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Extraction, production and purification of added value products from urban wastes — Part 2: Extraction and purification of PHA biopolymers

CCMC will prepare and attach the official title page.

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European foreword

This Workshop Agreement has been proposed by the DEEP PURPLE consortium (https://deep-purple.eu/), which is developing a Horizon 2020 project to move forward in the valorization of municipal biowaste into high value products

This CWA is a technical agreement, developed and approved by an open, independent Workshop structure within the framework of the CEN-CENELEC system, developed in accordance with the CEN-CENELEC Guide 29 "CEN-CENELEC Workshop Agreements" and with the relevant provisions of CEN-CENELEC Internal Regulations – Part 2.

This CWA was agreed on 2019-01-29 through a decision adopted by representatives of interested parties, approved and supported by CEN following a public call for participation made on 2022-04-17. It reflects the agreement only of the registered participants responsible for its content, and it does not necessarily reflect the views of all stakeholders that might have an interest in its subject matter.

The secretariat of the CEN Workshop that developed this CWA was the Spanish Association for Standardisation (UNE).

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Introduction

Wastewater treatment and organic fraction of municipal solid waste are responsible for the annual generation of up to 138 million tonnes of bio-waste, in the EU. It has been estimated that almost 75% of this waste is currently sent to incineration or landfilling, with an extraordinary environmental and economic cost associated. Moreover, a high percentage of this waste holds a great potential as a source of recycled materials or valuable component recovery source. Wastewater contains cellulose and nutrients that could be used as feedstock for many applications. Solid organic waste could be also an interesting source of materials for added value applications (e.g. ectoine, polyhydroxyalkanoates, biomethane, etc.), to complement their conventional valorisation routes (e.g fertilizers, biogas, etc).

Nowadays, bio-waste is usually processed by means of methods such as anaerobic digestion and composting for the production of biogas or compost to be used as fertilizer. Similarly, domestic wastewater treatment is often conducted in activated sludge systems, which present high operating costs and energy demand. These methods present a low performance, high carbon footprint and low recovery of nutrients and valuable components.

The European R&I project DEEP PURPLE has developed innovative processes of production and purification of different added value products. These processes are based on the recovery of bioproducts from the treatment of urban bio-waste as an inexpensive and sustainable carbon or/and nutrient source for biomass growth to be further transformed into high added-value products for different industrial sectors.

This workshop faces the standardization of the methodology extraction, production and purification of two added value products from urban waste: ectoine and Polyhydroxyalkanoates biopolymer (PHAs).

Polyhydroxyalkanoates (PHAs) include a range of various length hydroxy fatty acid polyesters which are naturally produced by some types of bacteria. Similar to polylactic acids (PLAs), PHA polymers are both bio-based and bio-degradable and therefore have attracted much attention over the last four decades as an alternative to commonly used non-degradable plastics. PHAs possess similar physical properties to polyethylene (PE), polypropylene (PP) and polyethylene terephthalate (PET) which currently dominate in the packaging industry. Currently, new abundant substrates are sought after to avoid the high price of monosaccharides and to achieve a circular economy which necessitates utilisation of various sustainable organic wastes for the production of commercial goods. Moreover, the concept of the carbon recovery at wastewater treatment facilities has been successfully verified and anaerobically grown purple photobacteria have been shown to produce PHAs at high concentrations.

One of the key steps in the PHAs polymer extraction process is the need for the cell to be disrupted, in order to recover the PHA granules, which have accumulated in the bacterial cell cytoplasm. The nature of the cell disruption can be divided into two categories: mechanical (high-pressure homogenisation and a bead mill) and non-mechanical methods (utilising chemicals to either digest the cellular material surrounding the PHA granules or to act as a solvent).

Since extraction process can represent as much as 50 % of the total cost of recovery of PHAs from the biomass, it was determined that it should provide high yield and incur low cost. However, yield and cost are not the only factors to consider. Chloroform extraction is used as a benchmark for the PHAs isolation methods as it allows the process to achieve the purest product after the polymer precipitation in cold ethanol or methanol, however there is a general concern over the hazardous nature of the chlorinated organic solvents (requiring compliance with strict operational H&S and disposal regulations) and also the high costs associated with organic solvents recovery.

1 Scope

The present document defines the process for extraction and purification of PHA biopolyesters using chlorinated solvent-free and wet chemistry methods from enriched biomass with a PHAs extraction efficiency > 80 % with a purity > 95 % and a molecular weight > 200 kDa.

