

# Utilization of protein-rich residues in biotechnological processes

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**Abstract** A drawback of biotechnological processes, where microorganisms convert biomass constituents, such as starch, cellulose, hemicelluloses, lipids, and proteins, into wanted products, is the economic feasibility. Particularly the cost of nitrogen sources in biotechnological processes can make up a large fraction of total process expenses. To further develop the bioeconomy, it is of considerable interest to substitute cost-intensive by inexpensive nitrogen sources. The aim of this mini-review was to provide a comprehensive insight of utilization methods of protein-rich residues, such as fish waste, green biomass, hairs, and food waste. The methods described include (i) production of enzymes, (ii) recovery of bioactive compounds, and/or (iii) usage as nitrogen source for microorganisms in biotechnological processes. In this aspect, the utilization of protein-rich residues, which are conventionally considered as waste, allows the development of value-adding processes for the production of bioactive compounds, biomolecules, chemicals, and materials.

**Keywords** Protein-rich residues · Protein hydrolysates · White biotechnology · Enzymes · Organic acids

## Introduction

The finiteness of fossil resources and negative ecological impact of fossil oil-based consumables and fuels caused increasing interest in sustainable, “green,” compounds by industries

and consumers. Due to the increasing preference to substitute petroleum-based compounds, various biotechnological processes for the production of bio-based compounds from residues of low value have been developed and intensively studied (Koutinas et al. 2014).

Many microorganisms are able to take up and utilize amines as organic nitrogen sources (Burkovski and Krämer 2002; Pleissner and Eriksen 2012; Pleissner et al. 2011; Richter et al. 2015). For instance, amino acids, belonging to the group of amines, can be linked together by a peptide bond to small peptides, polypeptides, and proteins. On the other hand, proteins and peptides can be hydrolyzed into amino acids and used as nitrogen sources for microorganisms in biotechnological processes. For instance, lactic acid bacteria are limited in their capability to synthesize amino acids, and thus strongly dependent on an external supply to build up peptides and proteins and eventually for growth (Ummadi and Curic-Bawden 2010).

A complex source conventionally used in fermentations is yeast extract (Tejayadi and Cheryan 1995). Other complex nitrogen sources with the potential to be used in fermentations are hydrolysates from protein-rich plants, vegetables, food residues in particular meat, animal tissues and body liquids, fish waste, feathers, cheese whey permeate, and grass press juice (Beaulieu et al. 2009; Klompong et al. 2012; Lasekan et al. 2013; Lazzi et al. 2013; Lü et al. 2007; Mark Hsieh et al. 1999; Papendiek and Venus 2014; Pleissner et al. 2013; Safari et al. 2012; Serrano-Carreón et al. 1993; Tejayadi and Cheryan 1995; Vasileva-Tonkova et al. 2007; Venus 2009; Zhao et al. 2014). The hydrolysis of protein-rich materials can be carried out using proteases, acids, and bases and at high temperature and pressure (Animox 2012; Brandelli 2008; Gao et al. 2006; Solaiman et al. 2011). Furthermore, a sub-critical water hydrolysis has been applied (Esteban et al. 2010). The hydrolysate contains a mixture of peptides, peptone, and amino acids.

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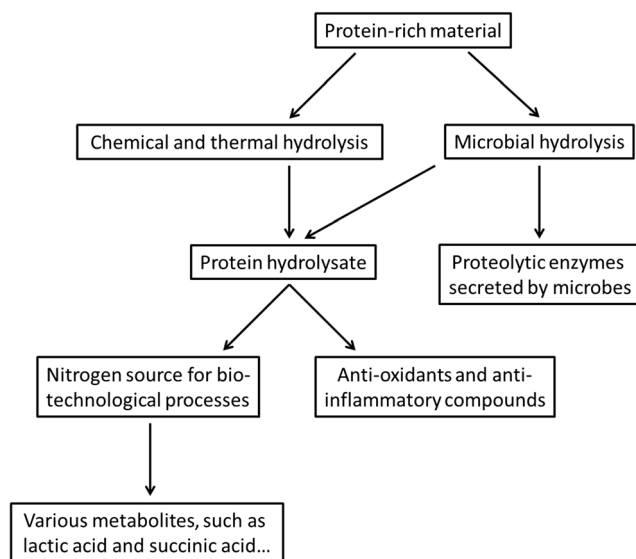
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The complexity of hydrolytic products may stimulate and improve the metabolic activity and growth of microorganisms (Pasupuleti et al. 2010). Protein-rich materials may further be used for the production of protein hydrolysates to be applied as feed, source of antibacterial and antioxidant agents, and plant growth regulator (Bataille and Bataille 1983; Benhabiles et al. 2013; Chalamaiah et al. 2012; Dalev 1994; Fakhfakh et al. 2013; Gunasekaran et al. 2014; Lasekan et al. 2013; Najafian and Babji 2012). Hence, protein-rich materials pose a great potential for the production of value-added products.

