

STRIVE

Report Series No.107

Resource-efficient Ireland – Biodegradable Plastic from Farm-waste Plastic

STRIVE

Environmental Protection
Agency Programme

2007-2013

Environmental Protection Agency

The Environmental Protection Agency (EPA) is a statutory body responsible for protecting the environment in Ireland. We regulate and police activities that might otherwise cause pollution. We ensure there is solid information on environmental trends so that necessary actions are taken. Our priorities are protecting the Irish environment and ensuring that development is sustainable.

The EPA is an independent public body established in July 1993 under the Environmental Protection Agency Act, 1992. Its sponsor in Government is the Department of the Environment, Community and Local Government.

OUR RESPONSIBILITIES

LICENSING

We license the following to ensure that their emissions do not endanger human health or harm the environment:

- waste facilities (e.g., landfills, incinerators, waste transfer stations);
- large scale industrial activities (e.g., pharmaceutical manufacturing, cement manufacturing, power plants);
- intensive agriculture;
- the contained use and controlled release of Genetically Modified Organisms (GMOs);
- large petrol storage facilities;
- waste water discharges;
- dumping at sea.

NATIONAL ENVIRONMENTAL ENFORCEMENT

- Conducting over 1200 audits and inspections of EPA licensed facilities every year.
- Overseeing local authorities' environmental protection responsibilities in the areas of - air, noise, waste, waste-water and water quality.
- Working with local authorities and the Gardaí to stamp out illegal waste activity by co-ordinating a national enforcement network, targeting offenders, conducting investigations and overseeing remediation.
- Prosecuting those who flout environmental law and damage the environment as a result of their actions.

MONITORING, ANALYSING AND REPORTING ON THE ENVIRONMENT

- Monitoring air quality and the quality of rivers, lakes, tidal waters and ground waters; measuring water levels and river flows.
- Independent reporting to inform decision making by national and local government.

REGULATING IRELAND'S GREENHOUSE GAS EMISSIONS

- Quantifying Ireland's emissions of greenhouse gases in the context of our Kyoto commitments
- Implementing the Emissions Trading Directive, involving over 100 companies who are major generators of carbon dioxide in Ireland.

ENVIRONMENTAL RESEARCH AND DEVELOPMENT

- Co-ordinating research on environmental issues (including air and water quality, climate change, biodiversity, environmental technologies).

STRATEGIC ENVIRONMENTAL ASSESSMENT

- Assessing the impact of plans and programmes on the Irish environment (such as waste management and development plans).

ENVIRONMENTAL PLANNING, EDUCATION AND GUIDANCE

- Providing guidance to the public and to industry on various environmental topics (including licence applications, waste prevention and environmental regulations).
- Generating greater environmental awareness (through environmental television programmes and primary and secondary schools' resource packs).

PROACTIVE WASTE MANAGEMENT

- Promoting waste prevention and minimisation projects through the co-ordination of the National Waste Prevention Programme, including input into the implementation of Producer Responsibility Initiatives.
- Enforcing Regulations such as Waste Electrical and Electronic Equipment (WEEE) and Restriction of Hazardous Substances (RoHS) and substances that deplete the ozone layer.
- Developing a National Hazardous Waste Management Plan to prevent and manage hazardous waste.

MANAGEMENT AND STRUCTURE OF THE EPA

The organisation is managed by a full time Board, consisting of a Director General and four Directors.

The work of the EPA is carried out across four offices:

- Office of Climate, Licensing and Resource Use
- Office of Environmental Enforcement
- Office of Environmental Assessment
- Office of Communications and Corporate Services

The EPA is assisted by an Advisory Committee of twelve members who meet several times a year to discuss issues of concern and offer advice to the Board.

EPA STRIVE Programme 2007–2013

Resource-efficient Ireland – Biodegradable Plastic from Farm-waste Plastic

(2008-ET-LS-1-S2)

STRIVE Report

Prepared for the Environmental Protection Agency

by

Biocatalysis Research Group, School of Biomolecular & Biomedical Science,
Centre for Synthesis and Chemical Biology, University College Dublin (UCD)
Conway Institute, and Earth Institute UCD; School of Chemical and Bioprocess
Engineering, Engineering and Materials Science Centre, UCD &
CRANN, School of Physics, Trinity College Dublin

Authors:

**Kevin O'Connor, Ramesh Babu, Eoin Casey, Shane Kenny, Maciej Guzik,
Trevor Woods, Gearóid Duane, Jasmina Nikodinovic, Aisling Hume
and Alexis Pacquit**

ENVIRONMENTAL PROTECTION AGENCY

An Ghníomhaireacht um Chaomhnú Comhshaoil
PO Box 3000, Johnstown Castle, Co. Wexford, Ireland

Telephone: +353 53 916 0600 Fax: +353 53 916 0699

Email: info@epa.ie Website: www.epa.ie

ACKNOWLEDGEMENTS

This report is published as part of the Science, Technology, Research and Innovation for the Environment (STRIVE) Programme 2007–2013. The programme is financed by the Irish Government. It is administered on behalf of the Department of the Environment, Community and Local Government (DECLG) by the Environmental Protection Agency (EPA) which has the statutory function of co-ordinating and promoting environmental research. The project was overseen by a steering committee composed of Professor Jim Field (University of Arizona, USA), Professor Alexander Steinbüchel (University of Muenster, Germany), Jean Clarke (DECLG), Lisa Sheils (EPA).

DISCLAIMER

Although every effort has been made to ensure the accuracy of the material contained in this publication, complete accuracy cannot be guaranteed. Neither the Environmental Protection Agency nor the author(s) accept any responsibility whatsoever for loss or damage occasioned or claimed to have been occasioned, in part or in full, as a consequence of any person acting, or refraining from acting, as a result of a matter contained in this publication. All or part of this publication may be reproduced without further permission, provided the source is acknowledged.

The EPA STRIVE Programme addresses the need for research in Ireland to inform policymakers 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.

EPA STRIVE PROGRAMME 2007–2013

Published by the Environmental Protection Agency, Ireland

ISBN: 978-1-84095-491-3

Online version

Price: Free

Details of Project Partners

Dr Kevin O'Connor

Ardmore House
School of Biomolecular and Biomedical
Science (SBBS), Centre for Synthesis and
Chemical Biology (CSCB), UCD Conway
Institute, and Earth Institute
University College Dublin
Belfield, Dublin 4, Ireland
Tel: +353 (01) 716 1307
Email: kevin.oconnor@ucd.ie

Dr Eoin Casey

School of Chemical and Bioprocess
Engineering (SCBE), Engineering and
Materials Science Centre
University College Dublin
Belfield, Dublin 4, Ireland
Tel: +353 (01) 7161877
Email: eoin.casey@ucd.ie

Dr Shane Kenny

Ardmore House,
SBBS, CSCB, UCD Conway Institute
University College Dublin Belfield, Dublin
4, Ireland
Tel: + 353 (01) 716 1361
Email: shane.t.kenny@ucd.ie

Trevor Woods

CRANN & School of Physics,
Trinity College Dublin,
College Green
Dublin 2, Ireland
Tel: +353 (01) 896 3470
Email: woodst@tcd.ie

Dr Ramesh Babu

CRANN & School of Physics
Trinity College Dublin
College Green
Dublin 2, Ireland
Tel: +353 (01) 896 2602
Email: babup@tcd.ie

Dr Alexis Pacquit

Ardmore House
SBBS, CSCB, UCD Conway Institute
University College Dublin
Belfield, Dublin 4, Ireland
Tel: +353 (01) 716 1515
Email: alexis.pacquit@ucd.ie

Dr Maciej Guzik

Ardmore House,
SBBS, CSCB, UCD Conway Institute
University College Dublin
Belfield, Dublin 4, Ireland
Tel: + 353 (01) 716 1309
Email: maciej.guzik@ucd.ie

Gearóid Duane

SCBE, Engineering and Materials Science
Centre
University College Dublin
Belfield, Dublin 4, Ireland
Tel: + 353 (01) 716 1361
Email: gearoid.duane@ucdconnect.ie

Dr Jasmina Nikodinovic

Ardmore House
SBBS, CSCB, UCD Conway Institute
University College Dublin,
Belfield, Dublin 4, Ireland
Tel: + 353 (01) 716 1309
Email: jasmina.nikodinovic@gmail.com

Table of Contents

Acknowledgements	ii
Disclaimer	ii
Details of Project Partners	iii
Executive Summary	vii
1 Background and Objectives	1
2 Research Approach and Results	3
2.1 A Biotechnological Solution to Plastic Waste: Converting Petrochemical Plastics to Biodegradable Plastic	3
2.2 What is Pyrolysis?	3
2.3 Polyhydroxyalkanoates	3
2.4 Converting Polyethylene to Polyhydroxyalkanoates	4
2.5 Improvement of the Conversion of Polyethylene to Polyhydroxyalkanoate through Bioprocess Manipulation	8
2.6 Bioprocess Analysis and Manipulation	8
2.7 Investigation of the Genes that encode Critical Pathway Enzymes for PHA Production in Bacteria	10
2.8 Introduction to Biodegradable Polymers and Nanocomposites	10
2.9 Polyhydroxyalkanoate	11
2.10 Biopolymer – mcl-PHA Blends	12
3 Output and Final Recommendations	15
3.1 Major Conclusions	15
3.2 Major Project Outputs	15
3.3 Final Recommendations	15
References	17
Acronyms and Annotations	20
Appendix 1 – Selected Outputs of Project	21

Executive Summary

The development of a green economy in Ireland that is based on innovation and creativity has the potential to not only push the country towards a more sustainable future but also offers tangible economic benefits by focusing on the growth of indigenous technologies and systems that can address a global issue. In order to move in this direction, the development of an infrastructure for recycling technologies coupled with a strategy to enhance market penetration by recycled products is required. It is also critical to maintain a continued focus on research and development to drive the innovation needed to build a green economy. The depletion of fossil resources and the need to reduce waste generation has brought resource efficiency into sharp focus in Europe (Resource Efficient Europe) and in Ireland (EPA strategy 2013–2015, Department of Environment Community and Local Government [DECLG] [2012]). In its communication 'Roadmap to a Resource Efficient Europe' the European Commission (EC) highlighted that there is a need to increase Irish resource efficiency four- to ten-fold by 2050 with significant improvements in the shorter term (EC, 2011b). Resource efficiency is at the heart of the European agenda for improving competitiveness and profitability while delivering sustainability and employment.

To improve resource efficiency, Ireland needs to develop new products and services, find new ways to reduce inputs, minimise waste, improve production processes and if possible create valuable environmental friendly products from them.

Currently, over 70% of the 480 thousand tonnes of petrochemical plastic waste managed annually on the island of Ireland (338,356 tonnes in the Republic and 144,000 tonnes in Northern Ireland) ends up in landfill (rx3, 2011). Within the recycling space in Ireland and globally there is a need to develop technologies to manage plastic waste so that their environmental impact can be minimised, the recycling rate is increased and that valuable environmental friendly products can be created from them.

The EPA is tasked as an environmental champion so that Ireland can achieve the objectives of the national waste-prevention programme and EC Waste Directive.

To this end, the EPA funded a research consortium led by University College Dublin (UCD) researchers to investigate novel methods for tackling the problem of waste plastic, which is predominantly polyethylene (PE), from the agricultural sector in a sustainable manner that could support the green economy.

