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Biopolymer Production from Irish Dairy Industry Wastewaters

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Comhshaol, Pobal agus Rialtas Áitiúil
Environment, Community and Local Government

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EPA Research Programme 2014–2020

Biopolymer Production from Irish Dairy Industry Wastewaters

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EPA Research Report

Prepared for the Environmental Protection Agency

by

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DISCLAIMER

The EPA Research Programme addresses the need for research in Ireland to inform policy and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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Executive Summary

This project set out to investigate the potential for generating bioplastics from dairy processing wastewater using an adapted mixed-microbial biomass process. The polymers in question were polyhydroxyalkanoates (PHAs), a diverse array of polyesters with industrially relevant thermoplastic properties, which have been shown to accumulate intracellularly in a wide array of bacterial species. To this end, a series of laboratory-scale sequencing batch reactors (SBRs) were established with microbial biomass samples from a dairy processing wastewater treatment plant. The SBRs were operated under conditions of "aerobic dynamic feeding", which involve sequential transitions of the biomass through periods of high and low carbon source availability referred to as a feast/famine regime. The process creates a selective pressure favouring microbial species capable of rapid uptake/storage of carbon sources in the feast phase that is independent of their growth rate. The accumulated carbon, typically in the form of a polyhydroxyalkanoate, is subsequently degraded during the famine phase, resulting in stable growth of the microbes across all stages of the aerobic dynamic feeding process. The goal is to develop a microbial biomass, enriched for PHA accumulators, that can subsequently be utilised in a fed-batch culture system to optimise PHA accumulation. From this work, the optimal conditions necessary for biomass adaptation/enrichment were established and found to be heavily influenced by carbon source composition and the key SBR parameter of sludge retention time. Extraction and chemical characterisation identified the polymer accumulating within the biomass as polyhydroxybutyrate (PHB), composed of 3-hydroxy-butyryl (C_4) monomers. During the adaptation phase, the biomass generated relatively low-level PHB accumulation of approximately 5% g PHB/g volatile suspended solids (VSS). However, subsequent fed-batch trials with defined media yielded 58% PHB with butyrate as the carbon source after 24 hours of incubation and 40% PHB with propionate as the carbon source after 4–6 hours. Inorganic nutrient consumption (phosphorus present as PO_4, nitrogen present as NH_3 and NO_3) was minimal in both the SBRs and the fed-batch trials, indicating that high inorganic nutrient loads, typical of dairy processing effluents, would require additional downstream remediation.

Efforts were made to isolate pure cultures of PHA-producing microorganisms from the aerobic dynamic feeding-enriched sludge using a range of complex and minimal media. Twenty-four phenotypically distinct isolates were identified and subjected to 16S rRNA gene polymerase chain reaction amplification and restriction fragment length polymorphism profiling. Comparison of the profiling patterns indicated that the isolates were composed of members of seven distinct species, and sequencing of representative 16S genes identified the species as *Xanthobacter, Kerstersia, Achromobacter, Defluvibacter, Azorhizobium* and *Alcaligenes*. All of these species have previously been linked with PHB production in the literature. Prior to assessing PHB production capacities, species were screened with degenerate primers for PHB synthase genes. Only the *Achromobacter* and *Defluvibacter* isolates were found to possess these genes. These isolates were subsequently cultured under phosphate-limited growth in Gotz minimal media with acetate, butyrate and propionate volatile fatty acids, respectively, to stimulate PHB production. It was observed that, in isolation from the sludge, these cultures accumulated relatively low levels of PHB. *Defluvibacter* achieved a maximum yield of 19% PHB with propionic acid, while *Achromobacter* accumulated 5% PHB in phosphate-limited growth on acetate. Efforts to cultivate other isolates in liquid media were unsuccessful. While inherent biases in the isolation and PHB induction experiments are recognised, our investigations indicated that the mixed community therefore appears to be more than the sum of its parts and a more effective vehicle of PHB production than any of the individual isolates sequestered from it in the present study.

Having identified optimal parameters for sludge adaptation and fed-batch production of poly-(3)-hydroxybutyrate under controlled media conditions, the project set out to assess the use of real-time dairy processing wastewater in the adaptation and fed-batch production phases. Anaerobic digester effluent was supplied by Kerry Ingredients (Listowel, Co. Kerry) during peak seasonal production at the plant. The specific use of anaerobic digester effluent reflects the biomass requirement for volatile fatty acids (e.g. acetate, butyrate, propionate) as the starting materials for polymer

synthesis as they are typically generated under anaerobic digestion conditions. Parallel SBRs were reseeded with fresh biomass samples and adapted under aerobic dynamic feeding conditions, with 4-day and 2-day solid retention times, respectively, receiving anaerobic digester effluent as the sole feedstock. Following 20 days of adaptation, stable PHB accumulation became established within the biomass at 2–5% g PHB/g VSS. Subsequent fed-batch trials to maximise PHA accumulation within adapted biomass samples yielded approximately 45% g PHB/g VSS from anaerobic digester effluent within 4–6 hours. Despite conclusive demonstration of the feasible bioconversion of effluent chemical oxygen demand to PHA, it is likely that the maximal yield did not reflect the maximum potential of the system, as volatile fatty acid concentrations (acetate, butyrate and propionate) were at relatively low levels within the anaerobic digester effluent during the fed-batch trials. Extraction and chemical analyses confirmed the dominant polymer to be PHB, although it is likely that higher volatile fatty acid concentrations could well contribute to co-polymer formation. The solid retention time length in the SBRs played an important role in fine-tuning the metabolic sensitivity of the biomass to PHA production in the fed-batch trials. The 2-day solid retention time-adapted biomass demonstrated the fastest response rates, in terms of both PHB accumulation and consumption, in subsequent trials. Any future bioprocess design for this technology would, therefore, have to achieve a balance between optimal PHA accumulation kinetics within the biomass and an appropriate timescale for polymer recovery.

The project also sought to assess the feasibility of PHB production by sludge biomass as an intermediary step in the synthesis of fatty acid methyl esters for potential biofuel applications. Extracted polymer was subjected to chemical derivatisation by acid reflux, rotary evaporation and fractional distillation with hydroxybutylmethyl ester ($C_5H_9O_3$) dominating the 61°C fractionated eluent. However, successful PHB to fatty acid methyl ester conversion at the laboratory scale could only be achieved through harsh chemical treatments with extensive time and energy demands that currently undermine the sustainability of the fatty acid methyl esters generated.

Life cycle analysis to assess the implications of efforts to scale up the laboratory production system indicated that, although the technology is promising, there are several challenges. A distorting factor in arriving at an accurate perspective on scale-up issues is the disparity in the readiness of the technology for the laboratory and the industrial processes. The analysis identified energy consumption as a significant barrier to technology development at present. The PHB end-product was attributed a commercial value of €5500/tonne, while the estimated energy consumption at 1–5kWh/L of wastewater constituted an unsustainable electricity cost of €64,000/tonne. Similarities were drawn with other bioconversion technologies that are currently hindered by negative energy balance issues, with the proviso that ongoing developments in associated technologies, policy developments and environmental drivers are likely to reduce these barriers, particularly when the processes in question offer a suite of beneficial impacts. Several recommendations are posited in Chapter 4 with respect to key challenges that need to be addressed in order to develop this wastewater to bioplastic bioconversion strategy further.

1 Introduction

1.1 Petrochemical Plastics: Consumption and Environmental Consequences

Direct, daily consumption of petroleum, exposure to upwards pricing trends, political visibility and global environmental advocacy have resulted in society at large becoming keenly aware of the need for a shift towards sustainable fuels (Delshad *et al.,* 2010). Less recognition, however, is given to our fundamental dependence on non-renewable petrochemicals for the provision of essential polymers/plastics, such as polyethylene terephthalate (PET), polyvinylchloride (PVC), polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyamide (PA). Global production of plastics has been reported at approximately 300 million tonnes in 2013, consuming 8% of world oil and gas outputs (Plastics Europe, 2014). Biodegradable polymers account for less than 1% of the global plastics market (Nampoothiri *et al.,* 2010). One third of annual petrochemical plastics production is directed towards disposable applications, with 99% of the materials being composed of xenobiotic compounds recalcitrant to biodegradation processes (Hopewell *et al*., 2009). Petrochemical plastics can persist for decades in natural environments and it is estimated that 18–25% of the total volume of landfill sites can be occupied by accumulated plastics solid waste (PSW) (Andrady, 2003). The US Environmental Protection Agency reported that approximately 12.5% of all municipal solid waste generated in the USA in 2010 was composed of PSW, equivalent to 3.13×10^7 tonnes (USEPA, 2010). A legacy of poor plastics waste management has also resulted in the extensive pollution of global marine environments. Widespread use of petrochemical plastics became established in the 1950s and within two decades significant particulate plastic dispersal within the world's oceans was being recorded (Colton *et al*., 1974). Recent studies reported an alarming upwards revision of the estimated scale of this problem and have prompted serious concerns over potential ecosystem/foodweb impacts (Law *et al*., 2010; Eriksen *et al*., 2014; Seltenrich, 2015). Therefore, a critical issue to address in any future shift towards a renewables-based, eco-sensitive economy will be the resourcing of sustainable, biodegradable polymers to meet society's critical need for such materials.

1.2 "Biopolymers": What's in a Name?

The terms "biopolymer", "bioplastic" and "bio-based plastic" appear interchangeably within polymer literature, suggesting universal properties common to the materials being discussed. However, these terms are generically applied to materials that can be (a) entirely biologically derived, (b) composed of a mixture of both petrochemical and biologically sourced materials, (c) composed solely of petrochemical monomers but offering a degree of biodegradability, or (d) composed of monomers whose origins are petrochemical but whose formation has involved a biological transformation via *in vitro* enzyme or microbial whole-cell-based steps. The biodegradable polyester poly-caprolactone provides a good example of instance (d). The requisite monomers for synthesis of this polymer, 6-hydroxyhexanoic acid and ε-caprolactone, can both be synthesised by *Acinetobacte*r sp. SE19 during the conversion of cyclohexanol to adipic acid (Thomas *et al*., 2002). The promotion of poly-caprolactone as a bioplastic is, therefore, *sensu strictu* appropriate, based on the use of whole-cell biotransformation steps in monomer production and the biodegradability of the end product. However, the starting substrate, cyclohexanol, is derived from petrochemical sources and represents an undesirable mobilisation of fossil fuel-derived CO_2 in the synthesis of this bioplastic. The American Society for Testing and Materials (ASTM) regards biodegradable plastic as any polymer that undergoes degradation by the biochemical activities of naturally occurring microorganisms. As a result, blends of petrochemical and biological materials, e.g. maize starch extruded with polystyrene, can be marketed as biodegradable, despite the fact that only the starch component of the polymer is amenable to biodegradation (Bhatnagar and Hanna, 1996). These incongruities have prompted commentators to argue that such products foster a detrimental misconception that all "biodegradable" plastics can simply be discarded into the environment without consequence. In reality, many such products have protracted degradation rates, even in sites with high microbial activity, such as composting facilities (Gross and Kalra, 2002; Kale *et al*., 2007).

1.3 Natural Polymer Alternatives

A significant research effort has been committed in recent decades to the development of eco-sensitive polymeric materials, with several natural materials being explored, including polysaccharides, poly-D-lactic acid (PLA) and PHAs (Gross and Kalra, 2002; Lindblad *et al.*, 2002; Flieger *et al.*, 2003; Widdecke, 2008). This project focused solely on PHAs, but a summary overview of the major alternatives is presented here in order to place PHAs within the wider biopolymer context.

1.3.1 Polysaccharides

Nature provides a range of polymeric arrangements based around a common glycosidic backbone in the form of starch, cellulose, hemicellulose and chitin. Despite the use of this common core structure, the precise structural and material properties of the resulting compound are quite different, lending themselves to varied applications (e.g. permeable films, loose packaging, injection moulded forms etc.).

Starch

Awareness in the 1970s of petrochemical polymer-related environmental pollution prompted interest in the incorporation of starch into polyethylene at levels of approximately 10% (Schrongen, 1993). As the field has progressed, increasing percentages of starch incorporation were achieved, culminating in materials with up to 90% of thermoplastic starch prepared with plasticisers (e.g. glycerol) to reduce brittleness (Mitrus *et al.*, 2009). 100% starch foams are commercially available for loose packaging applications, providing a renewable alternative to polystyrene (Bastioli *et al.*, 1998). Applications to date are focused on food films, packaging and compostable single-use materials, as well as agricultural uses in the controlled release of pesticides (Riley, 1983; Mitrus *et al.*, 2009). Technical challenges to broader usage relate to the hygroscopic nature of the polymer, which means that water absorption disrupts the ordering of the relative amylose and amylopectin components, causing swelling and disintegration (Li *et al.*, 2008). The majority of starch used in polymer production comes from a limited number of crops, primarily maize, sugar-cane and potato. In light of the negative scrutiny directed towards starch conversion to bioethanol, the question arises of whether a significant expansion of food crop diversion to polymer production would be welcome (Hill *et al.*, 2006). In 2006, bioethanol fermentation in the USA consumed 11% of the national maize crop, which rose to 20% in 2007 and 31% in 2008 (Widdecke, 2008). This rise, combined with the effect of poor weather on overall maize output, meant that the raw material price of maize in July 2008 was 300% higher than maize price listings in July 2006. In a world where population growth is approaching 1 billion per decade, the current acceptability of food starch diversion to industrial applications seems destined to come under negative pressure (UNFPA, 2011). As a result, it is difficult to envisage starch-based materials breaking out of their current market niche to occupy a dominant position in any long-term, renewable polymer strategy.

Cellulose

In contrast to starch, cellulose is a natural polysaccharide that can be sourced from non-food biomass. The non-branching linear arrangement of glucose units within cellulose offers considerable tensile strength and flexibility which are attractive features for biopolymer development. It is estimated that approximately 9 million tons of natural cellulose fibres could be produced from the cornhusk biomass generated annually in the USA (Reddy and Yang, 2005). At present, there are two major sources for cellulose polymer incorporations. The first involves the use of plant cellulose fibres in bio-composite formulations with materials such as glass, starch sources, plastics, resins and other synthetic fibres. A 100% renewably sourced and biodegradable granular thermoplastic, ARBOFORM, is produced by Tecnaro by combining lignin with other natural fibres such as flax and hemp (Widdecke, 2008). The second source involves the synthesis of cellulose by various bacterial species, including *Acetobacter, Acanthamoeba*, *Achromobacter* and *Zoogloea* (Petersen and Gatenholm, 2011). The polymer is identical to plant-derived cellulose but does not suffer from the disadvantages of lignin/hemicellulose binding. The current applications of bacterial cellulose are in niche biomedical areas which seek to exploit the nanofibre structural nature of the polymer, which has a diameter of approximately 100 nm (Bäckdahl *et al.*, 2006; Bodin *et al.*, 2010). Technical challenges to the increased use of cellulosic polymers include the hygroscopic nature of cellulose and the high energy and capital inputs required for the liberation of cellulose from its polyaromatic hydrocarbon lignin encapsulation.

Chitosan

Chitosan represents an emergent natural polysaccharide of technological significance which can be prepared by the deacetylation of chitin recovered from the shells of crustaceans (Coma *et al*., 2002; No *et al*., 2007). It is composed of β-1-4-linked glucosamine units and *N*-acetyl glucosamine residues and demonstrates antimicrobial activity against various bacterial and fungal species (Sebti *et al*., 2007). The functional properties and antimicrobial effects of chitosan are related to its degree of deacetylation and molecular weight. Due to its film forming property, low toxicity and allergenicity, chitosan applications are concentrated around the preparation of edible films and coatings (No *et al*., 2007). Current challenges in the exploitation of chitosan include extraction difficulties and its low solubility in neutral and/or alkaline aqueous solvents.

1.3.2 Microbial-derived polymers

Polylactic acid

As highlighted above for cellulose, microbial metabolic processes can also yield biological polymers of commercial interest. Microbial polysaccharides, which are primarily used for gelling applications in food, pharmaceutical and medical industries, can be of bacterial (dextran, xanthan and gellan) or fungal (pullulan and glucan) origin (Murano, 2000). However, the most economically successful bioplastic to date has been the condensation polyester polylactic acid (PLA), originally synthesised in the 1950s by DuPont, but now widely produced by many industrial groups (Pang *et al*., 2010). PLA resins have obtained approval from the US Food and Drug Administration and from European regulatory authorities for use in all food-type applications (Datta and Henry, 2006). Production of PLA and its variants involves microbial fermentations to produce either D- or L-lactic acid monomers, which are subsequently condensed by *in vitro* chemical methods (John *et al*., 2007). A potential economic and environmental advantage of PLA is the ability to use several waste materials (e.g. agri-wastes) as substrates for the monomer-yielding fermentation step (John *et al*., 2006; Pang *et al*., 2010). However, the homopolymeric (D-lactide) composition of PLA imparts a narrow range of properties/applications to the polymers. PLA is, therefore, often blended with non-renewable petrochemical additives to enhance key properties such as tensile strength and heat resistance (Widdecke *et al*., 2008; Balakrishnan *et al*., 2012).

Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHAs) are a highly promising group of polyester compounds which are synthesised by a diverse array of microbial species as intracellular carbon storage bodies. They offer a number of highly attractive bioplastic characteristics, such as sustainable production, complete biodegradability, highly diverse monomer compositions (> 150 monomers identified), broad thermoplastic and elastomeric properties and amenability to modification through the manipulation of growth substrates, growth conditions and production strains (Chen, 2009; Meng *et al*., 2014). The range of PHAs synthesised by bacteria reflects the diversity of metabolic routes known to contribute to their synthesis, summarised in Figure 1.1.

PHAs are broadly categorised into three groups based on acyl monomer lengths, with the majority being divided into the short chain length (C_2–C_6) and medium chain length (mcl) (C_6–C_{14}) groups. Short chain length monomer polymers are often crystalline and brittle in nature, while mcl monomer compositions yield polymers which are more flexible and offer a greater range of applications. Homo-, hetero- and block co-polymers can also be generated from the diverse monomers available, so that an extensive array of plastic compositions and properties can be generated. Commercial interest in PHAs emerged in the 1980s in response to concerns about rising crude oil prices and several small-scale PHA production facilities arose with a production capacity of 200–300 tons per annum (e.g. ICI, UK and Chemie Linz, Austria). The stabilisation of oil prices in the 1990s saw a diminished interest in the development of these polymers, coupled with the closure of many of the small-scale sites. However, renewed interest and research commitment has developed in recent years to the extent that global PHA production now approaches 100,000 tons per annum with further growth projected (Chen, 2009). The major challenge for the market expansion of PHA-based plastics relates to the end-product price of up to €3.5–5.0/kg, which is four- to five-fold higher than the price of the of equivalent petrochemical plastics they are designed to replace (Gurieff *et al*., 2007; Chanprateep, 2010). These costs reflect the high process costs associated with the production of these biopolymers in sterile bioreactors, with engineered pure cultures and high-purity substrates such as glucose or fatty acids. Efforts to bring down PHA production costs have recently focused on the use of mixed-microbial culture “open systems”, which obviate the costs/challenges

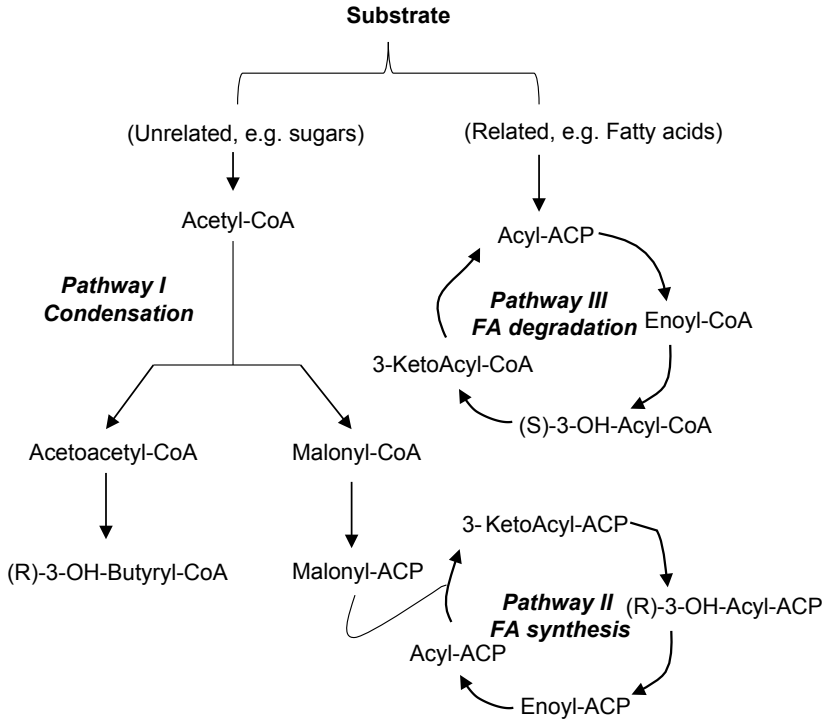

Figure 1.1. Major PHA accumulation pathways. Pathway I: condensation of acetyl-CoA moieties; pathway II: *de novo* FA biosynthesis from unrelated carbon sources; pathway III: fatty acid degradation (β-oxidation pathway).

associated with pure-culture aseptic bioreactor operation (Kleerebezem and van Loosdrecht, 2007). One promising strategy within this eco-engineering theme is that of aerobic dynamic feeding (ADF) (Serafim *et al*., 2004). The process involves feast/famine carbon cycling of a mixed-microbial culture (e.g. municipal sludge) to competitively enrich for microbial populations capable of PHA accumulation.

Figure 1.2 provides an example of the biochemical transformations associated with an enriched population under an ADF regime. Rapid biomass accumulation of the common short chain length polymer, polyhydroxybutyrate (PHB), occurs in the feast phase, which is subsequently mobilised during the famine period. With respect to the microbial ecology underpinning the adaptation process, the limited studies conducted to date have indicated an association with the emergence of particular species, e.g. *Plasticicumulans acidovorans,* though additional studies combining next generation sequencing approaches with spatial distribution/visualisation techniques across a range of feedstock physico-chemical characteristics would be required to comprehensively determine whether a common microbial community profile can be attributed to this process (Johnson *et al*., 2009; Tamis *et al*., 2014).

Following adaptation, biomass is subsequently exploited in fed-batch fermentations where the PHA accumulation capacity can be fully maximised. Such mixed culture systems also offer inherently diverse catabolic capabilities, allowing for incorporation of waste streams as low-cost substrates (Kim, 2000; Khanna and Srivastava, 2005; Serafim *et al*., 2008). Several industrial by-product feed-stocks have been investigated to date, which are often reflective of national agri-food sector activities, e.g. olive mill effluent (Dionisi *et al*., 2005), sugarcane molasses (Albuquerque *et al*., 2010), tomato cannery waste (Liu *et al*., 2008) and palm oil (Sudesh *et al*., 2011). The dairy industry represents a central pillar within the agri-food economies of Ireland and of multiple countries, with global milk production predicted to reach approximately 827 million tonnes by 2020 (Bojnec and Ferto, 2014; PMMI, 2014). Dairy processing activities, such as the production of milk powder, whey protein and cheese, consume a large percentage of global milk outputs and result in significant volumes of wastewater high in organic and inorganic nutrient loads (Demirel *et al*., 2005). In this study, we sought to investigate the potential for anaerobic digester effluent from an Irish dairy processing plant to act as a feedstock for sustainable PHA bioplastic production using a mixed-culture, ADF-dependent approach.

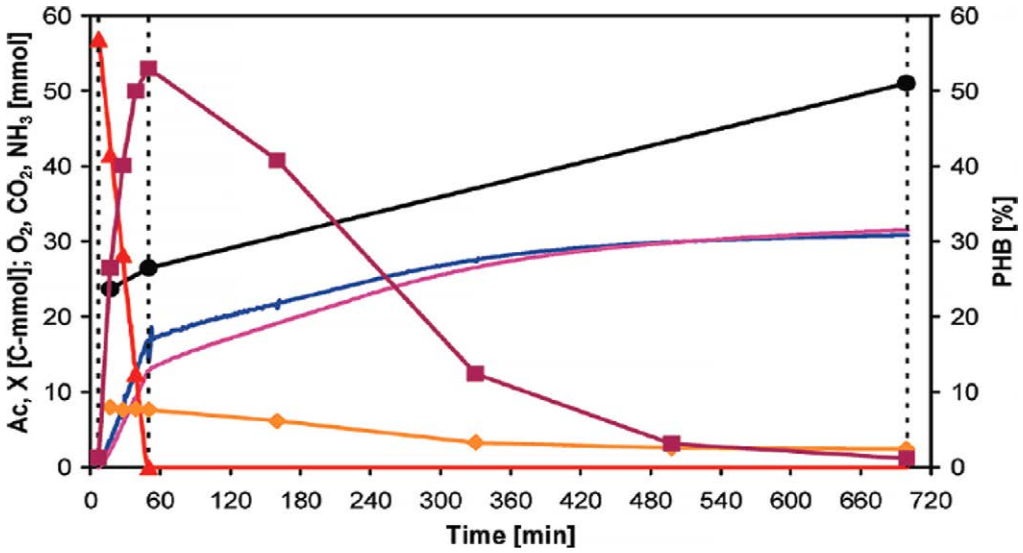

Figure 1.2. Sequencing batch reactor cycle. Red triangle, acetate [Ac (mmol)]; black circle, active biomass [X (C-mmol)]; magenta square, percentage polyhydroxybutyrate [PHB (%)]; blue line, cumulative CO_2 [CO_2 (mmol)]; pink line, oxygen uptake [O_2 (mmol)]; orange line, ammonia [NH_3 (mmol)]. Dotted lines indicate the timings of the feast (7–50 min) and famine phases (50–700 min). (Reproduced with permission from Johnson *et al.*, 2009. © 2009, American Chemical Society.)

1.4 Objectives

1.4.1 Set-up and optimisation of sequencing batch reactor systems for PHA production in mixed culture using an aerobic dynamic feeding strategy

The aim of this project was to conduct multi-reactor trials to assess the impact of operational variables, such as sludge retention time (SRT), hydraulic retention time, feed concentration, composition and dosing regimens, pH, aeration, etc., on biomass enrichment for PHA production by dairy industry-sourced, mixed-microbial cultures.

1.4.2. Application of real-time dairy processing wastewaters from varied plant process sampling points to assess system impact and model optimal aerobic dynamic feeding incorporation process

Optimised ADF parameters arising from objective 1.4.1 were re-established under real-time dairy processing effluent conditions to examine (a) the impacts of these parameters on ADF reactor enrichment for PHA accumulation and (b) PHA yields with this type of biomass in fed-batch production systems utilising real-time effluent as the sole source of carbon substrates.

1.4.3 Chemical characterisation of PHAs: polymer composition and conversion to fatty acid methyl esters

The properties, applications and economic potential of PHAs are heavily dependent on their monomer composition. Chemical characterisation of the extracted biopolymers was, therefore, proposed to determine the dominant polymer type(s) forming under the conditions imposed. In addition, objective 1.4.3 sought to determine the capacity for secondary conversion of the PHA produced to hydroxyl acyl methyl esters (HAMEs) to assess the potential for fuel additive applications.

1.4.4 Life cycle analysis of the PHA production process to assess sustainability at industrial-scale operation

In tandem with an investigation into the technical feasibility of dairy processing wastewater conversion to biopolymers, the research group sought to examine the implications of scale-up to evaluate the economic challenges of this process and the sustainability of the system in line with the optimisations determined in the technical objectives described above.

2 Materials and Methods

2.1 Aerobic Dynamic Feeding: Sequencing Batch Reactor Set-up and Operation

Following an extensive review of ADF literature, sequencing batch reactors (SBRs) with working volumes of 2.0 L were inoculated with microbial sludge from a mixed influent aeration tank at Kerry Ingredients dairy processing plant (Listowel, Co. Kerry) (Figure 2.1). Reactors were operated with an 8-hour batch cycle consisting of four sequential phases: feeding (5 minutes), aeration and mixing (420 minutes), settling (40 minutes) and effluent withdrawal (15 minutes). During the feed phase, a 300-mL volume of fresh media was supplied to reactors, with the corresponding volume removed during the effluent withdrawal phase, resulting in a hydraulic retention time (HRT) of 60 hours. Initial optimisation and characterisation of the reactor operational conditions were performed using a synthetic medium which was designed to reflect dairy wastewater carbon to nutrient ratios following an extensive review of reported nutrient profiles from a diverse array of dairy processing effluents. High organic and inorganic loads were, therefore, incorporated into the synthetic medium to assess the impact of ADF application/optimisation on the overall effluent nutrient profile. In addition, the use of synthetic media in the initial stage was designed to isolate the reactor systems from uncontrolled fluctuations in nutrient loads which frequently arise within the wastewater treatment plants during peak seasonal processing. While such fluctuations reflect the reality of on-site processing wastewater management, they obfuscate the characterisation of the impacts of the controlled system modifications under investigation (e.g. SRT, hydraulic retention time, aeration, suspended solids, etc.). System characterisation, initially based on synthetic media, allows investigators to determine a range of effective operational parameters within which the impacts of real-time wastewater can be assessed. The medium contained (per litre of distilled water) 4 g sodium acetate, 160 mg NH_4Cl, 600 mg $MgSO_4 \cdot 7H_2O$, 45 mg KH_2PO_4, 92 mg K_2HPO_4, 100 mg EDTA, 70 mg $CaCl_2 \cdot 2H_2O$ and 48 mg allylthiourea. The inclusion of allylthiourea resulted in the inhibition of nitrification, thereby preventing anoxic conditions (nitrate/nitrate formation), partially compensating for low levels of molecular oxygen. Biomass removal was conducted as required during the effluent phase to achieve relative SRTs of 28, 14, 4 and 2 days, as the trials progressed. In subsequent, real-time dairy wastewater incorporations into the SBRs, anaerobic digester effluent (20 L) was regularly collected from the Listowel plant during the peak processing period (February to October), supplemented with 4.5 mg allylthiourea per litre and stored at 4°C prior to feeding into SBRs. Gas chromatography (GC) profiling of effluent VFAs was performed by an accredited external agency (Exova, Ireland). Influent and effluent pumping was achieved using Watson Marlow 504S and 520S peristaltic pumps, respectively. Mixed liquor suspended solids (MLSS) and volatile suspended solids (VSS) were regularly determined by

Figure 2.1. Laboratory-scale sequencing batch reactors operated under aerobic dynamic feeding conditions.

standard methods (APHA, 2005). Mixing within reactors was achieved with stirring bars and VWR-Vs-C4 magnetic plates set at 180 rpm (VWR, Ireland). Oxygen was supplied at a rate of 1.5 L/min in both reactors using the combination of a Fisher pressure pump and airflow regulator valve (Fisherbrand, Ireland). pH values were not controlled but remained near neutral throughout and both reactors were maintained at room temperature of 18–20°C. Electronic sequencing of all equipment involved in reactor operation was achieved using manually programmed Termina TR601 Top2 digital timer switches (Theben, Germany).

2.2 Analytical Methods

Dissolved oxygen concentration (DO) in the reactors was monitored using a handheld Hanna HI 98186 DO probe (Hanna Instruments, UK). Reactor influent and effluent phosphorus present as PO_4 (PO_4-P), nitrogen present as NH_4 (NH_4-N), nitrogen present as NO_3 (NO_3-N) and chemical oxygen demand (COD) concentrations were determined using colorimetric assays which were quantified using a HACH Odyssey DR 2800 spectrophotometer (Hach Lange, Germany). Phosphorus present as PO_4 (PO_4-P) and NH_4-N concentrations were assayed with ammonium molybdovanadate and Nessler's reagent (Reagecon, Ireland), respectively, in accordance with the HACH DR 2800 standard protocols manual (using a wavelength of 430 nm). NO_3-N concentration was determined by the Nitratest method (Palintest, UK) of nitrate reduction to nitrite followed by the colour forming diazonium reaction (using a wavelength of 570 nm). COD was monitored by a potassium dichromate/sulfuric acid oxidation reaction (0–1500 mg/L vials, Reagecon) which was incubated for 2 hours at 150°C in a HACH COD reactor followed by spectrophotometric quantification. Organic acid concentrations were determined by high-performance liquid chromatography (HPLC) using an Agilent 1200 HPLC system with a refractive index detector and a REZEX 8 µL ROA-organic acid H^+ (8%) (300 × 7.8 mM) column (Phenomenex, USA) with a 0.01 N H_2SO_4 as the elution fluid, at a flow rate of 0.6 mL/min. The temperature of the column was maintained at 65°C.

2.3 Microscopic Analyses

Microscopic analyses of ADF sludge were conducted on a daily basis by phase contrast and fixed culture staining to monitor the sludge microflora for undesirable proliferations of organisms capable of destabilising the system (e.g. filamentous species, *Zooglea* and tetrads/cyanobacteria). Visualisation of polyhydroxyalkanoate accumulation in both reactor and fed-batch experiment cultures involved incubation of biomass samples with a 1% solution of Nile blue A lipophilic stain in dimethyl sulfoxide at 55°C for 10 minutes before transfer to a microscope slide (Ostle and Holt, 1982). Analyses of the sludge samples were performed using a Leica DM3000 epifluorescence microscope system, with an EL6000 metal halide external light source and a DFC490 8-mp, CCD digital camera (Leica Microsystems, Germany). Fluorescent visualisation was achieved with a Leica UV/violet range filter D, offering 335 nm and 455 nm excitation and emission wavelengths, respectively. Image capture and processing was performed with LAS software V3.1.0.

