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Research Paper

Epicoccum nigrum and *Cladosporium* sp. for the treatment of oily effluent in an air-lift reactor

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Abstract

The metalworking industry is responsible for one of the most complex and difficult to handle oily effluents. These effluents consist of cutting fluids, which provide refrigeration and purification of metallic pieces in the machining system. When these effluents are biologically treated, is important to do this with autochthonous microorganisms; the use of these microorganisms (bioaugmentation) tends to be more efficient because they are already adapted to the existing pollutants. For this purpose, this study aimed to use two indigenous microorganisms, *Epicoccum nigrum* and *Cladosporium* sp. for metalworking effluent treatment using an air-lift reactor; the fungus *Aspergillus niger* (laboratory strain) was used as a reference microorganism. The original effluent characterization presented considerable pollutant potential. The color of the effluent was 1495 mg Pt/L, and it contained 59 mg/L H_2O_2, 53 mg/L total phenols, 2.5 mgO_2/L dissolved oxygen (DO), and 887 mg/L oil and grease. The COD was 9147 mgO_2/L and the chronic toxicity factor was 1667. Following biotreatment, the fungus *Epicoccum nigrum* was found to be the most efficient in reducing (effective reduction) the majority of the parameters (26% COD, 12% H_2O_2, 59% total phenols, and 40% oil and grease), while *Cladosporium* sp. was more efficient in color reduction (77%).

Key words: *Epicoccum nigrum*, *Cladosporium* sp., metalworking effluent, air-lift reactor.

Introduction

One of the biggest environmental impacts of effluents is the oils they contain. Of these oily effluents, those produced by the metalworking industry are some of the most abstruse and difficult to treat and reuse because of the high variety and the complexity of the compounds present in this type of effluent (Runge and Duarte, 1990). Among these systems used to treat oily effluents, biologics are the ones that provide greater economic flexibility to improvements that result in effective and economically viable solutions to the treatment of these wastes (Mariano, 2001).

Bacteria, yeasts and filamentous fungi have been cited in the literature as effective change agents due to their ability to degrade a variety of organic substances commonly found in oily effluents (Tano-Debrah *et al.*, 1999; Mendes *et al.*, 2005; Van Der Merwe *et al.*, 2005). However, the lack of specificity in biological treatment means that this type of process is also sometimes ineffective. To solve this problem, the best approach is to perform biological treatment with microorganisms isolated from the effluent itself (autochthonous) to be treated, because the treatment process with these microorganisms (bioaugmentation) tends to be more effective since they are already adapted to the pollutants it contains (Hururay *et al.*, 1998).

Within this context, this work had as its main objective the treatment of a metalworking effluent with two autochthonous microorganisms, *Epicoccum nigrum* and *Cladosporium* sp., using the fungus *A. niger* (laboratory strain) as a reference microorganism.

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Materials and Methods

Effluent

We used the effluent from the demulsification step (oil-water separation) by acidification from the metalworking industry in the region of Vale do Paraiba in São Paulo State, Brazil. This step (demulsification) was performed by the industry for the removal of excess oil in the effluent. The watery part of the effluent after demulsification was collected so that the sample taken for the studies was representative of the whole. The total sample was 40 L (distributed into two 20 L bottles). The sampling volume was also divided in aliquots of 40 mL, 600 mL, 1.5 L, and 3.5 L, stored in plastic containers and kept in a cold chamber at -18 °C. However, prior to storage, separate 4 L bottles (divided into two 2 L bottles) were for set aside microorganism isolation. The isolation was performed through the technique pour-plate (Pelczar *et al.*, 1997).

Characterization of metalworking effluent

pH determination

The pH of the effluent sample was determined using an Analion PM608 pH meter with an Analion V627 electrode.

Color determination

Color was measured according to modified CPPA standard methodology (CPPA, 1975). In all determinations, the samples were first centrifuged for 15 min at 10,500 rpm and the pH was adjusted to 7.6 with NaOH 1 mol/L. The solution absorbance in the visible spectrum was determined at 465 nm against distilled water in a UV/VIS Hitachi U2000 spectrophotometer. The absorbance values were converted into milligrams of platinum per liter (mg Pt/L) according to Eq. (1).

$$\text{Color} = 500\frac{A_1}{A_2} \qquad (1)$$

where A_1 = Absorbance of a platinum - cobalt standard solution of 500 mg Pt/L, A_{465} = 0.132 and A_2 = Absorbance of the effluent, measured at 465 nm.

