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Research review paper

Green Biorefinery systems for the production of climate-smart sustainable products from grasses, legumes and green crop residues

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ABSTRACT

Grasses, legumes and green plant wastes represent a ubiquitous feedstock for developing a bioeconomy in regions across Europe. These feedstocks are often an important source of ruminant feed, although much remains unused or underutilised. In addition to proteins, these materials are rich in fibres, sugars, minerals and other components that could also be used as inputs for bio-based product development. Green Biorefinery processes and initiatives are being developed to better capitalise on the potential of these feedstocks to produce sustainable food, feed, materials and energy in an integrated way. Such systems may support a more sustainable primary production sector, enable the valorisation of green waste streams, and provide new business models for farmers. This review presents the current developments in Green Biorefining, focusing on a broad feedstock and product base to include different models of Green Biorefinery. It demonstrates the potential and wide applicability of Green Biorefinery systems, the range of bio-based product opportunities and highlights the way forward for their broader implementation. While the potential for new products is extensive, quality control approval will be required prior to market entry.

1. Introduction

The rapid expansion of the global population over the past century has been maintained, in part, by an increasingly efficient agriculture sector. Such practices have, however, damaged ecological systems worldwide, leading to increased greenhouse gas emissions, deforestation, loss of biodiversity and water pollution among other negative environmental impacts (Sanders et al., 2020). Living in the current era of climate and biodiversity emergencies, the major challenge of the 21st Century is ensuring that humanity can survive and thrive in a more sustainable way, which does not compromise the ecological boundaries of the planet (Steffen et al., 2015). The development of a sustainable bioeconomy, defined as an economy that relies on renewable natural resources to produce food, products, energy and services, is proposed as one strategy which can help the global population to meet many of these challenges and, in doing so, contribute to many of the United Nations 17 Sustainable Development Goals (Barrett et al., 2021; Cudlínová et al., 2017; Solarte-Toro and Alzate, 2021). This pathway may also offer the opportunity to decarbonize agriculture through waste reduction, production of renewable products and energy, and biogenic carbon storage (Awasthi et al., 2022; Bishop et al., 2021; Haveren et al., 2008; Sagues et al., 2020; Sanders et al., 2007). To support this transition, strategies

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and policies are being developed nationally, with over 50 countries worldwide already pursuing bioeconomy strategies (German Bioeconomy Council, 2018). In addition to broader bioeconomy strategies, other strategies impacting the bioeconomy, such as those focused on sustainable energy, novel protein and bio-based products are being developed (Clark and Lenaghan, 2020; Ebadian et al., 2020; OECD, 2013). The development of the bio-based products and value chains are also a key component of the European Union's Circular Economy Action Plan and the EU Green Deal which commits Europe to becoming a climate-neutral continent by 2050 (Thormann et al., 2021). Interest in the bioeconomy is also growing from societies' concern for meeting the increased demand for food and materials produced more sustainably, with an increasing number of well-informed consumers who are more inclined to purchase environmentally friendly products (Gaffey et al., 2021; Trigo, 2021).

Biorefineries are seen as key enabling technologies for the widespread implementation of the bioeconomy. A biorefinery is defined as the sustainable processing of biomass into a spectrum of marketable products and energy (Cherubini et al., 2009). Using this holistic approach, biomass can be converted using integrated processes into a variety of products, such as proteins, materials and energy, maximising the overall potential of the biogenic feedstocks. Furthermore, these systems allow for opportunities for valorisation of process by-products and waste streams (Pabbathi et al., 2022; Usmani et al., 2022). Several classification systems have already been proposed for categorising biorefineries (Cherubini et al., 2009; Lange, 2022). Green Biorefineries are a specific subset of biorefineries defined as multiproduct systems, that utilise green biomass, such as grasses, green crops and immature cereals, as a raw material for obtaining industrial products (Xiu and Shahbazi, 2015). Additional feedstocks can include legumes and green plant wastes (Kromus et al., 2005; Mandl, 2010). Part of the Green Biorefinery approach often involves extracting protein from the feedstock for animal or human consumption, with the residual pulp being used as cattle feed. In this case the driver is to develop additional animal feed from local protein sources, providing a homegrown alternative to imported soy which directly or indirectly causes deforestation (Damborg et al., 2019; Lange, 2022). However, further material or energy products may also be produced in a Green Biorefinery approach, including fibres for technical applications such as insulation or biocomposite production, bulk chemicals such as lactic and bioenergy typically in the form of biogas, many of which can potentially offer a renewable bio-based alternative to fossil derived materials and energy (Höltinger et al., 2014).

A number of previous Green Biorefinery review studies have been undertaken. An early review from Kromus et al. (2005) detailed the historic background, concepts and state-of-the-art for Green Biorefineries at that time. Mandl (2010) looked at the state-of-play of Green Biorefineries in Europe, exploring technologies, available grass-based feedstocks and alignment of products with the broader European economy. Sharma and Mandl (2014) conducted a review of Green Biorefineries including an itinerary of different processes and products, main players and future prospects. Xiu and Shahbazi (2015) conducted a review of Green Biorefineries in 2015, covering separation technologies and R&D activities, while also highlighting some of the key barriers to further development and commercialisation. In recent years there has been significant Green Biorefinery R&D activity, demonstration projects and commercial activity, which have implemented new processes and products and have also undertaken a deeper analysis of these products and systems. Given the fast-paced development of technologies and approaches, there is a knowledge gap when it comes to current status and trends for Green Biorefineries. Therefore, the current review provides an update on the recent developments of Green Biorefineries, feedstocks and products. The review expands beyond forage grasses and legumes as feedstocks, also exploring work undertaken on waste streams, such as sugar beet leaves, as feedstocks for Green Biorefineries. The outcomes of recent trial work on various products, including novel products, from Green Biorefineries of diversified feedstocks, is also included. The importance of establishing the sustainability of such systems is also recognised by the inclusion and analysis of recent life cycle assessments of Green Biorefinery systems.

2. Green Biorefinery feedstocks

When classifying feedstocks for biorefineries, Cherubini et al. (2009) distinguishes between dedicated feedstocks, such as grasses, sugar and starch crops and those which constitute residues, including crop residues and organic residues. Kromus et al. (2005) identifies "green biomass", mainly primary production resources, as the largest feedstock suitable for Green Biorefinery, including green plant materials such as grass and clover, with other potential secondary feedstocks including crop residues with green foliage and green juice-rich waste biomass. From a Green Biorefining perspective the interesting valuable components of fresh biomass are proteins, soluble sugars, and fibre fractions (i.e., cellulose, hemicellulose, and lignin), while other valuable ingredients may also be present. Some of these primary and secondary feedstocks are discussed within this section.

2.1. Primary Green Biorefinery feedstocks

Grass varieties in fresh or silage form, represent the most commonly used feedstocks for Green Biorefining (Pijlman et al., 2018; Ravindran et al., 2022; Ravindran et al., 2021). Diverse species of grass have been studied for the purposes of Green Biorefinery processes ranging from Perennial rye grass (*Lolium perenne*) and Italian rye grass (*Lolium multiflorum*) to Bermuda grass (*Cynodon dactylon*) and Elephant grass (*Pennisetum purpureum*) (French, 2019; Kromus et al., 2004; O'Keefe, 2010). Grass is a ubiquitous feedstock in regions around the world where it accounts for 70% of total land area (Taube et al., 2014). Grasslands can be classified into either (i) permanent grassland (PG) or (ii) temporary grasslands (TG). PG can be defined as grassland that has been untouched for the past five years or more without ploughing and has not been included in the crop rotation of the holding (De Vliegher and Carlier, 2007). Meanwhile, TG includes grassland that has not been ploughed for the past five years or less (Lesschen et al., 2014). Grass availability will vary depending on the use of the grassland. According to the figures obtained from Eurostat, the proportion of TG in EU is considerably less when compared to PG (Eurostat, 2018a, 2018b, 2018c). An exception to this statistic is the availability of TG in Finland, Denmark and Sweden where the ratio of TG to total grassland in these countries is 93%, 43% and 34% respectively (Fig. 1A). This would imply that grasslands in these countries are ploughed regularly and/or are part of crop rotation strategies reducing the availability of grass in the long term.

To help identify the EU countries that potentially have more grass accessibility in the long term for Green Biorefinery, an overview of Total Land Use (TLU), Utilised Agricultural Area (UAA) and grassland use across EU-28 for 2018 is presented in Table 1. From Table 1, Fig. 1B was created to represent, in percentage (%), the proportion of PG to TLU and UAA. It can be appreciated that, PG represented approximately 34% of the UAA and 14% of the TLU in the EU-28. Interestingly, Ireland, UK, Slovenia and Portugal, all had >50% of their arable land in the form of PG, while only Ireland had over 50% of total land use in the form of PG, and 90% of UAA. Additionally, 23 of the EU-28 countries, had >20% of their arable land in the form of PG, indicating the wide availability of grass-based feedstocks in different regions across the European continent in the long term. Changing current land use, i.e., switching from PG to TG for diverting grass for biorefinery purposes, could lead to to direct and indirect impacts such as, land carbon dioxide assimilation rates, or changes in soil carbon stocks and vegetation (Hughes and Qureshi, 2014). A properly assessed change can mitigate land use change impacts while improving the productive use of land (Aoun and Gabrielle, 2017).

While the components of grass biomass vary with environmental conditions, species, and mixture, typical contents of fresh grass in dry

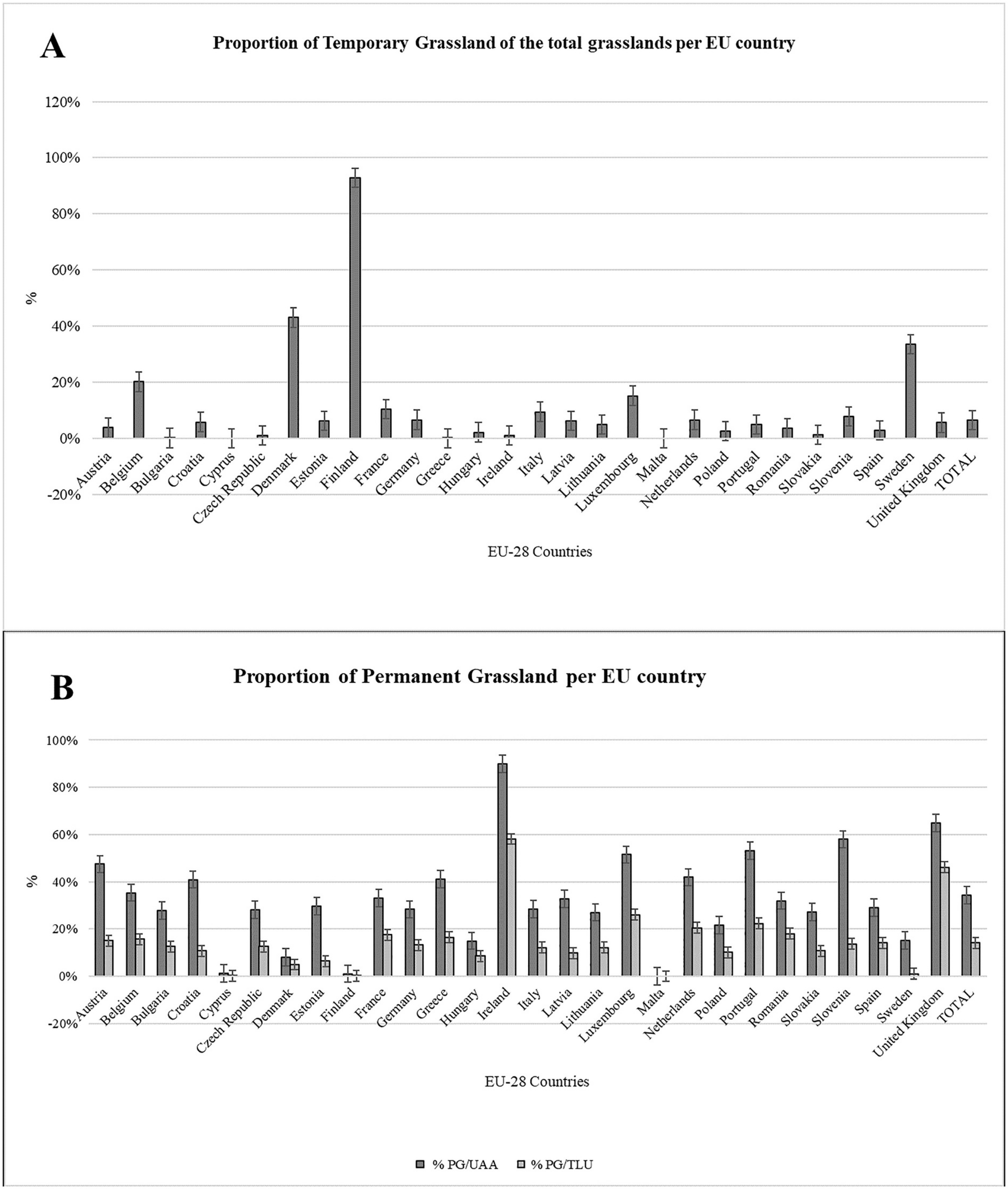

Fig. 1. Proportion of Temporary Grassland (A) and Permanent Grassland (B) per EU country.

matter (DM) range from 6 to 15% for protein, 20–55% for water soluble extracts containing 5–16% soluble sugars, and 38–65% for fibre components (cellulose 20–30%, hemicellulose 15–25%, Lignin 3–10%) (Mandl, 2010). Table 2 presents the constituents of different Green Biorefinery feedstocks, along with other primary and secondary feedstocks. Species with high crude protein (CP) content include white clover, red clover or cock's foot (24.9, 19.7 and 16.3–21.9% of CP per DM, respectively). Water soluble carbohydrates (WSC) are present at greatest proportion in feedstocks including leek leaves, Perennial ryegrass or cock's foot (250, 194–251 and 69.4–255.2 g/kg DM of WSC, respectively). Sugar beet leaves and potato leaves have as much Neutral Detergent Fibre (NDF) as alfalfa grass, ca. 30% DM, while other grasses, cock's foot, ryegrasses and clovers, present higher fibre content, ca. 36–58% (Table 2). Dry Matter and yield of the different feedstocks are represented in Table 2. Silage grass differs from fresh grass, containing amino acids instead of proteins and lactic acid generated by the fermentation of sugars during ensiling (Mandl, 2010). In the context of Green Biorefinery supply chains, ensiled grass can be stored and processed throughout the year, whereas fresh grass requires fast processing (typically within 24 h) and is therefore confined to months in which grass may be harvested (Keijsers and Mandl, 2010).

