1 Enzyme production from food wastes using a biorefinery concept:

2 a review

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15 ABSTRACT

16	According to FAO, one third of food produced globally for human consumption (nearly 1.3
17	billion tonnes) is lost along the food supply chain. In many countries food waste are currently
18	landfilled or incinerated together with other combustible municipal wastes for possible
19	recovery of energy. However, these two approaches are facing more and more economic and
20	environmental stresses. Due to its organic- and nutrient-rich composition, theoretically food
21	waste can be utilized as a useful resource for the production of enzymes through various
22	fermentation processes. Such conversion of food waste is potentially more profitable than its
23	conversion to animal feed or transportation fuel. Food waste valorisation has therefore
24	gained interest, with value added bio-products such as methane, hydrogen, ethanol, enzymes,
25	organic acids, chemicals, and fuels. The aim of this review is to provide information on the
26	food waste situation with emphasis on Asia-Pacific countries and the state-of-the-art food
27	waste processing technologies to produce enzymes.
28	

32 1. Introduction

33 Food waste (FW) is organic waste produced in food processing plants, domestic and

commercial kitchens, cafeterias, and restaurants. It accounts for a considerable proportion of
municipal solid waste all over the world [1]. According to FAO [2], nearly 1.3 billion tonnes

36 of foods including fresh vegetables, fruits, meat, bakery and dairy products are lost along the

- 37 food supply chain.
- 38

The amount of FW is continuing to increase due to the increase in population and economical 39 growth, particularly in Asian countries. The annual amount of urban FW in Asian countries 40 could rise from 278 to 416 million tonnes from 2005 to 2025 [3]. The highest absolute 41 amount per year was in China (82.8 Million tonnes (MT) followed by Indonesia (30.9 MT), 42 Japan (16.4 MT), Philippines (12 MT) and Vietnam (11.5 MT). However, the highest amount 43 of FW produced per capita was in New Zealand and Australia with 280 kg/year, while it was 44 45 around 120-130 kg in Southeast Asia other than Cambodia (173 kg/year). Although the absolute amount of food waste in China is the highest, the waste production per capita is the 46 lowest (61 kg/year), while the waste production per capita is 120 and 168 kg/year in 47 Singapore and Hong Kong, respectively [4, 5], showing that food wastage seems more 48 prevalent in high-income states. 49

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Food wastes can be practically dumped, landfilled, incinerated, composted, digested anaerobically and/or used as animal feed. In many Asian countries FW is still dumped with other household waste in landfills or dumpsites (Figure 1). Unfortunately, the capacity of the landfills is mostly surpassed due to a lack of waste management planning, so the environmental pollution (leachate, gas, odors, flies, vermin, and pathogens) poses serious problems [6]. Hence, there is a need for an appropriate management of FWs [7].

In order to reduce its volume, FW is traditionally incinerated with other combustible municipal wastes for generation of heat or energy, particularly in Japan and Singapore. It is generally favoured over landfilling with regard to overall energy use and emissions of gases contributing to global warming[8].However, it is an inappropriate approach for most lowincome countries due to the high capital and operating costs [6]. Moreover, incineration of FW can potentially cause air pollution [9].



65 Figure 1. Waste treatment methods in some Asia-Pacific countries.

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Another approach to handle biodegradable FW is composting which results in a valuable soil conditioner and fertilizer [10]. Composting facilities showed a relatively low environmental impact and a high economic efficiency compared to other treatment methods. The primary recycling method in Korea is composting (Figure 1). However, the high moisture content of FW causes remarkable levels of leachate which affects process performance by reducing 72 oxygen availability and weakening the pile strength [11]. In this case, high airflows for
73 aeration or excessive carbon ingredients are necessary for process control, which increase the
74 operational costs. Indeed, compost is more expensive than commercial fertilizers and the
75 available market for compost is not big [12].

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77 Anaerobic digestion is another alternative which yields methane and carbon dioxide as metabolic end products and therefore could be feasible from an economic and environmental 78 point of view because methane is used as an energy source [8]. Hirai, Murata [13] evaluated 79 80 the environmental impacts of FW treatment and found that utilising a methane fermentation process prior to incineration reduces approximately 70 kg CO₂eq/tonne waste of the global 81 warming potential, due to the substitution effect. The disadvantages of using FW as animal 82 feed are the variable composition and the high moisture content, which favors microbial 83 contamination [14]. To prevent this, animal feed is generally dried but greenhouse gas 84 emission increases depending on the energy usage during the drying process, which is related 85 to the water content of FW [9]. 86

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FW is mainly composed of carbohydrate polymers (starch, cellulose and hemicelluloses), 88 lignin, proteins, lipids, organic acids (Table 1). Total sugar and protein contents in FW are in 89 the range of 35.5-69% and 3.9-21.9%, respectively. Due to its inherent chemical complexity, 90 alternative treatment methods are currently studied and attention is being directed to 91 production of high value-added products such as biofuels, biodiesel, platform chemicals and 92 enzymes [15-23]. As a comparison, fuel applications (\$200-400/ ton biomass) and organic 93 acids, biodegradable plastics & enzymes applications (\$1000/ton biomass) are usually 94 creating more value compared to generating electricity (\$60-150/ton biomass) and animal 95 feed (\$70-200/ton biomass) [24]. 96

