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REVIEW ARTICLE

Valorisation of protein waste: An enzymatic approach to make commodity chemicals#

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Abstract Protein-rich waste is an abundantly available resource that is currently used mainly as animal feed and fertilizers. Valorisation of protein waste to higher value products, particularly commodity chemicals such as precursors for polymers, has attracted significant research efforts. Enzyme-based approaches, being environmentally-friendly compared to their chemical counterparts, promise sustainable processes for conversion of protein waste to valuable chemicals. This review provides a general overview on valorisation of protein waste and then further summarises the use of enzymes in different stages of the valorisation process—protein extraction and hydrolysis, separation of individual amino acids and their ultimate conversion into chemicals. Case studies of enzymatic conversion are presented for different amino acids including glutamic acid, lysine, phenylalanine, tyrosine, arginine and aspartic acid. The review compares the different enzyme reactors and operation modes for amino acid conversion. The emerging opportunities and challenges in the field are discussed: engineering powerful enzymes and integrating innovative processes for industrial application at a low cost.

Keywords amino acids, protein waste, reactor, conversion, commodity chemicals, enzymes

1 Introduction

As the ongoing rapid consumption of earth's natural resources by human activities has caused environmental concerns, there is an urgent need to engineer sustainable industrial processes and renewable materials. Bio-based resources and processes, environmentally attractive alternatives to crude-oil based routes, could potentially change the landscape of the chemical industry if they were made available at a reasonably low cost. Valorisation of protein-containing waste, a relatively under explored area, has attracted increasing research attention as a potential route to make chemicals [1]. Common sources of protein waste include distiller dried grains with solubles (DDGS) from maize & wheat, sugarcane, soybean, palm oil, grass and animal slaughter [2]. These wastes are produced as by-products of many industrial processes, exist in huge quantities and are currently used merely as animal feed and fertilizers. It is expected that with the increasing production of biofuels in the future, there will be an abundance of protein-containing waste [2]. It was estimated that if 10% of the global fuel demand was fulfilled by bioethanol and biodiesel, 100 million tonnes of protein-rich waste would be produced every year [3].

Proteins are composed of amino acids. The fundamental reason for considering amino acids as a raw material for chemicals is the structure of the amino acid itself. The structures of some amino acids are remarkably similar to a number of synthesised chemicals. In the petrochemical industry, some of the crude oil hydrocarbons are derivatised with target functional groups such as amines that are naturally present in amino acids. Therefore, it makes sense to directly convert amino acids to chemicals with the least amount of changes to their chemical structure. In proteins, there are 20 different amino acids with varying chemical properties determined by side chains. This diversity gives a wide choice of possible chemical precursors to make chemicals ranging from surfactants and lubricants to industrial solvents and precursors for plastics and polymers.

Conversion of protein waste to chemicals can be based on different approaches: chemical, microbial and enzy-

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matic methods. This review will first present an overview of valorisation of protein waste, followed by application of enzymes for conversion of protein-rich biomass to chemicals. The review will identify challenges and opportunities in this area, highlighting the need for powerful and yet inexpensive enzymes as well as efficient processes for valorisation.

2 Overview on valorisation of protein waste

To date most protein wastes are primarily used as animal feed or fertilizers. For example, DDGS, a by-product from the bioethanol industry, is mostly sold as cattle feed [2], although it is a poor nutrition source due to its low lysine and high fibre content [4]. Vinasse, a by-product from sugarcane-based bioethanol production, has been used as a fertilizer. However, such usage has been known to lead to ecological issues such as pollution in rivers as expected from any nitrogen containing fertilisers [5]. Furthermore, certain animal by-products cannot be used as animal feed or fertilisers as a regulation requirement by European Parliament because they may cause infectious diseases if they reach humans [6].

From an economic point of view, the use of biomass for the production of commodity chemicals gives it a higher value of approximately $1000 per tonne of biomass compared to its use in fuel production ($200−400 per tonne of biomass), as animal feed ($70−200 per tonne of biomass) and in electricity generation ($60−150 per tonne of biomass) [1,3,7]. Among these commodity chemicals, styrene, 1,2-ethanediamine and acrylic acid have an individual market of over one million tonnes worldwide per year. Alternative products such as flavour and drug intermediates have a higher value than commodity chemicals, but their market scales are too small to accommodate the sheer volume of protein waste. This review thus focuses on valorisation of protein waste towards commodity chemicals.

2.1 Sources and compositions of protein waste

A recent review has summarised possible sources of protein-rich waste that can be explored for bulk chemical production [2]. These sources range from industrial food waste and agricultural waste to animal slaughter waste. Depending on their origins, the amino acid composition of protein wastes can vary dramatically. Table 1 summarises the mass compositions of amino acids in some common protein-rich wastes. Glutamic acid is one of the most abundant amino acids across different sources of protein waste. For example, it can account for 27% and 25% of protein mass in gluten and oil palm kernel meal, respectively. Some amino acids can have a high content in one source but be low in another. For example, aspartic acid is highly rich in whey protein (10.5%), but is relatively low in wheat gluten (2.5%). Based on abundance of amino acids in the source of protein waste, different amino acids can be chosen as targets for conversion.