Aside from grasses, other Green Biorefinery studies have considered

Table 1
Total land use (TLU), Utilised Agricultural Area (UAA), Permanent Grassland (PG), Temporary Grassland (TG) and total grassland area for the EU-28 countries (2018 data).

EU-28	TLU (1000 ha)	UAA (1000 ha)	PG (1000 ha)	TG (1000 ha)	Total grassland (1000 ha)
Austria	8387.80	2653.84	1258.81	49.50	1308.31
Belgium	3066.60	1356.08	479.64	121.10	600.74
Bulgaria	11,099.60	5030.28	1399.04	2.30	1401.34
Croatia	5659.40	1485.65	607.56	37.40	644.96
Cyprus	925.30	132.44	1.59	–	1.59
Czechia	7887.10	3523.22	990.09	9.90	999.99
Denmark	4292.50	2632.50	212.70	160.80	373.50
Estonia	4533.60	984.67	292.23	19.20	311.43
Finland	33,841.10	2271.90	24.10	311.60	335.70
France	54,906.00	29,020.16	9593.99	1108.80	10,702.79
Germany	35,756.90	16,645.10	4713.40	329.80	5043.20
Greece	13,169.40	5288.05	2171.27	0.40	2171.67
Hungary	9301.20	5343.78	799.28	17.40	816.68
Ireland	6994.70	4516.04	4064.21	42.90	4107.11
Italy	30,207.30	12,908.75	3659.63	381.20	4040.83
Latvia	6458.60	1937.90	634.80	41.90	676.70
Lithuania	6528.40	2947.23	794.97	41.40	836.37
Luxembourg	259.50	131.56	67.71	12.10	79.81
Malta	31.60	11.58	–	–	–
Netherlands	3737.80	1822.40	763.79	53.60	817.39
Poland	31,192.80	14,539.55	3149.87	84.00	3233.87
Portugal	8910.30	3752.98	1992.61	103.30	2095.91
Romania	23,839.80	13,413.74	4288.41	157.70	4446.11
Slovakia	4903.50	1919.54	523.55	6.90	530.45
Slovenia	2027.30	477.93	277.17	23.20	300.37
Spain	49,850.20	24,201.91	7037.37	208.90	7246.27
Sweden	44,742.40	3000.39	455.14	229.80	684.94
United Kingdom	24,438.10	17,357.00	11,277.00	668.30	11,945.30
TOTAL	436,948.80	179,306.17	61,529.93	4223.40	65,753.33

the use of legumes, such as red and white clover (*Trifolium pratense* and *Trifolium repens*) or alfalfa/lucerne (*Medicago sativa*) (Damborg et al., 2019; Kamm et al., 2010; Stødkilde et al., 2021a). A major sustainability benefit of the inclusion of legumes is their ability to fix atmospheric nitrogen (N) via symbiosis with root nodule bacteria, *Rhizobium* spp., providing available N to grasslands (Harris and Ratnieks, 2021). This reduces the amount of N fertiliser required during the cultivation phase, which is important since N-based fertilisers represent the largest anthropogenic source of nitrous oxide (N_2O), a major greenhouse gas, while other factors such as nitrate (NO_3 − -N) leaching, nitric oxide (NO), and ammonia (NH_3) emissions, also have a negative impact on environment and climate (Gebremichael et al., 2021). Studies show that inclusion of clover within Perennial rye grass at >23% can reduce chemical nitrogen by 40% per hectare while providing similar yields of DM per hectare (Egan et al., 2018; McClearn et al., 2019). Furthermore, as demonstrated in Table 2, legumes can contain a high crude protein (CP) content, which is very interesting from a Green Biorefining perspective. In addition to legumes, additional research is being conducted to evaluate the potential of multi-species swards (MSS) grasslands, which combine traditional grasses, along with legumes and herbs (e.g., plantain, chicory, yarrow), with each species bringing different benefits to the sward (Cong et al., 2020; Moloney et al., 2020). Previous studies have demonstrated that MSS with no fertiliser provided similar yields to monoculture grassland receiving up to 300 kg/ha of chemical N fertiliser (Moloney et al., 2020).

The type of feedstock used can influence the potential for protein extraction in a Green Biorefinery. Thers et al. (2021) used the Cornell Net Carbohydrate and Protein System approach to investigate protein extraction in five forage species - white clover, red clover, alfalfa, Perennial rye grass, and tall fescue. The total crude protein recovery in leaf protein concentrate (LPC) was highest for the red and white clover and alfalfa, while a higher level of crude protein remained bound in the press cake in the case of the grasses. Work from Stødkilde et al. (2021b) has also investigated the protein recovery in Green Biorefineries through

Table 2
Table 1. Protein, carbohydrates and fibre content of primary and secondary green biomass for biorefineries

	Common name	Specific name	Yield (tDM/ha)	Dry Matter (%)	Crude Protein (% DM)	WSC (g/kg DM)	NDF (% DM)	References
PRIMARY FEEDSTOCKS	Cock's foot	*Dactylis glomerata*	5–13.5	20.7	16.3–21.29	69.4–255.2	58.52 ***	(Monteiro et al., 2020; Turner et al., 2012; Sanada et al., 2007)
	Perennial ryegrass	*Lolium perenne*	14.5–14.9	15.0–26.5	10.7–26.3 *	194–251	53.74 *	(Grogan, 2013; Meehan and Gilliland, 2019; Togeiro de Alckmin et al., 2020; Turner et al., 2012; Rivero et al., 2019)
	Italian ryegrass	*Lolium multiflorum*	0.9–20.3	18.1–18.3	15.0–16.2	143–265	40.0	(Einarsson et al., 2021; Yavuz et al., 2017; Cojocariu et al., 2008; Celen and Mohamed, 2021; Godlewska and Ciepiela, 2020)
	White clover	*Trifolium repens*	9–12	16.8	24.9	84–107	44.4	(Heuzé et al., 2019; Phelan et al., 2015)
	Red clover	*Trifolium pratense*	4–18	19.0	19.7	129.3	36.4–50.59	(Heuzé et al., 2015; Wróbel and Zielewicz, 2019)
	Alfalfa/Lucerne	*Medicago sativa*	16–27	23.21 *	18.3	48–72	31.4	(Heuzé et al., 2016; Karayilanli and Ayhan, 2016; Phelan et al., 2015)
SECONDARY FEEDSTOCKS	Sugar beet leaves	*Beta vulgaris*	Min. foliage (20%): 15.44 *	10–25.3	11.6–22.8	76.93 *	34.7–42.3	(EuroStat, 2021c; Tenorio et al., 2018); Aufrère et al., 2012; Bakshi et al., 2016; Tenorio et al., 2018;Kudoyarova et al., 2018)
			Max. foliage (39%): 30.64 *	10–30.4	11.6–35			
	Potato leaves	*Solanum tuberosum*	0.87**	11.7	14.0–14.9	81.5–88.5	28.6–35.9	(Torma et al., 2018;Ghosh et al., 2001; Bakshi et al., 2016; Eurostat, 2021b)
	Leek leaves	*Allium porrum*	0.24–0.36 *	11.85	1.5	250	–	(Bernaert, 2013; Biernacka et al., 2021; Boscher, 1981, EuroStat, 2021a)

* Estimated data.
** Leaves and stems.

selection of plant species and time of harvest, finding that plant species and spring cut harvest time affected nitrogen content distribution between the press cake and protein concentrates.

2.2. Secondary Green Biorefinery feedstocks

In addition to the main primary feedstocks for Green Biorefineries, certain underutilised land or residual or waste processing streams may also be considered as feedstocks for Green Biorefineries. These could potentially be used as standalone feedstocks but may also be used as supplemental feedstocks, in particular, where certain fresh primary biomasses such as grasses or legumes may not be available for part of the growing season.

In addition to pasture-based grasslands, grass can also be available in the form of roadside or verge grass, as well as grass from public spaces. These grasses grow back seasonally, and different regions adopt either a "cut-and-collect" or "cut-and-leave" management system depending on legislation, road safety rules and/or biodiversity goals (De Meyer and Guisson, 2021). In either scenario, cutting, collecting and/or processing of roadside grass brings a significant societal cost. As this feedstock is then readily available and usable for alternative applications, various Green Biorefinery initiatives have focused on using or including roadside grass as a feedstock within their studies (De Meyer and Guisson, 2021; Franchi et al., 2020a; Franchi et al., 2020b). Species found in roadside or verge grasses include *Dactylis glomerata* (Cock's foot), *Festuca arundinacea* (Tall fescue), *Lolium perenne* (Perennial rye grass), *Lolium x Festuca* (Festulolium), and *Phalaris arundinacea* (Red canary grass) (Meehan et al., 2017).

Leaves from sugar beet (*Beta vulgaris L*) are an example of a secondary residual green biomass feedstock suitable for use through Green Biorefining (De Visser and van Ree, 2016). The EU is the world's leading producer of sugar beet, being responsible for around half of the world's total production, with Northern European countries such as France, Germany, the Netherlands, Belgium and Poland being the most competitive production areas (European Commission, 2022b). Over the past five years the average sugar beet production area in the EU was 1.5 million hectares (ha) and 14.2 million tonnes of beet sugar were produced during the 2020/21 campaign, expected to increase to 16 million tonnes in 2022 (European Association of Sugar Manufacturers, 2021). Sugar beet leaves are a significant by-product of sugar beet harvesting, constituting between 20 and 39% of the total plant (Kiskini, 2017; Tenorio et al., 2018). Assuming a beet yield of 70 ton/ha (without leaves) then the leaf yield will be approximately 30% of that (21 ton/ha). Then 1.5 million hectares will generate approximately 31.5-million-ton leaf material for potential use. Beet leaves are normally left on the field after harvesting due to their fertiliser value. The fibres of the leaves contribute to the organic matter in the soil, however, three quarters of the valuable proteins are broken down during the first 3 months after harvest and the nitrate is leached out to the soil and some ammonia emitted to the atmosphere (Conijn et al., 2014). While beet leaves are sometimes used in applications such as animal feed, they could potentially be used for higher-value applications through biorefining (Kiskini, 2017; Skunca et al., 2021). Table 2 presents some main constituents of sugar beet leaves. According to Kiskini (2017), proteins are one of the main compounds present in sugar beet leaves, accounting for 25–35% of DM, while other major compounds include carbohydrates, ash, lipids and minerals, with phenolic compounds present in smaller amounts. While the moisture content of these leaves is up to 90%, the actual content of these compounds is therefore quite low; however, given the abundance and constituents of these leaves, their consideration for biorefinery purposes is well justified (Kiskini, 2017). Tenorio et al. (2018) estimates that the protein potential of beet leaves is significant, at 400–600 kg/ha of protein, which is quite comparable to the protein production of soy (450–600 kg/ha) and cereals (570 kg/ha).

