



Utilizing grass for the biological production of polyhydroxyalkanoates (PHAs) via green biorefining: Material and energy flows

Patterson, Tim; Massanet-Nicolau, Jaime; Jones, Rhys; Boldrin, Alessio; Valentino, Francesco Maria; Dinsdale, Richard; Guwy, Alan

Published in:
Journal of Industrial Ecology

Link to article, DOI:
[10.1111/jiec.13071](https://doi.org/10.1111/jiec.13071)

Publication date:
2021

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
Patterson, T., Massanet-Nicolau, J., Jones, R., Boldrin, A., Valentino, F. M., Dinsdale, R., & Guwy, A. (2021). Utilizing grass for the biological production of polyhydroxyalkanoates (PHAs) via green biorefining: Material and energy flows. *Journal of Industrial Ecology*, 25(3), 802-815. <https://doi.org/10.1111/jiec.13071>

General rights



Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Utilizing grass for the biological production of polyhydroxyalkanoates (PHAs) via green biorefining

Material and energy flows

Tim Patterson¹  | Jaime Massanet-Nicolau¹ | Rhys Jones¹ | Alessio Boldrin²  |
 Francesco Valentino³ | Richard Dinsdale¹ | Alan Guwy¹

¹ Sustainable Environment Research Centre (SERC), Faculty of Computing, Engineering and Science, University of South Wales, Pontypridd, Wales, UK

² Department of Environmental Engineering, Technical University of Denmark, Lyngby, Denmark

³ Department of Chemistry, "La Sapienza" University of Rome, Rome, Italy

Correspondence

Tim Patterson, Sustainable Environment Research Centre (SERC), Faculty of Computing, Engineering and Science, University of South Wales, Pontypridd, Wales, UK.
 Email: tim.patterson@southwales.ac.uk

Funding information

Research has been completed as part of WP1 of the Horizon 2020 Resources from Urban Bio-waste (ResUrbis) project (Grant Agreement 730349). The research presented is that of the authors and does not represent the views or opinions of the funding provider.

Editor Managing Review: Robert Anex

Abstract

The meat and dairy industry across Europe is dependent on the production of grass. However, faced with competing pressures to reduce the environmental impact of agriculture, a potential future reduction of meat and dairy consumption in western diets, and pressure to minimize food production costs, grass could be used to produce alternative products. The biological production of polyhydroxyalkanoates (PHA) by using grass as the primary carbon source in a novel mixed culture process has been studied. A total of 30,000 t of fresh grass would yield approximately 403.65 t of dried biopolymer granules. On the basis of this early stage, non-optimized process, the cumulative energy demand (CED) of PHA produced from waste grass and cultivated grass was found to be 248.4 MJ/kg and 271.8 MJ/kg, respectively, which is the same order of magnitude as fossil-carbon-based polymers. Improvements in volatile fatty acid yields, reduction in chemical and water inputs, and using residues to make other products will reduce the CED. Given the future requirement to produce polymers with little or no fossil-carbon feedstock, an optimized version of the process could provide a viable future production option that also contributes to the long-term sustainability of agricultural communities.

KEYWORDS

bioplastic, biorefining, grass, industrial ecology, polyhydroxyalkanoates (PHA), systems analysis

1 | INTRODUCTION

The growing of forage and fodder grasses is of critical importance to European Union (EU) agriculture because they provide the main feeds supporting the production of dairy and beef cows. Forage and fodder grass species typically used across Europe include varieties of ryegrass (e.g., westerworld ryegrass, perennial ryegrass, Italian ryegrass, or hybrid ryegrass), Timothy grass, white clover, and red clover.

Whilst grass is a mainstay of global agriculture there are a number of pressures acting at regional and national scales that suggest a possible future scenario where grass is grown for other purposes than for rearing animals. The UK dairy herd reduced from 2.6 million to 1.9 million animals

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *Journal of Industrial Ecology* published by Wiley Periodicals, Inc. on behalf of Yale University

between 1995 and 2015 with a corresponding reduction in dairy producers from 35,741 in 1995 to 13,815 in 2014 (Bate, 2016). The large resource and environmental burdens of rearing animals, primarily as a source of protein for human consumption, coupled with the human health impacts of over consumption of meat in many diets leads to the potential scenario of reducing intensive meat production in some regions (Chai et al., 2019).

Grass is also a component of municipal green waste (garden waste) which, in Europe, is predominantly generated during the summer months (May–September) from domestic lawns, municipal parks, and playing fields and the grass verges of roads. In the UK green waste is predominantly treated via composting with the resulting compost sold for horticultural/agricultural use. However, production of compost from green waste is net energy negative (Martínez-Blanco et al., 2010), generates greenhouse gas (GHG) emissions (Vergara & Silver, 2019), and, whilst providing the service of returning carbon and nutrients to land, is generally considered as a low value product.

Using grass as a feedstock for anaerobic digestion and the production of biogas has been considered as a potential option for rural diversification (Voelklein, Rusmanis, & Murphy, 2016), however, the economic value of biogas is also relatively low and the process only gains value from a small proportion of the overall input mass. As an alternative, biomass, in particular fresh or green biomass including many varieties of grass can be processed in what has become termed a “Green Biorefinery” (GBR) to produce multiple outputs including chemicals, manufacturing materials, food, and feed products (Mandl, 2010) many of which are of higher value than biogas, particularly where demand for low carbon products is increasing. Whilst the body of research work relating to GBR is growing, studies that focus on the industrial ecology of the concept including mass and energy balances and subsequent life cycle assessments relating to specific processes or products are limited.

The general feasibility of the biorefining of grass to produce proteins, amino acids, and lactic acid from liquid fractions and combustion material or low value fiber from solid fractions was investigated by Mandl (2010) and Sharma, Lyons, and McRoberts (2011). Sharma et al. (2011) concluded that a 60,000 t¹/year input biorefinery could be established in Northern Ireland, and, using a similar technical approach of solid/liquid separation Papendiek, Tartiu, Morone, Venus, and Höning (2014) identified that grass yields were a critical factor in achieving economic viability. A number of studies have considered the production and suitability of specific crops for biorefinery applications including high dry matter (DM) yield C4 grasses (maize, sugarcane, sorghum, miscanthus, and switchgrass) (Van der Weijde et al., 2013), reed canary grass and festulolium (Rancane, Lazdina, & Berzins, 2015), and white clover, red clover, Lucerne, perennial ryegrass, and tall fescue (Solati, Jørgensen, Eriksen, & Søgaard, 2017), concluding that plant breeding and harvesting strategies, nutrient applications, and the ability to realize additional value from use of residual fibers were all important factors in achieving economic viability. However, as these studies did not include a detailed assessment of material or energy flows only a general determination of the technical or economic viability or environmental burdens associated with the various processes could be made.

In investigating the economic viability of the GBR of grass in Ireland, O’Keeffe, Schulte, Lalor, O’Kiely, and Struik (2011a) identified that the value above production costs for the feedstock was highly dependent on the availability of biomass within the biorefinery catchment area (i.e., competition from other uses). In the first of a two part study, O’Keeffe, Schulte, Sanders, and Struik (2011b) investigated a number of GBR technical scenarios in an Irish context based on process mass and energy flows, and in a follow-up economic study O’Keeffe, Schulte, Sanders, and Struik (2012) concluded that the GBR approach was viable in Ireland based on the utilization of grass silage with the sale of fibers as the primary product, proteins as a secondary product with biogas production (from residues) providing an important contribution to the overall energy balance.

The German Federal Government Biorefineries Roadmap concluded that stability of feedstock supply and value, development of markets, technological development, and quantification of environmental burdens and benefits were all important factors in the development of a biorefining industry in Germany (The Federal Government of Germany, 2012), points that were further emphasized in a later study by Kamm, Schönicke, and Hille (2016). Similar conclusions were also made in an extensive review of the biorefining of biomass which also anticipated that the earliest deployment of the biorefinery concept would be for the production of fuels and chemicals that are directly compatible with existing hydrocarbon supply chains (Maity, 2015a, 2015b). The importance of the biomass production process and regional availability, along with the value realized by residual materials was also highlighted in several techno-economic studies, for example, Oleskiewicz-Popiel et al. (2012), Parajuli et al. (2015), Parajuli et al. (2016), and Cong and Termansen (2016). Whilst these studies provide a comprehensive overview of the possible future scope of biorefining, they do not provide detailed assessments of the material and energy requirements of the production processes and can therefore only determine the general feasibility of biorefining principles or of a particular aspect of the production process.

