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Review

# Sustainable bioconversion of food waste into high-value products by immobilized enzymes to meet bio-economy challenges and opportunities – A review

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ABSTRACT

Over the past few years, food waste has intensified much attention from the local public, national and international organizations as well as a wider household territory due to increasing environmental, social and economic concerns, climate change and scarcity of fossil fuel resources. On one aspect, food-processing waste represents a substantial ecological burden. On the other hand, these waste streams are rich in carbohydrates, proteins, and lipids, thus hold significant potential for biotransformation into an array of high-value compounds. Indeed, the high sugar, protein, and fat content render food waste streams as attractive feedstocks for enzymatic valorization given the plentiful volumes generated annually. Enzymes as industrial biocatalysts offer unique advantages over traditional chemical processes with regard to eco-sustainability, and process efficiency. Herein, an effort has been made to delineate immobilized enzyme-driven valorization of food waste streams into marketable products such as biofuels, bioactive compounds, biodegradable plastics, prebiotics, sweeteners, rare sugars, surfactants, etc. Current challenges and prospects are also highlighted with respect to the development of industrially adaptable biocatalytic systems to achieve the ultimate objectives of sustainable manufacturing combined with minimum waste generation. Applications-based strategies to enzyme immobilization are imperative to design cost-efficient and sustainable industrially applicable biocatalysts. With a deeper apprehension of support material influences, and analyzing the extreme environment, enzymes might have significant potential in improving the overall sustainability of food processing.

## 1. Introduction, current context, and drivers

In the food processing industries, waste is generated by the separation of target products from undesired by-products (Kwan et al., 2018). In recent years, massive research efforts have been made in utilizing food-processing waste (FPW) to produce high-value bioproducts. A sustainable bioconversion of food waste into valuable products not only offer economic advantages but also abates nuisances generated by food waste decomposition in the surroundings and landfills. The major requisite features that enable the use of food-based waste materials are shown in Fig. 1. Food-processing waste may be produced in liquid, solid, or semi-solid forms. Liquid wastes are generated because of the use of huge amounts of water for specific applications such as cleaning, sanitation, temperature regulation, transportation, cooking and as auxiliary water. The resulting effluent contains organic matter, suspended solids, nitrogen, fats, oils, and many other inorganic materials. Common liquid effluents comprised potato-processing wastewater, apple residue sludge, whey from the manufacturing of the cheese, yogurt and tofu, bakery, brewery, oil mill, and soda industry effluent, etc. On the other hand, major solid wastes are potato and tomato waste, waste bread, apple/grape pomace, and soybean curd residue. The solid food wastes are loaded with lignin, cellulose, starch, and monosaccharides (i.e., glucose and fructose) as compared to liquid food wastes containing nutrients in diluted form (Bilal et al., 2018; Hegde, Lodge, & Trabold, 2018).

Inevitable rising fossil fuel prices, dwindling natural resources and increasing prices of raw materials together with growing worldwide demand for energy, biofuels, chemicals, and biomaterials in our society are the key drivers to develop environmentally-friendly technologies based on cheaper and sustainable feedstocks to achieve such global target. Production of biofuels utilizing alternative feedstocks to conventional fossil raw materials, i.e. lignocellulosic raw materials has

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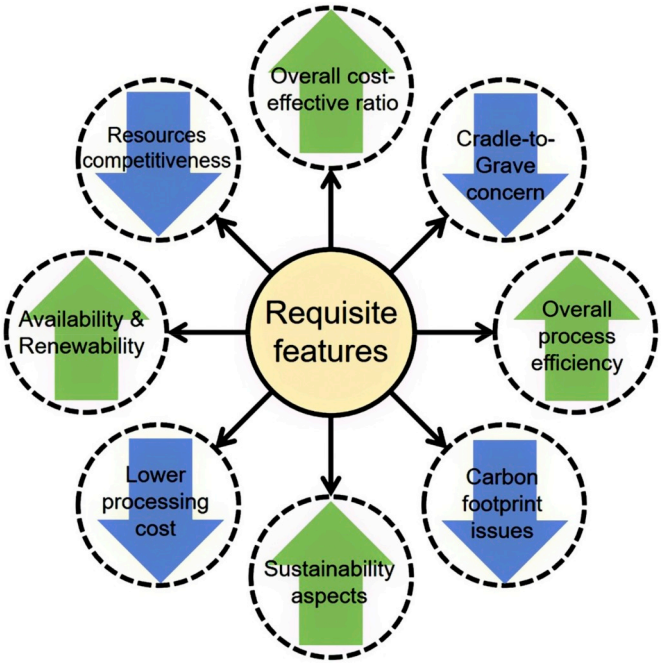


**Fig. 1.** Major requisite features that enable the use of food-based waste materials to develop high-value products of interests.

attracted particular interest over the last few years. Nevertheless, the high costs associated with the processing of lignocellulosic biomasses necessitate the exploration of inexpensive raw feedstocks to synthesize valuable compounds (Arevalo-Gallegos, Ahmad, Asgher, Parra-Saldivar, & Iqbal, 2017; Asgher, Wahab, Bilal, & Iqbal, 2016; Bilal, Asgher, Iqbal, Hu, & Zhang, 2017a, 2017b; Iqbal, Kyazze, & Keshavarz, 2013). Food waste is regarded as a raw material that possesses zero or low procurement cost contributing to the establishment of an innovative paradigm. Notably, household, institutional and industrial food waste has received accelerating research attention in the recent decade as a promising choice to treat these processing wastes, as well as, to valorize into useful products (Fig. 2). Carbohydrates, amino acids, and fats present in the industrial food waste offer an untapped opportunity, creating the waste a bourgeoning substrate for the synthesis of value-added products to replace petro-based chemical products (Chen, Maneerung, Tsang, Ok, & Wang, 2017; Iris et al., 2018). This approach would overcome not only the fuel supply limitations but also mitigates the wastes generated as a result of food processing industries in a cost-effective manner. Though production of industrially relevant compounds is explicitly a proficient opportunity to convert food waste substrates, careful and detailed assessment of the technology accompanied by optimizing processing economics is imperative before scaling-up and commercial implementation (Hegde et al., 2018).

## 2. Food waste— a rising global concern with dire environmental impact

The increasing global population at an exponential rate every year

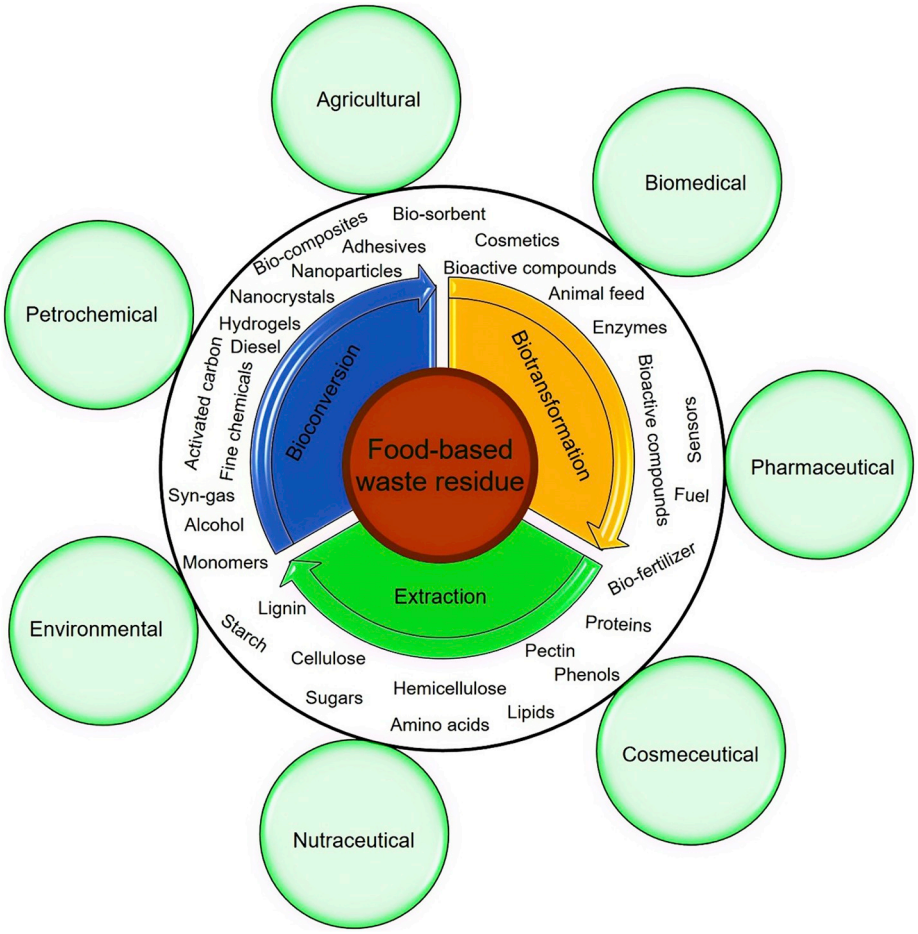


**Fig. 2.** Extraction, bioconversion, and transformation of food-based waste residue into value-added materials/products and their potential applications in different sectors of the modern world.

necessitate a huge demand for food and energy to entertain the societal needs. Rapid urbanization, inefficient food industry processes (processing, packaging, distribution, storage, transportation) in their current form and lack of effective waste management approaches result in the massive buildup of food waste. A preparatory study on food waste approximated that nearly 90 million tons of food waste are excluded from the food manufacturing industries every year (Monier et al., 2010). These organic food wastes exhibit pH variations, diverse composition and are characterized by high biological oxygen demand (BOD) and chemical oxygen demand (COD). Due to high water and nutritional content, such waste provides breeding grounds for disease-causing microorganisms on putrefaction leading to bacterial contamination, which in turn pose serious environmental problems. Further, the long-term persistence of untreated food waste in landfills ends up with a large amount of methane, a more destructive greenhouse gas than $CO_2$ that is considered another growing problem of climate change and global warming. The atmosphere is also adversely impacted as wasted food consumes many resources during manufacturing. In contrast to the middle- or higher-income regions, the developing countries are deteriorating more food due to lack of proper equipment, infrastructure, and sound harvesting acquaintance. Current in-practice strategies used in the food industry are incapable of overcoming the issues of waste management. Therefore, it is of great significance to manage the huge accumulation of such food waste. The concept of food waste conversion into bioenergy and other commodity products and chemicals is an intriguing research area with enormous potential for dealing with this problem.

