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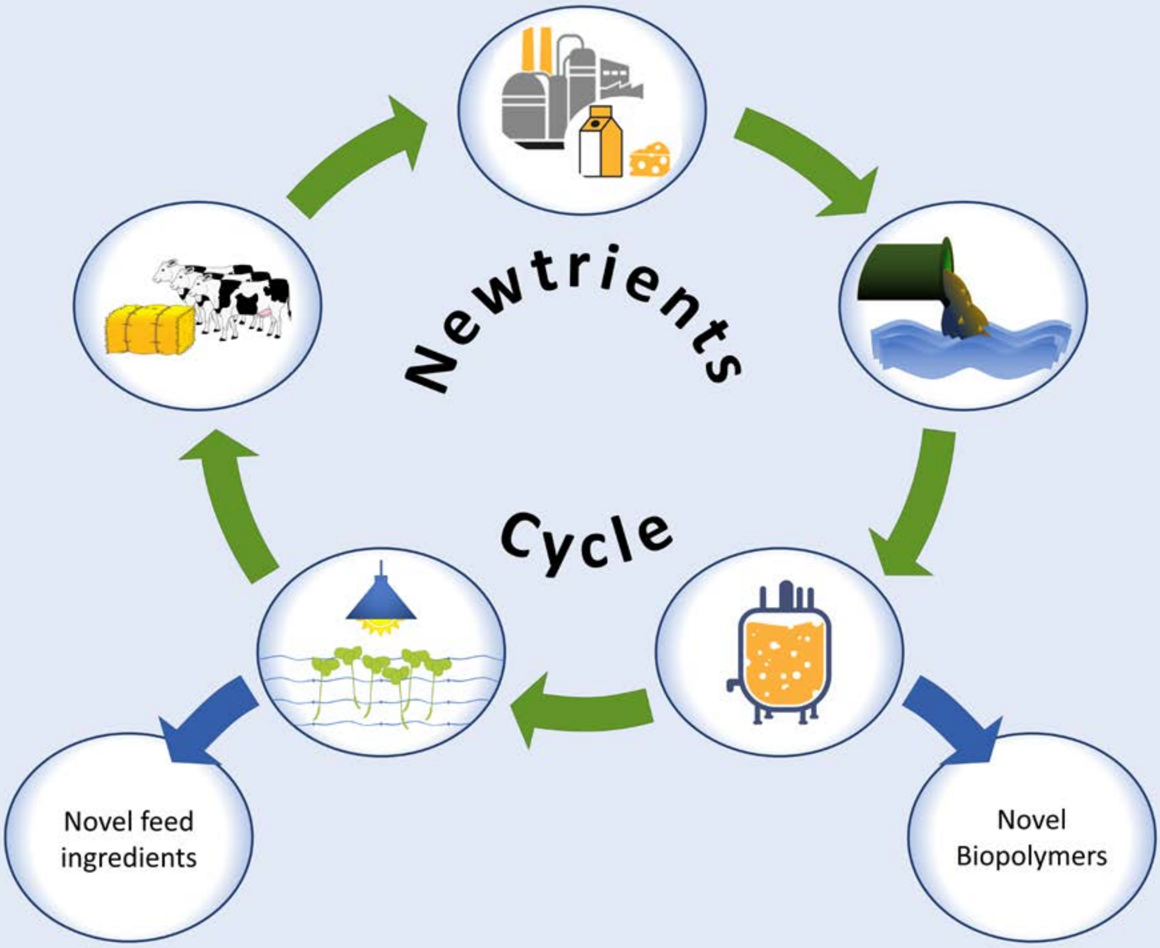
Innovative Valorisation of Dairy Processing Wastewater Using a Circular Economy Approach (Newtrients)

Authors: Éamonn Walsh, Lekha Margassery, Neil Coughlan, Roisin Broughton, Holger Kühnhold, Arno Fricke, Gavin Burnell, Maria O'Mahoney, David Wall, Paul Bolger, Niall O'Leary and Marcel A.K. Jansen



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Rialtas na hÉireann
Government of Ireland

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EPA RESEARCH PROGRAMME 2021–2030

Innovative Valorisation of Dairy Processing Wastewater Using a Circular Economy Approach (Newtrients)

(2016-W-LS-11)

EPA Research Report

Prepared for the Environmental Protection Agency

by

University College Cork

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The EPA Research 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.

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Executive Summary

The aim of the Newtrients project was to develop an innovative exemplar of a circular economy approach to the production of novel, value-added products from dairy industry wastewater. This was successfully achieved. Newtrients has shown that compostable bioplastics can be generated from dairy waste, rather than from fossil fuels. In addition, it was demonstrated that duckweed can be grown on dairy processing wastewater, producing protein-rich biomass for use as feed for farm animals and thus creating a closed loop for plant nutrients. Newtrients has developed and demonstrated innovative wastewater treatment technology, coupled with valorisation of waste, resulting in the production of clean water, novel value-added products, reduced CO_2 outputs, nutrient recycling and novel opportunities for rural industrial development.

Dairy processing wastewater represents a significant waste stream in Ireland and across the world; managing this waste constitutes a financial and technological challenge. Newtrients supports the objectives of the dairy industry to achieve value-added economic growth while simultaneously progressing towards a zero carbon and zero waste model. A shift to sustainable production and consumption is crucial for the transition to a sustainable society. Indeed, national and international policies (e.g. the Irish Government Circular Economy Bill 2021 and the EU Circular Economy Action Plan 2014) advocate a circular economy that focuses on long-term value retention and reductions in the linear use of raw resources. When waste is produced, the circular economy focuses on waste valorisation. The practical solutions arising from the development of green, circular economy approaches by Newtrients will inform policymakers, as well as the media and the wider public, about the scope to transition to a sustainable society.

By working with industry, Newtrients has developed a pioneering cascading system for valorising dairy wastewater that couples microbial-based technologies of anaerobic digestion and aerobic dynamic feeding with duckweed (Lemnaceae) cultivation. Each step in the cascading system contributes an identifiable, value-added output that carries a financial benefit:

1. by treating dairy processing wastewater to discharge standard;
2. by generating value-added and marketable products with new income potential for the dairy processing industry;
3. by reducing dependence on finite fossil resources.

Development of bespoke anaerobic digestion reactors revealed stable conversion of organic carbon present in wastewater into volatile fatty acids (VFAs), which are important chemical building blocks for a variety of applications. The formation of VFAs is associated with a greater reduction in CO_2 output than through traditional aerobic respiration of wastewater. The aerobic dynamic feeding reactor serves the circular economy through the production of biodegradable polymers derived from VFAs, and constitutes an innovative, sustainable route for the production of non-petrochemical-based polymers and compostable bioplastics with a lower environmental impact than those produced from petrochemical sources.

The duckweed reactors add value to wastewater through the production of protein-rich biomass with an amino acid profile close to the nutritional requirements of various farm animals, thus facilitating a new closed-loop rural animal feedstock industry. Duckweed reactors further contribute to sustainability by replacing imported soybean and by enabling nitrogen and phosphorus recycling, thus alleviating the need for energy-demanding urea production or the mining of limited rock phosphate. The developed stacked duckweed reactors offer several advantages in terms of year-round production and the environmental footprint of efficient wastewater treatment.

Overall, the integrated operation of the Newtrients cascading system results in the effective remediation of dairy wastewater, while generating products that can strengthen the local economy and create intricate new relationships between farmers, dairy processors

and the feed industry. Collaborative projects with the dairy processing industry are now required to progress the transfer of laboratory-based technology to industrial settings, in which the Newtrients system can be validated, demonstrated and integrated in wastewater treatment processes. However, the relevance of the developed Newtrients system is not limited to dairy processing. It is postulated that similar integrated systems can be used to valorise a range of waste streams, yielding a multitude of new, marketable products. It is recommended that a public portfolio of waste valorisation protocols be developed to facilitate the adoption of bespoke circular economy solutions by a wider range of companies. It is also recommended that further strategic work be undertaken in relation to broader techno-economic analyses of waste management, industry-based cradle-to-grave sustainability analyses, co-ordinated development of local authority approaches to end-of-life waste management for biopolymers and evaluation of consumer acceptance of waste-derived products and feedstocks.

1 Introduction

1.1 Sustainability, the Circular Economy and Waste

The World Commission on Environment and Development provided a wide-ranging definition of sustainability and sustainable development in its 1987 report, stating that "sustainability is meeting the needs of the present populace without compromising the needs of future generations" (Brundtland, 1987). Fitting with such a broad definition, sustainability covers a wide variety of concepts that have context-dependent applications. For example, in an ecological context sustainability refers to conservation of the biosphere (Chapin *et al*., 1996), while in socioeconomic scenarios it can be applied to the sustainability of rural living (Copus and Crabtree, 1996) and of urban water supplies (Krueger *et al*., 2020). These contexts are not independent; rather, the study of sustainability integrates ecological, societal and economic concerns (Chapin *et al*., 2010).

The EU has published several strategies for sustainable industrial growth (European Commission, 2011, 2012; De Besi and McCormick, 2015), focusing on creating sustainable economic growth through resource efficiency (European Commission, 2011). In March 2020, the European Commission adopted the new Circular Economy Action Plan. The EU's transition to a circular economy will reduce pressure on natural resources and will create sustainable growth and jobs. The Circular Economy Action Plan is one of the main building blocks of the European Green Deal. It aims to transform the EU into a modern, resource-efficient and competitive economy, ensuring that (a) there are no net emissions of greenhouse gases by 2050, (b) economic growth is decoupled from resource use and (c) no person and no place is left behind. A key deliverable of the European Green Deal is the EU action plan "Towards a Zero Pollution for Air, Water and Soil", which was adopted in May 2021. It aims for the reduction of air, water and soil pollution to levels no longer considered harmful to health and natural ecosystems, thereby creating a toxin-free environment.

The circular economy is an economic model that is nested within concepts of sustainability (Andersen, 2007). The circular economy is generally accepted to denote closed-loop production patterns, long-term value retention, reduction in the use of raw resources and reduction in waste production (Ghisellini *et al*., 2016; Morseletto, 2020). The global importance of circular economy approaches is reflected in a number of the United Nations Sustainable Development Goals (SDGs), but particularly in SDG 12 (responsible consumption and production).

Pressing economic and environmental concerns are fuelling an interest in closed-loop approaches, from the near-depleted supply of phosphate rock (Cordell *et al*., 2009; Elser and Bennett, 2011) to the finite supply of fossil fuels (Shafiee and Topal, 2009) and the unsustainable manner of food production (Jurgilevich *et al*., 2016). Consequently, the circular economy is promoted through initiatives such as the Circular Economy Action Plan from 2014 and a subsequent implementation report in 2017 (European Commission, 2015, 2017). European bioeconomy strategies also increasingly consider the concept of a circular bioeconomy (Stegmann *et al*., 2020), exploring the role that residues and wastes can play in developing circular and closed systems.

The Irish Government has drafted the "Whole of Government Circular Economy Strategy" (2021) to implement a circular economy, which will involve radical changes to production and consumption. The Strategy will implement many of the actions in the Irish Government's Waste Action Plan for a Circular Economy. A key provision of the Strategy will be the streamlining of processes for end-of-waste and by-products decisions, facilitating the use of waste as a resource.

Waste comprises a broad spectrum of materials, ranging from small-scale household waste to large-scale industrial waste (Pirani and Arafat, 2014; Ahmad *et al*., 2019). In the EU, waste is "any substance or object which the holder discards, intends to discard, or is required to discard" (European Union, 2008a). Legal definitions can create a scenario in which waste must be treated and discharged in a legally defined manner. To facilitate the development of a sustainable economy in which waste is treated as a resource, legislation must be amended to facilitate the valorisation of such waste.

1.2 The Dairy Industry

1.2.1 Dairy processing wastewater

The dairy industry is a significant component of the global economy (OECD and FAO, 2020). World milk production was estimated to be 852 million tonnes (Mt) in 2019, with predictions that it will increase to 997 Mt by 2029. The dairy sector is the EU's second biggest agricultural sector in terms of output value after the vegetable and horticultural plant sector, and before cereals. In 2016, European milk farmers produced 168 Mt of milk, 97% of which came from cows and 3% from ewes, goats and buffalo (Augère-Granier, 2018). The processing of milk results in a substantial amount of waste. Estimates of the amount of wastewater produced range from 0.2 to 11 L of wastewater per litre of milk processed (Bylund, 2015; Wang and Serventi, 2019). This large volume poses a significant financial and technological challenge for the industry.

Dairy processing wastewater is a complex mixture of organic and non-organic components. It contains considerable amounts of sugar (e.g. lactose; Carvalho *et al*., 2013), inorganic components (e.g. ammonia, nitrate and phosphate) and calcium, magnesium, potassium, iron, sodium and chloride (Malaspina *et al*., 1996; Ince, 1998). Concentrations of salts, such as NaCl, KCl and $CaCl_2$, can also be substantial (Carvalho *et al*., 2013), while small amounts of proteins, vitamins and detergents are often also present (Carvalho *et al*., 2013; Foroutan *et al*., 2019). The composition of the wastewater is quite changeable, varying significantly between production facilities (Slavov, 2017) and from day to day and season to season, within the same facility, due to differences in milk quality and the actual dairy products produced (Baskaran *et al*., 2003; Ryan and Walsh, 2016). Interestingly, dairy processing wastewater is derived from a quality-controlled feedstock, and it therefore does not normally contain pollutants such as pesticides, medicines and/or antibiotics.

1.2.2 Treatment of dairy processing wastewater

Dairy processing plants usually have on-site facilities to treat and dispose of generated wastewater (Chen *et al*., 2018; Ahmad *et al*., 2019). Wastewater released into the environment is subject to strict national (Schellenberg *et al*., 2020) and, in the EU, international emission limits (European Union, 2008b). To meet emission limits, dairy wastewater treatment facilities employ a complex mixture of physico-chemical and biological techniques to reduce the concentrations of pollutants (Ahmad *et al*., 2019; Wang and Serventi, 2019). Biological techniques include those that rely on aerobic processes, e.g. operating through activated sludge (Emerald *et al*., 2012) or sequential batch reactor (SBR) (Kushwaha *et al*., 2013), and those that rely on anaerobic processes, operating through anaerobic filters (Ince *et al*., 2000) or upflow anaerobic sludge blanket (UASB) (Latif *et al*., 2011).

The microorganisms use polysaccharides, protein and lipids as energy and nutrient sources (Amani *et al*., 2010). Furthermore, in aerobic systems, nitrification and denitrification result in the oxidation/reduction of ammonia to nitrogen gas (Farazaki and Gikas, 2019), while in anaerobic systems, anammox (anaerobic ammonia oxidising) bacteria convert ammonia to nitrogen gas (Third *et al*., 2001). Phosphorus is typically precipitated through the addition of metal salts (Bunce *et al*., 2018), with the precipitate being removed through filtering or settling.

Dairy processing industry wastewater discharges in Ireland are regulated under Integrated Pollution Prevention Control (IPPC) licences. A 2016 research report by the Irish Environmental Protection Agency (EPA) (Ryan and Walsh, 2016) showed that most processing facilities rely on aerobic waste treatment processes and use standard secondary treatment techniques such as bio-towers and activated sludge aeration. In several cases receiving waters comprise relatively small rivers; this further aggravates the risks to the environment, resulting in significant pressures. This report indicated that, in an international context, there are few "breakthrough technologies" under development in the area of dairy wastewater treatment. Most of the research and development work is focused on phosphorus removal and recovery, as well as on microbial fuel cell technology and hydrogen generation. This situation should be interpreted in the context of a recent EPA report on water quality (Trodd and O'Boyle, 2020), which highlighted that the pressure on water quality continues unabated in the Irish freshwater environment. The development of new wastewater treatment and resource recovery technologies, such as those being explored in the Newtrients project, is therefore innovative, topical and timely.

The treatment of dairy processing waste (Figure 1.1) is currently primarily focused on remediation, with limited valorisation centred on the extraction of biogas (Latif *et al*., 2011) and the use of sludge for land spreading (Ashekuzzaman *et al*., 2019). Promising alternatives are the production of organic acids (e.g. succinic acid, lactic acid and citric acid) for use as preservatives, acidifiers or flavour enhancers (Ahmad *et al*., 2019) and polyhydroxyalkanoates (PHAs) as precursors for bioplastics (Bosco and Chiampo, 2010; Dinesh *et al*., 2020). Valorisation of dairy wastewater can also be achieved using plants and algae (Hemalatha *et al*., 2019; Akansha *et al*., 2020), yielding protein, fine chemicals and biofuel (Ahmad *et al*., 2019; Hemalatha *et al*., 2019).

1.3 Microbial Valorisation of Dairy Processing Wastewater

While microbial approaches for remediation of wastewaters are well established and applied, the application of microbial communities to wastewater valorisation is still evolving (van Loosdrecht and Brdjanovic, 2014; Puyol *et al*., 2017). Advances in the capacity to characterise and correlate key community species with value-added bioconversion potential are creating new opportunities for the design of stable, bio-resource recovery systems (Nielsen, 2017). There has been much recent interest in the potential to produce products such as volatile fatty acids (VFAs) through a partial anaerobic digestion (AD) process (Peces *et al*., 2016). VFAs have a wide array of applications, such as the production of bioplastics and platform chemicals, which are currently obtained from fossil fuels (Puyol *et al*., 2017). PHAs are a class of promising biopolymers with commercially valuable thermoplastic properties. They can be synthesised intracellularly from VFAs by either pure cultures or mixed microbial communities. The use of mixed culture approaches in this context can dramatically reduce PHA production costs, as it does not require expensive axenic culturing conditions or media. As a result, recovery of PHAs from wastewater has become a major research focus globally (Mannina *et al*., 2020). Challenges to overcome in the optimisation of microbial technologies involve alignment of diverse wastewater properties with reactor operational parameters to maximise outputs and system robustness. Unlike the production of biogas, production of VFAs has not yet been developed at an industrial scale. In essence, the dairy sector could provide the platform for a renewable supply of VFAs owing to the availability of low-value waste streams. In addition, both AD and PHA production focus solely on the organic (carbon) fraction of the wastewater stream. Thus, to continue the cascading recovery model and address the considerable nitrogen and phosphate content of dairy wastewater, integration of an appropriate downstream recovery process for these components would be required, creating a significant opportunity for cultivation of protein-rich aquatic plant species.

1.4 Valorisation of Dairy Processing Wastewater Using Lemnaceae

Lemnaceae, i.e. duckweeds, is a family of free-floating aquatic angiosperms (Bog *et al*., 2020). *Lemna minor* is a common native species in Ireland and it can form dense mats on still and slow-moving water (Landolt, 1986). Lemnaceae growth rates are among the highest in the plant kingdom (Ziegler *et al*., 2015) with doubling time varying from 1.3 to 4.5 days (Ziegler *et al*., 2015). Under optimal conditions, this kind of growth can lead to more than 70 tonnes of dry matter being produced per hectare per year (tDW ha^{-1} y^{-1}) (Landolt and Kandeler, 1987). However, yields under field conditions typically range from 20 to 40 tDW ha^{-1} y^{-1} (Mohedano *et al*., 2012; Xu *et al*., 2012).

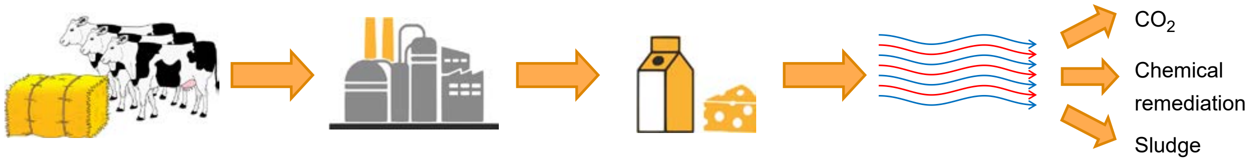

Figure 1.1. The linear dairy processing system. The processing of milk into dairy products creates wastewater, which is remediated using chemical processes, releasing CO_2 and creating sludge.

Rapid growth is associated with fast uptake of nitrogen, phosphorus and other plant nutrients. Consequently, duckweed species have been used for the remediation of nutrients from different types of wastewater, including swine manure effluent (Xu and Shen, 2011; Mohedano *et al*., 2012; Toyama *et al*., 2018), municipal and domestic wastewater (Iatrou *et al*., 2015) and industrial wastewater (Teixeira *et al*., 2014). Nitrogen removal rates on duckweed surface range from 0.124 to $4.4\,g\,N\,m^{-2}\,d^{-1}$ (Mohedano *et al*., 2012; Dinh *et al*., 2020) while phosphorus removal rates range from 0.014 to $0.59\,g\,P\,m^{-2}\,d^{-1}$ (Cheng *et al*., 2002; Dinh *et al*., 2020).