The result of this work is summarised in the following report, which describes the interdisciplinary approach used for converting post-consumer waste (PE) into value-added biodegradable material. Process chemistry, microbiology, bioprocesses engineering, chemical engineering, material science and nanotechnology were combined to convert PE to the biodegradable polymer polyhydroxyalkanoate (PHA). PE is used widely in many sectors of society including agriculture (in the form of films for applications such as silage wrapping, poly-tunnels and plastic mulch) and makes up the largest proportion of worldwide waste plastic. Farm-plastic waste, made of PE, was subjected to a two-step treatment to produce a biodegradable plastic. The researchers further improved their process through chemical and biological investigations and developed applications for the biodegradable polymer produced. The properties of the biodegradable plastic were then improved through the use of nanotechnology. The project results are now being exploited by a UCD spin-out company Bioplastech operating in the green technology space. Thus, job creation, new recycling technologies and environmentally friendly products are arising from research funding to build the waste plastic to biodegradable plastic technology at a larger scale in Ireland and internationally.

The major outputs of this project are patented technology and products, highly skilled university graduates who have gained employment in an Irish university (UCD) and the company (Bioplastech Ltd), which has licensed the innovative technology and will begin trials in 2013 to scale up the process. The research team has developed a process that could be described as 'upcycling' – essentially the creating of a high-value useable product from waste.

This is a unique world-first technology developed in Ireland, based on a brand new process, and it

demonstrates that Ireland – through investment and vision – can lead the way globally in environmental technologies. The development of technology and the creation of highly skilled jobs are in keeping with government policy for employment and wealth creation in Ireland through green growth.

Finally, the project described here is a microcosm of the effort and integration needed to address global challenges of waste conversion into value-added products. Ireland needs to take the next step and

develop a flagship waste refinery for the production of value-added products such as (bio)fuels, (bio)polymers and (bio)chemicals. This will facilitate the building of industry–academic partnerships that will drive technology development and innovation-driven Irish companies. In doing so, Ireland will take a giant leap forward towards developing and fulfilling a strategic vision for resource management, intellectual property development and protection, job creation, and high-value product exports.

1 Background and Objectives

Petrochemical-based plastics, produced worldwide on a multimillion tonne scale, pervade modern society as a result of their versatile and highly desirable properties. It is estimated that 58 million tonnes of plastic were produced in Europe in 2011. Over 480,000 tonnes of petrochemical plastic waste was generated in Ireland (338,000 in the Republic of Ireland and 144,000 in Northern Ireland) in 2009. Two-thirds of this waste went to landfill (rx3, 2011). Due to their recalcitrant nature, these plastics pose major waste-management challenges when they are disposed of (Alexander, 1975).

Polyethylene (PE) is produced more than any other polymer type, making up 29.1% of worldwide polymer production (European Commission [EC], 2011a). PE has a broad range of physico-chemical properties, which enable its use in a variety of products. These include heavy-duty commodities, such as water pipes, food containers, toys and detergent/chemicals-storage containers. PE is also widely used in film applications such as plastic bags, agricultural films, bubble wraps or multilayer and composite films (EC, 2011a; rx3, 2011; Plastics Europe, 2011). Like other petrochemical plastics the success of PE as a convenience bulk commodity polymer has led to post-consumer PE products becoming a major waste problem.

While Ireland has made great progress in improving its plastic-recycling rates since the 2000s and is meeting the requirements of European legislation, further improvements could be made in order to achieve better resource efficiency (rx3, 2011). Although plastic materials are readily recyclable, the current market for recycled plastic is small, saturated and the products are of low value (Al-Salem et al., 2009). This creates a barrier to plastic recycling and consequently plastic is landfilled rather than recycled. Indeed, the majority of plastic collected in Ireland for recycling is exported despite the availability of significant processing and reprocessing capacity here which could convert plastic waste into an intermediate value resource. The operation of the plastics market is complex and it is only when the export of plastic waste from Ireland becomes

increasingly expensive and as recipient countries increase charges that the market is likely to pay more attention to the possibilities of recycling and recovering more plastic waste within Ireland. New technologies to deal with plastic waste will have to emerge from Ireland and other European countries and this will make the European market more attractive. Thus, the conversion of petrochemical plastics into new value added commodities is desirable and of importance to the sustainable management of plastic waste.

The EU waste hierarchy of prevention, preparing for re-use, recycling, other recovery and finally landfill, which was established in the EC's Waste Framework Directive (European Parliament and Council of the European Union, 2008) (Fig. 1.1), prioritises the importance of waste prevention and re-use. However, the recycling and recovery of waste are far more preferable than landfill management. There are a number of possible solutions for managing post-consumer petrochemical plastics: (i) mechanical recycling; (ii) converting waste to energy (iii) conducting chemical/physical treatments to generate gases or liquid fuels; and (iv) combining chemical and biological treatment to produce a biodegradable plastic (as was done in the current research). In reality, all four approaches will most likely be used for waste plastic management.

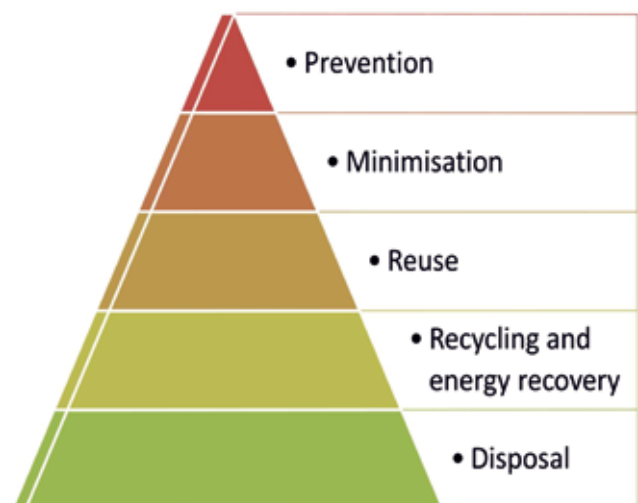


Figure 1.1. The waste-management pyramid.

The recycling of waste plastic for use as a material is viable for some applications, the viability of which is often dictated by the price of virgin plastics. The co-incineration of municipal waste with plastic allows for effective thermal treatment (incineration) of wastes but the energy is not always recovered.

The treatment of plastics to generate fuel oils is a well-established technology for certain applications and was only abandoned in the 1980s because of the low price

of crude oil. Due to the rising price of crude oil and the uncertainty with respect to its future supply, the major petrochemical industries (BP, Shell etc.) have recently returned to investigate this technology. Indeed, an Irish company Cynar Recycling is operating a technical plant in Portlaoise, Ireland, for the conversion of post-consumer plastic waste to fuels (Cynar, 2011) and is generating business through technology sales and implementation.

2 Research Approach and Results

2.1 A Biotechnological Solution to Plastic Waste: Converting Petrochemical Plastics to Biodegradable Plastic

While the products of pyrolysis are fuels, they can also be used as feedstock for bacteria that can make biodegradable plastic (Fig. 2.1). The combination of pyrolysis and bacterial fermentation to convert polystyrene (PS) and polyethylene terephthalate (PET) into the biodegradable plastic polyhydroxyalkanoate (PHA) was already uniquely realised by Dr O'Connor's research group before the start of the polyethylene to PHA project (EPA-funded project 2005-ET-LS-9-M3) (Ward et al., 2006; Goff et al., 2007; Kenny et al., 2008). Thus, the opportunity to integrate fuel and polymer production from waste exists.

2.2 What is Pyrolysis?

Pyrolysis is one of four common techniques used for generating fuels from plastic. The other techniques are 'hydrogenation', 'gasification' and 'chemolysis'. With hydrogenation, plastics waste is heated with hydrogen, and this 'cracks' the polymers into a liquid hydrocarbon. 'Gasification' involves the heating of plastics waste in air producing a mixture of carbon monoxide and hydrogen gases. This is used to produce new raw materials such as methanol. 'Chemolysis' is the chemical treatment of plastics for the production of raw materials for making the same plastics.

Pyrolysis converts plastics back to the constituents that were used to manufacture the plastic. This is known as 'feedstock recycling', although its classification as 'recycling' or 'recovery' may vary depending on the final use of the output fractions. Pyrolysis involves the heating of plastic waste in a vacuum producing a mixture of gaseous and liquid hydrocarbons not unlike petroleum. The pyrolysis oil that is generated can be used as a cheap fuel.

The composition of the pyrolysis oils produced from waste plastic is dependent upon the plastic supplied and the pyrolysis conditions. PE pyrolysis generates either a gas, liquid or solid (wax) like product depending upon the pyrolysis conditions used.

2.3 Polyhydroxyalkanoates

PHAs are a group of polyesters made by bacteria. PHAs occur naturally in the cytoplasm of bacteria where they act as a carbon and energy reserve and appear as white granules (Fig. 2.2). The most common PHA is polyhydroxybutyrate, which is a polymer of (*R*)-3-hydroxybutyric acid units (monomers).

PHAs are biological polyesters, which are made and degraded by bacteria that accumulate them but also by many bacteria that cannot make the polymer. Both the molecular weight and the monomer composition of

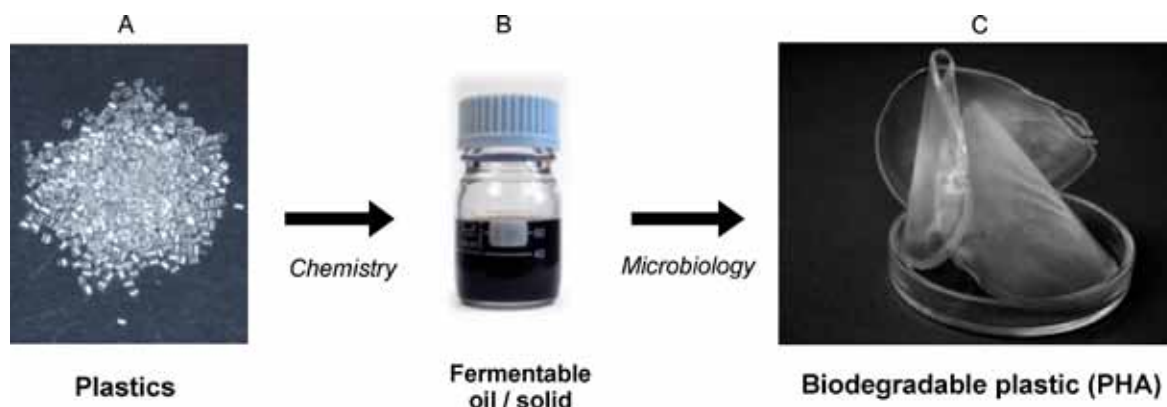


Figure 2.1. Process flow chart of the conversion of petrochemical plastics to the biodegradable plastic polyhydroxyalkanoate (PHA): (a) Plastic resin; (b) pyrolysis product; (c) PHA isolated from bacteria.

PHA affect the properties of the polymer (Fig. 2.3). The number of carbons in the side chain determines whether the polymer will be hard or elastic. Polyhydroxybutyrate (PHB) has only one carbon in the side chain and it is a hard brittle polymer.

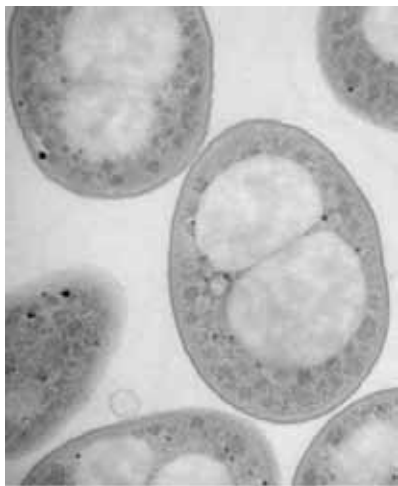


Figure 2.2. Transmission electron microscope of *P. putida* cells (grey) accumulating PHA (large white granules) in the cytoplasm.

Other PHAs can have up to 11 carbons in the side chain, making them elastomeric. A higher molecular weight bestows greater strength on a polymer. PHB generally has a much higher molecular weight than medium-chain-length PHA (mcl-PHA) and is thus a stronger polymer.