Hydrogen peroxide determination (H_2O_2)

The hydrogen peroxide (H_2O_2) concentration was determined according to the procedure adapted from Oliveira *et al.* (2001), based on the reaction between H_2O_2 and the metavanadate ion (VO_3^-). The reaction leads to the formation of ion peroxovanadate (VO_3+), which absorbs at 450 nm. Calibration curves were prepared from aqueous solutions of H_2O_2, there being a linear response range between 5 and 200 mg/L. For the determination, an aliquot of 1 mL of sample was added to 1 mL of ammonium metavanadate solution (NH_4VO_3) under constant agitation, and after 2 min, the absorbance was read at 450 nm on a UV/VIS Hitachi U2000 spectrophotometer. The H_2O_2 concentration of the sample was obtained by interpolation of the absorbance measured in the sample, in the calibration curve.

Chemical oxygen demand (COD), total phenols (TF), oils and grease (O&G) and dissolved oxygen (DO) determination

The COD, TF, O&G and DO (Winkler method) determinations were performed according to methods described in APHA (1998).

Chronic toxicity determination

To determine the chronic toxicity, we used the green alga *Pseudokirchneriella subcaptata*; the determination followed the methodology NBR 12648/05 (ABNT, 2005).

Biotreatment in an air-lift reactor

Treatments were performed with the selected microorganisms *Epicoccum nigrum* and *Cladosporium* sp., and compared to treatment without inoculum (S/I), for the observation of the possible oxidation of compounds of the effluent arising from the oxygenation and photolysis. For this, the microorganisms were transferred to tubes containing PDA (Potato Dextrose Agar) and incubated for 120 h in the dark at 28 °C. After this period, the culture was suspended in 10 mL of distilled water and autoclaved. Then, the cells were quantified in a Neubauer chamber, standardizing to a volume containing 10^8 spores. Each aliquot of the suspension was added to the air-lift reactor, containing 350 mL of raw effluent, in triplicate, with the pH adjusted to 6.5. The treatment was for 7 days at 28 °C and under a flow of air, previously filtered, 80-90 mL/min, controlled by rotameter. After treatment, the effluent was allowed to rest for 24 h, and the aqueous phase was collected for the determination of the selected parameters after treatment. Figure 1 shows a schematic diagram of the reactor that was used in this experiment.

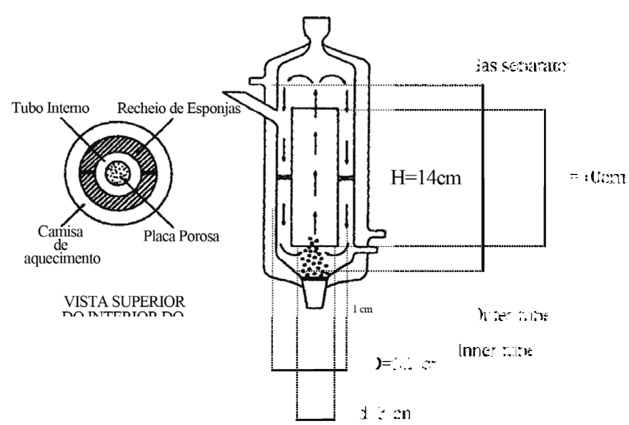

Figure 1 - Air-lift bioreactor.

Results and Discussion

Characterization of metalworking effluent

The results of the physicochemical characterization of the effluent are presented in Table 1. The results indicate that this was a highly polluting effluent, due to the high acidity (pH 1.7 ± 0.1) and coloration (1495 ± 149 mg Pt/L).

The coloration was associated with chromophore compounds present in the effluent. Disposal of this effluent may be harmful to the receiving body of water, since it increases the blockage of light, which consequently affect photosynthesis, and also hinders the transfer of atmospheric oxygen into the *aquatic* environment, thus reducing the dissolved oxygen content (CETESB, 2009).