Origin	рН	Moisture	Total solid	VS/TS	Total sugar	Starch	Cellulose	Lipid	Protein	Ash	References
Dining hall	NR	79.5	20.5	95.0	NR	NR	NR	NR	21.9	NR	Han and Shin [15]
Cafeteria	5.1	84.1	15.9	15.2	NR	NR	NR	NR	NR	NR	Kim, Oh [25]
Cafeteria	5.1	80.0	20.0	93.6	NR	NR	NR	NR	NR	1.3	Kwon and Lee [26]
MSW	NR	85.0	15.0	88.5	NR	NR	15.5	8.5	6.9	11.5	Rao and Singh [27]
Cafeteria	4.6-5	79.1	20.9	93.2	NR	NR	NR	NR	NR	NR	Ramos, Buitron [28]
Cafeteria	NR	75.9	24.1	NR	42.3	29.3	NR	NR	3.9	1.3	Ohkouchi and Inoue [29]
NR	NR	87.6	12.4	89.3	NR	NR	NR	NR	NR	NR	Kim, Oh [30]
Residents	4.9	80.8	19.2	92.7	NR	15.6	NR	NR	NR	NR	Pan, Zhang [21]
Dining hall	NR	80.3	19.7	95.4	59.8	NR	1.6	15.7	21.8	1.9	Tang, Koike [31]
Dining hall	NR	82.8	17.2	89.1	62.7	46.1	2.3	18.1	15.6	NR	Wang, Ma [32]
Restaurant	3.9	80.0	20.0	95.0	70.0	NR	NR	10.0	13.0	NR	Zhang, He [33]
Dining hall	5.6	82.8	17.2	85.0	62.7	46.1	2.3	18.1	15.6	NR	Ma, Wang [34]
Cafeteria	NR	61.3	38.7	NR	69.0	NR	NR	6.4	4.4	1.2	Uncu and Cekmecelioglu [35]
Food court	NR	64.4	35.6	NR	NR	NR	NR	8.8	4.5	1.8	Cekmecelioglu and Uncu [36]
Canteen	NR	81.7	18.3	87.5	35.5	NR	NR	24.1	14.4	NR	He, Sun [23]
Restaurant	NR	81.5	18.5	94.1	55.0	24.0	16.9	14.0	16.9	5.9	Vavouraki, Angelis [22]
Restaurant	NR	81.9	14.3	98.2	48.3	42.3	NR	NR	17.8	NR	Zhang and Jahng [37]

TabTable 1. Characteristics of mixed food waste.

99tal Solid, Total sugar, Starch, Cellulose, Lipid, Protein and Ash Contents were given in wt% on the basis of dry weight. Volatile solid contents were given as the **100**/VS ratio on total solid basis. NR: not reported.

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102 The critical stage of biomass bioconversion is saccharification, which hampers its

103 commercial use. For an efficient biomass conversion, carbohydrate components of FW

104 should be hydrolyzed to yield high concentrations of oligosaccharides and monosaccharides,

105 which are amenable to fermentation. Hence, there is an increasing interest on the production

106 of biomass saccharifying enzymes, mainly amylases and cellulases [38].

Commercial enzyme utilization increases the operational cost due to the purchase of these 108 enzymes on a regular basis. In addition, commercial enzymes are generally sold singly. 109 110 Therefore, mixtures of enzymes would have to be prepared from separate sources. Each commercial enzyme requires different operating conditions for the hydrolysis of their specific 111 substrates. Therefore, the process would either operate sub-optimally with a mix or take a 112 113 long time to carry out each enzyme step sequentially. However, the cost of enzyme 114 production could be reduced either by using low-cost raw materials and/or developing economical processing technologies. There are remarkable amount of publications on the lab-115 116 scale production of various industrial enzymes such as proteases, amylases, lignocellulosic enzymes and lipases using different types of FW. Therefore, this review summarizes and 117 discusses recent industrial enzyme production studies from FW. 118

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120 2. Enzyme Production

121 Enzymes are commonly used in many industrial applications due to their great selectivity for the substrates and their biodegradabilities. Besides they act under mild and environmentally 122 friendly conditions. Hence, enzyme production is one of the most important applications, 123 which serves to various industries. Research is continuing on the production of different 124 enzymes in solid-state fermentation (SSF) with the ultimate aims to obtain high activity 125 enzymes at lesser cost using low cost substrates and/or by improving economical processing 126 127 technologies. There are remarkable amount of publications on the production of various enzymes using different agro-industrial waste [39-42]. However, the main problem is the 128 recalcitrant nature, which resulted in low enzyme yields and expensive enzyme production. 129 The recalcitrant nature can be mitigated by some pre-treatment steps while the enzyme yields 130 can be enhanced by developing suitable fermentation conditions or by using genetically 131 modified microbial strains [43]. On the other hand, the enzyme production costs can be 132

reduced by developing suitable fermentation processes using FW, which has easily digestible 133 components. There are some publications reporting the production of different enzymes from 134 135 FW by using both solid and submerged fermentation systems (Tables 2 to 6). Various kinds of FWs were used to produce different enzymes such as proteases, cellulases, amylases, 136 137 lipases and pectinases particularly by using solid-state fermentation (SSF). SSF has several 138 advantages over submerged fermentation (SmF) as it requires less capital, lower energy, a 139 simple fermentation medium; it has superior productivity and produces less wastewater [44]. Moreover, an easy control of bacterial contamination and lower costs of downstream 140 141 processing make it more attractive. Dos Santos, Gomes [45] have evaluated SSFs efficiency for producing enzymes. It is appropriate for the production of enzymes, especially because of 142 the higher enzyme yields that can be obtained compared to submerged fermentation [46-48]. 143 144 SSF provides a similar environment to the microorganism's natural environment which provides better conditions for its growth and enzymes production [48]. However, there are 145 only a few reports on SSF bioreactor design in the literature. The large scale production of 146 enzymes using SSF is challenging because pH, temperature, aeration, oxygen transfer and 147 moisture content is difficult to control [44, 49]. 148

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150 2.1. Amylases

The amylase family has two major classes, namely α -amylase (EC 3.2.1.1) and glucoamylase (GA) (EC 3.2.1.3). α -amylase hydrolyses starch into maltose, glucose and maltotriose by cleaving the 1,4- α -D-glucosidic linkages between adjacent glucose units in the linear amylose chain [51] while glucoamylase hydrolyses the non-reducing ends of amylose and amylopectin to glucose [52]. Amylases have been widely used in the food, fermentation, textiles and paper industries [51]. They are also used for the pre-treatment of the agroindustrial and organic byproducts to improve the bioproduct yield in subsequent processes. Thereby, there is an

- 158 increasing interest on the production of amylases using cheap feedstocks [49]. High activity
- 159 amylases can be produced from various kinds of FWs such as kitchen refuse [49], potato peel
- 160 [47, 53], coffee waste [54] and tomato pomace [55] via the optimization of fermentation
- 161 using different microbial strains. However, it is not easy to compare the efficiency of the
- 162 processes as the produced enzymes' activities are defined differently (Table 2). The main
- 163 advantages of FW utilization for enzyme production are that fermentations do not require
- 164 harsh pre-treatments and extra nutrient supplements.
- 165
- 166 **Table 2.** Amylase production from food wastes.