2.4 Quantification of Polyhydroxybutyrate Production by Crotonic Acid Conversion and High-performance Liquid Chromatography

Polyhydroxybutyrate was quantified as crotonic acid by the conversion method first described by Law and Slepecky (1961) and modified by Karr *et al*. (1983). 10 mL biomass samples were collected, centrifuged and cell pellets frozen at –80°C. As required, samples were defrosted, re-suspended in 10 mL of distilled H_2O and an appropriate volume (usually 1–2 mL depending on sample VSS) containing 2.5–6.5 mg of biomass was subjected to sequential washes with distilled H_2O containing 50% and 75% ethanol and 98% undiluted ethanol. Samples were then centrifuged for 10 minutes at 6000 rpm, the supernatants removed and any residual ethanol allowed to evaporate at room temperature in a sterile laminar flow hood. Once residual ethanol had fully evaporated, the samples were digested in 1 mL concentrated sulfuric acid at 100°C for 30 min. Cooled samples were then diluted at a 5:1 ratio with 0.014 N sulfuric acid, filtered and analysed using an Agilent 1200 HPLC system with a refractive index detector, a REZEX 8 µL ROA-organic acid H^+ (8%) (300 mm × 7.8 mm) column (Phenomenex, USA) maintained at 65°C with 0.01N H_2SO_4 as eluent at 0.6 mL/min. Crotonic acid standards of 0.5 mM, 1 mM, 2.5 mM, 5 mM, 10 mM, 20 mM, and 40 mM were used as controls and absorbance was read at 210 nm. Results were expressed as w/w percentage PHB from the

active biomass (g PHB/g VSS). In order to assess PHB conversion rates to crotonic acid, samples of 500 mg pure PHB (Goodfellow, UK) were analysed using the same analysis protocol. A conversion efficiency of 72% was calculated and applied in PHB determinations from active biomass.

2.5 Fed-batch PHB Accumulation

Pulsed fed-batch trials were performed in triplicate with aerobic sludge acclimatised under relevant ADF conditions as described. Fed-batch culture induction experiments were conducted with 100 mL volumes of biomass harvested at the end of the ADF famine cycle, which was washed and resuspended in sterile phosphate-buffered saline to a final volume of 200 mL. Relative biomass concentrations were 1.25 g/L for the 14-day SRT, 1.0 g/L for the 4-day SRT and 0.8 g/L for the 2-day SRT biomass samples. All calculations were standardised to reflect the variance in starting biomass. Synthetic media-dependent PHB accumulation trials involved an initial 10 mL pulse of feed solution containing 50 mM VFA (acetate, propionate or butyrate) and 1.4 mM NH_4Cl, as appropriate to non-limited nitrogen conditions. In the case of PHB accumulations in fed-batch trials with real-time diary wastewater (conducted with non-ADF-adapted, 4-day and 2-day SRT-adapted biomass samples), the synthetic medium was directly replaced with 10 mL anaerobic digester effluent. The fed-batch cultures were incubated at 20–25°C in an orbital shaker (Excella E25, USA) operating at 200 rpm. Feed pulses were conducted at the beginning of the experiment and repeated after the first hour and at approximately 2-hourly intervals thereafter until completion (8–24 hours), dependent on carbon consumption efficiencies. Monitoring of VSS, residual carbon, PHB, NH_4-N, PO_4-P and dissolved oxygen were conducted at multiple intervals throughout the fed-batch trials.

2.6 Polyhydroxybutyrate Conversion to Fatty Acid Methyl Esters

Polyhydroxybutyrate (5g) was subjected to chemical conversion to fatty acid methyl esters using a modified application of the method proposed by Zhang *et al.* (2009). A 100 mL volume of 50:50 (v/v) chloroform:10% sulfuric acid/90% methanol was added and the mixture refluxed at 100°C for 72 hours. Following reflux, the solution was cooled and 20 mL of saturated (26% w/v) NaCl added and the mixture stirred for 10 minutes. The water phase was discharged with three 10 mL volumes of chloroform. The combined organic phase was dried over Na_2SO_4 and evaporated under vacuum (approximately 50 Kpa) in a rotary evaporator. The resulting translucent liquor was subjected to fractional distillation with 5°C stepwise increases in temperature from 30–95°C. Collected fractions were subjected to gas chromatography VFA analysis using a Hewlett Packard HP6890 series system with a Nukol 1.2 column (30 m × 250 mm) of fused silica with a bonded polyethylene glycol phase.

2.7 Culture-Dependent Microbial Investigation of Aerobic Dynamic Feeding-adapted Sludge for PHB Production

2.7.1 Pure culture isolation

Attempts were made to isolate pure cultures from the ADF-adapted sludge biomass to assess individual PHA accumulation capacities. Sludge biomass samples (1 g each) were diluted in sterile phosphate buffer solution by a factor of 1 in 10^{-7} and 100 mL aliquots plated on solid minimal media prepared by adding 15 g agar per litre of synthetic media influent (described in section 2.1). To support growth of a wide range of heterotrophic microbes, aliquots were also spread on nutrient-rich Luria Bertani (LB) agar plates composed of 10 g peptone, 10 g NaCl, 5 g yeast extract and 15 g agar per litre distilled H_2O. All plates were incubated for 2–5 days at 28°C. Colonies for isolation were selected based on various phenotypic characteristics on solid media and pure strains isolated by standard methods for further analyses.

2.7.2 16S RNA gene-based identification of isolates

Isolated colonies were assessed using 16S rRNA gene amplification, restriction fragment length profiling and sequencing/bioinformatics analysis. Isolated colonies were cultivated overnight in 10 mL of LB at 28°C with shaking at 145 rpm. 150 mL of the culture was boiled at 100°C for 10 minutes to release DNA and 5 mL used as a DNA template for polymerase chain reaction (PCR). Each reaction also contained 200 mM dinucleotide triphosphates, 1.5 mM $MgCl_2$, 5 mL 10 × NH_4^+ buffer (50mM), 100 pM primer 27F (5′-ABA GTT TGA TCM

TGG CTC AG-3′), 100 pM primer 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′), 0.25U BioTaq DNA polymerase and molecular biology grade H_2O to a total final volume of 50 mL. Thermocycling was performed with an MJ Research PTC-200 thermal cycler, operating under the following cycle parameters: 95°C for 5 minutes, followed by 35 cycles of 95°C for 1 minute, 54°C for 45 seconds and 72°C for 1 minute. Negative controls were provided by replacing the boiled culture sample with 5 µL of molecular biology grade H_2O. The positive control used DNA from a known bacterial strain as the template. Visualisation of the amplified fragments was achieved by electrophoresis using a 1% agarose gel followed by ethidium bromide staining and UV illumination. Amplified fragments were purified from the gel matrix by excision and extraction using the GenElute Kit (Sigma-Aldrich) in accordance with the manufacturer's instructions. The amplified fragments were subjected to digestion with the restriction endonuclease *Rsa* I (which has a four-base sequence specificity) for 30 minutes at 37°C in the buffer supplied by the manufacturer, and the resulting restriction fragments visualised on ethidium bromide-stained 1.5% agarose gels. Restriction profile similarities were used to group isolates and one 16s rRNA gene dsDNA sample from each group was sent for sequencing (GATC, Germany). Desktop DNA sequence assessment was performed with EditSeq (LaserGene, DNAStar) and online database comparisons were performed using the Basic Local Alignment Search Tool (BLAST) and the GENBANK database (NIH, USA).

2.7.3 Pure culture production of PHB in synthetic media

Two pure culture isolates, representative of the species *Achromobacter* and *Defluvibacter*, were selected for PHB induction studies. Duplicate 50 mL LB cultures of each were grown to an OD_{600} of approximately 0.5, pelleted by centrifugation at 6000 rpm for 5 minutes and the supernatants discarded. Pellets were washed in 50 mL of phosphate-limited Gotz minimal medium (0.132 g $(NH_4)_2SO_4$, 0.120 g $MgSO_4$, 0.174 g K_2HPO_4, 0.136 g KH_2PO_4, 0.015 g $CaCl_2$, 0.006 g $NaCl_2$, 0.002 g Na_2MoO_4, 0.0003 g $FESO_4$ per litre of distilled water, with 1 mL of vitamin solution added post autoclaving). Pellets were isolated again by centrifugation and resuspended in either 50 mL of phosphate-limited Gotz medium or 50 mL of normal Gotz medium (1.04 g K_2HPO_4, 0.53 g KH_2PO_4) in sterile 250 mL flasks. Test carbon sources were added to a final concentration of 20 mM and the culture flasks incubated for 4 hours. Eight test conditions were examined in total for each species: phosphate-limited or phosphorous-complemented growth with acetate, butyrate, propionate and lactose. PHB accumulation was quantified in each case by crotonic acid conversion and HPLC. Residual carbon in the supernatant was also assayed along with changes in culture biomass.

2.8 Life Cycle Analysis: A Perspective on Process Scale-up

The discrepancy in technology readiness levels (TRLs) of the laboratory-scale processes under investigation (TRL 2–4) and the target, established, high-performance industrial setting (TRL 9) undermine the capacity for a direct, economically quantitative life cycle analysis (LCA) of process scale-up and integration to be carried out. Despite this challenge, it is critical that technical feasibility of the technology be complemented by an informed perspective of its strengths and weaknesses relative to industrial-scale application. The goal of such an analysis is the exposure of key issues which much be addressed for further technology development towards industrial application. To this end, an evaluation of process outputs, materials applications and market value, energy consumption per unit wastewater and infrastructure requirements at industrial scale were conducted by member of the energy group at the Environmental Research Institute and School of Civil Engineering, UCC. Plant process schematics and wastewater treatment data were gratefully received from Sean Pender of Kerry Ingredients (Listowel, Co. Kerry).

3 Results and Discussion

3.1 Sequencing Batch Reactor Set-up and Operation with 28-day and 14-day Sludge Retention Times

Previous studies on PHA production by the ADF adaptation of sludge biomass have operated at SRTs of 1–20 days (Table 3.1). As short SRTs can introduce a pressure for high sludge turnover and downstream processing in a real-time plant, the investigators sought to examine a broad range of SRTs at both ends of the scale, incorporating 28-day, 14-day, 4-day and 2-day SRTs. The initial 28-day SRT was also employed to maintain an elevated mixed liquor suspended solids (MLSS) concentration (4.5–5 g/L) and potential culture diversity for pure culture isolations. Periodic microscopic analyses of sludge flocs revealed a stable morphology and the reactor system could be operated for an extended period (up to 10 months) before deterioration of sludge settling and pin floc formation began to emerge (Figure 3.1).

Chemical profiling of SBRs during this period also reflected stability within the systems with respect to reactor biomass and the organic/inorganic nutrient profiles of the reactor effluents (Figure 3.2). In summary, initial effluent COD concentrations were in the interval 165–1200 mg/L, nitrogen (ammonia), 3–5 mg/L, orthophosphate, 63–74 mg/L and pH ranged from pH 8 to 8.5. Isolated perturbations in effluent components typically reflected mechanical issues with equipment (pump failure, timer failure etc.), which were identified during daily monitoring and addressed accordingly. Temperatures were not actively regulated, but ranged from 20–23°C throughout the operational period.

Table 3.1. Substrates and variable sludge retention time employed in mixed culture systems for PHB production

Substrate	SRT (days)	Reference
Acetate	20	Beun *et al.* (2000)
VFAs from municipal sludge fermentation	10	Mengmeng *et al.* (2009)
Acetate	1–4	Johnson *et al.* (2010)
Fermented molasses	10	Albuquerque *et al.* (2011)
Acetate and lactate	1	Jiang *et al.* (2011)
Butyric acid	1	Marang *et al.* (2013)

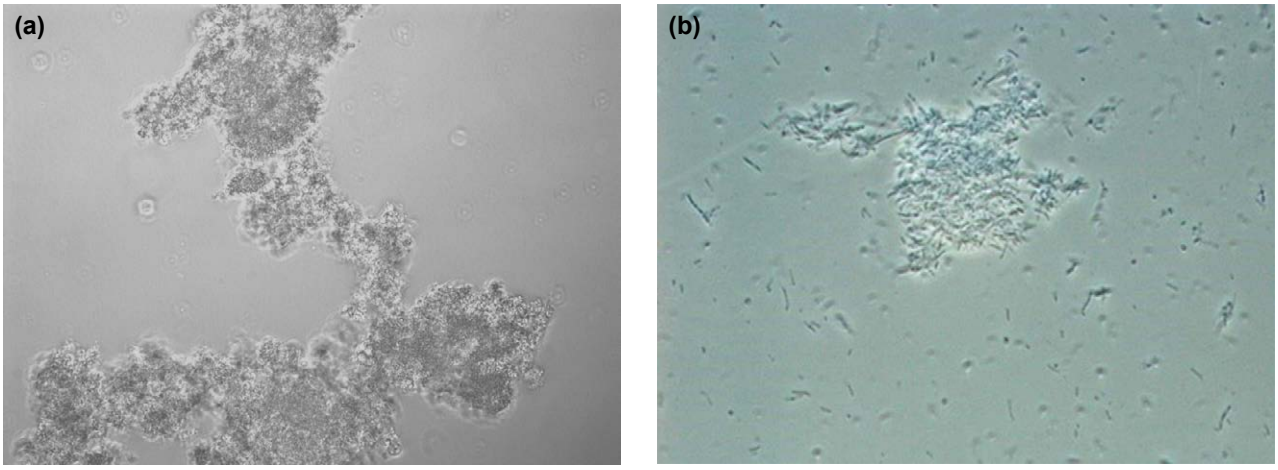

Figure 3.1. Sludge floc morphology in aerobic dynamic feeding reactors over 10 months. (a) Initial seed inoculum with compact flocs and good settling properties; (b) decreasing floc stability/pin floc formation after continuous reactor operation for 10 months, associated with poor sludge settling and wash-out.

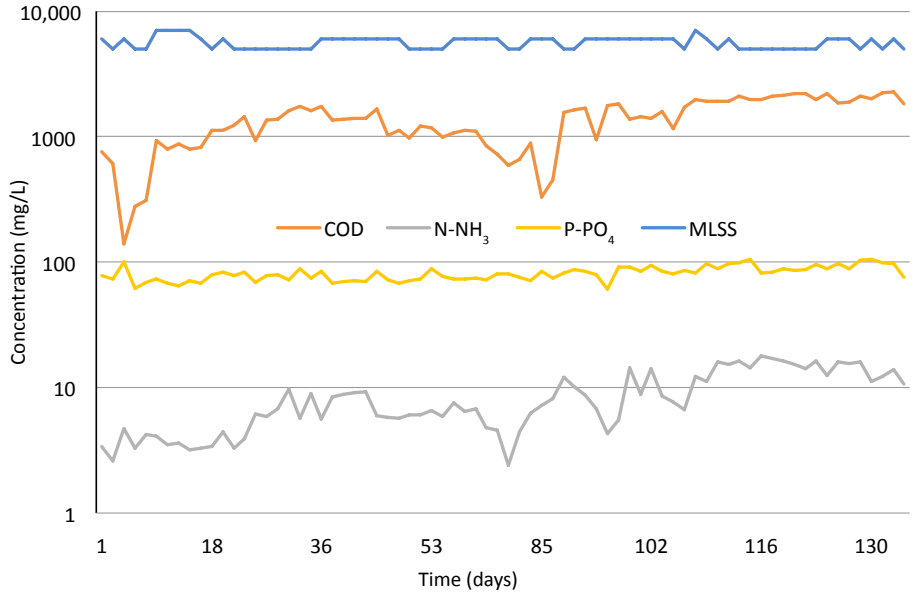

Figure 3.2. Routine chemical monitoring of mixed liquor suspended solids and effluent chemical oxygen demand, orthophosphate (PO_4) and ammonia (NH_3) components in 28-day SRT SBs (representative of 5 months of operation).

Preliminary assessment of PHA accumulation capacity within the dual reactors identified the emergence of intracellular fatty inclusions detected by Nile blue A staining and UV fluorescent microscopy after a period of 60 days of operation (Figure 3.3a,b). A key parameter for quantitative determination of PHB is active biomass concentration and VSS of 3.0–3.5 g/L were stably maintained in the 28-day SRT SBRs. HPLC analyses of crotonic acid conversion reactions with sludge biomass confirmed low-level PHB accumulations of 3–5% (g PHB/g VSS) in the biomass (Figure 3.3, panel C). Following the emergence of low-level PHA accumulation within the SBRs, the SRT of reactor 2 was reduced to 14 days, to enable comparative analyses of reactor performances and subsequent fed-batch culture PHB production. The reduction of the SRT to 14 days resulted in a corresponding decrease in stable MLSS and VSS to 3–3.5 g/L and 1.25 g/L, respectively. Such biomass reductions were in keeping with previous studies. Johnson and co-workers reported an ADF system active biomass concentration of 1.1–1.7 g/L with an SRT of 4 days (Johnson *et al*., 2010). Indeed, an SBR-ADF culturing system has been achieved with a 24-hour SRT and active biomass concentration of 0.5–0.6 g/L (Jiang *et al*., 2011). The decreased SRT/VSS conditions in reactor 2, however, did not affect sludge PHB accumulation capacity, also yielding 3–5% (g PHB/g VSS) when standardised samples were compared with reactor 1.

