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BIOECONOMY RESEARCH SYMPOSIUM: IRELAND

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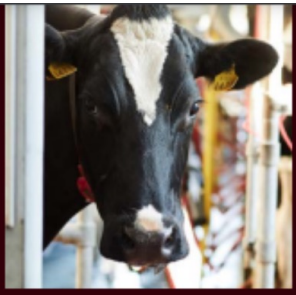
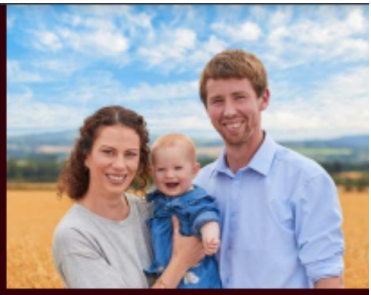


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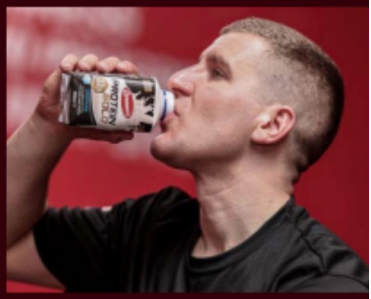


Farmland Afforestation: Forest Optimal Rotation Ages across Discrete Optimisation Objectives

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Abstract

Forestry is increasingly seen as having a multiplicity of functions, some of which may lead to tensions between the preferences of different forestry stakeholders. One expression of these differing preferences is in the area of optimal rotation length. This paper models the optimal rotation lengths of different aspects of the forest innovation system – the forest owner, the timber processor, the carbon sequestered, and the social value. A microsimulation framework is utilised to simulate the impact of alternative rotation lengths on different outcomes including timber volume, processor value of harvested timber, forest owner incomes, carbon emissions and incomes adjusted for a carbon value. The main conclusion is that the optimal rotation lengths vary quite significantly depending upon the stakeholder. We assess the impact of a forest owner harvesting their timber at a time that was financially optimal for them. The processor would have the smallest difference in their annual equivalised net present value (AENPV) from the forest owner. The biggest difference is with the optimal rotation length for carbon, with the AENPV of sequestered carbon being about 50% higher than for the forest owner's optimal rotation length. The differential for timber lies between the processor and the carbon. As a combination of private and carbon goals, the social return also lies between the processor and carbon amounts, the differential increasing with higher carbon values, reflecting an increasing importance of carbon. Additional survey results find that the financial optimum for a forest owner at a 5% discount rate differs substantially from the actual rotation length. These differing preferences for rotation length reflect the need for a systems-based approach to forestry policy, considering the needs of all the system's stakeholders.

The role of seaweed farming in a circular bio-based society: supply chain planning and sustainable business models

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Abstract

Seaweed (macroalgae) farming is being increasingly recognized for its potential in carbon sequestration, ecosystem services and wide applications for food and feed, fertilizers and biostimulants, along with cosmetics, pharmaceuticals, bioplastics and biofuels. However, the supply of seaweed in the European context is mostly based on the collection of wild resources and macroalgae cultivation is still in the early stages of development. The alignment of sustainable supply chains and business models could help to promote the development of responsible sourcing strategies for macroalgae and the emergence of cultivation systems. Through an adapted version of the business models canvas, the key building blocks of a sustainable business model for seaweed farming in Ireland were identified, which is driven by environmental and social values in an economically feasible way. The results indicate that responsibility for the environment is closely related to the prevention of impacts on ecosystems, while social aspects are associated with local community engagement alongside the societal benefits of final products. Economic feasibility is more connected to business diversification and partnerships with other marine-based stakeholders in what is related to knowledge sharing, production processes or facilities as well as market accessibility. The implications of this study can help to guide future research pathways and better decision-making of prospective growers, entrepreneurs, policymakers, sustainability standards and future research.

Optimising happiness for Ireland's developing bioeconomy: Assessing economic indicators within a social framework

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Abstract

For the developing Irish bioeconomy, it is important that sustainability is measured not only in terms of environmental impact but also in terms of human wellbeing. So what is important for the wellbeing of humans, and how can we measure this? This research attempts to further develop the social footprinting methodology by combining statistical industry-specific data with subjective wellbeing research taken from the World Happiness Reports. Specifically, we highlight the contribution of four main indicators - employment, income, perceptions of corruption and institutional trust - to overall wellbeing through Cantril ladder scores (subjective life satisfaction rated from 0-10). We then determine through a quantitative framework how economic aspects of the bioeconomy could affect the overall well-being scores in the case of Ireland. The development of the Irish bioeconomy is estimated to create 23,000 additional jobs in the agri-food sector by 2025, but we find that the contribution of these jobs to overall wellbeing is significantly impacted by how added value will be distributed. If the currently prevalent economic system is followed, and capital profits and incomes generated by the developing bioeconomy are disproportionately awarded to high-income groups, the increase to wellbeing is limited, if not negative due to increasing inequalities. On the other hand, by capping profit margins, maximising labour compensation, and prioritising communities with low-incomes and high unemployment rates, developing the bioeconomy can increase wellbeing in Ireland by up to 1.55 Cantril ladder points per job created. This research shows that in order for the developing bioeconomy to maximise social wellbeing, we cannot ignore current economic inequalities, and policies should prioritise capping profit shares and giving greater employment opportunities to low-income communities.

Biotransformation of monophenols to catechols by a bacterial oxidoreuctase

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Abstract

Catechols are versatile building blocks for the synthesis of high value chemicals such as pharmaceuticals, polymers, agrochemicals, flavours and fragrances. It can be produced by extraction from nature source, chemical synthesis and enzymatic synthesis. As an alternative method to chemical process, enzymatic synthesis shows several advantage such as higher purity and yield, lower energy cost, etc.. In this study, two catechols, 4-propylcatechol and 4-ethylcatechol are produced via the in vitro oxidation of 4- propylphenol and 4-ethylphenol, respectively, by a bacterial oxidoreductase. The gene encoding the oxidoreductase was overexpressed on vector pRset B under the control of T7 promoter in *E. coli* BL21(DE3). Kinetic characterisation of the purified enzyme with 4- propylphenol and 4-ethylphenol was undertaken using colorimetry. The Km value of the oxidoreductase was 0.44 mM for 4- propylphenol and 4.6 mM for 4-ethylphenol. The enzyme exhibits 6.6-fold higher catalytic efficiency (Kcat/Km) with 4- propylphenol than 4-ethylphenol. The biotransformation development was performed using crude cell extracts in both baffle flask and 1L bioreactor. The reaction was inhibited by excess addition of CFE or phenols. In flask biotransformation, the maximum concentration of CFE lysate required for biotransformation of 10 mM 4-propylphenol and 10 mM 4-ethylphenol was 0.1 mg/ml and 0.05 mg/ml, respectively. In addition, the maximum concentration of 4-propylphenol and 4-ethylphenol that the enzyme will transform was 20 mM and 30 mM, respectively. Based on the optimal reaction conditions tested in flask, the biotransformation was scaled up to a 1-L bioreactor where 90 mM (approx. 11 g/l) 4-ethylcatechol was produced using a fed batch process.

Growth of mushroom mycelia by submerged fermentation

Conor Ó Lochlainn
SBBS

Abstract

The objective of this project was to optimise growth conditions of *L. edodes* in continuous cultivation for the production of bioactives. This required understanding the growth of *L. edodes* and to optimise media for its growth. It was also necessary to understand the effect of media and growth on the production of β-glucans. Lastly, it was necessary to develop a continuous cultivation strategy for the strain in a fermenter. β-glucans are polysaccharides found in fungi and are an integral component of the cell wall. They possess several health benefits for humans and have been shown to possess antibacterial properties and to be effective against cancer, diabetes and cholesterol. Optimising growth media was done by altering media components in small-scale conical flask experiments and measuring the resulting biomass and β-glucan concentrations. Complex media components (e.g., corn steep liquor (CSL), yeast extract (YE) and malt extract (ME)) and simple media components (e.g., glucose, sucrose, KH_2PO_4) were tested. A media composition of 10g/l glucose, 15ml/l CSL and 3.3g/l KH_2PO_4 was chosen due to its relatively high resulting biomass and β-glucan composition. It was also chosen due to the wide array of nutrients that are present in CSL and due to the low cost and wide availability of glucose. In flask experiments, this media resulted in a biomass concentration of 6.3g/l and a β-glucan content of 9% after 10 days. A continuous cultivation strategy was then developed for *L. edodes* in fermenters. Initially, this was being done in an airlift bioreactor, however, the cultivation was impeded by large pellet size and adherence of the fungus to internal components of the fermenter. Cultivation was then carried out in a stirred-tank reactor. This was more effective since impellers prevented large pellet growth and adherence. The optimal conditions for STR growth were with an impeller tip speed of 1.3m/s and a temperature of 25ºC. Following on from this, various dilution rates were tested in the STR in order to determine the highest possible dilution rate that could be achieved without washout occurring. This included $0.02h^{-1}$, $0.025h^{-1}$, $0.03h^{-1}$, $0.04h^{-1}$. $0.025h^{-1}$ was the optimal dilution rate, with its biomass concentration and β-glucan composition plateauing after 8 days at 3g/l and 10%, respectively.