The chemical and biotechnology industry is keenly aware of the potential of PHA (as indicated by the vast number of industry patent applications relating to PHA production and applications). PHB, which was the first PHA identified (Lemoigne, 1926), has received the greatest attention in terms of industrial-scale production. ICI was the first company to produce PHB on a commercial scale and later Biopol™ was produced by Monsanto. Metabolix (USA), Tianan (China) and Kaneka (Japan) produce PHB copolymers. PHAs are both bulk commodity plastic used for films, bottles, surface coatings and in medicine. A particular subset of PHAs can be used as elastomers, as plastic coatings, and pressure-sensitive adhesives (Reddy et al., 2003).

2.4 Converting Polyethylene to Polyhydroxyalkanoates

Polyethylene is a non-degradable polymer and thus to convert it to a biodegradable polymer through a biologically mediated process requires innovation. The thermal treatment ('pyrolysis') of polyethylene to produce saturated and unsaturated hydrocarbons (Fig. 2.3) that make up polyethylene was the first step in the conversion process.

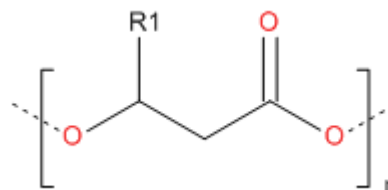


Figure 2.3. General structure of polyhydroxyalkanoates (PHAs). When R1 is CH₃ or C₂H₅ then the PHA is short-chain-length PHA (scl-PHA). When R1 = C₃H₇ to C₉H₁₉ then the PHA is medium-chain-length PHA (mcl-PHA).

These smaller molecules then act as substrates for microbial fermentation. The carbon present in the polyethylene pyrolysis products can be used by the microorganisms to build a new carbon polymer inside the microbial cells. The resulting polymer made by the microorganisms is a biodegradable polymer. The pyrolysis product was also modified to make it more accessible to bacteria for fermentation.

The conversion of pyrolysis and modified pyrolysis products to the biodegradable polymer PHA was initially studied using a broad range of bacteria in small-scale shake flasks (50 ml). The best performing bacteria were selected for further more detailed tests in 5, 15, and 30 L fermentors. The bacteria tested in the primary screening were known bacteria from the scientific literature that had previously been described as either accumulating PHA or were known to use hydrocarbons, which the team observed in its polyethylene pyrolysis product. Of 16 bacterial strains tested, 9 were able to grow on PE pyrolysis product, of which 4 accumulated low levels of medium-chain-length PHA (mcl-PHA, Table 2.1). The monomer composition of the PHA reflected the hydrocarbons being supplied to the bacteria with both even and uneven carbon chains present in the PHA (Fig. 2.5).

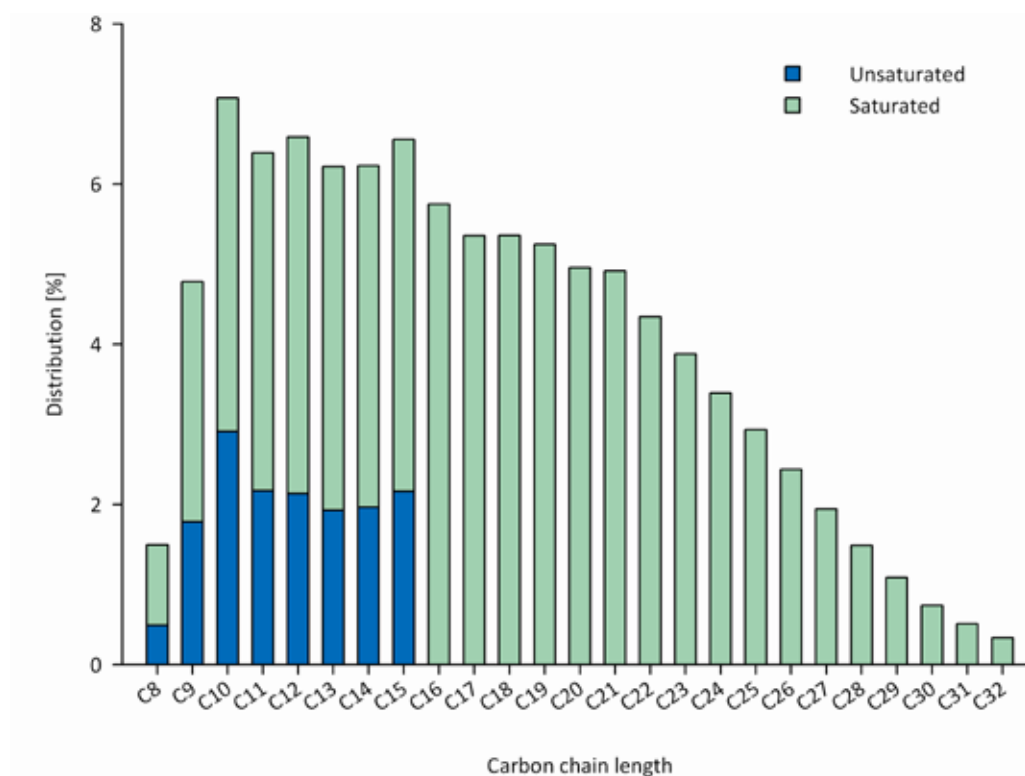


Figure 2.4. Distribution of saturated and unsaturated hydrocarbons in polyethylene (PE) pyrolysis product.

Table 2.1. Literature strains used in shake flask studies and their growth with polyhydroxyalkanoates (PHA) production from polyethylene (PE) pyrolysis product

Strain	CDW * [g L ⁻¹]	PHA [%CDW]	PHA [g L ⁻¹]
<i>A. calcoaceticus</i> BD413	0.20 ± 0.02	n.d	n.d
<i>A. calcoaceticus</i> RR8	0.12 ± 0.03	n.d	n.d
<i>B. cepacia</i> RR10	0.11 ± 0.01	n.d	n.d
<i>P. aeruginosa</i> 3924	0.18 ± 0.07	1.7 ± 0.3	< 0.01
<i>P. aeruginosa</i> GL-1	0.23 ± 0.02	9.8 ± 0.2	0.02 ± 0.00
<i>P. aeruginosa</i> PAO1	0.19 ± 0.02	n.d	n.d
<i>P. aeruginosa</i> RR1	0.29 ± 0.05	0.7 ± 0.1	< 0.01
<i>P. oleovorans</i> (Freitas et al., 2007)	0.32 ± 0.10	3.1 ± 0.3	0.01 ± 0.00
<i>P. putida</i> GO20	0.15 ± 0.09	n.d	n.d

*CDW = cell dry weight; n.d = not detected; PHA = polyhydroxyalkanoates; PE = polyethylene.

In parallel to the use of known bacterial strains, new naturally occurring PHA-accumulating bacterial strains were isolated from diesel-contaminated soil and tested for their ability to convert PE pyrolysis products to PHA. The soil sites chosen were both lightly and heavily contaminated with diesel and appeared as good

prospective environments for hydrocarbon-degrading bacteria to flourish. The isolation methodology applied in this study was a combination of different approaches to ensure successful isolation of novel hydrocarbon-degrading PHA producers (Fig. 2.6). In total, 82 different colonies were isolated out of which three were able

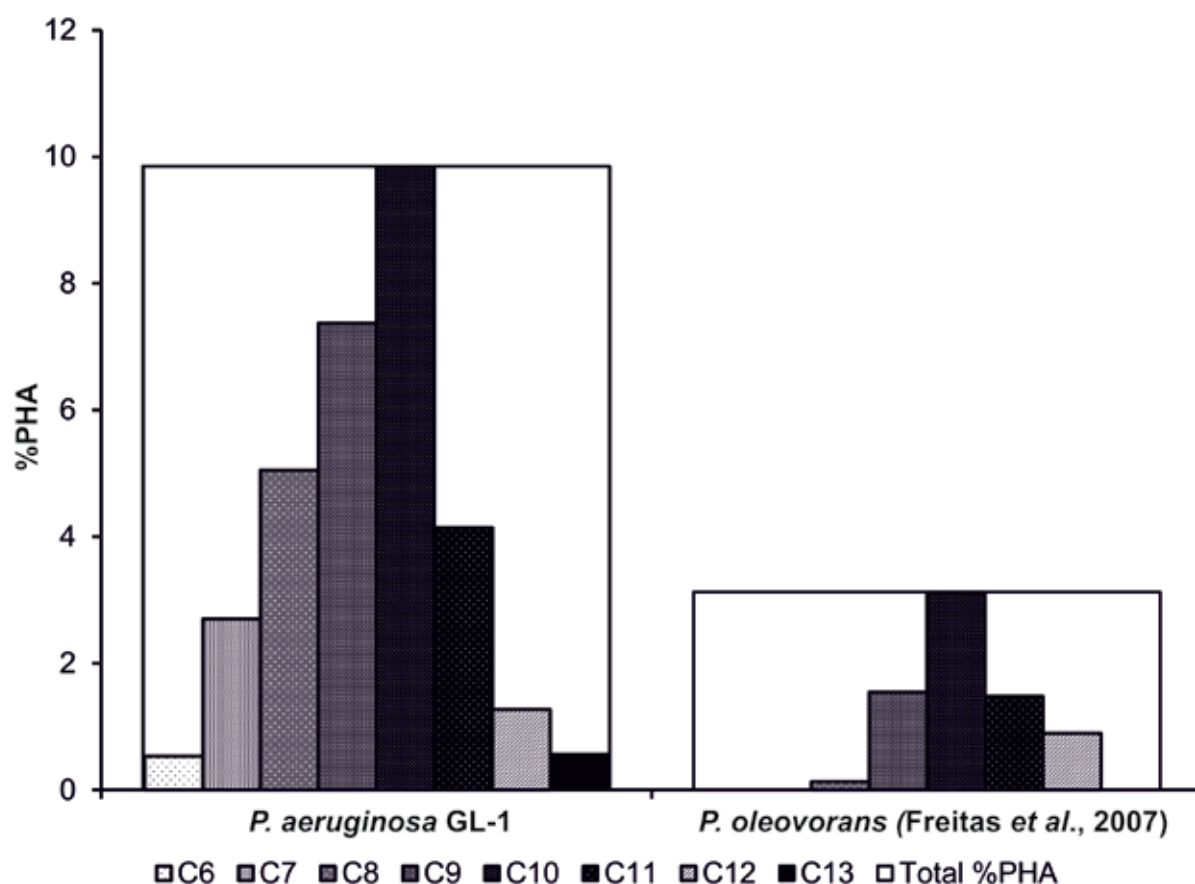


Figure 2.5. Monomer composition of medium-chain-length polyhydroxyalkanoates (mcl-PHA) accumulated from polyethylene (PE) pyrolysis product.

to utilise PE pyrolysis product as a carbon source and produce low levels of PHA. These strains were identified as two *Pseudomonas* spp. and one *Rhodococcus* sp.

Understanding that PE wax from the pyrolysis process is poorly bioavailable to microorganisms (because of its hydrophobic nature), a range of different surfactants were introduced in the liquid cultures in order to enhance the substrate uptake. A range of surfactants was tested, with the best performing strains from these reported in literature and tested here and, as well, with newly isolated microorganisms. One strain/surfactant combination enhanced both growth and PHA production from PE wax – *P. aeruginosa* GL-1 enhanced both biomass and polymer content 1.3 fold in the presence of saponin.

While the conversion of PE pyrolysis product to PHA was possible as described above it was not efficient enough, even in the presence of surfactants, to be scalable for fermentation studies. Thus, the pyrolysis product was modified chemically to make it more accessible to bacteria. The data and approach arising out of this method are the subject of a patent application and so we cannot disclose publicly this part of the process. Bacteria grew well and produced high levels of PHA from the modified pyrolysis product (MPP). The manipulation of the fermentation process with the MPP was undertaken with a view to maximising production of the biodegradable polymer PHA.

