Assessing the sustainability of emerging green biorefinery technology

A literature study

Cristian Wedgwood

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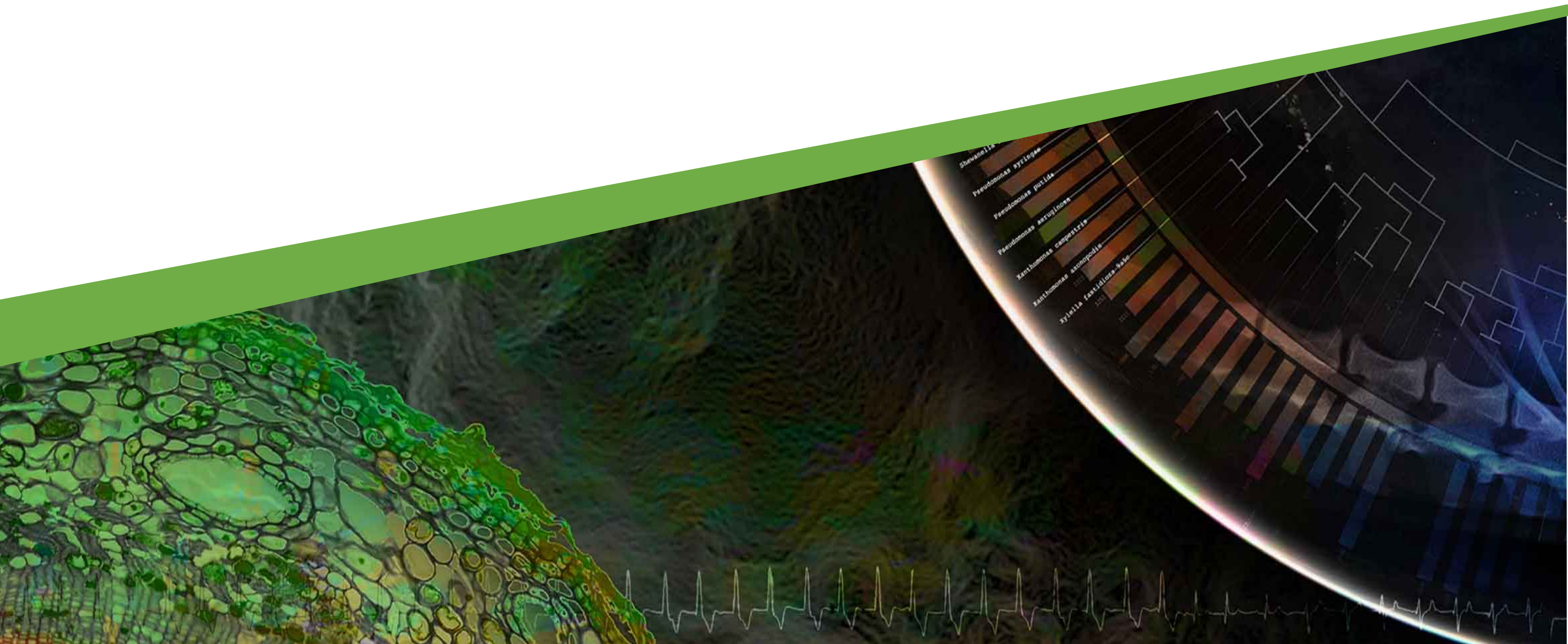

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Abstract

Green biorefinery (GBR) systems could play a pivotal role in enabling the transition to sustainable production systems by provisioning renewable bio-based product alternatives to existing non-renewable products, while avoiding increasing the pressure on environmental systems due to land use change free green feedstocks, such as crop residues, service crops and intermediate crops. Through the production of protein, for food or feed, biofuels, and fertilizers, GBR systems could provide a regional solution to address novel concerns of energy and food security driven by recent shocks to global supply chains. As an emerging technology, ongoing research into GBR system technology, covering process design, i.e. process sequence, operating conditions, and product development, i.e. secondary processing, expanding the product portfolio, and novel feedstocks is necessary. Technical, economic, and environmental analysis tools can help bridge the gap between innovation and market implementation and avoid unsustainable outcomes. Therefore, in addition to research into GBR innovations, there is a need for concurrent investigations into the technical, economic, and environmental performance of these systems and into tools that can synthesize these results into actionable insights.

Keywords: Green Biorefinery, Techno-Economic Assessment, Life Cycle Assessment

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1. Introduction

1.1. Biorefineries and the Sustainability Transition

As societies seek to transition to more sustainable systems of production and consumption, one strategy is to shift from product systems based on from finite extracted mineral and fossil fuel resources, to a system derived from renewable biological resources, e.g. plants, trees, fungus, algae (Richardson 2012). This approach however, is not without its challenges. **First,** biological resources are not de facto sustainable. In meeting societies growing demand for food (including the feed for the animals we consume), as well as forestry products, and energy, the pressure of modern biomass production systems on ecosystems lead to unsustainable outcomes such as deforestation, nitrogen pollution of water bodies, increased greenhouse gas emissions, freshwater depletion, among many others (Poore & Nemecek 2018; Crippa *et al.* 2021). **Second,** a shift to renewable bio-based alternatives would increase demand for biological resources, potentially driving further unsustainable outcomes from modern production systems, through competition with existing uses of biomass inducing land use change (Jeswani *et al.* 2020). In the long term, the unsustainable outcomes degrade the natural system's capacity to produce biological resources, undermining the sustainability of transition to bio-based alternatives (Nicolaidis Lindqvist *et al.* 2019). For this reason, a new generation of substrates is being developed to avoid the food vs. fuel dilemma and subsequent land use change (LUC). This new generation of LUC-avoiding agricultural feedstocks include crop residues, food processing wastes, additional biomass grown on abandoned or underutilized agricultural land, and service crops, i.e. crops grown for non-food purposes, whether to provide benefits to a farmer or to the environment (Prade *et al.* 2017a). **Third**, the popularity of polluting products systems is in part due to cost-effectiveness, e.g. cheap fossil fuels, and the utility provided by these materials, e.g. hygienic storage of food in plastic (Ghadikolaei *et al.* 2021; Nature Sustainability 2023). Therefore, in order to shift to renewable alternatives, the products must be competitive in terms of cost and the utility provided (Luchs & Kumar 2017). **Lastly**, while there are many valuable naturally occurring compounds in biomass, in order to produce similarly useful products, it is necessary to extract, transform and otherwise convert biological raw materials. Nowadays as oil refineries transforms crude fossil fuels into myriad fuels products commonplace in and critical to modern society, so too could the bio-based analogue, or biorefinery (Fernando et al. 2006).

In addition to the need to transition to sustainable production systems, recent shocks, such as war in Europe, have exposed vulnerability in global supply chains, particularly for energy, agri-food products and fertilizer (Arndt et al. 2023). While this has manifested in the development of alternative supply chains for fossil fuels and mineral products in the short-term, it has also concurrently spurred some European Union (EU) states to advance their development of renewable energy resources (Maliszewska-Nienartowicz 2024). In food systems, however, the EU has responded by delaying sustainability legislation, to better facilitate increasing production and to reduce burdens on producers, however, the European Commission (EC) the interdependence of sustainability and food security. Therefore, the EC encourages members to invest in efforts to reduce dependency on fossil fuels and fertilizers, including measures such as biogas production, carbon farming, and agro-ecological practices (Caprile 2022).

1.1.1. *Problem Statement*

In summary, efforts to enable a sustainable transition to a renewable bio-based production system should aim to (i) create cost-effective bio-based products that can (ii) match the demand and utility of existing non-renewable products, while (iii) improving the sustainability of existing biological production systems or at minimum, (iv) avoiding increasing the pressure on environmental systems through expanded production of biomass by (v) utilizing LUC free feedstocks. Additionally, efforts to enable a transition to renewable production should, if possible secure sources of energy, food, and fertilizer that minimize dependency on global supply chains.

From this perspective, biorefineries, particularly those designed to produce food, feed, fertilizer and biofuels are strategically positioned to play a critical role in achieving in part both of these priorities for future production systems. The aim of this paper is to review the biorefinery concept, particularly the green biorefinery, as well as introduce and review some of the tools used to identify which of these systems are promising for implementation and are in fact, sustainable.

1.2. What are green biorefineries?

The type of biorefinery this review is most concerned with is the green biorefinery concept as popularized by Soyez *et al.* (1998) and Kamm and Kamm (2004) as this concept includes use of emerging technologies that have the potential to produce renewable bio-based products and supply food, feed, energy, and fertilizer utilizing green LUC-free substrates. These substrates include both green, as in leafy, grass, or immature biomass and residues, wastes, or service crops, see Table 1.

Table 1: Potential sustainable green biorefinery substrates

Green substrates	**Sustainable substrates**
Grass	Grass or forage residues from land management, ecosystem services
Leaf biomass	Tuber/root crop residues (e.g. tops), low-quality leafy greens (e.g. kale)
Immature crops	Intermediate/cover crops

In the green biorefinery, the initial step is usually the mechanical or thermochemical disruption and fractionation of biomass into a protein rich organic juice and an organic fibre press cake/pulp. The process of fractionation, options for secondary processing, and potential products will be expanded on below in Chapter 2. However, the most common uses for the press cake is to be dried into pellets and baled for use as fodder, solid fuel, a raw fibre material, or substrate for chemical and biofuel production - via thermochemical or biochemical conversion processes. The press juice is concurrently separated further into valuable products and molecules, such as protein concentrates, which leaves a residual brown juice (Kamm & Kamm 2004). This juice can then be also used the production of biofuels and chemicals or used directly as a biofertilizer. In practice, many studies on protein green biorefineries focus on a single feedstock or a mix of similar feedstocks, e.g. grass mixes and forages. Additionally, depending on the treatment steps and secondary processing pathways selected, the range of platforms, processes, and products could expand far beyond that presented below.

1.3. Paper aims & structure

An early definition of the green biorefinery (GBR) implied that green biorefineries should aim to be sustainable, however, all production systems, even those considered sustainable, consume resources, involve emissions as well as trade-offs between outcomes of the three pillars of sustainability; economic, environmental, and social outcomes (Soyez *et al.* 1998). Additionally, within the GBR concept alone, there are numerous possible combinations of process, platform (the core link between feedstock and product), feedstock, and products (Cherubini *et al.* 2009). Systematically investigating emerging technologies considering technical, economic, environmental and social perspectives can provide an answer to the sustainability question as well as assist in identifying which potential GBR systems are promising for further development and implementation (Perrin *et al.* 2023). First, this paper will review GBR state of the art to determine what are the most prevalent GBR systems and emerging developments in process improvements, product developments, and secondary process integration including a review of examples from public and private sector trials. Second, this paper will review key aspects of technical sustainability assessments with a focus on tools for environmental, techno-economic evaluation in order to identify challenges to evaluating bio-based and emerging technologies, improvements to assessment methods, and lastly the recent publications on the results of previous sustainability assessments. Ultimately, the goal of this paper is to identify the current state of the art for GBR technological development as well as GBR economic and environmental performance, as well as identify any gaps in existing literature for expanding the sustainability assessments of GBR to integrate new process developments and feedstocks as well as sustainability assessment methods.