Waste streams from mammalian and fungal cell bioreactors as food in secondary bacterial fermentations

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Abstract

Over half a billion litres of waste media from cell culture bioprocess is produced every year across the world. Rather than disposing this as a waste, attempts to identify the potential value as a secondary fermentation feed have been investigated. Preliminary experiments in shake flask cultures showed that waste media from Chinese hamster ovary (CHO) cell culture supports *E. coli* fermentation, with recombinant protein expression at ~70% of that from cultures grown in rich microbiological media (LB). Is it possible that we could reuse waste media from all bioprocesses, including mammalian, fungal and microbial? We used 1L bioreactors in scaled-up fermentation experiments with waste media from CHO culture and fungal culture as a feed source. Our results show the *E. coli* cultures deliver very high recombinant protein yields of >1 g/L, equivalent to cultures grown in LB. In addition, we performed proteomic analysis using mass spectrometry to show that the waste media-fed *E. coli* have an active stringent response with a significant alteration of transcription factor activity. We analysed the chemical components of the waste media before and after culture using inductively coupled plasma mass spectrometry (ICP-MS) to identify nutrient depletion patterns. Planned experiments include using the nutrient depletion patterns to inform on supplementation of waste media for enhanced performance in future fermentations. Our data suggests a transcriptional rewiring in E. coli, a consequence of activation of the stringent response that simultaneously drives recombinant protein production. This represents a potentially significant finding in the context of valorisation of waste in bioprocessing.

Membrane Bioreactors to Produce Polyhydroxyalkaonate (PHA) from Gaseous Feedstocks

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Abstract

Polyhydroxyalkanoate (PHA) is a promising alternative to petroleum-derived plastics due to their comparable physical and chemical properties and biodegradability. Many microorganisms can produce PHA as an intracellular energy and carbon storage material. Microorganisms such as *Cupriavidus necator* can metabolize CO_2 as a carbon source and produce PHA when a mixture of H_2, CO_2 and O_2 gas is supplied. Thus, it is possible to produce PHA directly from CO_2 which would reduce greenhouse gas emissions. However, the optimum gas composition ratio for cell growth is 7:2:1 for H_2:O_2:CO_2 which is within the gas-explosion range. To eliminate the explosion risk, the oxygen concentration should be maintained below the lower explosion limit however this limits the growth and productivity due to oxygen limitation. Furthermore, gas fermentation faces substrate limitation due to the low solubility of gases in the culture medium. Membranes have the potential to achieve high gas transfer efficiencies at low gas supply rates due to the high specific surface area available for transfer. Thus, membrane bioreactors are promising reactor systems for gas fermentation processes. This project demonstrates the applicability of membrane bioreactors to deliver gaseous substrates to produce polyhydroxybutyrate (PHB), which is a type of PHA. To accomplish this, membrane bioreactors were constructed using 50 ml centrifugal tubes and gas-permeable polydimethylsiloxane membrane fibres with 760 µm outer diamater. The obtained membrane bioreactors are operated in batch mode where membranes are used for supplying gasesous substrates to maximize yield. The specific surface area for gas transfer is adjusted by optimizing the number of fibers used. The effect of operational conditions such as recirculation and gas flowrate is studied. Later, a fed-batch strategy is used to maximize PHB production by applying nitrogen limitation. PHB content within the biomass is increased from 8 ± 2% to 22 ± 2%. This is expected to be increase further by optimizing the conditions.

Propagation of selected yeasts strains on monolignol-analogues

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Abstract

Lignin is one of the most abundant polymers in the world. It is present in a wide range of plant materials. Lignin is a heterogenous polymer made of phenylpropanoids units, linked by C-O and C-C bonds. Sinapinic acid, ferulic acid and *para*-coumaric acid are monolignol-analogues that are breakdown products of lignin. Four microbial strains belonging to the *Saccharomycetales* order of the *Ascomycota* division showed sustained growth in the presence of sinapinic, ferulic and *para*-coumaric acid, respectively at different ranges of concentration in a media with yeast extract (YE) and soy peptone (SP) as amending components. The concentrations of the different aromatic carboxylic acids were between 20mM and 100mM (3.28-22.2 g/L); each aromatic carboxylic acid was tested individually. *Metschnikowia pulcherrima* achieved the highest biomass with 100mM sinapinic acid as the main carbon source (evaluated as OD at 600 nm). While *Debaryomyces hansenii* initially had a better biomass after 72h of growth, *Kluyveromyces marxianus*, outperformed *Debaryomyces hansenii* after 144 h of growth. *Debaryomyces hansenii* achieved the highest growth in the presence of 20 and 40mM of ferulic acid. *Metschnikowia pulcherrima* appeared to tolerate but not grow on ferulic acid and *para*-coumaric acid; *Pichia kudriavzevii* achieved the highest biomass in the presence of 20 mM of *para*-coumaric acid. HPLC analyses showed a complete consumption of 100 mM sinapinic acid by all the four strains in 72 h. The same analytical technique showed a maximum 25% reduction of 40 mM ferulic acid by *Metschnikowia pulcherrima* and no appreciable consumption of *para*-coumaric acid by any of the strains. Yeasts hold promise to consume mono-lignol derived compounds allowing for the production of yeast-derived bioactive and protein-rich biomass.

Thermal and photocatalytic hydrogenolysis of lignin model compounds for the generation of platform compounds

Raphaël Abolivier, James A. Sullivan
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Abstract
Lignin is a biopolymer built from phenolic units, it can be found in all plants/trees/grass at level of ~30 %. My research focuses on the valorisation of lignin through heterogeneous catalysis for the generation of value-added compounds (platform chemicals) such as phenol, benzene, acetophenone, etc. The conversion of lignin to these molecules is expected to become an important financial asset for the future of biorefineries. This research field is also aimed at the development of green alternatives to fossil fuels and to an overall reduced reliance on crude oil. This project focuses on the cleavage of ether bonds (through hydrogenolysis) within lignin model compounds. These are small molecules containing linkages (e.g. b-O-4; b-b, etc.) similar to the ones found in lignin. Their use for such study enables to gain insight into the chemistry of lignin through the generation of reliable and reproducible results. Both thermal and photochemical catalytic pathways have been investigated for this reaction over noble-free (e.g. Ni-based catalysts) catalysts. Different parameters such as, reaction temperature, H_2 pressure and metal loading percentage have been investigated. H-ZSM-5 and TiO_2 were chosen as catalytic support for thermal and photochemical catalysis, respectively, due to their commercial availability, cheap price, and well-known properties. Bimetallic-based catalysts (Ni & Cu) were found to be the most efficient material for the thermally induced hydrogenolysis of the investigated substrates (e.g. benzyl phenyl ether, 2-phenoxyacetophenone, etc.). It was also shown that the cleavage of ether bond within lignin model compounds can be performed without the need for high pressure H_2 (g) through photocatalysis.

Sustainable Strategies for the Synthesis of Organophosphorus Compounds

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Abstract
Organophosphorus compounds are an integral part of modern society. They find use as pharmaceuticals, agrochemicals, materials and within synthetic chemistry. Therefore, developing more sustainable ways of accessing these compounds is important as our society addresses the ongoing climate crisis. Here, we report two catalytic strategies for the synthesis of medicinally relevant organophosphorus compounds. The first strategy utilises organocatalysts to facilitate conjugate additions of amines to α,β-unsaturated phosphonates at ambient temperatures with up to 100% atom economy. Organocatalysis offers a more sustainable alternative to transition metal catalysis by using small organic molecules to catalyse reactions, reducing our society's reliance on often toxic and rare transition metals. The second approach uses photo-catalysed dicarbofunctionalisations of α,β-unsaturated phosphonates for the step-economic synthesis of complex organophosphorus compounds. Photo-catalysis uses visible light as a renewable energy source for reactions. 1,2-Dicarbofunctionalisation of alkenes are a powerful strategy for forming complex and valuable materials efficiently from simple starting materials. We aim to use this strategy for the synthesis of analogues of the antibiotic Fosmidomycin which has shown positive *in vitro* results against malaria.