Potato leaves are another example of a residual stream which may be harvested for use within a Green Biorefinery (Ravindran et al., 2021). An estimated 50 million tonnes of potatoes were produced in the EU-27 in 2021, with Germany, Poland, France, the Netherlands and Belgium collectively accounting for almost 75% of total production (Eurostat, 2021). It is estimated that approximately 4% of each potato harvested is in the form of stubble (leaves and stems), translating to around 2.2 million tonnes of stubble available in EU-27 in 2021 (Torma et al., 2018). The constituents of potato leaves based on literature are presented in Table 2, which shows a significant protein and carbohydrate content, which is interesting for biorefining. Additionally, Rodríguez-Pérez et al. (2018) recently profiled the constituents of potato leaves, identifying 109 compounds, including organic acids, amino acids and derivatives, phenolic acids, flavonoids, iridoids, oxylipins and other polar and semi-polar compounds. Such products may also be interesting from the perspective of value-added products in a Green Biorefinery. However, the toxic glycoalkaloid, solanine is often present in potato plant, sprouts and at potentially toxic levels for humans or animals, which would require removal during processing (Dalvi and Bowie, 1983).

Other potential by-products suitable for use in Green Biorefinery systems include hemp scrapes and leaves, kale and broccoli leaves, cassava leaves, maize stems, flax processing residues as well as residues and juices from fresh vegetable production (Ayele et al., 2021; Kromus et al., 2005; Prade et al., 2021).

3. Green Biorefinery process development

Green Biorefineries as described by Kromus et al. (2005) are integrated technologies and technology systems for the production of materials and energy from green plants and parts of green plants. While Xiu and Shahbazi (2015) described these as multiproduct systems, utilising green biomass as an abundant and versatile raw material for the manufacture of industrial products. The origin of chemical and biochemical scientific exploration of green plant leaves dates back to 1773 when the French chemists G. F. and H.M. Roulle reported obtaining protein extracts from alfalfa leaves, while the first modern industrial process for leaf protein extraction, the "Rothamsted process", was developed by N.W. Pirie in the 1960s (Kromus et al., 2005). In the early 2000s renewed interest in Green Biorefineries driven by a need for more sustainable products and energy took hold across Europe. This first wave of new Green Biorefining activity mainly took place within the R&D sectors of Austria, Germany and the Netherlands. In 2004, AVEBE, a consortium of R&D and industry partners from the Netherlands, developed a pilot process to separate a protein and fibre fraction from fresh grass (Mandl, 2010). The Green Biorefinery in Upper Austria, established in 2008, focused on the extraction of food grade lactic acid, amino acids and biogas from different species of ensiled grasses and legumes (Ecker et al., 2012). A demonstration facility was also developed at Havelland in Germany, focused on the integrated production of proteins, fermentation media, animal feed, and biogas (Kamm et al., 2010). On a commercial level, companies such as Newfoss, Grassa, Gramitherm and Biowert have been working to develop industry-led commercial or demonstration facilities across Europe (Höltinger et al., 2014; Mandl, 2010; Ravindran et al., 2021).

Recent years have seen a second wave of Green Biorefinery R&D and commercial activities, driven not only by the need for more sustainable materials, but also the potential to co-produce local and more sustainable forms of protein and potentially alleviate sustainability challenges within the agriculture sector. Such initiatives have been developed across Europe in countries such as the Netherlands, Denmark, Ireland, Belgium and Sweden (Damborg et al., 2019; Nynäs et al., 2021; Pijlman et al., 2018; Ravindran et al., 2021; Stødkilde et al., 2021b; Thiewes et al., 2019). Pilot and demonstration activities have been developed by organisations including Grassa, Aarhus University, Swedish University of Agricultural Sciences, Ghent University, and Munster Technological University with several recent R&D projects in the above countries such as GoGrass, Biorefinery Glas, Farm Zero C, Farm4More, Grassification, Green Valley, Grass Green Resources (GR3), Graskracht. Several of these projects involved the direct participation of key value chain actors, such as farmers and cooperatives, industry and regional government partners. Such Green Biorefining activities are also taking place in other jurisdictions including Ghana, Uganda and Indonesia, investigating novel green crops and residues such as elephant grass (*Pennisetum purpureum*), cassava (*Manihot esculenta*) and banana leaves (*Musa acuminate*) and rubber (*Hevea brasiliensis*) plantation residues (BIO4AFRICA, 2022; Sari et al., 2021).

3.1. Green Biorefinery processing

The majority of Green Biorefinery processes consists of a primary processing step of wet fractionation, to convert green biomass into a liquid fraction (press juice) and solid fraction (press cake). These two fractions are further processed using secondary processing (Keijsers and Mandl, 2010; Xiu and Shahbazi, 2015). An overview of some of the different products produced from press cake and press juice fractions is presented in Fig. 2. Some of the methods applied are described below.

3.1.1. Primary processing

Primary processing is an important first step in a Green Biorefinery. This step can make biomass components more accessible for subsequent conversion, facilitating the recovery of bio-based compounds and end products. Primary processing within Green Biorefineries usually involves some kind of mechanical wet fractionation which separates fresh or ensiled green biomass into press juice and press cake (Keijsers and Mandl, 2010; Xiu and Shahbazi, 2015). The juice fraction of green biomass is composed of sugars or water-soluble carbohydrates, proteins, organic acids, vitamins, minerals and other cell contents while the fibre rich press cake fraction contains cell wall components including cellulose, hemicellulose and lignin (Kamm et al., 2009). Depending on how efficient the wet fractionation is, the press cake will also contain soluble compounds that are not fully extracted or left on the still wet fibre after fractionation. In the case of fresh biomass, often this step is focused on the extraction of soluble protein into a green juice for subsequent extraction of LPC (Ravindran et al., 2021; Stødkilde et al., 2021b).

Several processes for mechanically pressing green leaves have been studied throughout the 20th century, including hammer mills, screw expellers, sugar cane rolls, ball mills, and rod mills, but none have proved to be ideal (Møller et al., 2021). More recent studies focused on the extraction of LPC have used different primary processing steps including, extrusion, single screw-pressing, pulping and pressing, and twin screw pressing in various chemical and microbiological combinations (Damborg et al., 2020; Koschuh et al., 2004; la Cour et al., 2019; Ravindran et al., 2021; Santamaria-Fernandez et al., 2019). The chosen separation technology is important, as the degree of cell disruption is crucial for high protein recovery in the press juice. Lower disintegration of the cell could result in a higher fraction of the protein remaining in the press cake fraction, which would require a secondary re-pressing to recover additional proteins (Santamaría-Fernández and Lübeck, 2020).

Mechanical processing has also been the primary processing approach used for fractionation of ensiled green feedstocks (Ecker et al., 2012; Kromus et al., 2005; Mandl, 2010). In such models the focus is usually on the extraction of a juice stream containing amino acids and lactic acid which can be further separated using secondary processing (Ecker et al., 2012; Mandl, 2010; Sharma et al., 2012). It has also been proposed that some initial processing of green biomass to reduce size and increase surface area may improve extraction. Such processes include grinding in a vertical hammer mill, or maceration using shredders or extrusion macerators (Møller et al., 2021). While mechanical processing has been the main type of primary processing applied, other pathways have been evaluated including thermal mechanical dewatering method, simultaneous application of a pulsed electric field, and superimposition of ultrasounds (Xiu and Shahbazi, 2015). Blanc and Arlabosse (2013) for example, developed a thermally-assisted mechanical dewatering process for the mechanical fractionation of green biomass which removed up to 83% of the inherent liquid fraction from alfalfa.

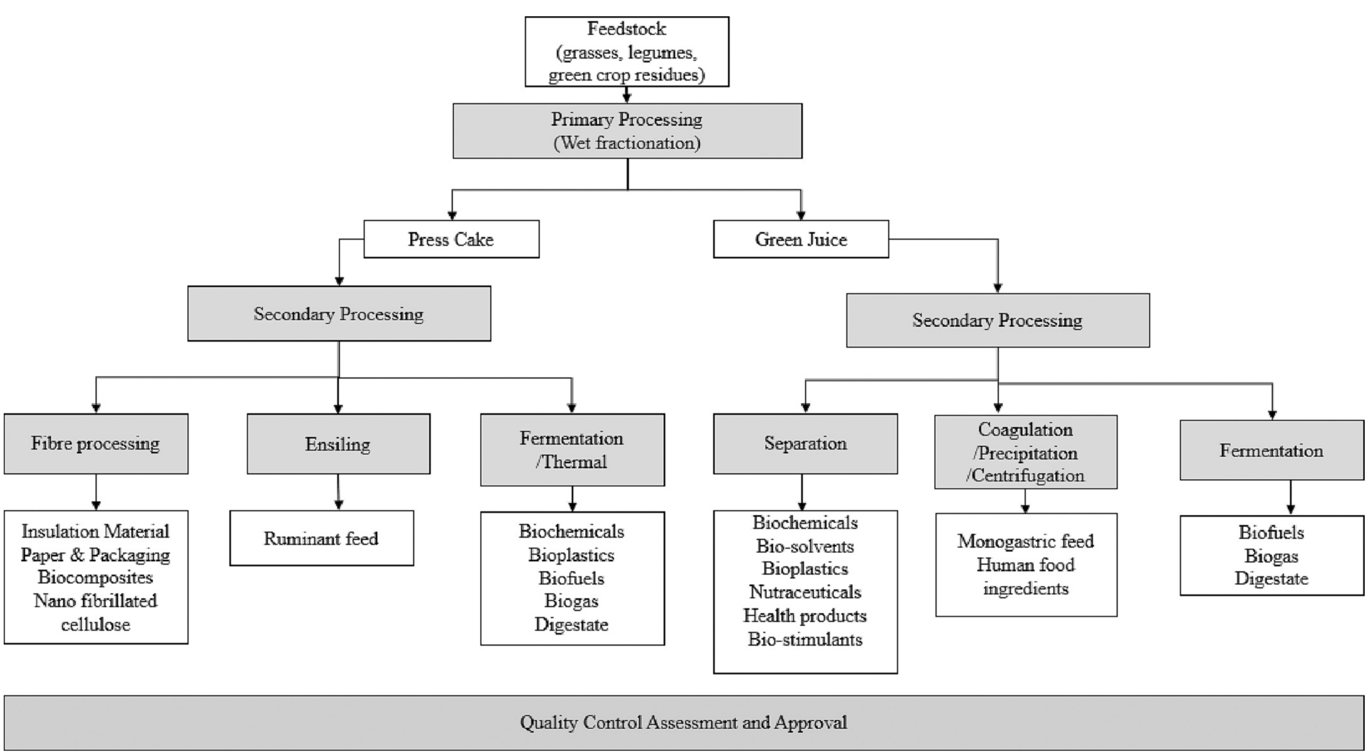

Fig. 2. An overview of some of the products produced from press cake and press juice fractions of Green Biorefineries. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.1.2. Secondary processing of press juice

Once the green juice has been extracted, the focus is usually on the extraction of value-added materials from the juice, or occasionally the direct use of juice in anaerobic digestion to produce biogas (Ecker et al., 2012; Kromus et al., 2005; Ravindran et al., 2021). In the case of press juice from fresh green leaves or grasses, the aim is often the precipitation of protein into a LPC. Proteins extracted from green leaves can be categorised into two types based on where they occur within the structure of the plant, i.e., chloroplast proteins and cytoplasmic proteins. Chloroplast proteins are insoluble lipoproteins that are commonly found in the thylakoid membranes of the chloroplasts (Diekmann et al., 2009). Naturally being green in colour due to the presence of pigments such as chlorophyll and carotenoids, these proteins destabilise and coagulate at lower temperatures and possess a strong grassy flavour (Fiorentini and Galoppini, 1983). Meanwhile, cytoplasmic leaf proteins are water-soluble, white in colour and relatively stable (Olvera-Novoa et al., 1990). These proteins when precipitated result in a white/creamy, tasteless and odourless precipitate (Fiorentini and Galoppini, 1983). The enzyme RuBisCO contributes to a major fraction of white protein concentrate obtained from leaves (Chiesa and Gnansounou, 2011). While the former is suitable for animal feed purposes, the latter is also well suited for human consumption, due to its taste- and colourless nature and high digestibility (Pojić et al., 2018; Ravindran et al., 2021). Protein precipitation strategies can be designed to obtain separate fractions of green protein and white proteins for various applications. However, the nutritional content of the extracted protein is also dependent upon the precipitation process (Santamaría-Fernández and Lübeck, 2020).

