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ORIGINAL PAPER

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# Chromium recycling of tannery waste through microbial fermentation

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**Abstract** An *Aspergillus carbonarius* isolate, selected from an established microbial culture collection, was used to study the biodegradation of chromium shavings in solid-state fermentation experiments. Approximately 97% liquefaction of the tannery waste was achieved and the liquid obtained from long-term experiments was used to recover chromium. The resulting alkaline chromium sulfate solution was useful in tanning procedures. A proteinaceous liquid was also obtained which has potential applications as a fertilizer or animal feed additive and has several other industrial uses. The *A. carbonarius* strain proved to be a very useful tool in tannery waste-treatment processes and chromium recovery in the tanning industries.

**Keywords** Tannery wastes · *Aspergillus carbonarius* · Chromium recycling · Solid-state fermentation · Bioremediation

## Introduction

Among the numerous waste pollutants, the chromium-containing tannery solid wastes known as shavings are of major concern, due to their high toxicity and the interest in chromium recovery. The utilization of chromium in tanning procedures accelerates the mobility and transport rates of chromium, which by far exceed the rates of natural cycle processes, resulting in serious problems in countries involved with the tanning industry [29]. Shavings are chromium-containing organic matter scraped from the reverse side of hides when an adjustment of thickness is performed. Sanitary landfills are reluctant to accept chromium-containing waste because of the possibility of trivalent chromium ($Cr^{3+}$) being oxidized to hexavalent chromium ($Cr^{6+}$), which consequently contaminates ground waters. $Cr^{6+}$ is more toxic and more mobile in the environment than $Cr^{3+}$ [24] and, as hazardous landfills are expensive, there is an urgent need to find an economically attractive and efficient method of disposal [30]. A different perspective in metal bioremediation is the microbial reduction of $Cr^{6+}$ to $Cr^{3+}$ by various bacteria [23].

Most published data on the reduction of the toxicity of tannery wastes and the recovery of chromium concern the alkaline, acidic or enzymatic hydrolysis of leather. These procedures are complex, time-consuming and expensive. Furthermore, they result in secondary pollution problems. Studies have been made on tannery waste-processing in order to obtain proteinaceous products and recycled chromium, using several chemical methods [2, 3, 4, 5, 6, 31]. Efforts to obtain chromium after the incineration of shavings did not result in a less toxic waste [9]. Other approaches focused on the extraction of chromium from shavings or chromium oxidation with moisturized air [28].

The use of microorganisms able to grow in highly concentrated chromium environments and transform the waste into an easily recycled bioproduct offers a promising perspective for successful chromium recovery. The present study was designed to develop a microbiological procedure for the biodegradation of tanning wastes in Greece using an *Aspergillus carbonarius* strain and also to recover chromium in order to reuse it in tanning procedures. The capacity of microbial biomass to recycle chromium offers the possibility of an effective and more economic alternative to conventional remediation strategies.

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## Materials and methods

### Microorganism

An *A. carbonarius* strain belonging to the University of Athens Culture Collection, under the name UACC 92, was selected. The strain was maintained in 30% glycerol suspensions and lyophilized in 2% skim milk cultures [12].

### Culture conditions

The effect of salt enrichment and heat treatment of the shavings on biodegradation was studied. Aliquots (20 g dry weight) of shavings were autoclaved at 121 °C, 101 kPa for 45 min, incubated at 30 °C for 24 h and then autoclaved again.

During short-term experiments, seven different conditions were tested: In the first five, the shavings were autoclaved twice, in the sixth only once and in the seventh the shavings were not autoclaved before inoculation. The humidity was adjusted by adding either a salt solution in distilled water, called basal salt solution (BM; all experiments except fourth, fifth), sterile tap water (second, fourth) or sterile distilled water (third, fifth). BM consisted of (per liter): 5.2 g $NaNO_3$, 3.0 g $KH_2PO_4$, 0.1 g NaCl, 0.1 g $CaCl_2$ and 0.5 g $MgSO_4{\cdot}7H_2O$. Inoculants ($10^7$ spores of A. carbonarius $g^{-1}$ dry shavings) were added to each solid culture, resulting in a final water content of 80% (w/w). The incubation temperature was maintained at 30 °C throughout each experiment. The biodegradation rate was determined by estimating the dry weight of the total waste mass after 12 days of incubation.

