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Behaviour of different anaerobic populations on the biodegradation of textile chemicals

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ABSTRACT

The anaerobic biodegradation of textile chemicals was evaluated with inocula grown under mesophilic ($37 \pm 2^\circ$ C) or thermophilic ($55 \pm 2^\circ$ C) conditions, on glucose (glucose-grown) or acetate (acetate-grown) as sole carbon sources. Wool dyebath chemicals (acetic acid, a liposomal surfactant, a synthetic amphoteric surfactant), single or as binary acetate–surfactant mixtures, were used as test carbon sources, in the presence or absence of Acid Orange 7 as model dye. First, the two mesophilic inocula (glucose- or acetate-grown) were compared relatively to lag-phase durations, specific biogas production rates, biogas yields and overall COD removal yields. In some runs, sulphide and/or the model dye were included, to test for inhibition effects. Then, the two glucose-grown inocula (mesophilic and thermophilic) were assessed in batch biodegradation tests with the same carbon feeds. The kinetics for substrate-COD and dye colour removal were described and quantified using a pseudo-first order model. The presence of dye had no effect on performance parameters for all substrates tested. Acetoclastic methanogens seemingly played an important role in biogas production from the liposomal additive, but less so from the synthetic surfactant. The association of acetate and surfactants apparently introduced mutual inhibitory effects on the rates of biogas production, substrate uptake and dye decolourisation.

1. Introduction

In addition to residual dyes, textile industry effluents usually contain a significant part of the auxiliary chemicals applied during the dyeing process, to improve its performance or confer specific characteristics to textile goods. Auxiliaries such as levelling agents, electrolytes (salts), dispersants, buffer systems and antifoam agents are added to the dyebath liquors and contribute to the high organic and inorganic loads. Surfactants are present at often high concentrations in the resulting wastewaters, with potentially adverse impacts on their treatment. In textile finishing, non-ionic (e.g., polyglycol ether) and anionic (e.g, alcohol sulphate) surface active agents are widely used. The available information on anaerobic biodegradation of non-ionic surfactants is scarcer than that reported for aerobic bioprocesses. Published studies indicate that the main factors affecting biodegradation of these chemicals are the size of the molecule and the degree of branching [1], with the presence of aromatic rings in the alkyl chain increasing its recalcitrance [2]. It is known that surfactants can damage cell membranes leading to inhibition of microbial activity. This inhibition effect depends on the chemical structure of the surfactant, which also determines the degree of interference on the interactions between cells and primary substrates or intermediates [3]. Recently, levelling agents based on liposomes have been introduced as dye carriers replacing the classical synthetic surfactants, with ecological benefits and advantages such as lower dyeing temperatures and improved final quality of the dyed textile. Liposomes are vesicular structures with an internal aqueous phase entrapped by a lipid bilayer. They are formed by surface active biological lipids which are the main constituents of cell membranes [4].

Following resource conservation trends, textile industries have been implementing water-saving measures, mainly by recycling between specific process stages or after on-site treatment. As a consequence, less wastewater is discharged but bearing heavier pollution loads. In this context, anaerobic digestion offers a viable treatment or pre-treatment alternative, able to address a wide range of chemical types and organic loads. Anaerobic processes can be run in the mesophilic or thermophilic temperature ranges and the microbial consortia can be adapted to the type of substrate being supplied to the system [5,6], providing different metabolic pathways to degrade complex and even recalcitrant chemicals. Processes operated at 55–65 °C generally display higher degradation rates (2–3 fold) when compared with mesophilic processes (<40 °C) [7]. This can be economically attractive, since smaller reactor volumes are necessary. According to Ahring et al. [8], in the thermophilic temperature range the oxidation of the methyl group

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of acetate to CO_2 and H_2 predominates over acetotrophic methanogenesis, indicating that CO_2-reducing methanogens are responsible for the majority of the methane produced.

Among the xenobiotics amenable to degradation in anaerobic conditions are azo dyes, which represent the major group (60–70%) of dyes currently employed in textile manufacture. Aside from their negative aesthetic effects when discharged in surface waters, these dyes may show toxicity to aquatic life, cause allergenic effects and be potentially carcinogenic or mutagenic to humans [9]. Azo dyes may be decolourised in anaerobic conditions by cleavage of the azo bond, via a non-specific and presumably extracellular process, in which reducing equivalents, biologically or chemically generated from an external electron donor, are transferred to the dye [10]. It has been reported, from studies carried out in mesophilic batch reactors, that azo bond reduction can be performed by acetate-fed cultures [11]. Santos [12] studied the decolourisation capacity of anaerobic granular sludge in the presence of methanogenic substrates (acetate, H_2/CO_2) at mesophilic and thermophilic temperatures. He observed that both substrates support cultures able to remove colour at mesophilic temperatures, but, for the thermophilic regime, a 4–6-fold reduction in the degradation rate was observed when acetate was used as substrate, in comparison with the culture fed with H_2/CO_2 [12].

Textile effluents can be discharged at high temperatures and undergo frequent, marked composition changes. Thus, it is important to understand how anaerobic consortia respond to such different feeds, in terms of biogas productivity and colour elimination. The present report contributes with some insights for this knowledge, with results from a case study typical of the wool dyeing sector. In this context, the performance of different anaerobic inocula for the use of acetate and two surface-active agents, of the amphoteric and liposomal types (representative of the chemicals employed in wool dyeing), as substrates for anaerobic biotreatment, allowing the decolourisation of a model azo dye, was assessed. Methanogenic activity (biogas production rate) tests were first carried out, in mesophilic conditions, using inocula previously grown on acetate or glucose, thus providing consortia with different proportions of acidogens and methanogens. Tests were carried out with single surfactant substrates, against an acetate control, or with binary mixtures of surfactants with acetate. A model azo dye was here tested as a possible inhibitor of methanogenesis, alongside with sulphide (a known inhibitor). Subsequently, mesophilic and thermophilic glucose-grown inocula were used in batch biodegradation tests, to study the kinetics of COD and dye decolourisation.