The aim of this mini-review is to provide a comprehensive insight in the utilization of protein-rich materials in biotechnological processes. The focus is particularly on residues to be considered of no or low value and appropriate to serve as alternatives to cost-intensive nitrogen sources and substrates for many microorganisms. Examples of fermentations are presented, which illustrate the potential of protein hydrolysates to be used in processes following the approach of white biotechnology for the production of enzymes, antioxidants and antibacterial agents, and value-added chemicals and materials.

### Utilization of protein-rich materials

The utilization of protein-rich materials can occur by the following approaches: (i) microbial hydrolysis of the material and recovery of enzymes secreted by microbes used, (ii) hydrolysis of the material and recovery of bioactive compounds from the hydrolysate, and/or (iii) hydrolysis of the material and use of hydrolysate as nitrogen source for microorganisms in subsequently carried out biotechnological processes (Fig. 1). The protein contents can vary between different materials derived from vegetables or animals (Table 1). Hair,



**Fig. 1** Possible utilization strategies of protein-rich materials

**Table 1** Widely used protein-rich materials

Protein-rich material	Protein content [%, w/w]	Reference
Soybean	36.5 <sup>a</sup>	(Young and Mebrahtu 1998)
<i>Lupinus albus</i>	38	(Mohamed and Rayas-Duarte 1995)
Whey	2.5–4 <sup>b</sup>	(Tejayadi and Cheryan 1995)
Stillage	57 <sup>c</sup>	(Wu et al. 1984)
Meat	≈20 <sup>a</sup>	(El 1995)
Food waste	11.4	(Pleissner et al. 2015)
Proteins from animal by-products	>90 <sup>d</sup>	(Winter et al. 2015)
Fish waste	14.2	(Solli et al. 2014)
Shrimp waste	≈40	(Benhabiles et al. 2013)
Wool	≈100 <sup>e</sup>	(Zoccola et al. 2009)
Horn	≈100 <sup>e</sup>	(Zoccola et al. 2009)
Feather	≈90 <sup>f</sup>	(Dalev 1994)
Hair	≈70	(Gehle et al. 1967)

<sup>a</sup> Protein-N multiplied by 6.25

<sup>b</sup> Protein-N multiplied by 6.38

<sup>c</sup> Protein-N multiplied by 5.7

<sup>d</sup> Related to a dry mater of ≈15 % (w/w)

<sup>e</sup> Based on amino acids

<sup>f</sup> Based on Kjeldahl-N

feather, horn, and wool contain a protein content of nearly 100 % (w/w), and thus are desirable nitrogen sources. Contrarily, food, meat and fish, wastes contain between 10 and 20 % (w/w) protein.

Some of the materials listed in Table 1 are waste streams and considered of no or low value. A utilization of these waste streams in biotechnological processes provides the opportunity to treat it in a way to exploit its full potential.