Residual materials	Microorganism	Pretreatment method	Fermentation mode & vessel type	Fermentation conditions	Duration (day)	Achievements
Potato peel	Bacillus subtilis	Dried, ground, sieved	SSF-250 mL flasks	40°C, pH 7, 65% MC, 10% (v/w) inoculum	2	α-amylase (600 U
Potato peel	Bacillus licheniformis	Dried, ground, sieved	SSF-250 mL flasks	40°C, pH 7, 70% MC, 10% (v/w) inoculum	2	α-amylase (270 U
Coffee waste	Neurospora crassa CFR 308	Ground, steamed	SSF-250 mL flasks	28°C, pH 4.6, 60% MC, 1 mm PS, 10 ⁷ spores/g ds,	5	α-amylase (6342
Potato peel	<i>Bacillus firmus</i> CAS 7	Dried, ground, sieved	SmF-250 mL flasks	35°C, pH 7.5, 1% S	2	α-amylase (676 U
Tomato pomace	Aspergillus awamori	Dried, milled, sieved	SSF-plate-type SSF bioreactor	28°C, pH 5	5	α-amylase (10.9 I
Bread waste	<mark>Bacillus</mark> caldolyticus DSM 405	NR	SmF- 1L flask with 100 ml working vol	<mark>30℃, pH 7</mark>	I	<mark>α-amylase (6.7 U</mark> /
Pea pulp	Bacillus caldolyticus DSM 405	None	<mark>SmF- flasks</mark>	70°C, 150 rpm	<mark>6</mark>	<mark>α-amylase (8.6 U/n</mark>
Food waste	Aspergillus niger UV-60	None	<mark>SmF-250 mL</mark> flasks	30°C, pH 5, 120 rpm, 5% <mark>I/S</mark>	<mark>4</mark>	GA (137 U/mL)
Bread waste	<mark>Aspergillus</mark> orvzae	None	SSF-petri plates	30°C, MC:1.8 (w/w, db), PS:20 mm, 10 ⁶ spore/gdS	<mark>6</mark>	<mark>GA (114 U/gdS)</mark>

167 S: substrate, SSF: solid state fermentation, SmF: submerged fermentation, I/S: Inoculum to substrate ratio,
 168 ds: dry substrate, MC: moisture content, PS: particle size, ds: dry solid, GA: glucoamylase

Wang, Wang [49] investigated the production of glucoamylase from FW by Aspergillus niger 169 170 UV-60 using SmF. They reported that the nutrient supplementation including yeast extract, (NH₄)₂SO₄, KH₂PO₄, MgSO₄.7H₂O, FeSO₄.7H₂O and CaCl₂ and particle size reduction had 171 no significant influence on the glucoamylase production. Maximum glucoamylase activity of 172 137 U/mL was obtained using 3.75% FW and 5% (v/w, 10⁶ spores/mL) inoculum at 30°C, 173 120 rpm for 96h. A reducing sugar concentration of 60.1 g/L could be produced from 10% 174 FW (w/v), within 125 min using the produced crude glucoamylase. Shukla and Kar [47] 175 produced high activity α -amylase from potato peels by SSF using two thermophilic isolates 176 177 of *Bacillus licheniformis* and *Bacillus subtilis*. Under optimal conditions (40°C, pH 7, using potato peels having 1000 µm particle size with 65-70% moisture content). Alpha-amylase 178 activities obtained by using B. licheniformis and B. subtilis were 270 and 600 U/mL, 179 respectively. In another study, α -amylase production from potato peels was conducted by 180 SmF using thermophilic isolate of alkaline tolerant Bacillus firmus CAS7 strain [53]. Under 181 the optimal conditions (at 35°C, pH 7.5 using 1% of substrate concentrations), 676 U/mL of 182 α -amylase which was optimally active at 50°C and pH 9 was obtained. Murthy, Madhava 183 Naidu(check the references style) [54] used coffee wastes as sole carbon source for the 184 synthesis of α-amylase in SSF using a fungal strain of *Neurospora crassa* CFR 308. α-185 amylase activity of 4324 U/g dry substrate was obtained using 1 mm particle size, 10^7 186 spores/g dry substrate, 60% moisture content at 28°C, pH 4.6. Steam pre-treatment improved 187 188 the accessibility of coffee waste and the α -amylase activity of 6342 U/g dry substrate was obtained. 189

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191 FW can be used to produce high activity amylases by using suitable microbial strains. In
192 some of the lactic acid production studies from FW, a saccharification step using commercial
193 amylases was conducted prior to the fermentation in order to improve and ease the

fermentation process [59, 60]. If the enzyme production step can be integrated to the 194 195 fermentation system, the process costs could be lowered. In a study of Leung, Cheung [61], 196 waste bread was used as sole feedstock in a biorefinery concept for the production of succinic acid (SA), one of the future platform chemicals of a sustainable chemical industry. 197 Waste bread was used in the SSF of Aspergillus awamori and Aspergillus oryzae to produce 198 199 enzyme complexes rich in amylolytic and proteolytic enzymes. The resulting fermentation 200 solids were added directly to a bread suspension to generate a hydrolysate rich in glucose and 201 free amino nitrogen. The bread hydrolyzate was used as the sole feedstock for A. 202 succinogenes fermentations, which led to the production of 47.3 g/L succinic acid with 1.16 g SA/g glucose yield, which is the highest succinic acid yield compared from other FW-derived 203 media reported to date. This consolidated process could be potentially utilised to transform 204 no-value FW into succinic acid. 205

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207 2.2. Lignocellulolytic enzymes