2. Materials and methods

2.1. Inocula

For the specific methanogenic activity (SMA) assays, mesophilic glucose-grown (MG) and mesophilic acetate-grown (MA) inocula were used. The original seed was granular sludge collected from a full-scale UASB (upflow anaerobic sludge bed) reactor treating a paper mill effluent. The inocula were previously adapted, during approximately 6 months, in mesophilic ($37 \pm 2\,^{\circ}C$) laboratory batch reactors fed with nutrient solutions supplemented with glucose (MG) or acetic acid (MA), both at 1.5 g/L, as sole carbon sources. Sulphate (Na_2SO_4, 30 mg/L) and sulphide ($Na_2S{\cdot}6H_2O$, 60 mg/L) were added to the MG and MA culture media, respectively, during this acclimatization period, as sulphur sources. The SMA flasks were inoculated with 15 mL of an inoculum at 21.0 ± 0.9 gVSS/L, in order to achieve a biomass concentration of 4.0 ± 0.2 gVSS/L in each flask.

For the batch biodegradation kinetics tests, the inocula were pre-incubated in laboratory batch reactors operating at $37 \pm 2\,^{\circ}C$ (MG) and $55 \pm 2\,^{\circ}C$ (TG), fed with the nutrient solution and supplements described above for the MG inoculum, for the same time period. The test reactors were seeded with these mesophilic or thermophilic inocula, to obtain a biomass concentration of 3 ± 1 gVSS/L.

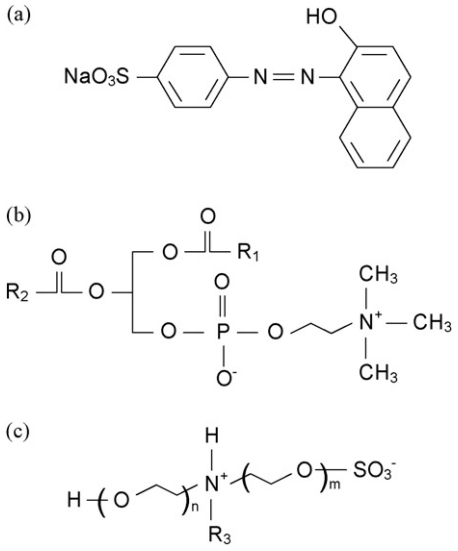

Fig. 1. Chemical structures of: (a) AO7 dye; (b) general structure of phosphatidylcholine (natural surfactant); (c) alkylamine poly(oxyethylene) ether sulphate (amphoteric surfactant). R_1, R_2 and R_3 are hydrocarbon chains and m and n are the numbers of oxyethylene (ethoxy) moieties.

2.2. Feed solutions

The feed solutions for inocula development, for SMA tests and for biodegradation tests, were all based on a mineral medium containing macro and micro nutrients, described elsewhere [13]. For the SMA tests, this basal medium was prepared in a phosphate buffer solution (4.0 g/L $Na_2HPO_4{\cdot}12H_2O$ and 0.4 g/L NaH_2PO_4, pH 7.2). Phosphate was selected, instead of bicarbonate, to minimize interferences in the biogas production measurements. For all other procedures, the basal medium was prepared in a $NaHCO_3$ (4–6 g/L) solution (pH 7–8). The model dye Acid Orange 7 (AO7), was added to some of the feed solutions at a concentration of 60 mg/L. This monoazo dye (85–95% purity) was supplied by Sigma (Spain) and its chemical structure is indicated in Fig. 1a. For both SMA and biodegradation tests, the carbon sources added to the basal medium were (single or in binary mixtures) acetic acid (1000 mg/L), purchased from Riedel de Häen (Germany), Archicolor Transfer (1000 mg/L), purchased from Archivel Technologies (Spain), and Avolan UL 75 (1000 mg/L), purchased from Bayer (Germany). The levelling agent Archicolor Transfer (AT) is a natural liposome, i.e., a biopolymer (Lipoid S-100 – soybean lecithin) with more than 95% of phosphatidylcholine (Fig. 1b). This phospholipid is widespread in nature and likely to be easily incorporated by a wide range of microbial biomass types. Chemically, Avolan UL 75 is an alkylamine polyglycol ether sulphate (amphoteric surfactant) (Fig. 1c) [14]. All other chemicals were purchased from Merck (Germany). In some of the SMA tests, sulphide ($Na_2S{\cdot}6H_2O$, 60 mg/L) was added to the feed solution in order to evaluate its influence on biogas production. In the biodegradation tests, the nutrient solution was supplemented with sulphate (Na_2SO_4, 1000 mg/L), as a common component of wool dyeing effluents.

2.3. Experimental procedure

SMA tests: The specific methanogenic activity tests, here limited to overall biogas production measurements, were performed

