Got Whey? The significance of cheese whey at the confluence of dairying, environmental impacts, energy and resource biorecovery

Giulianetti de Almeida, M.P.; Mockaitis, Gustavo; Weissbrodt, David G.

DOI
10.24404/5fdd3c22eaf7860008874c47

Publication date
2021

Document Version
Submitted manuscript

Published in
The Evolving Scholar

Got Whey?
The significance of cheese whey at the confluence of dairying, environmental impacts, energy and resource biorecovery

Maria Paula Giulianetti de Almeida[1,2], Gustavo Mockaitis[1*] and David G. Weissbrodt[2*]

[1] Faculty of Agricultural Engineering, University of Campinas, Brazil
[2] Department of Biotechnology, Delft University of Technology, The Netherlands
* Shared senior authorship and correspondence

ORCID and e-mails:
Maria Paula Giulianetti de Almeida (https://orcid.org/0000-0001-7113-7766; mp8109@gmail.com)
Gustavo Mockaitis (https://orcid.org/0000-0002-4231-1056; gusmock@unicamp.br)
David G. Weissbrodt (https://orcid.org/0000-0002-6313-1652; d.g.weissbrodt@tudelft.nl)
DOI https://doi.org/10.24404/5fdd3c22eaf7860008874c47

Abstract

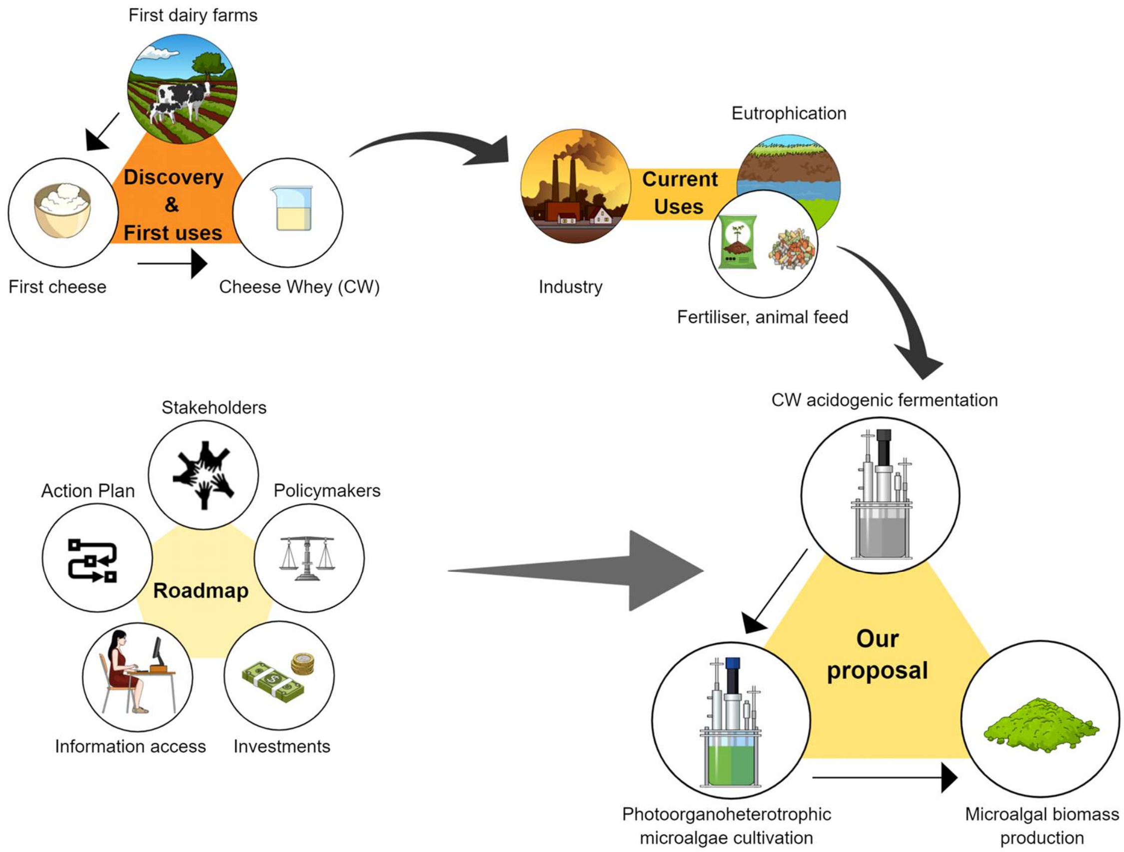
Milk discovery and processing enabled human settling and thriving in various settings. The discovery of cheese led to the production of whey as dairy by-product. Although it can find application in food, beverages, personal care products, pharmaceuticals and medical treatment, cheese whey is a massive dairying residue world-wide (154 $Mm^3 \cdot y^{-1}$) with high organic and nutrient loads. About 42% is used as low-value products as animal feed and fertilisers or even directly discharged in water streams, leading to ecosystem damage by eutrophication. Recycling and repurposing whey remains a challenge for remote locations and poor communities with limited access to expensive technology. Anaerobic digestion is proven and accessible for utilizing whey as substrate to produce biogas and/or carboxylates. Alternative processes combining anaerobic digestion and low-cost open photobioprocesses can foster the valorisation of cheese whey and capture of organics and nitrogen and phosphorus nutrients into a microalgal biomass that can be used as food and crop supply or processed into biofuels, pigments, antioxidants, among other value-added products. Awareness should be raised about the economic potential of cheese whey surplus by developing an action plan that *(i)* identifies stakeholders, *(ii)* sets goals and achieves solutions, *(iii)* decreases technology gaps among countries, *(iv)* enforces legislation and compliance, and *(v)* creates subsidies and foments partnerships with industries and other countries for the full valorisation of whey. We propose a closed-loop biorefinery implementation strategy to simultaneously mitigate environmental impacts and valorise whey resources.

Keywords: cheese whey, environmental impacts, resource valorisation, laws and regulations, information access, anaerobic and microalgal processes

Research Highlights

- Whey is a massive residue of dairy processing world-wide.
- When applied or discharged unhandled in the environment, whey leads to eutrophication.
- Action plans are needed to mitigate environmental impacts and capture whey resources.
- Low-cost alternatives combining anaerobic and microalgal processes can repurpose whey.
- We propose a scalable roadmap for the circularity of dairying, reaching remote communities.

Graphical abstract



Got Whey? *An integrated management of cheese whey should foster responsible solutions for environmental protection, energy production, and resource biorecovery in the dairying circular economy.*

[1]University of Campinas, Brazil and [2]Delft University of Technology, The Netherlands

1 Introduction

Milk has been in our daily lives for centuries. Unlike other mammals, humans make further use of this food past the lactation period. As our milk tolerance increased, so did the available dairy products. Yoghurts, cheese, spreads, among other products fill up our shelves.

In 1993, the California Milk Processor Board launched the 'Got Milk?' campaign encouraging milk consumption[a]. Celebrities and characters were its spokespersons. Good examples were Batman, Kermit the Frog, Elton John and Muhammad Ali to name a few[b]. They all displayed a milk moustache asking: "***Got Milk?***". The campaign was discontinued in 2014 but it is still parodied in movies, sitcoms, and cartoons. This tagline is a snowclone being easily recognisable regardless the variants.

Besides the direct consumption of milk, the production of cheese is another way to deal with milk the surplus. In the dairy industry, liquid whey is the remaining portion of milk after cheese or casein production [1] presenting a yellow/green colour with a blueish tinge depending on the type and quality of milk used [2]. Sweet whey results from the manufacturing of hard cheeses such as cheddar or Swiss cheese and is achieved by using rennet, a set of ruminant enzymes used in the coagulation process. The acidification of milk by *Lactobacillus* or addition of mineral acid (HCl or H_2SO_4 acid) in cheese making results in acid whey. Salty whey accounts for 2 to 5% of salted cheese production [3,4].

Generally, whey consists of water (90%), proteins (6.0 g L^{-1}), lactose (46- 52 g L^{-1}), dissolved salts, lactic acid, lipids, minor components (*e.g.*, citric acid, urea and uric acid) and B-complex vitamins [5]. Its main characteristics depends on its type (acid, sweet or salty), source of milk (*e.g.*, bovine, caprine, sheep, and camel), animal feed, livestock stage of lactation, time of the year and cheese making processes. Variances in milk casein and fat ratio can lead to cheese yield and quality fluctuation between seasons and locations influencing the quality of whey produced [3].

Every 100 L of milk yields about 12 kg of cheese or 3 kg of casein [6]. We can estimate a production of 87 L of whey per 100 L of milk. Large cheese-making plants can generate over a million litres of whey per day [1] and the volume of produced whey is rising annually [2]. Tsakali et al. [3] have demonstrated the global utilization of whey in 2010. Considering the amount of generated whey in cheese making [6], the whey global utilization balance [3], and the 2019 world cheese effective production and 2020 cheese production forecast [7], we can infer a total whey production of 154 Mm^3 $year^{-1}$. **Figure 1** depicts the global utilization of cheese whey for the year 2020 [3,6,7] and the growing world cheese production in tonnes from 1960 to 2020 [7,8].

After initial spray drying, acid and sweet whey can be precursors for value-added products in food, nutrition and pharmaceutical industries. Due to its high salinity, salty whey has limited use in industry [9]. Currently, about 42% of whey is used as animal feed, fertiliser or simply discarded [3]. Whey cannot be used as sole source of animal feed due to ruminants' dietary needs. The same is valid for liquid whey, which is temperature dependent becoming unsafe for consumption once warm [10]. Hence, we can envision other alternatives for its valorisation.

Soil application of whey or its direct discharge in water bodies are also not the best option as they result in severe environmental burdens. When used as a fertiliser, it acidifies the soil pH drastically and stabilization reaches as low as 2 units in pH scale [11]. Its discharge in water bodies can unfavourably lead to eutrophication processes [3].