Among different sources of protein waste, an interesting amino acid-rich source is cyanophycin. Cyanophycin is a nitrogen storage polypeptide commonly found in cyanobacteria [8], and strictly it is not a protein. Cyanophycin is composed of a poly L-aspartic acid backbone with L-arginine side chains. It has been reported that cyanophycin can be microbially converted into amino acids [9]. Due to its low solubility in water, cyanophycin can be conveniently separated from culture media [10], offering a significant advantage of easy separation.

2.2 Different approaches for valorisation of protein waste

Different methods have been explored for valorisation of protein waste. To date, most of these methods need to be further improved before commercial applications. Based on the action mechanism, these approaches include chemical, fermentation and enzymatic methods. A particular process of valorisation may use one or a combination of these methods.

2.2.1 Chemical approach

Proteins or amino acids can be converted to products via chemical approaches [18]. The chemical approaches can be based on either a simple reaction to make low-value products or multiple reactions to produce higher value chemicals. For example, the proteins in oil-free DDGS have been chemically acetylated and then compression moulded to form environmentally-friendly thermoplastics [19]. Combining the low value of DDGS and low cost of the simple reaction, this method has potential to deliver economically competitive thermoplastics.

Multiple chemical reactions are often carried out at different stages sequentially. One such example is the production of succinonitrile from glutamic acid [20]. The first stage was the synthesis of 3-cyanopropanoic amide from glutamic acid through three-step chemical reactions: esterification, decarboxylation and amidation. The second stage was dehydration of 3-cyanopropanoic amide to succinonitrile using a palladium (II) catalyst with a yield of 62%. However, this chemical process is expensive due to the high cost of palladium catalyst (approx. $2000 for 50 g from Alfa Aesar®) and the complex four-step reaction process.

2.2.2 Fermentation approach

Early study of the fermentation approach often used wild type microorganisms to make chemicals from amino acids and/or protein. For example, yeast *R. glutinis* was used to convert phenylalanine to *trans*-cinnamic acid with the

Table 1 Amino acid content of some common protein-rich wastes (mass % of amino acids in protein waste)[a)]

Amino acid	Whey protein	Wheat gluten[b)]	Soybean meal	Feather meal	Oil Palm kernel meal protein isolate[c)]	DDGS	Cyanophycin
Alanine	4.74	1.96	2.27	2.88	6.98	2.09	0
Arginine	2.23	2.27	3.77	6.76	5.20	1.32	46.1
Aspartic acid	10.5	2.53	6.09	4.18	11.3	1.99	39.2
Cysteine	1.51	0.12	0.86	–	5.00	0.58	0
Cystine	–	–	–	6.58	–	–	0
Glutamic acid	15.1	27.4	9.39	8.22	25.8	5.50	0
Glycine	1.65	2.55	2.20	5.18	4.53	1.16	0
Histidine	1.77	0.93	1.36	0.23	2.02	1.01	0
Isoleucine	4.57	3.02	2.28	3.94	3.40	0.91	0
Leucine	9.73	5.12	4.10	5.69	6.83	3.42	0
Lysine	8.40	3.07	3.23	1.54	5.12	1.09	0.6
Methionine	2.59	1.34	0.80	0.71	2.34	0.76	0
Phenylalanine	2.53	2.48	2.66	3.46	4.93	1.38	0
Proline	6.76	9.56	2.93	7.39	3.40	1.94	0
Serine	5.07	3.68	3.08	8.73	0.25	1.44	0
Threonine	4.89	1.79	1.67	3.45	5.32	1.19	0
Tryptophan	2.05	–	–	–	–	–	–
Tyrosine	3.01	2.17	1.75	–	3.16	0.91	0
Valine	4.56	2.34	2.38	5.30	4.93	1.47	0
Total amino acid	91.66	72.33	50.82	74.24	100	28.16	85.9
Reference	[11]	[12]	[13]	[14]	[15]	[16]	[17]

a) Amino-acid mass percentage obtained after acid hydrolysis of given protein waste. Note that the protein waste may also contain other components such as lipids and carbohydrates, and the total percentage of amino acids is thus less than 100%. The given data should be used as an estimated guide only because the composition of waste can vary significantly from different sources; "–"indicates that the particular amino acid was not quantified in the composition analysis, and it does not mean that the amino acid was not present
b) Amino acid content given in mmol/g in the original literature and has been converted into mass in this table
c) Amino acid content given relative to total protein content (75.6%) in the waste, same as originally reported in the literature

amino acid as the sole nitrogen and carbon source [21], and the *trans*-cinnamic acid secreted in the broth media was extracted by ether.

Metabolic engineering has been increasingly used in fermentation approach for valorisation of protein wastes. By changing metabolic pathways, the conversion yield of proteins/amino acids can be significantly improved. For example, both metabolically engineered *E. coli* and *C. glutamicum* have been used to make polyamines from proteins [22]. *C. glutamicum* has been engineered with different metabolic pathways to make putrescine, a monomer for polyamide production [23]. It was found that the ornithine decarboxylase pathway gave 40 times higher production yield than the arginine decarboxylase pathway. The significant difference demonstrates the importance of metabolic engineering in improving production yield.

