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Microbial diversity in a continuous system based on rice husks for biodegradation of the azo dyes Reactive Red 2 and Reactive Black 5

Jörgen Forss [a,*], Jarone Pinhassi [b], Markus Lindh [b], Ulrika Welander [a]

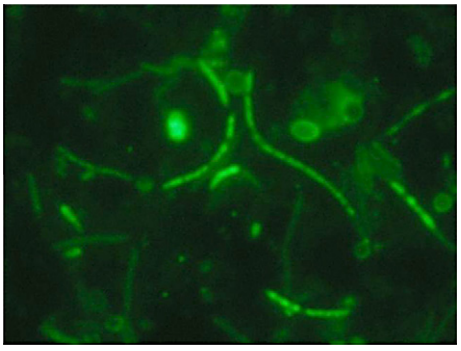
[a] School of Engineering, Linnæus University, SE-351 95 Växjö, Sweden
[b] Centre for Ecology and Evolution in Microbial model Systems, Linnæus University, SE-391 82 Kalmar, Sweden

HIGHLIGHTS

- Efficient degradation of two azo dyes by a cost-effective continuous process.
- Microorganisms analyzed by PCR, DGGE and identified by 16S rRNA sequencing.
- Several interesting species identified some known to produce azoreductase.
- Robust degradation performance over 80%, without any operational interruptions.
- Degradation monitored by LC/MS, no metabolites detected in the treated water.

GRAPHICAL ABSTRACT

Continuous biofilter with rice husks degraded azo dyes in a robust degradation performance over 80%. Molecular fingerprinting analysis (DGGE and 16S rRNA sequencing) revealed a diverse and dynamic bacterial community composition involved. Several of the species identified are known to form biofilm, to produce azoreductase and degrade other organic compounds. Capabilities most wanted for a bacterium to thrive in a biofilter treating textile wastewater. LC/MS analysis of the treated water leaving the system detected no metabolites.



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ABSTRACT

In the present study the degradation of two common azo dyes used in dye houses today, Reactive Black 5 and Reactive Red 2 was evaluated in biofilters. In two experiments, bioreactors performed over 80% decolorization at a hydraulic retention time of only 28.4 h with little production of metabolites. Molecular analyses showed a diverse and dynamic bacterial community composition in the bioreactors, including members of the *Bacteroidetes*, *Acinetobacter* (Gammaproteobacteria) and *Clostridium* (Firmicutes) that possess the capacity to reduce azo dyes. Collectively, the results indicate that the development of mixed bacterial communities from natural biomaterials contributes to an efficient and robust degradation performance in bioreactors even at high concentration of dyes.

1. Introduction

Today, fashion is a way of life, and bright and lasting colors are a vital part of clothing design. To meet this requirement, synthetic

* Corresponding author. Address: School of Engineering, Linnæus University, Vejdes Plats 6, SE-351 95 Växjö, Sweden. Tel.: +46 470 70 82 33; fax: +46 470 70 87 56.
E-mail address: jorgen.forss@lnu.se (J. Forss).

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dyes in a large variety of colors are manufactured. The Color Index International presently lists over 27,000 products (Society of Dyers and Colourists, 2010). Many different types of dyes are used, the most common being azo, anthraquinone and deoxidizing dyes. In 2003, textile dye consumption worldwide was 680,000 tons, representing a turnover value of 4.49 billion Euros (Sun FAITH China Ltd., 2004).

Dyes and metabolites from degraded dyes frequently have substantial negative impacts on the environment. Azo dyes can, for example, be degraded to aromatic amines, some of which have carcinogenic effects (Neumann, 2010). Consequently, the distribution of products containing azo dyes that can lead to any of 22 specified aromatic amines known to have carcinogenic effects is forbidden in the EU (EU-Commission, 2002). Also, a growing trend to produce organic and ecological clothing is placing new demands on manufacturing. In many developing countries, the increase in population and decrease in water resources are rendering clean water a valuable resource, as well as increasing the necessity for supervision of water-demanding industries. The textile industry uses substantial quantities of water that contain salt, fats, detergents, and dyes, when released. Many textile industries are located in growing economies, and it is therefore crucial to work with cost-efficient methods that can be implemented. One such method is adsorption, where cellulosic materials have proven their capability in adsorbing different dyes. Mondal (2008) summarizes the adsorption capability of some cellulosic material for a handful of different dyes, and suggests about 200–500 mg of dye g^{-1} of material. However, adsorption creates new waste fractions that require disposal. If burned, a minimum temperature of 800 °C is needed to completely oxidize the organic molecules. Chemical degradation leads to operational costs for the chemicals. Treatment with various filters (ultrafiltration, nanofiltration, and reverse osmosis) is very attractive, because these allow for the water to possibly be reused. However, the investment and operational costs are substantial. Biodegradation is a cost-efficient method that requires low investment and low operational costs. Nevertheless, there are difficulties in degrading azo dyes by traditional activated sludge treatment (Shaul et al., 1991). Although some aerobic bacteria can degrade azo dyes, anaerobic bacteria seem to be more efficient in breaking the azo bond; yet, no single bacterium has proven its ability to degrade a broad range of dyes (Dafale et al., 2010). Instead, it is expected today that a consortium of different species would be the best agent to degrade textile dyes. A similar consortium was used in earlier studies (Forss and Welander, 2011), however with no insights in the bacterial community involved. Thus, a critical challenge in the future is to investigate how bioreactors can be tuned so that microbial communities develop with a species composition that accomplishes efficient textile industry wastewater treatment.