Biorefinery production of intermediate chemicals and polymers has also been studied. The production of volatile fatty acids (VFA) as an intermediate of the anaerobic digestion process suggests that VFAs would be a good target for early biorefineries (Jagadabhi, Kaparaju, & Rintala, 2010; Yu et al., 2014). Koller et al. (2005) incorporated grass juice and silage juice as a nutrient source into a biopolymer production process utilizing *Wautersia eutropha* and found improvements in both productivity and process costs compared with either a minimum nutrient media or the supplementation with materials including corn steep liquor. Cerrone et al. (2016) investigated the production of medium chain length polyhydroxyalkanoates (mcl PHA) via VFAs produced from a grass feedstock in a leach bed reactor which were concentrated and utilized as a carbon source for the growth of three *Pseudomonas putida* strains that accumulated mcl PHA intracellularly. *Putida* CA-3 achieved the maximum growth of 1.56 g of biomass/L and maximum PHA yield of 39% of the cell dry weight (Cerrone et al., 2016). These studies focus on determining the technical feasibility of the conversion processes and therefore do not necessarily provide a comprehensive assessment of the feasibility of the overall production system.

¹ Throughout this article including all text, figures, and tables, the abbreviation “t” refers to metric tons (i.e., 1,000 kg).

Biorefinery pilot plants producing lactic acid, amino acids, and protein rich animal feeds have been operated, for example, Ecker et al. (2012) and Santamaría-Fernández et al. (2017), although no economic or environmental assessments of the processes were presented within the published studies.

1.1 | Study aims

This study aims to evaluate the potential of utilizing grass to produce high value products, specifically polyhydroxyalkanoate (PHA) biopolymers, in a biorefinery approach. The research aims to quantify material flows and energetic input requirements and establish the broad economic performance of the process to highlight areas where future technical research and policy support frameworks could be directed. The utilization of grass for biopolymer production is compared with the current non-food production state of the art process for deriving value from biodegradable material, namely biogas production by anaerobic digestion with subsequent conversion to electrical and thermal energy. Given that many elements of the PHA production process are still at laboratory scale the aim is not necessarily to deliver a definitive evaluation of life cycle inputs and outputs, but to highlight possible areas of the process that, if optimized, could significantly increase the potential for industrial deployment in the future. The study is based on a European context, however, findings would be relevant to other locations with grass fed meat and dairy production. To the authors' knowledge, this is the first time that such an evaluation has been undertaken for this process.

2 | METHODS

The components included within the studied system along with the system boundary is shown in Figure 1. Material balance of input biomass and conversion to biogas and digestate (for the reference case of anaerobic digestion), or conversion to VFAs and subsequent production of PHAs has been undertaken.

Electrical and thermal inputs into each sub-process are included in the evaluation. As urban grass waste is currently collected by municipal authorities in many parts of Europe regardless of the treatment route, the production and collection of this feedstock has not been included in the evaluation. Similarly, waste activated sludge (WAS) utilized in the process as a mixed culture microbial inoculum is assumed to be readily available from wastewater treatment plants (WWTP) and inputs for its production have not been considered. Inputs required for the agricultural production of grass as a crop are included for the case that utilizes cultivated grass. Digestate spreading and fertilizer displacement associated with digestate use is assumed to form part of a separate product system and is not considered here. The biorefinery concept allows for the recovery and utilization of residual fiber materials, however, due to limitations in data availability for both the recovery process and end use, this sub-process has not been included in this study. The organic acid production, mixed culture selection, and PHA production process stream is based broadly on that described in Valentino et al. (2017).

The study is not a formal LCA as not all life cycle stages of the product polymer are included (e.g., inputs/outputs for utilization, final disposal or recycling of the polymer are not considered), however, it is intended to be a useful analysis of the inputs and outputs of the specific production process described with the aim of identifying where process improvements can be made, and may be useful to those wishing to undertake a formal LCA of such a process. Given that the majority of the research included is still at laboratory scale and data on some aspects of the process is limited, no formal cut-off rules were applied to exclude inputs or outputs. Fossil cumulative energy demand (CED) is similarly limited to the *production* of the biopolymer based on primary energy inputs for thermal and electrical process requirements and treatment of effluents, CED of major material inputs (including upstream inputs), and, for options that require the use of grass grown as a crop, the cultivation and transportation of grass to the production plant is included. Other than the transport of cultivated grass to site, no other transportation of materials to the facility has been included. Transportation requirements for the production of crop-based biopolymers such as starch-based polyesters and polylactide are typically <1% of total production CED (Wernet et al., 2016). The CED presented for the biopolymer production process should therefore not be taken as representative of the full life cycle CED, but as broadly representative of the production process cumulative fossil CED based on early stage laboratory research. Largest uncertainties are likely to be associated with the energy inputs of a scaled up industrialized process as any optimization associated with this scale up has not been accounted for (e.g., possible efficiency savings in mixing energy and separation processes at larger scales, use of heat recovery, or integration with renewables or other industrial processes).

2.1 | Function and functional unit

The function of the process is to convert a mass of grass feedstock to an end product (either PHA for the system of study, or power and heat for the reference system of AD). To allow comparison between these systems a numerical input/output assessment of processes that treat 30,000 t

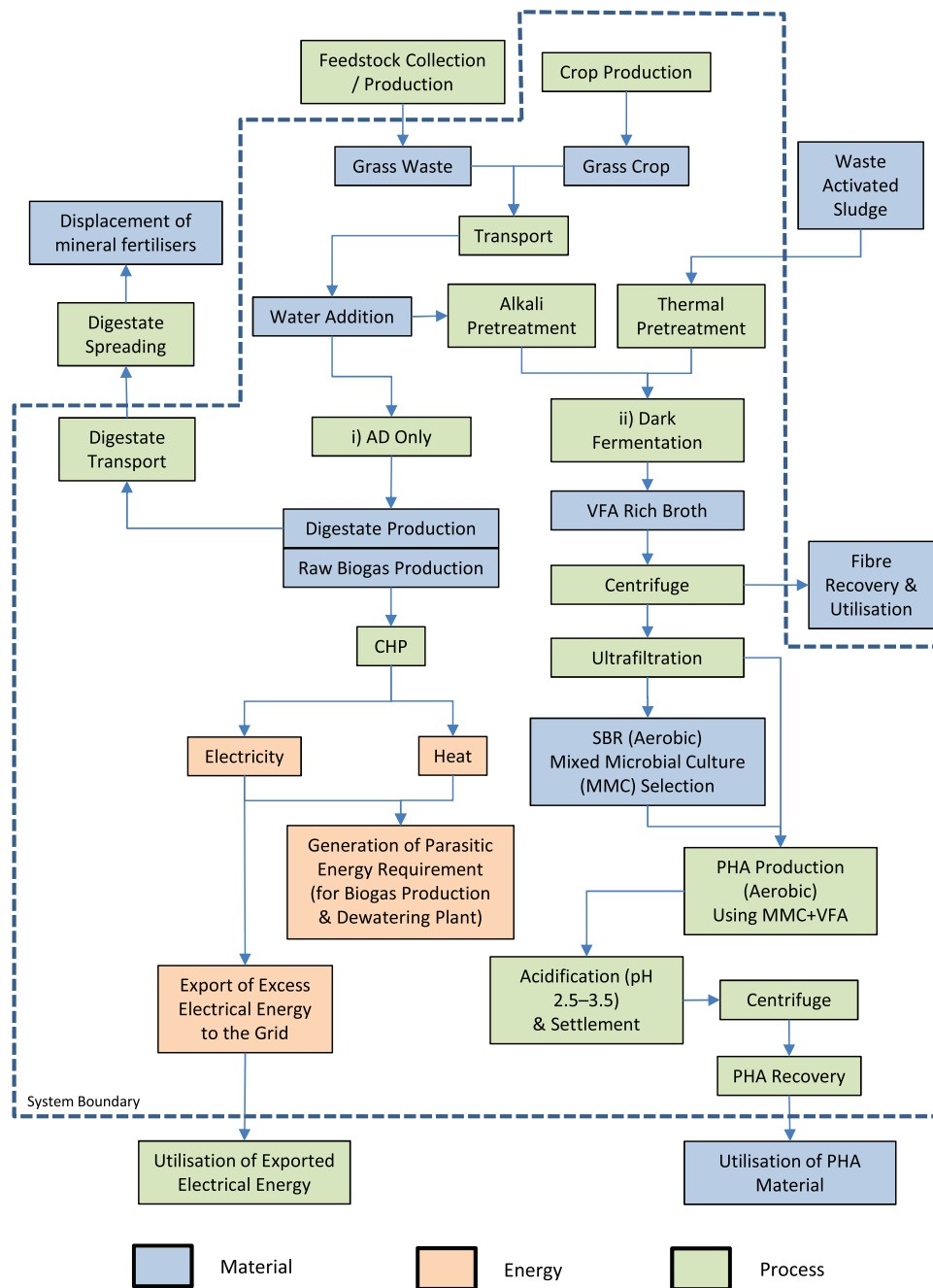


FIGURE 1 System components and boundary of PHA production process and reference system

