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Recovery of Nitrogen from Low-Cost Plant Feedstocks Used for Bioenergy: A Review of Availability and Process Order

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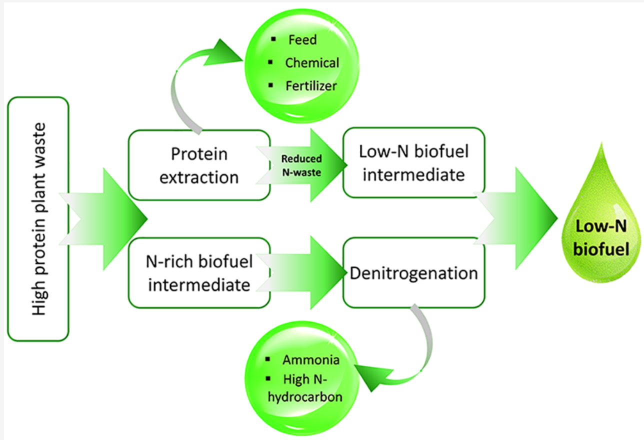
ABSTRACT: The cost of feedstock is often a major barrier to commercialization for lignocellulosic biofuels. Much of the research on biofuel and biobased chemical production has focused on the carbohydrate, lignin, and lipid components of biomass. There are numerous opportunities for the use of lower-cost feedstocks if more attention were devoted to the recovery and utilization of the protein and other N-containing components. This review compiles data from the last two decades on nonedible/nonpalatable agricultural residues, deoiled seedcakes, bioethanol and biogas residues, and food wastes, with a focus on the amounts and types of N-containing components. Given the challenges of removing N-containing compounds from biofuel intermediates postconversion, a case is made for the extraction and purification of proteins (and other N-containing compounds) from low-cost lignocellulosic biomass before conversion to fuel. The goal of such processes is to avoid difficult fuel upgrading steps while enhancing the opportunities for additional biorefinery products and sustainable nitrogen cycles.



1. INTRODUCTION

Lignocellulosic biomass, with an annual production of 150−170 Gt, is the most abundant biomass in the world with numerous applications for biofuels and bioproducts.[1] One of the primary barriers to commercialization of biofuels from lignocellulosic biomass is the high cost of the feedstock;[2,3] it is estimated that lignocellulosic biofuel production is two times more expensive than the average wholesale oil price (1.19−1.4 USD/L compared to 0.55 USD/L of wholesale heating oil).[4] According to the U.S. Department of Energy (DOE), the feedstock contributes the largest fraction (up to 67−81%) of the final fuel selling price.[5] Some alternatives to traditional crops are available to reduce production costs: low-cost dedicated energy crops growing on marginal/degraded lands,[6,7] food/feed processing residues, and wastes from the municipal and forest sectors.[8,9] These feedstocks have some advantages: lower processing costs (35−142 USD/t), continuous supply, local availability, and few existing applications.[10] A biorefinery using these feedstocks offers significant potential for a circular system for fuels and high-value products.[11]

In the last two decades, different lignocellulosic biorefinery approaches have been proposed based on different pretreatment strategies and fractionation of pretreated biomass into multiple streams (e.g., lignin, cellulose, hemicellulose, and their hydrolysates) for the generation of value-added products at different stages.[12−14] Biochemical processing approaches based on lignocellulosic-derived sugars are favored over other biorefinery models due to milder processing conditions and overall sustainability.[15,16] The majority of research on feedstocks for biofuel and biobased chemicals has focused on carbohydrates, lignin, and lipids (the CHO-containing fractions).[17,18] Cellulose-based biorefinery concepts have been developed.[19,20] Proteins and other N-containing fractions of biomass have received relatively little attention.[21,22]

After sugars, proteins and other N-containing compounds represent up to ∼16% of the dry weight of lignocellulosic biomass and are frequently the primary components in biomass waste and pretreatment byproduct streams.[2] In 2017, the global availability of agricultural residues and other solid wastes reached 2.45 Gt[23] and 2.12 Gt,[24] respectively. These materials are more likely to be disposed of by landfill or simple incineration than to be used for bioenergy—missing biorefinery opportunities and risking pollution of water, soil,

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Table 1. Current Availability, Biochemical Composition, N Content, and Higher Heating Value (HHV) of Different Agricultural Residues

feedstock	protein (wt %)	cellulose (wt %)	hemicellulose (wt %)	lignin (wt %)	N (wt %)	HHV (MJ/kg)	availability (Mt/yr)	ref
alfalfa stem	15.6	27.5−30.6	10.5−12.2	15.5−17.5	2.4	17.1		Gray et al.[53]
cassava peel	5.3	21.6	5.4		1.2	12.7	224.0[a]	Oladeji et al.[54] Ismadjia et al.[55]
date palm leaf	6.0	38.2	28.2	22.5	1.2		12.0	Sait et al.[56]
sugar cane leaf	6.5−13	27.6	19.1	11.9	1.8	14.6		Patil et al.[57]
sugar cane bagasse	18.2	33.3	30.1	26.4	1.6	20.0−24.0	250.0	Rabiu et al.[58]
sugar beet leaf	26.9	13−18	11−17	6.2		16.5	140.0	Godin et al.,[59,60] Aramrueang et al.[60]
barley straw	3.0−6.0	38.5	19.6	25.9	0.7	16.6	51.3	Chen et al.[61] Tye et al.[62]
tobacco residue	8.43	15.2	44.25	9.2	1.8	19.2	7.5	Pütün et al.[63]
milkweed stem	22.6	38.1		10.2	2.6	16.6		Campbell & Carr[64] Emon & Seiber[65]
rice straw	3.0−5.0	34.0−43.7	10.0−22.0	15.2−28.7	1.2	15.6	731.0	Satlewal et al.[66] Arshadi et al.[67]
cocoa pod husk	6.8−11.2	28.2	16.7	24.1	0.5	17.9		Olugosi et al.[68] Sandesh et al.[69]
guar bagasse	5.9	26.5		15.9	1.0	20.2		Audu et al.[70]
guayule bagasse	8.4−12.5	11.5−23.3	9.1−14.6	22.5−28.8	1.3−2.1	22.7−27.1	4.0[b]	Cheng et al.[71] Cornish & Schloman[72]
sorghum leaf	7.7	28.5	29.2	3.9		17.6	59.0	Rorke & Gueguim Kana[73] Reddy et al.[74]
chestnut cupulae	4.4	37.5	18.1	18.1	0.9	17.3		Kar & Keles[75]

[a]Total of fresh cassava leaves and peels. [b]It can be generated from 400 000 ha of guayule.

and air.[25−27] One particularly impactful missed opportunity is the recovery and reuse of nitrogen (N) for crop cultivation due to the low number of low-cost and efficient methods for protein extraction from lignocellulose[28,29] and the limited market demand for N-containing biorefinery byproducts.[30] Currently, the increased production of crops for biofuels requires production of more N-containing fertilizers using fossil-based energy,[31] which negatively affects the life-cycle benefits of those biofuels.[32] Landfilled/unused N-containing biomass resources create their own emissions of nitrous oxide (N_2O).[33] The production of chemicals containing -NH_2 functionalities requires the use of fossil-derived coreagents like ammonia (NH_3), while such functionalities can be found in protein-rich biomass.[34] Recycling and valorization of N-rich lignocellulosic biomass and wastes from agriculture, biofuel production, and food processing have the potential to reduce the costs of advanced biofuels, close the N cycle, and minimize the need for fossil-energy-derived fertilizers and chemical coreagents.

