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Monitoring of azo dye degradation processes in a bioreactor by on-line high-performance liquid chromatography

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Abstract

A technical solution and development of a method for on-line HPLC monitoring of bioreactor processes in a membrane reactor system are presented. Experiences in system design for the continuous coupling of a bioreactor system with capillary by-pass circuits using membrane flow cells and a dual HPLC system are reported. A continuously working integrated sample purification step by ultrafiltration with the membrane cell coupling is established. Using electrical switching valves and separated pumping and eluent systems, the dual HPLC system allows diode array detection as well as measurement of the refractive index. The application of the on-line HPLC monitoring system is demonstrated by measuring the anaerobic H-acid degradation kinetics. H-acid, 1-amino-8-hydroxynaphthalene-3,6-disulfonic acid, is one of the most important coupling components for a variety of direct, mordant, reactive dyes which remains in the process water and the textile dyeing effluents in high concentration.

Keywords: Process monitoring; Azo dyes; H-acid; Aminohydroxynaphthalenedisulfonic acid; Organic acids; Organosulfur compounds

1. Introduction

Worldwide, the problem of complete, irreversible decolorisation and detoxification of textile dyeing effluents is not well resolved [1–3]. It is known that large amounts of reactive dyes entering activated sludge sewage treatment plants will pass through unchanged and will be discharged into the environment together with their precursors and potentially carcinogenic degradation products such as aromatic amines [3]. Part of the problem is the lack of on-line analytical methods for direct control of aerobic and anaerobic degradation processes and mechanistic studies without distortions by fast consecutive thermal reactions or reactions with oxygen. Various stable approaches more or less suited for on-line monitoring have been tested recently [4–7].

High-performance liquid chromatographic (HPLC) analytical techniques are well-established laboratory methods. For process control in biotechnological processes indirect integral parameters such as pH, redox potential, bulk absorbances (for 436, 525 and 620 nm), total organic carbon (TOC) and adsorbable organic halogen (AOX) are normally used [2]. They are often not sufficient to describe the complex nature of process and waste water from dyestuff production. In addition, staining processes which have fluctuating compositions are rich in salt and contain high concentrations of by-products with up to 45% dyestuffs and their precursors such as H-acid [8]. The so-called H-acid, 1-amino-8-hy-

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droxynaphthalene-3,6-disulfonic acid, is one of the most important coupling components for a variety of direct, mordant and reactive azo dyes, that can couple in both acidic and alkaline environment [9,10]. It is used for example in the production of reactive azo dyes such as Reactive Black 5 and various Reactive Red dyestuffs.

The use of selective chromatographic separation techniques is necessary for the identification and quantification of individual substances, especially for the detection of toxic degradation products such as aromatic amines.

For the investigation of anaerobic degradation processes, on-line HPLC technique has been chosen in order to avoid distortion of the results of the bioreactor process due to thermal reverse reactions, consecutive reactions and reactions with oxygen [8,11].

2. Experimental

2.1. Chemicals, biomass and membranes

HPLC-grade LiChrosolv water and LiChrosolv gradient grade acetonitrile were obtained from Merck (Darmstadt, Germany). The ion pairing agent tetrabutylammonium hydrogensulphate (TBAHS) was purchased as puriss. (>99%) quality from Fluka (Buchs, Switzerland). TBAHS containing eluents were prepared 1 day before use and degassed by ultrasound. H-acid, disodium salt, was obtained from Aldrich (Steinheim, Germany). In the bioreactor H-acid was used in concentrations of 0.25 g/l dissolved in water.

Anaerobic–aerobic bacterial sludge for the bioreactor was obtained from a local municipal sewage treatment plant and was conditioned for several days and fed with glucose as a co-substrate so that the bacterial mass was kept constant.

In the membrane cells (F1 and F2 in Fig. 1) an ultrafiltration membrane Desal GH from Osmonics (Moers, Germany) with a molecular mass cut-off of 2500 has been used after preconditioning with methanol (5 min) or water (6 h) in order to separate the reactor efflux.