of grass per annum is presented, 30,000 t/year input being the average size of anaerobic digestion plants in Europe (Monson, Esteves, Guwy, & Dinsdale, 2007).

2.2 | Feedstock source

2.2.1 | Grass as a waste

Municipal “Green Waste” or garden waste comprises mixed biodegradable materials including, for example, grass cuttings, trimmings and leaves, and woody material such as sticks and branches. Waste masses are significant; in the UK a total of 4,580,013 t of municipal waste was composted in 2016 (Department of the Environment, 2019; Northern Ireland Environment Agency, 2017; Scottish Environmental Protection Agency (SEPA), 2017; Welsh Government, 2019), and, as food waste is increasingly being separately treated via anaerobic digestion the majority of this composted

material will increasingly comprise green/garden waste. Whilst separate collection of grass waste is only likely to be economically viable in a small number of cases, it could be feasible to sort this waste according to size. Boldrin and Christensen (2010) quantified that 75.6% of garden waste in Denmark comprised “small stuff,” approximately half of which would be considered as suitable for use as an organic feedstock, and that it typically had a total solids (TS) content of 60.9% and a volatile solids (VS) content of 41.5% of TS, and as such is a potentially valuable bioresource. As an indication for the UK, assuming that 75% of composted material is green/garden waste (3,435,009 t/year), this “small stuff” would represent 1,298,433 t of material, comprising 790,746 t of TS and 328,160 t of VS.

2.2.2 | Grass as a crop

Although there has been a general decline in agricultural grassland area over the past 40 years, it still accounts for over one third of the total land area utilized for agriculture in Europe. Not all of this area would be suitable for the production of grass crops required for biorefinery applications (i.e. high-quality crops). For example, in the United Kingdom of the total grassland area (11,233 thousand ha), 3,961 thousand ha comprises rough grazing that would not be suitable for biorefinery crop production, leaving 1,144 thousand ha of temporary grassland and 6,118 thousand ha of grassland over five years old (Department for Environment and Rural Affairs [DEFRA], 2017), the majority of which is likely to be suitable. On the assumption that 5% of this land could be utilized specifically for producing biorefinery crops without detrimentally impacting on other agricultural activities, this would provide 363.1 thousand hectares of grass producing land dedicated to an emerging biorefining industry.

UK average grass yield in the dairy industry is reported as 10.4 t DM/(ha year) (Dairy Development Centre, 2014), which at a TS content of 22% is equivalent to 47.24 t fresh matter per hectare. Organic farming practices achieve a lower yield (8 t DM/ha, or 36.6 t fresh matter per hectare) (Dairy Development Centre, 2014). On this basis, a yield of 40 t fresh matter per hectare (8.8 t DM/ha) is utilized within this study. The above area of 363.1 thousand hectares would therefore be capable of producing 3.19 million tonnes DM/year (14.42 million t fresh matter/year). Martínez-Pérez et al. (2007) calculated that the energy requirement for the cultivation of perennial ryegrass was 4,710 MJ/ha, however their study quoted a crop yield of 10–20 t fresh matter/ha. Based on the higher yield of 40 t fresh matter/ha, a conservative energy input of 12,560 MJ/ha (3,487.9 kWh/ha) is used, corresponding to 314 MJ/t fresh matter (87.19 kWh/t fresh matter).

In the studied PHA production system grass or grass waste is co-fermented with WAS sourced from a municipal WWTP. It is assumed that inputs associated with the production of the WAS are part of the wastewater treatment system process and burdens associated with its production are not included in this study.

2.2.3 | Feedstock characteristics

Feedstock is assumed to be equivalent to ryegrass, a grass that can be grown across much of northern Europe and regions with similar climatic conditions, generally analogous to grass produced to support current dairy production. Crop characteristics are dependent on climate, soil conditions, nutrient availability, and harvest regime, however, for the purposes of this study a total solids (TS) content of 22% and a volatile solids (VS) content of 19.6% (or 89% of TS) was considered as being broadly representative of fresh and ensiled ryegrass (Dairy Development Centre, 2014; Parkarinen, Lehtomäki, Rissanen, & Rintala, 2008; Vítěz et al., 2015).

Grass is co-fermented with thickened waste activated sewage sludge (WAS) with a TS and VS content of 5% and 3%, respectively.

2.3 | Pre-treatment

Due to high levels of structural carbohydrates present in grass (Kyazze et al., 2008), pre-treatment to enhance hydrolysis using NaOH was included to allow dark fermentation to take place within a short timescale (Cui & Shen, 2012; Massanet-Nicolau, Dinsdale, Guwy, & Shipley, 2015). For the purposes of this study it was assumed that 5 ml of 4% w/v NaOH was added per g 1 g of TS content of feedstock. Pre-treatment was not included in the configuration for anaerobic digestion of grass as the process retention time allows for hydrolysis under normal process conditions. WAS is pre-treated in a thermophilic (72°C) anaerobic fermentation stage with a hydraulic retention time of 2 days (Valentino et al., 2017).

2.4 | Dark fermentation (Organic acid production)

Data describing organic loading rates (OLRs) for a continuous process producing organic acids from grass is limited. Cui and Shen (2012) describe a laboratory batch experiment utilizing grass to produce acids with a loading rate of 15 g TS/L (13.34 g VS/L), however this would greatly

underestimate loading rates in a scaled up, continuous industrial process. Massanet-Nicolau et al. (2015) describe a pilot scale, semi-continuous process using pelletized grass with a dark fermentation stage optimized for hydrogen production and an OLR of 66 g VS/L.

For the purposes of this study alkali pre-treated grass and thermally pre-treated thickened WAS are combined in a volumetric ratio of 60:40, respectively, with recirculated fermentation output and fresh water added to achieve an OLR of 40.4 kg VS/m³/day (50 kg TS/m³/day). Fermentation retention time is 1.75 days with an operating temperature of 37°C.

Output from the process comprises an organic broth containing acetic acid (14.94 g/L), butyric acid (1.87 g/L), and propionic acid (9.5 g/L) based on the yields reported in Cui and Shen (2012) and a residual TS and VS content of 1.65% and 1.46%, respectively. The acids account for a COD equivalent of approximately 33.7 g COD/l.

2.5 | Acid separation

Solids and liquids present within the fermentation broth are subject to a two stage separation processes comprising centrifuge filter (5–10 μm) followed by ultrafiltration ceramic membrane (0.2 μm) to remove all residual suspended solids (Moretto et al., 2020). Cake from the centrifuge and membrane retained phase are assumed to comprise liquid proportions of 12.7% and 25% of the input liquid volumes, respectively, with liquids containing organic acids in proportion to the input concentrations. It is assumed that 75% of the cake can be effectively recovered and treated using anaerobic digestion to produce biogas that is subsequently utilized to produce heat and power. The remaining 25% of the solids and liquids are lost from the system (WWTP disposal). Energy consumed by the centrifuge and ceramic membrane is assumed to be 3.5 kWh/t and 12 kWh/t of input material, respectively (Fuchs & Drosch, 2010).

Filtrate from the separation process which contains 65.4% of the organic acids input to the separation stage is available for further processing. 34.4% of the filtrate is diverted to a sequencing batch reactor (SBR) for growth of a PHA accumulating culture, whilst the remaining 65.6% of filtrate is utilized directly as a carbon source in the PHA accumulation stage.