in 250 mL glass bottles, sealed with rubber septa and equipped with magnetic stirring. A volume of 56 mL of basal medium was introduced in each incubation bottle. They were then flushed with nitrogen gas for 5 min, thermostatized at 37 ± 2 °C for 30 min and inoculated with the mesophilic glucose-grown (MG) or mesophilic acetate-grown (MA) cultures. The inocula were previously centrifuged and washed twice with phosphate buffer solution at 37 °C, to remove residual substrate from the acclimatization step. The flasks were then flushed again with N_2 for 10 min, sealed and maintained in the dark for 12 h at 37 ± 2 °C, to promote metabolization of endogenous substrates. After this period, 4 mL of the substrate (single or mixtures, with or without dye or sulphide) stock solutions (pre-warmed to 37 ± 2 °C) were injected. The initial measured COD values ranged from 641 ± 32 mg/L to 1012 ± 48 mg/L for single substrates and from 1375 ± 52 mg/L to 1896 ± 84 mg/L for substrate mixtures. Individual pressure sensors (Oxitop®, WTW, Germany) on each bottle were used to measure the cumulative biogas pressure in the headspace till a constant pressure was reached (25–96 h), at 37 ± 2 °C. The estimated specific methanogenic activity (SMA) was calculated from the maximum slope of the time courses of pressure build-up. The number of moles of biogas produced was calculated, at work temperature and measured pressure in the Oxitop, by the ideal gas law equation. Values were expressed in litre of biogas (at 1 atm and 25 °C) per gram of volatile suspended solids per day. SMA values and direct pressure measurements (x) were further normalized (y) to an initial COD concentration of 1000 mg/L, assuming linear behaviour in the tested COD range, and considering the initial COD values (COD_i, mg/L) in each incubation flask $\left(y = (x/COD_i) \times 1000\right)$. A blank (basal medium in phosphate buffer) was used as control for each inoculum type. Biogas production in these control runs was always negligible. At the end of the incubation periods, samples were taken from the mixed liquor in each flask and filtered through glass microfibre membranes (Whatman GF/C, 1.2 μm average pore diameter), for subsequent COD and colour measurements. Tests were performed in triplicate.

Anaerobic biodegradation tests: Glass reactors of 500 mL capacity, with 400 mL of feed medium, were operated at 37 ± 2 °C and 55 ± 2 °C, for the biodegradation kinetic studies with the inocula previously grown on glucose in mesophilic (MG) and thermophilic (TG) conditions, respectively. The reactors were equipped with magnetic stirring and three sample ports for liquid feeding and sampling, biogas collection and sparging with nitrogen. All were thermostatized with a water jacket. Inocula washing, reactor inoculation and sparging with nitrogen followed the procedure outlined above for the SMA tests, excluding the 12 h endogenous incubation period. Mixed liquor samples (10 mL) were collected at reaction times 15 min and 3 h, 6 h, 12 h, 24 h, 36 h and 48 h, filtered (Whatman GF/C membranes), and subsequently used for pH, COD and colour measurements. Tests were performed in triplicate and dye-free runs were used as controls. The initial COD level ranges were similar to those used for the SMA tests.

The COD and colour profiles along time, measured in the batch reactors, were both modelled assuming pseudo-first order kinetics [15–17]. This approximation implies negligible biomass growth during the 48 h experimental period and substrate concentrations well below the saturation level. Curve fitting software was used to calculate first order rate constants for COD (k_{COD}, h^{-1}) and colour (k_c, h^{-1}) removal.

2.4. Analyses

The values of pH, volatile suspended solids (VSS) and soluble chemical oxygen demand (COD) in liquid samples were determined according to standard procedures [18]. The colour was monitored by UV–vis spectrophotometry (Lambda 6, PerkinElmer USA) by measuring absorbance at the wavelength of maximum absorbance of the dye in the visible region (482 nm) against distilled water. Absorbance measurements were then linearly correlated with dye concentrations according to the Lambert–Beer law (absorptivity 0.0590 ± 0.0008 L mg^{-1} cm^{-1}). Dye-free media and filtered samples from dye-free runs showed negligible absorbance at this wavelength.

3. Results and discussion

3.1. Specific methanogenic activity assays

Fig. 2 depicts the time courses of cumulative biogas production (normalized to an initial COD concentration of 1000 mg/L) for mesophilic, glucose-grown (MG) and acetate-grown (MA) inocula from different feed compositions, including acetate, Archicolor Transfer (AT) and Avolan UL 75 (Av). Calculated specific methanogenic activity (SMA) values (also normalized) are given in Fig. 3. Table 1 presents the overall values for specific COD removal rate and yield of biogas on substrate COD ($Y_{sbiogas}$). These values were calculated for the total incubation periods (24 h, 48 h or 96 h, depending on the substrate) and are not normalized. For each inoculum and substrate, the influence of the AO7 dye (60 mg/L) was assessed. The assays with sulphide (acetate-fed) were included to test the experimental system's sensitivity to a known inhibitor [19], which indeed, could occur in anaerobic reactors treating these effluents, as a result of the biological reduction of dyebath sulphate salts.

Comparing graphs (a) and (d) in Fig. 2, it is apparent that the MG inoculum exhibited lower acetoclastic activity, requiring an adaptation period to start biogas production with the acetate substrate. Also, this acetoclastic population seems to be the major target of sulphide inhibition, extending the lag phase of the MG inoculum and significantly lowering SMA values (Fig. 3) for the MA inoculum. Values of $Y_{sbiogas}$ for both inocula are also lower in the presence of sulphide (Table 1). Nevertheless, the overall COD removal rate values with either inoculum were not significantly affected by the presence of sulphide (Table 1), hinting that a significant fraction of the measured COD removal is due to sorption or incorporation into the biomass, without conversion to biogas. $Y_{sbiogas}$ values for acetate were more than twice higher for MA than for MG, with or without sulphide. Thus, the $Y_{sbiogas}$ and SMA test results, even in this limited biogas quantification mode (no methane analyses), seem to be sensitive to inoculum and feed characteristics. On the other hand, sulphide originated from biological sulphate reduction could improve colour removal by providing the electrons required for azo dye cleavage. The redox half potential of the S^0/H_2S couple at pH 7 and 25 °C is −250 mV [16], much lower than, for instance, the redox half potential for AO7 azo bond reduction, which is −100 mV [17].

