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Review

Waste biorefineries using filamentous ascomycetes fungi: Present status and future prospects



Xylanases

Cellulases

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Gluconic acid

Itaconic acid

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HIGHLIGHTS

G R A P H I C A L A B S T R A C T

Ethanol

 $\mathbf{\Lambda}$

Metabolites

Citric acid

Animal feed <

Human

consumption

- Filamentous ascomycetes can be cores of lignocellulosic "waste biorefineries".
- Filamentous ascomycetes are sources of cellulases and xylanases.
- Aspergillus spp. are producers of citric, gluconic and itaconic acids.
- Fusarium spp. and Neurospora spp. are potential good ethanol producers.
- Protein- and lipid-rich biomass as a second product of "waste biorefineries".

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АВЅТ Я А С Т

Filamentous ascomycetes fungi have had important roles in natural cycles, and are already used industrially for e.g. supplying of citric, gluconic and itaconic acids as well as many enzymes. Faster human activities result in higher consumption of our resources and producing more wastes. Therefore, these fungi can be explored to use their capabilities to convert back wastes to resources. The present paper reviews the capabilities of these fungi in growing on various residuals, producing lignocellulosedegrading enzymes and production of organic acids, ethanol, pigments, etc. Particular attention has been on *Aspergillus, Fusarium, Neurospora* and *Monascus* genera. Since various species are used for production of human food, their biomass can be considered for feed applications and so biomass compositional characteristics as well as aspects related to culture in bioreactor are also provided. The review has been further complemented with future research avenues.

➤ Fatty acids

Amino acids

Organic waste materials

Biorefiner

 \mathbf{v}

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1. Introduction

The cycles of e.g. carbon and nitrogen in nature have been tremendously affected by human activities. In nature, there is no waste, but just the resources for other species. However, the rapid exploration rate of Earth's resources has been steadily increased, giving rise to depletion concerns and to a rapid generation of waste products. The exploration of fossil fuels is of special relevance to the carbon cycle, since they have been the main source of energy and chemicals worldwide and probably the main culprit of the world rising CO₂ emissions (Ferreira, 2015). Similar facts are also valid for other elements such as nitrogen, phosphorus, and sulphur due to extensive human exploration of natural resources other than fossil fuels. Therefore, there is a need to find alternative sources of fuels and chemicals, to improve the processes involving the use of resources in order to produce more from less, and to cope with the increasing amount of waste materials via their conversion to value-added products in a "waste biorefinery" concept.

Research has armed society with diversified tools to give increasingly higher impetus to the production of fuels and chemicals within a biorefinery context as an alternative to refineries based on fossil fuels. Among the range of strategies developed, the biological treatment of a wide range of substrates to produce a panoply of value-added products occupies a relevant position. Microorganisms, as metabolically versatile biocatalysts, have been playing a crucial role in this regard where a variety of products including enzymes, antibiotics, organic acids, and human food have already been successfully produced (Gibbs, 2000). Thus, microorganisms are already directly contributing to the world economy. Nevertheless, as new microorganisms are being isolated worldwide, the range of substrates that can be used as well as the range of products that can be produced have steadily been increasing. Hence, the full potential of the microorganisms is far from reached.

Filamentous fungi, such as those belonging to the Zygomycetes and Ascomycetes groups, have made great contributions within a wide range of industrial sectors. A previous review article from our research group has put together some value-added products produced by zygomycetes using a wide range of substrates (Ferreira et al., 2013). Comparatively, the ascomycetes are a wider group of microorganisms where, reasonably, many of them have also been of special interest (Ferreira, 2015). The contribution of ascomycetes to the white biotechnology can be traced back to the production of antibiotics by Penicillium chrysogenum, which together with Aspergillus oryzae, are among the most studied filamentous fungi at industrial scale (Gibbs, 2000). Aspergillus spp. is involved in the production of many value-added products including enzymes such as amylase, protease, lipase, phytase, lactase and catalase. They can also be used for production of cellulase and xylanase where Trichoderma spp. are also used (Pandey et al., 2015). Besides, Aspergillus spp. are responsible for a major fraction of commercial production of organic acids including citric acid, gluconic acid, itaconic acid and they are also potential sources of malic and oxalic acid (Pandey et al., 2015). Moreover, chitosan, that can be used for production of superabsorbents (Zamani, 2010), can be obtained via hydrolysis of chitin from *Aspergillus* spp. cell walls and these ascomycetes can also be used for production of keratinase hydrolysates (Pandey et al., 2015). *Monascus* spp. have been important sources of pigments for the food industry and together with *Aspergillus* spp, *Fusarium* spp. and *Neurospora* spp. have been a source of different human food products (Ferreira, 2015). However, unicellular ascomycetes, that is, yeasts such as *Saccharomyces* spp, *Pichia* spp, and *Yarrowia* spp. have also been reported to be potential sources of organic acids (such as α -ketoglutaric acid, lactic acid, malic acid and pyruvic acid), polysaccharides such as β glucan, proteins such as collagen, polyunsaturated fatty acids, sterols (e.g. squalene) and lipids (e.g. ceramides) (Pandey et al., 2015).

The substrates for industrial production of value-added products by ascomycetes include mainly refined sugars (Pandey et al., 2015). Therefore, there has been an increasing interest to use other more cost-effective substrates such as industrial waste materials mostly based on industrial residuals and lignocelluloses. In this regard, ascomycetes can potentially play a crucial role as core biocatalyst in these "waste biorefineries" given their ability to produce enzymes that can break down these recalcitrant structures. By using filamentous ascomycetes, their biomass, normally rich in proteins and lipids, can represent another value-added product of the biorefinery. The filamentous growth enables easier biomass separation from the medium than that if unicellular microorganisms are used (Ferreira, 2015).

The present review reports recent developments regarding the production of organic acids and ethanol where such type of literature overview is not, to the best of our knowledge, available for the latter. Especially *Aspergillus, Fusarium, Neurospora,* and *Monascus* are considered. Taking into account the potential of the fungal biomass for the feasible establishment of "waste biorefineries", fermentation strategies and compositional characteristics are also provided. Moreover, the potential of the ascomycetes for production of cellulases and xylanases has been approached. Future research avenues for the production of the value-added products using ascomycetes have also been included.

2. Ascomycetes as core biocatalysts in "waste biorefineries

Filamentous ascomycetes such as *Aspergillus* spp., *Fusarium* spp., *Monascus* spp. and *Neurospora* spp. are versatile fungi able to grow in an expanding array of different substrates. The carbohydrates range from hexose and pentose monomers to disaccharides such as lactose (Angumeenal and Venkappayya, 2005), cellobiose (Bansal et al., 2014) and sucrose (Bari et al., 2009). In addition to be able to consume the monomers released during the hydrolysis of lignocellulosic materials, the ascomycetes can also grow on carbohydrate polysaccharides such as xylan, arabinan and glucan (Ferreira, 2015). By solid-state fermentation, they can even grow on non pretreated substrates such as wheat bran (Bansal et al., 2014) or wheat straw (Panagiotou et al., 2011).

