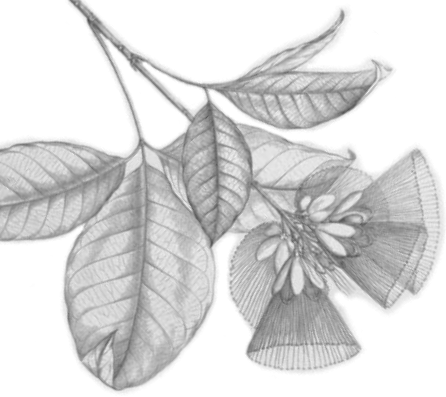

Filamentous Fungi as Promising Agents for the Biodegradation of Biosolids Compounds

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ABSTRACT

Wastewater Treatment Plant (WWTP) generate pasty wastes, known biosolids, which can be toxic and recalcitrant, motivating studies aiming at their degradation. Filamentous fungi were investigated to degradation of biosolids from a WWTP in the Goiânia, Goiás, Brazil. All grew in the presence of biosolids, being inhibited by increase in concentration, except SXS629, which increased proportionally to the concentration. All grew in the middle with biosolids at the original pH (10.5), although the correction (6.8) provided higher growth. Except SXS90, the others (SXS37, SXS615 and SXS628) degraded the biosolid, growing in medium containing biosolids as the only source of carbon; highlighting SXS628, whose growth in the biosolids exceeded the control. All evaluated isolates synthesize at least two prospected enzymes, especially SXS630 and SXS634, which synthesize all (carboxymethyl cellulase, tannase, polyphenoloxidase). This shows the potential use of these isolates (combined or not) in biotechnological processes aiming at the degradation of biosolids, especially SXS37.

Keywords: Activated Sludge; Bioremediation; Goiânia; Wastewater Treatment.

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With the constant population growth and the economic development of urban centers, there has been a significant increase in the production of solid and liquid waste. Unfortunately, even today, most domestic and industrial liquid wastes are dumped directly into the water bodies, polluting the water, making it unsuitable for consumption, and impacting the dynamics of aquatic ecosystems (Menezes 2013).

To minimize this situation, it is necessary to expand the Wastewater Treatment Plants (WWTP). Many Brazilian cities rely on WWTP, which operate with different technological systems (Ucker et al. 2012). In general, in these wastewater treatment systems, water returns to the springs with an acceptable degree of purity; however, the process results in the generation of a semi-solid waste, pasty and predominantly organic in nature, called sewage sludge or biosolids (Von Sperling & Gonçalves 2001).

This biosolid consists of a series of organic and inorganic compounds, such as sugars, proteins, heavy metals, chemical surfactants and traces of cellulose, hemicellulose, tannins, lignin and several other phenolic compounds. Some of these compounds are toxic and/or recalcitrant, which can cause health damage or even remain in the environment for many years (Santos 2009; Souza & Rosado 2009; Menezes 2013). For this reason, it is production and accumulation on a large scale has generated concerns, which has motivated different studies aiming at the degradation or the safe use of this residue (Brasil 2006).

Biosolid degradation may involve chemical, physical and/or biological processes. However, it is challenging to find a form of financially viable and environmentally beneficial treatment (Kamida et al. 2005). The biological treatment has become an efficient alternative in residues reduction, through the introduction of microorganisms. This technique can contribute to the reduction of biosolids within the treatment plant, minimizing transport costs and incorrect disposal, which can lead to contamination of soils and springs (Menezes 2013). However, this process requires studies on the chemical composition of biosolids and the efficiency of biological agents in their biodegradation.

More et al. (2010), Menezes (2013) and Menezes et al. (2017) have emphasized the use of fungi from natural ecosystems, which act in nutrient cycling, for the biodegradation of these compounds. These organisms produce enzymes, especially those of the lignocellulolytic system, capable of degrading complex compounds into simpler molecules. Among these enzymes, there are cellulases, polyphenoloxidases and tannases (Souza & Rosado 2009; Leonardo-Silva et al. 2018).

Cellulases are important part of cellulolytic enzymes complex, which act directly on the hydrolysis of cellulosic polysaccharides. One of them the carboxymethyl cellulase (CMCase), an endoglucanase that hydrolyzes bonds within the cellulose chain, releasing glucose, cellobiose and cellodextrins after your action (Oliveira 2014). They can be produced by fungi or bacteria, and those of fungal origin are predominant in commercial applications due to their high level of expression and secretion (Chang 2007).

Within the group of polyphenol oxidases, the ligninases has great prominence because they are enzymes that degrade the lignin through an oxidative process. These enzymes constitute a ligninolytic complex formed by the enzymes manganese peroxidase (MnP), lignin peroxidase (LiP) and laccase (Aguiar 2006). The MnP depends on Mn^{2+} which is oxidized by the enzyme Mn^{3+}, which in turn oxidizes organic substrates such as phenols and phenoxy radicals (Kuwahara et al. 1984). The LiP oxidizes lignin and its derivatives, in addition to non-phenolic compounds, by removing an electron from an aromatic nucleus, creating an unstable radical that undergoes numerous transformations leading to the disruption of the substrate molecule (Rodriguez & Durán 1988). And the oxidation of aromatic compounds and inorganic substances with concomitant reduction of dissolved oxygen are performed by the laccase (Durán et al. 1994).