2.2.3 Enzymatic approach

The use of enzymatic methods has several advantages over chemical methods because enzymes are highly selective and can be made from renewable sources. It has been suggested that protein waste can be converted into chemicals in three sequential steps: i) protein waste is hydrolysed to its constituent amino acids, ii) a target amino acid is isolated from the mixture and iii) the amino acid is converted to a target chemical [1]. An alternative to this process is to convert target amino acids to chemicals first and then isolate the products from the mixture. A detailed review of enzymatic conversion is presented in section 3.

3 Enzymatic conversion of protein waste into chemicals

3.1 Hydrolysis of proteins

Hydrolysis of proteins into amino acids is the first step in the process of converting protein waste to chemicals. It also helps extraction of proteins from insoluble protein waste such as keratin. Hydrolysis can be carried out using a chemical or enzymatic approach, or a combination of both.

3.1.1 Chemical hydrolysis

Chemical methods can be based on either acids or alkalis, and they have been extensively covered in literature [11–15,24–27]. Chemical methods often require extreme conditions and modify certain amino acids. For example, acid hydrolysis completely destroys tryptophan and partially damages tyrosine, serine and threonine [28]. Alkali hydrolysis can modify arginine, lysine, cysteine, serine and threonine [29].

3.1.2 Enzymatic hydrolysis

Proteinases can break down the peptide bond of proteins. Protein hydrolysis by proteinases is less energy intensive compared to chemical approaches because it can be carried out in mild conditions. Table 2 summarises a variety of enzymes for the hydrolysis process. In a recent study, a variety of proteases including Protex 40XL, Protex P, Protex 5L, Protex 50FP and Protex 26L were explored for hydrolysis and extraction of proteins in soybean meals [30]. The first three enzymes showed optimal activity under alkaline reaction conditions while the others under acidic conditions. Acidic reaction conditions were found to lower the protein's solubility because the pH was closer to the protein's isoelectric point. Therefore, the alkaline enzymes gave better protein extraction yields and were able to extract 90% of the protein. In another study, protein was extracted from wheat DDGS using Protex 14L, Protex 6L and Protex 51P. 57% of the proteins were broken down into smaller peptides using Protex 6L [31]. These methods gave a protein hydrolysate in the form of small peptides but not amino acids.

One important group of proteinase is keratinase which degrades insoluble keratin, the proteins in animal hairs, skin and chicken feathers. The great diversity of keratinases and their properties have recently been covered in an excellent review [32], and readers are recommended to refer to it for details on their properties and applications.

The main advantages of using enzymes over the traditional chemical methods are (1) the reaction can be carried out under relatively mild conditions, (2) the amino acids do not get damaged in the process [33]. However, a drawback of using proteases is that a single protease usage may give a mixture of peptides instead of amino acids. Thus a combination of enzymes may be required to fully hydrolyse proteins.

3.1.3 Combination of enzymatic and chemical hydrolysis

Combination of enzymatic and chemical hydrolysis can overcome disadvantages of the individual methods. A recent study has compared different hydrolysis methods to produce glutamic acid from wheat gluten [12]. A combination of enzymatic treatment and dilute acid treatment (1 mol/L HCl) avoided the use of high concentration of HCl (6 mol/L). In the first step, proteins were hydrolysed by enzymes at neutral pH, which released approx. 48% glutamic acid. In the second step, the protein solution was further treated by 1 mol/L HCl, giving a combined yield of 80%. The attempt to use a lower concentration of 0.1 mol/L HCl in the second step was not successful, suggesting more research are needed to enable hydrolysis at a milder condition.

3.2 Separation of individual amino acids

Hydrolysis of proteins leads to a mixture of amino acids whose composition can vary dramatically depending on their sources (Table 1). In theory, these amino acids can be isolated based on their size, solubility, hydrophobicity and electrochemical characteristics. For example, amino acids were separated into three different groups (acidic, basic and neutral) by electrodialysis using ion exchange

Table 2 Typical enzymes used in protein hydrolysis

Protein waste	Enzyme	Hydrolysis condition		Ref.
		pH	Temperature /°C	
Feather meals	Keratinase[a)]	Neutral to alkaline	40–60	[32]
Whey protein isolate	Corolase PP	8.0	50	[34]
Wheat gluten	Alcalase 2.4 L FG	8.5	55	[12]
	Validase FP concentrate	6.0	55	
	M Amano SD	7.0	40	
	Peptidase R	7.0	40	
	Flavourzyme 1000 L	7.0	55	
DDGS	Protex 14L	7.5	50	[31]
	Protex 6L	7.5	50	
	Protex 51P	7.5	50	

a) Keratinase is a large family of proteinase that degrades keratin and their optimal pH and temperature can vary significantly from different sources. See details in [32]

membranes [35]. In this method a problem occurred in the presence of arginine which interacted with cation exchange membranes and decreased separation efficiency of electrodialysis [36]. A specially designed electro membrane with a higher swelling degree was successfully used to overcome this problem [37]. Although chromatography technique is highly efficient in isolation of individual amino acids, it is unsuitable for large scale production of commodity chemicals due to the associated high cost. For practical applications at an industrial scale, there is an unmet need of amino acid isolation at a low cost.