## 3. Insight into the current practices for food waste management

The current approaches for food waste management included animal feed, composting, incineration, and landfill (Lin et al., 2013). Generally, animal feed is considered as the most cost-efficient valorization route for food supply chain waste but is sometimes restricted by regulatory concerns, as well as, the nature of the byproducts produced during the process. Composting, so-called land spread/injection is the most prevalent approach. It is an eco-friendly and highly acceptable practice since it diverts food waste from landfill and thus diminishes growers' necessities for (bio) fertilizers and water from the use of industrial effluents and solid waste. However, this kind of practice still suffers from the disadvantages of a relatively high cost. According to the studies of the UK Department of Environmental, Food and Rural Affairs (DEFRA), approximately 90% of food supply waste from the sites was valorized in some form such as 10% animal feed, around 75% of land spread agents, around 1.0% by anaerobic digestion or 4.0% incineration from the total amount of food waste, with 9.0% going to landfill. This data evidenced that the food sector in the UK is making a remarkable improvement towards zero organic and packaging waste going to landfirganic and packaging waste going to landfill by 2015 (Hollins, 2010). Dumping of the enormous amount of food waste in landfill is expensive and has a significant environmental concern, either with direct and indirect emission of greenhouse gases, i.e., methane and $CO_2$. Energy retrieve by incineration is not always feasible due to the loss of energy in evaporating the huge water content from the food waste. The employment of food waste as a soil enhancer is conceived to be appraised in coming years (Lin et al., 2013)

## 4. Food industry waste as sustainable raw materials

Food industry waste is particularly attractive for renewable energy scientists because of its predominant lignocellulosic nature, with abundant cellulose and lignin contents. Several reports have demonstrated various technologies to transform food waste, i.e., apple pomace and brewers' spent grain into biofuel (Liguori, Soccol, Porto de Souza Vandenberghe, Woiciechowski, & Faraco, 2015; Parmar & Rupasinghe, 2013). Enzyme-assisted biodegradation and biotransformation of cellulose and hemicelluloses release free glucose and xylose, respectively, which can be converted into fuel ethanol by fermentative microorganisms (Das et al., 2012). Additionally, on pyrolysis and anaerobic digestion, the lignin molecule potentially produces hydrogen and methane (Azadi, Inderwildi, Farnood, & King, 2013). Considering the limited fossil fuel reserves and mounting oil prices, food waste appears as a high renewable energy resource and reservoir of numerous added-value chemicals. It has recently been revealed that the manufacturing of bulk chemicals utilizing biomass waste is 3.5-times cheaper than transforming it into biofuel (Tuck, Pérez, Horváth, Sheldon, & Poliakoff, 2012). Bio-refinery is, in essence, an emerging concept in the field of biomass waste processing intending that all types of biomass-originated material can be converted into various kinds of energy, biofuels, chemicals, and materials by using a series of economically viable and low-environmental impact technological processes. The bio-refinery conception helps to extend collaboration between scientists/researches from diverse fields such as biochemistry, economics, environmental/material sciences, and chemical engineering to switch to a bio-based industry exploiting renewable resources for increased affordability (Cherubini, 2010; Clark & Farmer, 2009).

## 5. Enzyme as industrial biocatalysts — a societal need

Enzymes are water-soluble biocatalytic proteins that have been widely pursued in industrial processes. In the last decade, enzymes applications have dramatically increased, predominantly in the fields of biofuel production, food modification, laundry, biomedical, pharmaceutical research, and agro-industrial waste transformation (Adeel, Bilal, Rasheed, Sharma, & Iqbal, 2018; Amin, Bhatti, Bilal, & Asgher, 2017; Asgher, Wahab, Bilal, & Iqbal, 2017; Bernal, Rondriguez, & Martínez, 2018; Narancic, Davis, & Nikodinovic-Runic, 2015; Rehman, Bhatti, Bilal, & Asgher, 2016; Rehman, Wang, Bhatti, Bilal, & Asgher, 2017). Increasing biocatalysis demand for establishing new technological bioprocesses as a substitute for traditional chemical catalysts has substantially driven the need for engineered enzymes with unique biocatalytic and economic attributes. Therefore, the worldwide market for industrial enzymes has increased from 2.9 billion USD in 2008 to a projected 6 billion USD in 2016 (Beniwal & Sharma, 2014). Enzymes carry out chemical reactions with outstanding specificity, and high regio- and stereo-selectivity under very mild environmental conditions, leading to the setting up of more green, sustainable and eco-friendly chemical processes. However, enzyme-operating domain is quite limited that the natural sources-derived enzymes are often very proficient only at their ideal conditions, i.e., temperature, pH, and solvent characteristics. Nevertheless, under non-natural industrial bioprocessing conditions of low water activity, extreme pH, and temperature, etc. the enzymes catalytic activity is hindered. Until now, the prospect of enzymatic biocatalysis has not fully been exploited and searching out new and effective approaches to address the above-stated drawbacks is an extreme thirst for researchers and scientific community in this arena (Sheldon & Woodley, 2017).

## 6. Immobilizing enzyme for expanding the scope of implementation

Though the use of enzymes provides numerous advantageous features over traditional chemical catalysts in valorizing waste streams, lack of maintaining their catalytic activity and stability under the conditions typical of food processing waste streams such as non-neutral pH and higher temperatures is a major hurdle exists. The development of stabilized biocatalytic systems is therefore of high importance for amplifying industrial applications. Food waste streams are non-native and not favorable environments to enzymes, with conditions beyond their optimal working points. In recent years, many researchers have pursued immobilization as a noteworthy engineering tool to tailor and improve a plethora of enzyme catalytic features, such as activity,

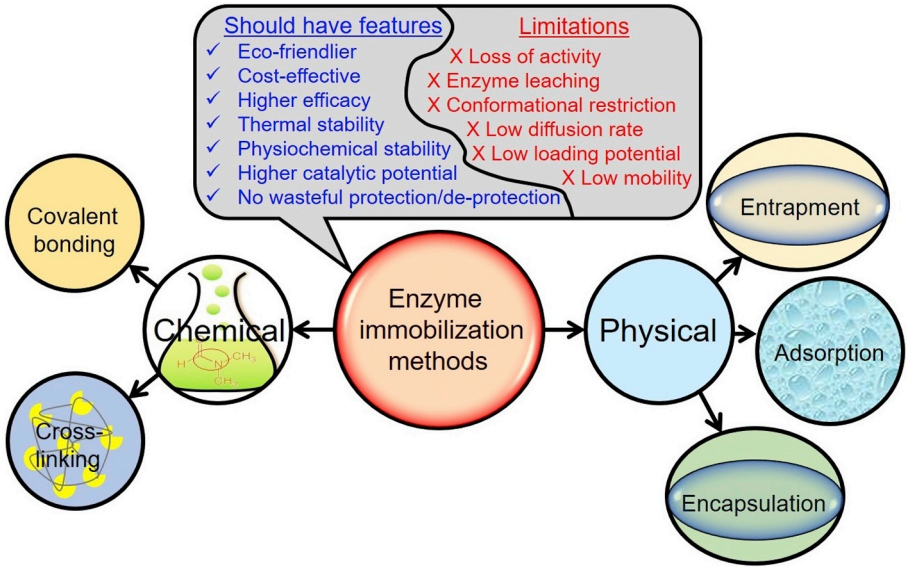


**Fig. 3.** Physical and chemical based enzyme immobilization methods along with considerable limitations and potentialities. Reprinted from Bilal et al. (2019) with permission from Taylor & Francis. Copyright (2019) Informa UK Limited, trading as Taylor & Francis Group.

selectivity, specificity, resistance to inhibitors, etc. (Bilal & Iqbal, 2019a; Bilal & Iqbal, 2019b; Bilal, Rasheed, Zhao, Iqbal, & Cui, 2018). Physical and chemical based enzyme immobilization methods along with considerable limitations and potentialities are shown in Fig. 3 (Bilal et al., 2017; Bilal, Asgher, Cheng, Yan, & Iqbal, 2019).

Enzyme immobilization can enable biocatalyst to act efficiently under these non-ideal conditions. For instance, the pH value of the acid whey generated during the production of strained yogurt is slightly acidic (less than 5.0), whereas certain glucose isomerases involved in sweetener production perform optimally at pH values between 7.0 and 8.5 (Mishra & Das, 2002; Bilal & Iqbal, 2019b). Moreover, temperature ranges around body temperature (37 °C) is the optimal temperature for many enzymes, the performance of these enzymes at elevated temperatures (50–70 °C) would allow use of greener reaction media (e.g., eutectic mixtures of choline chloride and urea), which are biodegradable and affordable (Abbott, Capper, Davies, Rasheed, & Tambyrajah, 2003). Furthermore, some substrates exhibit a denaturing effect on the enzyme itself, which is another challenge towards enzyme stability. For example, lipase possesses the ability to produce biodiesel from fatty acids and alcohols. Nevertheless, alcohols are recognized to be denaturants for lipase enzyme (Shimada, Watanabe, Sugihara, & Tominaga, 2002; Tan, Lu, Nie, Deng, & Wang, 2010). Immobilization of enzyme onto a solid support matrix enhance enzyme stability and catalytic performance under the non-ideal conditions of food processing waste streams by preventing structural deformation and maintaining its biologically active three-dimensional structure. Immobilization technology also facilitates repeated usability of the enzymes, which can help to trim down the overall costs of enzymatic bioprocesses. In this way, not only biocatalyst can be recovered from the complex reaction mixture, but it also maintains its significant original activity in multiple successive cycles.