Here, we critically reviewed and addressed cheese whey from its generation, discovery, first uses, characteristics, and valorisation potential. We provide solutions to prevent environmental impacts by anaerobic digestion or acidogenic fermentation of cheese whey followed by photobioprocesses for microalgal biomass production. We also propose a roadmap addressing (*i*) the need to bridge stakeholders together to tackle the problematic of cheese whey residues, (*ii*) the implementation of an action plan that will guide stakeholders into implementing cheese whey valorisation alternatives respecting a time frame, (*iii*) the importance of decreasing technology availability and affordability gaps among countries, (*iv*) the

[a] www.gotmilk.com

[b] https://www.ranker.com/list/celebrities-in-got-milk-ads/celebrity-lists

Citation: Giulianetti de Almeida *et al.*, *Got Whey*? The significance of cheese whey at the confluence of dairying, environmental impacts, energy and resource biorecovery, The Evolving Scholar, Preprint (2021)
[1]University of Campinas, Brazil and [2]Delft University of Technology, The Netherlands

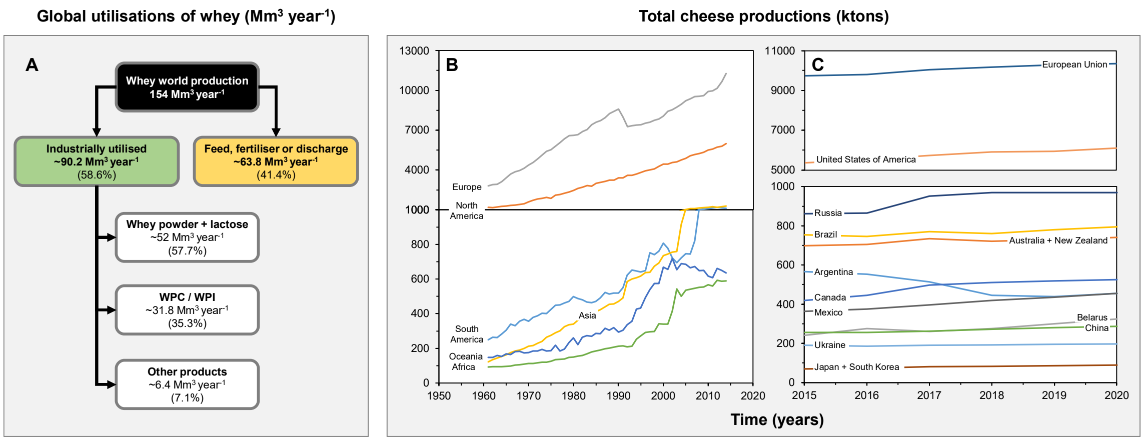


Figure 1. A) *Global utilization of cheese whey for the year 2020. Estimative was based on the amount of whey generated per kilo of cheese, the percentages of whey repurposed by industry and their remaining usage, and the forecast of cheese production for 2020* [3,6,7]*.* ***B)*** *Cheese production from 1961 to 2014 by continents. Geo-political changes were accounted throughout the decades* [8]*.* ***C)*** *Cheese production among the world biggest producers from 2015 to 2020 according to the USDA* [7]*. Values for the year of 2020 are forecasted.*

necessity of legislation enforcement and fomenting partnerships between countries and industries to help in this transition.

Considering the increasing demand of cheese and that the most relevant type of whey regarding production volume and economical value comes from cow milk processing, this article solely focuses on cheese whey derived from cow milk. Because of the need to safeguard natural ecosystems and since the potential of cheese whey for the production of value-added products is undeniable, we advocate: "***Got Whey?***".

2 From dairying discovery to the importance of cheese and the benefits of cheese whey

2.1 Historical evolution of milk processing

Archaeozoology has long speculated about the history of cheese. The Neolithisation was the transition from the semi-nomadic lifestyle to sedentary habits substituting a hunter-gathering culture to an agricultural and livestock one. This transition dates back to around 12,000 years before present (BP) in the Near East and Anatolia, spreading to the Middle East, the Caucasus, Europe, and finally reaching Africa [12].

Dairying in the in South-Eastern and the Near East Anatolia was intrinsically connected to the first domestication of animals dating from approximately 10,500 BP [12]. Dunne et al. [13] found similarities between the lifestyles of Holocene Sahara and North Africa and Neolithic Europe and Eurasia as they both relied upon ruminants as livestock before domesticating plants or setting farming communities.

The dairying profile required the culling of animals while they were babies to exploit the remaining milk. Also, the production of meat would require harvesting the animal when they reached their maximum weight, demonstrating that the exploitation of livestock was compatible with the milk production from the early Neolithic onwards [12].

Most mammals have the production of lactase down-regulated when the offspring is no longer dependent on milk for its survival. Once the levels of lactase have decreased, continuous consumption of milk caused lactose intolerance [12]. The Neolithic population minimised this disorder by processing milk into cheese, yoghurt, butter and other dairy products [12]. Genetic mutation about 7,000 to 8,000 BP in Europe and North Africa allowed the digestion of lactose by adults [14].

Dairying processing presented not only a measure to store and transport any milk surplus throughout the year but provided an alternative for lactose-intolerant people to consume milk byproducts. By producing cheese and yoghurt, most lactose was released with the whey [15].

Citation: Giulianetti de Almeida *et al.*, *Got Whey*? The significance of cheese whey at the confluence of dairying, environmental impacts, energy and resource biorecovery, The Evolving Scholar, Preprint (2021)
[1]University of Campinas, Brazil and [2]Delft University of Technology, The Netherlands

2.2 The use of whey

Dairying was a cornerstone for human settling during Neolithic. The discovery of cheese, yoghurt and derivatives resulted in whey as a by-product although its early use during the Neolithic is not well documented. Whey therapeutic purposes first began in 2410 BP with Hippocrates, continuing through the Middle Ages [16]. In Ancient Greece, whey was used as a skin balm or as a medicine [17]. Whey baths were famous from the nineteenth century until World War II [16].

The first clinic to use cheese whey as medicine was opened in Switzerland by Dr. Fréderic Hoffman in 1760, where its diuretic and laxative properties were recognised and used as medicine. Soon, other whey therapy clinics opened across Europe. Spas in central Europe served around 1.5 kg of whey per day to patients treating several different illnesses, from gout to arthritis and liver diseases [18].

Cheese whey became a fashionable drink in the mid-seventeenth century, including whey-borse (a broth), whey-butter, whey-porridge and whey-whig, a drink made with herbs [19]. Additionally, Scandinavian medieval population from the Norse made use of sour whey to pickle meats and also produced a Scandinavian 'whey' cheese with high lactose content (30%-35%) [20].

However, research on the nutritional aspects of whey started only in the nineteenth century. Whey was also used as animal feed in the early centuries [12]. Besides the use as animal feed, whey was also used as a fertiliser, irrigation water or dumped into water bodies [1–3,16].The nuisance caused by whey foul smell and high salinity makes it not the best fertiliser available.

Cheese whey disposal on land or in the municipal sewage system is not allowed in numerous locations. The high costs involved in whey collection, treatment and disposal by local governments, leaves small dairy farms financial struggling and with no choice but to consider disposal in hydric bodies when opportunity arises [21].

Milk processing was a driving force for human settlement allowing the discovery of dairy and livestock management. However, despite its many uses throughout the centuries [22], whey production still needs to be addressed. How can this panorama be changed?

3 Environmental impacts and management of cheese whey residues

Besides the uses and benefits of whey, a more integrated vision should address the environmental impacts resulting from the whey residue and its plain disposal in local waters [17]. Whey is the highest organic pollutant comprised by the wastewaters of the dairy industry. It presents an organic concentration as high as 50 to 80 g COD L^{-1} (in terms of chemical oxygen demand – COD) or 40 to 60 g BOD L^{-1} (expressed as biochemical oxygen demand – BOD) [23]. A small creamery can emit an average of 189 kg BOD d^{-1} load of raw whey as wastewater [24]. Whey treatment and recovery is paramount to valorise it and minimise its environmental burden.

3.1 Environmental burden and elevated costs of treatment of whey residues

Once in the water stream, cheese whey can unfavourably lead to eutrophication [25]. Besides organic matter, cheese whey is composed of organic nitrogen (0.2- 1.8 kg N m^{-3}) and mostly inorganic phosphorus (0.12-0.54 kg P m^{-3}) that drive algal bloom [25]. The discharge of untreated volumes of cheese whey residues can reach up to 3,800 L day^{-1} which is equivalent to the polluting strength of the sewage of 1,800 persons [24].

Whey must be collected by industrial and/or municipal sewage system for either decentralised treatment at the source or centralized treatment at the wastewater treatment plant. Treatments are often considered as an expensive procedure and might not be implemented if regulations are not enforced [2,24]. This can encourage producers, especially in developing countries or in remote locations, to discharge their whey residues directly in a water stream.

In 1988, Belloin [26] stated the difficulty in establishing costs for treating cheese whey and dairy wastewaters. Procedures depend on the plant size, quality of whey and geological and climatic factors [26]. An unpublished survey by Hughes et al. [27] stated that small cheese producers in the USA must only give proper treatment and disposal of whey for production over 5,000 kg per year with an average cost of 105.00 USD per

Citation: Giulianetti de Almeida *et al.*, *Got Whey*? The significance of cheese whey at the confluence of dairying, environmental impacts, energy and resource biorecovery, The Evolving Scholar, Preprint (2021)
[1]University of Campinas, Brazil and [2]Delft University of Technology, The Netherlands

ton disposed, leading to a substantial decrease in their profit margins.

Dairy producers face a lot of difficulties to process cheese whey into other value-added products. Alternatives to whey valorisation should be proposed and developed with existing ones (*i.e.*, spray drying transforming whey as animal feed, fertilisers and spirits or the disposal of whey in water bodies) [28]. The greatest obstacles for small-scale whey processing remain health and safety issues, especially due to its contamination and low shelf life [29].

The utilisation of whey as fertiliser presents disadvantages such as high organic and nutrient concentration, decrease of soil quality and productivity by acidification leading to environmental degradation [3]. Whey has little microbial stability and lactose has low water solubility, crystallising in low temperatures [30]. So, the farther is the distance from production to use site, the higher become the costs for temperature-controlled transportation of whey. Most times, these costs are passed onto cheese producers making whey fertiliser not economically viable [31].

3.2 *Paving the whey for an ecologically balanced, circular, and participative economy*

A successful implementation of change stems from engaging different stakeholders involved in the whey problematic towards a common goal for its sustainable use, treatment, and disposal [32]. In a circular economy, the whole usability of cheese whey should be considered, especially the part currently not absorbed by industry. Hence, its waste and pollution can be minimized. Understanding the social, political, economic, technological, legal and environmental aspects of whey from production to disposal is key to identify all stakeholders at different levels.

