA) production is a promising opportunity to recover organic carbon from waste streams. However, widespread application of waste-derived PHA as biodegradable plastic is restricted by expensive purification steps, high quality requirements, and a fierce competition with the conventional plastic market. To overcome these challenges, we propose a new application for waste-derived PHA, using it as bacterial substrate in self-healing concrete. Self-healing concrete is an established technology developed to overcome the inevitable problem of crack formation in concrete structures, by incorporating a so-called bacteria-based healing agent. Currently, this technology is hampered by the cost involved in the preparation of this healing agent. This study provides a proof-of-concept for the use of waste-derived PHA as bacterial substrate in healing agent. The results show that a PHA-based healing agent, produced from PHA unsuitable for thermoplastic applications, can induce crack healing in concrete specimens, thereby reducing the water permeability of the cracks significantly compared to specimens without a healing agent. For the first time these two emerging fields of engineering, waste-derived PHA and self-healing concrete, both driven by the need for environmental sustainability, are successfully linked. We foresee that this new application will facilitate the implementation of waste-derived PHA technology, while simultaneously supplying circular and potentially more affordable raw materials for self-healing concrete.

1. Introduction

Polyhydroxyalkanoate (PHA) has attracted widespread attention as an alternative to petrochemical-based plastics. PHA is biobased, completely biodegradable, and has thermoplastic properties (Lee, 1996). An opportunity to produce PHA cost-effectively is by using mixed microbial communities and organic waste streams as feedstock. These technologies diminish the relatively large expenses for raw substrates and sterilization (Kleerebezem and Loosdrecht, 2007), and consequently, avoid part of the waste disposal costs (Fernández-Dacosta et al., 2015). To date, a multitude of organic waste streams have been assessed successfully for PHA production in laboratory experiments (Rodriguez-Perez et al., 2018). Moreover, pilot projects, using industrial waste water or activated waste water sludge as feedstock, reached promising PHA productivities for achieving an economically viable process (Jia et al., 2014; Jiang et al., 2012; Tamis et al., 2014).

While the biotechnological process to produce PHA from waste is reasonably well-established, challenges remain in its conversion into a marketable thermoplastic. First of all, the purification costs are responsible for a large fraction of the total production cost due to high energy and chemical demand (Gurieff and Lant, 2007). A second challenge is to achieve a high and consistent quality product as required for commercially interesting plastics. More research is required to predict the relationship between raw material input, process parameters, and final mechanical properties of the produced PHA accurately (Laycock et al., 2013). Finally, introducing waste-derived PHA into the

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conventional plastic market is a lasting and complicated procedure. This is mainly caused by a lack of distribution channels, a lack of experience in bioplastic processing, and by the small scale at which PHA is currently produced compared to petrochemical plastics (Bengtsson et al., 2017; Iles and Martin, 2013).

In light of these challenges, we believe that a market entry of waste-derived PHA has a higher chance of success if the initial aim is not to produce bioplastics. Instead, the focus should be on new applications where minor fractions of impurities, and small variations in polymer characteristics are not regarded as problematic. Such a niche application can stimulate the introduction of waste-derived PHA into the market, while avoiding the obstacles and the complexity of the conventional plastic industry. Moreover, these applications can potentially exploit the unique properties of PHA (e.g., biodegradability) more effectively (Kleerebezem et al., 2015; Tamis, 2015).

For PHA, some innovative niche applications have been explored already, such as bio-based paper coating (Lauzier et al., 1993), bio-based glue (Pereira et al., 2019), and slow-release fertilizer/herbicide (Boyandin et al., 2017; Cao et al., 2019) . However, none of these applications are well-established yet. Therefore, we introduce and demonstrate a new application for waste-derived PHA, using it as bacterial substrate in self-healing concrete.

The technology of self-healing concrete was developed to overcome the almost inevitable problem of crack formation in aging concrete. Aggressive substances which enter through cracks can deteriorate the concrete, and decrease the service life of constructions considerably (Yang et al., 2004). In this way, the technology of self-healing concrete is capable of decreasing maintenance costs and increasing service life of constructions, and consequently, reducing part of the enormous amount of carbon dioxide emitted by the concrete industry (Quéré et al., 2018). Field trials of self-healing concrete structures have now demonstrated this technology at larger scale (Wiktor and Jonkers, 2016). However, for large-scale implementation there is still an urgent need for a more cost-effective production of healing agent (Silva et al., 2015).

The self-healing properties of the concrete are established through inclusion of a granulated healing agent in the concrete before casting. This healing agent consists mainly of a bacterial substrate, supplemented with a small fraction of bacterial spores and other essential nutrients. Once incorporated in the concrete, the spores become active when water intrudes due to crack formation. Upon activation the bacteria are able to metabolize the bacterial substrate to carbon dioxide. Due to the alkaline (pH of 12–13) and calcium-rich environment of the concrete, the carbon dioxide reacts to calcium carbonate. In this way, bacterial growth induces the formation of mineral precipitates in the crack, which decrease the water permeability of the concrete (Jonkers, 2007; Mors and Jonkers, 2017; Tziviloglou et al., 2016). In previous research, other bacterial substrates have been investigated, namely urea (Wang et al., 2015), peptone (Xu et al., 2018), different organic calcium acids (calcium lactate, calcium acetate, calcium gluconate, calcium formate) (De Belie et al., 2018), magnesium acetate (Palin et al., 2017), and a lactate derivative (Mors, 2015). Most substrates (e.g. lactate derivative) are obtained from valuable agro-industrial feedstock (i.e., sucrose or glucose), and potentially impose larger economic limitations to the product application than substrates derived from waste streams (Fernández-Dacosta et al., 2015; van den Oever et al., 2017).