There are several reviews addressing utilization of N-rich lignocellulose biomass for biofuels through thermochemical and biochemical conversions[35−38] and N (protein) recovery and conversion into high-value-added compounds such as food,[28,39] bioplastics,[40,41] adhesives,[42] pharmaceutical intermediates,[43] hydrolysates,[44,45] bulk chemicals,[21] and enzymes and bioactive compounds.[46,47] Previous studies on protein recovery have focused either on direct production of fuel or on protein extraction. A review with emphasis on the availability of low-cost feedstocks and N recovery before or after biofuel production, with the goal of commercialization of biofuel based on these feedstocks, is not still available. The purpose of this review is to provide the characteristics and availability of low-cost, N-containing biomass wastes relative to their potential as biofuel feedstocks and a perspective about how to best utilize them simultaneously as a N resource.

2. METHODS AND ORGANIZATION OF THIS REVIEW

The availability and composition of nonedible (or nonpalatable) lignocellulosic feedstocks that contain meaningful amounts of N (at least 0.5 wt % N on a dry basis) are grouped into agricultural residues, deoiled seedcakes from biodiesel production, residues from other biofuel processes (bioethanol and biogas), and food wastes. Algae and animal byproducts were specifically excluded from the review as these have been extensively reviewed elsewhere with respect to protein.[48−50] The forms of N in biomass-derived fuel intermediates and the challenges of N-containing compounds in fuels are presented. From there, schemes for removal of protein/nitrogen prior to conversion into biofuel intermediates are evaluated as a way to recover N for value-added use and avoid additional fuel intermediate upgrading.

3. POTENTIAL SOURCES OF LOW-COST PLANT FEEDSTOCK

3.1. Agricultural Residues As Feedstock.

Agricultural residues are carbon-based materials generated as a byproduct during harvest and processing of agricultural crops. Currently, the global annual production of agricultural residues is estimated at 150 Gt.[51] Not all agricultural residues can be collected for use. Some residues should remain on the soil to mitigate erosion, sequester carbon, and increase crop productivity by adding N to the soil. Too much residue, inefficient uptake, and/or inappropriate timing of residue application can lead to additional N_2O emissions. Agricultural soils emit approximately 4.2 Mt N_2O per year globally, approximately 52% of the total anthropogenic N_2O emissions.[52] The use of protein-rich agricultural residues for bioenergy and chemicals, therefore, must be a carefully managed trade-off. This section presents an overview of the potential feedstocks with the highest relative current (or future expected) production volumes: alfalfa stems, cassava leaves and peels, date palm leaves, sugar cane bagasse and leaves, sugar beet leaves and pulp, and guayule bagasse. Table 1 provides a summary of the characteristics of these and other high-N agricultural residues.

Alfalfa (*Medicago sativa* L.), the third most widely grown crop in the US., has been considered as a feedstock for biofuel, feed, and chemical production because it does not require annual reseeding and can reduce the nitrate concentrations in drainage water, prevent soil erosion, and reduce required agricultural inputs like fertilizer and pesticides.[76,77] In 2018, U.S. production of alfalfa averaged 52.6 Mt at 1.28 t/ha.[78] Alfalfa leaves, containing 26−30 wt % protein, are primarily used as a forage for livestock. Alfalfa leaf meal has been considered for human nutritional supplements. The relatively high lignin content and low digestibility of alfalfa stems suggest their use as a feedstock for biofuel production rather than for feed, even with their 10−20 wt % protein content.[79,80] The proportion of leaves in alfalfa hay has been estimated at 40−60 wt % based on the maturity of the plant. Since leaves and stems can be easily separated, utilization of stems for a second income stream would make the alfalfa more economically attractive. The stem fraction is also rich in cell wall polysaccharides that can be used as a source of fermentable sugars to produce ethanol and other bioproducts.[80−82]

Cassava (*Manihot esculenta* Crantz) is a tropical perennial root crop where the roots contain about 30% starch and very little protein (1−2%). Approximately 65% of global annual cassava output is processed for human consumption; the rest is used for bioethanol production and for the pharmaceutical industry.[83] The largest cassava processing waste streams are sludge, peels, and leaves, which are usually discarded. In 2008, the harvested area of cassava was approximately 1.87×10^5 km^2 with a yield of cassava leaves of 1.2 kt/km^2 with 20% protein, giving a crude protein potential of 15.5 Mt.[84,85] Cassava peels constitute about 19% of the fresh root weight.[83] Much of the interest in expanding the nonfood applications for cassava peels is the presence of toxic compounds, like cyanogenic glucosides and linamarin, at higher concentrations than in the root pulp. Direct disposal of cassava peels creates environmental hazards due to the release of hydrogen cyanide after hydrolysis by an endogenous linamarase.[86] Recent studies on the utilization of cassava peels have included use as a feedstock for activated carbon, absorbents,[87,88] supercapacitors,[89,90] and biofuels.[86,91]

The date palm (*Phoenix dactylifera* L.) is a tree adapted to arid and semiarid regions. Approximately 105 million date palm trees were being grown in 2014.[92] Saudi Arabia generates more than 200 kt/year of date palm biomass. Date palm trees generate approximately 12 Mt/year of waste biomass in form of dry leaves, stems, pits, and seeds.[93] Approximately 20 kg of dry leaves per tree is generated each year, containing 6% crude protein. The calorific value of leaf waste is low (16.4 MJ/kg) due to a high ash content,[94] and the relative lignin content is high (125 g/kg),[95] making date palm leaves unattractive for direct combustion or animal feed.

Sugar cane (*Saccharum officinale* L.), a tropical crop, is an important feedstock for bioethanol production. Because of their complex chemical composition and limitations on their use as fodder for animals, the leaves are generally burned in the fields, which damages the soil microbial diversity and raises environmental concerns.[96] A sizable portion (7−13% of the dry matter) of the leaves and tops is composed of protein. Sugar cane byproducts are currently used in production of enzymes, ethanol, xylitol, protein cells, and organic acids.[97−100] A study by Deepchand et al.[101] showed that sugar cane leaves can be a potential source of protein products. The high lignin content of sugar cane tops and leaves makes them a good target for pyrolytic bio-oil production.[102]

Sugar beet (*Beta vulgaris* L.) is a major sugar crop for food and bioethanol production. In the U.S., nearly 72 Mt (wet basis) of sugar beets were produced in 2010 with an average yield of 62 Mg/ha.[60] Sugar beet leaves account for approximately 38% of the plant mass with 3.2% protein; this represents a protein production potential of 4.5 Mt/year.[22] Sugar beet pulp, the solid remaining after sugar extraction, contains 10−15 wt % protein, 20−25 wt % cellulose, and 25−36 wt % hemicellulose. The high carbon content and pectin content (20−25 wt %) of sugar beet pulp make pulp a promising carbon source for production of biobased fuels and chemicals.[103,104]

Guayule (*Parthenium argentatum* A. Gray) is a woody shrub native to the southwestern U.S. and northern Mexico.[105] Guayule is a source of high-quality and hypoallergenic natural rubber (*cis*-1,4-polyisoprene).[106] Large amounts of two residues are produced during shrub processing: a liquid mixture of resin/low-molecular-weight rubber and a lignocellulosic bagasse.[105] Because of the low rubber yield from guayule (5−7 wt %), the value-added use of the resin and bagasse coproducts is important for the economic feasibility of guayule rubber; selling prices of \$1.00/kg and \$0.10/kg for resin and bagasse, respectively, would make guayule rubber more competitive with *Hevea* rubber.[107] Guayule bagasse constitutes approximately 70−80 wt % of the shrub biomass.[71] It is estimated that 4 Mt of lignocellulosic bagasse can be generated from 400 000 ha of guayule.[72] Guayule bagasse is mainly composed of cellulose, hemicellulose, lignin, residual resin, and various plant proteins and lipids; the composition varies substantially with extraction and pretreatment methods, cultivation site, harvest date, shrub strain, shrub age, and storage conditions.[71,108] Analysis of resin-free bagasse estimated the protein content at 22−24 wt %[71] with an attractive higher heating value of 18−24 MJ.[7071] Amino acids constitute around 18% of leaf and wood residues after resin and rubber removal.[109] Besides bioenergy applications,[110−112] other proposed applications for guayule bagasse include soil amendments,[113] termite-resistant composite boards,[114,115] paper,[116] and adsorbents for removal of contaminants from aqueous solutions.[117]

