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From Wastes to High Value Added Products: Novel Aspects of SSF in the Production of Enzymes

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21 **Abstract**

22 Solid state fermentation (SSF), a process that occurs in the absence or near
23 absence of water, has been used for the production of various high value added products
24 such as enzymes and other organic components. This paper reviews the recent studies
25 reported on the use of SSF for the production of enzymes; lipases, proteases, cellulases,
26 hemicellulases, ligninases, glucoamylases, pectinases and inulinases. The
27 microorganisms used for fermentation are mostly fungi and substrates are waste
28 materials from the agriculture and food industry. This shows the advantages of SSF
29 from an economical and environmental viewpoint. The paper provides an update on
30 several issues, *viz*. wastes, microorganisms and scale-up and control of the process of
31 fermentation in solid-state.

32

33 **Keywords:** Cellulases, enzymes, lipases, proteases, process scale-up, solid state
34 fermentation.

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36

37

1. Introduction

Solid-state fermentation (SSF) is a process of fermentation performed on non-soluble materials, namely the substrate, in the absence or near absence of water (Salihu et al., 2012). The substrate acts mainly as source of nutrients for the microorganisms responsible for the fermentation. There are various groups of microorganisms used in SSF depending on the final product considered necessary to be obtained from the fermentation. Among these, filamentous fungi, like species of *Aspergillus* and *Rhizopus* are the best adapted microbial species reported in most recent studies (Belmessikh et al., 2013; Cunha et al., 2012; Dhillon et al., 2011a,b; Thanapimmeth et al., 2012). The SSF process has been extensively used for the production of high value added products such as enzymes, biofuel, biosurfactants and biopesticides (Singhania et al., 2009).

In particular, for the production of enzymes, the fermentation is commonly conducted in a liquid medium containing the required dissolved nutrients (Colla et al., 2010). This process of fermentation is known as submerged fermentation (SmF), which presents the benefit of homogeneity of the culture media used and possibility of controling the parameters like temperature and pH. However, there are several advantages of SSF over the use of SmF (Mitchel et al., 2006). A comparison between SSF and SmF is presented in Table 1. SSF allows for the production of enzymes with higher activity and stability with lower water and energy demands. Additionaly, from the environmental and economical perspectives, the main advantage of SSF is related to lower volume of effluent produced, compared to SmF, and the possibility of carrying out the process under non-sterile conditions (Subramaniyam & Vimala, 2012). SSF uses low-cost waste products mainly from the sector of agriculture and food industries, such as wheat bran and peels of fruits and vegetables. These wastes can be used as ideal substrates for the microbial fermentation due to their rich contents of organic

components, which are considered as essential sources for carbon, nitrogen and many micronutrients that are important for the production of metabolites. Despite these advantages of SSF, the use of this type of fermentation in industrial processes is not widely applied due to challenges and limitations concerning monitoring, controlling and scaling-up of the process (Salihu et al., 2012; Sukumaran et al., 2010). For example, one of the critical issues regarding the latter is the inability to remove the heat excess generated by microbial metabolism during the fermentation. Another important disadvantage of SSF is the handling of solids on large scales, as 200 kg is reported to be the maximum weight of solids to be used in the fermentation on industrial scales. Both are considered being the main disadvantages of SSF for industrial applications.

The objective of this paper is to review the recent studies reported on the use of SSF of waste materials for the production of enzymes, as high value products. The paper provides an update on various aspects: wastes, microorganisms and SSF process scale up and control.

2. Wastes used in SSF

The wastes used in the processes of SSF for the production of enzymes are mainly of animal and plant origin from food industry. Table 2 outlines the wastes and microorganisms used in the processes of SSF and also the enzymes produced. In this section, a detailed description of the wastes of animal and plant origin used is also presented.

2.1. Wastes of animal origin

The waste materials of animal origin, including tannery solid wastes and cow dung, chicken feather and fish flour, have been mainly used in the production of only

proteases through SSF processes (Table 2).

Tannery solid wastes, in the form of raw hide trimmings and splits, limed and green animal fleshings, chrome shavings and hair wastes, are produced with huge quantities during leather manufacturing and are not usually used or under-used (Nalawade et al., 2009). Therefore, these waste materials are creating a solid waste disposal problem in tanneries. Kumar et al. (2009) studied the use of animal fleshings, the proteinaceous part of tannery solid wastes, as substrate for the production of aspartic protease by a *Synergistes* sp. It was suggested that there is a possibility to produce this enzyme by SSF using a cheap substrate and moreover, the enzyme obtained exhibited high stability in various organic solvents. Hair wastes have been also used in SSF for the production of proteases (Abraham et al., 2014). This waste mixed with raw sludge from wastewater treatment has been valorised by SSF without the inoculation with a pure microorganism. Alkaline protease was produced as a consequence of the degradation of hair by the microbial populations developed. Stabilized compost was another by-product of the process.

Cow dung, as an inexpensive waste material, has been evaluated as a substrate for the production of protease by *Halomonas* sp. through SSF (Vijayaraghavan & Vincent, 2012). A high production of halo-tolerant alkaline protease was obtained when compared with a substrate of wheat bran under the same process conditions. Accordingly, cow dung, which is characterised by its increased availability and low costs, might be used in future research as a key substrate in the production of protease enzymes. Keratin wastes such as chicken feather has been utilised in SSF by a feather degradating strain of *Bacillus subtilis* (Rai et al., 2009). The process conditions were optimised in order to maximise the yield of β-keratinase, which is a type of protease. This was one of the important studies that shows the successful use of a keratin waste

113 material in the production of enzymes. Fish flour, a fish processing by-product, mixed
114 with polyurethane foam has been used by *Aspergillus oryzae* for the production of a
115 proteolytic extract (Garcia-Gomez et al., 2009). This extract showed a higher enzymatic
116 activity, i.e. a higher degree of protein hydrolysis, when tested on fish muscle compared
117 to a commercially available enzyme. Therefore, it was concluded from the results of this
118 research that it was highly feasible to use fish flour as a substrate in the production of
119 proteolytic enzymes.

120

121 **2.2. Wastes of plant origin and food industry**

122 There are various types of wastes of plant origin and also of food industry that
123 have been used in processes of SSF for the production of enzymes (Table 2). These
124 waste materials include wastes of wheat and rice, such as wheat bran and rice husk,
125 peels and pomace of fruits and vegetables, sugarcane bagasse, soy and cotton wastes,
126 waste bread and brewery spent grain. Therefore, by using such a wide range of waste
127 materials, it was possible to obtain through the process of fermentation several types of
128 enzymes, i.e. lipases, proteases, cellulases, xylanases, pectinases, amylases and
129 inulinases.

130

131 **2.2.1. Wastes of vegetable oil**

132 Wastes of vegetable oil (oil cakes) have been used for the production of lipases,
133 proteases and xylanases through SSF. Lipase has been produced when cakes of edible
134 oil have been used as substrate. For instance, Colla et al. (2010) have been used soybean
135 oil cake as a substrate after adding about 10% rice husk for increasing the porosity of
136 the media that allows for oxygen transfer, in the presence of *Aspergillus* spp. as
137 inoculum. In comparison to SmF, SSF led to higher enzymatic activity of lipase. This

138 was due to the fact that in solid substrates the nutrients are more concentrate than in
139 liquid medium. This resulted in excellent cell-to-substrate interaction that consequently
140 led to a higher enzyme production. Another oil cake used for the production of this
141 enzyme was ground nut oil cake (Chaturvedi et al., 2010). It was shown the enzyme
142 production through the fermentation by *Bacillus subtilis* was highly affected by various
143 process conditions such as pH and moisture levels. It was found that the maximum yield
144 of lipase was at a moisture of 70% and pH of 8.0. Interestingly, the oil cake of *Jatropha*
145 *curcas*, a major energy crop in Thailand, has been used for the production of several
146 enzymes through SSF as mentioned in recent literature (Mahanta et al., 2008; Joshi &
147 Khare, 2011; Ncube et al., 2012). The outcome of the research performed was desirable,
148 as it was possible to obtain enzymes such as proteases, lipases and xylanases by using
149 various microorganisms of *Pseudomonas*, *Scytalidium* and *Aspergillus*. It is of high
150 importance to establish a beneficial disposal of this waste material, as it is characterised
151 by high contents of toxic compounds such as antineutrinos and phorbol esters (Ahmed
152 & Salimon, 2009).

153

154 **2.2.2. Wastes of wheat, rice, sugarcane and palm trunk**

155 Wastes of wheat and rice, sugarcane bagasse and oil palm trunk have been
156 mainly used as substrates for the production of cellulases through SFF. Therefore, it can
157 be observed that there is a view to developing low cost production systems for cellulase
158 enzymes.

159 Wheat bran materials, as a lignocellulosic material, among various kitchen and
160 agro-industrial wastes, such as corn cobs, peelings of fruits and sawdust, appeared to be
161 the best suited substrate producing appreciable yields of cellulase enzyme, in the
162 presence of an inoculum of *Aspergillus niger* and *Trichoderma reesei* (Bansal et al.,

163 2012; Dhillon et al., 2011). Interestingly, there was no need for a supplementation of
164 exogenous nutrients and therefore this research highlights the potential of wheat bran as
165 possible raw material for the enzyme production. Wheat bran and *A. niger* were also
166 evaluated for the production of cellulase and xylanase under SSF by Dhillon et al.
167 (2011), and enzyme yields were compared with SSF where the inoculation and substrate
168 were mixed with *Trichoderma reseei* and rice husk with a ratio of 1:1 and 2:3,
169 respectively. In this case, it was reported that mixed microbial cultures and waste
170 materials led to the production of higher amounts of enzymes.~~ than the use of a single~~
171 ~~microbial strain and wheat bran as a sole substrate. Mixed culture combinations have~~
172 ~~the ability to utilize the substrate, especially if there is more than one substrate, as~~
173 ~~energy sources are better used than in pure single strain cultures.~~ In addition, the
174 inclusion of rice straw provided an additional source for the carbon required by the
175 microorganisms used. This is in agreement with another research work that optimised
176 the production of cellulase by *Aspergillus funigatus* under SSF (Soni et al., 2010).
177 Moreover, wheat bran has been also used as a supplement to soybean hulls for the
178 production of cellulase using a mixed microbial culture of *Trichoderma reesei* and
179 *Aspergillus oryzae* (Brijwani et al., 2010). Mixed cultures clearly showed their
180 compatibility for hyper enzyme production.

181 Sugarcane bagasse, a waste product that is generated from the sugarcane
182 industry in huge amounts, has been used evaluated as a substrate for the production of
183 cellulase through SFF. Mekala et al. (2008) addressed the optimisation of environmental
184 parameters and media for the fermentation by using *Trichoderma reesei* for enhancing
185 the yield of the enzyme. A suitable SSF process has been developed for cellulase
186 production with this cheap biomass resource as substrate. In addition, Cunha et al.
187 (2012) have evaluated sugarcane bagasse as a substrate for the production of cellulase

188 through SSF and SmF. The fungus *Aspergillus niger* has been used as an inoculum in
189 both methods. It was shown that SSF was superior compared to SmF, as in this first
190 case the cellulase production was 3-fold higher. This was due to the fact that in SSF, the
191 nutrients are more concentrate than in liquid media used in SmF, as previously
192 explained (section 2.2.2.1). On the other hand cellulase production is controlled by
193 feedback, i.e. the more substrate available the higher the enzyme yield. The advantage
194 of SSF of this waste material is: first, this method is an economical process for the use
195 of a lignocellulosic waste that exhibits a long-standing difficulty in the costs associated
196 with the enzymatic hydrolysis of this material by other methods and second, SSF
197 developed can go a long way in bringing down the cost of cellulases, which will
198 eventually help to develop economical processes for bio-fuel production.

199 Direct utilization of complex untreated oil palm trunk, a cheap and abundant
200 material, for cellulases and xylanase production by lignocellulosic degrading fungi such
201 as *Aspergillus fumigatus* was evaluated under SSF (Ang et al., 2013). The palm trunk,
202 which was isolated from cow dung, was used as sole carbon source for the fungus
203 during the fermentation process. The ability to produce xylanases with high levels of
204 cellulases was also shown. However, in future studies, there is still a need for statistical
205 optimisation of all the parameters involved in the fermentation process.

206

207 **2.2.3. Wastes of fruit and vegetable industries**

208 The industry of fruits and vegetables is producing a high amount of wastes and
209 therefore, it is interesting to use these materials in processes of SSF. Peels and pomace
210 have been used for the production of enzymes. These enzymes include mainly
211 cellulases, xylanases, pectinases and proteases.

212 Potato peels have been determined as one of the best substrates among various

213 agro-industrial wastes for the production of alkaline proteases by *Bacillus subtilis*
214 (Mukherjee et al., 2008). Interestingly, citrus peels were chosen as a substrate for the
215 production of enzymes because it is an important agroindustrial by-product that offers
216 several carbon sources required for the growth of microorganisms and for the
217 production of phytases, pectinases and xylanases (Mamma et al., 2008).
218 Microorganisms readily use this waste in fermentations due to its rich composition,
219 especially due to its high content of organic matter, which is about 80%, being total
220 dietary fibres (above 50%) free sugars and pectin the main compounds. This
221 composition justifies the use of citrus peels as inducing substrate for the production of
222 multienzyme complexes, without the need for the addition of pectic materials as
223 inducers to the media used in the fermentation (Kang et al., 2004).
224 In addition, citrus peel is the major solid waste that is generated by the citrus
225 processing industry, which represents approximately more than the half of the fresh fruit
226 weight. Accordingly, the disposal of this by-product poses a big challenge to the fruit
227 industry, where this waste is mostly pelletised and employed as animal feed or pectin
228 precursor. This waste has been successfully used by Rodriguez-Fernandez et al. (2011)
229 for producing pectinase and xylanase by *Aspergillus niger* through SSF. The kinetics of
230 microbial growth related to the synthesis of the enzymes has been determined.
231 Moreover, citrus waste has been also utilised for the production of phytase by the same
232 fungus and a scale-up process was achieved (Rodriguez-Fernandez et al., 2012, 2013).
233 Pomace of fruits and vegetables has been recently used as substrate for the
234 production of protease and cellulase through the process of SSF. Apple pomace was the
235 substrate for obtaining cellulase through the fermentation by *Aspergillus niger* (Dhillon
236 et al., 2012a,b). Results showed a rapid bioproduction of fungal cellulase using this low
237 cost waste material especially with a supplementation of inducers such as lactose.

238 Tomato pomace has been also used as a substrate in SSF for the production of protease
239 by the same genus of fungi (Belmessikh et al., 2013). The use of this tomato waste
240 constitutes an efficient and inexpensive substrate for the enzyme production and a
241 suitable mean for the waste valorisation towards an attempt for reducing the ecological
242 impact.

