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Aspergillus oryzae Grown on Rice Hulls Used as an Additive for Pretreatment of Starch-Containing Wastewater from the Pulp and Paper Industry

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Abstract: From an industrial point of view, the use of microorganisms as a wastewater bioremediation practice represents a sustainable and economic alternative for conventional treatments. In this work, we investigated the starch bioremediation of paper mill wastewater (PMW) with *Aspergillus oryzae*. This amylase-producing fungus was tested in submerged fermentation technology (SmF) and solid-state fermentation (SSF) on rice hulls. The tests were conducted to assay the concentration of the reducing sugars on paper mill wastewater. The bioremediation of starch in the wastewater was carried out by *A. oryzae*, which proved capable of growing in this complex media as well as expressing its amylase activity.

Keywords: *Aspergillus oryzae*; rice hull; paper mill wastewater; bioremediation; amylase; solid-state fermentation (SSF)

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1. Introduction

Climate change, together with the growing population expected over the coming years, makes food production a crucial issue. In this context, prevention and minimization of food waste are recognized as key actions [1]. In addition, food waste is highly polluting as it leads to the misuse of resources and significant greenhouse gas emission levels. To address these issues, the adoption of the biorefinery concept (circular economy approach), particularly strengthening the agri-food waste biorefinery, is a strategic point not only to make the cost of the process economical but also to reduce the pressure on natural resources [2,3]. There is no explicit mention or definition of the valorization of food supply chain waste in the Waste Framework Directive. However, the objective of using waste for value-added production comes within the spirit of the directive [4,5].

A circular economy provides a different flow model, where no resources are wasted; on contrary, they are considered as feedstocks. In particular, open-loop material flow patterns bring new supplies of secondary materials into the raw material pool that can be reclaimed by other industries [6,7]. Rice hulls are the largest by-product of rice milling in producing countries and most of them are thrown away as a waste byproduct, which will undoubtedly have too many negative influences on the global environment. Worldwide production amounts to approximately 100 million tonnes per year [8]. Rice hulls, the lignocellulosic outer coats of rice, have been only considered as combustible to recover energy or animal bedding, because of their low nutritive value as animal feed [9]. Although rice hulls have long been identified as a source for energy production, experiences from large-scale rice husk firing are quite limited, because of the high quantity of ash (about 20%) [10].

Many efforts have been made to use rice hulls as feedstock to produce fermentable sugars, followed by ethanol fermentation, but the application on large scale is still affected by the out-of-market costs of pretreatments and saccharification [9]. The pretreatment process is considered the most expensive step in the valorization of lignocellulosic byproducts, where it can contribute to about 30% of the total cost. In fact, even those experiences have demonstrated that rice hulls can be more conveniently re-used as untreated material [11]. For example, promising research has been carried out on the use of rice hulls, as well as other agricultural lignocellulosic waste, as inexpensive and efficient biosorbent for heavy metals removal from contaminated wastewater [12]. From this perspective, rice hulls have also been used as solid support for the production of various fermented products and enzymes by solid-state fermentation (SSF) [13]. SSF is defined as the cultivation of microorganisms on inert carriers or on insoluble substrates that can, in addition, be used as carbon and energy source. The fermentation takes place in the absence or near absence of free water, thus being close to the natural environment to which microorganisms, especially fungi, are adapted [14].

SSF aims to bring the cultivated fungi or bacteria into tight contact with the insoluble substrate and thus to achieve the highest substrate concentrations for fermentation [15]. This technology results, although so far only on a small scale, in several processing advantages of significant potential economic and ecological importance as compared with submerged fermentation. SSF holds tremendous potential to produce enzymes, as amylases, proteases, or extracellular lipases by fungal strains belonging to the genus *Aspergillus*. Several experiences have been reported on the production of amylase and glucosidase by *Aspergillus niger* from sugarcane bagasse, corn cobs and rice hulls using SSF [16], production of amylase by *Aspergillus oryzae* on spent brewing grain as solid substrate in SSF [17].