To facilitate easier separation of amino acids, modifications of the amino acids have been explored. Such approaches often involve the selective conversion of a particular amino acid into an easy separable form. For such purpose, enzymes have been used to change amino acids' properties such as charges and solubilities. For example, lysine decarboxylase was added to a mixture of two basic amino acids—lysine and arginine, and selectively converted lysine into 1,5-pentanediamine which had a different charge and was subsequently separated from arginine by electrodialysis [38,39]. Similarly, glutamic acid decarboxylase was used to aid separation of a mixture of glutamic acid and aspartic acid [40]. The enzyme converted glutamic acid to less negatively charged γ-aminobutyric acid which was separated by electrodialysis using a mixed matrix membrane.

Despite these efforts, further research is needed to develop low-cost separation processes that can be operated at a large scale. Most reported methods dealt with a model system containing two or a few amino acids rather than a mixture of 20 amino acids. To date the available separation methods are still too expensive, making the overall valorisation process economically uncompetitive. As pointed out by Tuck et al, there is an urgent need of innovation in the isolation process [1].

3.3 Enzymatic conversion of amino acids to bulk organic compounds

Amino acids can be converted to different products such as flavours, drug intermediate or bulk chemicals. For example, amino acids can be converted to aromatic compounds using enzymes produced by lactic acid bacteria present in cheese [41], giving a particular variety of cheese its unique taste and flavour. These aromatic compounds are however not considered to be large-volume chemicals and will not be discussed further in this review. Note that the amino acids mentioned in this review are L-isomers unless stated otherwise. Table 3 summarises a few amino acids that have been enzymatically converted to chemicals, and they are reviewed as case studies in this section.

3.3.1 Glutamic acid

Glutamic acid, due to its high content in protein waste sources [2], is one of the top candidates for bulk chemical production. Two typical chemicals converted from glutamic acid (**1**), γ-aminobutyric acid (**2**) and α-ketoglutaric (**3**), are shown in Fig. 1. γ-Aminobutyric acid can be further used to make products including sedatives [59], acrylonitrile [60], succinonitrile [20] and *N*-methylpyrrolidone [61]. α-Ketoglutaric acid has been suggested for applications such as a protection against cyanide poisoning [62] and wound healing [63]. It can also be polymerised into poly(triol α-ketoglutarate), a biodegradable biomaterials [64].

Table 3 A summary of enzymatic conversions of amino acids to chemicals

Amino acid	Chemical	Enzymes	Source of enzyme	Ref.
Glutamic acid	γ-Aminobutyric acid	Glutamic acid decarboxylase	*E. coli* ATCC 11246	[42,43]
			E. coli DH5α	[44–47]
Glutamic acid	α-Ketoglutaric acid	Amino acid deaminase	*P. mirabilis* KCTC 2566	[48]
Glutamate	α-Ketoglutarate	Glutamate dehydrogenase	*C. symbiosum*	[49]
		NADH oxidase	*L. sanfranciscensis*	[49]
Lysine	5-Aminovaleric acid	Lysine α-oxidase	*T. viride*	[50]
Lysine	5-Aminovaleric acid	Lysine monooxygenase and 5-Aminovaleramide amidohydrolase	*P. putida* KT2440	[51]
Lysine	Cadaverine dicarboxylate	Lysine decarboxylase	Not disclosed	[52]
Phenylalanine	Cinnamic acid	Phenylalanine ammonia lyase	Not disclosed	[53]
Tyrosine	*Para*-hydroxycinnamic acid	Tyrosine ammonia lyase	*R. glutinis*	[54]
			P. chrysosporium	[54]
Arginine	Ornithine	Arginine amidinohydrolase	*B. subtilis* KY 3281	[10,55]
Aspartic acid	β-Alanine	Aspartate α-decarboxylase	*E. coli* W	[56,57]
Aspartate	β-Alanine	Aspartate α-decarboxylase	*C. glutamicum*	[58]

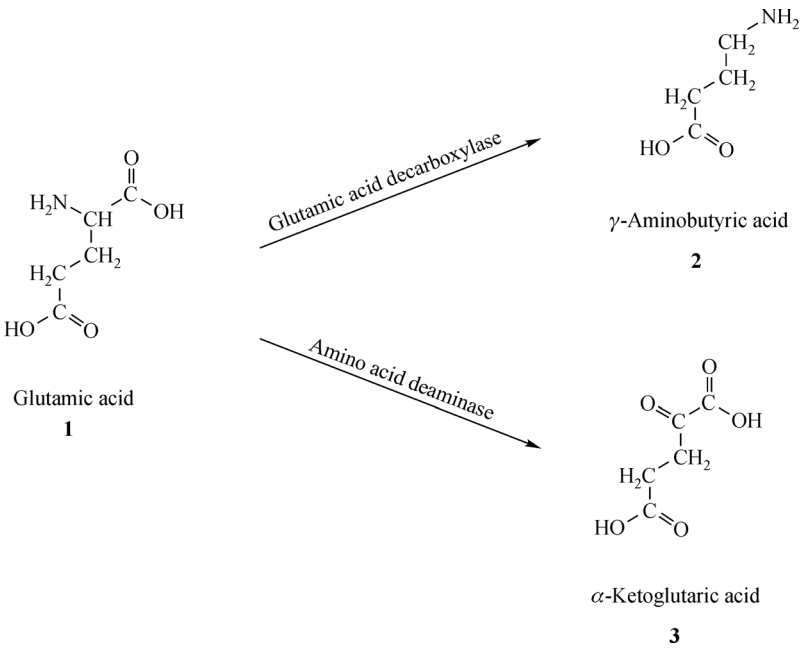

Fig. 1 Conversion of glutamic acid to γ-aminobutyric acid and α-ketoglutaric acid

