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INHIBITION OF ANAEROBIC DIGESTION BY TEREPHTHALIC ACID AND ITS AROMATIC BY PRODUCTS

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ABSTRACT

With 14.4 million tons produced in 1993, purified terephthalic acid (PTA) and dimethylterephthalate (DMT) are the main monomers used in the world polyester production. Even though there are ten industrial digestors treating the effluents of both products, little is known about the influence of their organic components, terephthalic (TA), p-toluic (p-tol), benzoic (BA) and phthalic (PA) acids as well as 4-carboxybenzaldehyde (4-CBA) on methanogenesis. This study shows that the concentrations of 4-CBA, p-tol and TA required to inhibit by 50% the hydrogenotrophic methanogenic activity are respectively 5.4, 34 and more than 100 mM. At the maximum concentration tested (10 mM), the inhibition of the acetoclastic methanogenic activity by the same compounds was less than 12%. The results indicate that at the concentration of TA, p-tol and 4-CBA found in PTA and DMT wastewater, no significant inhibition of the methanogenic activity should be observed for PTA but that the hydrogenotrophic activity could be reduced by as much as 30% in the case of DMT. The degradation of TA was completely inhibited by the presence of BA and glucose while the presence of phthalate had no effect. According to these results, it is concluded that it is at least possible to anaerobically treat the easily biodegradable compounds of these effluents (acetic, benzoic and formic acid) as they would not be significantly inhibited by TA, p-tol or 4-CBA. © 1997 IAWQ. Published by Elsevier Science Ltd

KEYWORDS

Anaerobic digestion, carboxybenzaldehyde, degradation , inhibition, p-toluic acid, terephthalic acid

INTRODUCTION

After a slow start-up, at the beginning of the 1980 decade, anaerobic digestion is being increasingly used for chemical and petrochemical wastewater treatment. Nowadays, at least 42 digestors distributed world-wide are operating on these kind of wastes (Macarie 1996). Anaerobic digestion is even on the way to become the conventional treatment form in the purified terephthalic acid (PTA) and dimethylterephthalate (DMT) industries. PTA (1,4-benzenedicarboxylic acid) and DMT are two of the main monomers used in the manufacture of polyester fibers, resins and films. In 1993, their production in the world reached 14.4 million tons per year and presented good perspectives of growth (Savostianoff and Didier, 1993). Both molecules are being obtained by a liquid phase air oxidation of p-xylene. PTA results however from a simple oxidation and a purification by hydrogenation, while DMT from a series of oxidations and esterifications with methanol

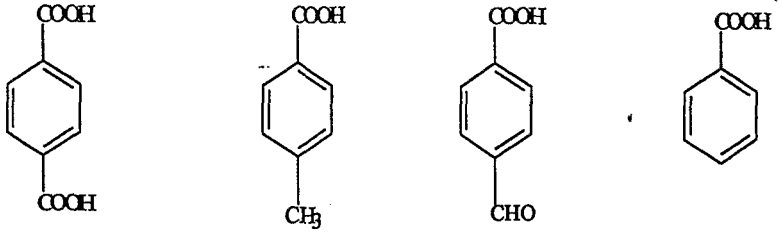
followed by a distillation (Bemis *et al.* 1982). The two processes generate wastewaters containing acetic (AA) and terephthalic acids (TA) as main pollutants and aromatic intermediates of p-xylene oxidation (p-toluic (p-tol) and benzoic (BA) acids) (Tab. 1, 2). Other aromatic compounds such as phthalic (PA, 1,2-benzenedicarboxylic acid), isophthalic (1,3-benzenedicarboxylic acid), o-toluic (2-methylbenzoic acid) and trimellitic (1,2,4-benzenetricarboxylic acid) acids may be present in low concentrations. They result from the oxidation of p-xylene impurities (like pseudocumene, ethylbenzene) or from side reactions (Ichikawa and Takeuchi, 1972, Roffia *et. al.*, 1984). DMT effluents contain also a high concentration of methanol, formic acid and formaldehyde.

Table 1. Characterization of the PTA and DMT wastewaters.