Community pressure led to change in legislation, either banning or restricting the disposal of untreated whey, toward improving its waste management [1,2,17]. However, environmental legislation was never the main issue regarding illegal and/or improper whey disposal. Environmental laws and policies started in the 1970's with the creation of the US Environmental Protection Agency and the first European environmental policy [33]. Countries among the biggest cheese producers [7], have legislation regarding freshwater conservation and management. They state that all agroindustrial residues and wastewaters must be treated. Those legislations are depicted in **Table 1**.

The United Nations' Environment Rule of Law divulged that although most countries have environmental conservation regulations, but few actually comply with them. This if often due to incomplete, irregular or ineffective enforcement [34]. Moreover, countries that favours the rational polluter model often have industries that fail to comply to regulations given that 'polluter pays'. Non-compliance can also originate from the difficulty in interpreting regulations due to overload of information, jargons and amendments or it results from the misconception that environmental regulations hinder economic growth and competitiveness [35].

Regarding cheese whey disposal, treatment, and valorisation, big dairy cooperatives are in most cases, responsible for further processing cheese into other products [3]. Micro-, small- and medium-producers have limitations due to the lack of infrastructure connecting them to the industry, the little sector R&D investments, the high cost *vs.* benefits to process cheese whey into value-added products and the few markets available to sell the recovered products. These factors are closely related to the location of production [36].

In Brazil, modern and artisanal cheese producers are scattered around the country and about 40% of produced cheese whey is not exploited [37]. Small dairy farms have higher costs to process whey, so alternatively they use it as animal feed or fertiliser or discharge it [3,37]. A similar situation occurred in the Basque region of Spain. For instance, projects like VALORLACT "whey to future" successfully implemented an action plan to recover whey over the territory. It resulted in the development of whey processing plants and production of 15 different value-added products for food and fodder. This project was subsidised by the European Union and counted with industrial partnerships [38].

Citation: Giulianetti de Almeida *et al.*, *Got Whey*? The significance of cheese whey at the confluence of dairying, environmental impacts, energy and resource biorecovery, The Evolving Scholar, Preprint (2021)
[1]University of Campinas, Brazil and [2]Delft University of Technology, The Netherlands

Table 1. *Available legislation on wastewater and water management in countries with higher cheese whey production. Implemented from [36]–[49].*

World biggest cheese producers	Water management legislations
Argentina	National constitution ART 121 civil code
Australia	Environmental Protection Act, 1993
	The National Waste Policy, 2009
Belarus	Water code of the republic of Belarus, 2014
Brazil	Water code, 1934
	National Solid Waste Policy, PNRS, 2010
Canada	Canada Water Act, 1985
	Wastewater Systems Effluent Regulations 2015 under the Fisheries Act 1985
China	The Water Law of the People's Republic of China, 1988
European Union	Waste Framework Directive (2006/12/EC)
	Urban Wastewater Directive (91/271/ EEC)
	Renewable Energy Directive (2009/28/EC)
	Circular Economy package (COM (2015) 614 final
Japan	The Water pollution control law, 1970
Mexico	National Water Law
New Zealand	National Policy Statement for Freshwater Management under the Resource Management Act 1991
Russia	Water code of the Russian Federation, 2006
South Korea	Water Quality and Ecosystem Conservation Act, 2009
Ukraine	The Water Code of Ukraine, 1995
United States of America	Clean Water Act, 1972

In 2019, the USA dealt with a surplus of 700,000 tons of cheese [53] by implementing a price support programme in which the government bought this surplus controlling the economy and avoiding the downfall of the American dairy industry [54]. However, one question remains: How did the country deal with the 4.60 Mm^3 of whey produced when they were having issues absorbing the cheese surplus?

Oftentimes, the implementation of environmental management and resource recovery plans by companies mostly relies on economic viability and/or business opportunities. The management of cheese whey residues is an excellent illustration of it. Economical support programmes should be implemented for small producers to collect, dispose, treat and possibly valorise cheese whey. At higher scale, market niches should be identified for the recovered products, if not directly re-used as resources or energy on the industrial site.

However, governance, regulations and law enforcement are not sufficient if the degree of knowledge and state-of-the-art facilities falls behind or is obsolete. Policymakers, industry, and dairy producers must join interests to implement regulations and research and development (R&D) for integrating cheese whey valorisation into a circular economy. This can be achieved by the implementation of progressive policies favouring renewable energies and material resource recovery from used streams rather than focusing only on prices and the understanding that low income countries transition can only be effective with financial and technological investments from high income countries [55].

3.3 Information access to drive mitigation, valorisation, and development engineering

Information access is crucial to any research field. In fact, scientific work is only made possible when we can find information that can either support or refute our initial hypothesis so we can tailor our work, achieve results and publish them reaching the scientific community. The handling of cheese whey by practitioners and local communities across the globe is hampered by failure of information access. Some known barriers to access information consist of but are not limited to *(i)* lack of critical thinking; (*ii)* language; (*iii)* libraries facilities; *(iv)* search engines and webhosts; *(v)* economical restraints and *(vi)* commercial sensitivity. Most times these barriers are interconnected and interdependent [56–63].

The lack of incentive to provide information of stakeholders' interests hinders them from perfecting important skills in R&D and in everyday life

Citation: Giulianetti de Almeida *et al.*, *Got Whey*? The significance of cheese whey at the confluence of dairying, environmental impacts, energy and resource biorecovery, The Evolving Scholar, Preprint (2021)
[1]University of Campinas, Brazil and [2]Delft University of Technology, The Netherlands

situations. Language barriers can also limit access of information to speakers of other languages than English. This can be a great obstacle when doing research [56] since significant information can become unknown or even obsolete because of lack of English fluency [58,59].

English is the *lingua franca* of science. However, most science is not made by native English speakers [58]. This fact leads to various assumptions and limitations. The lack of critical thinking due to language and cultural barriers is one of the main made assumptions [57]. Non-native English speakers and countries with research in dominant *lingua mater* are often the dark horses of the publishing race [63] regardless of the quality in their work. Scientists are encouraged to publish in English in order to make their research relevant, cited and known [58].

Some solutions to minimise language barriers issues are free, accurate online translations tools to engage readers, inclusive language texts reducing 'digital divide' [56], hosting exchange programmes between different institutions, access to international conferences and articles written in both *lingua mater* and *lingua franca* as offered by electronic libraries like Scientific Electronic Library Online - SciELO and PLOs One [58,59].

Most people have libraries as a primary place to study and research. In specific, undergraduates that do not have any practical research activities. According to Ugah et al. [60], a lot of facilities have obsolete, scarce and difficultly located sources of materials which can be unavailable for either consultation or lending. Libraries also face budget cuts to invest in their facilities, materials and staff [64]. Digital libraries can be an alternative to existing ones but they still present issues around web-hosts and domain names [56,60].

Another issue is the cost of subscriptions of academic journals. Some institutions especially in low income countries cannot afford them, limiting their research scope [65]. Search engines can be useful tools to search and retrieve documents from the internet [61]. However, it is important to improve and update their scientific content [60,62]. A great feature of the internet are databases with open-access material such as OCLC's Cooperative Online Resource Catalog, The Research Libraries Group (RLG), INFOMINE (Byrum), and other repositories as mentioned before. Other platforms like arXiv, ChemRxiv, BioRxiv and many alike function provide direct access to latest research via pre-prints. Institutional repositories and open access mega journals like The Evolving Scholar lately launched by Delft University of Technology in the Netherlands are important ways to convey the information in open access. Still, digital access relies on internet access, which remains a challenge for remote and marginalized areas and communities. In the present digitalisation era, key challenges need to be solved to promote effective information access and solutions for development engineering.

4 Clearing the whey: product, resource, and energy recovery

About 63.8 Mm^3 $year^{-1}$ of whey is currently not absorbed by industry [3]. The potential of valorisation with the manufacturing of value-added products can improve the sustainability of cheese processing [1]. Until recently, whey by-products were seen as low-value products. The lack of understanding of whey characteristics and functionality, together with its inconsistent performance in food system (*i.e.*, water and flavour binding, solubility and emulsification properties) and soy protein consolidated market limited the use of whey regardless available processing technology. This scenario has changed considerably since the initial process development of lactose downstreaming and its related value-added products [2,66].

The high concentrations of organic matter, nitrogen, and phosphorus in whey [67] render this residue into an interesting feedstock for resource and energy recovery. Alternatives for valorisation comprise of production of health and other industrial value-added products[1,2], phosphorus and nitrogen recovery [68], carbon capture [69], transformation by anaerobic digestion and fermentation processes [70], as well as other biotechnological processes for the valorisation of biomass, biofuel and biomaterials [71]. The following sections discourse these alternatives as well as our proposal to couple anaerobic digestion or acidogenic fermentation of cheese whey with photobioprocesses to biorecover energy and resources on top of safeguarding the natural environment.