Surface functional nanofibrous membranes for selective separation of chemicals of bioeconomic importance

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Abstract

Organic solutes like sugar mixtures, proteins, acids and so on are considered valuable and main targets in most of the biorefineries and used as building blocks for sustainable bioplastic production. Selective separation using membranes will hence play a significant role downstream processing methods and offering more scope for creating bioeconomy value chains. In this context, polymeric nanofibrous membrane modified with 2D nanomaterials and cyclodextrin derivatives (β-CDs) are fabricated for selectively separating out organic solutes that are of bioeconomic importance. Facile modification of nanofibers was carried out by means of novel surface functionalisation methods like in-situ crosslinking and electrospraying techniques to achieve active functional layer on the surface of nanoporous membrane. Incorporation of supramolecules like β-CDs has resulted in nanofibers with narrow average fiber diameter of as low as 200 nm. The electro-fluidic deposition of 2D nanomaterials like graphene, molybdenum disulfide (MoS2) has formed an active nanoporous layer over the nanofiber surface. Subsequent heat treatment step was also done post-electrospraying to develop stable and scalable functionalised nanofibrous membranes. SEM morphology and surface topography have revealed the presence of graphene and β-CDs containing surface layer has offered to tune the pore size in the range from 20 to 200 nm as desirable for separation of low molecular weight organic solutes. Cross-linking of bio-based cyclodextrin derivatives (β-CDs) has offered surface hydrophilicity as confirmed by the reduction in contact angle to 15.8° and 64.2° respectively for in-situ crosslinked and surface functionalised polyethersulfone (PES) nanofibers. Surface functionalisation has also increased the membrane flux rate despite having lower pore size, which implies that both solution-diffusion property and hydrophilicity of toroidal structured of β-CDs were offering improved membrane water permeability. Performance of functionalised nanofibrous membrane like graphene and β-CDs deposited PES was evaluated using 5L bench scale cross-flow filtration unit. Separation trials were performed on model lactose and salt solutions to study the flux and filtration efficiency in terms of % rejection of lactose, removal of total solids and % conductivity, Functionalised nanofibrous membrane has outperformed in terms of improved permeability with relatively lower trade-off to rejection rate signifying the importance of functional for realising superior filtration properties towards organic solute separation.

Prospects and challenges in exploring bioplastics for circular economy

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Abstract

The current global plastic waste management crisis, fuelled by the growing consumption of single-use fossil-based plastics needs no elaboration. With the vast expanse of plastic waste generated each year, the need of hour is to develop more cost-effective, high-performance bioplastics-based consumer products such as plastic bags, food packaging, etc. with good mechanical strength. In this backdrop, our aim is to develop biodegradable polymer composites appropriate for packaging film applications as an alternative to petroleum-based polymers. We developed novel blends of polylactic acid, polycaprolactone, polybutylene succinate, etc. and fabricated them with diverse nanofillers to produce multi-faceted polymer composites *via*. solvent-free melt-processing technique. Different weight percentages of nanofillers such as clay, 2D nanomaterials, calcium carbonate, etc. were incorporated into the blends to improve the mechanical and barrier properties, while retaining the transparency and improving composting characteristics of the composites. The structural perception of the composites was studied by SEM, XRD, FTIR, and thermal studies. The developed composites not only exhibited good mechanical, thermal and barrier properties, but also remarkable disintegration (a part of composability study) in current Irish composting conditions. Thus, the developed tough, biodegradable polymer composites with good retention of transparency can be worthy replacements for petroleum-based polymers as advanced, eco-friendly materials in the realm of packaging applications.

Performance optimisation of curly hollow membrane modules using CFD

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Abstract

Converting waste greenhouse gases such as CO2, CO, and CH4 into valuable products is an exciting opportunity that is gaining attention as we move from the linear economy to a more circular economy. Gas fermentation using bacteria as bio-catalysts is increasing in popularity as the reaction does not require extreme operating conditions and expensive catalysts. One major drawback of gas fermentation is that it is limited by gas to liquid mass transfer resulting in low productivity. This problem can be overcome by using membrane modules to increase gas to liquid mass transfer. The relationship between the membrane bioreactor system hydrodynamics and performance is examined in this study. The effect of curly hollow fibre membranes on the mass transfer compared to the straight membrane systems is investigated. Studies show that different configuration of fibres enhances flux from 53% to 92% depending on the module. In this project, there are three levels of investigation. The first one examines a single curly fibre (0.76 mm diameter) in a small tube (8 to 12 mm diameter). The second study compares multiple curly fibres in a small tube (8 to 12 mm) and multiple curly fibres (61 fibres) in a large tube (50 mm diameter). The experimental set-up is simulated using OpenFOAM software. Preliminary results from the single fibre systems showed that there is a range where the peak of the residence time distribution (RTD) curve is decreasing at a significant rate.

State of the art for Ammonia Removal and Recovery from Animal Manure

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Abstract

Gaseous agricultural emissions contribute to global warming and air pollution. Ammonia is a polluting toxic gas that also contributes to the formation of airborne particulate matter. Ammonia emissions from manure occur naturally, being accelerated by pumping and land application of slurries. At the same time, Ammonia is an important fertilizer needed to sustain agricultural production. Removing and recovering ammonia from manure wastes can reduce emissions while avoiding the need for fossil sources of ammonia fertilizer. The proposed study will investigate the techno-economics of ammonia removal and recovery from various animal manures. It will investigate factors that influence this process including technical parameters, e.g. ammonia concentration, contaminants in the manure, pH, and temperature as well as economic factors, process configuration, the scale of the process, transport, capital, and operational costs. As a first step in the study, a poster will be presented carrying out a review of the current state of the art and the latest innovative techniques of ammonia removal and recovery. These will be critically reviewed along with future challenges and prospects of ammonia evaluated. Extraction and purification of ammonia from animal manures, is sensitive to both technical and economic factors as well as their interactions. Future work will, therefore, develop an optimization model to determine the most suitable process and scale needed for a successful implementation of ammonia recovery in Ireland.

Mixed microbial cultures for polyhydroxyalkanoate production from plastic waste monomers

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Abstract
Polyhydroxyalkanoates [PHAs] are a class of biodegradable polyesters with melting and moulding properties similar to thermoplastics, and therefore have a broad range of applications. Microbes accumulate PHA as intracellular granules that serves as carbon and energy reservoirs under carbon excess and nutrient limiting conditions. Various microbes have been tailored to produce PHA from low-cost substrates. Plastic waste pollution is a global environmental problem. Thus, converting plastic wastes to PHA is one of the efficient ways to address the existing landfilled waste and reduce future landfilling thereby ensuring a circular economy of plastics. Processes like pyrolysis and enzymatic hydrolysis of mixed plastic waste produce a mixture of aromatic and aliphatic compounds. These monomers can then be used as carbon [C] feedstocks for microbial growth. Here, we aim to produce PHA from a mixture of plastic monomers, using mixed bacterial cultures. Various Pseudomonas strains were assessed for biomass [as cell dry weight, CDW] and PHA production using monomers as sole C substrates to determine growth rates. Mixed culture batch fermentation using mixed monomers yielded a maximum biomass of 0.53 ± 0.01 g/L CDW and a maximum PHA of just 0.02 ± 0.00 g/L with a growth rate of 0.21 ± 0.02 /h. Strategies to increase volumetric productivity are being developed and will be discussed.

Development of Convergent Biocatalytic Transformations for the Synthesis of Complex Alkaloids

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Abstract
Biocatalysis often involves the use of enzymes to transform simple, cheap, achiral materials into high value, chiral products. The selectivity, tuneability and efficiency associated with biocatalysts have led to their use in the pharmaceutical and fine chemical industry, offering a more attractive alternative to traditional catalysts. Biocatalysts enable more sustainable synthetic routes to the assembly of complex targets, with environmentally friendly reaction conditions and targeted valorisation of materials into industrially desired compounds. This research aims to combine biocatalysis with organocatalysis to develop convergent methodologies for the design of a hybrid bio-organocatalytic cascade. The use of transaminases alongside (*S*)-proline, in a cascade reaction is being investigated for the generation of 4-arylquinolizidine-2-ones and other alkaloid derivatives. The proposed cascade involves the biotransformation of cadaverine into the naturally occurring Δ^1-piperideine, followed by an (*S*)-proline catalysed enamine-iminium ion mediated Mannich-aza-Michael reaction, involving the use of arylideneacetones**.** The cascade has the potential to be significantly expanded by using alternative arylideneacetones, and incorporating additional enzymes to further functionalise the products, enabling access to a panel of natural products. The stereoselectivity and chemoselectivity of enzymes can enable a one-pot approach under mild conditions, forming complex alkaloid structures.

