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Detoxification and partial mineralization of the azo dye mordant orange 1 in a continuous upflow anaerobic sludge-blanket reactor

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Abstract In batch toxicity assays, azo dye compounds were found to be many times more toxic than their cleavage products (aromatic amines) towards methanogenic activity in anaerobic granular sludge. Considering the ability of anaerobic microorganisms to reduce azo groups, detoxification of azo compounds towards methanogens can be expected to occur during anaerobic wastewater treatment. In order to test this hypothesis, the anaerobic degradation of one azo dye compound, Mordant orange 1 (MO1), by granular sludge was investigated in three separate continuous upflow anaerobic sludge-blanket reactors. One reactor, receiving no co-substrate, failed after 50 days presumably because of a lack of reducing equivalents. However, the two reactors receiving either glucose or a volatile fatty acids (acetate, propionate, butyrate) mixture, could eliminate the dye during operation for 217 days. The azo dye was reductively cleaved to less toxic aromatic amines (1,4-phenylenediamine and 5-aminosalicylic acid) making the treatment of MO1 feasible at influent concentrations that were over 25 times higher than their 50% inhibitory concentrations. In the reactor receiving glucose as co-substrate, 5-aminosalicylic acid could only be detected at trace levels in the effluent after day 189 of operation. Batch biodegradability assays with the sludge sampled from this reactor confirmed the mineralization of 5-aminosalicylic acid to methane.

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Introduction

Azo dyes are one of the oldest industrially synthesized organic compounds. The azo dye production in the United States in 1985 amounted to over 10^8 kg, which was used, by textile, printing, drug and pharmaceutical industries (United States International Trade Commission 1986). Approximately 10 000 dyes are currently manufactured (Zollinger 1987) and it is estimated that at least 15% of these are released into the environment (Vaidya and Datye 1982). They occur in industrial effluent, groundwater, contaminated soils and sediments. These compounds are of concern because some of the dyes, dye precursors or their biotransformation products, such as the aromatic amines, have been shown to be carcinogenic (Kriek 1979; Longstaff 1983). Azo dyes are designed to be recalcitrant under typical product service conditions and it is this property, allied with their toxicity to microorganisms, that makes biological treatment difficult (Idaka et al. 1985; Ogawa et al. 1978).

Biological treatment systems may have promising applications for the removal of azo dye compounds since it is widely reported that the azo dyes are gratuitously reduced by anaerobic sludges, anaerobic sediments and anaerobic bacterial enrichment cultures (Brown and Hamburger 1987; Brown and Laboureur 1983a; Chung et al. 1992; Weber and Wolfe 1987). On the other hand, azo dye compounds are resistant to oxygenolytic attack. Pagga and Brown (1986) and Shaul et al. (1991) tested the degradation of more than 100 dyes in aerobic activated-sludge systems and found that only a few of them were actually biodegraded.

The anaerobic cleavage products of azo dye compounds (aromatic amines) are more easily degraded under aerobic conditions (Brown and Laboureur 1983b; Haug et al. 1991). The aromatic amines are generally not metabolized further under anaerobic conditions (Brown and Laboureur 1983a). Nonetheless, some aromatic amines such as the three isomers of aminobenzoate, 2-aminophenol, 4-aminophenol and 5-aminosalicylic acid (5-ASA) have been shown to be mineralized in methanogenic consortia (O'Connor and Young 1989, 1993; Razo-Flores et al. 1996).

Toxic compounds can be tolerated by methanogens in continuous upflow anaerobic sludge-bed (UASB) reactors if they are degraded or undergo biotransformations to less toxic products. Toxic chloro-, nitro- and azo- substitutions of aromatics are subject to reductive biotransformations in anaerobic environments (Field et al. 1995). Previously we reported that nitroaromatic compounds were on the average 500-fold more toxic than their corresponding aromatic amine analogues (Donlon et al. 1995). The reduction of the nitro- substituents during treatment in UASB reactors was shown to be responsible for a dramatic detoxification of several nitrophenols towards methanogens (Donlon et al. 1996).