Enzyme immobilization strategies have been widely studied, being hydrolases the most frequently reported, specifically lipases (Adlercreutz, 2013), carbohydrases (Dey, Kumar, Banerjee, Chandna, & Kuhad, 2016), and proteases (Yazid, Barrena, & Sánchez, 2016). However, the immobilization of non-hydrolytic enzymes such as ketoreductase from *Pichia glucozyma* (Dall'Oglio et al., 2017), Lignin peroxidase from *Schizophyllum commune* (Sofia, Asgher, Shahid, & Randhawa, 2016), manganese peroxidase from *Ganoderma lucidum* (Bilal & Asgher, 2015), laccase from *Trametes versicolor* (Asgher, Noreen, & Bilal, 2017a, 2017b), and ω-transaminase from *Escherichia coli* (Neto, Schürmann, Panella, Vogel, & Woodley, 2015) have also recently been reported with promising results. Selection of supporting matrix and immobilization method is critical to developing reusable, stable and durable immobilized biocatalytic systems. Therefore, the supporting material should be judiciously considered for immobilization. For example, lipolytic enzymes are reported to be hyper-activated upon immobilization onto hydrophobic materials (Manoel, dos Santos, Freire, Rueda, & Fernandez-Lafuente, 2015), whereas, $\beta$-galactosidases are found to diminish their activities by immobilization on hydrophobic materials (Wong, Talbert, & Goddard, 2013; Wong, Dai, Talbert, Nugen, & Goddard, 2014). Hydrogel microspheres show great potential for activity maintenance of $\beta$-galactosidase, but problems arise with the migration of the biocatalyst from the immobilization matrix (Zhang, Zhang, Chen, & McClements, 2016; Zhang, Zhang, Jiang, & Mu, 2016). Another important influence of enzyme immobilization is the potential to shift the optimal working conditions of the enzyme. It is revealed that the local pH environment surrounding enzymes can be manipulated by immobilization in various polymeric networks (with cationic and anionic materials shifting pH optima more acidic (Wentworth, Skonberg, Donahue, & Ghanem, 2004) and alkaline (Gonzalez-Saiz & Pizarro, 2001), respectively. Introduction of stabilizers or stabilizing groups, i.e. polyethylene glycol within a support carrier can also affect enzyme activity retention (Iyer & Ananthanarayan, 2008).

Generally, modified polysaccharides like agarose, alginate, agar-agar, and chitosan are often employed as support matrices for enzyme immobilization owing to their abundant availability and ease of surface functionalization options. Nonetheless, in the last decade, inorganic matrices such as porous silica and titanium dioxide have been appeared as alternative support materials because of their compatibility and high mechanical stability characteristics in different applications (Bernal, Sierra, & Mesa, 2014). Enzyme immobilization to a support material can be categorized as covalent bonding, adsorption, entrapment and crosslinking. Multipoint covalent bonding, where the enzyme molecule is associated with the functionalized carrier support through different amino acid residues, can attain high biomolecules stabilization (Fig. 4) (Bilal et al., 2019). Effective covalent binding is accomplished through surface modification of the support material with glutaraldehyde, genipin, epichlorohydrin or glyoxyl-containing compounds generating Schiff base between the functionalized support surface and the amino group of an enzyme that results in exceptional stabilization and repeated usability (Bernal et al., 2018). Most recently, a new covalent

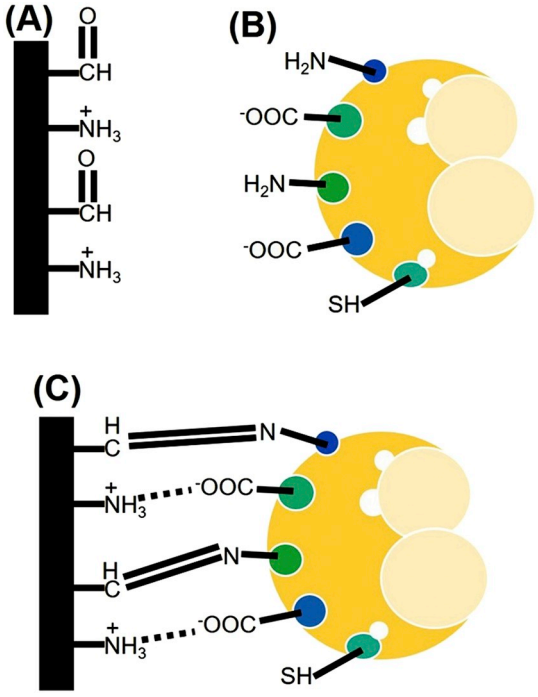


**Fig. 4.** Schematic representation of multipoint enzyme immobilization; A) support material with functional entities, B) enzyme with functional entities, C) multipoint enzyme immobilization. Reprinted from Bilal et al. (2019) with permission from Taylor & Francis. Copyright (2019) Informa UK Limited, trading as Taylor & Francis Group.

binding approach has been documented, where the support material is functionalized with vinyl sulfone groups that react with amine and thiol groups of the biocatalysts through Michael-type addition presenting outstanding results with alkaline pH-sensitive enzymes (Bernal et al., 2018). The fact revealed that controlling the enzyme support reactivity is critical to achieving biocatalysts with high catalytic performance and operational stability (Mateo, Palomo, Fernandez-Lorente, Guisan, & Fernandez-Lafuente, 2007). Further, the combination of adsorption and covalent bonding has appeared as a noteworthy approach to control enzyme orientation and promoting enzyme-support interactions during the immobilization process (Mateo et al., 2007). This combined immobilization strategy results in modified enzyme selectivity, which is of exceptional interest for chiral reactions (Guajardo, Bernal, Wilson, & Cabrera, 2015). Similarly, the co-localized immobilization of biocatalytic cascades comprising two or more types of enzymes exhibit short diffusional distances and consequently accelerate the rate of the reaction leading to the enhanced catalytic performance of the cascade than their soluble counterparts (You, Myung, & Zhang, 2012). Moreover, advanced metabolic pathways can be engineered by constructing artificial enzyme complexes, whose constituents can be derived from different host organisms with diverse properties and advantages.

## 7. Transforming food waste—deriving more value from waste by immobilized enzymes

The biomass conversion process determines the value of the substrate. Similarly, the operational cost and the value of the target products are the two major factors determining the feasibility of a biomass conversion process. Thus, it is important to appraise the recent trends and technological development in the conversion of food supply chain waste. A diversity of industrially pertinent and future platform chemical compounds such as biofuels, biopolymers, organic acids (succinic, malic, glutamic, fumaric, itaconic acids and 3-hydroxy propionic acid), enzymes, nutraceuticals, functional sugars (arabinitol and xylitol), and dietary fibers have been produced from the bioconversion of food industry waste (Galanakis, 2012). In this section, we present the state-of-the-art developments for valorizing food industry wastes into value-added products.

### 7.1. Carbohydrates as feedstocks/raw materials for enzymatic valorization

Carbohydrates demonstrate the leading carbon source in many processes that play a critical role in microbial metabolism to produce numerous fermentative products including bioethanol, hydrogen, lactase or oil (Carmona-Cabello, Garcia, Leiva-Candia, & Dorado, 2018). Food processing waste rich in carbohydrates is readily susceptible to enzymatic valorization by isomerases and hydrolases into valuable bioproducts such as prebiotics and sweeteners. Undeniably, some of the well-established and most lucrative processes in food and agricultural systems commenced with a carbohydrate-based feeding stock. Carbohydrates consisting of free sugars and crude fibers can be converted into different products. Free sugars characterize the monosaccharides that are naturally found in fruit extracts, honey, and fruit-waste streams. Though free sugars could be valorized into biofuels and many other bio-based biochemical, their economic assessments need to be critically inspected. It is demonstrated that the immobilized enzymes can be adapted and utilized to convert carbohydrates-rich food waste streams into value-added compounds.

#### 7.1.1. Utilization of monosaccharide and disaccharides

Owing to their great biodegradability and direct metabolic assimilation in different pathways, mono- and di-saccharides are recognized as the most attractive carbon-rich feedstock's in fermentative bioprocesses (Schröder, Selig, & Schönheit, 1994). Similarly, polysaccharides, i.e., starch, cellulose, hemicellulose, and pectin are present in enough concentration in food waste to be employed on an industrial scale. The concentration and fractionation of whey protein from casein, cheese, and yogurt manufacturing byproduct streams is a paramount example of food processing waste valorization. In 2004, Greek yogurt revealed only 1–2% share of the US yogurt market, and reached about 40% in 2015, representing a rapid increase in the manufacturing of Greek yogurt, and resultantly, the production of acid whey (Erickson, 2017). Since a significant amount of lactose-containing dairy waste streams generated every year, the majority of the research regarding valorizing monosaccharide and disaccharide-based food waste, therefore, focused on the enzyme-catalyzed transformation of lactose into useful products.