Lignocellulolytic enzymes are mainly produced by several fungi and are composed of 208 cellulases, xylanases and ligninases, which degrade the lignocellulosic materials. Cellulases 209 210 have many applications in various industries including food, animal feed, brewing and wine making, agriculture, biomass refining, pulp and paper, textile, and laundry [62]. The 211 bioconversion of cellulose to fermentable sugars requires the synergistic action of complete 212 cellulase system comprising of three enzyme classes: endoglucanases (EC 3.2.1.4) which act 213 randomly on soluble and insoluble cellulose chains, exoglucanases (cellobiohydrolases; EC 214 3.2.1.91) which liberate cellobiose from the reducing and non-reducing ends of cellulose 215 chains, and β -glucosidases (EC 3.2.1.21) which liberate glucose from cellobiose [63]. 216 Xylanases have many applications in food, feed, pulp and paper, brewing, wine making and 217 textile industries with or without concomitant use of cellulases [64]. The hydrolysis of xylans 218

mainly requires the action of endo- β -1,4-xylanase and β -xylosidase. However, the presence 219 of other accessory enzymes is needed to hydrolyse substituted xylans [65]. Lignin is an 220 221 undesirable polymer for biofuel production as it prevents the accessibility of plant derived polysaccharides. However, lignin derived materials can be used to develop valuable products 222 such as dispersants, detergents, drilling mud thinner, surfactants, coagulants and flocculants 223 224 (for sewage and waste water treatment), adhesives, graft polymers including polyurethanes, 225 polyesters, polyamines and epoxies and rubbers [66, 67]. In order to degrade lignin polymers ligninolytic enzyme systems composed by laccases, lignin peroxidases and Mn-peroxidase 226 227 are utilized.

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These enzymes are also used for the pre-treatment of the agroindustrial and organic by-229 products to improve the bioproduct yields in subsequent processes [68, 69]. Recent studies on 230 lignocellulosic enzyme production using different FWs and the achieved enzyme activities 231 are summarized in Table 3. Since the enzyme activity definitions are different in each study, 232 it is not an easy task to compare the achievements and detect the best method. However, 233 generally fungal SSF is the most preferred method due to its advantages over SmF [68-73]. 234 Krishna [71] reported that the total cellulase production from banana waste was 12 fold 235 higher in SSF than that obtained using SmF. However, Díaz, de Ory [74] reported that the 236 SmF resulted in higher xylanase production in comparison to SSF due to better aeration. 237 Umsza-Guez, Díaz [55] demonstrated a clear positive effect of aeration on xylanase and 238 carboxymethyl cellulase (CMCase) production using SSF in a plate-type bioreactor. 239

241 Table 3. Lignocellulosic enzyme production from food wastes.

Residual	Microorganism	Pretreatment	Fermentation	Fermentation	Duration	Achievements
materials		method	mode & vessel type	conditions	(day)	
Banana wastes	Bacillus subtilis	Dried, ground, acid	SSF-250 mL flasks	35°C, pH 7, 400 μm PS,	3	FPAse (2.8 IU/ds
	(CBTK106)	and alkali		70% MC,		(9.6 IU/g ds), Ce
		pretreatment		15% (v/w) I/S ratio		IU/g ds)

Grape pomace	Aspergillus awamori	Dried, milled, sieved	SSF- petri dishes	30°C, 10 g S, 5×10 ⁵ I/S, 60% MC	7	Xylanase (40.4 IU Cellulase (9.6IU/g
Apple pomace	Trichoderma sp.	Dried, crushed, sieved	SSF-250 mL flasks	32°C, 70% MC, 10 ⁸ spores/flask	6	Cellulase (5.8 U/g
Banana peel	Trichoderma viride GIM 3.0010	Dried, crushed, sieved	SSF-250 mL flasks	30°C, 65% MC, 10 ⁹ spores/flask	6	FPA(5.6U/g ds), (U/g ds), β-glucosi ds)
Tomato pomace	Aspergillus awamori	Dried, milled, sieved	SSF-plate-type SSF bioreactor	28°C, pH 5	5	Xylanase (195.9 I CMCase (19.7 IU
Carrot, orange, pineapple, potato peels, wheat bran	Aspergillus niger NS-2	Acid/base pretreatment	SSF-250 mL flasks	30°C, pH 7, 1:1.5 to 1:1.75 S/M ratio	4	CMCase (310 U/g U/gds), β-glucosid U/gds) using alka wheat bran
Apple pomace	Aspergillus niger NRRL-567	Drying, crushing, sieving	SSF-500 mL flasks	30°C, 1.7-2 mm PS, 75% MC, 10 ⁷ spores/g dS	7	FPase (113.7 IU/g (172.31 IU/gds), f (60.1IU/gds), Xyl IU/gds)
Grape pomace and orange peel	Aspergillus awamori	Dried, milled and sieved	SSF-petri dishes	30°C, pH 5, 70% MC, 4.5×10 ⁸ spores/g S.	15	Exo-PG (3.8 IU/g (32.7 IU/gds), Ce IU/gds)
Potato peel	Aspergillus niger	Dried, ground	SSF	30°C, 10 ⁷ spores/ g dS, 50% MC	3	FPase (0.015 U/m (0.023 U/mL), Xy U/mL)
Mango Peel	Trichoderma reesei	Alkaline pretreatment	SmF-250mL flasks	30°C, pH 7, 200 rpm	6	Cellulase (7.8 IU/
Passion fruit waste	Pleurotus pulmonarius	Dried, milled.	SSF-250 mL flasks	28°C in complete darkness	14	MnP (0.22 U/mL) (4.76 U/mL), β-G (2.96 U/mL), β-ga (6.21 U/mL)
Passion fruit waste	Macrocybe titans	Dried, milled.	SSF-250 mL flasks	28°C in complete darkness	14	Laccase (10.2 U/r (1.72 U/mL), End (0.27 U/mL)

242 S: substrate, SSF: solid state fermentation, SmF: submerged fermentation, I/S: Inoculum to substrate ratio, DS: dry substrate,

243 S/M: substrate to moisture ratio, MC: moisture content, PS: particle size, ds: dry solid, PG: polygalacturonase, CMCase:

244 carboxymethylcellulase, MnP: Manganese peroxidise, NR: Not reported.