### Production of enzymes

Many microorganisms, such as fungi (*Aspergillus fumigatus* and *Aspergillus flavus*) and bacteria (*Bacillus* sp. and *Streptomyces* sp.), are able to degrade keratinous substrates (e.g., hairs and wool) by the secretion of extracellular keratinases (Brandelli 2008). The secretion of extracellular keratinases seems to be triggered by either the presence of keratinous substrates or by a limitation in nitrogen and consequently the pressure for microorganisms to make non-conventional substrates available as nitrogen sources (Adigüzel et al. 2009; Wang and Shih 1999). *Streptomyces thermoviolaceus* has been found to secrete a thermostable and alkaline keratinase when cultured in the presence of feather, hair, nail, or collagen (Chitte et al. 1999). *Bacillus cereus* strains IZ-06b and IZ-06r secrete sequentially collagenolytic,

elastolytic, and keratinolytic proteases in peptide-limited cultures (Adıgüzel et al. 2009). First collagenolytic and elastolytic proteases are secreted in order to make organic nitrogen sources available. If this does not result in the release of utilizable nitrogen sources, keratinases to degrade keratin are secreted (Adıgüzel et al. 2009). *Chrysobacterium* sp. kr6 was found to produce keratinases when cultured in the presence of chicken feather (Fontoura et al. 2014). The use of keratinous substrates in biotechnological processes does not only result in the production of collagenolytic, elastolytic, and keratinolytic proteases with various industrially applications, such as in dehairing and deskinning processes in the leather industry, but also in the production of a hydrolysate to be used as source of bioactive compounds and/or nitrogen sources in biotechnological processes (Fontoura et al. 2014; Gupta and Rammani 2006).

### Production of bioactive compounds

Protein hydrolysates can lead to the production of antioxidative and antibacterial peptides. Antioxidative peptides are inactive within the sequence of the precursor protein and released and activated by hydrolysis (Chalamaiah et al. 2012). During the process of hydrolysis, peptide bonds are cleaved and active peptides are released, which are capable of sequestering oxygen radicals, chelating prooxidant metal ions and inhibiting lipid peroxidation in food systems (You et al. 2010). Antibacterial peptides usually have less than 50 amino acids and are also obtained by hydrolysis of proteinous material. Antibacterial peptides are involved in host defense mechanisms by interacting with bacteria (Najafian and Babji 2012).

When shrimp shell waste was demineralized and deproteinized in *Pseudomonas aeruginosa* A2 fermentation, 90 % of protein was hydrolyzed. The hydrolysate exhibited a 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity (Ghorbel-Bellaaj et al. 2012a). Similar results of demineralization and deproteinization were obtained when shrimp shell waste was treated in *Bacillus pumilus* A1, *Bacillus mojavencis* A21, *Bacillus licheniformis* RP1, *B. cereus* SV1, *Bacillus amyloliquefaciens* An6 or *Bacillus subtilis* A26 fermentation (Ghorbel-Bellaaj et al. 2012b). When fish meat, obtained from sardinelle, zebra blenny, goby, or ray, was hydrolyzed using *B. subtilis* A26, hydrolysates with antioxidant activities were produced. Furthermore, these hydrolysates showed antibacterial activities against Gram-positive bacteria (Jemil et al. 2014). Ruthu et al. (2014) used carb fish head and 10 % (w/w) dextrose for the fermentative production of a protein hydrolysate with antibacterial effects. The strains used in fermentations were *Enterococcus faecium* NCIM5335, *Pediococcus acidilactici* FD3, and *P. acidilactici* NCIM5368. Cai et al. (2015) hydrolyzed grass carb skin using Alcalase for 115 min at 52 °C and pH 8.5 and isolated antioxidant peptides. Furthermore, the production of angiotensin-

I converting enzyme inhibitory peptide by *Lactobacillus helveticus* LB10 was shown (Pan and Guo 2010). This enzyme inhibitor is used in medical treatments of hypertension. At an initial pH of 7.5, 39 °C, and 4 % (v/w) of skin milk inoculum, the predicted angiotensin-I converting enzyme inhibitory activity was 72.3 %.