243

244 **3. Microorganisms used in SSF**

245 This section deals with the research work performed on the microorganisms
246 used in the processes of SSF and the substrates and end-products obtained (Table 2).
247 The microorganisms used in the SSF processes for the production of enzymes are fungi
248 and bacteria, mainly *Aspergillus* spp. and *Bacillus* spp., respectively, which will be
249 discussed in detail.

250

251 **3.1. Fungi**

252 Fungi are the best adapted microbial species reported in most recent studies for
253 the production of enzymes through SSF. This is due to the ability of these
254 microorganisms to grow on surfaces of solid wastes and penetrate into the inter-particle
255 spaces of the substrates. The fungal hyphae can also penetrate some solid structure of
256 the matrix.The fungal genera used are *Aspergillus*, *Penicillium* and *Rhizopus* (Table 2).
257 The fungal genus *Aspergillus* has a broad range of species that have been used in the
258 processes of SSF. These species include *A. niger*, *A. oryzae*, *A. terreus*, *A. fumigatus*, *A.*
259 *foetidus*, *A. sojae* and *A. candidus*, where the most frequently used fungus is SSF is *A.*
260 *niger*, a filamentous mesophilic fungus. This fungus was used to produce a multi-
261 enzyme preparation containing pectinolytic, cellulolytic, and xylanolytic enzymes under
262 SSF process on citrus peels (Mamma et al., 2008). This process was enhanced by the

263 optimization of initial pH of the culture medium and moisture levels. Most importantly
264 is the water activity, which limits the microbial growth. After the SSF process, the
265 fermented substrate was either directly exposed to auto hydrolysis or new materials
266 were added, and the in situ produced multi-enzyme systems were successfully used for
267 the partial degradation of orange peel polysaccharides. Fermentable sugars were
268 liberated, which could be converted to bioethanol. In a more recent study on SSF using
269 the same substrate and fungus, the production of these enzymes were optimised based
270 on aeration conditions to allow for a sufficient amount of oxygen that is required for the
271 growth of the microorganism and the removal of CO_2 and metabolic heat (Rodriguez-
272 Fernandez et al., 2011). In addition, a mathematical model was applied to determine the
273 different kinetic parameters related to SSF.

274 *Aspergillus niger* was used in SSF for the production of citric acid and cellulase
275 enzyme, by using apple pomace and apple pomace ultrafiltration sludge, which are by-
276 products from the apple processing industry (Dhillon et al., 2011; Dhillon et al., 2012a).
277 The addition of 3-4% of ethanol and methanol to the apple pomace substantially
278 increased the values of the citric acid attained. The cellulase obtained, after its recovery
279 being optimized using various extraction solvents, was used for the saccharification of
280 apple pomace and brewer's spent grain. Sugarcane bagasse was also used for the
281 production of this enzyme by SSF, where a combination of SSF and submerged
282 fermentation was shown to be superior to the conventional submerged method due to
283 the improved assimilation of sugarcane bagasse and fungal growth morphology (Cunha
284 et al., 2012). The concentration of the substrate was fundamental in the comparison. The
285 germination of the fungi on a solid-state medium allowed for the development of a
286 dispersed filamentous form, which resulted in superior cell-to-substrate interaction and
287 accordingly a higher production of the enzyme. In addition, cellulase was produced by

288 using SSF of various wastes, agricultural and kitchen wastes such as corn cobs, carrot
289 peelings, composite, grass, leaves, fruit peels, rice husk, sugarcane bagasse, saw dust,
290 wheat bran and wheat straw (Bansal et al., 2012). Of all the substrates tested, it was
291 found that wheat bran is the most suited substrate for a high production of cellulase. *A.*
292 *niger* was also used in the production of proteases and lipases (Paranthaman et al.,
293 2009; Colla et al., 2010; Edwinoliver et al., 2010). For instance, Paranthaman et al.
294 (2009) studied the production of protease using rice brokens and rice mill wastes as
295 substrates in SSF. The protease obtained could be commercially used in detergents and
296 leather industry.

297 *A. oryzae* was used in SSF for the production of cellulase, proteases and
298 xylanases. Thanapimmeth et al. (2012) showed that it is feasible to use deoiled *Jatropha*
299 *curcas*, a major energy crop in Thailand used for biodiesel, seed cake as a substrate in
300 the process of SSF after the optimisation of the conditions of moisture, inoculum and
301 temperature. Recently, Pirota et al. (2013) used a new strain of *Aspergillus oryzae*
302 isolated from the Amazon rain forest in SSF processes in the production of xylanases.
303 The substate used was wheat bran and the production of xylanase was on a lab scale
304 with a possibility of scaling up of the process. *Aspegillus oryzae* was also used in mixed
305 cultures in the production of enzymes by SSF. This fungus was used with *Aspergillus*
306 *giganteus*, *Phanerochaete chrysosporium* and *Trichoderma virens* in SSF on cotton
307 seed-coat fragment waste as substrate (Csizar et al., 2007). The enzyme complexes
308 produced were composed of hydrolytic and oxidative enzymes, such as cellulases and
309 xylanases. *Aspergillus oryzae* was also used with *Aspergillus awamori* or *Trichoderma*
310 *reesei* in the production of glucoamylase and protease or cellulase enzymes via SSF,
311 using wheat bran which is a waste product of the wheat milling industry, or soybean
312 hulls as substrate, respectively (Du et al., 2008; Brijwani et al., 2010).

313

3.2 Bacteria

In general, bacteria are not widely used in the production of enzymes through SSF. The bacteria are mainly of the genus *Bacillus* (Table 2), specifically its species *subtilis*, *licheniformis*, *pumilus* and *firmus*, which have been used in the production of amylases, proteases, lichenases and xylanases (Mukherjee et al., 2008, 2009; Nimkar et al., 2010; Kapilan & Arasaratnam, 2011; Chaari et al., 2012). The waste materials used as substrate in the SSF were mainly agrowastes, such as potato peel and pea pomace, and chicken feather that is considered an animal waste by-product. *B. subtilis* was most often used in SSF processes. This bacterial species was used in SSF for the production of proteases and α-amylases.

Amylase was successfully produced using wheat and rice bran as substrate materials for the SSF process after optimisation of the various parameters such as pH and temperature (Nimkar et al., 2010). In addition, Mukherjee et al. (2009) found that potato peel, which is considered as a novel inexpensive substrate, was the best waste material among agro-industrial waste residues to be used for the production of amylase due to its high starch contents and the absence of mono-saccharides. This waste material was combined with other agrowastes such as grass and protein sources, which allowed for the production of protease by using the same species of *Bacillus* (Mukherjee et al., 2008). Recently, potato peel was also utilised by the bacterium *Bacillus firmus*, isolated from marine sediment of Parangipettai coast, to produce thermostable alkaline amylase by SSF process at optimised process conditions (Elayaraja et al., 2011).

335

4. Enzymes produced by SSF, their process conditions and applications

In general enzymes have been extensively produced by submerged fermentation

338 (SmF) and have been commercially available since many decades (Anwar &
339 Saleemuddin, 1998; Pandey et al. 2003; Queiroga et al., 2012). Recently, the production
340 of enzymes on solid state fermentation has been implemented in order to reduce the
341 costs involved, especially if residues are used as substrates, and enhance the field of
342 application (Sandhya et al., 2005; Kumar et al., 2009). However, there is indeed few
343 research work developed on bench scale solid substrate fermentation, as the majority of
344 this research was conducted on few grams of substrate materials, i.e. on a lab-scale. In
345 addition, some research work was performed in media with high moisture contents of
346 more than 70%, which might be due to some solid substrates that are able to retain high
347 moisture levels, whereas SSF is defined as fermentation being performed in the absence
348 or nearly absence of free water (Pandey et al. 2003).

349 This section is discussing in detail the following enzymes; lipases, proteases,
350 cellulases and xylanases and other enzymes, such as fucoidanase and pectinases that are
351 obtained through the process of SSF. In addition, there will be a detailed description on
352 the conditions of the fermentation process and the various applications of lipases,
353 proteases, cellulases and xylanases.

354

355 **4.1. Enzymes**

356 **4.1.1. Lipases**

357 Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are enzymes capable of
358 catalyzing the hydrolysis of triacylglycerols to glycerol and fatty acids at an oil-water
359 interface and reactions of esterification, transesterification and interesterification of
360 lipids (Sharma et al., 2001). In recent years, there has been an increasing interest in the
361 study of lipases mainly due to their potential applications to a wide range of industrial
362 sectors (Hasan et al., 2006; 2009). In the chemistry and pharmaceutical industries,

363 lipases are used in the production of surfactants, detergents and antibiotics; whereas in
364 the food industry these enzymes are used to synthesize emulsifiers and develop
365 flavours. Commercially useful lipases are typically obtained as microbial extracellular
366 enzymes. However, since lipases are products of industrial interest, their production
367 must be coupled with low cost processes. These enzymes would be economically
368 manufactured in processes of SSF that utilize residues as substrates and that give high
369 yields

370 The substrates used, which varied in amount from few grams up to 3 kg, in the
371 processes of SSF for the production of lipases are oil wastes, wheat bran and sludge
372 (Table 2). The activity of the lipase varies to a very high extent; the range of this
373 activity is from 4.5 to 120,000 U/g. Wastes of oil, such as solid wastes from the
374 production of vegetable oils (oil cakes), are one of the common waste materials used as
375 substrates for the production of lipase by SSF. Oil cakes are good supports for microbial
376 growth necessary during the process of SSF, because this waste has excellent sources of
377 proteinaceous nutrients needed for the microbial fermentation, i.e. requiring low or no
378 supplementation (Ramachandran et al., 2006). This waste material has also another
379 advantage. It is inexpensive and available in high amounts from the oil industry.

380 Oil cakes of coconut, ground nut, mustard, linseed and neem has been used as
381 substrates in SSF in the presence of *B. subtilis* for the production of extracellular lipase
382 on a lab-scale using 10 g of waste materials (Chaturvedi et al., 2010). It was observed
383 that the nature of the substrate significantly influenced the impact of initial moisture
384 content and therefore affected the process of SSF. The physical nature and water
385 holding capacity of the substrate are important criteria for its use in SSF process
386 because the moisture content is an important factor that determines the microbial growth
387 and activity of the enzyme. In another study of Edwinoliver et al. (2010), coconut oil

388 cake was also used and mixed with wheat bran and rawa for the production of lipase
389 through SSF in the presence of *A. niger*. The scaling up of the process was possible
390 from lab-scale to bench scale using up to 3 kg of wastes as substrates. The SSF process
391 led to a maximum activity of the lipase of 745.7 U/g. In addition, babassu cake
392 supplemented with sugar cane molasses as a substrate and the fungus *Penicllium*
393 *simplicissimum* were used for the production of lipase, on a lab-scale, with a maximum
394 activity of 314 U/g through SSF (Guturra et al., 2009). This fungus has been also used
395 in SSF for the production of lipase, but by using other oil wastes such as soybean cake
396 and castor bean waste showing an enzymatic activity of 317 and 80.24 U/g respectively
397 (Rigo et al., 2009; Godoy et al., 2011).

398 The oil cake of biodiesel crops, which contain about 50% oil called biocrude that
399 can be converted into biodiesel by esterification, are also used as substrates for the
400 production of lipase enzymes through SSF. Lipase was produced by using Niger oil
401 cake, as it is rich in various nutrients such as fatty acids and sugars, through SSF on a
402 lab-scale, where 5 to 10 g of wastes were used as substrate (Imandi et al., 2010). The
403 marine yeast *Yarrowia lipolytica* was used as an inoculum for the fermentation. There
404 was a low enzyme activity obtained, with a maximum of 26.42 U/g. Another biodiesel
405 crop called *Jatropha curcas*, a major energy crop in Thailand, was used by Mahanta et
406 al. (2008) for the production of lipase through SSF by *Pseudomonas aeruginosa*. The
407 seed cake supported good bacterial growth and enzyme production of an activity of 625
408 U/g, due to the composition of this cake that contains a high content of fat and fibres.

409 Winterisation residue from oil refinery and raw sludge were used as solid
410 matrices for the processes of SSF for lipase production on a bench scale using 2.5 kg of
411 waste materials, where the fermentation was dependent on the microbial consortium
412 present (Santis-Navarro et al., 2011). Winterisation residue was used a source of fat and

413 the sludge was added as co-substrate and inoculum. It was reported that the lipolytic
414 activity of the enzyme obtained reached a maximum of 120,000 U/g in the fermented
415 solid, which is substantially higher than activities reported in other research on SSF.
416 This highlights the possibility to work with solid wastes as effective biocatalysts, a topic
417 that has been scarcely treated in SSF literature.
418

419 **4.1.2. Proteases**

420 Proteases (EC 3.4.21-24), which are hydrolases that catalyze the cleavage of
421 peptide bonds in proteins, are a highly complex group of enzymes that differ in their
422 substrate specificity and catalytic mechanism (Sumantha et al., 2006; Turk, 2006).
423 These enzymes are classified into three main categories; alkaline, neutral and acid
424 proteases on the basis of pH range in which their activities are optimal. Therefore,
425 proteases are the most important industrial enzymes that account for about 60% of the
426 world market of industrial enzymes. The importance of these enzymes is reflected in
427 their tremendous applications in both physiological and commercial fields, for example
428 in detergent formulations, textile, food, and pharmaceutical industries (Queiroga et al.,
429 2012).

430 The preferred source of proteases is microorganisms, rather than plant and
431 animal tissues, to their broad biochemical diversity and their susceptibility to genetic
432 manipulation (Ellaiah et al., 2002). Among microbes, fungi as enzyme producers have
433 many advantages, since they could be mostly GRAS (generally regarded as safe) strains
434 and the produced enzymes are extracellular, which makes easy its recuperation from the
435 fermentation broth. Accordingly, the overall cost of the production of a complex group
436 of enzymes is very high, mainly due to low yield of enzymes because most of the costs
437 are related to the recovery and purification. Additionally, there are other high costs

438 associated with the substrates, i.e. the commercial media required (Singhania et al.,
439 2009). Therefore, development of novel processes to increase the yield of proteases
440 coupled with lowering down these costs are highly appreciable. Furthermore, proteases
441 produced by using commercial media possess undesirable flavours, which are
442 unsuitable for applications in food and pharmaceutical industries. Therefore, during the
443 recent years, efforts have been directed to explore the means to reduce the protease
444 production costs through improving the yield and the use of cost-free or low-cost
445 substrates such as agricultural waste materials in processes of SSF for the production of
446 proteases.

447 The waste materials used as substrates, which highly varied from 5 g to 1.4 kg,
448 in the processes of SSF for the production of proteases are mainly of plant origin, such
449 as potato peel, soy fibres, tomato pomace and wheat bran or of animal origin like
450 tannery solid wastes, chicken feather and cow dung (Table 2). The activity of the
451 enzyme obtained through SSF also highly varied from around 20 to more than 50,000
452 U/g.

