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CAZyme from gut microbiome for efficient lignocellulose degradation and biofuel production

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Over-exploitation and energy security concerns of the diminishing fossil fuels is a challenge to the present global economy. Further, the negative impact of greenhouse gases released using conventional fuels has led to the need for searching for alternative biofuel sources with biomass in the form of lignocellulose coming up as among the potent candidates. The entrapped carbon source of the lignocellulose has multiple applications other than biofuel generation under the biorefinery approach. However, the major bottleneck in using lignocellulose for biofuel production is its recalcitrant nature. Carbohydrate Active Enzymes (CAZymes) are enzymes that are employed for the disintegration and consumption of lignocellulose biomass as the carbon source for the production of biofuels and bio-derivatives. However, the cost of enzyme production and their stability and catalytic efficiency under stressed conditions is a concern that hinders large-scale biofuel production and utilization. Search for novel CAZymes with superior activity and stability under industrial condition has become a major research focus in this area considering the fact that the most conventional CAZymes has low commercial viability. The gut of plant-eating herbivores and other organisms is a potential source of CAZyme with high efficiency. The review explores the potential of the gut microbiome of various organisms in the production of an efficient CAZyme system and the challenges in using the biofuels produced through this approach as an alternative to conventional biofuels.

KEYWORDS
CAZyme, biofuel, biorefineries, ruminants, lignocellulose, rumen

1 Introduction

Microorganism with ubiquitous nature and high genetic diversity has evolved over millions of years to adapt to and form a unique ecological niche in different ecosystems on earth. A different group of microbes including bacteria, fungi, protozoa, viruses, etc. interacts together to form a complex microbial community termed microbiota which, along with their entire genetic content, is termed microbiome (Dekaboruah et al., 2020). These microbiomes are found in different environments such as soil, deep oceans, hot springs, etc. They are also found to be present in different living forms such as humans, insects, plants, animals, etc., and consist of all commensal, symbiotic, and pathogenic microbes living in association and interacting with their host organism (Wagg et al., 2019).

In recent years, various significant interests have evolved in the gut microbiome of different life forms since the microbiota is involved in the nutritional and metabolic activities that directly influence the health of the host. Earlier studies focused majorly on the composition of these microbiomes but the functional aspect of these microbiomes and their influence on the host health is analysed currently (Antwis et al., 2017; Coyte and Rakoff-Nahoum, 2019). Microorganisms in microbiomes interact with their host and among themselves to form synergistic relationships. It also determines the composition and influences not only where they reside but it also effects the physiology of their hosts (Foo et al., 2017; Contijoch et al., 2019). One such example is the gut microbiome of different organisms with various plant parts and polysaccharides as their nutritional source such as termites, ruminants, and other herbivores. The microorganisms are essential for the utilization of the saccharides trapped in this recalcitrant carbon source since the host lacks the enzymes required for their metabolism (Chettri and Verma, 2022a). Here the active microbial breakdown of dietary plant polysaccharides takes place and the gut of ruminants consists of plant polysaccharides, where 70% of the volume of the gut is devoted to microbial fermentation (Flint, 2020).

The gut microbiota from these organisms has become an interestingly attractive source of enzymes responsible for the degradation of lignocellulose also called the Carbohydrate Active Enzymes (CAZymes) (Wardman et al., 2022). The CAZymes have been divided into six major classes i.e., Glycosyl hydrolase (GHs), Glycosyl transferase (GTs), Polysaccharide Lyases (PLs), Carbohydrate Esterases (CEs) and Auxiliary Activities (AAs) which are involved in the hydrolysis, formation, and modification of glycosidic bonds and are essential for carbohydrate metabolism (Chettri et al., 2020). These CAZymes are of high industrial value and are also essential pertaining to the concept of sustainable development (Jakeer et al., 2020). High energy demand, emission of harmful pollutants from conventional fossil fuel and their limited availability has sparked research interest in alternate energy source in the form of biofuel which is generated using different carbon source with lignocellulose being considered the most abundant, economic and environmentally friendly option (Zhou et al., 2021).

Lignocellulose is the most copious renewable biomass on earth, and it is also considered a carbon-neutral source that decreases carbon dioxide emission and atmospheric pollution. It is the major component of different waste streams from different industries, agriculture, forestry and municipalities. Lignocellulose is composed of the following components: Cellulose, hemicelluloses and lignin which form a highly complex structure that is recalcitrant to degradation (Zhu et al., 2020). The CAZymes from various sources play a crucial role in the hydrolysis of the sugar polysaccharides in the lignocellulose for biofuel production. To increase the commercial viability of lignocellulose as an alternate energy source an efficient CAZyme system is required for which different ecosystems in nature are mined (Wertz and Béchade, 2020). Since the gut microbiome is such a prominent source and several studies indicate the potential of lignocellulose (Calusinska et al., 2020; Peng et al., 2021; Huang et al., 2022; Xiao et al., 2022), the present review examines the gut microbiome of herbivores and insects from this perspective. Previous review articles were limited to a specific part of organisms such as insects (Jang and Kikuchi, 2020; Bhujbal et al., 2021), or herbivores (Ozbayram et al., 2020; Chettri and Verma, 2022b). We searched the literature for prominent CAZymes from the guts of various organisms in the animal kingdom where a clear relationship between diet, gut flora composition, and CAZyme was found.