This growth versatility is arguably related to the different enzymes these filamentous ascomycetes can produce depending on the substrates they grow onto. This capacity entails high potential for these fungi to play core roles within "waste biorefineries" through valorisation of waste materials from different industrial sectors. For instance, filamentous ascomycetes can grow on agricultural left-overs such as wheat (Panagiotou et al., 2011) and corn (de Almeida et al., 2014) straw, wheat bran (Bansal et al., 2014), rice hulls (El-Metwally et al., 2015), sugarcane bagasse (Jabasingh and Nachiyar, 2011) or corn cobs (Panagiotou et al., 2011). The use of these materials would allow the full crop to be used for biotechnological applications as well as lower the pressure on the use of sugar-rich crops or starch-containing grains that can alternatively be used for human consumption (Ferreira, 2015). Filamentous ascomycetes could also be used for valorisation of wastes generated after handling of fruits and vegetables including banana, orange, pineapple, carrots, onions and potato peels (Bansal et al., 2014), empty fruit bunches from palm oil industry, or apple pomace (Dhillon et al., 2011b). Other examples of waste materials that filamentous ascomycetes can grow on include tea waste (Sharma et al., 2008), waste office paper (Ikeda et al., 2006), lactose-rich wastes from dairy industries such as cheese whey (Angumeenal and Venkappayya, 2005), cream or crème fraiche (unpublished results of the authors), and even spent grains from the brewery industry (Xiros and Christakopoulos, 2009). However, the economical feasibility of using ascomycetes for production of value-added products from lignocellulosic materials will arguably be limited by the level of expressed enzymes that will dictate the required time for hydrolysis and fermentation. Actually, finding microorganisms with natural powerful enzymatic machinery for fast degradation and fermentation of more recalcitrant structures has been a major research bottleneck.

Filamentous ascomycetes can also play a smaller role in already established industrial processes. For instance, side-streams namely thin stillage and whole stillage of the ethanol production process from sugar-rich or starch-rich agricultural crops can be converted to value-added products by filamentous ascomycetes (Ferreira, 2015). The inclusion of a new biological conversion step in an established facility is arguably faster to be realised than an entire new facility; some of the equipment is at place rendering lower investment costs (Lennartsson et al., 2014). For instance, if ethanol side-streams are converted to ethanol and biomass for feed by filamentous ascomycetes, the distillation column for the former, and the dryer for the latter, are already available.

Due to their metabolic versatility, filamentous ascomycetes can produce various value-added products from waste materials such enzymes, organic acids, ethanol and biomass for feed applications. The use of waste materials is of special importance either for reduction of the production cost of already available industrial products or for the establishment of new processes producing other value-added products. Clearly, filamentous ascomycetes detain high potential to be core biocatalyst in such "waste biorefineries". The range of possible substrates and produced valueadded products in filamentous ascomycetes-based "waste biorefineries" is presented in Fig. 1.

3. Fermentation with filamentous fungi

The cultivation of filamentous fungi is generally carried out via submerged fermentation (SmF) or via solid-state fermentation



Fig. 1. Potential substrates and produced value-added products in filamentous ascomycetes-based "waste biorefineries".

(SSF). The SmF has a wider biotechnological application probably due to better heat and mass transfer and culture homogeneity rendering it a more reliable, reproducible, flexible and easier to monitor character (Fazenda et al., 2008). However, constraints exist especially in larger scale. These constraints are mostly related to the morphology of the filamentous fungi: they can grow as uniform and long filaments evenly distributed through the medium or the filaments can get entangled into clumps or pellets (Gibbs, 2000). The rheological properties of the medium (particularly the viscosity) are influenced by the fungal growth form. When fungi give rise to dense mycelial suspensions, the medium is more viscous and so oxygen and other mass transfer resistances can become limiting factors. The problem is exacerbated when fungi are grown in stirred-tank reactors. The filaments can wrap around the internal parts of the bioreactor including impellers, baffles, pH. O₂, CO₂, or temperature probes leading to low-performance biotechnological processes (Gibbs, 2000). When fungi grow as pellets, the media are generally less viscous due to the lower impact of the pellets in the bulk medium (Gibbs, 2000). The growth of fungi as pellets is generally preferred for biotechnological application. However, pellets can suffer shear stress and, according to their size or fluffiness, hollow oxygen centre can arise for larger pellets and substrate and oxygen transfer rates limitations can occur when fungi grow as compact pellets (Gibbs, 2000). Generally, small fluffy pellets are the most suitable form for high-performance fermentations (Ferreira et al., 2013). For instance, the pellet form is viewed as the most advantageous fungal growth morphology for enhanced production of citric acid by Aspergillus niger (Dhillon et al., 2013b). Medium supplementation of lower alcohols, such as methanol has been reported to induce pellet morphology (Dhillon et al., 2011a).

When grown in bioreactors, the supplied air plays a crucial role for optimal cultivation performances. For instance, the production of organic acids as being dependent on the tricarboxylic acid cycle intermediates or on enzymes that use oxygen as substrate, is a strict aerobic process. Therefore, obtaining high oxygen transfer rates is crucial for good process performances. At bioreactor scale, higher oxygen transfer rates demand increased power input: for instance by faster stirring if a stirred-tank reactor is used (Okabe et al., 2009). Although stirred-tank reactors have widely been used in biotechnological processes, bubble columns have alternatively been constructed due to their simpler design, that is, absence of internal mechanical parts and so the constraints when cultivating filamentous fungi are considered to be lowered (Yoshida, 1988). Airlift bioreactors have also been developed which have an internal or external loop giving rise to a different mixing pattern in comparison to that in a bubble column. This, in turn, has been shown to lead to better oxygen and other mass transfer rates (Merchuk and Siegel, 1988). Using airlift bioreactors can represent an important step towards cheaper industrial processes since its energy demand is about one third of that needed for running a stirredtank reactor (Träger et al., 1989); the medium mixing is promoted by the supplied air and further medium density differences. The lower power requirement with concomitant better performances by an airlift bioreactor in comparison to a stirred-tank reactor has been shown by research towards gluconic acid production with Aspergillus terreus (Okabe et al., 2009). Nonetheless, the better substrate and oxygen transfer rates when using an airlift bioreactor instead of a bubble column might depend on the experiment setup. Ferreira et al. (2015) have reported similar performances when growing Neurospora intermedia in thin stillage for production of ethanol and biomass for feed using an airlift bioreactor and also when the bioreactor was used as a bubble column. Moreover, the supplied air is crucial for consumption of xylose, a very prominent pentose in lignocellulosic materials and that filamentous fungi can convert to ethanol, which is not possible under anaerobic conditions due to redox imbalance (Lennartsson, 2012). Besides, the production of enzymes in order to degrade more complex substrates entails higher energy expenditure by the cells. Thus, good access to ATP generating processes is required, stressing further the importance of the supplied air (Lennartsson et al., 2014).