Apart from starch and other carbohydrates, the main component of duckweed biomass is protein. Protein content varies between 15% and 35% of dry weight (Appenroth *et al*., 2017; Sun *et al*., 2020). Lemnaceae have a particularly favourable amino acid profile, closely matching the nutritional requirements of a range of species, including humans (Cheng and Stomp, 2009). Indeed, the use of Lemnaceae as a food source for animals is an established practice in some parts of the world, particularly in Asia (Leng, 1999). Recently, there has been an upsurge in interest in Lemnaceae as a high-quality, protein-rich feed and/or foodstock (Appenroth *et al*., 2015; Dinh *et al*., 2020), and particularly as a potential soybean replacement. Applications are backed up by feeding trials in cattle (Huque *et al*., 1996), goats (Reid, 2004), fish (Islam *et al*., 2004) and poultry (Anderson *et al*., 2011). The use of various Lemnaceae as a human food is practised in parts of Asia (Bhanthumnavin and McGarry, 1971) and more recently has been introduced in the USA and Israel; in both these countries modern cultivation, harvesting and processing technology have been developed (Yaskolka Meir *et al*., 2019). The combination of wastewater remediation with valorisation makes Lemnaceae species of interest for the creation of closed-loop, i.e. circular economy, systems. Valorisation is particularly attractive in the case of wastewater streams derived from food-grade resources, as is the case for dairy processing waste.

1.5 An Integrated Cascading System

Two key principles of circular economy strategies are (a) to maintain the value of products, materials and resources for as long as possible and (b) to “cascade” recovery by extracting high-value products from waste streams before lower value products. Cascading maximises resource effectiveness by using biomass in an order that creates the most economic value over multiple lifetimes. Cascading is central to the concept of integrated biorefineries, which focuses on the integration of multiple biomass conversion technologies to convert feedstocks into a range of value-added products (Ng *et al*., 2017). Cascading and biorefinery approaches have the potential to make circular bioeconomy processes more cost-effective and economically viable, thus catalysing the route to market for the underlying technologies. The Newtrients project deploys a cascading system for the valorisation of dairy processing wastewater using mixed-culture microbial bioconversions and Lemnaceae.

1.6 Aims of the Newtrients Project

The Newtrients project (see Figure 1.2) aims to:

1. deliver sustainable/green technology based on the integration of microbial and duckweed bioreactors for an Irish agri-food sector that is innovative, efficient and a global leader in environmentally sustainable production;
2. demonstrate a circular economy approach to dairy processing industry wastewater remediation, resulting in a paradigm shift from wastewater treatment to the closed-loop reuse of valuable components present in the effluent within the local and global economy, including:
   (a) production of high-protein duckweed biomass that can be used as a locally produced agricultural feed, replacing imported feedstocks;
   (b) production of biodegradable plastic as a replacement for fossil fuel-based plastics;
   (c) avoidance of the generation of large amounts of inorganic phosphorus in sludge;
   (d) recovery of nitrogen and phosphorus from dairy wastewater effluents.

Thus, the overarching vision of the Newtrients project is to underpin the development of a sustainable, innovative agri-food industry that generates novel products from waste and creates new opportunities for rural industries.

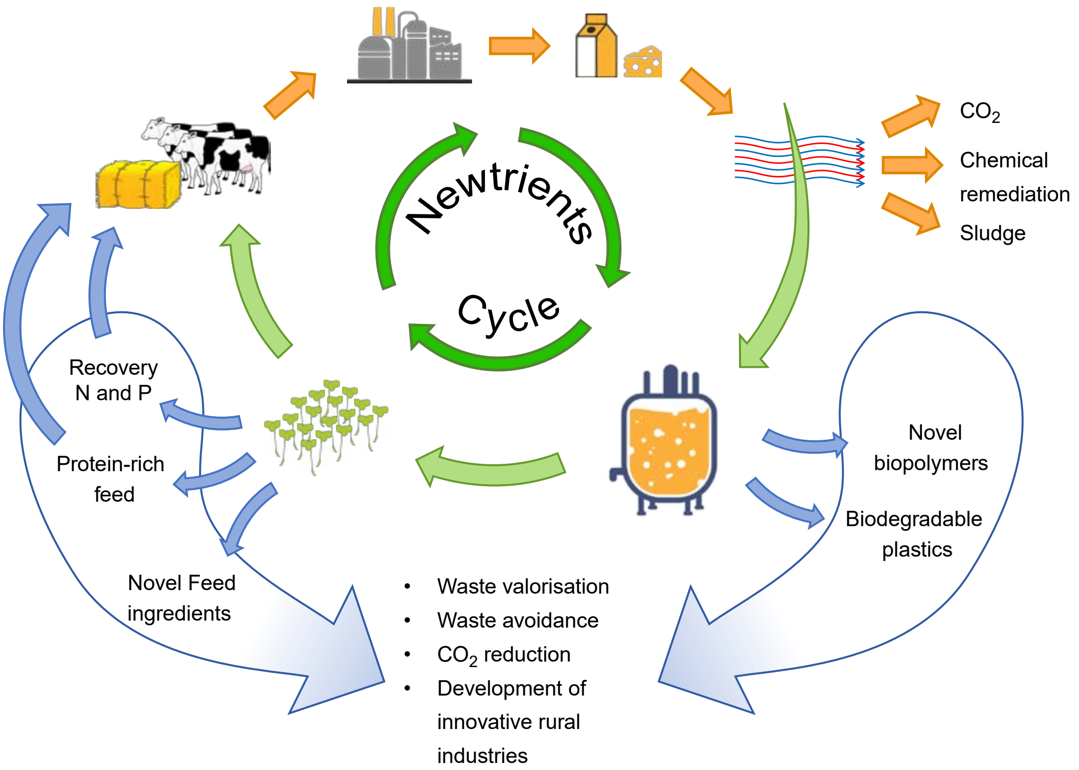

Figure 1.2. The Newtrients cycle. Changing the linear dairy processing system into a circular economy process.

Broadly, the Newtrients project will have positive environmental, economic and social impacts.
The environmental impacts include (a) reduced pollution and pressures on waterways through better wastewater treatment; (b) enhanced resource efficiency and reuse of vital resources, including water, nitrogen, phosphorus and carbon; and (c) reduced energy use and greenhouse gas emissions. The potential socioeconomic impacts include local circular economy loops for duckweed products, new jobs in bio-based industries and lower reliance on imports of protein supplements such as soybean in the agricultural industry.

2 Microbial Technologies for Remediation and Resource Recovery from Dairy Processing Wastewater

2.1 Introduction

Since the early 1900s wastewater treatment technology has used mixed microbial cultures to reduce the environmental and public health impacts of downstream discharge (van Loosdrecht and Brdjanovic, 2014). Modular systems have been adopted globally, often incorporating alternating aeration and non-aeration phases to achieve carbon (biochemical oxygen demand, BOD; chemical oxygen demand, COD) and nutrient (nitrogen and phosphorus) removal. Large dairy processing facilities typically integrate production processes with extensive on-site wastewater treatment plant infrastructure, combining chemical and biological remediation technologies. AD has proven to be of considerable value in dairy processing wastewater management (Hassan and Nelson, 2012). AD reactors can significantly reduce the organic fraction of the wastewater. In addition, sequential conversion of lipids, proteins and sugars to VFAs and ultimately methane gas can provide an energy offset through thermal energy recovery. However, AD reactor effluents still carry a significant nitrogen and phosphorus load; they also require downstream biological treatment processes together with chemical precipitation steps to meet final effluent discharge limits. While complete AD processes convert organic wastewater components to methane gas, truncated operation of the reactor cycle can favour the production of VFAs, which can be exploited as platform chemicals in resource recovery strategies (Peces *et al*., 2016).

The modified Ludzack–Ettinger (MLE) anoxic/oxic (A/O) process was developed for biological nitrogen removal and is commonly used in dairy processing wastewater treatment plants (Mulkerrins *et al*., 2003). More recent advances in bioreactor design have begun to examine single-reactor systems with capacities to remove carbon, nitrogen and phosphorus nutrient loads sequentially. One such emerging technology is the intermittently aerated sequencing batch reactor (IASBR), which combines oxic/anoxic cycling in a single reactor, reducing sludge production, aeration/energy costs and chemical precipitant inputs. IASBR systems have been used for the treatment of dairy processing wastewater with removal efficiencies of more than 95% for COD, nitrogen and phosphorus (Leonard *et al*., 2018).

Microbial communities can be enriched for species with specialist carbon storage-orientated metabolism. Certain bacteria can accumulate PHAs, which are fatty acid metabolism intermediates, in the form of intracellular condensation polyester granules. Purified PHAs demonstrate thermoplastic properties of commercial relevance and also offer promising capacities for biodegradation, either alone or in combination with polycaprolactone. Much PHA commercialisation has focused on bioengineered, pure cultures and expensive axenic, sterile cultivation systems. However, in recent years, research focus has shifted to the potential for mixed microbial communities to contribute to PHA synthesis using industrial waste streams as low-cost feedstocks. Mixed microbial communities in wastewater treatment systems can be enriched for species with PHA storage metabolism using a feast/famine feeding regime (referred to as aerobic dynamic feeding, ADF) that selects for the survival of species capable of PHA storage. The objective of this chapter is to investigate microbial technologies to remediate dairy wastewater and recover valuable resources, as indicated in Figure 2.1. Specifically, this analysis will start with raw dairy wastewater and, using a sequential reactor configuration, aims to generate VFAs and ultimately PHA polymers.

The four technologies described above, i.e. multi-stage MLE, single tank IASBR and AD and ADF reactors, were all employed in our evaluation of dairy processing wastewater valorisation. The three primary tasks can be summarised as:

1. conditioning of dairy wastewater using MLE and IASBR to use as feedstock for the cultivation of *Lemna* sp.;
2. AD seed biomass pretreatment for the selective enrichment of VFA-producing microbial species

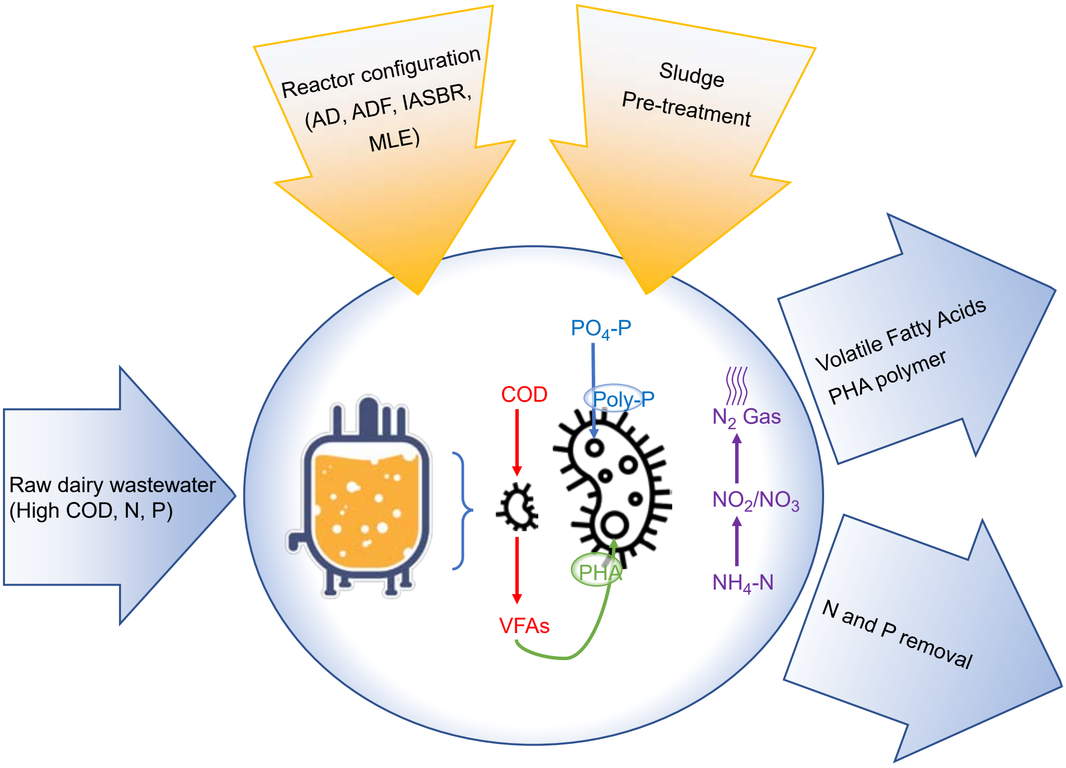

Figure 2.1. Using microbial technologies to remediate dairy wastewater and recover resources.

to increase PHA precursor output and limit conversion to methane;

3. promotion of PHA production in a coupled AD–ADF reactor chain.

All reactor configurations were operated at the laboratory scale, and analytical approaches included standard wastewater physico-chemical measurement methods for carbon, nitrogen and phosphorus compounds, pH, dissolved oxygen (DO) and volatile and suspended solids. Analytical approaches for VFA profiling and PHA quantification included gas chromatography and high-performance liquid chromatography (HPLC). Molecular characterisation of microbial community compositions from AD and ADF reactor biomass samples was performed through total DNA extraction, 16S ribosomal RNA gene library preparation and next-generation sequencing, followed by bioinformatics analyses of community diversity, functionality and robustness.

2.2 Conditioning Dairy Wastewater to Use as *Lemna* Feedstock Using MLE and IASBR

A potential resource recovery/valorisation outlet for dairy processing wastewater involves its use as a feedstock for the cultivation of protein-rich duckweed. However, the high organic nutrient loads of dairy wastewater exceed the tolerable ranges for growth of this aquatic plant. Thus, we evaluated whether modified operation of individual modules from traditional wastewater treatment trains and/or emerging single reactor remediation systems could achieve partial reductions in nutrient loads. Initial set-up and characterisation of this MLE system (Figure 2.2a) employed a synthetic dairy wastewater reported by Gil-Pulido *et al.* (2018). The system was operated for 160 days and the effects of manipulation of key parameters, such as total suspended solids, hydraulic retention time and DO, were evaluated for wastewater conditioning. Raw effluent from a local dairy processing plant was subsequently incorporated into the system for a further 100 days. The composition of the dairy wastewater was found to vary widely depending on the day-to-day operations of the source plant. COD ranged from 2500 to 7000 mg L^{-1}, PO_4-P from 150 to 300 mg L^{-1}, NH_4-N from 5 to 20 mg L^{-1} and pH from 2 to 12, although pH extremes were typically acute events. Figure 2.2b presents a schematic of the IASBR configuration and the key operational stages trialled for dairy wastewater conditioning. The IASBR system was operated on dairy processing wastewater

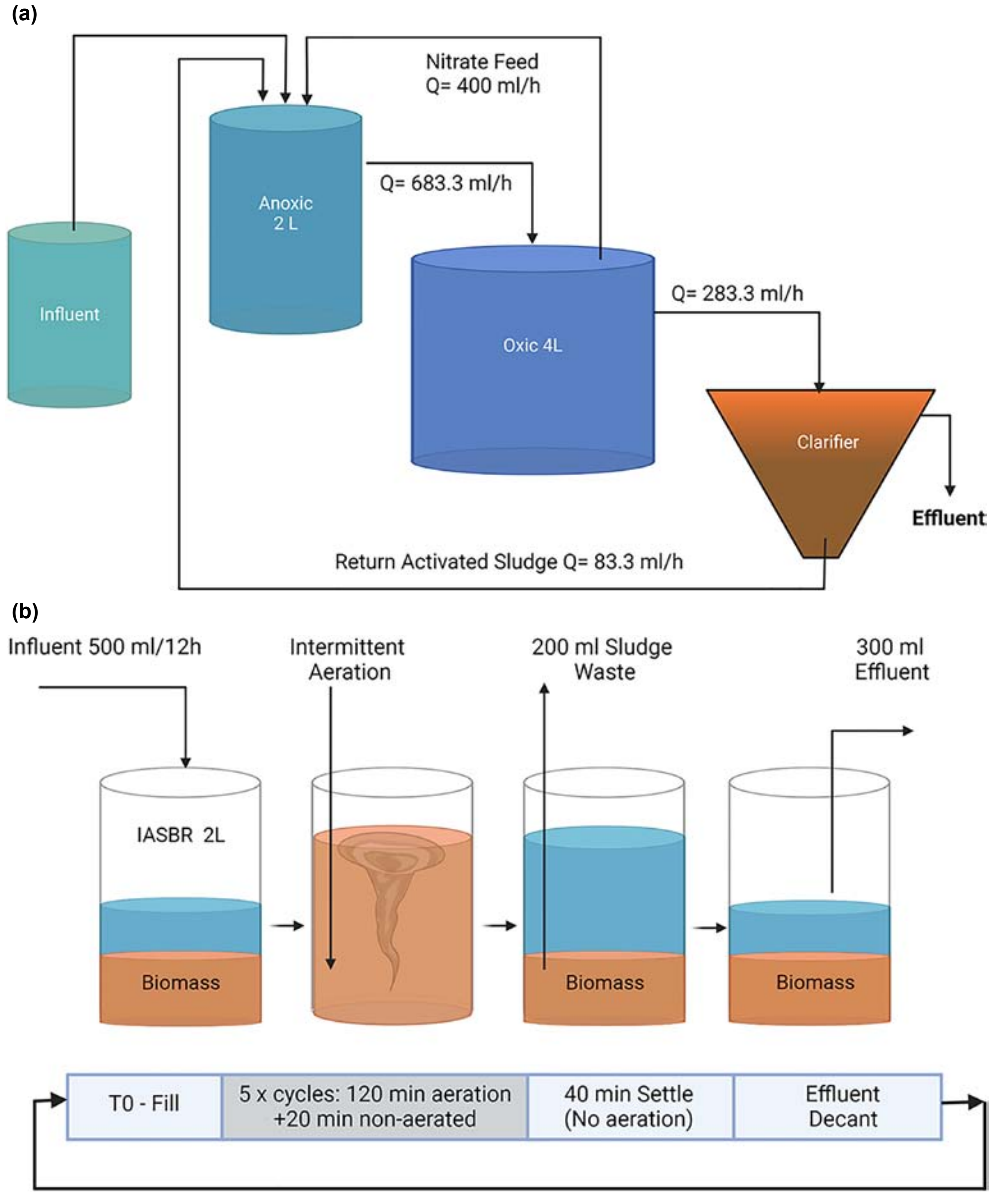

Figure 2.2. Dairy wastewater conditioning systems. (a) MLE configuration and (b) IASBR reactor and sequence operation. Q, flow rate.

only, in parallel with the MLE system exposure. Seed sludge for the reactor systems and dairy processing wastewater samples were obtained from an Irish dairy processing wastewater treatment plant in the Munster region.

The treatment of synthetic wastewater by the MLE system (Figure 2.3) shows promising nutrient removal, minimising COD while retaining residual nitrogen and phosphorus for *Lemna* cultivation. Optimal parameters determined for the MLE system synthetic wastewater for real-time dairy wastewater showed that robust nutrient removal was retained (Figure 2.3).

Average PO_4-P removal efficiency for MLE increased from 40% for synthetic wastewater to approximately 70% for raw dairy wastewater, while NH_4-N removal was comparable for both wastewaters. Similar performance outputs were also observed when dairy wastewater was subject to treatment in the IASBR single-reactor system (Figure 2.3). A notable issue was the low level of influent NH_4-N in the dairy wastewater (5–20 mg L^{-1}), which corresponded with off-peak seasonal production at the plant. At these low concentrations even the modest nitrogen removal efficiency of the MLE system yielded effluent with residual nitrogen levels below the optimal range of

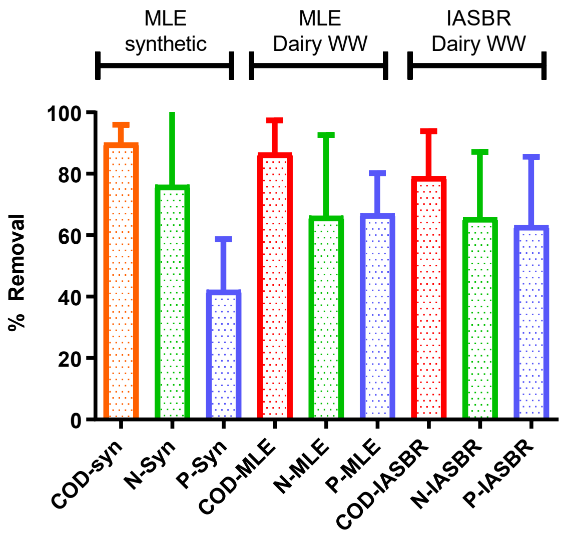

Figure 2.3. Comparison of percentage removal of COD (orange/red), NH_4-N (green) and PO_4-P (blue) from synthetic and raw dairy wastewaters (dairy WW) in the MLE and IASBR systems, respectively.