During long-term experiments, 200 g (dry weight) of shavings were autoclaved in a culture vessel at 121 °C, 101 kPa for 45 min, incubated at 30 °C for 24 h and then autoclaved again. An inoculant ($10^7$ spores $g^{-1}$ dry shavings) in an adequate quantity of BM was added, resulting in a final moisture content of 80% (w/w). The solid culture was incubated at 30 °C for 90 days. In both short- and long-term experiments, control solid cultures without inoculum were incubated.

### Analytical methods

Determination of total sugars was according to Dubois et al. [8]. Tannin content was measured according to Makkar et al. [24]. Depending on the nature of the sample, solid or liquid, the IUC-8 [19] and ISO 9174 [16] methods were used, respectively, for chromium analysis. Total ash and pH were measured by the ISO 4045 [13] and IUC 7 [20] methods, respectively. Moisture was determined by the IUC 5 method [18]. Total nitrogen and total protein were estimated by the IUC 10 method [21]. The ISO 4048 method [14] was used for total fat content. The chemical oxygen demand and biochemical oxygen demand during 5 days of the chromate-containing liquid phase were determined by the HACH [7] and ISO 5815 [15] methods, respectively. Determination of $Cr^{6+}$ was carried out according to the ISO 11083 method [17].

### Chromium diffusion method

For evaluation of the microbial resistance to a chromium gradient, made by diffusion of the metal salt $K[Cr(S0_4)_2]{\cdot}12H_20$, into the agar medium, the method of Abbas and Edwards [1] was employed.

### Chromium precipitation method and conversion into tanning agent

Recycling of chromium is based on precipitation as chromium hydroxide and redissolving this with sulfuric acid. The chromium-containing liquid phase obtained from long-term fermentation experiments was used to recover chromium, based on the procedure developed under the European Commission demonstration project "Recycling of chromium as tanning agent" (ACE88/GR/004/A21), modified as follows: precipitation of 500 ml of chromium-containing liquid sample was carried out in volumetric cylinders using magnetic stirring. Chromium was converted into insoluble $Cr(OH)_3$ by raising the pH to 10.5 with the addition of 50% (w/v) Na0H. Then, 5.6 g of Mg0 was added slowly with simultaneous heating at 55 °C. After 2–3 h of stirring, the sludge was left to settle at room temperature for 10 h and was then separated by filtration. The chromium-containing sediment was then redissolved with the addition of 68.5 ml of 10% $H_2SO_4$ (w/v), resulting in a 150-ml volume of acid chromium sulfate solution of pH 2.0–2.4.

### Statistical analysis

All points given on figures and in tables here are the means of three replicate samples. Minimum significant differences were calculated by analysis of variance using the Tukey–Kramer method [10, 26].

## Results

### Chemical composition of the shavings

An initial determination of the chemical composition of the solid tannery waste samples was performed, which was essential for their later exploitation via microbiological processes. All values given here are the means of three replicate samples. The chemical composition of the tannery waste samples was (per gram): $82.0 \pm 2.0$ mg protein, $2.0 \pm 0.3$ mg total lipid, $1.1 \pm 0.1$ mg tannin, $82.0 \pm 2.0$ mg ash, $268.2 \pm 13.0$ mg total nitrogen, $56 \pm 5\%$ humidity and pH $3.7 \pm 0.2$. Qualitative mineral analysis showed the existence (mainly traces) of $SO_4^{2-}$, $Cl^-$, K, Ca, Fe, Sr, Cu, Rb, Zn and Ag.

### Selection of microorganisms

Microbial screening was performed in order to examine the indigenous microflora. Neither fungi nor bacteria were isolated, even after enrichment of the waste with selective media (malt extract broth, nutrient broth).

As no indigenous microflora was isolated, a selection from the culture collection of the Microbiology Laboratory of Athens University was performed, using chromium tolerance as a criterion. Isolate UACC 92, closely related to *A. carbonarius*, was selected. The physiology of this strain, which presented extreme tolerance to chromium (Fig. 1) and had a broad enzyme system capable of carob tannin degradation in solid-state fermentation experiments (data not shown), had been previously studied. It grows at 25–35 °C and pH 4.0–7.5, with a maximum growth rate at 30 °C and pH 5.5–6.5.