	PTA		DMT	
	g/l	% COD	g/l	% COD
pH	4.5	-	1.6 - 3	-
COD	4.4 - 9.5	-	17 - 142	-
COD/BOD_5	1.4 - 1.7	-	1.14	-
TA	1.1 - 2.67	31 - 39	0.4 - 0.5	1.4 - 3.8
p-tol	0.3 - 0.7	10 - 19	1.14 - 2	8 -14
BA	0.05 - 0.45	1 - 13	0.05 -1.5	0.6 - 5.9
4-CBA	0.02 - 0.04	0.36 - 1	0	0
AA	0.5 - 2	5.3 - 30	2.5 - 41	11 - 61
Methanol	0	0	0.5 - 76	1.8 - 80
Formic acid	0	0	1.2 - 13	0.3 - 9.1
Formaldehyde	0	0	1.45 - 7.6	3.4 - 21

After: Leenheer *et al.* 1976, Reule 1990, Liangming *et al.* 1991, Macarie *et al.* 1992, Pereboom *et al.* 1994, Frankin *et al.* 1994, Sharma *et al.* 1994, TA= terephthalic acid, p-tol = p-toluic acid, BA= benzoic acid, 4-CBA= 4-carboxybenzaldehyde, AA= acetic acid.

Table 2. Some characteristics of the aromatics present in PTA and DMT wastewaters.



Name:	**Terephthalic acid**	**p-Toluic acid**	**4-carboxybenzaldehyde**	**Benzoic acid**
MW:	166	136	150	122
COD eq:	1.44	2.11	1.7	1.97
Water solub	0.0019 at 25°C	-	very soluble	0.2 at 17°C
(g/100g)	0.04 at 100°C	1.3 at 100°C	in hot water	2.2 at 75°C
pK:	3.54, 4.46	4.36	-	4.2

CODeq: g of COD exerted/g of compound.

In spite of the fact that 10 industrial reactors are treating these two types of effluents, there is still few information with regards to the impact that fluctuations in the concentration of their different organic components have on methanogenesis. In the case of PTA wastewater for instance, peak concentrations can reach double of the normal values (Lau 1978, Pereboom *et al.* 1994). It is already known that a high concentration of acetic acid inhibit benzoic and p-toluic acid degradation (Dolfing and Tiedje 1988, Macarie

and Guyot 1992) and that TA has no effect on BA degradation (Pereboom *et. al.* 1994). Recent experiments have indicated also that TA methanization can be affected by the presence of hydrogen as well as acetic and benzoic acid (Kleerebezem *et. al.*, 1996). Toxicity data are available only for benzoic, phthalic and terephthalic acid. The two first compounds have not shown any toxicity to acetoclastic methanogens until the highest concentration tested (7 g BA/l by Sierra-Alvarez *et. al.*, 1990, 4 g PA/l by Chou *et. al.*, 1978). Contradictory results have been reported for TA. While no inhibition to any of the species involved in its degradation was observed by some authors at concentrations as high as 5 g/l (Pereboom *et. al.*, 1994, Kleerebezem *et. al.*, 1996), others have found toxicity at concentrations as low as 0.5 g/l (Kuang and Wang, 1994) and 2.16 g/l (Macarie *et. al.*, 1992). The impact that the other aromatics, present in the wastewaters, might have on the acetoclastic and hydrogenotrophic methanogens remains completely unknown as well as, how, the different aromatics interfere in the degradation of the others.

The objectives of this paper are (1) to generate information about the toxicity of p-toluic acid and 4-CBA to the methanogenic bacteria, (2) to clarify the apparent contradiction on the toxicity data reported for TA, (3) to confirm that H_2, acetate and benzoate inhibit TA degradation and (4) to determine how phthalic acid influence TA degradation. All these data will be discussed with respect to the operation of the digesters.

MATERIAL AND METHODS

Biomass. Three different methanogenic granular sludge were used as inocula. The sludge of a full scale UASB reactor treating cheese wastewater and the sludge of other full scale UASB reactor operating on sewage served respectively to determine the toxicity of aromatic compounds to hydrogenotrophic and acetoclastic methanogens. These 2 sludges were not aclimated to any of the aromatics present in PTA or DMT wastewater prior to the experiment. The second sludge was however maintained on glucose and acetate before use. The test of TA degradation in the presence of other compounds was performed with a sludge, sampled originally from a lab scale reactor treating PTA wastewater, and fed several months in batch condition with a mixture of TA, 4-CBA and p-tol at 100 mg/l.