2. Green Biorefineries' world of platforms, processes, and products

2.1. The Green Biorefinery primary toolbox

Biorefinery processing begins when the harvester blade meets plant biomass as this is the moment when the operator begins the potentially long process from crop into product(s). Once the biomass reaches the GBR facility, however, the number of potential pathways for biorefining expand considerably. While it is not required, in practice, pre-treatment of plant biomass is commonplace to improve process performance. After any pre-treatment, the general process flow for protein fractionation methods follow the order; (1) separation of the protein rich organic juice from fibrous plant biomass, (2) precipitation and/or concentration of protein in the organic juice, and (3) if necessary, the separation of one or more protein fractions and possibly other compounds, i.e. chlorophyll, oligosaccharides, and phenolics (Moller *et al.* 2021; Pérez-Vila *et al.* 2024). There are a number of methods to separate protein fractions from biomass classified into the following types; physical – thermal and mechanical, biological, and chemical. ‘

2.1.1. *Common Protein Extraction Methods*

In a review on the extraction of protein for food/feed for non-ruminants, two general pathways for protein extraction from leafy biomass were identified, mechanical dewatering/juicing and alkaline extraction (Moller *et al.* 2021). In mechanical dewatering, the soluble proteins are separated in a juice from the initial plant biomass. While there are a number of methods to dewater biomass, in practice the usage of screw presses is commonplace in European pilot facilities (Moller *et al.* 2021). Prior to juicing, maceration of plant materials via various technologies can improve the performance of dewatering by disrupting cell walls (Moller *et al.* 2021). Screw presses can in effect both macerate and dewater plant biomass (Domokos-Szabolcsy *et al.* 2023). In alkali extraction, the pH of wet or dried biomass is raised to 7.5 increasing the solubility of proteins. This method is often paired with ammonium fibre expansion to disrupt cell walls (Bals & Dale 2011). Depending on the properties of the protein and biomass, other solvents can be employed (Kumar *et al.* 2021). In addition, there are a number of emerging technologies to assist solvent extraction methods, such as ultrasonic, microwave, pressure, and pulsed electric field. These emerging technologies are sometimes claimed to be sustainable extraction methods as they potentially avoid some of the drawbacks of conventional protein extraction methods, specifically, the use of harsh chemicals or energy intensive machinery (Herrero & Ibañez 2018). Multiple extraction methods may be employed concurrently, or in sequence to increase the gross protein yield and/or separate out undesirable solids, i.e. fibres, from the soluble fraction (Kumar *et al.* 2021). Additionally, undesirable and/or valuable non-protein soluble compounds, such as phenolics and can be extracted from the organic juice (Prade *et al.* 2021). The processes for separating other extractives overlaps with many of the solvent based methods used for protein separation. For example, when extracting dietary fibers, there are a number of extraction methods, both conventional and "green" extraction methods, although the most common method is alkali extraction (Dai & Mumper 2010; Buljeta *et al.* 2023).

2.1.2. *Green and White Protein Separation*

After extracting soluble proteins and other compounds "trapped" within the plant cells, and if necessary, separating out non-protein compounds, the resulting juice is further processed to concentrate or precipitate the proteins. In addition to dissolved solids, including proteins, the juice will often contain suspended solids, both insoluble proteins and other cell debris. The suspended solids can be removed from the juice using filtration (Corona *et al.* 2018b). Alternatively, soluble proteins can be coagulated using thermal, chemical, and biological methods. In the case of thermal coagulation, temperature is raised to between 60 to 80°C, whereas chemical and biological methods involve adjusting the pH with chemicals and lactic acid fermentation, respectively (Biswas & Purohit 2024). Once coagulated, the proteins can be separated from the juice with a centrifuge or filter. In order to fractionate separate proteins, e.g. "green" and "white" (named by the color of the fraction), differences in the coagulation properties of the two protein subfractions are used to coagulate the two fractions in sequence (Pérez-Vila *et al.* 2022; Biswas & Purohit 2024). For example, juice is first heated to 60°C to coagulate the green fraction that is separated out, via centrifuge, and then the remainder juice will be heated to 80°C or acidified to coagulate the remaining "white" protein fraction. Green protein, named due to its colour provided by the chlorophyll present in this fraction, tends to refer to chloroplastic proteins, whereas white protein tends to refer to cytoplastic proteins that remain after the green protein has been fractionated out. In theory, the green protein fraction contains mostly insoluble proteins leaving the remaining soluble cytoplastic proteins in the white protein fraction, however, in practice, green protein fractions often contain both soluble and insoluble proteins (Pérez-Vila *et al.* 2022). For the sake of clarity, cGPC will refer to the combined protein fraction from single stage fractionation, whereas GPC and WPC will refer to the respective, green and white protein fractions.

2.1.3. *Choice in the Fractionation Process & Emerging Topics*

The decisions made on feedstock, processes, process order and process operating conditions throughout the GBR system can influence which co-products are produced, at what quantity, and their quality. During the initial feedstock harvest and supply to the GBR, crop development, degree of maceration, time from harvest to process, and storage options such as ensiling will impact the subsequent processing in the GBR system and the product output and quality (McEniry & O'Kiely 2013; Andrade *et al.* 2024; Andrade *et al.* 2025). Across GBR publications, forage and grass feedstocks are one of the most commonly investigated, both fresh and ensiled (McEniry & O'Kiely 2013; dos Passos & Ambye-Jensen 2024). Additional feedstocks that have been used in the GBR process include harvest residues, such as sugar beet tops, kale and broccoli leaves, and intermediate crops such as cereals and oil crops (Tamayo Tenorio *et al.* 2016; de Visser & van Ree 2017; Muneer *et al.* 2021; Prade *et al.* 2021; Nynäs *et al.* 2024). Ensiling the biomass will both preserve biomass but also degrade the proteins into amino acids and produce lactic acid, lowering the feedstock´s potential as a food grade protein source (McEniry & O'Kiely 2013; Xiu & Shahbazi 2015; Perez Davila 2023). During the fractionation process, acidification as well as heat can denature proteins (unfolding of protein molecules), which impacts the structural functionality of protein products (Fiorentini & Galoppini 1983). In a comparison of the alkali and mechanical dewatering processes for protein separation, Bals and Dale (2011) found that alkali extraction had lower yields compared to the mechanical process. Repeated pressing of biomass can improve total protein yields, however, the quality of the protein decreases due to lowered digestibility, amino acid content on a dry matter basis, and dilution of total protein concentration (Hansen *et al.* 2022). This study also suggests that trial results at the lab scale are similar to results at the pilot scale. A recent review by Domokos-Szabolcsy *et al.* (2023) on green protein biorefineries found several studies agreeing with the potential for improving protein yields by repressing after adding water or alkali solution. In the case of alkali solutions, there is a risk of proteins denaturing if the pH rises above 8.0 (Domokos-Szabolcsy *et al.* 2023). A pulsed electric field applied prior to pressing of a grass mix increased juice and crude protein yield through electroporation, puncturing cell membranes (Guo *et al.* 2022). This was also tested at the pilot scale. While thermal precipitation carries a risk of damaging proteins due to overheating, thus negatively impacting functional properties, mechanical separation methods also generate heat and run the risk of overheating proteins (Domokos-Szabolcsy *et al.* 2023). Additionally, higher temperatures (over 35°C) during mechanical separation resulted in lower process yields (Domokos-Szabolcsy *et al.* 2023). In a study on the impact of biomass source and process parameters on fractionation, Nynäs *et al.* (2021),demonstrated that, in addition to variation between feedstocks, there was also variation in how feedstocks reacted to process conditions. This was present across separation and precipitation stages. For example, the authors found protein coagulation began at different pH levels for different feedstocks. A review of emerging techniques, Kumar *et al.* (2021), similarly found variation in optimal values for process parameters between different feedstocks. dos Passos and Ambye-Jensen (2024) found that alfalfa protein coagulation at 50°C was unfeasible, however temperature changes between 55°C, 60°C, and 65°C, led to different relative yields between WPC & GPC fractions, with a higher relative GPC yields at higher temperatures. Andrade *et al.* (2025) investigated the influence of the degree of maceration on protein, sugar, and lipid extraction, from different green biomasses, finding a higher degree of maceration did improve yields albeit at the cost of higher energy consumption.

While the general process scheme of pressing, precipitation, and protein separation is commonplace, there is still ongoing work to optimize the entire system in terms of harvest process, degree of maceration, number of pressings, use of chemical, biological or thermochemical methods to assist in protein extraction and coagulation, as well as whether to introduce additional fractionation steps to extract additional valuable compounds. These will impact protein and co-product yield as well as input use, such as electricity, chemicals, etc. Additionally, due to differences in optimal process conditions between feedstocks, as more feedstocks are considered for use in the GBR, feedstock specific investigations and optimizations could be required. Studies comparing fresh and ensiled biomass highlighted the benefits of year round production, enabled by ensiling, however, others have considered a mix of feedstocks to compensate for the lack of fresh biomass year round (O'Keeffe *et al.* 2011; McEniry & O'Kiely 2013; Cong & Termansen 2016).

2.2. The fractionation products – proteins & other coproducts

The protein fractionation system produces protein products alongside numerous other co-products. As discussed above, the exact co-products produced, their properties and their yields is dependent on the fractionation methods employed (Corona *et al.* 2018b; Nynäs 2018; Lubeck & Lubeck 2019; Balfany *et al.* 2023). Generally, protein fractionation systems produce a combined protein concentrate or separate green and white protein concentrates, a fibrous pulp/press cake residue, and liquid residues, brown juice. Additionally, various non-protein compounds within the plant biomass may also be extracted by the system to improve the quality of protein products and/or to generate a valuable side-stream, e.g. fibre or sugar extraction (Gaffey *et al.* 2023; Menon *et al.* 2024). Throughout the fractionation process, a number of intermediate products are created that if preferable to further processing, could be valorised directly. It is commonplace to lower the water content of the extracted protein products to help stabilize the product and decrease weight. In terms of the fate of the protein content in the initial biomass, the largest portion ends up in the pulp while the least ends up in the white protein fraction which has the lowest protein content of all the co-products in a 2 stage protein fractionation system (Nynäs *et al.* 2021; Prade *et al.* 2021).

In studies investigating the protein quality as food and feed, the concentrated protein fractions, i.e. the cGPC, GPC and WPC, had better protein digestibility than the pulp (Stodkilde *et al.* 2019; Nynäs *et al.* 2024). In the case of cGPC derived from alfalfa, better protein digestibility than the raw plant itself (Stodkilde *et al.* 2019). Across investigated plants and protein fractions, Stodkilde *et al.* (2019) found that their potential as feed to pigs and chickens was limited by their methionine and cysteine content, along with low lysine scores. Nynäs *et al.* (2024) presented amino acid profiles of different protein fractions and found suitable AA profiles and content in both GPC and WPC for use as animal and human food. Positive or at least neutral effects for animal digestibility and growth indicators of partially replacing soy and other feed ingredients, such as barley, with green protein concentrates derived from various feedstocks have been reported in trials on pigs, chickens, and cattle. In a feed trial of cGPC on pigs, the authors found that replacing soybean and sunflower meal did not adversely affect animal productivity (Stodkilde *et al.* 2023). Similarly, Ravindran *et al.* (2021) found that diets containing cGPC, partially replacing soy protein and barley, had positive effects on pig growth indicators. Stodkilde *et al.* (2021) found that at 8% cGPC inclusion, broilers were not negatively affected, unlike at other doses (16% and 24%), however, the authors highlighted the need for GBR process improvements to ensure a high protein content and improved digestibility of the cGPC. Others have investigated feeding the unprocessed green juice directly to pigs as a liquid feed. For instance, Keto *et al.* (2021) found no difference between experimental feeds including silage juice and the control diet across pig growth stage, meat quality, or pig gut health. There is potential for using protein concentrates as food or feed ingredients, however, studies on animal production have shown mixed results, as some noted the effect is negligible, or indicated the need for process improvements to improve protein quality (Keto *et al.* 2021; Stodkilde *et al.* 2021). Additionally, other studies have raised concerns about anti-nutritional properties found in protein fractions (Prade *et al.* 2021). As mentioned previously, one of the most common methods for protein precipitation results in the production of lactic acid, which can be recovered and valorized. Lactic acid is a well-established chemical building block with numerous applications in the food, chemical, cosmetic and pharmaceutical industries (Lubeck & Lubeck 2019).

2.2.1. *Residual By-Products*

Beyond the protein fractions, the pulp and brown juice are valuable by-products. Prade et al. (2021) determined that valorization of side streams was critical to overall GBR economic feasibility. Due to the high protein content of the pulp, there is a strong incentive to extract additional protein from this co-product. However, this pulp can be fed directly to ruminants or ensiled prior to feeding or further processing (Rinne 2024). Fibrous material is useful as feed for cattle and other ruminants, as they have the digestive capability to break down these recalcitrant materials. Fibres can also be useful as material. Nynäs *et al.* (2024) also found that the ratio of methionine and lysine, a critical feed quality indicator for lactating ruminants, was slightly above the recommended ratio in the pulp. Damborg *et al.* (2020) found that the pulp had similar in-situ rumen degradability, albeit with higher value of escape protein, indicating the pulp's value as a forage alternative. Kragbaek Damborg *et al.* (2019) tested the impact of protein fractionation, protein concentration, and different degrees of soybean meal substitution with GBR pulp on the quality of silage as feed for milking cattle. The authors found that partial substitution with cGPC did not negatively impact milk yield, dry matter intake or eating behaviour. Cattle fed with pulp silage mixes had higher milk yield, higher fat concentration, and higher protein concentration as well as improved digestibility of protein and fibre. Hansen *et al.* (2022) also tested ryegrass silage:cGPC feed ratios and similarly found, in general, grass pulp silage had improved fibre digestibility and protein value compared to unprocessed grass silage alone. Additionally, for late-harvested biomass, processing improved digestibility of the resulting feed whereas for early stage biomass there was no effect. Processing did not impact enteric methane emissions, however, crop developmental stage did. The study also investigated the effect of double pressing on pulp silage, finding single pressed pulp silage performed in between unprocessed and twice processed pulp silage. As an alternative to use as feed, existing grass-based biorefineries have utilized fibrous press cake to produce insulation materials (Gramitherm Europe SA ; Kromus *et al.* 2004; Corona *et al.* 2018a). The residual brown juice while commonly utilized for secondary processing, has been used as a fertilizer directly as well as to assist with the ensiling of pulp (Nyang'au *et al.* 2023).