By applying waste-derived PHA as bacterial substrate in self-healing concrete, we aim to maximize the added value of biodegradability by using waste-derived PHA as substrate for bacteria. Also, we envision that the purity and physicochemical properties of the PHA, such as monomer composition, molar mass distribution, and melting temperature, are of secondary importance in this application. Finally, using waste-derived PHA in concrete opens the possibility to use secondary resources in a bulk product, thereby contributing substantially to the principles of the circular economy. We propose that linking waste-derived PHA to self-healing concrete can boost the development of both technologies that aim for more effective use of resources. To our knowledge, this is the first time that waste-derived PHA technology is used in a civil engineering application, and the first time that a waste-derived material is used as main nutrient in self-healing concrete. The aim of this study was to introduce the concept of using waste-derived PHA as bacterial substrate in self-healing concrete, and to evaluate its feasibility.

To this end, PHA-rich biomass was received from a pilot plant that uses organic waste as raw material. The PHA was extracted and converted under laboratory conditions to a healing agent, and subsequently cast in concrete specimens. Three requirements were set to assess the performance of waste-derived PHA as bacterial substrate in self-healing concrete: 1) the ability of the self-healing bacteria to metabolize extracellular PHA, 2) the ability of PHA-based healing agent to induce mineral precipitation on the crack surface of concrete specimens, and 3) the ability of PHA-based healing agent to decrease the water permeability of cracks in concrete specimens. These conditions need to be met for a first validation of the proposed application.

2. Materials and methods

2.1. Source of PHA-rich biomass

The PHA-rich biomass was received from a pilot plant (Orgaworld/Paques, Lelystad, the Netherlands) where the organic fraction of municipal solid waste (OFMSW) is used as a raw material. The upstream production process of this pilot plant consists of a hydrolysis and acidification stage, a PHA producing biomass enrichment stage, and a PHA accumulation stage. The operating conditions of the enrichment reactor and the accumulation reactor are described by Mulders et al. (2020). The first part of the purification process was conducted on-site and consisted of centrifugation and oven-drying (20 h at 105 °C) of the PHA-rich biomass.

2.2. PHA extraction process

The dried PHA-rich biomass was processed further under laboratory conditions. The PHA was extracted using 1-hexanol as a solvent. An amount of 210 gram PHA-rich biomass was heated together with 1.6 L 1-hexanol to 140 °C. The material was incubated at this temperature for 30 min under continuous stirring (100 rpm). Subsequently, the 1-hexanol mixture was filtered to remove non-dissolved biomass, using a 1.2 μm filter paper (GE Healthcare Life Sciences, UK) that was placed in a Büchner funnel. Before filtering, the Büchner funnel and the filter paper were preheated to 105 °C in an oven. After filtering, the filtrate was allowed to cool down to room temperature under continuous stirring (100 rpm). The PHA precipitated during the cooling procedure. In a second filtration step, the PHA was filtered from the solution, using a cotton cloth. The purified PHA was subsequently vacuum dried at 50 °C for 24 h to remove traces of 1-hexanol.

2.3. Axenic growth experiment

The purified PHA was used as substrate in an axenic growth experiment to verify the growth of the self-healing bacterial strain, a *Bacillus cohnii*-related strain. To this end, three shake flasks were filled with 20 mL of carbon-free minimal medium consisting of 3.75 mM KNO_3, 3.7 mM NH_4Cl, 0.15 mM KH_2PO_4, 1 mM $MgCl_2$, 50 mM $NaHCO_3$, 50 mM Na_2CO_3, 1 mL/L trace elements solution according to Vishniac and Santer (1957) and 1 mL/L vitamin solution according to Phillips et al. (1993). In addition, 60 mg UV-sterilized PHA and 2 mL of an axenic *B. cohnii*-related culture (1.5×10^9 cells/mL) were added. As control experiments, flasks were prepared with the same medium, but supplemented with either PHA or the *B. cohnii*-related culture, again in triplicate.

Flasks were incubated at room temperature for 48 h while stirring at 200 rpm. Flasks with only PHA were incubated longer (11 days) to verify the axenic conditions of the experiment. Over time, the cell

concentration was measured with a BD Accuri C6® flow cytometer (BD Accuri cytometers, Belgium). Before measuring, samples were stained with 10 µL/mL SYBR® Green I (1:100 dilution in demineralized water) (Invitrogen, USA), by incubating the sample and the stain for 10 min at 35 °C in the dark. Flow cytometric measurements and data analysis were performed as described by Pinel et al. (2020).

2.4. Healing agent formulation

Purified PHA (5 g) was ground with a mortar and a pestle to a powder. Lyophilized spores (20 mg) of the Bacillus cohnii-related strain and additional nutrients in the form of yeast extract (102 mg) (Scharlau, Spain) were added to the PHA powder. The obtained mixture was melted for 30 s at 100 ± 10 °C and simultaneously flattened to a sheet with a thickness of 0.75 ± 0.25 mm. The sheet was kept for 2 days at room temperature to solidify, and after that ground to particles using a miniature grinding machine (Princess household supplies, Breda, the Netherlands) for 10 s. After grinding, the particles were sieved in order to obtain a fraction with a size between 0.5 and 1.0 mm. The larger faction (>1.0 mm) was ground again, and the smaller fraction (<0.5 mm) was first melted and solidified, and later ground again. Lastly, 4.3 g particles with a size between 0.5 and 1.0 mm were obtained, which was partly used as healing agent in the experiments below.