In this work, rice hulls have been used as support for SSF of the amylase-producing *Aspergillus oryzae* to verify the feasibility to add an amount of dried powder of *A. oryzae* adherent on rice hulls, as an additive in starch-containing wastewater treatments from pulp-and-paper mill [18]. Starch is presently the third most prevalent component by weight in papermaking, only surpassed by cellulose fiber and mineral pigments. It is used as a flocculant and retention aid, as a bonding agent, as a surface size, as a binder for coatings, and as an adhesive in corrugated board, laminated grades, writing paper, and other products. The starch-containing effluents generated by the papermaking industry are usually destined for the anaerobic digestion process or degraded by aerobes microorganisms in fluidized bed bioreactors. In this context, simple and low-cost strategies for accelerating starch digestion are desirable before both anaerobic and aerobic effluent treatments.

In a wider perspective, this application could represent a promising example of industrial symbiosis, where agri-food waste valorization can become the point of connection between two different supply chains, in which one company's waste is used as raw material by another company.

2. Materials and Methods

2.1. Microorganism and Its Maintenance

Aspergillus oryzae DSM 1862 belongs to the collection of microorganisms of the Life Sciences and Biotechnology Department of the University of Ferrara and was purchased from the DSMZ (Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany) company. It was propagated in potato dextrose agar medium (DIFCO, Wuerzburg, Germany), containing dextrose, 20 g/L, potato extract, 4 g/L, agar, 15 g/L added with chloramphenicol (Merck, Berlin, Germany), 50 mg/L for 72 h at 30 °C and stored in Petri dishes at 4 °C [19]. Amylase (CAS: 9000-90-2) lyophilized powder, $\geq$100 units/mg protein, was purchased from Merck, Berlin, Germany.

2.2. Inoculum Preparation

The liquid medium flasks (20 mL final volume) were inoculated with fungal cultures grown on PDA plates supplemented with chloramphenicol by aseptically transferring a

block of mycelium and spores (5 × 5 mm^2 area) of the plate culture into the flasks [20]. Three different growth media were tested: PDB medium (DIFCO, Wuerzburg, Germany) having the same composition as the PDA but without agar; malt extract medium (DIFCO, Wuerzburg, Germany) containing malt extract, 6 g/L, maltose, 1.8 g/L, dextrose, 6 g/L, and yeast extract, 1.2 g/L; paper mill wastewater, supplied by a local company. The cultures were incubated at 28 °C, 120 rpm for up to 96 h. All tests were carried out in triplicate for statistical significance.

2.3. Evaluation of Enzymatic Activity

From 20 mL inoculated flasks, 1 mL of the supernatant was taken immediately after inoculum and every 24 h until 72 h of growth. $(NH_4)_2SO_4$ sodium (657 mg) was added to each sample, once solubilized, the suspension was centrifuged (5600 RCF, 10 min). The precipitates were immediately resuspended in 10 mL of 100 mM acetate buffer containing 0.25% of starch at pH 5. Reaction samples (1 mL) were taken starting from T_0 (immediately after adding the enzyme), after 6 h, and every 24 h up to 72 h. The same enzymatic reaction protocol was applied to 10 mL of paper mill water instead of the solution containing starch. All tests were carried out in triplicate for statistical significance. All the collected samples were analyzed by the DNS assay for the quantification of reducing sugars.

2.3.1. Dinitrosalicylic Acid Method (DNS)

DNS reagent was prepared by dissolving dinitrosalicylic acid (0.2 g, 0.88 mmol), phenol (0.04 g, 0.42 mmol), sodium thiosulfate (0.01 g, 0.04 mmol) and sodium-potassium tartrate (4.0 g, 14.2 mmol) in 10 mL of NaOH 2% (W/V). Distilled water was added to this solution to a final volume of 20 mL.

For determination of reducing sugars, 2 mL of DNS reagent and 500 µL of distilled water were added to 500 µL of enzymatic reaction sample. The mixture was brought to a boil for 5 min and left to cool at RT. The absorbance was measured at 540 nm in a Shimadzu UV-1601 spectrophotometer.

The concentration of reducing sugars produced was calculated by comparison with the previously constructed calibration curve.

2.3.2. Solid-State Fermentation (SSF): Substrate Preparation

Fifty mililiter Erlenmeyer flasks containing 1 g of rice hulls were autoclaved (121 °C for 20 min). After cooling, the moisture content of rice hulls was brought up to 60% by the addition of 0.6 mL of a sterile water solution of KH_2PO_4 2 g/L, NaCl 1 g/L, $MgSO_4$, and $7H_2O$ 1 g/L.