Although tannases do not integrate the lignocellulolytic complex, they have great relevance in the degradation of recalcitrant compounds, since they promote the removal of tannins, which are substances responsible for the resistance of plants and other substrates to microbial attack (Scalbert 1991), thus hampering the biodegradation. Tannins have the ability to bind to proteins or combine with cellulose and pectin to form complexes, mostly insoluble (Pinto et al. 2005). In this perspective, this study aims to investigate the capacity of different filamentous fungi to degrade biosolids from chemically treated sewage.

MATERIALS E METHODS

BIOSOLID USED

The biosolid used in the assays was obtained from the WWTP Dr. Hélio Seixo de Britto, located in the city of Goiânia, capital of the State of Goiás, Brazil. The WWTP is responsible for collecting and treating 61% of the sewage produced in the city and works with a chemically assisted advanced primary treatment system, in which the treated effluent is later released in the Meia Ponte river spring, and the biosolid, previously treated with calcium hydroxide ($Ca(OH)_2$), in sufficient quantity to raise the pH to 12, is intended for agricultural properties for soil enrichment purposes.

The sample was collected in a polyethylene bag, which was placed in a styrofoam container and taken to laboratory, where part of the sample was dehydrated in a forced air circulation oven at 105 ºC for 24 hours, and crushed until a homogeneous powder was formed, and the other part was preserved in its original moisture and conditioned in a refrigerator at 4 ºC.

The biosolid was characterized by the WWTP, following the standardization recommended by the CONAMA Resolution 375/2005, art. 11 (Brasil 2005). For this, standard methodology was used to verify the presence of helminth eggs, mainly of *Ascaris* spp. (Yanko 1988), and for the physicochemical and microbiological analyzes through the Standard methods for the examination of water and wastewater (USEPA 2003).

DETECTION AND ISOLATION OF BIOSOLIDS FUNGI

To verify the presence and subsequent isolation of fungi from biosolids, a solution of 1 g of non-dehydrated biosolids diluted in 9 ml of physiological solution (0.09% NaCl) was used. The suspension obtained had the pH adjusted to 6.8 and was successively diluted to the ratio of 10^{-2}.

A 100 µl aliquot of suspension was inoculated into Petri dishes containing potato dextrose and agar medium (PDA) plus chloramphenicol (0.025 g L^{-1}). The inoculum was spread over the medium surface with a Drigalski handle. The suspension obtained from autoclaved biosolids was used the control.

The cultures were incubated in BOD at 25 ºC for 15 days, observed every two days for the appearance of fungal colony-forming units (CFUs), which were counted and categorized regarding their morphotype. Each colony of different morphotypes was isolated in new plates containing PDA growth medium in order to obtain pure cultures.

THE FUNGAL ISOLATES USED IN THE ASSAYS

The fungi used in the assays were selected from the collection of Fungi cultures from the Laboratório de Micologia Básica, Aplicada e Divulgação Científica at the Universidade Estadual de Goiás/CCET, which correspond to macromycetes species of different taxonomic groups, from different substrates in areas of the Cerrado biome. The isolates were reactivated from the culture collection through inoculation in PDA medium. In addition, fungi isolated from the biosolids (as described previously) were also used (Table 1).

Table 1. Fungal isolates used in the assays with biosolid from the Wastewater Treatment Plant (WWTP) Dr. Hélio Seixo de Britto, located in the city of Goiânia, Goiás. SJRP = Herbarium of the Universidade Estadual Paulista, campus of the São José do Rio Preto, HUEG = Herbarium of the Universidade Estadual de Goiás, SXS = Coleção de Culturas de Fungos do Laboratório de Micologia Básica, Aplicada e Divulgação Científica of the Universidade Estadual de Goiás. Bas = Basidiomycete, Asc = Ascomycete, WRF =White rot fungi, BRF = Brown rot fungi, Unk = Unknown.

Species/taxonomic and functional group	Voucher in collection		Origin
	Herbarium	Culture collection	
Inonotus rickii (Pat.) D.A. Reid/Bas/WRF	SJRP28714	SXS 37	On wood fragment, in the Ecological Station of the Northwest São Paulo. São José do Rio Preto-Mirassol, São Paulo, Brazil.
Gloeophyllum sp./Bas/WRF	SJRP28737	SXS 90	On wood fragment, in the Ecological Station of the Northwest São Paulo. São José do Rio Preto-Mirassol, São Paulo, Brazil.
Ganoderma stipitatum (Murrill) Murrill/Bas/WRF	HUEG11872	SXS 615	On living tree trunk, in urban area, Anápolis, Goiás, Brazil.
Lentinus tricholoma (Mont.) Zmitr./Bas/BRF	HUEG11869	SXS 624	On living tree trunk, Trilha do Tatu Ecological Reserve. Anápolis, Goiás, Brazil.
Hydnopolyporus palmatus (Hook.) O. Fidalgo/Bas/WRF	HUEG11866	SXS 628	On living tree trunk, Trilha do Tatu Ecological Reserve. Anápolis, Goiás, Brazil.
Phillipsia sp./Asc	HUEG11871	SXS 629	On dead tree trunk, Trilha do Tatu Ecological Reserve. Anápolis, Goiás, Brazil.
Indetermined (Morphotype1)/ Unk/Unk	-	SXS 630	WWTP Dr. Hélio Seixo de Britto. Goiânia, Goiás, Brazil.
Indetermined (Morphotype 2)/ Unk/Unk	-	SXS 631	WWTP Dr. Hélio Seixo de Britto. Goiânia, Goiás, Brazil.
Indetermined (Morphotype 3)/ Unk/Unk	-	SXS 632	WWTP Dr. Hélio Seixo de Britto. Goiânia, Goiás, Brazil.
Indetermined (Morphotype 4)/ Unk/Unk	-	SXS 633	WWTP Dr. Hélio Seixo de Britto. Goiânia, Goiás, Brazil.
Indetermined (Morphotype 5) /Unk/Unk	-	SXS 634	WWTP Dr. Hélio Seixo de Britto. Goiânia, Goiás, Brazil.