Building on the pioneering work of Pirie, who developed a large-scale process based on heat coagulation through direct steam injection of green plant juice at 70 °C, which resulted in an LPC with 60% protein content (Kromus et al., 2005). Several more recent studies have used variations of heat coagulation to precipitate the LPC (Damborg et al., 2020; Koschuh et al., 2004; la Cour et al., 2019). The most common method is to heat the juice to 70–90 degrees and separate the coagulated LPC by centrifugation (Stødkilde et al., 2021b), however, Damborg et al. (2020) used a two-step coagulation process for protein precipitation from green juices produced from red clover, white clover and Perennial rye grass, i.e., heating at 60 °C for 20–30 s for the precipitation of the green protein fraction, and the subsequent precipitation of the white protein fraction after heating at 80 °C for 20–30 s.

A recent review of primary processing technologies for the extraction of LPC from green biomass sources found that the protein recovery yield of green juice into LPC ranged from 25% to 87% (Santamaría-Fernández and Lübeck, 2020). Some of the most effective primary processing combinations included twin-screw pressing with heat coagulation (60 °C) and acidification (up to 87% yield) and twin-screw pressing with lactic acid fermentation (*L. salivarius*) (up to 86% yield) focused on alfalfa and timothy grass respectively.

Other investigated methods include acid precipitation, for example, using hydrochloric acid (HCl) which changes the solubility of the proteins in the green juice and can lead to their precipitation (Santamaría-Fernández and Lübeck, 2020). Lamsal et al. (2007) used a combination of heat coagulation at 55 °C and acid precipitation with HCl to pH 3.5 to obtain green and white protein concentrates from alfalfa. Koschuh et al. (2004) compared heat coagulation at 95 °C via steam injection followed centrifugation with ultrafiltration as methods for extraction of LPC from Perennial rye grass and alfalfa. The ultrafiltration method achieved a significantly higher crude protein recovery of 59% compared to coagulation (49%) in the case of Perennial ryegrass, with only marginal differences noted in the case of alfalfa. la Cour et al. (2019) investigated twin-screw pressing with addition of lignosulfonate, a by-product of the sulfite pulping process, as a flocculant for aggregation and precipitation of LPC from green juice of spinach, ryegrass-white clover mixture, ryegrass, and red clover. While the approach led to quite high levels of protein recovery up to 78%, the work also found that high levels of lignosulfonate were present in the LPC. The extraction of LPC from green biomass sources such as Perennial rye grass and grass clover mixtures has already been implemented at demonstration level by Grassa and Aarhus University (Ravindran et al., 2021; Stødkilde et al., 2021b). In Denmark, a commercial scale initiative launched in 2020, BioRefine Denmark A/S, will convert approximately 2000 ha of clover grass and lucerne into approximately 4000 tons of LPC and 25,000 tons of press cake using a centralized Green Biorefinery approach (DLF, 2020).

De Visser and van Ree (2016) investigated the extraction of protein from sugar beet leaves, combining a pressing step and a selective chemically aided coagulation stage, followed by several centrifugal and filtration stages. The extraction of food-grade RuBisCO protein from sugar beet leaves has been successfully implemented at demonstration scale in Dinteloord, the Netherlands (Nutrition Insight, 2019). Prade et al. (2021) investigated the production of a green juice from residual leaves of broccoli and kale through screw pressing with LPC thermally precipitated at 55 °C and separated by centrifugation, and white protein concentrated and later precipitated using acid precipitation and centrifugation. Kootstra and Huurman (2016) investigated the juice of Green Biorefinery processing of spinach for green and white protein extraction. After pressing, the spinach juice was treated by vacuum explosion and filtered. The filtrate was processed further by either adding activated carbon, or using a 50 °C heat treatment, or using a 50 °C heat treatment in presence of $CaCl_2$ to decolour the juice by removing unwanted material. Following these treatments, the liquid was centrifuged, and the decanted supernatant was filtered. The yield of white protein was approximately 8–10% of the original protein content of the spinach. Møller et al. (2021) notes that there has been limited work to extract white protein at scale to date.

Other high value ingredients may be extracted from the fresh Green Biorefinery juice stream which also contains lipids, lectins, sugars, free amino acids, dyes, hormones, enzymes, minerals, and other materials (Kromus et al., 2005). There are, however, limited studies regarding the feasibility to which some of these products may be co-produced. One recent study has found that it is feasible to co-produce press cake as a ruminant feed, alongside LPC, prebiotic sugars and minerals alongside press cake in a biorefinery approach (Ravindran et al., 2021; Menon et al., 2020). Following precipitation of LPC from the green juice, a short chain fructo-oligosaccharide (FOS) concentrate was extracted from the residual whey, with the residual stream applied as a fertiliser to land. Both the LPC and FOS have performed well under analysis compared to alternative products on the market (Ravindran et al., 2021; Menon et al., 2020). However, based on initial nitrogen, phosphorus and potassium levels, it was evident that the residual whey was not suitable for direct fertiliser application (Ravindran et al., 2022). According to Krenz and Pleissner (2022a), as most of the nutrients remain in the press cake following primary processing, the residual juice contains low amounts of nutrients, limiting the feasibility of applying sufficient fertiliser per area. In the same study anaerobic digestion was found to provide a suitable use for the residual grass whey (Ravindran et al., 2022). Kobbi et al. (2015) studied the extraction of protein hydrolysates from the pressed juice of fresh alfalfa leaves, which was subjected to ammonium sulphate precipitation and the brown juice, rich in white protein (RuBisCO), was recovered via isoelectric precipitation and further concentrated via lyophilisation. Pepsin was employed for the hydrolysis of RuBisCO. Prade et al. (2021) found significant contents of phenolic compounds of kale and broccoli leaves in the green juice fractions after pressing. The green juice following extraction of protein is also rich in nutrients and minerals. Feeney et al. (2020) investigated the direct application of this juice stream to land to investigate its bio-fertiliser and bio-stimulant properties, finding a similar performance in grass growth compared with slurry.

In the case of ensiled green biomass, previous work from the Green Biorefinery in Upper Austria investigated the extraction of lactic acid (LA) alongside amino acids (AA) from the green juice fraction of alfalfa silage (Ecker et al., 2012). Following screw-pressing, in which the press-cake passed to the biogas plant to serve as a substrate, the separated green juice was processed via ultrafiltration to remove impurities and a softening step to reduce cations before undergoing a hybrid process which included a two stage nanofiltration process to separate the AA (retentate) and LA (permeate). The LA permeate mixture was desalinated and concentrated using a two-stage electrodialysis (ED) process combined with reverse osmosis, while the AA mixture was cleaned and concentrated using an ion exchanger. Other studies have also investigated the separation of lactic acid from grass silage juice using a similar two-stage ED process, and by chromatography using neutral polymeric resin (Thang et al., 2005; Thang and Novalin, 2008). The production of amino acids and lactic acid in a Green Biorefinery has been implemented at pilot scale in Utzenaich, Austria (Ecker et al., 2012). Papendiek and Venus (2014) investigated optimal cultivation and fractionation processes for generating a fermentation medium from legumes for lactic acid production by *Bacillus coagulans*. With a focus on press juice from alfalfa as well as a clover-grass mixture, harvested across three sites, yield differences of up to 40% and 60% were recorded between the different locations. The final titer of lactate resulted in a higher product formation of about 80 g/l. Leiß et al. (2010) have also demonstrated the fermentative production of ammonium lactate, L-Lysine-L-lactate, from the fractionated press juice of a Green Biorefinery.

3.1.3. *Secondary processing of press cake*

Various approaches have been employed for the further refinement and use of press cake. According to Kromus et al. (2004), fibres from a Green Biorefinery may be used as a raw material in end products such as animal feed, insulation material, fibre boards, horticultural products, biocomposites, packaging material, additives for building material, gypsum boards, pulp and paper and bioenergy. If the press cake is to be used for feeding ruminants it is usually ensiled first to ensure stable storage and supply of feed as has been seen in various studies (Damborg et al., 2019; Pijlman et al., 2018). A study by Larsen et al. (2019) investigated the impact of ensiling on press cake produced from grass-clover and Perennial rye grass over 3 to 5 months. The press cake ensiled very well even though the sugar content was much lower than in the feedstock and the ensiling had a significant impact on the press cake composition, including a reduction in neutral detergent fibre (NDF), in-vitro digestibility of organic matter (IVOS), total content of AAs and true protein, but with an increase in free AAs. Beyond feeding applications, various studies and commercial activities have focused on the further processing of fibres into technical product applications. For such applications, the press cake usually requires a secondary treatment to remove the residual plant tissue and juice. According to Sharma and Mandl (2014), the press cake is initially cut to lengths of 8–10 cm, mixed with water at a ratio of 1:10 (mass/volume), and blended followed by further washing to remove residual juice. Sharma et al. (2012) investigated the use of press cake resulting from pilot scale rye grass biorefining trials, as feedstock for the production of nanofibrillated cellulose (NFC) using a four-step process including an NaOH pretreatment followed by mechanical and high-pressure microfluidization treatments. More recently Jebali et al. (2018) produced cellulose nanofibrils from fibres extracted from Marram Grass which grows naturally in nutritionally poor sand with low organic matter. A combination of conventional alkaline pulping and bleaching was used to extract fibres with a yield of approximately 50% DM of the plant, followed by high pressure homogenization resulting in mechanical disintegration of the pre-treated fibres producing nanosized fibrils with width around 5–8 nm with a yield exceeding 80%. These fibres can also be used as a form of cellulose in the production of paper and packaging. Thiewes et al. (2019) investigated the further processing of press cake fibres produced from roadside grass for use in blended composite materials. As the grass contain a large amount of sand and other pollutants it was washed until an ash content of ±14% was achieved, and dried until +/− 80% DM, before being further processed using a hammermill with a sieve of 2 mm and further processed into composite pellets using a pelletiser. Another application of press cake is in packaging. Alternative packaging sources may become increasingly relevant in a world which depends more on traditional lignocellulosic feedstocks for fuels and other materials in a bio-based economy (Keijsers and Mandl, 2010). In the Netherlands, the company NewFoss has developed a demonstration scale Green Biorefinery capable of processing up to 10 t/day grass collected from roadsides or nature. Newfoss uses a mild microbial extraction process with the main focus on the separation of clean fibres which are later converted into packaging products (Gursel et al., 2020). Biowert operates a commercial-scale Green Biorefinery for the extraction of clean fibres from grass silage. The facility has a yearly capacity of about 2000 t DM, and its main fibre products are grass fibre insulation material, and a natural fibre reinforced plastic or composite. After ensiling, the silage is mechanically processed and grass fibres are isolated through pulping, drying and pressing processes. (IEA Task 37, 2020).

4. Products from a Green Biorefinery

As indicated previously, depending on the feedstocks used, many new products can be produced from a Green Biorefinery. These include products for the food and nutraceutical markets, animal feed markets, biomaterials markets as well as the energy and fuel markets. An overview of some of these possibilities, based on the literature, is provided below. It is important to highlight that where novel products are being placed on the market, quality control assessment and approval is an essential requisite.

4.1. *Food and nutraceutical products*

As Green Biorefinery feedstocks often have a high CP content, this makes them suitable for protein extraction, which may be used as an animal feed, and potentially even in human food applications. Since the EU imports a large amount of feed annually (17 M ton of soybean meal in 2020) there is a growing concern around using such crops with human edible proteins as animal feed. However, Green Biorefinery of grasses has the potential to reverse this trend by producing human and animal grade proteins from widely abundant and native feedstocks (Mottet et al., 2017; van Zanten et al., 2016). In addition to food, depending on the green biomass composition, there is the potential for extraction of nutraceutical products.

4.1.1. *Food-grade leaf protein*

Møller et al. (2021) highlights a number of interesting properties associated with functional proteins from green biomass in food applications, including emulsification, gelation, foaming, and water holding capacity. Emulsifiers are important in the food industry for the production of foods such as mayonnaise, ice cream, sauces, and meat products such as frankfurters, bologna, mortadella, nuggets, sausage etc. While egg yolks and vegetable oils have emulsifying properties, emulsifiers for food processing are usually derived from soybean or sunflower oil. Chicken skin is another source of emulsifiers used in the food industry (Santos et al., 2020). In a recent study, Delahaije et al. (2022) developed an emulsion based on sugar beet leaf concentrate and soy protein isolate. A model was developed to predict the emulsion efficiency, stability, critical ζ-potential for stability against flocculation etc. Interestingly, the protein mixtures behaved similar to single protein-based emulsions. Separately, Ducrocq et al. (2020) investigated the use of RuBisCO extracted from sugar beet leaves as a promising plant protein to enrich wheat-based food without impairing dough viscoelasticity and protein polymerization, with results suggesting that RuBisCO actively participates in the formation of the dough protein network.

Møller et al. (2021) concluded that the leaf protein, RuBisCO, appears to be a potential gelation, foaming and emulsifying ingredient in foods as it performs at low concentration and relatively low temperature for gelation onset. It foams readily, and dependent on the pH in the environment, the foaming properties can compare to whey protein and soy protein performance. Møller et al. (2021) also noted that, in the context of food-grade protein and its functional properties, the majority of research on leaf protein studies focused on alfalfa, spinach, and sugar beet leaf proteins with no experimental papers found on grass or clover.