*7.1.1.1. Production of sweeteners and lactose-free milk.* As compared to sucrose (100%), lactose is not a valuable sweetener and exhibit a relative sweetness value of 16%. However, it can produce sweeteners with comparatively high sweetness values upon enzymatic conversion. Some of the resulting sweeteners possess additional health benefits due to their lower caloric value. Use of $\beta$-galactosidase to catalyze the hydrolysis of waste lactose into glucose (74% relative sweetness) and galactose (30% relative sweetness) is its most common application in lactose valorization. The $\beta$-galactosidase enzyme has been well investigated and used commercially to produce reduced-lactose and lactose-free milk. Reaction pathway for transgalactosylation and hydrolysis of lactose using β-galactosidase is shown in Fig. 5 (Panesar, Kaur, Singh, & Kennedy, 2018). For this purpose, several different carriers support, and immobilization strategies have been intended for the immobilization of $\beta$-D-galactosidase to enhance industrial manufacture of lactose-free/low-lactose foods. Recently, Wolf, Gasparin, and Paulino (2018) developed a modified Arabic gum-based hydrogel by cross-linking Arabic gum with acrylamide in the presence of potassium persulfate as an initiator. The resulting hydrogel was used to immobilize β-D-galactosidase for the hydrolysis of standard lactose and lactose contained in ultra-high temperature pasteurized (UHT) milk aiming to produce lactose-free milk. In contrast to free $\beta$-D-galactosidase, the hydrogel-immobilized enzyme

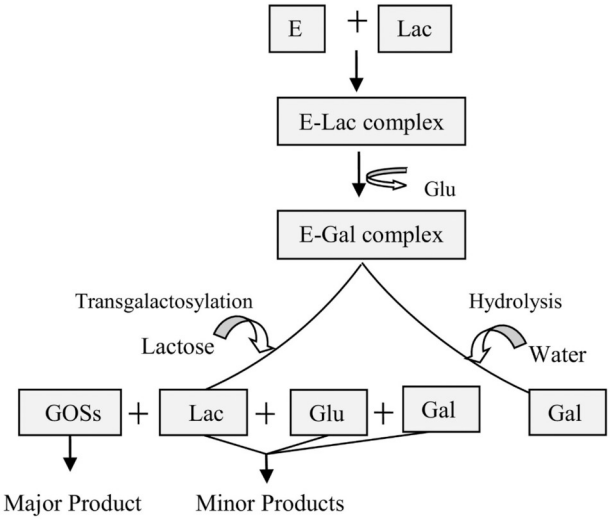


**Fig. 5.** Reaction pathway for transgalactosylation and hydrolysis of lactose using β-galactosidase. Reprinted from Panesar et al. (2018) with permission from Elsevier. Copyright (2018) Elsevier B.V.

derivative was found to be more efficient in the hydrolysis of both kinds of starch. Further, the immobilized β-D-galactosidase showed repeated use of lactose hydrolytic potentiality for three cycles without losing any significant enzyme activity. Lorenzen, Breiter, Clawin-Rädecker, and Dau (2013) investigated the potential of a bi-enzymatic system, i.e., *β*-galactosidase from *Kluyveromyces lactis* and glucose isomerase from *Streptomyces rubiginosus* to improve the sweetening power of lactose. Notably, the resulting enzymatic catalytic system efficiently converted lactose (lactose from skim milk, acid whey, and sweet whey) into glucose, galactose, lactulose, and galacto-oligosaccharides, and consequently, led to the production of yogurt with a greater sweetness perception. For the first time, Gennari, Mobayed, Volpato, and de Souza (2018) immobilized *β*-galactosidase enzyme from *Aspergillus oryzae* on aluminum-chelated powdered collagen. Collagen was also treated and modified with acetic acid, glutaraldehyde, and an amalgamation of aluminum and glutaraldehyde. Results showed that the storage stability of galactosidase immobilized on aluminum-chelated collagen and combined aluminum-glutaraldehyde collagen resulted in efficient biocatalytic system preserving about 60% of their original activities after three months at 4 °C. At the elevated temperature of 65, 68, 70, and 73 °C, these immobilized derivatives revealed greater half-life values than their soluble counterparts. Also, high lactose hydrolysis activity of the insolubilized derivatives in permeate and lactose solutions up to 50 and 60 cycles, respectively, in a batch reaction proposed aluminum-treated powdered collagen as promising support for *β*-galactosidase immobilization with food applications. Recently, concanavalin A cross-linked ca-alginate and gelatin spheres-encapsulated *β*-galactosidase from *K. lactis* resulted in more than 70% hydrolysis of cheese whey lactose accompanied by the decreased protein leaching (Mörschbächer, Volpato, & Souza, 2016). Similarly, silanized porous glass-immobilized a neutral *β*-galactosidase from *K. fragilis* demonstrated highly efficient lactose hydrolysis (86–90%) in whey permeate in a batch mode, as well as, in a continuous packed-bed bioreactor combined with full activity maintenance for five repeated cycles (Szczodrak, 2000). The findings persuasively manifest the outlook of immobilized β-galactosidase for

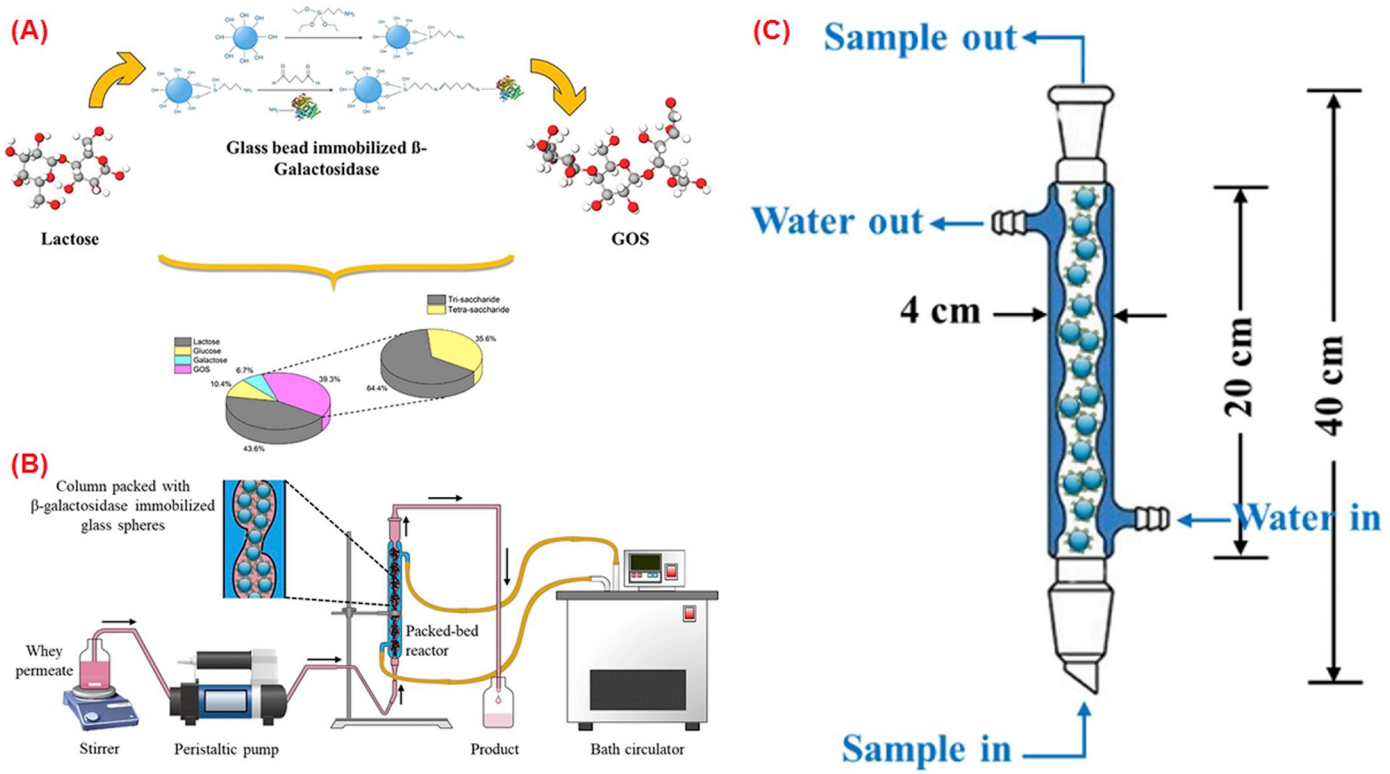


**Fig. 6.** (A) Covalent immobilization of the β-galactosidase enzyme on functionalized glass beads; (B) schematic illustration of the configuration of the experimental setup and (C) the continuous packed-bed reactor for lactose conversion, with dimensions. Reprinted from Eskandarloo and Abbaspourrad (2018) with permission from Elsevier. Copyright (2018) Elsevier Ltd.

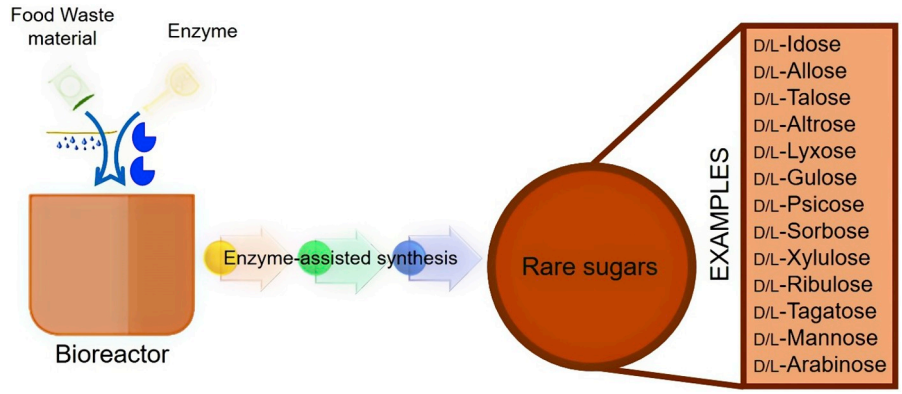


**Fig. 7.** A detailed process of rare sugar biosynthesis.

value-added re-utilization of lactose in multiple cycles of reusability.