5–50 mg L^{-1} required for active promotion of *Lemna* growth. In addition, the low nitrogen contents resulted in suboptimal carbon–nitrogen–phosphorus ratios (100:10:1), which are known to influence biological nutrient removal (BNR) system performance and the relative abundance of filamentous organisms in sludge. Despite these intermittent issues, MLE and IASBR both showed promise in their capacity for adjusting wastewater nutrient composition to facilitate *Lemna* growth. MLE-based infrastructure is operational in many large-scale dairy wastewater processing plants and is therefore ideally placed to be adapted for roles in conditioning wastewater in advance of resource recovery processes.

2.3 AD Seed Biomass Pretreatment for Selective Enrichment of Microbial Species to Produce Mixed VFAs for PHA Synthesis

Full-scale AD operation (Figure 2.4) proceeds through various metabolic stages to the principal end products of methane and carbon dioxide and is dependent on the sequential metabolism of key bacterial genera.

Aiming for a system where VFA end products are the primary outputs, a truncated AD process was trialled. The first step evaluated seed sludge pretreatments to enrich for acidogenic bacteria. Treatment of seed sludge with acid conditions (pH 2 × 24 h), high

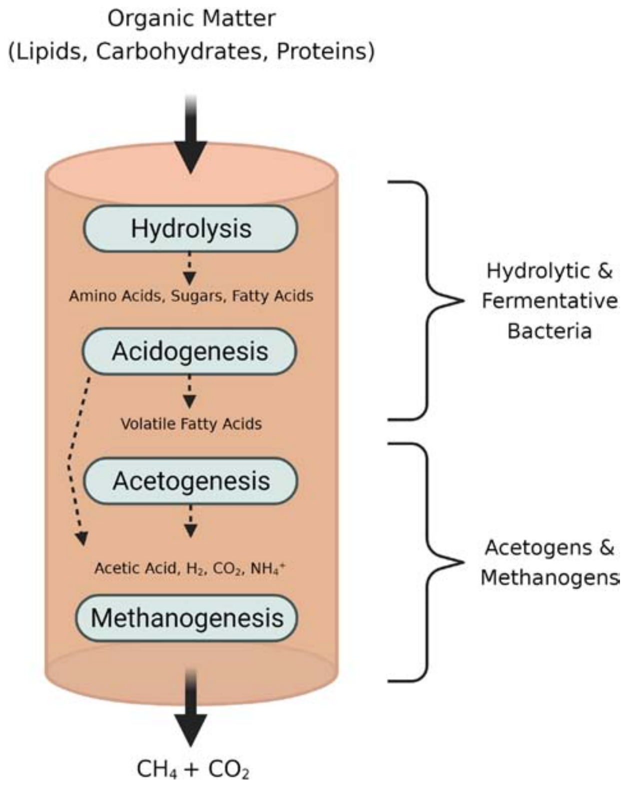

Figure 2.4. The AD process, intermediates and key microbes. Unlike the MLE and IASBR processes, which have aerobic stages, the anaerobic conditions in AD prevent respiration and full oxidation of soluble organic material to CO_2. The transformation of the organic materials proceeds through several sequential stages, enabling the potential for arresting the system at a selected stage to produce a desired product.

temperature (100°C × 1 h) or both may select for their survival while eliminating or reducing the complement of acetogens and methanogens that do not form spores. Figure 2.5a reports the total VFA outputs from four independent AD reactors seeded with sludge that was subjected to different pretreatments (see Figure 2.5 for details of the pretreatments). All reactors were found to produce peak VFA outputs at approximately 10 h; however, VFA production thereafter was sustained only in reactor R3, in which the seed sludge had been subjected to pH 2 for 24 h. In all other reactors VFAs were rapidly depleted, suggesting progression to acetogenesis and methanogenesis. VFA profiling by gas chromatography was also conducted over the course of one full hydraulic retention time in the R3 reactor. As shown in Figure 2.5b, acetic acid was found to be a minor component of the final VFA fraction, which was

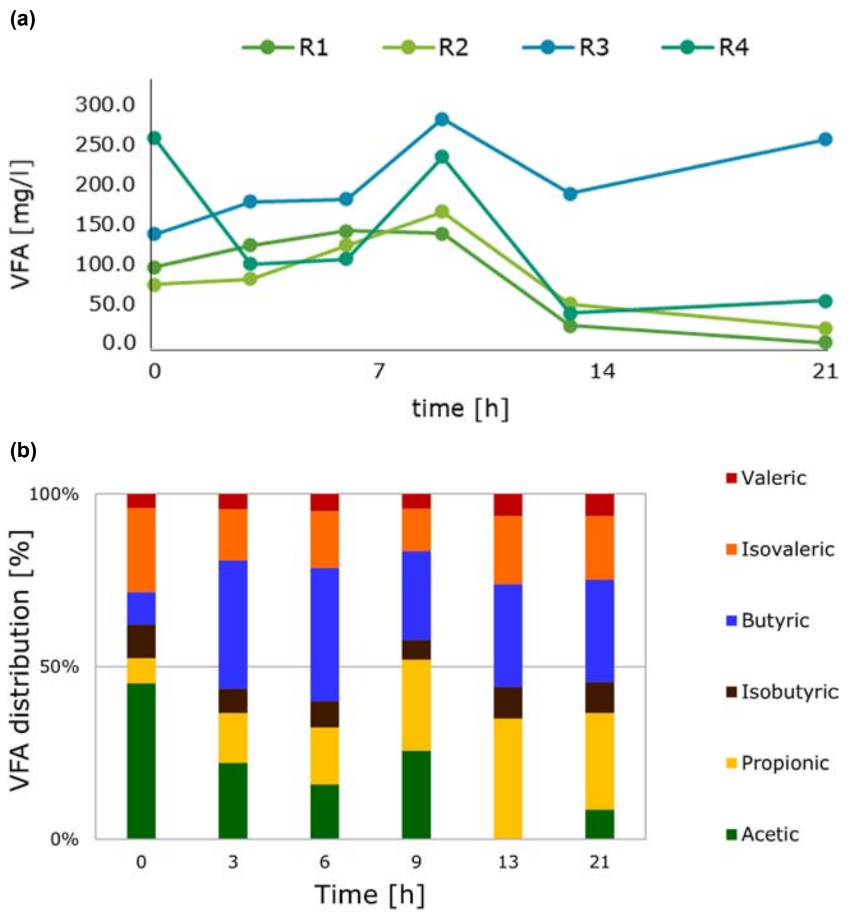

Figure 2.5. Seed sludge pretreatments and AD reactor VFA outputs. (a) R1, no pretreatment; R2, 100°C × 1 h; R3, pH 2 × 24 h; and R4, pH 2 + 100°C combined. (b) VFA fraction profile of reactor R3.

dominated by propionic, butyric and isovaleric acids. Importantly, this indicated the impact of pretreatment on successfully achieving a truncated operation of the AD reactor and the scope for producing diverse, longer chain VFAs using this process.

Experiments also compared VFA outputs from two industrially significant AD reactor configurations, namely a UASB and a continuously stirred tank reactor (CSTR) (Mao *et al*., 2015). Both reactor systems were established with seed sludge subjected to acidification at pH 2 for 24 h (R3 seed pretreatment conditions). The UASB design involved three 1-L, sequential, interlinked chambers receiving continuous wastewater at a feed rate of 3 L per day. The CSTR system comprised a 2-L sealed tank subject to pulse feeding at 12 h intervals totalling 1.5 L per day. A comparison of VFA outputs and profiles from the systems is presented in Figure 2.6.

The CSTR system was found to yield total VFAs of approximately 1500 mg L^{-1} from dairy wastewater fermentation, mainly consisting of acetic acid (60% total VFA) and propionic acid (33% total VFA). Total VFA yield from the UASB system was 25% lower than that from the CSTR system; however, the

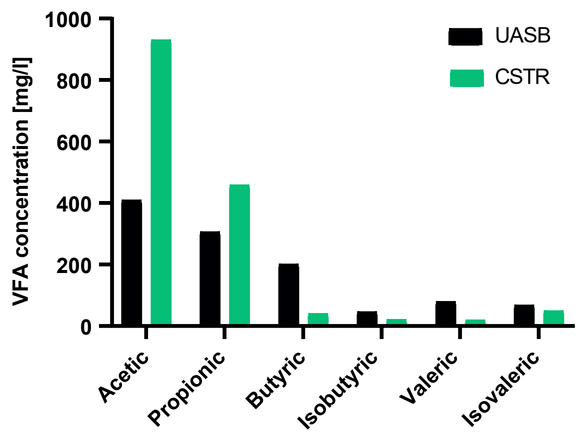

Figure 2.6. Comparison of dairy wastewater VFA outputs from UASB and CSTR AD configurations.

UASB system produced a VFA fraction with a broader distribution profile (acetic acid 36%, propionic acid 27%, butyric acid 18%, valeric acid 5%). Keeping in mind the goal of VFA valorisation through potential biopolymer accumulation, the greater yield and high acetic and propionic acid VFA compositions of the CSTR system effluent were preferred.

In addition to physico-chemical analyses of reactors to evaluate performance, biomass samples were collected at various points during the AD reactor adaptation and operation for molecular characterisation of the microbial communities (Figure 2.7). The AD reactor samples demonstrate important community shifts with respect to optimising

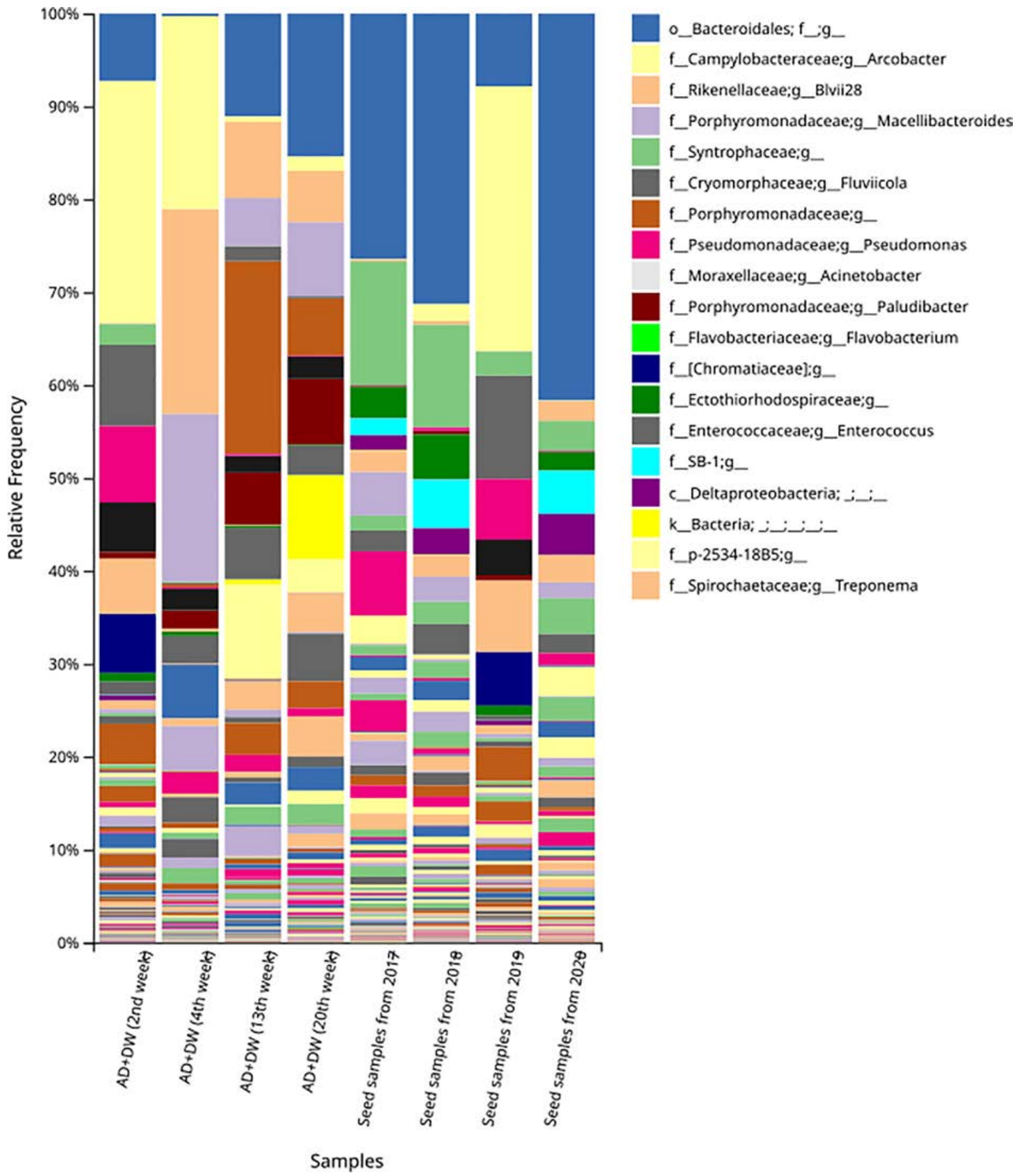

Figure 2.7. Molecular microbial ecology profiling of seed sludge and CSTR reactor community over time. Each column represents a single biomass sample and each stacked colour in a column represents a single bacterial family (identified in the key). c, class; f, family; k, kingdom; o, order.

and maintaining VFA production while minimising acetogenesis and methanogenesis. For example, Syntrophaceae family representatives, which are known to contribute to acetogenesis, constituted 15% of the initial seed sludge communities in seed samples from 2017 and 2018, but their presence was greatly reduced in the AD+DW (second week) community after acid pretreatment (Figure 2.7).

Pretreatment appeared to successfully exclude a major acetogenic family. In contrast, Porphyromonadaceae family-associated genera, which are known to produce acetate, propionate and butyrate, became established at 10–20% of community biomass in the later stages of reactor operation (Figure 2.7, AD+DW at 13th and 20th weeks). In summary, molecular profiling of the CSTR system for VFA production indicated stable reactor operation over relatively extended operational periods (3–5 months), underpinned by a resilient microbial community.

2.4 Promotion of PHA Production in a Coupled AD–ADF Reactor Chain

An ADF reactor system (Figure 2.8) was investigated for PHA biopolymer production from dairy wastewater with the goal of determining (a) the potential yields of PHA, (b) the dominant monomer compositions of PHAs produced, (c) the capacity for direct coupling of the AD and ADF systems with duckweed cultivation and (d) the molecular profile of PHA synthesis genes/pathways in sludge biomass for the predictive evaluation of PHA diversity. The ADF adaptation involved 2-L reactors seeded with 7 g L^{-1} biomass operated at 4 days solids retention time (SRT) and 2 days hydraulic retention time with 450 mL of VFA-enriched dairy wastewater pulse-fed in 8 h feast/famine cycles.

Chemical profiling of the ADF single-reactor system revealed significant, stable nutrient removal with efficiencies of 90% COD, 70% PO_4-P and 70–80% total nitrogen (TN). It is noteworthy, therefore, that, like the IASBR technology evaluated in section 2.1.1, the ADF single-reactor system may offer the dairy sector an additional, plug-in technology to expand wastewater remediation capacity. This also has relevance for the potential for duckweed cultivation. With respect to PHA biosynthesis, Figure 2.9 provides a sample evaluation during characterisation of batch production with synthetic media containing acetate. The sole PHA produced was polyhydroxybutyrate (PHB), a biopolymer with well-established commercial significance.

PHB evaluation by crotonic acid conversion and HPLC indicated that PHA accumulation capacity was successfully enriched in the ADF reactor. In the batch trial, the maximal yield of PHB was approximately 15% cell dry weight. However, further optimisations with synthetic media and VFA-enriched wastewater achieved yields of 38% cell dry weight PHB.

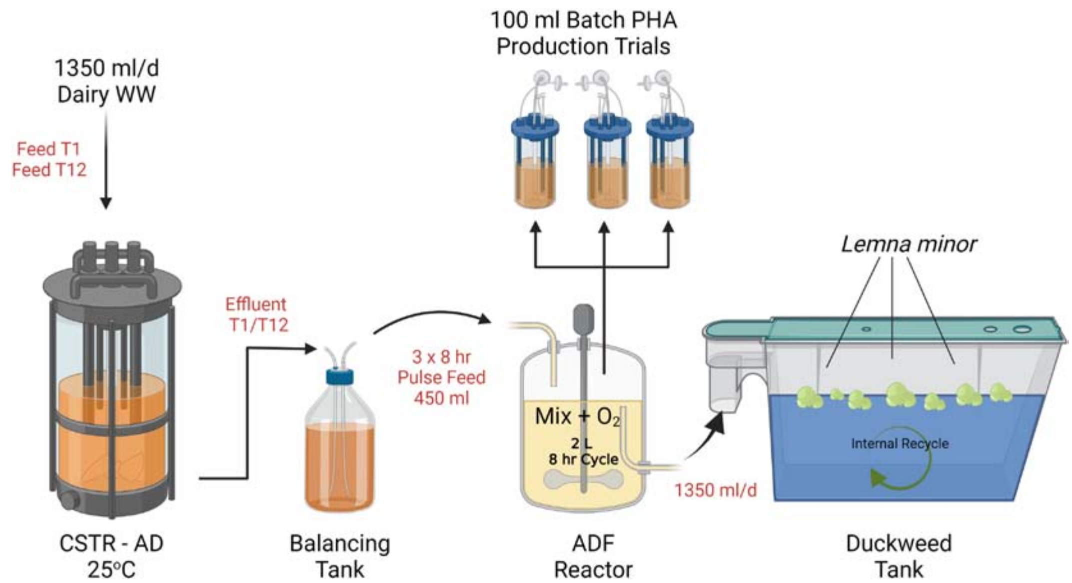

Figure 2.8. Schematic overview of proposed integration of AD–ADF–duckweed cascading system.

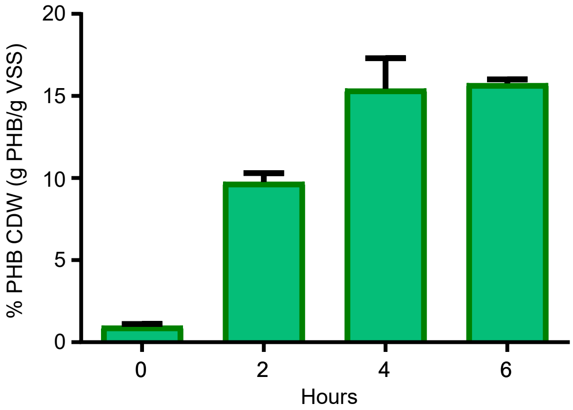

Figure 2.9. Chemical characterisation of batch PHA production with ADF-adapted biomass over 6 h with synthetic wastewater and acetate pulse feeding at 2-h intervals. CDW, cell dry weight; VSS, volatile suspended solids.

Direct coupling and stable operation of the AD and ADF reactors was also successfully achieved. The AD system provided an effluent enriched with acetic and propionic acids to the ADF unit, and these acids were fully consumed, together with 70–80% removal of nitrogen and phosphorus species. Another aim was to assess whether or not copolymers such as polyhydroxybutyrate–co-valerate were being produced, as these are economically more attractive than the short-chain-length PHB polymers. Bioinformatic analyses of genome sequences revealed that the ADF-adapted sludge community contained an enhanced abundance of PHB-specific synthesis genes (e.g. phbC) when compared with un-adapted seed sludge and AD biomass samples. The detection of this subset of PHA genes suggests that the metabolic capacity selected for by the ADF conditions, irrespective of feedstock, is wholly orientated towards short-chain-length PHB production.

In conclusion, a microbial-based system (AD–ADF) was developed to valorise dairy wastewater by initially producing platform chemicals (VFAs), which could be further synthesised to commercially significant biopolymers. The successful profiling of this system as outlined, in terms of both microbial and chemical characterisation, is fundamental to the future deployment of such technologies.

3 Remediation of Dairy Processing Wastewater by *Lemna minor*

3.1 Introduction

Dairy processing wastewater contains a variety of plant nutrients such as nitrogen, phosphorus and other minerals (Carvalho *et al*., 2013; Tikariha and Sahu, 2014). However, this does not necessarily imply that this wastewater is amenable to remediation using duckweed (Lemnaceae). Here, we demonstrate that dairy processing wastewater is a suitable growth medium for *L. minor* and can be remediated by this plant (section 3.2). The effects of operational parameters such as light intensity (section 3.3), plant density (section 3.4) and choice of duckweed clones (section 3.4) on nitrogen and phosphorus removal from wastewater are explored (Figure 3.1).

3.2 Dairy Processing Wastewater as a Suitable Growth Medium for *Lemna minor*

Remediation of dairy processing wastewater using duckweed (Lemnaceae) is proposed. Although duckweed species are tolerant of a wide range of conditions (Landolt and Kandeler, 1987), preliminary experiments by the research team at University College Cork showed no growth of duckweed on synthetic dairy wastewater. Thus, a study was conducted to explore (a) why duckweed did not grow on synthetic dairy wastewater and (b) how to overcome obstacles to plant growth.

