

Biominingalisation of azo dyes and their breakdown products in anaerobic-aerobic hybrid and UASB reactors

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Abstract Batch toxicity and biodegradability of two azo dyes (Siriusgelb and Siriuslichtbraun) has been investigated. It was found that the former azo dye was significantly less toxic to methanogenic sludge than the latter one (IC_{50} are equal to 3.55 and 0.41 g COD/l, respectively). Neither of the azo dyes was biodegradable under aerobic conditions but both dyes were readily decolourised and slowly mineralised in anaerobic environments. In order to optimise the treatment strategy, the anaerobic and aerobic phases were combined into one single unit called the anaerobic-aerobic hybrid reactor. The performance of this innovative reactor was tested with a synthetic wastewater containing Siriusgelb and ethanol at 30°C and 56% removal of azo dye COD was achieved at volumetric load of 0.3 g azo dye COD/l/day. The effluent COD content could be attributed to the presence of non-biodegradable autooxidation products of Siriusgelb breakdown intermediates. A continuous biominingalisation of 2-aminobenzoic acid (2-ABA) – intermediate of the anaerobic decomposition of Siriuslichtbraun – was studied in a UASB reactor at 30°C. A high (>90%) removal of 2-ABA was achieved under volumetric loads of 1.5 g 2-ABA COD/l/day. However, a further increase in volumetric load led to a decrease in 2-ABA removal, probably due to low attachment ability of the bacteria responsible for primary decomposition of 2-ABA.

Keywords 2-aminobenzoic acids; 5-aminosalicylic acids; anaerobic-aerobic hybrid reactor, Siriusgelb; Siriuslichtbraun, UASB reactor

Introduction

Azo dyes represent a major group of all the dyes produced world-wide (Carliell *et al.*, 1995). Approximately 10–15% of overall production is released into the environment mainly *via* wastewater (Tan *et al.*, 1999). This is very dangerous because some of the azo dyes or their breakdown products have a strong toxic, mutagenic or carcinogenic influence on the living organisms; therefore, the corresponding wastewaters should be treated before discharge. However, a majority of azo dyes are quite resistant to biodegradation under aerobic conditions and easily pass through conventional aerobic wastewater treatment systems. On the other hand, azo dyes are readily decolourised by splitting the azo bond(s) in anaerobic environments. In turn, the anaerobic breakdown products are more susceptible to biodegradation under aerobic conditions rather than under anaerobic conditions. These properties of azo dyes dictate the anaerobic-aerobic sequence in designing an efficient biominingalisation process. Two separate treatment steps are usually applied for this purpose. In order to optimise the treatment process and overall economics of the corresponding technology, we combined the anaerobic and aerobic phases into one single unit called the anaerobic-aerobic hybrid reactor (AnAHR) in this study. The advantages of this innovative design include reduced aeration costs and lower space requirements while offering substantial mitigation of a broad spectrum of recalcitrant xenobiotic contaminants (not only azo dyes) found in industrial wastewaters. The performance of the mesophilic (30°C) AnAHR was tested using the azo dye Siriusgelb and ethanol as donor of reductive equivalents. This was the first objective of this work. The second objective of the paper was to gain more insight into the continuous methanogenic degradation of one of the anaerobic breakdown products of azo dye Siriuslichtbraun – 2-aminobenzoic acid (2-ABA) – using a UASB reactor.

Materials and method

Azo dyes and their suspected breakdown products. Two commercially produced azo dyes (Siriusgelb GG, $C_{27}H_{18}O_7N_6Na_2$, MW=584 and Siriuslichtbraun 6RLL, $C_{35}H_{22}O_{13}N_6S_2Na_2$ MW=840) were delivered by the company “Pigment” (Tambov, Russia). The dye structural formulas are presented in Figure 1. 5-aminosalicylic acid (5-ASA) was purchased from Sigma-Aldrich (USA), and 2-ABA and 1,4-phenylenediamine (1,4-PDA) were purchased from Reakhim (Russia). All these compounds were of the highest purity available and were used without further purification.