2.6 | Development of PHA accumulating mixed culture

An aerobic SBR, initially inoculated with WAS and operating at a temperature of 26°C, is fed with a proportion (34.4%) of the liquid filtrate recovered from the post fermentation separation at a hydraulic retention rate of 1 day. The OLR to the reactor is 4.0 g COD (as VFAs)/L/day (Valentino et al., 2017) therefore requiring fresh water addition to dilute the input stream. Recirculation water from the PHA accumulation stage can be eventually used, provided that its soluble COD content does not affect the applied OLR in the SBR. Aeration is required at a rate of 31.2 m³ air/min (delivering 621.6 kg utilized O₂/day) with aeration assumed to provide sufficient mixing within the SBR (i.e., no additional mechanical mixing is required). Mixed culture output from the SBR is forwarded to the PHA accumulation stage.

2.7 | PHA accumulation

VFA rich filtrate from the post fermentation separation stage is combined with SBR cultured biomass in an aerobic PHA accumulation reactor operating at 26°C with an accumulation run time of 6 hrs. Aeration is provided at a rate of 676 m³ air/min with aeration assumed to provide sufficient mixing in the reactor. Numerous feeding and aeration strategies have been investigated, for example, Kshirsagar, Suttar, Nilegaonkar, Kulkarni, and Kanekar (2012), however, as a worst case scenario it was assumed that aeration was provided throughout a 6 hr accumulation phase. Three accumulations (i.e., 18 hrs) were accommodated for a given 24 hr period to allow sufficient time for turnaround of the reactor vessel.

The COD (VFA):COD (PHA) conversion ratio was 0.5502, and PHA was assumed to have a COD value of 1.7 g COD/g PHA (Valentino, Reis, Silva, & Majone, 2019). Overall PHA output from this stage was 496.95 tonnes/year (i.e., per input of 30,000 t of grass and 52,941 m³ of thickened WAS).

2.8 | Biopolymer separation and purification

The broth from the PHA accumulation phase containing cell material and accumulated PHA is subject to acidification to pH 3 using H₂SO₄ and allowed to settle for 12 hrs. Thickened slurry from the base of the settlement chamber is then recovered and centrifuged. It is assumed that 95% solids separation is achieved and that filter cake has a moisture content of 85%. Given this, filter cake comprising 472.1 t PHA, 944.2 t of non-PHA cell material (NPCM), and 1,203.9 m³ of water is available for further processing. Filtrate is assumed to be discharged to the waste water treatment system.

Removal of NPCM is achieved using a 1 hr NaOH (0.2 N) digestion at a temperature of 30°C (Choi & Lee, 1999) with 20 g of cell material (comprising PHA and NPCM) added per liter of digestion. A 10% loss of PHA (47.2 t/year) is assumed at this stage. Output from the digestion is then subject to a further separation by centrifuge to yield a filter cake and water. Filtrate, including residual PHA diluted in water is assumed to be discharged to the waste water treatment system.

The PHA rich filter cake is subject to a purification stage using 200 g H₂O₂ (9%) per 20 g of polymer. The polymer is then air dried and spray dried to a moisture content of 0.4%.

2.9 | Anaerobic digestion

Anaerobic digestion of grass as a single feedstock takes place in a single stage, mesophilic (37°C) reactor. OLR is 12 kg TS/m³/day, therefore requiring an addition of 454.55 L of water per m³/day to the reactor. Hydraulic retention time is 45 days. Methane production from pre-treated ryegrass considered in the study is 78.88 m³ CH₄/t wet weight, or 403 m³ CH₄/t VS added (Lehtomäki & Björnsson, 2006; Mähnert, Heiermann, & Linke, 2005; Vítěz et al., 2015).

3 | RESULTS

The major material and energy flows associated with the conversion of 30,000 t of grass via the process as described above are summarized in Figures 2a and 2b.

Figures 2a and 2b indicate that the processing of 30,000 t of grass with 52,941 m³ of thickened WAS using the process as described would yield approximately 403.65 tonnes of dried PHA granules (1 tonne of grass yields 13.45 kg of PHA). Unitary data providing inputs and outputs for the production of 1 kg of dried PHA is provided in Table S1 in Supporting Information. Notable material inputs include NaOH both for pre-treatment of the grass feedstock to allow a short HRT at fermentation stage (660 t 100% NaOH), and at PHA extraction stage for digestion of NPCM (566.1 t 100% NaOH), in addition to 4,036 m³ of 9% H₂O₂ for PHA purification. Several stages of the process also require the addition of water (e.g., grass pre-treatment—15,840 m³, SBR—177,413 m³, and PHA extraction—69,612 m³) that, whilst necessary to provide favorable conditions for that particular stage of the process given current knowledge, does incur additional heating, separation, and liquid disposal requirements, therefore reducing the efficiency of the process as a whole. In the configuration considered, liquid streams separated at the PHA recovery stages could be recycled as input water as PHA and NPCM concentrations are low (approximately 0.3 g/L) and the process is non-sterile. The balance of water requiring discharge to sewer for treatment is therefore reduced to 53,706 m³/year.

Based on the approximate availability of green waste in the UK suitable for input to the process (1,298,433 t of material, comprising of 790,746 t of TS and 328,160 t of VS) the above suggests that this could be utilized to produce 22,559 t of PHA. If 5% (363.1 thousand hectares) of UK agricultural grassland was dedicated to biopolymer production, the above result suggests that 195,420 t of PHA could be produced.

Table 1 shows that the largest energy inputs to the process are for aeration of the PHA accumulation reactor (5,961.5 MWh_e), pre-treatment of the WAS prior to fermentation (3,999.3 MWh_{th}), heating of the fermentation reactor (1,832 MWh_{th}), and post fermentation separation of solids and liquids (1,480.6 MWh_e). Some energy can be produced from the anaerobic digestion of solids recovered at the post digestion separation (5,843.7 MWh_{th} and 3,973.7 MWh_e), however, the process as considered in this study still requires an overall thermal energy input of 1,679.3 MWh_{th} and an electricity input of 5,952.1 MWh_e giving an overall energy input requirement of 7,631.4 MWh.

The CED of the major material inputs to the process is also significant, particularly for NaOH (6,060.7 MWh) and H₂O₂ (3,954.6 MWh). The CED associated with sulfuric acid is small due to the highly exothermic production process. Taking the CED of these materials into account in addition to the process energy inputs, the CED of the polymer product is estimated as 69.0 kWh/kg (248.4 MJ/kg) dried PHA where waste grass is used as a feedstock with no energy associated with its production within this process system. Where 30,000 t of grass is specifically cultivated, a further 2,615.7 MWh of energy is expended in its production and a further 19.7 MWh in its transport to the production facility (assuming a transport distance of 20 km), giving an overall CED of the product PHA of 75.5 kWh/kg (271.8 MJ/kg) PHA.

Table 2 provides an economic overview of the PHA production process as described in this study, compared with the option of utilizing grass material for the production of energy via anaerobic digestion (without financial subsidies) (Table 3). Market price for PHA has been reported as approximately €4.5/kg (Hart, Bluemink, Geilvoet, & Kramer, 2014; Koostra, Elissen, & Huurman, 2017) and therefore the total income from 403.65 t of PHA production is €1,816,425 per annum. Highest input costs are for electricity (€595,210) and NaOH (€357,162) and, taking the cost of major energy and material inputs into account, income from PHA exceeds basic process costs by €654,196.4. This is approximately comparable to the economic performance of anaerobic digestion where income from electricity and heat exceeds basic process costs by €713,250. Both of these figures assume that grass is sourced as a waste material with no cost (or gate fee) attributed to either biopolymer or biogas production. Where cultivated grass is utilized and costs for grass production (€27.12/tonne fresh matter) are included, basic process costs exceed income by €159,403.6/year and €100,350/year for biopolymer and anaerobic digestion, respectively. It is likely that capital equipment costs for the PHA