2.2. Equipment

The bioreactor system consisted of two parts: a 4-l heatable anaerobic glass reactor with nitrogen degassing (Fig. 1, B1) and a 3-l aerobic glass reactor (Fig. 1, B2). The thermal conditioning of the anaerobic reactor was controlled by a heat exchanger (Fig. 1, W1). The two reactor steps were connected by membrane cells (Fig. 1, F1 and F2) for retention of the biomass and large molecules and also for supplying the reactor efflux for the on-line HPLC analysis by integrated sample preparation. The anaerobic reactor content was continuously pumped (Fig. 1, P1) over the membrane cells (F1 and F2) in a cross flow mode. F1 and F2 could utilise different membranes, an ultrafiltration and a nanofiltration membrane for instance, to produce different permeates.

Using other connections the membrane device could also be used for on-line sampling of the aerobic reactor. However, we were primarily interested in an integrated anaerobic sample preparation where oxygen is excluded.

The permeate was recirculated into B1 or B2 (Fig. 1) and split into a 56-m by-pass circuit for the HPLC system remote from the technical laboratory, in practice, on another floor. The by-pass system was totally composed of polyether ether ketone (PEEK) tubings (1/16 in.; 1 in.=2.54 cm). A 4-ml PTFE safety cell (B3 and B4 in Fig. 1) with a HPLC filter frit (F3 and F4 in Fig. 1) was placed between the ultrafiltration membrane cell (F1 and F2 in Fig. 1) and the by-pass circuit pump (P3 in Fig. 1) in order to buffer volume stream fluctuations of the ultrafiltration membrane cell and also to collect gas bubbles before entering the by-pass circuit and the HPLC system.

LC analyses were performed with a dual HPLC system consisting of a Merck–Hitachi and a SFD Schambeck system. The Merck–Hitachi system was a LaChrom system (Darmstadt,Germany) and consisted of the following: a low pressure gradient pump L-7100, electrical six-way switching valve with 20-μl injection loop (on-line injection), autosampler L-7250 (off-line injection), column oven L-7450, diode-array detector L-7450A and an interface L-7000 for system control and data processing with the HSM software D-7000 under Windows NT 4.0. The