γ-Aminobutyric acid: Glutamic acid α-decarboxylase (GAD) was used to make γ-aminobutyric acid from glutamic acid [42]. GAD is a pyridoxal 5′-phosphate (PLP)-dependent enzyme with an optimal enzyme activity under acidic conditions [65]. The enzyme was expressed in *E. coli* [43], and the purified enzyme was then immobilised on two different materials: Eupergit by covalent bond formation and calcium alginate by gel entrapment [42]. It was found that the enzyme entrapped in calcium alginate performed better than immobilised on Eupergit. The conversion reaction was carried out in water without addition of buffer to make the process more economic. Using a fed-batch mode, a yield of 35 g γ-aminobutyric acid $L^{-1}\cdot h^{-1}$ was achieved [42]. The same salt buffer-free approach was used to make γ-aminobutyric acid in a batch mode by another group [44]. Using engineered GAD with hyper activity, the batch mode reached 100% conversion after 3 h, giving a yield of 34.3 g γ-aminobutyric acid $L^{-1}\cdot h^{-1}$ [44], which was close to the yield delivered by the fed-batch mode [42].

α-Ketoglutaric acid: Both whole cells and free enzymes have been used for production of α-ketoglutaric acid. In a study using whole cells biocatalytic technique [48], L-amino acid deaminase was expressed using *E. coli* and *B. subtilis* systems encoded with a gene from *P. mirabilis* KCTC 2566 [48]. The recombinant enzyme showed a higher specific activity in *B. subtilis* than in *E. coli*, possibly due to better protein refolding ability of the former [66]. A maximum yield of 4.65 g/L of α-ketoglutaric acid was obtained after optimisation of the conversion process. Immobilisation of whole cells was carried out using alginate to test reusability of the biocatalysts. It was found that the immobilised cells retained just about 25% of its activity after 4 cycles [48].

α-Ketoglutaric acid has also been produced from glutamic acid using purified enzymes [49]. The reaction was performed using a coupled enzyme system comprising glutamate dehydrogenase and NADH oxidase. The oxidation of glutamic acid was catalysed by glutamate dehydrogenase with simultaneous reduction of cofactor NAD^+ to NADH. In parallel, NADH oxidase regenerated NADH back to NAD^+ using molecular oxygen in the buffer as a co-substrate. This coupled enzyme system enabled continuous reaction without extra addition of NAD^+. It was observed that it took 18 h to completely convert 5 mmol/L glutamate to α-ketoglutarate. The slow production rate was due to feedback inhibition.

3.3.2 Lysine

As shown in Fig. 2, lysine (**4**) can be converted to 5-aminovaleric acid (**5**) or cadaverine (**6**). 5-aminovaleric acid is a precursor of valerolactam, a building block for nylon-5 production [67]. Cadaverine is used in industry for the production of polyamides, chelating agents and additives [68].

5-Aminovaleric acid: Lysine α-oxidase was used to oxidise lysine to produce 5-aminovaleric acid [50]. An aqueous solution of a mixture of lysine free base and lysine monohydrochloride was chosen as the buffer since the enzyme showed the best activity under this condition. A conversion yield of 95% was achieved after 5 days of reaction. The enzyme was also immobilised onto epoxy-activated Sepabeads EC-EP and could be reused [50].

