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Decolorization of dye Reactive Black 5 by newly isolated thermophilic microorganisms from geothermal sites in Galicia (Spain)

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ABSTRACT

In this study, thermophilic microbial strains from thermal spots in northwestern Spain displaying excellent decolorization capability were isolated. The research work tackled: (i) the ability of consortia to degrade a model di-azo dye Reactive Black at different pHs in flask cultures, obtaining that just neutral pHs licensed degradation levels near to 70%, (ii) the isolation of tree of the bacteria, which rendered possible reaching high levels of decolorization (80%) after just 24 h in aerobic conditions, and which were identified through 16S rRNA sequencing to possess high homology (99%) with *Anoxybacillus pushchinoensis*, *Anoxybacillus kamchatkensis* and *Anoxybacillus flavithermus*, and (iii) the cultivation of the isolates in a bench-scale bioreactor, which led to a decolorization rate two-fold higher than that obtained in flask cultures. Therefore, this work makes up the first time that a decolorization process of an azo dye by thermophilic microorganisms in aerobic conditions is investigated.

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1. Introduction

Management of water pollution is currently one of the major challenges for environmentalists. More than 10,000 different textile dyes, with an estimated annual production of 8×10^5 metric tonnes, are commercially available worldwide; about 50% of these are azo dyes [1,2].

Highly colored synthetic dye effluents from the textile, food, paper and cosmetic industries have contaminated water resources, and these contaminants are easily identifiable to the naked eye. The complex aromatic structures of the dyes are resistant to light, biological activity, ozone and other environmental degradative conditions [3]. The effluents may significantly affect photosynthetic activity in aquatic life due to reduced light penetration and increased chemical oxygen demand. Concerns have arisen because many of the dyes are made from known carcinogens, toxins and mutagens, such as benzidine and other aromatic compounds, and often involve the presence of metals, chlorides, and aromatic compounds [4].

Dyes can be classified according to several features, but one typical consideration is whether they are ionic or nonionic, as reported by Robinson et al. [5]. Ionic dyes are direct, acid and reactive dyes. Nonionic dyes refer to disperse dyes because they do not ionise in an aqueous medium. Direct dyes are the most popular class of dyes, owing to their easy application, wide color range, and availability at modest cost. Most direct dyes have di-azo and tri-azo structures. Azo dyes are the largest class (60–70%) of dyes, with the greatest variety of colors [6].

Importantly, conventional wastewater treatment remains ineffective in decolorizing these compounds. Several methods for the removal of dyes in textile wastewater have been implemented to overcome this problem. These methods have been classified into three categories, physical, chemical and biological, and they have been extensively reviewed [5,7–10]. Although physico-chemical techniques are commonly used [11–13], their major disadvantages are high cost, low efficiency, limited versatility, interference by other wastewater constituents and the handling of the waste generated. Microbial decolorization, being cost-effective, is receiving more attention for the treatment of textile dye wastewater [14].

Dye-bath effluent compositions vary depending on the type of fibers to be dyed. For instance, while wool dyeing involves effluents with acid pH, cotton dye entails neutral or alkaline conditions. Besides, these effluents, with a temperature range of 30–60 °C, exhibit high concentrations of dye stuff, biochemical oxygen demand, total dissolved solids, sodium, chloride, sulphate, hardness, heavy metals and carcinogenic dye ingredients. For this reason, the use of biological processes for their treatment requires the presence of microorganisms thriving in extreme conditions. Thermophilic microorganisms are amongst the most studied extremophiles and are gaining wide industrial and biotechnological interest due to the fact that they are well suited for harsh industrial processes. For this reason, thermal springs, solphataric fields,

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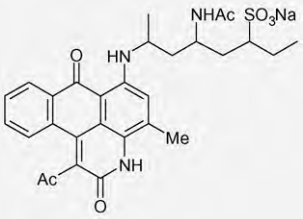
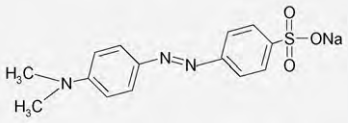
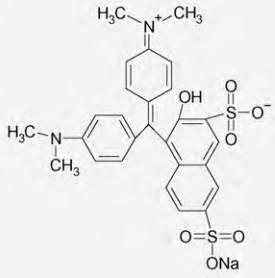
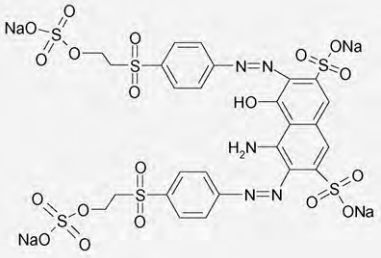
Table 1
Chemical structure, color index (C.I.) and wavelength at maximum absorbance of dyes.