2 Gut microbiome as a source of carbohydrate active enzymes

The gut of herbivorous animals is mainly the source of microorganisms that exhibit hydrolytic activity, allowing them to convert complex feed material into readily absorbable monomers. The gut of herbivorous animals, especially ruminants, is a rich source of microorganisms capable of producing carbohydrate-active enzymes (CAZymes) as they consume plant fibers and lignocellulosic plants. The gut microbiome of carnivores, on the other hand, specializes in the breakdown of proteins as an energy source and does not have CAZyme-producing organisms. Omnivores, such as humans, have certain bacteria in their gut that produce CAZymes because humans consume plants directly or indirectly.

2.1 Gut microbiome of ruminants

The rumen is the most important organ of ruminants with numerous and diverse microflora. There are many types of microorganisms belonging to different species (Zou et al., 2018). The rumen functions as an anaerobic digestive chamber that maintains a relatively constant pH of 6–7. It is inhabited by more than 5,000 species of microorganisms, 95% of which are bacteria while the remaining 3%–4% belong to archaea, protozoa, anaerobic fungi, viruses, and bacteriophages (Cholewińska et al., 2021). These microorganisms break down forage fibers (generally cellulose and hemicellulose) through enzymatic reactions in the rumen into monomeric compounds that can be easily digested and absorbed by the body (Chen H et al., 2021). The main function of the rumen

TABLE 1 List of Ruminants studied as source of CAZyme producing bacteria.

Name of ruminants	Major findings	References
Goat	Grain diet influence the structure of rumen microbiota, assure high population of several bacterial taxa that degrade plant fiber and starch, including *Butyrivibrio* (dominating), *Mogibacterium*, *Acetitomaculum* and *Anaerolineaceae* (unclassified)	Mao et al. (2016)
Young cattle	Dominating hemicellulose degrading bacteria phyla were *Bacteroidetes*, *Proteobacteria* and *Firmicutes* respectively that are influenced by saponins (camellia seed extract). *Prevotellaceae*, *Lachnospiraceae* and *Ruminococcaceae* groups were in abundance	Wang et al. (2019)
Yak	Dominating bacterial phylum that play key role in degradation of feed material as well as seemed potent source of CAZymes were *Bacteroidetes* (87%), *Firmicutes* (11.23%) and *Proteobacteria* (1.37%). At genus level, predominant bacterial populations were *Prevotella*, *Ruminobacter* etc.	Zou et al. (2019)
Dairy cow	16S rRNA gene sequence-based analysis of ruminal fluid microbiota results *Bacteroidetes* as dominating phylum (58.82%) followed by 37.60% *Firmicutes* and 5.51% *Actinobacteria*. *Prevotella*, *Ruminococcus*, and *Butyrivibrio* were the three most abundant genera	Huang et al. (2020)
Sheep	The rumen fluid of sheep contains *Bacteroidetes* as the dominating bacterial phylum followed by *Firmicutes* and *Proteobacteria*. The common bacteria include *Prevotella*, *Fibrobactor*, *Ruminococcus* etc.	An et al. (2020)
Buffalo	Metagenomic analysis of the rumen showed presence of *Bacteroidetes*, *Spirochaete* and an unidentified bacterial phylum	(Bohra et al., 2022; Cabral et al., 2022)

microflora is to convert plant food into energy. The resulting products are volatile fatty acids (VFAs) - mainly propionate and butyrate - and proteins. These are the main source of energy for the animals. This process has a direct impact on the physiological parameters of the animals, including production rates (De Nardi et al., 2016). Studies have shown that many important factors influence the abundance and composition of the rumen microbiota, for example, individual differences between animals, diet composition, and fineness-to-roughness ratio (Cholewińska et al., 2021).

The rumen of adult ruminants consists of many bacterial species involved in the breakdown of cellulose and lignin. Some common microorganisms are *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Prevotella ruminicola* (Liu et al., 2020), of which *F. succinogenes* is the most abundant and significant in the degradation of plant cellulose (Sha et al., 2020). The common microbiota of ruminants in the rumen and surrounding gut is summarized in Table 1.

A greater fraction of enzymes targeted against oligosaccharides is associated with bacteria present in the animal's digestive system, including β-glucuronidase, β-D-mannosidase, β-xylosidase, β-1,3-xylosidase, galactan 1, α-L-arabinofuranosidase, β-D-galactosidase, exo-β-glucosaminidase, arabinanase, xylanase, and 3-β-galactosidase (Sha et al., 2020).

2.2 Gut microbiome of other vertebrates

The gastrointestinal tract (GI) of vertebrates is a special environment that is composed of interactions that are ecologically conserved as well as an evolutionary progression between the host and resident microflora (Youngblut et al., 2019). However, the gut microflora varies greatly among animals, as the morphology of the GI tract differs among species (Siddiqui et al., 2022).

Approximately 90% of the fish gut microbiota is composed of Proteobacteria, Firmicutes, and Bacteroidetes. Some common bacterial genera are *Bacillus*, *Staphylococcus*, *Lactobacillus*, *Actinobacteria*, etc. (Clements et al., 2014). In fish, hydrolysis of β-glycosidic bonds of non-starch polysaccharides (derived from plant feeds) by CAZymes is scarce or absent. However, *in vitro* studies on several carbohydrate-active *Bacillus* species isolated from the hindgut of herbivorous fish showed cellulase and xylanase activity. This observation was supported by the presence of genes encoding specific extracellular CAZymes that help fish digest the non-starch polysaccharides of plant feeds (Serra et al., 2019).