In the test conditions, the effect of AO7 on the SMA tests was always negligible, within the experimental error. Biogas yields could have been affected by AO7 via either a metabolic inhibition effect or the azo bond reduction process competing with methanogenesis for the available reducing equivalents. However, it should be noted that, in tests 1–4 (Table 1), the reducing equivalents (H^+ and electrons) needed to completely decolourise 60 mg AO7/L correspond to only 0.63% of the acetate–COD in the feed solution. In the SMA tests with added dye, the ratio between the available reducing equivalents in the tested carbon source loads and those required for complete dye reduction (R_{na}) ranged from 117 to 338 ($R_{na} = (COD_i\ (g/L) \times 4/32)/0.6838 \times 10^{-3}$, where 0.6838×10^{-3} is the number of moles of electrons necessary to reduce 60 mg AO7/L), indicating that COD was always in large excess with respect to the dye. Thus, the competition effect was not detectable. This excess could also explain the high colour removal yields obtained

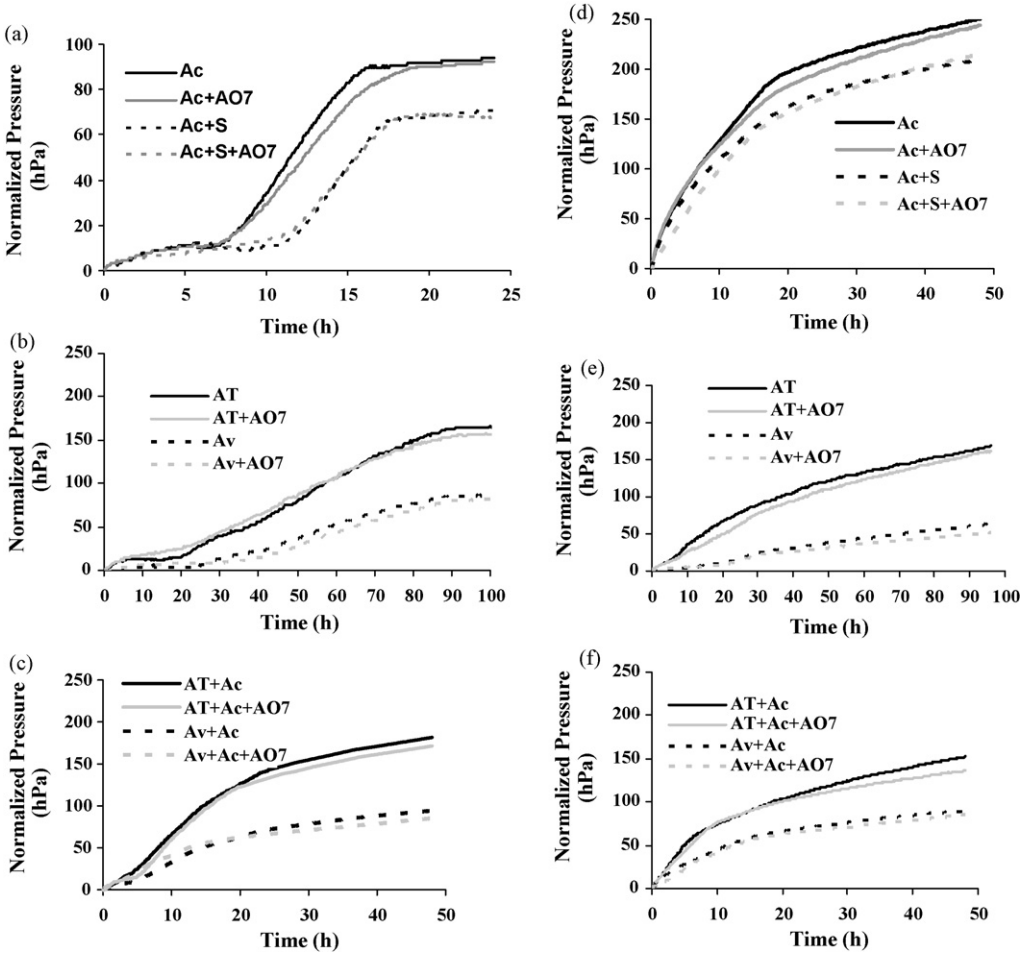

Fig. 2. Examples of time courses of cumulative biogas production (normalized to a COD of 1000 mg/L) for mesophilic, glucose-grown (MG; a–c) and acetate-grown (MA; d–f) cultures fed with different subtrates. Ac, acetate; AT, Archicolor Transfer; Av, Avolan UL75; S, sulphide; AO7, dye.

Table 1
Overall specific COD removal rate and yield of biogas on substrate for MG and MA populations, fed with different substrates. Standard deviations were calculated for triplicate assays and are shown in parenthesis. The values written in smaller size represent the incubation period taken to reach the maximum yield value measured (*h*). Assays 1, 3, 5, 7, 9 and 11 were with no dye added and assays 2, 4, 6, 8, 10 and 12 in the presence of 60 mg AO7/L. Initial COD values given exclude the dye. Biogas volumes are given at 1 atm and 25 °C.

Assay	Substrate	Initial COD (mg/L)	Specific substrate removal rate (overall) (mgCOD removed/(gVSS.d))				Yield of biogas on substrate (mL_{biogas}/gCOD removed)			
			MG		MA		MG		MA	
			without AO7	with AO7	without AO7	with AO7	without AO7	with AO7	without AO7	with AO7
1-2	*Acetate*	907 (34)	82 (5)	83 (7)	87 (4)	88 (5)	340 (25) 24	342 (27) 24	721 (76) 48	711 (95) 48
3-4	*Acetate + Na_2S*	921 (27)	81 (6)	81 (7)	83 (3)	87 (7)	265 (24) 24	256 (27) 24	637 (65) 48	640 (63) 48
5-6	*Archicolor Transfer*	641 (32)	28 (2)	29 (2)	24 (2)	25 (2)	507 (54) 96	492 (68) 96	630 (102) 96	588 (92) 96
7-8	*Avolan UL75*	1012 (48)	28 (4)	28 (2)	21 (2)	20 (4)	419 (87) 96	451 (65) 96	416 (66) 96	384 (76) 96
9-10	*Acetate + Archicolor Transfer*	1375 (52)	126 (7)	126 (7)	122 (10)	120 (8)	545 (46) 48	525 (43) 48	475 (53) 48	437 (50) 48
11-12	*Acetate + Avolan UL75*	1896 (84)	118 (9)	118 (10)	110 (10)	107 (9)	405 (44) 48	378 (49) 48	414 (54) 48	415 (68) 48