3.2. Deoiled Seedcakes As Feedstock. The product of oilseed crops after oil removal through extrusion or extraction is categorized as edible and nonedible seedcakes. According to the Food Safety and Standards Authority, 5−20% free fatty acid is considered edible oil, and anything outside of this range is considered nonedible oil.[118] Among the plants from which edible oil seedcakes are derived are soybean, coconut, sunflower, sesame, mustard, palm kernel, groundnut, cottonseed, canola, olive, Babassu palm, and rapeseed. These seedcakes have high nutritional value, with protein content ranging from 15 to 50 wt %, and are mainly used as animal feeds.[119] Nonedible oil seedcakes, such as Jatropha (*Jatropha curcas*), Karanji (*Milletia pinnata*, formerly *Pongamia pinnata*), neem (*Azadirachta indica*), castor (*Ricinus communis*), mahua (*Madhuca longifolia or M. indica*), cannot be used as animal

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Table 2. Biochemical Composition, N Content, and Higher Heating Value (HHV) of Different Deoiled Seedcakes

feedstock	protein (wt %)	cellulose (wt %)	hemicellulose (wt %)	lignin (wt %)	N (wt %)	HHV (MJ/kg)	ref
Jatropha curcas	50−60.0	59.4	18.2	22.3	10.6	13.5	Parekh et al.[124]
Karanji	17.0−21.0	56.1	17.5	26.4	5.5	17.0	Fu et al.[125]
neem		45.0	29.0	26.0	7.4	21.8	Mulimani & Navindgi[126]
rapeseed	16−24	27.7	36.6	4.9	6.9	19.8	Egües et al.[127] Ucar & Ozkan[128]
castor	31.0−35.4	9.6	26.6	21.9	8.5	21.5−23.4	Ferreira et al.[129] Castro et al.[130]
Mahua	16.0−30.0	60.4	16.2	20.2	3.3	21.0	Singh et al.[131] Volli et al.[120]
Pennycress	19.6−25.8				6.9	17−22	Selling et al.[132]
mustard	28.1−38.8				5.66	20.5	Volli et al.[120] Sarker et al.[133]
apricot kernel	23−31.4	44.2	24.7	24.6	6.1	22.8	Fadhil[134] Wadhwa & Bakshi[135]
black cumin	23.9	37.1	10.4	26.7	5.3	22.4	Sen & Kar[136] Thilakarathne et al.[137]
Camelina	36.3				6.2	22.8	Mullen et al.[138]

feed due to their toxicity from the presence of certain secondary plant metabolites. These secondary metabolites are produced by the plant for protection, sometimes acting as antioxidants and enabling the plants to grow in harsh environments. Table 2 summarizes the feedstock properties of nonedible deoiled seedcakes. Nonedible deoiled seedcakes with high nutrient components may be used as a source of plant nutrients (fertilizers), pesticides,[119] and biofuel production.[120−122] The utilization of the seedcakes after extraction of oil can substantially impact biodiesel production costs.[123] With their high initial calorific values, the seedcake is considered an ideal thermochemical conversion feedstock.

Jatropha curcas is considered one of the more ideal species for energy oilseed production on nonaerable lands. Unlike many other tropical plants, Jatropha is drought resistant and may grow at extreme conditions. Jatropha seed kernels contain 31−35% crude protein and 55−58% lipid;[139] 1−1.5 Mg of oil can be produced per hectare.[140] One kg of biodiesel can be produced from 4 kg of Jatropha seeds.[141] Pandey et al. predicted in the coming years that India will grow 20 million ha of Jatropha and produce about 20 Mt of seedcake, creating potential for value-added products.[142] The variations in protein content of Jatropha seedcakes are large and depend on the method of oil recovery. Achten et al. estimated an average of 58% protein on a dry matter basis.[143] Jatropha contains phorbol ester (phorbol-12-myristate-13-acetate), which is toxic to humans and animals. The toxicity of phorbol ester ranges from skin irritation to the production of tumors.[144] In addition to phorbol ester, Jatropha contains several antinutritional factors such as trypsin inhibitors, phytic acid, lectin, and saponin that raise safety questions for Jatropha around edible crops.[145] Jatropha leaves have been used as a fumigant for bed bugs, feed for silkworms, and clothing dyes; Jatropha latex and twigs have been used in medicine and its fruits in bioethanol production.[146,147] Some studies suggested the use of Jatropha seedcakes as a fertilizer, biopesticide/insecticide, and molluscicide because of the high levels of N-containing compounds, although a number of questions concerning the long-term and cumulative impacts of Jatropha seedcake on soils have not been addressed. Makkar et al.[148] suggested that the detoxified seedcakes can be used as a protein supplement for animal feed and aquaculture, albeit at higher feed prices due to the detoxification process. The high lignin content (45−47%) of Jatropha seed husks and shells, and their associated low digestibility and degradability, make them less suitable for biogas production.[148] Jatropha seedcake, on the other hand, contains 27% lignin and gave 60% higher biogas yield compared to cattle dung as a feedstock.[149,150] Blended Jatropha shell and seedcake biochar can be pelletized for pellet combustion fuel.[151−153] Some studies have also reported the conversion of Jatropha seedcake by hydrothermal liquefaction (HTL) or fermentation.[154,155]

Karanji (*Milletia pinnata*), a nonedible oilseed that can grow on marginal lands, has received significant attention as a legume plant because of its potential in biodiesel production and soil N fixation. A single tree can provide 9−90 kg of seeds, for a yield potential of 900−9000 kg seed/ha containing 25% oil. The remaining 75% seedcake has a low bulk density (0.3−0.35 g/cm^3). Karanji leaves have some applications as insect repellents in grain storage[156] and as a valuable animal lactation-promoting fodder.[157] Karanji seedcakes have high carbohydrate (42−56%), protein, and lignin contents.[158] The low ash content (2−4 wt %), and an absence of sulfur compounds, in Karanji seedcake make it a good candidate for biofuel production.[158] The presence of toxic flavonoids, such as karanjin, pongamol, phytates, tannins, protease inhibitors, glabrin, other polyphenolic compounds,[159] and a bitter taste, make Karanji seedcakes inedible for animals.[160] A potential biorefinery scheme for Karanji would be (i) conversion of the seed oil to biodiesel; (ii) conversion of the deoiled seed residue to bioethanol; and (iii) conversion of the bioethanol solid residues to biochar and bio-oil.[156] Sangeetha et al. suggested that Karanji seedcake could be a promising substrate for bacterial growth and enzyme production.[161]

Neem (*Azadirachta indica*) seeds grow on trees in tropical and semitropical regions and contain up to 40−50 wt % oil, with a potential to produce 350 Mt/year of oil.[162] Processing results in 50−60 wt % of the total seed weight as seedcake, along with glycerol. Deoiled neem seedcake is rich in protein (35%),[119] carbohydrates, minerals, and nitrogenous components, namely, azadirachtin.[162] As a biopesticide, deoiled neem cake improves nutrient availability, regulates weed growth, and controls nematodes.[163] The presence of azadirachtin, tetranortriterpenoid (an antifeedant), isoprenoids, and nimbidin

