



Treatment of delignification effluent with *Aspergillus* 2BNL1 and optimization of biomass production

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Abstract

Delignification effluent from a nitrocellulose industry was treated with *Aspergillus* 2BNL1. An experimental design was used to optimize the production of fungus biomass evaluating the influence of time and the contents of sucrose, sodium nitrate and effluent on fungal growth. *Aspergillus* 2BNL1 was able to decolorize between 70-80% of the effluent without co-substrate in 24 h. In addition to color reduction, TOC (total organic carbon) was also reduced by 55% (72 h). For biomass production the best initial contents of sucrose, sodium nitrate and effluent were 45 g L⁻¹, 3 g L⁻¹ and 35 % (v/v), respectively. The highest biomass concentration in the model was 5.35 g L⁻¹. Under the experimental conditions studied the effluent provided an increase in the production of *Aspergillus* 2BNL1 biomass.

Key words: Delignification effluent, fungal treatment, biomass production, nitrocellulose industry.

Introduction

Cellulose industry discharges into streams significant amounts of brown color effluents, which could cause serious environmental problems. The brown color originates in part from lignin derivatives. Biological processes, such as lagooning and activated sludge treatments, have been studied worldwide. However, these methods are not very effective to remove color and organic matter of effluents from cellulose industry¹⁴.

Since 1977, numerous researches have been conducted utilizing fungi for the treatment of effluents from cellulose industry but, on an industrial scale, none or just a small part of this knowledge has been used. Fungi have the capacity to metabolize lignin and its derivatives reducing the color, organic matter and toxicity of these effluents¹⁵. However, when fungi are employed on a large scale, it is difficult to produce enough biomass and to establish good aseptic conditions. Biomass production involves the optimization of culture media to supply the fungi with carbon, nitrogen and micronutrients necessary for growth. An alternative to the conventional culture media, which generally are costly, would be the utilization of nutrient sources from the effluent itself, which is inexpensive and more suitable for growth of microorganisms.

To obtain the best possible species to be used in new biotechnological processes, numerous species of wood-rotting fungi have been screened for rapid growth and high rates of lignin degradation⁵. *Aspergillus* 2BNL1 is a Deuteromycete isolated from activated sludge. In a screening experiment in our laboratory¹⁰ this fungus has proven to be highly able to reduce color, organic matter and toxicity of nitrocellulose industry effluent. The aim of the present work was to evaluate the efficiency of *Aspergillus* 2BNL1 in the treatment of effluent coming from the delignification process of a nitrocellulose industry employing an air-lift bioreactor and optimize the production of this fungus biomass (2BNL1). A 2⁴ experimental

design was used to evaluate the influence of time and the contents of sucrose, sodium nitrate and effluent on fungal growth.

Materials and Methods

Delignification effluent: The experiments were carried out employing a delignification effluent generated at the pulping process of a nitrocellulose industry which utilizes cotton as the raw material. The following physical and chemical properties were determined: color = 66923 CU, pH = 12, total phenol = 524 mg L⁻¹, TOC = 6,420 mg L⁻¹, total solid = 14,060 mg L⁻¹. The original cotton delignification effluent had its pH adjusted to 5 and was diluted with water in the ratio of 10% (v/v) before being used in tests.

Effluent treatment: The fungus used in this work was isolated from activated sludge and identified as *Aspergillus* 2BNL1. The strain was selected after screening a group of fungi for their ability to decolorize delignification effluent¹⁰. *Aspergillus* 2BNL1 was grown in Potato-Dextrose-Agar (PDA) medium in Petri dishes for 72 h at 28°C and then spores were suspended in sterile water¹³. Each flask (1000 mL, containing 200 mL of culture medium) was inoculated with the equivalent to 1x10⁵ spores mL⁻¹ culture medium (Potato Dextrose Broth - PDB). After 120 h, the pre-inoculum medium was filtered and the fungal biomass (20 g) was transferred to an air-lift bioreactor containing 350 mL of delignification effluent without extra carbon source. The treatment lasted 120 h. The air-lift bioreactor used in this investigation had an internal tube (riser) and an annular space as shown schematically in Fig. 1. The working volume was 350 mL and temperature (28°C) was controlled by water circulation through the annular jacket surrounding the reactional zone. In our work the aeration rate (400 mL min⁻¹) was measured by a flow meter.

Table 1. Level and factors used in the design.