SSF has attracted increasing interest over the years as an alternative to SmF processes for production of value-added products. SSF involves the growth of microorganisms on moist solid substrates similarly to their natural habitat, in the absence of free flowing water (Barrington et al., 2009). The degradation of the substrate will be catalysed by free extracellular enzymes or enzymes organised in cellosomes (Section 4), produced by the growing microorganism. Thus, SSF is influenced by the strain and substrate in addition to several process parameters including carbon and nutrient composition, moisture content, particle sizes, incubation temperature, pH and inoculum density (Bari et al., 2009; Pandey et al., 2001). Advantages of SSF include lower energy requirements and so lower operation costs, less wastewater produced (Pandev et al., 2001), and higher productivities (Sreedharan et al., 2016). Nonetheless, low surface aeration (the air provided and made available to growing ascomycetes on moisturised substrate surfaces is crucial for high process performances), temperature gradients, moisture variations and restricted gas exchange can hamper high SSF performances as reported for citric acid production by A. niger in a column bioreactor (Barrington et al., 2009). SSF has been used for food fermentation, enzyme production, mushroom production, mould-ripening of cheese and partial composting of agricultural residues (Barrington et al., 2009). Additionally, SSF has widely been used for production of enzymes for lignocellulose degradation that will be used in a second step where the monomers generated by the enzymes will be converted to value-added products such as ethanol using yeast. The production of a homogeneous and high volume of spores for conversion of glucose to gluconic acid by intracellular glucose oxidase can also be carried out by SSF (Ramachandran et al., 2006). Another example of application of spores produced by SSF includes those of *Trichoderma* spp. that function as biological control agents in agricultural and forest pest management. Trichoderma spp. shares around 50% of the market of fungal biological control agents. mostly as soil/growth enhancers (Verma et al., 2007). Fungal-based biological control agents detain broader spectrum regarding disease control and production yield compared to that of bacteria (Whipps and Lumsden, 2001).

4. Enzymes

Filamentous ascomycetes have gathered intense research interest due to the cocktail of enzymes they can produce when adequately induced. Their relevance is further stressed considering that the cheaper substrates sought to be used for production of value-added products are at a great extent lignocellulose- or starch-based substrates. Taking into account the available research during the last decade, the present review could be only based on production of some lignocellulolytic enzymes namely cellulases or xylanases. An overview was tentatively made upon different substrates and cultivation parameters towards improved production of these lignocellulolytic enzymes since they dominate the recent research by the four genera. The use of SSF for enzyme production seems to play a more prominent role when compared to the available research works using alternatively SmF. Here, filamentous fungi have advantage over unicellular microorganisms such as bacteria and yeasts due to their extracellular enzymatic system coupled with hyphal penetration (Sharma and Arora, 2015).

4.1. Cellulases and related enzymes

Filamentous ascomycetes are capable of growing both directly on lignocellulosic materials or after those had been through a Table 1

Enzyme activities for different filamentous ascomycetes when grown in different substrates under both solid-state fermentation (SSF) and submerged fermentation (SmF).

Ascomycete	Substrate	Mode of operation	Endoglucanase	Exoglucanase	β -Glucosidase	Xylanase	References
A. nidulans MTCC344	NaOH-pretreated sugarcane bagasse	SF, SSF	28.8 U/g	-	-	-	Jabasingh and Nachiyar (2011)
A. niger FGSCA733	Jatropha seed cake + 10% H ₂ SO ₄ -pretreated thatch grass	SF, SSF		3974.0 U/g ^a		6087.0 U/g	Ncube et al. (2012)
A. nidulans	NaOH-pretreated coir pith	SF, SSF	28.6 U/g	10.2 U/g	4.3 U/g		Jabasingh (2011)
A. niger NS-2	Wheat bran	SF, SSF	463.9 U/g	101.1 U/g	99.0 U/g		Bansal et al. (2014)
A. niger MA1	Rice hulls	SF, SSF	15.0 U/g	3.4 U/g	49.1 U/g		El-Metwally et al. (2015)
F. verticillioides	Corn straw	SF, SmF	8.0 U/mL	0.6 U/mL	0.3 U/mL	114.0 U/mL	de Almeida et al. (2014)
F. oxysporum F3	Wet exploded pre-treated wheat straw + corn cobs (1:2)	SF, SmF	9.1 U/mL	-	0.3 U/mL	34.3 U/mL	Panagiotou et al. (2011)
F. chlamydosporum	Sugarcane bagasse + wheat bran	SF, SSF	281.8 U/g	95.2 U/g	135.2 U/g	4720.0 U/g	Qin et al. (2010)
N. crassa DSM 1129	Wheat straw + wheat bran	SF, SSF	492.8 U/g	1.1 U/g	26.7 U/g	297.8 U/g	Dogaris et al. (2009)

SF-Shake-flasks.

^a Enzymatic activity for the whole cellulase system.

pretreatment step. In both cases, a second biocatalyst is often used to convert the released simpler sugars to value-added products. This is a very prominent case where Aspergillus spp. or Fusarium spp. hydrolyse lignocellulose-based substrates and yeast convert the released sugars to ethanol. The ascomycetes can also be used within the concept of consolidated bioprocessing (CBP) where the fungus is responsible for the hydrolysis of the substrate and conversion of the simple sugars to value-added products (Jouzani and Taherzadeh, 2015). The ascomycetes can also be used for isolation of cellulases and related enzymes retaining high enzymatic activities that can serve different purposes in a variety of industrial sectors. Cellulases can be used in textile, paper and pulp, food and animal feed, fuel, chemical, waste management, and pharmaceutical industries (Jabasingh and Nachiyar, 2011). The industrial production of cellulases is presently dominated by Trichoderma spp. and Aspergillus spp. (Pandey et al., 2015).

The complete hydrolysis of lignocellulosic substrates to monomeric sugars to be converted to value-added products is dependent on the availability of a consortium of different enzymes by the growing microorganism which directly imposes the first constraint for feasible industrial realisation. Such enzymatic consortium includes delignification enzymes (lignases), cellulose-hydrolysing enzymes (cellulases) and hemicellulose-hydrolysing enzymes (hemicellulases which includes variable enzyme classes where xylanases are of utmost importance due to xylan predominance) (Sreedharan et al., 2016). Ascomycetes are not generally good candidates for growth on lignocellulosic materials with high lignin contents when compared with white-rot fungi basidiomycetes (Kersten and Cullen, 2007). However, the comparatively slower growth in culture of the latter discourages their bioprospecting (Banerjee et al., 2010).

The produced cellulases in fungi are available extracellularly and have three components namely endoglucanase, exoglucanase, and β -glucosidase (Sreedharan et al., 2016). In a production as single-product context, aerobic fungi are preferred than anaerobic bacteria and fungi since their cellulase are extracellular, adaptive in nature and usually secreted in large quantities during growth. In contrast, cellulases in anaerobic bacteria and fungi are organised into tight multi-enzyme complex, often membrane bound as cellosomes, being difficult to recover individual active enzyme species (Gincy et al., 2008). Some recent examples of activity for those cellulase components plus xylanase, from different substrates, have been gathered in Table 1. The potential of the ascomycetes is therefore huge since the addition of enzymes mainly for hydrolysis of lignocellulose is always a hurdle for feasible scale up.