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Table 3. Biochemical Composition, N Content, and Higher Heating Value (HHV) of Other Bioenergy Waste Feedstocks Including Dried Distiller's Grains with Solubles (DDGS) and Digestates from Biogas Production

feedstock	crude protein (wt %)	crude fat (wt %)	ash (wt %)	N (wt %)	HHV (MJ/kg)	other (wt %)	ref
maize DDGS	28.7−32.9	8.8−12.4	3.9−9.8	5.3−8.1	18.8−21.7	crude fiber: 5.4−10.4	Bhadra et al.[197]
							Morey et al.[198]
sorghum DDGS	31.0	7.7	3.6		16.1	crude fiber: 9.8	Sotak et al.[199]
wheat DDGS	19.6−38.2	3.6−3.8	4.8−8.4	5.9	17.6	crude fiber: 6.8−8.0	Rasco et al.[200]
							Eriksson et al.[201]
cassava DDGS	5.6−14	0.2	11.7		14.3	crude fiber: 4.0−26.7	Taranu et al.[202]
							Sotak et al.[199]
oat DDGS	16.0	6.3				crude fiber: 5.7	Moreau et al.[192]
barely DDGS	17.7	2.5	5.7	0.5	21.3	acid detergen fiber: 30.3−31.8	Wu et al.[203]
rice DDGS	6.5−7.9	0.5−2.9	0.5			crude fiber: 2.8−3.5	Choi et al.[204]
triticale DDGs	33.2−34.5	4.5−4.7	4.4			fiber: 26.8−27.0	Chrenková et al.[205]
dairy manure digestate			27.4	3.5	13.3	cellulose: 23.5; hemicellulose: 17.5; lignin: 18.3	Posmanik et al.[206]
							Guilayn et al.[207]
swine manure digestate	16.3	7.8	9.7	1.4		cellulose: 59.3; hemicellulose: 14.7; lignin: 6.5	Vuppaladadiyam et al.[208]
food waste digestate			25.6	5.8		cellulose: 32.3; hemicellulose: 33.5; lignin: 13.4	Opatokun et al.[209]
wastewater treatment sludge digestate			35.9	5.3	15.2		Wang et al.[210]
digestate from mixed waste (60% barley silage, herbaceous silage, and poultry manure)		25.0−30.0	9.3	1.1	19.2	cellulose: 36.1−43.1; hemicellulose: 13.9−26.3; lignin: 21−27.9	Menardo et al.[211]
digestate from mixed waste (9% groats, 29% olive oil cake, 57% triticale silage, and 5% chicken manure)			8.7	1.6		cellulose: 22.7; hemicellulose: 19.4; lignin: 35.3	Sambusiti et al.[212]

preclude the use of neem cake as animal feed.[164,165] In studies of biofuel production from neem seedcakes, Dhanavath et al.[166] obtained 52.1 wt % bio-oil using pyrolysis. This yield was in good agreement with Volli et al.[120] for production of bio-oil from the deoiled neem cake by thermal pyrolysis.

Castor (*Ricinus communis*), a tropical plant that can be cultivated in tropical, subtropical, and temperate regions, is widely used in the manufacture of biodiesel, cosmetics, pharmaceuticals, and lubricants. The major castor-producing countries are India, China, Brazil, Russia, and Thailand, while the major importing countries are the U.S., Russia, and Japan. Apart from oil and biodiesel from the castor bean, 1.13 Mt/year of castor seedcake is produced globally.[167] India produces approximately 0.4 Mt of total castor seedcake.[168] The composition of castor seedcake varies with the method of oil extraction, storage conditions, and quality of the oilseeds. Castor seedcake is fairly protein-rich at 290−390 g/kg or 19.4−49.7% crude protein across several studies. Annongu et al. showed that both the decorticated and undecorticated forms of castor seedcake have high protein contents, 35.4 and 21.9%, respectively, while decorticated seedcake has a higher nutrition value.[169] The phytotoxin in castor is ricin, a water-soluble, heat-labile protein that is concentrated in the seeds.[3] The presence of ricin, ricinin (a toxic alkaloid), agglutinin, and allergen CB-1A makes the toxicity of the seedcake too high to be used as an animal feed.[170] Castro et al. showed that deoiled castor seedcake offers the potential for the production of multiple enzymes with applications in biofuels, such as amylases, cellulases, and xylanases.[130] Another study reported the use of castor seedcake for production of fertilizer and biodegradable materials by extraction of the proteins, which may represent an additional valued-added opportunity for castor in biorefinery concepts.[171]

Mahua (*Madhuca indica*), a tropical tree native to central and northern India, has an unusually high oil content (50−61%) with great potential for biodiesel production. Between 1983 and 1984, 55.5 kt of Mahua seed were produced in India, with every 4 kg of mahua seed producing 1 L of biodiesel and 3.5 kg of deoiled seedcake.[100] The Mahua deoiled seedcake constitutes 60% of the total biomass and contains 30% protein. Mahua seedcake is used in low-value applications: fertilizer, manure,[172] insecticide/pesticide,[173] hair wash,[131] and dye removal from wastewater.[174] Mahua seedcake is not edible without detoxification because of the presence of saponin compounds, which give a bitter taste and cause damage to the liver and kidneys. One exception is with use as fish feed; Alexander et al. did not identify any toxicity in fishes.[175] In recent years, Mahua seedcake has been used as a feedstock for pyrolysis and anaerobic digestion.[120,176−180] Gupta et al.[179] reported that detoxified Mahua seedcake showed significantly better results compared to the raw cake in biogas production, although utilization of raw cake for biogas was still a reasonably effective application.

Pennycress (*Thlaspi arvense* L.), also known as stinkweed or French weed, is a member of the Brassicaceae family, which is native to Eurasia and grows extensively in temperate North America. With high cold tolerance, a short lifecycle, high productivity (up to 840 L/ha oil and 1470 kg/ha press-cake),[181] high seed oil content (up to 38%), a tolerance for fallow lands and minimal agricultural inputs, and compatibility with existing agricultural infrastructure, pennycress is a good candidate for biodiesel production and extraction of value-added products.[2,182] The high protein (22−32%) and carbohydrate contents in pennycress cake make the cake a good candidate for biofuel production, while the presence of glucosnolate and allylthiocyanates (a toxic compound) limit its utility for human food or animal feed.[181] Uses of pennycress

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cake as a soil biofumigant or filler/reinforcement material for plastic and lignocellulosic composites have been considered.[183−185] Some studies have investigated pennycress presscake as a feedstock for pyrolysis and hydrotreating.[2,186−188] Pyrolysis oils derived from pennycress seed cake tend to be less acidic, more stable, and have higher energy content than those from most other types of lignocellulosic biomass due to their higher N content.

3.3. Other Bioenergy Residues As Feedstock. Distiller's dried grains with solubles (DDGS) are a residue from corn- and wheat-based bioethanol fermentation. In 2012, the U.S. ethanol industry produced 31.6 Mt DDGS from 114 Mt of corn, indicating that the economics of bioethanol production could be heavily influenced by the utilization of the DDGS.[189] The unfermented components consist of fiber, protein, lipids, minerals, and vitamins. The protein content is approximately 3-fold that of the corn kernels; however, DDGS is an incomplete animal feed based on amino acid analysis.[190,191] Corn and wheat are the predominant feedstocks for ethanol and DDGS, although a few other high-starch grains have been reported.[192] Table 3 shows the composition of different DDGS sources. The protein content of DDGS varies between 20 and 57%,[193] and accounts for 21−36% of the grain's total N. Protein contents for hard wheat fermentation products, including 29% for distillers' grains and 57% for centrifuged solids,[194] were higher than the corresponding fractions from corn.[195] Sorghum and wheat DDGS have a higher crude protein, higher ash content, lower energy content, and lower crude fat compared to corn DDGS (Table 3). Sludge obtained from cassava bioethanol production contained ∼35% protein by mass.[196]

The most common use of DDGS is for animal feed; however, there have concerns expressed regarding this application.[213] Heat-damaged DDGS (often identified by a darker color) has a poorer nutritional value and should not be fed to nonruminants.[214] Elevated phosphorus content in DDGS is excreted as manure that leads to some disposal problems. Animal consumption of DDGS can be hindered by difficult digestion due to the high fiber content, inconsistency of DDGS mineral content, and thiamine deficiency due to high sulfur.[215] Other potential uses of DDGS are fillers in biocomposites and bioplastics,[216] food additives from extraction of oil from DDGS,[217] fertilizers for plant growth,[218] and biofuel generation using thermochemical[219−222] and biochemical conversion methods.[35] Wet distillers' grains with solubles (WDGS) form directly as a byproduct of corn-based bioethanol fermentation and may be the better choice for HTL because HTL does not require drying, and an HTL reactor could be placed downstream at a bioethanol plant to mitigate problems from the short shelf life (4−7 days) and high transportation costs of WDGS. Toor et al.[223] obtained 34% (dry feedstock basis) biocrude oil from catalytic HTL of WDGS. The high oil content of corn-based DDGS makes it a potential feedstock for biodiesel production, calculated at up to 1.1 billion L/year.[224] Deoiled DDGS could then also be used as animal feed.