Stereoselective synthesis of 'superarmed' thioglycoside donors from 1,2-orthoesters

Zoe Beato, Xiangming Zhu
BiOrbic: Bioeconomy SFI Research Centre; School of Chemistry, University College Dublin

Abstract
The diversity and relative complexity of carbohydrates has made the synthesis of oligosaccharides a challenging task. However, the biological ubiquity of these molecules and their key roles in processes such as immunity and cell signalling mean their efficient chemical assembly is essential. A wide variety of monomeric building blocks called donors have been devised in the hopes of streamlining the process of oligosaccharide synthesis. One key advantage of sulfur-containing thioglycoside donors is their ability to be selectively activated in the presence of other glycosyl donors, making their use amenable to one-pot glycosylation strategies. The influence of aglycon identity, anomeric stereochemistry, and protecting group pattern on the reactivity of thioglycosides to activation has been well-reported, leading to an established continuum of reactivity for these donors. To simultaneously tackle the major challenges of protecting group manipulation and anomeric stereochemistry in S-glycoside synthesis, we report a Lewis acid mediated ring opening reaction utilising 1,2-orthoesters to deliver the 'super-armed' ethyl-2-acyl-3,4,6-tri-*O*-benzyl-1-thioglycosides of common monosaccharides. Boronic Lewis acids have been found to be superior to triflate-containing acids in promoting this reaction, as the harsh acidity of the triflates not only leads to cleavage of acyl groups, but also the inherent ability of triflates to catalyse epimerisation led to a mixture of α and β anomers. The use of sterically bulky groups has also proven crucial in minimising the formation of side products while delivering the stereopure products in good yields.

An investigation into the impact of the signal peptide of a biocatalytically active oxidoreductase

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Abstract
Oxidoreducatases are a class of biotechnologically important oxidising enzymes with applications in bioremediation, biocatalaysis and as biosensors. Oxidoreductases have been used to catalyse the hydroxylation of aromatic hydrocarbons to produce both monophenols and *o*-diphenols. A bacterial oxidoreducatase from *R. solanacearum* has been identified with the capacity to convert monophenols into catechols. This enzyme contains an N-terminal signal peptide (SP) predicted to direct it to the twin arginine translocase (TAT), a widely conserved but seldom used system which allows transport of folded substrate proteins from the cytoplasm into the periplasm. However, this system is inefficient for biotechnological processes due to the low number of pores per cell and the long transport time required per protein, often resulting in build-ups of insoluble protein matter when a protein targeted to this system is overexpressed. This study investigates the expression of the *oxidoreductase*, focusing on the impact of its SP. The gene encoding the oxidoreductase was expressed in *E. coli* BL21. Confirmation of the use of the TAT system by this enzyme was carried out by cell fractionation and affinity tagging the protein of interest. The impact of removing the SP, replacing the SP with SPs native to *E. coli* and expression of the enzyme in a host with the TAT translocase chromosomally deleted were all investigated to improve levels of soluble active enzyme produced.

From Linearity to Circularity – Creating Platform Chemicals from Thin Air

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Abstract

CO_2 is a by-product of many biobased processes like yeast fermentation and a major contributor to climate change. To integrate such processes in a circular (bio-) economy, CO_2 needs to be continuously recycled into the system. To tackle this, CO_2 can be utilised as a feed stock for biotechnological processes. *Cupriavidus necator* H16 fixes CO_2 in the presence of H_2 and can naturally produce the biodegradable plastic polyhydroxybutyrate (PHB) in nutrient limiting conditions. The CO_2-proxy formic acid can be used with *C. necator*, as it is easily produced from CO_2 and H_2 in electrochemical processes and has good solubility in water. The aim of this work is to improve growth on formic acid, particularly the maximum growth rate (μ_{max}), and to establish production of lactic acid. Growth improvements were achieved by subjecting *C. necator* to an adaptive laboratory evolution (ALE) campaign in a continuous fermentation. Periodical increases in the dilution rate exhibited evolutionary pressure for faster growing phenotypes. Thereby, a 1.8-fold increase of μ_{max} was achieved compared to the wildtype strain while biomass and PHB yield remained constant. Furthermore, in this project the product scope of *C. necator* on formic acid is to be expanded. Lactic acid can be produced from central metabolites by a lactate dehydrogenase (LDH). Several LDHs have been tested for expression, two of which are now under investigation for *in vitro* and *in vivo* lactic acid production. Current and future work revolves around the metabolic integration of a successful LDH for conversion to lactic acid.

Utilising Lignin Degradation Products for the Asymmetric Synthesis of (-)-Matairesinol

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Abstract

The past few decades have seen increased interest and fast-growing research on the valorisation of lignin. Lignin is a complex, amorphous, three-dimensional phenolic biopolymer, which constitutes one of the three primary components of lignocellulose, along with cellulose and hemicellulose. It is an integral part of plant cell walls, responsible for both providing structural rigidity, as well as protecting the plant against chemical or biological degradation. The composition of lignin is known to vary between different plant species and so the exact structure of native lignin remains unelucidated. However, what is known is that lignin is formed from the oxidative radical polymerisation of three phenylpropanoid units; *p*-coumaryl alcohol, sinapyl alcohol, and coniferyl alcohol. These three units are termed monolignols and are attached to one another to by a series of characteristic linkages which build up the lignin polymer. The nature of these phenyl-containing monomers makes lignin a potential sustainable feedstock for the production of non-fossil-based fuels and platform chemicals. So far, this feedstock has been vastly underutilised. To date, lignin valorisation strategies have focused primarily on depolymerising lignin into its aromatic degradation products, which include both the monolignol building blocks and their derivatives. These products have subsequently been applied to the synthesis of fuels and aromatic chemicals, such as vanillin and dimethyl sulfoxide. However, by virtue of their phenolic nature, the monolignols themselves display a range of bioactivities – such as antioxidant and antimicrobial – which can be exploited. This project looks at further derivatising the monolignols into natural products which have the potential to be of medicinal interest. In particular, through a series of chemical transformations, the monolignol coniferyl alcohol can be converted asymmetrically to the gamma-butryolactone natural product, (-)-Matairesinol, which has been reported to possess a wide range of biological activities, including anti-cancer, anti-fungal and antioxidant. This poster will present the results obtained to date in attempting to achieve this goal.

Reducing Food Waste during the Dry Ageing of Beef using IoT Technology

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Abstract

Advancements in sensor and communication technology offer the possibility for actors in the food supply chain to digitalise and modernise their processes and operations resulting in increased resource efficiency and reduced food loss and waste (FLW). The present study is part of the project: "REAMIT", which adapts and applies existing innovative technology, namely the Internet of Things (IoT) and Big Data analysis, to food supply chains in Nort-West Europe to reduce FLW and increase resource efficiency. Specifically, the study collects the experience and knowledge acquired in the pilot testing of an IoT monitoring and alerting system for the dry-ageing of beef in an abattoir in Ireland. This work aims at demonstrating the potential of such systems to reduce FLW in the supply chain, enhancing business efficiency and mitigating greenhouse gas emissions. After an evaluation of the company's requirements, a real-time monitoring and alerting system for anomaly detection during the dry-ageing process in their two refrigeration chambers were proposed. The ELT-2 Internal antenna (Elsys, Sweden) was selected as the platform for developing the IoT monitoring solution. This sensor was enclosed in an IP67-rated box which makes it suitable for extreme conditions; the ELT-2 also contained four built-in internal sensors, including temperature and humidity, which were used for monitoring of the environmental parameters in the dry-ageing chambers. For pilot testing, six ELT-2 sensors were fitted in the company's large chamber and four in the small one. All sensors were equally distributed at a distance of approx. 1.5 meters each, and in such a way that allowed for collecting data from different areas: closer to doors; closer to the refrigeration unit; and, in the large chamber, also in between the former two. The ELT-2 sensors transmitted data through LoRa communication signal to a gateway device, a Tektelic Kona Micro IoT Gateway (Tektelic, Canada), which in turn sent data to The Things Network cloud via an internet connection. The REAMIT partners, Whysor (Netherlands), developed a web-based dedicated dashboard for real-time monitoring and alerting, accessible from both computer and mobile phone. SMS alerting was provided by Amazon Simple Notification Service (SNS) and triggered when environmental conditions (i.e., temperature, humidity) exceeded the desired threshold for ensuring the safety and quality of the beef.

Increased production of polyhydroxyalkanoates (PHA) by overexpressing CrcZ and CrcY small RNAs in *Pseudomonas putida* KT2440 under various carbon and nitrogen growth conditions

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Abstract
Polyhydroxyalkanoates (PHAs) are biocompatible and biodegradable polymers, which can be synthesized and degraded by a wide variety of microorganisms. As a valuable plastic alternative, the synthesis of medium chain-length PHAs has been extensively studied in *Pseudomonas putida*. PHAs can be produced through fatty acid de novo synthesis and β-oxidation pathways. It has been shown that several genes in these pathways are under the regulation of carbon catabolite repression (CCR), which allows a fast adaptation of bacteria to the changing nutrient supplies. To understand how CCR regulate PHA synthesis in *P. putida* KT2440, two small RNAs CrcZ/CrcY putatively involved in this regulatory system had been investigated in this study. The strains overexpressing CrcZ and CrcY from pBT'T vector had 64.6% and 51.5% increased PHA levels compared to the wild type when glucose, and up to 92.3%and 38.0% higher when octanoate was used as a carbon and energy substrate. Furthermore, CrcZ and CrcY were deleted individually or in combination. Only △△CrcZ-CrcY produced 40% less PHA under the nitrogen-limiting condition with glucose as the single carbon source. Further analyses are being conducted to unravel the interplay of CRC, small RNAs and PHA synthesis in KT2440.