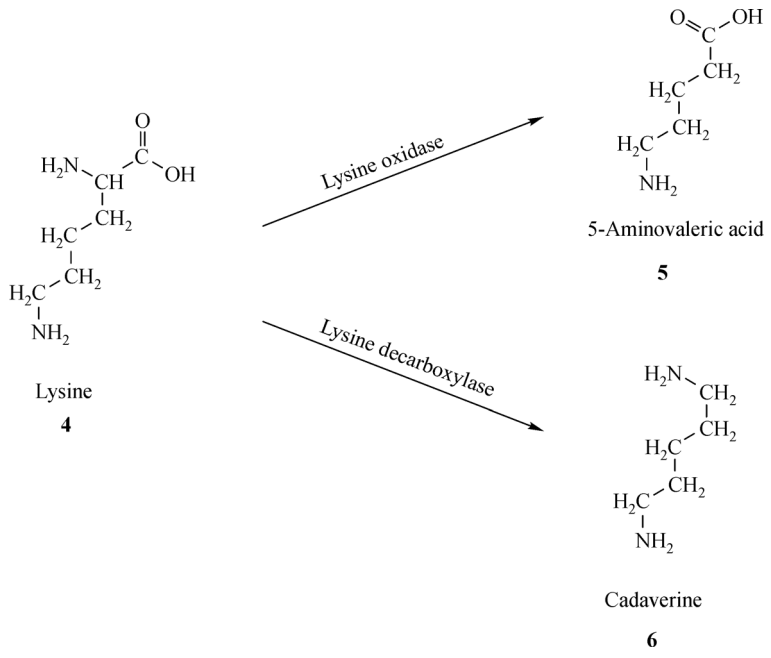

Fig. 2 Conversion of lysine to 5-aminovaleric acid and cadaverine

Liu et al. proposed an alternative method to produce 5-aminovalerate using a coupled system of two enzymes [51]. Lysine was first oxidised to form 5-aminovaleramide by lysine monooxygenase and then converted to 5-aminovalerate by 5-aminovaleramide amidohydrolase. Both enzymes were expressed using recombinant *E. coli* and purified before use. 87% of the lysine was converted to 5-aminovalerate after 12 h under optimised conditions, significantly shorter than the 5 days reported in [50]. However, some of the intermediate 5-aminovaleramide accumulated in the reaction mixture indicating that the second enzyme might have decreased activity due to deactivation or inhibition.

Cadaverine: Conversion of lysine to pentamethylene-diamine, commonly known as cadaverine, is also an attractive route. An enzymatic method using lysine decarboxylase to produce cadaverine dicarboxylate in the presence of dicarboxylic acid has been described in a patent [52]. The whole cell approach has also been reported to make cadaverine [68].

3.3.3 Phenylalanine and tyrosine

As shown in Fig. 3, phenylalanine (**7**) and tyrosine (**9**) can be converted to multifunctional aromatic compounds such as cinnamic acid (**8**) and *para*-hydroxycinnamic acid (**10**), both of which have a variety of industrial applications including production of liquid crystalline polymers, elastomers, adhesives, pharmaceuticals and biocosmetics [69,70].

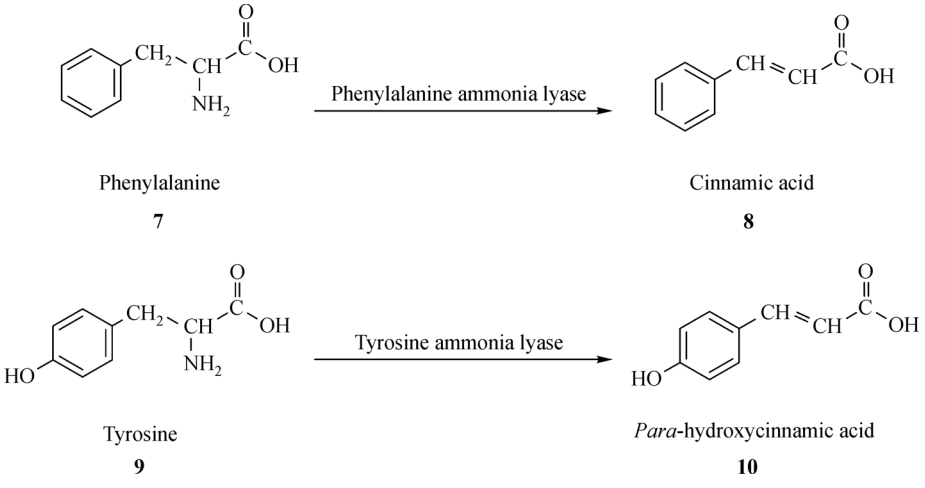

Fig. 3 Conversion of phenylalanine to cinnamic acid and tyrosine to *para*-hydroxycinnamic acid

A thermostable tyrosine ammonia lyase was used to make *para*-hydroxycinnamic acid from tyrosine [54]. This enzyme was produced in *E. coli* transformed with a gene from wood rotting fungus, *P. chrysosporium*, and could withstand 3–4 h exposure to 55−60 °C. In contrast, the enzyme from yeast *R. glutinis* lost activity under the same condition. The high thermostability of the enzyme from *P. chrysosporium* makes it an attractive choice because the higher reaction temperature increased both substrate solubility and enzyme activity.

3.3.4 Arginine

In a two-step reaction in Fig. 4, arginine (**11**) is hydrolysed by arginase (arginine amidinohydrolase) to ornithine (**12**), followed by decarboxylation into 1,4-diaminobutane (**13**), a precursor for nylon-4, 6.

Ornithine: In a study by Könst et al. [10], the reaction to form ornithine was carried out using both free and immobilised arginase from *B. subtilis*. Among three epoxy-activated matrices used for immobilisation, Sepabead EC-EP showed the best immobilisation results with enhanced stability for arginase. The second reaction, which converted the ornithine to 1,4-diaminobutane, was realised using ornithine decarboxylase expressed in *E. coli* using a gene from *T. brucei* [71].