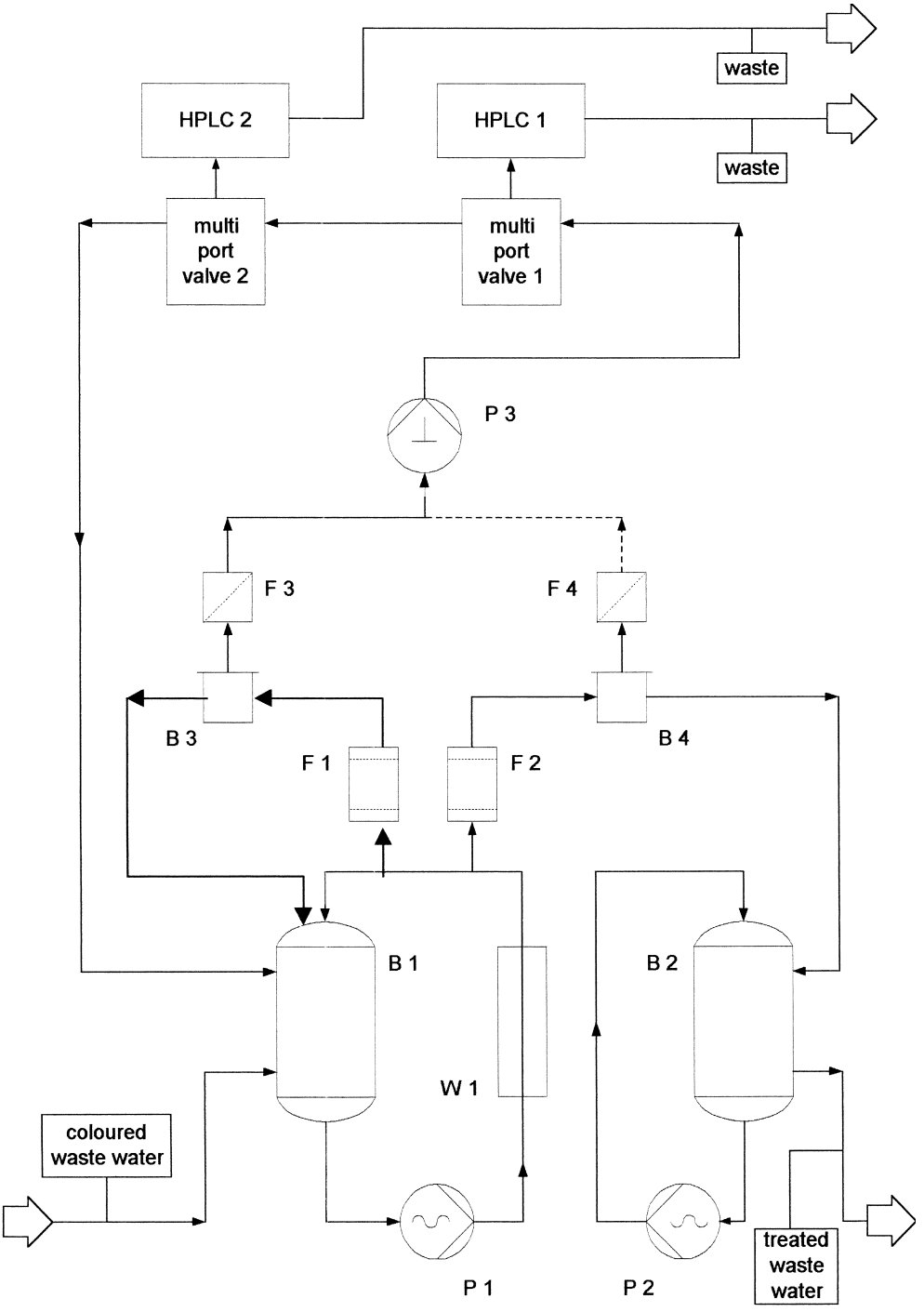

Fig. 1. Process and instrument flow sheet diagram: P1, P2, pumps; W1, heat exchanger, B1, B2, glass reactors; F1, F2, membrane cells B3, B4, safety PTFE cells; F3, F4, HPLC filter frits.

SFD Schambeck HPLC system (Bad Honnef, Germany) consisted of a low-pressure gradient pump SF 2100, an electrical six-way switching valve with 20-μl injection loop (on-line injection), a manual injection valve (off-line injection) and a refractive index detector RI 2000. All HPLC modules were connected by stainless steel or PEEK tubing with the lowest possible dead volumes.

The liquid in the by-pass circuit was transported by an isocratic pump (0.1–10 ml) from Knauer (Berlin, Germany) (P3 in Fig. 1) and continuously flushed by reactor fluid or water.

2.3. Column, stationary phase and LC conditions

The analytical column was a 125×4 mm LiChroCart HPLC cartridge with the reversed-phase material LiChrospher 60 RP-select B (5 μm particle size) from Merck. For the cartridges ManuCart column fittings for 4-mm inner diameter was used.

For azo dyes and their precursors, or degradation products, we developed a general gradient which gives a good resolution for quite a broad range of substances of different polarity and acidity: a mixture of acetonitrile–water with 6 m*M* TBAHS (10:90, v/v) was used as eluent A against pure acetonitrile as eluent B. From 0 to 25 min the gradient was run with a 2%/min slope for B. Then the acetonitrile content was increased by 10%/min for 4 min and kept at 90% for 4 min. Within 2 min a re-equilibration to 100% eluent A (10% acetonitrile) was completed. The whole run took 35 min and had to be carried out at constant temperature (40°C). Retention time variations were kept at less than 2% by the above thermostatting. Wavelengths were chosen between 200 and 800 nm in order to obtain spectra. H-acid was detected at 215 nm.