Source: Authors.

FUNGI TOLERANCE TO BIOSOLID

Fungi tolerance to biosolid was evaluated through mycelial growth in PDA medium plus biosolids at different pH values (6.8 and 10.5) and final concentration (5, 10 and 20%) in the medium. The inoculum consisted of a 1.2 cm diameter disc of the mycelial culture in PDA, which was deposited at a central point on the medium surface with biosolid. Petri dishes containing the cultures were incubated in a BOD incubator at 25 °C for 15 days and mycelial growth was evaluated by measuring the colony diameter on alternate days for a period of 15 days.

FUNGI CAPACITY IN DEGRADING BIOSOLID

To verify the ability of fungi to degrade the biosolid, using it as a nutrient source, we used the minimal medium (Pontecorvo et al. 1953) plus biosolid (final concentration of 20%) as the only source

of carbon. As a negative control, the minimal medium was used without additions, and as a positive control, the minimal medium was added with glucose (10 g L^{-1}). The pH was adjusted to 6.8. In these assays the fungal isolates that showed the greatest growth in the previous assays were used. The fungi were inoculated and the cultures incubated and evaluated as described in the previous item.

DIAGNOSIS OF FUNGAL ENZYMATIC ACTIVITY

The assays to verify the enzymatic production by the fungi studied were performed using the cup-plate method (Souza et al. 2008). For this purpose, was used minimal medium (Pontecorvo et al. 1953), with or without the respective substrate of the studied enzymes. That is, for the activity of carboxymethyl cellulase (EC 3.2.1.4), tannase (EC 3.1.1.20) and polyphenoloxidase (EC 1.10.3.1), it was added carboxymethyl cellulose, gallic acid or tannic acid (10 g L^{-1}), respectively. The fungi were inoculated and the cultures incubated as described in the previous item.

The positive result was observed through the appearance of the halo of degradation of the substrate around the colony, observed at the seventh day of growth, and the enzymatic activity determined by the calculation of the enzymatic index (EI), based on the sum of the diameter of the colony and the formed halo, dividing the result by the colony diameter value (Hankin & Anagnostakis 1975). Isolates that exhibited IE $\geq$ 2.0 were considered to be good producers of the enzyme evaluated (Lealem & Gashe 1994). For evaluation of CMCase activity, the halo of degradation was developed after the addition of congo red solution (2.5 g L^{-1}), prepared in 0.1 M Tris-HCl buffer, pH 8.0, followed by wash with NaCl solution (0.5 mol L^{-1}) (Teather & Wood 1982). The assays were conducted in triplicate and the results presented correspond to the average of three replicates.

RESULTS AND DISCUSSION

BIOSOLID CHARACTERIZATION

The solid fraction of the biosolid is called total solids (TS) and may be found in two forms: total dissolved solids (TDS) or total suspended solids (TSS), predominating the TSS. In both forms, the biosolid is subdivided into volatile solids (VS): organic fraction and fixed solids (FS): inorganic fraction (Jordão & Pessôa 1995). The VS/TS ratio is an indication of the degree of stability of biosolids, which according to the Brazilian legislation should demonstrate values lower than 0.7 (Brasil 2006).

The physicochemical analysis of the biosolids studied presented 31.78% of total solids and 58.63% of volatile solids, whose VS/TS ratio was 1.84 (Table 2). According to Costa et al. (2013) the volatile solids content allows an estimate of the amount of organic matter present in the biosolids. The value obtained in the studied biosolids resembles the value found by these authors (57.1%) when

evaluating the biosolids from the WWTP of Jundiaí, São Paulo, whose effluent was treated biologically, with the removal of the organic load carried out in two stages: aerobic digestion and anaerobic stabilization in decantation ponds. The fixed solids are related to the biosolid stabilization, which according to Sanepar (1999), must have at least 30% of fixed solids. The biosolids of the WWTP of Goiânia presented 41.37% (Table 2).

Table 2. Results of physicochemical analyses of biosolid from the Wastewater Treatment Plant (WWTP) Dr. Hélio Seixo de Britto, Goiânia, Goiás, Brazil. Sample collected in April 09th, 2018.

Parameter	Value		
	Without $Ca(OH)_2$	With $Ca(OH)_2$	Unit
pH	6.33	12.33	-
Total solids	28.59	31.78	%
Total volatile solids	67.56	58.63	%
Total fixed solids	32.44	41.37	%
Kjeldahl Nitrogen	32.76	29.40	g kg^{-1} dry basis
Total phosphorus	10.80	8.60	g kg^{-1} dry basis
VS/TS ratio	2.36	1.84	-

Source: WWTP Goiânia.