The digestive tract of birds' GI harbors a high density of microbial communities, up to 1011 CFU/g in the hindgut. As in the case of other vertebrates, the avian microbiota GI is dominated by Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria (Waite and Taylor, 2015). The dominant bacterial genera are *Enterococcus*, *Escherichia*, *Bacillus*, etc. (Siddiqui et al., 2022). A South American bird, the hoatzin (*Opisthocomus hoazin*), has unique nutritional characteristics. Unlike other birds, it carried out foregut fermentation in leaves and plants. As a result, its digestive strategy is more similar to ruminants than birds. Their forestomach microbiota is dominated by Firmicutes, Bacteroidetes, and Proteobacteria (Godoy-Vitorino et al., 2012). This may be an important source for isolating CAZyme-producing bacteria.

According to studies, only ~17% of vertebrate taxa are known in reptiles (Keenan et al., 2013). The most abundant phyla in the reptile gut are Proteobacteria, Bacteroidetes, Firmicutes, and Fusobacteria (Zhang et al., 2019). Some common bacterial genera are *Pseudomonas*, *Staphylococcus*, *Enterobacter*, *Aeromonas*, etc. (Siddiqui et al., 2022), while there is little information on the gut microbiome of amphibians. As in humans and mice, Bacteroidetes, Firmicutes, and Proteobacteria are the main components of the gut microbiome of amphibians. Some Flavobacteria are also observed (Colombo et al., 2015). There is no documentation supporting the presence of CAZyme-producing bacteria in reptiles and amphibians, as they are mostly carnivorous animals.

According to studies, only about 10% of vertebrate taxa are known to exist in mammals, particularly humans and mice (Keenan et al., 2013). The gut of mammals contains about 1,014 microorganisms (>1 kg). For example, human gut bacteria include predominantly species of the following genera: *Escherichia*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Lactobacillus*, *Bacteroides*, *Butyrivibrio*, *Bifidobacterium*, *Prevotella*, etc. various yeasts and other microorganisms may also be present (Nicholson et al., 2005). Most CAZymes are produced by members of the *Bacteroides* sp., *Butyrivibrio fibrisolvens*, *Clostridium hathewayi*, *Enterobacter cloacea*, *Escherichia coli*, *Klebsiella pneumoniae*, *Prevotella copri*, *Prevotella salivae*, etc. (Ghosh and Pramanik, 2021).

In addition to ruminants, CAZymes have also been studied in various mammals. For example, in an adult elephant, the members of the phylum Proteobacteria show a maximum CAZyme abundance of 91%, followed by Actinobacteria and Bacteroidetes each with a value of 4.6% and 2.5% respectively (Jakeer et al., 2020). The pseudo-ruminant dromedary camels have a rumen microbiota which is handy compared to ruminants. The bacterial community for CAZymes was *Prevotella* (23%), *Ruminococcus* (1%), *Bacteroides* (4%), *Butyrivibrio* (1%), and *Fibrobacter* (4%), (Hinsu et al., 2021). Researchers had identified an endonuclease with cellulase activity in a bacterial strain isolated from the intestine of a bamboo rat; a rich CAZyme family was also detected in the appendix microbiome. In lignocellulosic diets, the dominant microbial families in the gut include Muribaculaceae, Lachnospiraceae, Ruminococcaceae, and Desulfovibrionaceae (Xiao et al., 2022).

2.3 Gut microbiome of insects

Insects are the most diverse group of animals in different ecological niches. It is estimated that the insect gut contains microorganisms 10 times higher than their cells and their microbial genome is also 100 times their genes (Krishnan et al., 2014). The insect gut is inhabited by diverse microorganisms including bacteria (most abundant), fungi, archaea, protozoa, and viruses (Munoz-Benavent et al., 2021). The most common and abundant bacteria include Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Tenericutes (Brune and Dietrich, 2015).

Termites are one of the most successful groups of organisms that can degrade woody materials. Therefore, their gut is a potential source of CAZyme-producing bacteria (Krishnan et al., 2014). Wood-eating termites are generally higher termites in which Spirochaetes and Fibrobacteres are the dominant bacteria responsible for digesting lignocellulose. In lower termites, lignocellulose is degraded by symbiotic flagellates (Munoz-Benavent et al., 2021). Shipworms are a good example of wood-feeding organisms that contain multiple endosymbiotic bacteria capable of producing a large number of carbohydrate-active enzymes (CAZymes).

3 Industrial production of carbohydrate active enzymes

The commercial enzymes production, including CAZymes, can be classified into two phases, upstream and downstream processes. The upstream process involves the selection of a suitable microorganism/strain, followed by strain improvement and process development in order to optimize growth and enzyme production conditions (Zeng et al., 2020). The different substrates, fermentation conditions, and environmental factors such as pH, temperature, etc. are considered to maximize enzyme production. The optimized conditions will then be applied to flask/laboratory scale production, followed by a pilot plant that will eventually be scaled up to an industrial-scale fermenter. Novel approaches to fermenter design and mathematical modelling will be used for the upstream processes (Wehrs et al., 2019; Tarafdar et al., 2021).

Downstream processing includes recovery of the enzyme, concentration, and purification of the enzyme for various applications. Depending on the type of enzyme and the application, different techniques such as chromatography, phase separation, and, depending on the location of the enzyme, cell lysis are used (Yan et al., 2018; Cortes, 2020). The purified CAZyme is then used for enzymatic hydrolysis of lignocellulose to produce simple sugar molecules, which are then further used for biofuel production.