2.3.3. Solid-State Fermentation (SSF): Inoculum Preparation

Two different types of inocula were used: the first consisted of 1 mL of spore suspension (S) obtained by spraying 10 mL of sterile 0.1% Tween-80 solution on 7-day-old PDA Petri dishes containing *A. oryzae*. One milliliter of spore suspension was used to inoculate 50 mL Erlenmeyer flasks containing 1 g of rice hulls. The number of spores was quantified by carrying out serial dilutions and seeding 0.1 mL of each one on the PDA plate. Petri dishes were incubated for 4 days at 28 °C and then counted to establish the starting load.

The second type of inoculum consists of the supernatant of submerged liquid fermentation (SLF) and was obtained as follows: 6-day-old Petri dishes grown in PDA medium supplemented with chloramphenicol were used to inoculate a 50 mL Erlenmeyer flask containing 20 mL of PDB medium. After 4 days of growth, 1 mL of the medium was directly added to the rice hulls. The count of starting colonies was made following the same protocol used for spore suspension.

2.3.4. Solid-State Fermentation (SSF): Fermentation Conditions

For both inocula, the cultures were grown for 3, 5, 7, and 10 days. After fermentation, each SSF was resuspended in 10 mL of previously sterilized physiological solution and left

to stir at 100 rpm, RT for 2 h. One milliliter of suspension was then withdrawn, and the final microbial load (expressed in CFU/mL) was quantified by carrying out serial dilutions and seeding 0.1 mL of each one on PDA plates. Petri dishes were incubated for 4 days at 28 °C and then counted to establish the final microbial load. For the fermentation that provided the best microbial load, a subsequent drying step was carried out bringing the temperature to 45 °C for 48 h. All tests were carried out in triplicate for statistical significance. The relative standard deviation value for statistical analysis was also reported.

2.3.5. Solid-State Fermentation (SSF): Enzymatic Reaction on Paper Mill Water

After 6 days of *Aspergillus oryzae* growth of Petri dishes in PDA medium, a block of mycelium and spores (5 × 5 mm^2 area) of the plate culture with the fungus was used to inoculate a 50 mL Erlenmeyer flask containing 20 mL of PDB medium. The cultures were incubated at 28 °C, 120 rpm for up to 96 h. One milliliter of the supernatant was used to inoculate 50 mL Erlenmeyer flasks containing 1 g of sterile rice hulls added with 3 mL of a sterile water solution of KH_2PO_4 2 g/L, NaCl 1 g/L, and $MgSO_4 \times 7\ H_2O$ 1 g/L. The SSF was maintained in static conditions, at 28 °C for 10 days, and was then inoculated in 100 mL of paper mill water and kept under stirring at 100 rpm, 28 °C for 5 days. One milliliter of the supernatant was withdrawn and 657 mg of $(NH_4)_2SO_4$ sodium was added. Once solubilized, the suspension was centrifuged (5600 RCF, 10 min). The precipitates were immediately resuspended in 10 mL of paper mill water. In the case of the addition of amylase, 100 U was added to the reaction. Reaction samples were taken starting from T_0, every 24 h up to 72 h. All tests were carried out in triplicate for statistical significance. The relative standard deviation value for statistical analysis was also reported. All the collected samples were analyzed by the DNS assay for the quantification of reducing sugars.