With the addition of $Ca(OH)_2$, the total phosphorus content was reduced by approximately 20% (8.6 g kg^{-1} dry basis) (Table 2), lower than that reported by Araújo et al. (2009), which obtained the value of 16 g kg^{-1} for the activated biosolids from the WWTP of the city of Franca, São Paulo. The difference in this value may be related to the addition of ferric chloride in the treatment of biosolids from the WWTP of Goiânia, which was not used in the WWTP of Franca. Ferric chloride is a polyelectrolyte that functions as an efficient coagulant for removing of phosphorus in the liquid effluent (Brasil 2005). However, the use of this coagulant can generate a larger mass of biosolids, which occurs due to the large amounts of ferric chloride added, causing that compound to become part of the solid mass of the sewage biosolid (Cavalcanti 2009), increasing its volume and the final mass, besides concentrating the phosphorus in the biosolid.

The Kjeldahl nitrogen content with $Ca(OH)_2$ addition decreased from 32.76 to 29.4 g kg^{-1} dry basis (Table 2), similar to that found by Coelho et al. (2011) (31.8 g kg^{-1} dry basis) in the biosolids from the WWTP of the city of Jundiaí, São Paulo, being higher than the mean value reported by Santos (2005) (10.7 g kg^{-1} dry basis) for WWTPs in Brazil. This is due to the fact that the mineralization of the nitrogen compounds is slower, that is, the reduction of organisms' diversity that act in the organic-inorganic nitrogen conversion in the anaerobic environment.

The pH was close to neutrality (6.33) in the biosolid without $Ca(OH)_2$, becoming highly alkaline (12.33) after adding this compound (Table 2). This high pH due to the addition of $Ca(OH)_2$ at

the end of the treatment process aims to eliminate potential pathogenic microorganisms present in the biosolid.

In the microbiological analysis of biosolids after the addition of $Ca(OH)_2$, the total coliforms index (6.0 MPN g^{-1} of TS), of *Escherichia coli* (3.0 MPN g^{-1} of TS) and *Salmonella* spp. (0.0 MPN g^{-1} of ST) in 10 g of TS (Table 3), indicate that the biosolid is in accordance with standards of CONAMA Resolution No. 375 of 2006, and that the addition of $Ca(OH)_2$ is efficient in the elimination of microorganisms. This resolution classifies as Type B biosolids less than 10^3 MPN g^{-1} of TS and Type B biosolids, which presents more than 10^3 MPN g^{-1} of TS and less than 10^6 MPN g^{-1} of TS (Brasil 2006). Based on this, the biosolids of the Goiânia WWTP are classified as type B.

Table 3. Results of microbiological analyses of biosolid from Wastewater Treatment Plant (WWTP) Dr. Hélio Seixo de Britto, Goiânia, Goiás, Brazil. TS = Total Solids; MPN = Most probable number.

	Value		
Parameter	**Without $Ca(OH)^2$**	**With $Ca(OH)^2$**	**Unit**
Coliformes total	$8.4x10^6$	$6.0x10^6$	MPN g^{-1} of TS
Escherichia coli	$2.4x10^6$	$3.0x10^6$	MPN g^{-1} of TS
Salmonella spp.	Absence	Absence	in 10 g of TS

Source: WWTP Goiânia.

The parasitological characterization of the biosolids showed that the treatment was efficient, since the results of evaluation of the presence of viable and non-viable helminth eggs (*Ascaris* sp., *Hymenolepis* sp., *Toxocara* sp. and *Trichuris* sp.) were null and according to the CONAMA Resolution No. 375/06 (Brasil 2006).

DETECTION AND ISOLATION FUNGI FROM BIOSOLIDS

Table 4. Characterization of the fungi isolated of the biosolid from the Wastewater Treatment Plant (WWTP) Dr. Hélio Seixo de Britto, Goiânia, Goiás, Brazil. [1]Collection of Fungal Cultures of the Laboratório de Micologia Básica, Aplicada e Divulgação Científica of the Campus de Ciências Exatas e Tecnológicas at the Universidade Estadual de Goiás.

Morphotype	**Voucher[1]**	**Macromorphological aspect of the colony**			
		Color	**Texture**	**Density of mycelium**	**Additional observations**
1	SXS 630	White	Cottony	Sparse	Presence of yellowish exudate in the medium
2	SXS 631	White	Cottony	Dense	-
3	SXS 632	Cream	Cottony	Dense	Presence of blackish and rounded mycelial projections and yellowish exudate in the medium
4	SXS 633	White	Cottony	Dense	Presence of yellowish exudate in the medium
5	SXS 634	Cream	Cottony	Sparse	Presence of blackish globular structures developing on the mycelium

Source: Authors.