A wider range of natural polysaccharide-based substrates from the agro-industrial sector, e.g., lignocellulose, is available for biotransformation into industrially relevant and high-value products such as "biofuel" (Arevalo-Gallegos et al., 2017). CAZymes are used in biofuel production to convert this recalcitrant polysaccharide into fermentable carbohydrate monomers (Tingley et al., 2021). They offer a sustainable alternative to surmount the drawbacks linked with conventional chemical catalysts (Saini et al., 2022). Bacteria and anaerobic fungi are considered promising for lignocellulosic biofuel production due to their natural diversity of CAZymes (Saye et al., 2021). Thus, rumen isolates and their CAZymes provide an efficient system for

TABLE 2 List of CAZymes screened from different insect gut source.

Insect name	Name of enzyme	CAZyme family	Functional application	References
Termites (*Reticulitermes flavipes*)	1- β- glucosidase	GH1, GH3	Lignocellulose degradation	Corcoran et al. (2008); Krishnan et al. (2014); Scharf et al. (2010); Tartar et al. (2009)
	Esterase	Carboxyl esterase	Hemicellulose solubilization	Krishnan et al., 2014
	α-mannosidase	GH38, GH47	Mannose degradation	Tartar et al. (2009)
Rotschildia lebaeu (Lepidoptera)	Xylanase	GH8	Xylan degradation	Brennan et al. (2004)
Aedes aegypti	β-1,3-glucanase	GH16	β-1,3-glucan degradation	Souza et al. (2019)
Coptotermes formosanus	β -glucosidase	GH1	Cellulose degradation	Zhang et al. (2012)
Limnoria quadripunctata	Cellobiohydrolases	GH7	Lignocellulose degradation	Kern et al. (2013); Krishnan et al. (2014)
Lyrodus pedicellatus (shipworm)	Endo- β-1,4 -glucanase and β- glucosidase	GH9, GH45, GH1, GH13, GH2, GH18, GH31, GH5, GH10, and GH38	Lignocellulose degradation	Sabbadin et al. (2018)
Gastrophysa viridula	Endo-β-1,4-glucanase, endo-β-1,4-xyloglucanase	GH9, GH45	Plant polysaccharide degradation	Busch et al. (2018)
Odontotaenius disjunctus (wood feeding beetle)	Endocellulases, endoxylanases, cellobiohydrolases, cellodextrinases, β-glucosidases, β-xylosidases	GH1, GH3, GH5, GH8, GH10, GH43	Cellulose and xylan degradation	Ceja-Navarro et al. (2019)
	Polyphenol laccase, Fe-Mn superoxide dismutase and feruloyl esterase	AA10	Lignin degradation	

the degradation of lignocellulose for biofuel production (Patel et al., 2020).

4 Lignocellulose structure

Lignocellulose is a very complex and heterogeneous substance consisting of two main biopolymers, i.e., holocellulose and lignin. The holocellulose in turn comprises two different biopolymers, namely cellulose and hemicellulose (Smith, 2019). The ratio of these components can be different among species, species, and even within an individual plant depending on age, growth stage, and environmental parameters (Bajpai, 2016).

4.1 Cellulose structure

Cellulose is the predominant component of the lignocellulosic matrix and endows mechanical strength on the plant cell wall. Some bacterial, algal, and tunicate species are also known to produce cellulose (Seddiqi et al., 2021). It is an unbranched, linear homopolysaccharide composed of glucopyranose units linked by β-1,4-glycosidic bonds, with each monomer unit twisted 180° relative to its neighbours. The recurring unit of this biopolymer i.e. cellobiose consists of two adjacent glucose units linked by glucosidic bonds (C-O-C) at positions C1 and C4 (Chen P et al., 2021). The length of the cellulose chain depends on the degree of polymerization (DP), which can vary widely, from about 20 in synthetic cellulose to about 10,000 or more in cotton, plant fibres, and bacterial cellulose (Fang et al., 2020). The presence of two types of hydrogen bonds between the OH group of C3 and the oxygen atom of the pyranose ring within the same molecule and the oxygen of the hydroxymethyl group at the C6 position of a molecule and the oxygen atom of the glycosidic bond of the adjacent molecule gives cellulose fibrils a high axial rigidity. This crystal forms a fervent structure that cannot be penetrated by water or enzymes, making them insoluble in water. These intermolecular hydrogen bonds and van der Waals interactions promote the parallel stacking of multiple long-chain cellulose polymers, which form elemental fibrils that further group into microfibrils (Gupta et al., 2019). Microfibrils are further aggregated into cellulose fibres (Bajpai 2016). Within cellulose fibrils, a large percentage of cellulose is arranged in highly ordered crystalline forms, while a small portion of disordered cellulose chains exist in amorphous form (Gupta et al., 2019). Crystalline cellulose exists in several different allomorphs (cellulose I, II, III, and IV), of which cellulose I is the thermodynamically metastable and cellulose II has maximum stability (Baghaei and Skrifvars, 2020).