3. Results and Discussion

Aspergillus oryzae was selected as α-amylase-producer fungal strain for bioremediation pretreatment of starch from the paper mill wastewater. The wastewater used was derived from several processes of the pulp and paper industry. Thus, its characteristics depend on the type of process, type of wood materials, process technology involved, management practices, internal recirculation of the effluent for recovery, and the amount of water to be used in the specific process [21]. Mandal et al. reported the pH, TS, SS, BOD_5, COD, and color characteristics of wastewater at various pulp and paper processes [22]. In this work, we aimed to exploit the amylase activity of *A. oryzae* for the pretreatment of starch-containing wastewater. The pretreatment of the wastewater (in this case proposed through a biotechnological process) is necessary as a preliminary step that precedes the generally employed "fluidized bed reactors" bioremediation [23,24]. In particular, the purpose of this work was to evaluate the feasibility of using *Aspergillus oryzae* to eliminate starch from paper mill wastewater, proposing a bioremediation approach as pre-treatment (elimination of starch) of the wastewater given its subsequent further remediation steps. In the experimental design, the stringent operational needs—typical of the industry—were considered. To this end, attention was given to the amylase effect of the fungus in the wastewater, more than on the characteristics of the wastewater itself, or the fungal growth (which is, from an industrial point of view, assumed because of the observations of amylase activity). As mentioned, the composition of the wastewater undergoes great fluctuations in its composition due to various variables related to the paper processing processes. Our study, therefore, focused on the evaluation of the capacity of *Aspergillus* in the elimination of starch present in the wastewater. To do this, we decided to embrace the principles of the circular economy, using a production waste such as the rice hull, which, in this context, represented a raw material having a solid support role for the growth of *Aspergillus*, allowing us to obtain biomass through solid-state fermentation processes (SSF). Figure 1 reports the experimental strategy for the study of the pretreatment bioremediation feasibility by *A. oryzae*.

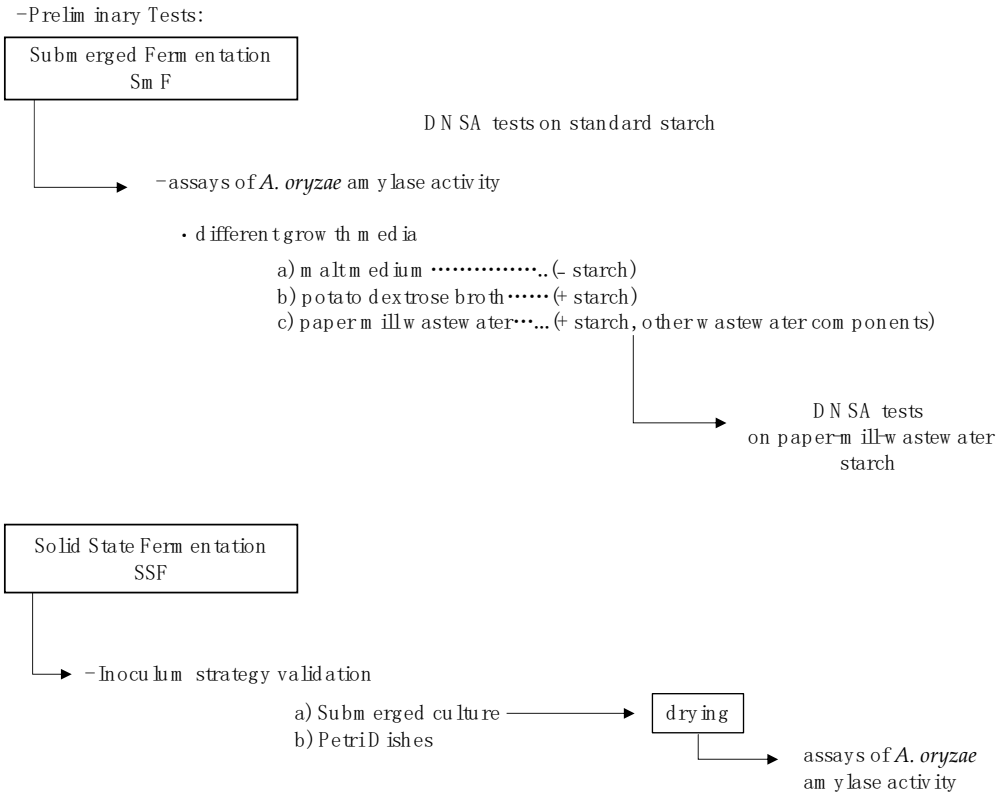

Figure 1. Draw chart of the experimental workflow of this study.