2.2.2. *Valuable Carbohydrates & Extracts*

Proteins are not the only compound within green biomass that has potential value. Dietary fiber along with other valuable compounds such as oligosaccharides (OS) have positive health effects when ingested by humans. Insoluble dietary fiber have shown positive effects on human health regarding diabetes and obesity, while soluble dietary fiber (SDF) has for colon health (Rout & Srivastav 2023). Ravindran *et al.* (2022) extracted fructo-oligogosaccharides (FOS, fructan), from rye grass juice prior to investigating biogas potential of remaining juice. The Dutch company Grassa also advertises a FOS dietary supplement as one of their GBR products (Grassa). Menon *et al.* (2024), investigated the potential for extracting FOS from brown juice for use as a pre-biotic. The authors found that the plant-derived FOS performed equally well as commercial FOS, e.g. synthesized via enzyme from sugar monomers. Oligosaccharides are carbohydrates composed of between 10 and 20 sugar monomers. They have numerous potential applications dependant on the type of oligosaccharides, from use for positive human health effects, such as promoting gut health and improving digestion as a pre-biotics, to use as food ingredient as a sweetener. Also their functional properties are of value, e.g. as gelling agent, and some are used for in pharmaceutical or cosmetic applications or used as binders in agricultural applications. The most common extraction methods for plant-derived oligosaccharides is water extraction or extraction with another solvent, such as ethanol (Patel & Goyal 2010). Plant phenolics are a broad category of compounds from including lignin, flavonoids, carotenoids, and tannins, which depending on the specific phenolic have potential for positive impact on human health or as building blocks for the production of various chemicals or bio-based materials. Solvent extractions are common, however, there is no universal extraction for all plant phenolics, due to the variety of phenolics, in terms of composition, degree of polymerization, association with other plant components, etc. (Dai & Mumper 2010).

2.2.3. *Brown Juice as Fertilizer*

In a report on the potential of grass GBR by products as substrate for biogas and other applications, Feeney *et al.* (2019) investigated the performance of brown juice, or grass whey as the authors refer to it, as directly applied fertilizer to a field. The authors suspected that brown juice may have biofertilizers potential due to residual nitrogen content and a potential biostimulant effect. In comparison to applications of manure, slurry and the control with no treatment, the manure treatment performed best, with brown juice a close second and both outperforming no treatment. Grassa, a Dutch company advertises a plant based fertilizer from the concentrated brown juice (Grassa). A commonplace practice for grass pelletization plants was to dewater biomass similar to the GBR concept and spread the residual juice as fertilizer (Andersen & Kiel 2000).

2.3. The toolbox for secondary processing

The possibilities for secondary processing of the GBR co-products grow beyond the relatively limited array of processing options commonly seen in the fractionation process for proteins. Both the pulp and brown juice are rich in carbohydrates, polymers and oligomers/monomers, respectively. When considering the full range of possibilities for processing carbohydrates via fermentation the array of platforms, processes, and products is extensive (Fernando *et al.* 2006; Calvo-Flores & Martin-Martinez 2022). Depending on the desired product (or mix), various thermochemical (e.g. pyrolysis, gasification or hydrothermal), or biochemical conversion processes (e.g. anaerobic or aerobic fermentation) can be employed (Cherubini *et al.* 2009; Gaffey *et al.* 2023). The toolbox for secondary processing is broad, therefore, the following sections will highlight only the possibilities generally followed by examples from GBR literature. Additionally, many of the conversion processes yield a mix of co-products that are interesting for biofuel and/or chemical synthesis (Bulushev & Ross 2011; Calvo-Flores & Martin-Martinez 2022). As with protein fractionation processes, the selected process, process sequence, and processing conditions play important roles in determining product yield and product quality. Additionally, as is the case for fermentation pathways, the choice of microorganism (bacteria, yeasts) determines the chemical and fuel outputs. In a review on "drop in" biofuels, i.e. biofuels interchangeable with existing fossil fuels, Kargbo *et al.* (2021) identified the primary pathways (across all feedstocks) as gasification to Fischer-Troph synthesis, pyrolysis to upgrading, hydrothermal liquefaction, and fermentation. The US Department of Energy identified hundreds of potential chemical building blocks and synthesis pathways, however, only 14 were identified as the most promising for developing a bio-based alternative (Werpy & Petersen 2024). These 14 were derived via syngas conversion, to H_2 or methanol, or sugar fermentation reactions to various C1-C6 precursor chemicals including carbon dioxide (C1), methane (C1), ethanol (C2), lactic acid (C3), succinic acid (C4), levulinic acid (C5), HMF (C6) (Calvo-Flores & Martin-Martinez 2022; Werpy & Petersen 2024). Of these, carbon dioxide, methane, and ethanol all have applications as fuels, or precursors to fuels.

2.3.1. *Thermochemical Conversion Processes & Products*

The primary processes used for biofuel and/or chemical production are thermochemical (dry) technologies, such as pyrolysis and gasification, or emerging wet hydrothermal technologies.

The advantage of thermochemical processes is that it converts all biomass constituents including lignin, considered more recalcitrant and less readily degradable (Bulushev & Ross 2011; Tekin *et al.* 2014). One of the drawbacks of pyrolysis is the low moisture content requirement, between 10 to 20%, which necessitates drying wet feedstock prior to ignition (Bulushev & Ross 2011). Additionally, dry thermochemical pathways are also energy intensive due to the high heating requirement to between 300-1000°C (Bulushev & Ross 2011). Pyrolysis can produce a liquid, called pyrolyic oil, tar, or bio-oil, a solid, or char, and gas mixture of carbon monoxide, hydrogen, methane, and carbon dioxide or syngas. The pyrolytic oil requires upgrading before it can be used as a fuel (Bridgwater 2012). Gasification, a higher temperature process, produces a gas that requires conditioning into syngas, as well as char. The sequential combination of gasification after pyrolysis can improve the quality of outputs, particularly the char and liquid (tar), while increasing the syngas yield of the process (Shah *et al.* 2022). The resulting char, called activated char, has a higher porosity and improved performance for non-fertilizer uses such as a feed additive or filtration (Ravenni *et al.* 2023). Compared to gasification and pyrolysis, hydrothermal liquefaction (HTL) conversion is carried out at relatively lower temperatures, 120-250°C, and allows the conversion of wet substrates (Tekin *et al.* 2014; Chaturvedi *et al.* 2020). Hydrothermal co-products are primarily a char, referred to as hydrochar, an waster-insoluble liquid, called bio-crude or bio-oil, an aqueous phase, and gas. HTL co-products must undergo a series of filtration and extraction with ether and acetone to fully separate out the solid char, a heavier acetone soluble bio-oil fraction and a lighter ether soluble bio-oil fraction from residual processing water (Tekin *et al.* 2014). HTL bio-crude can be further refined into hydrocarbons or valuable chemical precursors. Syngas from gasification, pyrolysis, and HTL is both a precursor from liquid fuels but also fuel additives, alcohols, and hydrocarbons (Tekin *et al.* 2014; Chaturvedi *et al.* 2020). Pyrochar, gaschar, and hydrochar (referring to the thermochemical process source) have numerous applications across chemical, water treatment, agricultural and other industries. Chars are interesting for use in agriculture as soil amendments, a store of carbon, and in the case of activated char, a feed supplement. Additionally, chars can be processed further to improve functional properties (Ravenni *et al.* 2023; Sani *et al.* 2023).

Investigations into the thermochemical conversion of GBR by-products from various feedstocks (forages and grasses) and have considered HTL, sequential HTL and AD, as well as sequential pyrolysis and gasification. While authors reported successful processing and high energy yield in products, they also identified trade-offs between biofuel product quality and losses of carbon to process water in the case of HTL or lower quality char in the case of sequential pyrolysis and gasification (Toor *et al.* 2022; Ravenni *et al.* 2023; Zoppi *et al.* 2023). Additionally process conditions, such as set temperature, feedstock moisture content (in the case of pyrolysis), the degree of maceration and in one study the crop maturity, all affected process outcomes. For HTL processing, pulp must be mixed with either brown juice or residual process water (Toor *et al.* 2022; Zoppi *et al.* 2023). Addition of HTL before AD reduced nitrogen flows to digestate with nitrogen ending up in the bio-crude and hydrochar, albeit with high carbon flows to biofuel products, bio-crude and biogas (Zoppi *et al.* 2023). In the case of thermochemical conversion, while there is the potential for utilizing GBR by-products, the selected process, process conditions, and sequence must be matched the feedstock and desired product mix. These studies reveal numerous tradeoffs between biofuel production and digestate or char production. Additionally, authors found trade-offs between energy consumption and the quality of biofuels produced.

2.3.2. *Lignocellulosic Fibre (LCF) & Carbohydrate Potential, Fermentation Processes, & Products*

In order of abundance in nature, primary constituents of plant fibres are cellulose, lignin, and hemicellulose (Calvo-Flores & Martin-Martinez 2022). While processing cellulose yields a structure is consistent across sources and methods, lignin and hemicellulose are more varied across sources and processing methods (Guadix-Montero & Sankar 2018; Calvo-Flores & Martin-Martinez 2022). Cellulose, of different purities, is valuable for various material applications or for further refining into smaller crystals, nanocellulose. Additionally, however, cellulose can also be used as feedstock for C6 (referring the number of H bonded to the carbon) sugar platform biorefining. While lignin is a valuable chemical feedstock with numerous applications both direct use, the traditional methods for separating out hemicellulose and cellulose produce a lignin, called technical lignin, which is high in impurities and sulphur content. This makes further processing difficult, therefore technical lignin is using combusted, however, new approaches are attempting to separate high-quality lignin without the downsides of technical lignin. Hemicellulose is used as a feedstock for C5 and C6 sugar platforms, and for various fuel and material applications (Calvo-Flores & Martin-Martinez 2022). Additionally, oligosaccharides can be produced by depolymerizing hemicellulose, e.g. xylan into XOS. While some fermentation process set ups simultaneously breakdown and ferment of complex carbohydrates, such as in simultaneous saccharification and fermentation, it is common to pre-treat the biomass to degrade the LCF into simple C5 or C6 sugars, to facilitate better fermentation performance (Kumar & Sharma 2017; Chaturvedi *et al.* 2020). In the case of simple sugars, however, such as those found in GBR brown juice, direct fermentation is possible without pre-treatment. As noted above, the choice of which bacteria to use in the reaction will determine the outputs and yields as well as process conditions. Additionally, some microorganisms have a preference for either C5 or C6 sugars, as is the case for traditional yeasts used for ethanol production. There is ongoing research into developing new bacterial strains to ferment both C5 and C6 sugars for ethanol, as well as other targeted compounds (Chaturvedi *et al.* 2020). While oligosaccharides have been extracted directly from plant biomass, FOS are typically produced using bacterial cultures (Patel & Goyal 2010).

Biowert has successful developed two material products from LCF, although they disregard protein fractionation entirely, directly processing grass to separate out cellulose, which is used to reinforce recycled polypropylene or polyethylene in extrudable or injection mouldable composite plastic polymers. Additionally, they previously marketed a grass fibre insulation material, although this is now unlisted on the company website (Biowert Industrie GmbH ; Schwinn 2019). Grass fibre insulation viability is unclear, however, as a separate producer still markets a grass fibre insulation product in the EU (Gramitherm Europe SA). Pihlajaniemi *et al.* (2020) investigated the production of single cell protein (SCP) from grass silage press cake using various pre-treatments to improve hydrolysis of fibre prior to SCP production. Others have investigated the production of ethanol using hydrolysed fibres. Xiu and Shahbazi (2015) in a review on green biorefineries identified that US investigations into GBR was driven primary by the desire to valorise protein by-products from the bioethanol production from grasses and forages. Thomsen *et al.* (2004) investigated the using brown juice as fermentation medium for L-Lysine at both the lab and pilot scale with a view towards upscaling to industrial production. Lubeck and Lubeck (2019) highlighted the potential for lactic acid fermentation to be applied to pulp and brown juice fractions. Andersen and Kiel (2000) investigated using brown juice as a medium for lactic acid fermentation. Andrade *et al.* (2023) simulated the sequential filtration then fermentation of brown juice to produce ethanol and improve process performance. While most research into LCF or sugar fermentation of GBR by-products have largely focused on ethanol or lactic acid production, there are emerging options for protein fermentation and when considering the full spectrum of fermentation possibilities, there is still many unrealized potential pathways. Fermented brown juice has also been investigated as a potential fertilizer or biostimulant (Bákonyi *et al.* 2020; Barna *et al.* 2021). Results from these experiments indicated that application of low concentrations of brown juice <2.5% led to significant improvements in seed germination indicators, as well as growth indicators, i.e. root and shoot length, number of leaves, compared to unfermented brown juice and the control (tap water).