3.2 Batch Culture Production of PHB with Aerobic Dynamic Feeding-adapted Sludge: Impacts of Sludge Retention Time and Varying Carbon Sources

Optimisation of PHB accumulation was subsequently performed in 24-hour fed-batch culture experiments, pulse-fed with varying carbon sources (sodium acetate, butyric acid or sodium propionate) added to a final concentration of 40 mM, or lactose added to a final concentration of 20 mM. High-performance liquid chromatography analysis of PHB accumulation under these conditions revealed striking differences between the 28-day and 14-day SRT biomass accumulation capacities (Figure 3.4). Surprisingly, sodium acetate-dependent PHB yields were quite low, achieving a maximum of only 16.6% PHB (g PHB/g VSS) for reactor 2 (14-day SRT), despite adaptation on sodium acetate feed. Serafim *et al*. previously reported a maximum accumulation of 67.5% during pulse feeding of ADF-adapted sludge with 180 mM acetate in an 8-hour batch cycle (Serafim *et al*., 2004). While this group employed higher concentrations of acetate than used in our study, such levels are not a prerequisite for high PHB accumulation. Similar yields of 70% PHB have also been reported at lower feed concentrations of 30 mM acetate fed-pulses in a 4-hour batch system (Johnson *et al*., 2010). High-performance liquid chromatography

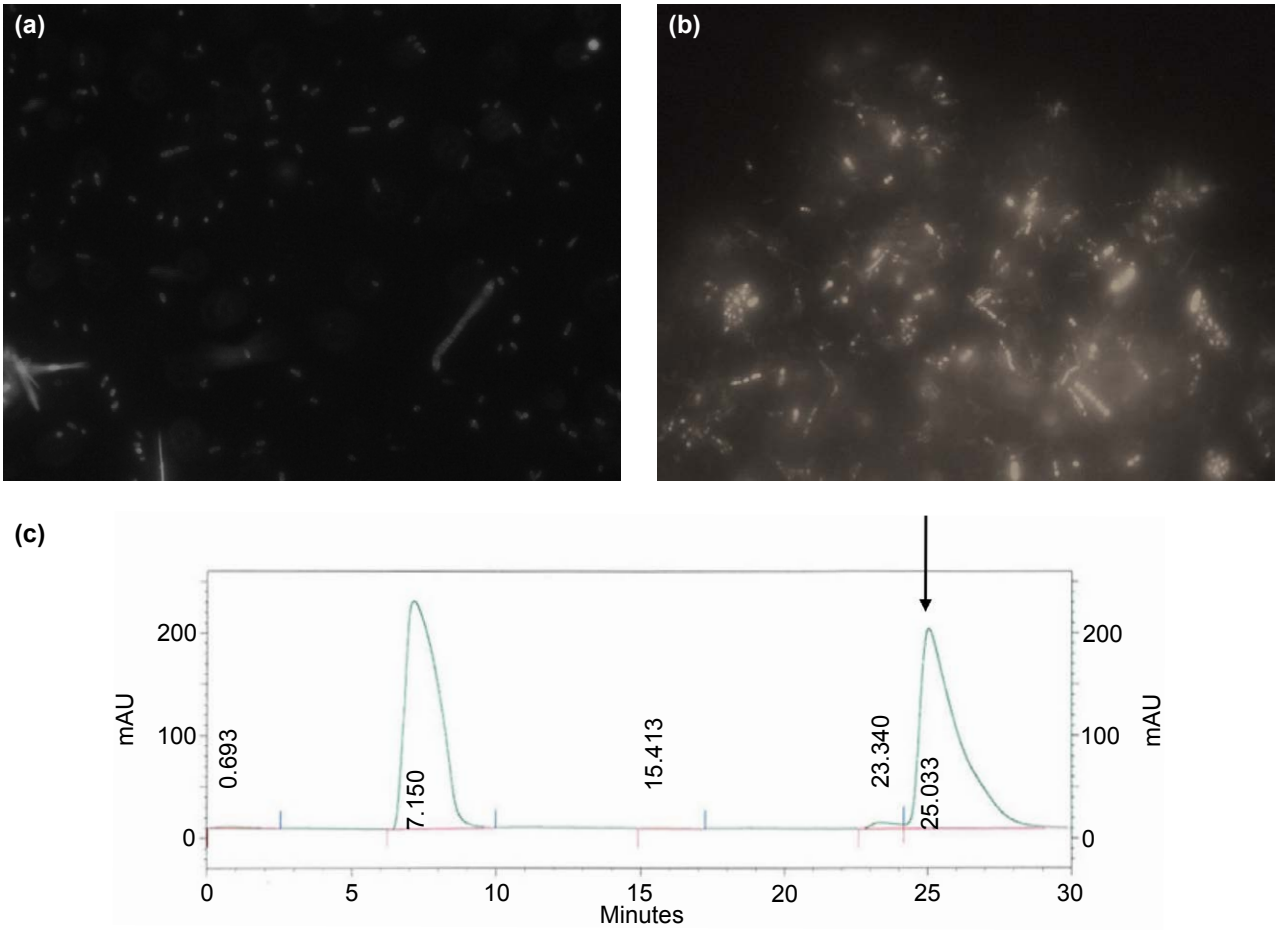

Figure 3.3. Fluorescence microscopy visualisation of PHA accumulation with Nile blue A lipophilic staining. (a) Original sludge biomass from the wastewater treatment plant; (b) aerobic dynamic feeding-adapted sludge biomass (after 60 days) (fluorescent granules are indicative of lipophilic inclusions); (c) high-performance liquid chromatography trace demonstrating a PHB-derived crotonic acid peak (indicated by the arrow) detected at 25.4 minutes.

analysis of residual acetate during the batch trial revealed a rapid consumption of the substrate in the early stages. In the absence of PHB formation, one must assume that this consumption reflects carbon utilisation rather than storage processes that are dominating the sludge physiology in response to the lengthy SRTs employed. Relative biomass development over 24 hours was determined for both sludge communities, with minimal overall VSS increases of 0.48% for reactor 1 and 1.65% for reactor 2.

The highest fed-batch PHB accumulation, 58.6% (g PHB/g VSS), was observed with 14-day SRT SBR samples pulse-fed butyric acid in contrast to acetate, where the lowest levels were seen. Maximal accumulation was observed towards the end of the 24-hour exposure, consistent with other studies that have demonstrated high polymeric intracellular accumulations following an increase in fed-batch experiment duration from 600 minutes (Marang *et al.*, 2013) up to 1400 minutes (Johnson *et al.*, 2010). Pulse feeding with propionic acid also contributed strongly to PHA accumulation in the shorter SRT biomass sample, yielding 40.3% (g PHB/g VSS), with peak accumulation occurring within a 4-hour period. In contrast, the 28-day SRT sample demonstrated poor PHB accumulation of approximately 10% in the same time period, despite notable consumption of propionate throughout the fed-batch experiment. On the basis of these findings, it is clear that SRT plays a critical role during ADF adaptation and that longer SRTs limit the adaptation response. Indeed, substrate consumption and storage rates in activated sludge are typically low at high SRTs and reflect the large percentage of inactive cells within sludge under such conditions (Dircks *et al.*, 2001). Thus, reducing the SRT is likely to streamline the relative ratio of inactive:active cellular fractions, rather than alter the

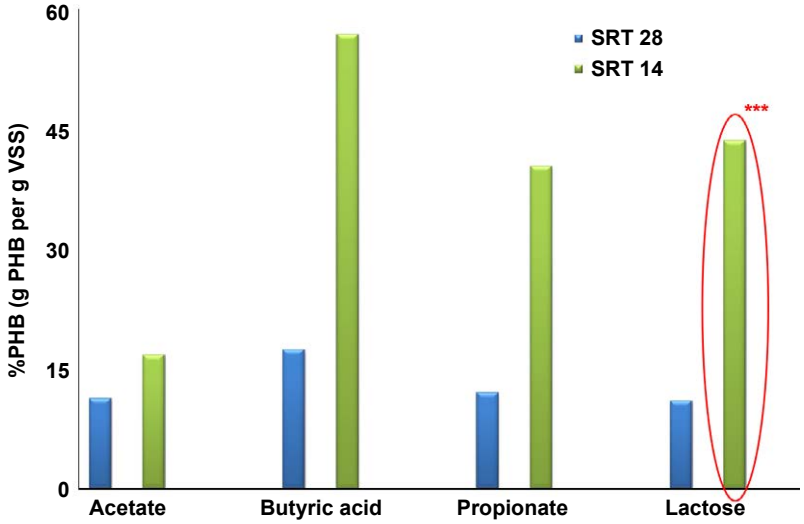

Figure 3.4. Maximal PHB yields in 24-hour batch cultures with 28-day and 14-day sludge retention time samples with respective carbon sources acetate, butyric acid, propionate and lactose. (*See text for further discussion of lactose-derived PHB finding.)**

diversity per se. Indeed, Cicek and co-workers reported sustained overall BIOLOG diversity within the microbial sludge of a membrane bioreactor despite SRT reductions from 30 days to 2 days (Cicek *et al*., 2001). ADF adaptation, therefore, is likely to have greater impacts at lower SRTs, as a larger active microbial population is exposed to its influence. In section 3.5.1, the impacts of further reductions of the ADF reactor SRTs to 4 or 2 days are discussed. An important practical consideration of this SRT fine-tuning with respect to "real world" implications is the consequence of high sludge turnover rates on infrastructure requirements and capital expenditure in any planned integration of this technology into a wastewater treatment process. These considerations are discussed in detail in section 3.6, which presents an engineering perspective on the challenges surrounding technology scale-up.

A surprising finding of our investigation was the generation of PHB following lactose pulse feeding. The inclusion of lactose in the study reflected the dairy plant origins of the sludge sample, as this disaccharide is found in milk at up to 8% w/v. In the case of sugar-enriched substrates, ADF systems reportedly require an intermediary, acidogenic fermentation step in order to cleave polysaccharides and generate VFA precursors amenable to PHA inclusion (Dias *et al*., 2006; Albuquerque *et al*., 2010). Jiang and co-workers recently reported >90% PHB accumulation in a mixed culture system fed a 1:1 mixture of lactate and acetate (Jiang *et al*., 2011). As a result, it was anticipated in our study that lactose batch experiments were unlikely to yield PHB and would, therefore, be an approximate control case for VFA batch experiments. Indeed, this was found to be the case for 28-day SRT-adapted sludge with minimal PHB accumulation detected (10.9% g PHB/g VSS). In stark contrast, however, biomass from reactor 2 (14-day SRT) was found to stably accumulate PHB up to a maximum of 43.5% within 4 hours when fed with lactose (Figure 3.4). Residual carbon levels in the 24-hour exposure did not provide a clear explanation of this phenomenon, as lactose levels appeared to persist stably in the batch media throughout. It should be noted that skimmed milk powder containing 55% lactose w/w was used to provide the disaccharide. While butyric acid can be present in milk, the residual fat component in the skimmed milk preparation used was <1% and should, therefore, be insufficient to support PHB accumulation to the levels observed. The second major component of skimmed milk is protein (approximately 40%), which is of benefit only in the provision of a source of nitrogen in PHB synthesis systems. Previous studies on the use of lactose as a carbon source for PHAs have focused on hydrolysed/non-hydrolysed whey supplemented with valeric acid, supplied to pure cultures or their genetically engineered derivatives (Koller *et al*., 2007; Pavolo *et al*., 2013). Under optimised conditions, these studies reported co-polymer accumulations of only 12–40%. In order to establish that lactose was not a source of PHB, fed-batch trials were repeated in which skimmed milk was replaced with a 100 mM solution prepared with anhydrous lactose. Batch cultures pulse-fed with this feed stock did not subsequently accumulate PHB

to any significant degree (approximately 3%), indicating that earlier observations reflect PHB accumulation from unknown constituents within the skimmed milk. The authors highlight this finding in order to outline the potential misleading outputs arising from skimmed milk powder use as a preparatory component for synthetic dairy wastewater in PHB studies. It was not determined whether the 3% accumulation arose from an endogenous substrate within the biomass inoculum; however, any such contribution would be deemed minimal.

3.3 Culture-dependent Investigation of Sludge Isolates for PHB Synthesis

Various solid media were employed to isolate pure culture representatives from within the 14-day SRT reactor biomass in an attempt to characterise individual key contributors to PHB accumulation. A total of 24 phenotypically distinct pure cultures were collected from the LB, E2 acetate and E2 skimmed milk media (Figure 3.5a). In order to reduce the potential for species degeneracy within the isolates, all 24 were subjected to molecular characterisation by 16S rRNA gene amplification and grouped by restriction fragment length polymorphism analysis (Figure 3.5a).

Through this process, the original 24 isolates were reduced to a core of seven potentially distinct species and representative 16S gene amplicons were subjected to Sanger sequencing. Bioinformatic analyses of the sequences returned identified the reactor isolates as species of *Rhizobiales, Achromobacter, Defluvibacter, Kerstersia* and *Xanthobacter*. All of the species identified were found to have been previously reported in the literature in association with PHA accumulation (Mergaert *et al*., 2001; Ratcliff *et al*., 2008; Rebah *et al*., 2009). A degenerate PCR approach was used to detect the presence of PHB synthase genes within these species to assess PHB accumulation potential. *phbC* genes could not be amplified from genomic DNA isolated from *Xanthobacter* and *Kerstersia Gyiorum*. Furthermore, growth of these cultures was very poor in E2 minimal media, Gotz minimal media, LB and/or nutrient broth. The *Achromobacter* and *Defluvibacter* isolates were found to contain *phbC* homologues and grew well in the media above, enabling induction studies into PHA accumulation. Phosphate-limited Gotz medium was used to induce PHB accumulation and contained respective carbon sources tested previously (acetate, butyrate, propionate, lactose). 19% w/w maximal PHB accumulation was achieved in the *Defluvibacter* isolate when propionate was the carbon source. The *Achromobacter* isolate was found to be less productive under the test conditions employed, achieving a maximal accumulation of 5.8% g PHA/g active biomass in response to acetate feeding under phosphate-limited conditions. While a degree of optimisation is likely to be achievable in additional iterations of this work, direct comparison with the mixed culture yields revealed the ADF-adapted sludge to be a far superior vehicle for PHB accumulation. While comprehensive molecular profiling of the microbial ecology of the mixed cultures did not represent a key objective of the project, such work should be considered in future iterations using next generation sequencing approaches. Determination of the key microbial species underpinning PHB production from wastestreams can offer significant benefits in terms of reactor performance and stability monitoring, particularly in conjuction with real-time treatment systems. Such data is likely to prove fundamental in any future promotion of PHB production at an industry level, based on documentation of the ecological robustness and metabolic versatility of the PHB synthesis population.

3.4 PHB Conversion to Fatty Acyl Methyl Esters

Figure 3.6 below presents a summary of the process for PHB conversion to fatty acyl methyl esters. It became clear early in the process that the chemical conditions necessary for stepwise fatty acid methyl ester conversion and recovery demanded high energy inputs which would undermine the sustainability of such downstream modification of wastewater-derived PHB.

The final step in the process, fractional distillation of the ester-like concentrate, yielded three separate fractions at 61°C, 68°C and 75°C, respectively. Gas chromatography analysis of these fractions revealed that all three contained hydroxybutyl-methyl ester (HBME)-like compounds ($C_5H_9O_3$) in a 1:0.7:0.5 ratio of concentrations. However, the 61°C sample combined the highest concentration HBME-like compounds with a low level of co-purified contaminants compared with the other two fractions (Figure 3.7). With respect to conversion efficiency, approximately 28% PHB to HMBE was achieved under the conditions employed.