Inhibition and cosubstrate tests. The hydrogenotrophic, acetoclastic and cosubstrate tests were performed respectively in 120 ml and 60 ml serum bottles filled with 10 and 20 ml of medium containing 0.863, 3.48 and 2.68 g VSS/l of sludge and 0.863 atm of H_2/CO_2 (80/20), 12 mM (0.72 g/l) acetate and 2.3 mM (0.4 g l) of TA as substrate. For the toxicity test, TA, p-tol and 4-CBA were added from neutralized concentrated anoxic mother solutions in order to get final concentrations between 0 and 100 mM. In the cosubstrate test, glucose, BA and PA were added respectively at 3.6 (0.65 g/l), 2.7 (0.34 g l) and 2.7 mM (0.45 g/l). Glucose was used as a precusor of hydrogen and acetate. The anaerobic technique and the medium number 1 described by Balch *et. al.* (1979) were used throughout the study to dilute the different granular sludges to the required VSS concentrations. All bottles were incubated in the dark at 36°C under agitation on an orbital shaker set up at 300 rpm for the hydrogenotrophic activity and 100 rpm for the other experiments. The hydrogenotrophic test was performed with a large atmosphere to liquid volume ratio and a high agitation speed in order to promote a strong hydrogen transfer from the gaseous to the liquid phase. The impact of BA, PA and glucose on TA degradation was monitored by following the concentration of all the compounds. The acetoclastic and hydrogenotrophic activities were determined by measuring acetate consumption and methane production respectively. The percentage of Inhibition (%I) was calculated as: $\%I = 100 * (K_O - K_I) / K_O$ were K is the specific methanogenic activity (as g AA/ g VSS.d or mmole CH_4/g VSS.d) subindex o for the control without aromatics and I for the bottles containing the inhibitors. All tests were done by triplicate. With the experimental conditions choosen, the concentration of acetate (12 mM) and dissolved hydrogen (0.5 mM at time zero, 0.2 mM at the end of incubation) were sufficiently high compare to the Km of the acetoclastic *Methanosaeta concilii* (1.2 mM, Patel, 1984) and of the hydrogenotrophic methanogens ($7.6\ 10^{-6}$ to 0.08 mM, Giraldo-Gomez *et al.*, 1992) to obtain a zero order kinetic.

Analyses. The volatile suspended solids (VSS) of the different sludges were determined on the pellet obtained after centrifugation of the samples at 5000 rpm as indicated in chapter 2540 of Standard Methods (Apha, 1992). The methane content of the serum bottle atmosphere and the concentration of acetate in their liquid

phase were measured by gas chromatography as previously described (Macarie and Guyot, 1992). Liquid samples were acidified to pH 2 before analysis. The concentrations of TA, BA and PA were followed by HPLC after filtration of the samples on a 0.22 µm membrane. The equipment consisted in a 10 µl loop injector, a membrane degasser, 2 constametric pumps (3200 and 3500), a µBondapak™ C18 µm column (300 x 4.5 ID mm) and a spectronic 4100 UV detector set at 254 nm. The column was maintained at ambient temperature. Two solvents were used to get a good resolution of the peaks (solvent A: 5% (V/V) acetic acid in water, solvent B: 5% (V/V) methanol in water.). They were pumped separately at the respective rate of 1.791 and 0.199 ml/min. Before use, the solvents were filtered on a 0.45 µm membrane and their pH adjusted to 3.5 with NaOH. Chromatograms were stored and integrated by an American Megatrend 486 DX computer equiped with a TCTalk software. Glucose was determined with a commercial enzymatic colorimetric test (GOD-PAP)

Chemicals. All reactives used for the culture medium were of analytical grade (disodium terephthalate and 4-CBA, Aldrich; p-toluic acid and sodium benzoate, Sigma and phthalic acid monopotassium salt (potassium biphthalate), Baker; minimum purity of 96%) except the peptone and yeast extract.