2 Materials and equipment

Materials

0.1 M Sulfuric Acid, 140 l

Water, 70 l

Anisole, 124 l

Iso-Propanol, 230 l

PHAs-enriched biomass, 7 kg

Cooling water

Equipment

500 l jacketed stirred reactor

Low pressure steam, 140 C

Disk-stack centrifuge for up to 500 l

Micro-filtration module for up to 500 l

Dryer or oven for 10 kg

Distillation system, 400 l

pH meter ± 0,2 pH Accuracy, 0,01 pH Resolution

Turbidity meter, 0 to 100 NTU

Thermometer, 0 to 200 °C

Gas Chromatography with VF-1 ms capillary CP8913 (30 m x 250 µm x 1 µm) column and Flame ionization detection (FID) (275 °C)

3 PHA extraction procedure

The process uses a low molarity acid digestion, followed by an anisole extraction and precipitation of the anisole/PHA mixture using either ethanol or a derivatised alcohol. The amount of PHAs amount contained in the biomass shall analysed using a Gas-Chromatography.

3.1 Chemical digestion of PPB biomass

The starting point of the process is the pre-digestion of the input biomass, which is a dried PPB with high PHA content. PHAs will be the final target of the following operations.

1 Weigh out the dried milled biomass (7,00 kg, 1 eq., 25,5% PHA) shall be charged to the reactor at 20 – 25 ºC.

2 The digestion solution 0,1 M H_2SO_4 (140 l, 20 vol., 50 g/l) should be charged with agitation (200-300 rpm) and at a rate to maintain reaction conditions.

3 The agitation shall be maintained while increasing the temperature to 60 – 70 ºC. Target temperature 65 °C.

4 The temperature shall be hold at 60 – 70 ºC for at least 3 hours with agitation (200-300 rpm). Agitation speed should be adjusted to suit.

5 The solution should be cooled to 20 – 25 ºC.

6 The biomass suspension shall be removed from the reactor and separate the solids from the liquors using centrifuge (3 500 rpm until liquors clear of solids). A sample of the liquors (~20 ml) and solids (~1 g) should be retained for analysis.

7 Deionised water (70 l, 10 vol., 100 g/l) at 20 – 25 ºC shall be charged to the reactor and agitate for 5 minutes. The solution shall be discharged to the centrifuge as a wash. A sample of the liquors (~20 ml) and solids (~1 g) should be retained for analysis. The solids shall be spinbed to dry the solid as much as possible. A sample of the liquors (~20 ml) and solids (~1 g) should be retained for analysis.

8 The solids shall be discharge from the centrifuge.

9 The solids shall be charged to an oven and dry until constant weight.

The digested biomass shall be calculated as:

$$Digested\ solids\,(\%) = \frac{Initial\ dried\ solids\,(g) - Final\ dried\ solids\,(g)}{Initial\ dried\ solids\,(g)} \times 100$$

Digested solids should be 10 % – 50 %.

An acidic pre-treatment does improve the recovery of the polymer but as a standalone digestion appears to result in less digestion than those observed with other digestion methods. However, use of acid in the process is milder, less expensive, and digestions using bleach were characterised by more foaming making operating the process more difficult in that instance.

3.2 Solvent extraction of PHA from digested biomass

Besides the acid-digested biomass as described in the previous section, a solvent extraction is necessary to release the PHA from the bacterial biomass.

The anisole, also known as methoxybenzene, a non-halogenated solvent produced from natural or synthetic routes is the most suitable solvent for extraction of PHA from PHA containing biomass. Anisole is suitable for the scale-up process, as it is non-chlorinated, relatively non-toxic and biodegradable.

A walk-in fume hood or ATEX facilities depending on the scale shall be used to ensure the safe handling of solvents. It will increase the capacity for PHA extraction from biomass and provided additional user safety when handling larger quantities of solvents.

Solvent extractions shall be performed with a filtration step to remove the non-PHA biomass from the PHA-anisole solution and centrifugation to isolate the solid PHA after alcohol precipitation. PHA isolated by filtration had a different physical nature than that isolated by centrifugation. Centrifugation produced pellets of PHA, while filtration produced a sheet of polymer.

The biomass solid should be weighed and charged to the reactor for the extraction phase. In this step the selected solvent, Anisole (98-99 %), shall be added into the reactor with a proportion of 1 litres of solvent every 50 g of solid.