Studies of microbial community composition using molecular biology techniques have been vital in developing efficient practices for sewage treatments and methanogenic processes, and have contributed considerably to our understanding of these processes (Ladygina et al., 2006; Levén et al., 2007; Sousa et al., 2008). In other areas, such as the degradation of dyes, knowledge of degradation routes and the microbial species involved is scarce. For example, Bafana et al. (2008) found that activated sludge from the treatment plant of a textile factory was dominated by Gammaproteobacteria and Deltaproteobacteria, though representatives of *Bacteroidetes*, Firmicutes and *Acinetobacteria* were also identified. Di Gioia (2008) used the microbial assemblage from an oxidation tank in a textile wastewater pretreatment plant to degrade 4-nonylphenol, with different Alpha-, Beta-, and Gammaproteobacteria being the most abundant among the isolated bacteria. The salt tolerant bacteria *Exiguobacterium sp.* TL and its impact on degradation of the dye Brilliant Scarlet GR were studied by Zhou et al. (2009). Interestingly, the process was bioaugmented with active sludge. Yang et al. (2012) identified several interesting species including *Clostridium sp.* in a combined anaerobic and aerobic treatment of printing and dyeing wastewater.

The present study investigates the biological degradation of the two azo dyes Reactive Black 5 and Reactive Red 2 by mixed microbial communities growing on lignocellulosic material. Two of the most common azo dyes used in dye houses today were selected. The objective of our study was two-fold: to quantify the degradation of the dyes and their metabolites and to determine how the community composition of the microflora changed along with the degradation process.

2. Methods

2.1. Chemicals

The dyes used in the experiments were Reactive Black 5 (CAS 17095–24–8) lambda max 597 nm and Reactive Red 2 (CAS 17804–49–8) lambda max 538 nm, their combined lambda max is 550 nm. The dyes were purchased from Sigma–Aldrich (Milwaukee, WI, USA) and yeast extract (no. 212750) from Difco (Detroit, MI, USA). The artificial wastewater (Ww I) contained 200 mg Reactive Black 5, 200 mg Reactive Red 2, and 1 g of yeast extract dissolved in 1 l of tap water, sterilized in an autoclave at 125 °C for 15 min. Supply flasks and tubing were checked for microbial growth daily. The high-quality tap water in Sweden is approved as drinking water and was here used to supply trace elements of metal.

2.2. System design and microorganisms

In all of the experiments, rice husks were used as support and a source of carbon for the microorganisms. The dry rice husks were originally from Bolivia. Ang et al. (2012) measured the surface area, pore volume and pore size of rice husks. The surface area reported was 21.2 $m^2\ g^{-1}$, the pore volume was 1.76×10^{-2} cc g^{-1} and the pore size 33.2 Å.

Experiments were performed at 20 °C. Experiment 1 with reactors 1 and 2 (R1/R2) and experiment 2 with reactors 3 and 4 (R3/R4) each had two reactors 300 mm height and 50 mm in diameter with a volume of 449 ml. The free water volume was 300 ml in each reactor. All reactors in the continuous systems had a drop inlet at the top and an outlet with ports for sampling at the bottom. The designation B is used for a background check of rice husks. The experiments' composition is shown in Table 1. A Watson Marlow pump was set at 5 rpm, producing a flow of 0.35 ml min^{-1} (21.1 ml h^{-1}). The hydraulic retention time in one reactor was 14.2 h. Steady-state condition was observed after the experiments had run for three weeks. In experiment 1 and the background experiment (B1/B2), the indigenous microflora inhabiting the rice husks was used as a source of microorganisms. In experiment 2, rice husks were used as a base and additional microorganisms were inoculated with rinse water from forest residues. Approximately 50 g of wood chips from forest residues were rinsed with a 0.9% sodium chloride solution during stirring for half an hour. After 15 min of sedimentation, the upper phase of the rinse water was used as inoculum for microorganisms in experiment 2. The forest residues were collected from the VEAB combined heat and power plant in Växjö, Sweden.

2.3. Chemical analyses

All samples were filtered through 0.45 μm filters (Sartorius AG, Goettingen, Germany). The samples were scanned from 190 nm to