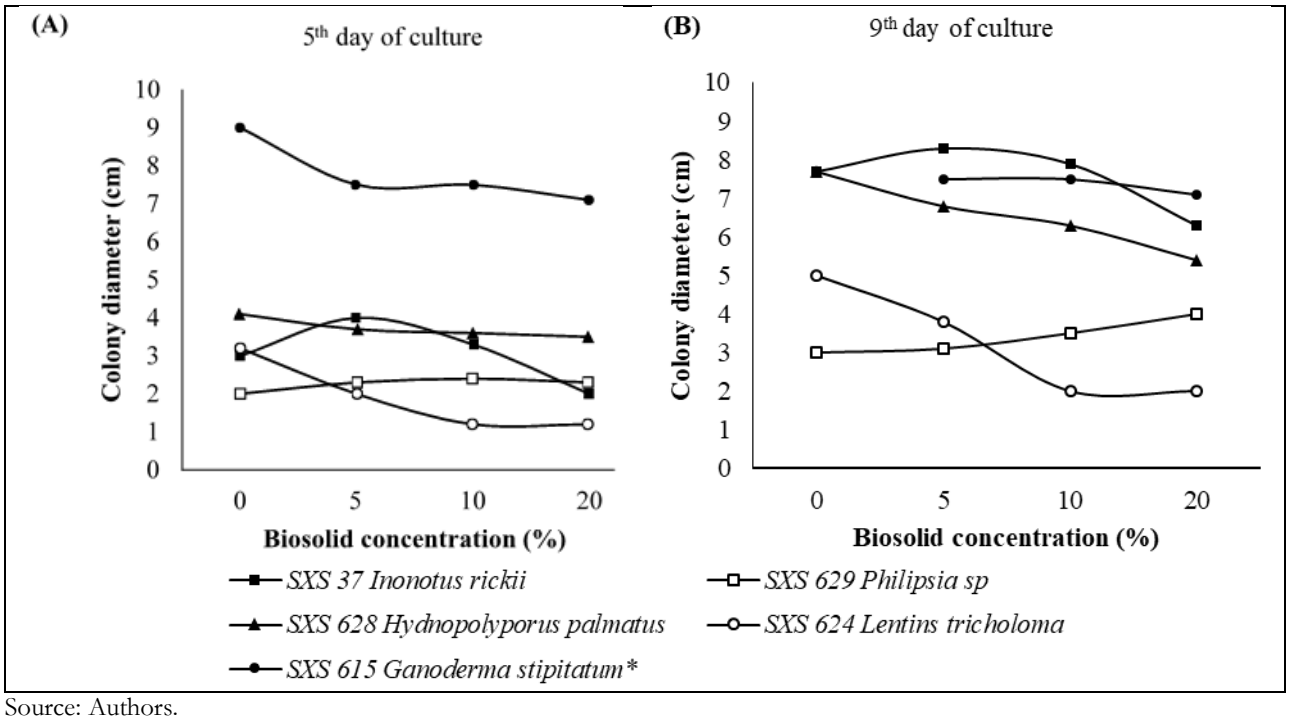
A total of 15 CFUs, distributed in five distinct morphotypes, all corresponding to filamentous fungi (Table 4), were obtained in the experiment. No CFUs were observed on the control plates. Santos

(2005), using procedures similar to those adopted in the present study, obtained 24 CFUs in biosolids from the WWTP Mangueira in Recife, Pernambuco, with pH around 6.6; which, according to Takashi (2003), is in the ideal range for fungal development, which is from 5.5 to 9.0, different from the alkaline pH (10.5) of the biosolid analyzed in this study. Therefore, it is possible that pH has been a limiting factor to the survival of fungi and consequently to the development of CFUs.

FUNGI TOLERANCE TO THE PRESENCE OF BIOSOLIDS

All fungi tested were able to grow in the presence of the biosolid. However, growth was inhibited proportionally to the increase of biosolids concentration, except for *Phillipsia* sp. (SXS 629), whose increase in colony diameter was proportional to the increase of biosolids concentration; and *Inonotus rickii* (SXS 37), whose growth in medium containing 5% biosolid was higher than growth in the negative control, but the growth decreased with the increase of the biosolids concentration (Figure 1).

Figure 1. Colonial growth of different fungal isolates in Potato Dextrose Agar medium with biosolids from the Wastewater Treatment Plant (WWTP) at different concentrations and pH 6.8. * On the 5th day of culture in the medium without biosolid, the colony of this isolate reached the whole extension of the plate, being no longer possible to evaluate the growth evolution.



Source: Authors.

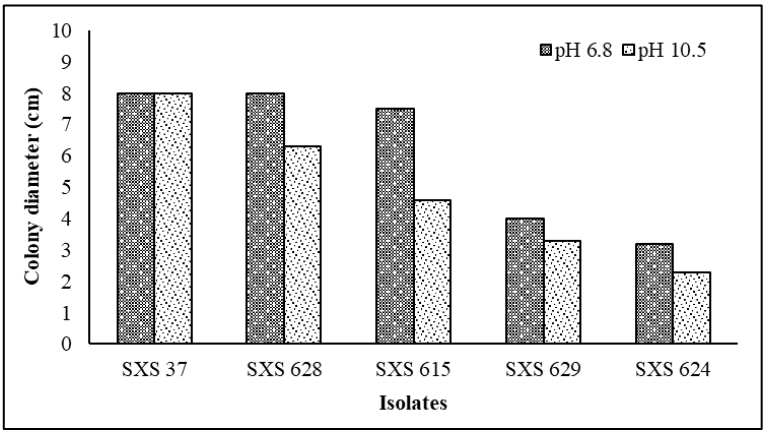
In addition to the effect on mycelial growth rate, the addition of biosolids in the medium caused morphological changes in some isolates. *Ganoderma stipitatum* (SXS 615), in the presence of the biosolid, presented more sparse, thin and hyaline hyphae. The isolate of *I. rickii* (SXS 37) showed hyphal spacing in the presence of biosolid, in all concentrations, besides the loss of the yellow/ochreish

coloration, when compared to the control. *Phillipsia* sp. (SXS 629) showed hyphal densification and irregular, nonconcentric mycelial growth, and slow growth in proportion to the increase in biosolids concentration (Figure 1). According to Schloter et al. (2003), Gomes & Pena (2016) and Ferreira et al. (2017), substrate composition, water availability and oxygen supply, as well as temperature and pH, influence the development of fungi. Also, according to Donini et al. (2005) and Marino et al. (2006), in addition to genetic factors of each fungal species, morphological alterations, such as different degrees of hyphal thickening, may be related to the capacity of metabolization of the substrate constituents, due to the ability to synthesize enzymes that degrade compounds to obtain carbon, nitrogen and other nutrients needed for its growth.