Sludges. Mesophilic sludge from a lab-scale UASB reactor treating vinasse wastewater (VSS concentration – 22 g/l; specific acetoclastic activity – 0.5 g COD/g VSS/day) was used for the batch experiments with azo dyes and their breakdown products. In the reactor experiments, this sludge was bioaugmented with the 5% addition of 5-ASA- and 2-ABA-adapted sludges (Kalyuzhnyi *et al.*, 1999) to the AnAHR and UASB reactor, respectively. Secondary sludge from Kur’yanovskaya municipal aeration station (Moscow) was used for aerobic batch experiments and as a seed sludge for development of attached biofilm in the AnAHR.

Mineral media. The basal N-supplemented medium contained (mg/l): NH_4Cl –280, $CaCl_2 \cdot 2H_2O$ –10, K_2HPO_4 –250, $MgSO_4 \cdot 7H_2O$ –100, EDTA–1, resazurin–0.2, $NaHCO_3$ –5000, H_3BO_3 –0.05, $FeCl_3 \cdot 4H_2O$ –2, $ZnCl_2$ –0.05, $MnCl_2 \cdot 4H_2O$ –0.05, $CuCl_2 \cdot 2H_2O$ –0.03, $AlCl_3 \cdot 6H_2O$ –2, $NiCl_2 \cdot 6H_2O$ –0.05, $Na_2SeO_3 \cdot 5H_2O$ –0.1; yeast extract–100; pH 7.2. The N-deprived medium contained the same components, except ammonium chloride was omitted. The micronutrients and vitamins solutions prepared as described by Razo-Flores (1997) were added to the media.

Batch experiments. The anaerobic batch assays were carried out at 30°C in serum bottles (0.12 l) as described elsewhere (Kalyuzhnyi *et al.*, 1999; Sklyar *et al.*, 1999). The aerobic batch assays were performed at 30°C in shake flasks (0.5 l) using a thermostatic rotary shaker “Lab-Shaker” (Switzerland, Basel, Adolf Kuhner AG) at 130 rpm. The final volume of the liquid phase in aerobic assays was 100 ml with the concentration of aerobic sludge at 1 g VSS/l. All the batch experiments were carried out in two or three replicates and the points shown in the graphs are the means of these replicates.

Reactors. The AnAHR made from transparent plastics consisted of an anaerobic lower compartment within which a dense bed of anaerobic granular or flocculent sludge was cultivated and retained (diameter – 6.8 cm, height – 85 cm, working volume – 2.6 l). A smaller aerated section (diameter – 6.5 cm, height – 23.5 cm, working volume – 1 l) was placed above the anaerobic compartment. The upper section was randomly packed with Rashig rings (2.5×2.5 cm) for development of attached aerobic biofilm. Air was continuously pumped to this section with a flow rate of 2 ml/min. The AnAHR was equipped with nine sampling ports along the reactor height. The overall experimental set-up for biomineralisation of azo dyes is depicted in Figure 2. The laboratory UASB reactor (diameter – 6.8 cm,