*7.1.1.2. Galacto-oligosaccharides production by immobilized enzyme.* Galacto-oligosaccharides (GOS) are promising and popular prebiotic ingredients that can be synthesized from lactose in dairy waste streams. These probiotics are potentially related to beneficial outcomes for human health. The enzyme-mediated catalytic conversion of lactose to GOS has become industrially significant (Gosling, Stevens, Barber, Kentish, & Gras, 2010); nevertheless, upgrading *trans*-galactosylation reaction efficiency and GOS biosynthesis to improve the quality of converted GOS are the several challenges that could improve this process. As portrayed in Fig. 6 (Eskandarloo and Abbaspourrad (2018), the enzymatic reaction of GOS production is carried out in two steps, where the first step implicates the generation of the enzyme-galactosyl intermediate with the exclusion of a glucose group. In the second step, the galactose moiety transfers to a nucleophile, perhaps a sugar molecule rather than water resulting in the formation of GOS (Zechel & Withers, 2001). As compared to free enzymes, immobilized biocatalysts offer numerous advantages of increased enzyme stabilization, the formation of products in a continuous flow mode, diminished bioprocessing costs, improved product quality, and purity, and excluding the necessity for enzyme separation from the reaction media (Palai, Singh, & Bhattacharya, 2014). The formation of product in continuous flow modes is substantially useful for efficient production process relative to the batch mode process. The enzyme-driven GOS production in continuous reactions using different bioreactor formats have been described (Harju, Kallioinen, & Tossavainen, 2012). Most recently, Eskandarloo and Abbaspourrad (2018) reported the conversion of whey permeates to GOS in a continuous packed-bed bioreactor by $\beta$-galactosidase covalently coupled onto 3-aminopropyl tri-ethoxysilane-functionalized glass beads. Results revealed an increased level of GOS production with the highest GOS yield of 39.3% accompanied by a 56.4% conversion rate of lactose following the 2nd cycle of reusability. Wide-working pH and enhanced thermal stabilities were the additional features of the immobilized enzyme than that to the free enzyme. The packed-bed bioreactor immobilized enzyme demonstrated potential recyclability dropping only about 4.6% of initial GOS yield after eight successive reusing cycles. Banjanac et al. (2016) assessed an increased transglycosidation activity by β-galactosidase from *A. oryzae* immobilized on silica nanoparticles. A significant improvement in GOS production from 30 g/L/h (by a free enzyme) to 90 g/L/h by nanoparticles-supported $\beta$-galactosidase suggest the usefulness of immobilized enzymatic system in increasing enzyme catalytic performance.

*7.1.1.3. Biosynthesis of rare sugars by immobilized enzyme.* Due to their low caloric value (0–2 kcal/g) and a high relative sweetening value (70–92%) compared to sucrose, rare sugars have recently, attracted increasing attention (Matsuo, Suzuki, Hashiguchi, & Izumori, 2002). According to the International Society of Rare Sugars (ISRS), rare sugars and their derivatives can be defined as “monosaccharides scarcely existing in nature” (Beerens, Desmet, & Soetaert, 2012; Shuang-Yan, 2012). Notwithstanding their lower natural abundance, rare sugars exhibit various known physiological/biological functions and particularly important for applications in cosmetics, pharmaceutical, and food, and flavor industries (Bilal, Iqbal, Hu, Wang, & Zhang, 2017; Li, Varanasi, & Relue, 2013; Wen, Zheng, Li, & Wang, 2016). Rare sugars are a promising alternative to sugar alcohols in sugar-free products since some of these sugars do not cause a cooling effect or gastrointestinal distress as commonly occurred using sugar alcohols (Granström, Izumori, & Leisola, 2007). After preliminary $\beta$-galactosidase-mediated lactose hydrolysis, rare sugars can be produced from glucose and galactose by the use of some additional enzymes. Prof. Izumori group carried out plenty of work on rare sugars and delineated numerous production pathways for rare sugars (Granström, Takata, Tokuda, & Izumori, 2004; Izumori, 2002), however, extensive research still needed, as many of the enzymes are not commercially produced. In spite of the great promise as reduced calorie sweeteners applications, limited research is focused on the immobilization of these enzymes. Fig. 7 depicts a simplified process for the biosynthesis of rare sugar. D-Psicose, carbon-3 epimer of D-fructose, is a rare sugar characterized by a low-calorie sweetener, activator of intestinal alpha-glycosidase enzymes, and hepatic lipogenic enzymes inhibitor (Baek, Park, & Lee, 2010. Its use helps to lower the hyperglycemia (type-2 diabetes) hyperlipidemia and obesity (Zhu et al., 2012). Given the significant biological and physiological functions with no side effects, Food and Drug Administration (FDA) as recommended D-psicose as “generally regarded as safe” (GRAS) (Zhang, Zhang, Chen, & McClements, 2016; Zhang, Zhang, Jiang, & Mu, 2016). Bioconversion of D-fructose to D-psicose production by D-psicose 3-epimerase family has been identified and biochemically characterized based on its substrate specificity (Kim, Hyun, Kim, Lee, & Oh, 2006; Kim, Park, & Oh, 2006). Immobilized *Agrobacterium tumefaciens*-derived D-psicose 3-epimerase onto Duolite A568 beads produced a high and stable amount of 441 g/L psicose sugar from the utilization of 700 g/L fructose after two h in a batch process. Moreover, the immobilized biocatalyst in a packed-bed bioreactor led to continuous production of 325 g/L psicose from the conversion of 500 g/L fructose substrate with a productivity of 527 g/L/h over a period of 236 h (Lim, Kim, & Oh, 2009). Also, the graphene oxide-immobilized ketose 3-epimerases nanobiocatalyst from *A. tumefaciens* demonstrated a significantly higher bioconversion efficiency up to 10 repeated use cycles as compared to its free counterpart (Fig. 8) (Dedania, Patel, Patel, Akhani, & Patel, 2017). The thermal stability of the immobilized enzyme was also improved with a half-life of 720 min at 60 °C, as compared to free enzyme, which displayed a half-life of only 3.99 min (Dedania et al., 2017). D-tagatose is a vital preparation used for relieving type-2 diabetes and has also

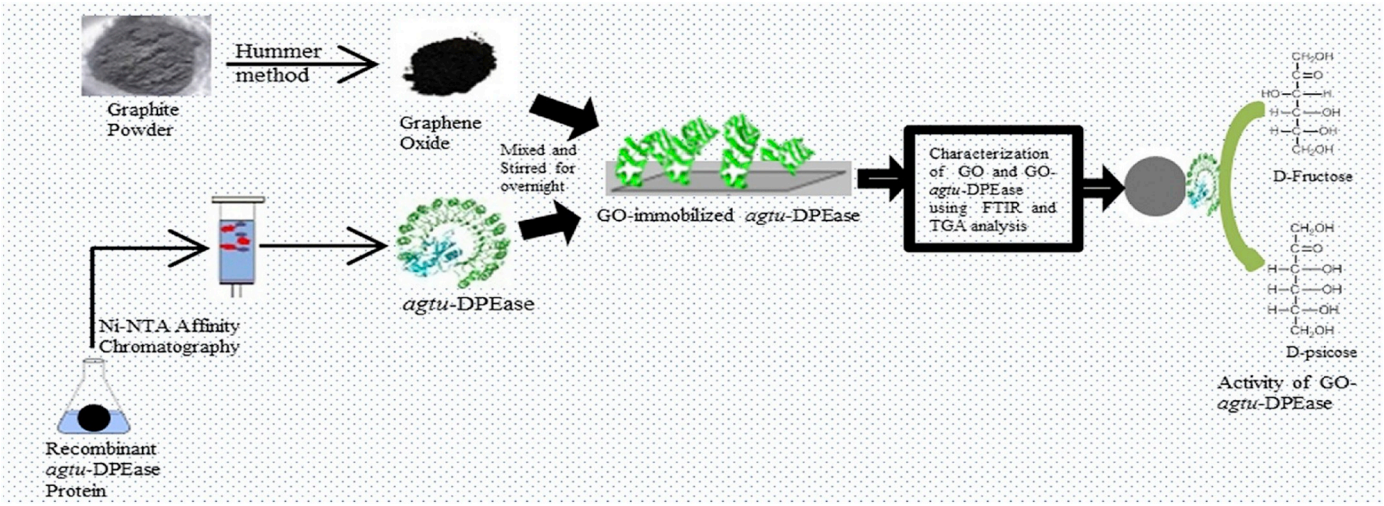


**Fig. 8.** Schematic representation of immobilization procedure and characterization of graphene oxide and graphene oxide-immobilized *Agrobacterium tumefaciens* D-psicose 3-epimerase. Reprinted from Dedania et al. (2017) with permission from Elsevier. Copyright (2017) Elsevier Inc.

been approved as a valuable food additive by the FDA, USA (Espinosa & Fogelfeld, 2010). For D-tagatose synthesis, Kim, Ryu, Kim, and Oh (2003) carried out the immobilization of a thermostable L-arabinose isomerase enzyme from *E. coli* in the alginate matrix to develop a most feasible biocatalytic system. In contrast to the free form of the enzyme that produced 37 g/L tagatose, the resulting alginate-beads immobilized enzymatic system yielded 58 g/L of tagatose from 100 g/L galactose after 90 h in a batch reaction mode that might be due to its higher stability. In a packed-bed bioreactor, the same biocatalysts led to a production of 50 g/L tagatose from 100 g/L galactose after 168 h under optimized galactose-isomerization reaction conditions.