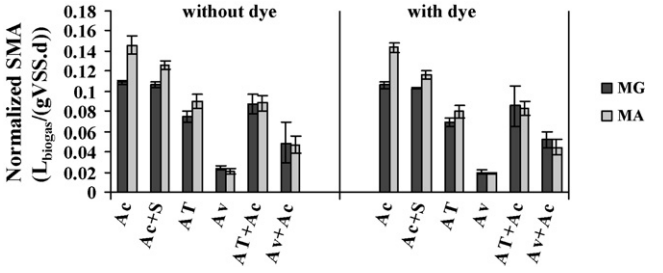

Fig. 3. Specific methanogenic activity (normalized to a COD of 1000 mg/L) values (SMA) for mesophilic, glucose-grown (MG) and acetate-grown (MA) cultures fed with different substrates. Ac, acetate; AT, Archicolor Transfer; Av, Avolan UL75; S, sulphide. Values are averages for three replicates with corresponding standard deviations (error bars).

with both cultures in the SMA experiments, ranging from 91% with the MG inoculum to 87% with the MA inoculum, for an incubation period of 48 h (data not shown). The metabolic pathway involved in azo dye decolourisation by MA cultures has been discussed by several authors. According to Santos, some mesophilic strains of methanogens incubated with acetate were able to reduce the dye Reactive Red 2 [20]. On the other hand, although inhibition by the dye was not detected in the present study, published results demonstrate that this is not always the case [21].

Fig. 2 shows the occurrence of lag phases for all test runs, except when acetate-containing substrates were fed to MA cultures. The surface active agents introduced the longest lag phases (Fig. 2b and e). With AT the MA culture adapts in 7 h while the MG culture lags for around 18 h. This result is accompanied by a better performance of the MA culture with AT, concerning SMA and $Y_{sbiogas}$, while the two inocula perform comparably with Av (Figs. 2 and 3 and Table 1). These results indicate a good capacity of the acetoclastic population for the conversion of AT to biogas. On the other hand, the overall specific COD removal rates for AT are similar for the two cultures (Table 1). This supports the idea that AT (much like acetate, as noted above) is removed from the solution by adsorption/incorporation onto the microbial granules, at essentially the same rate for the two inocula, but is much more efficiently converted to biogas by the inoculum having higher acetoclastic activity (MA). Nevertheless, the MG inoculum attains higher $Y_{sbiogas}$ values for AT than for acetate (Table 1), indicating the possibility of a non-acetoclastic route from AT to biogas. This could also be the reason for the lower $Y_{sbiogas}$ value for AT, with respect to acetate, when the MA culture is used.

Lipolysis of phospholipids (AT) generates fatty acids, glycerol, higher alcohols (e.g., choline, ethanolamine, serine) and phosphate. Glycerol and higher alcohols may be degraded by fermentative, acetogenic and methanogenic bacteria. Choline methyl groups are rapidly metabolised to trimethylamine by rumen microorganisms, which is further converted to methane [22]. Long-chain fatty acids are degraded by acetogenesis via β-oxidation to acetate and hydrogen [23,24]. Acetoclastic methanogenesis may thus be the preferable degradation pathway of phospholipidic compounds over hydrogenotrophic methanogenesis. The presence, in mesophilic inocula, of acetoclastic *Methanosarcina* sp. in high numbers (particularly in the MA culture), characterised by rapid growth and a versatile metabolism [25], could explain those results. However, the fatty acids resulting from lipolysis may also introduce an inhibition effect in acetoclastic methanogenesis [1].

The substrate Av leads to lag phase durations and values of the performance parameters not significantly different for the two tested inocula, suggesting that the activity of acetoclastic methanogens is not a limiting factor in the conversion of this substrate to biogas. Av also gives longer lag phases and lower SMA and $Y_{sbiogas}$ values than acetate or AT (Figs. 2 and 3, Table 1). An exception is the $Y_{sbiogas}$ values for the MG inoculum, which are higher for Av than for acetate. As with AT, non-acetoclastic methanogens thus seem to play a significant role in biogas production from this substrate. However, the overall substrate removal rates for Av are very close to those for AT, and similar with both inocula, again pointing to a sorption/incorporation mechanism as the first responsible for soluble COD removal. Considering its chemical nature (Section 2.2), Av may give origin to several types of anaerobic metabolites, arising from hydrolysis, acidogenesis and acetogenesis. The polyethylene glycol chain has been found to be the slowest part of synthetic surfactant molecules to be degraded under aerobic conditions [26]. A similar effect with anaerobic populations may explain why the biogas production rate values were lowest with Av and were apparently insensitive to the inoculum type. As far as possible inhibition effects are concerned, both acetogenic and acetoclastic anaerobic cultures have been found to suffer inhibition in the presence of alkylphenol ethoxylates, while the metabolic rates of fermentative and hydrogenotrophic microorganisms remained high [27]. Medium chain-length alcohol sulphates have been reported to cause inhibition of hydrolysis, acidogenesis and acetogenesis [28]. Acetic, propionic and butyric acids were found to be the main intermediates in the biodegradation of these substrates, with isobutyric and valeric acids as minor metabolites. Caproic, caprylic and capric acids were detected as specific alcohol sulphate metabolites [28]. These long chain fatty acids, namely capric and caprylic acids, may cause inhibition of acetoclastic methanogenesis. Synergistic effects between the several intermediates could also occur and contribute to enhance the inhibitory effect [1]. Since neither inoculum was adapted to Av, this surfactant itself or its metabolites were susceptible to cause inhibition.