In recent years, proteins from alfalfa leaves have been extensively investigated for the development of protein hydrolysates. Protein hydrolysates are small peptides formed when large protein molecules are subjected to controlled proteolytic enzyme hydrolysis followed by post hydrolysis processing to separate bioactive peptides. Protein hydrolysates have been garnering huge interest in the functional foods industry due to its various health benefits (Nasri, 2017). Alfalfa naturally contains 170 to 220 g of protein per kg DM. Moreover, the white protein extracted from alfalfa is constituted by 65% RuBisCO, and is also rich in all the essential amino acids (Apostol et al., 2017). Several studies have been conducted for the development of protein hydrolysates from alfalfa with various bioactive properties. For example, Cao et al. (2021) extracted proteins from alfalfa via alkali solubilisation and acid precipitation, which were then subjected to enzymatic hydrolysis employing six different proteases viz. papain, alcalase, neutrase, trypsin, pepsin and flavourzyme. The leaf protein hydrolysates thus obtained were tested for angiotensin I converting enzyme (ACE) inhibitory activity. ACE is related to blood pressure and hypertension and ACE inhibitors are medications which relax the arteries and veins. From the study, it was observed that the leaf protein hydrolysate obtained after treatment with papain enzyme resulted in maximum ACE inhibitory rate (92.33 ± 0.30%). Meanwhile, protein hydrolysates obtained from the hydrolysis of RuBisCO from alfalfa have also been reported to have antimicrobial activity. Kobbi et al. (2015) evaluated the antibacterial activity of alfalfa hydrolysates against gram-negative and gram-positive pathogenic bacteria. A total of 12 peptides were obtained as a result of this study, all of which exhibited excellent antibacterial activity against the pathogens tested. A later study was published by Kobbi et al. (2018) reporting the effectiveness of three novel peptides produced using alfalfa RuBisCO against *Listeria innocua*.

Green protein juice from alfalfa has also been used to produce protein hydrolysates with antioxidant properties. Kobbi et al. (2017) developed bioactive peptides by hydrolysing alfalfa RuBisCO using porcine pepsin. The RuBisCO hydrolysate thus obtained prevented linoleic acid oxidation and reduced ferric ions indicating strong antioxidant abilities.

4.1.2. Carbohydrate-based food and nutraceutical products

In addition to proteins, carbohydrate streams may be extracted from green biomass for use in various food or nutraceutical applications. In grasses, fructan reserves are mobilised from vegetative plant parts during seasonal growth, after defoliation during grazing (Kromus et al., 2005). Fructans have a number of potential applications including use within the food industry as a sweetener (D-fructose), as a feedstock for the production of chemicals and for use as dietary fibre, suppressing putrefying bacteria and selectively supporting bifidobacterial and lactobacilli in the colon (Kromus et al., 2005). A study from Abeynayake et al. (2015) indicated high levels of fructans as a mixture of oligosaccharides and polysaccharides with different degrees of polymerization (DP) within Perennial rye grass. A recent study from Menon et al. (2020) focusing on the extraction, purification and analysis of FOS concentrate from grass whey, demonstrated comparable prebiotic activity compared with commercially available standards of fructooligosaccharides and inulin. Hermansen et al. (2017) has discussed the extraction of xylooligosaccharides (XOS) from fibres following pre-treatment which has shown promising results in pig gut simulation trials in relation to healthy gut flora.

Meanwhile, iminosugars are sugar analogues that have been associated with several health benefits. These molecules interact with carbohydrate-processing enzymes thus being therapeutically important. For example, Esposito et al. (2020) reported that iminosugars can be useful as an anti-inflammatory agent in cystic fibrosis lung disease. Rodríguez-Sánchez et al. (2016) employed pressurised liquid extraction to obtain iminosugars from the leaves of *Aglaonema* spp. They subsequently identified two different iminosugar molecules viz. α-homonojirimycin and 2,5-dideoxy-2,5-imino-D-mannitol. The iminosugars derived from *Aglaonema* were associated with α-Glucosidase inhibition activity and Caco-2 cell viability indicating its use as a potential functional food ingredient.

In a recent study, Rudrangi and West (2020) investigated the potential of Prairie cordgrass as a suitable substrate for the production of xylitol using hydrolysis and fermentation. Xylitol is an industrially valuable chemical that is used as an artificial sweetener. Other varieties of grasses such as switch grass and Perennial prairie grass have also been reported to be an ideal substrate for the production of xylitol via fermentation (Dien et al., 2018; West, 2009).

4.2. Animal feed protein

As mentioned previously, products produced from Green Biorefineries are often suitable for use as animal feeds. Kromus et al. (2004) notes that green forage often provides an excess of protein for cows, and hence the possibility to biorefine protein to produce additional feed for monogastrics or humans is interesting.

4.2.1. Ruminant feed

In recent years the possibility to use Green Biorefinery press cake, containing part of the grass protein, as a feed for cows has grown in interest and has been the subject of a number of experiments.

When it comes to the utilisation of Green Biorefinery press cake as a feed for cattle, a study by Pijlman et al. (2018) in the Netherlands investigated the effect of feeding grass press cake to Holstein Friesian dairy cows, replacing 60% of the silage in the conventional diet, with both diets equally supplemented by concentrates. The results found that the cows fed with press cake had a significantly lower level of N and phosphorous (P) intake and tended to excrete 33% less N and P compared to the control diet. Furthermore, the percentage of dietary N and P excreted via milk was significantly higher for cows in the press cake treatment compared to the control sample. This is a positive outcome since press cake only contained about 60% of the CP in grass, with the remaining protein extracted into green juice. A number of factors can explain the improvement in efficiencies delivered by press cake produced from fresh grass including a reduction in conventional losses in DM which occurs during the usual drying and ensiling of grass to produce silage, an increased availability of grass constituents to cows within press cake made possible through a better unlocking of grass cells by biorefining, and a higher fraction of “resistant protein” contained within the press cake. Sanders et al. (2020) highlights the importance of such improvements in nitrogen use efficiency, combined with benefits offer through legumes, as offering a sustainable way of helping to feed a growing global population while staying within our planetary boundaries. In another study, this time in Denmark, Damborg et al. (2019) explored the potential of replacing grass-clover silage in the diets of Danish Holstein dairy cows with press cake resulting from biorefining of grass-clover. The results show that the daily energy-corrected milk was greater for cows receiving press cake diet (37.0 kg/d) compared with cows receiving unrefined grass–clover diets (33.4 kg/d). A more recent study from Serra et al. (2020) in Ireland, evaluated the effects of replacing the grass silage in Holstein Friesian dairy cow diets with biorefined Perennial rye grass press cake. The findings indicate that the replacement of grass silage with press cake did not have a negative impact on milk yield and milk quality. However, milk fat yield and milk solids yield was lower in the case of the press cake diet. Similar to Pijlman et al. (2018) a reduction in N and P excretion was observed among cows fed with the press cake diet compared to those fed with the grass silage diet. In addition, Serra et al. (2020) conducted in vitro analysis using the rumen simulation technique (RUSITEC) to investigate the impact of silage replacement with press cake on rumen methane production concluding that total gas and CH_4 production was not affected by treatment. A further study from Savonen et al. (2020) in Finland, evaluated the inclusion of press cake produced from silage (mixed timothy and meadow fescue) as an inclusion into the diets of Nordic Red cows. The study found that the cows maintained milk production at a 25% inclusion rate, but it showed some decline at 50%. Overall, the results to date indicate great potential to at least partly include press cake in dairy cow diets with improved nitrogen use efficiency and reduced losses.

4.2.2. Monogastric feed

The green juice separated from the press cake contains proteins that can be separated to leaf protein concentrate (LPC) serving as a feed for monogastrics, such as pigs and poultry, as well as pet food and in aquaculture, in addition to its potential use in human food. Santamaría-Fernández and Lübeck (2020) found that protein concentrations of the resulting LPC can vary significantly, from 18% to 89% DM based on the starting biomass and the extraction technique. A number of experimental trials have been conducted to evaluate LPC. In Denmark Stødkilde et al. (2021b) investigated the effect of including LPC extracted from organic grass clover, by pressing, heat coagulation, centrifugation and freeze drying, on growth performance and meat fatty acid profile of growing pigs. The trial found that the inclusion of grass-clover protein, with CP of 45% DM, within the diet did not affect feed intake or growth in starter, grower, or finisher pigs, and no difference was found in the slaughter weight of the animal. Interestingly, the percentage of meat at slaughter increased linearly with inclusion of biorefinery protein concentrate in the feed, while also increasing the content of omega-3 fatty acids. Separately in Ireland, Ravindran et al. (2021) investigated the use of Green Biorefinery LPC derived from Perennial rye grass as a protein additive in growing pig diets. The LPC had a CP content of 33.9% DM, with lower lysine compared with soybean meal but higher levels of methionine. LPC was included within a trial diet displacing mainly soybean meal (27%) and barley (25%) as well as wheat (8%) in a traditional 9-week-old weaner pig ration. Compared with the control batch, the pigs fed with the LPC demonstrated an increase in dry matter intake and daily weight gain of 8% and 6.44% respectively. In addition to pigs, Stødkilde et al. (2020) investigated the effect of biorefined grass-clover protein composition on organic broiler performance and meat fatty acid profile. The CP content of LPC was 36.2% DM which, like in Ravindran et al. (2021), had a higher methionine content, but lower lysine compared to soybean meal. The trial found that increasing levels of biorefined grass-clover protein concentrate reduced feed intake, growth and slaughter weight; however, at 8% inclusion, feed intake and performance were not affected. A lowered tocopherol content in meat from broilers fed with increasing grass clover protein demonstrated the need for increased amounts of antioxidants due to the high content of unsaturated fat. Overall, the preliminary results appear positive to include LPC as a feed for pigs, while further work is required to investigate the suitability for use in poultry diets. However, the protein concentration of the LPC is a key parameter for the feed digestibility, thus increasing the protein content in the LPC through efficient processing will also increase feed performance (Stødkilde et al., 2021b; Thers et al., 2021).

4.3. Biomaterials

Various biomaterials can be produced through Green Biorefineries since green biomass feedstocks are rich in a large amount of relevant bio-based compounds which may be extracted or converted into end products. The next subsections delve into these different biomaterials.

4.3.1. Biochemicals

Several biochemicals with wide application can be produced from Green Biorefinery feedstocks. In particular, work has been undertaken to investigate the production of bulk chemical lactic acid which can be extracted from the biorefinery juice fraction of grass silage (Mandl, 2010). LA is an important chemical with applications across various sectors including food, pharmaceutical and cosmetic industries, as well as being a monomer for the manufacture of biodegradable polymer polylactic acid, an alternative to traditional petrochemical polymers (Pereira et al., 2011). Ecker et al. (2012) investigated the extraction of LA alongside amino acids from alfalfa silage using advanced membrane separation (Ecker et al., 2012). The process found a partial separation of LA from grass juice, from a starting point of 32.6 g/L in silage juice a 54% product yield, 17.8 g/L of LA was achieved from this system. However other organic acids including acetic acid and butyric acid were not separated and therefore further purification of the product would be needed depending on the application and purity required. In another study, Thang et al. (2005) obtained a recovery of lactic acid in the range of 87–91% grass silage using a two-stage ED process. While in a separate study, Thang and Novalin (2008) obtained lactic acid from grass silage juice using neutral polymeric resin for chromatography procedures. At recovery yields of above 99.4%, purity ranging from 93.2% to 99.9% was possible. An analysis conducted by Ecker et al. (2012) utilising a Green Biorefinery LA solution in an esterification process to produce ethyl lactate, indicated that further purification of the LA-solution is not required for sufficient esterification rates. Ethyl lactate is a product of keen interest, since it is a biodegradable compound with good properties to be used as a green solvent in several applications, including pharmaceutical, fragrances, inks and coatings, and as a food additive (Pereira et al., 2011). In separate work, Thomsen et al. (2005) also demonstrated that brown juice from the green crop-drying industry and potato juice from the potato starch industry can be used as feedstock for LA production.

Another potential Green Biorefinery chemical building block are volatile fatty acids (VFA), produced as an intermediate from anaerobic digestion (Jagadabhi et al., 2010; Yu et al., 2014). These fatty acids include short chain fatty acids such as acetic, propionic, butyric, and valeric acid (Cerrone et al., 2014). Steinbrenner et al. (2022) demonstrated the co-production butyric acid, alongside methane from biorefining of grass silage, advancing beyond the traditional approach of direct silage anaerobic digestion and methane production.