2.3.3. *Anaerobic Digestion for Biogas & Digestate*

Anaerobic digestion (AD) is a multi-stage reaction that breaks down complex organic material before synthesizing intermediate chemicals and ultimately biogas, a mixture of carbon dioxide (Schnürer & Jarvis 2018). The bacteria used in anaerobic digestion reactions, can be sensitive to changes in reactor environment. Therefore carefully managing process conditions (temperature, loading rate, etc.), and formulating input substrate mixture (macronutrients, carbon/nitrogen ratio, dry matter content, trace elements, etc.), feeding rate, among other factors, can be essential to successful AD. Additionally, excessive production of ammonia NH+ in the AD reactor, from protein rich substrates, as well as the presence of lignin and other compounds, can produce inhibitory effects in the reaction (Schnürer & Jarvis 2018). Numerous reactor set-ups for AD are used across different industries and substrates to account with relatively wet or dry substrates (Vasco-Correa *et al.* 2018). Over the course of the AD reaction, various intermediates, such as volatile fatty acids, are produced which can be extracted and valorised (James *et al.* 2021). The primary products of AD are a biogas mixture of carbon dioxide and methane as well as residual organic material, called digestate. Biogas can be burned directly in a combined heat and power plant (CHP), however, often biogas is upgraded by removing carbon dioxide which results in a biomethane comparable to natural gas (Vasco-Correa *et al.* 2018; Chaturvedi *et al.* 2020). This biomethane can be used as a biofuel or in chemical processes. Alternatively, biomethane can compressed, for injection into a gas grid or use as a vehicle fuel. Alternatively, can be liquefied at low temperature to use as transportation fuel (Bauer *et al.* 2013; Vasco-Correa *et al.* 2018). While it is commonplace to the release carbon dioxide from the upgrading process, there are many promising alternatives for carbon storage or utilization in industrial processes (Bauer *et al.* 2013; Cordova *et al.* 2023). Lastly, the residual digestate is commonly used as a fertilizer directly due to the residual nitrogen, phosphorus, and potassium (N:P:K) content, however, further processing of digestate is also possible. For example, raw digestate is frequently separated into a N rich liquid fraction and a solid fraction (Carraro *et al.* 2024). In addition to the provision of N, recirculated digestate to fields can be a source of organic C with positive effects on plant productivity and soil organic carbon levels (Wang & Lee 2021). Additionally, digestate can be used as a feedstock for thermochemical conversion into char, bio-oil, etc. or other processes (Vasco-Correa *et al.* 2018; Wang & Lee 2021),

In addition to determining the biogas potentials of GBR pulp and brown, lab and pilot scale AD trials have also investigated numerous feedstocks, both fresh and ensiled, co-digestion of pulp and brown juice, digestion in different reactor types (anaerobic filter, UASB, and CSTR), as well as characterized the residual digestate to identify potential as a fertilizer (Delavy & Baier 2005; Martinez *et al.* 2018; Santamaria-Fernandez *et al.* 2018; Larsen *et al.* 2019; Feng *et al.* 2021; Nyang'au *et al.* 2023). The results of these trials show that the brown juice is most readily degradable, although if an acid precipitation process is used the pH needs correcting, whereas pulp was least productive and requires addition of water but co-digestion improved AD performance (Santamaria-Fernandez *et al.* 2018; Ravindran *et al.* 2022). Overall, GBR processing decreased the total methane yield compared to digesting unprocessed biomass by 50-81% varying by biomass source, however, additional extraction of FOS from the brown juice did not decease BMP substantially (Santamaria-Fernandez *et al.* 2018; Ravindran *et al.* 2022). Of the N:P:K entering biorefineries, a large share of these elements end up in the digestate, indicating potential as a fertilizer (Santamaria-Fernandez *et al.* 2019). In the case of nitrogen, despite N losses due to protein fractionation, over half input N ends up in pulp and brown juice and subsequently digestate, however, efforts to increase protein yields would reduce nitrogen flows to digestate. Feng *et al.* (2021), demonstrated the possibility of digesting brown juice, leaving protein separation at 60°C, in an anaerobic filter without additional heating. Ensiling pulp, with or without brown juice added, improved methane production when monodigested or co-digested with manure (Larsen *et al.* 2019; Nyang'au *et al.* 2023). Digestate from GBR pulp and brown juice, both fresh and ensiled, had high mineral N composition at 43% of total N content, and better N mineralization than controls (Santamaria-Fernandez *et al.* 2019; Nyang'au *et al.* 2023). Ravindran *et al.* (2022) found that due to low nitrogen levels but relatively high potassium, digestate from GBR by-products could be particularly interesting for potassium deficient soils. In addition to the lab or pilot trials of biogas and digestate potential, various commercial projects have integrated biogas production into the GBR system. Biowert co-digests residual organic material after cellulose extraction from grass into biogas, consumed onsite in a CHP, and a marketed biofertilizer *AgriFer (Schwinn 2019)*. This fertilizer has a N:P:K content at 0.65:0.22:0.20 in % content, low even compared to reported values from Ravindran *et al.* (2022), albeit with a very high percentage of plant available nitrogen at 72% of total N. In summary, numerous positive results exist for the potential of utilizing AD to produce biogas and digestate, however, there are particular process considerations for digesting pulp and high acidity brown juice, although ensiling, co-digestion both fractions or with manure may mitigate this. Little research has explored secondary processing of biogas and digestate fractions from GBR substrates, although other publications indicate existing GBR digestate products or the injection of biogas into the Danish gas grid (Schwinn 2019; Martinsen & Andersen 2020).

2.4. In summary – Primary & Secondary Processing in Green Biorefinery

Ultimately, as one of the aims of sustainable biorefining is to develop novel products or sustainable alternatives to existing products, therefore research must go beyond investigating what is possible and identify what is promising for commercial implementation. When considering what is theoretical possible to extract from green plant biomass, as well as produce via secondary refining, especially with fermentation, the number of possible combinations of feedstock, platform, processes, and products is impressive, but intractable. Reviewing the state of biorefinery processing, both primary and secondary, as well as possible products, has identified certain trends and challenges. In terms of feedstock, most studies and projects utilize forages or grass substrates, while some considered ensiling either the fresh biomass or pulp residues. There is limited research on alternative feedstocks identify promise for further investigation. The protein process while a common core of processes is present, there is still ongoing investigation into increasing process feasibility by increasing protein yields or extracting additional valuable fractions. Additionally, there is variation in the types of precipitation and protein filtration, as well as the final product and applications mix. Considering the secondary processing of GBR pulp and brown juice, while the potential is broad, in practice most research has been on fermentation pathways including ethanol, biogas, and other product fermentations, such as proteins and amino acids. Recently, investigations into thermochemical processing have demonstrated the viability of these techniques for GBR secondary processing, however, there are numerous trade-offs in the implementation. However, despite lack of clarity as to which secondary processing technique or process conditions are optimal, it is clear that fuels, fertilizers, and chemicals can be viably produced from GBR by-products. By restricting the GBR possibilities from the theoretically possible to what has been identified in research, the problem of identifying promising GBR concepts remains complex, however, it is more manageable. By applying tools to analyse the technical, economic, and environmental performance of potential GBR systems, it is possible to bridge the gap between basic lab research and commercial deployment, or at least guide that process to better outcomes.

3. Assessing the Sustainability of Biorefinery Systems

This chapter is focused primarily on tools for assessing sustainability of biorefineries, i.e. the technical, economic, environmental, and to a lesser extent social aspects. In practice, a sustainability assessment of technology or products should include each of these perspectives. However, the scope of this paper is primarily on the methods for technical, economic, and environmental analysis, although boundaries between tools are often blurred. The common tools of sustainability assessment are techno-economic analysis, financial and socio-economic analysis, and life cycle assessment (LCA). These are well-established methods, often with corresponding industry or international standards and methods. In research, however, these tools are evolving and there are numerous variants and expansions to these approaches. Section 3.1 discusses the primary tools for evaluating environmental sustainable, Section 3.2 discusses the LCA framework with a focus on considerations and challenges for biorefinery and bio-based material LCAs, while section 3.3 reviews technical and economic evaluation techniques. Section 3.4 describes tools for integrating technical, economic, and environmental analysis and other emerging tools for circularity and absolute sustainability assessment. Finally, sections 3.5

and 3.6 review the results of previous studies on the economic and environmental impact of GBR systems.

3.1. Life Cycle Assessment & Other Methods for Evaluating Environmental Sustainability

Life cycle assessment is a ubiquitous tool for evaluating the environmental impacts. The general framework used in academia, governments, and in the private sector is set in ISO 14040 (2006) and requirements and guidelines are set in ISO 14044 (2006). Within the field of life cycle assessments, or broadly methods that use a life cycle perspective, the tools have expanded to consider socio-economic indicators and circularity as well as linking to concepts such as carrying capacity and planetary boundaries (Bjørn *et al.* 2020; Cilleruelo Palomero *et al.* 2024). Other methods used in the quantification of environment impact are indicator methods, footprint methods, as well as hybrid economic-environmental costing methods (discussed in section 3.4). Indicator methods, including the footprint family of indicators, focus on one or a handful of environmental indicators and can easily be integrated with LCA impact assessment methods (Pawelzik *et al.* 2013; Matuštík & Kočí 2021). The life cycle perspective can also be applied in other analyses, e.g. economic, social, etc., without following the specific framework of the LCA tool. Environmental footprint methods, are a family of single topic indicators, the most prominent covering carbon, water, ecological (related to much publicized bio-capacity concept), energy, nitrogen, phosphorus, biodiversity, and land (Čuček *et al.* 2015). Generally, the footprint methods, while popular in scientific communication, e.g. usage in calculating Earth overshoot day, are less developed than LCA as tools and lack consistent definitions and methodologies across resource indicators (Matuštík & Kočí 2021). LCAs on the other hand are standardized, well known, used extensively across academia, and among other public and private stakeholder, and can easily integrate footprint indicators or other indicators. Therefore, the remainder of section 3.3 is dedicated to expanding on the LCA tool, key components, challenges of LCAs applied to biorefineries, emerging technologies, and novel approaches to address the shortcomings of the LCA method. Beyond LCA and indicator methods, there are other environmental economic tools that attempt to quantify the monetary value of costs and benefits of environmental impacts, sustainability indexes, and optimization methods (Čuček *et al.* 2015).

3.2. LCA Framework

Each LCA is composed of 4 phases: (i) goal and scope, (ii) inventory, (iii) impact assessment, and (iv) interpretation conducted in an iterative process, progressively reviewing and revising each stage as the analysis is conducted. For the purpose of this section, components of the LCA method are discussed only as they pertain to key considerations for LCA applied to biorefineries (Ahlgren *et al.* 2015; Gaffey *et al.* 2024a) and biobased materials (Pawelzik *et al.* 2013), see Table 2.