Firstly, the amylase activity of *A. oryzae* was tested with a submerged liquid fermentation technology (SmF). The preliminary steps of *Aspergillus* growth in submerged culture allowed us to test the effect that starch could have on the growth of the fungus and, consequently, on the production of amylase, as evidence of the observed hydrolysis of the starch supplied as a standard substrate. In a controlled context such as that of SmF, we were able to study the effects of the presence or absence of starch. Furthermore, we tested the system starting from a growth medium represented by the paper mill wastewater itself. The observations obtained justified the subsequent experiments allowing us to develop the SSF process. Therefore, solid-state fermentation (SSF) tests were conducted changing the inoculum type. In this experimental design, the dried inoculated rice hull will act as an additive for the bioremediation of starch in paper mill wastewater.

3.1. Preliminary Experiments: Amylase Activity Assays

The amylase activity of *A. oryzae* was preliminarily tested in SmF. DNS assays were performed for the quantification of reducing sugars. The indirect estimation of the amylase activity of *A. oryzae*, growth on malt medium, paper mill wastewater, and PDB, was tested on standard starch and paper mill wastewater (with and without STD starch).

The first test aimed to assess the ability of *A. oryzae* of growing independently by the presence or absence of starch in the media, testing the flexibility of this microorganism for bioremediation purposes. Therefore, two standard-medium (malt medium and potato dextrose broth—PDB), as well as the paper mill wastewater, were tested for the *Aspergillus* growth. The exoenzymes were purified and the amylase activity was tested on known quantities of standard starch. The results are shown in Figure 2.

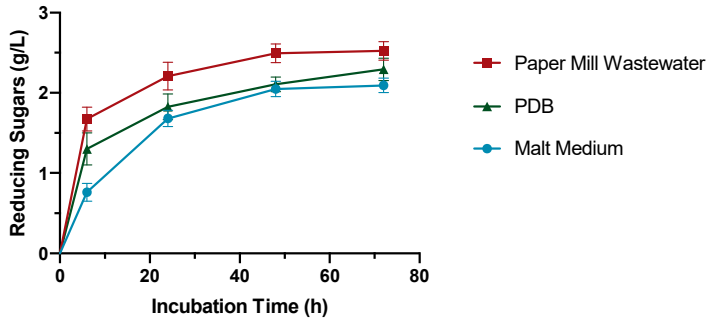

Figure 2. Effect of starch presence (paper mill wastewater and PDB) or absence (malt medium) on the concentration of reducing sugars. Assay of the reducing sugars after the hydrolysis of standard starch conducted with the purified enzymes from *A. oryzae* grown on PMW, PDB, and malt medium.

In our experimental design, we decided to exploit the parameter concerning the presence of reducing sugars, resulting from the hydrolytic activity of *Aspergillus* against starch. This parameter directed our experimental choices, allowing us to deduce the growth or not of *A. oryzae* as a function of the metabolic activity we found (specifically, its amylase activity). The industrial needs, which have guided our work, focused our attention on the feasibility of starch bioremediation by *Aspergillus*, justifying amylase activity as a key feature of the study of the process. As reported in Figure 2, the growth of *A. oryzae* seems to be not directly dependent on the presence of starch in the growth medium. Indeed, the quantity of reducing sugars, derived by the amylase activity of *A. oryzae* growth in presence of starch, or in its absence (malt medium), are comparable. However, in paper mill wastewater, the concentration of reducing sugars is higher in less time. In this context, probably, the presence of starch and additives in the paper mill wastewater could enhance the metabolic pathways of the expression of amylase. Moreover, essential for this work, the growth of *Aspergillus* is not hindered by a complex growth medium such as the paper mill wastewater.

Once the ability of the fungus to grow on paper mill wastewater was tested, as well as its production of amylase in this medium, the hydrolytic capacity of the enzymes produced by the fungus was evaluated, not on starch standards, but against the starches present in the paper mill itself. The results are shown in Figure 3.

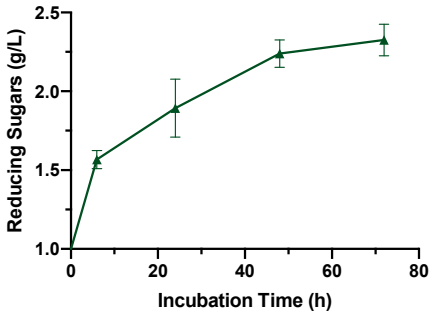

Figure 3. Reducing sugars from the amylase activity of *A. oryzae* in paper mill wastewater.