*7.1.1.4. Production of other prebiotics (lactulose, lactosucrose)*. Besides, conversion of lactose into sweeteners, its transglycosidation can give rise the beneficial health prebiotics such as lactulose, lactosucrose, etc. Lactulose is a disaccharide consisting of fructose and galactose monosaccharides. It is considered as a prebiotic, as well as, a therapeutic agent (Wu et al., 2017) in the treatment of constipation, *Salmonella* and encephalopathy (Bruzzese, Volpicelli, Squaglia, Tartaglione, & Guarino, 2006; Hernández-Hernández et al., 2012; Schumann, 2002). The formation of lactulose can occur by enzyme-catalyzed molecular rearrangement of lactose or the introduction of a β-glycosidic linkage between fructose and galactose (Kim, Hyun, et al., 2006, Kim, Park, & Oh, 2006). The ultimate yields of lactulose are determined by the source and properties of the enzyme. It is reported that immobilization technology may affect and improve the final yields of lactulose. Song, Lee, Park, and Kim (2013a, 2013b) investigated the production of lactulose from whey lactose both in batch and continuous systems by β-galactosidase from *K. lactis* immobilized on activated silica gel. The immobilized enzyme synthesized lactulose titer of 19.1 g/L in a continuous packed-bed reactor retaining more than 50% of its original catalytic activity after reusing ten successive cycles. In another study, the same authors used commercially available whey as a lactose source for lactulose synthesis by immobilized β-galactosidase and glucose isomerase. Under the optimized reaction conditions, lactulose production was markedly improved and recorded to be 7.68 g/L with a productivity of 0.32 mg/Uh using the immobilized enzyme. Moreover, the immobilized multi-enzyme system was recyclable for lactulose biosynthesis retaining 57.1% of its initial catalytic activity after seven repeated usages. The results indicate additional research to develop biocompatible immobilization approaches for β-galactosidase rendering it an economically feasible enzymatic processing of lactose-rich dairy waste streams (Song et al., 2013b). Of most recent, de Albuquerque et al. (2018) established the optimal operating conditions for enhanced biosynthesis of lactulose by *K. lactis* β-galactosidase immobilized on glutaraldehyde-activated chitosan support using cheese whey as a raw material. Under the optimized conditions, the developed biocatalytic system achieved the highest lactulose titer of 17.3 g/L in a short reaction time of 2 h, and the immobilized enzyme showed high operational stability. Lactosucrose is a synthetic trisaccharide made up of glucose, galactose, and fructose. It can be produced by enzymatic catalysis using lactose and sucrose as feedstocks. As compared to lactulose and GOS, lactosucrose presents a higher laxative threshold, and is extensively used in the manufacturing of functional foods for several specific health purposes (Silvério, Macedo, Teixeira, & Rodrigues, 2015). The lactosucrose prebiotic has been achieved by a new synthetic bioprocess using β-galactosidase covalently attached to chitosan microspheres. Thermal stability of the enzyme was considerably increased after the immobilization process, and elevated operating temperature conditions (50 °C, 64 °C) promoted the synthesis of lactosucrose. The microspheres-insolubilized biocatalyst revealed no losses in lactosucrose yield up to 30 repeated cycles (Duarte et al., 2017). These results evidence the versatility of the engineered β-galactosidase enzyme system to synthesize a range of high-value products depending on the processing conditions in multiple cycles of reuse. Though great opportunities exist to utilize lactose-containing dairy waste streams by carrier-bound enzymes effectively, continuous research is indispensable to assess the use of these systems in real-time practical applications and to enhance stabilization in multiple reactions cycles to compensate the cost of immobilized biocatalytic systems. Immobilized enzymes, also, to valorize monosaccharides and disaccharides present in food processing waste (whey, acid whey, and whey permeate), should also apply on other similar substrates such as wastewater treatment or fruit processing waste streams.

*7.1.2. Value-added utilization of polysaccharides*

Polysaccharides in waste streams from the processing of vegetable, fruit, and crustacean products are considered as attractive substrate candidates for enzymatic transformation. For example, potato-processing waste streams loaded with starch possesses an elevated BOD level (i.e., 5000 mg/L) that render it inappropriate to discharging into wastewater effluents without any proper treatment (Hadjivassilis, Gajdos, Vanco, & Nicolaou, 1997). Enzymes such as amylases, cellulases, and xylanases have shown the potential to convert these polysaccharides-harboring waste streams into numerous useful compounds comprising bioplastics, prebiotics biofuels, and sweeteners. The processing of corn, potato, rice, and sweet potato products generates a massive amount of starch-rich waste streams. It is reported that during potato processing,

approximately 16% of the starch is lost during washing and slicing that might be used to produce texture modifiers and plasticizers by lipase-catalyzed acylation reactions (Alissandratos & Halling, 2012).

*7.1.2.1. Hydrolysis of starch by immobilized enzyme.* In contrast to starch acylation by chemical catalysts, enzymatic methods provide great specificity and can be carried out under very mild reaction conditions. In food industries, starch is hydrolyzed to produce glucose syrup by the synergistic action of two enzymes, i.e., α-amylase, and glucoamylase. Generally, the process of enzyme-mediated breakdown of starch occurs in two steps. α-Amylase hydrolyses *α* (1–4) glycosidic linkage of starch in the first step by a process so-called "Liquefaction process" followed by "saccharification" in which glucoamylase breaks *α* (1–6) as well as *α* (1–4) glycosidic bonds to generate non-reducing ends (Jadhav & Singhal, 2014; Talekar et al., 2013). Nevertheless, the soluble form of enzymes not only produces secondary waste but also show the disadvantage of challenging retrieval and reusability. Carrier-bound enzymes have particularly been constructed to enhance catalytic stability of the enzyme in solvents allowing solubility of both acyl donor substrates and starch (Alissandratos & Halling, 2012). In recent years, immobilized enzymes as green biocatalysts have been widely attempted for starch hydrolysis to improve yield and processing performance. The multi-enzyme biocatalyst enables running in-vitro multi-step catalytic cascade reactions in a single pot. Salgaonkar, Nadar, and Rathod (2018) carried out the mixing of zinc acetate and two-methylimidazoles with enzyme mixture in one pot constructing an efficient combi-metal organic framework (combi-MOF) of *α*-amylase and glucoamylase for starch hydrolysis under biocompatible environment. Thermo-stability of the resulting combi-MOF in terms of half-life increased to 3-folds stability in the temperature ranging from 55 to 75 °C. After co-immobilization, the rate of starch hydrolysis by combi-MOF was improved, and the co-immobilized enzymes were recyclable for five successive cycles in a batch mode with retention of more than 50% remaining activity. In addition to this, combi-MOF exhibited a higher rate of starch hydrolysis for various starch sources such as rice, wheat corn, and potato) as compared to their free counterparts that might be owing to spatially co-localized multi-enzymatic systems. For example, Talekar et al. (2017) developed a highly efficient tri-enzyme magnetic nanobiocatalytic system by co-immobilizing α-amylase, glucoamylase and pullulanase on amino-functionalized iron oxide magnetic nanoparticles in the presence of GLU as a cross-linking agent and employed for the one-pot hydrolysis of starch. The as-prepared tri-enzyme nanobiocatalyst displayed greater resistance to alkaline pH values and higher thermal stability than that of the free enzymes. It also showed promising catalytic efficiency achieving 100% starch conversion as compared to free enzyme preparation, which showed 70% starch hydrolysis in a batch mode system. Interestingly, the newly developed biocatalytic system presented excellent recyclability retaining its complete original activity after eight repeated starch hydrolytic cycles. The results indicate the potential of the co-immobilized biocatalytic system for valorization bioprocesses necessitating manifold biotransformations. In another study, combined cross-linked enzyme aggregates (combi-CLEAs) of α-amylase, glucoamylase and pullulanase accomplished a 60% higher starch hydrolysis than that to the free form of the enzyme in a batch mode. In addition to improved pH and temperature stability, the resulting combi-CLEAs can be used for five consecutive starch hydrolysis cycles without any original activity (Talekar et al., 2013).

*7.1.2.2. Conversion of pectic substances.* Pectic substances (polygalacturonic acid, methyl-esterified polygalacturonic acids) extracted from vegetable and fruit wastes can be valorized to fillers, texturizers, thickeners, and glazes by the immobilized enzymes. D-galacturonic acid monomers are the main hydrolytic products of pectin and attempt to convert this monosaccharide into bio-based chemicals have been documented (Xu et al., 2018). Very recently, Wahab et al. (2018) covalently immobilized pectinase from *A. awamori* KX943614 onto alginate-agar microspheres and optimized the operating parameters such as glutaraldehyde, polyethyleneimine, loading time and enzyme units by applying the central composite design. Thermodynamic profile revealed a substantial enhancement of thermal stability against elevated temperature after gel immobilization. The immobilized pectinase biocatalyst showed a notable recycling ability retaining 60% of its initial activity after eight consecutive cycles suggesting an opportunity to deconstruct pectic-substrates for utilizing as building blocks for prebiotic oligosaccharides bioplastics manufacturing. An enzyme membrane reactors containing recombinant polygalacturonases immobilized on the electrospun chitosan-based nanofibrous membrane was tested for converting pectin-rich biomasses into fermentable sugars, which can be further used as feedstock for the production of biofuels and other bio-based chemicals. Results showed that the pectin-degraded hydrolysate contained some D-galacturonic acid monomers after the action of the immobilized polygalacturonase. The yield of reducing sugar was recorded to be 31.98 mg after one h with a conversion efficiency of 63.96%. Thus, the assembled bioreactor with attached polygalacturonases has great potential in the utilization of pectin-rich biomass as the feedstock for many valuable compounds (Xu et al., 2018).

*7.1.2.3. Conversion of lignocellulosic-based waste materials.* Lignocellulose or cellulose-based waste materials can be converted into valuable bioproducts including rare sugars, surfactants, and biofuels by enzymatic treatment. Lignocelluloses are complex heterogeneous natural composites that comprised of three main biopolymers, i.e., lignin, celluloses, and hemicelluloses. These materials are the principal constituent in sugarcane bagasse and field crop Stover; however, its value-added utilization necessitates a multi-enzymatic system owing to its recalcitrant chemical nature. Periyasamy, Santhalembi, Mortha, Aurousseau, and Subramanian (2016) successfully achieved a one-pot transformation of lignocellulosic biomass to fermentable sugars by combi-CLEAs of cellulose, xylanase, and *β*-1,3-glucanase synthesized using GLU as a cross-linking agent. The prepared carrier-free biocatalytic system was thermally more durable and retained about 70% of its original activity at an elevated temperature of 70 °C, whereas free enzyme showed only 30% activity under these conditions. Along with satisfactory storage stability, combi-CLEAs were active until the sixth reusability cycle. Finally, the combi-CLEAs led to an 83.5% hydrolysis of sugarcane bagasse into monosaccharides. Borges, Junior, Farinas, Giordano, and Tardioli (2014) carried out the immobilization of *β*-glucosidase by surface adsorption on polyacrylic resin as well as covalent coupling on glyoxyl-agarose. Results revealed that the polyacrylic resin- adsorbed biocatalysts *β*-glucosidase presented a pronounced activity for sugarcane bagasse degradation as compared to covalently immobilized β-glucosidase, signifying enzyme impairment following covalent bonding. Silica-amine functionalized iron oxide magnetic nanoparticles attached with cellulase, xylanase, and β-1,3-glucanase were recently fabricated by Periyasamy et al. (2018) to depolymerize cellulosic biomass into monomeric fermentable sugars (Fig. 9). The as-prepared magnetic nanoparticles displayed 95% thermal stability when incubated at 50 °C for two h, along with a prolonged storage stability profile (97%) than that to its native form. The recycling potential was recorded for eight repeated rounds with retention of higher than 70% of its original activity. Importantly, immobilized magnetic cellulolytic enzyme cocktails led to a 15% increase in carbohydrate digestibility on sugarcane bagasse and eucalyptus pulp as compared to the free form of the enzyme.