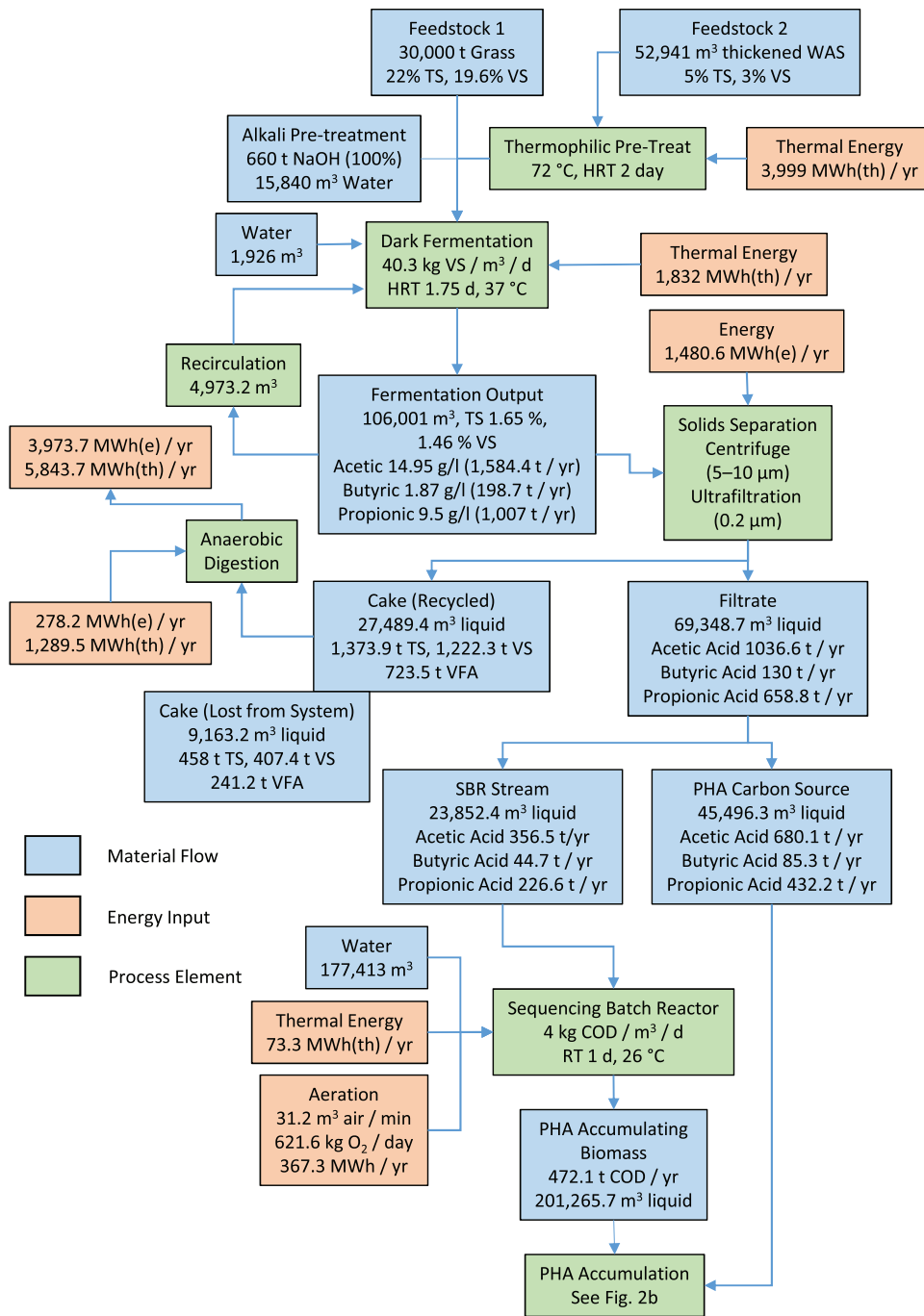


FIGURE 2 (a) Material and energy inputs fermentation → biomass accumulation. (b) Material and energy flows PHA accumulation → final product

production process will be higher than for AD given the larger number of process stages and the subsequent requirement for process monitoring and control.

4 | DISCUSSION

The above analysis indicates that overall CED of the PHA produced from this non-optimized process would be approximately 75.5 kWh/kg (271.8 MJ/kg) PHA. This is higher than, but in the same order of magnitude as, conventional hydrocarbon-based polymers produced in highly efficient industrial processes that are reported to have CED values of 72.7–79.85 MJ/kg (22.17 kWh/kg) for HDPE, 90–101.27 MJ/kg (28.12 kWh/kg) for polyurethane, and 51.7–59.01 MJ/kg (16.38 kWh/kg) for PVC (Galan-Marín, Rivera-Gómez, & García-Martínez, 2016; PRé Consultants, 2016)

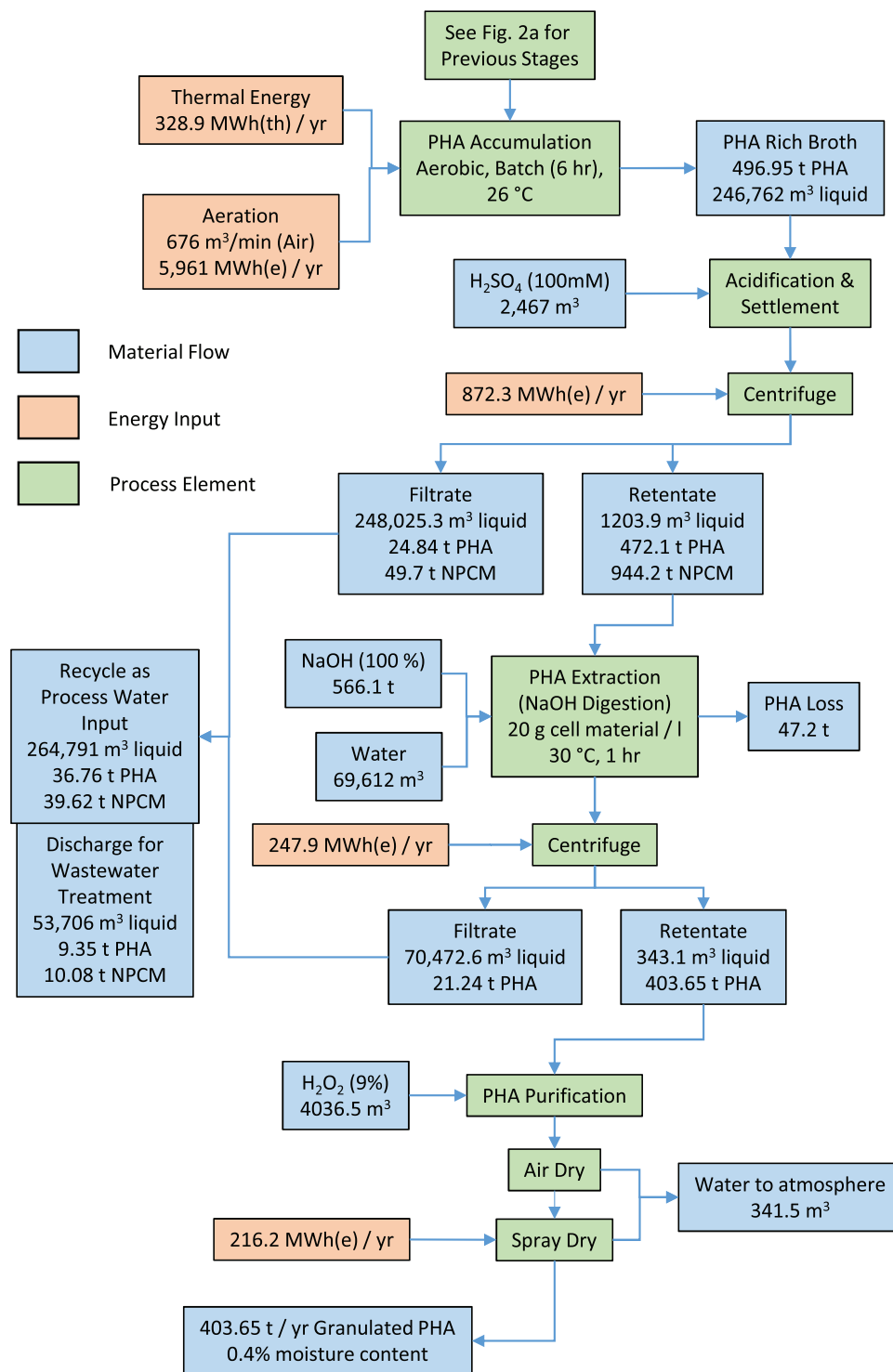


FIGURE 2 Continued

(reported as “embodied energy”), although it is acknowledged that the way in which CED correlates with overall life cycle impacts does vary between agricultural-based systems and fossil-based systems (Huijbregts et al., 2006). Given that the primary feedstock for the polymer is not based on fossil carbon, and that the process system investigated in this study is not yet optimized, this is considered to be an encouraging result. A 60% reduction in both energy and chemical inputs would be required to bring the PHA CED in line with existing fossil polymers based on the yields achieved, or lower reductions coupled with an increase in yield. Given a program of research, development, optimization, and industrialization, this is not an unreasonable goal.