Consequently, batch toxicity studies were conducted in order to compare the methanogenic toxicity of selected model azo dye compounds with that of the corresponding aromatic amines. Afterwards, the detoxification and degradation of the azo dye, Mordant orange 1 (MO1), in a continuous laboratory-scale UASB reactor was evaluated.

Materials and methods

Source of inoculum

Methanogenic granular sludge from a full-scale UASB reactor, treating wet oxidized petrochemical industry effluent of Shell Nederland Chemie (Moerdijk, The Netherlands), was used as inoculum. The sludge was washed to remove fines and stored at 4 °C before use. The sludge had not previously been exposed to any of the compounds being tested.

Medium

The basal medium used in all experiments was as described previously (Donlon et al. 1996). The micro-nutrients were supplemented to the media for the continuous column experiments at a 10-fold lower concentration.

Anaerobic toxicity assay

Specific acetoclastic methanogenic activity measurements were performed in 120 ml glass serum vials, containing 25 ml basal medium as described previously (Donlon et al. 1995). The maximum specific acetoclastic methanogenic activity of the control sludge was 420 mg methane expressed as chemical oxygen demand (COD) per gram of volatile suspended solids (VSS) per day. The exact toxic concentrations of the azo dyes were calculated taking into account the purity of the compound tested.

Continuous column experiments

The continuous experiments were performed in three separate glass UASB reactors with liquid volumes of 160 ml placed in a temperature-controlled room at 30 ± 2 °C. All reactors were inoculated with 20 g VSS/l anaerobic granular sludge. To start the experiment the granular sludge was initially fed with the medium containing a partially neutralized (pH = 6.0) volatile fatty acids (VFA) mixture (acetate/propionate/butyrate, 23 : 34 : 41 on a COD basis) at a concentration of 4 g COD/l during the start-up. After 1 month of operation, the three reactors received a subtoxic concentration of the azo dye (i.e. 0.01 mM MO1). This is referred to as the first day of the experiment, day 0 in the figures. Reactor 1 (R1) received no primary substrate (the control), the VFA feed was no longer supplied. Reactor 2 (R2) received glucose (1.3 g COD/l) in addition to the azo dye, and reactor 3 (R3) was fed the VFA mixture (1.5 g COD/l) in addition to the azo dye. The methane production was measured with 10-l Mariotte flasks filled with a 3% (w/v) NaOH solution to scrub out the carbon dioxide from the biogas.

Biodegradability studies

The biodegradation assay was conducted for the granular sludge sampled from the reactors (R2, R3) using a protocol outlined previously (Razo-Flores et al. 1996). The sludges were sampled from the reactors on days 0, 166 and 203. The concentrations of the target aromatic amines tested were 1.63 mM and 2.3 mM for the 5-ASA and 1,4-phenylenediamine (1,4-PDA), respectively. All results are reported as the mean value of triplicate serum vials.

Analyses

Methane and VFA were determined by gas chromatography as described previously (Sierra and Lettinga 1991). The pH was determined immediately after sampling with a model 511 pH-meter (Knick, Berlin, Germany) and a model N61 double electrode (Scot Gerade, Hofheim, Germany). All the other analytical determinations were performed as described in *Standard methods for examination of water and wastewater* (American Public Health Association 1985).

The MO1 concentration in solution was determined five times per week by monitoring azo-group absorbance at the absorbance maxima (373 nm). There was less than 5% interference from the cleavage products (5-ASA and 1,4-PDA) at this wavelength. The absorbance spectra of the compounds were recorded in 0.2 M phosphate buffer (pH 7.0) on a Spectronic 60 spectrophotometer (Milton Roy/Analytical Products Division, Ostende, Belgium) using a model 100-QS (Hellma Benelux, The Hague, The Netherlands) 1-cm quartz cuvette. All absorbance data are reported as the absorption of the medium containing aromatic compounds corrected for the absorption of the control medium (which contained no test compounds). Total aromatic amines were determined colorimetrically at 440 nm after reacting with 4-dimethylaminobenzaldehyde hydrochloride (Ehrlich Reagent) according to the method described by Oren et al. (1991). The expected absorbance units per millimole of MO1 removed was determined to be 3.055 by using an equimolar mixture of 5-ASA and 1,4-PDA as a standard.