Class	Dye	Structure	C.I.	λ_{max} (nm)
Poly R-478	Anthraquinone		–	520
Methyl Orange	Azo		13,025	466
Lissamine Green	Diphenylnaphthyl-methane		44,090	633
Reactive Black 5	Di-azo		20,505	597

abyssal hot vents ("black smokers"), active seamounts, smouldering coal refuse piles and hot outflows from geothermal and nuclear power plants have been screened worldwide to find the right metabolite for every application [15–19]. However, the use of thermophilic strains for textile dye decolorization is yet to be deeply investigated. Willets et al. [20] was the first to report on this issue, and since then, few papers have been found in the literature. Recently, Boonyakamol et al. [21] have reported the benefits of using thermophiles instead of mesophiles to decolorize a model anthraquinone dye, and dos Santos et al. have widely reported the necessity of introducing redox mediators to achieve high decolorization efficiencies of several azo dyes by using anaerobic thermophiles [22–27]. However, as the anaerobic degradation of azo dyes usually produces aromatic amines, which are carcinogenic and mutagenic, the aerobic treatment is the only safe method for the biodegradation of textile azo dyes, and thermophilic aerobic treatments is yet to be studied [28].

There are some benefits of working at high temperatures, such as reduced cooling costs, increased solubility of most compounds (except gases), decreased viscosity and a lower risk of contamination. However, there are also disadvantages, such as higher equipment corrosion problems, liquid evaporation, and substrate decomposition. Therefore, one of the most challenging and less studied aspects of culturing extremophilic microorganisms is the scaling-up of the process. Nevertheless, there are a few papers in which large-scale operations using thermophilic organisms have been addressed [24–26,29–31].

In this work, the model di-azo dye Reactive Black 5, one of the most commonly used reactive dyes for textile finishing, was selected as the target compound representing a dye pollutant of industrial wastewaters. Up to our knowledge, there are almost no reports dealing with thermophilic aerobic decolorization processes of azo dyes, so several hot springs in the northwest of Spain were screened to find thermophilic aerobic bacteria and consortia that could efficiently decolorize an effluent containing the model dye in aerobic conditions. The process was then scaled up from shake flasks to bench-scale bioreactors.

2. Experimental

2.1. Dyes

Poly R-478, Methyl Orange, Lissamine Green B and Reactive Black 5 were purchased from Sigma. The structure and the main characteristics of these dyes are shown in Table 1.

2.2. Sampling for strain isolation

The samples containing mud and water were collected during October (wet season) 2008 in four different locations of the

Table 2
Sampling site characteristics and growth and decolorization ability of consortia developed from Galician thermal springs.

Sampling sites			pH 4		pH 7.5	
Code	pH	T (°C)	Growth	% Removal (24 h)	Growth	% Removal (24 h)
BLO 1	7.54	47	+	15.6	++	76.4
BLO 2	7.31	48	+	16.8	++	72.7
BLO 3	7.32	44	+	16.4	0	0
BLO4	7.17	67	0	0	++	28.2
BCH1	7.91	60	0	0	0	0
BCH 2	8.07	64	+	14.3	++	25.8
BCH 3	8.01	49	+	14.1	++	49.2
BCH4	7.89	61	0	0	++	0
BCH5	7.86	53	0	0	0	0
BCH 6	8.00	53	0	0	++	89.5
BCH7	7.56	53	0	0	0	0
BTI 1	7.55	45	0	0	0	0
BTI 2	6.94	40	+	8.9	++	83.6
BBU 1	8.35	53	+	6.1	0	0
BBU 2	8.34	60	0	0	++	30.4
BBU3	7.92	66	0	0	0	0
BBU4	7.93	55	0	0	0	0

province of Ourense: as Burgas, Lobios, A Chavasqueira and Tinteiro. The pH and temperature of every sampling site was measured, and the data obtained are listed in Table 2. The samples were stored in sterile glass tubes with screw tops.

2.3. Strain isolation

Strain isolation was carried out by the 13-streak plate method, which consisted of a mechanical dilution of the samples on the surface of agar plates. Basal medium was composed of (g/L, in distilled water): 8 trypticase, 4 yeast extract, 3 sodium chloride, 20 agar, and 0.07 dye. In all cases, the pH was adjusted with NaOH (1 M) or HCl (1 M) (as indicated in the Results and Discussion section). The plates were incubated at 65 °C to screen for highly thermophilic microorganisms. Dyes were sterilised by filtration through a 20-μm filter and added to the autoclaved medium to avoid any possible alteration to the chemical structure of the dye. A positive result was a transparent halo around the colonies growing in plates stained with dye, indicating decolorization ability. Each isolate and consortium was named with letters referring to its origin (Chavasqueira, BCH; Tinteiro, BTI; Burgas, BBU; and Lobios, BLO).