Digestate is a byproduct of anaerobic digestion, representing the chemical composition of the feedstock after extraction of biogas. Digestates generally contain more cellulose, lignin, and nitrogenous compounds due to a slower consumption rate of these compounds by the anaerobic microorganisms compared to hemicellulose.[225] More information about the composition of different digestates is provided in Table 3. A biogas plant with a capacity of 500 kW, based on 10% dry matter feedstock, produces more than 10 kt/year of digestate.[226] In 2013, the European Union reported that 80 Mt/year of digestate were produced from 13 000 biogas plants and needed management or disposal.[227] The composition and quality of anaerobic digestates depend on the feedstock, operating conditions, and digester configuration. For example, digestate derived from yard waste contains more celluloses and hemicelluloses, while manure and sludge have more N-containing compounds. The moisture content of the digestate is influenced by the choice between a wet or dry anaerobic digestion process.[228] Logan et al. presented management strategies for digestates from municipal solid waste to address concerns about potential pollution, conservation of vulnerable zones, prevention of communicable diseases, and storage and application options.[228] Some solid digestates with a high N content (51−68%) can be used as a fertilizer to reduce costs, nitrate pollution, and soil carbon losses.[229−231] Solid digestates with lowerN contents (24−36%) are suggested for soil amendments.[232] Nkoa provides an extensive review of the benefits and effects of using digestates for fertilizer.[233] The high levels of water-soluble polyphenols/phenolic compounds in some liquid digestates may induce N immobilization in soil and inhibit seed germination.[232] Municipal solid waste digestates can contain plastic, timber, fibers (natural and synthesized textiles), grit or sand, metal fragments, and solid fruit residues that require special treatment before use as a fertilizer since toxic compounds, heavy metals, and undigested inorganic materials could be transported through the food chain and drainage water.[228] Digestate can have both phytostimulating and phytotoxic effects on plants.[234,235] Digestates can have higher NH_3 emission potential than undigested animal manures and slurries due to instability of the ammonium nitrogen (NH_4-N) at pH levels above neutral (7.5−8.5).[236]

In terms of bioenergy applications, Kratzeisen et al.[226] investigated digestate pellets as a solid fuel for combustion. Although the net calorific value of digestate pellets (15 MJ/kg) was comparable to the calorific value of wood, its high ash content, low ash melting point, slagging, and need for drying and dewatering steps made digestates disfavored for direct combustion and incineration.[237] Pyrolysis of digestates derived from different feedstocks has been studied by Opatokun et al.,[209] Neumann et al.,[238] Monlau et al.,[239] Hung et al.,[240] Bedoić et al.,[241] and Wei et al.[242] Recently, hydrothermal processes (carbonization, liquefaction, and gasification) have gained attention as a resource recovery option for digestates due to the high moisture content of the digestates and the wide range of compounds that can be converted thermochemically.[243−245]

3.4. Food Wastes As Feedstock. Food waste is one of the most abundant protein-rich wastes, accounting for ∼22% of landfill waste in the U.S.[246] Food waste can include residuals from large-scale commercial food processing (vinegar production residues, coffee and meat processing byproducts, etc.),[247,248] small-scale kitchen wastes, and uneaten prepared foods. According to the U.S. Environmental Protection Agency, 63.1 Mt of food was wasted in the U.S. in 2018, approximately 22.4 Mt more than what was wasted in 2017.[249] Food wastes contain substantial quantities of proteins, lipids, starch, micronutrients, bioactive compounds, and dietary fibers, making them a high priority for reuse as feeds,[39] biomaterials,[250,251] value-added compounds,[252] and biofuels.[253] Table 4 summarizes the composition of different

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Table 4. Biochemical Composition, N Content, and Higher Heating Value (HHV) of Different Food Wastes

feedstock	protein (wt %)	carbohydrate (wt %)	lipid (wt %)	cellulose (wt %)	hemicellulose (wt %)	lignin (wt %)	N (wt %)	HHV (MJ/kg)	ref.
spent coffee grounds	18.0	67.6	2.0	13.0	42.1	25.0	15.5−16.7	21.8	Karmee et al.[256]
									Marx et al.[257]
mango seed kernels	6.3	32.2	13.3	55.0	20.6	23.8	0.2	15.9	Nzikou et al.[258]
									Ganeshan et al.[259]
									Henrique et al.[260]
mango peels	2.1	26.5		9.1	14.5	4.2		16.3	Imran et al.[261]
									Orozco et al.[262]
banana peels	10.1	68.5	5.0	12.1	10.1	2.8	1.3	18.8	Pathak et al.[263]
tomato pomace	19.3	25.7	5.9	29.1	13.5	57.4	2.8	25.1	Chiou et al.[264]
watermelon rinds	13.5	48.5	4.5	20.0	23.0	10.0	0.8	12.7−19.2	Jawad et al.[265]
									Ebikade et al.[266]
olive pomace	6.7	49.0	10.9	37.4	33.9	28.6	1.4	22.3	Chiou et al.[264]
orange peels	9.1	80.7	4.1	11.9	14.4	2.1			Orozco et al.[262]
potato peels	18.5	73.5	0.5	44.2	2.7	22.4	3.0	16.6	Martinez-Fernandez et al.[267]
municipal food waste	21.4	25.8	20.5	24.7	1.8		3.4	20.9	Bayat et al.[253]
cauliflower leaves	21.8			40.0	50.0	3.0	4.0	10.8	Stella Mary et al.[268]

food waste streams. To be considered a viable source of protein (often the most expensive/critical component of a feed), food waste must have high protein content on a mass basis, have a well-balanced essential amino acid composition, and have had any toxic or allergic substances removed.[39] Prandi et al.[254] characterized the N fraction of 39 food waste streams to assess their potential for valorization. In addition to familiar protein-rich food wastes (dairy, egg, and cereal wastes), they identified leek leaves, parsley wastes, and mushroom wastes as good candidates for feed proteins. Orange peels, Belgian endive leaves, and berry waste were protein-rich but had limited nutritional value. The high amounts of phytotoxic and/or antinutrient compounds (e.g., caffeine, tannins, and polyphenols) in coffee byproducts (spent coffee, husks, and pulp) have limited their direct use for soil and feed applications even though the byproducts contain high levels of protein (13.6%) and nonprotein nitrogenous compounds (3.7%).[255] Among nonfeed applications, such food wastes can be used for soil improvers, biopolymers, biofuels, and biocomposites.[256]

4. CHALLENGES OF NITROGEN IN BIOFUEL INTERMEDIATES

Concerns about closing the N cycle, in addition to closing the carbon cycle, have prompted some to argue for the specific use of proteins (over carbohydrates) in biofuel production with the goal of coproducing and recycling NH_3 for plant nutrition.[269] There has also been much attention paid to N-based fuels, such as NH_3 and urea, which bypass the hydrocarbon generation process completely.[270] The same two issues that must be managed for N-based fuel utilization, namely, the toxicity of certain N-compounds in (by)products and the potential for nitrogen oxide (NO_x) emissions during combustion,[271] dictate the removal of N from carbon-based fuels, whether these fuels are fossil- or biomass-derived. For crude oils, a N content greater than 0.25 wt % is indicative of the need for further refining. In general, 25−55% of the N in biomass distributes into the bio-oil phase during pyrolysis, resulting in a 2−8 wt % N content; 20−40 wt % of the N in biomass is recovered in the HTL biocrude oil, resulting in a 3−6 wt % content.[36,37] Amines, amides, nitriles, and N-heterocycles (pyridines, pyrroles, piperazines) are the main nitrogenous compounds in HTL biocrude oils and pyrolysis bio-oils because of Maillard reactions between amines and carbohydrates.[272] N is particularly difficult to remove from the heterocyclic compounds such as pyrazines, pyrroles, quinolines, and pyridines.[273]