The MO1 concentration as well as authentic standards of 4-nitroaniline and MO1 were determined periodically by reverse-phase high-pressure liquid chromatography (HPLC) (Spectra-Physics model SP8810, Thermo Separation Products, Breda, The Netherlands). A C18 reverse-phase column (Chromosphere 18, Chrompack, Bergen op Zoom, The Netherlands) was used to separate individual compounds, which were detected using a Kratos Superflow 773 UV detector (Separations, H. I. Ambacht, The Netherlands). Absorbance was detected at 280 nm. The solvent phase was methanol and 1% acetic acid (40 : 60, v/v). The solvent flow was 0.5 ml/min. Aromatic amines 1,4-PDA and 5-ASA could

not be determined by the HPLC method and, consequently these compounds were analysed by GC-MS.

Identification of aromatic amines by GC-MS

1,4-PDA and 5-ASA were extracted from the liquid phase with freshly distilled ethyl acetate. In order to make 5-ASA amenable to GC-MS analysis, a derivatization step was performed using the method described by Brook and Chan (1983). For this purpose, chlorotrimethylsilane was added to give the methyl ester of 5-ASA (5-ASA-ME). 4-Bromoanisole at a concentration of 0.55 mM was used as the internal standard. All the samples were analysed on an HP5970B quadrupole mass spectrometer coupled to an HP5890 gas chromatograph (Hewlett Packard, Palo Alto, USA). The identification of the aromatic amines was achieved by comparison of retention times and mass spectra to data of respective standard compounds.

Chemicals

Chemicals were purchased from either Jansen Chimica (Tilburg, The Netherlands), Merck (Darmstadt, Germany) or Sigma (Bornem, Belgium). All the chemicals were of the highest purity commercially available. None of the chemicals was purified further.

Results

Effect of N-substituted aromatic structure on methanogenic inhibition

The inhibitory effects of eight N-substituted aromatic compounds on the activity of acetoclastic methanogenic bacteria were evaluated in this study. The inhibition caused by each compound was tested at various levels covering non-toxic to completely inhibitory concentrations of the compounds. Table 1 summarizes the 50% inhibitory concentrations (IC) of the aromatic compounds evaluated in this study and outlines the relationship between the azo dye and the reduced cleavage products. Figure 1 shows the aromatic structure of the azo dyes used in this study. The type of N substitution had a profound effect on their toxicity. The least toxic compounds were the aromatic amines: 1,3-phenylenediamine (1,3-PDA), 1,4-PDA and 5-ASA. The azo compounds and the nitroanilines were more toxic. The most toxic compound tested was MO1 having a 50% IC of 0.014 mM.