2.5. Preparation of concrete specimens

The concrete specimens were prepared using an adapted version of the method described by Palin et al. (2016). In fact, mortar was chosen for this experiment as a substitute for concrete; however, we will refer to concrete in the rest of the publication. Three concrete specimen series were prepared, each consisting of 7 cylinder-shaped pieces (diameter 35 mm; height 60 mm). A series was prepared without healing agent (further referred to as negative control specimens); a series was prepared with a currently marketed healing agent (further referred to as positive control specimens) containing a lactate derivative (Basilisk, Delft, the Netherlands) (Mors and Jonkers, 2017); and a series was prepared with healing agent containing PHA (further referred to as PHA specimens) (see Section 2.4). Each specimen series was prepared by homogeneously mixing the following ingredients: 110.5 g Portland cement (CEM I 42, 5 N), 55.3 g tap water, 109.4 g sand (grainsize of 1–2 mm), 112.7 g sand (grainsize of 0.5–1 mm), 69.6 g sand (grainsize of 0.25–0.5 mm), 39.8 g sand (grainsize of 0.125–0.25 mm), and 3.0 g healing agent with a size of 0.5–1.0 mm (not added to the negative control specimens). The resulting concrete mixtures were cast in a plastic cylinder-shaped mold, which was designed such that the obtained specimens contained two opposite grooves (2 mm wide and 3 mm deep) running along their sides. All specimens were cured inside the plastic mold at room temperature for 28 days.

2.6. Crack formation and incubation

After curing for 28 days, a crack was made in every specimen with a servohydraulic testing machine (Instron Corp., Canton, MA). A compressive load was applied until the specimens split from groove to groove. After splitting, the crack width was fixed at 0.4 mm using spacers (2.4 mm wide and 3 mm deep) that fitted in the grooves. Details of this procedure have been presented by Palin et al. (2016). However, instead of using temporary Perspex spacers, specifically developed permanent spacers were used. The spacers were secured with a universal glue, which was allowed to dry for 24 h at room temperature. Specimens were incubated under humid conditions (>95% RH) at 20 ± 2 °C for 56 days.

2.7. Crack evaluation

Before and after incubation, the cracks at the top and bottom of the specimens were imaged with a stereomicroscope (Leica MZ6, Nussloch, Germany). The photos before incubation were used to measure the crack width and to calculate the average crack width, making use of imaging analysis software (ImageJ).

Environmental scanning electron microscopy (ESEM) was conducted to analyze the precipitates formed on the crack surface using a Philips XL30 Series under Back-scatter electron mode (BSE). As preparation, an incubated specimen was broken into its two halves directly before analysis, such that the crack surface was completely exposed and precipitates could be examined. The stereomicroscopic and ESEM photos acquired were used to link the visual observations to the functional properties of the specimens (i.e., water permeability experiment in Section 2.8).

2.8. Water permeability experiment

Three specimens from each series (negative control, positive control, and PHA) were assessed on their water permeability without being subjected to an incubation period. The average of these specimens represent the initial water permeability for all specimens belonging to this series. Four other specimens from each series were assessed on their water permeability after incubation under humid conditions; these specimens provide the final water permeability. In addition, the incubated specimens were dried at 36 °C for 7 days before the final water permeability was tested.

The permeability of the specimens was measured using a method developed by Palin et al. (2016). A schematic representation of the experimental set-up is shown in Fig. 1. Accordingly, specimens were placed in a permeability cell and then attached to the bottom of a 1 m column. At the top of the column a water reservoir was placed. Water, which flowed from the reservoir through the column and finally through the crack of the specimens, was collected in buckets and weighted at 5, 10, and 15 min. The water level in the reservoir was manually kept constant during the measurements. In doing so, the crack experienced an almost constant water pressure of 0.1 bar.

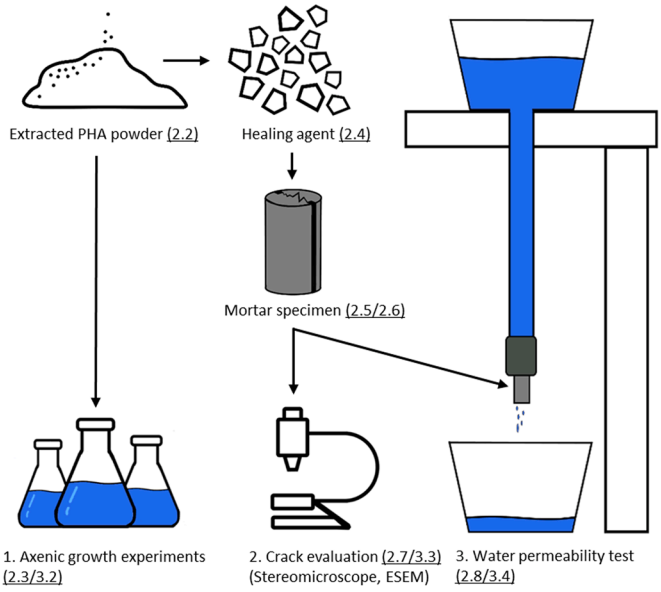

Fig. 1. Experimental flow-chart of this study and a schematic representation of the experimental set-up of the water permeability test. The underlined numbers refer to the sections of the Materials and Methods and Results and Discussion where this step of the flow-chart is described. The three experimental outputs of this study (1, 2, and 3) are shown at the lower part of the chart.