The high COD (9147 ± 514 mg/L) was attributed to the presence of a high content of dissolved organic matter. Several authors have described metalworking effluents to have high COD values. Cheng *et al.* (2006) determined a COD of 8,000 mg/L in a metalworking effluent. Van der Gast and Thompson (2004) found a COD of approximately 10,000 mg/L in a similar effluent. Monteiro (2006) characterized a metalworking effluent similar to the one used in the present study and determined a COD of approximately 3,700 mg/L. Oily effluents generally have complex and highly variable characteristics, whether physical, chemical or biological, and their decomposition increases the COD, thus reducing the OD, and causes harmful changes in aquatic ecosystems (CETESB, 2009).

Hydrogen peroxide (H_2O_2) was found at a concentration of 59 ± 1 mg/L. Its presence in this effluent type is common because it is used as a fungicide and bactericide in cutting oils to prevent the contamination of the cutting fluid (Canter, 2008; Peres *et al.*, 2008).

The high concentration of total phenolics (5.4 ± 0.4 mg/L) was a limiting characteristic of the effluent, since under CONAMA (2012) resolution 430/11, the maximum permitted concentration is 0.5 mg /L. The toxicity of phenolic compounds in oily effluents has been extensively studied and it is well-established that the presence of these contaminants at levels of mg/L significantly impairs the receiving body of water (Guerra, 2001). Moreover, the phenolic pollutants may also cause toxicity to the microorganisms used in biological treatment systems of oily effluents (Mishra *et al.*, 1995).

Table 1 - Physico-chemical properties of the effluent.

Parameters	Results	Standard*
Temperature	25 °C**	< 40 °C
pH	1.7 ± 0.1*	5 - 9
Color (mg Pt/L)	1.495 ± 149*	75
COD (mg/L)	9.147 ± 514	ND
H_2O_2 (mg/L)	59 ± 1	ND
Total phenols (mg/L)	5.4 ± 0.4*	0.5
DO (mg/L)	2.5 ± 0.2*	≥ 5
O&G (mg/L)	887 ± 55*	20
C. Tox (Tox. factor)	1667	ND

* - value not allowed by federal and state legislation.
** - value allowed by legislation.
ND - Value not defined in legislation.

The industrial effluents usually contain more than one type of pollutant phenolic, and those with more complex structures are often more toxic than the phenol molecule (Zhou and Fang, 1997). Another undesirable characteristic of these contaminants is the fact that they can react with chlorine, producing chlorophenols and polychlorophenols, which are carcinogenic compounds (Colarieti *et al.*, 2002).

The low concentration of DO (2.5 ± 0.2 mg O_2/L) was certainly related to the high COD, which consequently reduces the DO concentration, a common characteristic of oily effluents. Ekundayo and Fodeke (2000) determined a DO of 5 mg/L in a receiving tank of various oily effluents, including those from the metalworking industry. Ferreira *et al.* (2000) found an DO less than 1 mg/L in an untreated effluent from an oil refinery, while Conceição *et al.* (2005) detected a higher level of DO in a refinery oily effluent, at a level of approximately 7 mg /L, thus showing that the DO level is quite variable in different oily effluents generated by different industries.

In this work, we also determined the O&G concentration (887 ± 55 mg/L), which, according to the methodology in item 2.4.8., are associated with soluble substances in n-hexane. These substances include fatty acids, animal fats, soaps, greases, oils, waxes, and mineral oils (CETESB, 2009). Similar results of the O&G concentration in oily effluents are reported in the literature. Damato *et al.* (1997) reported 871 mg/L of O&G in an effluent containing cutting fluid. In another study, Tessaro (2008) found a concentration of 9942 mg/L of O&G in a metalworking effluent. Furthermore, Schoeman and Novhe (2007) worked with a similar effluent, and determined a concentration of 19,794 mg/L.

The high O&G value and the effluent color, but also the low DO value and pH, led to an initial interpretation that the effluent could be highly toxic. That was confirmed by the results of the chronic toxicity test on the green alga *Pseudokirchneriella subcaptata*, which showed a toxicity factor (ToxF) of 1667. Generally, algae are 50% more sensitive than invertebrates and fish in toxicological studies using industrial effluents (Hartmann, 2005). Monteiro (2006) observed a ToxF of approximately 1,300 when conducting chronic toxicity tests with *P. subcaptata* using the same type of effluent.