3.2.1 Materials and methods

Experiments were conducted using a stock culture of *L. minor* – Blarney (Rutgers Duckweed Stock Cooperative, RDSC, 5500), held under optimum conditions (Walsh *et al*., 2020). The composition of the synthetic wastewater was detailed by Tarpey (2016) and modified here by increasing or decreasing specific nutrients. The study consisted of *L. minor* short-term (7 days) growth experiments on 100 mL of synthetic dairy processing wastewater, with plants grown on optimised half-strength Hutner’s medium as a control.

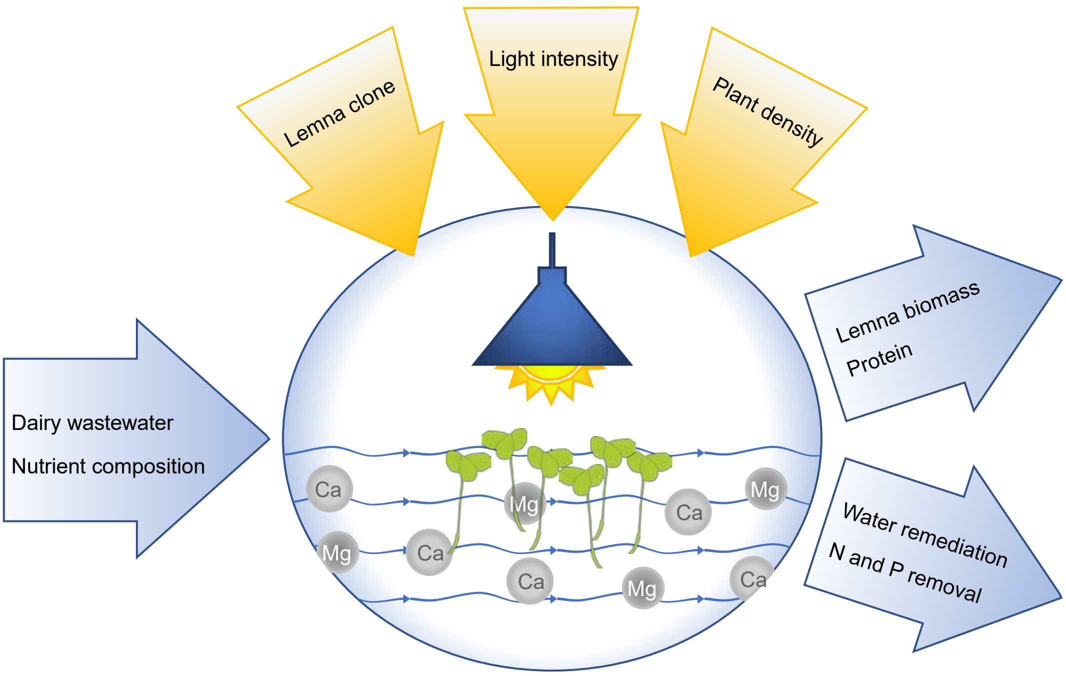

Figure 3.1. Schematic overview of the role of the duckweed *L. minor* (Lemnaceae) in the remediation of dairy processing wastewater.

Growth was monitored as the increase in wet weight and expressed as relative growth rate (RGR).

3.2.2 Results and discussion

Cultivation of *L. minor* on synthetic dairy wastewater resulted in poor growth rates, which occurred within days, indicating toxicity. A systematic study of the chemical composition of the synthetic medium indicated that a number of components (iron, manganese, sodium, chloride and sulfate) were present in non-optimal concentrations, potentially having negative impacts on growth. However, changing the concentration of individual elements did not improve growth.

The concentration of calcium in synthetic dairy processing wastewater is low (0.014 mM) compared with the optimal range (0.2–20 mM) for *L. minor*. Furthermore, the calcium to magnesium ratio (Ca to Mg) in synthetic wastewater is skewed towards magnesium, causing a "relative" lack of calcium. To investigate potential calcium deficiency, *L. minor* plants were grown on medium with increasing amounts of calcium (0.014–1.21 mM), at a constant magnesium concentration (0.2 mM), increasing the Ca to Mg ratio from 1:14.6 to 6.1:1. Addition of calcium to the synthetic dairy processing medium resulted in a significant increase in RGR (Figure 3.2a). Further investigations explored the importance of the Ca to Mg ratio, at a constant Ca concentration (0.12 mM), and with magnesium concentrations decreasing from 4.9 to 0.2 mM, yielding Ca to Mg ratios from 1:41.2 to 1:1.6. Growth of *L. minor* progressively increased as the concentration of magnesium increased (Figure 3.2b).

Negative effects on RGR can be attributed to the rise in magnesium concentration and/or the decrease in the Ca to Mg ratio. High concentrations of magnesium (> 5mM) have been reported to be toxic to Lemnaceae (Walsh *et al.*, 2020). To explore this, concentrations of both calcium and magnesium were increased while maintaining the same Ca to Mg ratio. Under these conditions, the RGR remained fairly stable (Walsh *et al.*, 2020), emphasising the importance of the Ca to Mg ratio. The observed antagonism between calcium and magnesium relates to cellular uptake and metabolism (van Dam *et al.*, 2010). It is concluded that dairy processing wastewater is a suitable growth medium for *L. minor*; however, monitoring and possibly amending the Ca to Mg ratio is required as part of operational management.

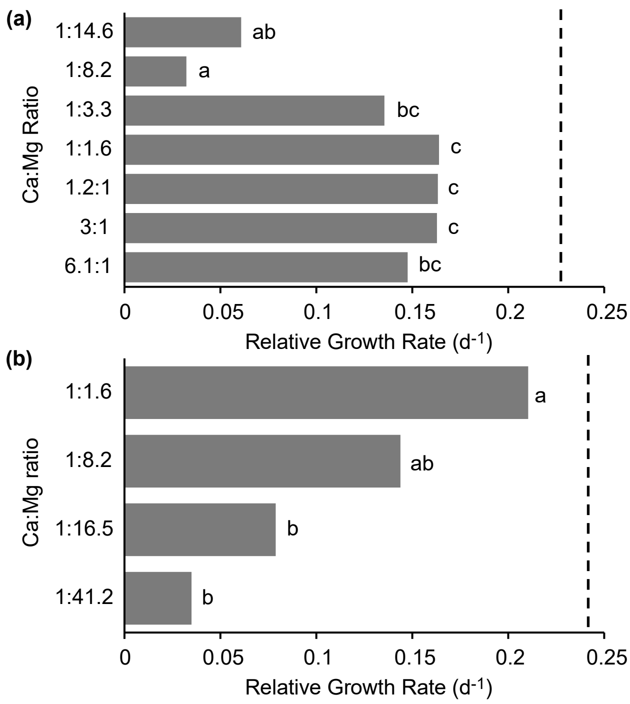

Figure 3.2. RGR of *L. minor* grown on wastewater with various Ca to Mg ratios (bars) and on half-strength Hutner's medium (dashed line). Ratios were modified by (a) varying the concentration of Ca at constant Mg concentration of 0.2 mM or (b) varying the concentration of Mg at constant Ca concentration of 0.12 mM. Unmodified wastewater has a Ca to Mg ratio of 1:14.6. Bars that do not have the same letter differ significantly from one another ($p < 0.05$).

3.3 Light Dependence of *Lemna minor*-mediated Remediation of Dairy Processing Wastewater

Phytoremediation is a low-cost and sustainable purification technique of effluents (Akansha *et al.*, 2020). The effectiveness of duckweed-based phytoremediation of nitrogen- and phosphorus-containing compounds in wastewater is linked to the duckweed growth rate (Cheng *et al.*, 2002). Accordingly, phytoremediation operational parameters should be optimised to maximise duckweed growth. Here, the interactive effects of light intensity and media composition were explored to advance the development of high-output, indoor duckweed remediation systems.

3.3.1 Materials and methods

The duckweed strain used was *L. minor* – Blarney (RDSC 5500), kept under laboratory conditions (Walsh *et al.*, 2020). In initial experiments, plants were grown in stationary containers (100 mL) under 10 different light intensities between 10 and 850 μmol m^{-2} s^{-1} on synthetic dairy wastewater (Tarpey, 2016). In follow-up experiments, three light intensities (50, 200 and 850 μmol m^{-2} s^{-1}) were used for detailed assessments, and medium was either synthetic wastewater or optimised half-strength Hutner's medium (Hutner, 1953).

L. minor was also grown in an upscaled, recirculating tank system at three different light intensities (100, 300 or 900 μmol m^{-2} s^{-1}). In this system, synthetic dairy wastewater was pumped from a lower sump tank to an upper duckweed tank from where it drained back into the sump. The system contained 11.7 L of medium across two equally sized tanks.

The RGR of *L. minor* was calculated in line with Connolly and Wayne (1996). Total nitrogen (TN) and total phosphorus (TP) in media were quantified using a Hach spectrophotometer and/or using cadmium reduction (TN) and Murphy and Riley (TP) methods.

3.3.2 Results and discussion

Stationary remediation system

Growth of *L. minor* peaked at 50 μmol m^{-2} s^{-1}, followed by a gradual decrease in RGR as light intensity increased (Figure 3.3a). This is an unusual light response, as growth of Lemnaceae typically increases with intensity up to 400–600 μmol m^{-2} s^{-1} (Paolacci *et al.*, 2018). Indeed, plants on half-strength Hutner's medium displayed a significant increase in RGR with increasing light intensity, which was not apparent for plants on synthetic wastewater. Chlorophyll *a* fluorescence measurements revealed that, under higher light intensities, the efficiency of light utilisation was significantly lower in plants grown on synthetic wastewater than in those grown on Hutner's, indicating a medium-specific impairment of photosynthesis (Walsh *et al.*, 2021b). Thus, we conclude that growth

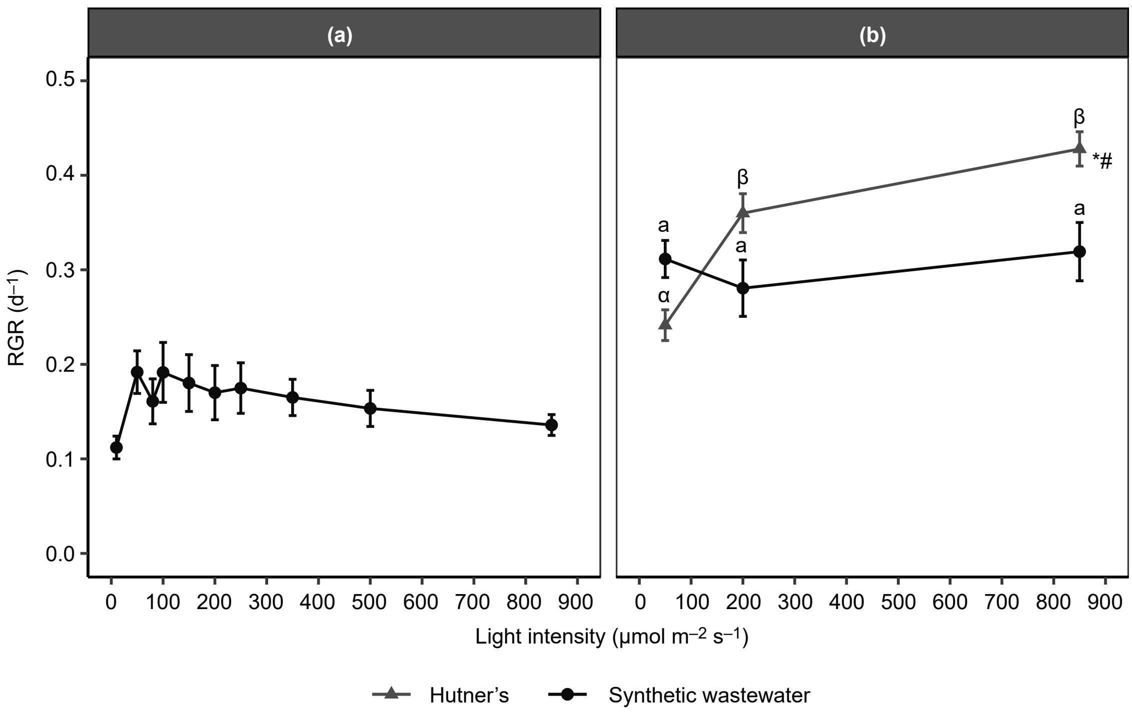

Figure 3.3. Mean (± standard error) fresh biomass RGR (d^{-1}) of (a) *L. minor* grown on synthetic wastewater under 10 light intensities and (b) *L. minor* grown under three different light intensities on either wastewater or half-strength Hutner's medium. An asterisk (*) denotes an effect of media at $p < 0.05$, while a hash symbol (#) denotes an effect of light intensity at $p < 0.01$. Points that do not share the same letter (i.e. "a" vs "b" in synthetic wastewater, or "α" vs "β" in Hutner's medium) significantly differ from one another ($p < 0.05$). Reproduced from Walsh *et al.* (2021b), with permission.

is subject to interactive effects of light intensity and medium composition (Figure 3.3b).

Overall, *L. minor* grown on synthetic wastewater displayed higher TN removal rates than *L. minor* grown on half-strength Hutner's medium (Figure 3.4a). This may relate to the type of nitrogen in the medium. Synthetic wastewater contains ammonia and urea, whereas Hutner's medium contains nitrate. Ammonia is more readily taken up than nitrate by *L. minor* (Landolt and Kandeler, 1987). Plants grown on synthetic wastewater displayed similar TN removal rates at all light intensities, despite fluctuations in RGR, and this may indicate "luxury" (non-essential) uptake of nitrogen by the plants.

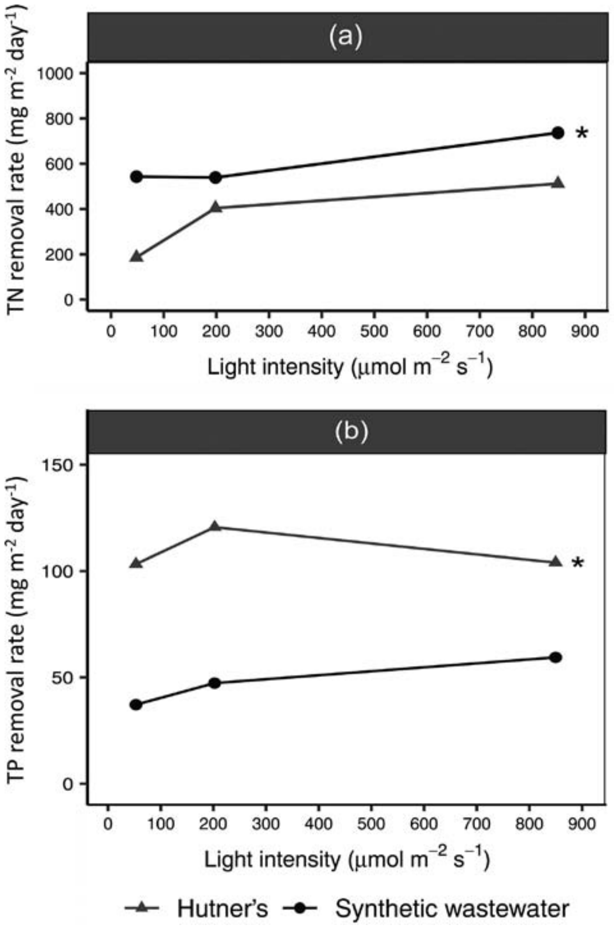

Figure 3.4. Mean (± standard error) values for (a) TN and (b) TP removal rate (mg N m^{-2} d^{-1}) from synthetic wastewater or half-strength Hutner's medium under three different light intensities. An asterisk (*) denotes a significant effect of media (two-way analysis of variance, $p < 0.05$). Reproduced from Walsh *et al.* (2021b), with permission.

The *L. minor* TP removal rate on Hutner's medium was double that on wastewater (Figure 3.4b). As Hutner's medium contains substantially more phosphorus than synthetic dairy wastewater (93 mg L^{-1} and 10.9 mg L^{-1}, respectively), luxury phosphorus uptake is likely. Light had no major effect on phosphorus uptake.

Recirculating remediation system

In recirculating tanks, RGR and TN removal rate did not display significant light dependency (Figure 3.5). Algae and microbes in recirculating tanks may have contributed to a modest increase in TN and TP removal rates at higher light intensities without an associated increase in *L. minor* RGR (Figure 3.5) (Zhao *et al*., 2015). However, the relatively high density of duckweed in the system (60%) is likely to have negated a substantial effect of algae (Roijackers *et al*., 2004). The lack of light dependency implies that indoor systems do not require expensive investment in high-capacity lighting systems.

3.4 Plant Density Influences the Efficacy of Wastewater Remediation by *Lemna minor*

Wastewater remediation by duckweed is a surface process. Typically, a higher duckweed plant density increases the potential for uptake of nitrogen and phosphorus (Xu and Shen, 2011). Yet, once duckweed surface cover is complete, individual colonies overlap and shade each other. Such crowded conditions have a negative impact on growth (Driever *et al*., 2005; Frédéric *et al*., 2006) and cause senescence and the release of nutrients back into the water (Ceschin *et al*., 2019). Understanding the relationships between plant surface density, biomass yield and net nutrient uptake is required to develop the operational parameters of duckweed-based remediation systems.

3.4.1 Materials and methods

L. minor – Blarney (RDSC 500) was first grown on synthetic dairy wastewater (Tarpey, 2016) for 7 days at eight density conditions (10%, 20%, 30%, 40%, 50%, 60%, 70% and 80% plant coverage of medium surface area). Medium was half-strength Hutner's (Hutner,1953) or a dairy processing wastewater (Tarpey, 2016). For both experiments, the specific growth rate (SGR) of *L. minor* was calculated in

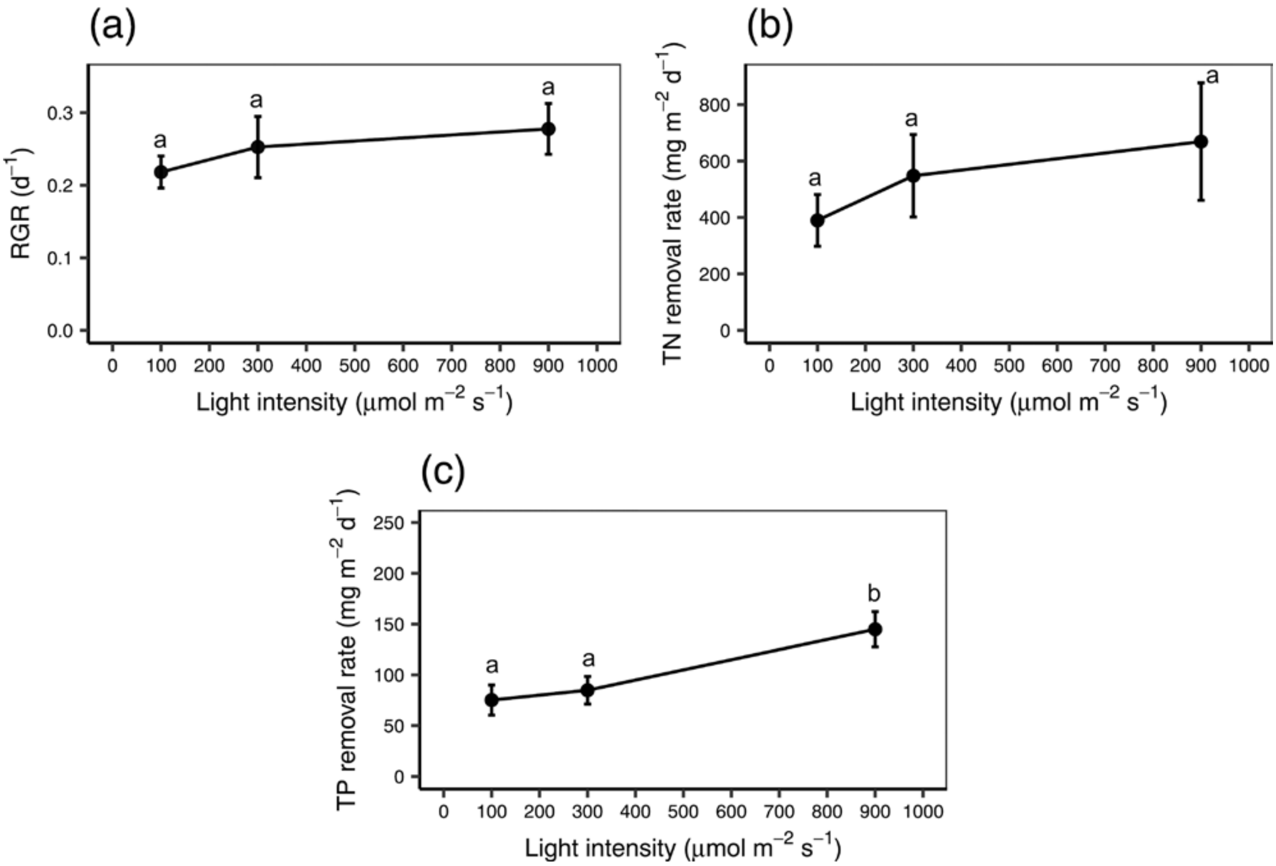

Figure 3.5. Mean (± standard error) (a) RGR, (b) TN removal rate (mg N m^{-2} d^{-1}) and (c) TP removal rate (mg P m^{-2} d^{-1}) of *L. minor* grown on wastewater under three different light intensities in recirculating tanks. Points that do not share the same letter (i.e. in panel c), significantly differ from one another ($p < 0.05$). Reproduced from Walsh *et al.* (2021b), with permission.

line with Chaiprapat *et al*., (2005). TN and TP in the medium were measured on the first and final day of each experiment. For TN and TP analysis, Hach tests LCK138 (range 1–16 mg L^{-1} TN) and LCK348 (range 0.5–5 mg L^{-1} PO_4-P) were used, respectively. Where concentrations were out of range, samples were diluted.