Level	-1	0	+1
Sucrose (g L ⁻¹)	15	30	45
Sodium nitrate (g L ⁻¹)	1	2	3
Effluent % (v/v)	15	25	35
Time (h)	72	96	120

Table 2. Results of the 2⁴ experimental design for optimization *Aspergillus* 2BNL1 biomass production.

Test n ^o	X1	X2	X3	X4	Biomass (g L ⁻¹)
1	0	0	0	0	3.30
2	-1	-1	1	1	3.26
3	-1	1	-1	-1	2.02
4	1	-1	-1	-1	3.47
5	1	1	1	1	5.14
6	-1	1	1	1	3.95
7	1	-1	1	1	5.09
8	1	1	1	-1	5.32
9	-1	-1	-1	-1	1.88
10	1	1	-1	1	5.12
11	-1	1	-1	1	2.90
12	-1	1	1	-1	3.16
13	1	-1	1	-1	3.90
14	1	-1	-1	1	2.91
15	1	1	-1	-1	4.75
16	-1	-1	-1	1	2.05
17	-1	-1	1	-1	2.33
18	0	0	0	0	3.45

[X1] = sucrose content; [X2] = sodium nitrate content; [X3] = delignification effluent content; [X4] = growth time

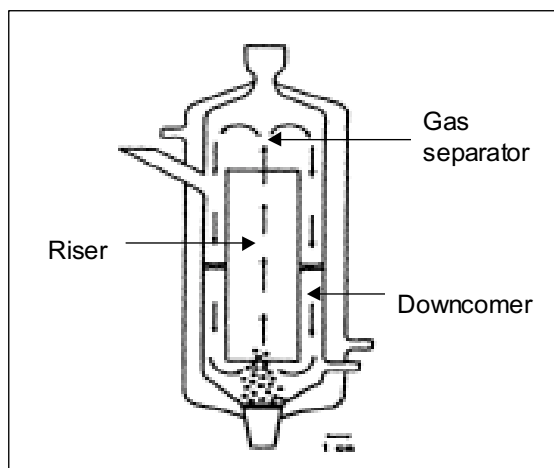


Figure 1. Schematic diagram of the air-lift bioreactor.

Table 3. Variables affecting the biomass production of *Aspergillus* 2BNL1 as revealed by the 2⁴ experimental design

Variables	Estimated effects ± standard error
Average	3.56 ± 0.03 *
X1	1.77 ± 0.05 *
X2	0.93 ± 0.05 *
X3	0.88 ± 0.05 *
X4	0.45 ± 0.05
X1.X2	0.30 ± 0.05
X1.X3	-0.08 ± 0.05
X1.X4	-0.24 ± 0.05
X2.X3	-0.19 ± 0.05
X2.X4	0.02 ± 0.05
X3.X4	0.23 ± 0.05

*Significative effects at the 5% level (t = 12,706).

[X1] = sucrose content; [X2] = sodium nitrate content; [X3] = delignification effluent content; [X4] = time

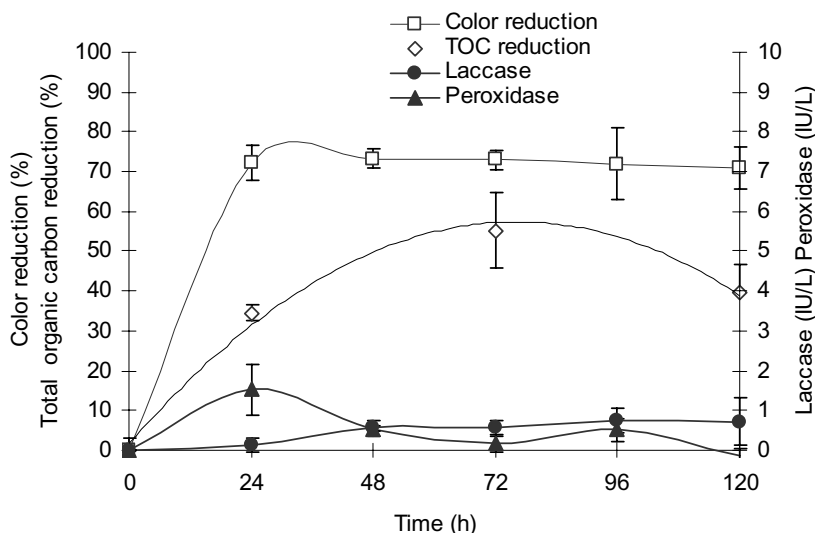


Figure 2. Color and TOC reduction and phenoloxidases production during the treatment of a delignification effluent with *Aspergillus* 2BNL1.