Table 2: Key considerations for biorefinery and bio-based material LCAs organized by LCA phase

LCA Phase	Gaffey *et al.* (2024a)	Ahlgren *et al.* (2015)	Pawelzik *et al.* (2013)
Goal & scope	• Functional unit • System boundary	• Goal definition • Functional unit	• Land use • Allocation

Inventory	• LCA type • Allocation • Product use & end of life • Inventory data	• Allocation - output • Allocation – feedstock	• LCA type
Impact Assessment	• Method/indicators • Land use change • Biogenic carbon	• Biogenic carbon • Timing of emissions • Land use change	• Biogenic carbon • Soil organic carbon (SOC) • Water use • Soil degradation
Interpretation	• Sensitivity & uncertainty analysis		• Assessment framework

3.2.1. *Goal Definition, Functional Unit, System Boundary, LCA Type & Allocation*

In the first phase, key attributes of an LCA are defined; the purpose, which determines the scope, the type of LCA, consequential or attributional, the system boundaries, the functional unit, and reference flow. The first methodological consideration is the goal definition and research question formulation, as this determines both functional unit, which defines the function of the system, and LCA type, which in turn influences allocation and system boundaries (Ahlgren *et al.* 2015). When investigating biorefineries, the goal may be to investigate the best use of land or feedstocks, the impact of a biorefinery product or products, or identify the impact of the biorefinery (Ahlgren *et al.* 2015). For each of these goals, the functional unit would be defined differently, in terms of feedstock quantity or production area, product quantity, product function, such as energy content, or a multi-functional unit, a biorefinery or product mix (Ahlgren *et al.* 2015; Gaffey *et al.* 2024a). In the case of GBR studies, single functional units applied have covered feedstock, e.g. cultivation area (Karlsson *et al.* 2015) or input feedstock (Corona *et al.* 2018b), product, e.g. quantity of cGPC (Skunca *et al.* 2021; Khoshnevisan *et al.* 2023; Chan *et al.* 2024; Gaffey *et al.* 2024b), animal feed (Franchi *et al.* 2020; Stodkilde *et al.* 2023), or amino acid (Prieler *et al.* 2019), product function, e.g. animal growth (Cong & Termansen 2016; Tallentire *et al.* 2018). Many of the protein quantity functional units, also included a quality parameter, such as cGPC with a minimum protein content, or dry matter content. Parajuli *et al.* (2017a) and (Parajuli *et al.* 2018), both used a multi-functional unit, ethanol energy content and lactic acid quantity, and cow and pig weight, respectively. LCA type, attributional or consequential, is determined by what type of question the study seeks to answer, whether that is an accounting of the impact associated with a product or system (attributional), or the consequences of taking a policy decision (consequential), such as the opening of a GBR (Bjorn *et al.* 2018b). Gaffey *et al.* (2024a) found that ALCA were most commonly type of biorefinery LCA, followed by CLCA and then studies using both methods concurrently. These different approaches drastically change results: Parajuli *et al.* (2017a), compared both types applied to a lactic acid and ethanol GBR and found the CLCA method resulted in substantially lower emissions than the ALCA. The discrepancy of results between LCA types is driven in part by differences the data used in CLCAs and

ALCAs and methods to delimit study system boundaries and handle multi-functionality (Bjorn *et al.* 2018b).

System boundaries determine what is and isn't included in a study and range from the narrow factory entry to exit, or "gate to gate," to the full life cycle of raw material production to product disposal, or "cradle-to-grave." Gaffey *et al.* (2024a) found that "cradle-to-gate" were the most common system boundary followed by "gate-to-gate" applied in biorefinery studies indicating that the actual product use and disposal is frequently disregarded. Additionally, there are two components to an LCA system, the foreground, as in the object of the study, i.e. product or system, and the background, the secondary production systems providing raw materials to the foreground (Bjorn *et al.* 2018b). The second consideration for system boundary is whether the system should include avoided emissions from products displaced, or increased environmental burdens from production necessary to produce feedstock displaced to the biorefinery system, i.e. land use change, see below. In the case of ALCA, these avoided products and opportunity costs are disregarded. As many of the products and co-products of biorefineries are developed specifically to replace unsustainable alternatives, these credits can substantially improve (lower) the environmental impact for GBR systems, even bringing the net impact below zero emissions, as in net avoided emissions (Corona *et al.* 2018b).

Another consideration in defining system boundaries between ALCA and CLCA, is how to handle multi-functional or multi-product systems. As most biorefineries are multi-functional or multi-product, in order to fairly compare the environment impact of a single product to a multi-product system if possible, the system is expanded to add multi-functionality to the single product system. It is also possible to subtract a system to make the multi-functional system, a single product system (Ahlgren *et al.* 2015; Gaffey *et al.* 2024a). Multi-functionality applies to feedstock production systems as well, as cropping systems produce both harvestable product, the primary economic activity, and crop residues, sometimes classified as a co-product, others as a waste. While system expansion is preferred over allocation for ALCA and required for CLCA, system expansion can be difficult with agri-food systems as most alternatives are also generated by multi-product systems, e.g. straw and grains (Dominguez Aldama *et al.* 2023). This also raises a challenge with avoided product consumption, as a decrease in consumption of a multi-functional product will decrease the supply of the secondary products. Therefore, when quantifying avoided emissions credits, consideration of secondary feedback effects due to multi-functionality is necessary (Bjorn *et al.* 2018b). For example, soybean meal is co-produced with an oil, so reductions in soybean meal consumption leads to increase in palm oil production to compensate for lost soy oil supply (Dalgaard *et al.* 2007). Allocation between products is ideally determined on a physical relationship between co-products, such a relative mass yield or energy content, or if not, with an economic relationship(Bjorn *et al.* 2018b). Others have proposed using alternatives factors, beyond mass or energy, as the basis for allocation. For example, Brankatschk and Finkbeiner (2014) proposed allocating impacts in agricultural LCAs on the basis of Cereal Unit, a composite measure of the protein and energy content of feeds. Michiels *et al.* (2021) used the relative N content in the allocation of fertilizer products. Parajuli *et al.* (2017a) used economic allocation due to the difficulty of using a physical relationship between due to different relevant physical unit for co-production of energy (bioethanol, biogas, energy in CHP) and mass products (lactic acid, protein, silage), as is the case for many biorefineries. Khoshnevisan *et al.* (2023) similarly used economic allocation factors in their LCA of cGPC, although they also calculated mass and energy factors, for comparison. Allocation of impacts to cGPC was 74%, 3%, and 14% in the case of economic, mass, and energy allocation, respectively. The large variability of these allocation factors highlights that for ALCAs of protein GBRs, selection of suitable allocation method is necessary to avoid under- or overestimating associated environmental impact. Gaffey *et al.* (2024a) found that for biorefineries economic allocation was the most common method, followed by mass, or a mix of both. The allocation of impacts between products from primary system and wastes, such as with cropping systems and residues, has often been considered as wastes, defined by ISO 14044, as intended for disposal absent intervention (Dominguez Aldama *et al.* 2023). This has been used as justification for zero allocation of impacts from the primary system to wastes, other than impacts incurred in the collection and processing of waste. The accuracy of applying zero allocation to wastes for crops, is controversial as residues have value as sources of nutrition as feed, however, there is still not a universally accepted position on this. Additionally, removal of residues can reduce soil carbon inputs which should be accounted for in carbon balances, see 3.2.3 below (Ahlgren *et al.* 2015). The final distinction between ALCA and CLCA is in the data required. As CLCA and ALCA have a different set of goals, they require different sets of data. As CLCA assess the consequences of a decision, they use marginal production data, e.g. which producers increase/decrease to changes in demand. ALCA, on the other hand, use average production data (Bjorn *et al.* 2018a). Table 3 summarizes the key distinctions between ALCA and CLCA.

Table 3: Differences between CLCA & ALCA in data used and handling of multi-functionality or avoided products

Topic	Consequential LCA	Attributional LCA
Multi-functionality	System Expansion Only	Expansion, then allocation
Avoided Products, Opportunity Costs	Included	Depends, if needed to address multi-functionality
Data Used	Marginal	Average

3.2.2. *Inventory Data*

After defining goal, system boundary, functional unit, LCA type, the next step of the LCA is to gather necessary inventory data, that is the inventory of inputs, outputs and emission flows associated with the provision of the functional unit. Data used in LCAs range in specificity/quality from very high, as direct measurements or scaled, high, derived from modelling, to medium, process/location specific database or data from literature, low, generic database values, and very low, expert judgement (Bjorn *et al.* 2018a). The challenge in LCAs, including studies on biorefineries, is quality data can be difficult or time-intensive to collect, therefore in practice, lower quality data can be used to supplement high quality data. Gaffey *et al.* (2024a) found that only 28% of biorefinery studies included some primary data, while half used modelling tools, while nearly all studies used database or literature values. While modelling tools are prevalent in biorefinery studies, compared to chemical refining, tools are relatively immature. Considering GBR studies, some authors use primary data (Andrade *et al.* 2023; Khoshnevisan *et al.* 2023; Andrade *et al.* 2025), some used a mixture of primary data with other techniques (Prieler *et al.* 2019; Gaffey *et al.* 2024b) while some relied on models supplemented with literature values (Karlsson *et al.* 2015; Cong & Termansen 2016; Corona *et al.* 2018a; Corona *et al.* 2018b; Parajuli *et al.* 2018). Finally a few studies relied on process or country specific databases and literature values for foreground systems (Parajuli *et al.* 2017a; Parajuli *et al.* 2017b; Chan *et al.* 2024) although in the case of Chan *et al.* (2024), the author cited their own previous work that used primary data. Section 3.2.5 will highlight the specific challenge of data collection for emerging technologies.

3.2.3. *Land Use Change, Soil Organic Carbon, Biogenic Carbon, Removal Rates*

The utilization of biomass with existing applications, such as food or feed, as substrate can induce additional biomass production elsewhere to compensate for the change in demand, so called land use change (Tonini *et al.* 2015). There are two types of land use change, direct land use changes (dLUC), observable changes in a system, and indirect land use change (iLUC), the market induced changes by increases/decreases in biomass demand (Ahlgren *et al.* 2015)(Ahlgren et al. 2015). Direct LUC such as from the conversion of forest to agriculture can increase emissions. However, in practice not all LUC is negative, studies on the shifting of agricultural system to introduce a more sustainable cropping system can have positive effects on environmental indicators such as increasing soil organic carbon (Parajuli *et al.* 2017b; Prade *et al.* 2017b) or mixed effects (Cong & Termansen 2016). dLUC, are observable and can be directly included in the assessment, however, the challenge with iLUC is that changes are unobservable, therefore modelling is required (Ahlgren *et al.* 2015). There are numerous approaches for integrating iLUC emissions, into LCA impact assessment methods (Pawelzik *et al.* 2013; Tonini *et al.* 2015). While Ahlgren *et al.* (2015) identified iLUC as key consideration in biorefinery studies, Gaffey *et al.* (2024a) found only 8% of biorefinery studies included dLUC and another 8% included iLUC calculations, although the majority of CLCA included iLUC effects. Part of the reason for disregarding iLUC is due to uncertainty in measurements, debate as to whether it is real, and concerns about double counting (Pawelzik *et al.* 2013; Ahlgren *et al.* 2015; Gaffey *et al.* 2024a). In GBR studies, some evaluated a direct land use change to cropping system, (Cong & Termansen 2016; Parajuli *et al.* 2017b), some considered iLUC explicitly (Parajuli *et al.* 2018; Khoshnevisan *et al.* 2023), while some assumed productivity increases could compensate for increased biomass demand (Karlsson *et al.* 2015), some did not mention LUC (Prieler *et al.* 2019; Skunca *et al.* 2021), however, that is likely due to a limited scope or a small set of process performance indicators (Ravenni *et al.* 2023; Gaffey *et al.* 2024b; Andrade *et al.* 2025).

Related to the question of land use change, are the paired considerations of soil organic carbon and biogenic carbon, as in the carbon stored in soils and biomass (and bio-based products), respectively (Pawelzik et al. 2013). Both act as carbon sinks and sources of carbon. As biomass grows, carbon is absorbed from the atmosphere, stored in plant components, both above and below ground and ultimately, when the plant is harvested, any residual plant biomass degrades releasing carbon into soils and the atmosphere. Traditionally, biogenic carbon emissions to the atmosphere are considered neutral, or as zero net change, as carbon incorporated into the plant will equal carbon eventually emitted (Pawelzik *et al.* 2013; Ahlgren *et al.* 2015). One of the challenges with biogenic emission neutrality, is that in the context of biorefinery products, the period between incorporation into biomass and eventual degradation ranges from short-term, e.g biomass, to long-term, e.g. construction materials (Ahlgren *et al.* 2015). Gaffey *et al.* (2024a) found only 7% of biorefinery studies considered biogenic carbon flows, whereas the rest assumed neutrality. (Ahlgren *et al.* 2015) distinguished 3 categories of biogenic carbon cycles, (i) LUC, emissions, and sequestration, (ii) bioenergy carbon cycle, and (iii) biogenic storage in products. Only in cases of bioenergy production, accounting for biogenic carbon can be omitted. Additionally, while from a system perspective biogenic carbon emissions may be neutral over time, the removal of biomass can lead to a local decrease in SOC. Studies on crop residue utilization in biorefineries identified potential negative effects from the residue removal on soil quality, through loss of SOC and increased nitrogen emissions (Cherubini & Ulgiati 2010; Cong & Termansen 2016; Barrios Latorre *et al.* 2024a; Barrios Latorre *et al.* 2024b). There are a number of methods for incorporating SOC and biogenic calculations into LCAs (Pawelzik *et al.* 2013). Biorefineries, however, can enable circular flows of nutrients, for example, through digestate and biochar application, back to soils potentially compensating for losses from biomass removal. Therefore, such affects are necessary to account for when determining the life-cycle emissions of biorefinery systems.