Kromus et al. (2005) noted that fructans extracted from grasses or leaves through biorefining could serve as feedstock for development of biochemicals. Through hydrolysis to D-fructose and subsequent dehydration, the chemical intermediate hydroxymethyl furfural may be produced. Other potential chemicals include ethanol, organic solvents and furan-based chemicals (Kromus et al., 2005).

Alternatively, green leaf nutrient concentrate is rich in palmitic acid, linoleic acid and linolenic acid. These can be separated by steam distillation and may be used as ingredients in cosmetic products. In addition, dyes, vitamins and other phytochemicals can be obtained from both green leaf nutrient concentrate and press cake (Kromus et al., 2004; Thomsen et al., 2005). In particular, carotene (vitamin A) and xanthophyll can be separated by steam distillation and can be considered for use in the cosmetic and food sectors.

4.3.2. Bioplastics and biocomposites

Green Biorefining may also be used to produce bioplastic and biocomposite materials. Cerrone et al. (2014) has demonstrated the use of a two-stage anaerobic digestion of grass silage press juice to produce VFA's which can be used to obtain medium chain length polyhydroxyalkanoate (mcl-PHA). Anaerobic digestion of ensiled grass was conducted using a recirculated leach bed bioreactor resulting in the production of a leachate, containing 15.3 g/L of VFAs ranging from acetic to valeric acid with butyric acid predominating (12.8 g/L) (Cerrone et al., 2014). Based on the selected *Pseudomonas putida* strain to accumulate PHA from VFA's, 39% of the cell dry weight was accumulated as PHA and this was composed predominantly of 3-hydroxydecanoic acid. Separately, Cerrone et al. (2015) also confirmed that mannitol rich ensiled grass press juice can be used as a renewable carbon substrate for obtaining PHA. Other authors such as Patterson et al. (2021) have produced PHAs from grass using novel mixed cultures. In their study, co-fermentation of grass or grass waste and waste activated sludge coming from municipal wastewater treatment plants was used for PHA extraction with biogas produced from the process residues. According to Patterson et al. (2021) a total of 30,000 t of fresh grass, or 750 ha assuming a grass yield of 40 t fresh matter (8.8 tDM/ha), would yield approximately 403.65 t of dried biopolymer granules. PHA can be used in a variety of sectors including agriculture, aerospace, biomedical sector and cosmetics. Moreover, PHAs are used in conductive bioplastics, toys, textiles, buildings industry, fertiliser mulches and pellet for soil application PHA polymers have also been used as microparticles in drug delivery, cardiac valves and surgical sutures, artificial skin and artificial organ reconstruction (Abd El-malek et al., 2020).

NFC production was studied by Sharma et al. (2012) in which press cake fibre was extruded from rye grass and silage processing. NFC can be used as a replacement for conventional fillers, such as glass, aramid and carbon fibres for manufacturing high strength polymer composites. Specifically, they developed a cascading process for the fractionation of NFC from the press cake where four different variations of NFC cellulose were obtained according to different granulation and other properties. Based on the analysis, of composites containing NFC and residual hemicelluloses, Sharma et al. (2012) indicated that the product could be used for manufacturing high strength and high value products, such as packaging, drain pipes, gutters and flower pots. Jebali et al. (2018) was able to extract cellulose nanofibrils with width around 5–8 nm from Marram Grass indicating use as a nanofiller in a wide range of applications such as nanocomposites, papermaking, packaging and environmental remediation.

At commercial level, the company Biowert produces biocomposite granulates by blending 30–50% grass fibre with 50–70% recycled polyolefine. These granulates are suitable for injection moulding and can be used to manufacture consumer goods, such as decking tiles (IEA Task 37, 2020). The Grassification project tested press cake produced from roadside grass, along with dried grass as an input for making grass fibre pellets as a half-finished product for biocomposite production. The analysis evaluated the mechanical of the press cake product in blends with High-density polyethylene and found that this fibre offers comparable mechanical properties to dried grass fibres (Thiewes et al., 2019). The findings indicated that biorefining should be performed if the liquid fraction can be further processed leading to a valuable co-product (Thiewes et al., 2019).

4.3.3. Insulation and building materials

Kromus et al. (2004) highlighted that grass silage press cake fibres can be used as natural fibre insulation material in the form of insulation mats and boards as well as loose fill materials. According to Corona et al. (2018) the press cake is initially dried to 92% DM and then mixed with Borax to increase fire resistance, to comply with building fire and safety standards (Corona et al., 2018). The product could replace conventional insulation panels such as panels from mineral wool. The company Gramitherm based in Switzerland and Belgium has promoted their commercial insulation material produced using grass cellulose fibres with the juice stream supplied to biogas units to produce energy for the process (Gramitherm, 2022). Keijsers and Mandl (2010) indicated that these insulation panels based on press cake offer similar thermal conductivity to stone wool, polystyrene and flax-based insulation panels, but with significant reductions in Global Warming Potential (CO2-eq). Biowert also supplies press cake in the form of blow-in insulation material (Sharma and Mandl, 2014).

Building materials could also be produced from these fibres. King et al. (2013) investigated the use of fibrous grass silage press cake within building materials and how this could decrease shrinkage cracking for low strength building materials. The press cake was used as a fibre reinforcement additive within clay and cementitious mortars. Interestingly, the study found that press cake outperformed polypropylene fibres in reducing the likelihood of cracking caused by early-age constrained shrinkage in cementitious specimens.

4.3.4. Packaging materials

Green Biorefinery fibre may also be used as a renewable form of cellulose in packaging applications. Such fibres are produced at demonstration scale by the company Newfoss, and these are predominantly used in the packaging industry. Packaging manufacturer Huhtamaki integrate grass fibres within their GreeNest eggbox range in a 50% by weight mixture with recycled paper, which are available in supermarkets including Albert Heijn and Jumbo (Gursel et al., 2020). In addition, press cakes of alfalfa, along with reed canary grass, wild mix grass, and cock's foot, have all been used to make packaging. Additionally, it has been demonstrated that grass cardboards are both more affordable and a higher quality than wastepaper which is re-worked (Kromus et al., 2004). According to De Visser and van Ree (2016) certain Green Biorefinery feedstocks, such as sugar beet leaves are less suitable in technical applications such as paper, composite or building materials production as they do not have a fibre fraction of sufficient strength for such applications.

4.4. Energy applications from biorefinery residuals

When establishing the European Green Deal in 2019, the 27 EU Member States committed to reducing emissions by at least 55% by 2030 compared to 1990 levels. Specifically for energy, the European Commission proposed to increase to 40% the share of renewables in the energy mix by 2030 while decreasing the use of wood for energy purposes (European Commission, 2021). In May 2022, the commission further updated their ambitions upwards, in the REPower EU Plan. This plan aimed at a greater resilience in regards with energy vis-à-vis Russia and set dedicated goals towards renewable energy, including biogas, targeting 35 billion cubic metres of biomethane production by 2030, replacing the need for import of natural gas (European Commission, 2022a). These initiatives result in an increased need for using biomass residues for energy production so as not to overburden agricultural systems and avoid the food vs. fuel debate.

Moreover, when operating a biorefinery, energy production from residues enables the reduction of fossil fuel dependency and results in a more sustainable process (De Jong et al., 2009). Therefore, ideally, a Green Biorefinery would be arranged in a way that one or more residues are used to produce renewable energy. The main process used in previous studies is anaerobic digestion, as these are streams rich in organic molecules, but bioethanol production and combustion of the press cake have also been proposed. The following sections summarise the main findings of energy production in a Green Biorefinery and the way forward to maximising the value created from the used resources.

4.4.1. Biogas

In a Green Biorefinery process, two main streams have been investigated for biogas production: the press cake derived from the first separation step of the green biomass into fibres and green juice, and the brown juice obtained after protein precipitation of the green juice. Both press cake and brown juice are mainly composed of proteins and carbohydrates, suitable for anaerobic digestion, but while the press cake is a fibre-rich material composed of complex carbohydrates (cellulose and hemicellulose), the brown juice is a diluted stream composed of more available mono and oligosaccharides (Santamaría-Fernández et al., 2018). This difference in composition affects both the preferred process configuration for anaerobic digestion and the obtained biogas yields, as discussed in the following sections. The produced biogas can be used in a combined heat and power system or can be further upgraded to biomethane – which is equal and equivalent to natural gas in quality, to the point that it can also be injected in the natural gas grid and directly replace natural gas.

4.4.1.1. Juice. The brown juice, obtained after protein precipitation, is a common residual stream of the Green Biorefinery process. Therefore, several studies have investigated its use as a feedstock for energy production via anaerobic digestion, thus increasing the energy efficiency of Green Biorefineries (Njakou Djomo et al., 2020).

Because the brown juice is an acidic stream (pH $\leq$ 4) with a high chemical oxygen demand (COD) content, the use of a continuous stirred tank reactor (CSTR) may result in the inhibition of the anaerobic digestion process. Therefore, different reactor configurations have been proposed to better cope with this stream. Martinez et al. (2018) proposed the use of an up-flow anaerobic sludge blanket (UASB) reactor for the anaerobic digestion of brown juice (Table 3). UASB uses immobilised microorganisms, resulting in process robustness towards fluctuations in temperature, pH and substrate concentration and the possibility of short hydraulic retention times (HRT). However, the highest methane yield achieved with UASB in the 2018 study was significantly lower than in the biomethane potential test, indicating that the used HRT might not have been sufficient to allow for complete degradation of the organic load present in the brown juice. Moreover, a reduction in granule size of the activated sludge was observed, indicating a lack of stability in the process.

A recent study by Feng et al. (2021) proposed the use of an anaerobic filter (AF) reactor for biogas production from brown juice (Table 3). This reactor consists of a fixed bed where a bacterial biofilm grows in porous media, resulting in high biological activity, high organic loading rates (OLR), and short retention times compared to conventional anaerobic digesters. Even with the proposed configuration, acidification was still observed with the initially tested OLR of 3.8 kg COD m^{-3} d^{-1}, which was attributed to the lack of acclimatisation of the inoculum to this new substrate. After reducing the OLR and gradually increasing it, stable biogas production was obtained when the initially tested OLR was reached once again. Splitting the daily feeding into two equal portions also resulted in better reactor operation possibly due to the reduced impact of the acidic pH in the buffer capacity of the reactor.

The need for inoculum adaptation observed by Feng et al. (2021) is also highlighted by other studies, indicating that this is an important aspect of obtaining a robust and stable anaerobic digestion process when using brown juice as the sole substrate (Martinez et al., 2018; Santamaría-Fernández et al., 2018). Another possible strategy for coping with the difficult characteristics of the brown juice is the co-digestion with the press cake. The digestion of a 1/1 ratio of these two streams (on VS basis) resulted in a better methane yield than the sole digestion of either stream, indicating that this might be a suitable strategy for improving the yields obtained with the press cake and stabilizing the digestion process of the brown juice (Santamaría-Fernández et al., 2018). Other co-digestion strategies might also be suitable, such as blending the brown juice with animal manure, which enjoys a higher buffer capacity; nevertheless, no studies on the topic have been found and this strategy still requires testing.

In a Green Biorefinery, potentially different green biomasses might be processed in the same facility. Therefore, the influence of the feedstock on the methane potential of the brown juice could be an important parameter affecting the viability of the process. Santamaría-Fernández et al. (2018) investigated the anaerobic digestion of four brown juices coming from different green biomass. The authors observed that the methane yield per kg of VS was very similar between the feedstocks (Table 3), varying from 429 to 475 L CH_4 $kgVS^{-1}$. Moreover, when evaluating other reports in the literature, a relatively narrow range of methane yields was observed, between 307 and 544 L CH_4 $kgVS^{-1}$ (Table 3), even though both feedstock and digestion configuration were highly variable between the studies. This is a promising indication that brown juices from different origins can be processed in the same digester with similar yields if the feeding is adjusted on the basis of VS content.

After protein precipitation, the brown juice might still be rich in dietary fibres in the form of fructooligosaccharides (FOS), which can be recovered from it and result in a de-FOS whey residual stream (Ravindran et al., 2022). The anaerobic digestion potential of the brown juice against the de-FOS whey was evaluated by Ravindran et al. (2022) and only a 5% reduction in the biomethane potential was observed after FOS recovery (Table 3). These results indicate that more products can potentially be obtained from Green Biorefineries while still maintaining their potential for renewable energy generation.

4.4.1.2. Press cake. Depending on the main products targeted in a Green Biorefinery, e.g., lactic acid or proteins for monogastric animals or human consumption, the press cake can also be considered as a residual stream for use in energy production via anaerobic digestion.

Steinbrenner et al. (2022) studied the influence of different ensiling conditions on the butyric acid formation and biomethane potential of the obtained press cake in a grass Green Biorefinery (Table 3). The authors did not observe a significant influence of ensiling temperature (20 °C or 37 °C) on the biomethane potential of the press cake except in the case of the control treatment without any additives; in this case, ensiling at the higher temperature resulted in a slight increase in the biomethane yield. The addition of water and/or $CaCO_3$ to the grass during ensiling also did not affect the biomethane yield of the resulting press cake.