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246 The effects of process parameters such as incubation temperature, pH, moisture content,

247 particle size of the substrates, nutrient supplementation, inoculum size and different substrate

248 pre-treatment methods on enzyme production have been investigated. In general, the

249 optimum conditions in SSF depend not only on the microorganism employed, but also greatly

250 on the type of substrate. The incubation time, pH, temperature, particle sizes and water

251 content of the medium should be optimized when the substrate and microorganisms are

252 chosen. Some FWs require extra nutrients [55, 70, 72], while some others can be used as sole

253 nutrient to produce high titers of cellulases [68, 73, 75]. Dhillon, Kaura [70] analysed the effects of different inducers on cellulase and hemicellulase production by Aspergillus niger 254 NRRL-567 using apple pomace as a substrate. The higher filter paper cellulase (FPA) and β -255 glucosidase activities of 133.68 \pm 5.44 IU/gram dry substrate (gds) and 60.09 \pm 3.43 IU/gds, 256 respectively were observed while using CuSO₄ and veratryl alcohol. Similarly, higher 257 xylanase activity of 1412.58 ± 27.9 IU/gds was observed with veratryl alcohol after 72 h of 258 259 fermentation time while the higher CMCase activity of 172.31 ± 14.21 IU/g ds was obtained with lactose after 48 h of incubation period. Sun, Ge [72] have also reported that the cellulase 260 261 production using SSF was markedly improved by supplementing lactose and corn-steep solid to the apple pomace. 262

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The effects of nutrients and other process parameters on cellulase production from banana 264 waste by Bacillus subtilis (CBTK 106) was also evaluated by Krishna [71]. The optimal 265 FPAse of 2.8 IU/g dry substrate, CMCase activity of 9.6 IU/g dry substrate and cellobiase 266 activity of 4.5 IU/g dry substrate were obtained at 72 h incubation with media containing heat 267 pretreated banana fruit stalk, (NH₄)₂SO₄, NaNO₃ and glucose. Saravanan, Muthuvelayudham 268 [69] investigated the cellulase production from mango peel using Trichoderma reesei and 269 reported that avicel, soybean cake flour, KH2PO4, and CoCl2·6H2O have positive influences 270 on cellulase production. Cellulase activity was to 7.8 IU/mL using the optimum nutrient 271 272 concentrations of 25.3 g/L avicel, 23.53 g/L soybean cake flour, 4.9 g/L KH₂PO₄ and 0.95 g/L CoCl₂ 6H₂O which was determined by response surface methodology. 273

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Díaz, de Ory [74] reported that the cellulase production was inhibited at high concentration of
reducing sugars when grape pomace was used as substrate. They avoided this problem by
adjusting the nutrients composition of grape pomace by supplementing orange peel, which is

a pectin, cellulose and hemicellulose rich substrate inducing cellulase production. The synthesis of xylanase and cellullase increased using the mixed type substrate compared to whole grape pomace. Umsza-Guez, Díaz [55] have reported that the xylanase production from tomato wastes using SSF system is activated by Mg^{2+} , but strongly inhibited by Hg^{2+} and Cu^{2+} .

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284 The effects of substrate pre-treatments on cellulase and xylanase production have been studied [69, 71]. Bansal, Tewari [68] studied the effects of acid and base pre-treatment on 285 286 cellulase production from different FWs including carrot peelings, orange peelings, pineapple peelings, potato peelings and wheat bran using SSF. The pretreated substrates are well suited 287 for the organism's growth, producing high titers of cellulases after 96 h without the 288 289 supplementation of additional nutritional sources. Yields of cellulases were higher in alkali treated substrates compared to acid treated and untreated substrates except in wheat bran. Of 290 all the substrates tested, untreated wheat bran induced the maximum production of enzyme 291 components followed by alkali treated composite kitchen waste and potato peelings. Krishna 292 [71] investigated the effects of acid, alkaline and heat pre-treatment on cellulase production 293 from banana waste using Bacillus subtilis. Although cellulase production was not affected by 294 alkali or acid treatment, it increased by 6.84 fold using pressure-cooking under controlled pH. 295 Pressure cooking of plant materials at a controlled pH could result in greater substrate 296 accessibility for microbial growth. Moreover, it did not result in the formation of 297 monosaccharide degradation products, such as furfural and hydroxymethyl furfural, which 298 otherwise inhibit the cellulases [77]. 299

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Besides cellulases and xylanases, ligninases were also produced from FWs by white rotfungi. Zilly, dos Santos Bazanella [76] studied the oxidative and hydrolytic enzymes

production by SSF of yellow passion fruit waste using white-rot fungi *Pleurotus ostreatus*, *Pleurotus pulmonarius, Macrocybe titans, Ganoderma lucidum*, and *Grifola frondosa*. Under
the conditions used, the main enzymes produced by the fungi were laccases, pectinases, and
aryl-β-D-glycosidases (β-glucosidases, β-xylosidases, and β-galactosidases). The yellow
passion fruit waste was as good as wheat bran, which is the most commonly used substrate
for white-rot fungi cultivation.

309

310 Biorefineries need to develop their indigenous enzyme production processes along with their 311 existing processes as commercial enzyme production systems are still expensive to incorporate in biorefineries [78]. As can be seen from the studies above, some strains are 312 producing different lignocellulosic enzymes from food wastes simultaneously. These enzyme 313 cocktails can be used to hydrolyse biomass effectively at low cost for their conversion to 314 biofuels, platform chemicals and biodegradable films. To further improve the hydrolysis, 315 different strains can be used to produce enzyme solutions with different hydrolytic acivities. 316 Besides, some engineered strains can be used to improve the saccharification yield. 317

318 **2.3 Pectinolytic enzymes**

319 Pectinolytic enzymes, i.e. pectinases degrade pectin polymers in a sequential and synergic way, by depolymerisation and deesterification reactions. Complete degradation of pectin 320 requires endo- and exo-acting polygalacturonases and pectin- and pectate lyases as well as 321 enzymes that cleave the rhamnogalacturonan chain, the rhamnogalacturonases [79]. 322 Pectinases are widely used in food industry particularly for juice and wine production and 323 many other conventional industrial processes, such as textile, plant fiber processing, tea, 324 coffee, oil extraction, treatment of industrial wastewater [46, 80, 81]. The production of 325 pectinases is mainly conducted via fungal SSF particularly by using Aspergillus strains [79]. 326 For industrial implementation, pectinases can be produced from pectin-containing wastes, 327

such as citrus and orange wastes [82-84], apple pomace [85, 86], grape pomace [75] and
many other fruit residues [87] without any harsh pre-treatment owing to the nature of these
substrates and the low moisture content [80, 87]. Hours, Voget [86] investigated the pectinase
production from apple pomace by SSF using *Aspergillus foetidus*. The medium composition,
temperature and type of apple pomace used affected the enzyme production. After 36h
culture at 30°C with organic nitrogen supplemented apple pomace medium, an enzyme
activity of 1,300 U/g was obtained (Table 4).