The fungal species that have fast and robust mycelial growth, accentuate the production of metabolites (Oliveira 2008), and consequent assimilation of the constituents provided by the substrate. This relationship is noticeable in the different isolates studied, since the substrate containing biosolids may present toxic and/or recalcitrant compounds, hindering their break down by species with limited enzymatic apparatus, which would cause the mycelium to be sparser, in search of other sources of nutrition. On the other hand, species with enzymatic apparatus capable of degrading these compounds, would present hyphalic densification (Ruegger & Tauk-Tornisielo 2004).

When evaluating the influence of pH on fungal growth in the presence of biosolids, it was found that, most of the isolates, despite being able to grow in the presence of biosolids without the pH adjustment in the medium, when adjusting the pH to 6.8, the growth was significantly higher, except for the isolate *I. rickii* (SXS 37), whose growth was not influenced by the change in the pH evaluated (Figure 2).

Figure 2. Mycelial growth of different fungal isolates, in the 13th day of culture in Potato Dextrose Agar medium with biosolids (20% and pH 6.8 and 10.5) from the Wastewater Treatment Plant (WWTP) Dr. Hélio Seixo de Britto, Goiânia, Goiás, Brazil.

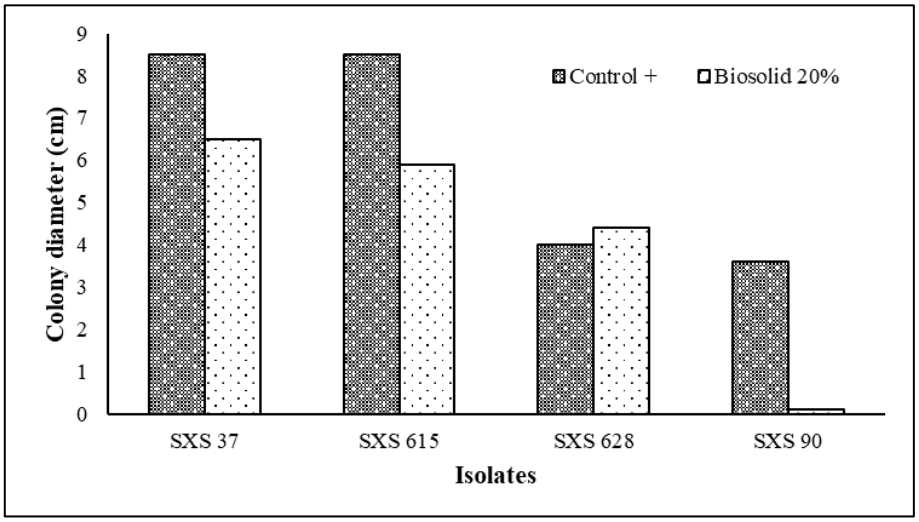


Source: Authors.

FUNGAL CAPACITY IN DEGRADING THE BIOSOLID

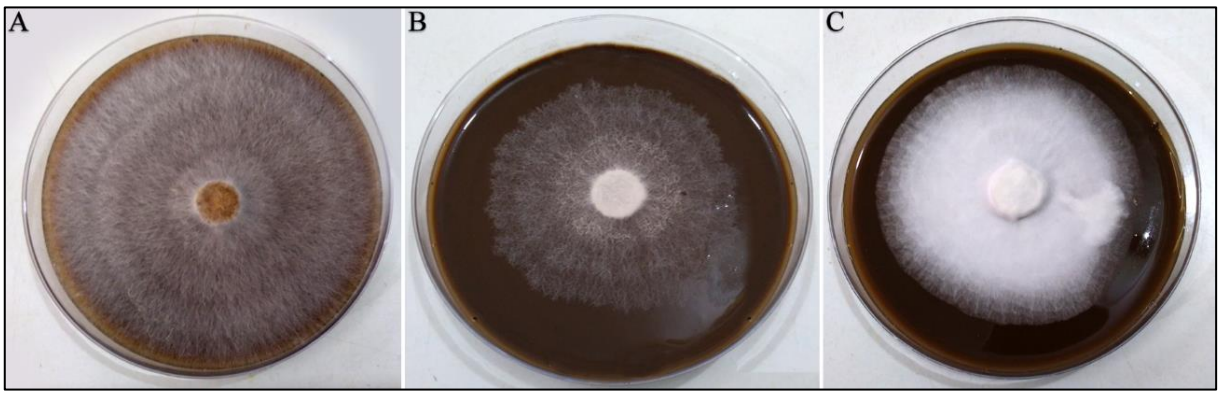
With the exception of the isolate *Gloeophyllum* sp. (SXS 90), the other isolates *I. rickii* (SXS 37), *G. stipitatum* (SXS 615) and *H. palmatus* (SXS 628) were able to grow in medium containing biosolids as the only source of carbon, which indicates that these fungi are capable of degrading the biosolids, using in their metabolism the components present in the biosolids (Figure 3 and Figure 4). The ability of fungi to quickly adapt their metabolism to different carbon sources is an essential factor for their survival. This ability is due to the production capacity of extracellular enzymes (Silva & Esposito 2004).

