

Dye decolourization by newly isolated thermophilic microorganisms

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In this work, various northwestern Spain hot springs have been explored as potential sources of dye-degrading thermophilic microorganisms. The sites were selected based on their average water temperature, which was in all cases higher than 50°C. Water and mud samples were collected, and strains isolation were carried out by the streak plate method using a medium supplemented with the model reactive dye, Reactive Black 5. First, the capacity of specific isolated strains and consortia of indigenous microorganisms to degrade this dye was checked on agar plates. Then, the biological process was carried out in shake flasks, and the decolourization degree provided by each strain and consortium was ascertained through spectrophotometric measurements. Very promising degradation values (around 80%) were detected for some of the assayed consortia, at temperatures of 65°C. The results supported the interest of investigating thermophilic strains as a potential alternative to mesophilic microorganisms in microbial dye decolourization.

1. Introduction

Synthetic dyes are widely produced and used in many different industries, including the textile, cosmetic, paper, leather, pharmaceutical and food industries. Moreover, these industries consume substantial volumes of water and chemical products associated with the dyeing process. More than 10,000 different textile dyes with an estimated annual production of 8×10^5 metric tonnes are commercially available worldwide, and about 50% of these are azo dyes (Leena and Selva Raj, 2008; Szygula et al. 2008).

Conventional wastewater treatment has been found to be ineffective to solve the problem caused by the release to the environment of coloured synthetic dye industrial effluents. The complex aromatic structure of the dyes is resistant to light, biological activity, ozone and other degradative environmental conditions (Kilic et al. 2007). Alternative methods, either physical, chemical and biological, have been proposed for the removal of dyes in textile wastewater (Lucas and Peres, 2009; Rodriguez Couto et al. 2002) The commonly used physico-chemical techniques present some drawbacks, such as high cost, low efficiency, limited versatility, interference by other wastewater constituents and the need to handle the resulting waste. On the other hand, microbial decolourization appears as an attractive cost-effective option. In this context, the use of

thermophilic microorganisms would allow to operate at high temperature, thus with increased solubility of most compounds (except gases) and diffusion rates, as well as decreased viscosity.

Nowadays, thermophilic microorganisms are amongst the most studied extremophiles and are gaining wide industrial and biotechnological interest due to the fact that their enzymes are well suited for harsh industrial processes. For this reason, natural and artificial places with hot temperatures have been screened worldwide in order to find the right metabolite for every application, for instance, thermal springs, solfataric fields, abyssal hot vents (“black smokers”), active seamounts smouldering coal refuse piles and hot outflows from geothermal and nuclear power plants (Torkamani et al, 2008; Kublanov et al, 2009).

Despite the benefits described above, there are also inconvenients such as higher equipment corrosion problems or liquid evaporation. Therefore, one of the most challenging and less studied aspects in the culture of extremophilic microorganisms is the scaling-up of the process. However, there are few papers in which the large scale operation with thermophilic organisms has been tackled (Domínguez et al, 2005; Deive et al, 2009).

In this work, several hot springs in the northwestern of Spain have been screened to find thermophilic strains and consortia which can efficiently decolorize an effluent containing the model azo dye Reactive Black 5, and the process has been scaled-up from shake flasks to bench-scale bioreactors.

2. Materials and Methods

2.1 Origin of the isolates

The samples containing water and mud collected for this work were taken from several hot springs in the province of Ourense (Spain): As Burgas, Lobios, A Chavasqueira and Tinteiro. They were stored in sterile glass tubes with screw tops.

2.2 Liquid and plate media

For liquid cultures the media used was a complex basal one containing (g L^{-1} , in distilled water) 10 casein peptone, 5 yeast extract and 10 sodium chloride. The pH is initially adjusted at 7.0. The medium was autoclaved at 121°C for 20 min. Reactive black dye was added at final concentration of 70 mg L^{-1} after having been sterilized through microfiltration ($0.20 \mu\text{m}$ pore size). In the plate medium, agar (20 g L^{-1}) was added to medium described above. The submerged cultures were carried out in 250 mL Erlenmeyer flasks with 50 mL of medium. The flasks were inoculated (3% v/v) with the samples or with previously obtained cell pellets, and incubated in an orbital shaker at 65°C and 100 rpm.

2.3 Detection of decolourization ability

Plates containing the culture medium described above, supplemented with Reactive Black 5, were inoculated and incubated at 65°C . The positive result is a transparent halo around the colonies that growth in plates stained with Reactive black dye, indicating the decolourization ability.

2.4 Isolation method

The isolation was achieved by the 13 streak plate method, which consisted of a mechanical dilution of the samples on the surface of the plates. In all cases the pH was adjusted at 7.0 and the plates were incubated at 65 °C, which were the average values of the environmental conditions in the different thermal springs that were sampled.

2.5 Bubble bioreactor

It consisted of a jacketed glass column, which is 4.5 cm in internal diameter and 20.0 cm high (working volume: 300 ml). Temperature was maintained at 65°C by circulation of thermostated water. Humidified air was supplied in a continuous way at 300 mL/min.

2.6 Analytical method

Biomass concentration was measured by turbidimetry at 600 nm and the obtained values were converted to g cell dry wt/L using a calibration curve.