4.2 Hemicellulose structure

Unlike cellulose, hemicellulose is a heterogeneous polymer of 5-C sugars (arabinose, xylose, and rhamnose), 6-C sugars (galactose, mannose, and glucose), and uronic acids (D-galacturonic acid, 4-O-methylglucuronic acid, and D-glucuronic acid) (Ayeni et al., 2015). These monosaccharides are arranged in either homopolymeric or heteropolymeric configurations with small side branches linked by β-(1,4) and β-(1,3) glycosidic linkages (Bajpai, 2016). The backbone of hemicelluloses is arranged in different compositions depending on biomass type. Agricultural biomass such as straw, grasses, and hardwood hemicelluloses are mainly composed of xylan, while glucomannan is the major component in softwood hemicelluloses (Bajpai, 2016). In addition to a common 1,4-linked β-D-xylopyranose units backbone, they may comprise acetic acid, arabinose, ferulic acid, glucuronic acid or its 4-O-methyl ether, and p-coumaric acid (Qaseem et al., 2021).

4.3 Lignin structure

Lignin is one of the most abundant terrestrial biopolymers, surpassed only by cellulose and accounting for a significant proportion of the lignocellulosic biomass. It is an amorphous heteropolymer that is not composed of carbohydrates and has a complex structure resulting from the oxidative coupling reaction between three monolignols (phenylpropane unit): p-coumaryl alcohol, sinapyl alcohol, and coniferyl alcohol. In the lignin polymer, these modified phenylpropanoid monomers are recognized as p-hydroxyphenyl (H-lignin), syringyl (S-lignin), and guaicyl (G-lignin) units. Alkyl-alkyl, alkyl-aryl, and aryl-aryl ether linkages are the primary linkages used for the polymerization of phenolic monomers (Ralph et al., 2019).

4.4 Lignocellulose degradation

The complex protective barrier of lignin and hemicellulose surrounding cellulose along with the crystalline structure of cellulose makes it difficult to break down lignocellulose into its components. Various chemical, physicochemical, physical, and biological pretreatment methods play an important role in depolymerizing lignocellulosic biomass into its major components. Biological pretreatment by microorganisms is a cost-effective and environmentally friendly approach (Kumar et al., 2020).

The various enzymes produced by these organisms target the lignocellulosic components to degrade them. Enzymes involved in lignin polymerization are broadly classified into two classes based on their mode of action: Lignin-modifying enzymes (LMEs) and lignin-degrading auxiliary enzymes (LDAs). LMEs include lignin peroxidase, manganese peroxidase, versatile peroxidase, laccases, and dye-degrading peroxidase. Rather than degrading lignin themselves, LMEs assist LDAs in the degradation process. Glyoxal oxidase, glucose dehydrogenase, aryl alcohol oxidase, cellobiose dehydrogenase, and pyranose-2-oxidase are lignin-degrading auxiliaries involved in various steps of lignin degradation (Sharma et al., 2020). The complex structural heterogeneity and chemical diversity of hemicellulose require the synergistic activity of a wide range of enzymes for its effective hydrolysis. The cellulase enzyme system includes three soluble extracellular enzymes: 1, 4-β-endoglucanase (EC 3.2.1.4), 1, 4-β-exoglucanase (cellobiohydrolase) (EC 3.2.1.91), and β-glucosidase (β-D-glucoside glucohydrolase or cellobiase) (EC 3.2.1.21) (Nandy et al., 2021). Absolute hydrolysis of cellulose to glucose is only possible through the synergy of the three enzymes mentioned above or thorough mineralization to H_2O and CO_2 (Popovic et al., 2019).

The isolates from the rumen possess these CAZymes, which have been explored by several researchers for the degradation of lignocellulose (Table 2).

4.5 Production of biofuels

In recent decades, biofuel production has been explored worldwide as a sustainable substitute to fossil fuels because it is cost-effective, eco-friendly, and has an abundant substrate. Based on substrate source and the technical aspect of production, biofuels have been categorised into four generations (Rai et al., 2022) (Table 3). However, to date, more than 90% of bioethanol produced worldwide is derived from edible crops such as sugarcane, beets, corn, etc., which ultimately affects food security and leads to inflation (Choudhary et al., 2020). The United States and Brazil rank first and second, respectively, in the production of bioethanol and biodiesel (Sajid et al., 2021). In 2019, the U.S. recorded bioethanol production of 15.8 billion gallons, representing about 54% of global production. The second largest producer, Brazil, recorded bioethanol production of 35.6 billion gallons from sugarcane and corn, which accounted for 30% of global production during the same period. In 2019, the US recorded biodiesel production of 2.5 billion gallons, followed by Brazil with 5.8 billion litres (Sajid et al., 2021). Lignocellulosic biomass includes non-food energy feedstocks used as an alternative to cane sugar or corn-starch for biofuel production and is referred to as second-generation biofuel (Choudhary et al., 2020; Rai et al., 2022). However, the presence of lignin is a barrier to the expansion of

TABLE 3 Comparison of first, second, third, and fourth generation biofuels.