In the tests with mixed carbon sources, acetate + AT and acetate + Av, the MG culture (Fig. 2c) exhibited a lag phase duration comparable to that of the run fed with only acetate (Fig. 2a, without sulphide), indicating that the culture adapts first to acetate. However, the advantage of the MA inoculum in shorter adaptation periods is not confirmed for SMA or $Y_{sbiogas}$ values. No significant differences in SMA between MA and MG were found (Fig. 3 and Table 1). For acetate + AT, the $Y_{sbiogas}$ values were actually lower for the MA than for the MG inocula, despite the opposite had occurred for the same substrates fed separately (Table 1). The overall specific substrate removal rate values for mixed substrates are very close to the sums of their values for single substrates (Table 1), suggesting that they are independently removed, again most likely by an initial step of sorption/incorporation. But, the values of SMA and $Y_{sbiogas}$, are not additive (Table 1). For the MA inoculum, biogas yield values for the mixtures are consistently lower than those for any of the isolated substrates.

The presence of the surface-active agents thus seems to partially impair the capacity of both inocula to convert acetate to biogas, possibly due to the inhibitory effects mentioned above towards the acetoclastic population. In this case, the MG inoculum, presumably richer in non-acetoclastic methanogens, would perform better (as observed from $Y_{sbiogas}$ values, Table 1). Inhibition effects such as those here noted are important factors to investigate, since substrate mixtures are likely to occur in dyebath effluents. These are further complicated by the batchwise nature of textile processing, with frequent additive changes, making culture adaptation difficult.

3.2. Batch kinetic studies in mesophilic and thermophilic conditions

The time courses of soluble COD and AO7 concentrations were followed in batch reactors fed with single and mixed substrates (the same as in Section 3.1) and inoculated with glucose-grown, mesophilic and thermophilic cultures (MG and TG, respectively).

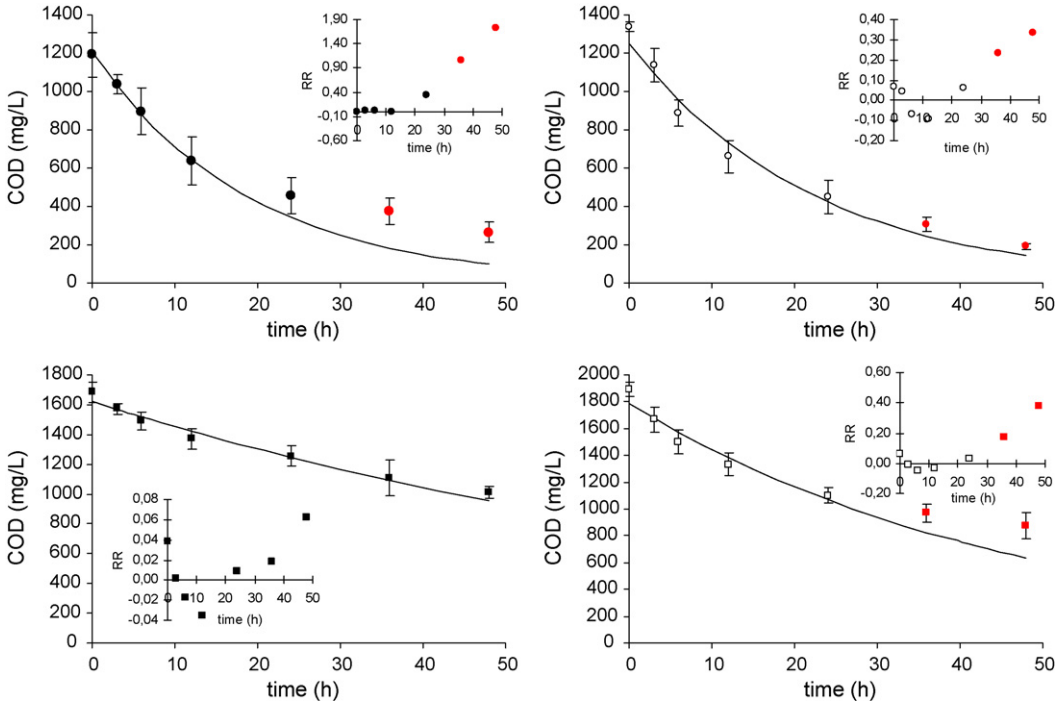

Fig. 4. Examples of the time course of residual COD for MG (●, ■) and TG (○, □) cultures fed with different substrates. Average values of three replicas and correspondent standard deviations (error bars) are given. Lines represent the best-fit first-order model and relative residual (RR or $\Delta y/y$), are also displayed. The fill red marks indicate values that were not used in the fitting. Substrates tested: acetate and Archicolor Transfer (●, ○); acetate and Avolan UL75 (■, □). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The soluble COD consumption measured during an incubation period of 48 h is the overall result of substrate incorporation into the biomass and partial bioconversion, including (as noted in the SMA experiments for MG) lag phases for biogas production. However, this overall process could be described by a pseudo-first order kinetic model (Fig. 4), with coefficients of determination (r^2) ranging from 0.957 to 0.999 (5–7 experimental data points). The decolourisation of the azo dye AO7 was, by means of spectral analyses in the UV–vis region and chromatographic detection of one of its metabolites (sulphanilic acid), found not to involve signifi-