Consecutive Photochemical Reactions Enabled by a Dual Flow Reactor Coil Strategy

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Abstract
In the last decade topics such as eco-friendliness and environmental sustainability are increasingly becoming important due to the detrimental effects of chemistry on climate change. Continuous flow chemistry has increased in popularity over the last 20 years due its contributions to sustainability, scalability, and reproducibility over batch synthesis. The wide implementation of flow rectors impacting the synthesis of industrially relevant molecules in academia and industry in a cost effective and sustainable synthesis. Continuous flow photochemistry is seen by many as a powerful tool to not only enable the effective generation of target molecules exploiting light as a traceless reagent, but moreover to improve the sustainability and scalability of these processes. Our group recently reported on a photochemical method to generate substituted quinolines from amino chalcones. In this project we demonstrated the use of a dual coil photoreactor for the effective telescoping of light driven- continuous processes which has not been reported to date. Specifically, this new strategy is demonstrated for the generation of quinolines and their late-stage functionalisation via a diacetyl-catalysed Minisci reactions via the use of LED's. During our studies we optimised the Minisci reaction prior to its integration into the telescoped reaction. The resulting process showed good tolerance for aliphatic and aromatic moieties in the 2-position of the quinoline intermediate. The devised telescoped photochemical process enabled a library of highly substituted quinolines to be synthesized in good yields (22-70%) coupled with varying ethers such as THF, 2-Me-THF, dioxane and Et2O.

Natural capital and ecosystem services of Irish dairy farms

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Abstract

My work is part of the project named "Farm Zero C", aiming to have a net zero carbon dairy farm with improved biodiversity. Natural capital (NCA) is being accounted for and ecosystem services (ES) are being quantified (some also valued economically) to show improvements at the farms. This is being done initially for a demonstration farm, Shinagh Farm, in west Cork and the framework developed will then be applied to 10 other replicator farms. NCA is a way of measuring and reporting the stocks and flows of natural resources (water, soils, plants, animals). The rationale is that, since nature is important to society and the economy, we have to measure the stocks and flows of natural assets and make sure they are managed ain a sustainable way. ES are the contributions of healthy ecosystem to human welfare. The NC stocks and the ES stemming from them are quantified and valued using different ecological or economic techniques, depending on the type of stock or service. A healthy stock of natural capital will provide ES which will results in benefits to the farmer (crops, forage, sequestration of carbon in soil and plants, amenity value of the farm). First of all, a quantification in biophysical terms is carried out (tonnes of carbon sequestered, tonnes of pollutants purified, etc.). A valuation in monetary terms can then be carried out. The Farm Zero C project will follow the System of Economic and Environmental Accounting framework, which is the United Nations system of NCA which is the accepted international standard. This allows to highlight changes in the extent of stocks of natural capital year-by-year and the corresponding flows of ecosystem services (the contribution of nature to our welfare), as well as any improvement or deterioration in their condition. The extent and condition of the habitats at Shinagh are being quantified using data from fieldwork already carried out at Shinagh (soil sampling, habitat assessment), from remotely sensed data and from literature. The stocks of organic carbon will then be quantified. The following ecosystem services are being considered: Forage provision, Carbon sequestration, Water purification, Habitat provision, and Amenity value

The Asymmetric Synthesis of Sterically Hindered α-Allyl-α-aryl N-Heterocycles Using DAAA

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Abstract

Many natural products and pharmaceuticals possess quaternary α-aryl stereocentres. Allylic groups are a well-studied functional handle that finds excellent use in natural product synthesis and other synthetic applications. Nitrogen-containing heterocycles are ubiquitous in biologically relevant compounds, and as such are common structural motifs in many natural products and pharmaceuticals. Therefore, the study of methods for the highly enantioselective synthesis of α-allyl, α-aryl stereocentres in nitrogen-containing compounds, particularly in lactams, is desirable. These products can be accessed from the Pd-catalysed decarboxylative asymmetric allylic alkylation (DAAA) of α-aryl β-amido-allyl esters using chiral *P*,*P*-Trost-ligands. The DAAA reaction is a mild, but powerful synthetic tool in the enantioselective generation of quaternary stereocentres. Although well studied, the scope of the DAAA reaction has generally been limited to stereocentres bearing small alkyl groups. Starting with our report in 2016 on α-aryl cyclopentanones, our group extended the scope of this catalysis to a substrate that contained a bulky α-aryl motif. Since this first report, we have continued to develop the DAAA reaction for other α-aryl containing substrates. The development and optimisation of the DAAA for the synthesis of 6- and 5-membered α-allyl, α-aryl lactams will be described. The model substrate of the 6-membered lactam has been successfully synthesised and converted to the desired product in good yields and high levels of enantioselectivities. A substrate scope containing 14 examples has been synthesised, with variations to the lactam protecting group and identity of the α-aryl moiety generated. The scope of this DAAA has been carried out, with results of up to 72% yields and *ee*s of up to 82% have been obtained. The model substrate of the 5-membered lactam has also been successfully synthesised, with optimisation of the DAAA for this substrate ongoing.

Endogenously produced 2-phenylethanol inhibits biofilm growth in *Cunninghamella echinulata*

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Abstract
Some fungi are models of mammalian metabolism, and thus a potential ethical alternative to animal testing in drug development. The filamentous fungus *Cunninghamella echinulata* transforms a broad spectrum of xenobiotic compounds to mammalian-equivalent metabolites. Under certain conditions this fungus can adhere to a surface and grow as a biofilm. This enables the fungus to catalyse repeated biotransformations and allows the liquid fraction to be easily decanted and processed. Previously identified signalling molecules produced by other *Cunninghamella spp*., such as 3-hydroxytyrosol and tyrosol, do not affect the biofilm growth of *C. echinulata*. This suggests that a different molecule(s) is employed for regulating biofilm growth. Here we report that 2-phenylethanol is produced in much higher concentrations when planktonic cultures of *C. echinulata* than when the fungus is grown as a biofilm. The effect of exogenously added 2-phenylethanol on biofilm growth was studied in both solid and liquid media. This demonstrated that the presence of 2-phenylethanol inhibits biofilm growth of *C. echinulata*, but does not affect planktonic growth. The addition of exogenous 2-phenylethanol on established caused detachment of biofilm. Therefore, we conclude that this molecule is produced by the fungus to regulate biofilm growth.

Semi-synthesis of fluorinated iturin A analogues with improved antifungal activity

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Abstract

The number of pharmaceuticals and bioactive compounds containing fluorine has significantly increased over the last years. This is due to fluorine's ability to act as an isostere of C-H and C-OH moieties, to alter the hydrophilic/hydrophobic properties and pKa values of the parent molecule and to increase metabolic stability. Iturin A is a cyclic lipopeptide, produced by Bacillus subtilis, with strong antifungal activity against known fungal pathogens that affect the roots of cereal crops, such as Fusarium graminearum and Gaeumannomyces tritici. Its structure contains seven α-amino acids and one β-amino acid with a lipid side chain. Its mechanism of action, even though not fully elucidated, involves the formation of hydrogen bonds between the phenol and the sterols of the fungal membrane with the subsequent pore formation by the lipid side chain. There is also evidence for the formation of reactive oxygen species (ROS) that are involved in cell damaging. It was reasoned that the addition of a trifluoromethyl group on the Tyr residue would increase the hydrogen bond forming capacity, giving enhanced bioactivity, increased lipophilicity and increased metabolic stability. Moreover, fluorine atoms can also be exploited for characterisation techniques, such as 19F NMR, for studying the mode of binding of iturin A to the fungal membrane. Here we investigate different synthetic and biosynthetic methods for installing a trifluoromethyl group on the Tyr residue for bioactivity studies. These include a precursor directed biosynthesis approach and a late-stage modification approach. In the former strategy, trifluomethylated tyrosine is synthesised and feeding studies with known concentrations of the non-natural amino acid are performed, so that it can be incorporated by bacterial biosynthesis. In the late-stage modification approach the trifluoromethylation reaction is performed directly on the purified natural lipopeptide. Lastly, antifungal assays against Fusarium graminearum, including 96-well plate assays and disk diffusion assays to assess the bioactivity of the natural and fluorinated lipopeptides are presented.