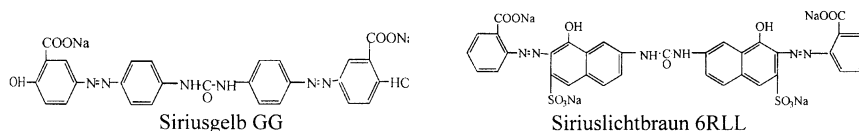


Figure 1 The structural formulas of azo dyes used

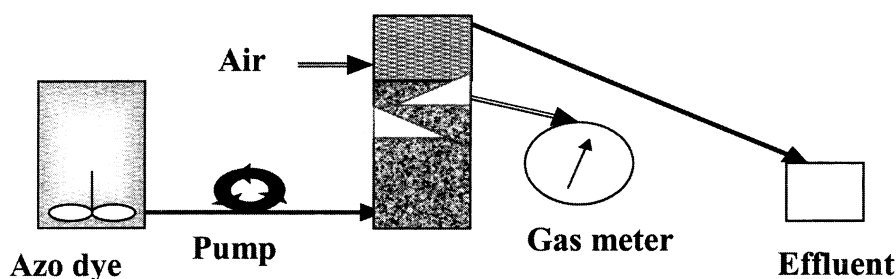


Figure 2 The experimental set-up for biomineralisation of azo dyes in the AnAHR

height – 85 cm, total working volume – 2.5 l) also made from transparent plastics and equipped with six sampling ports along the reactor height was used for continuous mineralisation of 2-ABA. The operating temperature of $30 \pm 1^\circ\text{C}$ was maintained by placing both the reactors into a “TS-80” thermostat (Mashzavod, Odessa, USSR). Feeding of the reactors was provided by a “P-3” peristaltic pump (Pharmacia, Sweden).

Assessment of sludge kinetic parameters in reactor conditions. For *in situ* determination of sludge kinetic characteristics, the UASB reactor was temporarily operated in batch-mode. Before starting the experiments, the reactor was not fed (but flow was maintained with effluent recycle) for 1–2 days in order to deplete all remaining biodegradable COD. At time zero, the concentration of 2-ABA was set to 1.4 g COD/l and its depletion was monitored. The substrate depletion data were fitted to the integrated Michaelis-Menten equation using non-linear least-squares analysis (Kalyuzhnyi *et al.*, 2000).

Analyses. Gas composition, ethanol and volatile fatty acids (VFA) were analysed by gas chromatography (Sklyar *et al.*, 1999). Azo dyes and their breakdown products were quantified by their maximal VIS- and UV-absorbance at 506 (Siriuslichtbraun), 375 (Siriusgelb), 330 (5-ASA), 310 (2-ABA) and 302 (1,4-PDA) nm using a Shimadzu UV-1202 spectrophotometer (Japan). Ammonia concentration was monitored spectrophotometrically with Nessler reagent at 425 nm (Kalyuzhnyi *et al.*, 1999). Biogas production from the reactors was recorded by a wet gas meter “GSB-400” (Gaspribor, USSR). All gas measurements are expressed at 0°C and standard pressure (760 mm Hg). Feed input in reactors was monitored by measuring the accumulated outflow on a daily basis. All other analyses were performed as described previously (Kalyuzhnyi *et al.*, 1999).

Results and discussion

Batch experiments with azo dyes

As some azo dyes can exert an inhibitory effect on bacteria (Razo-Flores, 1997), toxicity assays with Siriusgelb and Siriuslichtbraun were undertaken with regard to acetoclastic methanogenic activity of anaerobic sludge. The results are shown in Figure 3, from which one can conclude that Siriusgelb (IC_{50} is equal to 3.55 g COD/l) is significantly less toxic for methanogenic sludge than Siriuslichtbraun (IC_{50} is equal to 0.41 g COD/l). It is likely that the presence of the sulphonated groups in Siriuslichtbraun (Figure 1) has a more detrimental influence on the sludge acetoclastic activity. An increase of acetoclastic activity at low concentrations of Siriusgelb (Figure 3a) can be explained by the presence of 5-ASA (anaerobic breakdown product of this azo dye), which was shown to have a stimulating effect on acetoclastic methanogenesis (Kalyuzhnyi *et al.*, 1999).

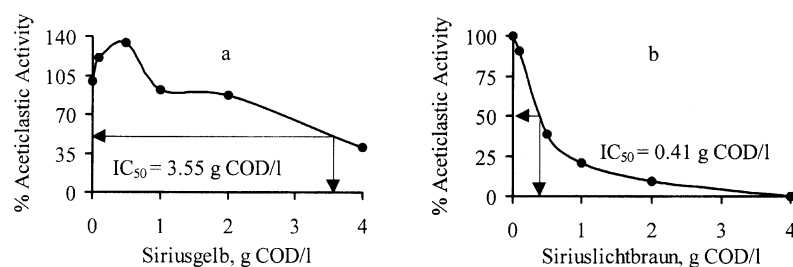


Figure 3 Influence of concentrations of tested azo dyes on acetoclastic methanogenic activity of anaerobic sludge (calculated from the slope of the steepest linear segment of cumulative methane concentration curve, initial acetate concentration – 2.1 g COD/l; sludge concentration of 1 g VSS/l; the activity of the sludge not treated with an azo dye was taken as 100%)