At industrial scale, SmF is the preferred strategy due to aforementioned advantages, despite the long fermentation times with low production in comparison to that when SSF is used. The latter has more recently been dominating research towards production of cellulases and xylases due to lower costs and higher enzyme production (Sreedharan et al., 2016). A panoply of different lignocellulosic substrates has been studied for enzyme production using both strategies. Research towards production of those enzymes by ascomycetes in focus in this review, has at all times been dominated by studies using Aspergillus spp. most likely due to the fact of being a well-known industrial microorganism. Over the past decade the cellulase activities obtained when using Aspergillus spp. ranged from 3 to 321 U/mL where the highest values (83-321 U/mL) were obtained during SmF using wheat or maize straw as substrates whereas coir waste, banana peel and grass gave rise to the lowest activity values (3-12 U/mL) (Sreedharan et al., 2016). It is worth mentioning that among those substrates just coir waste suffered a pretreatment step before enzyme production during Aspergillus spp. growth. As shown in Table 1, the cellulase system produced by some Fusarium spp. when grown on wheat straw or pretreated wheat straw gave rise to around 10 U/mL. Over the last decade, cellulase activities of Aspergillus spp. when grown in SSF have been within the range 3–581 U/g of dry substrate, where the highest values were obtained when using corn stover or wheat straw as substrates and the lowest values achieved when grass, wheat bran, or combinations of wheat bran with rice straw or wheat straw (Sreedharan et al., 2016). However, as it can be observed in Table 1, higher cellulase activities were obtained with wheat bran or its combination with wheat straw when other Aspergillus spp. as well as Fusarium spp. and Neurospora crassa were used. Xylanase activities are comparatively higher than cellulase activities and also varies among substrate and strain used (Table 1).

Clearly, the production of enzymes is dependent on various factors including substrate, strain, cultivation strategy, medium supplementation, and cultivation parameters. Moreover, the existence or not of a pretreatment step, in order to remove the lignin and to make the cellulosic component more accessible to the fungus, can also influence the final production of enzymes. Such improvement has been reported for sugarcane bagasse (Jabasingh and Nachiyar, 2011) and coir pith (Jabasingh, 2011) although for the latter, 11 days were needed to achieve the highest enzyme activity. Several studies over the years have been focused on the optimisation of cellulase and xylanase production by the ascomycetes genera here in focus under SSF and SmF considering

temperature, pH and medium supplementation. Since constraints such as poor aeration and decreased dissipation of heat are verified in SSF, the weight or bed height of the substrate has also been included in the optimisation studies. Some interesting outputs include the increase in xylanase production by Aspergillus nidulans when Jatropha curcas seed cake was supplemented with 10% of acid-pretreated thatch grass or ammonium chloride, whereas no positive influence on cellulase concentration was observed (Ncube et al., 2012). Bansal et al. (2014) have carried out an extensive study on the influence of supplementation of wheat bran for production of cellulase by a strain of A. niger. The influence of a wide range of carbon, nitrogen, phosphate, sulphate, and surfactant sources as supplementation to wheat bran on cellulase production has been investigated. Supplementation with cellobiose led to the highest activity of endoglucanase, whilst cellulose led to the highest activity of exoglucanase and salicin to the highest B-glucosidase activity. Peptone as nitrogen sources lead to the highest activity of endoglucanase, whereas tryptone led to the highest amount of exoglucanase and β -glucosidase. Among the phosphate sources examined, K₂HPO₄ had a stimulatory effect on the production of cellulase. When testing different sulphate sources, MnSO₄ led to the highest activity of endoglucanase, whilst $CuSO_4$ led to the highest amount of exoglucanase and $(NH_4)_2SO_4$ to the highest activity of β -glucosidase. Surfactants are thought to alter the permeability and fasten the secretion of enzymes (Deswal et al., 2011); triton-X enhanced the production of endoglucanase and β -glucosidase, whilst SDS enhanced the activity of exoglucanase. Activities reported in Table 1 relate to the optimised medium via supplementation of wheat bran. When Bansal et al. (2014) increased the amount of wheat bran from 5 to 1000 g either in shake-flasks or shallow trays decreased enzyme activities were observed. The crude enzyme were used for saccharification of various steam-pretreated lignocellulosic residues including dry potato peels, carrot peels, composite waste mixture, orange peels, onion peels, banana peels, pineapple peels where cellulose conversion efficiencies of 92-98% were achieved.

de Almeida et al. (2014) have optimised conditions for production of the cellulase system in Fusarium verticillioides using corn straw as substrate, where sodium nitrate was found to have a positive effect at a corn straw concentration of around 5% and the cultivation time for optimum enzyme activity was within 140-150 h in SmF. In contrast, sodium nitrate had no significant effect on xylanase activity. An aspect found by the authors and worth reporting was that lactose was found to have a negative effect on endoglucanase and xylanase activities, whereas it is a known cellulase inducer in Trichoderma species (Fekete et al., 2008). Panagiotou et al. (2011) have tested different ratios of wetexploded pretreated wheat straw and corn cobs where the highest cellulase and xylanase activities were found at a ratio of 1:2 using Fusarium oxysporum. Qin et al. (2010) have also reported cellulase and xylanase production by Fusarium chlamydosporum when grown in sugarcane bagasse together with wheat bran. Dogaris et al. (2009) have obtained the highest activities of cellulases and hemicellulases with N. crassa when using a mixture of wheat straw and wheat bran (25/5, w/w), ammonium sulphate as nitrogen source, initial pH of 5.0 and initial moisture of 70.5% (w/w). Cellulolytic and hemicellulolytic abilities have also been found in N. intermedia which was able to degrade sugar polymers such as glucan, xylan and arabinan present in the fibres of thin stillage, a byproduct from the ethanol industry (Ferreira et al., 2015). Cellulases and xylanases produced by ascomycetes genera in focus seems to be stable at a wide range of temperature and pH where maxima activities have been reported within the ranges of 25-80 °C and pH 3.0–9.0, respectively.

Altogether, ascomycetes are undoubtedly a potential source of enzymes either for enzyme cocktail preparation or for application within a biorefinery context where value-added products are produced from the enzymatically released simpler sugars. However, there seems to be a lack of research studies regarding the hydrolysis of lignocellulosic materials using SSF or SmF at larger scales. Moreover, higher enzyme activities were reported for agricultural wastes such as wheat straw, wheat bran or corn stover than those reported for other lignocellulosic substrates. Therefore, optimisation studies are also needed in order to show a potential wider application of ascomycetes cellulases and xylanases. Nonetheless, taking into account the importance of a direct use for the lignocellulose-based materials increasingly generated, extensive research is expected to be performed in order to hopefully fill in those gaps.

5. Ascomycetes for ethanol and organic acids

Due to their enzymatic capabilities, filamentous ascomycetes have widely been investigated for production of metabolites such as ethanol and organic acids using mostly lignocellulose-based materials. Nowadays, the entire commercial production of organic acids including citric, gluconic and itaconic acids is ensured by Aspergillus spp. using, at great extent, refined sugars (Pandey et al., 2015). Therefore, the possibility to produce these organic acids within, for instance, lignocellulose-based "waste biorefineries" would lower the overall production costs with concomitant increase in the range of possible applications. The same holds for the production of ethanol industrially dominated by the unicellular ascomycete Saccharomyces cerevisiae using sugar-rich or starchrich agricultural crops (Ferreira, 2015). Recent research towards production of ethanol has been carried out using Fusarium spp. and Neurospora spp. (Table 2), whilst Aspergillus spp. expectedly still dominates the recent research towards production of organic acids (Table 3). Bacteria can also be used for production of ethanol or organic acids from lignocellulose-based substrates but the capacity of producing the necessary concentrations of enzymes for complete saccharification of pretreated substrates is still limited to fungi (Amore and Faraco, 2012).