The data retrieved from this experiment was used to calculate the reduction of the water flow (*RWF*) as follows:

$$RWF = \frac{W_{i,\text{average}} - W_{f,n}}{W_{i,\text{average}}} * 100\%$$

where $W_{i,\text{average}}$ is the average (out of three specimens) amount of water that flowed through the cracks of non-incubated specimens (every specimen is measured in triplicate); $W_{f,n}$ is the amount of water that flowed through the crack of an incubated specimen (every specimen is measured in triplicate).

2.9. PHA characterization

The PHA after extraction was analyzed for purity and monomer content (hydroxybutyrate (HB) and hydroxyvalerate (HV)) using gas chromatography. The method is described in detail by Johnson et al. (2009). In brief, the PHA was hydrolyzed and esterified in the presence of concentrated HCl, propanol, and dichlorethane with a ratio of 1/4/5 (v/v/v) for 2 h at 100 °C. The formed esters, which accumulated in the organic phase, were analyzed by a gas chromatograph (model 6890 N, Agilent, USA). However, a mixture of methyl-3-hydroxybutyrate and methyl-3-hydroxyvalerate (Sigma Aldrich, USA) was used as standard due to a reduced purity of the commonly used standard (P(3HB-co- 3HV) with 12 mol% 3-HV (Sigma Aldrich, USA), as shown by Burniol-Figols et al. (2020).

A differential scanning calorimeter (DSC) measurement was performed to measure the glass transition (T_g) and melting (T_m) temperature using a Perkin Elmer DSC-7. The PHA sample was heated from 25 to 140 °C at a rate of 10 °C/min. After 1 min at 140 °C, the sample was cooled to −20 °C at the same rate. In a second run, the sample was heated again to 140 °C at the same rate. All steps were performed under a nitrogen atmosphere.

A thermogravimetric analysis (TGA) was performed to measure the decomposition temperature (T_d) using a Perkin Elmer TGA 8000. The PHA sample was heated from 30 °C to 350 °C at 10 °C/min under a nitrogen atmosphere.

A gel permeation chromatography (GPC) measurement was performed to measure the molecular weight distribution of PHA using a Shimadzu Prominence GPC system equipped with a Shodex LF-804 column. Tetrahydrofuran (THF) was used as the eluent at a flow rate of 1 mL/min at 40 °C. Before injection, the PHA sample was dissolved in chloroform at a concentration of 3 mg/ml, and subsequently filtered. Data of the refractive index detector was quantified with a universal calibration of monodisperse polystyrene standards with the help of LabSolutions software.

3. Results and discussion

3.1. Characterization of the PHA polymer

The results of the characterization of the PHA polymer are shown in Table 1. The purity of the extracted PHA used for the experiments was high. The fraction of HV monomers (36% wt%) resulted in a relatively low melting temperature (T_m) (175–180 °C for pure PHB**).** The value of the molecular weight (M_w) appeared to be very low, probably caused by prolonged drying of the PHA-rich biomass and extracting at relatively high temperatures (typically between 200 and 3000 kDa) (Laycock et al., 2013). According to Kanesawa and Doi (1990), the tensile strength starts to decrease at a M_n of 70 kDa, and approaches zero around a M_n of 17 kDa. Furthermore, the high polydispersity index (PDI) revealed a heterogeneous polymer in terms of molecular weight (typically between 1.5 and 2.0). This suggests that the quality of the PHA polymer in this study is unsuitable for plastic applications. It should be noted that the purification method adopted in this study was not optimized for any of the polymer characteristics.

Table 1
Purity and physicochemical characteristics of the PHA polymer used for experiments in this study.

Purity and physicochemical characteristics of PHA	
Purity (wt%)	97.4
HV (wt%)	36
T_g (°C)	−4
T_m (°C)	89
T_d (°C)	269
M_w (kDa)	47
M_n (kDa)	18
PDI (Mw/Mn)	2.6

3.2. Axenic growth experiment

An axenic growth experiment was conducted to verify the PHA-consuming ability of the *B. cohnii*-related strain (Section 2.3 of Materials and Methods). The shake flasks which contained PHA and the *B. cohnii*-related strain showed a 100-fold increase in cell concentration after 48 h (blue series; Fig. 2). In addition, microscopic analysis of the suspension revealed that the PHA particles were covered by mobile bacterial cells (Fig. 3). No growth was observed in absence of PHA (red series; Fig. 2). This implies that the growth in the shake flasks incubated with the *B. cohnii*-related strain and PHA was directly related to PHA consumption. Also, no growth was observed in absence of the *B. cohnii*-related strain as inoculum (gray series; Fig. 2), not even after 11 days (data not shown). This implies that the growth in the shake flasks incubated with the *B. cohnii*-related strain and PHA cannot be caused by contaminating species. Together, these results supply evidence that this *B. cohnii*-related strain is capable of utilizing extracellular PHA as substrate.