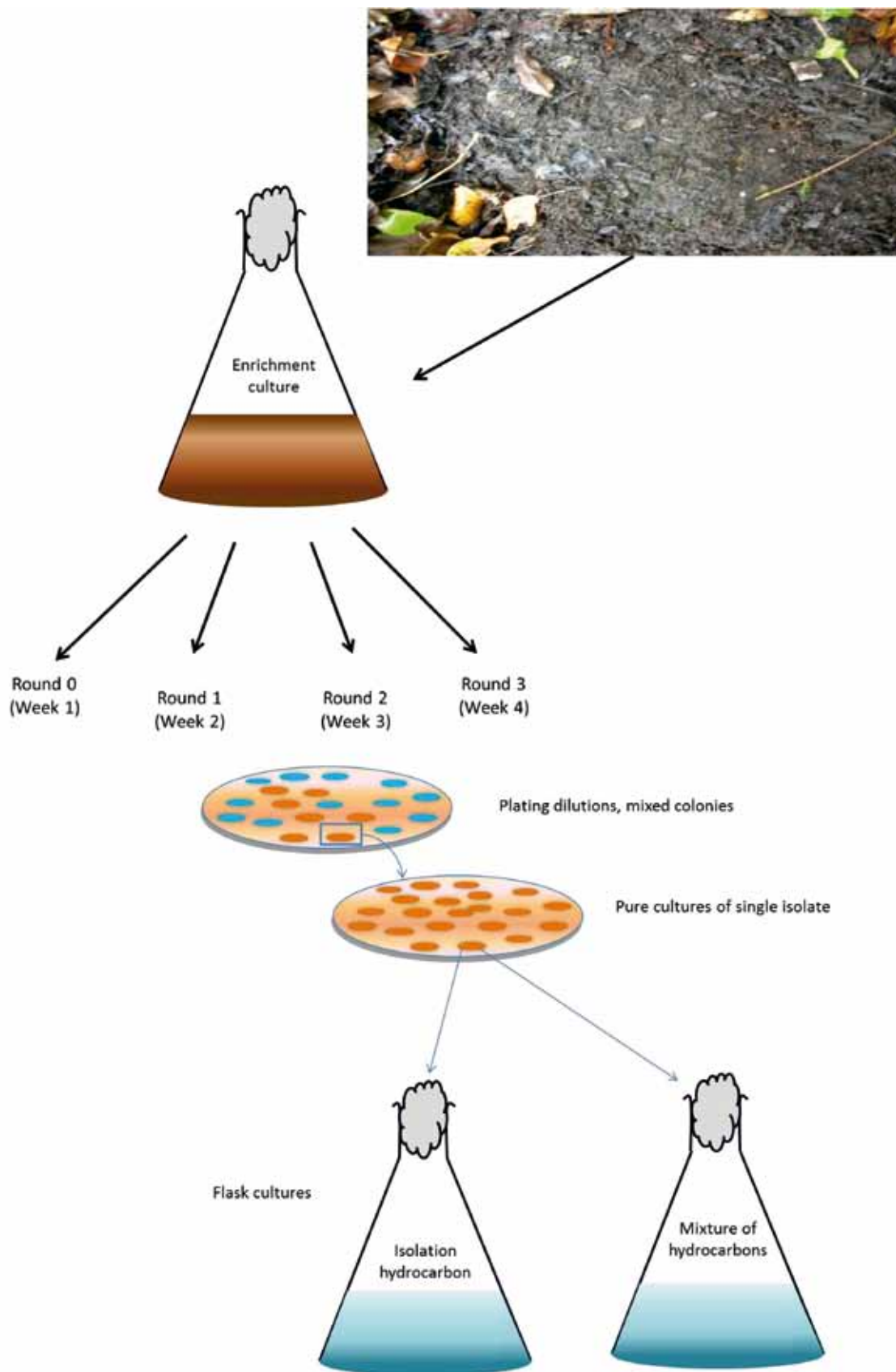


Figure 2.6. Procedure for isolating new bacterial strains capable of growth with polyethylene pyrolysis product from soil. First soil sample was sieved and submitted for liquid enrichment culture. Every week a sample of enrichment culture was plated on solid media, distinct colonies were picked up and submitted for growth and polyhydroxyalkanoate (PHA) accumulation experiments in shake flasks.

2.5 Improvement of the Conversion of Polyethylene to Polyhydroxyalkanoate through Bioprocess Manipulation

Modified PE pyrolysis product was a much better substrate for bacterial growth and PHA production, so this was used as the starting material to maximise PHA productivity in fermentors. Many industrial fermentation processes require rapid growth to a high-cell density. Fed-batch fermentations are considered to be one of the most efficient ways of achieving this goal (Elbahloul and Steinbüchel, 2009; Lee et al., 1999; Sun et al., 2007; Lee, 1996). In several studies where high-cell density culture has been applied to mcl-PHA production a significant improvement in both mcl-PHA and biomass levels has been observed when compared to flask-scale experiments (Sun et al., 2009; Diniz et al., 2004; Kellerhals et al., 1999b). The improved levels of control over variables such as pH and aeration contribute to the improvements in these processes (Lee et al., 1999).

Furthermore, the ability to feed substrates in a controlled manner while monitoring growth conditions continuously through online sampling has led to a higher productivity (Elbahloul and Steinbüchel, 2009; Sun et al., 2006; Lee et al., 2000; Maclean et al., 2008).

A critical stimulus for PHA production by many bacteria is a limitation in a critical nutrient such as nitrogen or phosphorous. Due to this knowledge many substrate-feeding strategies for fed-batch fermentations incorporate an initial phase of fast biomass accumulation followed by nutrient-limiting stage for PHA accumulation (Goff et al., 2007; Kellerhals et al., 1999a; Bourque et al., 1995). However, some mcl-PHA producers do not require such a nutrient limitation and are able to accumulate significant levels of polymer (Durner et al., 2001; Kessler et al., 2001; Sun et al., 2007).

During high-cell density-aerobic processes the high oxygen demand is a common problem (Riesenberg and Guthke, 1999; Sun et al., 2007; Follonier et al., 2012). To avoid or delay oxygen limitation, which prevents achievement of higher-cell densities and productivities, the oxygen uptake rate of the culture should be limited (Maclean et al., 2008).

In order to improve the mechanistic understanding of the process and to aid in process improvement, a mathematical model (see [Fig. 2.7](#)) was constructed of the fermentation process. A description of a system using mathematical concepts and language, it can help to explain a system, study the effects of different components and make predictions about behaviour. It can also aid in gathering system information as the lack of agreement between theoretical models and experimental measurements often leads to important advances as better theories are developed.

Commercially available substrates were used to develop the fermentation model. Once the process for high cell density cultivation of *P. putida* KT2440 with high mcl-PHA content was established, the mathematical predictive model was used which served as a good substrate for biopolymer production.

The first stage of the bioprocess development aimed to maximise biomass (growth) of the bacteria and subsequently the focus was on maximising the mcl-PHA content of each bacterial cell. Finally, the scale-up of this process from 5 L to 30 L was investigated.

2.6 Bioprocess Analysis and Manipulation

The mathematical model allowed experiments to be simulated. The model predicted system behaviour accurately ([Fig. 2.7](#)). As a result, experiments could be simulated using the model instead of being performed in reality. Once the simulation was performed and analysed the wet experiment was then performed. This resulted in huge time saving as a wet experiment was simulated using the model in a matter of seconds, where it would take >48 hours to perform it in reality. The optimisation routine implemented for the process is shown in [Fig. 2.8](#). It was seen that the key to improving the process was finding the ideal way to feed the fatty acid mixture to the cells. Approximately ten different feeding strategies were tested with the model. Amongst these were strategies from the literature and also novel strategies created with the model.

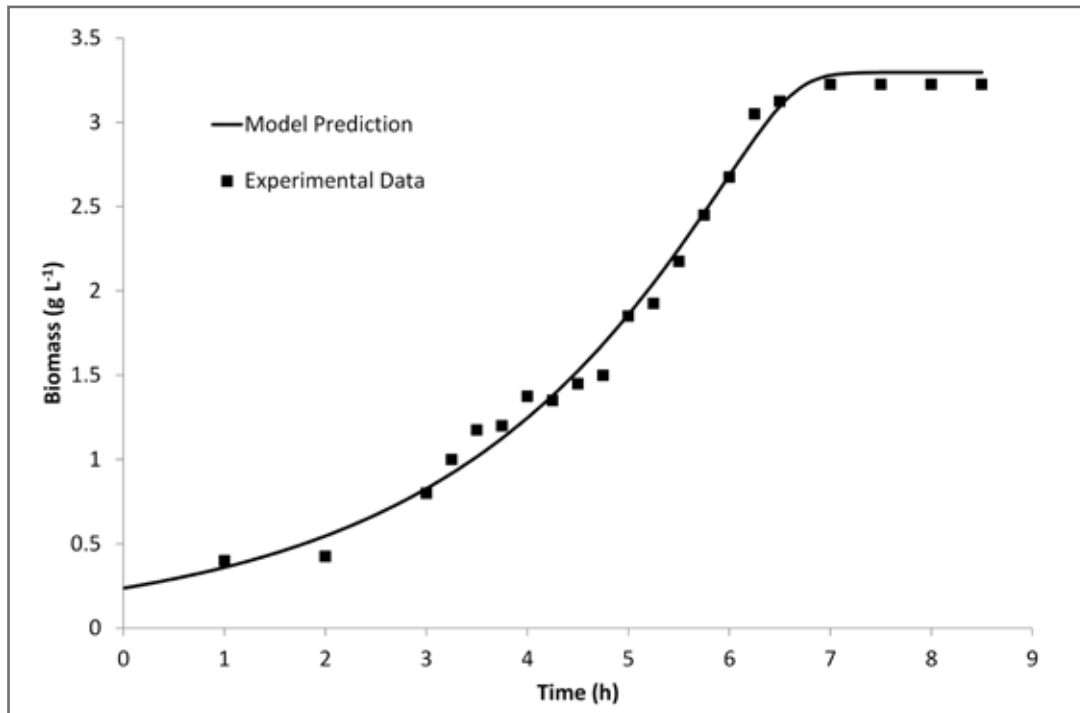


Figure 2.7. Comparison between model prediction and experimental data for fermentation experiment.

The three best performing feeding strategies were then implemented experimentally. This meant that only three separate experiments would have to be performed instead of over ten. This reduced the experimental workload significantly and meant that only promising experiments were conducted.

Of the three feeding strategies executed experimentally, the one which yielded the best results was novel and further developed/improved with the model. The key factor for measuring process performance was PHA productivity. The ultimate goal is to make the PHA productivity as high as possible. A high volumetric PHA productivity ($\text{g L}^{-1} \text{h}^{-1}$) means that not only is a lot of PHA being produced, but it is being produced quickly.

As can be seen in [Table 2.2](#) the novel strategy led to a 16-fold improvement in PHA productivity when compared to results obtained before its use (2.6 vs $0.16 \text{ g L}^{-1} \text{h}^{-1}$). It was also 13% higher than the highest productivity reported in the literature to date (2.6 vs $2.3 \text{ g L}^{-1} \text{h}^{-1}$), even though more complex and expensive methods and substrates for fermentation were used to achieve the literature results (Maclean et al., 2008). After process development, approximately 360 g of PHA was produced in the 5 L fermentor.