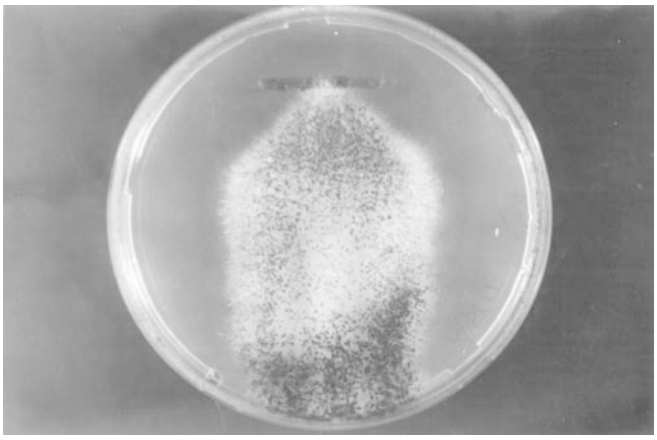


**Fig. 1** Evaluation of the *Aspergillus carbonarius* strain resistance to a chromium gradient, according to the method of Abbas and Edwards [1]

## Liquefaction of tanning waste in short-term experiments

The effect of salt enrichment and autoclaving of the shavings on biodegradation was studied. Degradation and liquefaction of the solid waste was achieved within 12 days when salt enrichment and double-sterilization of the waste were both carried out before inoculation (Fig. 2).

Solid waste was used in dry form (dried at 60 °C for 4 days). When it was used without autoclaving, the fungal strain was not able to act within a short time-period. Slight degradation was noticed after 3 months of incubation. Total liquefaction of the solid waste occurred after 9 months only if the initial volume of the solid waste was up to 20 g. Preliminary experiments performed at different incubation temperatures showed that the highest degradation of solid waste was achieved after 12 days at 30 °C. (Fig. 3).

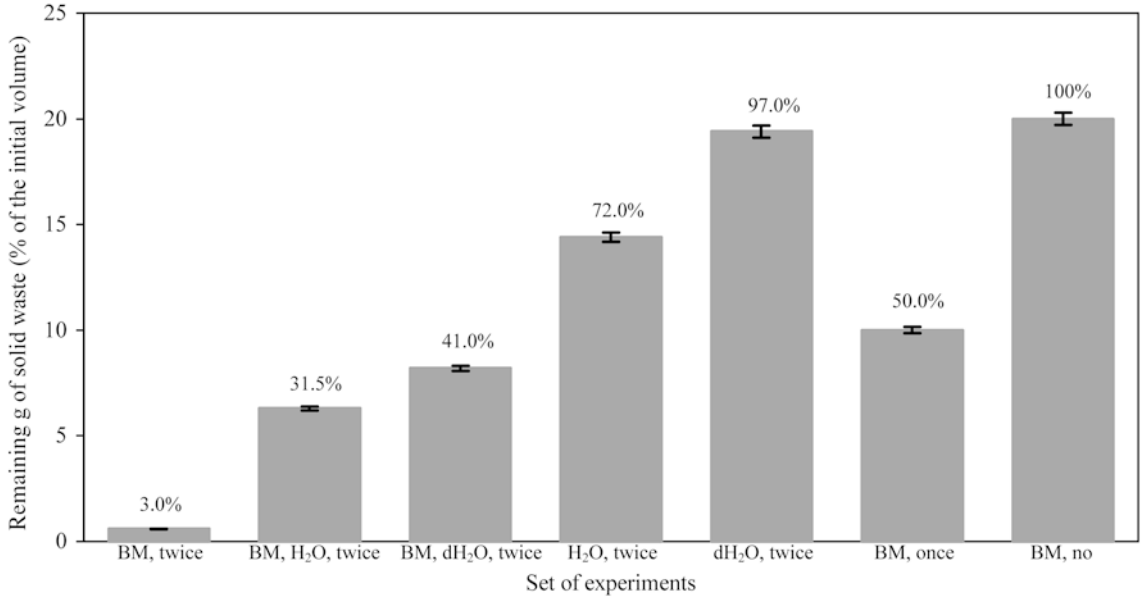
**Fig. 2** Effect of salt enrichment and sterilization on the degradation and liquefaction of solid tannery waste. Experimental design: *BM* humidity was adjusted by adding basal salt solution, $H_2O$ humidity was adjusted by adding sterile tap water, $dH_2O$ humidity was adjusted by adding sterile distilled water, *twice* shavings were autoclaved twice, *once* shavings were autoclaved once, *no* shavings were not autoclaved before inoculation

## Liquefaction of tanning waste in long-term experiments

Long-term experiments were designed to estimate the time for and percentage of shavings degradation. BM was used to adjust the moisture content and to provide the necessary trace elements. Spore germination occurred within the first 10–15 h. After 10–12 days of incubation, the solid substrate began to liquefy and, at the end of incubation time (after 90 days), the solid waste was almost totally liquefied. By that time, lysis of the mycelium was observed and only spores appeared in the culture. After 90 days of incubation, the culture was centrifuged at 22,000 *g* for 30 min at 4 °C. The sediment was desiccated and its chromium concentration was determined. The liquid phase was analyzed and the chromium was precipitated and converted into chromium sulfate, which is used in tanning procedures (Table 1).