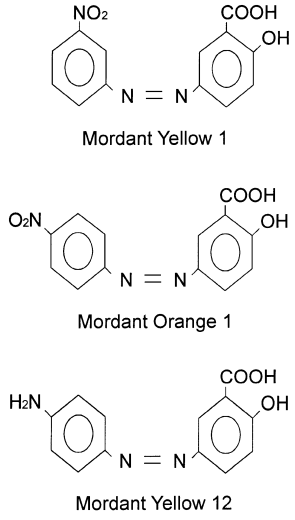

Fig. 1 Azo dye compounds used in this study

Continuous anaerobic treatment of MO1 in UASB reactors

Three laboratory-scale UASB reactors were operated in order to investigate the continuous anaerobic treatment of MO1. The reactors (R1, R2, and R3) were initially started up after a 1-month period of adaptation to VFA. After this adaptation period the reactors were initially fed with subtoxic concentrations of MO1 (0.01 mM). The concentration of MO1 in the influent was increased periodically after at least 20 hydraulic retention times (hydraulic retention times were approximately 0.32 days) and when more than 75% removal of MO1 and the cosubstrate COD had been obtained. The rationale behind this approach is that the sludge can withstand higher concentrations of the incoming dye by cleaving the azo bond to form less toxic aromatic amines. The reactors differed from each other in the selection of the cosubstrate; R1 (the control) received no cosubstrate, whereas R2 and R3 received glucose and VFA mixture respectively. Glucose and the VFA mixture were chosen as model wastewater substrates deemed necessary for providing the electrons for the reduction of the azo compounds.

Table 1 Toxicity of selected azo dye compounds and their suspected cleavage products to acetoclastic methanogens. *50%IC* compound concentration that caused 50% inhibition of the methanogenic activity, *NA* not applicable

Compound name	M_r (Da)	50% IC (mM)	Theoretical reductive biotransformation products
Mordant orange 1	287	0.014	5-ASA, 4-NA, 1,4-PDA
Mordant yellow 1	287	0.063	5-ASA, 3-NA, 1,3-PDA
Mordant yellow 12	279	0.250	5-ASA, 1,4-PDA
3-Nitroaniline (3-NA)	138	0.03	1,3-PDA
4-Nitroaniline (4-NA)	138	0.017	1,4-PDA
1,3-Phenylenediamine (1,3-PDA)	108	65.7	NA
1,4-Phenylenediamine (1,4-PDA)	108	30	NA
5-Aminosalicylic acid (5-ASA)	153	2.9	NA

R1 was able to remove the azo dye for 20 days with the production of aromatic amines. The yield of aromatic amines was based on the total aromatic amines, which were fairly constant yielding 3 absorbance units/mmol MO1 eliminated. Azo dye removal exceeded 60% at concentrations up to 0.041 mM for 20 days. The concentration of MO1 was increased stepwise to 0.26 mM for an additional 30 days. During this period there was a major decrease in the azo dye removal to less than 10% with a parallel decrease in total aromatic amine production (results not shown). In addition, 4-nitroaniline, which previously had not been detected in the reactor effluent, was observed in the HPLC analyses (data not shown). Owing to the fact that MO1 was not being reduced completely, the concentration of both MO1 and 4-nitroaniline increased. Finally, complete failure of the reactor occurred as shown by the lack of methane production. It is likely that the endogenous substrate present in the sludge was initially contributing the reducing equivalents to reduce the azo bond. The endogenous substrate was probably exhausted after 50 days, causing an incomplete reduction, as shown by the accumulation of 4-nitroaniline, just prior to the reactor failure.

The operational parameters and treatment efficiency during the continuous operation of R2 and R3 at the end of the reactor operation are listed in Table 2. The operational conditions and treatment performance of R2 are shown in Fig. 2. From day 75 to day 166, MO1 was greatly reduced (at least 90%) in this reactor. The total aromatic amines produced were fairly constant, yielding approximately 3 absorbance units/mM MO1 eliminated, indicating a stoichiometric yield of the aromatic amines (Fig. 2A). The reduction of the parent azo compound to the less toxic daughter aromatic amines ensured that a detoxification of the influent occurred as was demonstrated by the glucose degradation (86.6% COD removal) and methane production (79.8% of the incoming cosubstrate COD). By use of HPLC and GC-MS, 4-nitroaniline was not detected as a cleavage product that accumulated. From day 189 onwards, the total aromatic amine concentration decreased, indicating that part of the amines produced by the reduction were converted further (Fig. 2A). Effluent samples collected in this period were subjected to derivatization conditions and were analysed by GC-MS. The GC-MS spectra confirmed the presence of 1,4-PDA while only trace levels of 5-ASA-ME were detected (results not shown). These results indicated biological removal of the 5-ASA.