In order to produce sufficient quantities of plastic to allow analysis of its properties and to test the reliability of the process developed at 5 L scale, it was decided to scale it up to 20 L. [Table 2.2](#) shows that there was only a slight drop in PHA productivity from 2.6 to $2.5 \text{ g L}^{-1} \text{h}^{-1}$

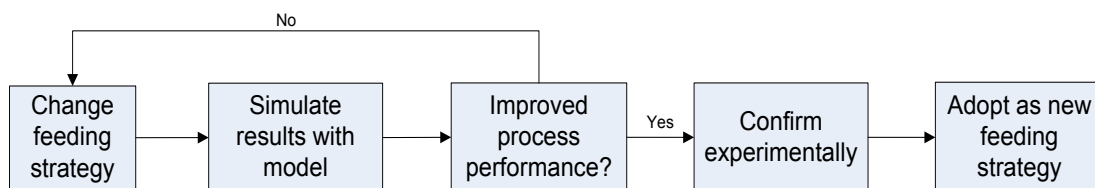


Figure 2.8. Process optimisation routine.

Table 2.2. Summary of optimisation results.

Fermentation	Time [h]	CDW [g L ⁻¹]*	PHA [%]	PHA [g L ⁻¹]	PHA productivity [g L ⁻¹ h ⁻¹]
Before model	48	17.5	45	7.9	0.16
Best strategy	34	127.7	70	89.8	2.6
Novel – 20 L	32	110.4	72	80.9	2.5

* CDW = cell dry weight; n.d – not detected; *CDW = cell dry weight; n.d = not detected; PHA = Polyhydroxyalkanoates; PE = polyethylene.

when the scale was increased. Over 1.1 kg of PHA could be produced per run at 20 L scale. These results suggest that the process currently has the potential to be scaled up for further studies. As sufficient quantities of PHA plastic could be produced, it was isolated from cells and then sent to the material scientists (Dr Ramesh Babu) at Trinity College Dublin (TCD) for analysis to determine its properties and develop it for future applications.

2.7 Investigation of the Genes that encode Critical Pathway Enzymes for PHA Production in Bacteria

To gain an insight into the molecular biology of PHA accumulation by bacteria, two different genes were deleted, both reportedly linked to one of the metabolic routes for PHA production (Nelson et al., 2002). The investigation of gene function can help gain insights into PHA accumulation and potentially design better microorganisms in the future for PHA production. Previous investigations have shown that the polymer content and/or its composition can be affected by removing genes in the PHA production pathway (Lu et al., 2003; Liu et al., 2011; Park et al., 2003; Fiedler et al., 2002; Hume et al., 2009; Park et al., 2005).

The genes deleted did not affect the growth of the bacterial strain *P. putida* KT2440 with a large range of carbon substrates (nutrients). However, PHA production in this gene deletion strain was affected significantly. An increase in biomass was observed when *P. putida* KT2440 gene deletion strain was grown on substrates with longer carbon chains (C11 to C16) when compared to the wild type strain. Interestingly, this biomass increase corresponded to the decrease in PHA accumulation. Thus, the enzymes encoded by these genes in *P. putida* KT2440 are not essential but do contribute to the production of PHA.

2.8 Introduction to Biodegradable Polymers and Nanocomposites

Biodegradable polymers can offer important contributions to modern society by reducing the dependence on fossil fuels and the related environmental impacts. As a result, there is worldwide demand for replacing the petroleum-derived raw materials with renewable resource-based raw materials. While the terms 'biobased polymers' and 'biodegradable polymers' are used extensively (and sometimes interchangeably), there is a difference between the two. A biodegradable polymer is a material that undergoes deterioration of its physical and chemical properties and completely degrades under the influence of microorganisms to carbon dioxide, methane and water. While many biobased polymers can be biodegradable, this is not always the case: for example, biobased polyethylene is not biodegradable: it is called biobased because a biologically renewable substrate was used for its production such as sugar cane. Similarly, biodegradable polymers are not always biobased: polycaprolactone, for instance, is a fossil-based biodegradable polymer. To be considered compostable, materials such as plastics must meet a recognised standard such as EN 13432 (European Parliament and Council of the European Union, 2005).

The first generation of biodegradable polymers focused on deriving the polymers from fossil-fuel-based materials, which includes aliphatic and aromatic polyesters such as polycaprolactone and polybutylene succinate. The focus has shifted in recent years due to significant breakthroughs in biotechnology. Biodegradable polymers similar to conventional polymers can be produced by bacterial fermentation processes by synthesising the building blocks (e.g. succinate) from renewable resources, including lignocellulosic biomass (starch and cellulose) and organic waste.

There are three principal ways to produce biobased and/or biodegradable polymers using renewable resources:

- 1 Use natural polymers with partial modification (e.g. starch = biobased and biodegradable);
- 2 Produce biobased monomers by fermentation or conventional chemistry followed by polymerisation (e.g. polylactic acid [biobased and biodegradable], polybutylene succinate (biobased and biodegradable), polyethylene (biobased not biodegradable);
- 3 To produce biobased/biodegradable polymers directly using bacterial fermentation (e.g. polyhydroxyalkonates biodegradable, which can be biobased or fossil based depending on the starting material).

Most biobased and biodegradable polymers have good mechanical and chemical properties with regards to industry requirements. However, they must compete with petrochemical polymers that have been in development for over 60 years and so often lack the qualities of existing conventional polymers. This is why research into these new biobased and biodegradable polymers is central to making advancements. Biodegradable polymers such as polylactic acid (PLA) have great commercial potential for bioplastic, but some of the properties such as brittleness, low heat distortion temperature, low gas permeability, low melt viscosity for further processing and so on restrict their use in a wide range of applications. Since the 1980s, nanotechnology has been widely applied to polymeric materials, following the discovery that nanocomposites could be made from polymer and layered nanoclays (Okamoto, 2004).

The most popular process is to introduce nanoscale particles such as layered silicates (nanoclays), carbon nanotubes, metal nanoparticles and cellulose nanowhiskers into the polymer matrix to produce polymer/nanoparticle composites (called polymer nanocomposite) by conventional melt-processing techniques. Nanoclays are basically hydrous aluminium silicates with dimensions in the order of 1 nm thick, 150 nm wide and around a micron long with a high aspect ratio (Utracki et al., 2007). Nanoclays

can be obtained from both natural and synthetic sources and are found in vast deposits around the world. Thanks to innovative research (Sinha Ray and Okamoto, 2003), nanotechnology has also been successfully used to produce biodegradable polymer nanocomposite materials with high performance and improved mechanical properties. Among all the polymer nanocomposite technologies, polymer-nanoclay composites are of particular interest because of their demonstrated ability to produce polymer nanocomposites with enhanced properties on an industrial scale for various applications. The improvement in the properties can be obtained at lower nanoclay content (<5 wt%).

Unmodified, natural and purified nanoclays are extremely hydrophilic (water loving) and fundamentally incompatible with most polymers. This hinders the dispersion of nanoclays within the polymer host and requires nanoclay modification to enhance compatibility with polymer and nanoclay. The conventional way to overcome this problem is to modify the nanoclays with surfactants (soaps) to enhance the compatibility. The chemistry of the surfactant dictates the improvement in the properties of polymer nanocomposite. For the purpose of the project, synthetic and natural nanoclays were sourced from various commercial suppliers around the world.

2.9 Polyhydroxyalkanoate

More than 150 PHA monomers have been identified as the constituents of PHAs. Such diversity allows for the production of biobased polymers with a wide range of properties which can be used for a variety of applications. PHB has received the greatest attention in terms of industrial-scale production, with companies in both the US and China producing PHB and PHB copolymers. However, the type of PHA produced in this project (medium-chain-length PHA) has not been well studied nor developed for applications. This has provided the project partners with the impetus to proceed with new potential developments with the polymer.

Table 2.3. Material properties of various biodegrade polymers along with mcl-PHA polymers produced in the study.

Polymer	Tg [°C]	Tm [°C]	Mn [kDa]	Mw [kDa]	PolyD
mcl-PHA (from the Project)	-44–40	36–40	40–43	140–200	1.7–2.5
PHB	-5–5	170–180	100–500	up to 1500	1.5–3.0
PHO	-33.1	58.0	119	286	2.4
PLA	60.0	175.0	28–192	50–500	1.8–2.6
PCL	-60	60	10–15	14–25	1.4–1.6
PVA	32	–	–	–	–
PBS	-41.0	113.1	30	70	2.5

mcl-PHA = medium-chain-length polyhydroxyalkanoates, Mn = average molecular weight, Mw = weight average molecular weight, n.d. = not detected, PBS = polybutylene succinate, PCL = polycaprolactone, PLA = polylactic acid, PHB = polyhydroxybutyrate, PHO = polyhydroxyoctonate, PolyD = Mw/Mn = polydispersity, PVA = polyvinyl acetate, Tg = glass transition temperature, Tm = melting temperature.

The current project focuses on mcl-PHA which has fundamentally different physical, thermal and mechanical properties to conventional PHB polymers (Table 2.3). The temperature at which the PHA polymer produced in this project becomes glasslike (the ‘glass transition temperature’) is around -40°C with temperatures at which the polymer melts (becomes liquid) at between 33.1°C and 36°C. The temperature at which thermal degradation/destruction of the polymer occurs is in the range of 276.6 and 293.9°C. The polymers produced have molecular weights in the range of 140–200 kDa (weight average molecular weight) with a polydispersity index of 1.7–2.5, which is common for bacterial mcl-PHAs produced by fermentation process.

2.10 Biopolymer – mcl-PHA Blends

While most biodegradable polymers have great potential due to their properties and biodegradability the development of these polymers as end products is challenging. Biodegradable and biobased polymers cannot be accommodated in all types of processing techniques used for traditional polymer materials, and do not meet specific property requirements such as tensile strength, elongation, biodegradability, and so on, for some applications.

It is well known that polymer/polymer blending is an effective method for improving the properties of one or both of the components, or for preparing new polymeric

materials with improved properties at low cost. Generally, there are two ways to produce polymer composites: (i) mixing them in the solutions and (ii) blending the components in the molten state. The latter is much more favourable in industry, since the production process can take place in conventional processing equipment with high production rates.

The current project was designed to prepare blend composites of various commercial biopolymers with mcl-PHA produced from PE waste. Mcl-PHA:biodegradable polymer blend composites were produced using commercial aliphatic polyesters, and naturally occurring polymers to improve the mechanical properties of both polymers. All the composites were prepared on lab-scale melt-processing equipment (Brabender). The mcl PHA blend composites showed improvement in elongation and toughness. The use of nanoclays resulted in further improvements in the mechanical properties of the mcl-PHA blends. Shore hardness is a surface property of the polymer blend, which determines the plastic deformation of the polymer blend and softness of the material. Shore hardness of the mcl-PHA blend composites with prepared (various) amounts of aliphatic polyester was measured using shore D type durometer. From the data it was evident that polymer blends with mcl-PHA are softer, which indicates that these novel blends can be processed at lower temperatures, which can reduce the energy consumption.

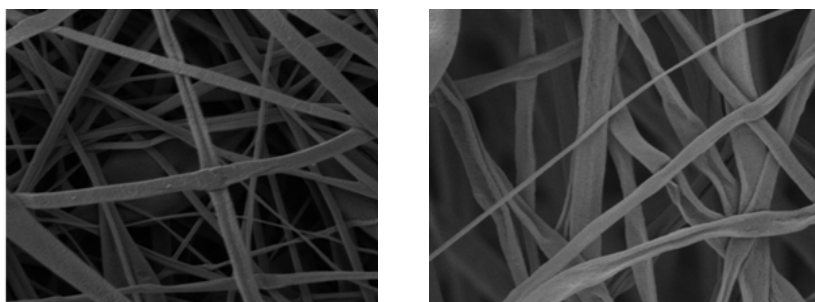


Figure 2.9. Electro-spun nanofibres produced from medium-chain length polyhydroxyalkanoate (mcl-PHA) blends.