RESULTS AND DISCUSSION

Toxic levels of 4-CBA, p-tol and TA to methanogenesis

All aromatic compounds used during this test were added as sodium salts. Sodium is known to be toxic to methanogens at concentrations higher than 200 mM (Kugelman and Chin, 1971). This upper limit was reached only in the cultures receiving 100 mM disodium terephthalate. The aromatic toxicity could then be evaluated independently to that of sodium.

Hydrogenotrophic methanogenesis. Figures 1 and 2 show the effect of 4-CBA, p-tol and TA on the methane production from H_2/CO_2. 4-CBA presented the strongest inhibitory effect followed by p-tol and TA.

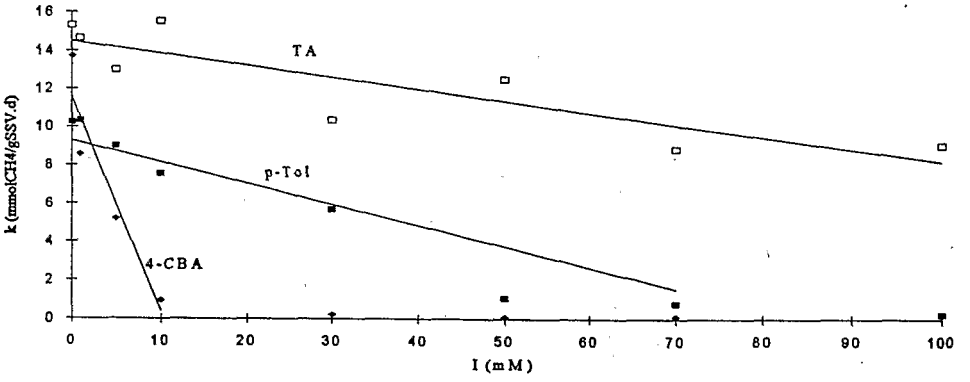


Figure 1. Hydrogenothrophic methanogenesis inhibition by TA, p-Tol y 4-CBA considering a zero order kinetics. The lines are lineal adjustments with $r^2 = 0.738$, 0.888 and 0.896 respectively.

The concentration for which they caused 50% inhibition (IC_{50}) were respectively 5.4 (0.8 g/l), 34 (4.6 g/l) and more than 100 mM (16.6 g/L). This order of inhibition (4-CBA > p-tol > TA) is in agreement with the increase of toxicity observed by others (Sierra-Alvarez and Lettinga, 1991) when a formyl, a methyl and a carboxyl group are branched on a benzene ring. Formyl radicals are highly reactives due to the existence of the π bond between the carbon and the oxygen, the polarization of this bond and the presence of 2 free electrons on the oxygen. They can participate in reactions of substitution and nucleophilic additions. Particularly, they may react with the amino groups of proteins and denaturate them. Methyl substituents are

not reactives, but increase the lipophilicity of the molecules. It is then logical that the IC_{50} obtained for 4-CBA (4-formylbenzoic acid) and p-toluate (4-methylbenzoic acid) were lower than the one reported by Sierra-Alvarez and Lettinga (1991) for the inhibition of acetoclastic methanogens by benzoate (not toxic at 57.3 mM). It should be noticed that the presence of a second carboxyl group on TA did not increase its toxicity compared to BA and that this is in accordance with the absence of toxicity of its isomer, phthalic acid, at a concentration as high as 24 mM (Chou *et al.*, 1978).

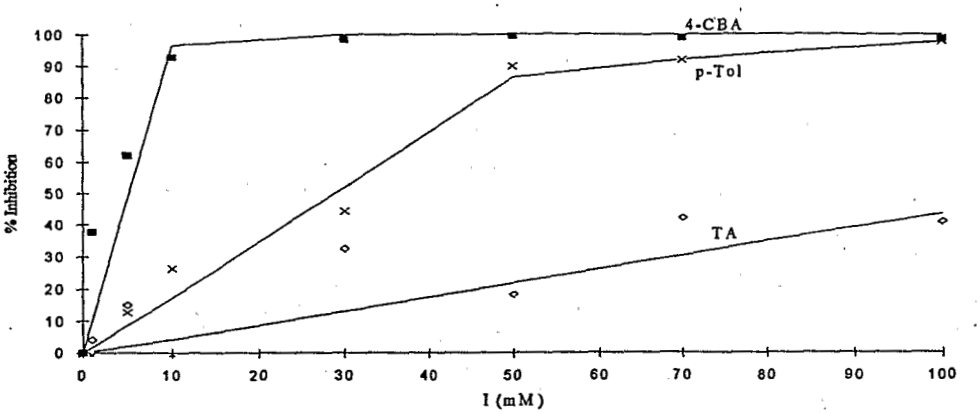


Figure 2. Relative inhibition of hydrogenothrophic methanogenesis by TA, p-Tol and 4CBA.