One of the goals for sustainability of bio-based materials is to avoid degrading the productive capacity of natural systems, however, in the context of biomass removal and LUC, losses of SOC, chemical degradation, such as nutrient loss, and the demand for more substrates, a critical question is; what rate of biomass removal can satisfy sustainable feedstock demand while maintaining ecosystem quality? Therefore it is necessary to determine the sustainable rate of biomass removal, as defined by the rate at which residues can be removed without negatively affecting the long-term health of agricultural systems, i.e. zero net SOC losses. Additionally, research is ongoing into strategies for increasing SOC levels, such as the incorporation of intermediate crops into crop rotations, to enable higher sustainable rates of biomass removal (Barrios Latorre *et al.* 2024b). One factor complicating carbon circularity is that while ploughing down crop residues or cultivating intermediate crops as green manure may be beneficial to soil fertility, this form of carbon contributes less to SOC than more recalcitrant forms of carbon. Others have taken research into sustainable rates of biomass removal, a step forward to compensate for biorefinery co-products, such as char and digestate flows back to soils. Andrade Díaz *et al.* (2023) explored biofuel biorefinery systems to determine if co-product application, i.e. char in thermochemical processes and digestate in AD pathways could compensate for biomass removal and enable increased residue utilization. Barrios Latorre *et al.* (2024b) investigated the removal of intermediate crops and/or residue integrated with AD production and digestate application to soils to determine effects to SOC. While there was spatial variation in their results accounting for local factors such as soil characteristics, both found C rich by-product incorporation, and in the case of Barrios Latorre *et al.* (2024a); Barrios Latorre *et al.* (2024b), addition of intermediate crops into crop rotations could enable higher rates of residue removal without losses to SOC (Andrade Díaz *et al.* 2023).

3.2.4. *Impact Assessment Method*

There are numerous methods for calculating environmental impact, some specializing in one or several impact categories. Additionally, as noted above, indicators developed independent of

LCA methods can be easily incorporated into the LCA tool. Many of the most prominent impact assessment methods consider numerous midpoint environmental impact categories, i.e. land use, water use, GHG emissions, eutrophication, acidification, the link in the chain of damages from potentially hundreds of emission flows, to calculate a handful of endpoint categories, broad aggregate categories such as damage to environment, human health, etc. (Rybaczewska-Błażejowska & Jezierski 2024). The trade-off between endpoint and midpoint methods is that while the composite endpoint indicator provides ease of interpretation, it sacrifices transparency into the individual impact categories, risking shifting environmental burden between impact categories. Other trade-offs include higher uncertainty in endpoints as well as risks from inaccurate weighting of midpoints in the calculation of endpoints (Bare *et al.* 2000). Calculation methods, impact category definition, geographic range, etc. vary across methods for impact assessment. This variation leads to difficulty in comparing results generated with different methods, although in some cases the relative ranking of results has remained consistent despite differences in calculation methods and in turn, emission values (Rybaczewska-Błażejowska & Jezierski 2024). Gaffey *et al.* (2024a) found global warming potential (GWP) was almost universally used, followed by other common categories, in order of decreasing use, such as eutrophication potential, acidification potential, human toxicity, eco-toxicity, resource depletion and ozone depletions. The land use category or land occupation, was used in a quarter of biorefinery LCAs, while the impact land transformation (or LUC) impact category was included in only three (Gaffey *et al.* 2024a). The challenge with limiting the number of impact categories as well documented trade-offs may be ignored in an assessment. Additionally, as is the case with land use change or biodiversity, there can be a lack of consistent definition, lack of adoption in LCA studies, or omissions in indicator methods (Pawelzik *et al.* 2013; Winter *et al.* 2017; Gaffey *et al.* 2024a). (Pawelzik *et al.* (2013) identified biodiversity inclusion in bio-based material LCAs as a key consideration. While some of shared drivers and pressures on biodiversity outcomes are included in calculations for common impact assessment categories, such as GHG emissions, or acidification, others such as ionising radiation consider only human health (Winter *et al.* 2017). Similarly, others have raised concerns about inadequate consideration freshwater use and other biotic indicators in bio-based LCAs (Pawelzik *et al.* 2013; Nicolaidis Lindqvist *et al.* 2019). While standard impact assessment indicators cover a broad range of impacts and estimation methods, studies must consider whether a selected indicator or set of indicators is suitable for the study object and goal.

3.2.5. *Prospective Assessment of Emerging Technology*

According to the IEA Bioenergy organization, green biorefineries are emerging technologies with a TRL as between 5-7, or the technology demonstration phases (Annevelink *et al.* 2022). Therefore, LCAs of GBRs must anticipate the environmental impact of a future commercial GBR. The typical LCA is *ex post* or conducted on existing technology, however, there is a subset of LCA methods that have adapted for the unique challenges of evaluating emerging technology. The ex-ante LCA, which is a subset of prospective LCAs, is an LCA tailored to evaluating the potential future impact of a product's or service's life cycle, at such a time when it is an established technology. Many challenges for conducting an ex-ante LCA arise from needing to model the production system of interest at a point in the future (Piccinno *et al.* 2016).

Prospective LCAs expand on the estimate future production parameters to also predict the future state of background conditions (e.g. electricity production mix – nuclear, solar, wind, gas, oil, coal) and predict future comparable alternative technologies (Piccinno *et al.* 2016; Thonemann *et al.* 2020). Additionally, *ex ante* LCAs exacerbate existing challenges in conducting traditional LCAs such as comparability of studies , functional unit, impact assessment, as well as issues with data availability, quality, scaling up process data and uncertainty (Thonemann *et al.* 2020). Due to a general lack of production data There are a number of methods for scaling up lab or pilot data, or simulating a process with engineering software, among many others (Piccinno *et al.* 2016; Thonemann *et al.* 2020). Parvatker & Eckelman (2019) evaluated methods for estimating inventory data for chemical processes, characterizing approaches from highly accurate but data and time intensive, such as process simulations to least accurate but with low data or time requirements, such as using proxies. Concluding that there was no universal methodologies, the authors instead provided a decision tool for selecting the appropriate method Erakca *et al.* (2024). When modelling future background systems, authors often rely on existing projections for things like electricity supply, or increasing technology efficiency, however, some background processes lack data on future conditions (Thonemann *et al.* 2020).

3.2.6. *Circularity and Absolute Life Cycle Assessment*

One critique of the traditional LCA is that it fundamentally is about efficiency or producing the most with the least amount of emissions. It can identify the comparatively better system, the portion of global emissions associated with a system, or the consequences of implementing a system on emissions, however, it makes no determination of whether the product is fundamentally unsustainable (Bjørn *et al.* 2020). Two approaches address this shortcoming by (i) considering circularity of flows in an LCA or (ii) determining the absolute sustainability of a system. Circular economy approaches address sustainability by attempting to maximize the material/nutrient flows that are recycled in a system and minimize the use of virgin raw material. Cilleruelo Palomero *et al.* (2024) analysed indicators of circularity, circular index and material circularity indicator (MCI), in activities throughout the popular EcoInvent database and applied these results to a case study to determine the degree of circularity of a product, providing a novel approach to quantifying the ratio of virgin material and waste produced to product yield. Gallo *et al.* (2023) proposed an approach to integrating a circularity assessment phase into an LCA by utilizing standard energy and waste indicators collected for Environmental Product Declarations (EPD) to calculate MCI.

Absolute life cycle assessment conceptually is an LCA where the environmental impact is bounded within a limited space for emissions, represented by planetary boundaries. In other words, if society is to hold emissions to below safe limits, what share of scarce emissions can be generated by an activity. This introduces a novel dimension to the LCA, that there is a total limit to emitting activities and therefore, a limit to the amount of the activity being studies. There are two core challenges when conducting the absolute LCA; (i) determining the limit of polluting activities and (ii) determining what share across all polluting activity should be permissible for a single industry or activity, e.g. share of GDP, utility to society (Bjørn *et al.* 2020). Absolute LCA is an emerging topic, albeit an important one as it integrates assessments of limited scale, such as a single product, to a macro-scale goal, e.g. keeping pollution within levels to achieve below 1.5°C warming. A 2020 review of absolute LCA studies, identified cases where was absolute environmental impact approaches were applied to analyse a future product in future background conditions (Bjørn *et al.* 2020).

3.3. Technical, Financial, Cost-Benefit & Other Methods for Evaluating Technical & Economic Feasibility

3.3.1. *Techno-Economic Analysis*

Techno-economic feasibility assessment is a tool for evaluating emerging technology in terms of both technical feasibility and preliminary economic viability. The value of early stage assessments is to identify early on barriers to operation, possible sizing given limitations such as capacity and feedstock availability, product yields and wastes/by-products, and whether a project is promising, or at minimum what factors limit feasibility (Van Dael *et al.* 2015). Van Dael *et al.* (2015) proposed a standard methodology for techno-economic assessment (TEA) that includes an iterative cycle of market study, designing process diagrams for calculating energy and mass balances, economic evaluation, risk and possibly extension into environmental analysis. The parameters for the TEA are split between the technical; *inputs*, *technology*, *output*, and the economic; *investment costs* as in capital expenditures (CAPEX), *operational costs* (OPEX), *revenue,* and *financial*, as in tax, interest, etc. OPEX includes variable costs, that vary with production rate and fixed costs that are independent of production rates. Van Dael *et al.* (2015) recommend that a sensitivity or risk analysis is undertaken to account for uncertainty of early stage values. Louw *et al.* (2023) reviewed TEAs of biorefineries and identified the typical technical factors considered were feedstock, product types, conversion processes, downstream processing, number of products, integration, centralisation, novel plant or expansion, energy source, and scale. Estimating variable costs usually requires significant effort, however, this is necessary as ultimately variable costs can make up a large proportion of total operating costs (Turton *et al.* 2008). The final step of a financial analysis is to use estimated costs and revenues for a project to calculate common financial indicators to evaluate projects/investments, such as net present value (NPV), return on investment (ROI), internal rate of return (IRR), minimum acceptable rate of return (MARR), payback period, and minimum selling price (MSP), see Table 4.

Table 4: Definitions of common project financial indicators

Term	Definition	Term	Definition
NPV	Present value of all future revenues, discounted with the discount rate	MARR	Internal benchmark discount rate that if IRR > MARR, investment is made
ROI	Ratio of the present value of total revenues and costs	Payback period	Time required for investment costs to be repaid
Discount Rate	% defining time value of money, as in money today > money tomorrow	MSP	Minimum price for products that results in IRR > MARR
IRR	Discount rate % that results in an NPV of 0		

3.3.2. *(Socio-) Economic Analysis*

Although often used interchangeably, the critical difference between financial analysis methods and economic analysis is that financial analysis generally focuses solely on the financial performance from the perspective of the owner/operator, whereas economic analysis considers the perspective of society. For clarity, it is best to refer to economic analysis as socio-economic analysis to highlight this distinction. This distinction manifests in a number of ways, most importantly, the inclusion of opportunity costs, externalities, such as pollution, but also in the disregard of transfer payments (taxes and subsidies), the present value of future cash flows, even the prices used to calculate input costs (Martinsen & Andersen 2020). While financial costs and revenues are a significant part, the analysis extends to non-cash benefits and costs, such as opportunity costs, including those that are realised outside the primary actors in an activity, individual producers and consumers, so-called externalities. Once all financial revenues and costs have been converted to socio-economic benefits and costs and externalities, opportunity costs, and other aspects have been estimated, the social net present value of all benefits and costs is calculated. In theory, societies tend to have a long-term perspective prioritizing maintaining economic activity over time, which is reflected, in a lower discount rate than the private sector (Sartori *et al.* 2014). In their 2014 guidelines for evaluating projects for public financing, the EC considers the results of both financial and economic analysis. Specifically, public financing is given to projects that have a negative financial NPV (net losses to company) but a positive economic NPV (net benefit to society), as in projects that are not able to self finance but have positive society benefits (Sartori *et al.* 2014). Conversely, combining financial and socio-economic assessment can identify projects that may be interesting for an investor but detrimental to society at large.