3.4.2 Results and discussion

The absolute yield of plant biomass, as well as the removal of TN and TP (mg) from wastewater, increased with increasing plant density (Figure 3.6a, c,e). In contrast, the SGR and TN and TP removal rates per frond surface area (mg N m^{-2} d^{-1}) decreased as density increased (Figure 3.6b,d,f). The data show that, for remediation purposes, high plant densities are desirable on account of higher TN and TP removal. However, decreases in “per plant” growth and TN and TP removal rates are of concern, particularly at very high densities when plants can become senescent and release nutrients back into the medium. Therefore, the management of duckweed-based remediation systems should consider density-dependent growth effects and nutrient removal.

3.5 Lemnaceae Clones Display Different Remediation Efficiencies

The potential of duckweed to remediate wastewater varies for different duckweed species (Toyama *et al*., 2018). However, differences between clones of the same species can be as significant as between distinct species (Ziegler *et al*., 2015); for example, doubling times of *Spirodela polyrhiza* clones varied between 1.8 and 4.1 days (two-fold difference in growth rate). Here, we explore whether or not duckweed growth rates and remediation of dairy processing wastewater can be optimised by choosing specific duckweed clones.

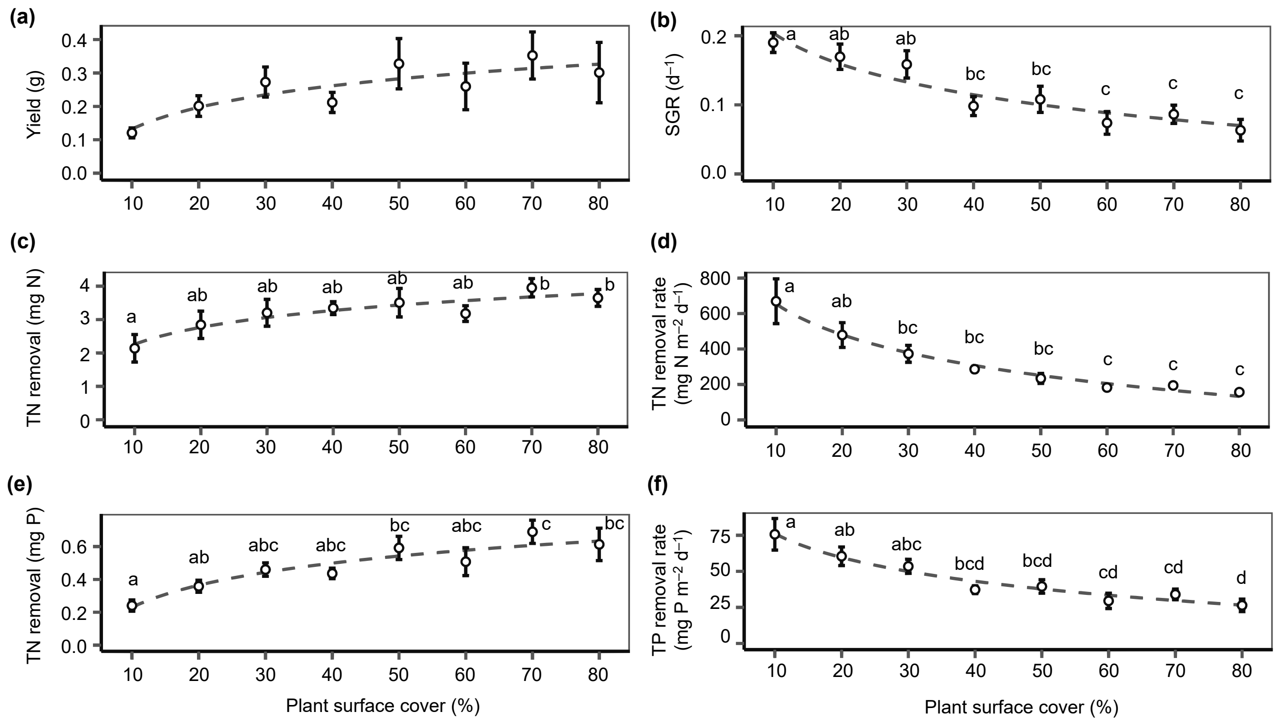

Figure 3.6. Mean (±standard error) values with natural log trendline of (a) yield (g), (b) SGR (d^{-1}), (c) TN removal (mg), (d) TN removal rate per frond surface area ($mg\,m^{-2}\,d^{-1}$), (e) TP removal (mg) and (f) TP removal rate per frond surface area ($mg\,m^{-2}\,d^{-1}$) for *L. minor* grown on synthetic wastewater at different plant densities. Points that do not share the same letter significantly differ from one another ($p<0.05$). Adapted from Walsh *et al.* (2021a); licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

3.5.1 Materials and methods

Clones of *L. minor* and *L. minuta* were collected throughout the south of Ireland (Table 3.1). Clones MJ109–MJ111 were collected from dairy processing facilities, clones MJ112–MJ116 from waters with saltwater influence and clones MJ309 and MJ117 from streams in an intensively farmed region. Additional clones were from stock cultures

Table 3.1. International Steering Committee on Duckweed Research and Applications (ISCDRA) accession identifier, clonal name, sampling location and identity of *Lemna* clones

ISCDRA ID (RDSC ID)	Clonal name	Origin – sampling location	Coordinates	Species identity
MJ100 (5500)	Blarney	Stock (*L. minor*)	–	*L. minor*
MJ302 (5571)	Charleville	Stock (*L. minuta*)	–	*L. minuta*
UD103	Hungary	Stock (*L. gibba*)	–	*L. gibba*
MJ109	Mitchelstown A	Clarifying tank, dairy wastewater facility	52.2717123, –8.2805116	*L. minor*
MJ110	Mitchelstown B	Thickening tank, dairy wastewater facility	52.2717123, –8.2805116	*L. minor*
MJ111	Ballineen	Tank, dairy wastewater facility	51.7318492, –8.9811663	*L. minor*
MJ309	Fermoy	Blackwater river near Fermoy	52.145377, –8.162454	*L. minuta*
MJ112	Midleton	Owenacurra river at Midleton	51.9164163, –8.1761566	*L. minor*
MJ113	Tourig River	Tourig river near Youghal	51.9745581, –7.9073607	*L. minor*
MJ114	Whiting Bay A	Stream entering sea at Whiting Bay	51.9524439, –7.7736677	*L. minor*
MJ115	Whiting Bay B	Stream entering sea at Whiting Bay	51.9527918, –7.7736767	*L. minor*
MJ116	Cuskinny Marsh	Cuskinny Marsh near Cobh	51.8603034, –8.2674446	*L. minor*
MJ117	Shournagh River	Shournagh river, Blarney	51.9697820, –8.6716304	*L. minor*

at University College Cork (MJ100 Blarney and MJ302 Charleville) or Debrecen University, Hungary (UD103 Hungary). Here, the term clone is used for any clone, regardless of species.

Collected clones were characterised (i.e. genetically barcoded) through sequencing of the intergenic spacers *atpF-atpH* and *psbK-psbI* and morphological analysis (Bog *et al*., 2020). Subsequently, clones were grown on synthetic dairy wastewater (Tarpey, 2016) and RGR and protein content (Bradford, 1976) were measured. The removal of TN and TP from the medium was measured using Hach tests LCK138 (range 1–16 $\text{mg}\,\text{L}^{-1}$) and LCK348 (range 0.5–5 $\text{mg}\,\text{L}^{-1}$), respectively.

3.5.2 Results and discussion

Because of their reduced structure, different duckweed species can appear similar (Landolt, 1986). Therefore, intergenic spacer sequences were compared with established species identity. Clones MJ302 and MJ309 were unambiguously identified as *L. minuta* (Table 3.1). However, identical *psbK-psbI* and *atpF-atpH* sequences for *L. minor* and *L. japonica* precluded unambiguous identification of clones MJ109 to MJ117. Morphological analysis (Bog *et al*., 2020) was used to complement genetic barcoding and these clones were identified as *L. minor* (Figure 3.7).

The RGR (d^{-1}) differed significantly between duckweed clones (Figure 3.8a). Furthermore, the protein content varied significantly, ranging from 27.5% to 40% of dried biomass (1.1–1.6% of fresh biomass), which compares well with commonly found values of 25–40% (Mohedano *et al*., 2012).

TN removal rates expressed per gram of duckweed ($\text{mg}\,\text{P}\,\text{g}^{-1}\,\text{d}^{-1}$) or per m^2 of duckweed ($\text{mg}\,\text{P}\,\text{m}^{-2}\,\text{d}^{-1}$) varied substantially between clones and were similar to those in the literature (Mohedano *et al*., 2012; Zhao *et al*., 2015). In contrast, TP removal rates did not vary significantly between clones (Figure 3.8e,f). Variability between clones is a natural occurrence that derives

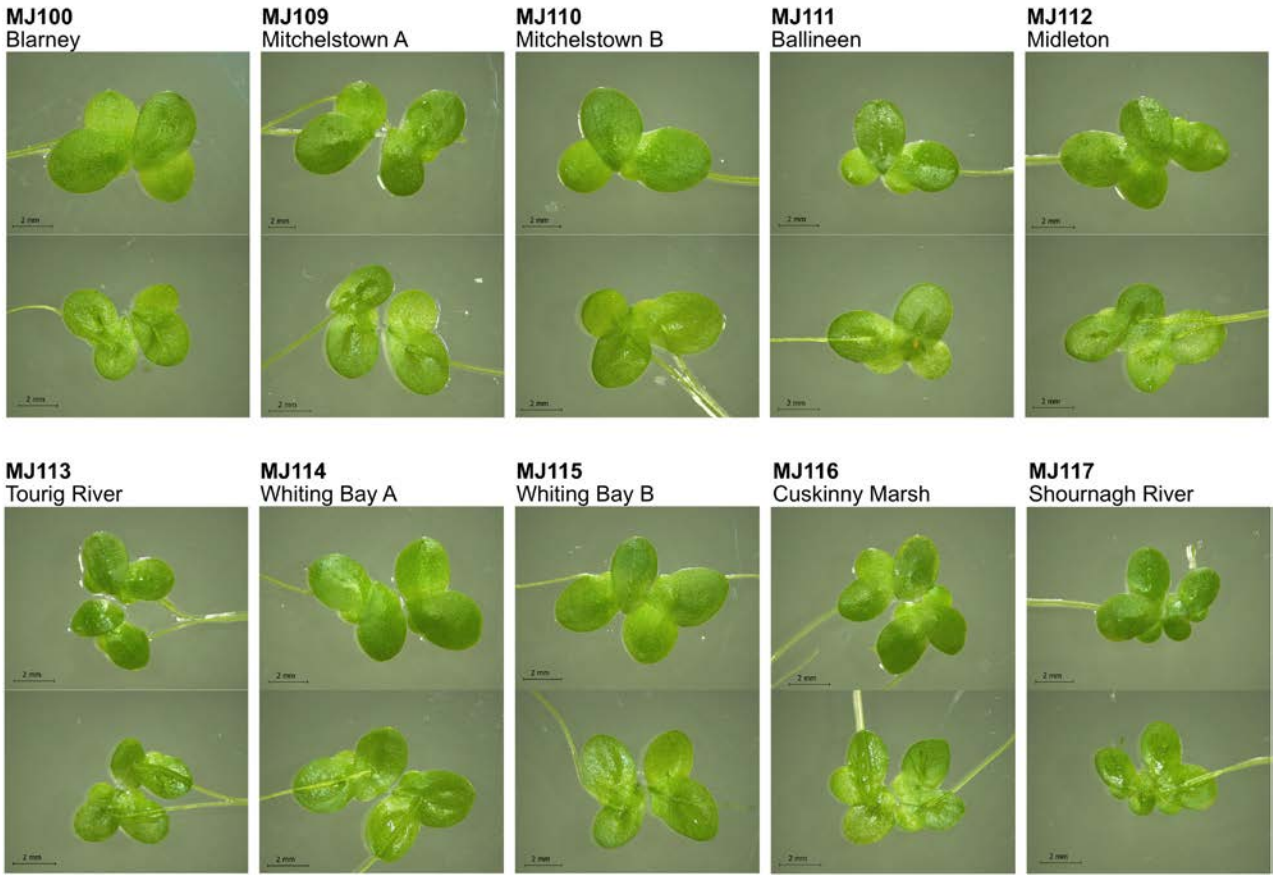

Figure 3.7. Adaxial (top row) and abaxial (bottom row) sides of *L. minor* clones showing the lack of surface colouration typical of *L. japonica*. Reproduced from Walsh *et al*. (2022); licensed under CC BY-NC-ND 4.0 (https://creativecommons.org/licenses/by-nc-nd/4.0/).

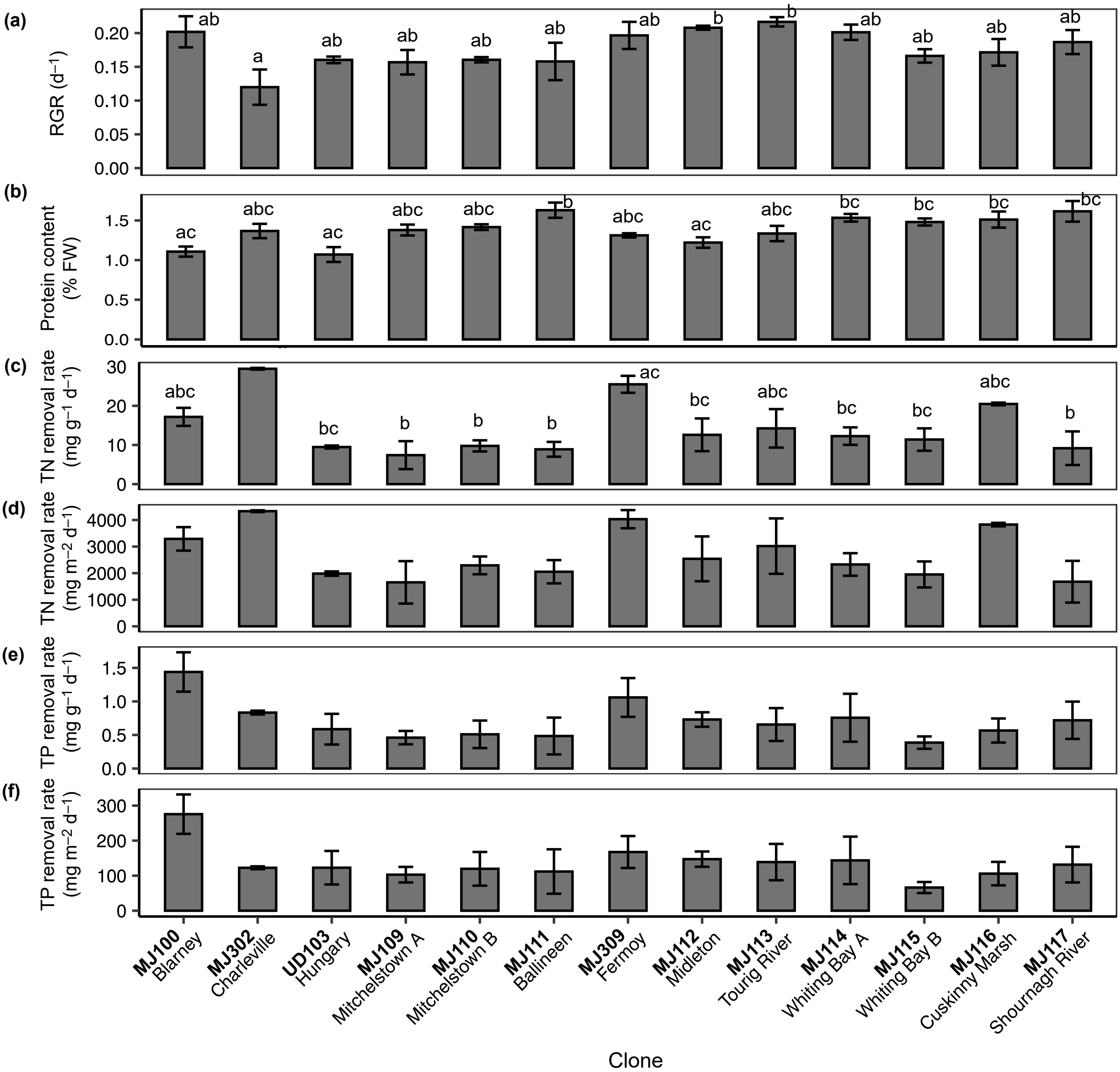

Figure 3.8. Mean (±standard error) (a) RGR (d^{-1}), (b) protein content (% of fresh biomass), (c) TN removal rate per g of duckweed ($mg\,N\,g^{-1}\,d^{-1}$), (d) TN removal rate per m^2 of leaf area ($mg\,N\,m^{-2}\,d^{-1}$), (e) TP removal rate ($mg\,P\,g^{-1}\,d^{-1}$) and (f) TP removal rate ($mg\,P\,m^{-2}\,d^{-1}$) for clones of duckweed grown on synthetic dairy wastewater. Bars that do not share the same letter are significantly different from each other ($p<0.05$). When no letters are included, rates were not found to be significantly different. Reproduced from Walsh *et al*. (2022); licensed under CC BY-NC-ND 4.0 (https://creativecommons.org/licenses/by-nc-nd/4.0/).

from random genetic drift and/or adaptation to local conditions (Sandler *et al*., 2020).

Principal component analysis was used to explore patterns between traits (Figure 3.9). Interestingly, *L. minor* clones from wastewater treatment plants (4, 5, 6 on the right-hand side of the graph) converged with slower TP removal, but higher protein content. The standard *L. minor* clone, MJ100 (Blarney), is positioned to the left, showing good growth and TN and TP removal, but low protein content. The data show an antagonism between nitrogen and phosphorus uptake, with some of the best clones for nitrogen uptake being less efficient in phosphorus uptake, as well as between growth rate and protein content. As no clone combined high nutrient removal with high biomass and protein content, developers

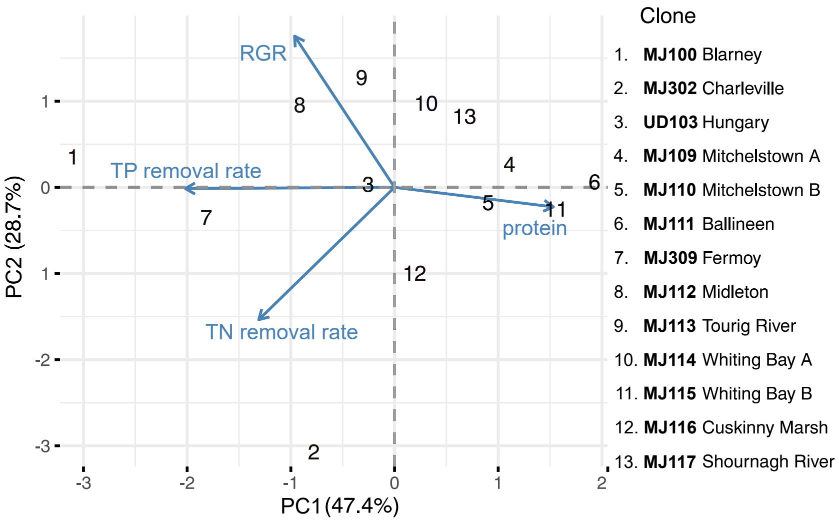

Figure 3.9. Principal component analysis of four parameters [RGR, protein content (%), TN removal rate (mg N g^{-1} d^{-1}) and TP removal rate (mg P g^{-1} d^{-1})] measured on 13 duckweed clones. Numbers (1–13) are linked to a clone, as shown in the key. Each arrow corresponds to a measured parameter. Reproduced from Walsh *et al.* (2022); licensed under CC BY-NC-ND 4.0 (https://creativecommons.org/licenses/by-nc-nd/4.0/).

of remediation systems must choose the primary objective in wastewater treatment.