453 Several studies on the utilisation of residues of plant origin in the production of
454 proteases through SSF were carried out on wheat bran. Merheb-Dini et al. (2010) used
455 the microorganism *Thermomucor indicae-seudaticae* in the presence of wheat bran and
456 wheat bran mixed with casein at a ratio of 80:20 respectively for the production of
457 protease with an enzymatic activity of 168 U/g. In addition, wheat bran has been used as
458 substrate in the production of protease with a maximum activity of 5-20 U/g by a fungal
459 strain of *Schizophyllum commune* and *Myceliophthora* sp. (Boyce & Walsh, 2012;
460 Zanphorlin et al., 2011). Mukherjee et al. (2008) screened various agro-industrial and
461 kitchen waste materials of plant origin, such as oil cake, wheat and rice bran, grass,
462 banana leaves, potato peels and used tea leaves, for the use as substrate for protease

463 production through SSF by *Bacillus subtilis*. It was found that the substrates of potato
464 peel and grass led to the production of proteases with the highest protease activity of up
465 to 2,383 U/g. In another study by Abraham et al. (2013), the effect of three agro-
466 industrial residues was examined; hair waste, coffee husk and soy fibre. Soy fibre
467 presented the highest yield for protease production showing an enzymatic activity of
468 47,331 U/g. Recently, tomato pomace was used in a comparative study of protease
469 production by cultivating *Aspergillus oryzae* in SSF and submerged fermentation
470 (Belmessikh et al., 2013). The results obtained showed a highest enzymatic activity of
471 21,309 U/g in case of the process of SSF. There were recent few studies on the
472 utilisation of residues of *Jatropha curcas* (oil cake), which is a major energy crop that
473 cannot be used in nutrition or animal feed due to its toxicity. Mahanta et al. (2008) and
474 Thanapimmetha et al. (2012) investigated the potential ultilisation of this oil cake as
475 substrate for protease production by *Pseudomonas aeruginosa* and *Aspergillus oryzae*,
476 respectively. The results demonstrated that the utilisation of this waste material for the
477 enzyme production was a viable approach, with an activity of about 2,000 up to 14,000
478 U/g. Moreover, Chutmanop et al. (2008) compared the use of *Jatropha* oil cake with
479 wheat and rice bran as substrates in SSF for the production of proteases under the same
480 fermentation conditions and by using the same inoculum of *Aspergillus oryzae*.
481 Interestingly, it was found that the protease activity produced by the oil cake was 30-
482 40% higher than that of wheat and rice bran, due to the fact that this cake has a very
483 high protein content that can be utilised by the microorganism for the production of the
484 enzyme.

485 Residues of animal origin, tannery waste and cow dung, have been utilised in
486 SSF process in the research work of Kumar et al. (2009) and Vijayaraghavan et al.
487 (2012), respectively. Tannery solid wastes, which consist of hide trimmings and limed

488 animal fleshing, was considered as a proteinaceous substrate for the production of
489 proteases, with activities up to 755 U/g, under SSF by using *Synergistes* sp. Similarly,
490 hair waste from the tanning industry mixed with raw sludge from waste water treatment,
491 without the need for inoculations of pure microorganisms, were valorised for the
492 production of protease, where a maximum enzymatic activity of 56,270 U/g was
493 reached (Abraham et al., 2014). Cow dung was used in the presence of an inoculum of
494 *Halomonas* sp. leading to the production of proteases of a relatively high activity of
495 1,351 U/g, which was substantially higher compared to other waste materials of plant
496 origin that have been used under the same process conditions.

497

498 **4.1.3. Cellulases and Xylanases**

499 Cellulose and xylan are the first two most abundant natural biopolymers, which
500 are most dominating agricultural wastes (Zhang, 2008). The lignocellulosic biomass of
501 most plants consist of mainly cellulose (a homologous polymer of glucose linked by β-
502 1-4 glycosidic bonds); lesser hemicelluloses (a heterologous polymer of 5- and 6-carbon
503 sugars with sugar acids) that contains principally xylan; and finally lignin (a complex
504 aromatic polymer). Cellulose, only its amorphous form, is synergistically hydrolysed by
505 a complex enzyme system named as cellulases; such as cellobiohydrolase or
506 exoglucanase, carboxymethylcellulase or endoglucanase and cellobiase or β-glucosidase
507 (EC 3.2.1.91, 3.2.1.4 and 3.2.1.21 respectively), while the degradation of xylan requires
508 various enzymes; essentially endo-1-4,-β-xylanase (EC 3.2.1.8) and to some extent β-
509 xylosidase, α-glucuronidase, α-L-arabinofuranosidase and acetylxylan esterases (Maki
510 et al., 2009; Van Dyk & Pletschke, 2012). The lignocellulosic biomass, as it can be
511 degraded, it is a renewable and abundant resource in agricultural industry, with an
512 appropriate treatment, with great potential for bioconversion to value-added

513 bioproducts. Therefore, cellulases and xylanasse are now considered as a major group of
514 industrial enzymes that have various industrial applications.

515 Techniques of fermentation, due to their economic and environmental
516 advantages, have been widely used for a feasible production of cellulases and xylanase
517 (Subramaniyam & Vimala, 2012). The most frequently used techniques are SmF and
518 SSF, where the latter being the most beneficial due to the use or recycle of wastes that
519 are cheap and highly available.

520 As previously mentioned, cellulose and xylan are present in plants and therefore
521 the substrates used for the production of the enzymes of cellulases and xylan are only of
522 plant origin (Table 5). These substrates are wastes of soybean, wheat, rice, corn, cotton,
523 sugarcane bagasse and fruits such as apple, as well as residues from wood industries.
524 The yield of the cellulases represented for 3 enzymes as activities of filter paper (FPase)
525 for cellobiohydrolase or exoglucanase, carboxy methylcellulase (CMCase) for
526 carboxymethylcellulase or endoglucanase and β-glucosidase (BGase) for cellobiase or
527 β-glucosidase. The yield for xylanase is shown for the activity of endo-1-4,-β-xylanase.

528 Soybean hulls have been used as a substrate for the production of cellulases and
529 xylanase through SSF by a mixed culture of *A. oryzae* and *Trichoderma reesei*
530 (Brijwani et al., 2010, 2011). The maximum enzymatic activity obtained was 101 and
531 505 U/g for the carboxymethyl cellulase and xylanase, respectively. Results revealed
532 that the additional use of wheat bran in the substrate positively affected the enzymatic
533 activities obtained through the fermentation process. The SSF process was proven to be
534 a valuable technique for producing a system of cellulases and xylanase enzymes with
535 balanced activities, which were able to efficiently saccharify lignocellulosic biomass.
536 Wheat bran, untreated and without any supplements, as a sole substrate has been also
537 evaluated for the production of cellulolytic enzymes through SSF by using the same

538 microbial culture as inoculum. For instance, Bansal et al. (2012) and Dhillon et al.
539 (2011) achieved an enzymatic activity for carboxymethyl cellulase and xylanase up to
540 about 300 and 2,700 U/g.

541 Wastes of rice, such as the straw and husk, have been recently utilised as
542 substrate materials during the fermentation by *A. oryzae* and *Trichoderma reesei* for the
543 production of enzymes. Rice straw supplemented with wheat bran in the ratio of 3:2
544 resulted in the highest enzymatic activity of up to 132 U/g for carboxymethyl cellulase,
545 whereas the xylanase reached a very high activity of 3,106 U/g (Dhillon et al., 2011). In
546 similar studies, the fungus *Aspergillus fumigatus* has been used as inoculum for the
547 process of fermentation, where a cellulolytic activity of up to 251 U/g for β-glucosidase
548 enzyme and 2,782 U/g for xylanase have been reported (Soni et al., 2010).

549 The feasibility of using apple pomace for cellulase production under SSF was
550 evaluated. The fermentation by *Trichoderma* sp. and a supplement of lactose and corn-
551 steep solid allowed for obtaining of an enzyme activity with a maximum of 7.6 U/g for
552 the exoglucanase (Sun et al., 2010). This activity substantially increased to above 130
553 U/g and an activity of carboxymethyle cellulase of about 150-170 U/g was also
554 reported, using an inoculum of *Aspergillus niger* and especially when lactoserum, which
555 is a source of lactose, was added as a moistening medium (Dhillon et al., 2012 a,b).
556 There was also a high activity of xylanase of 2,619 U/g obtained.

557

558 **4.1.4. Other Enzymes**

559 In addition to the enzymes of lipase, protease, cellulases and xylanase that were
560 discussed in detail in previous sections (4.1-4.3), there are other enzymes obtained
561 through SSF processes. These enzymes include mostly glucoamylase, pectinase and
562 inulinase, which will be discussed in this section. There has been also few research

563 studies performed on the production of certain proteolytic enzyme with a mycotoxin
564 hydrolytic activity, named as ochratoxin A (OTA)-hydrolysing enzyme, and
565 fucoidanase (Abrunhosa et al., 2011; Rodriguez-Jasso et al., 2013, respectively). The
566 latter is able to hydrolyse marine hetero-polysaccharides, called as fucoidans, that have
567 a wide range of biological activity, e.g. anticoagulant, antithrombotic and
568 antiproliferative activities.
569

570 **4.1.4.1. Glucoamylase**

571 Glucoamylase belongs to the amylases enzymes that hydrolyse polysaccharides,
572 such as starch and its degradation products, into molecules of glucose, maltose and
573 dextrin. Amylases are one of the important enzymes in the industry due to their diverse
574 applications, e.g. in the food (bakery products), paper, textiles, pharmaceutical and
575 detergents industries (Botella et al., 2009). These enzymes are classified into α-amylase
576 (EC 3.2.1.1), β-amylase (EC 3.2.1.2) and glucoamylase (EC 3.2.1.3), which is known as
577 amyloglucosidase or γ-amylase (Norouzian et al., 2006). This enzyme, which
578 hydrolyses α-1,4- and α-1,6-glucosidic linkages at the non-reducing ends of
579 polysaccharides, has been recently produced through SSF by using species of the fungal
580 genus *Aspergillus*. Melikoglu et al. (2013) utilised waste bread as a substrate for the
581 production of this enzyme. At optimum process conditions, such as a moisture of 60%
582 and an incubation period of 144 h, it was possible to obtain an activity of glucoamylase
583 of up to 114 U/g. Moreover, protease enzyme was also obtained through the process of
584 fermentation. Accordingly, this study shows that waste bread could be successfully used
585 as a primary substrate for obtaining enzymes. In another study, the production of
586 glucoamylase was presented by using substrates of agro-residues of rice wastes, wheat
587 bran, cotton seeds, corn steep solids, sugarcane bagasse and edible oil cakes (Zambare,

588 2010). The optimisation of the SSF process showed that the highest enzyme activity
589 obtained of ~ 2,000 U/g was with a substrate of wheat bran at a moisture content of 50%
590 and pH of 6, after an incubation of 120 h.
591

592 **4.1.4.2. Pectinases**

593 Pectinases consist of endo- and exo-polygalacturonases (EC 3.2.1.15 and
594 EC 3.2.1.67/82, respectively) and are enzymes that degrade pectin, a complex
595 heteropolysaccharide containing galacturonic acid residues that is a principal
596 component of the middle lamella and primary cell wall of higher plants (El-Sheekh et
597 al., 2009). These enzymes are therefore of great importance to the food industry as they
598 are predominantly used in the clarification of juices, as well as to textile and plant fibre
599 processing industries. In addition, pectinases are applied as food additive for
600 monogastric animals, such as food for pets.

601 An economical and feasible alternative for the production of pectinases is SSF,
602 where it has been found that species of the fungus *Aspergillus* are one of the
603 microorganisms that are able to produce these enzymes during the fermentation. Demir
604 and Tari (2014) found that wheat bran, among various agro industrial wastes, was the
605 most suitable substrate for the production of polygalacturonase using *Aspergillus sojae*.
606 The optimum process conditions that favoured the enzyme production were 4 days of
607 fermentation time at a temperature of 37°C and initial moisture of 62% which resulted
608 in an enzyme activity of up to 536 U/g. In addition, waste products of citrus fruits were
609 used as substrates for the production of pectinases by *Aspergillus niger*. The feasibility
610 of using citrus peels was evaluated in a bench-scale bioreactor (Rodriguez-Fernandez et
611 al., 2011). A mathematical model was applied to determine the different kinetic
612 parameters related to the enzyme production through SSF. The best conditions for

613 pectinase production were at 60% initial moisture and a pH of 5.0 at and 30°C. The
614 maximum activity of pectinase of up to 265 U/g was produced after a fermentation time
615 of 3 days. Ruiz et al. (2012) utilised lemon peel pomace as substrate in a laboratory
616 scale bioreactor at the same condition but with a moisture content of 70%. Results
617 showed that high levels of pectinase activities were obtained, up to a maximum of more
618 than 2,000 U/g, which suggested this process as very promising for pectinase
619 production.
620

621 **4.1.4.3. Inulinase**

622 Inulinases, most commonly known as endo-inulinase (EC 3.2.1.7), are enzymes
623 that hydrolyse inulin into fructose (Chi et al., 2009). The application of these enzymes
624 are in the production of high fructose syrups and fructoligosaccharides, which are
625 compounds with high nutritional values and therefore can be used in low-calorie diets
626 and as a source of dietary fibres in food preparations. Although the inulinases could be
627 obtained from vegetable and animal sources, microorganisms such as *Aspergillus,*
628 *Kluyveromyces* and *Staphylococcus* are the best sources for the commercial production
629 of inulinases. This is due to their easy production and high yields obtained. SSF could
630 be one of the useful approaches for the production of these enzymes. *Kluyveromyces*
631 *marxianus* was utilised as inoculum for the fermentation in recent studies. Dilipkumar et
632 al. (2013) obtained inulinase using pressmud as substrate, where parameters like air
633 flow rate and particle size were optimised, leading to a maximum enzyme activity of ~
634 300 U/g. Sugarcane bagasse was also used as a substrate for the production of the
635 enzyme with a maximum activity of 590 U/g (Astolfi et al., 2011). The optimised
636 temperature and moisture was 30C and 65% respectively, at a fermentation time of 24 h.
637 The study showed the technical feasibility of the process of production of inulinase

638 through SSF.

639

640 **4.2. Process conditions**

641

642 The production of enzymes through SSF required the study of process

643 conditions suitable for the enzyme production, such as temperature, incubation time,

644 pH, moisture content, and types and inoculum levels of pure strain bacteria or fungi

645 added or other sources of microorganisms, e.g. raw sludge.

646 **4.2.1. Lipase**

647 These conditions for the production of lipase were at temperatures of about 20-

648 30 or higher than 45°C, neutral pH and moisture levels of 50 to 75% (Table 3). The

649 microorganisms used as inoculum required for the fermentation are fungi such as

650 *Aspergillus* and *Penicillium*, *Yarrowia* yeast and *Bacillus* and *Pseudomonas* bacteria.

651 Depending on the scale, i.e. amount of substrate used, the optimum temperature for the

652 fermentation was 20 up to 45°C for an incubation period of 2 to 20 days, at pH values of

653 4.5-8 and moisture levels of 50-70%.

