Journal of the University of Chemical Technology and Metallurgy, 41, 2, 2006, 153-156

BIOBLEACHING OF LIGNIN IN LINEN BY DEGRADATION WITH *TRICHOSPORON CUTANEUM R57*

N. Georgieva, L. Yotova, R. Betcheva, H. Hadzhiyska, I. Valtchev

University of Chemical Technology and Metallurgy
8 Kl. Ohridski, 1756 Sofia, Bulgaria
E-mail: neli@uctm.edu

Received 17 January 2006
Accepted 10 March 2006

ABSTRACT

The use of lignin degrading enzymes from Trichosporon cutaneum R57 strain in flax fiber treatment was studied. The whiteness of enzymatically processed fibers was significantly improved and the residual quantity of nondegraded lignin was less than obtained with chemical processing.This is particularly evident in the cases when hydrogen peroxide was used in addition to enzyme treatment.

Key words: ligninolytic enzymes, Tr.cutaneum, enzymatically processed fibres,laccase, Mn-peroxidase.

INTRODUCTION

In the last few years, fungi and bacteria which are able to degrade lignocellulosic compounds have been researched. Wood is composed of three important constituents: cellulose, lignin and hemicelluloses. Until recently the only known ligninolytic microorganism has been white rot fungus Ph.chrysosporium, but some new data have showed that filamentous yeast from the genus Trichosporon can also produced Laccase and Mn-peroxidase, which are important enzymes, involved in lignin cleavage [1]. Lignin is the second most abundant renewable compound on earth. In addition, lignin is waste component in the pulp industry. Lignin is poorly biodegradable due to its hydrophobicity and complex randomly structured molecule lacking regularly repeated hydrolysable bonds. The best degraders of lignin are white rot fungus Ph.chrysosporium, that produce extracellular peroxidases, such as laccases, Mn-peroxidase and lignin peroxidase [2-4]. These enzymes can be used for biopulping, biobleaching, biotransformation and bioremediation. The relationships between lignin cleavage and decolorization of linen are usually studied. The role of laccases, MnP, H_2O_2 and fungal secondary metabolites on biobleaching activity by whole cultures will be elucidated. In this process the roll of the microorganisms used to be considered. But the extent to which this process correlated depends on the efficiency of the microorganism species. Most of the investigators conclude that a new species can be found to increase the efficiency of lignin degradation.

The isolated microorganisms Trichosporon cutaneum R57 showing high ligninolytic activity will be inoculated in culture media containing milled linen as substrate, in order to determine the viability of the degradation in industrial delignification processes. In vivo delignification experiments will be run in parallel to physiological studies in order to investigate the role

of physiological factors which affect the ligninolytic activities of fungi in whole cultures [5, 6].

This study investigated the bleaching of raw flax fibers by *Tr.cutaneum R57* culture.

EXPERIMENTAL

Yeast strain and media. The filamentous yeast *Trichosporon cutaneum R 57* – strain N2414 was supplied from the National Bank of Industrial Microorganisms and Cell Cultures, Bulgaria [7]. The investigated strain was grown out in a medium containing (g dm^{-3}): $(NH_4)_2SO_4$ – 4,0; $MgSO_4.7H_2O$ – 0,02; KH_2PO_4 – 1,7; $Na_2H_2PO_4.2H_2O$ – 0,75; $CaCl_2$ – 0,002; $FeSO_4.2H_2O$ – 0,001; $MnSO_4.H_2O$ – 0,001; tiamin.HCl – 0,001. The medium was sterilized at 120°C for 20 min. The glucose solution (20 g dm^{-3}) was sterilized separately at 110°C for 15 min. and was added to the growth medium. All chemicals were supplied by Merck (Germany).

Cultivation conditions. For *Trichosporon cutaneum R57* starter cultures were prepared by inoculating 100 cm^3 liquid medium with a loopful of colony growing on an agar surface and incubating for 18 hours on a rotary shaker (180 rpm at 30°C). For experimental cultures, 100 cm^3 of medium was inoculated with 5 cm^3 of the starter culture (to an initial biomass concentration of approximately 0.1 mg dry mass.cm^{-3}) and incubated on the shaker for 96 hours at 30°C. Benzyl alcohol (0,5cm^3 dm^{-3}) was added after 24th h. The biomass was separated at the end of the cultivation. Experiments on linen fibers treatment were conducted with the resulting concentrated culture liquid (CL).

Enzymatic assays. The CL contained the enzymes of the lignin degradable complex - extra cellular enzymes, such as Laccase and manganese-peroxidase. Laccase activities in the supernatants of fungal cultures were determined spectrophotometrically as described by [8] and manganese-peroxidases were determined as described by [9].

Treatment of the linen fiber. Unbleached flax fibers from "Rylski len-AD" Bulgaria were placed in a vessel containing 90 cm^3 CL and incubated at pH 7 and, 45°C with shaking from 1 to 48 h. Some samples were subsequently additionally bleached with 1% H_2O_2 out the weight of fibers (o.w.f) for 1 h. at 25°C. Separately a sample bleached with 1%H_2O_2 (o.w.f) at 80°C for 1 hour was prepared and was used as a reference for the bleached flax fibers. All samples were treated with H_2O_2 in bath ratio (M = 1:10) without shaking.