2.4. Flask culture

Unless otherwise stated, submerged aerobic cultures were carried out in 250-mL Erlenmeyer flasks with 50 mL of a basal medium (the same indicated in Section 2.3 without agar) [31]. The pH was initially adjusted to 7.5, and the medium without dye was autoclaved at 121 °C for 20 min. The flasks were inoculated (3%) with the hot spring samples or with previously obtained cell pellets, which were then incubated in an orbital shaker (Innova 44, New Brunswick Scientific,) at 65 °C and 100 rpm. A number of samples higher than 6 were taken, each one with a volume of 1 mL.

2.5. Bioreactor culture

The bubbling bioreactor consisted of a jacketed glass column 4.5 cm in internal diameter and 20 cm high (working volume: 300 mL) and it is schematically shown in Fig. 1. It was filled with the basal medium described above containing 0.07 g/L of Reactive Black 5. The temperature was maintained at 65 °C by circulation of thermostatted water, and the pH was adjusted to 7.5. The bioreactor was inoculated with previously obtained cell pellets (obtained after centrifugation for 10 min at 5000 × g and dried under vacuum) at a concentration of 3% (v/v) [31]. Humidified air was supplied continuously at 300 mL/min (hydraulic retention time = 0.017 h), and samples were taken regularly during the experimental period (24 h).

2.6. Culture sample preparation and decolorization analysis

Cells were harvested by centrifugation (10 min, 5000 × g), and the supernatant was reserved for decolorization analysis. Decolorization was measured spectrophotometrically (Unicam Helios β, Thermo Electron Corp.) from 300 to 750 nm, calculated by measuring the area under the plot and expressed in terms of percentage. D (% removal) = $(I_i - I_f)100/I_i$, where I_i and I_f are initial and final area of the dye solution, respectively [32]. Each decolorization value was the mean of two parallel experiments. Abiotic controls (without microorganism) were always included. The assays were done in duplicate, and the experimental error was less than 3%.

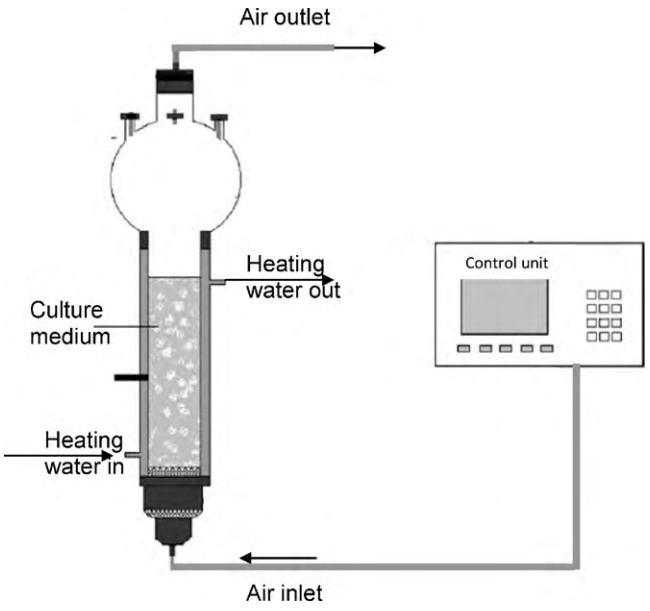


Fig. 1. Schematic diagram of the bubble bioreactor used in this study.

2.7. Cell growth determination

Biomass concentration was measured by turbidimetry at 600 nm, and the obtained values were converted to grams of cell dry weight per litre using a calibration curve.

2.8. Genetic identification of the selected strains

Isolates were identified by 16S rRNA sequencing. Degenerate primers based on the conserved sequence of 16S rDNA were used to amplify 16S rDNA from isolated bacterium using PCR. The forward primer was 5′-AGA-GTT TGA TC/TA/C TGG CT-3′ (Invitrogen), and the reverse primer was 5′-TAC GGC/T TAC CTT GTT ACG ACT-3′ (Invitrogen). PCR-amplified fragments were purified on Microspin columns (Amersham Farmacia Biotech, Piscataway, NJ, USA), and cycle sequencing was done in a GeneAmp PCR system 2400 (Applied Biosystems) thermocycler. Multiple alignment of sequences was created by ClustalX, version 1.81 [33], which were subsequently compared with sequences in public databases of GeneBank (http://www.ncbi.nlm.nih.gov/) with BLAST, version 2.2.6.