3.3.5 Aspartic acid

As shown in Fig. 5, decarboxylation of aspartic acid (**14**) produces *β*-alanine (**15**) (3-aminopropionic acid), an intermediate that can be converted to nitrogen containing chemicals such as acrylamide or acrylonitrile. L-aspartate *α*-decarboxylase was used to convert aspartic acid to *β*-alanine [56]. This enzyme was highly stable and retained activity after 1 h at 70 °C or 24 h at 60 °C. However, the enzyme had low activity due to low turnover numbers of the enzyme (2390 s^{-1}) [56]. In a separate work by Shen et al. [58], L-aspartate *α*-decarboxylase from *C. glutamicum* was used for the same conversion. It was found that the enzyme was mostly active at 55 °C, but lost 38% activity after 12 h. A direct comparison of the enzymes from these two sources is difficult because the two studies used different reaction conditions. Nevertheless, the enzyme from *C. glutamicum* seems to have a higher activity at room temperature, but is less thermostable, compared to the one from *E. coli.* This difference suggests that engineering of the enzyme is needed to achieve both high activity and stability in a single enzyme.

4 Processes for enzymatic conversion

In addition to activities and physical properties of enzymes, another important consideration is the operation modes used in the enzymatic conversion. While both free and immobilised enzymes can be selected for the valorisation, some reactor types such as a fixed bed will require immobilised enzymes. Enzyme immobilisation technologies are matured technologies and are extensively covered in the literature [72,73], and will not be repeated in this review. Instead, we will focus on the compatibilities of

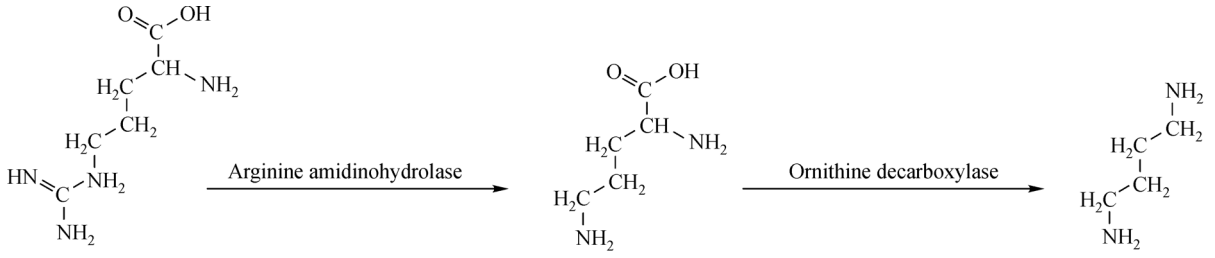

Fig. 4 Conversion of arginine to ornithine to diaminobutane

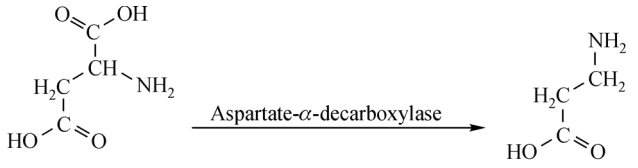

Fig. 5 Conversion of aspartic acid to *β*-alanine

the form of enzymes with the intended reactors and operation modes.

The operation modes for an enzyme-based reaction include stirred tank reactors, membrane reactors, packed bed reactors and fluidised bed reactors [74,75]. The choice of the type of reactor depends on the intended process, nature of the chemical reaction and the enzyme's vulnerability to inhibition by external factors [76]. For details, readers are recommended to read some excellent reviews published previously [75,77,78]. Herein, we present analyses of reactors for their specific application for amino acid conversion.

An important consideration in determining the type of reactor for a given system is how to achieve the optimised reaction conditions (pH, temperature, and concentration of substrates and products). Usually, a salt-buffer may be used in small reactors to control pH values. However, using buffer for pH control for scale-up operations is not economically viable. Furthermore, the use of buffer complicates the purification of the final product [42,44,46]. Thus, a reactor that can achieve optimised reaction conditions at the lowest possible cost is highly desirable.

4.1 Stirred tank reactor

The stirred tank reactor is the simplest reactor commonly used for enzymatic reactions [74]. This reactor system is often used in a batch mode for enzymatic conversions of amino acid including glutamate [44,49], aspartate [56], and lysine [50]. One disadvantage of the batch mode is that reaction conditions such as pH and substrate concentration changes with time, decreasing activity of enzymes as it departs from the optimal condition. The second disadvantage is that some enzymes can be inhibited by the substrate, product or other substances present in the reaction media. For example, aspartate-α-decarboxylase, the enzyme that converts aspartic acid into β-alanine, is inhibited by high substrate concentration [58]. Batch mode has a high concentration of the substrate and a high concentration of the product at the early and late stage of reaction, respectively, and these conditions are unfavourable if there are substrate/product inhibitions.

To overcome these two problems, a fed-batch mode can be used instead of the batch mode. As substrate is gradually fed into the reactor, the substrate concentration can be kept low to minimise its inhibition effect on enzyme. Reaction conditions such as the pH can also be retained in a desired range. Using a fed-batch mode, Shen et al. attenuated the enzyme inhibition by aspartic acid and retained pH at an optimum value, delivering a high conversion yield of 97.2% [58]. Their results demonstrate the importance of operation mode in enzymatic conversion of amino acids.