Acetoclastic methanogenesis. The toxicity was tested only until 10 mM (1.66 g TA/l, 1.36 g p-tol/l, 1.5 g 4-CBA/l). In this range, the 3 compounds presented a similar behaviour and caused less than 12% inhibition (data not shown). At the same concentration, the hydrogenotrophic activity was already reduced by 90% for 4-CBA and 25% for p-tol. The acetoclastic methanogens appear then to be less sensitive to this kind of molecules than the hydrogenotrophic one. In a previous study, Patel *et al.* (1991) showed that *Methanosaeta concilii*, the main acetoclastic methanogen found in anaerobic digesters, and *Methanospirillum hungatei*, both sheathed bacteria, were more resistant to sodium benzoate than unsheathed methanogens. Except *Methanospirillum hungatei*, all hydrogenotrophic methanogens are unsheathed bacteria. This morphological difference could then be the base for the different resistance observed here. The difference may come also from the low biomass amount (0.868 g/l) used during the hydrogenotrophic test in order to have a hydrogen consumption rate lower than the physical rate of hydrogen transfer from the gaseous to the liquid phase. Actually, the ratio of toxicant to biomass was 4 time higher in the hydrogenotrophic than the acetoclastic test.

Potential inhibitory level in real conditions of digester operation. The previous results indicate that at the concentrations normaly found in PTA wastewater (0.27 mM 4-CBA, 5.14 mM p-tol, 16 mM TA, Tab. 1), but also during peak conditions (5.5 mM p-tol, 30 mM TA, Pereboom *et al.*, 1994), the aromatic compounds, once neutralized, should not affect significantly the methanogenic activity of the digesters (less than 10% inhibition of both the hydrogenotrophic and acetoclastic activity). This is consistent with the observations of Pereboom *et al.* (1994) that highly fluctuating concentrations of TA and p-tol had no influence at all on the breakdown of benzoate and acetate by a 7000 m^3 UASB reactor operating on PTA wastewater. The situation could be different in the case of DMT effluents since the amount of p-toluate (14.7 mM) is theoretically sufficient to inhibit by 30% the hydrogenotrophic and by more than 10% the acetoclastic activities. The consequences on digester performances could be dramatic because formate and acetate represent up to 70% of the COD of this effluent (Tab. 1). It must be emphasized however that all the toxicity test were performed with unaclimated sludge and that the inhibitory levels in real conditions with adapted sludge should be lower.

Degradation of TA in presence of other compounds.

The sludge used for this experiment was able to metabolize respectively disodium terephthalate and sodium benzoate at the rate of 17 and 87 mg / g VSS. day. As previously observed by Kleerebezem *et al.* (1996), the

presence of benzoate and of a sugar completely inhibited TA degradation (Fig. 3). The degradation recovered after the complete consumption of BA, but not in the case of glucose.

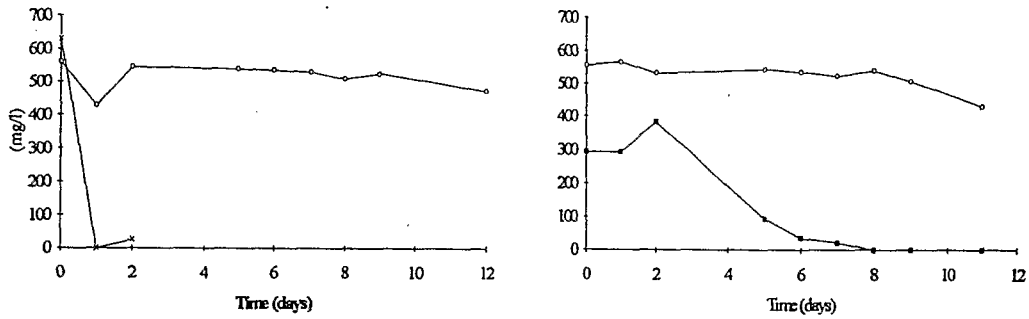


Figure 3. TA (O) degradation under the presence of glucose (x) and sodium benzoate (■).