In summary, it would appear that the feasibility of producing HBME from PHB in a wastewater context is

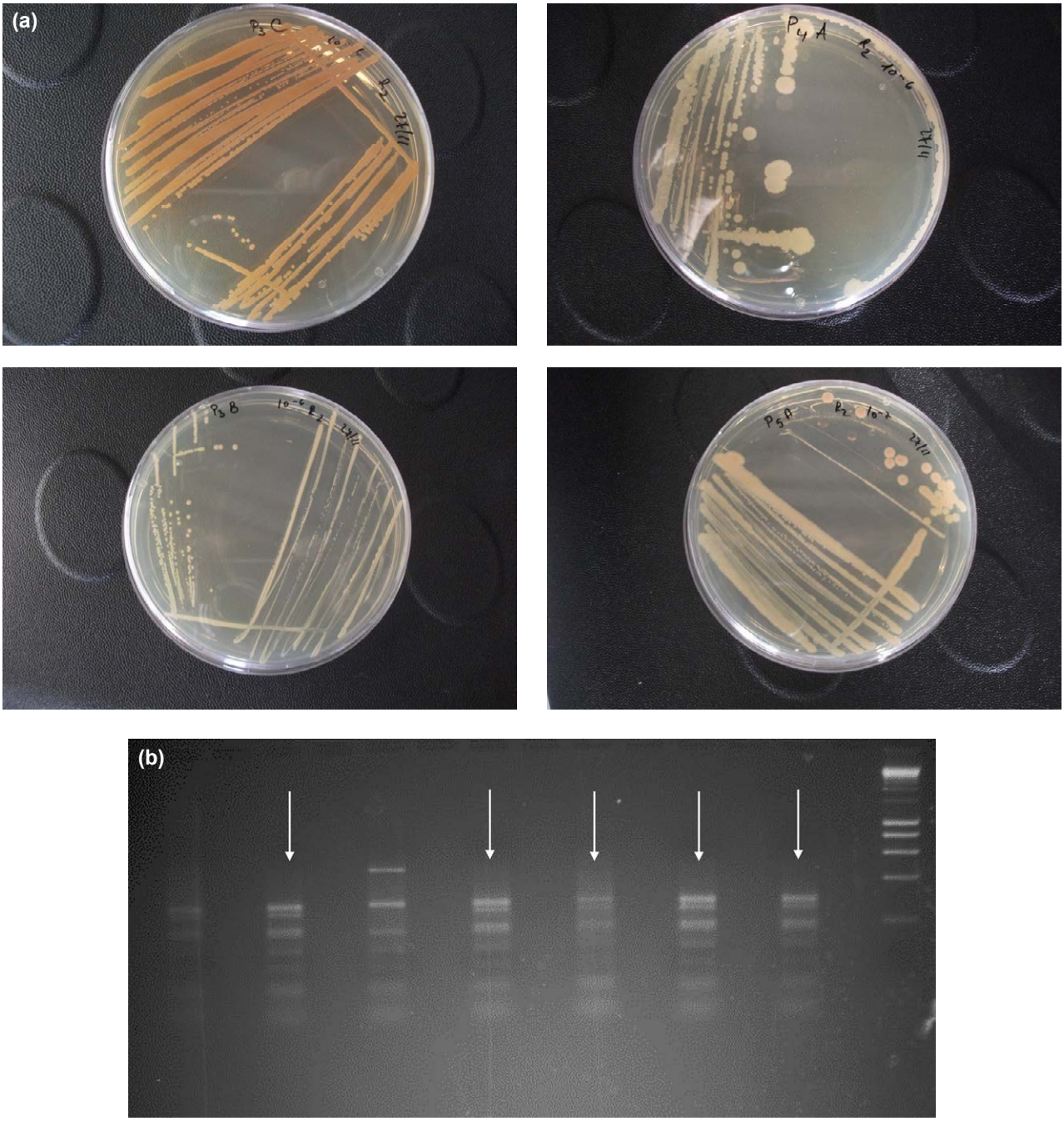

Figure 3.5. Pure culture isolation from aerobic dynamic feeding-adapted sludge. (a) Representative, phenotypically distinct pure culture isolates on skimmed milk minimal media; (b) Restriction fragment length profiles of culture isolates demonstrating species diversity. White arrows indicate samples with similar RFLP patterns, indicating that the same species is present. Lane numbers indicate sample number; M indicates molecular weight markers (1 kb DNA ladder, Biolabs).

not supported given the low conversion efficiency and high energy consumption profile presented here. This perspective is reinforced when one examines the likely downstream biofuel applications for HBME as a fuel conditioner to displace ethanol. Ethanol is problematic in that its hygroscopic characteristics have impacts on transport and use. However, HBME combustion yields approximately 50% of the energy of gasoline (approximately 21 MJ/kg compared with 40 MJ/kg). Therefore, conditioning gasoline with HBME, even at moderate levels of 10–15% would result in a significant decrease in the fuel energy output. Wang and co-workers compared MJ/kg yields of gasoline blends (5, 10, 15 and 20%) with ethanol and HBME and reported that, at

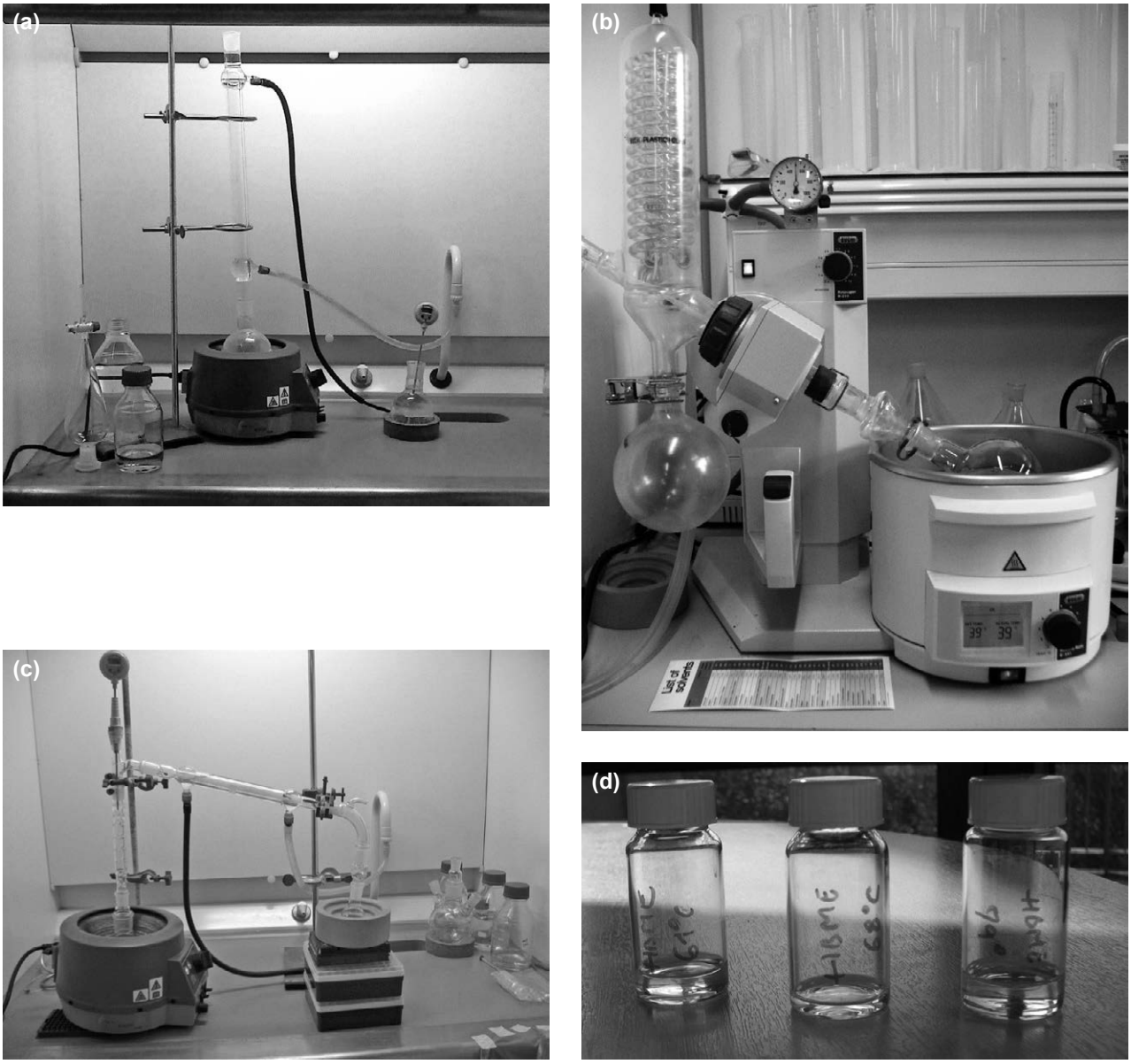

Figure 3.6. PHB chemical conversion to fatty acid methyl esters and recovery. (a) 72-hour reflux of PHB at 60°C; (b) 24-hour vacuum rotary evaporation to concentrate esters; (c) fractional distillation of ester components at 35–95°C, 8–10 hours; (d) fractions collected at 61°C, 68°C and 75°C.

each ratio tested, HBME yielded approximately 2 MJ/kg less than the ethanol equivalent (Wang *et al.*, 2010). In order to offset this energy deficit, one could argue the "greener credentials" of PHB production from waste-water feedstock. However, the present study suggests that mixed-culture provision of PHB as an intermediate for HBME synthesis currently faces several technical barriers which must first be resolved. These include (1) maximising biomass PHB yields (> 90%), (2) low-energy PHB recovery strategies from sludge and (3) low-energy conversion of PHB to HBME. It is envisaged that ongoing research endeavours in this field will continue to bridge these gaps between feasibility and practicality as new technological advances become available.

Gas chromatography analyses of samples derived from cultures with high propionate feed ratios also showed low levels of methyl ester derivatives of 3-hydroxyvalerate (3HV). While the trace levels detected were insufficient for commercial promotion, a clear potential exists for further optimisation to achieve production of the industrially significant co-polymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)]. Characterisation of mixed-culture PHA properties by Lemos and co-workers previously

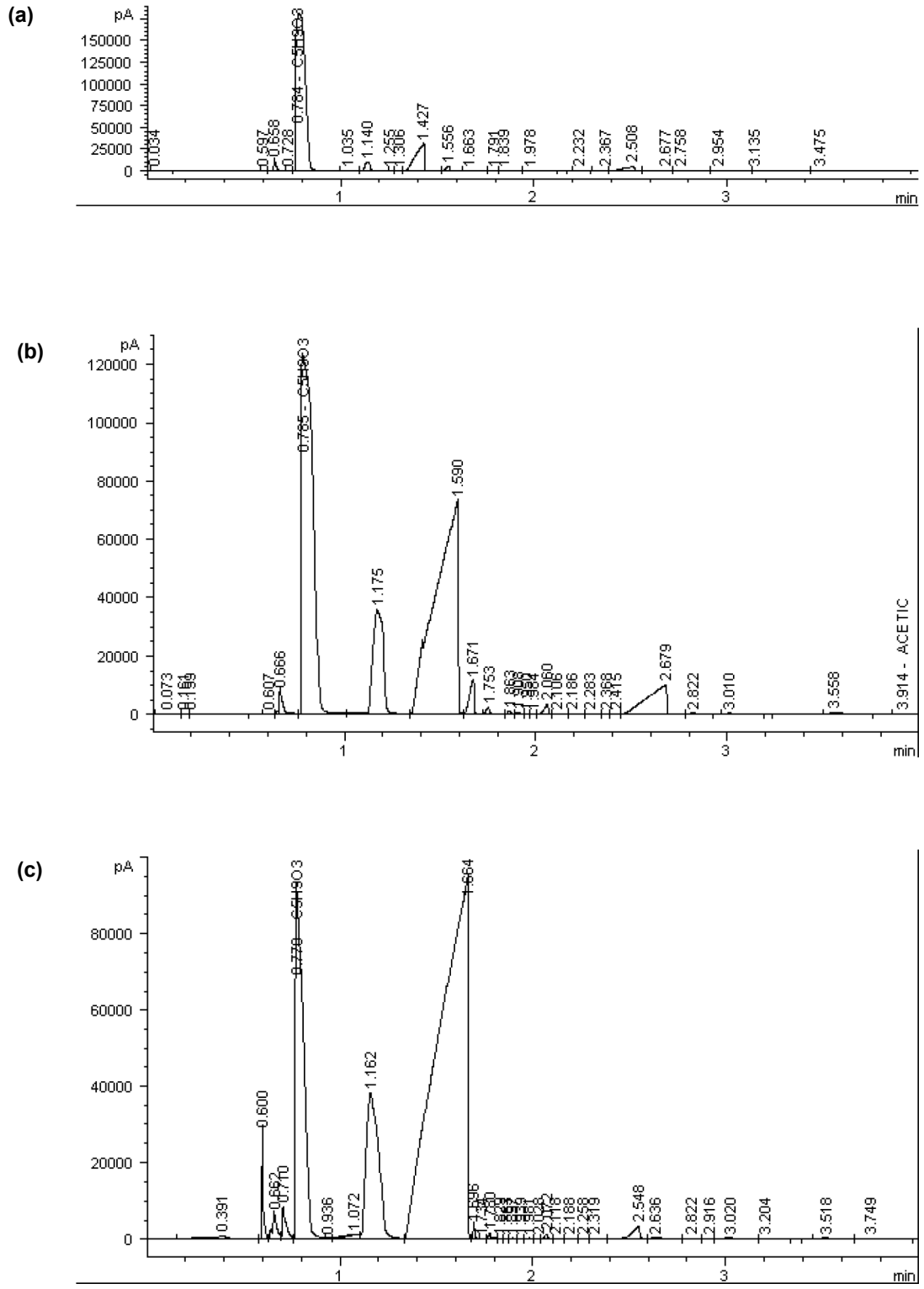

Figure 3.7. Gas chromatography analysis of distilled fractions at (a) 61°C, (b) 68°C and (c) 75°C.

identified the production of P(3HB-co-3HV), where the HV content was determined by the feed composition (Lemos *et al*., 1998). It is possible, therefore, that the low HV content detected in our study simply reflects a compositional limitation imposed by the wastestream tested. There is clearly scope for further investigation of this aspect of the study to fully determine the commercial potential for P(3HB-co-3HV) production from dairy processing effluents. The capacity to analyse P(3HB-co-3HV) production directly in mixed and pure culture systems is also of industrial significance in terms of future technology selection/development. Gas

chromatography–mass spectrometry (GC-MS) analysis did not identify hydroxyhexanoate C6 monomers despite the capacity within the test parameters to detect heptanoic methyl derivatives had they been present. On the basis that there was minimal hydroxyvalerate (C5) synthesis and no detectable hydroxyhexanoate (C6) formation, the authors did not extend the run further to test for longer mcl monomers (C_{6-14}). The authors noted that synthesis of mcl monomers is theoretically not beyond the bounds of the system, in terms of either the likely metabolic capability of a mixed sludge or the potential availability of suitable substrates in dairy effluent feeds. Yang and co-workers previously reported the synthesis of hydroxydodecanoate (HDD) monomers in tandem with butyrate and valerate using municipal sludge subjected to ADF adaptation (Yang *et al*., 2013). Notably, however, the percentage mol of HDD was 3.42%, compared with 62.41% HB and 34.17% HV. In addition, at the gene level, the authors reported cloning of 80 PHB synthase genes from the system, 95% of which were found to be related to short chain length (C_3–C_5) synthases. While further studies would be required to establish the relevance of this phenomenon across diverse ADF settings, the work of Yang *et al*. suggests mcl PHA synthesis may represent a minor output of such systems (Yang *et al*., 2013).

3.5 Investigation of Real-time Dairy Processing Wastewater Incorporation into Aerobic Dynamic Feeding Reactors for PHB Enrichment and Production

3.5.1 Reactor set-up, 4-day and 2-day sludge retention times and gas chromatography profiling of anaerobic digestion effluent

In the latter stages of the laboratory-scale investigation, the original SBRs were decommissioned and two new reactors set-up with fresh sludge samples from the dairy processing plant taken at the beginning of the peak dairy production season (February to October). The SBRs were operated for several months under ADF conditions, with real-time, anaerobic digester effluent from the dairy wastewater processing plant acting as feedstock. Acetic, propionic and butyric acids are typically generated by acidogenic fermentation processes, such as an anaerobic digestion (AD) step within wastewater treatment regimes. Anaerobic digestion effluent from the dairy plant was, therefore, deemed to be an ideal feedstock for the real-time wastewater trials. Gas chromatographic VFA profiling of the AD effluent at the dairy wastewater treatment plant revealed that > 90% of VFAs detected were attributable to acetate (approximately 16 Cmmol/L), propionate (approximately 8 Cmmol/L) and butyrate (approximately 3 Cmmol/L) during the sampling period (Figure 3.8). It should be noted that the degree of acidification was not determined for the AD effluent. The potential for non-VFA component promotion of competitive growth by non-PHB accumulating populations must, therefore, be acknowledged as a potential limiting factor in maximising PHB yields from the system. The reactors were operated with 2-day and 4-day SRTs which resulted in VSS becoming stabilised at 0.80–0.90 g/L in both cases. The active biomass levels were in keeping with previous studies by Johnson and co-workers, who reported VSS concentrations of 1.1 g/L under similar operational conditions (Johnson *et al*., 2010). Routine, qualitative Nile blue A lipophilic staining and fluorescence microscopy revealed the emergence of enhanced polyester-accumulation capacities within the adapted biomass following approximately 20 days of acclimatisation under ADF conditions. Biomass PHB extractions, crotonic acid conversion and HPLC analyses confirmed low levels of PHB accumulation, 2–5% g PHB/g VSS, for both reactors. Residual carbon profiling revealed the rapid consumption of VFAs within the first hour of each cycle, suggesting a 1:7 feast to famine ratio over the course of each 8-hour cycle.

3.5.2 Fed-batch culture PHB accumulation with synthetic media and acetate as the sole carbon source

Initial fed-batch trials with the AD effluent-adapted sludge utilised synthetic media as previously described (section 3.2) with acetate as the sole carbon source. Relatively low biomass PHB yields of approximately 13% (g PHB/g VSS) were observed (Figure 3.9 and Table 3.2) with only a three-fold difference observed between non-ADF-adapted sludge and the 2-day or 4-day SRT ADF-adapted samples. The low polymer yields are similar in nature to the 28-day and 14-day SRT sample trial, where synthetic wastewater with acetate had been used both as the SBR influent and the batch culture media. Therefore, the poor performance with acetate as a substrate does not appear to be dependent on the ADF adaptation media, or the length of SRT in the adaptation phase. As Figure 3.9 demonstrates, no significant difference was evident in

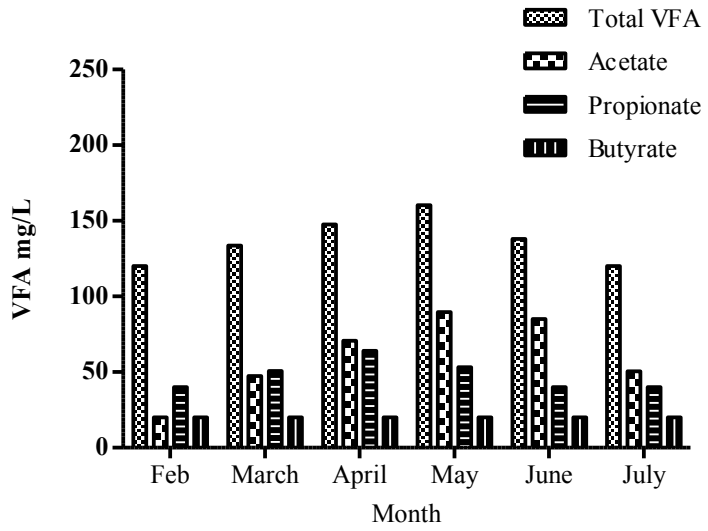

Figure 3.8. Representative gas chromatographic profiles of the anaerobic digester effluent VFAs during peak dairy processing season.