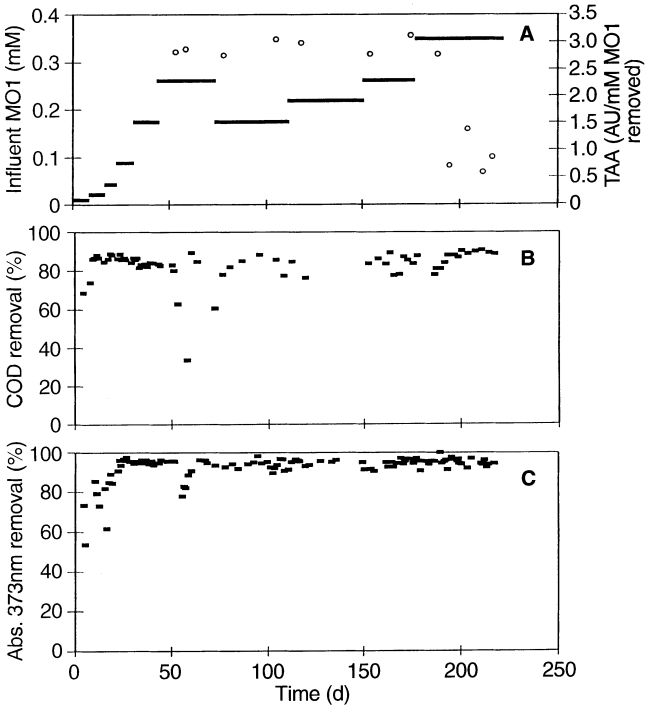

Fig. 2A–C Operational parameters and removal efficiencies during the continuous anaerobic treatment of Mordant orange 1 (*MO1*) when glucose was employed as cosubstrate: **A** ▬ MO1 concentration in the influent, o total aromatic amines (*TAA*) produced; **B** percentage removal of glucose in terms of chemical oxygen demand (*COD*); **C** percentage MO1 removal as determined by loss in UV absorbance at 373 nm

Table 2 The operational parameters and treatment efficiency during the continuous operation of the upflow anaerobic sludge-blanket reactors (160 ml) treating Mordant orange 1 at the final period of reactor operation (days 174–217). *COD* chemical oxygen demand, *VFA* volatile fatty acids

Parameter	Reactor 2	Reactor 3
Operational parameters		
Influent cosubstrate (g COD/l)[a]	1.42	0.91
Conc. influent Mordant orange 1 (mM)	0.35	0.18
Organic loading rate cosubstrate (g COD $l^{-1}day^{-1}$)	4.16	2.88
Mordant orange 1 load (mmol/$l^{-1}day^{-1}$)	1	0.57
Hydraulic retention time (days)	0.34	0.31
Efficiency		
COD removal (%)[b]	86.6 ± 3.9	81.8 ± 5.5
Methane (% CODin)[c]	79.8 ± 11.1	58.3 ± 20
VFA effluent (% CODin)	1.2 ± 0.5	2.6 ± 2.1
Azo dye removal (%)		
Absorbance[d]	95.1 ± 1.6	91.8 ± 1.3
HPLC	> 99	> 99

[a]Cosubstrate: reactor 2, glucose; reactor 3, VFA mixture
[b]Removal of cosubstrate in terms of COD elimination
[c]Percentage conversion of influent cosubstrate COD to methane
[d]Azo dye absorbance removal indicates loss in azo group absorbance at 373 nm

Reactor 3 gave high VFA removal efficiencies (81.8% on a COD basis) and methane production (58.3% of the incoming cosubstrate COD) over the course of the experimental period (Table 2). The reduction of MO1 to 5-ASA and 1,4-PDA was attributed to the high MO1 removal efficiencies (91%), even after concentrations had been increased to 0.174 mM (Fig. 3). Again it was observed that 4-nitroaniline could not be detected as a cleavage product by use of the HPLC and GC-MS methods. The concentration of total aromatic amines produced was approximately equivalent to that expected from a stoichiometric recovery of aromatic amines during the whole period (Fig. 3A). The GC-MS spectra data confirmed the presence of 1,4-PDA and 5-ASA-ME (results not shown). 5-ASA-ME was present at much higher concentration than in R2. Consequently, no further conversion of the aromatic amines was observed although the reactor was in operation for 217 days.