TABLE 1 Primary energy requirement of 403.65 t of PHA produced from 30,000 t of grass

Energy input/material input	Energy input/(output) (MWh/year)
Thermal process energy	
WAS pre-treatment	3,999.3
Dark fermentation heating	1,832.0
Heating of AD reactor for recycling of solids and VFA	1,289.5
Heat production from AD of recycled solids and VFA	(5,843.7)
Heating of SBR	73.3
Heating of PHA accumulation reactor	328.9
Thermal energy subtotal	1,679.3
Conversion factor to primary energy (industrial boiler)	0.78
Primary energy required for thermal inputs (PET)	2,152.9
Electrical process energy	
Post fermentation separation (two stage)	1,480.6
Power generation from AD of recycled solids and VFA	(3,973.7)
Stirring and pumping associated with AD plant	278.2
Aeration of SBR	367.3
Aeration of PHA accumulation reactor	5,961.5
Post acidification separation	872.3
Post NPCM digestion separation	247.9
Spray dry	216.2
Estimate of process pumping energy (5% of other electrical inputs)	471.2
Effluent treatment by activated sludge (Tchobanoglous, Franklin, & Stensel, 2003)	30.6
Electrical energy subtotal	5,952.1
Conversion factor to primary energy	0.38
Primary energy required for electrical inputs (PEE)	15,663.4
CED material inputs	
NaOH (Vellinga et al., 2015)	6,060.7
Sulfuric acid (Vellinga et al., 2015)	46.4
H ₂ O ₂ (Wernet et al., 2016)	3,954.6
Total CED of process inputs (PEM)	10,061.7
CED of PHA produced (waste grass as feedstock) ((PET + PEE + PEM) / 403.65)	69 kWh/kg PHA (248.4 MJ/kg PHA)
Primary energy associated with cultivation of perennial ryegrass (Martínez-Pérez et al., 2007)	2,615.7
Energy used to transport grass to refinery (20 km) (Wernet et al., 2016)	19.7
Total primary energy for grass production and transport (PEG)	2,634.4
CED of PHA produced (using cultivated grass as feedstock) ((PET + PEE + PEM + PEG) / 403.65)	75.5 kWh/kg PHA (271.8 MJ/kg PHA)

Furthermore, for the modeled system all energy burdens are allocated to the biopolymer as this is the only product considered at this early stage. The inclusion of other value products such as those derived from fibers in future iterations of the model would result in burdens being divided between these products, therefore proportionally reducing the allocation of burdens to the PHA. This allocation would normally be conducted on an economic or mass basis. It is noteworthy that the production of polymers from fossil resources is already subject to allocation procedures at the multi-output oil refining stage.

Results also suggest that where waste grass is available in sufficient quantities, an optimized process could be financially viable. If grass is to be cultivated specifically as a raw material for biopolymer production a high level of process optimization is required before financial viability will be achieved, and the potential provision of financial incentives in the early to mid-term stages of deployment may be required (as has been seen for energy production via anaerobic digestion in Europe). In the system presented, separated solids are treated via anaerobic digestion. However, as

TABLE 2 Income and basic process costs of PHA production from waste and cultivated grass

Item description	Value	Rate	Total (€)
<i>Income</i>			
PHA production	403,650 kg	€4.5/kg	1,816,425
<i>Costs</i>			
Electrical inputs	5,952.1 MWh	€0.1/kWh _e	(595,210)
Thermal inputs	1,679.3 MWh	€0.025/kWh _{th}	(41,982.5)
NaOH	1,226.1 t	291.3 €/t (320 USD/t)	(357,162)
Sulfuric acid	24.19 t 98% H ₂ SO ₄	127.4 €/t (140 USD/t)	(3,057.6)
H ₂ O ₂	363.28 t 100% H ₂ O ₂	341.2 €/t (375 USD/t)	(123,951)
Trade effluent discharge	62,870 m ³	0.65 €/m ³	(40,865.5)
<i>Subtotal of costs</i>			(1,162,228.6)
Balance (Income – Costs)			654,196.4
Grass production	30,000 t	€27.12/t £24/t fresh matter (AHDB, 2019)	(813,600)
Balance including grass cultivation			(159,403.6)

TABLE 3 Income and basic process costs of biogas production from waste and cultivated grass

Item description	Value	Rate	Total (€)
<i>Income</i>			
Grass (methane produced)	2,366,315.72 m ³ CH ₄		
Grass (electrical energy)	7,981.12 MWh _(e)	0.06/kWh	478,867
Grass (thermal energy)	11,736.92 MWh _(th)	0.02/kWh	234,738.4
WAS (methane produced)	419,830 m ³ CH ₄		
WAS (electrical energy)	1,400.3 MWh	0.06/kWh	84,018
WAS (thermal energy)	2,059.27 MWh	0.02/kWh	41,185
Digestate	Cost neutral		
<i>Subtotal of income from AD</i>			838,808
<i>Costs</i>			
Thermal energy input	4,870.60 MWh	0.02/kWh	(97,412)
Electrical energy input	469.1 MWh	0.06/kWh	(28,146)
<i>Subtotal of costs</i>			(125,558)
Balance (Income – Costs)			713,250
Grass production	30,000 t	€27.12/t £24/t fresh matter (AHDB, 2019)	(813,600)
Balance including grass cultivation			(100,350)

identified by previous research, for example, Cong and Termansen (2016), the use of separated solids including fibers in higher value products such as composite materials may significantly improve economic viability. Furthermore, as a result of multi-product allocation the CED (and other life cycle burdens) allocated to PHA would also be reduced where other products are generated from the biorefinery process.

The initial energy and economic assessment of the process therefore suggests that there is indeed merit in continuing to develop and optimize the process in order to develop a sustainable biopolymer production process based on utilization of waste grass, or, potentially, based on standard agricultural practices as a response to any future reduction in demand for dairy and meat production in developed economies.

Based on the results of the energy balance and economic analysis, areas where researchers developing the process should concentrate include:

1. Reduction of NaOH use for pre-treatment of grass. This could be achieved by extending hydraulic retention time (leading to larger vessel size and heating requirements) or alternative pre-treatments.

2. Reduction of liquid inputs throughout the process, particularly at fermentation, PHA accumulation, and PHA recovery stages. This would lead to reduced separation energy, and lower energy requirements for heating and aeration.
3. Optimizing organic acid yield at the fermentation stage. Options could include feedstock pre-treatment, enhanced process control including limitation of inhibitory conditions and the use of specialized microbes.
4. Reducing chemical inputs at the PHA recovery and purification stages. In addition to the use of different solvents and detergents options include biological and enzymatic purification, or tailoring the production, recovery, and purification process to match the technical requirement of the end user to reduce unnecessary processing.

As research continues in these areas and more reliable data becomes available for a more optimized process it is important that life cycle assessments, both attributional and consequential, are completed to better understand the burdens and benefits associated with the production of PHA using this approach, including the potential to replace dairy production with feedstock for biopolymer production. The potential use of recovered materials such as fibers for incorporation into novel composite products as opposed to anaerobic digestion/spreading to land could provide significant benefits and should be considered in future studies. Compliance with or adjustment of regulatory frameworks related to the use of biopolymers derived from waste materials for specific end uses (e.g., food packaging), particularly where organic biosolids are utilized in part of the production process, is also a point for future consideration.