Continuous stirred tank reactor (CSTR) is another option for a stirred tank reactor. Lammens et al. compared a CSTR and fed-batch reactor for the conversion of glutamic acid into γ-aminobutyric acid [42]. Although each tank had a conversion yield of only 80%, a cascade of three CSTRs gave a total conversion yield of 99%. The high overall yield of a series of CSTRs, however, came at a price: the reaction rates of the second and third stages were very low due to lowered concentrations of the substrate (smaller than the K_m value of the immobilised enzyme), decreasing the volumetric productivity for CSTRs. In contrast, a simple fed-batch reactor achieved a better productivity as the substrate concentration could remain higher [42]. However, it should be noted that there is an accumulation of the product in a fed-batch mode, and it may not be a suitable mode if enzyme is inhibited by the product.

4.2 Membrane reactor

A membrane reactor uses a semipermeable membrane to retain the enzyme while allowing permeability of the substrates and products in or out of the reactor. A rather unique feature of the membrane reactor is that it can separate the final product from the free enzyme through careful reactor design and a selection of suitable membranes. Könst et al. used a membrane reactor to convert arginine to ornithine in a continuous mode [10]. A cellulose ultrafiltration membrane with a 10 kDa cut off was used to retain the free enzyme. Compared to the same enzyme immobilised on Sepabeads EC-EP used in the same membrane reactor, the conversion using free enzyme shows a similar operational stability. It was reported that after 72 h operation, neither the free nor immobilised enzyme showed a significant loss in activity, suggesting immobilisation was not necessary in this membrane reactor. Therefore, the membrane reactor offers the advantage in saving the cost of enzyme immobilisation in comparison to processes that use immobilised enzymes to reuse enzyme.

4.3 Fixed bed reactor

Since the operation of the fixed bed reactor requires the reaction solution to pass the enzyme-loaded bed, enzymes have to be immobilised on particles before being used in a fixed bed reactor. Recently, Teng et al. encapsulated glutamate α-decarboxylase into calcium alginate beads which were then packed into a glass column [79]. A reactant solution containing glutamic acid was pumped through the column. The product, γ-aminobutyric acid from the column reached a steady state concentration of 0.54 mmol/L. The concentration of the product was lower than that predicted based purely on the enzyme kinetics. A possible reason for this was likely to be the diffusion limitations of the feed and product molecules in and out of alginate beads in the column, which was expected to decrease the overall conversion rate.

Although other reactors such as fluidised bed reactors have been used for various other enzymatic conversions [74], their use in enzymatic amino acid conversions have not been sufficiently explored yet.

5 Perspectives and conclusions

Literature reports have proven the concept of enzymatic valorisation of protein wastes into chemicals. Among different valorisation strategies, enzymatic approach has the potential to bring high value products in an environmentally friendly manner. However, the enzymatic approach is still in an early stage towards practical applications, and there are a number of challenges that need to be overcome before it can be broadly used on an industrial scale.

The first challenge is to engineer economically competitive enzymes that are tailored for amino acid conversion. This requires the engineered enzyme to have the following attributes: i) A high selectivity to convert a specific amino acid to the final product or intermediates, ii) A high activity to enable fast reactions, iii) A high stability under variable reactor conditions for operation over a prolonged period, and iv) A recoverable form that allows reuse of enzymes (through either enzyme immobilisation or a membrane process). Protein engineering, particularly directed evolution, is expected to play an important role to bring enzymes such attributes [80]. Given that there are 20 amino acids in the mixture of protein hydrolysis solution, a library of enzymes with each targeting a specific amino acid will be needed to fully utilise all amino acids existing in protein waste.

The second challenge is to achieve reasonable value of valorisation at a low cost. While it is desirable to get the most value out of protein wastes, a process design that targets only those products of highest price may not necessarily be the best strategy. High value products, such as fine chemicals or drug intermediates, have a relatively small market and do not require large volumes of protein wastes. For this reason, Tuck et al. have suggested that production of bulk chemicals should be given a higher priority than fine chemicals in valorisation of protein waste [1]. The valorisation process needs to be engineered to operate at a low cost in order to make production of bulk chemicals viable. It not only requires cheap enzymes at a large quantity, but also requires further engineering to produce reusable enzymes in immobilised forms to stay cost-competitive. New immobilisation strategies, perhaps by better control of the surface orientation of the enzymes, could be a promising option [81,82].

The third challenge is to increase the efficiency of the overall valorisation process. The current valorisation approach requires multiple steps including protein hydrolysis, separation of the mixture of amino acids, and conversion of individual amino acids to chemicals. Although it is technically possible to realise each step, the efficiency of the overall process is still very low. In addition to cost considerations, the designed process will need to have a low carbon footprint compared to existing methods to allow long term sustainability. While incremental innovation will be helpful here, disruptive inventions are needed to have a true breakthrough in process development. A smart way that can integrate the multiple steps to intensify the processes on an industrial scale is urgently needed. For example, integration of the separation and conversion steps could dramatically improve the efficiency of the overall process. These challenges present many opportunities for chemical engineers to take new approaches to come up with game-changing inventions towards cost-competitive and sustainable processes for valorisation of protein waste.

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