Organocatalytic Approaches Towards the Asymmetric Synthesis of Saturated Nitrogen Heterocycles

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Abstract
Cyclic nitrogen-containing compounds are commonly encountered in natural products. In addition, they are also a frequently found structural motif in numerous active pharmaceutical ingredients, with a recent article identifying that 59% of small molecule-based drugs contain a nitrogen heterocycle. From these, drugs containing the piperidine (6-membered) or pyrrolidine (5-membered) saturated ring motifs are ranked number one and five respectively, in terms of their prevalence. As a result, methods to construct this valuable type of nitrogen heterocycle are of vital importance to both the synthetic and medicinal chemistry communities. Furthermore, as the vast majority of medicinally relevant piperidines and pyrrolidines contain chiral centres around the saturated ring, particular emphasis has recently been placed on the ability to produce these structures stereoselectively. Our work in this area is specifically focused on the development and amelioration of a series of recently reported chiral thiocarbamate-catalysed bromoaminocyclization reactions towards the asymmetric synthesis of 2-substitued 3-bromopyrollidines and piperidines from their corresponding aminoalkene derivatives. To date, this methodology has been solely employed successfully for bromine-based haloaminocyclizations and requires conditions (-78 ˚C, 5 days) that are not suitable for the sustainable mass production of these compounds. Our primary aim involves expanding the scope of this highly enantioselective procedure to facilitate haloaminocyclizations involving the other halogens; fluorine, chlorine and iodine. In addition, it is also our desire to render this transformation more amenable to a large-scale production setting by developing more proficient thiocarbamate-based catalyst that are capable of retaining excellent enantioselectivities at higher temperatures than realised to date.

Asymmetric Synthesis of Sterically Hindered α-allyl, α-aryl Heterocycles

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Abstract
The development of asymmetric C-C bond formation via palladium-catalysed allylation reactions has greatly accelerated the evolution of the field of asymmetric catalysis. Methods for the synthesis of α-allyl, α-aryl stereocentres in nitrogen-containing heterocycles is desirable as many pharmaceutical and biologically relevant compounds contain C(sp^3)-quaternary stereocentres. These motifs can be accessed via Pd-catalysed decarboxylative asymmetric allylic alkylation (DAAA) of α-aryl β-amido-allyl esters. The development and optimisation of the DAAA reaction for the synthesis of α-allyl, α-aryl quinolinones is described. The model substrate has been successfully synthesised and transformed into the desired product in moderate conversions and shows promising values for *ee*, however, optimisation of the reaction is ongoing.

Mind the Gap: Investing the Effects of Macromolecular Crowding on the PHA Pathway of *Cupriavidus necator*

Kate McKeever and Gerard Cagney

Abstract

Issues surrounding the overuse of polyethylene terephthalate (PET) plastic products are well established and an area of focus in the BiOrbic circular bioeconomy initiative. Specifically, the replacement of nonbiodegradable plastics with biocompatible alternatives is under intense study and already implemented in the market. Polyhydroxyalkanoates (PHAs) are a naturally occurring bioplastic produced by many bacteria. *Cupriavidus necator* is a chemolithoautotroph of interest as a consumer of carbon dioxide to produce biofuel and other valuable products. Over 90% of the dry cell weight of *C. necator* can comprise PHA granules, which accumulate intracellularly. Molecular crowding refers to high local concentrations of biomolecules in the cell (proteins, carbohydrates, lipids etc.). The effect of crowding on multistep enzyme pathways is understudied compared to classical enzyme studies, which have historically favoured dilute aqueous environments. Understanding how the cell adapts to crowding is of fundamental interest, but also of potential relevance to expanding the range of industrially relevant enzyme processes. We will investigate the effect of macromolecular crowding on the global *C. necator* proteome, as well as the PHA pathway using biologically agents that can mimic crowding such as glycol or dextran. The response of the proteome to the presence of crowding agents will be monitored using a new approach termed thermal proteome profiling (TPP). TPP can map conformation changes induced by crowding events on a proteome-wide scale. The assay relies on producing melting curves that reflect the differential protease sensitivity of proteins as they are heated in a thermal gradient. The melting point of proteins can vary depending on factors that influence conformation including physiological state and interactions with other molecules. We will investigate the effect of crowding agents on the *C.necator* proteome, initially using cell lysate. Multiple agents will be used to investigate the effect of concentration, molecular weight, and crowding agent chemistry, on the global 'meltome'. We anticipate that this new approach will give insight into the response of *C.necator* to crowding events, as well as the specific response of the PHA pathway.

Asymmetric Synthesis of Quaternary α-Aryl Stereocenters in Benzofuranones Using Decarboxylative Asymmetric Allylic Alkylation

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Abstract

Benzofurans are a common motif found in natural products and biologically active compounds, some of which contain anti-inflammatory and anti-cancer properties. A typical approach to install these structures is to start with the corresponding benzofuranone which can then be converted to the benzofuran by reduction and subsequent dehydration. Quaternary α-aryl stereocenters are found in nature and have been shown to have interesting properties. Recent efforts with the benzofuran/benzofuranone motifs have focused on the installation of aryl groups at the α-position to generate sterically hindered all-carbon quaternary stereocenters in the resulting products and so methodologies to install these are desired. These products can be accessed from the Pd-catalysed decarboxylative asymmetric allylic alkylation (DAAA) of α-aryl β-keto allyl esters using chiral *P,P*-Trost ligands. This methodology uses mild conditions to generate motifs difficult to access by other means, generating highly desired quaternary stereocenters in an asymmetric fashion. However, the scope of these attempts are typically limited to small alkyl chains or functionalities distant from the reactive centre. The Guiry group has already extended the scope previously to other α-aryl containing substrates via the use of aryl lead reagents to install the bulky groups on the reactive centre of the desired β-keto allyl ester before applying to the DAAA reaction which insofar has observed high reactivities and enantioselectivities. The development and optimisation of the synthesis of the model substrate is described along with the optimisation of the DAAA reaction for the 5-methoxybenzofuranone analogue which has displayed good yields and enantioselectivities with research into the substrate scope still ongoing.

What is Known from the Existing Literature about Consumer Perspectives on Bio-based Products? A Scoping Review

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Abstract

Today, to overcome sustainability challenges, in all their social, economic, and environmental dimensions, it is essential to shift from a society based on mass consumption, uncontrolled waste generation, and dependent on fossil resources, towards a better society based on the efficient use of natural renewable resources, new production and consumption behaviour, waste reduction, reuse, and valorisation. The transition toward a bio-based society is at an early stage, and little knowledge exists about consumer behaviour toward Bio-based products. Since the development of the bioeconomy relies on new knowledge and technologies and faces risks and uncertainties, cultivating the consumption of Bio-based products is a formidable challenge for policymakers and managers who strive to develop the bioeconomy at the local, national and international levels. As consumers are important economic actors and their behaviour plays a pivotal role in supporting the successful transition to a bio-based economy, this research mainly focuses on consumer-related studies to determine different perspectives concerning bio-based products and factors influencing consumers' purchasing decisions in the available literature. This scoping review used the methodological framework for scoping review recommended by Arksey and O'Malley (2005) and Levac et al. (2010). This study searched relevant academic articles from electronic databases and grey literature for pertinent studies. This search covered studies published in English from the inception of each database within a 20-year timeframe (2002-22). For the study selection part, all the studies were screened and refined by titles and abstracts for assessment against the eligibility criteria. In consequence, we identified studies published with varying populations the world over that investigated consumer acceptance and factors affecting their consumption behaviour. We identified a wide range of determinants regarding consumer acceptance of Biobased products and categorized them into three major groups; Psychological, Marketing and Socio-demographic characteristics.

The *Cunninghamella elegans* CYPome club: enzymes that are seen but not heard

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Abstract

Cunninghamella spp. is recognized as a mammalian model of drug and xenobiotic metabolism. These species can catalyse phase I and phase II of drug metabolism. Cytochromes P450 (CYPs) are enzymes mostly responsible for phase I (oxidative) metabolism; although, the function of these enzymes is not thoroughly studied in vitro. In silico analysis of the genome sequence of *C. elegans* identified 32 genes putatively coding for CYPs. However, the low sequence homology of CYPs restrains a prediction of a function based on a sequence. Furthermore, CYP activity is tied with their cytochrome reductases (CPRs). Heterologous expression in a suitable host is the best option to study their substrate specificity. Cyp5313D1 produced in *P. pastoris* converted flurbiprofen to 4′-hydroxyflurbiprofen, the same metabolite was produced by *C. elegans* cultures. Furthermore, co-expression of CYPs cyp5208A3 and cyp5313D1 with one of the three CPRs led to improved biotransformation of various xenobiotic substrates (transfluthrin, β-cyfluthrin and λ-cyhalothrin, ibuprofen, diclofenac). Most recently two other CYPs were studied, cyp5205A8 and cyp5313E1. Bifunctional cyp5205A8, containing its own reductase domain, was successfully produced in *E. Coli* BL21. However, after incubation with various xenobiotic substrates, no biotransformation was observed. Subsequently, cyp5205A8 and cyp5313E1, together with one of the three CPRs, were transformed in *P. pastoris*, and its production tested. Identification of the substrate activity of these enzymes could lead to a reduction in animal use and provide a more sustainable route to drug metabolite production.