5.1. Ethanol

Ethanol, or ethyl alcohol, is a volatile, flammable, and colourless liquid. Its production at commercial scale is dominated by USA and Brazil that produce annually 50 billion and 23 billion litres of ethanol, respectively (ePURE, 2014). The dominant feedstock in the USA is corn, whereas sugarcane dominates in Brazil. The potential as an additive or a replacement of gasoline in the transport sector has been the main driving force behind the production of ethanol from renewable feedstocks. Nonetheless, ethanol finds applications in a variety of industries including chemical, cosmetic, pharmaceutical and medical, as well as the automotive and beverage sectors (ePURE, 2014).

Research has been performed on ethanol production using *Fusarium* and *Neurospora* species (Table 2). *Aspergillus* species was also used in ethanol production. However, they are mainly used for production of enzymes for further conversion to ethanol using yeast. *Fusarium* and *Neurospora* are used within a context of consolidated bioprocessing (CBP) where the biocatalyst is responsible for the production of enzymes, saccharification of substrate carbon sources to monomeric sugars and further fermentation to ethanol (Jouzani and Taherzadeh, 2015). The advantage of using filamentous fungi such as ascomycetes becomes clear due to their ability to assimilate and convert xylose released during hydrolysis of lignocellulosic materials to ethanol (Ferreira, 2015). In contrast, baker's yeast, the most widely used biocatalyst for industrial production of ethanol, it unable to consume pentose sug-

Table 2

Examples of performance of ascomycetes towards the production of ethanol under submerged fermentation.

Microorganism	Medium	Mode of operation	Production (g/L)	Yield (g/g)	Productivity (g/L/h)	References
F. oxysporum F3	7.5% (w/v) alkali-pretreated brewer's spent grain	2 L STR	≈8.00	0.11	0.07	Xiros and Christakopoulos (2009)
F. verticillioides	20 g/L glucose	SF	9.44	0.47	0.05	de Almeida et al. (2013)
	20 g/L xylose		5.68	0.46	0.03	
	10 g/L glucose + 10 g/L xylose		5.60	0.50	0.05	
	45 g/L pre-treated sugarcane bagasse		4.6	0.31	0.03	
F. oxysporum MTCC 284	Sequentially dilute acid and dilute alkali pretreated tapioca stem (32 g/L)	SF	8.64	0.27	0.05	Magesh et al. (2011)
F. oxysporum F3	20 g/L cellulose	6 L STR anaerobic	7.00	0.35	0.04	Panagiotou et al. (2005)
F. oxysporum F3	Wet exploded pre-treated wheat straw (110 g/L)	SF, anaerobic	4.9	0.04	0.03	Panagiotou et al. (2011)
N. crassa DSM 1129	8% w/v dilute-sulphuric acid pretreated sweet sorghum bagasse	SF, anaerobic	8.1	24.8% of theoretical yield based on cellulose and hemicellulose	0.04	Dogaris et al. (2012)
N. intermedia CBS 131.92	7.5% dilute-phosphoric acid pretreated wheat bran	SF	10.1	0.135 g/g of dry biomass loading (95% theoretical yield based on glucan)	0.21	Nair et al. (2015)
N. crassa DSM 1129	75 g/L NaOH-pretreated brewer spent grain	2 L STR	74.0	0.17 g/g (36% of the theoretical yield based on glucose and xylose)	0.77	Xiros et al. (2008)
N. intermedia CBS 131.92	15.6% solids whole stillage + 1 FPU/g SS cellulase	SF	8.7	0.06	0.18	Bátori et al. (2015)
N. intermedia CBS 131.92	9% solids thin stillage	SF	5.0	0.16 g/g of reduced solids	0.14	Ferreira et al. (2014)
N. intermedia CBS 131.92	9% solids thin stillage	26 L ALB, continuous $(0.1 h^{-1})$	5.0	0.303 g/g reduced solids	0.57	Ferreira et al. (2015)

Table 3

Performance of ascomycetes towards production of different organic acids using different experiment set-ups.

Microorganism	Medium	Mode of operation	Titre (g/L)	Yield (g/g)	Productivity (g/L/h)	References
Citric acid						
A. niger NRRL 567	100 g apple pomace, SSF Apple pomace sludge (56.2 g/L carbohydrates)	SF, SSF SF, SmF	127.9 g/kg 18.2	0.13 0.32	1.33 g/kg/h 0.13	Dhillon et al. (2011b)
A. niger NRRL 322	HCl-pretreated jack fruit carpel fibre supplemented with glucose (140 g/L)	SF, SmF	73.9	0.53	1.03	Angumeenal and Venkappayya (2005)
A. niger NRRL 567	3 kg apple pomace + rice husk (1% w/w)	12 L rotating drum type solid-state bioreactor	294.2 g/kg	0.29	2.45 g/kg/h	Dhillon et al. (2013a)
A. niger NRRL 567	270 g wet peat moss (80% moisture) + 40 mL glucose (250 g/L)	0.89 L column reactor	123.9 g/kg	17.8%	0.43 g/kg/h	Barrington et al. (2009)
A. niger NRRL 567	Apple pomace ultrafiltration sludge (66.0 g/L carbohydrates)	7.5 L STR	40.3	0.61	0.31	Dhillon et al. (2013b)
Gluconic acid						
A. niger NCIM 548	Deproteinised whey (9.5% lactose) + 0.5% glucose	SF, SmF	69.0	0.69	1.44	Mukhopadhyay et al. (2005)
A. niger IAM 2094	Enzymatically hydrolysed waste office paper (glucose adjusted to 50 g/L)	SF, SmF	46.0	0.92	0.64	Ikeda et al. (2006)
	Enzymatically hydrolysed waste office paper (glucose adjusted to 120 g/L)	SF, repeated batch	110.0	0.92	1.53	
	Enzymatically hydrolysed waste office paper (glucose adjusted to 120 g/L)	0.8 L turbine blade reactor, repeated batch	72.0 (average)	0.60 (average)	0.99 (average)	
A. niger ARNU-4 (mutant strain)	Tea waste + 20% molasses	SF, SSF	82.2	0.88	0.28	Sharma et al. (2008)
A. niger ORS-4.410 (mutant strain)	Rectified grape must (120 g/L glucose)	Sf, SmF	73.2	0.81	0.51	Singh et al. (2005)
	Rectified banana must (120 g/L glucose)	Sf, SmF	69.3	0.72	0.48	
	Hexacyanoferrate-treated sugarcane molasses (120 g/L glucose)	SF, SmF	60.3	0.61	0.41	
Itaconic acid						
A. terreus TN 484-M1	$140\mbox{ g/L}$ sago starch hydrolysed with HNO_3 to pH 2.0	3 L STR	48.2	0.34	0.40	Dwiarti et al. (2007)
A. terreus LU02b	Glucose (180 g/L)	15 L STR	86.2	0.86	0.51	Kuenz et al. (2012)

ars. CBP is normally a two-stage process including a first aerobic saccharification followed by ethanol production under oxygenlimited conditions. Reasonably, the success of CBP will be dependent on the strain used (Ferreira, 2015). Among the genus Fusarium, F. oxysporum is the species most widely used for production of ethanol but F. verticillioides, F. equiseti and F. acuminatum have also been investigated. Substrates for ethanol production included brewer spent grain where 60% of the theoretical yield based on total glucose and xylose content was achieved (Xiros and Christakopoulos, 2009). A high-affinity transporter for hexose (Hxt) has been characterised in F. oxysporum where high expression levels were observed at low glucose concentrations, a common situation under CBP. The transporter was further reported to transport both C5 and C6 sugars (Ali et al., 2013). F. oxysporum has also been used for production of ethanol from wheat straw as pure culture or co-cultured with S. cerevisiae. Whilst the production of ethanol was similar between F. oxysporum and the mixed culture, the productivity was three times higher when the latter was used (Panagiotou et al., 2011). The source of nitrogen has also been reported to influence the production of ethanol and alcohol dehydrogenase activity where higher values were registered when urea was used instead of yeast extract with F. equiseti and F. acuminatum (Anasontzis et al., 2011).