Numerous reviews have been written on denitrogenation of biomass-derived fuel intermediates, with or without catalysts, and with or without the use of hydrogen gas, adsorption, supercritical fluids, or hydrogen-donor solvents.[274,275] Often, N is eliminated as NH_3 gas, which has the potential to be recycled as a fertilizer.[276] Catalytic hydro-denitrogenation is one of the most efficient approaches to removing heteroatoms from HTL/pyrolysis oils; the H and C atoms that had been associated with the N atoms remain in the upgraded oil. For separation processes like adsorption and solvent extraction, whole N-containing compounds are removed, leading to easier processes but higher C and H mass losses.[277] The viability of any given N removal process depends on the quantity and identity of the N-containing compounds to be removed. Cheng et al.[38] reviewed catalytic denitrogenation of pyrolysis bio-oil from high-protein biomass, including thermochemical and physicochemical denitrogenation methods. Leng et al.[37] did not recommend adding catalyst during pyrolysis or catalytic denitrogenation of pyrolysis bio-oil because of the low activity, low selectivity, coking, and leaching problems and instead suggested the use of minerals already present in the high-ash, protein-rich biomass and the use of NH_3 as the reaction atmosphere. In a review of catalytic hydrotreating and adsorptive denitrogenation methods, Li et al.[278] reported that adsorptive denitrogenation is promising for removal of N from microalgae bio-oil. More recently, bio-oil upgrading by means of supercritical fluids has been explored.[279,280] Alternatively, biofuel intermediate fractions high in N-containing compounds, such as pyradines, quinolines, and indoles, may have potential for chemical, pharmaceutical, and polymer production.[281]

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Table 5. Common Techniques for Extraction of Protein from Plant-Based Feedstocks

extraction method	protein content/ recovery (wt %)	advantage(s)	disadvantage(s)	refs
Chemical treatment				
alkaline extraction	15–95	- Simple process	- Hazardous waste production	Gao et al.[312]
		- No sophisticated equipment	- Low selectivity	Hou et al.[311]
		- Easy scale-up		
organic solvent extraction	23–63	- Relatively inexpensive	- Low selectivity	Watanabe et al.[313]
			- Low protein recovery	
			- Hazardous waste production	Capellini et al.[314]
			- Undesirable byproduct production	
			- High extraction temperature	
Physical treatment				
ultrasound-assisted	15–87	- High extraction yield	- Longer processing times	Kumar et al.[296]
		- High purity	- Denaturation and aggregation of protein at higher intensities	Bedin et al.[307]
		- Lower energy requirements		
				Gençdağ et al.[298]
		- Lower solvent consumption		
microwave-assisted	28–70	- Shorter processing times	- Energy-intensive technique	Varghese and Pare[315]
		- Minimal target compound degradation		
		- No additional filtration	- High extraction temperature	Görgüç et al.[316]
pulsed electric field-assisted	1.8–50	- Nonthermal extraction (extraction at room temperature)	- Low extraction yield	Sarkis et al.[317]
		- Very short processing times (<1 s)	- Low level of technology readiness	Parniakov et al.[318]
		- Extraction of pure compounds		
high-pressure fluid-assisted	30–65	- Low solvent consumption	- High energy demand	Di Domenico Ziero et al.[308]
		- High selectivity	- Expensive	
		- High yield and purity		
Biochemical treatment				
enzyme-assisted extraction	43–96	- Moderate extraction temperatures	- High enzyme cost	Gençdağ et al.[298]
		- High protein solubility	- Long extraction times	Nadar et al.[309]
		- Suitable for large-scale production	- Inconsistent yield	
		- Use of nonexplosive solvents	- Enzyme sensitivity to process conditions	
		- No hazardous waste		
		- High extraction yield		
		- Compatible with different procedures		
		- Proteins suitable for human consumption		

5. UPSTREAM NITROGEN RECOVERY FROM BIOMASS

Rather than removing N from fuel intermediates after conversion, recovery of protein (and other N-containing compounds) before conversion may be more advantageous for process efficiency and for the number of products from a biorefinery. Removal of N generally occurs in three (sequential) processes: (1) cell disruption and isolation to extract proteins, (2) protein purification/recovery, and (3) protein hydrolysis to amino acids.[282]

Several protein extraction methodologies and conditions have been reported for integrated biorefineries.[11,283] Chemical methods include alkaline extraction,[284,285] aqueous ammonia (AFEX) extraction,[286,287] buffer extraction,[288] organic solvent extraction,[289] and combinations of these methods.[290,291] Alkaline extraction is commonly used for extracting proteins from agriculture residues, deoiled seedcakes, and DDGS with protein recoveries of 82–91.1%, 15–36%, and 60–79%, respectively.[292−294] The efficacy of protein extraction using chemical methods depends on the nature of the biomass, solid-to-solvent ratio, temperature, pH, and process time.[295] A recent review by Kumar et al. summarized the effects of the sample-to-solvent ratio, alkali concentration, time, and temperature for several protein extractions from plant.[296] A pH of 8–14, temperature of 30–95 °C, time of 30 min to 4 h, and NaOH/KOH as alkali for pH adjustment were identified as conditions to achieve maximum protein yields.[283,297,298] The chemical extraction of proteins, in particular, organic solvent extraction, is suitable for biomass containing aromatic amino acids and proteins with nonpolar/polar side chains and lipid-binding ability.[296]

Physical methods of protein extraction include ultrasound-assisted, microwave-assisted,[299] hydrothermal,[300,301] supercritical fluid,[302] mechanical fractionation, and ultrafine milling with electrostatic separation as the most conventional method for lignocellulosic materials.[303] Contreras et al. reviewed physical protein extraction methods from agricultural and food residues based on dry and nondry extraction conditions and use of different protein extraction/recovery methods.[283] Ultrasound-assisted extraction is widely reported for extraction of proteins from soybean wastes, sunflower meal and deoiled seedcakes, and defatted rice bran.[304,305] Up to 88% protein

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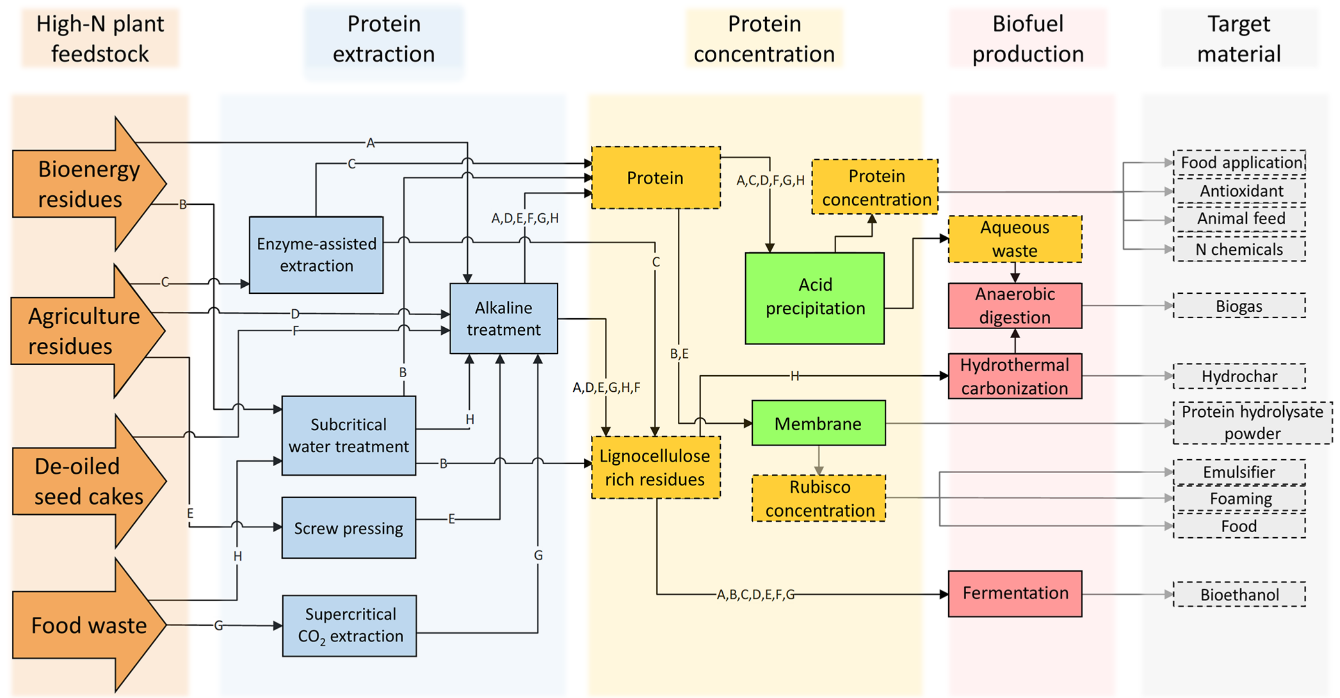