Engineered Mutants of *Pseudomonas putida* KT2440 to direct the production of S-Polyhydroxyalkanoate (PHA)

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Abstract
(R)-Polyhydroxyalkanoate (PHA), a versatile biobased polyester, is synthesised naturally by the common soil bacterium, *Pseudomonas putida* KT2440. PHA is biodegradable on land and in the sea. It can be used to produce a wide range of sustainable everyday products and is a viable substitute for fossil fuel-based plastics. It is currently used for high value products such as implant coatings, sutures, and drug delivery systems due to its biocompatibility. The objective of this work is to synthesise novel PHAs and to expand its industrial applications. To do this, slight alterations are engineered into the structure of the primary enzyme responsible for the bio catalysis of PHA, PHA synthase1 (PhaC1). PhaC1 is a vital factor in determining the type of PHA synthesised by this bacterium. PHA is naturally and exclusively synthesised in its (R) enantiomeric form. Key amino acid residues in this enzyme are substituted to alter its activity and to drive the production of the rarer (S) enantiomer of PHA. The first key stage of this work used CRISPR-Cas9 gene editing techniques to delete *pha*C1 from the *P. putida* KT2440 genome creating the novel Δ*pha*C1/ *P. putida* KT2440. 48-hour flask cultures and subsequent gas chromatography analysis revealed that the deletion of the *pha*C1 gene resulted in creation of a PHA deficient *P. putida* KT2440 strain. Selected PhaC1 amino acid variants were cloned into a pBT'T mcs vector and subsequently transformed into Δ*pha*C1/ *P. putida* KT2440 The monomeric constituents of these mutants are being examined along with yields and their ability to synthesise the rarer (S) enantiomeric form of PHA. Parallel to this, a novel method using surface plasmon resonance (SPR) is being developed to measure PhaC1 activity in vitro. This biophysical assay will used to identify the best (S)-PHA producers. Once developed the substrate for this assay, 3-hydroxydecanoyl-CoA, which is bio-synthesised, will be introduced to the immobilised PhaC1 mutants and their activity quickly assessed. Bioplastics are not only viable alternatives to petrochemically derived plastics but present an opportunity exists to create novel and profitable applications and products for a variety of industries.

Bioproduction of 2,5-Furandicarboxylic Acid via Metabolic Engineering

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Abstract
2,5-Furandicarboxylic Acid (FDCA) is a molecule functionally similar to terephthalic acid, one of the monomers of the ubiquitous plastic, polyethylene terephthalate (PET). FDCA can be biobased and can be used to create polyethylene furanoate (PEF), an alternative to fossil based plastics. However, synthesis of FDCA is limited by the high cost of production. Biotechnology offers an alternative route to plastic production by using fewer toxic reagents and milder conditions, while starting with waste stream derived feedstocks. *Pseudomonas umsongensis* GO16, which has the native capacity to metabolise PET monomers as growth and energy substrates, has been used to upcycle these monomers to polyhydroxyalkanoate biopolyesters. Building on its ability to convert PET monomers to valuable compounds, this bacterium is currently being explored as a microbial chassis for the synthesis of FDCA. A precursor in the synthesis of FDCA is 5-hydroxymethylfurfural (HMF). It was found that *P. umsongensis* GO16 can utilise this substrate as a sole carbon and energy substrate. Further work has identified the genetic basis for this, and a CRISPR/Cas9 system has been used to disrupt this pathway for FDCA accumulation. A biotransformation system is currently being developed to allow for the production of this potential terephthalic acid replacement.

Social Acceptance of Bioeconomy: A Scoping Review

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Abstract

The bioeconomy has gained importance over the last decade and is presented as a solution to societal challenges. It aims to enable sustainable economic growth by utilizing renewable natural resources. In the bioeconomy domain, the question of social acceptance is vital, as this transition requires focusing not only on technological innovation but also on involving society. Understanding their perspectives and effects on transition pathways is essential to finding the right approach and innovations that align with societal needs. Consequently, it increases understanding of the bioeconomy and improves trust among society. There is an increasing volume of literature reviews reporting on the importance of social acceptance but it's time to build on the previous studies to help understand the behaviour and society acceptance and how they can affect in bioeconomy transition to help policymakers and government act on implementing bioeconomy and create a society which accepts and supports bioeconomy. Therefore, a scoping review has been conducted to gather and map the available research on the acceptance of the bioeconomy. It answered what is known from the existing literature about the social acceptance of the Bioeconomy. Moreover, it allowed a broad and structured exploration to examine the affective factors on acceptance and identified gaps in the existing literature.

Valorising Urban Organic Waste Streams Through Agrochemical Extraction and Organic Acid Production via Cascading Biorefinery Approach

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Abstract

The CircBioCityWaste project is based on the vital principle of the 'circular bioeconomy' focusing on the sustainable, resource-efficient valorisation of anaerobic digested (AD) digested urban waste stream (mainly, organic fraction of municipal waste (OFMSW), dairy processed sludge (DPS), and food waste) to produce bio-based agrochemicals (biostimulants and biofertilisers) and organic acids via the cascading biorefinery concept. The project focuses on an 'end-of-waste' approach to producing deliverables for sustainable agriculture, promoting plant growth, and improving soil health while keeping circular bio-economy aspects in focus. The biorefinery starts with digestate collection, characterisation, development of pre-treatment methods, and novel extraction technologies for agrochemicals and bio-fertilizers recovery. The potential for organic acid production from digestate will be examined via microbial fermentation and optimised. The residual sludge will undergo biochemical and mineral profiling and be evaluated for use as solid fertiliser. The impact of both biostimulants and biofertilisers will be benchmarked against the current best agronomic practice in commercially important plants under growth room conditions. Additionally, CircBioCityWaste will evaluate the sustainability of multiproduct biorefinery and assesses the economic, social and environmental impacts of the developed products/materials or processes using life-cycle assessment methodologies. To end a comprehensive perspective on the opportunity of exploiting urban waste as a valuable resource will be scrutinised.

Design of a biocatalyst for the production of 3-hydroxybyric acid

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Abstract
3-hydroxybutyric acid (3HBA) is a commodity chemical, used as a building block for the synthesis of fine chemicals, such as, vitamins and bioplastics. The chemical synthesis of 3HBA requires pure methyl 3-oxobutanoate substrate and a Ru_2Cl_4 as catalyst, thus it is expensive. This work investigated the enzymatic route for the synthesis of 3HBA using glucose as a starting substrate. Two synthetic operons were designed to produce (*R*) and (*S*)3HBA enantiomers (RHBA and SHBA, respectively) using glucose feedstock. These operons were cloned in pET45b(+) vector and include the genes of a hydrolase from *Bacillus cereus*, a β-ketothiolase and (*S*)-hydroxy butyril-CoA dehydrogenase from *Ralstonia eutropha*, for SHBA production. For RHBA the two first genes were the same, but an (*R*)-hyydroxy butyril-CoA dehydrogenase from *R. eutropha* was used to produce (*R*)3HBA. It was observed that 3HBA production was accompanied by high acetate accumulation in the supernatant, thus likely pulling carbons away from the desired product. To reduce acetate accumulation, we deleted *eutD* gene of the producer strain, *E. coli* BL21(DE3), that encodes a phosphotransacetylase previously shown to be involved in the acetate formation. Acetate production was reduced for (*R*)3HBA production in Δ*eutD* strain, however this appeared to also affect the target product yield, which was 1.4-fold reduced. In (*S*)3HBA producing strain, acetate accumulation was 0.7-fold increased in Δ*eutD* strain, while the target product yield remained the same. Further optimisations aim to balance the carbon flow towards the target product.

Conducting LCA of emerging technologies at low technology readiness levels (TRL) to make way for pilot scale

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Abstract

Life cycle assessment (LCA) is recognized as a potential tool to evaluate environmental impacts of emerging technologies from "cradle to grave" and to facilitate decision making in the policy and research communities. The funding agencies have been asking for LCA of newly proposed projects i.e. from ideation stage. LCA of an emerging technology at low TRLs (1-3) is distinct from traditional LCA since the evaluation precedes the product life cycle. In this study, LCA of three case studies are conducted at the same TRL level (1-3). In case study 1, *Hericium erinaceus* was used as a substrate with enzymes and sugars to grow fungal biomass in a laboratory scale aerobic, submerged fermentation system, and β-glucan was subsequently extracted. Attributional LCA was conducted to identify environmental impact hotspots so that informed decisions could be made for the design of scaling from TRL3 (laboratory proof of concept) to TRL6 (demonstration in industrially relevant environment). For case study 2, biomolecule called fucoidan is produced from brown seaweed. Fucoidan is used in pharmaceutical, cosmetic products and as a supplement. It represents a proportion of the mass in fresh brown seaweed. It requires investment of time and energy to isolate the product. The aim is to reduce the processing time and thereby reduce the environmental burden. The intended application of the study is to quantify the environmental impact at all stages of the life cycle of the product to ensure sustainable production. In case study 3, a comparative life cycle assessment of two production system for obtaining nanocellulose on a laboratory scale is conducted. The conventional process use sulphuric acid, and the novel method use ionic liquids (p- toluenesulphonic acid (PTSA):choline chloride). The system boundary for all three case studies is "cradle to laboratory gate" with FU-1kg of product produced. Climate change (kg CO2 eq) is evaluated and hotspots are identified at laboratory scale. A common hotspot observed for all three case studies is the production of raw materials i.e. the upstream processes. These may also play a contributive role while upscaling the technology whereas for the hotspots which were identified in the foreground process can be avoided. The aim is to identify "hotspots" from the early stage of process development. It has been observed that conducting LCA at early stages can facilitate technology developers to understand the implications of design choices on future consequences. Thus it can prevent regrettable investments and avoid environmental burdens.