3.6 Conclusions

Dairy processing wastewater is a suitable medium for growth of *L. minor*. Imbalances in the Ca to Mg ratio negatively affect duckweed growth and they therefore may need amending to achieve remediation. Attempts to accelerate remediation by using higher light intensities showed a media-dependent effect of light intensity. Higher light intensities have no benefit for the remediation of synthetic dairy wastewater, minimising investment in lighting systems. Plant density is an important parameter, with the highest overall TN and TP removal rates occurring at the highest tested plant density (80%), although growth rates and nutrient uptake rates per plant were slowest at this density. Further optimisation can be achieved using specific duckweed clones, maximising growth, protein content or phosphorus and nitrogen uptake. However, it is unlikely that remediation approaches can be optimised for both high nutrient removal and high protein yield, i.e. some clones are better for remediation of wastewater, while others show particularly strong valorisation. Thus, there is considerable scope to improve the duckweed-mediated remediation process, but operators of duckweed-based wastewater remediation systems need to prioritise their key objectives.

4 Large-scale Indoor Bioreactors for Duckweed-mediated Wastewater Remediation

4.1 Introduction

There is a need to develop more sustainable food production methods that deliver high-quality produce in line with the principles of the circular economy. The concept of using Lemnaceae as a sustainable source of high-quality nutrition has gained worldwide interest. To date, most duckweed cultivation occurs outdoors in ponds or canal-based systems. However, indoor systems have significant benefits in the context of urban farming (Tibbetts, 2019) or in industrial settings where continuous growth is required (see Figure 4.1).

Indoor duckweed bioreactors enable the temperature, light spectrum, photoperiod, humidity and other climatic factors to be controlled and optimised; they also provide opportunities to grow duckweed reliably irrespective of weather and climate. However, there is a lack of basic data on the construction and operation of large-scale, indoor duckweed cultivation systems in the literature. A key difference between indoor and outdoor systems is the cost of floor space. Consequently, for indoor use in the horticultural industry, multilevel, i.e. stacked, cultivation systems have been developed, increasing production per square metre. Duckweed species are highly suitable for such stacked cropping, given their two-dimensional structure. The literature review in section 4.2 highlights key considerations and exciting novel opportunities for the development and operation of closed-loop, large-scale, indoor duckweed cultivation systems (Figure 4.2). The development and operation of a prototype stacked duckweed bioreactor is outlined in section 4.3.

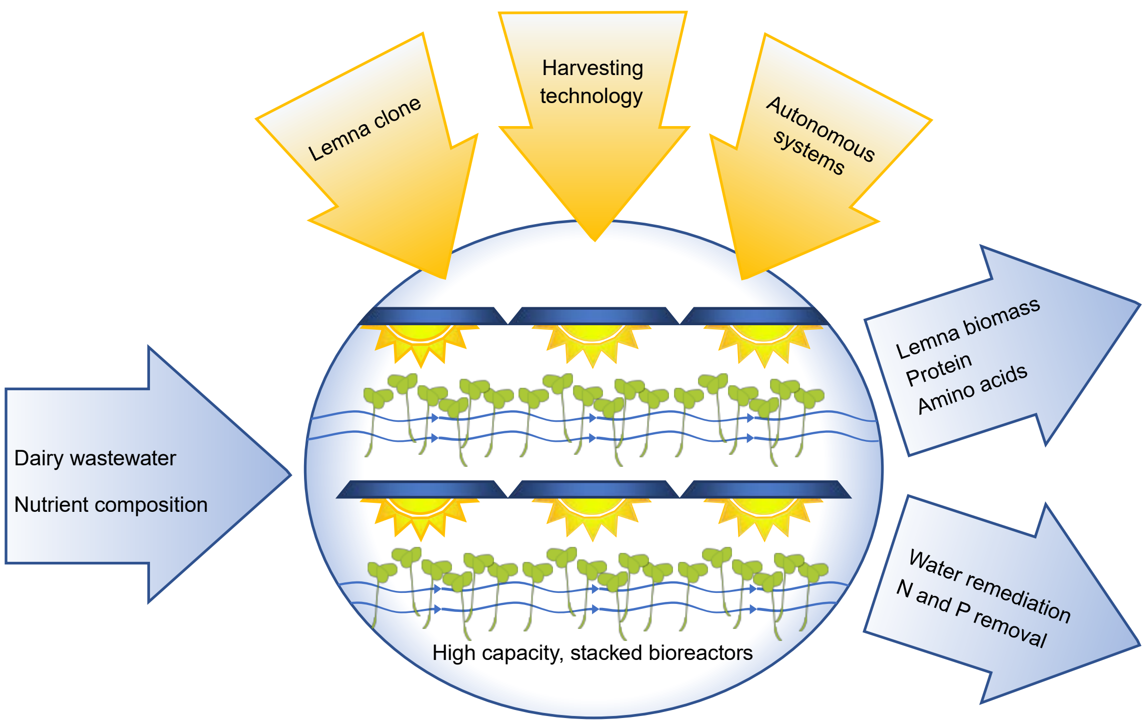

Figure 4.1. Schematic overview of the scope and development of an indoor stacked duckweed (*L. minor*) growth system, its function in the production of *Lemna* biomass and the remediation of dairy processing wastewater.

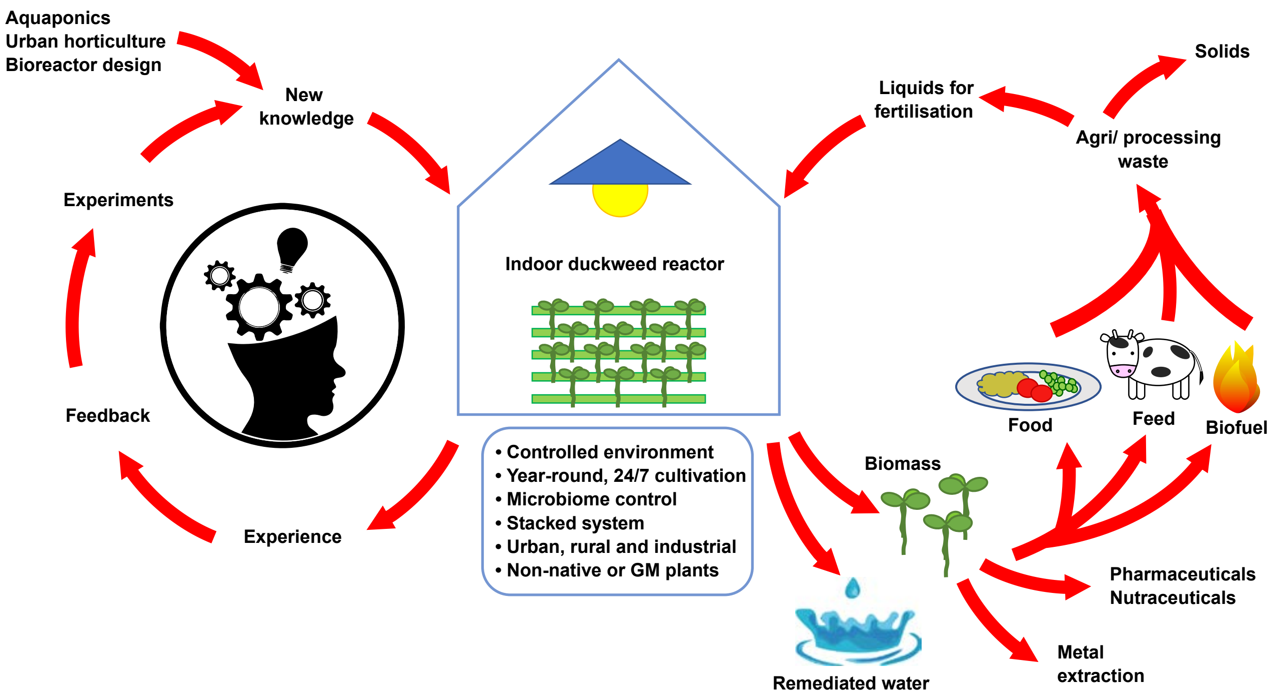

Figure 4.2. Infographic of the development and operation of closed-loop indoor duckweed cultivation systems, in line with the principles of the circular economy concept. Reproduced from Coughlan *et al.* (2022); licensed under CC BY-NC-ND 4.0 (https://creativecommons.org/licenses/by-nc-nd/4.0/).

4.2 Challenges and Opportunities for Large-scale, Indoor Cultivation of Lemnaceae

4.2.1 Cultivation system design

We hypothesise that it is possible to cultivate more than 15 m^2 of duckweed per square metre of floor space by using stacked duckweed cultivation systems. Recent advances in vertical farming and controlled-environment agriculture can underpin the development of stacked duckweed systems. For example, automated sensor-supported systems, linked to artificial intelligence and robotic management, can optimise the allocation of resources and reduce operating expenses (Roberts *et al.*, 2020). There are thus exciting opportunities to adopt novel technologies into large-scale duckweed cultivation.

4.2.2 Cultivation operational conditions

An advantage of indoor duckweed systems is the accurate control of key cultivation parameters. While the actual control of conditions is straightforward, it requires a thorough knowledge of plant requirements. However, the effects of parameters such as photoperiod, light intensity, light spectrum, plant density, medium temperature, flow rate and depth are largely unknown, particularly in the context of potential interactions (e.g. Walsh *et al.*, 2020). Exploiting the benefits and opportunity of indoor systems therefore requires extensive research and development work.

4.2.3 Identification of optimal duckweed species and clones

Different Lemnaceae species display divergent growth and development, even when cultured on the same medium (Zhao *et al.*, 2014). In addition to such interspecific differences, intraspecific differences between clones of the same species have been well documented (Ziegler *et al.*, 2015). Accordingly, duckweed species and clones should be selected based on their ability to align with the purpose of a bioreactor systems, for instance the assimilation of possible contaminants and the generation of nutritious biomass.

4.2.4 Exploiting the duckweed microbiome

The duckweed microbiome can have complex effects on duckweed physiology and growth (O'Brien *et al.*, 2020). Plant growth-promoting bacteria accelerate growth by modulating levels of plant hormones, fixing of nitrogen and/or increasing the bioavailability of phosphorus or iron. The impact of plant growth-promoting bacteria depends on the specific combination of duckweed species and the composition of the growth medium. There is now an emerging perspective that growth and/or remediation by an indoor duckweed system can be optimised by customising the microbiome.

4.2.5 Harvesting technologies suitable for indoor cultivation systems

Regular harvesting maintains duckweed in a physiological state of rapid growth and increases the total biomass yield (Kufel *et al.*, 2018). Regular harvesting is also important for phytoremediation, as it removes pollutants such as nitrogen and phosphorus from the medium (Walsh *et al.*, 2021a). Automated robotic surface skimmers and conveyor belt extraction systems have been used for outdoor duckweed harvesting (Timmerman and Hoving, 2016). However, in indoor, stacked duckweed systems the gap between individual duckweed layers (typically 5–10 cm) means that harvesting equipment needs to be miniaturised. Furthermore, harvest technologies should be able to function in an automated capacity in an indoor multitiered system. Current developments in horticultural harvesting include automated, optical systems that inspect the crop, assess suitability for harvesting and harvest the product with high precision (Birrell *et al.*, 2020). There are thus exciting novel opportunities to adopt similar high-tech systems in indoor, large-scale duckweed cultivation.

4.3 A Pioneering Stacked Bioreactor for Indoor Cultivation of Lemnaceae

4.3.1 Materials and methods

A vertically stacked system for duckweed cultivation

A recirculating, vertically stacked system for indoor cultivation of *L. minor* was designed and constructed (Figures 4.3 and 4.4). The system consists of four stacked stainless-steel trays and a sump tank, operated with a combined capacity of 600 L of medium (Figure 4.3). Trays are suspended within a supporting stainless-steel framework and medium is pumped from the sump tank to the top tray (tray 1). A gravity-fed flow drains tray 1 into tray 2, tray 2 into tray 3, and so on. Medium exits the final tray (tray 4) to be returned into the sump tank. Medium depth can be varied between 25 and 100 mm using flow regulators. Above each tray, a combination of red and blue LED (light-emitting diode) strip lighting yields a maximum intensity of 150 μmol m^{-2} s^{-1}.

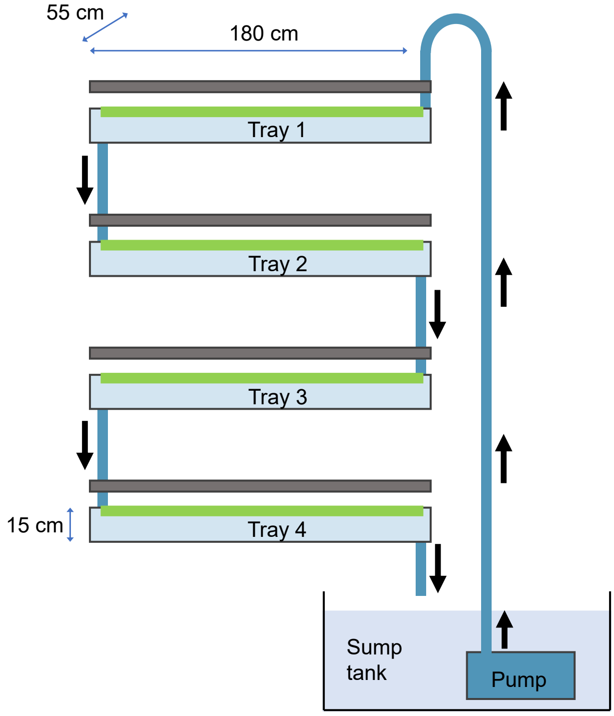

Figure 4.3. Design of a vertically stacked system for indoor cultivation of *L. minor*. The system consists of four stacked stainless-steel trays (180 cm × 55 cm × 15 cm) and an operating volume of 600 L. Medium is pumped from the sump tank to the top tray, after which gravity drives the flow back into the sump tank. LED strip lighting above each tray facilitates autotrophic growth.

Operation of a stacked duckweed reactor

Hydroponics medium (General Hydroponics, Santa Rosa, CA) was used for the cultivation of *L. minor*. This medium contains 36.2 mg L^{-1} nitrogen and 1.78 mg L^{-1} phosphorus. A flow rate of 2.5 L min^{-1} was maintained throughout the system, giving a retention time for each tray of 30 minutes. To assess growth, 60% surface cover of *L. minor* – Blarney (RDSC 5500) was placed in each tray. Biomass growth was determined on the seventh day and the RGR was calculated (Connolly and Wayne, 1996). The TN and TP content of the

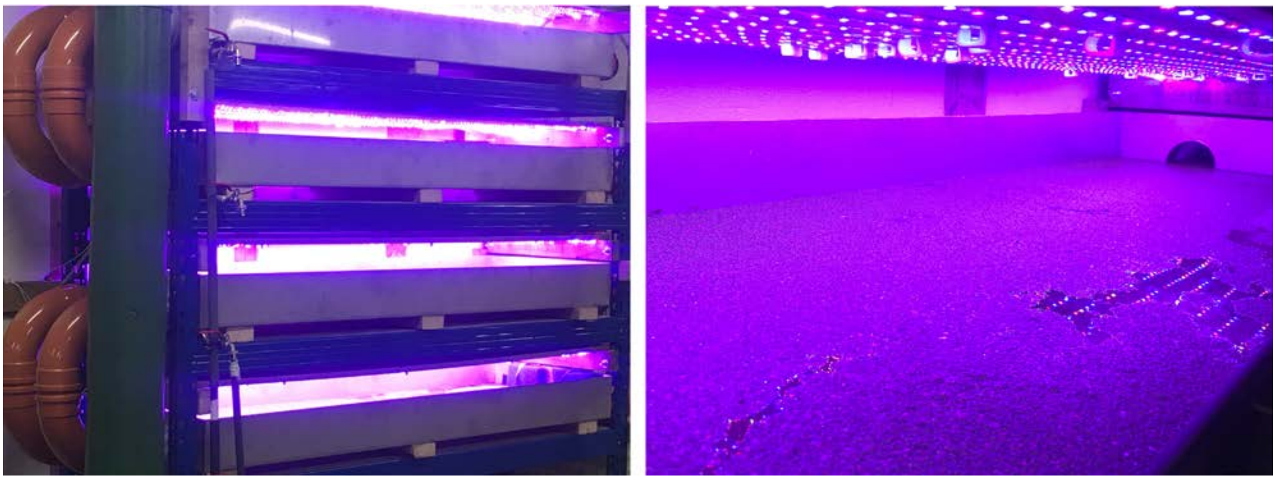

Figure 4.4. Vertically stacked system for indoor cultivation of *L. minor*. The four trays stacked above each other (left) and duckweed floating in one of the trays with LED lighting above (right).

medium was quantified using Hach tests LCK138 (1–16 mg L^{-1}) and LCK348 (0.5–5 mg L^{-1}), respectively. When concentrations were outside the recommended range, samples were diluted.

4.3.2 Results and discussion

Biomass growth with RGR values of 0.17 d^{-1} was noted across all trays (Table 4.1) and values were in the expected range, given the use of a non-optimised medium. Growth was accompanied by removal of TN and TP from the medium. By the end of the 7-day experiment 75% of TN (27.3 mg L^{-1}) and just 10% of TP (0.18 mg L^{-1}) remained in the medium. Although measured TP concentrations were at the edge of the validity range of the used measurement kit, we concluded that the TP pool in the wastewater had been substantially depleted. The measured duckweed TP removal rates were comparable to the lower range reported in the literature, and this is likely to reflect the lack of TP towards the end of the experiment. Duckweed TN removal rates were also in the lower range of those reported (Mohedano *et al.*, 2012). It is likely that the low concentration of the remaining phosphorus at the end of the experiment impeded duckweed growth and thereby nitrogen removal. These data show the scope for indoor stacked remediation; they also show that imbalances between bioavailable TN and TP, unless addressed, will result in a surplus of the element that is present in excess. Optimisation of operational parameters of the bioreactor is expected to substantially increase growth and remediation capacity.

4.4 Conclusions

Enhanced food security is dependent on the development of innovative sustainable agriculture and aquaculture practices. Duckweed species are increasingly considered as an alternative high-quality feed source for livestock, fish and even humans; they also have potential for phytoremediation, resource

Table 4.1. Biomass growth rate and TN and TP removal from a 600 L duckweed incubator. Tray 1 is the top tray, while tray 4 is the bottom tray of the incubator. Means plus standard errors are shown

Tray number	Biomass RGR (d^{-1})	TN removed (mg m^{-2} d^{-1})	TP removed (mg m^{-2} d^{-1})
1	0.170 ± 0.001	–	–
2	0.170 ± 0.001	–	–
3	0.171 ± 0.001	–	–
4	0.170 ± 0.001	–	–
Average	0.170 ± 0.001	372.9 ± 117.2	48.0 ± 11.4

"–", not measured.

recovery services and biofuel production. Development of indoor growth systems is subject to technological challenges, but it also comes with new opportunities, particularly in the development of autonomous systems. It has been shown in this study that a pioneering stacked, recirculating growth system can yield good growth of *L. minor*, associated with removal of both TN and TP.

5 The Newtrients System – Integrating Microbial Digestion with Duckweed Cultivation

5.1 Wastewater Remediation

Disposal of dairy processing wastewater is restricted by emission limits (European Union, 2019) and therefore wastewater must be remediated at a considerable cost to the industry. The complex composition of dairy waste, and in particular the combination of organic and inorganic nutrients, necessitates a complex and multistage wastewater management system. Various microbial systems are effective in removing organic components (Charalambous *et al*., 2020), including sugars, fat and protein. In contrast, duckweed species tend to be particularly effective in the removal of residual nitrogen and phosphorus, i.e. polishing (Cheng and Stomp, 2009). In this study, a number of integrated solutions for the clean-up of dairy processing wastewater were trialled. Specifically, we explored which combinations of microbial wastewater treatment are compatible with duckweed-based polishing of nitrogen and phosphorus, and which result in effective remediation (Figure 5.1).

5.1.1 Materials and methods

Dairy processing wastewater was sourced from a commercial dairy processing facility. At the facility, aqueous waste from several production lines was collected in a balancing tank before being fed into an AD. The 45,000-m^3 AD removes a high percentage (~90%) of BOD and total suspended solids while producing energy-rich biogas. Wastewater from the balancing tank was used as a feedstock for experimental microbial fermentation. Wastewater from the balancing tank and AD was used for duckweed growth.

Experimental microbiological wastewater treatment

A number of different microbial reactor types were developed and explored within the study, as outlined below.

An A/O reactor was constructed using two reactor vessels, one anoxic and one oxic (as per Carrera *et al*., 2004). A clarifier separated solids from the liquid effluent. Sludge was returned to the treatment system to maintain suspended solids at approximately 4 g L^{-1}.