### Use of protein hydrolysate for the production of biomolecules, chemicals and materials

Protein hydrolysates obtained using various hydrolytic methods from various protein-rich materials have been used in biotechnological processes as alternatives to cost-intensive nitrogen sources (Table 2). A large number of the processes described in literature deal with the production of lactic acid. This is probably due to the capability of the lactic acid bacteria to utilize various complex nitrogen sources. Fitzpatrick and O’Keeffe (2001) used whey protein hydrolysate, obtained by hydrolyzing the retentate from ultrafiltration of whey using a serine protease from *B. licheniformis*, together with permeate in *L. helveticus* lactic acid fermentation. They emphasized on evaluating the minimum amount of whey protein hydrolysate required to obtain high lactose conversion and lactic acid yields. The goal was to keep raw material cost low and to avoid costly purification steps of lactic acid in order to remove impurities introduced by the hydrolysate. It was found that a 3–4 % (w/w) whey protein hydrolysate, accounting for 0.06–0.09 % (w/w) nitrogen, is appropriate to convert 50 g L<sup>-1</sup> lactose into lactic acid within 30–40 h. It was, however, admitted that the amount of whey protein hydrolysate required depends on the protein concentration of the retentate. The retentate used in their study had a protein content of 10 % (w/w). Gao et al. (2006) utilized fish wastes in lactic acid fermentations. They first grinded the waste material and used autoclavation at 121 °C for 20 min as pretreatment. Afterwards, supernatant and residue were separated. The pH of residues was then adjusted to 1 by adding 6 M HCl and the suspension autoclaved again at 121 °C for 20 min (Gao et al. 2006). The supernatants of pretreatment and acid hydrolysis were mixed and used as low-cost nutrient source in *Lactobacillus rhamnosus* fermentation. The authors reached a lactic acid concentration of 79 g L<sup>-1</sup> within 29 h when they added 5.1 g L<sup>-1</sup> nitrogen to 100 g L<sup>-1</sup> glucose. This fermentation was remarkably faster than control fermentations carried out in the presence of 20 g L<sup>-1</sup> yeast extract; however, it was also indicated that the addition of fish waste hydrolysate results in higher separation costs due to impurities introduced (Gao et al. 2006). An interesting study on reducing nutrient cost in lactic acid fermentations was published by Ma et al. (2014). They used hydrolyzed excess sludge from a sewage disposal plant, which was rich in protein, lipid, and carbohydrate, and carried out non-sterile *Bacillus coagulans* lactic acid fermentations. This innovative approach links