3. Results and discussion

3.1. Obtaining consortia with decolorization ability

The use of thermophilic microbial consortia to decolorize effluents is considered an accelerated and effective degradation strategy [34,35], so the search for thermophilic consortia able to decolorize model dyes marked the onset of this work. Four different dyes, such as Poly R-478, Methyl Orange, Lissamine Green B and Reactive Black 5, were selected to qualitatively check the decolorization ability on plate of thermophilic consortia from Galician hot springs. Although in all cases several degrees of decolorization were observed, Reactive Black 5 was chosen as model di-azo dye to approach this study. Many researchers have focused decolorization studies in the following two ways. One is the decolorization of various dyes by an individual strain and the other is the decolorization of a single dye by various strains [36–38]. In this study, we tried to demonstrate the feasibility of a decolorization process using different thermophilic consortia and isolates in aerobic conditions for one only model dye (Reactive Black 5).

The next stage of the work included a preliminary screening of viable dye concentration values allowing high levels of growth and decolorization rate. There are a variety of published studies addressing different dye concentrations of synthetic effluents with concentrations ranging from 0.000022 to 5 g/L. As many dyes are visible in water in concentrations as low as 0.001 g/L and processing textile effluents have concentrations ranging from 0.01 to 0.2 g/L [39], plate cultures at Reactive Black concentrations of 0.01, 0.03, 0.07, 0.1 and 0.2 g/L were performed, and it has been visually observed high cellular growth and decolorization ability in 2 days at concentrations under 0.07 g/L, being 0.1 g/L the minimal inhibitory concentration. Due to this, 0.07 g/L was the concentration chosen for further investigation.

Cultures were carried out at different pH values (4, 7.5 and 9) by inoculating the flasks with the samples collected from the selected thermal springs. Growth was only detected at acid and neutral pH, and the results of decolorization are shown in Table 2. The consortia growing at pH 4 rendered very low levels of decolorization. We observed that several consortia growing at pH 4 possessed a mycelium-type morphology because this pH value is more suitable for fungal growth.

Additionally, dye biosorption could be visually identified, indicating that the dominant mechanism of dye removal by the fungus was probably bioaccumulation. pH is one of the important parameters affecting the biosorption and bioaccumulation potential, and acidic values seemed to favour dye biosorption. In this sense, there are many studies concluding acidic pHs for achieving maximum dye uptake with living fungi, as those reported by Ikramullah et al. and Taskin and Erdan for Reactive Red and Reactive Black, respectively [40,41].

The results obtained can be explained in terms of the net charge due to the functional groups existing on the microorganismal surface. At acidic pH values, there is more interaction between the negatively charged dye molecules and positively charged binding sites on the biosorbent surface. This fact was also reported by Akar et al. [42] and by Iqbal and Saeed [43] in studies on dye biosorption by the macro-fungus *Agaricus bisporus* and by the white-rot fungus *Phanerochaete chrysosporium*, respectively. Increasing sorption capacity with decreasing pH was also reported for algae, bacteria and yeasts [44–46].

On the other hand, in light of the results listed in Table 2, there were four consortia that yielded over 70% of decolorization within 24 h of treatment at 65 °C. These results are better than those obtained by Tony et al. [47]. They reported less than 40% of decolorization with several azo dyes when a consortium developed from a textile wastewater treatment plant was used. We also used a temperature higher than that reported by Dos Santos et al. [26]. They reported decolorization values of the dye Reactive Red 2 ranging from 79% to 95% when a thermophilic consortium was grown at 55 °C in thermophilic bioreactors at a HRT of 2.5 and 10 h, respectively. Therefore, since all the data found in the literature related to thermophilic decolorization of azo dyes tackles anaerobic processes, our results underscore the importance of going beyond the technical aspects and trying to isolate and identify the microorganisms involved in the decolorization process.

3.2. Isolation and genetic identification of dye-decoloring microorganisms and process modelling

Agar plate cultures streaked from the consortia yielding the highest decolorization levels allowed us to isolate three valuable strains of bacteria with decolorization potential. The colonies showed the typical appearance of bacteria, and they presented fast growth (less than 1 day), which is usual in this kind of microorganisms. The three isolated strains were identified by 16S rRNA sequencing. The degenerate primers based on the conserved sequence of 16S rDNA were used to amplify 16S rDNA from the isolated bacteria through PCR. The 16S rRNA sequences of BCH6st, BLO1st and BLO2st exhibited the highest similarity (99%) to those of *Anoxybacillus pushchinoensis*, *Anoxybacillus kamchatkensis* and *Anoxybacillus flavithermus*, respectively. None of the isolated strains had ever been reported as dye-decoloring microorganisms, and there are just a few papers describing growth characteristics or metabolite production [48–50].