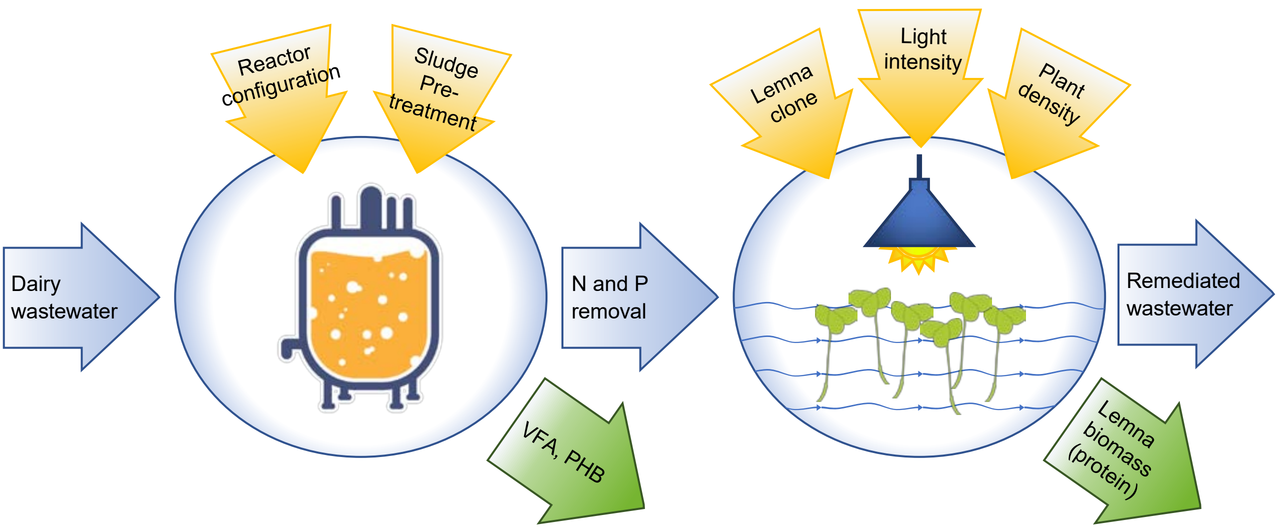

Figure 5.1. The integration of microbial technologies (commercial AD, laboratory-based A/O, IASBR and ADF reactors) and duckweed growing systems to achieve dairy wastewater remediation and resource recovery.

The effluent provided the feedstock for downstream polishing using a duckweed cultivation system.

A laboratory-scale IASBR was constructed using a vessel running on a 12-h cycle (Gil-Pulido *et al*., 2018). Two-hour aeration periods were followed by 20-minute non-aeration periods for a total of 12 h, followed by a settling phase of 40 minutes. Following settling, 300 mL of effluent was removed for treatment through duckweed cultivation.

A laboratory-scale ADF reactor was constructed to generate a so-called feast and famine treatment, in which a short period of excess carbon is followed by a longer period of starvation, which promotes accumulation of PHA (Serafim *et al*., 2004). Reactors were operated on 8-h automated cycles of feeding and aeration for 7 days, after which effluent was removed for duckweed polishing.

Duckweed cultivation on dairy processing wastewater

Duckweed was grown on the following types of wastewater (see Figure 5.2):

- untreated commercial dairy processing wastewater;
- effluent from a commercial AD;
- A/O effluent;
- IASBR effluent;
- ADF effluent.

The culture system comprised *L. minor* – Blarney (RDSC 5500) cultivated under controlled conditions. The RGR was assessed as detailed in Walsh *et al*. (2020).

Water quality measurements

For the assessment of remediation in A/O and IASBR systems, measurements of COD, NH_4^+, NO_3^- and PO_4^{3-} were made using a Hach DR2800 spectrophotometer. For the full physico-chemical assessment (pH, BOD, COD, total solids, TN, NH_3-N, NO_3^--N, NO_2^--N, TP, PO_4^{3-}, Na, Cl, K, Ca, Mg, Fe, Zn, Cu, Mn) of untreated wastewater, AD effluent, ADF effluent, duckweed reactor effluent and samples of wastewater/effluent were analysed by a validated laboratory.

5.1.2 Results and discussion

Commercial dairy wastewaters integrated with duckweed cultivation

Duckweed growth trials on untreated dairy processing wastewater led to poor growth. Surface slime – caused

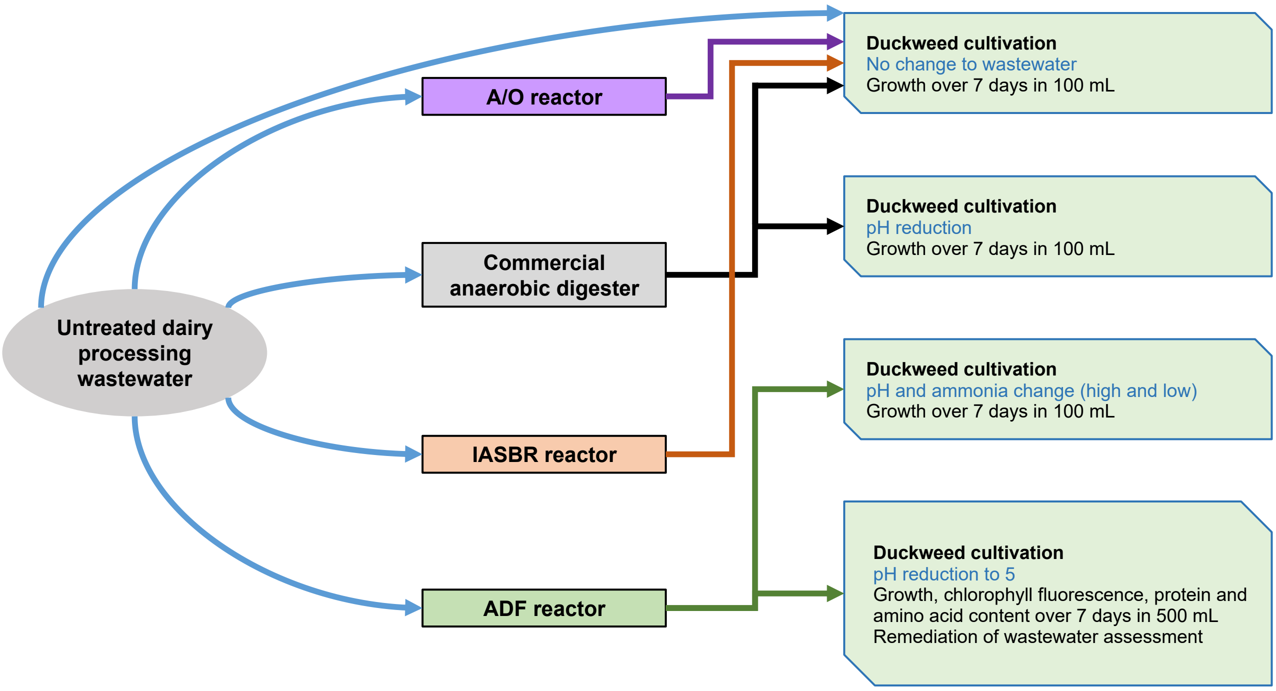

Figure 5.2. Integrated microbial and duckweed remediation of dairy processing wastewater.

Table 5.1. Characteristics and composition of untreated dairy wastewater, AD effluent, ADF effluent and duckweed reactor effluent

Parameter	Untreated wastewater (mean ± SD)	Commercial AD effluent (mean ± SD)	ADF effluent (mean ± SD)	Duckweed reactor effluent (mean ± SD)
pH	6.5 ± 0.5	8.5 ± 0.2	8.9 ± 0.1	7.9 ± 0.3
BOD (mg L^{-1})	1496 ± 594	33.2 ± 3	22.3 ± 18	9 ± 3.9
COD (mg L^{-1})	2663 ± 459	292.3 ± 181	60 ± 32	77 ± 43
TN (mM)	7.8 ± 1.6	6.7 ± 0.8	1.4 ± 0.6	0.3 ± 0.1
NH_3-N (mM)	2.7 ± 0.8	5.1 ± 0.6	0.6 ± 0.3	0.001 ± 0.0001
NO_3^--N (mM)	BD	BD	0.8 ± 0.4	0.009 ± 0.01
TP (mM)	1.0 ± 0.2	0.91 ± 0.003	0.5 ± 0.1	0.3 ± 0.1
PO_4^{3-}-P (mM)	0.8 ± 0.2	0.86 ± 0.02	0.4 ± 0.1	0.3 ± 0.1

BD, below detection (< 0.0007 mM); SD, standard deviation.

by the high load of organic material, microbial growth and the stationary nature of the system – restricted duckweed growth. This is consistent with previous research (Broughton, 2019).

AD effluent contains substantially less COD and BOD than raw wastewater (Table 5.1) and the pH tends to be high (> 8). *L. minor* growth on AD effluent was poor; however, once the pH was reduced to 5, an improved RGR (0.18 d^{-1}) was measured.

A/O and IASBR systems integrated with duckweed cultivation

The A/O and IASBR systems removed up to 98% of COD from untreated dairy processing wastewater (Table 5.2), as well as most NH_3-N, NO_3^--N and PO_4^{3-}-P. Manipulation of the A/O and IASBR systems to limit nitrogen and phosphate removal led to biomass instability, poor settling and washout of microbial aggregates (flocs) (Broughton, 2019). Growth of duckweed depends on the presence of nitrogen and phosphate, so plant growth rates were modest, as expected given the nutrient depletion.

Table 5.2. Removal efficiencies in A/O and IASBR systems

Parameter	Removal (%)	
	A/O system	IASBR system
COD	80–98	60–97
NH_3-N	20–99	4–94
NO_3^--N	100	100
PO_4^{3-}-P	50–95	30–80

ADF effluent retained substantial amounts of nitrogen and phosphorus (Table 5.1). However, initial trials on ADF effluent wastewater showed poor *L. minor* growth, particularly at high ammonia concentrations (Figure 5.3). This was attributed to a combination of high pH (8–9) and ammonia. After neutralisation to pH 5.0, good biomass growth with RGR of more than 0.2 d^{-1} was observed (Figure 5.3).

Impacts of an integrated ADF and duckweed-based reactor on wastewater quality

There are indicative ranges for emissions to water bodies ("allowable daily concentrations") based on

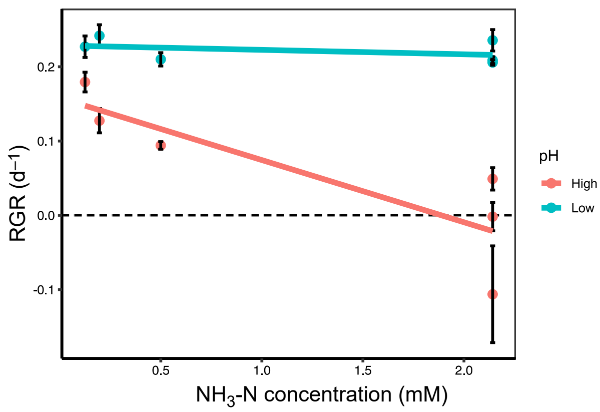

Figure 5.3. Mean RGR (d^{-1}) ± standard error of *L. minor* grown on ADF wastewater effluent vs concentration of NH_3-N (mM) in ADF wastewater effluent. "High" pH denotes values from 8.6 to 8.8 and "low" pH denotes values from 4.9 to 5.1. Trendlines are fitted as per multiple linear regression analysis.

the use of “best available techniques”. These are: 25–100 mg L^{-1} COD, 4–50 mg L^{-1} TN (0.2–3.6 mM) and 0.2–2 mg L^{-1} TP (0.006–0.06 mM) (European Union, 2019). The untreated wastewater from a commercial facility had high concentrations of BOD, COD and total solids, which were, however, substantially reduced by an ADF reactor (Table 5.1). As expected, the duckweed reactor did not contribute to the removal of COD and BOD.

The concentration of TN was reduced in the ADF reactor effluent (by 83%); however, the concentrations of NH_3 and NO_3^- together were still substantial (Table 5.1). The duckweed system effectively removed NH_3 and NO_3^- by up to 99% and overall TN by 81% (Table 5.1). Thus, a duckweed reactor can be used to reduce TN following pretreatment in ADF, with the main nitrogen species, NH_3 and NO_3^-, being reduced to near undetectable levels.

TP in wastewater was not reduced to below EU emission limits following passage through the ADF reactor and duckweed cultivation. On average, the ADF reactor removed half of the phosphorus (Table 5.1). The duckweed reactor reduced TP and PO_4^{3-} by a further third (Table 5.1). Duckweed requires nitrogen and phosphorus in a 5:1 ratio (Güsewell, 2004). However, TN and TP in ADF reactor effluent were present in a 2:1 ratio. As a result, nitrogen was rapidly depleted, after which growth slowed, leaving significant amounts of phosphorus (mostly PO_4^{3-}) in the wastewater. The remaining phosphorus requires further intervention. A possible solution is the use of a second, nitrogen-rich waste stream, for example cow’s urine, to improve the nitrogen to phosphorus ratio (Ramanna *et al*., 2014).

5.1.3 Conclusions

Dairy processing wastewater has been remediated by a combination of microorganism-based reactors and duckweed cultivation. The combination of an ADF reactor with subsequent duckweed cultivation removed nearly all organic compounds and TN from the wastewater. However, significant amounts of TP remained; it is likely that this TP could be removed by the duckweed system, but it would require some addition of nitrogen to maintain the optimal nitrogen to phosphorus ratio for duckweed growth.

5.2 Valorisation of Dairy Processing Wastewater

Dairy processing wastewater contains large amounts of organic components such as sugars and fats, as well as inorganic nutrients including nitrogen and phosphorus. Although modern wastewater treatment systems can capture the energy content of dairy processing wastewater, for example through AD, in general there is a lack of capture of other components. Applying the principles of the circular economy to wastewater treatment can stem the loss of organic and inorganic components through resource recovery. Key principles of the circular economy are (a) to maintain the value of products, materials and resources for as long as possible and (b) to “cascade” recovery by extracting high-value products from waste streams before lower value products. Here, a novel integrated approach to valorising dairy processing wastewater is presented. This approach comprises microbial-driven fermentation with duckweed cultivation. Microbial activity converts organic compounds into VFAs, which can be packaged by bacteria into bioplastic materials referred to as PHAs (Serafim *et al.*, 2004), while duckweed-based systems convert nitrogen and phosphorus into high-protein feedstock.

5.2.1 Materials and methods

Untreated (raw) dairy processing wastewater was sourced from a commercial dairy processing facility.

Acidogenic fermentation for wastewater treatment

Anaerobic digestor sludge was collected from a commercial dairy processing facility and subjected to a pretreatment at pH 2 to select for spore-forming acidogenic bacteria. Pretreated sludge was used to seed 2-L CSTR systems operated at 25°C with pulse feeding with raw wastewater at 12-h intervals and stirring for 30 minutes every 5 h.

ADF for wastewater treatment

ADF reactors had an operational volume of 2 L and were seeded with untreated anaerobic digester sludge. The reactors were operated on an 8-h cycle consisting of an initial feed with acidogenic fermenter effluent followed by continuous overhead mixing and aeration, which ceased after 7 h. Biomass was allowed to settle under gravity for 40 minutes before clarified supernatant was withdrawn for duckweed cultivation.

Cultivation of duckweed on dairy processing wastewater

Duckweed (*L. minor* – Blarney, RDSC 5500) was grown on ADF effluent in 100 ml vessels in a controlled growth room (Walsh *et al.*, 2020). Prior to use, the pH of ADF effluent was reduced to pH 5. The increase in plant biomass growth was measured and a RGR was calculated. The protein content was determined using the Bradford method while the amino acid composition was determined using HPLC with an ultraviolet light absorption detector (HPLC-UV; Infinity, Agilent Technologies).

5.2.2 Results and discussion

Microbial biotransformations of dairy processing wastewater

The anaerobic CSTR system seeded with pretreated sludge produced high yields of mixed VFAs (in the order of 1500 mg L^{-1}) from raw dairy wastewater, which compared favourably with other studies on food industry waste stream valorisation by acidogenic fermentation (e.g. Montiel-Jarillo *et al.*, 2021). The VFA profile comprised 60% acetic acid and 33% propionic acid, which are valuable precursors in PHA homopolymer and copolymer synthesis. Downstream ADF adaptation successfully enriched for PHA-accumulating microbes within the sludge community with maximum yields of 35% (g PHB g^{-1} cell dry weight) achieved with VFA-enriched synthetic wastewater. In the case of direct feeding with acidogenic fermentation effluent, yields were approximately 16% (g PHB g^{-1} cell dry weight). PHB, a short-chain-length polymer with potential uses in bulk applications (e.g. packaging films), was the sole polymer generated in this system, and genetic analyses of the sludge community confirmed that PHB synthesis pathways were dominant. The yield achieved requires further optimisation for commercial exploitation. Despite this, the yields reported here are substantially higher than recently reported outputs from a trial of dairy wastewater acidogenesis and batch-feeding with pure culture PHA-producing bacteria (Pagliano *et al.*, 2020). The importance of these achievements is that, in addition to PHA synthesis, VFAs are platform chemicals which can feed into a range of applications across the chemical, agricultural and food sectors, and they may, therefore, present additional opportunities for dairy wastewater valorisation (Ramos-Suarez *et al.*, 2021).

Duckweed biomass and composition

The potential value of the duckweed biomass, generated on pH-adjusted ADF effluent, relates to both protein content and amino acid profile (Appenroth *et al.*, 2017). *L. minor* protein content was on average 38.5% of dry weight (Table 5.3), which compares well with literature values of 25–40% for duckweed. The total essential amino acid content (a key aspect of protein value) as a percentage of protein content was 20% (Table 5.3). Overall, the amino acid profile of biomass raised on ADF effluent was slightly less favourable than values found in the literature, with isoleucine, lysine, phenylalanine and valine levels being lower than those reported in the literature (Cheng and Stomp, 2009).

5.2.3 Conclusions

Proof-of-concept of waste valorisation was demonstrated. Production of PHB was substantial and can feed into an existing market. Moreover, VFAs as platform chemicals can feed into a range of applications across the chemical, agricultural and food sectors; additional opportunities for dairy wastewater valorisation may therefore arise. Duckweed with a protein content of 40% of dry matter was successfully grown on effluent from an ADF reactor. This is promising, given that the cultivation system is far from optimised. The monetary value of the valorisation is difficult to assess; however, it will comprise the following three components: (1) the value of the wastewater remediation (see section 5.1); (2) the value of generated PHBs, VFAs and/or duckweed biomass; and (3) environmental and reputational benefits for the industry. A large-scale, outdoor project cultivating duckweed in Ireland resulted in yields of around 40 tonnes of dry weight per year (M.A.K. Jansen, University College Cork, unpublished data). This equates to an estimated 16 tonnes of protein, with scope for use as a replacement for soybean. As a feedstock, the value of 40 tonnes of duckweed

Table 5.3. Biomass parameters, including key amino acids, for *L. minor* – Blarney (RDSC 5500) grown on ADF effluent adjusted to pH 5, and compared with literature values for *L. minor* and soybean

Parameter	Mean (±SE)	Literature values for *L. minor*[a]	Literature values for soybean[b]
3-day RGR (d^{-1})	0.09 (±0.014)	–	–
Protein content (% FW)	1.54 (±0.22)	–	–
Protein content (% DW)	38.5 (±5.5)	–	–
Essential amino acid (% of total protein)	20.0 (±0.7)	–	–
Histidine ($g.100g^{-1}$ protein)	1.6 (±0.06)	1.5	2.6
Isoleucine ($g.100g^{-1}$ protein)	1.5 (±0.08)	3.7	5.3
Leucine ($g.100g^{-1}$ protein)	5.0 (±0.2)	7.3	8.1
Lysine ($g.100g^{-1}$ protein)	3.7 (±0.07)	5.0	6.7
Phenylalanine ($g.100g^{-1}$ protein)	2.8 (±0.1)	4.4	5.6
Threonine ($g.100g^{-1}$ protein)	2.7 (±0.2)	4.0	3.9
Valine ($g.100g^{-1}$ protein)	2.7 (±0.03)	4.6	5.6

[a]Appenroth *et al.* (2017).
[b]van Etten *et al.* (1959).
"–", not compared; DW, dry weight; FW, fresh weight; SE, standard error.

ranges between €80,000 and €200,000; the wide range reflects a volatile, nascent market. Furthermore, these values include the cost of drying, which can be considerable. Rapid developments in protein extraction technology, as practised by duckweed farmers in the USA, negate the need for transport and drying, and constitute an important enabling step in commercial duckweed cultivation. While these numbers need to be interpreted in the context of a developing market, they underline the potential to generate value from waste. Waste valorisation is technically possible, and focus should now move to optimisation, upscaling and especially embedding within an industry context.