Biotreatment in an air-lift reactor

This stage of the work involved treatment with the selected microorganisms (*E. nigrum and Cladosporium* sp.) and treatment without inoculum (W/I) to observe a possible contribution of compound oxidation due to photolysis and aeration in the treatment system.

The aim of the study was to assess which microorganism was more effective in the treatment of wastewater from the cutting fluid, using an oxygenated (air-lift) system. The advantages of using an “air-lift” reactor are the simplicity of the equipment, thus facilitating its handling, the decrease of shear to cells, thus avoiding their disruption, good mixing and better asepsis during long operations, due to the elimination of a stem shaker (Gouveia *et al.*, 2000).

The treatments lasted for 7 days, containing 350 mL of effluent with the pH adjusted to 6.5 and 28 °C. The results of these treatments are shown in Tables 2 and 3.

The treatment without inoculum (W/I) reduced H_2O_2 to 92 ± 2% and increased the DO to 228 ± 3%. The H_2O_2 consumption was possibly brought about by photolysis, due to the *natural light* that the reactor received during treatment. The dissolved oxygen increase in the sample after treatment was expected since O_2 was added during the process; additionally, this may have increased through H_2O_2 decomposition (Suznjevic *et al.*, 1997). The treatment without inoculum still reduced the sample color by 10 ± 2%; this may have occurred by the destruction of chromophore groups and/or mineralization of organic compounds, respectively, by hydroxyl radicals formed by the possible decomposition of H_2O_2 (Mattos *et al.*, 2003). However, in relation to other determined parameters after treatment, the system without inoculum did not have a significant influence.

Table 3 presents the results obtained with biological treatment in the air-lift reactor with *autochthonous* fungi in the effluent (*E. nigrum* and *Cladosporium* sp.) and the reference fungus *A. niger*.

Following wastewater treatment in the air-lift reactor, it was observed that the pH effectively increased on average by 10 ± 2% after treatment. This increase in pH may be associated with the decomposition of fatty acids present in the sample, by the enzymatic action of fungi (Mendes *et al.*, 2005).

Table 2 - Post-treatment parameters in the air-lift bioreactor in effluent without inoculum (W/I).

Parameters post-treatment	Value	Reduction (%)**
pH	6.4 ± 0.1	2 ± 2
Color (mg Pt/L)	1345 ± 15	10 ± 2
COD (mg/L)	9056 ± 54	1 ± 1
H_2O_2 (mg/L)	4.7 ± 0.1	92 ± 2
Total phenols (mg/L)	5.2 ± 0.2	4 ± 4
DO (mg/L)	8.2 ± 0.1	228 ± 3*
O&G (mg/L)	860 ± 18	3 ± 2
C. Tox (Tox. factor)	1634	2

* - percentage increase.

Table 3 - Post-treatment effluent parameters in the air-lift bioreactor with *A. niger*, *E. nigrum* and *Cladosporium* sp.

Parameters post treatment	Microorganisms								
	A. niger			E. nigrum			Cladosporium sp.		
	Value	Effective reduction (%)	Total reduction (%)	Value	Effective reduction (%)	Total reduction (%)	Value	Effective reduction (%)	Total reduction (%)
Ph	7.0 ± 0.1	10 ± 2*	2 ± 1*	7.0 ± 0.1	10 ± 2*	2 ± 1*	6.7 ± 0.1	4 ± 2*	1 ± 1
Color (mg Pt/L)	430 ± 5	68 ± 4	71 ± 3	430 ± 5	68 ± 4	71 ± 3	309 ± 3	77 ± 4	79 ± 4
COD (mg/L)	7154 ± 150	21 ± 1	22 ± 1	6701 ± 175	26 ± 1	27 ± 1	8241 ± 140	9 ± 1	10 ± 1
H_2O_2 (mg/L)	4.6 ± 0.1	2 ± 1	92 ± 2	4.1 ± 0.1	12 ± 3	93 ± 2	4.3 ± 0.1	8 ± 3	93 ± 2
Total phenols (mg/L)	3.2 ± 0.1	39 ± 4	41 ± 2	2.1 ± 0.1	59 ± 4	61 ± 2	2.9 ± 0.1	44 ± 4	46 ± 2
DO (mg/L)	7.8 ± 0.2	5 ± 2	212 ± 5*	7.4 ± 0.3	10 ± 2	196 ± 8*	6.9 ± 0.1	16 ± 4	176 ± 3*
O&G (mg/L)	783 ± 15	9 ± 4	12 ± 3	516 ± 10	40 ± 2	42 ± 1	602 ± 15	30 ± 4	32 ± 3
C. Tox (Tox. factor)	1487	9	11	850	48	49	899	45	46

* - percentage increase.