The ability to decolorize the di-azo dye at a concentration of 0.07 g/L was experimentally ascertained through cultivation in Erlenmeyer flasks, and the UV spectra obtained (Fig. 2) clearly reflects the removal of the color. This strategy allowed us to perform an in-depth study of the biological characteristics of bacterial growth and decolorization. The cell growth and decolorization modelling could be a valuable tool for characterising the relationship between the metabolites triggering dye decolorization and the biomass. In this sense, kinetic behaviour is an extremely important subject in the implementation of any biological process, as it can ease the control, which is crucial when operating at industrial scale. Moreover, modelling the behaviour of bacteria in biological reactions will allow us to anticipate their responses to certain environmental conditions, to select the operation mode that ensures the quality of the desired product, and to facilitate an efficient

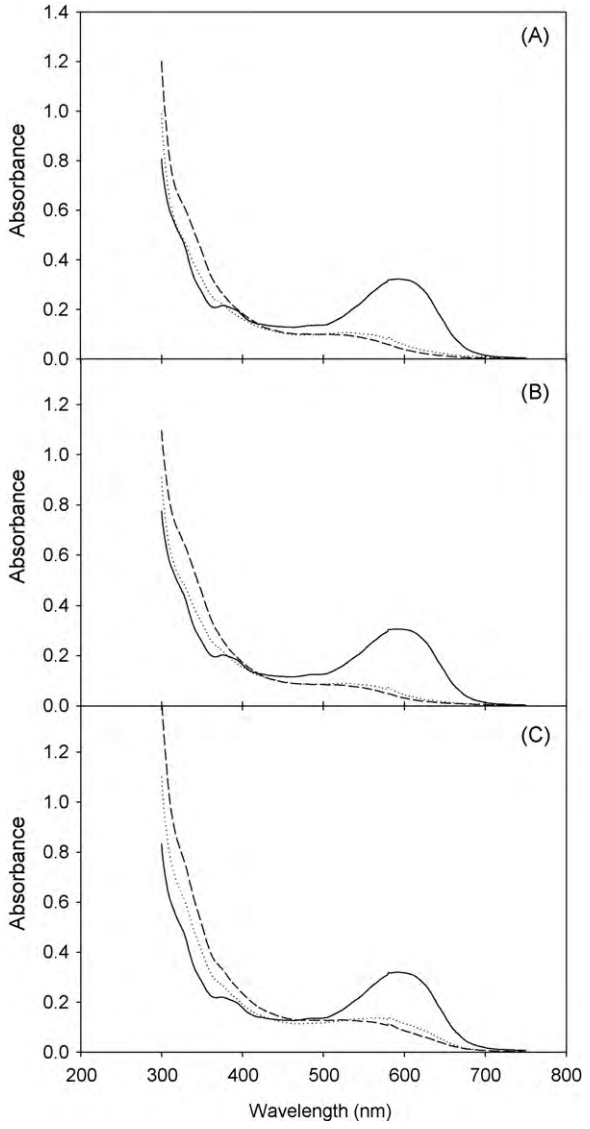


Fig. 2. UV spectra at time 0 (continuous line), 18 (dotted line) and 42 h (dashed line) of flask cultures of: (A) *Anoxybacillus pushchinoensis*, (B) *Anoxybacillus kamchatkensis*, and (C) *Anoxybacillus flavithermus*.

process design, by avoiding costly, time-wasting steps and minimising the amount of spoiled batches to reject and wastes for disposal. The interest is even greater because no data addressing the modelling of this kind of thermophilic processes have been published.

The performance of the biological process, shown schematically in Fig. 3, was adequately modelled by fitting to the logistic equations, in which X and D are the biomass and the decolorization or removal degree at a specific moment of the culture time (t), respectively, X_0 and X_{max} are the initial and maximum biomass concentrations, respectively, D_0 and D_{max} are the initial and maximum decolorization percentage, respectively, μ_m is the maximum specific growth rate, and μ_D is the maximum specific decolorization rate.

$$X = \frac{X_{\max}}{1 + e^{[\ln((X_{\max}/X_0)-1)-\mu_m t]}} \quad (1)$$

$$D = \frac{D_{\max}}{1 + e^{[\ln((D_{\max}/D_0)-1)-\mu_D t]}} \quad (2)$$

Adjusting experimental data to models was done by an iterative procedure, based on the Marquardt–Levenberg algorithm, which seeks the values of the parameters that minimise the sum of the squared differences between the observed and predicted values of the dependent variable, using Sigma Plot 8.0 software.

A visual inspection of the results indicates that the decolorization process mainly occurred during the first 12 h of biological treatment. Notably, one of the isolates (*A. flavithermus*) reached 83% decolorization within this short period of time at a temperature of 65 °C. It is clear that these isolates are a promising alternative for decolorization treatment of textile dye effluents, discharged at elevated temperatures, because the typical treatment time required for carrying out the biological process is often longer than 1 day [51]. Barragan et al. [52] studied three bacterial strains (*Enterobacter* sp., *Morganella* sp. and *Pseudomonas* sp.), both separately and in combination. As in our work, they found that none of the strains was capable of growth in a liquid medium using dye as the sole carbon source or supplemented with glucose. Growth only occurred when the liquid medium was supplemented with peptone, yeast extract and urea, as observed here. Indeed, bacterial growth for color removal in a liquid medium usually requires complex organic sources, such as yeast extract, peptone or a combination of complex carbon sources and carbohydrates.