Several studies have compared the biomethane potential of fresh or ensiled biomass to the press cake to further justify the biorefinery setup and indicate that more value could be created when opting for the refinery configuration rather than just digesting the unprocessed biomass. Nevertheless, conflicting results have been observed. For grass silage, Steinbrenner et al. (2022) observed that, in most cases, there was no significant difference between the biomethane potential of the silage and the different press cakes. However, one condition resulted in a slightly higher and two treatments resulted in a slightly lower

Table 3
Biomethane yields obtained with varied streams processed from Green Biorefineries using different biomass sources.

Biomass	Stream	Methane yield	Mode (duration)	Reference
60% rye grass and 40% clover	Brown juice	409.6 L CH4 $kgVS^{-1}$	Continuous (5.5 days)	(Feng et al., 2021)
Red clover and clover grass	Brown juice	307 L CH4 kg VS^{-1}	Continuous (3 days)	(Martinez et al., 2018)
Red clover	Brown juice	428.7 L CH4 $kgVS^{-1}$	Batch (55 days)	(Santamaría-Fernández et al., 2018)
	Press cake	218.6 L CH4 $kgVS^{-1}$		
Clover grass	Brown juice	464.4 L CH4 $kgVS^{-1}$		
	Press cake	295.6 L CH4 $kgVS^{-1}$		
Alfalfa	Brown juice	456.7 L CH4 $kgVS^{-1}$		
	Press cake	239.9 L CH4 $kgVS^{-1}$		
Oilseed radish	Brown juice	475.0 L CH4 $kgVS^{-1}$		
	Press cake	374.7 L CH4 $kgVS^{-1}$		
Clover grass	31% Press cake +69% Brown Juice	238 L CH4 $kgVS^{-1}$	Continuous (20 days)	(Santamaria-Fernandez et al., 2020)
Grass	Press cake	± 350 L CH4 $kgVS^{-1}$	Batch (35 days)	(Steinbrenner et al., 2022)
	Press cake	300 L CH4 $kgVS^{-1}$		
Perennial rye grass	Brown Juice (grass whey)	544 L CH4 $kgVS^{-1}$	Batch (21 days)	(Ravindran et al., 2022)
	De-FOS whey	520 L CH4 $kgVS^{-1}$		

biomethane yield for the press cake when compared to the silage, with no apparent trend for these results. When conducting a similar study with several biomass streams, Santamaría-Fernández et al. (2018) observed consistent results across all tested samples, with an average reduction of 25% in the biomethane potential of the press cake when compared to the fresh biomass. This is in agreement with Ravindran et al. (2022), who also observed a reduction of 38% in biomethane yield when comparing the press cake to the fresh biomass of Perennial rye grass. This reduction in biomethane potential is usually associated with the loss of easily convertible organics to the green juice, while the press cake has more recalcitrant organic molecules (lignin, cellulose and hemicellulose) that result in a lower biomethane yield per kg VS. However, it might be that the changes undergone during ensiling contributed to a higher biomethane yield for the press cake that compensated for the loss of the green juice, explaining the opposite results observed by Steinbrenner et al. (2022), as ensiling has been reported to increase biomethane yields compared to the untreated biomass (Herrmann et al., 2011).

Similar to what was observed for the brown juice, the different studies in the literature report similar biomethane yields for varied press cakes (Table 3). Biomethane potentials between 218 and 375 L CH_4 $kgVS^{-1}$ were reported for different feedstocks and anaerobic digestion conditions, once more indicating the potential to process varied biomass streams in the same digester by adjusting the feeding rate according to the volatile solids content of the used substrate.

4.4.1.3. Digestate as fertiliser. Nutrient recycling for the production of bio-based fertilisers that can replace synthetic fertilisers can also reduce natural gas dependency (Saju et al., 2022), enhancing the environmental gains obtained from a Green Biorefinery, as the Haber-Bosch process currently used for the production of synthetic N fertilisers has a high energy expenditure based on fossil fuels. Furthermore, the revised Fertilising Product Regulation (FPR) which came into effect as of July 16, 2022, will open the doors for producing all types of soil enhancers, fertilising products and biostimulants from bio-based and recycled sources. Nevertheless, the application as a fertiliser of the digestates generated from Green Biorefinery streams has not been widely studied. Santamaria-Fernandez et al. (2020) evaluated the nutrient profile of the digestate obtained after anaerobic co-digestion of a mixture of press cake and brown juice from clover grass. The C:N ratio in the digestate produced was about 7, indicating a low risk for N immobilisation upon application. N mineralization was also observed, with an increase in the ratio of NH_4^+ to total-N from 9.4% in the influent to 43% in the digestate. Finally, an N:P:K ratio of 4:1:12 was obtained, which indicates that this digestate can be a good K source.

Even though not much information is available in the scientific literature, several companies implementing a Green Biorefinery concept have fertiliser as one of the proposed products, albeit not always from digestate or not from mono-digestion of the Green Biorefinery residues. Grassa, a Dutch company processing high-quality grass into several products, indicates that the grass juice after protein and FOS recovery can be used as a fertiliser mainly as a K source (Grassa BV, 2022). As the study of Ravindran et al. (2022) indicated that the brown juice after FOS recovery still contains a significant biomethane potential, it might be feasible to further use it as a biogas substrate and then apply the generated digestate as fertiliser, further expanding the diversity of products obtained and enabling a more sustainable energy production in the process. Biowert, a Grass Biorefinery in Germany, uses the green juice as substrate in their biogas plant together with local co-substrates such as food waste and slurry and the digestate is applied as a fertiliser by local farmers (IEA Task 37, 2020).

Direct use of Green Biorefinery brown juice as fertiliser has been proposed by a demonstration plant in Germany (Kamm et al., 2010). However, when evaluating the green juice from roadside grass clippings as fertiliser also without any pre-processing, the Grassification project observed that the C:N ratio of this stream was imbalanced, causing N immobilisation by soil organisms due to the elevated sugar content of this stream (Souza and Scott, 2020). Therefore, it seems useful to either recover the sugar fraction from the brown juice, as done by Grassa, or perform the anaerobic digestion of this stream to convert the easily available sugars into biogas before applying it as a fertiliser, in that way recovering more products from the same stream and avoiding risks on N immobilisation when using the juice as fertiliser.

4.4.2. Bioethanol

Bioethanol production from the press cake in a Green Biorefinery has been investigated by a few studies. However, its use for generating energy for the Green Biorefinery itself would be less straightforward and it would probably be established as an additional product from the refining process. Nevertheless, it might be possible to integrate bioethanol and biogas production by the anaerobic digestion of the stillage stream resulting from bioethanol distillation (Cesaro and Belgiorno, 2015), even though no study to date has investigated this configuration in a Green Biorefinery.

The press cake of cattail, an aquatic plant, was used for bioethanol production after ultrasonication by either simultaneous saccharification and fermentation or separate hydrolysis and fermentation (Rahman et al., 2015). Both processes yielded low biomass to ethanol conversion rates (12% of the theoretical value), which were attributed to insufficient pretreatment of cattail before the enzymatic hydrolysis. The same group further investigated the conversion of the press cake of miscanthus after diluted sulfuric acid pretreatment and found a higher conversion rate of 88% of the theoretical value in a simultaneous saccharification and fermentation configuration, indicating that bioethanol production from the press cake of a Green Biorefinery is possible with the correct choice of biomass and pretreatment combination (Xiu et al., 2017). The green juice obtained from miscanthus was also investigated as a substrate for the growth of *Saccharomyces cerevisiae* and the authors observed a higher ethanol yield from the miscanthus press cake when using the juice-cultured cells, probably due to the higher cell count obtained in this culture compared to the cell count obtained when using the commercial medium (Boakye-Boaten et al., 2016).

4.4.3. Bioenergy carriers via thermal processing

Even though AD is the main energy production process used in Green Biorefineries, thermal processes have also been studied for converting green biomass into energy. However, the fractionation step of the biomass when aiming for the production of solid fuel for combustion from the fibre fraction usually involves a mashing step, i.e., the biomass is mixed with warm water at 40 °C before being processed in a screw press for fractionation in a process called "integrated generation of solid fuel and biogas from biomass – IFBB" (Hensgen et al., 2011).

Grasses, for instance, have only a slightly lower calorific value than wood; however, they contain high concentrations of N, S, Cl, and K and ash, which can cause problems during combustion (Krenz and Pleissner, 2022b; McEniry et al., 2012). McEniry et al. (2012) indicated that the fractionation of grass via the IFBB process resulted in a slight increase of the calorific value of the press cake (±18 MJ/ kg DM) when compared to unfractionated silage (±17 MJ/ kg DM) while reducing its content of minerals, which were transferred to the juice fraction. These results were in line with two other more recent studies processing grass for roadside verges and from sports fields using the same IFBB process (Nitsche et al., 2017; Piepenschneider et al., 2016). Therefore, when using green biomass for combustion, the Green Biorefinery process not only would allow for better value creation from the biomass but would also result in a more suitable material for energy generation.

5. Sustainability aspects of green biorefineries

This chapter reviews sustainability analyses undertaken for Green Biorefineries and highlights the importance of including temporal aspects with such assessments as well as a higher number of impact categories. The conclusion of the techno-environmental assessment study of the Green Biorefinery concept is that increasing product yields can bring higher environmental benefits than lowering energy consumption. Under favourable market conditions Green Biorefineries are economically viable, even without supporting policy measures, while the key factor, influencing the purchase of bio-based products is their final market price.

5.1. Environmental studies

5.1.1. Production of raw material for Green Biorefinery

Parajuli et al. (2017) performed a Life Cycle Assessment (LCA) analysis of producing maize, grass-clover, rye grass, and straw from winter wheat as biomass feedstocks for biorefinery, which included the following impact categories: Global Warming Potential (GWP_{100}), Eutrophication Potential (EP), Non-Renewable Energy (NRE) use, Potential Freshwater Ecotoxicity (PFWTox) and Potential Biodiversity Damages (PBD). The rye grass, grass-clover and maize had the highest results for the NRE use (MJ eq/t DM) and GWP_{100} (in kg CO_2 eq, including contribution from soil carbon change) for the production of 1 ton of dry matter (t DM), while straw had the lowest results. The hotspots for the NRE use were related to the diesel use for field operations and agro-chemicals production, while the carbon footprint hotspots were associated with the nitrous oxide emissions and emissions related to the production of agro-chemicals (including N-fertiliser), and influenced by the inclusion of the contribution from soil organic carbon (SOC) changes (due to the SOC change around 35% of net Greenhouse Gas (GHG) emissions related to rye grass and grass clover were mitigated). The grass-clover, rye grass and maize had the highest results for the EP calculated per t DM, while straw had the lowest. On the other hand, straw had the highest values for the PFWTox (CTU_e/t DM), while maize had the highest negative impact to the biodiversity, due to the highest score for the PBD (expressed as Potentially Disappeared Fraction - PDF). Parajuli et al. (2017) has proven the importance of the inclusion of the higher number of impact categories for environmental assessment, due to the variations in ranking of the production of different biomasses using different environmental impact categories.

5.1.2. Techno-environmental assessment of a Green Biorefinery concept

Corona et al. (2018) conducted a techno-environmental assessment of the Green Biorefinery concept, combining process simulation and life cycle assessment at an early design stage. The study investigates alternative alfalfa-based Green Biorefinery product applications, such as human and animal-grade protein from juice fraction and animal feed or composite material from the press-cake fraction, with each product assumed to displace a conventional product. Using a combination of EPD (Environmental Product Declaration), ReCiPe (RIVM and Radboud University, CML, and PRé Consultants) and ILCD (International Reference Life Cycle Data System) impact assessment methods the following impact categories were included: GWP, EP, NRE use, Agricultural Land Occupation (ALO), and PFWTox. The study has proven that maximising product yield represents the most significant parameter for environmental optimisation, which can bring higher environmental benefits than lowering energy consumption, as it can be seen in the case when the products' protein content is raised through the use of either two-step fractionation, enhanced protein separation, or both. However, biological precipitation produces poor protein yields that cannot be substituted by a reduction in energy use, and this is the reason why after the biological precipitation an optimisation of the separation step should be implemented by using, for example, membrane technology. The usage of press-pulp was found to be another critical environmental optimisation parameter. When press-pulp is used instead of conventional materials like mineral wool, important energy-related impact categories savings are made (GWP and NRE).