335

Residual materials	Microorganism	Pretreatment method	Fermentation mode & vessel type	Fermentation conditions	Duration (day)	Achievements
Apple pomace	Aspergillus foetidus NRRL 341	None	SSF- petri dishes	30°C, pH 4, 10 ³ I/S	2	Pectinase (1300 U
Citrus waste	Aspergillus foetidus NRRL 341	None	SSF- petri dishes	30°C	2	Pectinase (1641 U
Apple pomace	Aspergillus niger	None	SSF- 15L horizontal solid state stirred tank reactor	35°C	3	900 AJDA U/mL
Grape pomace	Aspergillus awamori	Milled, sieved	SSF- petri dishes	30°C, 60% MC	1	Exo-PG(40U/g S U/g S)
Orange bagasse	Botryosphaeria rhodina MAMB- 05	Dried, ground	SSF-125 mL flask	28°C	6	Pectinase (32 U/r Laccase (46 U/m)
Orange waste	Aspergillus giganteus CCT3232	NR	SmF-Flask	30°C, pH 6, 120 rpm, 1.10 ⁷ spores/mL	3.5	Exo-PG (48.5 U/
Fruit residues (apple, lemon peel, grape skin & tamarind kernel)	Aspergillus flavipes FP-500	Dried, milled, sieved	SmF-Flask	37°C, pH 3.5-5.5, 150 rpm, 1.10 ⁶ spores/mL	3	Endopectinase (6 Pectinlyase (5 U/ Exopectinase (4.8 Rhamno-galactur U/mL)
Fruit residues (apple, lemon peel, grape skin & tamarind kernel)	A. terreus FP- 370	Dried, milled, sieved	SmF-Flask	37°C, pH 3.5-5.5, 150 rpm, 1.10 ⁶ spores/mL	3	Endopectinase (3 Pectinlyase (33 U Exopectinase (4.8 Rhamno-galactur U/mL)
Tomato pomace	Aspergillus awamori	Dried, milled, sieved	SSF-plate-type SSF bioreactor	28°C, pH 5	5	Exo-PG (36.2 IU

336 Table 4. Pectinolytic enzyme production from food wastes.

Lemon peel pomace	Aspergillus niger Aa-20	Dried, ground	SSF- column-tray bioreactor	30°C, 70% MC, 194 mL/min AFR, 2–0.7 mm PS	4	Pectinase (2.18 U
Passion fruit waste	Macrocybe titans	Dried, milled.	SSF-250 mL flasks	28°C in complete darkness	14	Pectinase (1.72 U
Orange peel	Aspergillus niger URM5162	Dried, ground	Fixed bed bioreactor	25°C, 3.105 spores/mL	7	Endo-PG (1.18 U Exo-PG (4.11 U/

337 S: substrate, SSF: solid state fermentation, SmF: submerged fermentation, I/S: Inoculum to substrate ratio, AFR: air flow

338 rate, DS: dry substrate, MC: moisture content, PS: particle size, ds: dry solid, PG: polygalacturonase, CMCase:

339 carboxymethylcellulase, NR: Not reported.

340

341 In another study, pectinolytic enzyme production from citrus waste was studied using Aspergillus foetidus for SSF [83]. Yeast extract and mineral salt addition improved the 342 activity up to 1,600-1,700 U/g after 36 h of culture. Berovic and Ostroversnik [85] reported 343 344 that the pectolytic enzyme production from apple pomace using SSF with Aspergillus niger was induced and/or improved by supplementing the media with other cheap nutrients such as 345 soya flour, wheat bran, wheat corn and whey. They also mentioned that the highest activity 346 was obtained using 38% moisture content and moisture content is very important in enzyme 347 production. Whereas, Ruiz, Rodriguez-Jasso [46] reported that the 70% moisture content 348 349 gave the highest pectinase activity using lemon peel pomace. Botella, Diaz [80] evaluated the feasibility of grape pomace for the production of exo-polygalacturonase by Aspergillus 350 *awamori* in SSF fermentation. The particle size of the substrate did not influence the enzyme 351 352 production like it was reported by Hours, Voget [86] while the addition of extra carbon 353 sources and the initial moisture content of the grape pomace were found to have a marked 354 influence on the enzymes yields. In another study, Giese, Dekker [84] carried out the production of pectinases from orange waste by Botryosphaeria rhodina MAMB-05 using 355 both SSF and SmF with and without adding nutrients. Orange bagasse with a solid 356 concentration of 16% (w/v) provided good microbial growth and the highest pectinase titre 357 (32 U/mL) was obtained using SSF without adding extra nutrients. 358

Aeration is another important parameter affecting the pectinase production. Umsza-Guez, Díaz [55] reported that the forced aeration has negative effects on exo-PG synthesis, reducing to half of its activity in multi-layer packed bead reactor. MacIel, Ottoni [89] obtained the maximum endo- and exo-PG activities of 1.18 U/mL and 4.11 U/mL, respectively, using the reactors without aeration. A system without aeration is advantageous since it is easier to implement and economical.

366

The pH value of the medium can also affect the pectinase production. Martínez Sabajanes, Yáñez [87] investigated the effect of different substrates (apple, lemon peel, grape skin & tamarind kernel) and fungi (*Aspergillus flavipes* FP-500 and *Aspergillus terreus* FP-370) on the production of pectinases. The highest activities were obtained using lemon peel. In both strains, acidic pH values and high carbon source concentration favoured exopectinase and endopectinase production, while higher pH values and low carbon source concentration promoted pectin lyase and rhamnogalacturonase production.

374

375 In summary, fruit wastes are superior substrates to produce high titers of pectinolytic

area enzymes using either SSF or SmF. Process parameters including medium pH, temperature,

377 composition, inoculum size, moisture content and particle size of the substrate and aeration

378 highly depend on the utilized substrate and microbial strain. Statistical experimental designs

379 can be employed to optimize the fermentation conditions by evaluating the effects and

380 interactions of the different parameters that rule a biochemical system.