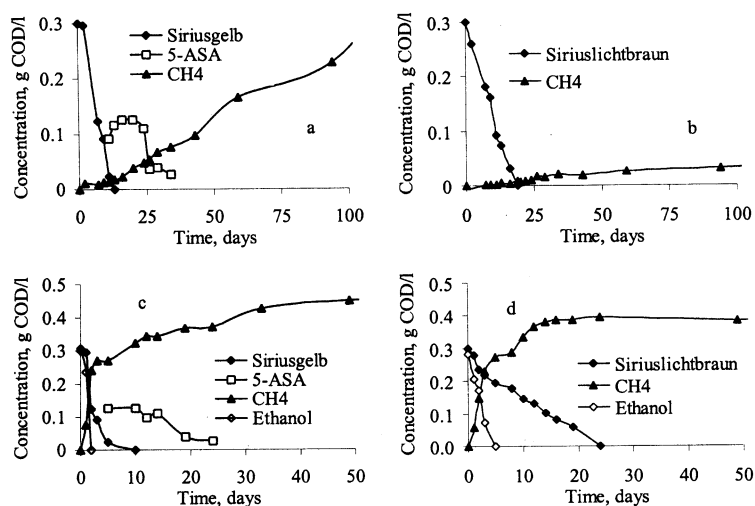


Figure 4 Degradation patterns of Siriusgelb and Siriuslichtbraun in the absence (a and b) and presence (c and d) of ethanol (sludge concentration of 1 g VSS/l). Net methane production was calculated by subtracting background methane production in the controls (without any exogenous substrate) from that in the test vials

With regard to biodegradability, it was found that neither of the azo dyes tested was degradable under aerobic conditions (duration of incubation with intensive shaking was seven days) but both dye were readily decolourised and even slowly mineralised in anaerobic environments (Figures 4a–b). Addition of ethanol as a donor of reductive equivalents practically did not enhance the decolouration rates of either azo dye but seemed to stimulate anaerobic degradation of Siriuslichtbraun (Figures 4c–d). However, independently on the presence or absence of ethanol, the decolouration rate of Siriusgelb was higher than that of Siriuslichtbraun. The slower rate of decomposition of ethanol in the presence of Siriuslichtbraun (Figure 4d) compared to Siriusgelb (Figure 4c) is in accordance with the high toxicity of the former dye towards anaerobic sludge (Figure 3b).

Spectral scans of Siriusgelb digestion liquors revealed a transient increase of optical density at 330 nm (days 5–15, Figures 4a and c). That can be attributed to intermediate accumulation of 5-ASA, which has an absorbance maximum exactly at this wavelength. A subsequent gradual decrease of 330 nm absorbance accompanied by methane accumulation (days 16–30, Figures 4a and c) is consistent with the decomposition of 5-ASA. This is in agreement with recent findings that this compound is completely biodegradable under methanogenic conditions (Razo-Flores, 1997; Kalyuzhnyi *et al.*, 1999). The other suspected product of anaerobic Siriusgelb decomposition (Figure 1) – 1,4-PDA – also seems to be slowly degraded under these conditions, because the methane and ammonia recoveries

approached the theoretically expected ones (Figure 4a). However, our batch assays with only 1,4-PDA using the same sludge did not show any methane production during the incubation period, which was as long as four months. Razo-Flores (1997) also did not find any conversion of 1,4-PDA under methanogenic conditions throughout his batch and continuous experiments. Therefore, the issue of methanogenic biodegradability of 1,4-PDA needs further study.