N. crassa is the most widely used among the Neurospora genus for ethanol production. The fungus has been used for production of ethanol from sweet sorghum bagasse, the lignocellulosic solid residue obtained after extraction of sugars from sorghum stalks, where 24.8% of the theoretical yield based on cellulose and hemicellulose was achieved (Dogaris et al., 2012). It should be noted, however, that higher solid loading was used for pretreatment (16%) and fermentation (8% w/v). N. crassa has also been studied for production of ethanol from alkali-pretreated brewer spent grain where 36% of the theoretical yield based on glucose and xylose was obtained. No negative effect of pretreatmentoriginated inhibitors such as acetic acid of lignin phenolic compounds was found on the fungus performance since no accumulation of glucose and xvlose was observed (Xiros et al., 2008). Zhang et al. (2008) have proved the link between xylose fermentation and supply of oxygen in N. crassa. Intracellular enzyme activities of xylose reductase, xylitol dehydrogenase and xylulokinase, the first three enzymes in xylose metabolic pathway, decreased with the increase in oxygen limitation, leading to a decreased xylose uptake. Nair et al. (2015) have cultivated N. intermedia in dilute-acid pretreated wheat bran in separate hydrolysis and fermentation where 95% of the theoretical yield based on wheat bran glucan was achieved using a 7.5% (w/v) solid loading. N. intermedia has also been evaluated for production of ethanol from residuals from the 1st generation bioethanol process namely whole stillage and thin stillage, waste streams originated after ethanol distillation (Bátori et al., 2015). When grown in 15.6% solids whole stillage, an ethanol concentration of around 9 g/L was obtained with addition of cellulase, which was around the double of ethanol produced when no enzyme was added. However, further studies have shown that the addition of cellulase helped the most at converting left saccharides, in the liquid fraction, to monomers. Therefore, a pretreatment step is hypothesised to be needed in order to improve the ethanol production (Bátori et al., 2015). When grown in 9% solids thin stillage, the liquid fraction after whole stillage centrifugation, *N. intermedia* could produce around 5 g/L of ethanol enzymatically un-aided (Ferreira et al., 2014). When set under continuous cultivation at 0.1 h^{-1} dilution rate using a 26 L airlift bioreactor, the same ethanol concentration was achieved (Ferreira et al., 2015).

Taking into account that the addition of enzymes to the process of ethanol production from lignocellulosic materials still constitutes a relevant economic burden, it is expected that the research using filamentous fungi with relevance for CBP will continue at high pace. From the data gathered in Table 2, there seems to be a need of a potent microorganism that will lead to higher ethanol yields and productivities. Genetic tools might give an important input in this regard towards the isolation of mutants with better performances. However, this may also hamper the use of the biomass for feed applications (Section 6).

5.2. Weak organic acids

5.2.1. Citric acid

Citric acid, an intermediate of the tricarboxylic acid cycle with annual growth demand, is mostly used in the food industry (>70%) but it has also applications in phytoremediation of heavy metals and manufacturing of biodegradable polymers for medical applications (Dhillon et al., 2011a). A. niger is nowadays used for large scale production of citric acid under submerged fermentation using beet or cane molasses, sucrose or glucose syrup as substrates (Soccol et al., 2006). Citric acid production has also been studied using yeast; however, comparatively lower yields are obtained due to the production of isocitric acid. Citric acid produced by synthetic route cannot compete with that produced by A. oryzae due to the higher price of the starting materials in comparison to that of citric acid (Papagianni, 2007). The production of citric acid is the second largest fermentation product after industrial ethanol fermentation (Dhillon et al., 2013a). However, as an effort to lower citric acid production costs, other more cost-effective substrates have been investigated. Those have included cheese whey, rape seed oil, whey permeate, date syrup, banana extract, maize zea, orange processing waste, yam bean starch, rape seed oil, carob pod, and root crops (Angumeenal and Venkappayya, 2005). More recent research insights regarding citric acid yields and productivities from different substrates using A. niger strains are presented in Table 3. Dhillon et al. (2011b) reported an increase of citric acid production both in SSF and SmF of apple pomace and its sludge, respectively, when 3% (v/v) of ethanol and 4% (v/v) of methanol were added to the media in comparison to the absence of these alcohols. Methanol (3% (v/v)) was also shown to increase the production of citric acid by about 40% when A. niger was grown in apple pomace ultrafiltration sludge. The addition of methanol also induced pellet growth whilst A. niger presented filamentous growth when the alcohol was absent. Rheological studies were carried out where the former gave rise to less viscous non-Newtonian broths, whilst the latter gave rise to a medium with non-Newtonian pseudoplastic behaviour (Dhillon et al., 2013b). In addition, several optimisation research studies have been carried out that took into account the influence of different parameters on the production of citric acid by A. niger strains grown in different substrates. Dhillon et al. (2013a) reported optimum conditions for citric acid production from apple pomace using a rotating drum type solid-state bioreactor regarding methanol concentration (3% (v/v)), agitation frequency, aeration and incubation time. The authors also reported optimum conditions for extraction of citric acid (294 g/kg dry weight) regarding extraction time (20 min), agitation rate (200 rpm), and extraction volume (15 mL). Bari et al. (2009) reported the optimised conditions for citric acid production using empty fruit bunches, generated by palm oil industries during extraction of oil, regarding sucrose, minerals and inoculum concentrations; a maximum production of 338 g/kg of dry empty fruit bunches was achieved. Karthikeyan and Sivakumar (2010) reported the maximum citric acid production (170-180 g/kg dry weight) using banana peel after optimisation of the moisture, pH, temperature, spore concentration and incubation time. Barrington et al. (2009) have optimised citric acid production in a semi-continuous process using a column bioreactor where they studied the aeration rate, bed depth and temperature.