Figure 3. Mycelial growth of different fungal isolates in minimal medium (Pontecorvo et al. 1953) with biosolids (20%) from the Wastewater Treatment Plant (WWTP), Dr. Hélio Seixo de Britto, Goiânia, Goiás, Brazil. Biosolids are the only source of carbon. The positive control corresponds to the minimal medium with glucose (10 g L^{-1}) and the negative control to the minimal medium without additions. The final pH was 6.8. There was no growth in the negative control. Data were recorded on the 11th day of cultivation.



Source: Authors.

Figure 4. Colonies of different fungal isolates on the 15th day of growth in minimal medium (Pontecorvo et al. 1953) with biosolids (20%) from the Wastewater Treatment Plant (WWTP) Dr. Hélio Seixo de Britto, Goiânia, Goiás, Brazil, as the only source of carbon. A: *Inonotus rickii* (SXS 37). B: *Ganoderma stipitatum* (SXS 615). C: *Hydnopolyporus palmatus* (SXS 628).



Source: Authors.

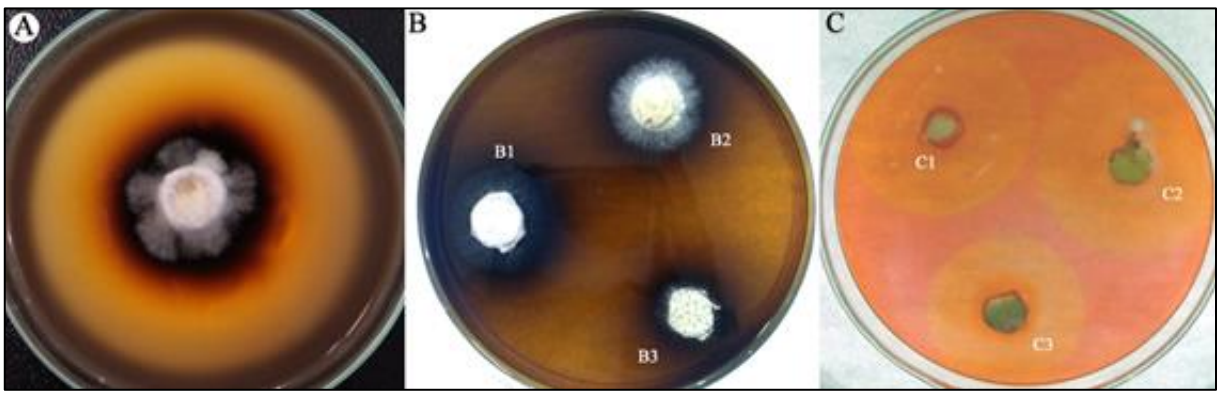
Although in *I. rickii* (SXS 37) and *G. stipitatum* (SXS 615) isolates, the growth was lower than in the positive control (medium with glucose as carbon source), *H. palmatus* isolate (SXS 628) showed greater growth in the medium containing biosolids (Figure 3) than in the positive control. This indicates a preference for biosolids over glucose, demonstrating a high degradation potential of this compound. No fungus was able to grow in the absence of carbon source (negative control).

I rickii (SXS 37) showed less dense mycelium and *H. palmatus* (SXS 628) showed mycelial densification in the medium containing biosolids as the only source of carbon, when compared to the control. These morphological changes may be related to the production of enzymes, as mentioned previously. However, the use of biosolids as the only source of carbon enhances the ability of these isolates to degrade the compounds of the medium, including recalcitrant ones, suggesting a high efficiency in the degradation of the present compounds in the biosolid, by these two isolates.

DIAGNOSIS OF FUNGAL ENZYMATIC ACTIVITY

The positive result was observed through the appearance of the halo of degradation of the substrate around the colony (Figure 5). All the evaluated isolates were able to synthesize at least two of the prospected enzymes. Isolates SXS 630 and SXS 634 showed positive results for all enzymes. The largest EI of tannase (5.3) was detected in *I. rickii* (SXS 37), followed by SXS 632 (2.4). For polyphenol oxidase, stood out the isolates *I. rickii* (SXS 37) (EI = 3.4), followed by *H. palmatus* (SXS 628) (2.8), SXS 632 (2.7) and *Gloeophyllum* sp. (SXS 90) (2.6). Finally, for the carboxymethyl cellulose, whose EI values were lower in comparison to the other enzymes evaluated, highlighted *Phillipsia* sp. (SXS 629) and *Gloeophyllum* sp. (SXS 90) who presented EI of 4.0 and 2.1, respectively (Table 5).

Figure 5. Detection of enzymatic activity of fungal isolates through formation of degradation halo of substrate on the 7th day of growth. A: Tannase activity on isolate of *Hydnopolyporus palmatus* (SXS 628). B: Polyphenol oxidase activity on isolates B1: Morphotype 4 (SXS 633); B2: Morphotype 2 (SXS 631) and B3: Morphotype 3 (SXS 632). C: CMCase activity on isolate C1: *Lentinus tricholoma* (SXS 624); C2: *Gloeophyllum* sp. (SXS 90) and C3 *Phillipsia* sp. (SXS 629).



Source: Authors.

Table 5. Enzymatic activity of the fungal isolates studied, detected on the 7th day of growth in minimal medium with the respective substrate of the enzyme. CMCase = carboxymethyl cellulase; Øc = colony diameter (cm); Øh = diameter of the degradation of halo (cm); EI = enzymatic index according to Hankin & Anagnostakis (1975). * Voucher in the Collection of Fungi Cultures of the Laboratório de Micologia Básica, Aplicada e Divulgação Científica da Universidade Estadual de Goiás. ND = not detected.