To support these findings, the genomic sequence data of *B. cohnii* was explored. The key enzyme required for extracellular PHA consumption is an extracellular PHA depolymerase, because a PHA molecule cannot be transported across the cell membrane as a polymer (Mukai et al., 1993). According to the GenBank database, *B. cohnii* is in the possession of an extracellular PHA depolymerase, like many *Bacillus* species. (Accession no. AST93050.1 - NCBI) (Knoll et al., 2009). Hence, it was presumed that the *B. cohnii*-related strain used in this study also must possess an extracellular PHA depolymerase enzyme.

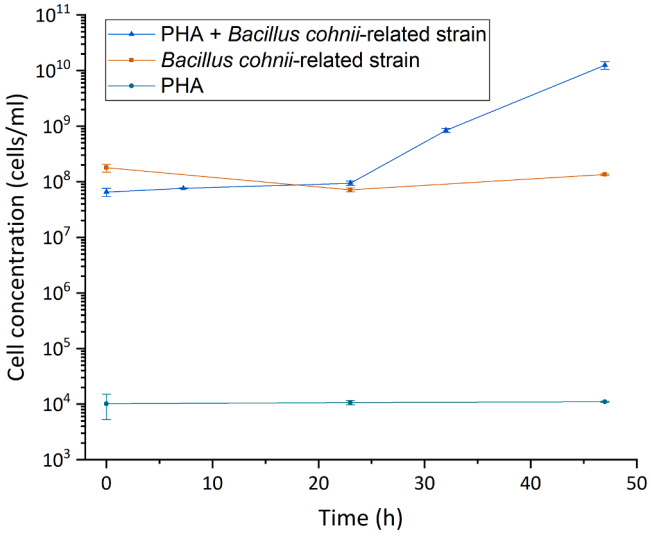

Fig. 2. Flow cytometric cell counts of axenic growth experiments in shake flasks. All three shake flask series were carried out in triplicate.

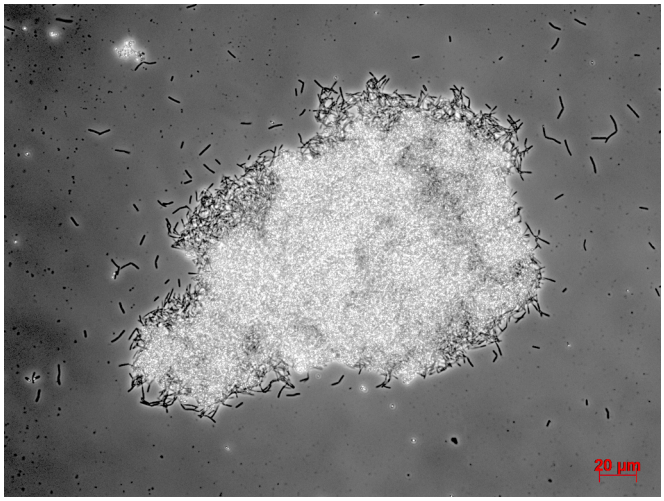

Fig. 3. A microscopic photo of a PHA particle surrounded by cells of a Bacillus cohnii-related strain. Phase contrast image at 400x magnification.

3.3. Crack evaluation

Different series of mortar specimens were prepared and incubated (see Sections 2.4, 2.5, and 2.6 of Materials and Methods). Before incubation, the crack widths of the specimens were analyzed with a stereomicroscope. The average crack widths (out of 7 specimens) were as follows: 0.46 ± 0.05 mm for all negative control specimens (no healing agent included), 0.48 ± 0.05 mm for all positive control specimens (lactate derivative based healing agent included), and 0.46 ± 0.04 mm for all PHA specimens. Although this was slightly higher than the intended 0.4 mm, the differences of the average crack width between the different treatments were very small.

Stereomicroscopic photos were taken of the same crack before and after incubation (see Fig. 4, left and middle column). The crack of the negative control specimen (Fig. 4, row A) shows some degree of healing, although the crack opening is still clearly visible. This can be explained by so-called autogenous healing, a process which occurs in conventional, mainly young concrete structures primarily due to continuous hydration of cement (Van Tittelboom and De Belie, 2013). This process allows for the healing of the smallest cracks (widths of 0.1–0.2 mm) (Rooij et al., 2013). In the crack of the positive control specimen (Fig. 4, row B) and the PHA specimen (Fig. 4, row C) substantially more precipitation is visible. Here, the cracks are completely covered with precipitate, indicating the efficacy of the self-healing concrete technology.

The ESEM photos of the crack surface of incubated specimens (see Fig. 4, right column) reveal that the surface was indeed covered with a mineral precipitate consisting of cubic and rhombohedral shaped crystals, probably formations of calcium carbonate. The crystals formed in the positive control and the PHA specimens (Fig. 4, row B and C) were considerably larger than the crystals formed in the negative control