### *7.2. Valuable utilization of lipids as substrates*

Valorization of waste lipid is significant in terms of economic and environmental impact. Waste oil can be converted into high-value

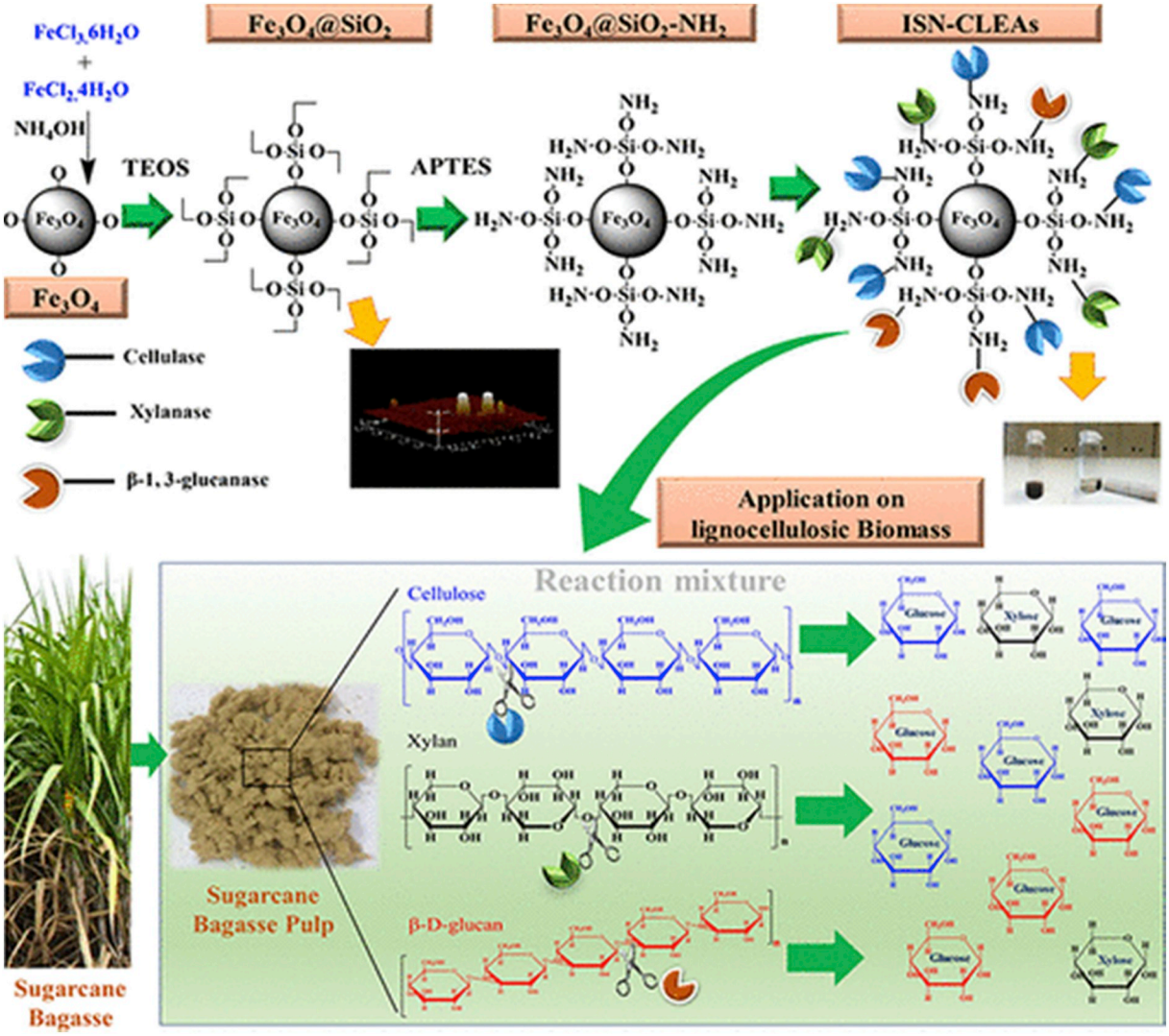


**Fig. 9.** Cellulase, xylanase, and β-1,3-glucanase-bound silica-amine functionalized iron oxide magnetic nanoparticles and their application as a biocatalyst for the depolymerization of cellulosic biomass into monomeric. Reprinted from Periyasamy et al. (2018) with permission from American Chemical Society. Copyright (2018) American Chemical Society.

products such as surfactants, lubricants, and biodiesel by the enzymatic treatment. Among different applications, the lipases-mediated transformation of biodiesel from waste oil is the most extensively studied. For example, Babaki, Yousefi, Habibi, and Mohammadi (2017) obtained an elevated level of biodiesel from waste cooking oil using a multi-enzyme system developed by covalently immobilizing Lipase (from *Rhizomucor miehei*) and lipase B (from *Candida antarctica*) onto epoxy-functionalized silica. The influences of different factors such as enzyme ratio, the ratio of *t*-butanol to oil, water adsorbent content and reaction duration were studied and optimized by response surface methodology under a central composite design. A high yield of fatty acid methyl esters (FAME) (91.5%) was recorded after ten h of reaction time. Another study investigates the biodiesel production by lipase-mediated hydroesterification of waste cooking oil as a feedstock in two steps. The hydrolysis and esterification steps were carried out by the immobilized *Thermomyces lanuginosus* and *C. antarctica* lipase B as biocatalysts, respectively. Complete hydrolysis of acylglycerols was achieved after 12 h, whereas the conversion was about 90% after six h of enzymatic treatment in the esterification step. Moreover, the immobilized enzymes could be recycled five times in 10 h reaction batches (Vescovi et al., 2016). Ferrero, Rojas, Argaraña, and Eimer (2016) designed a hybrid biocatalyst by immobilizing *Pseudomonas fluorescens* lipase on Ca, and Na modified mesoporous SBA-15 supports for environmentally and economically viable biodiesel production from waste oils processing. The Ca-modified SBA-15 based biocatalytic system displayed almost 90% of FAEE yield at 37 °C and 180 rpm. However, recovery and reusability experiments are required to determine durable stability and reprocessing. As compared to the immobilization with an MCM-41, and a mesostructured cellular foam (MCF) material as support materials, SBA-15-immobilized lipase demonstrated much higher activity in the biotransformation of unrefined wasted cooking oil to biodiesel at room temperature. Up to 80.1 and 71.8% of FAME yield and specific activity was achieved after 60 h of reaction at 28 °C using SBA-15-immobilized lipase (Zhang et al., 2014). Arumugam and Ponnusami (2017) produced biodiesel from waste sardine oil by transesterification catalyzed by lipase immobilized on activated carbon. About 94.55% of methyl ester yield was obtained using the optimized conditions of 9:1 (mol/mol) methanol to oil ratio, 10% water content and 30 °C. The immobilized enzyme was recycled five times without any substantial loss in activity.

Polymeric resins have also been tried for lipases immobilization to produce biodiesel, fatty acids, and surfactants from waste oils. As an illustration, Li, Wang, Faiza, Yang, and Wang (2017) described a novel and highly effective approach to biosynthesize biodiesel from high-acid

content waste cooking oil by immobilizing *Malassezia globosa* lipase on an epoxy-functionalized resin. After 8 h of reaction time, the resulting lipase showed efficiency to reduce the free fatty acid content from 28.69 to 0.05%. Following the biotransformation process, the residual triglycerides were chemically converted into FAME with a final conversion efficiency of more than 98%. Covalent immobilization of *P. cepacia* lipase on epoxy-acrylic resin support exhibited high catalytic specific surface and ensured easy recovery, regeneration, and re-utilization of the biocatalyst (Lopresto et al., 2015). Lecitase Ultra immobilized on Amberlites XAD2 and XAD4 through physical entrapment was used in the valorization of an acidic food-derived residue to produce monoacylglycerols under batch and continuous flow conditions. Results revealed a moderate conversion (50–60%) capability of the entrapped biocatalyst for food waste residue accompanied by improved stability under continuous flow conditions (Gonçalves et al., 2013). Regardless of the great promise of lipases for the transformation of waste cooking oil to biodiesel, further studies optimizing the support materials and experimental design are required to achieve high conversion efficiencies and activity retention for several successive reaction cycles. Though immobilized enzyme cost is considered as a major obstacle for commercial implementation, a recent study suggested the cost of the support material as the biggest cost hurdle for enzymatic biodiesel production rather than the enzyme cost (Budžaki, Miljić, Sundaram, Tišma, & Hessel, 2018). Increased catalytic activity in several repeated cycles can overcome the cost of the biocatalysts (Budžaki et al., 2018); however, additional research investigation is indispensable on bio-renewable resources for enzyme immobilization.