As suggested by Aftring *et al.* (1981), terephthalic acid degradation in anaerobiosis proceeds probably through a decarboxylation of TA to BA (reaction 1), BA being subsequently degraded by a syntrophic association similar to that described for *Syntrophus buswellii* (Mountfort *et al.*, 1984) (reaction 2). The two reactions are probably carried out by one or two different microorganisms.

$TA^{2-}_{aq} + H_2O_l$ -----> $BA^-_{aq} + HCO_3^-{}_{aq}$ $\Delta G^{\circ\prime} = -19.86$ kJ (1)

$BA^-_{aq} + 7\,H_2O_l$ -----> $3\,acetato^-_{aq} + 3\,H^+_{aq} + HCO_3^-{}_{aq} + 3\,H_{2\,g}$ $\Delta G^{\circ\prime} = +63.32$ kJ (2)

$TA^{2-}_{aq} + 8\,H_2O_l$ -----> $3\,acetato^-_{aq} + 3\,H^+_{aq} + 2\,HCO_3^-{}_{aq} + 3\,H_{2\,g}$ $\Delta G^{\circ\prime} = +43.21$ kJ (3)

The decarboxylation step is thermodynamically favorable under standard conditions while the conversion of BA to acetate and H_2 is a highly endergonic process. As a consequence, equation 2, but also the global conversion of TA to H_2 and acetate (reaction 3) becomes exergonic only when acetate and H_2 are at very low concentrations (The Gibbs free energy of the reactions were calculated from the ΔG_f° tabulated by Thauer *et al.* (1977) except for TA^{2-}_{aq} and BA^-_{aq}. Their ΔG_f° at 25°C were estimated to be respectively equal to -547.58 and -217.77 kJ/mol using the contribution group method proposed by Mavrovouniotis, 1991). The fermentation of glucose by methanogenic granular sludge results usually in the production of H_2 and acetate which may accumulate in the serum bottles and cause an increase of ΔG in reaction 3 inhibiting TA degradation. Calculations of the ΔG of this equation using the *in situ* conditions indicate however that such inhibition is improbable since the concentration of acetate or H_2 required to reach endergonic conditions (100 atm of H_2 if [AA] = 0.1 mM, 100 mM AA if H_2 pressure = 0.1 atm) are unlikely to be obtained from the fermentation of 0.5 g glucose/l. The in situ ΔG of TA decarboxylation was also highly exergonic (-29.33 kJ/reaction = $\Delta G^{\circ\prime}$ + RT ln ($[BA^-]\,[HCO_3^-] / [TA^{2-}]$)) for the experimental conditions used (36°C, pH 7, $[TA^{2-}]$ = 2.285 mM, $[BA^-]$ 3.345 mM, $[HCO_3^-]$ = 16.7 mM) and more than 310 M of benzoate would have been required to obtain a positive ΔG. The previous comments indicate that thermodynamics are not sufficient to explain the observed inhibitions which are probably more the result of product inhibition or diauxic growth as hypothesized by Kleerebezem *et al.* (1996). Diauxic growth preferentially on BA than TA is however difficult to justify since the $\Delta G^{\circ\prime}$ of reaction 3 is less endergonic than that of reaction 2 indicating that TA should be a privilegiated substrate.

TA degradation has been difficult to obtain in presence of BA and acetate as shown in these batch experiments. Similar results have been obtained in lab and full scale reactors operating on real or artificial PTA and DMT wastewaters (Reule, 1990, Pereboom *et al.*, 1994, Kleerebezem *et al.*, 1996). A similar behaviour has been observed for p-toluate. The suggestion of Macarie and Guyot (1992) and Kleerebezem *et*

al. (1996) to use a two stage reactor could be a good solution for this type of effluents, the first stage being used to eliminate the compounds inhibiting TA and p-tol degradation and the second to remove the aromatics.