Citation: Giulianetti de Almeida *et al.*, *Got Whey*? The significance of cheese whey at the confluence of dairying, environmental impacts, energy and resource biorecovery, The Evolving Scholar, Preprint (2021)
[1]University of Campinas, Brazil and [2]Delft University of Technology, The Netherlands

***Table 2. A**) Cheese whey downstreamming processes, available technologies, products spectra and current applications. Adapted from [1,66,72,74]. **B**) Price of derived whey products in tonnes. Prices in Euro refers to products from France, Germany and The Netherlands and Poland. Prices fluctuate according to location and period. Maximum price was considered. Data source: CLAL Consulting - Dairy Economic[c].*

A

Cheese whey processing and applications in pharma, nutrition, health & personal care, food and feed

Physical separation	Protein separation	Protein modification
Membrane separation (PF, MF, RO, NF) High hydrostatic pressure Pulse electric field Microwave Ultrasound	Spray drying Extrusion texturisation Carbon dioxide precipitation	Enzymatic modification Chemical modification
Whey powder (WP) Dry whey powder (DWP) Whey powder concentrate (WPC) 35% Whey permeate α-, β-lactoalbumin Lactose Casein	Whey powder concentrate 80% Lactose pharma grade Whey protein blends (WPI + WPC) Pure whey isolate Minerals	Lactic acid Bovine serum albumin (BSA) Other bioactive compounds (immunoglobulins, lactoferrins, glycomacropeptides, transferins, lactoperoxidase, lysosymes)

B

Price of whey-derived products

Products	US market (USD ton^{-1})	European market (EUR ton^{-1})
Casein (acid)	7,766	7,002
Casein (rennet)	7,865	7,091
Dry whey powder (DWP)	613	742
Lactose (non-pharmaceutical)	816	735
Whey (animal feed)	477	690
Whey powder concentrate 34%	2,315	2,087
Whey powder concentrate 80%	8,100	4,450

4.1 Health benefits fostered processes to recover whey

The first attempts to concentrate dry whey started in the 1920's. Technologies involved conventional hot roller milk driers, heating whey until a concentration liquid is obtained, cooling whey until it solidifies following a tunnel extrusion and combining spray drying and rotary drum drying [72]. Due to the hygroscopic nature of lactose to this day some processes are still rather costly, especially for small and medium size cheese producers [30]. Despite this, the hot drum drying process is still one of the most used processes for whey powder production [16]. Whey as animal feed or fertiliser present lower prices compared to other value-added products obtained from whey such as whey powder concentrate or isolate. **Table 2** depicts the different techniques currently used in whey processing its added value products, applications and prices of some by-products.

Whey by-products became commodities of interest for nutritional, pharmaceutical, medical industries giving that its proteins and peptides components present nutritional value and antimicrobial, anti-viral, anticarcinogenic and anti-oxidant properties [2,3,66]. As technology evolved, protein separation and modification enabled the discovery of new uses for whey such as isolates and other bioactive compounds [73]. Current technologies for cheese whey processing therefore notably consist of physical separations and bioengineering for proteins recovery and modification.

4.2 Anaerobic digestion and acidogenic fermentation to prime the biorecovery of cheese whey resources

Anaerobic digestion of cheese whey has been studied [75] regardless its trend to acidify. According to Malaspina et al. [76], the high biodegradability (~99%) of cheese whey, pH reduction (below 5), and low bicarbonate alkalinity (50 meq L^{-1}) can

[c] www.clal.it

[1]University of Campinas, Brazil and [2]Delft University of Technology, The Netherlands

lead to operational difficulties [77]. However, the high organic content of cheese whey makes it suitable for energy recovery via biogas production by anaerobic digestion [78]. The efficiency of the bioprocess relates to parameters like the substrate feed, temperature, pH, hydraulic retention time [79].

Acidogenic fermentation of cheese whey is an interesting alternative to anaerobic digestion. Methanogenesis can be stopped after the conversion of whey by fermentative microorganisms to accumulate hydrogen and volatile fatty acids (VFAs) [23]. Anaerobic digestion without production of biogas is an opportunity for the valorisation of VFAs via the carboxylate platform [80].

The acidogenic fermentation of cheese whey can be driven by inoculum pre-treatment (*e.g.*, physical, biological) [81,82], lowering the hydraulic retention times (*i.e.*, between 2 to 5 days) [83], and controlling pH (*i.e.*, below 7.0 to 3.3) [84], selecting acidogens to outcompete methanogens. Other fermentation processes can also valorise cheese whey. These processes can be performed either in axenic pure-culture systems or via mixed-culture fermentation in non-sterile open systems [85,86].

Some of the products obtained from cheese whey valorisation are short-, mid- and long-chain organic acids [87,88], intracellular storage products (*i.e.*, polyhydoxyalkanoate and polyhydroxybutyrate) [89,90], bioplastics [91], biohydrogen [92], bioethanol [93] and biobutanol [94]. Other innovative bioprocesses involve the conversion of VFAs into electricity or other value-added products using bioelectrochemical systems (*i.e.*, microbial fuel cells and microbial electrosynthesis cells) [95,96].

4.3 Co-digestion of whey

Anaerobic co-digestion is a process where different substrates from agricultural farming, manure, municipal, food and industrial wastes are combined in anaerobic digestion to optimise parameters such as temperature (30-50°C), pH (5-7), organic matter concentration, nutrients availability, alkalinity and C/N (25 to 35:1) ratio. Consequently, the overall biogas yield is increased and resource recovery is facilitated, diverging from waste disposal in landfills and leading to environmental and financial benefits [97,98].

Synergy between substrates is paramount for higher biogas production. Anaerobic co-digestion process with proteins can increase biogas production and halt inhibition by excess of ammonia, although this synergy is yet to be proved in full scale reactors [99].

The co-digestion of cheese whey has been studied combining with other substrates such as animal manure [77], food waste [100], other wastes [101], and microalgae [102]. Currently, there are some anaerobic digestion plants using cheese whey as substrate for their processes [103]. The anaerobic digestion of cheese whey seems a sound bet for repurposing the current surplus of the whey.

4.4 Light-based valorisation of cheese whey using photobioprocesses: harnessing eutrophication in bioprocess boundaries

We advocate for new biorecovery process alternatives coupling the acidogenic fermentation of cheese whey into short and mid-chain VFAs production prior to feeding into algal ponds, photoactivated sludge systems, or photobiotechnologies to produce a photoorganoheterotrophic microalgal biomass. This biomass can be processed into an outlet of products of industrial interest of higher value than biogas.

Although most hydrogen production results from “dark fermentation” processes performed by chemoheterotrophic bacteria and microalgae [92], it can also occur in the presence of light. This process is known as biophotolysis, comprising direct and indirect biophotolysis and photofermentation [104]. In direct biophotolysis, water is oxidized into hydrogen and oxygen in presence of light during photosynthesis by photoautotrophic microalgae. In indirect photolysis, hydrogen is the product of the reduction of organic compounds by photosynthetic bacteria, cyanobacteria and microalgae [104].

Photofermentation is a process where anoxygenic photosynthetic bacteria (*i.e.*, green sulfur bacteria, purple-sulfur bacteria and purple non-sulfur bacteria) [105,106] uses alternative reduce compounds as electron donors (*e.g.*, hydrogen sulfide, organic acids and carbon sources) nitrogenase and light as energy source to synthesise hydrogen [107]. Biological water-gas shift is performed by hydrogenogenic carboxydotrophic bacteria that oxidises carbon monoxide while cat-

Citation: Giulianetti de Almeida *et al.*, *Got Whey*? The significance of cheese whey at the confluence of dairying, environmental impacts, energy and resource biorecovery, The Evolving Scholar, Preprint (2021)
[1]University of Campinas, Brazil and [2]Delft University of Technology, The Netherlands

Table 3. Microalgae biomass applications considering different uses and products spectra. Compiled from [122–126].

Microalgal biomass applications			
Fine chemicals	Fatty acids Carotenoids Antioxidants Vitamins Other bioactive compounds	**Industrial**	Pharmaceutical Aquaculture Animal feed Biofertiliser
Drug screening	Antimicrobial agents Antiviral drugs Anticancer drugs	**Environmental**	Pollutants removal Wastewater co-digestion CO_2 mitigation Biochar
Commercial	Human health Nutrition Cosmetics Pigments Recombinant proteins Stable isotopes Biochemicals	**Biofuels**	Biodiesel Bioethanol Biobutanol Biosyngas Biogas Electricity Heat

alysing the water-gas shift reaction [107,108], producing hydrogen. The biological water-gas shift can be an alternative for the current chemical one used for syngas production [108]. Cheese whey have been used both in dark [75] and photofermentation [109] processes. It also served as substrate for microalgae cultivation. Given microalgae photosynthetic and lipid production efficiency, photofermentation processes using VFAs as carbon source for biomass production can give a more profitable use for the 63.8 Mm^3 $year^{-1}$ of cheese whey currently used as animal feed, fertilizer or discharged in water streams.

4.5 Synergetic interactions between bacterial and microalgal consortia to valorise whey

Compared to other biofuels feedstocks microalgae cultivation is advantageous as they can be cultivated in arid land [110] and brackish or high strength waters [111]. They can remove nitrogen and phosphorus from wastewaters simultaneous [112] and mitigate carbon dioxide, given their photosynthetic efficiency [113].

Microalgae carbon metabolism can be photoautotrophic, (photo)heterotrophic and mixotrophic [114,115]. Heterotrophic microalgae are an economic attractive since they are light independent [114] being employed in municipal and agroindustrial wastewater treatment [116]. Mixotrophic microalgae displays both photoautotrophic and (photo)heterotrophic regime [117]. Due to respiration, mixotrophic microalgae have reduced photoinhibition, improved growth rate and reduced biomass night losses[118]. Current industrial application dwells in the production of unsaturated fatty acids (*e.g.*, omega-3 fatty acids or arachidonic acid), antibiotics and pigments, such as carotenoids [119]. However, their carbon assimilation and growth mechanisms still needs elucidation [120]. **Table 3** shows the value-added products obtained from microalgae and their respective uses. Although some studies investigated microalgal growth on cheese whey [102] and cheese-whey-related products (*e.g.*, dairy waste, digested cheese whey, second cheese whey, permeate) [121] as well as co-digestion processes [116], there are few studies having tailored the biovalorisation of cheese whey by combining microalgal-bacterial mixed-culture biotechnologies [127].

Microalgal mixed-culture bioprocesses have been studied notably for the anaerobic digestion of microalgae [128], lipids and high storage compounds production and accumulation [129], as well as co-evolution [130] and signal transduction for microalgae-bacteria cell growth [131]. These studies elicit the importance of microbial ecologic relationships for biosynthesis via mixed-culture photo biotechnologies.

The symbiotic relationships between microalgae and bacteria is important with respect to the exchange of substrates (*e.g.*, CO_2-O_2 exchange between bacteria and microalgae, bacterial cobalamin supply to auxotrophic microalgae), signalling transduction (*e.g.*, quorum sensing, growth inhibition or stimulation by exudates release), or horizontal gene transfer [132].

Microbial ecology still presents various knowledge gaps regarding the study and comprehension of microalgal-bacterial symbiosis [133].

Citation: Giulianetti de Almeida *et al.*, *Got Whey*? The significance of cheese whey at the confluence of dairying, environmental impacts, energy and resource biorecovery, The Evolving Scholar, Preprint (2021)
[1]University of Campinas, Brazil and [2]Delft University of Technology, The Netherlands

The knowledge on bacto-microalgal chemical interactions is still scarce. The advent of 'multi-omics' (*e.g.*, meta- genomics, transcriptomics proteomics, lipidomics, metabolomics) is now providing key analytical means to elucidate them.