381

There is no industrial scale FW biorefinery facility currently in operation. However, there are some studies reporting the technical advances and engineering challenges of orange and lemon waste biorefineries [90, 91]. Direct utilization of citrus peel as animal feed is the

simplest option, requiring little infrastructure or investment, while increasing the value of the 385 waste material significantly [91]. However, citrus peel contains many high value compounds 386 387 such as pectin and D-limonene [92]. Pectin is frequently used in food processing, while Dlimonene is an important essential oil for cosmetics, foods and pharmaceutical industries. D-388 limonene can be extracted using suitable solvents. The biomass left over after limonene 389 390 extraction, mainly consists of pectin and lignocellulose, is an excellent source for pectinolytic 391 and lignocelluloytic enzyme production and for the growth of microorganisms to generate 392 high value products such as industrial enzymes, ethanol, methane and single cell proteins. 393 Moreover, the residual biomass i.e. lignin can be used as an energy source.

394

395 2.4. Proteases

Proteases are also one of the most important commercial enzyme groups because of their 396 wide range use in food, pharmaceutical, detergent, dairy and leather industries [39, 41, 93, 397 398 94]. Some fungal strains such as Aspergillus, Penicillium and Rhizopus and bacteria of genus *Bacillus* have been reported as the active producers of proteases [39, 57, 95]. Although the 399 protease production from agro-industrial wastes has been studied in detail using both SSF and 400 401 SmF, the investigations on the utilization of FWs has not been comprehensive. The studies reporting protease production from several FWs are listed in Table 5. Khosravi-Darani, 402 Falahatpishe [95] used a newly isolated alkalophilic Bacillus sp. in SmF of date wastes 403 without any pre-treatment. High activity protease production (57420 APU/mL) was obtained 404 at pH 10, 37°C and the enzyme was reported to be thermostable, indicating its possible 405 utilization in industrial applications. Afify, Abd El-Ghany [96] investigated the production of 406 proteases from potato waste in a submerged system using S. cerevisiae and studied the 407 utilization of remained solid waste as a biofertilizer for plant development. The highest 408 enzyme activity (360 U/mg) was obtained using a fermentation medium containing 15 g 409

potato waste, at initial pH 6.0, 20°C for 72 h. There are some studies reporting the 410 production of high activity proteases using fishmeal and shrimp wastes. In a study of Gupta, 411 412 Prasad [93], fishmeal from sardine and pink perch were evaluated as a sole carbon and nitrogen sources in the medium for alkaline protease production by Bacillus pumilus MTCC 413 7514. The protease obtained in medium containing only fish meal (4,914 U/mL) was nearly 414 415 two times higher than that using basal medium (2,646 U/mL). The protease production was 416 enhanced to 6,966 U/mL and 7,047 U/mL when scaled up from flask to 3.7 and 20 L fermenters, respectively, using fish meal as the sole source (10 g/L). The crude protease was 417 418 found to have dehairing ability in leather processing, which is bound to have great environmental benefits in leather industry. In another study, a powder was prepared from 419 shrimp wastes and tested as growth substrate for the production of protease by P. aeruginosa 420 MN7 [97]. P. aeruginosa MN7 was found to grow and over-produce proteolytic enzymes 421 (15,000 U/mL) in media containing only SWP as microbial growth substrate. Although there 422 423 are few reports on protease production from FW, the appreciable protease activities obtained on different FW residues highlighted the potential of these wastes. 424

425

426 Besides its potential utilization in many industrial applications, proteases produced from FW

427 can be also used for biorefining different biomasses. Koutinas, Malbranque [98] evaluated an

428 oat-based biorefinery for the production of lactic acid as well as other value-added by-

429 products, such as β -glucan and antioxidant-rich oil bodies using *Rhizopus oryzae*. During the

430 process, *Rhizopus oryzae* produced a range of enzymes (glucoamylase, protease,

431 phosphatase) during the hydrolysis of complex macromolecules in oat. The utilization of

432 waste biomass and in-situ produced enzyme cocktails in such a biorefining strategy could

433 lead to significant operating cost reduction as compared to current industrial practices for

434 lactic acid production from pure glucose achieved by bacterial fermentations.

Residual materials	Microorganism	Pretreatment method	Fermentation mode & vessel type	Fermentation conditions	Duration (day)	Achievements	References
Date waste	Bacillus sp. 2-5	Heat treatment & filtration	SmF-125 mL flask	37°C, pH 10, 125 rpm	2	57420 APU/mL	[95]
Potato waste	Saccharomyces cerevisiae	NR	SmF- 250 ml flask	28°C	5	360 U/mg	[96]
Fish meal	<i>Bacillus pumilus</i> MTCC 7514	None	SmF-20L bioreactor	30°C, pH 7.5	2	7.05 U/mL	[93]
Waste bread	Aspergillus oryzae	None	SSF-petri plates	30°C, MC:1.8 (w/w, db), PS:20 mm, 10 ⁶	<mark>6</mark>	83.2 U/gdS	<mark>[58]</mark>
Cuttlefish by-products	Vibrio parahaemolyticus	Heat treatment, pressing, grinding, drying at 80°C o/n, powdering	SmF- 250 mL flasks	37°C, pH 8.7, 200 rpm	I	2487 U/mL	<mark>[99]</mark>
Shrimp waste	Pseudomonas aeruginosa MN7	Heat pretreatment (100°C, 20 min), drying, grinding	SmF- 250 mL flasks	37°C, 200 rpm	<mark><1</mark>	15000 U/mL	<mark>[97]</mark>

Table 5. Protease production from food wastes.

436 SmF: submerged fermentation, SSF: solid state fermentation, MC: moisture content, PS: particle size, S: substrate, o/n: overnight, NR: Not reported.