5 | CONCLUSIONS

PHA produced from the early stage, non-optimized, and single product process has a CED higher than, but of the same order of magnitude as, fossil-based polymers. Reduction in chemical and water use and utilization of fibers in products would increase process efficiency, reduce CED and is required to reduce process costs. Given the future requirement to continue to produce polymers whilst eliminating fossil carbon feedstocks, the process shows great promise and could also provide a pathway to maintain grass production and sustain agricultural communities in a low carbon future.

ACKNOWLEDGMENTS

The authors would like to thank the funders and all the project partners for providing the background data used in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Tim Patterson  <https://orcid.org/0000-0001-5123-4665>

Alessio Boldrin  <https://orcid.org/0000-0001-5622-7632>

REFERENCES

- AHDB. 2019. Forage Cost Calculator, Agriculture and Horticulture Development Board (AHDB), Beef and Lamb. Retrieved from <http://beefandlamb.ahdb.org.uk/returns/tools/forage-cost-calculator/>.
- Bate, A. (2016). UK Dairy Industry Statistics. Briefing Paper Number 2721. Published by the House of Commons Library.
- Boldrin, A., & Christensen, T. H. (2010). Seasonal generation and composition of garden waste in Aarhus (Denmark). *Waste Management*, 30, 551–557.
- Cerrone, F., Choudhari, S. K., Davis, R., Cysneiros, D., O'Flaherty, V., Duane, G., ... O'Connor, K. (2016). Medium chain length polyhydroxyalkanoate (mcl-PHA) production from volatile fatty acids derived from the anaerobic digestion of grass. *Applied Microbiology and Biotechnology*, 98(2), 611–620.
- Choi, J., & Lee, S. Y. (1999). Efficient and economical recovery of poly(3-hydroxybutyrate) from recombinant *Escherichia coli* by simple digestion with chemicals. *Biotechnology and Bioengineering*, 62(5), 546–553.
- Chai, B. C., van der Voort, J. R., Frolinik, K., Eliasdottir, G. G., Kloss, I., & Perz-Cueto, F. J. A. (2019). Which diet has the least environmental impact on our planet? A systematic review of vegan, vegetarian and omnivorous diets. *Sustainability*, 11(15), 4110. <https://doi.org/10.3390/su11154110>
- Cong, R. G., & Termansen, M. (2016). A bio-economic analysis of a sustainable agricultural transition using green biorefinery. *Science of the Total Environment*, 571, 153–163.
- Cui, M., & Shen, J. (2012). Effects of acid and alkaline pretreatments on the biohydrogen production from grass by anaerobic dark fermentation. *International Journal of Hydrogen Energy*, 37, 1120–1124.
- Dairy Development Centre. (2014). Grass value—what is the value of grass, summary of results. Retrieved from <https://dairy.ahdb.org.uk/resources-library/technical-information/grass-management/welsh-dairy-supply-chain-value-of-grass/#.XZ89EXdFxVU>
- Department for Environment and Rural Affairs (DEFRA). (2017). Agriculture in the United Kingdom 2016. Retrieved from <https://www.gov.uk/government/statistics/agriculture-in-the-united-kingdom-2016>

- Department of the Environment, Food and Rural Affairs (DEFRA). (2019). ENV18 - Local authority collected waste from households from January 2010 to March 2018—England data. Retrieved from <https://www.gov.uk/government/statistical-data-sets/env18-local-authority-collected-waste-annual-results-tables>
- Ecker, J., Schaffenberg, M., Koschuh, W., Mandl, M., Böchzelt, H. G., Schnitzer, H., ... Steinmüller, H. (2012). Green biorefinery upper Austria—Pilot plant operation. *Separation and Purification Technology*, 96, 237–247.
- Fuchs, W., & Drosch, B. (2010). *Technologiebewertung von Gärrestbehandlungs- und Verwertungskonzepten*. Wein, Austria: Universität für Bodenkultur Wien. ISBN: 978-3-900962-86-9
- Galan-Marin, C., Rivera-Gomez, C., & Garcia-Martinez, A. (2016). Use of natural-fiber bio-composites in construction versus traditional solutions: Operational and embodied energy assessment. *Materials*, 6(6), 465. <https://doi.org/10.3390/ma9060465>
- Hart, N. R. d., Bluemink, E. D., Geilvoet, A. J., & Kramer, J. F. (2014). Bioplastic uit slib. Verkenning naar PHA-productie uit zuiveringsslib (Grondstoffenfabriek). stowa Rapport 10. ISBN 978.90.5773.649.0. Retrieved from <https://www.stowa.nl/sites/default/files/assets/PUBLICATIES/Publicaties%202014/STOWA%202014-10.pdf>
- Huijbregts, M. A. J., Rombouts, L. J. A., Hellweg, A., Frischknecht, R., Jan Hendriks, J. A., van de Meent, D., ... Struijs, J. A. (2006). Is cumulative fossil energy demand a useful indicator for the environmental performance of products? *Environmental Science and Technology*, 40, 641–648.
- Jagadabhi, P. S., Kaparaju, P., & Rintala, J. (2010). Effect of micro-aeration and leachate replacement on COD solubilisation and VFA production during mono digestion of grass-silage in one-stage leach-bed reactors. *Bioresource Technology*, 101, 2818–2824.
- Kamm, B., Schönlicke, P., & Hille, C. (2016). Green biorefinery—Industrial implementation. *Food Chemistry*, 197, 1341–1345.
- Koller, M., Bona, R., Hermann, C., Horvat, P., Martinz, J., Neto, J., ... Braune, G. (2005). Biotechnological production of poly(3-hydroxybutyrate) with *Wautersia eutropha* by application of green grass juice and silage juice as additional complex substrates. *Biocatalysis and Biotransformation*, 23(5), 329–337.
- Koostra, M., Elissen, H., & Huurman, S. (2017). PHA's (Polyhydroxyalkanoates): General information on structure and raw materials for their production. Application Centre for Renewable Resources (ACRRES), Wageningen UR, Report 727. Retrieved from <https://edepot.wur.nl/414011>
- Kshirsagar, P., Suttar, R., Nilegaonkar, S., Kulkarni, S., & Kanekar, P. (2012). Scale up production of polyhydroxyalkanoate (PHA) at different aeration, agitation and controlled dissolved oxygen levels in fermenter using *Halomonas campisalis* MCM B-1027. *Journal of Biochemical Technology*, 41, 512–517.
- Kyazze, G., Dinsdale, R., Hawkes, F. R., Guwy, A. J., Premier, G. C., & Donnison, I. S. (2008). Direct fermentation of fodder maize, chicory fructans and perennial rye grass to hydrogen using mixed microflora. *Bioresource Technology*, 99(18), 8833–8839.
- Lehtomäki, A., & Björnsson, L. (2006). Two stage anaerobic digestion of energy crops: Methane production, nitrogen mineralisation and heavy metal mobilisation. *Environmental Technology*, 37, 209–218.
- Mähnert, P., Heiermann, H., & Linke, B. (2005). Batch- and semi-continuous biogas production from different grass species. *Agricultural Engineering International: The CIGR Ejournal*, Manuscript EE 05 010. VII, 1–11. Retrieved from <https://cigrjournal.org/index.php/Ejournal/article/view/609/603>
- Maity, S. K. (2015a). Opportunities, recent trends and challenges of integrated biorefinery: Part I. *Renewable and Sustainable Energy Reviews*, 43, 1427–1445.
- Maity, S. K. (2015b). Opportunities, recent trends and challenges of integrated biorefinery: Part II. *Renewable and Sustainable Energy Reviews*, 43, 1446–1466.
- Mandl, M. G. (2010). Status of green biorefining in Europe. *Biofuels, Bioproduction and Biorefining*, 4, 268–274.
- Martínez-Blanco, J., Colón, J., Gabarrell, X., Font, X., Sánchez, A., Artola, A., & Rieradevall, J. (2010). The use of life cycle assessment for the comparison of biowaste composting at home and full scale. *Waste Management*, 30, 983–994.
- Martínez-Pérez, N., Cherryman, S. J., Premier, G. C., Dinsdale, R. M., Hawkes, D. L., Hawkes, F. R., ... Guwy, A. J. (2007). The potential for hydrogen enriched biogas production from crops: Scenarios in the UK. *Biomass and Bioenergy*, 31, 95–104.
- Massanet-Nicolau, J., Dinsdale, R., Guwy, A., & Shipley, G. (2015). Utilising biohydrogen to increase methane production, energy yields and process efficiency via two stage anaerobic digestion of grass. *Bioresource Technology*, 189, 379–383.
- Monson, K. D., Esteves, S. R., Guwy, A. J., & Dinsdale, R. M. (2007). Digestion of biodegradable municipal wastes: A review. ISBN: 978-1-84054-157-1. Retrieved from <https://www.walesadcentre.org.uk/media/1050/anaerobic-digestion-of-bmw-compressed-part1.pdf>
- Moretto, G., Russo, I., Bolzonella, D., Pavan, P., Majone, M., & Valentino, F. (2020). An urban biorefinery for food waste and biological sludge conversion into polyhydroxyalkanoates and biogas. *Water Research*, 170, 115371.
- Northern Ireland Environment Agency. (2017). Northern Ireland local authority collected municipal waste management statistics, 2016/17 annual data. Retrieved from <https://www.daera-ni.gov.uk/articles/northern-ireland-local-authority-collected-municipal-waste-management-statistics>
- O'Keeffe, S., Schulte, R. P. O., Lalor, S. T. J., O'Kiely, P., & Struik, P. C. (2011a). Green biorefinery (GBR) scenarios for a two cut silage system: Investigating the impacts of sward botanical composition, N fertilisation rate and biomass availability on GBR profitability and price offered to farmers. *Biomass and Bioenergy*, 35, 4699–4711.
- O'Keeffe, S., Schulte, R. P. O., Sanders, J. P. M., & Struik, P. C. (2011b). Technical assessment for first generation green biorefinery (GBR) using mass and energy balances: Scenarios for an Irish GBR blueprint. *Biomass and Bioenergy*, 35, 4712–4723.
- O'Keeffe, S., Schulte, R. P. O., Sanders, J. P. M., & Struik, P. C. (2012). Economic assessment for first generation green biorefinery (GBR): Scenarios for an Irish GBR blueprint. *Biomass and Bioenergy*, 41, 1–13.
- Oleskowicz-Popiel, P., Kádár, Z., Heiske, S., Klein-Marcuschamer, D., Simmons, B. A., Blanch, H., ... Schmidt, J. E. (2012). Co-production of ethanol, biogas, protein fodder and natural fertilizer in organic farming—Evaluation of a concept for a farm-scale biorefinery. *Bioresource Technology*, 104, 440–446.
- Papendiek, F., Tartiu, V. E., Morone, P., Venus, J., & Hönig, A. (2014). Benefit for farmers to produce press juices from legumes for chemical industries. Paper presented at the 22nd European Biomass Conference and Exhibition, Hamburg, Germany.
- Parajuli, R., Dalgaard, T., Jørgensen, U., Adamsen, A. P. S., Knudsen, M. T., Birkved, M., ... Schjørring, J. K. (2015). Biorefining in the prevailing energy and materials crisis: A review of sustainable pathways for biorefinery value chains and sustainability assessment methodologies. *Renewable and Sustainable Energy Reviews*, 43, 244–263.
- Parajuli, R., Kristensen, I. S., Knudsen, M. T., Mogensen, L., Corona, A., Birkved, M., ... Dalgaard, T. (2016). Environmental life cycle assessments of producing maize, grass clover, ryegrass and winter wheat straw for biorefinery. *Journal of Cleaner Production*, 142, 3859–3871.
- Parkarinen, O., Lehtomäki, A., Rissanen, S., & Rintala, J. (2008). Storing energy crops for methane production: Effects of solids content and biological additive. *Bioresource Technology*, 99, 7074–7082.
- PRÉ Consultants B.V. (2016). SimaPro v.8.2.3. See <https://pre-sustainability.com/>
- Rancane, S. K. A., Lazdina, D., & Berzins, P. (2015). Biomass yield and chemical composition of perennial grasses for energy production. Paper presented at the 14th International Scientific Conference, Engineering for Rural Development, Jelgava, Latvia.