Proteomic Investigation of Polyhydroxyalkanoate Granules in Pseudomonas putida KT2440

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Abstract

Polyhydroxyalkanoates (PHAs) are biodegradable polyesters composed of (R)-3-hydroxy fatty acids. PHAs are produced by a wide range of bacteria under conditions of nutrient imbalance and are stored intracellularly in the form of water-insoluble granules, serving as carbon and energy stores that can be degraded in times of starvation. These granules are complex subcellular structures and electron microscopy data has shown that the hydrophobic polyester core is surrounded by a boundary layer of proteins known as granule associated proteins (GAPs). GAPs are believed to comprise PHA synthesis, structural and regulatory proteins. Phasins, a group of low-molecular-weight proteins, make up the majority of GAPs, constituting up to 5% of the total cellular protein. Whilst there has been substantial research focused on understanding the PHA metabolism pathways, only 2 phasins (PhaF and PhaI) have been characterised in *Pseudomonas putida KT2440* to date. Additionally, the exact composition and surface structure of PHA granules as well as the processes that take place during granule biosynthesis and degradation remain unknown. This may be because some proteins involved in PHA metabolism may have yet to be characterised. This study aims to completely characterise the PHA-ome, including GAPs using proteomic techniques. Multiple PHA inducing conditions were used to stimulate PHA synthesis in KT2440 and changes that occur across the whole proteome were analysed in order to identify proteins that are significantly dysregulated or exclusively present under PHA-accumulating conditions. Since the proteomic analysis of isolated PHA granules is prone to false-positive results due to the artificial binding of proteins during cell disruption, we performed a whole cell comparative proteomics study using a PHA-negative mutant to identify background changes.

NxtGenWood: Converting wood-based phenols to value added products

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Abstract

Lignin, one of the most abundant natural polymers on the planet, is also one of the most underutilized due to its recalcitrant nature. Our project aims to investigate novel ways to valorize lignin into value- added products utilizing bacterial cultures as biocatalysts. In order to achieve this, different bacterial species were selected from the literature due to their reported ability to use lignin derived phenols. The bacteria were tested for growth on the three main phenolic acids present in lignin (ferulic, sinapic and *p*-coumaric acid). The results from these experiments showed that *Pseudomonas* species such as *P. putida* KT, F1 and S1, as well as *Sphingobium* sp. SYK-6 showed the most promise for growth on phenolic acids. We then improved the growth of some of the strains on ferulic and *p*-coumaric acids using Adaptive Laboratory Evolution (ALE). Here we systematically increased the concentration of *p*-coumaric acid in the medium the strains were exposed to. After three weeks we obtained a mutant that was able to grow at a faster growth rate and was tolerant to higher concentrations of ferulic and *p*-coumaric acid. This bacterium also shows promising growth with sinapic acid (which is the more recalcitrant out of the three phenols) after a further ALE experiment. It is therefore a promising candidate for revalorization of all three lignin based phenols. Future experiments will be focused on developing a process using the optimized bacterial strains for the biotransformation of lignin derived phenols into value-added products.

Maximising plastic waste upcycling in *Pseudomonas* species

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Abstract
Polyethylene terephthalate (PET) is a plastic which mostly used in single use packaging and synthetic fibres. Its short shelf-life, durability, and recalcitrant nature hugely contribute on plastic pollution. Previously, up-cycling PET into valuable products using micro-organism was suggested as a promising tool to tackle plastic waste problem. PET can be depolymerised into terephthalic acid (TA) and ethylene glycol (EG) by enzymes, and then micro-organism such as Pseudomonas umsongensis GO16 utilise TA as a feedstock to produce polyhydroxyalkanoates (PHAs). PHAs are bacterial carbon and energy storage polyesters usually accumulated under nutrient limitation stress. PHAs can be used in various applications from its biodegradable, thermoplastic, and biocompatibility properties. While the proof-of-concept for the biotechnological strategy to upcycle PET was demonstrated, the efficiency of TA metabolism remains a challenge. In P. umsongensis GO16, TA can be degraded by tph genes into protocatechuate (PCA). Depending on which position of aromatic ring of PCA opens, the final products to TCA cycle can be varied. In this project, we deleted pcaGH genes to disable ortho cleavage pathway from GO16 and introduced PCA-2,3-para or 4,5-meta cleavage pathway. It was confirmed that GO16 did not grow with TA as a sole carbon source when pcaGH genes were deleted and it recovered its growth when para or meta-cleavage pathway was adopted.

Engineering *Pseudomonas umsongensis GO16* for Production of tailored Short-Chain-Length-co-Medium-Chain-Length (SCL-co-MCL) PHA Copolymers

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Abstract

Plastic pollution issues are seen around the globe and are only getting worse as time goes on. “Bioplastics” are viewed as an alternative to petrochemical plastics, and ideally they are biobased and biodegradable. One of these bioplastics, which meet both requirements, are polyhydroxyalkaonates (PHAs), bacterial intracellular polyesters with varied physical properties dependent on the monomer composition. Pseudomonas umsongensis GO16 is a unique bacterium due to its ability to produce PHA monomers of varied carbon (C) length, short-chain length (SCL) C4-C5 and medium-chain length (MCL) C6-C12, thereby offering a potential to tailor PHAs to have specific material characteristics. In this project, GO16 was grown with carbon-unrelated sodium terephthalate (Na2TA), and carbon-related sodium octanoate (NaOct) or sodium butyrate (NaBut) and its PHA accumulation capacities were investigated. NaOct supported accumulation of PHA with majority of C8 monomer and a smaller fraction of C4, while NaBut supported accumulation of PHA with majority of C4 and smaller fraction of C8 monomer. These two PHAs have a combination of SCL- and MCL-PHA characteristics i.e. strength and elasticity. As the PHA are accumulated in form of intracellular granules, two SCL- and MCL-PHA granule associated proteins were identified and were fused to yellow fluorescent protein (YFP) and cyan fluorescent protein (CFP) to investigate the subcellular localization of SCL- and MCL-PHA in GO16. Furthermore, to produce tailored SCL-co-MCL (C4-co-C6) PHA copolymers, four PHA synthesis genes had been identified according to the preference in forming SCL or MCL PHA polymers. The CRISPR/Cas9 is being used to engineer the metabolic pathways of GO16 to produce SCL-co-MCL PHA copolymers in the future.

Upcycling of PET waste into industrially relevant higher-value products

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Abstract
Plastic waste management is a challenge with global impact. Polyethylene terephthalate (PET), the most abundant plastic used in textiles and packaging, is here of particular interest. Recently there have been reports on enzymatic depolymerization of PET into its monomers ethylene glycol (EG) and terephthalic acid (TA), by, e.g., PETase, MHETase, and PHL7. Those findings opened up major opportunities for the biological upcycling of PET waste into higher-value products. Our focus is on transforming post-consumer PET to polyurethanes (PU). We aim for the microbial metabolization of EG and TA and the subsequent production of PU building blocks such as adipic acid (AA) and hydroxyalkanoyloxy-alkanoates (HAAs) in a one-step process. We enabled the metabolization of EG and TA by genetic engineering in Pseudomonas putida KT2440, a strain natively unable to grow on those monomers. P. putida KT2440 harbors the catabolic pathway for EG degradation via the glyoxylate carboligase, which is repressed by the transcriptional regulator GclR. The catabolic route for TA encoded by the tph operon was taken from Pseudomonas umsongensis GO16 and via the Tn7 system introduced into the genome of P. putida KT2440. The strain was further modified for the production of AA and HAAs. Secretion was enabled by integrating the respective synthesis pathway, either plasmid-based or genome integration. For the synthesis of AA, the reverse adipate degradation pathway was integrated and combined with the TA degradation pathway. Our study serves as a proof of concept for the valorization of PET waste to a product that is currently produced using petrochemical resources, therefore showing potential for decreasing the environmental impact of PU synthesis.

Notes:


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