Mineralization of aromatic amines by sludge sampled from the UASB reactors

The sludges sampled from R2 and R3 at various times during the continuous experiments were assayed for the conversion of 5-ASA and 1,4-PDA to methane in batch assay experiments. There was no conversion of 1,4-PDA to methane in any of these samples. On the other hand, complete mineralization of 5-ASA to methane in batch assays from sludge sampled from R2 on days 166 and 203 was observed (results not shown). The conversion of the 5-ASA-COD to methane-COD was more than 90% of the theoretical methane production expected for both sludges. In contrast, the lag phase of 5-ASA degradation was reduced from 21 to 5 days for the sludges sampled on days 166 and 203 respectively. This fact indicated that a complete sludge adaptation to the 5-ASA degradation had occurred. The seed sludge and the sludge sampled from R3 were unable to convert 5-ASA to methane.

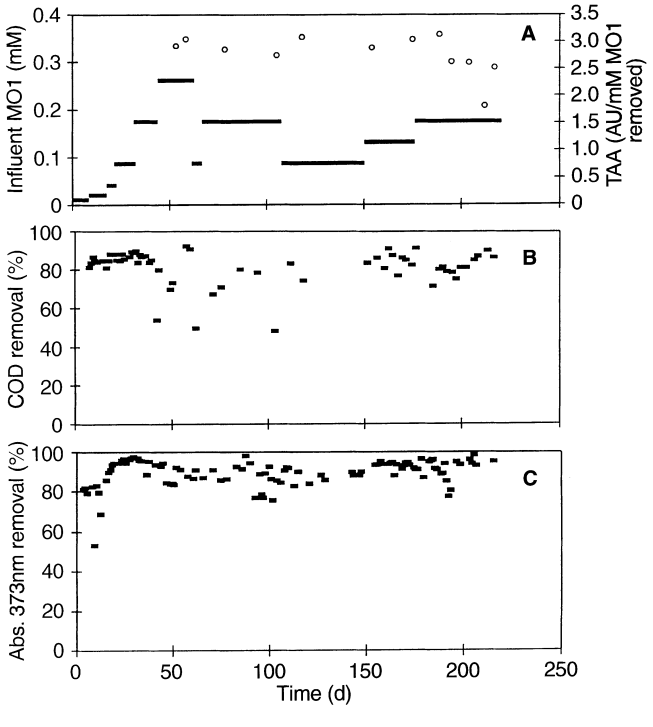

Fig. 3A–C Operational parameters and removal efficiencies during the continuous anaerobic treatment of Mordant orange 1(*MO1*) when volatile fatty acids were employed as cosubstrates: **A** ▪ MO1 concentration in the influent, o total aromatic amines (*TAA*) produced; B percentage volatile fatty acids removal in terms of COD; **C** percentage MO1 removal as determined by loss in UV absorbance at 373 nm

Discussion

Toxicity of N-substituted aromatic compounds

In our study, the azo dyes and the nitroanilines were clearly very toxic to methanogens, with 50% IC values ranging from 0.014 mM to 0.25 mM. Of the three azo dyes tested, Mordant yellow 12 was the least toxic, probably because this dye does not contain a nitro-functional group. Seshadri et al. (1994) studied the inhibitory effects of some azo dyes on COD and dye removals in anaerobic fluidized-bed reactors. They found that Acid orange 10 and Acid orange 8 dye concentrations of 0.037 mM and 0.051 mM, caused a significant inhibition in dye and COD removal respectively.