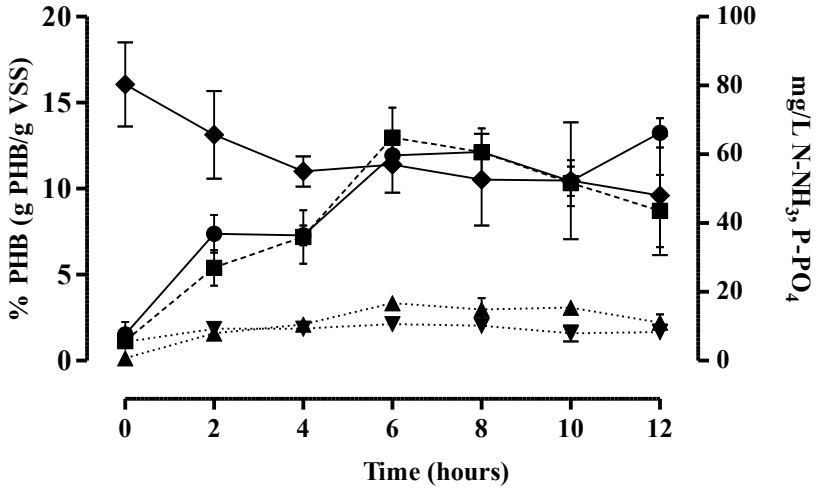

Figure 3.9. PHB accumulation in acetate pulse-fed, batch trials. Non-adapted industrial sludge biomass adapted under aerobic dynamic feeding conditions with real-time anaerobic digester effluents (▲), and 4-day (●) and 2-day (■) bioreactor SRTs. NH_3-N, (▼) and PO_4-P (♦) levels were also monitored. Maximum (average) polyhydroxybutyrate accumulation is outlined for each biomass sample in Table 3.2.

Table 3.2. Maximum (average) polyhydroxybutyrate accumulation in acetate pulse-fed, batch trials

Biomass sample	Non-adapted	2-day SRT	4-day SRT
Maximum PHB yield	3.34 ± 0.16	12.97 ± 1.75	13.26 ± 0.86

the 2-day and 4-day SRT reactor samples in terms of either the maximum yield, or the time-dependent profile of PHB accumulation. Relative rates of change of other monitored characteristics (phosphate, nitrogen, VSS levels and residual acetate) were also found to be comparable. Johnson and co-workers have previously reported the impact of C:N ratios on fed-batch PHB accumulation, indicating that excess nitrogen (< 8 Cmol/Nmol) resulted in reduced PHB accumulations, which peaked within 4–5 hours in fed-batch systems (Johnson *et al*., 2010). However, C:N ratios in our experiments were consistently > 20 Cmol/Nmol. It remains unclear at present whether this outcome is due to a specific lack of capacity following adaptation, or if it is a result of some, as yet unidentified, source of inhibition under the test conditions.

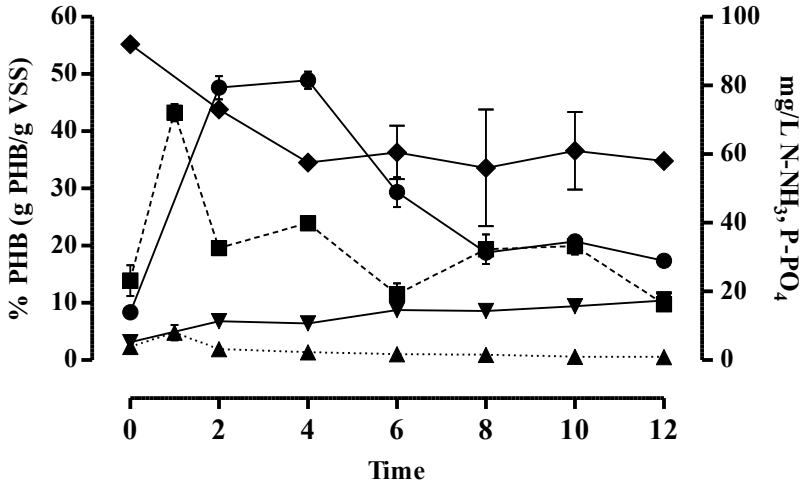

Figure 3.10. PHB accumulation with dairy wastewater anaerobic digester effluent. Non-adapted industrial sludge biomass adapted under ADF conditions with real-time anaerobic digester effluents (▲), and 4-day (●) or 2-day (■) bioreactor SRTs. NH_3-N, (▼) and PO_4-P (♦) levels were also monitored. Maximum (average) polyhydroxybutyrate accumulation is outlined for each biomass sample in Table 3.3.

Table 3.3. Maximum (average) polyhydroxybutyrate accumulation using dairy wastewater anaerobic digester effluent

Biomass sample	Non-adapted	2-day SRT	4-day SRT
Maximum PHB yield	4.83 ± 1.34	43.25 ± 1.49	48.28 ± 1.64

3.5.3 Fed-batch culture PHB accumulation with anaerobic digestion effluent as sole media

Figure 3.10 and Table 3.3 present the impact of anaerobic digester effluent incorporation into the fed-batch trials. PHB accumulation was not significant in non-ADF-adapted biomass subjected to AD effluent pulsed-feeding over a 12-hour period, with 4.83 ± 1.34% g PHB/g VSS representing the maximal yield. However, 10-fold higher PHB accumulations of approximately 45% g PHB/g VSS were achieved with both the 2-day and 4-day SRT-adapted sludge samples. A recent pilot-scale 3-step continuous ADF system (acidogenic fermentor, ADF SBR for PHB synthesis and separate biological treatment reactor) similarly reported approximately 43% PHB accumulation from ice-cream processing wastewater carrying a moderate overall organic load of 1320 mg/L COD (Chakravarty *et al*., 2010). The differing SRT lengths did not appear to contribute to variances in overall PHB yields; however, peak accumulation in biomass adapted under the shorter 2-day SRT occurred within the first 1–2 hours of operation and decreased rapidly thereafter. It has previously been reported that shorter SRTs are capable of selecting for younger populations with rapid metabolic turnover rates, which is consistent with our observations (Johnson *et al*., 2010). In contrast, the 4-day SRT biomass sample fed with the same wastewater samples demonstrated peak, stable accumulation over the 2- to 4-hour period of growth, which may offer greater stability in terms of downstream processing opportunities for PHB recovery. The relative rates of change among other profiled characteristics [VSS (approximately 0.066 mg/hour), phosphate and ammonia] were found to be comparable throughout the 12-hour fed-batch experiments, irrespective of SRT length.

The maximum yield of approximately 50% g PHB/g VSS achieved with unmodified AD effluent provides a strong platform for future development; however, several challenges exist. As highlighted in Table 3.4, dairy processing wastewaters can vary greatly in organic and inorganic nutrient loads and resulting C:N ratios. Previous studies on ADF have demonstrated the importance of such nutrient concentrations/ratios in the optimisation of the SBR adaptation of biomass to achieve high species densities of PHA accumulators (Dias *et al*., 2006; Serafim *et al*., 2008).

Table 3.4. Organic and inorganic characteristics of various dairy processing wastewater streams

Origin	COD (mg/L)	Fats (mg/L)	TKN (mg/L)	P_t (mg/L)	pH	TSS (mg/L)	VSS (mg/L)	C:N	Reference
DP	4000	400	55	45	8–11	675	635	73	Kasapgil *et al*. (1994)
DP	4000	–	200	60	5–9	–	500	20	van den Berg (1984)
DP	2926	294	36	21	6.7	–	–	81	Gutierrez (1991)
Whey	6600	–	650	650	4–6	–	2000	10	van den Berg (1984)
DP	2125		70	100	9.8	280	250	30	Monroy *et al*. (1985)
DP	4500	350	60	50	–	800	–	75	Craggs *et al*. (2000)
DP	1750	–	75	9.1	–	400	355	23	Koyuncu *et al*. (2000)
CP	4430	754	18	14	7.32	1100	–	246	Koyuncu *et al*. (2000)
YB	1500	–	63	7.2	–	191	–	24	Koyuncu *et al*. (2000)
DP	1150–9200	–	14–272	8–68	6–11	340–1730	255–830	33–82	Demirel *et al*. (2005)
Whey	68,814		1462	378	–	–	–	47	Demirel *et al*. (2005)
DP	3380	260	51	22	7.9	830	750	66	Latif *et al*. (2011)

DP, dairy processing; CP, cheese production; YB, yoghurt and buttermilk processing; TKN, total Kjeldahl nitrogen; P_t, total phosphorus; TSS, total suspended solids; VSS, volatile suspended solids.

In large-scale dairy facilities with convergent waste streams from multiple processes, it may prove difficult to achieve consistent, ideal nutrient loads for optimal ADF operation. Indeed, GC profiling of VFAs in the anaerobic digester, as presented in Figure 3.8, revealed considerable variation in acetic and propionic acid concentrations during the sampling period. It may well be the case that the maximal yield of 45% PHB did not reflect the actual maximal capacity of the adapted sludge, with a limitation in the availability of suitable VFAs impeding greater yield. Further investigations with wastewaters from other facilities would be required to arrive at a conclusive position on this point.

3.6 Life Cycle Analysis: A Perspective on Process Scale-up

3.6.1 Complexity of study

Life cycle analysis is an evolving instrument within the dairy sector at present, which is striving towards a position of standardisation for assessing the sustainability of products and processing. Multiple challenges exist in relation to such standardisation when the goal is a comprehensive “cradle-to-grave” overview of any product’s overall sustainability criteria (IDF, 2010). In such scenarios, account must be taken of the whole chain, from farming practices right through to consumer use of products and endpoint disposal routes. At each stage in the chain, data may be gathered regarding a multitude of potential impacts, including energy consumption, toxicity, water stress, land use, climate change etc. (Flysjö, 2011; Vergé *et al*., 2013). Finally, the modelling software (e.g. GaBi, SimaPro, OpenLCA) and the strategies employed in its use must ensure that data processing combines maximal sensitivity with minimal uncertainty (Broekema and Kramer, 2014; Aguirre-Villegas *et al*., 2015). As a result, many analyses are unable to facilitate cradle-to-grave assessments and tend to focus on singular impacts arising within truncated aspects of the whole chain, for example “cradle-to-gate greenhouse gas (GHG) emissions” or “gate-to-grave carbon footprint” analyses (UN FAO, 2010). At present, the Irish dairy processing sector is largely uncharted with regard to life cycle analyses, although several national research groups are collaboratively engaged with industry stakeholders in addressing elements of this field (http://www.dairywater.ie). Against this backdrop, it is impossible to evaluate the full extent of the mitigation of environmental impact from the redirection of wastewater nutrient loads to bioplastics production described in this project. As a result, our assessment is necessarily couched within the scope of dairy processing wastewater remediation for which energy balance data could be extrapolated.

The process of treating wastewater to a quality standard suitable for discharge to local water systems can vary substantially. Using LCA, the energy or economic performance of such processes can be evaluated. LCA methodology combines the relative inputs and outputs of the system and can potentially identify areas where method improvements or new technologies

may be employed to support a more cost-effective or energy-efficient process. Efficiency in wastewater treatment is evaluated in terms of the specific water quality. This is typically analysed through the reduction of biochemical oxygen demand (BOD). Different stages of the treatment process will remove certain fractions of BOD. The treatment process should be designed so that the removal of BOD is achieved with minimal energy use and with least cost but is still to the required quality standard. The treatment system should also minimise the production of sludge, as this can be a particular environmental burden and financially expensive to treat.

A schematic diagram of the wastewater treatment system related to the study in question is shown in Figure 3.11. The wastewater from the dairy plant enters through a dissolved air floatation (DAF) system, and then undergoes an AD process. The effluent then typically undergoes further treatment in a biotower, followed by a sequence of anoxic and oxic zones, and the process finishes with a clarifier treatment. Each step of the wastewater treatment process is designed to eliminate a fraction of the total BOD. For bioplastics (polyhydroxybutyrate or PHB) production, an extra process step would be added to the treatment system following AD and prior to the biotower stage, as shown in Box 1 in Figure 3.11. The PHB production process requires VFAs from the effluent of the digester. The effective BOD reduction from the production of bioplastics must be accounted for within the overall wastewater treatment process, as it will have a knock-on effect on the existing downstream processes.

Undertaking an LCA from the outset of this scenario is highly complex; this is due to the wastewater treatment process and PHB production process studied having a huge difference in scale. The production of PHB, as indicated in a laboratory setting, must be integrated with a fully developed dairy wastewater treatment facility. This identifies a vast difference in TRLs. The production of bioplastics, in this case, is at a research stage, to prove feasibility and is thus at TRL2-TRL4, while the treatment of wastewater is a fully developed operation (TRL9). A graphic representation of the TRL scale is shown in Figure 3.12. It is, therefore, extremely difficult to generate meaningful comparisons from an economic LCA perspective.

The specifications of the original wastewater treatment process would need to be studied in great detail to carry out an effective LCA. This would include all the input and output parameters identified in Figure 3.11. A

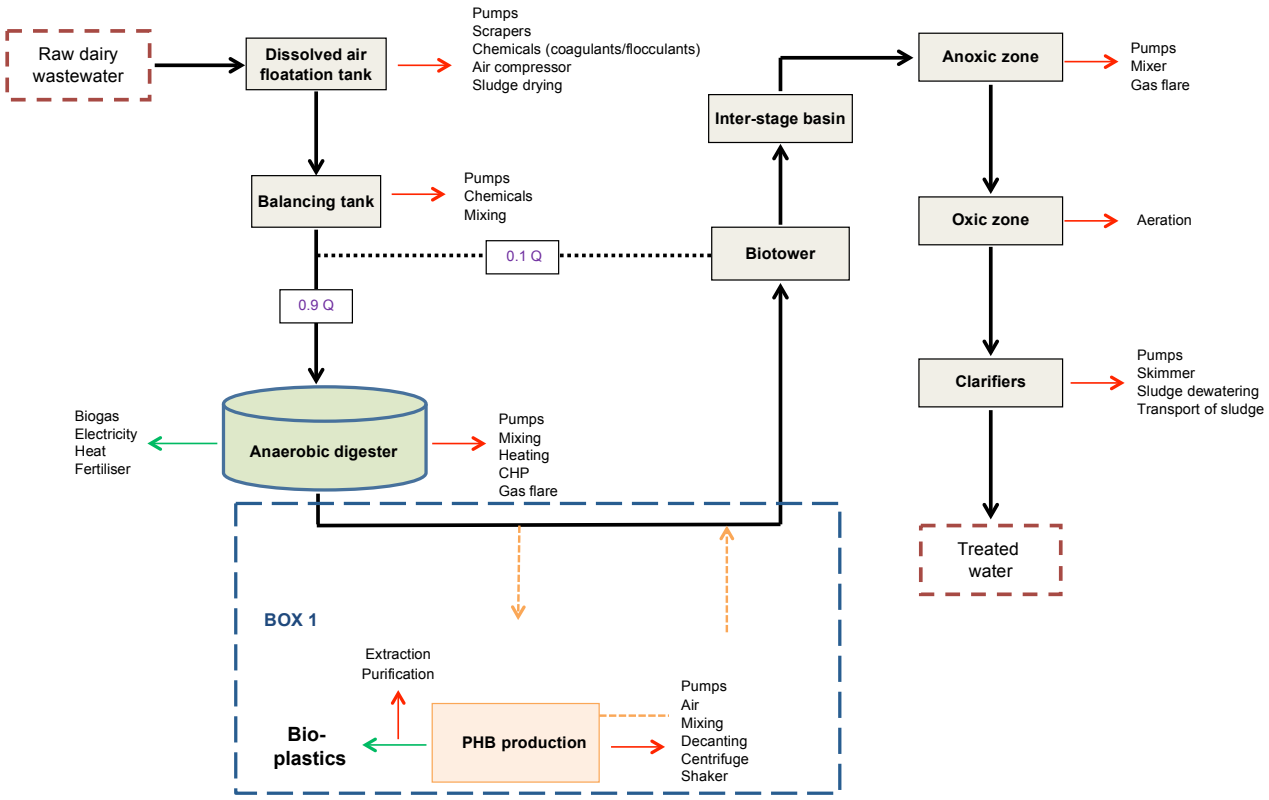

Figure 3.11. Schematic diagram of the wastewater treatment process with PHB production.