654 The optimisation of the production of lipase through SSF has been studied,

655 mainly on a lab-scale, using experimental designs such as Plackett-Burman and central

656 composite designs. Rigo et al (2009) studied the lipase production through SSF by using

657 *Penicillium* sp. and soybean meal as substrate. Initially, the effect of different carbon-to-

658 nitrogen ratios (C/N ratios) on lipase production was evaluated and it was considered

659 6.11 as optimum. Furthermore, the optimum conditions found were at a temperature of

660 20°C for a fermentation period of 5 days. Higher lipase activities were found in a wide

661 range of pH from 4.0 to 9.0, with a pH of 7 as optimum by using the substrate of

662 soybean and *Penicillium* sp. as inoculum. The moisture level used during SSF was 75%.

663 In another study by Mahanta el al. (2008), the production of lipase through SSF by

664 using oil cake and *Pseudomonas aeruginosa* has been optimised for moisture content
665 (29 - 80%), incubation time (24 - 144 h) and pH (6.0 - 8.5). It was observed that the
666 optimum moisture level was at 50%. A higher level of moisture content causes a
667 decrease in porosity, development of stickiness and an increase in the chances of
668 contamination and, accordingly, a decrease in the gas exchange occurs. A lower level of
669 moisture led to sub-optimal microbial growth during the fermentation and a lower
670 degree of the swelling of substrate. The highest yield of the enzyme production was at
671 120 hours of incubation and there was no significant effect of pH on the lipase
672 production. Imanti et al. (2010) have used *Yarrowia* yeast in the fermentation process
673 and reported that the moisture content was optimised at a level of 60%. The incubation
674 time at which the highest lipase production was obtained was 96 hours as longer periods
675 led to the depletion of nutrients, accumulation of toxic end products, and the change in
676 pH or loss of moisture and shorter incubation times were not sufficient for the microbial
677 growth and hence the lipase production. The effect of pH on the lipase production in the
678 presence of *Bacillus subtilis* as inoculum was studied by Chaturvedi et al. (2010). The
679 lipase activity increased when increasing the pH from 6 to 8 and on further increase of
680 pH to 9 and 10, the lipase activity decreased. This shows that the optimum pH for the
681 lipase production was around a pH of 8.

682 In another approach of optimisation, Garlapati et al. (2010) have used modelling
683 combined with optimization as two vital steps for maximizing the efficacy of SSF.
684 Response Surface Methodology (RSM), a statistical technique which generates a
685 mathematical model, coupled with Differential Evolution, which is an optimization
686 technique, have been used. This approach has been used to maximise the lipolytic
687 activity by *Rhizopus oryzae* through SSF. The maximum lipase activity was observed at
688 35°C, 5.28, 60% and 116h for temperature, pH, moisture and incubation time,

689 respectively. These obtained results of optimization were experimentally validated and
690 it was suggested that the developed model and optimization appear to be useful for the
691 design and control of the extracellular lipase production through SSF by using this
692 microorganism.

693 **4.2.2. Protease**

694 The process conditions for the production of proteases were at mesophilic
695 (30°C) up to thermophilic temperatures (50°C), pH levels of 6-8.5 and moisture levels
696 of about 50% (Table 4). Fungi, such as species of *Aspergillus*, and bacteria, mainly
697 *Bacillus subtilis* were the most predominant microorganisms used for the production of
698 proteases. Concerning the scale of the production of the proteases, most of research
699 work was done on a lab scale by using a maximum of 25 g of substrate, where an
700 Erlenmyer falsk was used as a reactor (Merheb-Dini et al., 2010; Zanphorlin et al.,
701 2012; Boyce & Walsh, 2012; Vijayaraghavan et al., 2012). However, in recent research
702 work by Abraham et al. (2013, 2014), a 4.5 l air tight reactor was used, working under
703 near-adiabatic conditions, allowing for the use of 1.25 kg of solid substrate.

704 The initial moisture content required may vary depending upon the type of
705 substrates and microorganisms used. However, it has to be considered that the keystone
706 in SSF is to remove the metabolic heat produced during the fermentation in order to
707 maintain constant moisture levels during the process, when saturated air is used for the
708 cooling. The optimisation of the moisture content has been studied in the processes of
709 SSF for the production of enzymes. For example, Mukherjee et al. (2008) found that
710 50% initial moisture contents of the substrates of potato peels and grass were optimum
711 for the production of protease by *B. subtilis*, whereas the optimum moisture in the case
712 of wheat bran was 30%. Moreover, when *Jatropha* seed cake was used as substrate in
713 the fermentation by *A. oryzae* at different levels of moisture of 45 to 55%, an optimum

714 moisture content of 45% was reported (Thanapimmetha et al., 2012).

715 There has been an optimisation for the source of carbon and nitrogen required by
716 the microorganisms used as inoculum for the production of proteases during the process
717 of SSF. In most processes of SSF, maltose and xylose were the optimum sources for
718 carbon and yeast and beef extract, sodium nitrate and peptone for nitrogen, respectively.
719 Mukherjee et al. (2008) tested several sources of carbon and nitrogen required for the
720 growth of *B. subtilis*. The carbon sources were glucose, fructose, galactose, maltose,
721 sucrose and lactose, being maltose the best source for obtaining the maximum enzyme
722 activity of ~ 1,100 U/g, whereas the activities were sequentially 50 and 400 for
723 galactose and lactose and glucose, fructose and sucrose. Additionally, it was found that
724 beef extract, followed by yeast extract, rather than ammonium salts and casein, and
725 served as the best nitrogen sources producing enzyme activities of ~ 1,400, 1,000, 420
726 and 400 U/g, respectively. In a study by Mahanta et al. (2008) using *Jatropha* seed cake
727 as substrate and *Pseuodomanas aeruginosa* as inoculum, it was also found that the
728 enrichment with maltose compared to other sugars led to an increase in the production
729 of protease. The best nitrogen source was peptone, where ammonium chloride and
730 sodium nitrate were also tested. Recently, carbon sources such as glucose, lactose,
731 trehalose, maltose, xylose and starch, and nitrogen sources such as gelatin, ammonium
732 nitrate, peptone, yeast extract, urea and casein were evaluated for the fermentation by
733 *Halomonas* sp. when a substrate of cow dung was used (Vijayaraghavan et al., 2012).
734 The optimum enzyme production was achieved with a combination of xylose and yeast
735 extract.

736 There have been different statistical methods used for the optimisation of various
737 parameters, rather than individual optimisation, in the processes of SSF for the
738 production of proteases. By adjusting the conditions to optimum levels, the protease

739 production increased up to 5 times compared to non-optimised experiments. Belmessikh
740 et al. (2013) used the experimental designs of Plackett Burman and the Central
741 Composite design for the study of the effect of five enrichment factors (wheat bran,
742 casein, ammonium nitrate, sodium chloride and zinc sulphate) on the enzyme
743 production with a substrate of tomato pomace by *A. oryzae*. It was reported that only
744 two factors, casein and sodium chloride, had a significant effect on the production. This
745 was due to the fact that during the fermentation process, casein could provide intact
746 peptides that were necessary in the induction, whereas sodium chloride might have had
747 a role in the protection of the enzyme from denaturation. In addition, the fermentation
748 time was also optimised to 96 hours for the optimum production of protease. The
749 optimised SSF led to a higher production of protease by about 1.5 times than non-
750 optimised processes. Furthermore, optimization via Taguchi method was performed to
751 evaluate the effect of five factors on the protease production by *A. oryzae*
752 (Thanapimmetha et al., 2012). The effect of three different levels of five factors,
753 including initial moisture content of the substrate used (*Jatropha* seed cake), inoculum
754 size, temperature, type of porous substrate and fermentation time, were examined.
755 These levels were as the following; moisture content (45%, 50% and 55%), inoculums
756 size (1%, 5%, 10%), temperature (25°C, 30°C, 35°C), porous substrate (*Jatropha* oil
757 cake, *Jatropha* oil cake mixed with coconut cake and *Jatropha* oil cake mixed with
758 cassava bagasse, both mixtures with a ratio of 4:1), and time (84, 96 and 108 h). The
759 optimum conditions for the protease production of up to a maximum of 14,273 U/g
760 were 45% moisture content, 10% inoculum size, 30°C incubation temperature, *Jatropha*
761 cake mixed with cassava bagasse as porous substrate at 84 h of fermentation time. This
762 statistical approach provided a satisfactory outcome in defining the optimal conditions,
763 as the optimised process led to an increase of 4.6 times in the protease yield. Rai et al.

764 (2009) reported the application of RSM for the optimization of the media composition
765 for β-keratinase production by *Bacillus subtilis* using chicken-feather as substrate. The
766 factors studied were the fermentation time (24 h, 48 h, 72 h, 96 h and 120 h), initial
767 moisture content of the substrate (33%, 43%, 50%, 60%, 67% and 75%),
768 supplementation with co-carbon sources (glucose, fructose, galactose, maltose, sucrose,
769 lactose and starch at 10%) and co-nitrogen sources (NH_4Cl, $NaNO_3$, yeast extract, beef
770 extract, casein and peptone at 1%) were studied. The optimized culture conditions were
771 at a time of 71h, 50% moisture and with maltose and sodium nitrate as the best co-
772 carbon and co-nitrogen sources, respectively. The results showed that the optimisation
773 led to a 5-fold increase in the enzyme obtained, up to 95.3 U/g, compared to non-
774 optimized conditions.

775 **4.2.3. Cellulases and Xylanases**

776 The temperatures, pH and moisture used for the fermentation were mostly
777 mesophilic (30°C) or slightly thermophilic (45 or 50°C), 4 to 7 and 50 to 80%,
778 respectively (Table 5). The process conditions of pH and moisture were not controlled
779 in a lot of studies. The enzymes are produced by a variety of microorganisms including
780 bacteria, actinomycetes and fungi. However, in recent research works, fungi of the
781 generea *Trichoderma* and *Aspergillus* have been reported as the most important
782 microorganisms used as inocula for the process of SSF. Moreover, the effect of pre-
783 treatment of the substrate used on the production of enzymes was studied.

784 Bansal et al. (2012) evaluated various process parameters during the
785 fermentation by *Aspergillus niger* of agriculture and kitchen waste residues for the
786 production of cellulase complex. The effect of acid and alkali pre-treatment of
787 substrates used was studied. The alkali treatment led to increased yield of enzymes
788 when the wastes, especially potato peels, were utilised as substrate compared to

789 untreated waste materials. This was mainly due to the fact that alkaline pre-treatment
790 dissolve lignin present in the lignocellulosic waste and expose the cellulose and
791 hemicellulose fractions for enzyme and microbial actions. Moreover, untreated
792 substrates contain a variety of nutrients may probably have an inhibitory effect on the
793 fermentation process and thus leading to a lesser production of enzymes. However, in
794 the case of using wheat bran as substrate, the untreated waste induced the highest
795 production of enzyme components. In addition, it was demonstrated that appreciable
796 levels of enzymes could be produced over a wide range of temperatures (20-50°C) and
797 pH (3.0-8.0), with an optimum of 30°C and 6.5 respectively, at initial moisture contents
798 of 60%. These results were in agreement with other research work performed with the
799 aim of producing cellulase. The same optimum pH of 6.5 was found to be the best pH
800 for the enzyme production by the *A. niger* and when using municipal solid wastes as
801 substrate (Gautam et al., 2011). The temperature of 30°C was found to be optimum for
802 incubation of the fungus used as inoculum for the production of enzymes. Brijwani et
803 al. (2010) reported 30°C as the optimum temperature during the fermentation using
804 soybean hulls and wheat bran as substrates by *Aspergillus oryzae* and *Trichoderma*
805 *reesei*, for the production of cellulase and β-glucosidase. This temperature, together
806 with the optimised moisture and pH of 70% and 5 respectively, was used for scale-up
807 processes and a further experimental analysis using novel bioreactor for the production
808 of cellulase complex enzymes (Brijwani et al., 2011). In addition, Sun et al. (2010)
809 found 32°C as the optimum temperature when evaluating the feasibility of using apple
810 pomace as a substrate for cellulase production by *Trichoderma* sp.
811 Thermophilic temperatures of about 50°C were also found to be optimum in the process
812 of SSF for the production of cellulase complex enzymes by *Aspergillus fumigatus*,
813 where lignocellulosic wastes were used as substrate. For instance, Liu et al. (2011)

814 optimised the cultivation conditions and results showed that for cellulases, both endo-
815 and exoglucanase; the best conditions were at a temperature of 50°C, in the presence of
816 an initial moisture of 80% and a pH of 4.0. Soni et al. (2010) reported the optimisation
817 of cellulase production at 45°C, where the culture produced maximal levels of enzyme
818 activity on a medium containing rice straw and beef extract as carbon and nitrogen
819 source, respectively. It was also concluded that optimisation of the process of
820 fermentation by mixing different substrates is a strategy for improvement of the
821 production of cellulase enzymes.
822
823 **4.3. Applications**
824 **4.3.1. Lipase**
825 Lipase enzyme produced by SSF has great biotechnological potential
826 applications, mainly due to the thermophilic and thermostable properties. The enzyme
827 has various applications in oil, pharmaceutical, food and chemical industries (Sharma &
828 Hasan, 2006; Salihu et al., 2012). Recently, lipase produced by SSF was used in
829 synthesis reactions, food applications and treatment of waste water (Table 6).
830 In synthesis reactions, lipases have an important application in the field of
831 bioenergy, especially for the production of biodiesel which is currently an expanding
832 sector in research and on industrial level. Lipase obtained through SSF of sugarcane
833 bagasse and sunflower oil cake by *Burkholderia cepacia* was used to catalyse the
834 synthesis of biodiesel in a fixed-bed reactor (Salum et al., 2010, Liu et al., 2013). This
835 synthesis was through the ethanolysis of soybean oil in a medium free. It was possible
836 to achieve a biodiesel yield of about 90% after 46 hours of reaction. Compared with
837 some commercial lipases, this process avoids the need for expensive processing steps
838 such as enzyme recuperation and immobilization and co-solvent separation and

839 therefore has potential to decrease the costs associated with enzyme-catalyzed synthesis
840 of biodiesel. In addition, lipase produced by SSF, using *Rhizopus* sp. as a thermotolerant
841 fungus, was used as a catalyst for the enzymatic esterification of oleic acid and ethanol
842 (Martinez-Ruiz et al., 2008). Olive oil and perlite were used as an inducer and inert
843 support, respectively. The results demonstrated that the lipase can be successfully used
844 for the synthesis of ethyl oleate, with high etherification rates and substrate conversion,
845 over short reaction periods under conditions when ethanol is in excess. Similarly,
846 Hernandez-Rodriguez et al. (2009) showed that in addition to the lipase produced by
847 *Rhizopus* sp., the enzyme produced by the thermophilic fungus *Rhizomucor* sp. through
848 SSF can be used in the ethyl oleate synthesis reaction.