Isolate*	Enzymatic activity								
	CMCase			Polyphenol oxidase			Tannase		
	Øc	Øh	EI	Øc	Øh	EI	Øc	Øh	EI
Inonotus rickii SXS 37	ND	ND	ND	0.7	2.4	3.4	0.7	3.7	5.3
Gloeophyllum sp. SXS 90	2.0	4.2	2.1	0.7	1.8	2.6	ND	ND	ND
Lentinus tricholoma SXS 624	3.0	4.0	1.3	0.7	1.7	2.4	ND	ND	ND
Hydnopolyporus palmatus SXS 628	ND	ND	ND	1.5	4.15	2.8	6.7	12.1	1.8
Phillipsia sp. SXS 629	0.7	2.8	4.0	0.7	2.1	3.0	ND	ND	ND
Morphotype 1 SXS 630	2.1	3.5	1.6	1.9	2.7	1.4	1.9	2.8	1.5
Morphotype 2 SXS 631	ND	ND	ND	1.7	2.7	1.6	2.1	2.5	1.2
Morphotype 3 SXS 632	ND	ND	ND	0.8	2.2	2.7	0.9	2.2	2.4
Morphotype 4 SXS 633	ND	ND	ND	1.3	2.1	1.6	1.5	2.1	1.4
Morphotype 5 SXS 634	2.5	2.5	1.0	3.3	3.5	1.1	0.8	1.9	2.4

Source: Authors.

With the exception of the isolates SXS 630, 631 and 632, the other isolates evaluated presented EI ≥ 2.0 for at least one of the investigated enzymes (Table 5), which allows them to be considered, according to Lealem & Gashe (1994), good producers of the enzyme in question. In this way, we have as good producers of CMCase the isolates SXS 90 and 629, of polyphenol oxidases the isolates SXS 37, 90, 624, 628, 629, 632 and of tannases the isolates SXS 37, 632 and 634. These results suggest that these isolates (combined or not) are potential candidates to act on the biodegradation of biosolid components.

FINAL CONSIDERATIONS

Five different morphotypes of filamentous fungi were detected in the biosolids. These and all the other fungi from culture collection tested were able to grow in the presence of biosolids; however, the higher the biosolids concentration, the greater the growth inhibition, except for *Phillipsia* sp. (SXS 629), whose growth was stimulated by the presence of biosolids. The isolates *H. palmatus* (SXS 628) and *I. rickii* (SXS 37) were slight affected by the presence of the biosolid.

The biosolid pH was also a conditioning factor of the fungal growth, because although all the tested isolates were able to grow in medium containing the biosolid at its original pH (10.5), the pH correction of the medium (to 6.8) provided greater growth. In this sense the isolate of *I. rickii* (SXS 37) was the least affected, as it showed high growth rates in both pH conditions. This tolerance to pH is very favorable regarding the minimization of costs with the adjustment of pH in a system of great scale.

With the exception of *Gloeophyllum* sp. (SXS 90), a brown rot fungus, the other isolates tested (*I. rickii* SXS 37, *G. stipitatum* SXS 615 and *H. palmatus* SXS 628), which are white rot fungi, were able to

grow in medium containing biosolids as the only source of carbon, thus showing that were able to degrade the biosolids, especially *H. palmatus* (SXS 628), whose growth in the biosolids exceeded the growth in the control medium.

The prospection of the enzymatic activity showed that all the evaluated isolates were able to synthesize at least two of the prospected enzymes, especially SXS 630 and SXS 634, which showed positive results for all the enzymes (carboxymethyl cellulase, tannase and polyphenol oxidase). Although complementary tests are necessary, the results demonstrate the potential for the use of these fungal isolates (combined or not) in biotechnological processes, aiming at the degradation of the biosolids in question, especially the isolate *I. rickii* (SXS 37) that showed favorable results in all evaluated aspects.

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Fungos Filamentosos como Agentes Promissores da Biodegradação de Compostos Biossólidos

RESUMO

Estações de tratamento de esgoto (ETEs) geram resíduo pastoso, conhecido como biossólido, podendo ser tóxico e recalcitrante, o que motiva estudos visando sua degradação ou aproveitamento seguro. Foram investigados fungos filamentosos na degradação do biossólido de uma ETE de Goiânia/Goiás. Todos cresceram na presença do biossólido, sendo inibidos pelo aumento da concentração, exceto SXS629, que cresceu proporcionalmente à concentração. Todos cresceram no meio com biossólido no pH original (10.5), mas a correção (6.8) proporcionou maior crescimento. Exceto SXS90, os demais (SXS37, SXS615 e SXS628) degradaram o biossólido, crescendo em meio contendo-o como única fonte de carbono; destaque para SXS628, cujo crescimento no biossólido superou o do controle. Todos isolados avaliados sintetizam pelo menos duas enzimas prospectadas, destaque para SXS630 e SXS634, que sintetizam todas (carboximetilcelulase, tanase, polifenoloxidase). Isso mostra o potencial para utilização desses isolados (combinados ou não) em processos biotecnológicos visando à degradação do biossólido, com destaque para SXS37.

Palavras-Chave: Biorremediação; Lodo Ativado; Tratamento de Esgoto; Goiânia.

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