5.2.2. Gluconic acid

p-Gluconic acid, a polyhydroxycarboxylic acid, is used in food, feed, beverage, textile, pharmaceutical and construction industries. The worldwide demand is fulfilled mostly by submerged fermentation with A. niger which reach yields of 98% using glucose (Singh and Kumar, 2007). Similar to production of citric acid by yeast, the use of bacteria for production of gluconic acid has also been investigated, but it is limited by the side production of oxogluconic acids (Ramachandran et al., 2006). In fungi, gluconic acid is produced via dehydrogenation of p-glucose catalysed by glucose oxidase (Singh and Kumar, 2007). Singh and Kumar (2007) have reviewed the main aspects of gluconic acid production namely the influence of carbon sources, concentration of salts, inorganic nitrogen sources, aeration, modulators (vegetable oils, H₂O₂, and starch), pH, and cultivation type (Smf and SSF). Further research for gluconic acid production with A. niger has included other substrates namely cheese whey, grape and banana must, waste office paper, tea waste and sugarcane molasses where product yields within the range 0.60–0.92 g/g were obtained (Table 3). The need of substrate clarification (removal of heavy metals and presented in Table 3 as rectified medium) that can impact the final medium nutrients is viewed as a constraint for feasible application (Singh and Kumar, 2007). The influence of oxygen was clearly shown by (Ikeda et al., 2006) since an increase of four times in gluconic acid was achieved when pure oxygen was used instead of air in a turbine blade bioreactor. Sharma et al. (2008) reported a maximum for gluconic acid concentration using tea waste after optimisation of concentration of molasses, salts and yeast extract, temperature, pH, inoculum size and aeration rate. Ramachandran et al. (2006) have used glucose oxidase retained in conidiospores of A. niger grown in SSF using buckwheat seeds for conversion of glucose to gluconic acid. The reaction rate was found to be 1.5 g/L/h with 102 g/L of gluconic acid produced out of 100 g/L glucose consumed. The reaction time was 100 h and the molar yield of around 93%. More recently, the production of gluconic acid from A. terreus has been reported from glucose with a conversion efficiency of 0.7 mol/mol of glucose consumed. The strategy was to set the pH at 6.5, since at low pH A. terreus produces itaconic acid (Dowdells et al., 2010).

5.2.3. Itaconic acid

Aspergillus spp., namely A. itaconicus and A. terreus, can also be sources of itaconic acid, an unsaturated dicarboxylic acid with high potential to be used as a chemical building block. Itaconic acid has the potential to function as a substitute for acrylic and methacrylic acid for production of polymers (Okabe et al., 2009). Itaconic acid possesses two carboxyl moieties which give it the ability to act as a co-monomer in the manufacture of polymers. Its applications areas are mainly in the plastics, papers and adhesives industries (Li et al., 2013). Itaconic acid production is provided via fermentation more commonly with A. terreus from molasses or glucose with production normally lower (>80 g/L) than that for citric acid with A. niger (>200 g/L) (Blumhoff et al., 2013). Production of itaconic acid has been studied in bacteria, yeast, other fungi (A. niger) and potato plants but comparatively lower yields were obtained (Klement and Büchs, 2013). In Aspergillus spp. itaconic acid is most likely produced via a decarboxylation of cis-aconitate catalysed by a cisaconitate decarboxylase (Klement and Büchs, 2013). Several aspects of itaconic acid production including regulation, producing microorganisms, effects of reactor design, oxygen supply, substrate and cultivation mode have been reviewed by Klement and Büchs (2013) and Okabe et al. (2009). The required medium components for production of itaconic acid are similar to those needed for production of citric acid, including high concentration of glucose (7.5–15%) and magnesium sulphate, low nitrogen and phosphorus, low but adequate levels of zinc, copper and iron, and limited

manganese (around 10 ppb) (Li et al., 2012). A doubled productivity in itaconic acid has been achieved when both enzymes aconitase and cis-aconitate decarboxylase were expressed in the mitochondria of A. niger in comparison when both enzymes were expressed in the cytosol (Blumhoff et al., 2013). In another study, Li et al. (2013) observed an increase in itaconic acid production by A. niger at low DO levels (10-25%) after overexpression of the haemoglobin domain. By other A. niger strains, Li et al. (2013) have also increased the amount of itaconic acid by deleting the production of oxalic acid. Expression of the A. terreus itaconic acid cluster consisting of the cadA gene (encoding a cis-aconitate decarboxylase), mttA gene (encoding a putative mitochondrial transporter) and the mfsA gene (encoding a plasma membrane transporter) results in A. niger strains producing over twenty-fivefold higher levels of itaconic acid and a twenty-fold increase in yield compared to a strain expressing only CadA (van der Straat et al., 2014). Some medium optimisation studies have also been carried out. Li et al. (2012) reported that copper was positively correlated with improved itaconic acid production and that the optimal conditions for itaconic acid clearly differ from conditions for citric- and oxalic acid production. Other studies reported optimised production of itaconic acid based on concentration of sago starch hydrolysate, glucose, corn steep liquor and salts as well as pH and temperature values (Dwiarti et al., 2007; Kuenz et al., 2012). Rao et al. (2007) also reported production of 24.5 g/L itaconic acid from Jatropha seed cake.

Altogether, research towards citric acid has kept going at a high pace and it is expected to continue in the future, whereas such intensity has not been observed for gluconic and itaconic acids. This aspect is related to the fact that there is a continuous growth demand for citric acid whereas such growth has not been observed for gluconic and itaconic acids. It is hypothesised that unless other high-value applications for gluconic and itaconic acid are unveiled that compensate for the production cost, the research towards production of these two organic acids is expected to continue at a comparatively lower pace.

6. Cell mass

Naturally, the feasibility of biorefineries will depend on several factors where the variety of the products contributes to a great extent. The macroscopic growth of ascomycetes into filaments leads to an opportunity to produce a second value-added product from fermentation when the primary goal was to optimise the production of e.g. enzymes, ethanol or organic acids. The filamentous character of ascomycetes biomass allow easier separation from the medium that can be simply carried out using a sieve-like separator. In contrast, for separation of bacterial or yeast biomass a centrifuge needs to be applied increasing the operation costs of the industrial process. Moreover, based on their biomass composition regarding lipid and protein contents, ascomycetes biomass can be considered for animal feed or human consumption (Ferreira, 2015). This second value-added product can play a crucial role for feasible establishment of biorefineries using more complex substrates such as those based on lignocellulose.

6.1. Ascomycetes biomass for human consumption

Several ascomycetes have been widely used for production of human food products. *Aspergillus* spp. have been used for production of indigenous Japanese foods and drinks such as sake, shoyu, miso and vinegar (Ferreira, 2015). Moreover, *Aspergillus conidia* are used as starters in the food industry during the first step of fermentation to digest ingredients such as steamed rice, soybean and wheat, and its conidiation regulatory pathway has been a matter of research (Ogawa et al., 2010). Fusarium venenatum is perhaps one of the most known filamentous fungi within the food industry given its use for production of myco-protein for human consumption sold under the trade name Quorn[®]. This myco-protein is produced in 150,000 L pressure-cycle bioreactors in a continuous flow process. The final product is prepared by mixing the myco-protein paste with a binding agent (egg albumin) to align the mycelia into a fibrous network with a similar texture to that of meat. Quorn® products range from chunks and mince to sausages, burgers, fillets and steaks (Wiebe, 2002). Monascus spp. have been used for production of red rice with anti-hypertensive effects on humans (Seraman et al., 2010). Neurospora is used for production of the human food oncom (soybean-based presscake), koji (an enzyme cocktail which is used as a starter for production of various fermented food such as soy sauce), Iban people in Borneo collect the orange fungus from burnt-down hilly rice fields and also use it as food, to prepare a fermented beverage from cassava, and it is normally present in Roquefort cheese in Southern France (Ferreira, 2015). Altogether, several ascomycetes are well-known microorganisms for humans and due to their massive use for production of human food products they are considered as GRAS (generally regarded as safe) microorganisms. This can be an important property if their biomass is used for animal feed or human consumption.