Contrarily to glucose and BA, the presence of phthalic acid had no effect on the degradation of TA. PA was not degraded during the 12 days of incubation (Fig. 4). This shows that a sludge adapted to metabolize one of the phthalate isomers (o, m, p) is not necessarily co-adapted to the degradation of the others. Specific overloading of phthalic acid should not affect the operation of digesters treating PTA and DMT wastewaters.

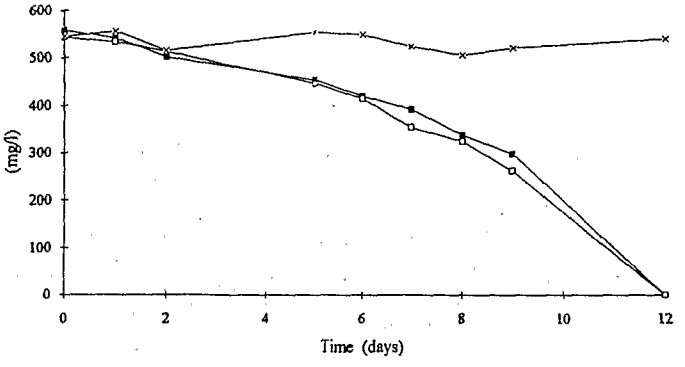


Figure 4. TA degradation in the presence of potassium biphthalate (□) and alone (■) show the same kinetics. PA remains undegraded (x) and non-inhibitory.

CONCLUSIONS

The easily biodegradable compounds (acetic, benzoic and formic acid) found in PTA and DMT wastewaters can be methanized without problem during anaerobic treatment since their degradation is not significantly inhibited by 4 carboxylbenzaldehyde as well as terephthalic and p-toluic acid. For PTA wastewater, COD removals of 40-50% may then be readily obtained at full scale. Higher COD removals would imply terephthalic acid degradation which can be obtained only after prior removal of acetic and benzoic acids from the effluent. A two stage reactor may then be recommended in order to attain such objective.

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REFERENCES

Aftring, R. P., Chalker, B. E. and Taylor, B. F. (1981). Degradation of phthalic acids by denitrifying, mixed cultures of bacteria. *Appl. Environ. Microbiol.*, 41 (5), 1177-1183.

APHA, AWWA and WPCF. (1992). *Standard Methods for the Examination of Water and Wastewater*, 18th edition, Washington D. C.

Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R. Wolfe, R. S. (1979). Methanogens: reevaluation of a unique biological group. *Microbiol. Rev.*, **43** (2), 260-296.

Bemis, A. G., Dindorf, J. A., Horwood B., Samans C. (1982). Phthalic acids and other benzene polycarboxylic acids. In: *Kirk Othmer Encyclopedia of Chemical Technology*. H. F. Mark, D. F. Othmer, C. G. Overberg, G. T. Seaborg, M. Grayson and D. Eckroth (eds)., Vol 17, John Wiley and Sons, New York. USA, pp. 732-777.

Chou, W. L., Speece, R. E., Siddiqi, R. H. and McKeon K. (1978). The effect of petrochemical stucture on methane fermentation toxicity. *Prog. Wat. Technol.*, **10** (5/6), 545-558.

Dolfing, J. and Tiedje, J. M. (1988). Acetate inhibition of methanogenic, syntrophic benzoate degradation. *Appl. Environ. Microbiol.*, **54** (7), 1871-1873.

Frankin, R. J., Koevoets, W. A. A., Versprille, A. I. (1994). Application of the Biobed system for formaldehyde containing dimethylterephthalate (DMT) wastewater. In: *Poster Papers, VII Int. Symp. on Anaerobic Digestion*. 23-27 January 1994, Cape Town, South Africa, pp. 244-247.

Giraldo-Gómez, E., Goodwin, S., Switzenbaum, M. (1992). Influence of mass transfer limitations on determination of the half saturation constant for hydrogen uptake in a mixed culture CH_4-producing enrichment, *Biotechnol. Bioeng.*, **40** (7), 768-776.

Ichikawa, Y. , Takeuchi Y. (1972). Compare pure TPA processing. *Hydrocarbon Process.*, **51** (11), 103-108.