**Table 2** Biotechnological production processes based on hydrolysates from various protein-rich materials

Protein source	Carbon source	Strain	Product	Reference
0.088 % (w/w) nitrogen supplied by whey protein hydrolysate <sup>a</sup>	50 g L <sup>-1</sup> lactose supplied by whey protein permeate and hydrolysate	<i>L. helveticus</i>	99.5 % lactose converted into lactic acid, lactic acid yield 96 %	(Fitzpatrick and O'Keefe 2001)
5.1 g L <sup>-1</sup> nitrogen supplied by fish waste <sup>b</sup>	100 g L <sup>-1</sup> glucose	<i>L. rhamnosus</i>	79 g L <sup>-1</sup> lactic acid	(Gao et al. 2006)
1 % (w/w) excess sludge hydrolysate <sup>c</sup>	100 g L <sup>-1</sup> glucose from tapioca starch	<i>B. coagulans</i>	79 g L <sup>-1</sup> lactic acid, productivity 2.8 g L <sup>-1</sup> h <sup>-1</sup>	(Ma et al. 2014)
Animal byproducts <sup>d</sup>	120 g L <sup>-1</sup>	<i>B. coagulans</i>	93.2 g L <sup>-1</sup> lactic acid	(Winter et al. 2015)
≈2 g L <sup>-1</sup> nitrogen from Lucerne press juice (untreated)	55 g L <sup>-1</sup> glucose	<i>L. paracasei</i>	47.2 g L <sup>-1</sup> lactic acid, yield 91 %	(Vodnar et al. 2010)
1.3 g L <sup>-1</sup> of crude protein from dairy manure <sup>e</sup>	240 g L <sup>-1</sup> glucose	<i>R. oryzae</i>	Lactic acid yield 57.2 %	(Yao et al. 2009)
Food waste hydrolysate <sup>f</sup> containing 0.28 g L <sup>-1</sup> free amino nitrogen	Food waste hydrolysate <sup>f</sup> containing 31.9 g L <sup>-1</sup> glucose	Recombinant <i>E. coli</i>	29.9 g L <sup>-1</sup> succinic acid	(Sun et al. 2014)
Food waste hydrolysate <sup>f</sup> containing 0.28 g L <sup>-1</sup> free amino nitrogen	Food waste hydrolysate <sup>f</sup> containing 31.9 g L <sup>-1</sup> glucose	<i>A. succinogenes</i>	24.1 g L <sup>-1</sup> succinic acid, 13.7 g L <sup>-1</sup> byproducts	(Sun et al. 2014)
Food waste hydrolysate <sup>f</sup> containing 0.3 g L <sup>-1</sup> free amino nitrogen	Food waste hydrolysate <sup>f</sup> containing 17.9 g L <sup>-1</sup> glucose	<i>C. vulgaris</i>	1.1 g L <sup>-1</sup> lipids containing among other 0.2 g L <sup>-1</sup> alpha-linolenic acid	(Lau et al. 2014)
Food waste hydrolysate <sup>f</sup> containing 1.9 g L <sup>-1</sup> free amino nitrogen	Food waste hydrolysate <sup>f</sup> containing 106.9 g L <sup>-1</sup> glucose	<i>C. pyrenoidosa</i> <sup>j</sup>	9 g L <sup>-1</sup> lipids containing among other 2.7 g L <sup>-1</sup> alpha-linolenic acid	(Pleissner et al. 2015)
30 g L <sup>-1</sup> spent yeast (autolyzed)	30 g L <sup>-1</sup> spent yeast untreated and glycerol	<i>C. curvatus</i>	19 g L <sup>-1</sup> lipids for biodiesel production	(Ryu et al. 2013)
Bakery waste hydrolysate <sup>g</sup> containing 0.2 g L <sup>-1</sup> FAN	Baker waste hydrolysate <sup>g</sup> containing 62.5 g L <sup>-1</sup> glucose and 20 g L <sup>-1</sup> fructose	<i>H. boliviensis</i>	2.6 g L <sup>-1</sup> polyhydroxybutyrate	(Pleissner et al. 2014b)
5 g L <sup>-1</sup> meat and bone meal hydrolysate <sup>h</sup>	≈13 g L <sup>-1</sup> glycerol	Recombinant <i>E. coli</i>	0.04 g L <sup>-1</sup> cyanophycin	(Solaiman et al. 2011)
5 g L <sup>-1</sup> meat and bone meal hydrolysate <sup>i</sup>	≈13 g L <sup>-1</sup> glycerol	Recombinant <i>E. coli</i>	0.11 g L <sup>-1</sup> cyanophycin	(Solaiman et al. 2011)

<sup>a</sup> Obtained by enzymatic hydrolysis

<sup>b</sup> Pretreatment of fish waste at 121 °C for 20 min, afterwards acid hydrolysis of residues at 121 °C for 20 min

<sup>c</sup> Excess sludge formed during the aerobic biological treatment process of municipal sewage was hydrolyzed at 121 °C for 20 min

<sup>d</sup> Hydrolysis carried out at 180–220 °C for 25–40 min and 50 and 75 bar (Animox 2012)

<sup>e</sup> Untreated dairy manure used

<sup>f</sup> Fungal hydrolysis was carried out in submerged fermentation using extracellular enzymes secreted by *Aspergillus awamori* and *Aspergillus oryzae*

<sup>g</sup> Hydrolysis was carried out using a multienzym solution obtained from *A. awamori* and *A. oryzae*

<sup>h</sup> Enzymatic hydrolysis was carried out using Alcalase 2.4 L for 4 h at pH 8.5 and 50 °C, followed by the addition of Flavourzyme and incubation for 4 h at pH 7 and 50 °C

<sup>i</sup> Alkaline hydrolysis carried out using 0.1 g Ca(OH)<sub>2</sub> at 85 °C for 16 h

<sup>j</sup> Fed-batch process

wastewater treatment to white biotechnology. In their study, a lactic acid concentration of 79 g L<sup>-1</sup> and a productivity of 2.8 g L<sup>-1</sup> h<sup>-1</sup> were obtained in the presence of 1 % (w/w) excess sludge hydrolysate and 100 g L<sup>-1</sup> glucose (Ma et al. 2014). Yao et al. used untreated crude protein from dairy manure in order to supply an inexpensive nitrogen source in *Rhizopus oryzae* lactic fermentations (Yao et al. 2009). They

used 1.3 g L<sup>-1</sup> crude protein and 240 g L<sup>-1</sup> glucose and achieved a lactic acid yield of 57.2 %. Another example dealing with the use of non-conventional nitrogen sources in lactic acid fermentations was provided by Vodnar et al. (2010) who applied lucerne press juice. Using lucerne press juice, no hydrolysis is needed in order to make nitrogen compounds available for microorganisms. In the study by Vodnar et al. (2010),