Types	Substrate	Process	Product	Merit	Demerit	Ref
First-generation biofuel	Edible parts of plants (sugar beet, corn sugar cane, grains, vegetable oil, and seeds)	Produced through fermentation of sugars or starches, or cellulose with the action of enzymes and microorganisms, Transesterification of oil seeds	Bioethanol, biodiesel, corn ethanol, sugar alcohol	Less flammable and emit less harmful carbon than fossil diesel	Impact food security, soil quality and may leads to inflation. Not cost competitive with existing fossil fuels	Viesturs and Melece (2014); Rai et al. (2022)
Second-generation biofuel	Non-edible plants or non-edible lignocellulosic biomass of the plants, kitchen and agricultural wastes	Produced through effective pretreatment of biomass followed by hydrolysis, fermentation and saccharification	Ligno-cellulosic ethanol, bioethanol, biobutanol, biodiesel	Relatively cost effective and do not impact land uses, food security	Removal of lignin is complex, challenging as well as costly process and require effective pretreatment processes	(Rai et al. (2022); Tsegaye et al. (2019)
Third-generation biofuel	Derived from aquatic autotrophic organism (microalgae)	Produce by obtaining lipid content of algal biomass and processed through transesterification	Biodiesel bioethanol, hydrogen	High biomass productivity and do not require any farm land. Able to grow throughout the year under various harsh condition	Large volumes of CO_2 and water are required	(Lee and Lavoie (2013); Rai et al. (2022); Shuvashish et al. (2015)
Fourth-generation biofuel	Genetically modified photosynthetic microorganisms (cyanobacteria, fungi, algae)	Microbes are modified genetically in order to increase lipid content	Biodiesel, bioethanol, biobutanol, methanol	Modification enables fastest growth rate, higher yield, low cost and less chances of contamination	May causes environmental and health-related risks due to lateral gene transfer from residual by-product	(Abdullah et al. (2019); Rai et al. (2022); Shokravi et al. (2019)

biofuel production and commercialization (Tran et al., 2019). Therefore, pretreatment of biomass is required to remove the lignin component and increase the accessibility of cellulose and hemicellulose for hydrolysis and fermentation by increasing the porosity and surface area of the substrates (Tsegaye et al., 2019). Currently, there are various pretreatment methods for lignocellulosic biomass, which can be broadly classified into physical, chemical, physiochemical, and biological pretreatments. Physical pretreatment methods include biomass size reduction, extrusion, microwave irradiation, and ultrasonic treatment (Tayyab et al., 2018). Chemical pretreatment includes acid pretreatment, alkaline pretreatment, ozonolysis pretreatment, and organic solvent pretreatment (Rahardjo et al., 2021). Ammonia fiber explosion (AFEX) pretreatment, liquid hot water pretreatment, steam explosion pretreatment, and supercritical CO_2 pretreatment fall under physiochemical pretreatment (Tran et al., 2019; Rahardjo et al., 2021). Nowadays, biological pretreatment with microorganisms is gaining a lot of attention due to its energy efficiency, environmental friendliness, and the fact that no or very few unwanted by-products are generated (Rahardjo et al., 2021). However, biological pretreatment is quite expensive and requires more time (Tayyab et al., 2018; Rahardjo et al., 2021).

The ability of the gut microbiome to produce CAZymes is also a focus of interest worldwide to find an optimal microbial candidate with different enzymatic mechanisms for the depolymerization of lignocellulose. The ability of gut microbes to degrade lignocellulose was discussed in the previous section. The resulting hydrolysis products, which include simple saccharides, can be used for biofuel production (Rabee et al., 2022) evaluated the higher ethanol production associated with the hydrolysis of barley straw by camel and sheep rumen juice containing *Ruminococcus*, *Saccharofermentans*, and *Butyrivibrio*. The physicochemical properties of biodiesel produced by *Meyerozyma caribbica* SSA1654, a manganese-dependent peroxidase-producing oleaginous strain isolated from the guts of the wood-eating termites *Coptotermes formosanus* and *Reticulitermes chinenesis* were thoroughly investigated by (Ali et al., 2021). In this study, CAZyme activity (β-glucosidase, xylanase) was also determined along with a coupled pathway of dye degradation for biodiesel production (Li et al., 2020). reported the presence of 136 genes of CAZyme from five subfamilies in *B. velezensis* LC1, a symbiotic bacterium isolated from the intestine of *Cyrtotrachelus buqueti*. This report also provides crucial insight into the potent ability of *B. velezensis* to convert lignocellulosic biomass to ethanol (Ben Guerrero et al., 2015). described the endoglucanase and xylanase activities of *Cohnella*, *Paenibacillus*, *Acinetobacter*, and *Klebsiella* bacteria associated with the gut digestome of *N. aquilinus* and *C. fulviceps* termites suitable for bioethanol production (Deka et al., 2013). conducted a batch simultaneous saccharification and fermentation experiment using the enzyme cellulase isolated from *B. subtilis* along with *Z. mobilis* and wild grass as the substrate to determine the different pretreatment methods by determining the ethanol yield coefficient (g ethanol/g substrate) (Corcoran et al., 2008). conducted a study on a two-step process of hydrolysis and fermentation to produce ethanol using sugarcane bagasse by microorganisms found in the rumen fluids of dairy cows.

5 Assessment of the feasibility of the application of biofuels

Although biofuels have several advantages compared to fossil fuels, economic and environmental viability are the most important issues that need to be critically analysed in order for biofuel to replace conventional fuels. The life cycle analysis of these biofuels provides information on this aspect of biofuels and helps in policy-making for sustainable production and application of biofuels. In addition, the technological aspect of biofuel application also needs to be reviewed.

5.1 Life cycle analysis

It is generally assumed that biofuels emit fewer pollutants during their life cycle compared to fossil fuels. However, the environmental impact of the energy crops used and the type of lignocellulosic treatment method used must also be examined to determine the overall impact of biofuels. Since there are limited land resources available for energy crop production, in addition to the need for already scarce freshwater, threats to food security and reduction in crop nutrient content are major concerns for biofuel production (Joshi et al., 2017). Therefore, for large-scale production of biofuels, a systematic assessment of their benefits is essential. For this purpose, LCA is used, which provides an overview of the impacts of a production system by collecting data on inputs and outputs and assessing their impact on the environment (Bilal and Iqbal, 2020) (Figure 1).