Additionally, from the parameters obtained by fitting to the logistic model, and shown in Table 3, it can be concluded that the maximum specific growth rates (μ_m) varied significantly from one strain to another, and all of them were significantly higher than those obtained by Bedwell and Goulder [53] or Ostrowski et al. [54]. Moreover, the analysis of D_{max} confirmed that all the isolates achieved decolorization percentages higher than 65% in short periods of time.

A new insight into the kinetic behaviour of the biological process is proposed here. The decolorization degree is given as a function of the growth rate and the biomass on the basis of the model reported by Marques et al. [55]. This classic model considered the relationship between cell growth an product formation, and in this case, the product could be any metabolite or enzyme with oxidant/reducing activity triggering the decolorization process.

$$D = D_0 + mX_0 \left\{ \frac{e^{\mu_m t}}{[1.0 - (X_0/X_{\max})(1.0 - e^{\mu_m t})]} - 1.0 \right\} + n \left(\frac{X_{\max}}{\mu_m} \right) \ln \left[1.0 - \left(\frac{X_0}{X_{\max}} \right) (1.0 - e^{\mu_m t}) \right] \quad (3)$$

This algorithm allows us to define the decolorization efficiency as a function of the growth rate ($m = 0$), a function of the biomass ($n = 0$) or a function of both parameters ($m \neq 0$ and $n \neq 0$). The model is suitable for describing the decolorization response by the different microorganisms, as can be concluded from the adequate accuracy of the regression coefficients (higher than 0.90). All the fit-

Table 3
Parameters defining the logistic model that characterises the growth and the decolorization of isolates from Galician thermal springs in flask cultures.

Strain	X_0 (g/L)	X_{max} (g/L)	μ_m (h^{-1})	R^2	D_0 (% Rem.)	D_{max} (% Rem.)	μ_D (h^{-1})	R^2
A. pushchinoensis	0.05	0.84	0.17	0.99	2.05	66.25	0.51	0.99
A. kamchatkensis	0.04	0.59	0.25	0.99	0.49	75.27	0.78	0.99
A. flavithermus	0.06	0.53	0.36	0.97	2.85	83.83	0.59	0.99