After 45 days of incubation, the biomass consisted of spores and vegetative mycelium and, after 90 days, the mycelium was almost totally self-lysed and the biomass consisted only of spores. After 90 days, only 7.5 g of the sediment had not been converted. Almost 97% of the initial 200 g of solid waste was converted into liquid.

Preliminary experiments were then performed to find out whether the remaining liquid could be used as an inoculum in a new solid fermentation. A quantity equal to 50 ml was used to inoculate 200 g of solid waste. The same percentage of liquefaction was achieved in only 60 days (data not shown).



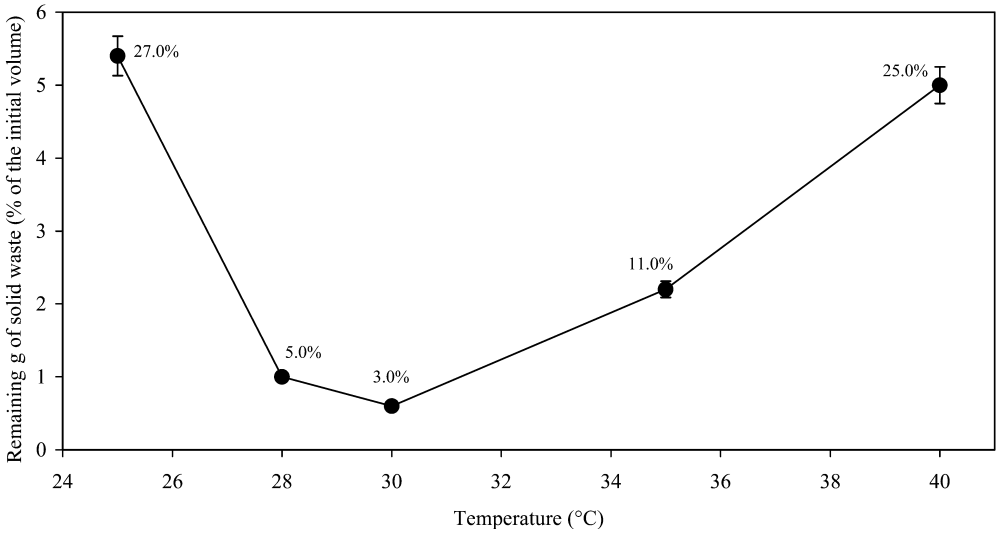



**Fig. 3** Effect of incubation temperature on the degradation and liquefaction of solid tannery waste

**Table 1** Analysis of sediment and chrome liquid phase from solid cultures. *COD* Chemical oxygen demand, $BOD_5$ biochemical oxygen demand during 5 days

| | After 45 days | After 90 days |
|---|---|---|
| Sediment | | |
| Dry weight | 16 g | 7.5 g |
| Ash | 120 mg $g^{-1}$ | 136 mg $g^{-1}$ |
| $Cr^{3+}$ | 24 mg $g^{-1}$ | 28 mg $g^{-1}$ |
| Chrome liquid phase | | |
| pH | 6.5 | 8.1 |
| $Cr^{3+}$ | 0.9 g $l^{-1}$ | 1.9 g $l^{-1}$ |
| COD | 122 mg $l^{-1}$ | 82 mg $l^{-1}$ |
| $BOD_5$ | 46 mg $l^{-1}$ | 38 mg $l^{-1}$ |

Another set of experiments was performed to check the influence of the initial solid waste volume on the liquefaction rate. Liquefaction was achieved at a higher rate in small volumes up to 200 g. Above this quantity and up to 5 kg, the time needed was 90 days.