3.3.3. *Life-Cycle Perspective on Economic Performance*

Life cycle costing is the evaluation of the total costs of a system across the life cycle of a project, giving an estimate for the total cost of ownership (TCO), including the cost to dispose. There are three types of life cycle costing (LCC), conventional LCC, environmental/external LCC, and societal LCC. The conventional LCC, is analogous to the financial analysis considering a single actor perspective, such as a business operator or consumer and including only internal/private costs and benefits, whereas environmental LCC, and society LCC, begin to integrate external factors, such as cost of pollution, or cost to society, into the life cycle cost perspective. These extensions to LCC are analogous to environmental economic or socio-economic analysis, respectively (Rödger *et al.* 2018).

3.4. Integrated TEA & LCA, spatial tools and dynamic modeling

Sustainability assessment of biorefineries usually include an assessment of feedstock potential for conversion and supply, quantifying process mass balances and flows, and lastly impact to sustainability components (Parajuli *et al.* 2015). For example, the techno-economic and environmental assessment used by the IEA Bioenergy Tast 42, consists of two parts, description of biorefinery including mass and energy balances along with costs, followed by a value chain assessment, essentially a cradle-to-gate assessment of cumulative energy demand, GHG emissions along with profits (Bacovsky *et al.* 2023). The previous sections, have introduced and reviewed critical tools and frameworks for the design and assessing economic and environmental sustainability. While it is valid to investigate technical, economic, and environmental sustainability separately, and synthesize results at a later stage, approaches for integrating LCA and TEA have been developed and applied, including for early stage technologies (Mahmud *et al.* 2021). The challenges with directly integrating TEA and LCA are the lack of established software tools, lack of coherence on scope, i.e. TEAs very site specific, while LCAs consider a broad life-cycle, functional units, and lack of data on early design stages, however, the methodological challenges can be overcome. Pérez-Almada *et al.* (2023)identified the potential for integrated environmental and techno-economic assessment framework for biorefineries to leverage the power of technical process design, modelling and optimization with economic and environmental life cycle data to understand the trade-off between process design choices and economic and environmental outcomes. There are numerous approaches to biorefinery design with the most prominent being conceptual design and superstructure optimization. While each framework takes a different approach to identifying promising biorefinery process systems, both already integrate economic and extending this to include environmental data is possible (Aristizábal-Marulanda & Cardona Alzate 2018). García-Velásquez *et al.* (2018) applied an integrated TEA and LCA approach in to evaluate different levels of integration in energy biorefineries, calculating economic and environmental across four scenarios of an increasingly integrated, in terms of energy and mass flows, biorefinery. Two of the weaknesses of LCA approaches for integration with integrated TEA and LCA is the lack of absolute sustainability assessments and regionalized LCA impacts, however, as discussed in 3.2.7, the absolute LCA can address this challenge (Pérez-Almada *et al.* 2023).

3.5. Economics Performance of (Green) Biorefinery Systems

3.5.1. *Multi-product Systems*

At the end of the 20th century, in the United States biorefineries were proposed as a solution to help rural communities achieve full long-term employment by providing the crucial transformative step between agricultural production and the economy's demand for diverse products beyond foodstuffs (Van Dyne *et al.* 1999). In theory a "phase III" biorefinery could adapt to shifting demands for products by adjusting processes and yields to optimise for a different output mix and also compensate for varying supplies of feedstocks (Van Dyne *et al.* 1999). In practice, having multiple product lines increases complexity but can protect the business performance from market fluctuations. However, in one case, a LCF biorefinery found that feedstock supply and composition was functionally constant. Therefore, changes in the output mix and volume of the primary products was not possible (Rodsrud *et al.* 2012). This created an issue as demand for each of the product was not in sync with each of the fixed output volumes (Rodsrud *et al.* 2012). For many biorefinery systems, a multi-product approach is the only way to generate sufficient revenues to break even (Gaffey *et al.* 2023). Additionally, Prade *et al.* (2021) identified the need to develop additional product streams to improve the feasibility of protein GBR systems. The optimization model in Höltinger *et al.* (2013) indicated while the revenues of the most promising grass GBR system, i.e. technical fiber production, was predominately from a single product stream, the runner up GBR system, i.e. amino acid production system, generated notable income streams from multiple product categories, both materials and energies, a true biorefinery. Most prominent concepts for GBR systems, both at the pilot and commercial, pursue the valorisation of multiple product streams, although at the pilot scale, this is primarily a mix of cGPC, lactic acid, biogas and digestate (Kromus *et al.* 2004; O'Keeffe *et al.* 2012). Commercial endeavours for GBR produce a mix of materials and/or biofuel products (Grassa ; Schwinn 2019).

3.5.2. *Promising GBR Processes & Systems*

While Höltinger *et al.* (2013) considered protein GBRs the third best process system for GBR deployment in Austria, this system relied on an earlier protein GBR concept where the GBR system was dependent on the provision of pulp to pelletization drying plants, i.e. sale of fibre for pelletization was the largest constituents of protein GBR revenues. While Prade *et al.* (2021) highlighted the low protein yield as a drawback to GBR systems, recent investigations on extensive mechanical maceration of feedstocks agreed with earlier research by Bals and Dale (2011) which showed that mechanical protein fractionation was an efficient processing technique (Andrade *et al.* 2025). Andrade *et al.* (2025) successfully increased protein yields through more advanced maceration albeit with high energy consumption. Additionally, a pair of studies in from Ireland indicated the potential for protein GBR deployment, with both grass and silage GBR performing similarly in the economic assessment (O'Keeffe *et al.* 2011; O'Keeffe *et al.* 2012). Theses study also cast doubt on whether more advanced processing after protein fractionation, in this case lactic acid extraction, was justified due to higher energy demands and costs. These studies did raise the issue that fresh grass systems lack year round supply of biomass, instead showing a preference for stable supply of ensiled biomass. This agreed with earlier findings by Kromus *et al.* (2004) on the challenges of fresh biomass utilization in GBRs. Cong and Termansen (2016) proposed an alternative approach, the shifting of biomass from fresh to ensiled in colder months. They found protein GBRs yielded positive economic outcomes for both pig farmers and refinery operators due to decreased feed costs and biorefinery profits from the sale of biogas, insulation fibres, and feed. In their comprehensive report on the economic performance of GBRs for feed (protein and fibre), and biogas, the authors analysed the financial and socio-economic performance of small and large-scale systems (Martinsen & Andersen 2020). The financial analysis, indicated that small-scale facilities are profitable, whereas large-scale are not as the investment costs per unit input was unexpectedly higher for larger facilities. This was due to the assumption that small-scale facilities required investment in expanding reactor capacity at existing facilities, whereas large-scale facilities would require construction of a novel plant. These results highlight that extensive investment in additional processing options can result in a net loss to the system despite the creation of an additional product stream. This is particularly significant for the Danish case, as public subsidies for biogas production are a significant part of the reason for the positive economic for the small-scale plant. The authors also note that due to low margins, even minor fluctuations in revenues or costs could result in a negative economic result. When converting the financial analysis to a socio-economic analysis, both cases produce negative results, indicating that while the small-scale plant may have limited value to investors, it is questionable as to whether society and governments subsidize GBRs, at least those that fit the assumptions of this study. In a recent optimization study in Sweden on the potential of a shift to grass GBR feedstock cultivation by farmers as a response to low value cereals with grasses for GBR, the authors found positive economic results for both farmers, from feedstock sales, and plant operators, from feed sales and substrate sales to AD (Balaman *et al.* 2023). These results, however, were dependent on low grass price, and unlike Martinsen and Andersen (2020) did not include compensation to farmers to shift production to grasses or the investment cost of biogas plant, instead generating an income stream from substrate sales. These results highlight, that the conditions for a switch to GBR substrate cultivation (induced by unfavourable cereal prices and subsidies for grass cultivation) and the lack of competing demand (maintaining low prices) for GBR substrates may factor into the successful deployment of GBRs. Additionally, externalizing the cost (and revenues) for secondary processing could improve profitability in some cases.

3.5.1. *Novel Feedstocks*

In addition to the grass and grass silage, economic studies have considered harvest and processing residues of kale and broccoli leaves, as well as various intermediate crops; buckwheat, phacelia, hemp, oilseed radish (Muneer *et al.* 2021; Prade *et al.* 2021). Of the harvest residues, kale is considered most promising, as there is a need for technical develop in the co-collection of broccoli tops to avoid additional on field costs (Prade *et al.* 2021). Muneer *et al.* (2021) considered the breakeven cost required for GPC for different intermediate crops, grown with and without fertilizer. All crop varieties grown in the 2018 season, produced a GPC breakeven price below the threshold value of 2€ per kilogram for bulk protein sales. GPC produced in the 2017 season, however, required a breakeven price above the threshold, indicating that these proteins would need to be marketed as high value proteins, up to 10€ per kilogram. While these studies indicate the potential for alternative iLUC free feedstocks for GBRs, they also highlight that developing novel feedstocks may require concurrent development of best practices and innovations in cultivation and harvest methods.

3.5.2. *2 Stage Fractionation for GPC and WPC*

In additional to novel feedstocks, both Muneer *et al.* (2021) and Prade *et al.* (2021) considered 2-stage protein GBRs, with the sale of WPC, GPC, and pulp. While the economic analysis Muneer *et al.* (2021) indicated that at least in some cases, i.e. 2018, a 2-stage GBR could sell GPC at bulk prices (2€ per kilogram) and compensate for the net loss from WPC and pulp sales alone. Prade *et al.* (2021) compared the performance of the 2-stage GBR with a 1-stage GBR for cGPC and a GBR system for milling dried biomass into a food additive powder. For both harvest residues, the 2-stage GBRs were not economically viable due to low white protein yields and performed worse than the 1-stage GBR. However, neither of the protein GBR systems produced profits. Only the milling of kale produced a profit. These results indicate that a second protein product stream does not necessarily compensate for additional costs varying in part by process performance and feedstock choice.

3.5.3. *Research Gaps*

Existing research on the economic feasibility of protein GBR systems is marked by somewhat conflicting results, indicating at times, positive outcomes for operators and farmers, while at others, a worse alternative to better GBR concept or possibly an economic drain to society. Of course, the conflicting results do not imply one conclusion is wrong, additional variation is likely in part driven by study particulars, e.g. country, product mix, type of GBR, system boundaries, and type of study. This does indicate, however, a need for further investigation to identify or verify in which conditions, protein GBRs can be successful. Studies mostly considered pelletization or AD of GBR by-products, and grass or forage feedstocks. Additionally the breadth of technological studies in GBR process development, both in terms of initial processing, e.g. improvements in yield and sequential product extraction, and secondary processing options, dwarfs the technological scope of GBR economic asssesments. In summary, there is a gap in knowledge for economic assessments of advanced secondary processing, beyond AD, within a GBR, including high value fibres application. A recent review on the current state of LCF valorization, such as the fibre cake, highlighted that while existing studies demonstrate that high value chemicals compounds can be produced cost competitively, more research on scaled up processes must be conducted (Blasi *et al.* 2023). Existing commercial scale viability has been demonstrated for LCF ethanol as well as thermochemical conversion of LCF into bio-crude, biochar and syngas. However, in the case of second-generation LCF facilities commercial scale implementation faces key challenges. The authors identified high capital costs, limited ability to switch feedstocks and products, intermittent supply of biomass, as well as process specific issues as the barriers to second generation LCF deployment (Blasi *et al.* 2023). Lastly, additional analysis into alternative feedstocks, and 2-stage protein separation, and in general expanded comparison of alternative GBR schemes.