Similarly, spectral scans of Siriuslichtbraun digestion liquors showed an absorbance increase in the vicinity of 310 nm during days 15–40 (data not shown). This can be attributed to the formation of 2-ABA having an absorbance maximum at this wavelength. However, the quantification of 2-ABA concentrations from spectral data was masked by the presence of Siriuslichtbraun, which has a second absorbance maximum in this range (at 306 nm). A subsequent gradual decrease of 310 nm absorbance (data not shown) was accompanied by methane production (Figures 4b and d) though both these processes were rather slow in the absence of ethanol. Thus, methane formation in the Siriuslichtbraun system likely originated from decomposition of 2-ABA because complete anaerobic biodegradation of the latter compound has been shown in many studies (Razo-Flores, 1997; Kalyuzhnyi *et al.*, 1999). The other suspected product of anaerobic Siriuslichtbraun breakdown (Figure 1) – sulphonated aminoxy-naphthalene – does not seem to be biodegradable under the conditions imposed.

Continuous biomineralisation of Siriusgelb in an AnAHR

Though anaerobic biomineralisation of Siriusgelb occurs under anaerobic conditions, it proceeds rather slowly. To enhance this process, the AnAHR was applied and its operational performance was investigated using a synthetic wastewater containing Siriusgelb (0.3 g COD/l) and ethanol (0.82 g COD/l) as co-substrate (Figure 5). It should be noted that throughout the entire experimental run, only traces (if any) of ethanol and acetate (very rarely) were detected in the upper part of the anaerobic zone of the AnAHR. This suggests that the conversion of ethanol to methane was already complete in this zone and the measured COD content of the samples taken from the upper part of the anaerobic compartment as well as those of the AnAHR effluent represented only non-degraded azo dye and its breakdown products.

During the first 18 days, when the azo dye loading rate (ADLR) was 0.09 g COD/l/day using a HRT (hydraulic retention time) of approximately 3.4 days (Figure 5a), azo dye treatment efficiency (TE) in the anaerobic compartment was 51% and the overall TE of the AnAHR was 71% (Figure 5b). After an increase of ADLR to an average value of 0.18 g

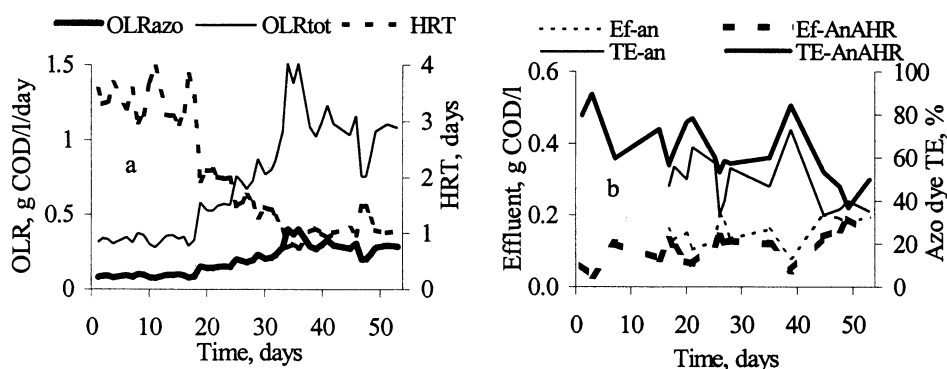


Figure 5 Operation performance of AnAHR treating a Siriusgelb (0.3 g COD/l) synthetic wastewater supplemented with ethanol (0.82 g COD/l)

COD/l/day for the period from day 19 to day 32 (Figure 5a), azo dye anaerobic and overall TEs dropped slightly and were on average 50 and 64%, respectively (Figure 5b). In the final stage of this experiment (day 33 onwards), the ADLR was further increased to 0.3 g COD/l/day keeping the HRT around 1 day (Fig. 5a). This resulted in a further drop of both TEs – 44 and 56% (on average) for the anaerobic compartment and the entire AnAHR, respectively (Figure 5b). Negligible absorbancies at 375 nm were observed in the reactor effluent throughout the entire experimental run indicating complete decomposition of Siriusgelb. However, contrary to the anaerobic batch tests, complete decolouration of the effluent did not occur. Rather, it remained slightly rose in colour compared to the intensive brownish-yellow colour of influent. A transient accumulation of 5-ASA was detected in the anaerobic compartment of the AnAHR but not in the effluent. The 5-ASA concentrations peaked (until 0.06 g COD/l) immediately after increases of ADLR and then gradually decreased if the ADLR was kept constant. This observation suggests a stepwise adaptation of anaerobic sludge for decomposition of 5-ASA. As can be seen in Figure 5b, a majority of the azo dye COD was removed in the anaerobic compartment and the aerobic section had a relatively minor impact on the overall TE. The aerobic removal, as a percentage of the influent, varied between 20 and 30%. Such low TEs achieved in the aerobic step as well as effluent colouring can be attributed to the fact that the breakdown products of anaerobic Siriusgelb decomposition (5-ASA and 1,4-PDA) are readily autooxidised to coloured polymeric products upon exposure to air (Razo-Flores, 1997). These autooxidation products are often complex humic compounds that are non-biodegradable. Incomplete recovery of ammonia (data not shown) also supports the above-mentioned supposition about the inclusion of generated aromatic amines in these persistent polymeric products.