As reported in Figure 3, *A. oryzae* has proven capable of biodegrading the starches present in the paper mill wastewater. In just 6 h, the amount of reducing sugars found in the medium amounted to 1.6 g/L.

3.2. Solid-State Fermentation (SSF)

The physiological and genetic properties of the microorganisms could make SSF advantageous against SmF biotechnology [13]. Thus, we tested the SSF of *A. oryzae* starting with the choice of the right inoculum strategy. Furthermore, the SSF is the best strategy of bioremediation from an industrial point of view.

The solid rice hull was inoculated with the supernatant of *A. oryzae* growth in SmF (paper mill wastewater) compared to the inoculum of physiological solution in contact with the fungus growth on Petri dishes. To assay the effectiveness of the respective inocula, both suspensions, derived from SmF and Petri dishes, were collected for the CFU quantification. The CFU/mL were, respectively, 3×10^6 and 3×10^4.

The two inocula were used in the SSF of the *A. oryzae* rice hull. Several incubation times, 3 to 10 days, were tested. The results concerning the CFU/g of rice hull are shown in Table 1.

Table 1. CFU/g of rice hull for several incubation times. Inoculum strategies tested: SmF and Petri dishes. The results are shown as average, and the standard deviation is shown.

Days	CFU/g $_{\text{rice hull}}$	
	SmF	Petri Dishes
3	$8 \times 10^5 \pm 0.03$	$3 \times 10^4 \pm 0.04$
5	$3 \times 10^6 \pm 0.05$	$5 \times 10^4 \pm 0.05$
7	$4 \times 10^7 \pm 0.04$	$5 \times 10^5 \pm 0.03$
10	$4 \times 10^8 \pm 0.03$	$5 \times 10^6 \pm 0.02$

As reported in Table 1, the most interesting inoculation method, at 10 days, is the inoculation from SmF. Although the SmF inoculum has fewer initial CFUs, it proved to be the best on analysis after 10 days. This is due to the presence of active fungi in the SmF inoculum, rather than the Petri dish inoculum. In fact, in the latter, only spores of the fungus are present, which typically need more time for the regeneration of the metabolically active fungus [25]. Furthermore, this is an advantage from the industrial point of view, where the management of a liquid inoculum does not determine obstacles in process development.

In the industrial management of the solid-state fermented product for bioremediation purposes, its use in dry form is interesting. Although the normal moisture content of the SSF is 80%, for the storage and use of *Aspergillus* on rice hulls in an industrial context the dry form is the most advantageous in terms of process management. Therefore, we tested the capacity of *Aspergillus oryzae* of producing amylase in paper mill wastewater also after a drying step. The test involved the quantification of reducing sugars as evidence of the amylase activity. In 6 h, 1.3 ± 0.2 g/L of reducing sugars were found as proof of maintained amylase activity.

Finally, the SSF was tested in paper mill wastewater. The reducing sugars concentration was assessed after 72 h of treatment. The results are shown in Figure 4.

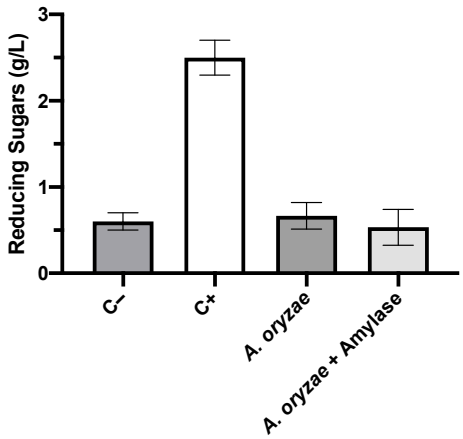

Figure 4. Effect of *A. oryzae* on the starch bioremediation of paper mill wastewater (72 h of incubation). C–, untreated PMW; C+, PMW treated with amylase STD.