One major problem with many biodegradable polymer composites, especially PHB-based polymers, is the ageing of mechanical properties over a period of time. The mechanical properties of mcl-PHA blend composites were monitored over a 9-month period during the course of the project. Composites prepared with mcl-PHA retained their mechanical properties without a significant decrease in the elongation, toughness and other mechanical properties.

Electro-spun fibres are of high interest in applications ranging from regenerative medicine and drug delivery, through scaffolds and nanocomposites and to energy storage and biomedical devices and new technical applications. Electro-spinning, an electro-hydrodynamic process, is a versatile and promising platform technology for the production of nanofibrous materials consisting of diverse polymers and polymer

composites. Mcl-PHA blends developed in the project were processed using an electro-spinning process to produce nanofibre mats, which have potential application in scaffolds. The fibres produced by this process are in the nano and micrometer scale ([Fig. 2.9](#)). The mechanical properties of the nanofibres indicated that blends with mcl-PHA had improved mechanical properties over biopolymers alone

Polymer blend composites prepared with various compositions of PHA and nanoclays were evaluated for their *in vitro* cytotoxicity using human lung epithelial cells ([Fig. 2.10](#)). The preliminary results indicated that blend composites did not show any toxicity toward human lung epithelial cells, which indicates that blend composites may have potential applications for making medical devices that can be used for humans.

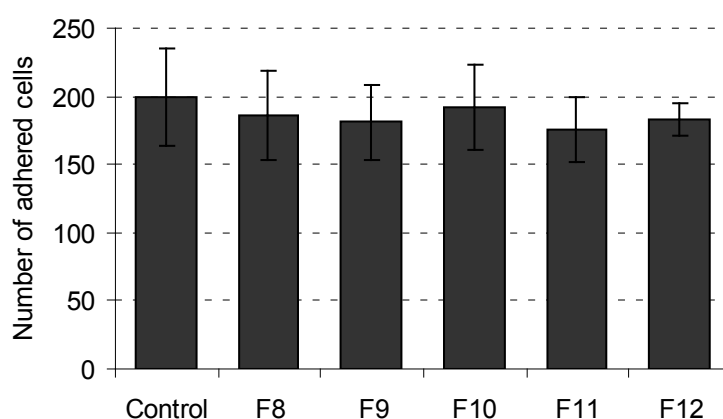


Figure 2.10. Lung epithelial cell viability on various medium-chain length polyhydroxyalkanoate (mcl-PHA) blend composites for 24 hrs. As part of nano reinforcement, mcl-PHA blend composites were prepared to study the effect on mechanical and electrical properties of the blend composites. The initial results indicated the addition of graphene could improve the mechanical properties.

The potential applications of PHA include its use in medical devices and other related applications. As a result, it is very important to understand the toxicity of this polymer towards human cells. *In vitro* cytotoxicity of PHA was evaluated using lung epithelial human cells.

The analysis showed no difference in cell viability or adhesion between cells incubated in the presence or absence of mcl-PHA (Fig. 2.11). Thus, mcl-PHA is non-toxic to the human epithelial cells.

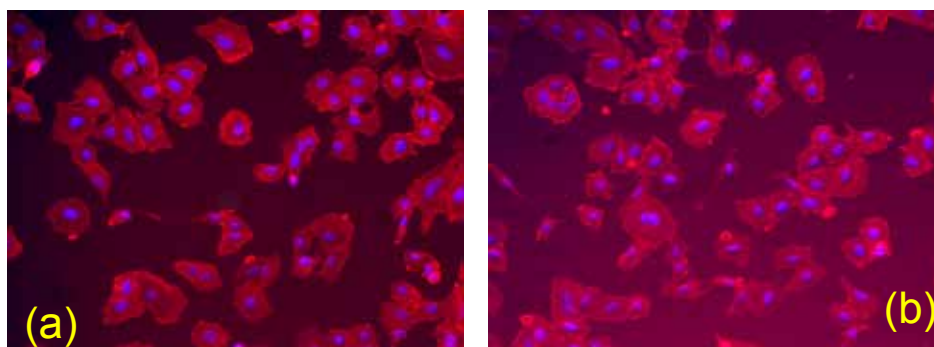


Figure 2.11. Viability cell staining images of (a) blank samples and (b) medium-chain-length polyhydroxyalkanoate exposed to epithelial cells for 2 hrs.

3 Output and Final Recommendations

3.1 Major Conclusions

Ireland faces a significant global challenge to manage its resources, one of which is its waste. From this comes an important opportunity to develop environmental technologies and products. Such developments can act as a platform for a reduced dependency on imported or fossil resources, economic growth and progress towards a sustainable Irish society.

The Waste Framework Directive (European Parliament and Council of the European Union, 2008) and the Europe 2020 objective for a resource-efficient economy are clear indicators of the intent at European level to improve the management of all resources, including waste, which will bring economic, environmental and social benefits. A key enabler of this objective will be the development of new technologies and products with reduced environmental impacts.

Global challenges require new thinking and the combination of expertise. The bringing-together of Irish researchers with diverse skills from multiple disciplines through significant EPA funding was essential to developing a world-first 'polyethylenylene to biodegradable plastic' technology.

A second critical tier to the complex team-building required for technology development is the industry–academic interactions. While both parties often have different objectives and timelines common ground can be found to create a meaningful partnership.

This project used the team's academia–industry partnerships to shape and develop a technology to convert waste farm-based polyethylene to a biodegradable polymer (polyhydroxyalkanoate) using a three-step process, but this technology is applicable to any source of polyethylene. The biodegradable polymer product was processed for applications as an adhesive and in films. The conversion technology and polymer product are being developed by a UCD spin-out company Bioplastech for implementation in Ireland and internationally.

Interactions between the research community, industry, and government agencies and departments (e.g. DECLG and EPA) inform partners about the policy and market landscape in which they are operating and about wider technological developments. Technology development is not an overnight success and requires adaptation, flexibility and vision from all parties.

3.2 Major Project Outputs

- Global first for the conversion of farm-plastic polyethylene to the biodegradable polymer;
- Protected intellectual property for the polyethylenylene waste to biodegradable plastic technology;
- Development of applications for the biodegradable product using nanotechnology;
- Protected intellectual property for uses of the biodegradable polymer;
- Project technology has been licensed to a UCD spin-out company Bioplastech for commercial development.

3.3 Final Recommendations

- The conversion of non-degradable plastics to biodegradable plastics is possible. Such technologies can contribute to the efforts at a national and European level to recover waste as a resource and divert waste from landfill. The technology has the potential to address the global issue of plastic waste management which is of paramount importance socially (rx3, 2011; Plastics Europe, 2011) and politically (European Commission, 2011a).
- Scale-up facilities in Ireland are non-existent for environmental technologies. This is the single biggest barrier to the development of technologies and products beyond laboratory level and into the market place.

- The support of scale-up activities for environmental technologies such as that described herein are critical if such a technology is to emerge from the laboratory into the industrial sphere.
- The integration of disciplines and technologies is critical in order to address complex issues and process chains such as waste management. Integrated flagship projects involving multiple disciplines and players need to exist in Ireland to build capability and capacity. Such integration allows the development and improvement of one component of a technology that can inform the development of another so that integration has a greater chance of success.
- The development of an integrated flagship waste refinery in Ireland for the production of value added products such as (bio)fuels, (bio)polymers and (bio)chemicals can become a reality with the development of industry/academic partnerships.
- A waste refinery in Ireland would enable universities and industry to come together to eco-innovate, which is a key objective of the Eco-innovation action plan.

References

- AL-SALEM, S. M., LETTIERI, P. & BAEYENS, J. 2009. Recycling and recovery routes of plastic solid waste (PSW): a review. *Waste Management*, 29, 2625–43.
- ALEXANDER, M. 1975. Environmental and microbiological problems arising from recalcitrant molecules. *Microbial Ecology*, 2, 17–27.
- BOURQUE, D., POMERLEAU, Y. & GROLEAU, D. 1995. High-cell-density production of poly- β -hydroxybutyrate (PHB) from methanol by *Methylobacterium extorquens*: production of high-molecular-mass PHB. *Applied Microbiology and Biotechnology*, 44, 367–76.
- CYNAR 2011. The Cynar Technology, <http://www.cynarplc.com> (access date 1 May 2011).
- DEPARTMENT OF THE ENVIRONMENT, C. L. G. 2011. Our Sustainable Future. A Framework for Sustainable Development for Ireland.
- DEPARTMENT OF THE TAOISEACH 2008. Building Ireland's Smart Economy. A Framework for Sustainable Economic Renewal.
- DINIZ, S. C., MARILDA KEICO TACIRO, M. K., GOMEZ, J. C. & DA CRUZ PRADELLA, J. G. 2004. High-Cell-Density cultivation of *Pseudomonas putida* IPT 046 and medium-chain-length polyhydroxyalkanoate production from sugarcane carbohydrates. *Applied Biochemistry and Biotechnology*, 119, 51–69.
- DURNER, R., ZINN, M., WITHOLT, B. & EGLI, T. 2001. Accumulation of poly((R)-3-hydroxyalkanoates) in *Pseudomonas oleovorans* during growth in batch and chemostat culture with different carbon sources. *Biotechnology and Bioengineering*, 72, 278–88.
- ELBAHLOUL, Y. & STEINBUCHER, A. 2009. Large-Scale Production of Poly(3-Hydroxyoctanoic Acid) by *Pseudomonas putida* GPo1 and a Simplified Downstream Process. *Applied Microbiology and Biotechnology*, 75, 643–51.
- EUROPEAN COMMISSION 2011a. Plastic waste in the environment. *BIO Intelligence Service*, 1, 171.
- EUROPEAN COMMISSION 2011b. Roadmap to a Resource-efficient Europe. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions, Brussels 20.09.2011.
- EUROPEAN PARLIAMENT & COUNCIL OF THE EUROPEAN UNION 2005. Requirements for packaging recoverable through composting and biodegradation – Testing scheme and evaluation criteria for the final acceptance of packaging. *Official Journal of the European Union*, C (44).
- EUROPEAN PARLIAMENT & COUNCIL OF THE EUROPEAN UNION 2008. Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives. *Official Journal of the European Union*, L 312/3.
- FIEDLER, S., STEINBÜCHEL, A. & REHM, B. 2002. The role of the fatty acid B-oxidation multienzyme complex from *Pseudomonas oleovorans* in polyhydroxyalkanoate biosynthesis: molecular characterization of the *fadBA* operon from *P. oleovorans* and of the enoyl-CoA hydratase genes *phaJ* from *P. oleovorans* and *Pseudomonas putida*. *Archives of Microbiology*, 178, 149–60.
- FOLLONIER, S., HENES, B., PANKE, S. & ZINN, M. 2012. Putting cells under pressure: A simple and efficient way to enhance the productivity of medium-chain-length polyhydroxyalkanoate in processes with *Pseudomonas putida* KT2440. *Biotechnology and Bioengineering*, 109, 451–61.
- FREITAS, F., PAIS, J., OLIVEIRA, C., ALVES, V. D., OLIVEIRA, R. & REIS, M. A. M. 2007. Co-production of a galactose-rich polysaccharide and polyhydroxyalkanoates by a *Pseudomonas* strain using glycerol as carbon source. *European Symposium on Biopolymers*, Turkey, 1, 109.
- GOFF, M., WARD, P. G. & O'CONNOR, K. E. 2007. Improvement of the conversion of polystyrene to polyhydroxyalkanoate through the manipulation of the microbial aspect of the process: A nitrogen feeding strategy for bacterial cells in a stirred tank reactor. *Journal of Biotechnology*, 132, 283–6.
- HUME, A. R., NIKODINOVIC-RUNIC, J. & O'CONNOR, K. E. 2009. *FadD* from *Pseudomonas putida* CA-3 is a true long-chain fatty acyl coenzyme A synthetase that activates phenylalkanoic and alkanoic acids. *J Bacteriol.*, 191, 7554–65.
- KELLERHALS, M., HAZENBERG, W. & WITHOLT, B. 1999a. High cell density fermentations of *Pseudomonas oleovorans* for the production of mcl-PHAs in two-liquid phase media. *Enzyme and Microbial Technology*, 24, 111–16.
- KELLERHALS, M., KESSLER, B. & WITHOLT, B. 1999b. Closed-loop control of bacterial high-cell-density fed-batch cultures: Production of mcl-PHAs by *Pseudomonas putida* KT2442 under single-substrate and cofeeding conditions. *Biotechnology and Bioengineering*, 65, 306–15.