Continuous biomineralisation of 2-ABA in a UASB reactor

As the adapted sludge could use nitrogen from 2-ABA (Kalyuzhnyi *et al.*, 1999), the continuous experiments were started with this substrate as a unique source of carbon and nitrogen. After a week, the reactor reached a TE higher than 95% (Figure 6b) at a 2-ABA loading rate (2-ABA LR) of 0.9 g COD/l/day and HRT of 3.5 days (Figure 6a). Complete recovery of methane and ammonia was almost achieved, and no other components except 2-ABA and traces (if any) of acetate were detected in the effluent. A further increase of 2-ABA LR to 1.4 g COD/l/day (on average) by decreasing the HRT to 2–2.5 days resulted in a drop in TE to around 50% (days 25–35, Figure 6). The effluent contained only 2-ABA and acetate, which was present at a level close to the detection limit (0.01 g COD/l). As the applied 2-ABA concentrations (until 3 g COD/l) are not inhibitory for anaerobic sludge (Kalyuzhnyi *et al.*, 1999), such a deterioration of reactor performance might be attributed to a possible exhaustion

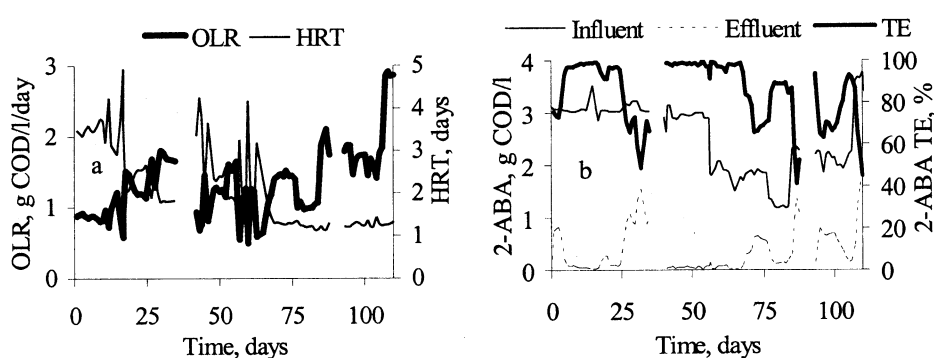


Figure 6 Operation performance of UASB reactor treating 2-ABA

of some important cell components needed for decomposition of 2-ABA. To restore a necessary level of these components, 2-ABA was replaced by ethanol in the reactor feed at day 36. After renewing the reactor feed with only 2-ABA, the TE was again high (>90%) for days 40–66 (Figure 6b). Average 2-ABA LR and HRT applied during this period were 1.4 g COD/l/day and 2.4 days, respectively (Figure 6a). However, a decrease of HRT to 1.3 days, keeping the average 2-ABA LR at almost the same level (1.3 g COD/l/day) using a decrease of influent 2-ABA concentration to around 2 g COD/l, led to a drop of TE to 62% (on average) for days 67–76 (Figure 6). A further decrease of influent 2-ABA concentration to 1.2 g COD/l keeping a HRT at almost the same level (1.2 days) resulted in a restoration of TE to 80% (days 77–84, Figure 6). Finally, the influent 2-ABA concentration was increased to 2.3 g COD/l, which immediately led to a sharp drop in TE (days 85–88, Figure 6).