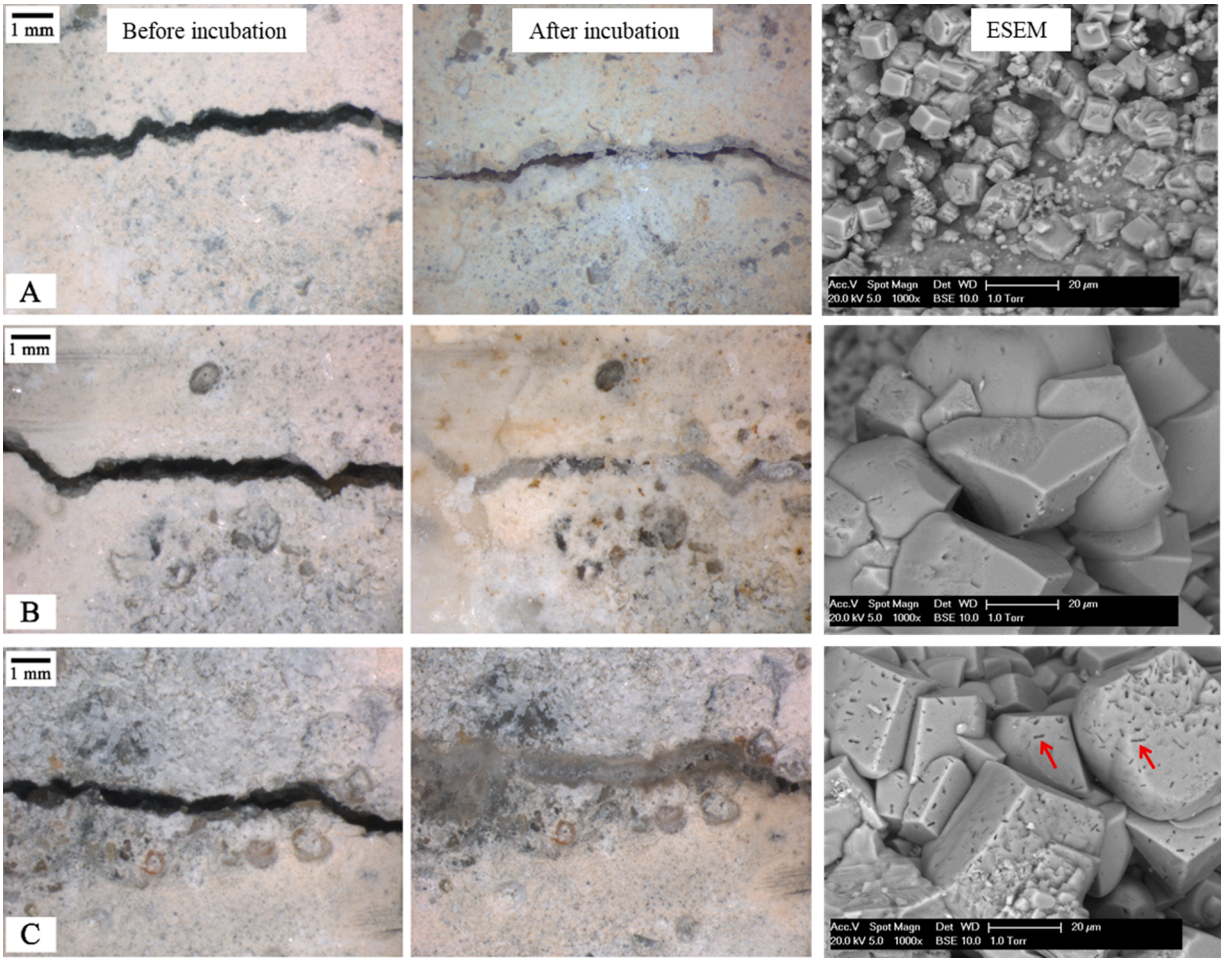

Fig. 4. Stereomicroscopic and ESEM analysis of the cracks. Row A) Negative control specimen, without healing agent; Row B) Positive control specimen, with healing agent composed of a lactate derivative; Row C) PHA specimen, with healing agent composed of waste-derived PHA. The stereomicroscopic photos in the left column depict the cracks before incubation, the stereomicroscopic photos in the middle column depict the crack after incubation, and the ESEM photos in the right column depict precipitates on the surface of an incubated, freshly broken specimen.

specimen (Fig. 4, row A), again indicating the efficacy of the self-healing concrete technology. A remarkable observation were the presence of many rod-shaped cavities (±1 μm by ±4 μm) on the crack surface of the PHA specimen (Fig. 4, row C), indicated by the red arrows. These cavities resemble the bacterial imprints observed in other studies which examined microbially induced precipitation (Cacchio et al., 2003; Tziviloglou et al., 2016).

While choosing the photos, a representative selection has been made. However, the obtained photos cover only a minor fraction of the total amount of crack length incubated, and could therefore be slightly biased. Therefore, the photos should be regarded as an indication of the effectiveness of crack healing and not as definite proof. The water permeability test, on the other hand, quantifies the effectiveness of crack healing of the whole crack of multiple specimens (see Section 3.3). Moreover, the most important functionality of self-healing concrete is assessed by measuring water permeability.

3.4. Water permeability experiment

The water flow through the crack was measured before and after incubation of the specimens (see Section 2.8 of Materials and Methods). The water flow before incubation ($W_{i,average}$) was as follows: 7.6 ± 0.9 mL/s for the negative control specimens, 6.7 ± 0.9 mL/s for the positive control specimens, and 6.7 ± 0.8 mL/s for the PHA specimens. These values demonstrate, in accordance with the average crack width, an acceptable degree of homogeneity between and within the specimen series.

The strategy of using different sets of specimens for measuring the initial and final water permeability is based on the assumption of homogeneity between the samples. However, this assumption is only valid to a certain extent. In an ideal experiment, the self-healing capacity of an individual specimen should be measured. Nevertheless, the current strategy was chosen to exclude the risk of flushing out healing agent or other reactive components from the crack before incubation.