6.2. Ascomycetes biomass for feed

Animal feed products are, at great extent, based on soybeans. However, animal feed products can also be produced as a second value-added product from biorefineries. An example is the DDGS (distillers dried grains with solubles) originating from drying of solids that are separated after distillation of ethanol in biorefineries using cereals such as corn or wheat as feedstocks. Examples of markets for animal feed can include poultry, cattle, chicken, and fish among many others. Ascomycetes can play an important role either replacing soybean-based feeds or by improving the animal feeds already produced by biorefineries (Ferreira, 2015). Four ascomycetes including strains of A. orvzae, F. venenatum, M. purpureus and N. intermedia were studied for production of biomass from thin stillage, a side-stream from the bioethanol industry which is also used for the production of DDGS. During production of ethanol, S. cerevisiae is unable to consume all monomers or saccharides from starch hydrolysis or to hydrolyse and consume pentose-based substrates found in the undegraded bran that end up in the whole stillage and so in the thin stillage after centrifugation (Ferreira, 2015). Therefore, due to their enzymatic and pentose assimilation abilities, ascomycetes were used in order to potentially consume those remaining carbon sources. Biomass ranges of 12–19 g/L containing 50-60% (w/w) protein were obtained during shake-flasks cultivation (Ferreira et al., 2014). N. intermedia was used in another study where the aeration rate and reactor design effect were tested during cultivation in thin stillage using 2 m high airlift reactor and bubble column of 26 L capacity. The biomass production of around 5 g/L was similar at both reactor designs and increased to 9 g/L as aeration was increased from 0.5 to 2 vvm. The biomass was found to be composed of 50% (w/w) protein and 12% (w/w) lipids. All nine essential amino acids to humans were found in N. intermedia biomass and accounted to 40% of its amino acid composition: the amino acid profile was quite similar to that of the DDGS. Moreover, its lipid profile was rich in omega-3 and -6 fatty acids. The process was also studied in continuous mode using a 26 L bubble column where dilutions rates of up to $0.2 h^{-1}$ could be applied without cell wash-out; maximum biomass production rate of around 370 mg/L/ h was achieved (Ferreira et al., 2015). The current process can have an important impact on the final characteristics of the final animal feed from the company since from the 15 g/L of solids, containing 5% (w/w) protein, consumed by N. intermedia, around 5 g/L of biomass containing 50% (w/w) protein is obtained (Ferreira et al., 2015). The current process has been scaled up using a 80 m^3 bioreactor with similar performance and future prospects include a further scale up using a 1000 m³ bioreactor (Ferreira, 2015). The production of A. oryzae biomass has also been studied using whole stillage (stream that gives rise to thin stillage after centrifugation); around 6 g/L of biomass containing 42% (w/w) protein were obtained (Bátori et al., 2015). Biomass production has more recently been investigated by our research group using various dairy products. Although it is not surprising to report that A. oryzae could assimilate lactose, N. intermedia has also been found to be able to assimilate this saccharide which has not, to the best of our knowledge, been reported. Interestingly, when A. oryzae was cultivated in fat-rich media such as cream or crème fraiche the ascomycete preferred to hydrolyse fat than assimilating lactose. This hydrolysis was supported by the amounts of glycerol released. During a pH screening using cream, the amount of glycerol increased from 0.2 g/L at pH 4.3 to around 17 g/L at pH 7 (unpublished results). Further studies are ongoing in order to identify the fatty acids released to evaluate their potential for animal feed supplementation. Therefore, there is a potential for dairy industries to produce biomass for feed in addition to their main product building so a true biorefinery.

Considering that the biomass of filamentous ascomycetes is rich in proteins and lipids and that essential amino acids and fatty acids to humans constitute an important fraction of their profiles (Ferreira, 2015), its use for human feed cannot be neglected. Due to their use for production of fermented human foods since ancient times, the biomass of filamentous ascomycetes can constitute a potential source of human dietary supplements. Therefore, intensive research towards production of biomass or of a specific biomass component for feed or human consumption is expected in the future using an increasingly wider range of substrates.

7. Future prospects

The potential of lignocellulosic substrates for production of chemicals and fuels is expected to dominate research efforts through the years due to their availability as industrial wastes in spite of refined sugars. Their recalcitrant structure calls for the discovery of microorganisms with super-potent enzymatic machineries or the construction of such microorganisms using genetic tools. The ideal lignocellulose-based biorefineries would be those employing consolidated bioprocessing with naturally robust microorganisms and so, bioprospecting will play a crucial role regarding the discovery of such potent microorganisms. Since such microorganisms are not available at the present, the successful conversion of monomeric sugars present in lignocellulosic materials relies on a pretreatment step increasing though the overall process costs. Thus, a feasible lignocellulose-based industrial process has to represent a true biorefinery producing as many as possible different value-added products. Solid-state fermentation is widely used for biological treatment of lignocellulosic waste materials particularly for preparation of enzymatic cocktails. However, studies are still lacking regarding the use of SSF at larger scales for process potential clarification. It is constantly stated that cultivation in SSF has limitations for scaling up due to the space that would be needed as well as physical constraints such as reaching sufficient aeration and dissipation of heat and the maximum solid loading possible before decreased performances are observed. The design of new bioreactors for solid-state fermentation circumventing these constraints would result in a major research boost. Therefore, processes reaching industrial scale using submerged fermentation are expected to maintain their dominance.

The use of filamentous fungi as core catalysts in lignocellulosebased biorefineries represents a heavy contributor in order to reach feasible industrial processes. Beyond of being very diversified and so the probability of finding microorganisms capable of producing a specific chemical or fuel at high yields being very high, filamentous fungi promote directly the production of at least a second value-added product of the biorefinery namely biomass. Reasonably, the use of food-grade filamentous fungi will ease the public acceptance regarding the use of their biomass for feed or food applications in comparison to bacteria or geneticallymodified microorganisms. Filamentous fungi such as ascomycetes are already an industrial source of value-added products such as organic acids or biomass for human consumption using mostly refined sugars. Therefore, the replacement of refined sugars by theoretically more cost-effective and environmentally-friendly lignocellulosic wastes will depend if the value-added products produced outweigh the costs needed for running the plant. In here, substrate- and strain-specific developments towards optimised pretreatment steps coupled with optimised preparation of enzyme cocktails can play an important role. Finally, the wider application of filamentous fungi will also be dependent on robust strategies for control of their morphology under SmF since it influences the process performances towards the production of the desired product.

8. Conclusions

Ascomycetes are arguably potential biocatalysts for conversion of a wide range of waste materials to value-added products. Their ability to synthesise enzymes for degradation of lignocellulosebased materials will most probably give them great attention by the scientific community for production of organic acids, ethanol and enzymes since they are the first-choice substrates towards cheaper processes. Their biomass rich in protein and lipids has high potential to push forward the establishment of new biorefineries or improvement of already running facilities.

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