Figure 1. Example mass flow diagrams for low-cost lignocellulosic feedstock coupling protein extraction and conversion to produce a suite of target biorefinery materials. Letters A−H represent pathways described in literature studies.

recovery has been reported for fruit seeds and plant leaves using ultrasound-assisted extraction, depending on the type of ultrasonic reactor (bath or probe), sonication frequency, and power.[306] Bedin et al. compared ultrasound-assisted and microwave-assisted extraction methods for alkaline protein extraction from rice residues; ultrasound-assisted showed the highest yield (12.1%) and protein content (75%).[307] Ultrasound-assisted extraction, with either chemical extraction or novel methods, is the most recommended combined method to improve the protein yields.[296] Di Domenico Ziero et al. reviewed protein extraction under subcritical and supercritical water as an efficient and environmentally friendly method.[308]

Biochemical methods of protein extraction, using single or multiple enzymes, have recently gained attention. Because of the rigid cell walls and high lignin contents in agriculture and processing wastes, cell disruption is one of the main challenges for protein extraction. Enzyme-assisted extraction can further increase protein yields.[290] Carbohydrolases, proteases, and pectinase have been used to release proteins from lignocellulose biomass, often with superior quality, lower viscosity, better thermal stability, and higher solubility compared to extraction without enzymes.[298] Protein yields, enzymes used, and optimized extraction parameters were reviewed by Contreras et al.[283] and Nadar et al.[309] A combination of enzyme-assisted extraction with physical extraction often increases extraction efficiency (and economic viability) depending on cell wall rigidity, chemical composition, protein structure, and storage conditions.[21] Enzyme-assisted processes can be performed under mild conditions (pH of 6−8 and temperatures of 40−60 °C) without the presence of toxic chemicals and to the desired degree of hydrolysis.[300]

Table 5 summarizes protein extraction techniques with their protein yields and recoveries, and advantages and disadvantages. Alkaline treatment is the most common and affordable approach to extract proteins from agricultural and food residues.[310] Alkaline extraction at high pH (generally pH > 9) can result in 90% recovery of the original proteins. Chemical extraction methods, however, are time-consuming, energy-consuming, and less economical. The destruction of amino acids, like lysine and cysteine, and the formation of cross-linked amino acids lead to lower overall quality, meaning that alkaline treatment is not widely used in food production.[311] Extraction of proteins with organic solvents showed low protein recovery and quality compared to alkaline extraction. Enzyme-assisted extraction and ultrasonic-assisted extraction are effective for thermally sensitive proteins since they do not require high temperatures like microwave-assisted methods. Physical extraction methods have shorter processing times and lower unit costs compared to conventional enzymatic extraction. Ultrasound-assisted extraction before enzymatic extraction is one of the most preferred and feasible methods for a large-scale biorefinery.[298]

Protein purification after extraction usually depends on the physical and chemical properties of the target proteins. Acid precipitation,[319] isoelectric precipitation coupled with electrolyzed water treatment,[313] membrane separation,[320] hydrophobic interaction chromatography,[321] and electrophoresis[322] are the most common methods to separate and purify proteins from agricultural and processing wastes. A large amount of nonproteinaceous compounds are also present in the protein precipitate.[323] Extraction of proteins from oilseed cakes has been done using hydrothermal and enzyme-assisted processes, followed by ultrafiltration and isoelectric precipitation, electrolyzed water treatment, alkaline precipitation, or acidic precipitation.[41,284,324] Extraction of proteins from DDGS and food wastes has been reported using alkaline,[292] hydrothermal,[301] and enzyme-assisted extraction, or coupling

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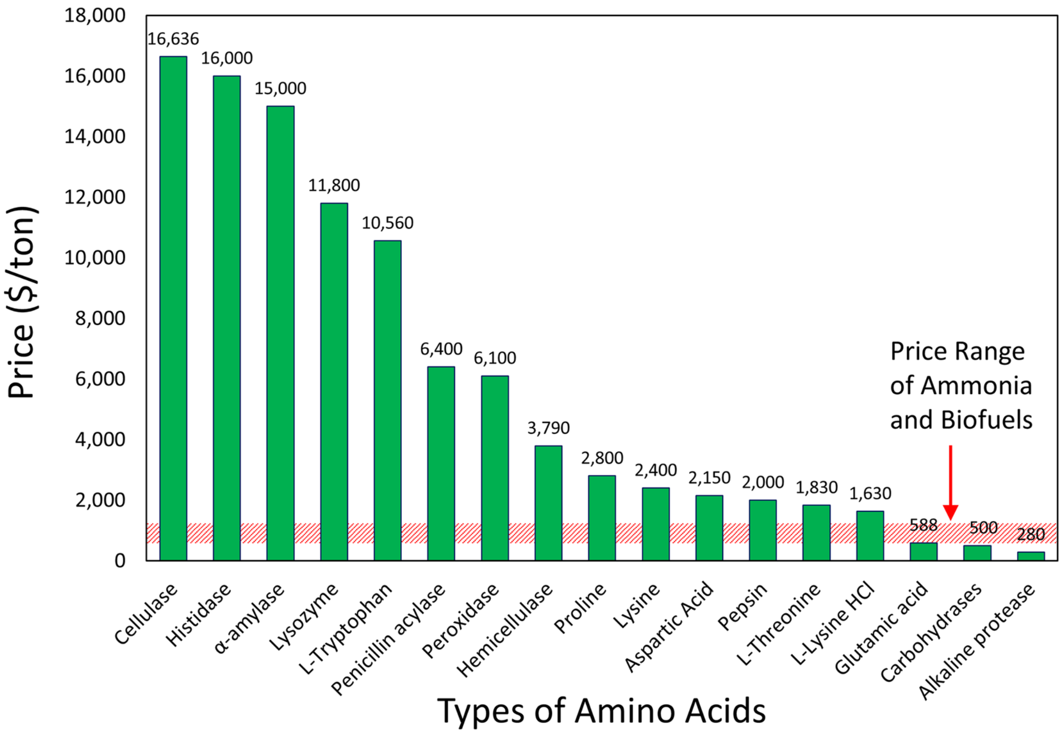

Figure 2. Prices of enzymes and amino acids derived from organic wastes as compared with the approximate prices of NH_3 and biofuels (red slash-line ribbon) on a per ton basis.[338,345,346]

AFEX pretreatment and enzymatic extraction followed by acid precipitation.[34]