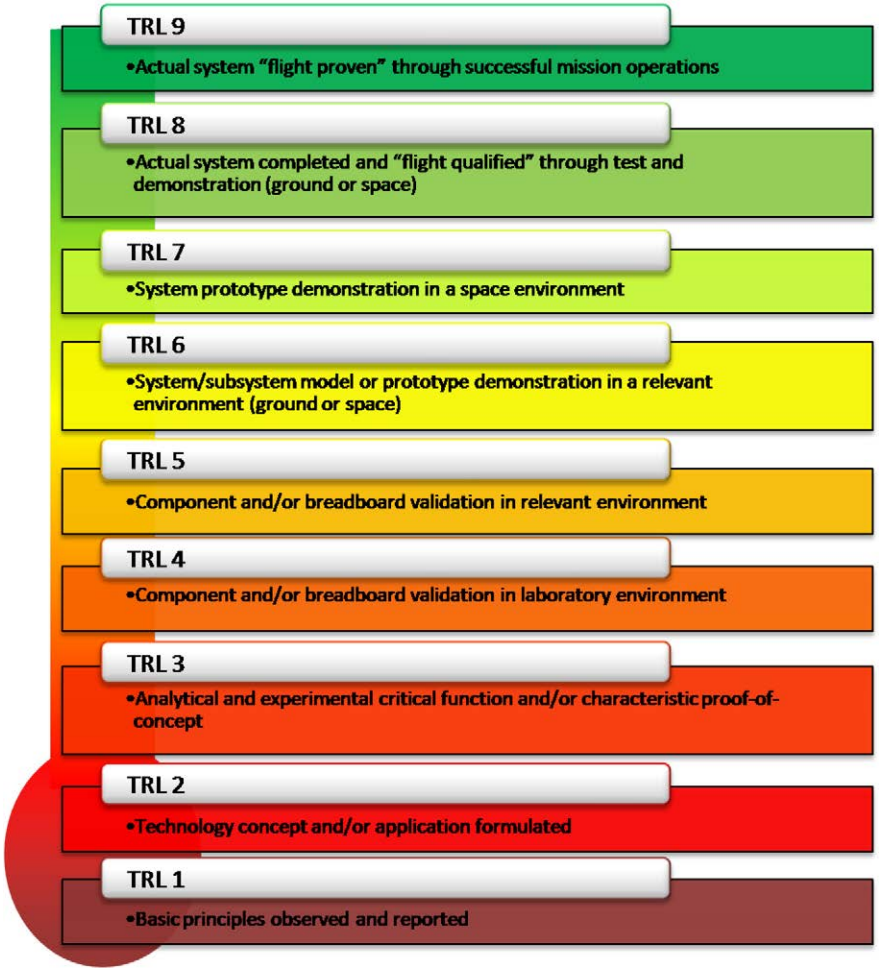

Figure 3.12. Technology readiness levels (TRLs) 1–9 (image from https://www.nasa.gov/directorates/heo/scan/engineering/technology/txt_accordion1.html).

generic set of numbers has been provided for the flow rates on-site and the BOD reduction from each step for the purposes of this study. A comprehensive LCA of the wastewater treatment set-up alone would be substantial, requiring additional data, such as the energy requirements for each pump and rising main system, each mixer, compressor, the quantity of each chemical used etc. An evaluation would have to be made for the trade-off between the biogas produced on the site and how this could be used for on-site electricity and/or heat. Production of fertiliser from the sludge generated on-site would need to be accounted for. The economic value of the bioplastic produced and its effect on reduced BOD at the wastewater treatment site would need to be ascertained. The scope of such a study is far in advance of what was achievable given the time assigned in the work package and data readily available for this study. However, the following objectives were outlined for this work package in order to advance knowledge should such an LCA be conducted:

1. assess the production, use and costs of bioplastics;
2. quantify the plastic produced from VFAs;
3. establish what options are available for extraction of PHB;
4. calculate how much PHB can be obtained from the process;
5. undertake energy consumption calculations in terms of kWh required to treat 1 L of wastewater;
6. assess the scale of vessel infrastructure required for PHB production by the process described.

3.6.2 Production, use and costs of bioplastics

PHB is a fully biodegradable polyester with reported piezoelectricity and physico-chemical characteristics similar to that of polypropylene (Knowles *et al.*, 1991; Díez-Pascual and Díez-Vicente, 2014). It has barrier

properties akin to PVC and PET, which means it is suited to industry use in producing bags, cups, foils and textile fibres (Bugnicourt *et al*., 2014). PHB can also be used in medicine in surgical implants (Brzeska *et al*., 2012). However, the use of PHB is not straightforward. A major drawback is the higher production costs of PHBs compared with those for synthetic plastics. Most bioplastic production facilities are small-scale plants which means that manufacturers do not benefit from economies of scale. High raw material costs along with high processing costs (e.g. purification) contribute to the poor economic feasibility of PHB production. It is believed that approximately 30% to 40% of the final PHB cost is related to raw materials (Chanprateep, 2010), so it is of high importance to find cheaper feedstock materials to increase PHB profitability, such as the anaerobic digester effluent described in this study.

Mitsubishi Gas Chemical Company Inc. has a PHB production facility in Japan producing 10,000 tonnes of its product (Biogreen) per annum (Chanprateep, 2010). In 2010, Metabolix Inc. opened the first commercial-scale plant producing bioplastics in the USA. This plant had a capacity to produce 50,000 tonnes per annum of its PHB-based product (Mirel). According to Kosior *et al*., this bioplastic could replace high-density polyethylene (HDPE) and low-density polyethylene (LDPE) products (Kosior *et al*., 2006).

As of 2010, the market price of Mirel was US $5.5/kg and the market price for Biogreen ranged between €2.5 and €3.0/kg (Chanprateep, 2010). According to a recent report from the California Department of Resources Recycling and Recovery in the USA, for a small facility, PHP plastics had to be sold at a minimum of US $1.2/kg to break even (Roland-Holst *et al*., 2013). These prices can be compared to those for polypropylene and LDPE. According to the Platts Global Petrochemical Index (PGPI) the price in the market for polypropylene in January 2015 was US $1538 per US ton (US $1.53/kg) and the price for LDPE in that same month was US $1550 per US ton (US $1.55/kg) (Platts, 2015).

3.6.3 Reported PHB yields from a range of substrates

Previous studies have examined the yields of PHB available from particular substrates. A recent study suggests PHA yields are 0.5 g PHA/g COD VFAs and 0.22 g PHA/g COD ethanol (Tamis *et al*., 2014). These results are comparable with Table 3.5, which is taken from a previous review paper on PHB yields (Lee, 1996; Salhizadeh and van Loosdrecht, 2004).

3.6.4 Extraction processes for PHB

Currently, the production of PHAs from single strains using sterilised substrates is about five times more expensive than standard petrochemical plastics (€5–6/kg compared with €1/kg). The downstream process (DSP) comprises several stages (e.g. cell pre-treatment, polymer extraction and post-treatment for purification); it is expensive and may represent up to 50% of the total production costs (Samori *et al*., 2015a). For mixed-microbial cultures in waste sludge, the extraction and purification costs may be further increased, as these cultures are more resistant to cell hydrolysis than single strains grown in a defined

Table 3.5. Effect of substrate cost and PHB yield on the production cost of PHB

Substrate	Price (US$/kg)	Yield (g PHB/g substrate)	Substrate cost (US$/kg PHB)
Glucose	0.493	0.38	1.35
Sucrose	0.295	0.40	0.72
Methanol	0.180	0.43	0.42
Acetic acid	0.595	0.38	1.56
Ethanol	0.502	0.50	1.00
Cane molasses	0.220	0.42	0.52
Cheese whey	0.071	0.33	0.22
Hydrolysed maize starch	0.220	0.185	0.58
Hemicellulose hydrolysate	0.069	0.20	0.34

Table 3.6. Strategies for polyhydroxyalkanoate extraction from mixed-microbial cultures [adapted from Samori *et al.* (2015b)]

Extraction approach	PHA recovery (%)	PHA MW (MDa)
Acidic treatment followed by acetone (125°C, 2 h)	n.d.	0.2–0.5
HCL (3M, 15 min) followed by $CHCL_3$ (37°C, 3 days)	n.d.	0.2–0.4
HCL (2M, 10 min) followed by $CHCL_3$ (37°C, 72 h)	n.d.	0.2–0.5
NaClO (5% Cl_2, 24 h) or NaOH (1M, 24 h)	80–100	0.3–0.5
Acetone (reflux, 3 h) followed by $CHCl_3$ (reflux, 16 h)	n.d.	0.2–0.4
$CHCl_3$ (100°C, 2 h)	n.d.	0.4–0.9
Acetone (reflux, 3 h) followed by CH_2Cl_2/H_2O (reflux, 30 min)	18–30	2.2
$CHCl_3$ (20 h)	n.d.	2.1–3.4
$CHCl_3$ (Soxhlet)	n.d.	0.1–0.4
$CHCl_3$ (20 h)	n.d.	0.1–0.9

n.d., not determined.

medium and there is less accessibility for the solvent to polymer granules (Samori *et al*., 2015b). A recent study suggests a cost of €1.4–2.0/kg for DSP during mixed culture production of PHA from wastewater (Fernandez-Dacosta *et al*., 2015). Table 3.6 above highlights some diverse strategies for PHB extraction and the relative molecular weights of the polymers generated.

As can be seen from the protocols outlined in Table 3.6, it is clear that polymer extraction strategies employed to date focus on either organic solvent extraction (with or without acid predigestion) or strong oxidant/base applications for cell hydrolysis and polymer release. The use of such harsh solvents clearly imposes an impact on the sustainability criteria for the polymers generated, in addition to having an impact on the molecular weight/quality of the isolated polymers. Advances in this area have been reported, with the successful use of "green solvents", such as dimethyl carbonate, to achieve >65% polymer recovery, with 98% purity and molecular weights (MWs) in the region of 1.2 MDa (Samori *et al*., 2015b). In a recent comprehensive review of the chemomechanical properties of PHAs, Laycock and co-workers identified PHAs with MWs <0.4 MDa as being associated with detrimental impacts on the polymer properties (Laycock *et al*., 2014). From this perspective, many of the traditional extraction processes identified in Table 3.6 result in MW distributions straddling this lower threshold. It is anticipated that further research in this field will continue to deliver efficient, eco-sensitive, economically viable extraction methodologies to address the challenge of PHB recovery from mixed-microbial cultures.

3.6.5 How much PHB can be obtained from the process?

With an estimated flow of 4000 m^3/day and a VSS content of 0.9 g/L, the total VSS per day can be calculated at 3600 kg. If a production rate of approximately 50% PHB (g PHB/g VSS) is maintained, the total quantity of PHB produced per day is 1800 kg. This equates to approximately 657 tonnes PHB per annum. Using the higher price of PHB from Mirel (US $5.5/kg), this gives an annual gross value of approximately €3.2 million. A true cost analysis, assessing the potential bioplastic value against the cost of production, would require more information on the extraction process. This is likely to be the critical phase and more research needs to be undertaken.

3.6.6 Importance of energy consumption per unit of PHB

Table 3.7 shows the energy consumption for the laboratory-scale process to produce PHB. It is estimated that a maximum of 5 kWh of electrical energy are required to treat 1 L of wastewater entering the laboratory system. From Figure 3.11, taking an average flow of 4000 m^3/day of wastewater exiting the digester and entering Box 1 for PHB production (as calculated by data obtained from the plant), this would equate to an electrical consumption of 20 GWh/day. This is a massive electricity input; however, this is an evaluation based on a scale-up from a laboratory demonstration unit to a fully operating facility and so the estimate must be taken on its merits. Since it is a scale-up, it also represents an upper extreme, as it implies uninterrupted operation. If

Table 3.7. Energy consumption: kWh required to treat wastewater for PHB production

Process stage	Actual power calculation for 2 L wastewater						
	Time (h)	Voltage (V)	Current (A)	Power (W)	VA	Power factor (cosϕ)	Energy (W_eh)
Adaptation							
Pump	0.833	230	0.6			0.8	92
Air	7	230	0.6			0.8	773
Mixing				30		0.8	210
Decanter	0.25				110	0.8	22
Production							
Centrifuge	0.3333			230		0.8	77
Pump	0.5833	230	0.8			0.8	86
Shaker	12	230	4			0.8	8832[b]
Control[a]							
Control	20				18	1	360

The following assumptions were made: a monophasic system and a power factor of 0.8; 12 hours per cycle of production phase and 8 hours per cycle of adaptation phase. The formula used for calculations was $P = V \times I \times \cos\varphi$, where P is power (kWh), V is voltage (volts), I is current (amperes) and cosϕ is the power factor. Data used to calculate energy were taken from the equipment's name plates.

[a]**Three controllers, each of 6 volt-amperes.**

[b]**The most energy demanding equipment.**

the electrical input were realistically to operate at 40% capacity, the energy requirement is still exceedingly high at 8 GWh/day. Again, laboratory-scale demonstrations may not be an accurate reflection of the process in terms of energy requirements. For example, the mixing incubator/shaker represents approximately 85% of the electrical input (6.8 GWh/day). Improved methods to reduce the energy intensive shaking process would need to be established for a viable production system. If it is assumed that the electrical energy consumption could be reduced to a value of the amount of energy used in the laboratory demonstration unit excluding the energy use of the shaker, then 420 GWh per annum would be associated with production of 657 tonnes of PHB. This equates to 640 MWh/tonne of PHB. If we consider an electrical cost of approximately 10c/kWh then the electricity price is €64,000/tonne of PHB. With a revenue of €5500/tonne PHB, this is not commercially realistic. This serves to highlight the need for an efficient energy input system. The integration of future technologies will be vital for PHB production and could enhance the attractiveness of such systems. For instance, in the coming years, electricity production in Ireland through wind turbines producing an excess of instantaneous demand could be tied into PHB production systems. Approximately 7–14% of renewable electricity will be curtailed by 2020 in Ireland (McGarrigle *et al*., 2013). Future policy drivers may also play a role. There will inevitably be a move away from oil-derived plastic to more environmentally friendly bioplastics. This will be further encouraged from a GHG emissions perspective. However, it is important that the potentially high production costs of bioplastics are highlighted at this early stage. By comparison, similar issues have been highlighted in the energy balance of microalgal biodiesel, where it is suggested that the energy consumption for microalgal biomass production amounts to six times the energy produced in the microalgal biodiesel (Stephenson *et al*., 2010).

3.6.7 Size of vessel required for PHB production

As indicated in the PHB production process description, there is a requirement for a minimum 2-day retention time. This is considered a biomass adaption phase which can optimise the production of PHBs. Using a very simplified calculation, the 2-day retention time will require a vessel of 8000 m^3 (assuming a flow of 4000 m^3/day over 2 days). This vessel would need to be developed on-site at the plant. This is not untypical of the size of vessel already found at the wastewater treatment plants but would be an added cost and would require extra space on-site.

3.6.8 Conclusions on perspective of commercial application of bioplastics

There is a considerable future market for bioplastics; however, the technology is at a very early stage of development (TRL2–4). Mass yields of 38% to 50% PHB per unit mass of ethanol and acetic acid, respectively, are obtainable. The asset value of PHB is of the order of €5500/tonne. Examining a particular wastewater treatment process which treats 4000 m^3 per day, there is potential for production of 660 tonnes per annum which could be equivalent to €3.2 million per annum. This does, however, involve construction of a 8000-m^3 vessel.

Barriers to the industry include the energy input to the system required to produce and extract bioplastics. Data on these issues is not readily available but a simple scale-up of a laboratory system, allowing for optimistic assumptions, still suggests unsustainably high levels of electricity consumption per unit of bioplastic produced. Green energy systems (such as microalgal biodiesel) remain at low technology levels due to the high energy inputs involved in extracting lipids from dilute algal solutions (Stephenson *et al*., 2010). It is suggested by the authors that similar problems will be found for bioplastics.

These findings are in line with a previous LCA analysis of mixed culture PHB production which compared this technology with biogas production from food processing wastewater with 3 g/L COD loadings (Gurieff and Lant, 2007). Table 3.8 presents a summary of the LCA outputs with respect to cash flow, energy consumption and non-renewable CO_2 emission equivalents. Trade waste in this setting represents the "zero option" equivalent of maintaining the status quo of the wastewater management system.

The study concluded there was an internal return rate (IRR) of approximately 20% for PHB production, which was below the IRR threshold of 25% that is indicative of an economically attractive venture. Much of the limitation was linked with high energy consumption as reflected in both the costs and the elevated CO_2 emissions associated with PHB production. It is worth noting, however, that the majority of polymer production technologies suffer from high CO_2 emission levels. Furthermore, PHB replacement of high-density polyethylene actually imparts a nine-fold higher displacement of CO_2 in kg/unit equivalents when compared with biogas displacement of the natural gas CO_2 equivalents. Given the advances in polymer extraction and role that development plays in reducing the cost of technologies, it is quite likely that the IRR goal of 25% is currently achievable, with additional research committed to raising mixed-culture bioplastic production technology up the TRL ladder. It is recommended, therefore, that a demonstration system be constructed in collaboration with relative industry stakeholders to assess energy inputs and potential for the optimisation of energy input per unit of bioplastic produced.

Table 3.8. Life cycle analysis comparison of PHB and biogas production versus trade waste options in the treatment of a food processing wastestream

Product	PHB	Biogas	Trade waste
Income (US$ per annum)			
Production	4,218,546	0	0
Savings	1,195,740	2,455,965	0
Costs (US$ per annum)			
Resources	992,217	728,817	3,265,460
Energy	1,325,226	374,490	416,100
Environmental (kg CO2 equivalent per annum)			
Resources	2,562,225	3,391,358	0
Energy	32,021,506	9,134,657	9,588,695
Totals			
Cash flow	3,096,843	1,352,658	−3,681,560
Total CO_2 equivalent	34,583,731	12,526,015	9,588,695

Adapted from Gurieff and Lant (2007), based on 2005 US economic figures.