849 In food applications, lipase produced in SSF by *Rhizopus oryzae* and *Rhizopus*
850 *microsporus*, on a mixture of sugarcane bagasse and sunflower seed meal, was used in
851 interesterification processes of oils to produce fat products with desirable properties
852 (Rasera et al., 2012). This enzyme was able to catalyze the interesterification of a
853 mixture of palm stearin, palm kernel oil and a concentrate of triacylglycerols enriched
854 with omega-3 polyunsaturated fatty acids. This application could be suitable for the
855 production of edible fat products such as margarines and shortenings with low
856 production costs. Another application of the lipase produced by SSF was in the
857 bioremediation of the waste cooking oil (Kumar et al., 2012). The enzyme was
858 produced by *Penicillium chrysogenum* in the presence of wheat bran and waste grease.
859 The results showed that the enzyme could be employed for the bioremediation of used
860 cooking oil such as soya, canola, sunflower and corn oil that contain polyunsaturated
861 oils, which degrade to toxic compounds upon heating.

862 Wastewater has been treated by a lipase enzymatic preparation, with 0.1% (w/v)
863 of solid enzymatic preparation at 30ºC for 24 h, produced by *Penicillium* sp. during

864 solid-state in an anaerobic digester (Rosa et al., 2009). The waste water that was from
865 the dairy industry contained 1200 mg oil and grease per litre. The oil and grease
866 hydrolysis resulted in a final free acid concentration eight times higher than the initial
867 value. This approach showed the importance of the application of enzymatic
868 preparations obtained by SSF in the treatment of fatty wastewater, with high
869 efficiencies, using anaerobic reactors. In addition, Damasceno et al. (2012) used of a
870 lipase produced by SSF with *Penicillium simplicissimum* using babassu cake as
871 substrate. This enzyme, with a concentration of 0.19% (w/v), was combined with a lipid
872 biosurfactant of 114 mg/L, at 33ºC, produced from *Pseudomonas aeruginosa* and used
873 for the methane production by anaerobic treatment of a wastewater with a high fat
874 content from a poultry processing plant. These results showed the synergistic effect of
875 these two bio-products on the hydrolysis of fats from the effluent, with the potential to
876 treat a poultry processing effluent rich in oils and greases, without using a flotation step.
877 Thus, this approach allowed for the elimination of the problem of generating solid waste
878 and enhancing the production of methane.

879 **4.3.2. Protease**

880 Alkaline protease produced by SSF processes has been used as an inclusion in
881 detergent formulations, where the suitability of such an enzyme in this application
882 depends on certain factors such as enzyme stability and compatibility with detergent
883 components (Venugopal et al., 2006). In addition, the enzyme should be thermostable
884 and it is preferred to have the ability to act as a detergent component at different
885 temperatures, including room temperature. Another application of this enzyme was in
886 the process of dehairing of goat and cow hides. This enzymatic process of dehairing
887 could lead to the consumption of less water and harmful chemical reagents used in
888 traditional methods. Therefore the alkaline protease produced through SSF could have

889 potential applications in detergent formulations as well as in the leather processing
890 industry.
891 Mukherjee et al. (2008) applied the produced alkaline protease by *Bacillus*
892 *subtilis* through SSF process, as an additive in laundry detergents. The protease showed
893 the ability to function in a broad range of temperatures, i.e. high thermal resistance and
894 remained active at room temperature, high stability and compatibility with commercial
895 detergents. It was observed that the enzyme retained 33-90% of its original activity at
896 37°C in the presence of commercial detergents. In addition, it was observed that the
897 enzyme obtained was free of any undesirable flavour, which could be advantageous for
898 further applications in food and pharmaceutical industries. Vijayaraghavan et al. (2012)
899 evaluated the effect of alkaline protease obtained through SSF, by *Halomonas* sp., on
900 surfactants, detergents, solvents and goat hide. The enzyme was remarkably stable on
901 surfactants, such as Tween-20, Triton X-100 and Brij-35 displaying 112%, 202% and
902 178% activity respectively. There was also a high stability observed on various
903 commercial detergents and organic solvents, such as ethanol, acetone and methanol,
904 with an activity range from 61 to 224% and 49 to 263%, respectively. In addition, the
905 protease effectively dehaired goat hides. This property of the enzyme found as highly
906 significant since most of commercial dehairing proteases are produced by *Bacillus*
907 bacteria (Subba et al., 2009). Recently, Abraham et al. (2014) have also shown that the
908 protease produced through SSF by the microbial populations developed on the hair solid
909 wastes biodegradation process can be used as a satisfactory alternative for the dehairing
910 of cow hides.
911 Acid proteases produced by SSF processes have applications in the sector of
912 food science and technology, where recently these enzymes have been used in the field
913 of milk and dairy industry. Merheb-Dini et al. (2010) reported the application of an acid

914 protease, produced from a new and local strain of *Thermomucor* and using only wheat
915 bran as substrate, in the hydrolysis of bovine casein of milk and the investigation of its
916 peptide profile obtained for a better understanding of the proteolytic activity of the
917 enzyme. Results revealed that the acid protease exhibited high milk-clotting activity and
918 low proteolytic activity. These properties might encourage future experiments by using
919 this microbial enzyme on cheese production where the enzyme could be used as a
920 substitute for animal rennin. The advantages of using such a microbial protease are
921 mainly related to the low cost production of such an enzyme since in industrial
922 applications the minimisation of costs is of a crucial importance. Another application of
923 the protease in the field of dairy industry was investigated by Boyce and Walsh (2012).
924 The enzyme produced by *Schizopyllum commune* was used to remove an industrial-like
925 milk fouling deposit (containing about 35% minerals) from stainless steel. This
926 experiment imitated the cleaning-in-place (CIP) operations that use acidic and alkaline
927 solutions in cleaning of various equipments used in the dairy industry, especially heat
928 transfer surfaces used during thermal treatments of milk where milk deposits are
929 continuously formed. The results of this research work showed that suitable cleaning
930 was achieved using this enzymatic cleaning procedure without the use of
931 environmentally harmful and corrosive chemicals.

932 **4.3.3. Cellulases and xylanase**

933 Cellulases and xylanase have major and numerous industrial applications, such
934 as in pulp and paper manufacture as well as in the textile industry for polishing of
935 fabrics and laundry detergents for improving fabric softness. For example, Das et al.
936 (2013) used these enzymes, which were produced through optimised processes of SSF,
937 for the deinking of waste pulp of laser printed paper, i.e. mainly the removal of
938 chromophores and hydrophobic compounds. In addition, cellulase enzymes are used in

939 the extraction process of fruit and vegetable juices, starch processing and formulations
940 used for animal feeds (Dhillon et al., 2012a,b; Singhania, et al., 2009). Cellulases have
941 found promising applications for non-specific hydrolysis of chitosan to produce
942 chitooligosaccharides with low molecular weight, which showed high antibacterial
943 activity (Xia et al., 2008).

944 From biotechnological perspectives, the most important and recent application
945 of cellulases and xylanase produced through SSF is in the generation of potentially
946 sustainable energy sources such as sugars and biofuels or, specifically, bio-ethanol.
947 These enzymes are used to hydrolyse cellulosic waste materials to sugars that can be
948 fermented, usually by yeasts, to bioethanol and/or biofuel compounds. It is shown that
949 there is a wide potential to develop a simple biological process to produce ethanol from
950 a variety of lignocellulosic substrates, i.e. by hydrolyzing and fermenting carbohydrates,
951 which are considered as waste materials produced in huge amounts especially in the
952 agro-industrial sector. Liu et al. (2011) directly applied the cellullase enzymes, in their
953 crude form, obtained through SSF processes in the hydrolysis of corn stover. The
954 hydrolysates, reducing sugars obtained, were further used as a substrate for the
955 production of ethanol through the fermentation by *Saccharomyces cerevisiae*. The same
956 biofuel was produced through SSF by sequential saccharification of corn fibre where
957 fermentation by the yeast was allowed leading to the production of ethanol (Rasmussen
958 et al., 2010). SSF followed by buffered anaerobic incubation converted a substantial
959 fraction of corn fibre into harvestable reducing sugars, through the action of cellulases
960 and xylanase obtained from the process of fermentation. The sugars released were
961 fermented with or without the yeast to yield bio-ethanol, in the presence of the
962 cellulolytic fungi used for SSF, where the highest yield was obtained in case of utilising
963 yeast in the process. Several improvements to the production of ethanol were suggested,

964 i.e. optimising the growing conditions such as moisture, pH, temperature and inoculum
965 used. Similarly, in a study by Sukumaran et al. (2009), it was shown that ethanol can be
966 produced using the saccharification of three different feed stock; rice straw, sugarcane
967 bagasse and water hyacinth biomass, followed by the yeast fermentation. It was reported
968 that the highest sugar yield and subsequent ethanol production was in the case of using
969 rice straw. Interestingly, crude unprocessed cellulase obtained, which was not high in its
970 yield, was sufficient to produce ethanol from wheat straw in simultaneous
971 saccharification and fermentation by the yeast (Lever et al., 2013). Therefore, the
972 findings of this research could suggest that using SSF of lignocellulosic wastes may be
973 employed instead of commercial enzyme manufacture, which has usually the
974 disadvantage of a production that is associated with high costs.
975

976 **5. Process Scale-up and Control of SSF**

977 Most research work performed reporting the production of enzymes through
978 SSF use production on a laboratory scale, i.e. batch mode in shaken flasks where few
979 grams of substrate is added. There are technological and operational constrains that
980 limit the scaling-up of the process of fermentation. These constrains are mainly related
981 to the removal of the excess heat formed and the temperature control during
982 fermentation, and also the agitation of solids and handling techniques required for solid
983 substrates (Singhania et al., 2009). Table 7 summarises various aspects of lab scale vs
984 large scale SSF processes. Therefore, large-scale production of enzymes has not yet
985 been proven feasible. However, there are a considerable number of studies focusing on
986 the use of bioreactors in SSF studies at pilot scale for the production of protease and
987 lipase enzymes (Edwinoliver et al., 2010; Santis-Navarro et al., 2011; Abraham et al.,
988 2014). According to a recent review by Thomas et al. (2013), few SSF processes have

been developed at industrial scale: delignification of biomass, dyes bioremediation or *Jatropha* cake detoxification. All these processes have a common objective of enhancing enzymes production, although the enzymes are not targeted as a product, but their effect on the biomass is sought.

In general, there are some basic steps required to scale-up the production of enzymes through SSF (Salihu et al., 2012). Firstly, there is a need to choose suitable microorganisms and substrates, which have been reviewed in the current paper (sections 2 and 3). Secondly, it is required to study various process parameters, e.g. optimisation of moisture, pH and inoculum used. These were discussed in detail concerning the production of lipase, protease and cellulases (sections 4.1-4.3). Thirdly, the scale-up process is performed, which depends mainly on operating conditions (aerations, mass and heat transfer) and process control (Singhania et al., 2009; Li et al., 2013) ~~and optimisation studies~~. A last step might be the study of the technical, environmental and economical viabilities of the process developed.

5.1. Challenges of Process Scale-up

The main aspects of scaling-up the production of enzymes through SSF include challenges and recent advances. SSF is difficult to scale-up due to the three-phase heterogeneous nature of the substrate and the existing gradients inside the reactor in temperature, pH, moisture, oxygen and inoculum (Rodriguez et al., 2010, Salihu et al., 2012). In addition, the absence of free water during the fermentation leads to poor heat removal and accessibility of nutrients resulting in slow microbial growth which might lead low or no production of enzymes obtained at the end of the fermentation process. On the other hand, difficult agitation of solid substrates might occur which leads to physical and chemical heterogeneous distribution. Moreover, the heat generated due to

1014 the metabolic activities of microorganisms is in most cases an inconvenient for
1015 biotechnological processes especially when the optimum growth of microorganisms is
1016 affected and a large part of the enzymes produced during SSF can be heat-denatured.
1017 Another important challenge of scale-up processes is the control of pH within the
1018 system during the fermentation, as this control is required to manage the growth of
1019 microorganisms and the subsequent production of enzymes. Therefore, the control of
1020 heat transfer is one of the major crucial issues in the design and operation of large-scale
1021 SSF fermenters. There is also a need to firstly, monitor on-line the parameters
1022 throughout the process, such as temperature and pH (Ali & Zulkali, 2011). Most
1023 importantly, oxygen consumption and the carbon dioxide evolution are important
1024 measurements because they represent the best way of monitoring the growth of
1025 microorganisms inside the reactor. As a more sophisticated and no-invasive proposal,
1026 Jiang et al. (2012) successfully monitored physical and chemical changes at a 100L
1027 pilot bioreactor using FT-NIR spectroscopy coupled with vector data description, thus
1028 avoiding chemical analysis Secondly, it is needed to adequately mix the substrates
1029 within the fermenter without negatively affecting the growth of microorganisms as well
1030 as the substrates used.
1031

1032 **5.2. Recent Advances on Process Scale-up**

1033 There are a number of bioreactors that have been designed to overcome the
1034 problems of scale up. Commonly used SSF bioreactors are classified into four types
1035 based on the pattern of aeration and/or agitation system employed (Mitchel et al., 2006).
1036 These types are tray, packed-bed, rotating and stirred-drum and forcefully aerated
1037 agitated reactors. Each of these types have their own advantages and disadvantages,
1038 which promoted the necessity to develop novel bioreactors with better design in order to

1039 solve major problems related to the scale-up processes for the production of enzymes
1040 through SSF. There are few recent studies on the overcoming of some of the
1041 disadvantages and limitations regarding the scaling-up processes. An extensive analysis
1042 on the design and operation of bioreactors in SSF has been published in the recent
1043 review by Thomas et al., (2013). Also Yoon et al. (2014) describe in detail the use of
1044 bioreactors for cellulase production.

1045 Scaling up of the process of SSF for the production of enzymes from waste
1046 material was shown to be successful. Edwinoliver et al. (2010) scaled up the process of
1047 lipase production from 10 g up to a level of 3 kg, using *A. niger* as inoculum. The
1048 strategy of scaling-up included the transfer of the optimised process conditions
1049 developed at laboratory level to pilot production level, where temperature and moisture
1050 were online monitored and controlled during the process of fermentation. Another
1051 strategy for the scaling-up was mainly depending on control of air flow intensity as a
1052 key factor during the production of phytase (Rodriguez-Fernandez et al., 2012). At a 10-
1053 fold scale-up, from 2 to 20 kg drum bioreactors with a paddle agitation, the control of
1054 the air flow intensity was required to maintain the temperature constant during the
1055 fermentation, as well as to cool the fermenter at late stages or to allow for the removal
1056 of metabolic heat generated. In addition, the air flow was able to provide oxygen that is
1057 considered as a crucial factor for the growth of microorganisms.