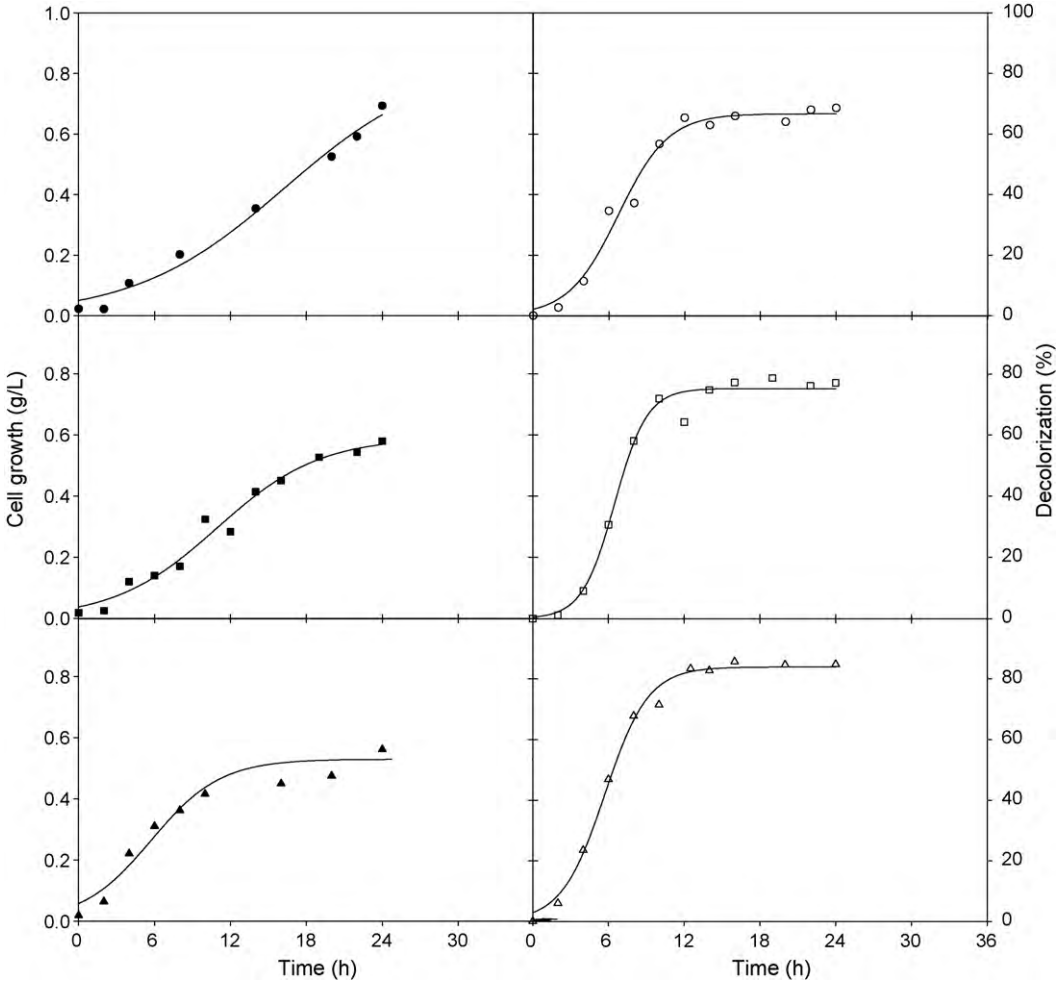


Fig. 3. Cellular growth (full symbols) and dye decolorization (%) (void symbols) in flask cultures: *A. pushchinoensis* (●, ○), *A. kamchatkensis* (■, □), and *A. flavithermus* (▲, △). Symbols: experimental data; solid line: logistic model.

ting parameters are listed in Table 4. Bearing in mind these results, significantly different kinetic behaviours could be concluded for the different strains. It is noteworthy that *A. pushchinoensis* possessed the ability to degrade the azo-dye structure primarily based on the growth rate, whereas *A. kamchatkensis* and *A. flavithermus* showed a clear dependence on biomass production. These findings indicate that kinetic behaviour is a critical issue that must be addressed when a novel strain is studied, and it can be concluded that the decolorization efficiencies provided by *A. kamchatkensis* and *A. flavithermus* are higher at increased levels of cell growth. In agreement with this fact, Silveira et al. [56] reported that, based on SEM analysis, greater biomass production is needed to promote better dye color removal. Thus, there is a need for greater biomass production, as all the biomass produced seemed to be attached to the mineralised dye. Khehra et al. [57] found that the bioaccumulation process is directly linked to biomass production for strains isolated from soil or sludge from textile dye waste.

Table 4
Parameters obtained by regression of the model described in Eq. (3).

Strain	m (% Rem./g)	n (% Rem./g h)	D_0 (% Rem.)	R^2
A. pushchinoensis	0	2.25	0	0.93
A. kamchatkensis	63.20	3.19	7.86	0.90
A. flavithermus	176.25	0	11.75	0.95

3.3. Scaling-up of the decolorization process

The results obtained at the bioreactor scale and plotted in Fig. 4 are in accordance with previous experiments in shake flasks, as the elevated levels of dye degradation are relatively fully maintained. Although the particular hydrodynamic setting in the bioreactor can lead to limitations in the suitable operation ranges, the conditions proposed in this process permit successfully approaching the scaling-up of the biological treatment without significant operational problems.

Table 5
Parameters defining the logistic model that characterises the growth and the decolorization of the isolates from Galician thermal springs in bioreactor cultures.

Strain	X_0 (g/L)	X_{max} (g/L)	μ_m (h^{-1})	R^2	D_0 (% Rem.)	D_{max} (% Rem.)	μ_D (h^{-1})	R^2
A. pushchinoensis	0.05	0.77	0.29	0.98	0.70	43.56	1.34	0.98
A. kamchatkensis	0.04	0.38	1.47	0.99	1.75	75.10	0.82	0.99
A. flavithermus	0.02	0.54	0.55	0.99	6.22	79.83	0.39	0.97