Even though studies about microalgal-bacterial symbiosis in anaerobic digestion processes are increasing (*e.g.*, biomass, biofuels, value-added products production, CO_2 mitigation or wastewater treatment), there are few studies on scale-up reactors since conditions might differ than in lab scale [134].

Some of the bottlenecks to overcome in mixed-culture processes regarding microalgae-bacteria interactions are *(i)* the costs and energy requirement of microalgae biomass harvesting, *(ii)* the complex microecosystem and its dynamics that can shift in a short span of time, and *(iii)* the algal-bacterial biofilm preventing light going beyond the photic zone [134]. Despite these hurdles, microalgal-bacterial mixed-culture processes have been studied as a polishing step after anaerobic digestion, biomass production from wastewaters, biofuels production and reactor [135].

4.6 Phosphorus and nitrogen removal and recovery from cheese whey

The prevention of eutrophication usually goes via the biological/chemical removal of phosphorus and nitrogen from wastewaters [136]. Bioprocesses for removing nutrients from municipal and industrial wastewater have been studied and operated extensively worldwide [137]. Technologies using biofilms and granular sludge enabled intensification and integration processes of wastewater treatment plants [138].

In the context of high-loaded streams such as agroindustrial ones, the combination of anaerobic digestion and subsequent digestate polishing for nutrient removal is a standard [139]. This technological combination has been implemented for treating cheese whey in anaerobic digestion or co-digestion processes [140].

The demand for fertilisers is constantly increasing. Phosphorus, a non-renewable resource, is currently extracted from geological deposits of phosphate rocks or phosphorites [141], whilst nitrogen, a highly stable gas present in atmosphere, is obtained by costly chemical reactions [142]. Hence, anaerobic digestion of high-strength wastewaters combined with nitrogen and phosphorus recovery processes is a feasible alternative.

Phosphorus can be recovered by sedimentation, enhanced biological phosphorus removal (*i.e.*, by phosphorus-accumulating organisms) or chemical precipitation (*i.e.*, with aluminium or iron salts into insoluble phosphates compounds) [143]. Nitrogen recovery uses energy from ammonia producing atmospheric nitrogen, followed by the Haber-Bosch process reversing the previous reaction. Other technologies for nitrogen recovery are struvite precipitation, adsorption, ammonia stripping, the combination of air stripping and absorption, membrane distillation and membrane gas separation [144]. Struvite ($NH_4MgPO_4{\cdot}6H_2O$) production is a well-established process to recover phosphorus and nitrogen by crystallisation [141]. The low water solubility of whey and its high N and P concentration is an advantage for struvite precipitation [140]. Most struvite recovery studies focus on municipal waste water [145] or source separated-urine [146]. However, struvite precipitation has its drawbacks. Phosphorus removal increases the amount of sludge and decreases digesters pipelines diameters leading to operational problems. In addition, its recovery reduces the overall costs of anaerobic digestion processes as well as the costs of sludge handling, disposal and scaling [143]. Phosphorus can also be recovered as vivianite ($Fe_3(PO_4){\cdot}8H_2O$) which is more thermodynamically favoured than struvite precipitation. Although the reaction is more thermodynamically favoured than struvite precipitation and vivianite high aggregated-value, it does not separate easily from sludge. Current technologies for vivianite recovery are chemical precipitation and magnetic separation due to its paramagnetism [147].

5 Outlook: A roadmap for the full valorisation of whey and mitigation of environmental impacts

Cheese production and whey management are interdependent. The cheese demand increases yearly. Therefore, whey management must be addressed. Technological advances enabled whey down-streaming, making an inexpensive

Citation: Giulianetti de Almeida *et al.*, *Got Whey*? The significance of cheese whey at the confluence of dairying, environmental impacts, energy and resource biorecovery, The Evolving Scholar, Preprint (2021)
[1]University of Campinas, Brazil and [2]Delft University of Technology, The Netherlands

dairy by-product into a sought commodity. However, this is not valid all over the world.

About 42% of whey annual production is still regarded as a low-value product. We proposed the production of short and mid-chain VFAs from cheese whey coupled anaerobic processes for microalgal biomass production. This alternative accounts for the acidification trend of cheese whey in anaerobic digestion processes and the feasibility of photoorganoheterotrophic microalgal growth.

Cheese whey and its derivatives are currently studied for biogas and bioethanol production. Microalgae cultivation using sole whey as a substrate can form an attractive alternative for environmental resource biorecovery, besides mitigating eutrophication into bioprocess boundaries. Cheese whey can be valorised by acidogenic fermentation and production of microalgae biomass in anaerobic coupled processes. Cheese whey coupled anaerobic and photo bioprocesses can eventually lead to a whey biorefinery in the following decade. Controlling metabolic routes to produce specific interest products, understanding the symbiotic relationship between microalgae and bacteria, and achieving the best C/N ratio for co-digestion are some of the knowledge gaps to be filled. Surplus whey will be the substrate for anaerobic digestion processes for either the production of VFAs or biogas combined with phosphorus and nitrogen recovery. The VFAs produced would serve as carbon sources in photoorganoheterotrophic processes for microalgae biomass production which would be further processed into biofuels, high value-added products. Biogas production could generate heat and electricity and biofuels. Both processes allow struvite precipitation recovering phosphorus and nitrogen that together with anaerobic digestion and microalgae biomass digestate can be turned into fertilisers. **Figure 2** illustrates these scenarios.

Mitigation and valorisation tracks for cheese whey processing can only become effective solutions when stakeholders are identified and an action plan is carefully crafted. It can help building dialogue for knowledge transfer and utilization, solution design, and informed decisions. Hence, regulations and policies can be enforced in a way that benefit especially small-producers.

Scalable, implementable and user-friendly technologies should be made available where it is most needed, the remote regions and communities. This is valid independently of the development level of countries. Enforcing knowledge development, regulation and technology for remote locations is a widespread issue across low, middle and high-income countries. Consequently, governments must implement incentive programmes encour-aging compliance, giving subsidies for whey repurposing and fomenting partnerships with industries or other countries that have the means and know-how to help this transition.

It is certain that achieving the full valorisation of cheese whey is not an easy task. Raising awareness about this issue is paramount to showcase the economic potential of transforming whey surplus into value-added products. The action plan can become a reality within a couple of years in low and middle-income countries and even in less time in high income ones. Each phase of the plan can then be implemented according to its degree of difficulty and financing.

Here, we addressed the importance of cheese whey from its discovery to current days at the confluence of dairying, environmental impacts, energy and resource biorecovery. We pinned issues that hinders whey full valorisation and alternatives to promote it. Information access, identification of stakeholders, setting an action plan that envisions minimising countries technology availability and affordability gaps, as well as promoting legislation implementation and governance to valorise cheese whey and safeguard the environment world-wide.

Acknowledgements

This work was funded by CAPES PDS scholarship (CAPES PDS 88882.435082/2019-01) and CNPq (CNPq 166460/2017-6). Maria Paula Giulianetti de Almeida obtained additional support comes from CAPES PSDE scholarship (CAPES PSDE 88881.190603/2018-01) for an international sandwich PhD period at TU Delft. David Weissbrodt was funded by the start-up grant of the TU Delft Department of Biotechnology.

Author contributions

M.P.G.d A. conceptualized the critical review and wrote the manuscript with direct core inputs by D.G.W and G.M. The roadmap was designed by M.P.G.d A., D.G.W. and G.M. by confronting ideas, concepts and solutions to technological, economical, regulatory, societal, and educational outcomes. All authors read, edited, and provided critical feedback to the manuscript.

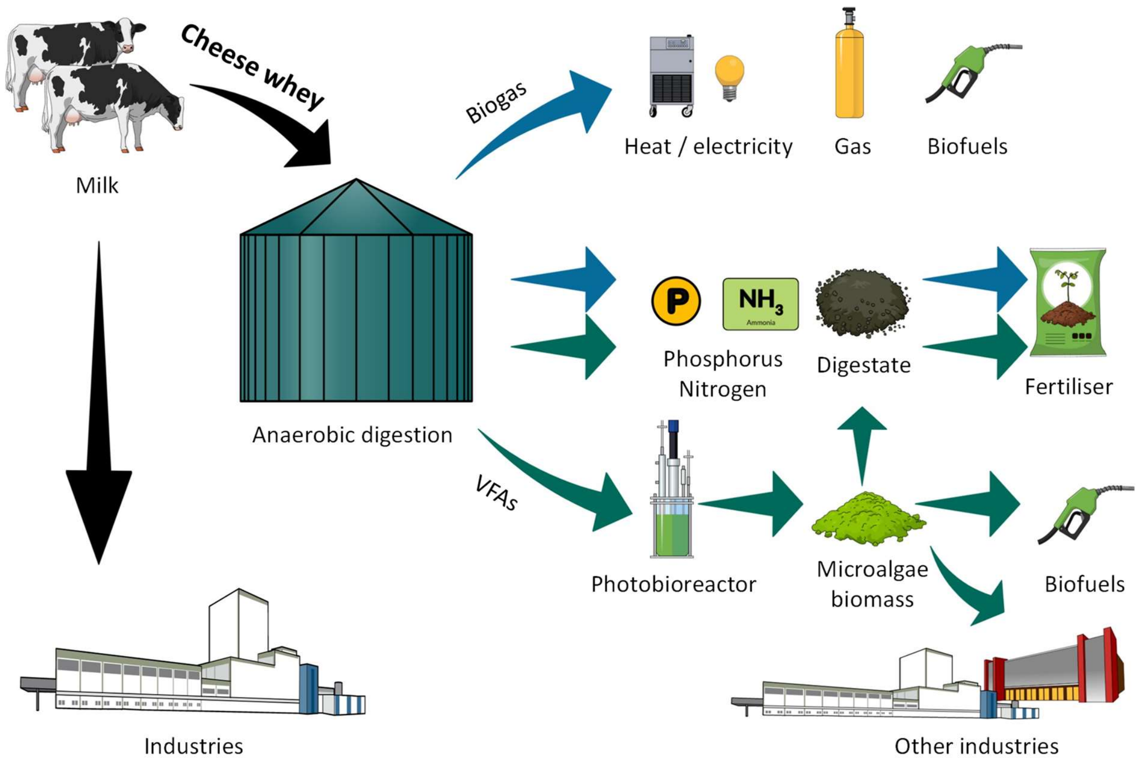


Figure 2. *Scenarii for the full valorisation of cheese whey. The surplus of whey can undergo to (i) biogas production generating heat, electricity, gas and biofuels, (ii) VFAs production that would serve as a carbon source for photoorganoheterotrophic processes. Microalgae biomass could serve as raw material for the production of biofuels or be absorbed by industry for the production of high added-value products. Struvite precipitation is possible regardless of chosen pathway. Anaerobic digestion and microalgal biomass harvesting digestates together with phosphorus and nitrogen recovery can be turned into fertilisers.*

Competing interests statement

The authors share no conflict of interest.