After purification, proteins are frequently hydrolyzed into free amino acids and oligopeptides using acidic, alkaline, subcritical water, or enzymatic-assisted methods. The acidic and alkaline approaches have some drawbacks, such as the risk of solvent leakage, difficulty in reactor design, degradation of some amino acids, and the formation of salt wastes.[325] Subcritical (and supercritical) water hydrolysis is considered a green process for simultaneous extraction and hydrolysis of protein but requires high energy inputs and infrastructure investment.[308] Enzyme-assisted approaches are less energy-intensive and more environmentally friendly than other techniques and can be used alone or in combination with other methods.[326] Glutamic acid is the most abundant nonessential amino acid derived from the hydrolysis of plants and a top candidate for production of bulk biobased chemicals such as *N*-methylpyrrolidone, *N*-vinylpyrrolidone, and acrylonitrile.[327] Amino acids derived from high-protein waste streams can be used directly as precursors to some value-added chemicals using decarboxylation and deamination to form nitrogenous compounds such as amines, (cyclic) amides, or nitriles. Further details of these methods are available in the study by De Schouwer et al.[21]

6. CONVERSION PATHWAYS FOR LOW-COST, N-CONTAINING BIOMASS

Several studies have reported on protein extraction in biorefinery schemes. Figure 1 shows example pathways for the various fractions of biomass from starting materials to final products. Kehili et al. reported on protein extraction during recovery of carotenoids from tomato peels and seeds; supercritical CO_2 was used to recover carotenoids, followed by alkaline protein extraction before hydrolysis of the cellulose and hemicellulose to produce bioethanol; 90% of the initial *β*-carotene content and 30% of the initial protein content were recovered.[328] Bals et al. recovered proteins from switchgrass using aqueous NH_3 and then hydrolyzed the remaining sugars to produce bioethanol; they reported a protein recovery of 87% and a sugar recovery of 74%.[286] Chiesa et al. considered three routes for protein extraction from dry biomass during production of cellulosic ethanol: before feedstock pretreatment, after feedstock pretreatment, and after saccharification. They reported that protein extraction from fresh leafy biomass has higher yield and quality compared to extraction from dry biomass.[28] Sanders et al. extracted amino acids from potato starch processing waste to be used for fermentative production of acrylonitrile and urea.[329] Dang et al. studied recovery of collagen protein powder from chromium leather scrap waste; the extract contained different amino acids and low concentrations of mineral salts that can be used as a biofertilizer.[330] Since the extraction denatures the collagen fibers and enables degradation by anaerobic microorganism, collagen protein extraction pretreatment can be used for biogas production from leather waste.[331]

The extraction of proteins prior to thermochemical processing (pyrolysis, HTL, and hydrothermal carbonization (HTC)) has been investigated.[332−334] Massaya et al. described a multiple-product process for spent coffee grounds. A series of hydrothermal processes were used to obtain an antioxidant aqueous extract containing chlorogenic acids, polyphenolics, and polysaccharides. Proteins (21.8−32.8 wt %) were then recovered from the residual cake using alkaline extraction and acid precipitation. The final solid residuals were converted to hydrochar using HTC.[334] HTC-char after N recovery had a higher HHV content (32−37 MJ/kg vs 29−36 MJ/kg) and a higher burnout temperature (518 °C vs 452 °C). Arauzo et al. described a biorefinery approach for brewer's spent grains in

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which proteins were first extracted using alkaline pretreatment and acid precipitation, followed by HTC. Extraction of the protein allowed for a higher C/N ratio and lower ash content in the hydrochar.[335] Integrated protein extraction with pyrolysis has been explored for microalgae and sewage sludge with promising results,[48,300,336,337] but little is available on the pretreatment of lignocellulosic biomass to remove protein before pyrolysis.

7. CHALLENGES AND PERSPECTIVES

To date, few fuel upgrading catalysts have been shown to be effective at denitrogenation of biocrude oil, as most of the N is contained in aromatic compounds (e.g., pyridine derivatives), the condensed structures of which are extremely stable and require a large amount of energy to break down. Even if N can be completely removed from biocrude oil, the energy consumption for the formation of the N-free biofuel and the NH_3 are unlikely to be compensated by market benefits: 500−700 USD/t for the biofuel and 300−900 USD/t NH_3 (which fluctuate dramatically due to the unstable supply and demand).[338] To minimize the energy input into the biorefinery, more attention should be paid to development of milder and more efficient techniques for removal of N-containing compounds before severe processing conditions can form stable N-containing condensed products in the biomass. The global markets for amino acids, and in particular, glutamic acid, are expected to reach 43.55 and 22.55 billion USD, respectively.[339,340] Most organic waste-derived amino acids have much higher market values than those for biofuels or NH_3 (Figure 2). The potential market demands and higher values for amino acids should allow higher cost and greater energy consumption for protein extraction and amino acid production prior to biomass conversion into bioenergy, and simultaneously support the whole biorefinery. Future research, therefore, should focus more on the development of delicate chemical or biochemical processes for protein extraction from biomass, the preserve the original protein structures, and avoid cross-linking to enable better hydrolysis into amino acids.[341] Even though more severe techniques (hydrothermal conversion and supercritical solvent extraction) may extract protein with higher yields,[342] the quality of proteins may deteriorate (e.g., loss of functional groups), leading to lower productivity and selectivity, functionality failure within the desired amino acid products, or lower quality downstream value-added polymer products (e.g., polyurethane).[21,343,344] Careful attention needs to be paid to the trade-off between protein yield and protein quality in order to achieve the highest atom efficiency in the ultimate products.

8. CONCLUSION

Substantial amounts of plant biomass materials are available at low cost that contain valuable protein and nitrogenous compounds. Use of these materials in food and feed applications is limited by the presence of inedible or nonpalatable constituents. The lignocellulosic fractions of these biomass sources are good targets for reduced-cost biofuels production if the N-containing compounds can be removed—ideally for other value-added use. Separation of N from biofuel intermediates is difficult due to the types of N bonds created during biomass conversion. Rather than denitrogenation of biofuel intermediates, researchers should devote more efforts to the preconversion removal of N through protein extraction methods so that these lignocellulosic biomass resources can be better utilized to address waste management, renewable energy, and N cycling issues.

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The authors declare no competing financial interest.

Biographies

Hengameh Bayat is a Ph.D. candidate in chemical engineering at New Mexico State University (NMSU). She holds a B.S. degree in chemical engineering and an MS degree in renewable energy engineering from Iran. Her research interests include thermochemical conversion of waste material, nutrient recovery methods, and wastewater treatment.

Feng Cheng received a B.S. degree in chemical engineering from Beijing University of Chemical Technology and a Ph.D. in chemical engineering from New Mexico State University (NMSU). He completed postdocs on the utilization of guayule biomass at NMSU and on catalytic hydrothermal liquefaction of food waste at Worcester Polytechnic Institute. He is currently a postdoctoral research associate at University of Wisconsin−Madison working on lignin valorization. His research interests include upcycling of organic wastes and reactor engineering.

Mostafa Dehghanizadeh is a Ph.D. candidate in chemical engineering at New Mexico State University (NMSU). He received his B.S. and M.S. degrees in chemical engineering from Iran. His research focuses on utilization of different kinds of biomass, designing and conducting experiments in biomass conversion and extraction, and characterizing products from biomass.

Catherine Brewer is an associate professor in the Department of Chemical & Materials Engineering at New Mexico State University. She received a B.S. degree in chemistry from Indiana University of Pennsylvania and a Ph.D. in chemical engineering and biorenewable resources & technologies from Iowa State University. She completed a postdoc on the science and engineering of biochars at Rice University. Besides thermochemical conversion of biomass for fuels, her research interests include brewing, alternative crops, and soil quality.

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■ ABBREVIATIONS

AFEX, aqueous ammonia extraction; DDGS, dried distiller's grains with solubles; Gt, gigaton; HHV, higher heating value; HTC, hydrothermal carbonization; HTL, hydrothermal liquefaction; NO_x, nitrogen oxides; WDGS, wet distiller's grains with solubles

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