6 Conclusions, Recommendations and Future Perspectives

The circular economy is widely seen as a critical framework to transform food and material production and consumption, through the matching of consumption with resource regeneration (European Commission, 2015, 2017). A circular bioeconomy can make more efficient use of renewable resources, while supporting growth and employment. However, the transition from policy to practice is still at an early stage, and there is a lack of understanding and agreement concerning the concept of a circular economy, as well as a lack of readily available examples demonstrating the application of circular economy principles to industrial waste streams, i.e. a gap exists between the objectives of circular economy policies and practical applications. The key achievement of the Newtrients project has been to develop an exemplar of a circular economy approach for the production of new products and resources from dairy industry wastewater (Figure 6.1). This was achieved through collaboration with the dairy industry, thus grounding the project in commercial reality and narrowing the gap between laboratory-based research and commercial applications.

In this report, the Newtrients team has pioneered an innovative, circular economy approach to valorising dairy processing wastewater. We have demonstrated the potential of an integrated, cascading system that couples the microbial-based technologies of AD and ADF with duckweed cultivation. Each step in the cascade contributes a clearly identifiable, value-added output and also delivers broader impacts for sustainability within the waste remediation process and beyond. Specifically, Newtrients has demonstrated the potential of dairy processing wastewater as a feedstock for bioconversion to bioplastics and protein-rich biomass (section 5.2).

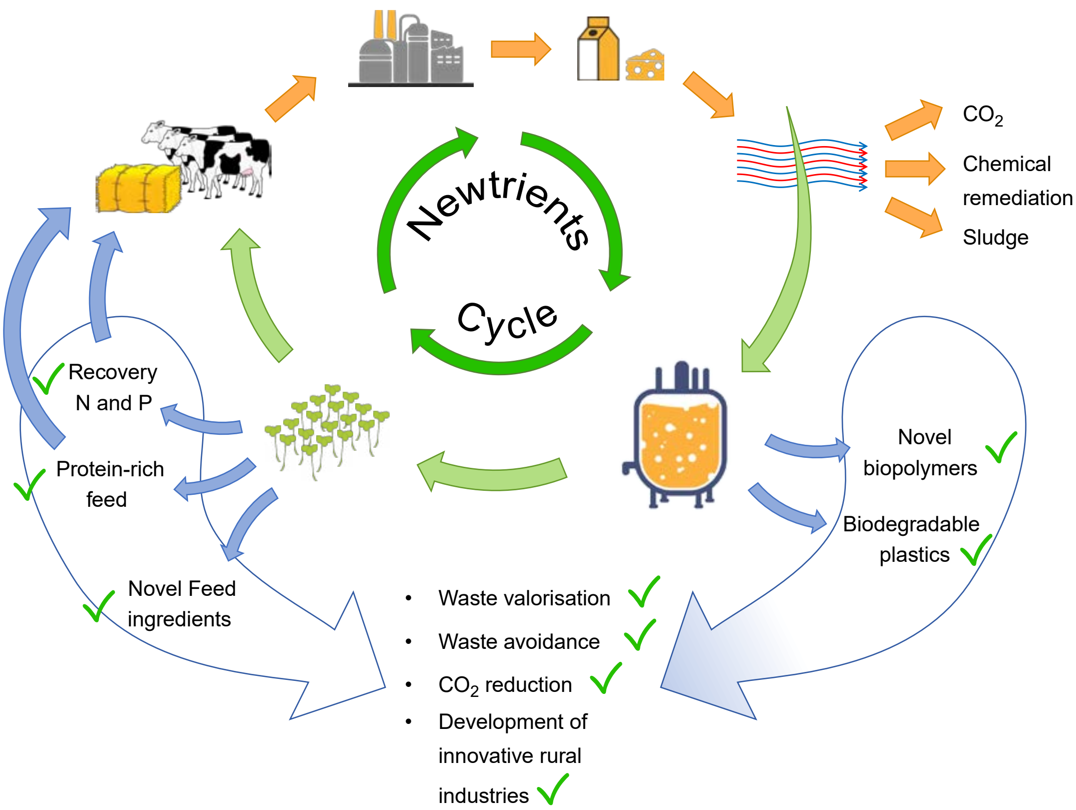

Figure 6.1. The Newtrients cycle and its potential for a green economy.

Recommendations (policy and technical)

- The definition of the concept of a "circular economy" remains ambiguous and the development of standardised terminology is recommended to facilitate communication.
- It is recommended that a portfolio of circular economy case studies is developed to strengthen the impact of the EU Circular Economy Action Plan 2014, the subsequent EU implementation report in 2017 (European Commission, 2015, 2017) and the Circular Economy Bill Ireland 2021.

The developed Newtrients cascading system has three valorisation focal points:

1. cost-effective regeneration of polluted wastewater;
2. generation of value-added and marketable products;
3. a decreased need to tap into finite resources of fossil fuels and fertilisers.

Preconditioning of AD reactor biomass and operation at short hydraulic retention times revealed the capacity for stable and robust conversion of organic carbon loads to VFAs. VFA production through partial AD offers the dairy sector new market opportunities as providers of a range of high-value chemicals, sustainably derived from renewable, low-cost feedstocks rather than from current petrochemical origins. This is associated with a decrease in CO_2 outputs, achieved when traditional wastewater treatment plant aerobic respiration steps are replaced by VFA production. The ADF reactor within the cascade was also found to serve both circular economy and sustainability aims through the production of biodegradable polymers from the derived VFAs, and simultaneous high-capacity nutrient removal of carbon, nitrogen and phosphorus. Beyond the immediate opportunity of producing value-added products, biopolymer production also results in wider sustainability impacts in terms of creating sustainable routes for the production of non-petrochemical-based polymers and providing compostable bioplastics with a reduced environmental impact. Thus, Newtrients has developed a robust, single-tank, sequencing batch microbial bioreactor that not only offers several sustainable advantages in terms of infrastructure, energy and chemical demands, but also has the potential to deliver cost efficiencies to dairy wastewater treatment in tandem with feedstock conditioning opportunities for downstream bioconversions (Chapter 2).

Recommendations (technical and policy)

- It is recommended that industry, state bodies and universities develop further supports to facilitate testing of promising proof-of-concept ideas under semi-commercial conditions.
- It is recommended that further research and development explores the integration of newly developed technology into commercial practice at dairy processing plants. This research should be carried out in collaboration with industry partners.

The duckweed reactors add value through the production of protein-rich biomass with an amino acid profile close to the nutritional requirements of humans and various farm animals, thus facilitating a new closed-loop rural feed industry. Duckweed reactors also contribute to environmental sustainability by replacing the importation of soy from outside the EU (which is linked to substantial greenhouse gas emissions) and limiting exposure to the volatility of the international market for soybean. Furthermore, recycling of nitrogen and phosphorus back into the feed chain alleviates the need for energy-demanding urea production (responsible for 2% of world fossil fuel consumption) and non-sustainable mining of limited rock phosphate resources. Newtrients has shown that protein-rich *L. minor* can be effectively raised on dairy processing wastewater; furthermore, the project has developed a large-scale indoor bioreactor that has the potential to deliver efficient wastewater treatment (Chapters 3 and 4).

Finally, the integrated operation of the Newtrients cascading system results in the remediation of wastewater, with very high percentages of BOD, COD, TN and TP removed, in many cases to within allowable emission limits (section 5.1). Thus, Newtrients has demonstrated alternative wastewater treatment technology, coupled with valorisation of waste (Chapter 5).

Many companies have stated their ambitions to be zero carbon and circular in the coming decades (usually by 2040 or 2050) and have put in place targets to underpin these ambitions. Meeting the targets will require transformative change by all companies; the food and beverage sector will be no different. The onus is now on the food and beverage sector to improve management of its by-products, to recycle nutrients when feasible and to reduce overall fossil fuel use. The integrated cascading process developed by the Newtrients process serves as an exemplar of how companies could achieve circularity with their wastewater streams, which represent a key leakage of resources from their production facilities. Industries are aware of their corporate social responsibility and the need to engage in sustainable ventures for societal good. While much emphasis has been placed on scope 1 emissions (onsite fuel combustion) and scope 2 emissions (electricity generation), the reduction in scope 3 greenhouse gas emissions (indirect emissions) in industry is a far more complex prospect and will be of significant importance in the future in terms of full decarbonisation. Nutrient recycling, such as that exemplified in the production of bioplastics and duckweed, can potentially reduce chemical fertiliser input in agriculture and thus provides a novel method to reduce scope 3 emissions. Newtrients supports the objectives of the dairy industry to achieve value-added economic growth while simultaneously progressing its goals to be a zero carbon and zero waste industry.

Recommendations (policy, industry, technical, socioeconomic)

- It is recommended that the dairy industry explores wastewater management as a novel diversification strategy, to ensure that dairy production can remain competitive on an international scale and to ensure its place as a contributor to vibrant rural communities.
- It is recommended that further analysis maps the full scope for potential reductions in CO_2 emissions following implementation of the Newtrients cycle (i.e. decreased need for fossil fuels for polymer, fertiliser and soybean production), thus supporting the industry objective to be a zero carbon and zero waste industry.
- It is recommended that further socioeconomic analysis maps the full impacts of implementation of the Newtrients cycle on the dairy industry and the rural economy.
- It is recommended that a cost–benefit analysis be carried out on the Newtrients process to benchmark costs (and benefits) against conventional wastewater treatment; this would provide a road map for further development of the process into a commercial product.

It has long been argued that solutions to inform better decisions on environmental challenges require research at the boundaries of scientific disciplines. Sustainability and environmental challenges cannot be solved by any single discipline; instead, they require the efforts of multiple disciplines along with external stakeholders to bring usable knowledge to bear on these research challenges. The achievements of Newtrients were dependent on effective interdisciplinary collaboration, integrating environmental sciences, energy engineering, microbiology and plant biology within a single project. Newtrients has demonstrated that interdisciplinary teams of scientists and engineers working together on shared goals can provide integrated and holistic solutions to circular economy and sustainability challenges. Newtrients enabled the postgraduate and postdoctoral researchers working on the project to be exposed to a wide range of disciplinary approaches within the project team; Newtrients has also provided a broader and richer training experience for them than could have been achieved within their own discipline.

However, further efforts are required to draw on broader inputs from relevant stakeholders across academia, industry, environmental groups, local authorities and the general public in relation to waste valorisation, i.e. transdisciplinary research. Newtrients has demonstrated the technical feasibility of dairy processing wastewater valorisation; however, work remains to position our strategic approach in relation to broader techno-economic analyses of waste management, industry-based cradle-to-grave sustainability analyses, co-ordinated development of local authority approaches to end-of-life waste management for biopolymers, and evaluation of consumer acceptance of waste-derived products and feedstocks. Finally, a review of the waste regulatory framework will be required to ascertain that the use of dairy processing waste as a resource does not contradict current Irish (A Resource Opportunity – Waste Management Policy in Ireland) and EU (Waste Framework Directive, 2008/98/EC) waste management regulations.

Findings present the state of the art for dairy processing wastewater valorisation, as well as a clear proof of concept. Conceptual and technical advances will inform the dairy processing industry about opportunities to achieve value-added economic growth while simultaneously progressing its goals to be a zero carbon and zero waste industry. However, the relevance of the developed processes is not limited to the dairy processing industry. The Irish Government's common agricultural policy (CAP) strategy plan for 2023–2027, combined with ambitious targets to reduce agricultural greenhouse gas emissions under the Climate Action Plan (2019), will result in transformative change for Irish agriculture in the coming decade. The Newtrients project shows the way forward by local production of high-protein feed that can reduce dependence on imported soybean and strengthen the local economy by creating intricate new relationships between farmers, dairy processors and the feed industry. Similarly, the bioconversion of the organic carbon fraction of dairy wastewater to VFAs generates platform chemical feedstocks for several alternative value-added end products, thus boosting the development of novel chemical, food, agricultural and pharmaceutical industries (Ramos-Suarez *et al.*, 2021), and ultimately rural development.

Recommendations (policy and socioeconomic)

- It is recommended that funding bodies strengthen support for interdisciplinary research consortia that address the key challenges of the current era.
- It is recommended that national policy promotes a strategy for multistakeholder-engaged centres of excellence for co-ordinated development of end-to-end sustainable, circular economy approaches across key industry sectors. It is suggested that these efforts might be focused on particular waste streams and bring together multiple large industries and potential end users in collaborative ventures on shared environmental challenges.
- It is recommended that current national waste regulations are reviewed to ascertain compatibility with the possibilities offered by a circular economy.
- It is recommended that waste valorisation strategies are integrated with evaluations of consumer support for derived biomaterials/feedstocks.
- It is recommended that local authorities are supported in the development and implementation of dedicated waste management streams for biodegradable polymers.

The Newtrients cascading system comprises AD, ADF and duckweed reactors. As detailed in this report, the Newtrients system has been validated under laboratory conditions (i.e. technology readiness level, TRL, 4). As a follow-up, the described system needs to be validated and demonstrated in an industrial "dairy processing" environment, with a large-scale prototype being operated (i.e. TRL5–7). However, rather than a rigid set of relationships between AD, ADF and duckweed reactors units, it is hypothesised that waste valorisation will increasingly become a highly bespoke process whereby an increasing range of waste streams will be valorised by an increasing range of technologies, yielding a multitude of new products. In this sense, the achievements of the Newtrients project apply to industry in general, as the team has shown a shift in the paradigm from waste as a problem to waste as a resource. Conversely, the achievements of the Newtrients project are not stand-alone; they will need to be integrated into an ever-increasing range of waste valorising technologies developed across the globe. Following on from the Newtrients project, aspects of the Newtrients system are already further developed as part of the EU-funded BRAINWAVES project (https://www.ucc.ie/en/brainwaves/); however, further integration of developed concepts within the European research area is needed to maximise the benefits of Newtrients.

Importantly, the Newtrients team has visualised a possible outcome from the various circular economy

Recommendations (policy and technical)

- It is recommended that the Newtrients system is validated and demonstrated in a relevant dairy-processing environment.
- It is recommended that the relevance of the Newtrients approach to a broad range of waste streams is mapped and the most feasible opportunities advanced through research and demonstration.
- It is recommended that a public portfolio of circular economy protocols is assembled to assist companies in developing bespoke solutions for waste valorisation.
- It is recommended that development of the Newtrients system is progressed by attracting further EU funding.

approaches, including the Irish Government Circular Economy Bill 2021, the European Commission policies of 2015 and 2017, and the United Nations SDG 12 “Responsible Consumption and Production”. The outcome of the Newtrients project is of direct importance for the dairy processing industry (which was involved as end users in the project), but is also intended to similarly inspire other waste-producing industries. Indeed, the Newtrients project actively reached out to the wider public, the farming community, industry in general and policymakers not only to inform and inspire, but also to build a base for public acceptance and implementation (see, for example, https://www.ucc.ie/en/newtrients/ for details). It is believed that the visualisation of practical solutions arising from research and development of green, circular economy approaches will inform policymakers, as well as the media and the wider public, about the scope to transition to a sustainable economy and society, and inspire a vision of a more sustainable society.

Recommendation (policy)

- It is recommended that the importance of circular economy approaches, such as the Newtrients cycle, is highlighted when addressing the United Nations SDGs such as SDG 3 (zero hunger), SDG 6 (water and sanitation), SDG 11 (sustainable cities and communities), SDG 12 (responsible production and consumption) and SDG 13 (climate action).
- It is recommended that the development of novel circular economy approaches is widely shared with:
  - the wider public, including consumer organisations, environmental interest groups and the education sector, to generate interest and acceptance of novel technologies;
  - the dairy processing and other relevant industries, to increase interest in further bespoke development and commercial implementation;
  - policymakers, to underpin the continued development of effective policy measures.

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Abbreviations

AD	Anaerobic digestion
ADF	Aerobic dynamic feeding
A/O	Anoxic/oxic
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
CSTR	Continuously stirred tank reactor
DO	Dissolved oxygen
EPA	Environmental Protection Agency
HPLC	High-performance liquid chromatography
IASBR	Intermittently aerated sequencing batch reactor
MLE	Modified Ludzack-Ettinger process
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
RDSC	Rutgers Duckweed Stock Cooperative
RGR	Relative growth rate
SBR	Sequential batch reactor
SDG	Sustainable Development Goal
SGR	Specific growth rate
TN	Total nitrogen
TP	Total phosphorus
UASB	Upflow anaerobic sludge blanket
VFA	Volatile fatty acid

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

Is féidir obair na Gníomhaireachta a roinnt ina trí phríomhréimse:

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

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

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

Ár bhFreagrachtaí

Ceadúnú
Déanaimid na gníomhaíochtaí seo a leanas a rialú ionas nach ndéanann siad dochar do shláinte an phobail ná don chomhshaol:
- saoráidí dramhaíola *(m.sh. láithreáin líonta talún, loisceoirí, stáisiúin aistrithe dramhaíola)*;
- gníomhaíochtaí tionsclaíocha ar scála mór *(m.sh. déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta)*;
- an diantalmhaíocht *(m.sh. muca, éanlaith)*;
- úsáid shrianta agus scaoileadh rialaithe Orgánach Géinmhodhnaithe *(OGM)*;
- foinsí radaíochta ianúcháin *(m.sh. trealamh x-gha agus radaiteiripe, foinsí tionsclaíocha)*;
- áiseanna móra stórála peitril;
- scardadh dramhuisce;
- gníomhaíochtaí dumpála ar farraige.

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

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

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

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

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

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

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

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

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

Bainistíocht agus struchtúr na Gníomhaireachta um Chaomhnú Comhshaoil
Tá an ghníomhaíocht á bainistiú ag Bord lánaimseartha, ar a bhfuil Ard-Stiúrthóir agus cúigear Stiúrthóirí. Déantar an obair ar fud cúig cinn d'Oifigí:
- An Oifig um Inmharthanacht Comhshaoil
- An Oifig Forfheidhmithe i leith cúrsaí Comhshaoil
- An Oifig um Fianaise is Measúnú
- Oifig um Chosaint Radaíochta agus Monatóireachta Comhshaoil
- An Oifig Cumarsáide agus Seirbhísí Corparáideacha

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

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Innovative Valorisation of Dairy Processing Wastewater Using a Circular Economy Approach (Newtrients)

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Identifying Pressures

There are pressing economic and environmental concerns regarding the imbalance between resource consumption and regeneration. These concerns relate to the over-exploitation of key resources, such as wood, minerals and water, and the consequences for food supply and the climate. Many valuable resources are currently lost through wasteful production processes, which generate waste streams where treatment, not recovery, is the primary focus. Sustainable production and consumption are now seen as crucial for delivering a more sustainable society. Circular economy models focus on closed-loop production patterns, long-term value retention, and reductions in the linear use of raw resources and production of waste. Waste valorisation is an area in which there are tangible opportunities for resource recovery while lowering waste remediation costs, reducing greenhouse gas emissions and instigating innovative business models. In Ireland, dairy processing wastewater represents a significant waste stream and a financial and technological challenge for the industry. The Newtrients project successfully demonstrated a paradigm shift in how wastewater is treated by developing a pioneering cascading system for valorisation of dairy wastewater, based on circular economy principles.

Informing Policy

In the past decade, the European Union has published several strategies for sustainable industrial growth based on circular economy principles. The "Europe 2020" strategy focuses on creating sustainable economic growth through resource efficiency. Furthermore, the European Commission's report *Innovating for Sustainable Growth* provides a basic framework for better resource efficiency and a roadmap towards a low-carbon economy. Circular economy approaches have also been strongly advocated in the Irish Government Circular Economy Bill 2021 and as part of the United Nations Sustainable Development Goal 12 (responsible consumption and production). In response, numerous industries are in the process of developing zero-carbon, circular economy-based operational models. Yet, there is an urgent need to underpin policy-inspired initiatives with tangible, technical examples of how companies can achieve circularity. Newtrients has successfully demonstrated the potential for developing novel, practical and financially attractive solutions to pressing environmental challenges. These solutions can inspire further development of circular economy approaches within commercial, policy and public arenas.

Developing solutions

Newtrients has successfully developed an integrated system coupling microbial-based technologies of anaerobic digestion and aerobic dynamic feeding with duckweed cultivation. Each step in this cascading system contributes a clearly identifiable, value-added output, but also delivers broader impacts for sustainable waste remediation. The overall value of the Newtrients cascading system is that it (1) is resource efficient, (2) generates value-added and marketable products, and (3) reduces dependence on finite fossil fuel resources. The stable conversion of organic carbon loads in dairy wastewaters to volatile fatty acids and other chemical building blocks constitutes an innovative, sustainable route for the production of non-petrochemical-based polymers, including bioplastics. The recycling of nitrogen and phosphorus in wastewater yields protein-rich biomass and enables a closed-loop rural feed industry. Overall, the Newtrients cascading system results in the effective remediation of dairy wastewater while generating products that can strengthen the local economy and create intricate new relationships between farmers, dairy processors and innovative rural industries.

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