*Lactobacillus paracasei* efficiently utilized  $2 \text{ g L}^{-1}$  nitrogen from lucerne press juice and produced around  $50 \text{ g L}^{-1}$  L-lactic acid from  $55 \text{ g L}^{-1}$  glucose within 20 h. The optical purity of L-lactic acid was 97 %. Winter et al. (2015) used animal by-products as cheap source of peptones to be used as nitrogen source in *B. coagulans* lactic acid fermentation. Hydrolysis was carried out at 180–220 °C for 25–40 min and 50–75 bar (Animox 2012). The application of the obtained hydrolysate did not negatively affect lactic acid fermentation, and after around 30 h, more than  $90 \text{ g L}^{-1}$  was obtained from  $120 \text{ g L}^{-1}$  glucose (Table 2).

The organic fraction of municipal solid waste can be rich in proteins due to wasted food. Food waste usually consists of meat, noodles, vegetables, and fruits, which can be hydrolyzed to create carbon and nitrogen-rich fermentation feedstocks (Pleissner and Lin 2013) (Table 2). An economic way to hydrolyze starch and protein in food waste, in order to treat and utilize food waste, and for providing an inexpensive fermentation medium, is fungal hydrolysis (Kwan et al. 2015). Fungal hydrolysis refers to a process where fungal biomass (*Aspergillus awamori* and *Aspergillus oryzae*) added to a blend of food waste secretes extracellular glycolytic and proteolytic enzymes. Up to 90 % of initial food waste can be hydrolyzed using this approach (Pleissner et al. 2014a). The advantage is that no cost-intensive enzymes are required. About 10 % (w/w) of food waste can be proteins and under consideration that 1.3 billion tons appear globally per year, food waste is a remarkable protein source to be used in fermentations (Pleissner et al. 2014a). Sun et al. (2014) used food waste hydrolysate, consisting of  $0.28 \text{ g L}^{-1}$  free amino nitrogen, as feedstock for the fermentative production of succinic acid and obtained promising results. When *Actinobacillus succinogenes* was used in fermentations,  $24.1 \text{ g L}^{-1}$  succinic acid and  $13.7 \text{ g L}^{-1}$  of by-products were produced from  $31.9 \text{ g L}^{-1}$  glucose. However, when a recombinant strain of *Escherichia coli* was used,  $29.9 \text{ g L}^{-1}$  of succinic acid was obtained from  $31.9 \text{ g L}^{-1}$  glucose (Table 2).

Food waste hydrolysate is not only an appropriate nutrient source for bacteria but also for microalgae and yeasts (Table 2) (Lau et al. 2014; Pleissner et al. 2015; Ryu et al. 2013). For instance, Pleissner et al. (2015) presented a process for fatty acid feedstock preparation as an integrated process in mixed restaurant food and bakery wastes treatment. In their study, fed-batch cultivation of the heterotrophic microalgal strain *Chlorella pyrenoidosa* was carried out using a food waste hydrolysate containing  $106.9 \text{ g L}^{-1}$  glucose and  $1.9 \text{ g L}^{-1}$  free amino nitrogen as feed. More than  $50 \text{ g L}^{-1}$  biomass was produced within 5 days containing  $9 \text{ g L}^{-1}$  lipids and  $2.7 \text{ g L}^{-1}$  alpha-linolenic acid. Lau et al. (2014) applied food waste hydrolysate as nutrient medium in *Chlorella vulgaris* batch fermentation. The hydrolysate contained around  $18 \text{ g L}^{-1}$  glucose,  $0.5 \text{ g L}^{-1}$  fructose,  $0.3 \text{ g L}^{-1}$  free amino nitrogen, and  $0.2 \text{ g L}^{-1}$  phosphate. From the biomass produced, they obtained  $1.1 \text{ g L}^{-1}$  lipids and  $0.2 \text{ g L}^{-1}$  alpha-linolenic