The effective color reduction was similar with the three fungi. Using *Cladosporium* sp., the reduction was 77 ± 4%, while for *E. nigrum* and *A. niger* this was 68 ± 4%.

The effective COD reduction was more significant when the effluent was treated using *E. nigrum*, with a 26 ± 1% reduction. Using the reference fungus *A. niger*, this reduction was smaller, reaching 21 ± 1%. Van der Merwe *et al.* (2005) studied the biological treatment of oily effluents, with a pH around 5.5 and a temperature of 30 °C. However, these authors inoculated the effluent with a fungal consortium, and achieved a maximum COD reduction of 51%.

The effective H_2O_2 reduction was more intense using the fungus *E. nigrum* (12 ± 3%), while *A. niger* had virtually no effect on reducing this parameter.

In all treatments with the autochthonous microorganisms, the concentration of total phenolics (TP) in the effluent achieved reductions above 40%. The microorganism *E. nigrum* caused the greatest reduction (59 ± 4%), compared to 44 ± 4 and 39% ± 4% achieved with *Cladosporium* sp. and *A. niger*, respectively.

It was observed that the treatment without inoculum was associated with an increase in DO, possibly due to the addition of the same during treatment. However, taking into account treatment with the microorganisms, reductions in DO levels were seen at the end of treatment. Treatment with the fungus *Cladosporium* sp., with more effective consumption of oxygen, reduced the DO concentration by 16 ± 4%. However, when treatment was performed with the fungi *E. nigrum* and *A. niger*, the DO of the effluent was effectively reduced by 10 ± 2% and 5 ± 2% respectively.

The treatments performed with the effluent autochthonous microorganisms were also more effective in O&G reduction than the treatment that used the reference fungus *A. niger*. Using *E. nigrum* and *Cladosporium* sp., the effective reductions were 40 ± 1% and 30 ± 4%, respectively. However, with *A. niger*, the effective reduction was only 9 ± 4%.

This possibly occurred due to *autochthonous* microorganisms are already adapted to the effluent under study. Tano-Debrah *et al.* (1999) treated a sample of oily effluent for 7 days at 30 °C and pH 7, with the inoculation of $5x10^8$ spores/mL of different microorganisms (no identification) and obtained 86% O&G degradation, increasing the DO of the sample by 30% and reducing its COD to 60%.

E. nigrum and *Cladosporium* sp. also effectively and more significantly reduced the chronic toxicity (C. Tox.) of the effluent compared to the reference microorganism *A. niger.* While *E. nigrum* and *Cladosporium* sp. effectively reduced the C. Tox. to 48% and 45%, respectively, *A. niger* reached an effective reduction of 9%. We observed that the effective chronic toxicity reductions achieved by the three fungi (*A. niger*, 9%; *E. nigrum*, 48% and *Cladosporium* sp., 45%) were similar when compared with the effective reductions in oils and greases (9 ± 4%, 40 ± 2% and 30 ± 4%). Therefore, the oil and grease constituents of the effluent may be one of the main factors that determine the chronic toxicity.

Conclusion

The characterization of crude oily wastewater showed that, in terms of the determined parameters, it had a high pollution potential. Biotreatment in an air-lift type reactor reduced all determined parameters, except pH, which increased on average by 10%. The fungus *Epicoccum nigrum* was more effective in reducing most of the determined parameters (COD, H_2O_2, TP, O&G and C. Tox.). However, *Cladosporium* sp. was more effective in color reduction. These are, therefore, microorganisms with a high potential to reduce pollution-related parameters in this effluent type compared with the reference microorganism *A. niger*.

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