Analytical measurements. The bleaching effect of the treatments was measured by Datacolor equipment and CIELab parameters lightness (L), color difference (DE) and lightness difference (DL) were to evaluate the effectiveness of enzyme treatment. Untreated flax fibers were used as a reference sample.

The content of lignin in samples studied was characterized by c– Kappa Number assay. The Kappa number was measured by SCAN method (SCAN C 1:00). This Standart specifies the method for determining the degree of delignification. However, it should be noted that there is relationship between kappa number and lignin content [10].

RESULTS AND DISCUSSION

The yeast strain *Trichosporon cutaneum R57* examined in this study was periodically cultivated in a liquid synthetic medium as detailed under Materials and Methods. Benzyl alcohol was added at the 24th hour in a stationary phase of the culture development. It is supposed that benzyl alcohol acts as an inductor in producing lignin degrading enzymes [11]. The biomass is separated at the end of the cultivation and a number of experiments on linen fiber treatment were conducted with the resulting nutrient medium. The CL contains the enzymes of the lignin degradable complex; these are extra cellular enzymes. A native non-concentrated

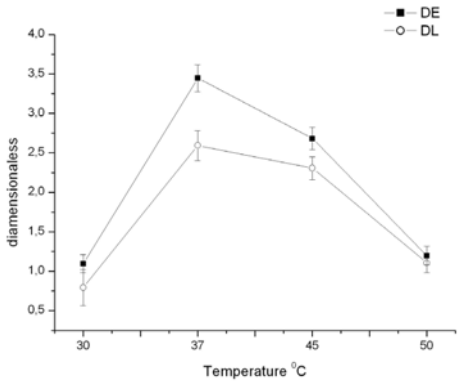

Fig. 1. Flax fibers whiteness by treatment with concentrated CL from *Tr cutaneum R 57(*E = 198 U/mg, 45ml.dm^{-3}, M 1:10, Ph 7, 1h).

Table 1. Linen treatment with non-concentrated CL of *Tr.cutaneum R57.*

Duration of treatment (h)	DE	DL	χ - Kappa number
0	1,50	1,48	18,8
2	1,64	1,52	18,2
4	1,97	1,88	16,7
96	3,42	2,90	11,8

Table 2. Linen treatment with concentrated CL of *Tr.cutaneum R57.*

Duration of treatment (h)	DE	DL	χ - Kappa number
0 (no enzymatic treatment)	1,61	1,47	15,2
2	0,88	0,62	13,5
4	1,51	1,12	13,6
96	2,57	2,38	14,08

Table 3. Treatment of linen fiber with concentrated CL of *Tr.cutaneum R57* and subsequent whitening with H_2O_2.

Duration of treatment (h)	L without H_2O_2	L with H_2O_2	χ - Kappa number without H_2O_2	χ - Kappa number with H_2O_2
0 (no enzymatic treatment)	60,29	87,38	15,2	7,2
1	60,24	86,76	13,3	6,2
5	60,10	83,44	13,6	6,1
24	58,48	84,56	14,08	6,5
48	61,23	85,71	14,01	6,3

Symbols used:
L – CIELab Lightness
DE – CIELab Colour Difference
DL - CIELab Lightness Difference
H - kappa number
h – time in hours
Tr – Trichosporon cutaneum
CL – culture liquid
o.w.f – out of weight of fibers
M – bath ratio
rpm – revolution per minute

enzyme solution was used in the first stage of the linen treatment experiment. The specific enzyme activity varies from 3 to 5 U mg^{-1}. The data is shown in Table 1. Table 2 shows the data from linen treatment with concentrated enzyme solution and the activity was found to be 179.22 U mg^{-1}.

The data reveals that the highest degree of linen fiber lightness was obtained with a treatment time of 96 hours for the non-concentrated enzyme solution. The highest degree of lightness in the experiments with concentrated enzyme solution was obtained at the second hour. Higher values of color differences (DE) and lightness differences (DL) indicated a more efficient treatment and a resultant higher degree of lightness. Lower values of the Kappa number showed that a smaller quantity of non degraded lignin had remained in linen fibers. The smallest quantity of non degraded lignin measured in both experiments occurred after 2 hours of linen fiber treatment. Table 3 shows the results from different times of treatment of linen fibers and subsequent treatment with hydrogen peroxide (H_2O_2).

As it can be seen from the data, the highest degree of lightness was obtained at the 48th hour in both variants with and without hydrogen peroxide, while the use of hydrogen peroxide resulted in significantly increased brightness values. The smallest quantities of lignin were found in the samples at the 5th hour of the treatment. Most probably, readsorption of a part of degraded lignin fragments occurs at the 5th hour. The results shown in this study were obtained at an optimum pH 7.0.

Temperature is always a very important component of the enzyme application. Temperature dependences of the target color characteristics DE and DL are given on Fig.1. Enzyme preparation from *Tr.cutaneum R57* showed the most effective bleaching properties at the temperature 37 ^{0}C, which coincide with the optimal temperature for strain growth.

CONCLUSIONS

The results from our experiments reveal a clear tendency: the degree of lightness is significantly increased

and the amount of non-degraded lignin is decreased by treating linen fibers with an enzyme complex obtained from *Trichosporon cutaneum R57*. This is particularly evident in the cases when hydrogen peroxide was used in addition to enzyme treatment. It is obvious that the enzyme complex secreted by *Tr.cutaneum R57* has a good potential for applying in linen fibers pretreatment.

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