4 Recommendations

Dairy processing wastewaters present a considerable remediation challenge for the industry due to high nutrient loads ranging from 3–70 g/L COD, 0.05–5 g/L total nitrogen and 20–100 mg/L total phosphorus (Table 3.1). Treatment schemes for the remediation of these wastewaters typically include AD processes which combine low operational energy input demands and reduced biomass/sludge production with methane and hydrogen gas outputs as potential on-site, combustible fuels (Banu *et al*., 2007). In the present study, we demonstrate the technical feasibility of carbon capture from dairy wastewater anaerobic digester effluent to produce a commercially relevant biodegradable polyester, polyhydroxybutryate. The PHB polymer generated is chiefly suited to low-cost, high-volume applications, such as packaging, due to the brittle characteristics of its short chain monomers (Philip *et al*., 2007). At present, approximately 35% of petrochemical plastics use worldwide is directed to single-use applications such as packaging (Al-Salem *et al*., 2009) which gives rise to non-biodegradable plastic solid waste and the environmental impacts associated with this waste, discussed in section 1.1. Synthesis of an eco-sensitive, biodegradable plastic substitute, such as PHB, from a widely available, agri-food waste stream is, therefore, a highly attractive alternative. The dairy industry represents a central pillar within the agri-food economy of multiple countries, with global milk production predicted to reach approximately 827 million tonnes by 2020 (Bojnec and Ferto, 2014; PMMI, 2014). Dairy processing activities, such as the production of milk powder, whey protein and cheese, consume large percentages of global milk outputs and require 2–6 m^3 of water per tonne of milk. This results in significant volumes of wastewater high in organic and inorganic nutrient loads (Demirel *et al*., 2005). The capacity to redefine wastewater in the dairy processing sector as a potential feedstock is of particular significance in the current climate with the discharging of milk quotas in 2015. National development strategies in many states have targeted the major growth in milk and milk-related product outputs with significant expansion anticipated in Ireland, Austria, Belgium, Denmark, Germany, France and the Netherlands. In Ireland alone, a 50% increase in output by 2020 is being predicted, with a projected range of 31–65% linked to various performance and profitability scenarios (Donnellan *et al*., 2015). 90% of milk production in Ireland is directed to processing into high-value end products such as fats, butters and powders by dairy processing plants. Against this backdrop, the authors believe that biopolymer production from dairy waste streams demonstrated in this study is a promising technological application which warrants further research commitment. It offers a multitude of positive impacts, including waste remediation, carbon capture and GHG offsets, sludge reduction, petrochemical plastics displacement, sustainable commercial materials provision, low environmental impacts with respect to production and/or end-of-use disposal. While feasibility has been demonstrated in this study, several critical research requirements are recommended to build upon this preliminary work and develop the technology beyond the laboratory scale.

1. *Yield improvement*. A maximal yield from AD effluent of approximately 50% PHB was achieved in the study. However, the authors are conscious that this may reflect a limitation in the availability of VFAs for PHB production in the effluent. All fed-batch studies revealed the greatest PHB responses to be linked to butyrate and propionate availability, while GC analyses of the AD effluent identified acetate as the primary VFA. Therefore, a potential conflict exists between efficiency of the anaerobic digestor for COD degradation and the demand for longer chain VFAs for PHB synthesis. Optimised PHB yields of approximately 90% g PHB/g VSS would need to be delivered in order to approach economic viability. In any future iteration of the work, attention should be given to the inclusion of a dedicated primary acidogenic fermentation step.

2. *Extraction technology development*. Minimising the costs of the recovery of intracellular PHA inclusions represents an ongoing challenge in the commercialisation of these materials (Madkour *et al*., 2013). Currently, primary routes to recovery from sludge include alkali digestion or organic solvent extraction. The alkali method imposes a production cost of €1.4/kg PHB, a global warming potential of 2.4 kg CO_2-eq/kg PHB and non-renewable energy use of

106 MJ/kg PHB. The solvent-based process is less commercially competitive, with production costs of €1.95/kg PHB, 4.30 kg CO_2-eq/kg PHB and 156 MJ/kg PHB. However, solvent extraction yields a higher purity polymer output (Fernandez-DeCosta *et al*., 2015). Further work must, therefore, be directed to identifying additional cost- and energy-saving strategies for bioplastic extraction to bring the commercial profile of wastewater-derived PHB in line competitively with petrochemical equivalents production.

3. *Challenging energy consumption*. The laboratory-scale system has proven an effective platform to demonstrate technological feasibility; however, it presents a real challenge to direct scale-up assessment. The chief discrepancy lies in the disparate TRLs of the laboratory-scale system and the real-time plant as the point of application. Energy consumption has been identified as the chief economic barrier to development of this technology, based on a direct scale-up of the laboratory system. A pilot-scale production system should form a key component of any future research-led development of this technology, where a more realistic assessment of energy inputs and the incorporation of emergent technology synergies could be achieved. A full-scale, comprehensive LCA of integration of the pilot system into a real-time plant is also recommended in order to supplement energy balance perspectives with the full scope of economic impacts.
4. *Polymer chemomechanical profiling*. Laycock *et al*. (2014) highlighted the need to address the limited research data available regarding the "link between compositional distribution, process parameters, feeding strategies and final mechanical properties for mixed culture PHA. It is important to demonstrate that the chemomechanical properties are predictable and may be controlled and within desirable limits for specific practical applications". Future work on wastestream bioconversions to PHB or co-polymer derivatives should, therefore, seek to contribute these material characterisations to demonstrate viability to industrial stakeholders. This work should also encompass comparative assessment of pure-culture versus mixed-culture PHA properties, which are known to differ considerably.
5. *Assessing public perspective*. Finally, public perspectives on the use of wastewater-derived polymers in particular applications, e.g. food packaging, may present societal challenges to future market implementation. In tandem with scientific and engineering developments, attention should also be given in future work to assessing the social attitudes towards "green" polymers, their implications and any perceived resistances to their various applications.

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Abbreviations

AD	Anaerobic digestion
ADF	Aerobic dynamic feeding
BOD	Biological oxygen demand
Cmmol	Millimoles in relation to carbon
COD	Chemical oxygen demand
DO	Dissolved oxygen
DSP	Downstream process
GC	Gas chromatography
GC-MS	Gas chromatography–mass spectrometry
GHG	Greenhouse gas
HBME	Hydroxybutyl methyl ester
HDD	Hydroxydodecanoate
HPLC	High-performance liquid chromatography
IRR	Internal return rate
LB	Luria Bertani
LCA	Life cycle analysis
LDPE	Low-density polyethylene
mcl	medium chain length
MLSS	Mixed liquor suspended solids
NH_3-N	Nitrogen present as NH_3
NH_4-N	Nitrogen present as NH_4
NO_3-N	Nitrogen present as NO_3
P(3HB-co-3HV)	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PCR	Polymerase chain reaction
PET	Polyethylene terephthalate
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PLA	Poly-D-lactic acid
PO_4-P	Phosphorus present as PO_4
PSW	Plastics solid waste
PVC	Polyvinylchloride
SBR	Sequencing batch reactor
SRT	Sludge retention time
TRL	Technology readiness level
VSS	Volatile suspended solids

AN GHNÍOMHAIREACHT UM CHAOMHNÚ COMHSHAOIL

Tá an Ghníomhaireacht um Chaomhnú Comhshaoil (GCC) freagrach as an gcomhshaol a chaomhnú agus a fheabhsú mar shócmhainn luachmhar do mhuintir na hÉireann. Táimid tiomanta do dhaoine agus don chomhshaol a chosaint ó éifeachtaí díobhálacha na radaíochta agus an truaillithe.

Is féidir obair na Gníomhaireachta a roinnt ina trí phríomhréimse:

Rialú: *Déanaimid córais éifeachtacha rialaithe agus comhlíonta comhshaoil a chur i bhfeidhm chun torthaí maithe comhshaoil a sholáthar agus chun díriú orthu siúd nach gcloíonn leis na córais sin.*

Eolas: *Soláthraímid sonraí, faisnéis agus measúnú comhshaoil atá ar ardchaighdeán, spriocdhírithe agus tráthúil chun bonn eolais a chur faoin gcinnteoireacht ar gach leibhéal.*

Tacaíocht: *Bímid ag saothrú i gcomhar le grúpaí eile chun tacú le comhshaol atá glan, táirgiúil agus cosanta go maith, agus le hiompar a chuirfidh le comhshaol inbhuanaithe.*

Ár bhFreagrachtaí

Ceadúnú

Déanaimid na gníomhaíochtaí seo a leanas a rialú ionas nach ndéanann siad dochar do shláinte an phobail ná don chomhshaol:

- saoráidí dramhaíola *(m.sh. láithreáin líonta talún, loisceoirí, stáisiúin aistrithe dramhaíola)*;
- gníomhaíochtaí tionsclaíocha ar scála mór *(m.sh. déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta)*;
- an diantalmhaíocht *(m.sh. muca, éanlaith)*;
- úsáid shrianta agus scaoileadh rialaithe Orgánach Géinmhodhnaithe *(OGM)*;
- foinsí radaíochta ianúcháin *(m.sh. trealamh x-gha agus radaiteiripe, foinsí tionsclaíocha);*
- áiseanna móra stórála peitril;
- scardadh dramhuisce;
- gníomhaíochtaí dumpála ar farraige.

Forfheidhmiú Náisiúnta i leith Cúrsaí Comhshaoil

- Clár náisiúnta iniúchtaí agus cigireachtaí a dhéanamh gach bliain ar shaoráidí a bhfuil ceadúnas ón nGníomhaireacht acu.
- Maoirseacht a dhéanamh ar fhreagrachtaí cosanta comhshaoil na n-údarás áitiúil.
- Caighdeán an uisce óil, arna sholáthar ag soláthraithe uisce phoiblí, a mhaoirsiú.
  - Obair le húdaráis áitiúla agus le gníomhaireachtaí eile chun dul i ngleic le coireanna comhshaoil trí chomhordú a dhéanamh ar líonra forfheidhmiúcháin náisiúnta, trí dhíriú ar chiontóirí, agus trí mhaoirsiú a dhéanamh ar leasúchán.
- Cur i bhfeidhm rialachán ar nós na Rialachán um Dhramhthrealamh Leictreach agus Leictreonach (DTLL), um Shrian ar Shubstaintí Guaiseacha agus na Rialachán um rialú ar shubstaintí a ídíonn an ciseal ózóin.
- An dlí a chur orthu siúd a bhriseann dlí an chomhshaoil agus a dhéanann dochar don chomhshaol.

Bainistíocht Uisce

- Monatóireacht agus tuairisciú a dhéanamh ar cháilíocht aibhneacha, lochanna, uiscí idirchriosacha agus cósta na hÉireann, agus screamhuiscí; leibhéil uisce agus sruthanna aibhneacha a thomhas.
- Comhordú náisiúnta agus maoirsiú a dhéanamh ar an gCreat-Treoir Uisce.
- Monatóireacht agus tuairisciú a dhéanamh ar Cháilíocht an Uisce Snámha.

Monatóireacht, Anailís agus Tuairisciú ar an gComhshaol

- Monatóireacht a dhéanamh ar cháilíocht an aeir agus Treoir an AE maidir le hAer Glan don Eoraip (CAFÉ) a chur chun feidhme.
- Tuairisciú neamhspleách le cabhrú le cinnteoireacht an rialtais náisiúnta agus na n-údarás áitiúil *(m.sh. tuairisciú tréimhsiúil ar staid Chomhshaol na hÉireann agus Tuarascálacha ar Thascairí)*.

Rialú Astaíochtaí na nGás Ceaptha Teasa in Éirinn

- Fardail agus réamh-mheastacháin na hÉireann maidir le gáis cheaptha teasa a ullmhú.
- An Treoir maidir le Trádáil Astaíochtaí a chur chun feidhme i gcomhair breis agus 100 de na táirgeoirí dé-ocsaíde carbóin is mó in Éirinn.

Taighde agus Forbairt Comhshaoil

- Taighde comhshaoil a chistiú chun brúnna a shainaithint, bonn eolais a chur faoi bheartais, agus réitigh a sholáthar i réimsí na haeráide, an uisce agus na hinbhuanaitheachta.

Measúnacht Straitéiseach Timpeallachta

- Measúnacht a dhéanamh ar thionchar pleananna agus clár beartaithe ar an gcomhshaol in Éirinn *(m.sh. mórphleananna forbartha)*.

Cosaint Raideolaíoch

- Monatóireacht a dhéanamh ar leibhéil radaíochta, measúnacht a dhéanamh ar nochtadh mhuintir na hÉireann don radaíocht ianúcháin.
- Cabhrú le pleananna náisiúnta a fhorbairt le haghaidh éigeandálaí ag eascairt as taismí núicléacha.
- Monatóireacht a dhéanamh ar fhorbairtí thar lear a bhaineann le saoráidí núicléacha agus leis an tsábháilteacht raideolaíochta.
- Sainseirbhísí cosanta ar an radaíocht a sholáthar, nó maoirsiú a dhéanamh ar sholáthar na seirbhísí sin.

Treoir, Faisnéis Inrochtana agus Oideachas

- Comhairle agus treoir a chur ar fáil d'earnáil na tionsclaíochta agus don phobal maidir le hábhair a bhaineann le caomhnú an chomhshaoil agus leis an gcosaint raideolaíoch.
- Faisnéis thráthúil ar an gcomhshaol ar a bhfuil fáil éasca a chur ar fáil chun rannpháirtíocht an phobail a spreagadh sa chinnteoireacht i ndáil leis an gcomhshaol (*m.sh. Timpeall an Tí, léarscáileanna radóin).*
- Comhairle a chur ar fáil don Rialtas maidir le hábhair a bhaineann leis an tsábháilteacht raideolaíoch agus le cúrsaí práinnfhreagartha.
- Plean Náisiúnta Bainistíochta Dramhaíola Guaisí a fhorbairt chun dramhaíl ghuaiseach a chosc agus a bhainistiú.

Múscailt Feasachta agus Athrú Iompraíochta

- Feasacht chomhshaoil níos fearr a ghiniúint agus dul i bhfeidhm ar athrú iompraíochta dearfach trí thacú le gnóthais, le pobail agus le teaghlaigh a bheith níos éifeachtúla ar acmhainní.
- Tástáil le haghaidh radóin a chur chun cinn i dtithe agus in ionaid oibre, agus gníomhartha leasúcháin a spreagadh nuair is gá.

Bainistíocht agus struchtúr na Gníomhaireachta um Chaomhnú Comhshaoil

Tá an ghníomhaíocht á bainistiú ag Bord lánaimseartha, ar a bhfuil Ard-Stiúrthóir agus cúigear Stiúrthóirí. Déantar an obair ar fud cúig cinn d'Oifigí:

- An Oifig um Inmharthanacht Comhshaoil
- An Oifig Forfheidhmithe i leith cúrsaí Comhshaoil
- An Oifig um Fianaise is Measúnú
- An Oifig um Cosaint Raideolaíoch
- An Oifig Cumarsáide agus Seirbhísí Corparáideacha

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag comhaltaí air agus tagann siad le chéile go rialta le plé a dhéanamh ar ábhair imní agus le comhairle a chur ar an mBord.

EPA Research Report 190

Biopolymer Production from Irish Dairy Industry Wastewaters

Authors: Anca Minescu, David Wall, Jerry Murphy, Alan Dobson and Niall O' Leary

Identifying Pressures

This report examines issues surrounding non-sustainable plastic production, use and disposal and highlights the challenges associated with limited commercial uptake of wholly biodegradable polymers. The report also examines dairy processing wastewater in the context of a rapid expansion in Irish milk production/processing following the 2015 removal of quotas. These pressures inform the research undertaken in this study, which sought to demonstrate the use mixed microbial cultures to produce commercially significant bioplastics from dairy processing wastewater feedstocks.

Informing Policy

This research successfully demonstrated the technical feasibility of polyhydroxyalkanoate bioplastic accumulation from real-time dairy processing wastewater in a laboratory setting. The broader implications of status quo, trade waste practices versus dairy wastewater valorisation opportunities are also presented together with recommendations for future research focus and the merits for pilot scale trials. Life cycle analysis of the laboratory process identifies key hurdles to commercialisation in addition to limitations in data collation from industry r.e. comprehensive cradle to grave sustainability modelling. In summary, this study offers broad, cross-policy significance as it integrates waste to feedstock reclassification, environmentally sustainable material provision, petrochemical polymer replacement and non-renewable CO2 displacement.

Developing Solutions

This study identified optimised operational parameters for an aerobic dynamic feeding reactor strategy to achieve microbial bioplastic accumulation from dairy processing wastewater feedstocks. Polyhydroxybutyrate was the primary biopolymer generated, but the observation of additional, commercially significant co-polymers suggested further opportunities to develop the process further. Energy profiling and extrapolations of process scale up identified a clear need for pilot scale trialling of the system in order to advance the technology readiness level and facilitate comprehensive demonstration of commercial viability and sustainability.

Comhshaol, Pobal agus Rialtas Áitiúil
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