Previously we have shown that aromatic nitro- substituents are responsible for severe methanogenic toxicity. Aromatic amines, in contrast, were less inhibitory compounds: their 50% IC values were between 2.9 mM and 65.7 mM (Donlon et al. 1995). An example of the increased toxicity of azo compounds compared to their aromatic amine cleavage products can be observed for MO1. This dye is over 200-fold and 2140-fold more toxic than 5-ASA and 1,4-PDA respectively. This indicates that the reduction of azo dyes, known to occur in anaerobic environments (Brown and Hamburger 1987; Brown and Laboureur 1983a; Weber and Wolfe 1987), would be responsible for their dramatic detoxification with respect to methanogens.

Anaerobic treatment of Mordant orange 1 in UASB reactors

The application of high-rate reactors, such as the UASB reactors, has proven to be capable of treating various wastewaters bearing toxic aromatic compounds with a high degree of efficiency and stability (Lettinga et al. 1991; Wu et al. 1993). The effective treatment regimes cited for nitroaromatics rely on the gratuitous reduction of these compounds in the presence of suitable electron donors to products of lower toxicity (Donlon et al. 1996). Consequently, it was of interest to examine the cosubstrate requirement for the azo group reduction. Both glucose and the VFA mixture supported azo dye

reduction for the entire duration of the continuous experiment. In the reactor to which no cosubstrate was fed, some azo dye reduction occurred in the initial period of operation when low MO1 loading rates were applied. The reducing equivalents for this conversion were presumably due to endogenous substrates in the anaerobic sludge. Eventually the endogenous substrate would be expected to become exhausted, which might explain why the R1 performance slowly dropped over time, failing completely by day 50. The presence of 4-nitroaniline as an incomplete reduction end-product in R1, prior to its failure, is analogous to the observations reported in a recent paper by Cheng et al. (1996), where they described an incomplete reduction of 2,4-dinitrotoluene to nitro-aminotoluenes by an anaerobic culture in the absence of cosubstrate.

The MO1 loading that could be tolerated was almost two times higher in R2 than in R3, indicating that glucose was a better cosubstrate than VFA for azo dye reduction. Glucose has previously been shown to be a good donor of reducing equivalents for the cleavage of azo dyes (Haug et al. 1991; Roxon et al. 1966). Glucose could act as donor of reducing equivalents to enzyme cofactors [e.g., via NAD(P)H or reduced $FAD(H)_2$] transferring electrons for azo dye reduction by specific enzymes. Roxon et al. (1967) reported that both NADH and NAD(P)H are active electron donors for the reduction of tartrazine in whole-cell suspensions of *P. vulgaris*. Gingell and Walker (1971) found that reduced flavins play an important role in azo reduction in *Streptococcus faecalis*. Azo reduction under anaerobic conditions may represent a fortuitous, non-enzymatic reduction by enzymatically generated reduced flavins, whereas inhibition by oxygen is due to the poor regeneration of the oxidized form (Gingell and Walker 1971). Acetogenic and methanogenic bacteria in the granular sludge also contain metallocofactors, such as F_{430} and vitamin B_{12}, which perhaps could reduce the azo dyes non-specifically, similarly to what has been found for the reductive dechlorination of the chlorinated aliphatics (Wackett and Schanke 1992).

The establishment of a glucose-degrading consortium could also possibly supply the 5-ASA-degrading organism(s) with essential compounds, e.g. vitamins or cofactors, not present in the VFA-degrading consortia. A cross-feeding pattern of this kind could also explain the very strict demand for complex carbon sources, like yeast extract or proteose peptone, found for an anaerobic 2-aminophenol-degrading consortium (Bisaillon et al. 1993).

Highly toxic concentrations of MO1 could be tolerated in the continuous UASB reactors fed with cosubstrate (0.35 mM and 0.18 mM for R2 and R3 respectively). These concentrations exceeded the 50%IC of the MO1 for methanogenic bacteria by 25- and 13-fold in R2 and R3 respectively. Nonetheless, the methanogenic consortia appeared to be healthy since the cosubstrate was degraded, at moderate organic loading rates with a high conversion efficiency, to methane. Detoxification of MO1 was clearly taking place in the UASB reactors. The reducing equivalents produced during cosubstrate degradation were used to support the complete reduction of MO1 to less toxic breakdown products.