1058 Recently, there were studies on the scaling up of SSF process for the production
1059 of ethanol. Soni et al. (2013) demonstrated that a rotary drum reactor can be directly
1060 scaled up to a larger capacity up to 100 L, by using SSF optimised operating conditions
1061 obtained at laboratory levels, using flask batch modes. The results showed that the
1062 scale-up process is feasible and has commercial potential, especially when the substrate
1063 used, which was sugarcane bagasse, was pre-treated with alkali prior to the process of

1064 fermentation. Interestingly, Li et al. (2013) reported an advanced SSF technology,
1065 which is capable of overcoming most problems associated with the scale-up and large-
1066 scale fermentation processes. An efficient system for the control of mass and heat was
1067 connected to a continuous solid-state rotary drum fermentation reactor, developed by
1068 the research group, where a newly developed microbial strain was used that allowed for
1069 the shortening of the time of fermentation.

1070 Different approaches for an easily scalable process have been reported by Santis-
1071 Navarro et al., (2011) and Abraham et al. (2014). In these approaches, the temperature
1072 was not controlled and enzymes were produced in a batch fermentation process similar
1073 to the composting. The temperature rose to thermophilic values due to heat released and
1074 decreased to ambient values during the fermentation of the readily biodegradable
1075 matter, which has been consumed.

1076 Recently, in other SSF applications, there were also studies on the scaling up of
1077 SSF process for the production of bioethanol. Lin et al. (2013) demonstrated that a
1078 rotary drum reactor can be directly scaled up to a larger capacity up to 100 l, by using
1079 SSF optimized operating conditions obtained at laboratory levels, using flask batch
1080 modes and the thermotolerant yeast *Kluyveromyces marxianus* as an inoculum for the
1081 fermentation. The results showed that the scale-up process is feasible and has
1082 commercial potential, especially when the substrate used, which was sugarcane bagasse,
1083 was pretreated with alkali prior to the process of fermentation. The alkali pretreatment
1084 of this substrate allows for a direct carbon source for the growth of microorganisms in
1085 the SSF system (Chandel et al., 2012). Interestingly, Li et al. (2013) reported an
1086 advanced SSF technology, which is capable of overcoming most problems associated
1087 with the scale-up and large-scale fermentation processes. An efficient system for the
1088 control of mass and heat was connected to a continuous solid-state rotary drum

1089 fermentation reactor, developed by the research group. A newly developed microbial
1090 strain of *Saccharomyces cerevisiae* was used that allowed for the shortening of the time
1091 of fermentation of a substrate of sweet sorghum stems.
1092
1093 **6. Final Remarks**
1094 In summary, this review provides an update on recent studies that are dealing
1095 with the use of SSF for the production of enzymes, and it especially covers issues
1096 related to wastes, microorganisms and scale-up and control of the process of
1097 fermentation. The main focus was on the production of lipases, proteases, cellulases,
1098 xylanases, glucoamylases, pectinases and inulinases. For the process of fermentation,
1099 the inocula used were mostly fungi, like various species of *Aspergillus*, and substrates
1100 were waste materials obtained from the sector of agriculture and food industry. The use
1101 or recycle of these wastes, which are very cheap and highly available in big amounts,
1102 shows a high benefit of SSF from the economical and environmental perspectives.
1103 Accordingly, SSF presents a substantial advantage over SmF, which has been
1104 extensively used for the production of commercially available enzymes since many
1105 decades. Nevertheless, the majority of research conducted on SSF was on lab-scale,
1106 whereas the large scale/commercial production of enzymes is still not developed
1107 because of constraints related to the scaling-up of the process. For instance, the absence
1108 of free water during the process leads to poor mixing and heat removal that results in
1109 slow microbial growth and a subsequent low or no production of enzymes. Recent
1110 developments are based on transfer of the optimised process conditions developed at
1111 laboratory level to larger scales, control of heat and mass transfer and on-line
1112 monitoring of the parameters, such as temperature and pH, where air flow has been
1113 shown to be crucial for the maintenance of constant temperatures and microbial growth.

1114 **References**

1115 Abraham, J., Gea, T. & Sanchez, A. (2013). Potential of the solid-state fermentation of
1116 soy fibre residues by native microbial populations for bench-scale alkaline
1117 protease production. Biochemical Engineering Journal, 74, 15–19.

1118 Abraham, J., Gea, T. & Sanchez, A. (2014). Substitution of chemical dehairing by
1119 proteases from solid- state fermentation of hair wastes. Journal of Cleaner
1120 Production, 74, 191-198.

1121 Abrunhosa, A., Venancio, A. & Teixeira, J. (2011). Optimization of process parameters
1122 for the production of an OTA-hydrolyzing enzyme from *Aspergillus niger* under
1123 solid-state fermentation. Journal of Bioscience and Bioengineering, 112, 351-
1124 355.

1125 Ahmed, W.A. & Salimon, J. (2009). Phorbol ester as toxic constituents of tropical
1126 *Jatropha Curcas* seed oil. European Journal of Scientific Research, 31, 429-436.

1127 Ang, S.K., Shaza, E.M., Adibah, Y., Suraini, A.A. & Madihah, M.S. (2013). Production
1128 of cellulases and xylanase by *Aspergillus fumigatus* SK1 using untreated oil
1129 palm trunk through solid state fermentation.
1130 Process Biochemistry, 48, 1293-1302.

1131 Anwar, A. & Saleemuddin, M. (1998). Alkaline Proteases: a review. Bioresource
1132 Technology, 64, 175-183.

1133 Astolfi, V., Joris, J., Verlindo, R., Oliveira, J.V., Maugeri, F., Mazutti, M.A., de
1134 Oliveira, D. & Treichel, H. (2011). Operation of a fixed-bed bioreactor in batch
1135 and fed-batch modes for production of inulinase by solid-state fermentation.
1136 Biochemical Engineering Journal, 58, 39-49.

1137 Bansal, N., Tewari, R., Soni, R., & Soni, S.K. (2012). Production of cellulases from
1138 *Aspergillus niger* NS-2 in solid state fermentation on agricultural and kitchen

1139 waste residues. Waste Management, 32, 1341-1346.
1140 Belmessikh, A., Boukhalfa, H., Mechakra-Maza, A., Gheribi-Aoulmi, Z. & Amrane, A.
1141 (2013). Statistical optimization of culture medium for neutral protease
1142 production by *Aspergillus oryzae*. Comparative study between solid and
1143 submerged fermentations on tomato pomace. Journal of the Taiwan Institute of
1144 Chemical Engineers, 44, 377–385.
1145 Botella, C., Diaz, A.B., Wang, R., Koutinas, A. & Webb, C. (2009). Particulate
1146 bioprocessing: a novel process strategy for biorefineries. Process Biochemistry,
1147 44, 546–555.
1148 Boyce, A. & Walsh, G. (2012). Identification of fungal proteases potentially suitable for
1149 environmentally friendly cleaning-in-place in the dairy industry. Chemosphere,
1150 88, 211–218.
1151 Brijwani, K., Oberoi, H.S., & Vadlani, P.V. (2010). Production of a cellulolytic enzyme
1152 system in mixed-culture solid-state fermentation of soybean hulls supplemented
1153 with wheat bran. Process Biochemistry, 45, 120-128.
1154 Brijwani, K., Vadlani, P.V., Hohn, K.L., & Maier, D.E. (2011). Experimental and
1155 theoretical analysis of a novel deep-bed solid-state bioreactor for cellulolytic
1156 enzymes production. Biochemical Engineering Journal, 58–59, 110-123.
1157 Chaari, F., Kamoun, A., Bhiri, F., Blibech, M., Ellouze-Ghorbel, R., Ellouz-Chaabouni,
1158 S. (2012). Statistical optimization for the production of lichenase by a newly
1159 isolated *Bacillus licheniformis* UEB CF in solid state fermentation using pea
1160 pomace as a novel solid support. Industrial Crops and Products, 40, 192-198.
1161 Chaturvedi, M., Singh, M., Man C.R. & Pandey, S. (2010). Lipase production from
1162 *Bacillus subtilis* MTCC 6808 by solid state fermentation using ground nut oil
1163 cake as substrate. Research Journal of Microbiology, 5, 725-730.

1164 Chi, Z.M., Chi Z., Liu G.L., Zhang T. & Yue L.X. (2009). Inulinase-expressing
1165 microorganisms and applications of inulinases. Applied Microbiology and
1166 Biotechnology, 11, 81-89.
1167 Chutmanop, J., Chuichucherm, S., Chisti, Y. & Srinophakun, P. (2008). Protease
1168 production by *Aspergillus oryzae* in solid-state fermentation using agroindustrial
1169 substrates. Journal of Chemical Technology and Biotechnology, 83, 1012–1018.
1170 Colla, L.M., Rizzardi, J., Pinto, M.H., Reinehr, C.O., Bertolin, T.E. & Costa, J.A.
1171 (2010). Simultaneous production of lipases and biosurfactants by submerged and
1172 solid-state bioprocesses. Bioresource Technology, 101, 8308-8314.
1173 Csizar, E., Szakacs, G. & Koczk, B. (2007). Biopreparation of cotton fabric with
1174 enzymes produced by solid-state fermentation. Enzyme and Microbial
1175 Technology, 40, 1765–1771.
1176 Cunha, F.M., Esperança, M.N., Zangirolami, T.C., Badino, A.C. & Farinas, C.S. (2012).
1177 Sequential solid-state and submerged cultivation of *Aspergillus niger* on
1178 sugarcane bagasse for the production of cellulose. Bioresource
1179 Technology, 112, 270-274.
1180 Damasceno, F.R.C., Cammarota, M.C. & Freire, D.M.G., 2012. The combined use of a
1181 biosurfactant and an enzyme preparation to treat an effluent with a high fat
1182 content. Colloids and Surfaces B: Biointerfaces, 95, 241-246.
1183 Das, A., Paul, T., Jana, A., Halder, S.K., Ghosh, K., Maity, C., Das Mohapatra, P.K.,
1184 Pati, B.R. & Mondal, K.C. (2013). Bioconversion of rice straw to sugar
1185 using multizyme complex of fungal origin and subsequent production
1186 of bioethanol by mixed fermentation of *Saccharomyces*
1187 *cerevisiae* MTCC173 and *Zymomonas mobilis* MTCC 2428. Industrial Crops
1188 and Products, 46, 217-225.

1189 Demir, H. & Tari, C. (2014). Valorization of wheat bran for the production of
1190 polygalacturonase in SSF of *Aspergillus sojae*. Industrial Crops and Products,
1191 54, 302-309.
1192 Dhillon, G. S., Brar, S. K., Kaur, S., Metahni, S. & M'Hamdi, N. (2012a). Lactoserum
1193 as a moistening medium and crude inducer for fungal cellulase and
1194 hemicellulase induction through solid-state fermentation of apple pomace.
1195 Biomass and Bioenergy, 41, 165-174.
1196 Dhillon, G.S., Kaur, S., Brar, S.K. & Verma, M. (2012b). Potential of apple
1197 pomace as a solid substrate for fungal cellulase and hemicellulase bioproduction
1198 through solid-state fermentation. Industrial Crops and Products, 38, 6-13.
1199 Dhillon, G.S., Oberoi, H.S., Kaur, S., Bansal, S. & Brar, S.K. (2011). Value-addition of
1200 agricultural wastes for augmented cellulase and xylanase production through
1201 solid-state tray fermentation employing mixed-culture of fungi. Industrial Crops
1202 and Products, 34, 1160-1167.
1203 Dilipkumar, M., Rajamohan, N. & Rajasimman, M. (2013). Inulinase production in a
1204 packed bed reactor by solid state fermentation
1205 Carbohydrate Polymers, 96, 196-199.
1206 Du, C., Lin, S., Koutinas, A., Wang, R., Dorado, P. & Webb, C. (2008). A wheat
1207 biorefining strategy based on solid-state fermentation for fermentative
1208 production of succinic acid. Bioresource Technology, 99, 8310–8315.
1209 Edwinoliver, N.G., Thirunavukarasu, K., Naidu, R.B., Gowthaman, M.K., Kambe, T.N.
1210 & Kamini, N.R. (2010). Scale up of a novel tri-substrate fermentation for
1211 enhanced production of *Aspergillus niger* lipase for tallow hydrolysis.
1212 Bioresource Technology, 101, 6791-6796.
1213 Elayaraja, S., Velvizhi, T., Maharani, V., Mayavu, P., Vijayalakshmi, S. &

1214 Balasubramanian, T. (2011). Thermostable -amylase production by *Bacillus*
1215 *firmus* CAS 7 using potato peel as a substrate. African Journal of Biotechnology,
1216 10, 11235-11238.
1217 Ellaiah, P., Srinivasulu, B. & Adinarayana, K. (2002). A review on microbial alkaline
1218 proteases. Journal of Scientific and Industrial Research, 61, 690–704.
1219 El-Sheekh, M.M., Ismail, A.S., El-Abd, M.A., Hegazy, E.M., El-Diwany, A.I. (2009).
1220 Effective technological pectinases by *Aspergillus carneus* NRC1 utilizing the
1221 Egyptian orange juice industry scraps. International Biodeterioration and
1222 Biodegradation 63, 12–18.
1223 Garcia-Gomez, M.J., Huerta-Ochoa, S, Loera-Corral, S. & Prado-Barragán, L.A.
1224 (2009). Advantages of a proteolytic extract by *Aspergillus oryzae* from fish flour
1225 over a commercial proteolytic preparation. Food Chemistry, 112, 604–608.
1226 Garlapati, V.K. & Banerjee, R. (2010). Optimization of lipase production using
1227 differential evolution. Biotechnology and Bioprocess Engineering, 15, 254-260.
1228 Gautam, S.P., Bundela, P.S., Pandey, A.K., Khan, J., Awasthi, M.K. & Sarsaiya, S.
1229 (2011). Optimization for the production of cellulase enzyme from municipal
1230 solid waste residue by two novel cellulolytic fungi. Biotechnology Research
1231 International, 1, 1–8.
1232 Gutarra, M., Godoy, M.G., Maugeri, F., Rodrigues, M.I., Freire, D.M. & Castilho, L.R.
1233 (2009). Production of an acidic and thermostable lipase of the mesophilic fungus
1234 *Penicillium simplicissimum* by solid-state fermentation. Bioresource
1235 Technology, 100, 5249-5254.
1236 Godoy, M.G., Gutarra, M.L., Castro, A.M., Machado, O.L. & Freire, D.M. (2011).
1237 Adding value to a toxic residue from the biodiesel industry: production of two
1238 distinct pool of lipases from *Penicillium simplicissimum* in castor bean waste.

1239 Journal of Industrial Microbiology & Biotechnology, 38, 945-953.

1240 Hasan, F., Shah, A. & Hameed, A. (2006). Industrial applications of microbial lipases.
1241 Enzyme Microbiology and Technology, 39, 235-251.

1242 Hasan, F., Shah, A. & Hameed, A. (2009). Methods for detection and characterization
1243 of lipases: a comprehensive review. Biotechnology Advances, 27, 782–798.