- Santamaría-Fernández, M., Molinuevo-Salces, B., Kiel, P., Steinfeldt, S., Uellendahl, H., & Lübeck, M. (2017). Lactic acid fermentation for refining proteins from green crops and obtaining high quality feed product for monogastric animals. *Journal of Cleaner Production*, *162*, 875–881.
- Scottish Environmental Protection Agency (SEPA). (2017). Scottish household waste generation and management statistics. Retrieved from <https://www.sepa.org.uk/environment/waste/waste-data/waste-data-reporting/household-waste-data/>
- Sharma, H. S. S., Lyons, G., & McRoberts, C. (2011). Biorefining of perennial grasses: A potential sustainable option for Northern Ireland grassland production. *Chemical Engineering Research and Design*, *89*, 2309–2321.
- Solati, Z., Jørgensen, U., Eriksen, J., & Søgaard, K. (2017). Dry matter yield, chemical composition and estimated extractable protein of legume and grass species during the spring growth. *Journal of the Science of Food and Agriculture*, *97*, 3958–3966.
- Tchobanoglous, G., Franklin B., & Stensel, H. D. (2003). *Wastewater Engineering, Treatment and Reuse* (4th ed.). New York, NY: McGraw Hill.
- The Federal Government of Germany. (2012). “Biorefineries Roadmap” as part of the German Federal Government action plans for the material and energetic utilization of renewable raw materials. Retrieved from https://ec.europa.eu/knowledge4policy/publication/biorefineries-roadmap_en
- Valentino, F., Sagastume, F. M., Campanari, S., Vilano, M., Werker, A., & Majone, M. (2017). Carbon recovery from wastewater through bioconversion into biodegradable polymers. *New Biotechnology*, *37*, 9–23.
- Valentino, F., Reis, M., Silva, F., & Majone, M. (2019). Report on optimization of PHA production at pilot scale. Horizon 2020 Project 730349 Resources from Urban Biowaste (RESURBIS), Deliverable 2.5. Unpublished. Summary available at https://www.resurbis.eu/deliverable_2.5
- Van der Weijde, T., Kamei, C. L. A., Torres, A. F., Vermerris, W., Dolstra, O., ... Trindade, L. M. (2013). The potential of C4 grasses for lignocellulosic biofuel production. *Frontiers in Plant Science*, *4*(107), 1–18.
- Vellinga, T. V., Blonk, H., Marinussen, M., Zeist, W. J., Van, B., I. J. M., & De, S. D. (2015). Agri-footprint LCI Database v.2.0. Blonk Consultants B.V., The Netherlands.
- Vergara, S. E., & Silver, W. L. (2019). Greenhouse gas emissions from windrow composting of organic wastes: Patterns and emissions factors. *Environmental Research Letters*, *14*, 124027.
- Voelklein, M. A., Rusmanis, D., & Murphy, J. D. (2016). Increased loading rates and specific methane yields facilitated by digesting grass silage at thermophilic rather than mesophilic temperatures. *Bioresource Technology*, *216*, 486–493.
- Vítěz, T., Koutný, T., Geršl, M., Kudělka, J., Nitayapat, N., Ryant, P., ... Maraček, J. (2015). Biogas and methane yield from grass. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, *63*(1), 143–146. Retrieved from <https://doi.org/10.11118/actaun201563010143>
- Wernet, G., Bauer, C., Steubing, B., Reinhard, J., Moreno-Ruiz, E., and Weidema, B., 2016. The ecoinvent database version 3 (part I): overview and methodology. *The International Journal of Life Cycle Assessment*, [online] *21*(9), 1218–1230. Retrieved from <http://link.springer.com/10.1007/s11367-016-1087-8>
- Welsh Government. (2019). Annual waste collected for reuse/recycling/composting (tonnes) by material and source (2016/17). Retrieved from <https://stats.wales.gov.wales/Catalogue/Environment-and-Countryside/Waste-Management/Local-Authority-Municipal-Waste>
- Yu, L., Bule, M., Ma, J., Zhao, Q., Frear, C., & Chen, S. (2014). Enhancing volatile fatty acid (VFA) and bio-methane production from lawn grass with pretreatment. *Bioresource Technology*, *162*, 243–249.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Patterson T, Massanet-Nicolau J, Jones R, et al. Utilizing grass for the biological production of polyhydroxyalkanoates (PHAs) via green biorefining: Material and energy flows. *J Ind Ecol*. 2021;25:802–815.

<https://doi.org/10.1111/jiec.13071>