3. Results and discussions

An anaerobic–aerobic bioreactor was connected to a dual HPLC system by a 56-m by-pass circuit via a membrane filtration cell and a volume stream buffer security cell (Fig. 1). With this coupling an integrated sample purification step by ultrafiltration was tested. Using electrical switching valves and separated pumping and eluent systems, the HPLC system allowed diode array detection as well as measurement of the refractive index for substrate measurements like glucose. Care was taken to avoid contamination of refractive index system and the bioreactor by the ion-pairing reagent of the diode array system. This is because injections into both detection systems were carried out in parallel at the same time. For one bioreactor run the described analytical on-line system worked automatically for more than 100 h without any disturbances such as memory peaks, pressure increase or clogging. A more classical on-line HPLC approach via autosampling equipment, which can carry out pipetting, filtration and dilution of a sample [6] could not be used in our case because of the large amount of solid particles suspended in the bioreactor liquid. On-line immunoextraction precolumns coupled to HPLC analysis [4] could also not be used because of the complex bioreactor matrix. Besides the ultrafiltration it is important to flush the by-pass capillaries continuously at 0.3–1.0 ml/min of reactor efflux to give a quick exchange between reactor liquid and by-pass circuit and also to prevent precipitation of elemental sulfur. In addition to high sulphate concentrations in the reactor, in combination with hydrogen sulfide, which occurs under anaerobic conditions, the aggregation of traces of dissolved colloidal sulfur could be prevented in this manner as long as air and light were excluded from the sample liquids.

The kinetics of anaerobic degradation of H-acid were studied by this on-line set-up in the presence of microbiological sludge and textile waste water. Fig. 2 shows a typical chromatogram of H-acid in the ultrafiltration permeate. The selected membrane, DESAL GH with a molecular mass cut-off of 2500 retains undegraded azo dyes very well. This is a function of molecular mass but also of size of solvatation sphere which is related to the charge of the molecules.

Without biomass and textile waste water, the chromatogram of a clean HPLC system shows only one main peak of H-acid.

A concentration of H-acid 95% less than the initial concentration of 0.25 g/l was measured in the ultrafiltration permeate from the anaerobic bioreactor that had been purged with nitrogen (Fig. 3). This is not only due to anaerobic degradation. The ultrafiltration membrane turned out to be permeable for

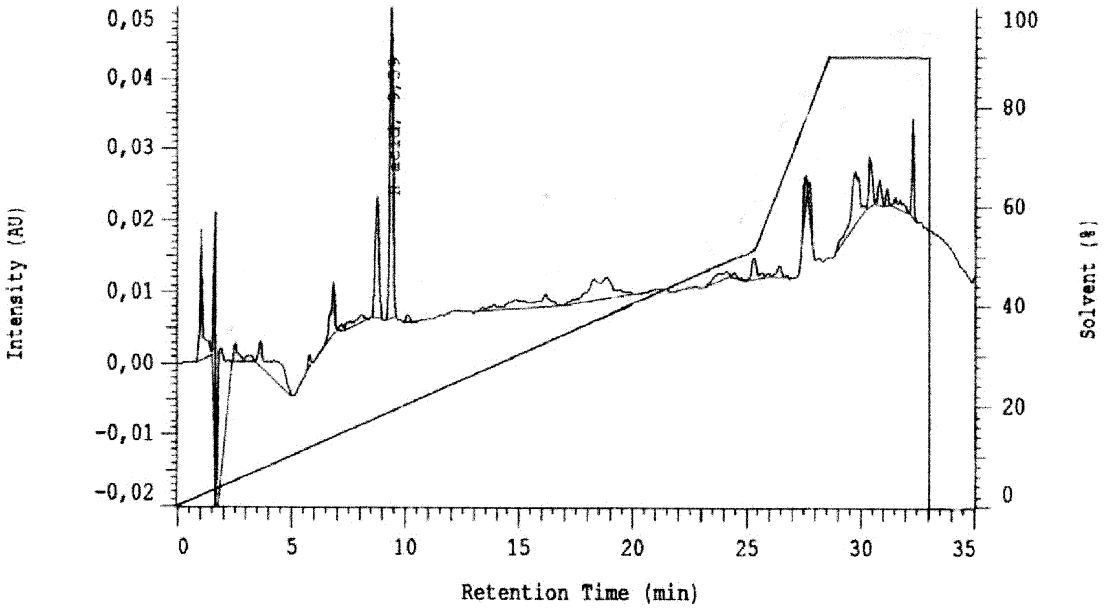

Fig. 2. Chromatogram of H-acid in the ultrafiltration permeate from the anaerobic reactor: injection volume 20 μl, retention time 9.39 min, concentration 0.02 g/l of original 0.25 g/l after 11 h biomass treatment.