1244 Hernandez-Rodriguez, B,. Hernández-Rodríguez, B., Córdova, J., Bárzana, E. &
1245 Favela-Torres, E. (2009). Effects of organic solvents on activity and stability of
1246 lipases produced by thermotolerant fungi in solid-state fermentation. Journal of
1247 Molecular Catalysis B: Enzymatic, 61, 136-142.

1248 Imandi, S.B., Karanam, S.K. & Garlapati, H.R. (2010). Optimization of Process
1249 Parameters for the Productionof Lipase in Solid State Fermentation by *Yarrowia*
1250 *Lipolytica* from Niger Seed Oil Cake (*Guizotia Abyssinica*). Journal of Microbial
1251 & Biochemical Technology, 2, 28-33.

1252 Jiang, H., Liu, G., Xiao, X., Mei, C., Ding, Y., Yu, S. (2012). Monitoring of solid-state
1253 fermentation of wheat straw in a pilot scale using FT-NIR spectroscopy and
1254 support vector data description. Microchemical Journal, 102, 68-74.

1255 Joshi, C & Khare, SK. (2011). Utilization of deoiled *Jatropha curcas* seed cake for
1256 production of xylanase from thermophilic *Scytalidium thermophilum*.
1257 Bioresoure Technology, 102, 1722-1726.

1258 Kang, S.W., Park, Y.S., Lee, J.S., Hong, S.I. and Kim, S.W. Production of cellulases
1259 and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass.
1260 Bioresoure Technology, 91, 153-156.

1261 Kapilan, R. & Arasaratnam, V. (2011). Paddy Husk as Support for Solid State
1262 Fermentation to Produce Xylanase from *Bacillus pumilus*. Rice Science, 18, 36-
1263 45.

1264 Kumar, A. G., Venkatesan, R., Rao, B.P., Swarnalatha, S. & Sekaran, G. (2009).
1265 Utilization of tannery solid waste for protease production by *Synergistes* sp. in
1266 solid-state fermentation and partial protease characterization. Engineering Life
1267 Science, 9, 66–73.
1268 Kumar, S., Mathur, A., Singh, V., Nandy, S., Khare. S.K. & Negi, S. (2012).
1269 Bioremediation of waste cooking oil using a novel lipase produced by
1270 *Penicillium chrysogenum* SNP5 grown in solid medium containing waste grease.
1271 Bioresource Technology, 120, 300-304.
1272 Lever, M., Goen, Ho. & Cord-Ruwisch, R. (2013). Simplifying cellulase production by
1273 using environmental selection pressures and recycling substrate. Environmental
1274 Technology, 34, 471–475.
1275 Li, S., Li, G., Zhang, L., Zhou, Z., Han, B., Hou, W., Wang, J. & Li, T. (2013). A
1276 demonstration study of ethanol production from sweet sorghum
1277 stems with advanced solid state fermentation technology. Applied
1278 Energy, 102, 260-265.
1279 Liu, Y., Li, C., Meng, X. & Yan, Y. (2013). Biodiesel synthesis directly catalyzed by
1280 the fermented solid of *Burkholderia cenocepacia* via solid state fermentation.
1281 Fuel Processing Technology, 106, 303–309.
1282 Liu, D. et al. (2011). Thermostable cellulase production of Aspergillus fumigatus Z5
1283 under solid-state fermentation and its application in degradation of agricultural
1284 wastes. International Biodeterioration and Biodegradation, 65, 717-725.
1285 Liu, J. & Yang, J (2007). Cellulase Production by *Trichoderma koningii* AS 3.4262 in
1286 Solid-State Fermentation Using Lignocellulosic Waste from the Vinegar
1287 Industry. Food Technology and Biotechnology, 45, 420-425.
1288 Mahanta, N., Gupta, A. & Khare, S.K. (2008). Production of protease and lipase by

1289 solvent tolerant *Pseudomonas aeruginosa* PseA in solid-state fermentation using
1290 *Jatropha curcas* seed cake as substrate. Bioresource Technology, 99, 1729–
1291 1735.
1292 Mamma, D., Kourtoglou, E. & Christakopoulos, P. (2008). Fungal multienzyme
1293 production on industrial by-products of the citrus-processing industry.
1294 Bioresource Technology, 99, 2373–2383.
1295 Martinez-Ruiz, A., García, H.S., Saucedo-Castañeda, G. & Favela-Torres, E. (2008).
1296 Organic Phase Synthesis of Ethyl Oleate Using Lipases Produced by Solid-state
1297 Fermentation. Applied Biochemistry and Biotechnology, 151, 393-401.
1298 Mekala, N., Singhania, R., Sukumaran, R. & Pandey, A. (2008). Cellulase production
1299 under solid-state fermentation by *Trichoderma reesei* RUT C30: statistical
1300 optimization of process parameters. Applied Biochemistry and Biotechnology,
1301 151, 121-131.
1302 Melikoglu, M., Lin, C. & Webb, C. (2013). Stepwise optimisation of enzyme
1303 production in solid state fermentation of waste bread pieces. Food and
1304 Bioproducts Processing, in press, http://dx.doi.org/10.1016/j.fbp.2013.04.008.
1305 Merheb-Dini, C., Gomes, E., Boscolo, M. & da Silva, R. (2010). Production and
1306 characterization of a milk-clotting protease in the crude enzymatic extract from
1307 the newly isolated *Thermomucor indicae-seudaticae* N31 (Milk-clotting
1308 protease). Food Chemistry, 120, 87–93.
1309 Mitchell, D.A, Krieger, N. & Berovic, M. (2006) Solid-State Fermentation Bioreactors:
1310 Fundamentals of Design and Operation. Heidelberg, Germany: Springer.
1311 Mukherjee, A.K, Adhikari, H. & Rai, S.K. (2008). Production of alkaline protease by a
1312 thermophilic *Bacillus subtilis* under solid-state fermentation (SSF) condition
1313 using *Imperata cylindrica* grass and potato peel as low-cost medium:

1314 Characterization and application of enzyme in detergent formulation
1315 Biochemical Engineering Journal, 39, 353–361.
1316 Nalawade, P.M., Kamble, J.R, Late, A.M., Solunke, K.R. & Mule, M.B. (2009). Studies
1317 on Integrated Use of Tannery Wastewater, Municipal Solid Waste and Fly Ash
1318 Amended Compost on Vegetable Growth. International Journal of Agriculture
1319 Sciences, 1, 55-58.
1320 Narra, M., Dixit, G., Divecha, J., Madamwar, D. and Shah AR. (2012). Production of
1321 cellulases by solid state fermentation with Aspergillus terreus and enzymatic
1322 hydrolysis of mild alkali-treated rice straw. Bioresource Technology, 121, 355-
1323 361.
1324 Ncube, T., Howard, R.L., Abotsi, E.K., van Rensburg, J. & Ncube, I. (2012). *Jatropha*
1325 *curcas* seedcake as substrate for production of xylanase and cellulase by *Aspergi*
1326 *llus niger* FGSCA733 in solid-state fermentation. Industrial Crops and Products,
1327 37, 118-123.
1328 Nimkar, M.D., Deogade, N.G. & Kawale, M. (2010). Production of α-amylase from
1329 *Bacillus subtilis* & *Aspergillus niger* using different agro waste by solid state
1330 fermentation. Asiatic Journal of Biotechnology Research, 1, 23-28.
1331 Norouzian, D., Akbarzadeh, A., Scharer J.M. & Young M.M. (2006). Fungal
1332 glucoamylases. Biotechnology Advances, 24, 80–85.
1333 Pandey, A. (2003). Solid-state fermentation. Biochemical Engineering Journal, 13, 81-
1334 84.
1335 Paranthaman, R., Alagusundaram, K. & Indhumathi, J. (2009). Production of Protease
1336 from Rice Mill Wastes by *Aspergillus niger* in Solid State Fermentation. World
1337 Journal of Agricultural Sciences, 5, 308-312.
1338 Pirota, R., Tonelotto, M., Delabona, P., Fonseca, R., Paixão, D., Baleeiro, F., Neto, V.,

1339 Farinas, C. (2013). Enhancing xylanases production by a new Amazon Forest
1340 strain of *Aspergillus oryzae* using solid-state fermentation under controlled
1341 operation conditions. Industrial Crops and Products, 45, 465-471.
1342 Queiroga, A. C., Pintado, M.E. & Malcata, X. (2012). Search for novel proteolytic
1343 enzymes aimed at textile and agro-industrial applications: An overview of
1344 current and novel approaches. Biocatalysis and Biotransformation, 1, 154–169.
1345 Ramachandran, S., Singh, S.K., Larroche, C., Soccol, C.R. & Pandey, A. (2006). Oil
1346 cakes and their biotechnological applications – a review. Bioresoure
1347 Technology, 93, 169-174.
1348 Rasera, K. Rasera, K., Osório, N., Mitchell, D., Krieger, N. & Ferreira-Diasa, S. (2012).
1349 Interesterification of fat blends using a fermented solid with lipolytic activity.
1350 Journal of Molecular Catalysis B: Enzymatic, 76, 75-81.
1351 Rasmussen, M.L., Shrestha, P., Khanal, S., Pometto, A.L. & van Leeuwen, H. (2010).
1352 Sequential saccharification of corn fiber and ethanol production by the brown rot
1353 fungus *Gloeophyllum trabeum*. Bioresource Technology, 101, 3526–3533.
1354 Rai, S.K., Konwarh, R. & Mukherjee, A.K. (2009). Purification, characterization and
1355 biotechnological application of an alkaline-keratinase produced by *Bacillus*
1356 *subtilis* RM-01 in solid-state fermentation using chicken-feather as substrate.
1357 Biochemical Engineering Journal, 45, 218–225.
1358 Rigo, E., Ninow J.L., Polloni, A.E., Remonatto, D., Arbter, F., Vardanega, R., Oliveira,
1359 D., Treichel, H., Luccio, M. (2009). Improved lipase biosynthesis by a newly
1360 isolated *Penicillium* sp. grown on agricultural wastes. Industrial Biotechnology,
1361 5, 119-126.
1362 Rodriguez, L.A., Toro, M.E., Vazquez, F., Correa-Daneri, M.L., Gouiric, S.C. &
1363 Vallejo, M.D. (2010). Bioethanol production from grape and sugar beet pomaces

1364 by solid-state fermentation. International Journal of Hydrogen Energy, 35, 5914-
1365 5917.
1366 Rodriguez-Fernandez, D.E., Rodriguez-Leon, J.A., de Carvalho, J.C., Sturm, W.C. &
1367 Soccol, C.R. (2011). The behavior of kinetic parameters in production of
1368 pectinase and xylanase by solid-state fermentation. Bioresource Technology,
1369 102, 10657-10662.
1370 Rodriguez-Fernandez, D.E., Parada, J.L., Medeiros, A., de Carvalho, J.C., Lacerda,
1371 L.G., Rodríguez-León, J.A. & Soccol, C.R. (2013). Concentration by
1372 ultrafiltration and stabilization of phytase produced by solid-state fermentation.
1373 Process Biochemistry, 48, 374-379.
1374 Rodriguez-Jasso, R.M., Mussatto, S.I., Sepulveda, L., Agrasar, A., Pastrana, L.,
1375 Aguilar, C.N. & Texeira, J.A. (2013). Fungal fucoidanase production by solid-
1376 state fermentation in a rotating drum bioreactor using algal biomass as substrate.
1377 Food and Bioproducts Processing, in press,
1378 http://dx.doi.org/10.1016/j.fbp.2013.02.004.
1379 Rosa, D.R., Duarte, I.C., Saavedra, N.K., Varesche, M.B., Zaiat, M., Cammarota, M.C.
1380 & Freire, D.M. (2009). Performance and molecular evaluation of an anaerobic
1381 system with suspended biomass for treating wastewater with high fat content
1382 after enzymatic hydrolysis. Bioresource Technology, 100, 6170-6176.
1383 Ruiz, H.A., Rodriguez-Jasso, R.M., Rodriguez, R., Contreras-Esquivel, J.C. & Aguilar,
1384 C.N. (2012). Pectinase production from lemon peel pomace as support and
1385 carbon source in solid-state fermentation column-tray bioreactor. Biochemical
1386 Engineering Journal, 65, 90-95.
1387 Salihu, A., Alam, Z., Abdulkarim, I. & Salleh, H. (2012). Lipase production: An insight
1388 in the utilization of renewable agricultural residues. Resources, Conservation

1389 and Recycling, 58, 36-44.
1390 Salum, T.F.C., Villeneuve, P., Barea, B., Yamamoto, C.I., Côcco, L.C., Mitchell, D.A.
1391 & Krieger, N. (2010). Synthesis of biodiesel in column fixed-bed bioreactor
1392 using the fermented solid produced by *Burkholderia cepacia* LTEB11. Process
1393 Biochemistry, 45, 1348-1354.
1394 Sandhya, C., Sumantha, A., Szakacs, G. & Pandey, A. (2005). Comparative evaluation
1395 of neutral protease production by *Aspergillus oryzae* in submerged and solid-
1396 state fermentation. Process Biochemistry, 40, 2689–2694.
1397 Santis-Navarro, A., Gea, T., Barrena, R. & Sanchez, A. (2011). Production of lipases by
1398 solid state fermentation using vegetable oil-refining wastes. Bioresource
1399 Technology, 102, 10080–10084.
1400 Sharma, R., Chisti, Y. & Banerjee, U.C. (2001). Production, purification,
1401 characterization, and applications of lipases. Biotechnology Advances, 19, 627–
1402 662.
1403 Singhania, R.R., Patel, A.K., Soccol, C. & Pandey, A. (2009). Recent advances in solid-
1404 state fermentation. Biochemical Engineering Journal, 44, 13-18.
1405 Soni, R., Nazir, A. & Chadha, B. S. (2010). Optimization of cellulase production by a
1406 versatile *Aspergillus fumigatus fresenius* strain (AMA) capable of efficient
1407 deinking and enzymatic hydrolysis of *Solka* floc and bagasse. Industrial Crops
1408 and Products, 31, 277-283.
1409 Subba, C., Sathish, T., Ravichandra, P. & Prakasham, R.S. (2009). Characterization of
1410 thermo- and detergent stable serine protease from isolated *Bacillus circulans* and
1411 evaluation of eco-friendly applications. Process Biochemestry, 44, 262–268.
1412 Subramaniyam R. & Vimala R. (2012). Solid state and submerged fermentation for the
1413 production of bioactive substances: A comparative study. International Journal

1414 of Science and Nature, 3, 480-486.

1415 Rodriguez-Fernandez, D.E., Rodríguez-León J.A., de Carvalho, J.C., Karp, S.G., Sturm,
1416 W., Parada, J.L. & Soccol, C.R. (2012). Influence of airflow intensity on phytase
1417 production by solid-state fermentation. Bioresource Technology, 118, 603-606.

1418 Sukumaran, R.K., Patel, A.K., Larroche, C. & Pandey, A. (2010). Advancement and
1419 comparative profiles in the production technologies using solid-state and
1420 submerged fermentation for microbial cellulases. Enzyme and Microbial
1421 Technology, 46, 541-549.