### 7.3. Valuable utilization of proteins

Proteolytic enzymes can hydrolyze protein waste from a variety of food industry waste sources such as dairy, soybeans, grains, oilseeds, and eggs into value-added chemicals, e.g., polymer precursors. Dairy waste, in particular, whey protein, has been hydrolyzed by immobilized trypsin. Glutaraldehyde-Activated agarose support aspartic protease has shown whey protein concentrates (WPC) hydrolyzing potential to form antioxidant peptides. In addition to enhanced thermal stability at 40–50 °C, the immobilized enzyme also presented remarkable reusability, preserving more than 50% of its original activity after ten repeated catalytic cycles. The immobilized enzyme derivative showed a higher affinity for hydrolysis of α-lactalbumin protein than that to β-lactoglobulin, indicating that immobilization can change the selectivity and cleavage affinity of the biocatalyst (Rocha, Kise, Rosso, & Parisi, 2017). Similarly, lignocelluloses have been demonstrated as a renewable support for immobilizing different enzymes such as rice straw, spent grain, coconut fiber, and brewery granular waste have been used for the immobilization of invertase, laccase, lipase, and trypsin, respectively (da Silva et al., 2012; D'Souza & Godbole, 2002; Rocha, Gonçalves, & Teixeira, 2011). Aiming to obtain peptides with bioactive potential from cheese whey, trypsin was recently immobilized corncob powder as low-cost lignocellulosic support functionalized with GLU, glyoxyl groups, and iminodiacetic acid glyoxyl. All the immobilized enzyme derivatives were thermally stable at higher temperatures than that to their counterparts. The glyoxyl and iminodiacetic aldehyde-glyoxyl activation produced a 15.46% and 12.49% degree of hydrolysis, with activity retention of 91%, and 87%, and 15.46%, respectively, in the batch experiments. Additionally, the trypsin-glyoxyl immobilized biocatalytic system was also tested in a packed bed reactor providing an average hydrolysis degree of 23%, which was found to be suitable for the manufacturing of bioactive peptides (Bassan et al., 2016a, 2016b). Soy protein has also been regarded as a substrate for waste valorization by proteases. Wang et al. (2014) developed chitosan-coated magnetic nanoparticles-based carrier support using GLU as a cross-linking agent for the effective immobilization of alkaline protease. The immobilized biocatalysts showed the enhanced activity, and a considerable broadening of the optimal pH and temperature profile range in contrast to the free enzyme. After immobilization, the thermal stability was also significantly improved than free-state of the enzyme. The $Fe_3O_4$-chitosan conjugated alkaline protease exhibited the highest hydrolytic activity to be 18.38% after 140 min in comparison to 17.50% for the free enzyme. It retained almost 86% of its initial catalytic activity after ten continuous reaction batches suggesting a promising candidate for the soy protein hydrolysis.

## 8. Environmental impact and cost assessment of immobilized enzymes for implementation viewpoint

While the immobilized enzyme biocatalysis is an emergent area of technology, early-stage critical economic as well as environmental assessment of the immobilized enzyme-mediated bioprocesses are essential to determine their industrial suitability (Harding, Dennis, Von Blottnitz, & Harrison, 2008; Olofsson, Barta, Börjesson, & Wallberg, 2017; Raman, Ting, & Pogaku, 2011; Tufvesson, Fu, Jensen, & Woodley, 2010). In this framework, life-cycle assessments (LCAs) and techno-economic analyses are considered powerful tools as an increasing number of immobilized enzymes are evaluated for commercial-scale biocatalytic processes (Chapman, Ismail, & Dinu, 2018). LCAs are widely used to analyze material and energy consumption, as well as waste and emissions, which in turn measure the environmental impact and sustainability of a process. The use of biocatalysts in industrial processes is often associated with minimum energy consumption, chemical inputs, and waste streams. For instance, the amount of steam required to preheat feedstock is substantially reduced by the enzyme-driven biodiesel production due to the milder reaction conditions. It also circumvents the environmental-related issues, including human toxicity, climate change, and ozone depletion (Harding et al., 2008). Immobilized enzyme-assisted processes have also been envisioned to further abate the environmental burden of free enzyme-catalyzed processes (Raman et al., 2011). In order to develop an optimally sustainable process, Raman et al. (2011) carried out LCA on biofuel production from alkali catalyst, free and immobilized lipases. Owing to milder reaction conditions, both free and immobilized forms of lipases reduced process energy consumption when scaled up to 1000 kg annual production. Additionally, the immobilized lipase system also improved the free-enzyme catalyzed process because of its multiple reuses (Raman et al., 2011). The overall decrease in material and energy consumption by enzymatic processes due to reduced energy consumption reveals that biocatalytic processes are potentially economically lucrative, and environmentally friendly (Chapman et al., 2018).

Regarding economics, the global market for industrial enzymes surpassed $US3.3 billion in 2010 and is expected to rise around $4.5 to $5 billion by 2015 (World Enzymes to 2015, 2011). This market is prevailed largely by hydrolases (e.g., amylases, cellulases, lipases, and proteases). Use of biocatalysts predominantly for diagnostic, pharmaceutical, and research purposes accounted for about $2.4 billion in 2010. Revenue of enzymes (including the immobilized form of the enzymes) for biocatalysis was valued at $160 million in 2010 and anticipated to reach nearly $230 million by 2015 (World Enzymes to 2015, 2011). It is incorrect to speculate that the high costs of industrial enzymes are the major driving force for designing an immobilized form of the biocatalysts (Rozzell, 1999). The true cost of most industrial enzymes is ranged from $50 to $500 per kg enzyme protein, which represents a minor component of over-all processing economics (Horn, Kumar, Liese, & Kragl, 2008; Rozzell, 1999). For instance, the total cost of enzymes for starch-based ethanol is about 1 cent per liter, and the additional enzyme immobilization related expenses might be retrieved from enzyme recycling. The actual cost from an immobilized enzyme depends on the number of times the enzyme is recycled, which fluctuates for the specialty to bulk chemicals production (Horn et al., 2008; Rozzell, 1999). In conclusion, the scale-up implementation of immobilized enzyme technology necessitates a good understanding of both technological and economic aspects, as well as a good perception

of the larger market forces at play (DiCosimo, McAuliffe, Poulose, & Bohlmann, 2013; Tufvesson, Fu, et al., 2010; Tufvesson, Lima-Ramos, Nordblad, & Woodley, 2010a; Tufvesson, Lima-Ramos, Nordblad, & Woodley, 2010b).

## 9. Current challenges, concluding remarks and opportunities

Circular economy based sustainable development has now achieved a significant role in the global agenda. The concept of the circular economy is grounded on waste minimization, resource efficiency, valorization, and recycling. Food industry waste, an underutilized resource, is a key focus area in the circular economy that can be converted into useful products (Imbert, 2017). In this background, immobilized biocatalytic enzyme systems represent a distinctive technological approach for the valuable utilization of food processing waste and augmenting economic and environmental sustainability of food production. Nevertheless, significant challenges remain with their larger-scale applicability requiring additional comprehensive research efforts to overcome these obstacles. Shortlisting of an ideal immobilization method among numerous available methods combined with various operational requirements in terms of waste stream conditions is a major challenge for food waste stream valorization. In addition to differential behaviors of various enzymes upon immobilization, the cost is another significant hurdle in adopting immobilized biocatalytic systems in waste valorization. Approximately, 47% of the cost of an immobilized enzyme system is related to the cost of the immobilization support matrix (Tufvesson, Fu, et al., 2010). The deployment of purified enzymes instead of whole-cell or crude extract additionally raises the cost of biocatalysis. Consequently, economical carriers, or carrier-free immobilized enzyme systems, i.e., CLEAs along with immobilized enzyme systems utilizing whole-cell, or crude extracts need to be investigated. The cost of the immobilized biocatalytic system, as well as demand and pricing for naturally derived ingredients, are the key drivers for food waste valorization. Therefore, the economics of waste stream valorization by the immobilized enzyme technologies need to be more systematically taken into account by the scientists and industrial community before their implementation.

Application of emerging synthetic biology tools that enable site-directed immobilization can increase the enzyme stability in non-ideal environments, or contribute to subunit stabilization against denaturation (Andler & Goddard, 2018). Apart from the utilization of low-cost support materials, immobilized biocatalyst must preserve activity over multiple successive cycles to diminish the cost per use. Though many researchers have developed biocatalysts with high activity retention and potential repeatability, the activity of many immobilized systems significantly decreased after several consecutive cycles. Stringent experimental characterization of the stability of immobilized enzyme systems against both activity loss and leaching is essential to reveal their true performance in an industrial application.

In contrast to single-enzyme processes, multi-enzymatic biocatalytic systems demonstrate great promise for increasing conversion efficiencies and allowing more effectively catalyzing value-added bioconversion processes. A meticulous characterization of the immobilized enzyme kinetics and diffusion-reaction mechanisms will update the essential apprehension of the immobilized enzyme systems for real-time practical applications. Further, industrial conditions are quite complex than true conditions that can considerably affect catalytic performance and stabilization of enzymes. This phenomenon is especially true for multi-enzyme systems, where sensitivity to solvents, inhibitors, and water activity, and their co-factors or co-substrates requirements must be considered for each enzyme. In this perspective, rational design approaches by selecting an immobilization method based upon the complete understanding of the enzyme source, immobilization methods, and application conditions should be used to employ carrier-supports biocatalysts efficaciously. Rationally designed enzymes are believed to have a profound potential to improve food sustainability by producing an array of valuable bioproducts and thus put a favorable impact on managing food waste accumulated because of food processing. Further, genetically modified enzymes can display enhanced catalytic performance when applying to food applications. Indeed, interdisciplinary teams associations such as biochemistry, molecular biology, genetic/enzyme engineering, agricultural economics, food science, and food regulatory authorities are necessary to drive enzyme-assisted applications for the commercial-scale valorization of the waste stream.

## Conflict of interest

Authors declare that they do not have a conflict of interest in any capacity including competing or financial.

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