Analytical methods: The color of the effluent was determined according to CPPA methods³. Activity of phenoloxidases (laccase and peroxidase) IU L⁻¹ was measured using syringaldazine as a substrate, with and without oxygen peroxide¹². TOC (total organic carbon) was measured according to the standard procedures described by ISO (1987).

Optimization of *Aspergillus* 2BNL1 biomass production: In order to verify the influence of sucrose and sodium nitrate (g L⁻¹), effluent (% v/v) and time (h) on the biomass production (Y), a 2⁴ experimental design and two repetitions in the central point were employed. Table 1 shows the level and factors used in the design.

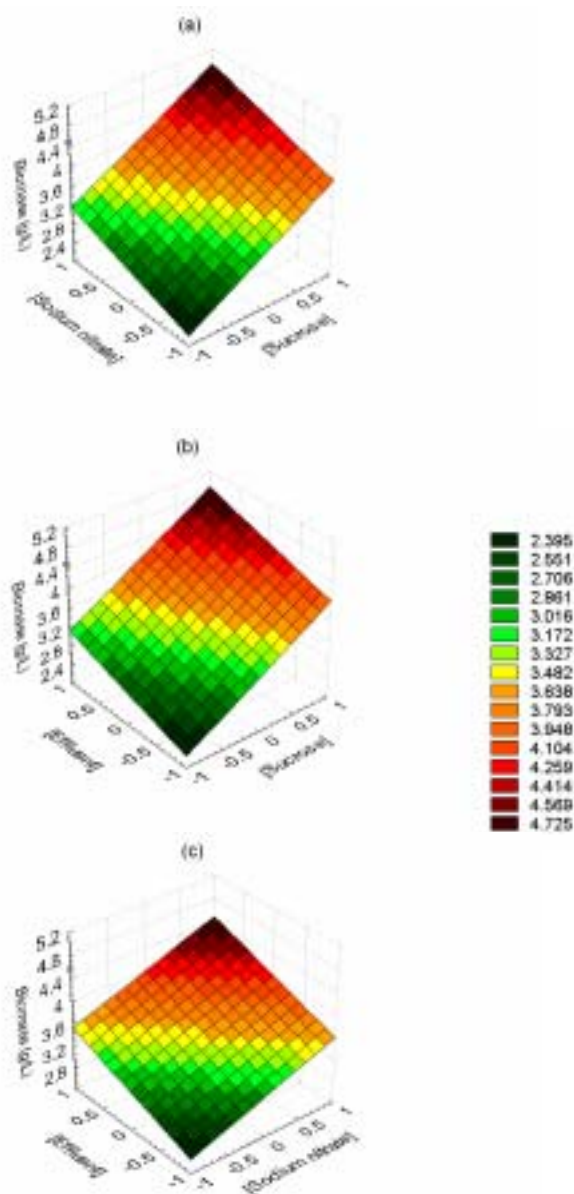


Figure 3. Influence of the contents of sucrose, sodium nitrate and effluent on fungal growth.

For each of the four factors, high, center and low set points coded into +1, 0 and -1, respectively were selected. All the 16 experiments were performed as well as two assays representing the central point (coded value 0). A statistical model for an optimized biomass production was determined by the response regression procedure. The model is expressed by Equation 1: $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3$, where Y = response variable, b_0 , b_1 , b_2 and b_3 = regression coefficients X_1 , X_2 and X_3 = variables under study. The statistical analysis was performed using STATGRAPHICS statistical software version 6.0 and STATISTICA program version 5.0.

Biomass quantification: *Aspergillus* 2BNL1 biomass was determined gravimetrically after drying cells at 80°C.

Results and Discussion

Effluent treatment with *Aspergillus* 2BNL1: During the incubation of the effluent with the biomass in the air-lift

bioreactor, the murky brown color of the effluent became much lighter. The decolorization process, TOC reduction and phenoloxidases production were quantified and the results are shown in Fig. 2.

The decolorization was more rapid during the first 24 h, stabilizing after 48 h, when the maximal rate (72%) was attained. Souza et al.¹⁰ also observed an equal decolorization rate in a screening study in which *Aspergillus* 2BNL1 was more efficient than the well-known *Lentinus edodes* UEC 2019.

Although an appreciable TOC reduction (55%) was observed in 72 h, this value was not enough to make the delignification effluent dischargeable into the environment. This type of effluent probably contains a significant recalcitrant fraction that apparently does not degrade under the conditions used in this experiment.