5.1.3. Grass-based Green Biorefinery

5.1.3.1. Extraction of amino acids from grass silage. Prieler et al. (2019) conducted a comparative LCA analysis for different Green Biorefinery options, which focused on the extraction of amino acids (AA), from grass silage. Using a functional unit of 1 kg of amino acids produced, and the CML method of impact assessment, the study compared seven scenarios with comparison to reference products. The study found that the global warming potential per kg AA derived from Green Biorefinery ranged between 11 and 16 kg CO_2eq/kg AA, which was lower than some reference products such as skimmed milk powder and soybean isolate at 23 and 20 kg CO_2eq/kg, respectively. The integration of biogas within the biorefinery scenarios reduced the GWP related to electrical and thermal energy demand.

5.1.3.2. Proteins derived from fresh grass for application in chicken feed. Franchi et al. (2020b) investigated the sustainability of Green Biorefinery-derived protein feed derived from fresh grass, as an alternative to soybean meal, in which, the functional unit is a meal of chicken feed with the same raw protein content. In terms of feedstock for the biorefinery, 50% is assumed to be natural grass, which is available from maintenance of nature reserves, and 50% is cultivated grass. The consequences of by-products are taken into account by means of crediting. The results using ReCiPe End-Point environmental indicators, show a lower overall environmental impact for the grass protein scenario compared to the soy protein scenario, with over 100 times lower impact to Damage to Human Health and Damage to Eco-system, and also lower impact on Damage to Resource Availability. The process that appears to be most impactful in the grass biorefining is the cultivation of switchgrass. This means that if the percentage of cultivated grass could be reduced and replaced with an increased percentage of grass, the results could be further improved.

5.1.3.3. Silage grass fibre insulation produced through biorefining. In another study, Franchi et al. (2020b) compared silage grass fibre insulation produced through biorefining to stone wool insulation, by using one insulation panel of 1200 mm × 600 mm with a λ-value of 0.037 W/mK as a functional unit. The substitution approach was chosen for produced by-products (heat and electricity from residual streams of the fibre production). The comparative results were showcased as ReCiPe endpoint environmental impact categories, and they presented a favourable environmental performance from grass biorefinery insulation panels in comparison to stone wool insulation, including lower impact on Damage to Human Health, Damage to Ecosystems and Damage to Resource Availability.

5.1.4. Sugar beet leaves-based Green Biorefinery – RuBisCO protein extraction

Skunca et al. (2021) conducted the LCA analysis of the extraction and isolation of RuBisCO protein from sugar beet leaves associated with the GreenProtein project (funded by the Bio-Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme), which has established a demonstration plant in Dinteloord, the Netherlands. The environmental impact categories were calculated using the IMPACT 2002+ method. The functional unit (FU) was set to 1 kg of protein powder containing RuBisCO (87.72 kg of sugar beet leaves is transformed into a functional unit). For the whole system of RuBisCO protein extraction and isolation from sugar beet leaves, which included seven subsystems (milling and extraction (also included transport), heat treatment, centrifugation, microfiltration, ultrafiltration, chromatography, and spray drying), GWP results were 16.41 kg CO_2-eq., Ozone Layer Depletion (OLD) results were 1.21 mg CFC-11-eq., Energy Demand (ED) results were 205.24 MJ, EP results were 4.73 g PO_4 P-lim, Acidification Potential (AP) results were 620.76 g SO_2-eq., while Land Use (LU) results were 0.19 m^2 org. Arable. The study found that the main environmental hotspot was the usage of electricity, while a sensitivity analysis has demonstrated that mitigation options for optimization of environmental impacts should be focused on the energy pinch approach for spray drying.

5.1.5. Dynamic sustainability assessment tool

Timma et al. (2020) introduced a dynamic sustainability assessment tool, which combines temporal soil carbon modelling and system dynamics. The tool was validated through the case study for sustainability analysis of agriculture and Green Biorefineries supply chains in Denmark. The development of the Danish agricultural sector was simulated by examining three policy scenarios and evaluated according to the carrying capacity of the ecosystem until 2050, set as 1.4 livestock units per hectare. The first scenario (reference scenario), related to the behaviour of the system in the next 30 years under the current development and without any form of policy intervention, demonstrated that the agricultural sector would exploit the ecosystem beyond its carrying capacity soon after 2030. The second scenario, associated with the limitation of the animal production development by the defined ecosystems' carrying capacity, showed that the agricultural system will still exceed the carrying capacity due to inertia of the development and further reduction of the agricultural area. The third scenario, connected to the introduction of the biorefineries (within the carrying capacity), which would provide local protein from alfalfa for animal feed and substitute imported soy protein, demonstrated that there would be six times less land area available for alfalfa than required to deliver protein locally. In the Timma et al. (2020) study the soil carbon gains showcased the difference in results between using constant and temporal soil carbon modelling values, while the variable for impact assessment also contained time dynamics (from the system dynamics model), consequently showing the application for the dynamic sustainability assessment tool. The conclusion of the study is that the usage of the temporal aspects should be incorporated into sustainability assessments, since the results from the dynamic sustainability assessment tool presented a more precise projection of future development in comparison with the assessment using only constant soil carbon modelling values.

5.2. Economic aspects

In order to compare the economic viability of Green Biorefinery concepts to biogas plants, Höltinger et al. (2014) developed a mixed integer programming model, which is spatially explicit, and maximises total producer surpluses of Green Biorefinery supply chains depending on resource endowments by selecting optimal plant sizes and locations. The results of the model demonstrated that the Green Biorefineries could use significantly more biomass in comparison to biogas plants, leading to higher regional prices of feedstock. The Monte-Carlo simulation (which analysed impacts of uncertain model input parameters on model outputs) proved that the economic viability of Green Biorefineries largely depends on the selected process layout and the main products prices. The conclusion of the Höltinger et al. (2014) study is that under favourable market conditions Green Biorefineries are economically viable with average profits between €15 and €115 t^{-1} feedstock input, even when supporting policy measures are excluded. As noted by Höltinger et al. (2014) and Thiewes et al. (2019), the combination of multiple products is important to achieving the economic viability of a Green Biorefinery.

Gaffey et al. (2021) conducted the quantitative study in order to understand the purchasing intentions, motivations and drivers of 18–75-year-old Irish and Dutch consumers in relation to the bio-based products. This research also assessed the willingness to pay a "green premium", which is the extra price a buyer is willing to pay for the enhanced emotional or the strategic performance of the bio-based product in comparison to the price of the traditional product, which has the same technical performance (Carus et al., 2014). Results of the study proved that consumers in the Netherlands and Ireland have a relatively positive view in regard to the bio-based products, while the larger share of Irish consumers would prefer buying bio-based products than fossil-based products in comparison with Dutch consumers. In addition, consumers from Ireland have a slightly more positive perception that their choices can be valuable for the environment, and they are more willing to pay a green premium for bio-based products. In the Netherlands and Ireland, a large green premium is most likely to be paid for disposable products and cosmetics and personal care. The conclusion of the Gaffey et al. (2021) research is that the key factor, which influenced the purchase of bio-based products in both countries, was the price.

6. Future research and development activities

From this review, it is the viewpoint of the authors that progress is being made with regards to the development of Green Biorefinery processes to help resolve challenges which exist in the world today. However, the review also indicates that further work is required to optimize Green Biorefinery models and prepare them for real-world implementation. The authors therefore recommend a number of further research and development activities to enhance the development of the sector:

1. **Greater focus on waste feedstocks for Green Biorefineries** – This review has outlined the potential for inclusion of dedicated feedstocks as well as by-product or waste feedstocks for biorefineries. While some research activities have focused on by-product and waste feedstocks, the vast majority focus on dedicated feedstocks. In the view of the authors, by-product and waste feedstocks for Green Biorefineries should be explored more broadly, as they can offer significant benefits for Green Biorefineries including continuity of supply chain during certain unproductive months (e.g., when fresh grass will not be available), a low-cost opportunity to valorise waste streams, and the opportunity to reduce the overall environmental footprint of the model through inclusion of by-products and wastes.
2. **Greater research on value-added co-products**– The authors recommend greater research on potential value-added co-products and their applications to support the range of products produced by Green Biorefineries. While the majority of research and development in Green Biorefineries has focused on the production of bulk products (e.g., fibres and proteins) this review has already highlighted a range of extracted ingredients and products suitable for use in high value application such as nutraceuticals, cosmetics and health care. The integration of value-added materials, even in low volumes, can make a significant impact on the overall viability of biorefineries. In addition, this review indicates that further research is required to understand the potential of producing functional protein from widely available grasses and clover. The development and commercialisation of theses materials will help to improve the economic viability and uptake of these models.
3. **Integration of Green Biorefineries with renewable energy systems** – Some initiatives are already underway to explore the use of green feedstocks, such as grass silage, as a substrate for biogas production via anaerobic digestion. This review underscores the potential to integrate Green Biorefineries with anaerobic digestion, wherein products such as proteins or fibre-based materials may be extracted with the resulting by-product or residual streams being used a substrate or co-substrate for anaerobic digestion. This model on one hand ensures that the full potential of the biomass is delivered in various applications, but also helps to support a more sustainable system, as heat and electricity produced from biogas can be supplied to meet the biorefinery needs. The integration of these approaches may help to reduce pressure and dependency on governments to provide renewable energy subsidies for biogas production. From this review, the authors recommend that further work be undertaken to understand the potential of Green Biorefinery by-products as a co-

substrate with other waste materials, such as animal slurries or food waste. Furthermore, work should be undertaken to understand how such a model may benefit from the future integration of other forms of renewable energy, such as wind or solar.

4. **Greater research on sustainability aspects of Green Biorefineries** – Based on this review, the number of sustainability assessments for Green Biorefineries appears to be relatively limited, making it difficult to compare options for implementation. The authors recommend that further research, such as life cycle assessment along with studies focusing on economic and social sustainability aspects of Green Biorefineries, be undertaken to assess these options. Given the wide replication potential for this technology within pasture-based agriculture in many countries, it would be very useful to quantify the potential impacts or benefits related to national targets, such as emission reduction targets. In addition, the authors recommend that further work should be taken to improve the sustainability of Green Biorefineries, looking at various aspects including;

- Feedstocks (e.g., through integration of waste materials or multi-species swards grasslands)
- Technologies (e.g., integration of low emission and resource efficient technologies and renewable energies)
- Products (e.g., displacement of emission-intensive products on the market with more sustainable Green Biorefinery alternatives)

5. **Greater research on suitable business models and business cases** – From this review, there is little research looking at suitable business models and business cases for Green Biorefineries. This is an important aspect, since grasslands in many cases are farmer-owned, and are often under some current form of economic activity. Green Biorefineries, are a widely replicable bioeconomy model in Europe and globally, even at smaller economies of scale, and while they may complement certain agricultural sectors very well, in many cases they will require at least a partial diversification from existing agricultural activities. Understanding and assessing the different business models through which Green Biorefineries could be implemented, for example through co-operatives or privately owned models, is an important step towards implementation. In addition, the authors feel that the development of robust business cases to justify the transition for farmers and other stakeholders, is critical to supporting this change.

7. Conclusion

Green Biorefineries are a very promising pathway for enhancing the utilisation of green biomass derived from abundant sources, such as grasslands, as well as green crop residues. Using these approaches, grass and other green feedstocks can be converted in multiple feed, material and energy products using an integrated systems approach, which enhances its resource efficiency. Recent advances have shown great promise for Green Biorefineries to improve the protein efficiency of grasses, legumes and crop residues, to make more sustainable protein available for animals and humans, while co-producing beneficial high-value ingredients, as well as sustainable bio-based materials and energy for use in everyday life. In this way, Green Biorefineries can offer a particular opportunity for addressing sustainability challenges in grassland agriculture, while opening up new diversification opportunities for farmers. While the replication potential of these models is vast, based on the availability of feedstock, and the need for more efficiently produced materials and energy, further research is required to optimize these Green Biorefineries as integrated systems, which are economically and environmentally sustainable.

CRediT authorship contribution statement

James Gaffey: Conceptualization, Writing – original draft, Writing – review & editing. **Gaurav Rajauria:** Conceptualization, Writing – original draft, Writing – review & editing. **Helena McMahon:** Conceptualization, Writing – original draft, Writing – review & editing. **Rajeev Ravindran:** Conceptualization, Writing – original draft, Writing – review & editing. **Carmen Dominguez:** Conceptualization, Writing – original draft, Writing – review & editing. **Morten Ambye-Jensen:** Conceptualization, Writing – original draft, Writing – review & editing. **Macella F. Souza:** Conceptualization, Writing – original draft, Writing – review & editing. **Erik Meers:** Conceptualization, Writing – original draft, Writing – review & editing. **Marta Macias Aragonés:** Conceptualization, Writing – original draft, Writing – review & editing. **Dubravka Skunca:** Conceptualization, Writing – original draft, Writing – review & editing. **Johan P.M. Sanders:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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