- KENNY, S. T., RUNIC, J. N., KAMINSKY, W., WOODS, T., BABU, R. P., KEELY, C. M., BLAU, W. & O'CONNOR, K. E. 2008. Up-cycling of PET (Polyethylene Terephthalate) to the biodegradable plastic PHA (Polyhydroxyalkanoate). *Environmental Science & Technology*, 42, 7696–701.
- KESSLER, B., WEUSTHUIS, R., WITHOLT, B. & EGGINK, G. 2001. Production of microbial polyesters: Fermentation and downstream processes. *Advances in Biochemical Engineering/Biotechnology*, 71, 160–82.
- LEE, S. Y. 1996. High cell-density culture of *Escherichia coli*. *Trends in Biotechnology*, 14, 98–105.
- LEE, S. Y., CHOI, J. & WONG, H. H. 1999. Recent advances in polyhydroxyalkanoate production by bacterial fermentation: mini-review. *International Journal of Biological Macromolecules*, 25, 31–6.
- LEE, S. Y., WONG, H. H., CHOI, J., LEE, S. H., LEE, S. C. & HAN, C. S. 2000. Production of medium-chain-length polyhydroxyalkanoates by high-cell-density cultivation of *Pseudomonas putida* under phosphorus limitation. *Biotechnology and Bioengineering*, 68, 466–70.
- LEMOIGNE, M. 1926. Products of dehydration and polymerization of β -hydroxybutyric acid. *Bull. Soc. Chem. Biol.*, 8, 770–82.
- LIU, Q., LUO, G., ZHOU, X. R. & CHEN, G. Q. 2011. Biosynthesis of poly(3-hydroxydecanoate) and 3-hydroxydodecanoate dominating polyhydroxyalkanoates by beta-oxidation pathway inhibited *Pseudomonas putida*. *Metabolic Engineering*, 13, 11–7.
- LU, X., ZHANG, J., WU, Q. & CHEN, G.-Q. 2003. Enhanced production of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) via manipulating the fatty acid [beta]-oxidation pathway in *E. coli*. *FEMS Microbiology Letters*, 221, 97–101.
- MACLEAN, H., SUN, Z., RAMSAY, J. & RAMSAY, B. 2008. Decaying exponential feeding of nonanoic acid for the production of medium-chain-length poly(3-hydroxyalkanoates) by *Pseudomonas putida* KT2440. *Canadian Journal of Chemistry*, 86, 564–69.
- NELSON, K. E., WEINEL, C., PAULSEN, I. T., DODSON, R. J., HILBERT, H., MARTINS DOS SANTOS, V. A. P., FOUTS, D. E., GILL, S. R., POP, M., HOLMES, M., BRINKAC, L., BEANAN, M., DEBOY, R. T., DAUGHERTY, S., KOLONAY, J., MADUPU, R., NELSON, W., WHITE, O., PETERSON, J., KHOURI, H., HANCE, I., LEE, P. C., HOLTZAPPLE, E., SCANLAN, D., TRAN, K., MOAZZEZ, A., UTTERBACK, T., RIZZO, M., LEE, K., KOSACK, D., MOESTL, D., WEDLER, H., LAUBER, J., STJEPANDIC, D., HOHEISEL, J., STRAETZ, M., HEIM, S., KIEWITZ, C., EISEN, J., TIMMIS, K. N., DÜSTERHÖFT, A., TÜMMER, B. & FRASER, C. M. 2002. Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440. *Environmental Microbiology*, 4, 799–808.
- OKAMOTO, M. 2004. Biodegradable polymer/layered silicate nanocomposites: a review. *Journal of Industrial and Engineering Chemistry*, 10, 1156–81.
- PARK, S. J., CHOI, J.-I. & LEE, S. Y. 2005. Engineering of *Escherichia coli* fatty acid metabolism for the production of polyhydroxyalkanoates. *Enzyme and Microbial Technology*, 36, 579–88.
- PARK, S. J., PARK, J. P., LEE, S. Y. & DOI, Y. 2003. Enrichment of specific monomer in medium-chain-length poly(3-hydroxyalkanoates) by amplification of *fadD* and *fadE* genes in recombinant *Escherichia coli*. *Enzyme and Microbial Technology*, 33, 62–70.
- PLASTICS EUROPE 2011. *Plastics – the Facts 2011*. An analysis of European plastics production, demand and recovery for 2010. *PlasticsEurope AISBL*
- REDDY, C., GHAI, R., RASHMI & KALIA, V. 2003. Polyhydroxyalkanoates: an overview. *Bioresource Technology*, 87, 137–46.
- RIESENBERG, D. & GUTHKE, R. 1999. High-cell-density cultivation of microorganisms. *Applied Microbiology and Biotechnology*, 51, 422–30.
- RX3 2011. *The Irish Recycled Plastic Waste Arisings Study*. rx3, 130.
- SINHA RAY, S. & OKAMOTO, M. 2003. Polymer/layered silicate nanocomposites: a review from preparation to processing. *Progress in Polymer Science*, 28, 1539–641.
- SUN, Z., RAMSAY, J., GUAY, M. & RAMSAY, B. 2007a. Fermentation process development for the production of medium-chain-length poly-3-hydroxyalkanoates. *Applied Microbiology and Biotechnology*, 75, 475–85.
- SUN, Z., RAMSAY, J., GUAY, M. & RAMSAY, B. 2009. Enhanced yield of medium-chain-length polyhydroxyalkanoates from nonanoic acid by co-feeding glucose in carbon-limited, fed-batch culture. *J. Bacteriol.*, 143, 262–7.
- SUN, Z., RAMSAY, J. A., GUAY, M. & RAMSAY, B. A. 2006. Automated feeding strategies for high-cell-density fed-batch cultivation of *Pseudomonas putida* KT2440. *Applied Microbiology and Biotechnology*, 71, 423–31.
- SUN, Z., RAMSAY, J. A., GUAY, M. & RAMSAY, B. A. 2007b. Carbon-limited fed-batch production of medium-chain-length polyhydroxyalkanoates from nonanoic acid by *Pseudomonas putida* KT2440. *Applied Microbiology and Biotechnology*, 74, 69–77.
- SUN, Z., RAMSAY, J. A., GUAY, M. & RAMSAY, B. A. 2007c. Fermentation process development for the production of medium-chain-length poly-3-hydroxyalkanoates. *Applied Microbiology and Biotechnology*, 75, 475–85.

UTRACKI, L. A., SEPEHR, M. & BOCCALERI, E. 2007. Synthetic, layered nanoparticles for polymeric nanocomposites (PNCs). *Polymers for Advanced Technologies*, 18, 1–37.

WARD, P. G., GOFF, M., DONNER, M., KAMINSKY, W. & O'CONNOR, K. E. 2006. A two step chemo-biotechnological conversion of polystyrene to a biodegradable thermoplastic. *Environmental Science and Technology*, 40, 2433–37.

Acronyms and Annotations

CDW	Cell dry weight
DECLG	Department of Environment Community and Local Government
EC	European Commission
mcl-PHA	Medium chain length polyhydroxyalkanoates
Mn	Average molecular weight
MPP	Modified pyrolysis product
Mw	Weight average molecular weight
n.d	Not detected
PBS	Polybutylene succinate
PCL	Polycaprolactone
PE	Polyethylene
PET	Polythene terephthalate
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PHO	Polyhydroxyoctonate
PLA	Polylactic acid
PVA	Polyvinyl acetate
Tg	Glass transmission temperature
Tm	Melting temperature.
TCD	Trinity College Dublin
UCD	University College Dublin

Appendix 1 – Selected Outputs of Project

Research Outputs (Peer-reviewed Publications)

Three manuscripts have been prepared for submission. However, due to commercially sensitive information and data, these publications cannot yet be made public until appropriate patents have been filed. Invention disclosures have been made to NovaUCD in collaboration with TCD technology transfer office and we are waiting further instruction. We expect to be in a position to publish in the second half of 2013.

Conference Presentations (Oral)

Event	Authors	Title
2nd European Medical Polymers Conference, Society of Plastics Engineers (SPE) Polymer Processing Research Centre, Queen's University Belfast, Ireland, 07–09 September 2010.	Trevor W. Woods, Werner J. Blau, Kevin O'Connor, Ramesh P. Babu	Mechanical, thermal, morphological and biodegradation properties of polylactide/polyhydroxybutyrate blends
8th Annual EPA Post-graduate Seminar at the Dublin Conference Centre, 11 November 2010.	S. T. Kenny, M. Guzik, J. Nikodinovic-Runic, W. Kaminski, T. Woods, R.P. Babu, C.M. Keely, K.E. O'Connor	Conversion of waste products to value added biodegradable plastic
Green chemistry/Environmental Technologies Conference organised by the Society for Chemical Industry (SCI) in Dublin City University, 15 April 2010.	Shane Kenny M. Guzik, J. Nikodinovic-Runic, Woods, R.P. Babu, K.E. O'Connor	The upcycling of waste to the biodegradable polymer PHA
'Novel nanostructured polymeric materials for food packaging and beyond' conference (Part of European COST programme) in Espoo (Finland), 15–16 September 2011.	Trevor W. Woods, Werner J. Blau, Kevin O'Connor, Ramesh P. Babu	Thermal and mechanical properties of PLA/PHB nanoclay composites
European Medical Polymers Conference, Queen's University Belfast, 7–9 September 2010. Organised by the Society of Plastics Engineers.	Trevor W. Woods, Werner J. Blau, Kevin O'Connor, Ramesh P. Babu	Mechanical, thermal, morphological and biodegradation properties of polylactide/polyhydroxybutyrate blends
2011 European Symposium on Biopolymers (ESBP 2011), co-hosted by UCD and TCD, 27–29 September 2011, Science Gallery, Trinity College, Dublin	S. T. Kenny, M. Guzik, J. Nikodinovic-Runic, W. Kaminski, T. Woods, R.P. Babu, C.M. Keely, K.E. O'Connor	The conversion of waste to the biodegradable polymer PHA
Polymer Processing Society Americas Conference 2012, Niagara, Ontario, Canada, 21–24 May 2012, under the 'Biodegradable and Sustainable Polymers' section of the conference.	Trevor W. Woods, Werner J. Blau, Kevin O'Connor, Ramesh P. Babu	PLA-PHB nanoclay composites
International Symposium on BiPolymers (ISBP 2012), Cairns, Australia, 7–10 October 2012. Two oral presentations given.	Maciej Guzik, Shane Kenny, Stephen O Connor, Emilia Szwej, William Gallagher, Jasmina Nikodinovic, Annete Byrne, Marc Devocelle, Ramesh Babu, Trevor Woods, Manfred Zinn, Qun Ren, and Kevin O'Connor Maciej Guzik, Shane Kenny, Ramesh Babu, Trevor Woods, Manfred Zinn, Qun Ren, and Kevin O'Connor	Applications of PHA and PHA monomers Bioconversion of aliphatic hydrocarbons to a biodegradable polymer - polyhydroxyalkanoate (PHA)