References

1. Božanić, R., Barukčić, I., Kl, J. & Tratnik, L. Possibilities of Whey Utilisation. *Austin J Nutr. Food Sci* **2**, 1036–1042 (2014).
2. Smithers, G. W. Whey and whey proteins-From 'gutter-to-gold'. *Int. Dairy J.* **18**, 695–704 (2008).
3. Tsakali, E., Petrotos, K., D'Allessandro, A. & Goulas, P. A review on whey composition and the methods used for its utilization for food and pharmaceutical products. in (ed. Centre, C. R.) (Conference: 6th International Conference on Simulation and Modelling in the Food and Bio-Industry FOODSIM, 2010).
4. Early, R. *Technology of dairy products*. (Springer US, 1997).
5. Hammam, A., Tammam, A., Elderwy, Y. & Hassan, A. Functional Peptides in Milk Whey: An Overview. *Assiut J. Agric. Sci.* **48**, 77–91 (2017).
6. Archer, R. H. *Whey Products*. https://nzic.org.nz/app/uploads/2017/10/3G.pdf (1998).
7. USDA Foreign Agricultural Services. *Dairy: World markets and trade*. https://www.fas.usda.gov/data/dairy-world-markets-and-trade (2019).
8. Food and Agriculture Organization of the United Nations. FAOSTAT - Livestock processed. http://www.fao.org/faostat/en/?#data/QP.
9. Kapoor, R. & Metzger, L. E. Evaluation of salt whey as an ingredient in processed cheese. *J. Dairy Sci.* **87**, 1143–1150 (2004).
10. El-Shewy, A. A. Whey as a Feed Ingredient for Lactating Cattle. *Sci. Int.* **4**, 80–85 (2016).
11. Ketterings, Q., Czymmek, K., Gami, S., Godwin, G. & Ganoe, K. *Guidelines for Land Application of Acid Whey. Department of Animal Science Publication Series*

http://nmsp.cals.cornell.edu/publications/files/AcidWheyGuidelines2017.pdf (2017).
12. Leonardi, M., Gerbault, P., Thomas, M. G. & Burger, J. The evolution of lactase persistence in Europe. A synthesis of archaeological and genetic evidence. *Int. Dairy J.* **22**, 88–97 (2012).
13. Dunne, J. *et al.* First dairying in green Saharan Africa in the fifth millennium bc. *Nature* **486**, 390–394 (2012).
14. Callaway, E. Pottery shards put a date on Africa's dairying. *Nature* (2012) doi:10.1038/nature.2012.10863.
15. Evershed, R. P. *et al.* Earliest date for milk use in the Near East and southeastern Europe linked to cattle herding. *Nature* **455**, 528–531 (2008).
16. Tunick, M. H. Whey Protein Production and Utilization: A Brief History. in *Whey Processing, Functionality and Health Benefits* (ed. Charles I. Onwulata Ph.D.; Peter J. Huth) 400 (Wiley-Blackwell, 2008). doi:10.1002/9780813803845.
17. Ramos, O. L. *et al.* Whey and Whey Powders: Production and Uses. *Encyclopedia of Food and Health* 498–505 (2016) doi:10.1016/B978-0-12-384947-2.00747-9.
18. Pien, J. Utilisation des Sérum de Fromagerie et des Lacto-Protéines dans l'Alimentation. *Lait* **227**, 228 (1943).
19. Ayto, J. *The Diner's Dictionary: Word Origins of Food and Drink*. (Oxford University Press, 2012).
20. Kosseva, M. R. & Webb, C. *Food Industry Wastes Assessment and Recuperation of Commodities*. (Academic Press, 2013). doi:10.1016/C2011-0-00035-2.
21. Montecchio, D., Yuan, Y. & Malpei, F. Hydrogen production dynamic during cheese whey Dark Fermentation : New insights from modelization. *Int. J. Hydrogen Energy* **43**, 17588–17601 (2018).
22. Smithers, G. W. Whey-ing up the options – Yesterday, today and tomorrow. *Int. Dairy J.* **v. 48**, 2-14–2015 v.48 (2015).
23. Saddoud, A., Hassaïri, I. & Sayadi, S. Anaerobic membrane reactor with phase separation for the treatment of cheese whey. *Bioresour. Technol.* **98**, 2102–2108 (2007).
24. Marwaha, S. S. & Kennedy, J. F. Whey—pollution problem and potential utilization. *Int. J. Food Sci. Technol.* **23**, 323–336 (1988).
25. Prazeres, A. R., Carvalho, F. & Rivas, J. Cheese whey management: A review. *Journal of Environmental Management* vol. 110 48–68 (2012).
26. Belloin, J. C. *Milk and dairy products : production and processing costs*. (Food and Agriculture Organization of the United Nations, 1988).
27. Hughes, P., Risner, D. & Meunier-Goddik, L. Whey to Vodka. in *Whey - Biological Properties and Alternative Uses* (IntechOpen, 2018). doi:10.5772/intechopen.81679.
28. Opio, C. *et al. Greenhouse gas emmission from ruminant supply chains - A global life cycle assessment*. (2013).
29. Lustrato, G. *et al.* Cheese whey recycling in traditional dairy food chain: effects of vinegar from whey in dairy cow nutrition. *Acetic Acid Bact.* **2**, 8 (2013).
30. Miracco, J. L., Alzamora, S. M., Chirife, J. & Ferro Fontan, C. *On the Water Activity of Lactose Solutions*.
31. Robbins, C. W. & Lehrsch, G. A. Cheese Whey as a Soil Conditioner. in *Handbook of Soil Conditioners* (eds. Wallace, A. & Terry, R. E.) (Marcel Dekker Inc., 1998).
32. Ellen MacArthur Foundation. *Delivering the Circular Economy - A Toolkit for Policymakers*. https://www.ellenmacarthurfoundation.org/assets/downloads/publications/EllenMacArthurFoundation_PolicymakerToolkit.pdf (2015).
33. Neil Gunningham. Environment Law, Regulation and Governance: Shifting Architectures. *J. Environ. Law* **21**, 179–212 (2009).
34. UNEP. *Environmental Rule of Law: First Global Report*. https://www.unenvironment.org/resources/assessment/environmental-rule-law-first-global-report (2019).
35. Spence, D. B. The Shadow of the Rational Polluter: Rethinking the Role of Rational Actor Models in Environmental Law Recommended Citation. (2001) doi:10.15779/Z388X38.
36. Alves, M. P., Moreira, R. de O., Júnior, P. H. R., Martins, M. C. de F. & Ítalo Tuler Perrone, A. F. de C. Whey: technologies for co-products production. *Rev. Inst. Laticínios Cândido Tostes* **69**, 212–226 (2014).
37. Trindade, M. B. *et al.* Cheese whey exploitation in Brazil: A questionnaire survey. *Food Sci. Technol.* **39**, 788–791 (2019).
38. VALORLACT. *Comprehensive Use of the Whey Generated by the Dairy Industry in the Basque Country VALORLACT*. http://valorlact.eu/ (2016).
39. The Law Library of Congress & Rodriguez-Ferrand, G. National constitution ART 121 civil code. *Legislation on Use of Water in Agriculture: Argentina* (2013).
40. Government of South Australia. South Australia legislation. https://www.legislation.sa.gov.au/index.aspx.
41. CIS Legislation. Water Code of the Republic of Belarus of April 30, 2014 No. 149-Z. https://cis-legislation.com/document.fwx?rgn=68800 (2014).
42. Ministry of the Environment. Brazilian legislation platform. https://www.gov.br/mma/pt-br.
43. Government of Canada. Justice laws website. https://laws-lois.justice.gc.ca/eng/acts/.
44. Zhifang, X. China's water law and environment. *Can. Water Resour. J.* **16**, 275–282 (1991).
45. European Union. EU law. https://europa.eu/european-union/law_en.
46. Japan - Ministry of The Environment. Water Pollution Control Law Law No. 138 of 197. http://www.env.go.jp/en/laws/water/wlaw/index.html (1970).
47. Secretary of Environment Natural Resources and Fisheries. *Mexican Official Standard NOM-001-ECOL-1996*. (United States of Mexico).
48. New Zealand - Ministry for the Environment. The National Policy Statement for Freshwater Management (Freshwater NPS) Provides Direction on How Local Authorities Should Carry Out Their Responsibilities Under the Resource Management Act 1991 for Managing Fresh Water. http://www.mfe.govt.nz/fresh-water/regulations/national-policy-statement-freshwater-management (2014).
49. CIS Legislation. Water Code of the Russian Federation of June 3, 2006 No. 74-FZ. https://cis-legislation.com/document.fwx?rgn=13410 (2006).
50. Republic of Korea - Ministry of Environment. *Water Quality and Ecosystem Conservation Act*. (Korea Legislation Research Institute, 2005).
51. Legal Services Online. The Water Code of Ukraine. 06–44 http://yurist-online.com/en/kodeks/009.php.
52. United States of America. Clean Water Act. *Senate and House of Representatives of the United States of America in Congress* (1972).