In LCA, the goal is first defined along with the system boundary (stage of agriculture, biofuel production, etc.), then the inputs and outputs are listed, and then the impact of the system on predefined environmental processes such as ozone depletion, global warming, etc. Is quantified, and finally, the result is interpreted (Morales et al., 2015). The LCA provides an understanding of the impacts of biomass conversion techniques to create awareness among researchers, industry, and policymakers to develop strategies for sustainable biofuel production (Standardization, 2006). Both the economic and environmental impacts of biofuels are evaluated at LCA, using net energy production, overall reductions in pollutant production throughout the cycle, social and economic feasibility, and food security impacts as criteria (Weldu and Assefa, 2016; Mayer et al., 2020).

Numerous studies with different lignocellulosic biomasses have been investigated for biofuel production and the associated risk according to different LCA models. The first approach to LCA for lignocellulosic biofuel production is the study of lignocellulosic briquettes and pellets, followed by different pre-treatment and fermentation strategies. A study was conducted with lignocellulosic biomass in different parts of Latin America to investigate its impact on freshwater eutrophication, cumulative energy demand, land use and soil acidification, and water consumption, apart from global warming. Among the different samples, Chilean pellets were the most efficient in terms of global warming with a −68.7 g CO_2 equivalent, while Brazilian charcoal briquettes had the least impact with 103 g CO_2 equivalent per functional unit. However, when looking at the overall impact, urban forest waste was the most efficient, while charcoal briquettes and pellets from palm branches were the worst (Silva et al., 2022).

The LCA study conducted by (Prasad et al., 2016) of the different pretreatment methods for lignocellulose showed that out of the four methods selected, i.e. organosolv (OS), liquid hot water (LHW), dilute acid (DA) and steam explosion (SE), LHW was the most efficient as twice as much sugar was released and CO_2 emissions were significantly reduced although water consumption was higher. DA proved to be the least efficient, considering that a treatment temperature of 60°C and a duration of 12 h are required, which are maintained using electricity, resulting in an increased environmental impact.

In addition, LCA has found that the impact of various factors is case and region-specific and depends on geographical conditions such as water availability, farming practices, major energy sources, etc. (Murali and Shastri, 2022). In the case of sugarcane bagasse, the strategy of dilute acid pre-treatment followed by simultaneous saccharification and fermentation was found to be the most efficient method of ethanol production with higher yield and low biomass requirement and CO_2 generation of 0.132 kg eq. per MJ. It was also found that the major step associated with CO_2 release is the steam generation, which accounts for about 85%. It is proposed to replace natural gas to reduce the amount of CO_2 released to 0.019 kg eq. per MJ. The amount of water required for the entire process was 232–672 L for each litre of ethanol produced, most of which came from agricultural water. The ammonia fibre explosion (AFEX) pretreatment method had the largest impact. The main reason is the large demand for ammonia and the related recycling problem (Murali and Shastri, 2022).

5.2 Economic and technological impacts

In addition to assessing the environmental impacts of biofuels, it is also important to consider the economic and technological limitations of their application. The first major hurdle is the technical and economic aspect of the type of biomass, collection, storage, processing, and related logistics. The mode of transportation, seasonal change-related variation in the biomass with increased volume: density ratio, and the distance of the field from the factory all contribute to the total transportation cost. The need for specific equipment to pre-treat the biomass, the cost of the enzymes required, the generation of unwanted by-products, the down streaming of the biofuel, and the neutralization of toxic substances in the effluent also have a direct impact on biofuel production costs (Balan, 2014; Mungodla et al., 2019).