In the results shown in Figure 4, the negative control refers to untreated paper mill wastewater. As expected, no reducing sugars were found. Indeed, in this context, the absence of *A. oryzae* and the following lack of amylase in the PMW suggest that no reducing sugars were found (except for those naturally present in the paper mill wastewater). On the contrary, reducing sugars were found when commercial amylase was added to the wastewater (as reported with positive control). This testifies that, in the PMW, starch is present and can be a substrate of the commercial amylase provided, validating the experimental design. If *A. oryzae* is supplied to the wastewater, it produces amylase which allows it to use the reducing sugars obtained from the hydrolysis of starch as a carbon source for its cellular metabolism. To evaluate this observation, the last column refers to the treatment of paper mill wastewater with *Aspergillus* and commercial amylase. The absence of reducing sugars supports that these are exploited by *Aspergillus* as metabolic substrates.

4. Conclusions

Aspergillus oryzae is a fungal strain widely exploited as an amylase producer. In this work, we aimed to study and test this fungus for the bioremediation of starch in industrial paper mill wastewater. For this purpose, submerged fermentation technologies (SmF) and solid-state fermentation (SSF) were studied. *A. oryzae* was found to grow on non-conventional media such as the paper mill wastewater. The SSF of *A. oryzae* was performed on rice hulls. In the bioremediation (as pretreatment) of paper mill wastewater, to remove starch, the fungus maintains its amylase activity and uses reducing sugars as metabolic substrates. This study opens new perspectives for the bioremediation of industrial effluents such as pulp-and-paper mill wastewater using *A. oryzae*.

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References

1. Costa, S.; Summa, D.; Semeraro, B.; Zappaterra, F.; Rugiero, I.; Tamburini, E. Fermentation as a Strategy for Bio-Transforming Waste into Resources: Lactic Acid Production from Agri-Food Residues. *Fermentation* **2021**, *7*, 3. [CrossRef]
2. Elmekawy, A.; Diels, L.; De Wever, H.; Pant, D. Valorization of Cereal Based Biorefinery Byproducts: Reality and Expectations. *Environ. Sci. Technol.* **2013**, *47*, 9014–9027. [CrossRef]
3. Zappaterra, F.; Summa, D.; Semeraro, B.; Buzzi, R.; Trapella, C.; Ladero, M.; Costa, S.; Tamburini, E. Enzymatic Esterification as Potential Strategy to Enhance the Sorbic Acid Behavior as Food and Beverage Preservative. *Fermentation* **2020**, *6*, 96. [CrossRef]
4. Dessie, W.; Luo, X.; Wang, M.; Feng, L.; Liao, Y.; Wang, Z.; Yong, Z.; Qin, Z. Current Advances on Waste Biomass Transformation into Value-Added Products. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 4757–4770. [CrossRef] [PubMed]
5. Semeraro, B.; Summa, D.; Costa, S.; Zappaterra, F.; Tamburini, E. Bio-Delignification of Green Waste (GW) in Co-Digestion with the Organic Fraction of Municipal Solid Waste (OFMSW) to Enhance Biogas Production. *Appl. Sci.* **2021**, *11*, 6061. [CrossRef]
6. Neves, A.; Godina, R.; Azevedo, S.G.; Pimentel, C.; Matias, J.C.O. The Potential of Industrial Symbiosis: Case Analysis and Main Drivers and Barriers to Its Implementation. *Sustainability* **2019**, *11*, 7095. [CrossRef]
7. Zappaterra, F.; Costa, S.; Summa, D.; Semeraro, B.; Cristofori, V.; Trapella, C.; Tamburini, E. Glyceric Prodrug of Ursodeoxycholic Acid (UDCA): Novozym 435-Catalyzed Synthesis of UDCA-Monoglyceride. *Molecules* **2021**, *25*, 5966. [CrossRef] [PubMed]
8. Economou, C.N.; Aggelis, G.; Pavlou, S.; Vayenas, D.V. Single Cell Oil Production from Rice Hulls Hydrolysate. *Bioresour. Technol.* **2011**, *102*, 9737–9742. [CrossRef]
9. Wei, G.Y.; Gao, W.; Jin, I.H.; Yoo, S.Y.; Lee, J.H.; Chung, C.H.; Lee, J.W. Pretreatment and Saccharification of Rice Hulls for the Production of Fermentable Sugars. *Biotechnol. Bioprocess Eng.* **2009**, *14*, 828–834. [CrossRef]
10. Glushankova, I.; Ketov, A.; Krasnovskikh, M.; Rudakova, L.; Vaisman, I. Rice Hulls as a Renewable Complex Material Resource. *Resources* **2018**, *7*, 31. [CrossRef]