The final solution with 40 g/l of solid (initial biomass loading) should be then agitated 200-300 rpm and heated at 100-130 °C for 2-3 h.

1 The dried digested biomass (5,250 kg, 0,75 eq., 25-50% PHA) shall be charged to the reactor followed by Anisole (98-99%, 117 l, 16,7 vol., 60 g/l) at 20 – 25 °C with agitation (200-300 rpm). The reactor should be inert with nitrogen.

2 Agitation shall be maintained while increasing the temperature to 100 – 130 °C, over 5-30 minutes. Target temperature should be 120 °C.

3 Temperature shall be maintained at 100 – 130 °C for 3 hours with agitation (200-300 rpm). Target temperature should be 120 °C.

4 The solution should be cooled 40 – 45 °C. This is below the flash point of anisole (45,5 C).

5 The solution shall be discharged to a filter (≤5-13 μm pore size) to remove solids from the liquors. A sample of the liquors (~20 ml) and solids (~1 g) should be retained for analysis.

6 Anisole (7,00 l, 1 vol) should be charged to the reactor as a rinse and allow to warm up to 40 – 45 °C then the rinse should be used as a wash of the filter. This rinse should be retained as a wash for the addition of the PHA solution. This is to improve the PHA recovery by rinsing any residual PHA from the system and the biomass.

7 The reactor should be cleaned out and the filter ensuring all solids have been removed.

8 The PHA-anisole solution should be recharged to the reactor and heat to 70 – 80 °C before applying vacuum to remove half of the total volume (Target 61 l, 8,7 vol, 115 g/l). The solution should be cooled to 40 – 45 °C.

9 The concentrated PHA solution should be discharged from the reactor and be split into enough portions, so it fits into reactor available for the precipitation.

3.3 PHA precipitation in alcohol

After extraction of the PHA from the biomass with anisole, PHAs shall be precipitated by the addition of PHA/anisole mixture to alcohol, followed by isolation by a centrifugation step of 3 500 rpm for 15 mins at room temperature or filtration. The ratio alcohol to anisole of at least 3:1 shall be used for reaching the higher rate of recovery.

Alcohol precipitation process requires either a centrifugation or filtration step to recover pure PHA after precipitation. Without this precipitation step there may be issues with concentrating the PHA anisole solution as it forms a PHA-anisole gel on cooling.

1 *Iso*-propanol (IPA) shall be charged to the reactor (87,5 l, 12,5 vol., 40 g/l) and should be heated to 40-45 ºC and agitated (200 – 300 rpm) before recharging a portion of the anisole solution (30,5 l) to the reactor dropwise (over 10 minutes) maintaining 40 – 45 ºC. The PHAs polymer shall form a solid. The solution should be agitated for 30 minutes.

2 The PHA-*iso*-propanol-anisole suspension should be cooled to 0–20 ºC, over at least an hour maintaining slow agitation.

3 The suspension shall be discharged to the centrifuge to remove the solid (desired product) from the liquors.

4 Repeat steps 1 and 3 until all the anisole solution has been charged to *iso*-propanol.

5 With the last precipitation, the liquors from step 3 should be charged to the reactor as a wash and through the centrifuge (3 500 rpm for 30 minutes) to recover solids.

6 *Iso*-propanol should be charged (58,3 l, 11,2 vol., 120 g/l) to the reactor as a final rinse, washing through the centrifuge as a final wash. If solids remain in the reactor use the collected IPA/anisole liquors (step 3 and step 6) as a wash.

7 The solids shall be discharged from the centrifuge onto a pre-weighed drying tray and dry in an oven at 80 ºC until constant weight.

8 The reactor and the filter (or centrifuge) should be cleaned for the new batch (either anisole > 100 C or 1 M NaOH heating to digest residual polymer. If that doesn't remove the polymer, chloroform will dissolve the PHA, but this should be avoided.

9 Anisole (124 l) from *iso*-propanol (233 l) shall be separated in a rectification column. At 0,5 atm. condenser is at 58-63 ºC and boiler at 125-130 ºC. 5 stages are needed and a reflux ratio of 0,2.

The PHAs recovery yield shall be calculated as:

$$PHAs\ recovery\ \ yield = \frac{Initial\ biomass\,(grams) \times PHAs\ content\,(\%) - Final\ product\,(g) \times PHAs\ purity\,(\%)}{Initial\ biomass\,(grams) \times PHAs\ content\,(\%)}$$

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