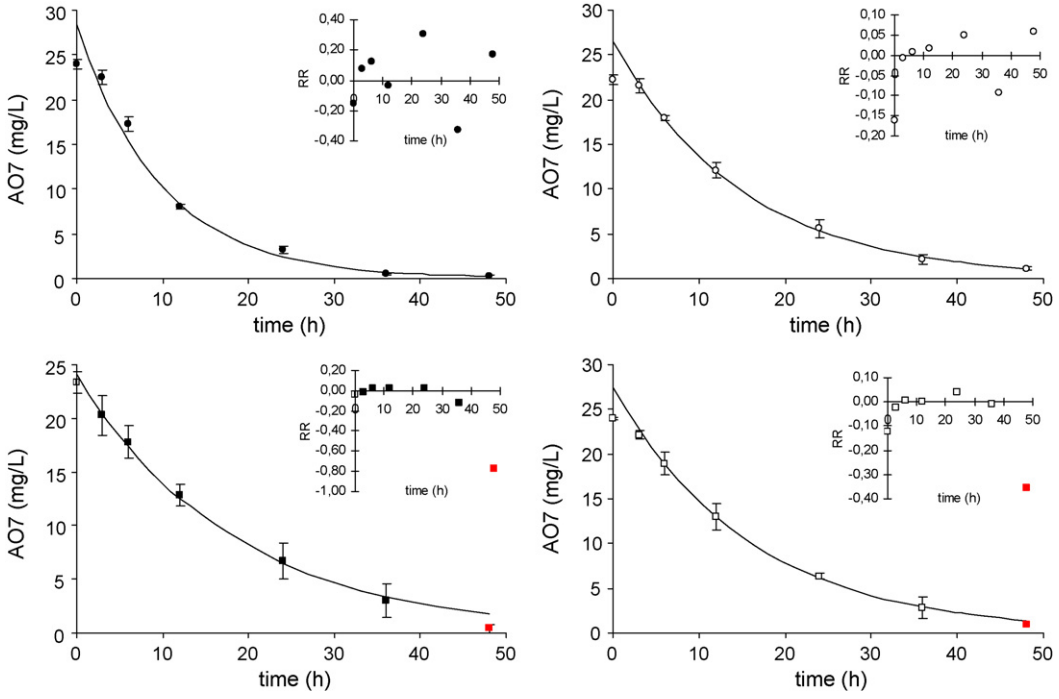

Fig. 5. Examples of the time course of residual dye (AO7) concentration for MG (●, ■) and TG (○, □) cultures fed with different substrates. Average values of three replicas and correspondent standard deviations (error bars) are given. Lines represent the best-fit first-order model and relative residuals (RR or $\Delta y/y$) are also displayed. The fill red marks indicate values that were not used in the fitting. Substrates tested: acetate and Archicolor Transfer (●, ○); acetate and Avolan UL75 (■, □). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

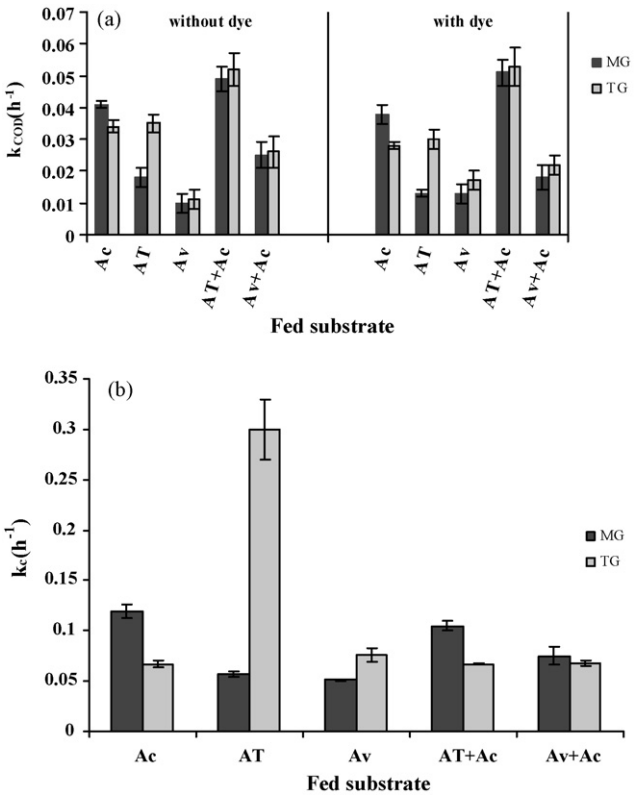

Fig. 6. Pseudo-first order kinetic constants for COD, k_{COD}, (a) and colour, k_C, (b) removal in assays performed with glucose-grown, mesophilic (MG) and themophilic (TG) cultures fed with different substrates. Ac, acetate; AT, Archicolor Transfer; Av, Avolan UL75. Values are averages for three replicates with corresponding standard deviations (error bars).

cant sorption onto the biomass. For the initial concentration used, the time course of dye removal could also be approximated as a pseudo-first order process (Fig. 5), with r^2 values from 0.965 to 0.998 (5–7 experimental data points). The calculated values of the pseudo-first order constants for the two processes (k_{COD} and k_c) are given in Fig. 6.

The k_{COD} values in Fig. 6a show that the presence of the dye had no significant effect on the apparent kinetics of substrate consumption, for any of the tested carbon sources. Also, comparing k_{COD} and k_c values, it can be noted that decolourisation was a much faster process than COD removal, at both temperatures. These results point to a simple, electron transfer reaction, probably extracellular, as the mechanism of AO7 azo bond reduction in the tested batch bioreactors. The source of reducing equivalents for azo bond reduction in anaerobic systems and the possible electron transfer reactions involved have not yet been completely established, but the possibility of both fermentative and methanogenic populations promoting dye reduction has been shown [11,12,20]. Santos et al. [20] have also found that, in a methanogenic consorcium, H_2 was a much better electron donor for azo dye decolourisation than acetate, particularly in the presence of a specific inhibitor of methanogenesis. Thus, the amount and rate of reducing equivalent production from the fed substrate, as well as the ability to convey them to the azo bond, diverting them from methanogenesis, seem to be important aspects of azo dye decolourisation in anaerobic systems [12].