Conference Posters

Event	Authors	Title
International Symposium on BioPolymers (ISBP 2010), Stuttgart, Germany, 3–7 October 2010.	M. Guzik, A. Hume, J. Nikodinovic-Runic, K.E. O'Connor	Investigation of the role of β -oxidation upper pathway genes in PHA accumulation
UCD Festival of Research and Innovation; Earth Sciences, Energy & the Environment, Dublin, 8 December 2010.	S. T. Kenny, M. Guzik, J. Nikodinovic-Runic, W. Kaminski, T. Woods, R.P. Babu, C.M. Keely, K.E. O'Connor	Conversion of waste products to value added biodegradable plastic
EPA National Research Conference 2010, Croke Park, June 2010. Two posters presented.	Jasmina Nikodinovic Runic, Shane T. Kenny, Maciej Guzik, Kevin O'Connor. Shane T. Kenny, Jasmina Nikodinovic Runic, Walter Kaminsky, Ramesh P. Babu, Kevin O'Connor	The conversion of BTEX compounds by single and defined mixed cultures to medium chain length polyhydroxyalkanoate The upcycling of waste to the biodegradable Polymer Polyhydroxyalkanoate (PHA)
1st European Congress of Applied Biotechnology, Berlin, Germany, 25–29 September 2011.	G. Duane, M. Guzik, S. Kenny, E. Casey, K. O'Connor .	Mathematical modelling of a bioprocess for PHA production
2011 European Symposium on Biopolymers (ESBP 2011), co-hosted by UCD and TCD, 27–29 September 2011, Science Gallery, TCD. Two posters presented.	Maciej Guzik, Siofra Kelly, Shane Kenny, Kevin E. O'Connor S. T. Kenny, M. Guzik, J. Nikodinovic-Runic, W. Kaminski, T. Woods, R.P. Babu, C.M. Keely, K.E. O'Connor	Isolation of new hydrocarbon degrading bacteria capable of polyhydroxyalkanoate (PHA) accumulation The upcycling of waste to the biodegradable Polymer Polyhydroxyalkanoate (PHA)
EPA-STRIVE Research Conference 2012, TCD, 28 June 2012.	Maciej Guzik, Siofra Kelly, Shane Kenny, Kevin E. O'Connor	Isolation of new hydrocarbon degrading bacteria capable of polyhydroxyalkanoate (PHA) accumulation

Conference Organisation

- The 2011 European Symposium on Biopolymers conference (ESBP 2011) was organised by the members of this project and co-hosted by UCD and TCD from the 27–29 September 2011, in the Science Gallery, TCD.

Awards

- Maciej Guzik was awarded a prize for his poster (2nd Place) at the 2010 UCD Festival of Research and Innovation; Earth Sciences, Energy & the Environment, Dublin, 8th December 2010 for his poster titled 'Conversion of waste products to value added biodegradable plastic. S. T. Kenny, M. Guzik, J. Nikodinovic-Runic, W. Kaminski, T. Woods, R.P. Babu, C.M. Keely, K.E. O'Connor'.
- Shane received the Best Poster Award at the 2011 European Symposium on Biopolymers (ESBP 2011) held at the TCD Science Gallery, TCD on the 27–29 September 2011, for his poster titled 'The conversion of waste to the Biodegradable Polymer PHA' S. T. Kenny, M. Guzik, J. Nikodinovic-Runic, W. Kaminski, T. Woods, R.P. Babu, C.M. Keely, K.E. O'Connor'.

Thesis Submissions

- Based on his work on this research programme, Maciej Guzik successfully passed his *Viva Voce* on 10 September 2012. His PhD title is 'Chemo-biotechnological conversion of polyethylene to the biodegradable polymer polyhydroxyalkanoate'.

Media/Publicity

- Project website http://www.ucd.ie/biocatal/biocatal/PE_to_PHA.html
- Magic Materials Expo in TCD Science Gallery opened for four-week from 15 September until 14 October 2012 – 'What makes a material magical? An ability to change shape before your eyes, to turn from a liquid to a solid or to be one of the lightest materials on earth and yet also one of the strongest? MAGICAL MATERIALS explores the properties of some of the world's most mysterious materials, giving you an opportunity to investigate and experiment at the cutting edge of material science.'
<http://sciencegallery.com/blog/2012/09/unleash-your-superpowers-launch-magical-materials>



An Ghníomhaireacht um Chaomhnú Comhshaoil

Is í an Ghníomhaireacht um Chaomhnú Comhshaoil (EPA) comhlachta reachtúil a chosnaíonn an comhshaol do mhuintir na tíre go léir. Rialaímid agus déanaimid maoirsiú ar ghníomhaíochtaí a d'fhéadfadh truailliú a chruthú murach sin. Cinntimid go bhfuil eolas cruinn ann ar threochtaí comhshaoil ionas go nglactar aon chéim is gá. Is iad na príomhnithe a bhfuilimid gníomhach leo ná comhshaol na hÉireann a chosaint agus cinntiú go bhfuil forbairt inbhuanaithe.

Is comhlacht poiblí neamhspleách í an Ghníomhaireacht um Chaomhnú Comhshaoil (EPA) a bunaíodh i mí Iúil 1993 faoin Acht fán nGníomhaireacht um Chaomhnú Comhshaoil 1992. Ó thaobh an Rialtais, is í an Roinn Comhshaoil, Pobal agus Rialtais Áitiúil.

ÁR bhFREAGRACHTAÍ

CEADÚNÚ

Bíonn ceadúnais á n-eisiúint againn i gcomhair na nithe seo a leanas chun a chinntiú nach mbíonn astuithe uathu ag cur sláinte an phobail ná an comhshaol i mbaol:

- áiseanna dramhaíola (m.sh., líonadh talún, loisceoirí, stáisiúin aistrithe dramhaíola);
- gníomhaíochtaí tionsclaíocha ar scála mór (m.sh., déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta);
- diantalmhaíocht;
- úsáid faoi shrian agus scaoileadh smachtaithe Orgánach Géinathraithe (GMO);
- mór-áiseanna stórais peitreal;
- scardadh dramhuisce;
- dumpáil mara.

FEIDHMIÚ COMHSHAOIL NÁISIÚNTA

- Stiúradh os cionn 2,000 iniúchadh agus cigireacht de áiseanna a fuair ceadúnas ón nGníomhaireacht gach bliain
- Maoirsiú freagrachtaí cosanta comhshaoil údarás áitiúla thar sé earnáil - aer, fuaim, dramhaíl, dramhuisce agus caighdeán uisce
- Obair le húdaráis áitiúla agus leis na Gardaí chun stop a chur le gníomhaíocht mhídhleathach dramhaíola trí chomhordú a dhéanamh ar líonra forfheidhmithe náisiúnta, díriú isteach ar chiontóirí, stiúradh fiosrúcháin agus maoirsiú leigheas na bhfadhbanna.
- An dlí a chur orthu siúd a bhriseann dlí comhshaoil agus a dhéanann dochar don chomhshaol mar thoradh ar a ngníomhaíochtaí.

MONATÓIREACHT, ANAILÍS AGUS TUAIRISCIÚ AR AN GCOMHSHAOIL

- Monatóireacht ar chaighdeán aer agus caighdeáin aibhneacha, locha, uiscí taoide agus uiscí talaimh; leibhéal agus sruth aibhneacha a thomhas.
- Tuairisciú neamhspleách chun cabhrú le rialtais náisiúnta agus áitiúla cinntí a dhéanamh.

RIALÚ ASTUITHE GÁIS CEAPTHA TEASA NA HÉIREANN

- Caimníochtú astuithe gáis ceaptha teasa na hÉireann i gcomhthéacs ár dtiomantas Kyoto.
- Cur i bhfeidhm na Treorach um Thrádáil Astuithe, a bhfuil baint aige le hos cionn 100 cuideachta atá ina mór-ghineadóirí dé-ocsaíd charbóin in Éirinn.

TAIGHDE AGUS FORBAIRT COMHSHAOIL

- Taighde ar shaincheisteanna comhshaoil a chomhordú (cosúil le caighdeán aer agus uisce, athrú aeráide, bithéagsúlacht, teicneolaíochtaí comhshaoil).

MEASÚNÚ STRAITÉISEACH COMHSHAOIL

- Ag déanamh measúnú ar thionchar phleananna agus chláracha ar chomhshaol na hÉireann (cosúil le pleananna bainistíochta dramhaíola agus forbartha).

PLEANÁIL, OIDEACHAS AGUS TREOIR CHOMHSHAOIL

- Treoir a thabhairt don phobal agus do thionscal ar cheisteanna comhshaoil éagsúla (m.sh., iarratais ar cheadúnais, seachaint dramhaíola agus rialacháin chomhshaoil).
- Eolas níos fearr ar an gcomhshaol a scaipeadh (trí cláracha teilifíse comhshaoil agus pacáistí acmhainne do bhunscoileanna agus do mheánscoileanna).

BAINISTÍOCHT DRAMHAÍOLA FHORGHNÍOMHACH

- Cur chun cinn seachaint agus laghdú dramhaíola trí chomhordú An Chláir Náisiúnta um Chosc Dramhaíola, lena n-áirítear cur i bhfeidhm na dTionscnamh Freagrachta Táirgeoirí.
- Cur i bhfeidhm Rialachán ar nós na treoracha maidir le Trealamh Leictreach agus Leictreonach Caite agus le Srianadh Substaintí Ghuaiseacha agus substaintí a dhéanann ídiú ar an gcrios ózóin.
- Plean Náisiúnta Bainistíochta um Dramhaíl Ghuaiseach a fhorbairt chun dramhaíl ghuaiseach a sheachaint agus a bhainistiú.

STRUCHTÚR NA GNÍOMHAIREACHTA

Bunaíodh an Ghníomhaireacht i 1993 chun comhshaol na hÉireann a chosaint. Tá an eagraíocht á bhainistiú ag Bord lánaimseartha, ar a bhfuil Príomhstíúrthóir agus ceithre Stíúrthóir.

Tá obair na Ghníomhaireachta ar siúl trí ceithre Oifig:

- An Oifig Aeráide, Ceadúnaithe agus Úsáide Acmhainní
- An Oifig um Fhorfheidhmiúchán Comhshaoil
- An Oifig um Measúnacht Comhshaoil
- An Oifig Cumarsáide agus Seirbhísí Corparáide

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag ball air agus tagann siad le chéile cúpla uair in aghaidh na bliana le plé a dhéanamh ar cheisteanna ar ábhar inní iad agus le comhairle a thabhairt don Bhord.

Science, Technology, Research and Innovation for the Environment (STRIVE) 2007-2013

The Science, Technology, Research and Innovation for the Environment (STRIVE) programme covers the period 2007 to 2013.

The programme comprises three key measures: Sustainable Development, Cleaner Production and Environmental Technologies, and A Healthy Environment; together with two supporting measures: EPA Environmental Research Centre (ERC) and Capacity & Capability Building. The seven principal thematic areas for the programme are Climate Change; Waste, Resource Management and Chemicals; Water Quality and the Aquatic Environment; Air Quality, Atmospheric Deposition and Noise; Impacts on Biodiversity; Soils and Land-use; and Socio-economic Considerations. In addition, other emerging issues will be addressed as the need arises.

The funding for the programme (approximately €100 million) comes from the Environmental Research Sub-Programme of the National Development Plan (NDP), the Inter-Departmental Committee for the Strategy for Science, Technology and Innovation (IDC-SSTI); and EPA core funding and co-funding by economic sectors.

The EPA has a statutory role to co-ordinate environmental research in Ireland and is organising and administering the STRIVE programme on behalf of the Department of the Environment, Heritage and Local Government.



ENVIRONMENTAL PROTECTION AGENCY
PO Box 3000, Johnstown Castle Estate, Co. Wexford, Ireland
t 053 916 0600 f 053 916 0699
LoCall 1890 33 55 99
e info@epa.ie w <http://www.epa.ie>



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