Decolourization was determined spectrophotometrically using decrease percentage of initial absorbance at maximum visible absorbance (597 nm).

Genetic identification of the isolated microorganisms was carried out by 16S rRNA sequencing. The degenerated primer based on conserved sequence of 16S rRNA was used to amplify 16S rRNA from isolated bacterium using PCR.

3. Results and Discussion

3.1 Origin of the isolates

Several hot springs in Galicia (Northwest Spain) were considered, and those with the highest emerging temperatures were selected for sampling. Every sample is named with letters referred to its origin (Chavasqueira BCH, Tinteiro BTi, Burgas BBU and Lobios BLO). Shake flask cultures with Reactive Black 5 inoculated with different samples was carried out. As it is shown in Table 1, four consortia were able to decolourize Reactive Black 5 at high extent after 24 hours. It is outstanding that the decolourization degree obtained in this work by the consortia BCH6 and BTi (higher than 80%) is higher than the reported in the literature by other bacteria and fungi, operating in harsh conditions at high temperature (65°C) and pH 7.

Table 1 Decolourization after 24 h

Sample	Growth	Decolourization (%)
Control	0	0
BCH 2	++	25.8
BCH 3	++	49.2
BCH 4	++	0
BCH 6	++	89.5
BTi 2	++	83.6
BBU 2	++	30.4
BLO 1	++	76.4
BLO 2	++	72.7
BLO 4	++	28.2

From those four consortia it was only possible to isolate three strains in plates: BCH6, BLO1 and BLO2. Most of the colonies show typical appearance of bacteria, and they present fast growth (less than 1 day), what is usual in this kind of microorganisms.

3.2 Genetic identification of the selected strain

The screening for dye-decolouring microorganisms in several hot springs in northwestern Spain led to the isolation of four very promising strains, which were able to efficiently remove the colour in shake flask cultures. The isolated strains were identified by 16S rRNA sequencing. The degenerated primer based on conserved sequence of 16S rRNA was used to amplify 16S rRNA from the isolated bacterium through PCR. The 16S rRNA sequence exhibited the highest similarity in three of them to the following bacteria (100% homology): *Anoxybacillus pushchinoensis* (BCH6), *Anoxybacillus kamchatkensis* (BLO1) and *Anoxybacillus flavithermus* (BLO2).

3.3 Bioreactor culture

The selection of an adequate bioreactor is crucial to carry out the scale-up of the process, since the success of the decolourization process proposed depends on it. The most important factor that must be taken into account to design a bioreactor is the reduction of operational problems. In this case, a bubble bioreactor was employed to develop the decolourization of Reactive Black 5 in batch and fed-batch culture mode.

Figure 1 shows the decolourization degree (expressed as a percentage) of the three strains identified. The results indicated that the decolourization pattern of the studied strain is the same, showing a similar decolourization degree to that obtained operating in flask cultures after 24 hours of culture.

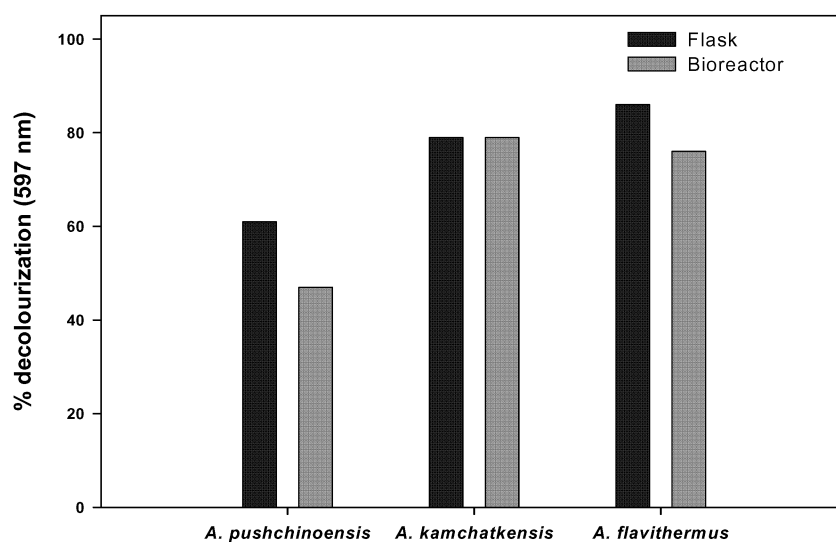


Figure 1 Comparison between flask and bioreactor culture scales with the three strains isolated and identified.

4. Conclusions

Eight thermophilic consortia which ability to decolorize dyes were found from several hot springs in northwestern Spain, and four of them showed remarkable decolourization degree in submerged cultures. From these consortia three strains were isolated and identified as *Anoxybacillus pushchinoensis*, *Anoxybacillus kamchatkensis* and *Anoxybacillus flavithermus*. Suitable culture conditions have been defined for the biological process. Moreover, culture in a bubble bioreactor was successfully undertaken, and a decolourization degree similar to that raised in shake flask cultures was obtained. Therefore, the selected strains appear to be an attractive option for the treatment of industrial effluents contaminated with dyes.

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