53. Raphelson, S. Nobody Is moving our cheese: American surplus reaches Record high. *WJCT* https://www.npr.org/2019/01/09/683339929/nobody-is-moving-our-cheese-american-surplus-reaches-record-high (2019).
54. USDA - Agricultural Marketing Service. Pre-solicitation announcement for trade mitigation purchase of dairy products. (2019).
55. Kemp-Benedict, E. 3 key steps to a more sustainable economy. *World Economic Forum - Future of Economic Progress Sustainable Development* https://www.weforum.org/agenda/2015/09/3-key-steps-to-a-more-sustainable-economy/ (2015).
56. Kralisch, A. & Mandl, T. Barriers to Information Access across Languages on the Internet: Network and Language Effects. in *Proceedings of the 39th Hawaii International Conference on System Sciences* (IEEE, 2006). doi:10.1109/HICSS.2006.71.
57. Fell, E. V & Lukianova, N. A. British Universities : International Students ' Alleged Lack of Critical Thinking. *Procedia - Soc. Behav. Sci.* **215**, 2–8 (2015).
58. Meneghini, R. & Packer, A. L. Is there science beyond English? *EMBO Rep.* **8**, 2–6 (2007).
59. Landa, L. G. G. Academic Language Barriers and solutions. *Curr. Issues Lang. Plan.* **7**, 61–81 (2006).
60. Ugah, A. D. Obstacles to Information Access and Use in Developing Countries. *Libr. Philos. Pract.* 6 (2007).
61. Hussain, A. Search Engines as an Effective Tool for Library Professionals. *J. Libr. Inf. Technol.* **35**, 389–397 (2015).
62. Lawrence, S. & Lee Giles, C. Accessibility of information on the web. *Nature* **400**, 107–109 (1999).
63. Di Bitetti, M. S. & Ferreras, J. A. Publish (in English) or perish : The effect on citation rate of using languages other than English in scientific publications. *Ambio* **46**, 121–127 (2017).
64. Soria, K. M. Factors Predicting the Importance of Libraries and Research Activities for Undergraduates. *J. Acad. Librariansh.* **39**, 464–470 (2013).
65. Research Information Network. *Overcoming barriers: access to research information content*. www.rin.ac.uk (2009).
66. Onwulata, C. & Huth, P. J. *Whey processing, functionality and health benefits*. (Wiley-Blackwell, 2008).
67. Tsitouras, A., Basu, O., Al-ghussain, N. & Delatolla, R. Kinetic effects of anaerobic staging and aeration rates on sequencing batch moving bed biofilm reactors: carbon, nitrogen, and phosphorus treatment of cheese production wastewater. *Chemosphere* **252**, 126407 (2020).
68. Marazzi, F., Bellucci, M., Fantasia, T., Ficara, E. & Mezzanotte, V. Interactions between microalgae and bacteria in the treatment of wastewater from milk whey processing. *Water (Switzerland)* **12**, 1–13 (2020).
69. Imtiaz-Ul-IslamHong, M., Hong, L. & Langrish, T. CO2 capture using whey protein isolate. *Chem. Eng. J.* **171**, 1069–1081 (2011).
70. Singh, R. S. Biotechnological Approaches for Valorization of Whey. in *Advances in Industrial Biotechnology* (eds. Singh, R. S., Pandey, A. & Larroche, C.) 443–478 (IK International Publishing House Pvt. Ltd, 2016).
71. Stamatelatou, K., Antonopoulou, G., Tremouli, A. & Lyberatos, G. Production of gaseous biofuels and electricity from cheese whey. *Ind. Eng. Chem. Res.* **50**, 639–644 (2011).
72. Gillies, M. T. *Whey processing and utilization : economic and technical aspects*. (Noyes Data Corp, 1974).
73. Onwulata, C. & Huth, P. J. *Whey processing, functionality and health benefits*. (Wiley-Blackwell, 2008).
74. Park, Y. W. *Bioactive components in milk and dairy products*. (Wiley-Blackwell, 2009).
75. Antonopoulou, G., Stamatelatou, K., Venetsaneas, N., Kornaros, M. & Lyberatos, G. Biohydrogen and methane production from cheese whey in a two-stage anaerobic process. *Ind. Eng. Chem. Res.* **47**, 5227–5233 (2008).
76. Malaspina, F., Cellamare, C. M., Stante, L. & Tilche, A. Anaerobic Treatment of Cheese Whey with a Downflow-Upflow Hybrid Reactor. *Bioresour. Technol.* **55**, 131–139 (1996).
77. Gelegenis, J., Georgakakis, D., Angelidaki, I. & Mavris, V. Optimization of biogas production by co-digesting whey with diluted poultry manure. *Renew. Energy* **32**, 2147–2160 (2007).
78. González Siso, M. I. The biotechnological utilization of cheese whey: A review. *Bioresource Technology* vol. 57 1–11 (1996).
79. Patel, P., Desai, M. & Madamwar, D. Biomethanation of cheese whey using anaerobic upflow fixed film reactor. *J. Ferment. Bioeng.* **79**, 398–399 (1995).
80. Cadavid-Rodríguez, L. S. & Horan, N. J. Production of volatile fatty acids from wastewater screenings using a leach-bed reactor. *Water Res.* **60**, 242–249 (2014).
81. De Gioannis, G. *et al.* Biohydrogen production from dark fermentation of cheese whey: Influence of pH. *Int. J. Hydrogen Energy* **39**, 20930–20941 (2014).
82. Girotto, F., Lavagnolo, M. C., Pivato, A. & Cossu, R. Acidogenic fermentation of the organic fraction of municipal solid waste and cheese whey for bio-plastic precursors recovery – Effects of process conditions during batch tests. *Waste Manag.* **70**, 71–80 (2017).
83. Sultana, M. *et al.* Effect of hydraulic retention time , temperature , and organic load on a horizontal subsurface flow constructed wetland treating cheese whey wastewater. *J. Chem. T echnol. Biotechnol* (2015) doi:10.1002/jctb.4637.
84. Venetsaneas, N., Antonopoulou, G., Stamatelatou, K., Kornaros, M. & Lyberatos, G. Using cheese whey for hydrogen and methane generation in a two-stage continuous process with alternative pH controlling approaches. *Bioresour. Technol.* **100**, 3713–3717 (2009).
85. Roukas, T. & Kotzekidou, P. Lactic acid production from deproteinized whey by mixed cultures of free and coimmobilized Lmtobacillus casei and Lmtococcus Zactis cells using fedbatch culture. *Enzyme Microb. Technol.* **22**, 199–204 (1998).
86. Guo, X. M., Trably, E., Latrille, E., Carrère, H. & Steyer, J.-P. Hydrogen production from agricultural waste by dark fermentation: A review. *Int. J. Hydrogen Energy* **35**, 10660–10673 (2010).
87. Turkmenoglu, S. Organic Acids Production from Cheese Whey. (Middle East Technical University, 2006).
88. Calero, R., Lagoa-Costa, B. C., Kennes, C., Fernandez-Feal, M. C. & Veiga, M. C. Volatile fatty acids production from cheese whey: influence of pH, solid retention time and organic load rate. *J. Chem. Technol. Biotechnol.* **93**, (2018).
89. Nath, A., Dixit, M., Bandiya, A., Chavda, S. & Desai, A. J. Enhanced PHB production and scale up studies using cheese whey in fed batch culture of Methylobacterium sp. ZP24. *Bioresour. Technol.* **99**,