### Chromium precipitation and conversion into tanning agent

The chromium liquid phase obtained from long-term fermentation experiments was used to recover chromium. From each 500 ml of chromium liquid sample, 68 g of chromium precipitate sludge and 400 ml of proteinaceous supernatant were obtained. The proteinaceous filtrate was desiccated, resulting in a product that could be used as a high-nitrogen-content fertilizer or in several other industrial procedures. Characteristics of the proteinaceous liquid and the chromium sulfate solution are shown in Table 2. In this form, chromium can be reused in tanning procedures. It was important that no toxic $Cr^{6+}$ was obtained in the proteinaceous liquid.

**Table 2** Qualitative and quantitative characteristics of the proteinaceous liquid and basic chromium sulfate solution

| Content | Proteinaceous liquid | Chromium sulfate solution |
|---|---|---|
| Proteins | 68.4 g $l^{-1}$ | 34.2 g $l^{-1}$ |
| Total nitrogen (Kjeldhal) | 12.1 g $l^{-1}$ | 6.1 g $l^{-1}$ |
| $Cr^{3+}$ | 0.028 g $l^{-1}$ | 6.3 g $l^{-1}$ |

## Discussion

In recent years, several chemical procedures have been reported for the digestion of commercial chrome shavings [3, 32]. The detoxification of tannery wastes has focused on the use of enzymes or the combination of alkaline treatment and enzymatic hydrolysis [30]. McLean et al. [25] characterized a *Pseudomonas synxantha* bacterial strain capable of reducing $Cr^{6+}$ to an insoluble precipitate, removing the toxic chromium from solution. The use of microorganisms for the treatment of solid tannery wastes has not yet been achieved. The lack of growth of indigenous microorganisms in the waste can be attributed to the nature of the waste. Chrome shavings are small particles, in a variety of shapes, mainly consisting of collagen cross-linked with complexes. Stabilization of the skin is achieved by the formation of metal ion-mediated coordinated crosslinks in the protein, involving side-chain carboxyl sites of aspartic and glutamic acids in the collagen [27, 31]. This crosslinking of the collagenous matrix by different polymeric $Cr^{3+}$ species has been shown to impart collagen with both hydrothermal stability and stability against enzymatic degradation [11].

The physiology and enzymatic system of the *A. carbonarius* strain used were extensively studied by Lambraki et al. [22]. This strain is capable of degrading polyphenolic substances in carob beans and it grows

over a wide range of pH and temperature [22]. In this study, we show that it has the ability to utilize collagen and convert the solid waste into liquid. Although it is difficult to elucidate the mechanisms underlying disruption of the collagenous matrix, perhaps among the broad range of enzymes of *A. carbonarius* there is a collagenase able to act even in the presence of complexes.

Although the waste contained a high amount of protein, *A. carbonarius* strain UACC 92 was unable to grow without added salt. The degradation capability increased gradually from distilled water to tap water and finally to BM. It is evident that the addition of salts proved essential in the ability of the microorganism to degrade the waste.

The chemistries of $Cr^{3+}$ and collagen have been studied extensively and much is known about the need for efficient and effective tanning. However, there is little information about the reactions involving collagen during the chrome tanning [11]. We found that double-autoclaving was crucial for the microorganism to degrade the solid waste. Further studies have to be performed in order to examine the effect of sterilization on the stability of the collagen complex. It seems certain that modification of the structure of the complex allows higher collagenase activity.

The results of the 90-day experiments showed that the initial volume of the waste affected the liquefaction rate for volumes up to 200 g. Above that, the estimated time for waste liquefaction was 90 days. Thus, the biotechnological treatment of 1 t of shavings could give 37.5 kg (dry weight) of sediment and 9.5 $m^3$ of chromate liquid waste as a product. Almost 97% of the initial shavings were liquefied. The liquefaction rate was also affected by the inoculum. If the inoculation was performed using liquefied product from a previous solid culture, liquefaction of the same percentage was achieved in only 60 days. This was probably due to adaptation by the microorganism to the specific culture conditions.

In the tanning industry, reduction of the waste volume is very important. The proteinaceous solution resulting from the liquid waste after a suitable chemical process has a high nitrogen content and might be used for fertilizer, feed additive or silage production; and the basic chromium sulfate solution resulting from the same procedure could be used in tannery procedures or sold to chemical suppliers [2].

The almost complete hydrolysis of the solid waste collagen by this chromium-resistant strain seems to be an effective solution for protecting the environment from tannery waste, as no toxic $Cr^{6+}$ is formed. This innovative method, which can be carried out at minimal cost, opens a new perspective in shavings utilization.

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