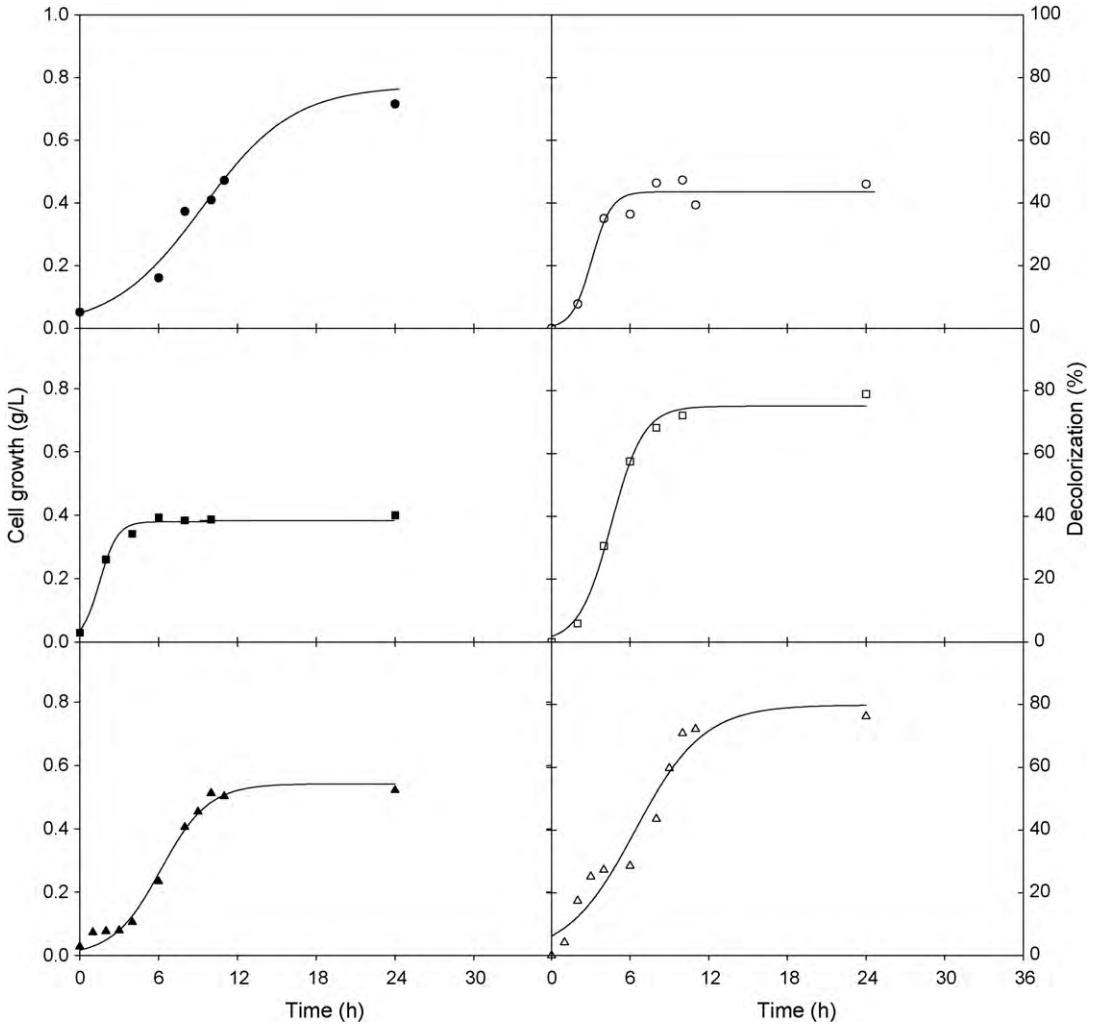


Fig. 4. Cellular growth (full symbols) and dye decolorization (%) (void symbols) in bioreactor cultures: *A. puschinoensis* (●, ○), *A. kamchatkensis* (■, □), and *A. flavithermus* (▲, △). Symbols: experimental data; solid line: logistic model.

The decolorization degree and cell growth were ascertained with the logistic model detailed above. The fitting parameters obtained from the experimental data are listed in Table 5. As shown, the maximum specific growth rate was abruptly increased when operating at bioreactor than in flask cultures, which agrees with the investigations performed by Deive [58]. Additionally, the maximum decolorization attained was quite similar to those yielded in flask cultures, except in the case of *A. pushchinoensis*, where a reduction of 30% was recorded. Furthermore, analysis of the data of biomass and decolorization percentage led us to the hypothesis that the dye was being co-metabolised with the complex organic source (peptone and yeast extract). Two opinions have been argued for many years in relation to the roles of dyes in biological processes: one deems that dyes are not carbon sources, whereas another deems the contrary, and the variability is explained in terms of the different microbial characteristics involved [59,60]. Our results also show that a certain concentration of carbon source (such as yeast extract or peptone) was necessary for the decolorizing process. The effects of some other carbon sources on bacterial decolorization performance have been studied. Lactate, peptone, succinate, yeast extract, and formate enhance decolorization, whereas sucrose and dextrin have lower decolorization activities [61].

In summary, the influence of the operating conditions in a bubble bioreactor together with the characteristics of the microorganisms are determining factors in the development of the biological decolorization process. We conclude that the treatment process in a bench-scale bioreactor proposed in this work has been successfully developed.

4. Conclusions

This study reports a novel decolorization process by thermophilic bacteria isolated from Spanish hot springs in aerobic conditions. The promising results obtained from three isolated thermophilic strains belonging to the *Anoxybacillus* genus (more than 80% decolorization in less than 12 h) led us to develop the process at the bioreactor scale. A deeper examination of the biological-process kinetics allowed us to analyse the influence of operational variables and selected strains on the decolorization percentage, and it also allowed us to classify the decolorization as primarily based on biomass or growth rate.

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