3.6. Environmental Impact of GBRs

3.6.1. *Cropping System Changes & Feedstock Choice*

In studies evaluating the environmental performance of different potential green GBR feedstock production systems, authors have investigated willow, spring barley, alfalfa, clovergrass, ryegrass, and festulolium (Parajuli *et al.* 2017b; Corona *et al.* 2018b). Corona *et al.* (2018b) found that among forage and grass feedstocks, alfalfa feedstock systems outperformed (lowest emission) the alternative in most impact categories (except agricultural land occupation) both before and after crediting avoided emissions from GBR products. The authors considered both negative impacts from indirect LUC and increases in SOC. While they similarly found alfalfa outperformed alternatives in greenhouse gas emissions and increased SOC, Parajuli *et al.* (2017b) found that willow and the reference cereal system performed better in other impact categories. This study evaluated a change in cropping system from spring barley to willow or alfalfa. Parajuli *et al.* (2018) evaluated how a diversion of some feed biomass to an integrated GBR and AD system, finding that this change in biomass utilization improved the sustainability of the agri-food system. Other studies have considered the variation of GBR results for different feedstocks including, in some cases, accounting for differences in quality, such as maturity or cut date (Skunca *et al.* 2021; Ravenni *et al.* 2023; Andrade *et al.* 2025). These studies expand the potential GBR feedstocks evaluated for environmental impact to include sugarbeet leaves, carrot leaves, leaf radish, chicory, brussel sprouts, and yellow mustard (Skunca *et al.* 2021). Andrade *et al.* (2025) found that of the forage feedstocks considered, alfalfa processing required the least energy consumption. Ravenni *et al.* (2023) found that the products of alfalfa pulp pyrolization and gasification demonstrated the lowest potential for global warming mitigation of the forage feedstocks evaluated. Some studies have considered the environmental impact of a change in cropping systems coupled with GBR. Cong and Termansen (2016) investigated a switch from a cereal (reference system) to grass cultivation. They found that biorefining grass for animal feed led to mixed environmental results, with improvements in energy use, land use, and nitrogen leaching, albeit with higher overall nitrogen emissions from the higher fertilization rate of grass. Skunca *et al.* (2021) was the only study to evaluate sugarbeet tops in the context of GBR, finding only alfalfa RuBisCo performed better (lower emissions) than sugarbeet tops RuBisCo. However, Tonini *et al.* (2015), compared various crop residues including sugarbeet tops for use in ethanol and biogas biorefineries. They found that sugarbeet tops use was associated with significant emissions, in part due to emissions allocated from cultivation and from iLUC to replace lost feed supply. Chan *et al.* (2024) considered two alternative market responses to shifting grass use as GBR feed, shifting straw use to feed from green manure and LUC to increased grass cultivation. They found straw was the least emitting alternative in most impact categories, except GWP due to the loss of SOC from straw removal and increase in SOC from LUC to grass. This result demonstrates the importance of market study to identify responses to changes, including investigating multiple potential responses. Considering the feedstocks evaluated in GBR systems as well as the impact of changing cropping systems, it is clear that changes to cropping systems can have mixed results, however, in general, alfalfa is a promising feedstock while sugarbeet tops have mixed results when accounting for LUC. Additional research is warranted on potential feedstocks, particularly less studied emerging options, considering both changes to cropping systems and biomass uses, and the impact of GBR processing.

3.6.2. *Comparing GPC Protein Emissions to Alternatives*

In their assessment of different diets on the climate impact of pigs, Stodkilde *et al.* (2023) found a lower climate impact from pigs fed with a local diet including GPC compared to an imported diet containing soybean meal. This study, however, did not model GBR processing and this result was based entirely off the higher feed efficiency of GPC, lowering overall consumption. Tallentire *et al.* (2018) found that among novel sources of feed protein, GPC had generally positive results performing better than most alternatives in GHG & land use, while performing worse in terms of N & P emissions. Skunca *et al.* (2021), compared the GWP of GPC derived from various GBR feedstocks and found that only alfalfa and sugarbeet top GPC performed similarly to existing protein feeds, as in the GWP per kilogram RuBisCo fell in the range of reported values for the existing protein sources. Khoshnevisan *et al.* (2023) found that the product environmental footprint (PEF) of clover GPC performed better than soy alternatives in most impact categories and in the weighted composite score. Overall, studies find GPC performs favourably compared to soy alternatives, however, this may be dependent on feedstock as well as GBR process used.

3.6.1. *Avoided Emissions*

The products from the secondary processing of by-products have the potential to contribute positively to improving GBR system environmental outcomes. Ravenni *et al.* (2023), highlighted the potential for pyrolysis and gasification of GBR pulp from various forage feedstocks to mitigate or avoid GHG emissions from fossil fuel use, through C sequestration by biochar, and the provision of biofuels and process heat. Khoshnevisan *et al.* (2023), found that substitution effects from biomethane and fertilizer produced in an integrated GBR and AD plant, compensated for nearly half of the clover cGPC product footprint. The GBR for feed proteins modelled in Franchi *et al.* (2020), produced FOS and fibres by-products that credited the grass cGPC with the avoided emissions from sugar and feed production contributing to the positive results for cGPC compared to soy protein. Chan *et al.* (2024) found that the credit from co-production of oxygen by an electrolysis system for provisioning hydrogen to biogas upgrading, contributed a larger avoided impact than avoided natural gas, and fertilizer use. Other studies similarly found meaningful effects from the substitution of GBR products (Parajuli *et al.* 2017a; Corona *et al.* 2018b). However, the magnitude of the substitution can depend on the product mix, on market conditions, which the avoided activity, GBR feedstock, and process conditions.

3.6.2. *GBR Hotspots*

Hotspot analysis identifies challenges across the GBR life cycle, in particular with biomass cultivation, but also with energy or chemical intensive processes, and changes induced due to shifts in biomass allocation, i.e. from feed to GBR substrate. Parajuli *et al.* (2018), found the carbon emission hotspots, in an integrated GBR animal production agri-food system, were feed cultivation and animal production. Franchi *et al.* (2020) similarly identified substrate cultivation as the primary contributor to cGPC results. Kamp *et al.* (2019) also identified cultivation as a major contributor to emergy (embodied energy) results, in part due to diesel use and the indirect labour used to produce agricultural machines. Khoshnevisan *et al.* (2023) found that in the production of grass clover cGPC that the largest contributor to environmental impacts were the feedstock cultivation stage, due farm emissions from slurry application, fuel use, and liming. In terms of the biorefinery stages, the largest contributor of emissions was from drying and fuel use. The anaerobic digestion stage contributed the least to the result. Andrade *et al.* (2025) evaluated energy consumption at each stage of the biorefinery process finding that biomass maceration and drying stages have the highest energy demand in biorefinery process. Although this system had a high degree of maceration in all scenarios. In a GBR process for amino acid extraction, Prieler *et al.* (2019) found that while biomass cultivation represented the highest share of impacts, chemical use in the ion exchange and amino acid scrubbing represented the second highest share of emissions. While feedstock cultivation remained significant, Chan *et al.* (2024) found that the emissions associated with the change in biomass use, contributed the largest share of emissions in cases where straw use compensated for a loss in grass feed.

3.6.3. *GBR System Results*

Studies most frequently investigate feed protein GBR systems, with either heat or biological precipitation, along with the use of by-products as substrate for AD. However, some studies considered systems outside that GBR process design. Prieler *et al.* (2019) compared the GBR production of AA in an integrated facility to a decentralized, satellite-hub model with juice dewatering and concentration prior to transit from satellite facility. While this decentralized model performed worse than the integrated AA GBR, the authors suspect that at that scale, the satellite-hub model may be necessary to decrease the total weight of materials transported. Parajuli *et al.* (2017a) compared the standalone GBRs for bio-ethanol or lactic acid with an integrated ethanol and lactic acid GBR. The authors found this combined system performed better (lower emissions) than the standalone GBRs. The above studies highlight the potential of integrating secondary processing with protein GBRs to lower emissions. Corona *et al.* (2018a) compared different protein GBR concepts and product mixes considering both biological and thermal precipitation options, as well as both, a 1-stage feed protein fractionation and a 2-stage fractionation. 2-stage fractionation methods had the highest and third highest energy consumption levels, depending on pulp use, however, when accounting for increased protein yield, 2-stage fractionation performs better than 1-stage fractionation. Additionally this study determined that biological precipitation methods yielded less protein than thermal precipitation. Chan *et al.* (2024) considered a number of novel approaches to secondary processing, considering in scenarios, the potential for SCP cultivation using AD by-products, use of electrolysers to supply hydrogen for biological biogas upgrading. Choosing to digest pulp, as opposed to using as feed, along with brown juice, therefore maximizing biomethane and oxygen (from the electrolyser) production was significant in the result.

3.6.4. *Summary*

Studies on GBR environmental impact ranged from process level investigations, both at the initial processing and secondary processing stages, in what is better described as technical-environmental assessments reporting single eco-efficiency indicators, e.g. energy use, process emissions, global warming mitigation potential (Andrade *et al.* 2023; Ravenni *et al.* 2023; Andrade *et al.* 2025). Most studies evaluated GBR system-level often including secondary processing and cultivation stages. Other studies take a macro/meso level perspective covering a fully integrated agri-food system of feed/feedstock cultivation, GBR for feed protein and AD substrates or scenarios for widespread implementation of GBR systems in Denmark (Parajuli *et al.* 2018; Kamp *et al.* 2019). Process level results have highlighted the importance of process efficiency, and process conditions, including initial feedstock choice and composition in determining process environmental outcomes. GBR system level results provide insight into process and system wide hotspots, as well as how choices in process selection, GBR product mix, by-product use, and cropping system changes effect results. In the case of the integrated agri-food system, the addition of GBR and AD (including upgrading) systems reduced the environmental impact of the entire system, suggesting that from a macro perspective, integrated GBR and AD systems can improve overall sustainability of food systems. The country scale assessment was able to quantify the scale of clover production (7% of Danish farmland) and GBR deployment (100 GBRs) necessary to displace 20% of soy imports, highlighting the need for and challenges of wide-spread GPR deployment. These studies highlight the utility of applying alternative types of studies at different scales in promoting a broader understanding of GBR systems.

3.6.5. *Research Gaps*

The challenge with sustainability assessments is that they are always context specific, as in they pertain to a specific GBR system; feedstock choice, process choice and sequence, product mix, by-product use, and method choice, such as study type, system boundaries and impact assessment method. While this provides valuable sustainability information, it both complicates comparison to other results, and leaves significant gaps in knowledge. For these reasons, there remains a broad need for more assessments to both confirm existing results but also to investigate if those results remain true if changes are made to the GBR system, e.g. in feedstock selection, processing severity, product mix and by-product use. Considering the range of technical research on GBR process, such as FOS extraction and LCF utilization, there is a need for environmental sustainability assessments to further integrate and compare emerging alternatives. Lastly, considering the range of economic assessments and environmental assessments, there is a need to integrate these studies. For example, results from economic analysis provide a mixed picture on integrating GBR into AD systems, under some conditions, there were positive results to GBR operators, however, under others, the result was negative (Martinsen & Andersen 2020; Balaman *et al.* 2023). Environmental studies, however, almost universally highlighted the benefits to system sustainability from using GBR as substrates for AD. While environmental sustainability studies have highlighted at times the benefits of advanced processing, both within the protein fractionation process, e.g. 2-stage protein fractionation, as well as in secondary uses of by-products, there may be trade-offs with economic results. For this reason, the largest research gap is in the lack of integrated or paired analysis of GBRs considering both environmental and economic sustainability.

4. Conclusions

While the green biorefinery concept is still developing, there are some common choices or trends in GBR system designs, in terms of feedstocks, processes, and products. These are generally in line with foundations laid by the influential 1998 "Grüne Bioraffinerie" techno-ecological concept for sustainable regional production and value creation processes (Soyez *et al.*). Generally, many GBRs uses forage or grass biomass, fresh or ensiled, processed into organic juice via maceration, solid-liquid separation, and then protein extraction for a single "feed" protein fraction and potentially lactic acid. Process residuals, pulp and brown juice, are then used directly, as animal feed, materials or fertilizer, or processed further via anaerobic digestion or fermentation to produce a mix of biofuels, chemicals, and fertilizers. While this core set of GBR designs alone represents a promising set of bio-based products, despite generally positive environmental impact results, particularly for "feed" proteins and integration with biogas production, economic results have been mixed, and indicating a need for improvements. In addition to research on improving the performance of this core, there is ongoing development into extracting additional product streams, such as a "food" protein and FOS, additional feedstocks, such as crop residues, and secondary processing options. The limited environmental studies considering these options indicate their potential to further increase the sustainability performance of green biorefineries. However, the need remains to evaluate these developments considering economic and environmental sustainability from a broad perspective, including the entire biorefinery value chain from cultivation to use. Often sustainability assessments are only conducted after technical development; however, the tools exist to evaluate innovations at not only an early stage but also considering technical, economic, and environmental perspectives. By combining these perspectives, sustainability tools can help identify trade-offs, and guide promising technology developments towards outcomes that are good for business, society, and the environment. Ultimately, the goal of green biorefining is to develop sustainable alternatives to products associated with negative environmental impacts, whether that is soy protein, fossil fuels, mineral fertilizers or other chemicals and materials.

While the details how to best achieve that goal are still up for debate, it clear that biorefining of green feedstocks has the potential to be a powerful tool for improving economic outcomes for farmers, lowering emissions from agriculture, and providing a renewable and regional supply of energy, food, and fertilizer.

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