In the present kinetic study experiments, most of the dye reduction took place during the first 20 h of incubation. As the used inocula were glucose-adapted, at least part of this period corresponds to the lag phase of biogas production reported in Section 3.1. For the thermophilic inoculum, adaptation could have been faster but this is not confirmed for acetate as substrate, as the MG culture displayed higher degradation rates than the TG culture (Fig. 6a). This is possibly due to the different types of acetoclastic methanogens present in each of the anaerobic consortia. According to Zinder et al., at mesophilic temperatures, rapidly growing acetoclastic *Methanosarcina* sp. are present in high numbers [25]. However, at higher temperatures (55 °C) another type of acetoclastic methanogenic culture is present, *Methanothrix* sp. [29]. The lower growth rate of *Methanothrix* sp. could explain the observed lower removal rate of acetate. On the contrary, with AT as the carbon source, the thermophilic inoculum exhibited faster COD consumption kinetics while, with Av and the two mixed substrates, the TG culture was only slightly faster, but not significantly different from the MG culture (Fig. 6a). The higher removal rate of AT could be attributed to its phospholipidic composition, since lipid degradation is favoured with thermophilic bacteria [8]. As both temperatures used in the present study are higher than the transition temperature of liposome lipids (below 0 °C), the continuous fluid state of these lipids maintains the vesicles without structural alterations [4]. However, high temperatures should cause an increase in fluidity, by decrease of molecular order (viscosity decrease). Thus, in the thermophilic range the interactions between liposomes and cell walls could be favoured, resulting in higher uptake rates. On the other hand, this could also enhance the inhibitory effect of AT on acetoclasts suggested in Section 3.1. Such inhibition could explain why the k_{COD} values with acetate + AT are not significantly higher for TG than for MG and are both lower than would be expected if the two substrates were uptaken independently (Fig. 6a). For instance, Ching-Shyung and Lettinga found that physicochemical surface associations between oleate and acetate-utilizing methanogens played an important role in intoxication of the latter [30]. For Av and acetate + Av, a similar reasoning can be applied in what concerns inhibitory effects, possibly worsened by the chemical nature of Av. The same authors pointed out that thermophilic acetate-utilizing methanogens seem to be more susceptible to inhibition effects than the mesophilic acetate-utilizing methanogens [30].

In the biodegradation tests, the production of reducing equivalents (for AO7 reduction) from acetate could have occurred through synthrophic oxidation, which is more favourable in the thermophilic range ($\Delta G_{25\,^\circ C}$ = +104 kJ/mol; $\Delta G_{55\,^\circ C}$ = +90.2 kJ/mol) [12]. In this range, the production of H_2 from the methyl group of acetate becomes much more important [8,31]. In thermophilic conditions acetate seems to contribute with 63–66% of the CH_4 generated, a percentage relatively lower than that found in mesophilic reactors, in which 60–90% of the methane is derived from the methyl group of acetate [32]. The development of an efficient hydrogenotrophic population, presumably already present in glucose-adapted inocula, would have made the H_2 reducing equivalents unavailable for dye reduction. The balance between H_2 production and consumption, with acetate as primary substrate, seemed to result in lower reducing equivalent availability at higher temperatures, since, for all tests in the presence of acetate, decolourisation was slower with the TG than with the MG population (Fig. 6b). However, with the surface active agents as single substrates, particularly AT (Fig. 6b), k_c values are significantly higher in thermophilic conditions. As mentioned above, these measurements took place mostly during a period when, presumably, methanogens were undergoing adaptation (biogas lag phase lasting 20 h for MG cultures, Fig. 2b). Thus, the reducing equivalents would have resulted mostly from the fermentative phase, as also observed by Santos et al. [20]. Moreover, it has been reported that the H_2 content resulting from the acidogenic step is higher for thermophilic than for mesophilic consortia [24]. The results in Fig. 6b suggest, therefore, that the better decolourisation performance of the TG inoculum with the single, AT and Av substrates derives from faster acidification, producing

reducing equivalents which were not readily used for methane production. Supplementing these substrates with acetate apparently eliminates this advantage of the thermophilic population (Fig. 6b). This could indicate that the fermentative pathway of AT and Av produces acetate, thus being negatively affected by its presence in the medium, particularly if the acetoclastic population responsible for its removal is inhibited.

4. Conclusions

The present study shows that different anaerobic consortia can be adapted, in reasonable time (1–2 days), to the conversion to biogas of varied substrates likely to constitute the major part of the organic load of wool dyeing effluents. Also, high decolourisation yields of a typical acid azo dye were shown to be obtainable with different substrate combinations, for all inocula. The results point to some basic ideas to be thus explored:

- Of the two surface-active agent types tested, the liposome-based additive generally led to faster uptake (particularly in thermophilic conditions) and better performance in biogas production (lag phase, SMA, biogas yield on substrate–COD) than the polyglycol ether-based additive; also, the former apparently involved acetoclastic methanogenesis as a rate-limiting step in its conversion to biogas, while the latter's conversion was not affected by the proportion of acetoclasts in the inoculum; these results suggest that the uptake and/or fermentative steps are rate-limiting in the biomethanation of the polyglycol surfactant, possibly due to the slow degradation of the ethoxylate chain.
- The fermentative step in the anaerobic conversion of the surface-active agents tested seems to contribute with most of the reducing equivalents for azo dye decolourisation; this result was apparent from the high colour removal yields obtained during the expected adaptation period of the inocula to the new substrates, with better performances at higher temperature; with acetate as the single substrate, the source of reducing equivalents is unclear, but less efficient in the thermophilic range.
- The association of acetate with either of the surface-active agents led to poorer performance, in terms of organic load removal, biogas production and decolourisation, than would have been expected from the correspondent results obtained with each of the mixture components fed separately; also, the thermophilic advantage observed with either surfactant for COD uptake and dye reduction kinetics is lost with acetate supplementation; thus, both surfactants seem to inhibit acetate conversion to biogas and, on the other hand, the presence of acetate appears to slow down surfactant biodegradation; the latter could be a thermodynamic effect, if the fermentative pathway for the surfactants produces acetate.

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