11. Vaskalis, I.; Skoulou, V.; Stavropoulos, G.; Zabaniotou, A. Towards Circular Economy Solutions for the Management of Rice Processing Residues to Bioenergy via Gasification. *Sustainability* **2019**, *11*, 6433. [CrossRef]
12. Rizzuti, A.M.; Lancaster, D.J. Utilizing Soybean Hulls and Rice Hulls to Remove Textile Dyes from Contaminated Water. *Waste Biomass Valorization* **2013**, *4*, 647–653. [CrossRef]
13. Hölker, U.; Höfer, M.; Lenz, J. Biotechnological Advantages of Laboratory-Scale Solid-State Fermentation with Fungi. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 175–186. [CrossRef] [PubMed]
14. Zambare, V. Solid State Fermentation of Aspergillus oryzae for Glucoamylase Production on Agro Residues. *Int. J. Life Sci.* **2010**, *4*, 16–25. [CrossRef]
15. Rob, B.; George, R.; Yovita, S.P.R.; Marisca, J.H.; Margreet, H.; Ana, L.; Kenneth, G.A.D.; Maarten, A.I.S.; Jan, D.; Yang, Z.; et al. Aspergillus oryzae in Solid-State and Submerged Fermentations. *FEMS Yeast Res.* **2002**, *2*, 245–248.
16. Aliyah, A.; Alamsyah, G.; Ramadhani, R.; Hermansyah, H. Production of α-Amylase and β-Glucosidase from Aspergillus niger by Solid State Fermentation Method on Biomass Waste Substrates from Rice Husk, Bagasse and Corn Cob. *Energy Procedia* **2017**, *136*, 418–423. [CrossRef]
17. Febe Francis, A.; Sabu, K.; Madhavan Nampoothiri, G.S.; Pandey, A. Synthesis of A-Amylase by Aspergillus oryzae in Solid-State Fermentation. *J. Basic Microbiol.* **2002**, *42*, 320–326. [CrossRef]
18. Kamali, M.; Khodaparast, Z. Review on Recent Developments on Pulp and Paper Mill Wastewater Treatment. *Ecotoxicol. Environ. Saf.* **2015**, *114*, 326–342. [CrossRef] [PubMed]
19. Zappaterra, F.; Costa, S.; Summa, D.; Bertolasi, V.; Semeraro, B.; Pedrini, P.; Buzzi, R.; Vertuani, S. Biotransformation of Cortisone with Rhodococcus rhodnii: Synthesis of New Steroids. *Molecules* **2021**, *26*, 1352. [CrossRef] [PubMed]
20. Krishna, C.; Nokes, S.E. Influence of Inoculum Size on Phytase Production and Growth in Solid-State Fermentation by *Aspergillus Niger*. *Trans. Am. Soc. Agric. Eng.* **2001**, *44*, 1031–1036. [CrossRef]
21. Pokhrel, D.; Viraraghavan, T. Treatment of Pulp and Paper Mill Wastewater—A Review. *Sci. Total Environ.* **2004**, *333*, 37–58. [CrossRef] [PubMed]
22. Mandal, T.; Bandana, T. Studies on Physicochemical and Biological Characteristics of Pulp and Paper Mill Effluents and Its Impact on Human Beings. *J. Freshw. Biol.* **1996**, *4*, 6.
23. Kadwe, B.; Khedikar, I.; Hardas, C. Treatment of Starch Wastewater from Cardboard Packaging Industry Treatment of Starch Wastewater from Cardboard Packaging Industry. *IOSR J. Eng.* **2019**, 80–83.
24. Kumar, A.; Chandra, R. Biodegradation and Toxicity Reduction of Pulp Paper Mill Wastewater by Isolated Laccase Producing Bacillus cereus AKRC03. *Clean. Eng. Technol.* **2021**, *4*, 100193. [CrossRef]
25. Gougouli, M.; Koutsoumanis, K.P. Relation between Germination and Mycelium Growth of Individual Fungal Spores. *Int. J. Food Microbiol.* **2013**, *161*, 231–239. [CrossRef] [PubMed]