The initial and final water flow values were used to calculate the reduction of water flow (*RWF*) (shown in Fig. 5). The PHA specimens have a significantly higher mean *RWF* than the negative control specimens (88 ± 1.3% compared to 55 ± 3.0%) and a slightly higher mean *RWF* than the positive control (88 ± 1.3% compared to 81 ± 11.3%). This shows that the PHA specimens possess a healing capacity which is significantly higher than the autogenous healing process in the negative control specimens. Moreover, the healing capacity is comparable to the currently marketed lactate derivative based technology represented by the positive control specimens. These findings are in line with the microscopic analysis of the crack mouths in Section 3.3.

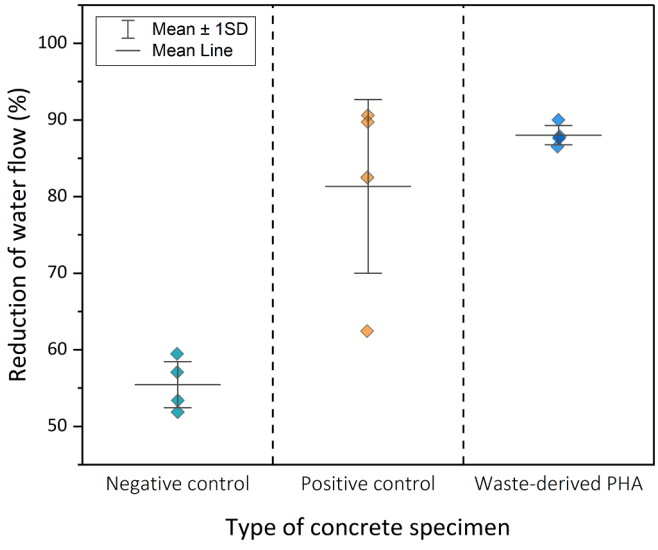

Fig. 5. Reduction of water flow (RWF) of concrete specimens after 56 days of incubation under humid conditions. Negative control specimens do not contain healing agent; positive control specimens contain healing agent composed of a lactate derivative; PHA specimens contain healing agent composed of PHA derived from organic waste. The whiskers represent ±1 standard deviation.

Fig. 5 also displays that none of the samples reached complete water tightness (*RWF* = 100%) after 56 days of incubation. Although the microscopic photos reveal complete healing of some of the cracks, the formed precipitates are not able to completely withstand the water pressure in the experimental set-up. This observation is in accordance with other publications with similar experimental set-ups. For example, Tziviloglou et al. (2015) show that the *RWF* of specimens with a significantly smaller average crack width (0.35 mm) no complete water tightness is achieved after 56 days (*RWF* = 98%). Larger crack widths (0.6 mm) were established by Palin et al. (2017). They describe that a *RWF* of 93% was reached after 56 days of submersion in artificial seawater, where it should be noted that seawater can enhance the autogenous healing capacity (Palin et al., 2016).

As in Section 3.3, it appears that autogenous healing is a significant factor in the healing capacity of the cracks. This widely studied phenomenon has also been observed and quantified by other authors (Edvardsen, 1999; Reinhardt and Jooss, 2003). Aldea et al. (1999), recorded a water permeability reduction of 56% in normal strength concrete in samples without any healing agent, comparable to the results in this study (55 ± 3.0%). Autogenous healing is mainly caused by the continuation of the cement hydration reaction in the period after casting (i.e., 28 days). Aging concrete structures, which are usually facing the problem of cracking, will possess this type of autogenous healing capacity to a smaller extent. Therefore, we can expect that the relative difference in healing capacity between PHA-based healing agent and negative control specimens will increase even further, as the concrete ages.

Another noticeable observation in Fig. 5 is the relatively large scatter of the values of the positive control specimens. The crack width of the specimen which forms the outlier (*RWF* = 62.5%) is slightly above the average for this series (0.51 mm). Another specimen in this series has a crack width of 0.56 mm and reaches a *RWF* of 89.7%. Therefore, it is unlikely that this deviation is merely caused by increased crack width. Another explanation is the intrinsic, stochastic factor linked to a healing agent consisting of particles. This means that a formed crack has a certain probability that it will hit an embedded particle, which affects the degree of healing.

3.5. Summary of results: waste-derived PHA can be applied as healing agent in concrete

The presented data suggest that self-healing concrete is a promising new application for waste-derived PHA. The self-healing bacterial strain (*B. cohnii*-related) has the metabolic machinery present to consume extracellular PHA. Moreover, healing agent composed of waste-derived PHA induces crack healing in concrete structures, while simultaneously reducing the water permeability of the cracks. The obtained results are comparable to the performance of the currently marketed lactate derivative based technology.

3.6. Functional properties of concrete

When additives are mixed with concrete, the influence on the functional properties of the concrete must be within the acceptable range. It is known that the compressive strength and/or the cement hydration reaction of concrete can be critically affected by the components of healing agent (i.e., PHA, spores, yeast extract, and unknown impurities in the PHA) (Basaran Bundur et al., 2015; Luo and Qian, 2016). However, Mors and Jonkers (2017) demonstrate that the compressive strength of concrete containing a comparable healing agent (i.e., a lactate derivative based healing agent; the positive control in this study) is not compromised after 28 or 56 days of curing. Nevertheless, future

research should confirm the compatibility of concrete with healing agent composed of waste-derived PHA.

3.7. Specifications of the PHA polymer

In this study, PHA with a high purity (97.4%) was cast in the concrete. However, a high purity generally requires an extensive and costly purification process. On the other hand, a large fraction of impurities might have an adverse effect on the functional properties of the concrete. However, it is possible that the melting step during the particle formulation will neutralize the effect of the impurities by encapsulating them. Altogether, it would be interesting to study which fraction of impurities still allows for a healing agent which is concrete compatible. Implementing this idea can reduce the costs for PHA purification.