only 10% of the H-acid in spite of the much higher membrane exclusion limit. This was proven by using the bioreactor without biomass. Without biomass there was no further removal of H-acid after establishing a 10% permeation (Fig. 3). With biomass about the half of this 10% has been degraded (Fig. 3), presumably microbiologically. Adsorption steps are assumed to be involved in microbiological degradation processes [10] but they are not likely to be responsible alone for the decrease of H-acid in that time scale.

The on-line measurements gave standard deviations of about 5–20% while off-line investigations did not give reproducible results. Standard deviations

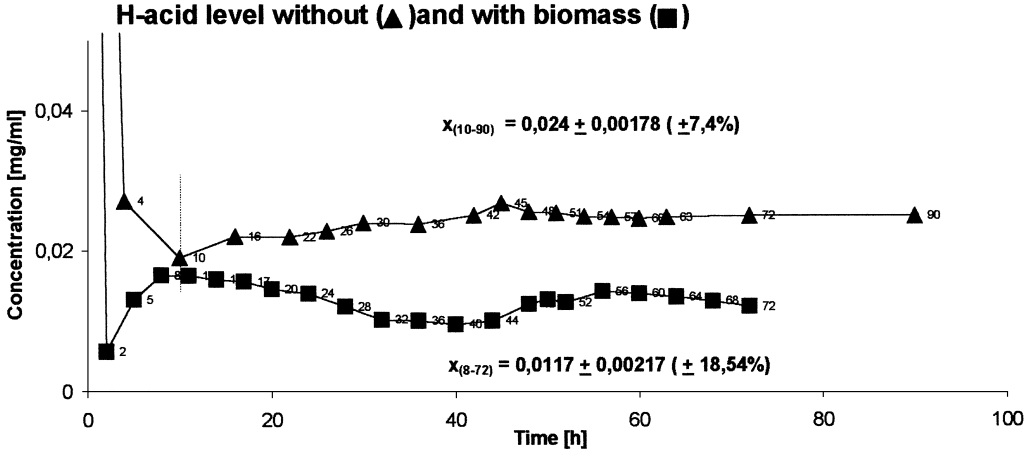

Fig. 3. Concentration of H-acid in the ultrafiltration permeate from the anaerobic bioreactor with and without biomass.

were higher than 200% because of sample alterations by subsequent thermal reactions or reactions with oxygen.

The on-line sampling system has a dead volume of about 1 ml for capillaries and about 4.5 ml for the safety cell. Every 35 min analyses can be done so that the approach fits to kinetic studies in real-time as long as changes are expected in the time scale of hours and days. This is the usual case for the slower biodegradation processes of textile industry process and waste water. For faster fermentation processes and on-line monitoring of process HPLC production methods quick sensors can be used [5].

4. Conclusions

The objective of this study was to evaluate the performance of an on-line HPLC method with ultrafiltration and by-pass coupling for the detection of azo dyes and selected precursors of these dyestuffs for monitoring their biological anaerobic degradation in waste waters of the textile industry. Experiences in system design for the continuous coupling of a bioreactor-membrane system with a dual HPLC system are illustrated for automatic analysis over several days.

The industrial drivers of productivity, quality and environmental impact have pushed technical developments of process analytical chemistry [12]. The studies are an example for a successful HPLC process monitoring. They emphasise the need for further investigations with respect to identification of more suitable membranes with favourable permeation and selectivity for on-line analysis. With increasing separation and identification strength of the chromatographic system they also emphasise the use of open in-line filter probes without retention for any dissolved molecules.

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