437 2.5. Lipases

After proteases and carbohydrases, lipases (EC 3.1.1.3) are considered as the third largest 438 439 group based on total sales volumes [100]. They are widely used for several applications in food, detergent, cosmetics, organic synthesis and pharmaceutical industries. They are 440 catalysing the hydrolysis of triacylglycerols to di- and mono- acylglycerols, fatty acids and 441 glycerol [42, 101, 102]. They are also able to catalyze alcoholysis, acidolysis, aminolysis, 442 443 esterification and transesterification under certain conditions [103]. Phospholipases are a sub class of lipases that catalyse the hydrolysis of one or more ester and phosphodiester bonds of 444 445 glycerophospholipids. They vary in site of action on phospholipid which can be used for the modification/production of new phospholipids for some applications in oil refinery, health, 446 food manufacturing, dairy and cosmetics industries [104]. 447

448

Most of the research has been concentrated on high activity extracellular lipase production by 449 using both SmF and SSF via a wide variety of microorganisms including bacteria, fungi, 450 yeast and Actinomyces [42, 102, 105, 106]. Several strains of commercial lipase producing 451 fungi are quite dominant, including Rhizopus, Rhizomucor, Aspergillus, Geotrichum, 452 Yarrowia and Penicillium species [107]. Recently, the production of lipase investigated by 453 several researchers using different FWs as substrates [101] or by supplementing FWs as 454 inducer [108, 109]. Alkan, Baysal [101] investigated the production of lipase from melon 455 waste by SSF using *Bacillus coagulans*. The highest lipase production (78.1 U/g) was 456 achieved after 24 h of cultivation with 1% olive oil enrichment at 37°C and pH 7.0 by 457 supplementing sodium dodecyl sulphate (Table 6). The best results were obtained by 458 supplementing starch and maltose (148.9 and 141.6 U/g, respectively), whereas a rather low 459 enzyme activity was found in cultures grown on glucose and galactose (approximately 118.8 460 and 123.6 U/g, respectively). Enzyme was inhibited by Mn^{2+} and Ni^{2+} by 68% and 74%, 461

respectively. By contrast, Ca²⁺ enhanced enzyme production by 5%. In a study of 462 Dominguez, Deive [108] investigated the biodegradation of waste cooking oil and its 463 464 application as an inducer in lipase production by *Yarrowia lipolytica* CECT 1240. The addition of waste cooking oil to the medium led to a significant augmentation in extracellular 465 lipase production by yeast, compared to oil-free cultures. Papanikolaou, Dimou [109] 466 explored the effects of different Aspergillus and Penicillium strains on lipid accumulation and 467 468 lipase production using the waste cooking oil as substrate. In carbon-limited medium, the highest amount of biomass (18 g/L) with a lipid content of 64% was obtain using Aspergillus 469 470 sp. ATHUM 3482, while the highest extracellular lipase activity (645 U/mL) was obtained by Aspergillus niger NRRL 363. The studies above have indicated the possibility of FWs 471 utilization either as substrates or inducers for lipase production. Lipase production can be 472 further improved using mutant or engineered strains. 473

474

Lipases are also used for biodiesel production from crude oil and fats [112] either in free or
immobilized form. Lipase production processes from FW can be integrated in a biodiesel
biorefining process to decrease the transesterification cost. Besides lipases, phospholipases
are used for oil degumming and improving the efficiency of fatty acid yields [113]. Although
there is no report on phospholipase production using FWs, a process for the production of
various types of phospholipases from FWs can be developed using suitable strains.

481

Table 6. Lipase production from food wastes.

Substrate	Microorganism	Pretreatment method	Fermentation mode & vessel type	Fermentation conditions	Duration (day)	Achievements	References
Banana waste, melon waste, watermelon waste	Bacillus coagulans	None	SSF-Flasks	37°С, рН 7	1	148.9 U/g S from melon waste	[101]
Waste cooking oil	Y.lipolytica CECT 1240	None	SmF- 5L stirred tank bioreactor with 3L working vol, fb	30°C, 400 rpm	6	0.93U/mL	[108]
Waste cooking olive oil	Aspergillus and Penicillium strains	Filtration	SmF-250 mL flasks	28°C, pH 6, 200 rpm	3	645 U/ mL	[109]
Olive oil cake	Y.lipolytica NRLL Y-1095	Alkaline pretreatment (3% NaOH) 20°C o/n	SSF-150 mL Erlenmeyer flasks	<mark>30°С, рН 7, 55% МС</mark>	<mark>4</mark>	40IU/g S	[114]
Tri-substrate (wheat bran, wheat rawa and coconut oil	A.niger MTCC2594	None	SSF-3*1kg tray type bioreactor	<mark>30°C, 60% MC</mark>	<mark>4</mark>	745.7 IU/gdS	[115]
Seafood processing waste	Bacillus altitudinis	Drying (80°C o/n)	SSF-Flasks	50℃, pH_8, 80% <mark>MC</mark>	<mark>3</mark>	2U/gdS (Esterase)	[116]
Tuna by-products	Rhizopus oryzae	Heat pretreatment (100°C 20 min) and filtration	SmF- 1L flasks	30℃, pH 6, 150 rpm	3	23.5 IU/mL	[117]
Wheat bran with 2% olive oil	Aspergillus flavus	None	SSF-Flasks	<mark>29℃, pH 7, 65% MC</mark>	<mark>4</mark>	121.4 U/gdS	<mark>[118]</mark>
Wheat bran with 2% olive oil	Aspergillus niger J1	None	SmF- 500 mL flasks	30℃, pH 6, 100 rpm	8	1.46 U/mL	[119]
Wheat bran with 2% olive oil	Aspergillus niger J1	None	<mark>SSF- flasks</mark>	<mark>30℃, pH 6, 65% MC</mark>	7	1.46 U/mL	[119]

484 S: substrate, ds: dry substrate, SSF: solid state fermentation, SmF: submerged fermentation, fb: fed-batch, *Y. lipolytica: Yarrowia lipolytica*, MC: moisture content, o/n:overnight.

487

488 3. Conclusions

The management of FWs has posed a serious economic and environmental concern. The publications discussed above indicated that a wide range of high titres industrial enzymes can be produced from various FWs The produced enzymes can be used for some industrial applications. Moreover, these enzyme production processes can be consolidated with other value-added product development processes to create FW biorefineries.

494

So far, all developed biorefinery processes for the conversion of FW into ethanol and other 495 value-added products have only been achieved at bench-top and pilot levels. There is no 496 industrial scale FW biorefinery facility currently in operation. Therefore, it is not possible to 497 conduct an economical analysis for the proposed biorefinery systems. However, considering 498 499 the cost of defined medium preparation in current commercial enzyme processes, the utilization of low or no cost waste biomass for biorefining could lead to significant reductions 500 501 in operating costs. However, difficulties and costs associated with the collection/transportation of FW should also be taken into account. Optimization and scale up 502 studies need to be carried out in order to exploit for large-scale applications. 503

504

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508

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