As described in Section 3.1, the PHA used in this study has a low molecular weight with a broad mass distribution. From the perspective of bioplastic manufacturing, this polymer would have a low quality. Nevertheless, this study demonstrates that the specification are sufficient for an application in self-healing concrete. This finding opens possibilities for PHA batches unsuitable for plastic applications in future production facilities. However, it should be pointed out that the polymer needs to meet some specifications, such as the ability to be processed into particles which can withstand the frictional forces of concrete mixing, and the ability of the particles to be consumed by the self-healing bacteria. Future trials with different batches of PHA comprising a range of physicochemical characteristics (HV content, molar mass distribution, melting temperature) must determine the impact of polymer properties on both the particle formulation process and the self-healing capacity.

3.8. General considerations for value chain development of waste-derived materials

The necessity for a more circular approach for wastewater treatment has been underlined by the scientific community for many years now. In this view, wastewater is regarded as resource, and the wastewater treatment plant is regarded as a resource recovery facility, where water, energy, nutrients, and products are collected in a centralized manner (Guest et al., 2009; Van Loosdrecht and Brdjanovic, 2014). As a consequence, waste water treatment moves from a process with a single objective to a more complicated and multidisciplinary operation, involving novel aspects, such as product and value chain development (Bozkurt et al., 2017). A closer examination of other potential technologies to recover waste-derived materials, such as extracellular polymeric substances (EPS), medium chain fatty acids (MCFA), cellulose, and struvite, reveals that their level of implementation in the wastewater sector is still limited, as it is for PHA. Moreover, it appears that the challenges underlying this limited success show similarities between the different waste-derived materials (Kehrein et al., 2020).

A challenge, which is applicable to almost all waste-derived materials, is the small amount of resources available in waste streams. Because organic waste streams are a side-product of an agro-industrial process, the quantity is not available in a scalable amount, in contrast to conventional agricultural or fossil resources. This means that the quantity of product that can be generated from a waste stream will be much smaller than in conventional production processes. Therefore, the advantage of optimization by scaling, which is crucial for the production of commodities, is limited for waste-derived materials (Kleerebezem et al., 2015). This emphasizes that the production of waste-derived PHA applied as bioplastic is such an ambitious undertaking.

Another limitation associated with waste-based production processes is the quality of the feedstock compared to sugar- or fossil-based processes. All waste streams have compositional fluctuations to a certain degree which can result in a variable amount and type of impurities in the final product. Moreover, for EPS, PHA, and MCFA variations in waste water composition result in variations in the chemical composition of the product itself, which brings along uncertainty about the market value. However, important to realize is that quality is defined by the final application (Valentino et al., 2017). Not all applications need the highest specifications, as it is envisioned for the usage of EPS as brick additive (van der Roest et al., 2015), the usage of cellulose as aggregate for asphalt (Visser et al., 2016), or, as proposed in this study, the usage of PHA in self-healing concrete.

A critical obstacle for widespread implementation of waste-derived materials are the final production costs. Most resource recovery processes are not cost-effective because of high operational and/or investment cost compared to conventional industrial production. When PHA is applied as high-quality bioplastic, high purity and mechanical requirements result in a complex and expensive process. Another example is phosphate recovery as struvite, which can be applied as fertilizer. Here, a high chemical and energy demand for the production of struvite results in a high price compared to conventional phosphate fertilizer production (Le Corre et al., 2009). EPS production has a clear advantage at this point, because no additional investments in the upstream process are required when an aerobic granular sludge reactor is in place (Lin et al., 2015). On the other hand, product yields and product purities in the upstream process are significantly lower compared to other resource recovery processes described here.

Due to the factors described above, waste-derived materials experience a strong competition with the conventionally produced materials. The exploration of new application routes can form a solution to these problems. Waste-derived materials used in niche applications will encounter less competition when their unique selling points are utilized. Fortunately, the number of examples of these new applications in literature increases. Waste-derived cellulose has proven useful as soil conditioner (Ruiken et al., 2013) and insulation material (Eijlander and Mulder, 2019). And, although understanding of the chemical structure of EPS is still minimal, different applications have been proposed and tested, such as flame retardant (Kim et al., 2020), paper coating (Lin et al., 2015), and cement coating (Zlopasa et al., 2014).

For PHA, a number of innovative applications have been explored, as described in the Introduction section. With this study, we would like to add the use of PHA as healing agent in self-healing concrete to the list of innovative applications for waste-derived PHA. In our opinion, more creativity in establishing connections between developments that aim for resource recovery, and developments that use recovered resources is one of the main challenges we are facing in the transition to a more circular economy.

4. Conclusions

This study demonstrates the potential of a new application for waste-derived PHA, as bacterial substrate in self-healing concrete. The experimental data shows that a PHA-based healing agent produced from PHA unsuitable for thermoplastic applications, induces crack healing in concrete specimens, and consequently, reduces the water permeability of the cracks. Although questions for future work remain, this study provides a proof-of-concept which successfully connects two separate fields of sustainable engineering. We foresee that this new application may counteract some of the key challenges for the large-scale implementation of waste-derived PHA, while simultaneously supplying circular and potentially more affordable raw materials for the production of self-healing agent.

CRediT author statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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