5749–5755 (2008).
90. Colombo, B., Sciarria, T. P., Reis, M., Scaglia, B. & Adani, F. Bioresource Technology Polyhydroxyalkanoates (PHAs) production from fermented cheese whey by using a mixed microbial culture. *Bioresour. Technol.* **218**, 692–699 (2019).
91. Ryan, M. P. & Walsh, G. The biotechnological potential of whey. *Rev. Environ. Sci. Biotechnol.* **15**, 479–498 (2016).
92. Davila-Vazquez, G., Alatriste-Mondragón, F., de León-Rodríguez, A. & Razo-Flores, E. Fermentative hydrogen production in batch experiments using lactose, cheese whey and glucose: Influence of initial substrate concentration and pH. *Int. J. Hydrogen Energy* **33**, 4989–4997 (2008).
93. Christensen, A. D., Kádár, Z., Oleskowicz-Popiel, P. & Thomsen, M. H. Production of bioethanol from organic whey using Kluyveromyces marxianus. *J. Ind. Microbiol. Biotechnol.* **38**, 283–289 (2011).
94. Raganati, F. *et al.* Butanol production by bioconversion of cheese whey in a continuous packed bed reactor. *Bioresour. Technol.* **138**, 259–265 (2013).
95. Antonopoulou, G., Stamatelatou, K., Bebelis, S. & Lyberatos, G. Electricity generation from cheese whey using a microbial fuel cell. in *CHISA 2008 - 18th International Congress of Chemical and Process Engineering* (ed. Engineering, C. S. of C.) (2008).
96. Rabaey, K. & Rozendal, R. A. Microbial electrosynthesis — revisiting the electrical route for microbial production. *Nat. Rev. Microbiol.* **8**, 706–716 (2010).
97. Hagos, K., Zong, J., Li, D., Liu, C. & Lu, X. Anaerobic co-digestion process for biogas production : Progress , challenges and perspectives. *Renew. Sustain. Energy Rev.* 1485–1496 (2016) doi:10.1016/j.rser.2016.11.184.
98. Nghiem, L. D., Koch, K., Bolzonella, D. & Drewes, J. E. Full scale co-digestion of wastewater sludge and food waste: Bottlenecks and possibilities. *Renew. Sustain. Energy Rev.* **72**, 354–362 (2017).
99. Castro-Molano, L. del P., Escalante-Hernández, H., Lambis-Benítez, L. E. & Marín-Batista, J. D. Synergistic effects in anaerobic codigestion of chicken manure with industrial wastes. *DYNA* **85**, 135–141 (2018).
100. Gomez-Romero, J., Gonzalez-Garcia, A., Chairez, I., Torres, L. & García-Peña, E. I. Selective adaptation of an anaerobic microbial community: Biohydrogen production by co-digestion of cheese whey and vegetables fruit waste. *Int. J. Hydrogen Energy* **39**, 12541–12550 (2014).
101. Maragkaki, A. E. *et al.* Improving biogas production from anaerobic co-digestion of sewage sludge with a thermal dried mixture of food waste, cheese whey and olive mill wastewater. *Waste Manag.* **71**, 644–651 (2018).
102. Markou, G. & Georgakakis, D. Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review. *Appl. Energy* **88**, 3389–3401 (2011).
103. Clearfleau. Cost Effective Treatment of Dairy Processing Residues. https://clearfleau.com/cost-effective-treatment-of-dairy-processing-residues/.
104. Yu, J. & Takahashi, P. Biophotolysis-based Hydrogen Production by Cyanobacteria and Green Microalgae. in *Communicating Current Research and Educational Topics and Trends in Applied Microbiology* (ed. Méndez-Vilas, A.) 79–89 (2015).
105. Chen, J. *et al.* Photosynthetic bacteria-based technology is a potential alternative to meet sustainable wastewater treatment requirement ? *Environ. Int.* **137**, 1–19 (2020).
106. Warthmann, R., Cypionka, H. & Pfennig, N. Photoproduction of H2 from acetate by syntrophic cocultures of green sulfur bacteria and sulfur-reducing bacteria. *Arch. Microbiol.* **157**, 343–348 (1992).
107. Najafpour, G. D., Shahavi, M. H. & Neshat, S. A. Assessment of biological Hydrogen production processes: A review. in *IOP Conference Series: Earth and Environmental Science* vol. 36 (2016).
108. Alfano, M. & Cavazza, C. The biologically mediated water-gas shift reaction: Structure, function and biosynthesis of monofunctional [NiFe]-carbon monoxide dehydrogenases. *Sustain. Energy Fuels* **2**, 1653–1670 (2018).
109. Pandey, A., Srivastava, S., Rai, P. & Duke, M. Cheese whey to biohydrogen and useful organic acids: A non-pathogenic microbial treatment by L. acidophilus. *Nat. Sci. Reports* **9**, 1–9 (2019).
110. Zeng, X., Danquah, M. K., Chen, X. D. & Lu, Y. Microalgae bioengineering: From CO2 fixation to biofuel production. *Renewable and Sustainable Energy Reviews* vol. 15 3252–3260 (2011).
111. Wang, Y. *et al.* Perspectives on the feasibility of using microalgae for industrial wastewater treatment. *Bioresour. Technol.* **222**, 485–497 (2016).
112. Aslan, S. & Kapdan, I. K. Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecol. Eng.* 64–70 (2006) doi:10.1016/j.ecoleng.2006.04.003.
113. Jeong, M. Carbon Dioxide Mitigation by Microalgal Photosynthesis. *Bull. Korean Chem. Soc.* **24**, 1763–1766 (2003).
114. Kim, S., Park, J., Cho, Y. & Hwang, S. Growth rate , organic carbon and nutrient removal rates of Chlorella sorokiniana in autotrophic , heterotrophic and mixotrophic conditions. *Bioresour. Technol.* **144**, 8–13 (2013).
115. Crane, K. W. & Grover, J. P. Coexistence of mixotrophs , autotrophs , and heterotrophs in planktonic microbial communities. *J. Theor. Biol.* **262**, 517–527 (2010).
116. Pittman, J. K., Dean, A. P. & Osundeko, O. The potential of sustainable algal biofuel production using wastewater resources. *Bioresour. Technol.* **102**, 17–25 (2011).
117. Cheirsilp, B. & Torpee, S. Enhanced growth and lipid production of microalgae under mixotrophic culture condition: Effect of light intensity, glucose concentration and fed-batch cultivation. *Bioresour. Technol.* **110**, 510–516 (2012).
118. Cheirsilp, B. & Torpee, S. Enhanced growth and lipid production of microalgae under mixotrophic culture condition : Effect of light intensity , glucose concentration and fed-batch cultivation. *Bioresour. Technol.* **110**, 510–516 (2012).
119. Perez-Garcia, O., Escalante, F. M. E., de-Bashan, L. E. & Bashan, Y. Heterotrophic cultures of microalgae: Metabolism and potential products. *Water Research* vol. 45 11–36 (2011).
120. Yang, C., Hua, Q. & Shimizu, K. Energetics and carbon metabolism during growth of microalgal cells under photoautotrophic, mixotrophic and cyclic light-autotrophic / dark-heterotrophic conditions. *Biochem. Eng. J.* **6**, 87–102 (2000).
121. Tsolcha, O. N. *et al.* Treatment of second cheese whey effluents using a Choricystis-based system with simultaneous lipid production. *J. Chem. Technol. Biotechnol.* **91**, 2349–2359 (2016).

Citation: Giulianetti de Almeida *et al.*, *Got Whey*? The significance of cheese whey at the confluence of dairying, environmental impacts, energy and resource biorecovery, The Evolving Scholar, Preprint (2021)
[1]University of Campinas, Brazil and [2]Delft University of Technology, The Netherlands

122. Rizwan, M., Mujtaba, G., Memon, S. A., Lee, K. & Rashid, N. Exploring the potential of microalgae for new biotechnology applications and beyond: A review. *Renewable and Sustainable Energy Reviews* vol. 92 394–404 (2018).
123. Caporgno, M. P. & Mathys, A. Trends in Microalgae Incorporation Into Innovative Food Products With Potential Health Benefits. *Front. Nutr.* **5**, 58 (2018).
124. Khan, M. I., Shin, J. H. & Kim, J. D. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microb. Cell Fact.* **17**, 36 (2018).
125. Spolaore, P., Joannis-Cassan, C., Duran, E. & Isambert, A. Commercial applications of microalgae. *J. Biosci. Bioeng.* **101**, 87–96 (2006).
126. Abo, B. O., Odey, E. A., Bakayoko, M. & Kalakodio, L. Microalgae to biofuels production: A review on cultivation, application and renewable energy. *Rev. Environ. Health* **34**, 91–99 (2019).
127. Riaño, B., Blanco, S., Becares, E. & García-González, M. C. Bioremediation and biomass harvesting of anaerobic digested cheese whey in microalgal-based systems for lipid production. *Ecol. Eng.* **97**, 40–45 (2016).
128. Gonzalez-Fernandez, C., Sialve, B. & Molinuevo-Salces, B. Anaerobic digestion of microalgal biomass: Challenges, opportunities and research needs. *Bioresour. Technol.* **198**, 896–906 (2015).
129. Schnurr, P. J., Espie, G. S. & Allen, D. G. Algae biofilm growth and the potential to stimulate lipid accumulation through nutrient starvation Bioresource Technology Algae biofilm growth and the potential to stimulate lipid accumulation through nutrient starvation. *Bioresour. Technol.* **136**, 337–344 (2013).
130. Ramanan, R., Kim, B. H., Cho, D. H., Oh, H. M. & Kim, H. S. Algae-bacteria interactions: Evolution, ecology and emerging applications. *Biotechnology Advances* vol. 34 14–29 (2016).
131. Dao, G.-H. *et al.* Enhanced microalgae growth through stimulated secretion of indole acetic acid by symbiotic bacteria. *Algal Res.* **33**, 345–351 (2018).
132. Kouzuma, A. & Watanabe, K. Exploring the potential of algae/bacteria interactions. *Curr. Opin. Biotechnol.* **33**, 125–129 (2015).
133. Kim, J., Jung, H. & Lee, C. Shifts in bacterial and archaeal community structures during the batch biomethanation of Ulva biomass under mesophilic conditions. *Bioresour. Technol.* **169**, 502–509 (2014).
134. Zhang, B. *et al.* Microalgal-bacterial consortia : From interspecies interactions to biotechnological applications. *Renew. Sustain. Energy Rev.* **118**, 109563 (2020).
135. Posadas, E. *et al.* Microalgae-based agro-industrial wastewater treatment : a preliminary screening of biodegradability. *J. Appl. Phycol.* **26**, 2335–2345 (2014).
136. Lee, W. S., Chua, A. S. M., Yeoh, H. K. & Ngoh, G. C. A review of the production and applications of waste-derived volatile fatty acids. *Chem. Eng. J.* **235**, 83–99 (2014).
137. Jenkins, D. & Wanner, J. *Activated sludge - 100 years and counting*. (IWA Publishing, 2014).
138. Nicolella, C., van Loosdrecht, M. C. M. & Heijnen, J. J. Wastewater treatment with particulate biofilm reactors. *J. Biotechnol.* **80**, 1–33 (2000).
139. Vaneeckhaute, C., Lebuf, V., Michels, E., Belia, E. & Vanrolleghem, P. A. Nutrient recovery from digestate : Systematic technology review and product classification. *Waste and Biomass Valorization* **8**, 21–40 (2017).
140. Escalante, H., Castro, L., Amaya, M. P., Jaimes, L. & Jaimes-Estévez, J. Anaerobic digestion of cheese whey: Energetic and nutritional potential for the dairy sector in developing countries. *Waste Manag.* **71**, 711–718 (2018).
141. Desmidt, E. *et al.* Global phosphorus scarcity and full-scale P-recovery techniques: A review. *Crit. Rev. Environ. Sci. Technol.* **45**, 336–384 (2015).
142. van der Hoek, J. P., Duijff, R. & Reinstra, O. Nitrogen Recovery from Wastewater: Possibilities, Competition with Other Resources, and Adaptation Pathways. *Sustainability* **10**, 18 (2018).
143. Parsons, S. A. & Smith, J. A. Phosphorus removal and recovery from municipal wastewaters. *Elements* **4**, 109–112 (2008).
144. Beckinghausen, A., Odlare, M., Thorin, E. & Schwede, S. From removal to recovery : An evaluation of nitrogen recovery techniques from wastewater. *Appl. Energy* **263**, 114616 (2020).
145. Le Corre, K. S., Valsami-Jones, E., Hobbs, P. & Parsons, S. A. Phosphorus Recovery from Waste by Struvite Crystallisation: A Review. *Crit. Rev. Environ. Sci. Technol.* **39**, 433–477 (2009).
146. Wilsenach, J. A. Ã., Schuurbiers, C. A. H. & Loosdrecht, M. C. M. Van. Phosphate and potassium recovery from source separated urine through struvite precipitation. *Water Res.* **41**, 458–466 (2007).
147. Wu, Y. *et al.* Potentials and challenges of phosphorus recovery as vivianite from wastewater: A review. *Chemosphere* **226**, 246–258 (2019).

Citation: Giulianetti de Almeida *et al.*, *Got Whey*? The significance of cheese whey at the confluence of dairying, environmental impacts, energy and resource biorecovery, The Evolving Scholar, Preprint (2021)
[1]University of Campinas, Brazil and [2]Delft University of Technology, The Netherlands