1422 Sukumaran, R.K., Singhania, R.R., Mathew, G.M. & Pandey, A. (2009). Cellulase
1423 production using biomass feed stock and its application in lignocellulose
1424 saccharification for bio-ethanol production. Renewable Energy, 34, 421-424.

1425 Sumantha, A., Larroche, C. & Pandey, A. (2006). Microbiology and industrial
1426 biotechnology of food grade proteases-a perspective. Food Technology and
1427 Biotechnology, 44, 211–220.

1428 Sun, H., Xiangyang, G, Zhikui, H. & Peng, M. (2010). Cellulase production by
1429 *Trichoderma* sp. on apple pomace under solid state fermentation. African
1430 Journal of Biotechnology, 9, 163-166.

1431 Thanapimmethaa, A., Luadsongkrama, A., Titapiwatanakunc, B. & Srinophakun, P.
1432 (2012). Value added waste of *Jatropha curcas* residue: Optimization of protease
1433 production in solid state fermentation by Taguchi DOE methodology. Industrial
1434 Crops and Products, 37, 1– 5.

1435 Thomas, L., Larroche, C., Pandey, A. (2013). Current developments in solid-state
1436 fermentation. Biochemical Engineering Journal, 81, 146-161.

1437 Turk, B. (2006). Targeting proteases: successes, failures and future prospects. Nature
1438 Reviews. Drug Discovery, 5, 785–798.

1439 Vishwanatha, K.S., Appu Rao, A.G. & Sridevi, A.S. (2009). Characterisation of acid
1440 protease expressed from *Aspergillus oryzae* MTCC 5341. Food Chemistry, 114,
1441 402-407.
1442 Vijayaraghavana, P. & Vincent, G.S.P. (2012). Cow dung as a novel, inexpensive
1443 substrate for the production of a halo-tolerant alkaline protease by *Halomonas*
1444 sp. PV1 for eco-friendly Applications. Biochemical Engineering Journal, 69,
1445 57– 60.
1446 Venugopal, M. & Saramma, A. (2006). Characterization of alkaline protease from
1447 *Vibrio fleevialis* strain VM10 isolated from a mangrove sediment simple and its
1448 application as a laundry detergent additive. Process Biochemistry, 41, 1239–
1449 1243.
1450 Xia, W., Liu, P. & Liu, J. (2008). Advance in chitosan hydrolysis by non-specific
1451 cellulases. Bioresource Technology, 99, 6751-6762.
1452 Yoon, L.W., Ang, T.N., Ngoh, G.C., Chua, A.S.M. (2014) Fungal solid-state
1453 fermentation and various methods of enhancement in cellulase production.
1454 Biomass and Bionergy, 67, 319-338.
1455 Zambare, V. (2010). Solid State Fermentation of *Aspergillus oryzae* for Glucoamylase
1456 Production on Agro residues. International Journal of Life Science, 4, 16-25.
1457 Zanphorlin, L.M., Cabral, H., Arantes, E., Assis, D., Juliano, L., Da-Silva, R., Gomesa,
1458 E. & Bonilla-Rodrigueza, G.O. (2011). Purification and characterization of a
1459 new alkaline serine protease from the thermophilic fungus *Myceliophthora* sp.
1460 Process Biochemistry, 46, 2137–2143.
1461 Zhang, Y. (2008). Reviving the carbohydrate economy via multi-product lignocellulose
1462 biorefineries. Journal of Industrial Microbiology Biotechnology, 35, 367-375.
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1464 **Tables**

1465

1466 Table 1. A comparison between SSF and SmF for the production of enzymes, showing
1467 the main advantages and disadvantages

1468

Factor	SSF	SmF
Substrate	No-cost materials, e.g. waste products	Very expensive media ingredients
Inoculum	Not necessary	Essential
Aseptic conditions	Not needed	Essential
Moisture	No free water	Liquid media required
Agitation	Very difficult	Easy
Process Control (T, pH)	Difficult	Easy
Contamination	Less chance	High risk
Enzyme Yield	Very high	Low
Downstream processing	Easy, cheap, not time consuming	Very difficult, very expensive
Liquid waste	Not produced	High quantities
Scale up	Difficult, new design equipments needed	Easy, industrial equipments available
Volume and costs of equipments	Small reactors can be used, low costs	Large-scale reactors required, very high costs

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Table 2. Types of various waste materials used in the SSF processes for the production of enzymes, including the microorganisms if present in these processes.

Category of waste	Types of waste	Microorganisms	Enzymes	References
Wastes of animal origin	Fish flour	*Aspergillus niger*	Proteases	Garcia-Gomez et al., 2009
	Chicken feather	*Bacillus subtilis*		Rai et al., 2009
	Cow dung	*Halomonas* sp.		Vijayaraghavan et al., 2012
	Hair waste	nc		Abraham et al., 2013, 2014
	Tannery solid waste	*Synerggistes* sp.		Kumar et al., 2009
Wastes of plant origin and food industry	Oil wastes	*Aspergillus niger*, *Bacillus subitilis, Penicillium* spp.	Lipases, Amylase	Colla et al., 2010; Chaturvedi et al., 2010; Edwinoliver et al., 2010, Godoy et al., 2011
	Wheat wastes	*Aspergillus oryzae*	Protease	Thanapimmeth et al., 2012
		Rhizopus oryzae	Lipases	Garlapati & Banerjee, 2010
		Apergillus spp., *Trichoderma reesei*	Cellulases, Xylanase	Dhillon et al., 2011; Bansal et al., 2012 ; Farinas et al., 2011 ; Pirota et al., 2013
		Aspergillus sojae	Pectinase	Demir & Tari, 2014
	Rice wastes	*Apergillus* spp.	Cellulase	Soni et al., 2010; Dhillon et al., 2011
		Aspergillus niger	Proteases	Paranthaman et al., 2009
		Aergillus fumigatus	Cellulase	Liu et al., 2011
		Aspergillus terreus	Cellulase	Narra et al., 2012
		Bacillus pumilus	Xylanase	Kapilan & Arasaratnam, 2011
	Soy wastes	*Aspegillus niger*, *Penicillium* sp.	Lipases	Rigo et al., 2009; Colla et al., 2010
		nc	Proteases	Abraham et al., 2013
		Aspergillus oryzae, *Trichoderma reesei*	Cellulases, Xylanase	Brijwani et al., 2010, 2011
	Peels of fruits and vegetables	*Aspergillus niger*	Cellulase, xylanase, pectinase, phytase	Rodriguez-Fernandez , et al., 2011, 2012, 2013, Mamma et al., 2008

Sugarcane bagasse	*Bacillus subtilis*	Proteases	Mukherjee et al., 2008, 2009
	Bacillus firmus	Amylase	Elayaraja et al., 2011
	Aspergillus niger	Cellulases	Cunha et al., 2012, Mekala et al., 2008
	Kluvyromyces marxianus	Inulinases	Astolfi et al., 2011
Cotton wastes	*Aspergillus* spp.	Hydrolytic & oxidative enzymes	Csiszar et al., 2007; Liu et al., 2011
Pomace of fruits and vegetables	*Aspergillus* spp.	Proteases, cellulases	Belmessikh et al., 2013; Dhillon et al., 2012a,b
	Bacillus licheniformis	Lichenase	Chaari et al., 2012
Grape waste	*Pleurotus eryngii*	Lignolytic enzymes	Akpinar et al, 2012
Oil palm trunk	*Aspergillus fumigatus*	Cellulases, Xylanses	Ang et al., 2013
Waste bread	*Aspergillus awamori*	Amylases, proteases	Melikoglu et al., 2013
Agrowastes	*Bacillus subtilis*	Amylase	Mukherjee et al., 2009; Nimkar et al., 2010
Winterisation residues, sludge	nc	Lipases	Santis-Navarro et al., 2011

nc: not controlled

Table 3. Substrate, process conditions, microorganisms and enzyme activity of lipase produced by SSF

Substrate	Process conditions				Microorganisms	Enzyme Activity (U/g)	Reference
Type	Amount (g)	T (°C)	pH	Moisture (%)*			
Coconut oil cake, wheat bran, wheat rawa	Up to 3000	30	nc	60	*Apergillus niger*	745.7	Edwinoliver et al., 2010
Mix of oil cakes	10	30	8	70	*Bacillus subtilis*	4.5	Chaturvedi et al., 2010
Niger seed oil cake	10	30	6.4-6.8	60	*Yarrowia lipolytica*	26.42	Imandi et al., 2010
Babassu cake	10	30	nc	70	*Penicillium simplicissimum*	314	Gutarra et al., 2009
Castor bean waste	20	30	nc	nc	*Penicillium simplicissimum*	80.24	Godoy et al., 2011
Soybean meal	10	20	7	75	*Peniciullium* sp.	317	Rigo et al., 2009
Soybean meal, rice husk	50	30	4.5	60	*Apergillus niger*	25.22	Colla et al., 2010
Jatropha curcas seed cake	5	30	7	50	*Pseudomonas aeruginosa*	312.5	Mahanta et al., 2008
Wheat bran	4	35	5.28	60	*Rhizopus oryzae*	96.52	Garlapati & Banerjee, 2010
Winterisation residue, sludge	2,500	Higher than 45	7	50	nc	120,000	Santis-Navarro et al., 2011

nc: not controlled
* Initial moisture content of the substrate(s) used

Table 4. Substrate, process conditions, microorganisms and enzyme activity of protease produced by SSF.

Substrate		Process conditions			Microorganisms	Max enzyme activity (U/g)	Reference
Type	Amount (g)	T (ºC)	pH	Moisture (%)*			
Potato peel, grass	100	50	8	50	*Bacillus subtilis*	2,383	Mukherjee et al., 2008
Fish flour, polyurethane foam	30	30	nc	50	*Aspergillus niger*	120.78	Garcia-Gomez et al., 2009
Soy fiber residues	1250	nc	8.5	40-60	Nc	47,331	Abraham et al., 2013
Tomato pomace	10	30	6.8	60	*Aspergillus oryzae*	21,309	Belmessikh et al., 2013
Jatropha seed cake	5	30	6	50	*Pseudomonas aeruginosa*	1818	Mahanta et al., 2008
	25	30	nc	45	*Aspergillus oryzae*	14,273	Thanapimmetha et al., 2012
Wheat bran, casein	5	45	nc	60	*Thermomucor indicae-seudaticae*	167.6	Merheb-Dini et al., 2010
Wheat bran	5	nc	nc	nc	*Myceloiphthora* sp.	19.8	Zanphorlin et al., 2011
	10	24-40	nc	50	14 Fungal strains	5.05	Boyce & Walsh, 2012
	few grams	27	acidic	60	*Aspergillus oryzae*	8.3×10^3	Vishwanatha et al., 2009
Chicken feather	5	50	8	50	*Bacillus subtilis*	95.3	Rai et al., 2009
Cow dung	5	37	8	50	*Halomonas* sp.	1,351	Vijayaraghavan et al., 2012
Tannery solid waste	5	37	6	50	*Synergistes* sp.	755	Kumar et al., 2009
Hair wastes	1400	nc	8.5	40-60	nc	56,270	Abraham et al., 2014

nc: not controlled
* Initial moisture content of the substrate(s) used

Table 5. Substrate, process conditions, microorganisms and enzyme activity of cellulases and xylanase produced by SSF.

Substrate		Process conditions			Microorganisms	Enzymes		Reference
Type	Amount (g)	T (°C)	pH	Moisture (%)*		Type	Max activity (U/g)	
Soybean hulls	10,240	30	5	70	*Aspergillus oryzae*, *Trichoderma reesei*	FPase CMCase Bgase Xylanase	5.39 58.57 18.36 242	Brijwani et al., 2011
Soybean hulls, wheat bran	100	30	5	70	*Aspergillus oryzae*, *Trichoderma reesei*	FPase CMCase Bgase Xylanase	10.78 100.67 10.71 504.9	Brijwani et al., 2010
Rice straw	5	45	7	75	*Aspergillus fumigatus*	FPase CMCase Bgase Xylanase	3.37 98.5 250.9 2,782	Soni et al., 2010
Rice straw, wheat bran	10	30	nc	nc	*Aspergillus oryzae*, *Trichoderma reesei*	FPase CMCase Bgase Xylanase	35.8 132.34 33.71 3,106	Dhillon et al., 2011
Apple pomace	10	30	nc	70	*Trichoderma* sp.	FPase	7.6	Sun, 2010
Apple pomace, rice husk	40	30	nc	75	*Aspergillus oryzae*	FPase CMCase	133.68 172.3	Dhillon et al., 2012b
Wheat bran	5	30	nc	40-57	*Trichoderma reesei*	FPase CMCase Bgase	4.55 135.44 21.49	Sukumaran et al., 2009
Rice husk, wheat bran	30	30	nc	nc	nc	FPase CMCase	6.3 26	Hu et al., 2011
Apple pomace, lactoserum	40	30	nc	nc	*Aspergillus niger*	FPase	130.4	Dhillon et al., 2012a

						CMCase	148.9	
						Bgase	90.1	
						Xylanase	2,619	
Rice husk, wheat bran & straw, corncob, kitchen wastes	5	30	6.5	60	*Aspergillus oryzae*, *Trichoderma reesei*	FPase CMCase Bgase	15.9 297 33.2	Bansal et al., 2012
Rice, wheat & cotton straw, corncob	20	50	4	80	*Aspergillus fumigatus*	FPase CMCase	144.6 526.3	Liu et al, 2011

FPase: Filter paper activity for cellulase
CMCase: Carboxy methyle cellulase
BGase: β-glucosidase

nc: not controlled
* Initial moisture content of the substrate(s) used

Table 6. Applications of lipase, protease and cellulases and xylanase enzymes

Enzyme	Applications	References
Lipase	Oil, pharmaceutical, food and chemical industries: synthesis reactions (biodiesel production), food applications (interesterification of oils) and treatment of waste water	Sharma & Hasan, 2006; Salihu et al., 2012; Damasceno et al., 2012 ; Raser et al., 2012
Protease	Detergent formulations, dehairing (leather industry), dairy industry	Venugopal et al., 2006; Abraham et al., 2014 ; Merheb-Dini et al., 2010
Cellulases and Xylanase	Paper manufacture, textile industry, bioethanol production	Das et al. (2013); Liu et al. (2011) ; Lever et al. (2013)

Table 7. Conditions needed for lab scale vs. large or commercial scale SSF

Condition	Lab scale	Large Scale
pH control	Possible through pH adjustment	Not possible
T control, Heat Removal	Easy, Possible through controlled temperature water bath	Possible by managing aeration, costly, possible presence of T gradients along the solid matrix in bed reactors.
Handling of solid substrates	Very easy	Very difficult
Inoculation	Easy, not expensive	Very high costs, difficult homogenization.
Agitation	Very easy	Possible in some reactors configurations such as rotatory drums, high energy cost.
Aeration	Sufficient	Moderate-high, high energy costs