To avoid the possible exhaustion of some important cell components needed for decomposition of 2-ABA, ethanol was added to the reactor feed together with 2-ABA in the COD ratio of 0.5:1 (days 99–110, Figure 6). It is seen that after ethanol addition, TE on 2-ABA gradually increased to 90% at day 105 (Figure 6b) and ethanol was completely degraded to methane. Hence, the presence of a readily biodegradable compound like ethanol in the wastewater did not inhibit anaerobic degradation of 2-ABA. However, a further increase of influent 2-ABA concentration from 2.1 to 3.7 g COD/l resulting in an 2-ABA LR of 2.9 g COD/l/day led to a substantial drop of TE on 2-ABA to less than 50% (days 106–110, Figure 6). In order to have a deeper understanding of the processes occurring in the UASB reactor, the sludge kinetic characteristics with regard to degradation of 2-ABA were assessed *in situ*, i.e., under reactor conditions (days 111–115). The corresponding values of V_m and K_m were found to be 1.77 ± 0.09 g COD/l/day and 0.41 ± 0.03 g COD/l, respectively. The relatively low value of V_m explains why 2-ABA was not completely degraded at high 2-ABA LRs. A simple estimation using the assessed kinetic parameters and the average reactor concentration of 2-ABA (e.g. 1 g COD/l) gives a maximum assimilative capacity of the sludge with regard to this substrate of around 1.3 g COD/l/day. This estimation is in accordance with the data of Figure 6, where it can be seen that high TEs (>90%) were usually observed at 2-ABA LRs of less than 1.5 g COD/l/day. Thus, the hypothesis about an exhaustion of some important cell components needed for decomposition of 2-ABA does not seem to be relevant. The most probable reasons for the observed values of V_m include both low growth rates of the bacteria responsible for primary decomposition of 2-ABA and low attachment ability of these bacteria. The latter factor seems to be a prevailing one because a long-term (>3 months) continuous feeding of the reactor with 2-ABA as a sole substrate would have to enrich the sludge by the 2-ABA-degrading bacteria. Meantime, relatively low HRTs applied (especially during last 1.5 months of this experiment) probably led to the increased wash-out of these bacteria resulting in the low observed values of V_m .

Conclusions

The following conclusions can be drawn based on this study.

- The azo dyes Siriusgelb and Siriuslichtbraun were found to be not biodegradable under aerobic conditions but both dyes readily decolourised and even slowly mineralised under anaerobic conditions. 5-ASA and 2-ABA were detected as intermediates of their anaerobic decomposition, respectively. The toxicity assays performed with both the azo dyes with regard to acetoclastic activity of anaerobic sludge showed that Siriuslichtbraun was significantly more toxic for methanogenic sludge than Siriusgelb (IC_{50} are equal to 0.41 and 3.55 g COD/l, respectively).
- An innovative reactor construction where the anaerobic and aerobic phases were combined in one single unit called an AnAHR is proposed for the treatment of azo dyes as well as other aerobically persistent xenobiotic contaminants. The performance of the

AnAHR was tested with a synthetic wastewater containing Siriusgelb and ethanol as co-substrate at 30°C. Almost complete decolouration of the influent and 56% removal of the azo dye COD was achieved using a HRT of 1 day and volumetric loading rate of 0.3 g azo dye COD/l/day. The effluent contained no ethanol or acetate and its COD content could be attributed to the presence of non-biodegradable autooxidation products of Siriusgelb breakdown intermediates. Further research is needed to assess the feasibility of this reactor concept for treatment of industrial wastewater containing persistent compounds.

- According to our best knowledge, this study constitutes the first report about the continuous anaerobic biomineralisation of 2-ABA (one of the typical breakdown products of anaerobic decomposition of azo dyes) used as a unique source of carbon and nitrogen. High (>90%) removal of 2-ABA was achieved using volumetric loading rates around 1.5 g 2-ABA COD/l/day. A drop in 2-ABA removal at higher loads was attributed to a low attachment ability of the bacteria responsible for primary decomposition of 2-ABA.

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