The phenoloxidase activity values were low and consistent with those previously reported for pulp and paper effluent treatments^{4,6-10}. Although the fungi of the *Deuteromycetes* class, do not commonly present peroxidase activity, *Aspergillus* 2BNL1 showed a maximal activity of this enzyme (1.5 IU L⁻¹) in the first 24 h. However, after 48 h, the peroxidase activity was replaced by laccase activity.

Optimization of *Aspergillus* 2BNL1 biomass production: An essential requirement for the effluent treatment with fungi is the biomass production. In order to determine the influence of time and the contents of sucrose, sodium nitrate and effluent on fungal growth a 2⁴ experimental design was used (Table 2). The biomass production was between 1.50 and 5.32 g L⁻¹. The main effects and their respective interactions calculated from the data of Table 2 are shown in Table 3.

The standard error for the effects in Table 4 was 0.053. Barros Neto et al.² only consider significant (95% confidence) the effects with values higher than $t_{\alpha, \nu} \cdot s$. The $t_{\alpha, \nu}$ value is t test for ν freedom degree. In this study the t test, for 1 freedom degree (95% confidence), was 12.706. It can be concluded that only the effects higher than 0.67 were significant.

The linear effects of sucrose (X_1), sodium nitrate (X_2) and effluent (X_3) were significant and a linear model was adjusted (Equation 2): $Y = 3.56 + 0.88X_1 + 0.47X_2 + 0.44X_3$. A model is mathematically satisfactory when it presents high regression coefficients and the lack-of-fit is not significant. Table 4 shows the variance analysis, ANOVA, for the linear effects shown in Equation 2.

The F test (Fisher) was used to evaluate the regression and the lack-of-fit of the model. The relation between SM_R and SM_I was 28.49, a number higher than the observed in the table of test $F_{3,14}$ (3.34) which proved that the regression was significant (95% confidence).

The model can also be described as descriptive since the calculated F (28.64) was 8.6 times higher than the observed in the table of test $F_{3,14}$. The relation between SM_{If} and SM_{pe} was 0.87, a number lower than that observed in the table of test $F_{5,9}$ (3.48), which proves that the lack-of-fit was not significant. The absence of lack-of-fit, the significant regression and the variance percentage explained of 86% showed that the model, presented in Equation 2, was adequate to explain the studied region.

The maximum biomass production predicted by the model (5.35 g L⁻¹) corresponded to the point defined by the contents of sucrose ($X_1 = 1$), sodium nitrate ($X_2 = 1$) and effluent ($X_3 = 1$)

Table 4. Analysis of variance for evaluation of the model (Equation 2).

Source of variation	Sum of squares	df	Mean square (MQ)	F-ratio	F- 95 % confidence
Model	19.11	3	6.37	28.49	3.34
Residual	3.13	14	0.22		
Lack-of-fit	1.02	5	0.20	0.87	3.48
Pure error	2.11	9	0.23		
Total	22.24	17			
% of explained variance 85.99					
% of explainable variance 90.59					

corresponding to 45 g L⁻¹, 3 g L⁻¹ and 35% (v/v), respectively. This high biomass content was higher than those reported by Souza et al.¹¹ and Ambrósio and Campos-Takaki¹.

An experiment was carried out with the following variables: sucrose X1=0; sodium nitrate X2=0 and effluent content X3=2 (30 g L⁻¹, 2 g L⁻¹ and 45% (v/v), respectively) in order to generate more information about the correlation between biomass production and effluent content. The experimental result was 5.51 g L⁻¹, while the result predicted by the model was 4.43 g L⁻¹. This lack-of-fit was probably due to quadratic interactions.

From the experiments it was evident that the effluent content had a positive influence on the biomass production. Hemicellulose derivatives, as xylose and arabinose, are the main carbon sources in the effluent. Other experiments on the production of high contents of biomass using the delignification effluent as much as possible as a substrate are underway.

Conclusions

The present study is the first one to demonstrate the ability of *Aspergillus* 2BNL1 to decolorize and reduce the organic matter of nitrocellulose effluent in an air-lift bioreactor. This fungus was able, in the absence of co-substrate, to decolorize the effluent by 70-80% in 24 h. In addition to TOC reduction, COD was also reduced by 55% (72 h).

The best initial contents of sucrose, sodium nitrate and effluent for biomass production were 45 g L⁻¹, 3 g L⁻¹ and 35 % v/v, respectively. The highest biomass concentration predicted by the model was 5.35 g L⁻¹. Under the experimental conditions used in this work, the effluent provided a significant increase in biomass production, proving, therefore, to be a suitable substrate for *Aspergillus* 2BNL1 growth.

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