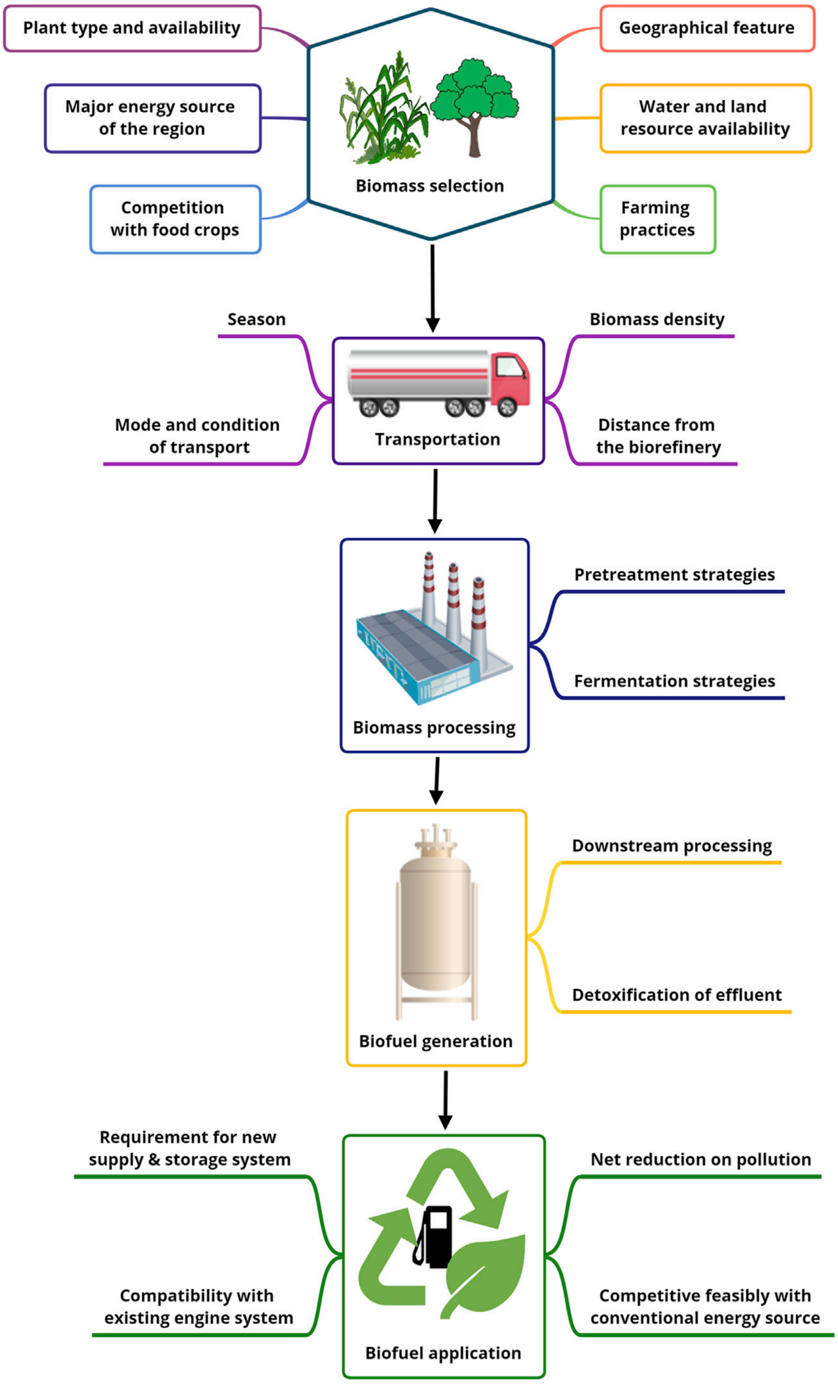

FIGURE 1
Schematic representation of the various points of Life cycle analysis for biofuel production from biomass.

Once biofuel is produced, the other major hurdle is its transportation, distribution, and compatibility with current engine systems. Biofuels require different systems to handle the fuel than the existing ones, so new pipelines and storage facilities need to be constructed (Kesieme et al., 2019). Although pipelines are the most efficient way of transporting fuel among the different transport models, it is observed that biofuels lead to pipeline corrosion (Gabetta, 2021). Although many countries have started using bioethanol as a fuel because it is compatible with gasoline, has a high-octane rating, and increases vehicle performance, ethanol has a lower energy value compared to gasoline, which increases fuel costs (Masum et al., 2015). The biofuel can also have a negative impact on engine durability, where the biofuel can precipitate, clog filters, or it can leak and dilute lubricating oils, damaging the engine. In addition, the effects of biofuels on other engine system components, such as fuel injectors, are not fully known (Joshi et al., 2017).

Since the currently available engine system has been developed over the years to achieve maximum efficiency concerning conventional fuels, biofuels are not fully compatible with them. The strategy for processing biofuels to increase their compatibility with these engine systems has a drastic impact on overall costs and pollutant emissions. Therefore, further development of engine technology may be an alternative to this. However, there has been increased interest in developing hybrid engines for vehicles that can run on both conventional fuels and biofuels (Bergthorson and Thomson, 2015).

6 Conclusion

Scientific developments in biological research related to microbial culture techniques have provided new insights into the microbiology and composition of the microbiome of many organisms that have come a long way. A major advance has been the study of the microbiome of various organisms, analyzing the diversity, function, importance, and conditions associated with dysbiosis. The gut microbiome of ruminants, termites, and other organisms that feed on plants consists of microbes with an efficient CAZyme system to utilize lignocellulose as a nutrient source. In the highly selective environment of these ecosystems, microorganisms with an efficient CAZyme system have evolved and have been studied with great success for the degradation of lignocellulose. Current trends show an increasing research interest in the gut of various organisms with the isolation and utilization of CAZymes for biofuel production. These enzymes, along with other strategies, have high potential as a future energy source while reducing waste management and pollution.

7 Challenges and future prospect

Although much of the current energy supply still comes from fossil fuels, lignocellulose will become an important substitute in the near future. CAZymes from the gut of herbivorous organisms may provide a revolutionary and environmentally friendly approach to this recalcitrant carbon source. However, a major challenge in isolating these novel microbes and CAZymes is the limitation associated with culture-dependent methods: Not all isolates can be grown on plates. In addition, most gut microbes are strict anaerobes, so more complex equipment is required for their growth and maintenance. In addition, the composition of the host microflora depends on other factors such as age, nutrition, health status, etc. The limited knowledge about the nutritional and environmental factors required for the growth and CAZyme production of gut microflora can be elucidated by culture-independent techniques such as metagenomics, transcriptomics, proteomics, and so on. Technological advances in high-throughput screening, computational studies and mathematical modelling, novel genetic and protein engineering techniques, etc., are now being used to maximize the potential of CAZyme for the gut microflora.

Author contributions

AV conceived of the presented idea. DC, SN, and UK wrote the manuscript. AV provided critical feedback, supervised the study, and helped shape the review.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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