The results from this study showed that UASB reactors could handle high azo dye loading rates up to 1 mmol MO1 l^{-1} day^{-1}. These loading rates are at least a 10-fold improvement over those obtained in previous studies with anaerobic reactors. Seshadri et al. (1994) used an anaerobic fluidized-bed reactor for the treatment of selected azo dyes. The authors applied azo dye loading rates of 0.1 mmol Acid orange 8 $l^{-1}day^{-1}$. Whereas, Fitzgerald and Bishop (1995) applied azo dye loading rates of 0.017 mmol Acid orange 10 $l^{-1}day^{-1}$ to an anaerobic fixed-fluidized-bed reactor.

Mineralization of aromatic amines

The GC-MS results combined with the chemical assay for total aromatic amines and the anaerobic batch biodegradability assays indicated that 5-ASA was being mineralized in the glucose reactor. In the batch experiments assaying the conversion of 5-ASA to methane by sludge sampled from R2, a specific mineralization rate of 0.23 mmol g VSS^{-1} day^{-1} was observed. This rate was much greater than the maximum MO1 sludge loading rate used in the continuous-column experiment which, when expressed in terms of 5-ASA equivalents, amounted to 0.033 mmol g $VSS^{-1}day^{-1}$. Consequently we concluded that the removal of 5-ASA cleaved from MO1 in R2 was due to its anaerobic degradation.

In the case of the other MO1 breakdown product, there was no conversion of 1,4-PDA to methane in any of these sludge samples. The elimination of 1,4-PDA in aerobic sludge has been previously documented (Alexander and Lustigman 1966; Pitter 1976). Consequently, an aerobic post-treatment step would be required for the complete mineralization of the azo dye MO1. Coupled anaerobic-aerobic systems have proven to be successful in achieving the complete biodegradation of azo dyes. In such systems, azo dyes are reduced anaerobically, followed by subsequent aerobic degradation of the aromatic amines produced (Field et al. 1995; Haug et al. 1991).

Previously it was assumed that azo cleavage products would be recalcitrant to anaerobic degradation (Brown and Hamburger 1987; Haug et al. 1991; Seshadri et al. 1994), and that subsequent aerobic mineralization would be required. However, this study demonstrates that one such azo cleavage product, namely 5-ASA, is in fact biodegradable in methanogenic consortia under the operational conditions of a continuous anaerobic reactor. This finding has important implications for the environment since this compound is an important building block of many azo dyes and thus constitutes a widespread contaminant of aqueous ecosystems. The proposed pathway for the partial mineralization of

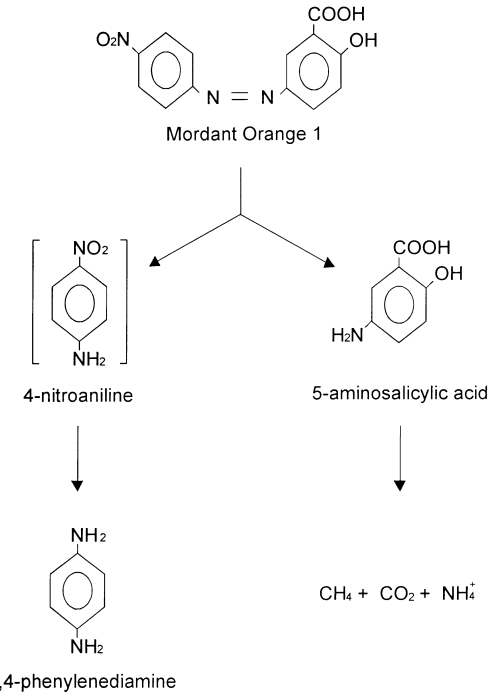

Fig. 4 Proposed scheme detailing the anaerobic breakdown of the azo dye MO1 by granular sludge present in the reactor receiving glucose as cosubstrate

MO1, determined for the reactor receiving glucose as primary substrate, can be depicted schematically as shown in Fig. 4.

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