acid. It should, however, be admitted here that the growth rate of *C. pyrenoidosa* and *C. vulgaris* was strongly dependent on the food waste hydrolysate concentration applied. *C. pyrenoidosa* did grow much slower at glucose concentration above  $30 \text{ g L}^{-1}$  (Pleissner et al. 2015). *C. vulgaris* exhibited a reduced growth rate when the cultivation was carried in food waste hydrolysate exceeding the abovementioned concentrations (Lau et al. 2014). Hence, preliminary experiments are required in order to identify the appropriate food waste hydrolysate concentration to be used in *Chlorella* sp. fermentations. As indicated, the biomasses of both algal strains are sources of the polyunsaturated fatty acid alpha-linolenic acid, which is an essential fatty acid for mammals, and thus a desired food and feed supplement. However, it should be admitted here that food and feed supplements produced from waste streams may pose risks for animal and human health. For instance, the EU prohibited the use of animal protein materials as farmed animal feed due to the bovine spongiform encephalopathy problem (García et al. 2005). This restriction may also apply for the use of food and feed supplements produced from food waste via biotechnological processes. Furthermore, the presence of heavy metals and dioxins in organic waste makes an analysis of fermentation products necessary (García et al. 2005).

Ryu et al. (2013) presented another approach to produce fatty acids from inexpensive feedstocks. They used untreated spent yeast as nitrogen source in fermentations of the oleaginous yeast *Cryptococcus curvatus*. The hydrolysis of spent yeast was carried out by the process of autolysis. In the study of Ryu et al. (2013), a biomass concentration of  $50.4 \text{ g L}^{-1}$  and a total fatty acid concentration of  $19 \text{ g L}^{-1}$  were obtained when  $30 \text{ g L}^{-1}$  spent yeast was supplied with glycerol as carbon source. Another product obtainable from the fermentative treatment of food waste hydrolysate, and in particular bakery waste hydrolysate, is polyhydroxybutyrate, a highly wanted bio-plastic (Table 2). Pleissner et al. (2014b) reported the fermentative production of  $2.6 \text{ g L}^{-1}$  polyhydroxybutyrate using *Halomonas boliviensis* cultured in bakery waste hydrolysate containing  $62.5 \text{ g L}^{-1}$  glucose,  $20 \text{ g L}^{-1}$  fructose, and  $0.2 \text{ g L}^{-1}$  free amino nitrogen.

The quality of nitrogen source is particularly important when the production of a desired product requires special amino acids. Solaiman et al. (2011) used hydrolysates of meat and bone meal for the fermentative production of cyanophycin, a poly(arginyl-aspartate) biopolymer. A recombinant *E. coli* strain with the ability to intracellularly store cyanophycin was employed. Interesting enough, using meat and bone meal hydrolysates, Solaiman et al. (2011) obtained 33–35 % of a crude cyanophycin product compared to the product when reference casamino acids were used. The result is particularly of interest as it shows that waste streams can be utilized for the supply of special amino acids (Table 2).

## Conclusions and future perspectives

Protein-rich materials are appropriate substrates for the production of enzymes, hydrolysates with antioxidant and antimicrobial activities, and hydrolysates rich in organic nitrogen to be used as nitrogen sources in biotechnological processes. The presented examples of biotechnological processes for the production of various value-added products illustrate the potential of protein-rich materials to serve as complex nitrogen sources for many microorganisms. In particular, the use of keratinous materials, such as hair, wool, and horn, is not only an effective utilization approach but also a treatment to minimize the amount of those recalcitrant materials, which are conventionally considered as waste. It should, however, be noted that the application of complex nitrogen sources in biotechnological processes may complicate downstream processing. Complex nitrogen sources may contain impurities, which need to be removed in order to obtain pure products. Hence, additional purification steps may be necessary. Nevertheless, the low cost of residues may counterbalance the increased process costs. It can be concluded that the number of industrial processes based on protein-rich materials will increase in the future due to economic benefits.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

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