Kleerebezem, R., Mortier, J., Hulshoff Pol, L.W. and Lettinga, G. (1996). Anaerobic pre-treatment of petrochemical effluents: terephthalic acid wastewater. In: *Proc. 2nd Spec. Conf. on Pretreatment of Industrial Wastewaters*. In Press

Kuang, X. and Wang, J. (1994). Anaerobic biodegradability of terephthalic acid and its inhibitory effect on anaerobic digestion. *J. Environ. Sci.*, **6** (2), 180-188.

Kugelman, Y. J. and Chin, K. K. (1971). Toxicity, synergism and antagonism in anaerobic waste treatment processes. *Adv. Chem. Ser.*, **105**, 55-90.

Lau, C. (1978). Staging aeration for high efficiency treatment of aromatic acids plant wastewater. *Proc. 32nd Ind. Waste Conf. Purdue University*, pp. 63-74.

Leenheer, J. A., Malcolm, R. L., White, W. R. (1976). Investigation of the reactivity and fate of certain organic components of an industrial waste after deep well injection. *Env. Sci. Technol.*, **10** (5), 445-451.

Liangming, X., Yuxiang, C. and Xiangdong, Z. (1991). The anaerobic biological treatment of high strength petrochemical wastewater by hybrid reactor. In: *Proc. Int. Conf. Petrol. Ref. Petrochem. Process.* September 11-15, H. Xianglin (ed.), Int. Acad. Pub., Beijing, China, pp. 120-126.

Macarie, H. and Guyot, J. P. (1992). Inhibition of the methanogenic fermentation of p-toluic acid (4-methylbenzoic acid) by acetate. *Appl. Microbiol. Biotechnol.*, **38**, 398-402.

Macarie, H. (1996). Anaerobic digestion, an adequate technology for the treatment of some chemical and petrochemical wastewaters. In *Memorias del IV Taller Latinoamericano Sobre Tratamiento Anaerobio de Aguas Residuales*. 19-22 de Noviembre, O. Rojas, L. Acevedo (eds.), Universidad Industrial de Santander, Bucaramanga, Colombia. pp. 313-323. (in spanish).

Macarie, H., Noyola, A. and Guyot, J. P. (1992). Anaerobic treatment of a petrochemical wastewater from a terephthalic acid plant. *Wat. Sci. Tech.*, **27** (7), 223-235.

Mavrovouniotis, M. L. (1991). Estimation of standard Gibbs energy changes of biotransformation. *J. Biol. Chem.* **266** (22), 14440-14445.

Mountfort, D. O., Brulla, W. J., Krumholz, L. R., Bryant, M. P. (1984). *Syntrophus buswellii* gen. nov. spec. nov., a benzoate catabolizer from methanogenic ecosystems. *Int. J. Syst. Bacteriol.*, **34** (2), 216-217.

Patel, G. B. (1984). Characterization and nutritional properties of *Methanothrix concilii* sp. nov., a mesophilic, aceticlastic methanogen. *Can. J. Microbiol.*, **30**, 1383-1396.

Patel, G. B., Agnew, B. J. and Dicaire, C. J. (1991). Inhibition of pure cultures of methanogens by benzene ring compounds. *Appl. Environ. Microbiol.*, **57** (10), 2969-2974.

Pereboom, J. H. F., De Man, G. and Su, Y. T. (1994). Start-up of full scale UASB-reactor for the treatment of terephthalic acid wastewater. In: *Poster Papers, VII Int. Symp. on Anaerobic Digestion*. 23-27 January 1994, Cape Town, South Africa. pp. 307-312.

Reule, W. (1990). Methane from chemical industry wastewater. *Chem. Ind.* (Dusseldorf), **113** (9), 20, 22, 24

Roffia, P., Calini, P. and Motta, L. (1984). Byproduct identification in the terephthalic acid production process and possible mechanism of their formation. *Ind. Eng. Chem. Prod. Res. Dev.*, **23**, 629-634.

Savostianoff, D. and Didier, R. (1993). DMT-TPA. Asia advances towards a crushing domination. *Informations chimie*, **352**, 119-129.

Sharma, S., Ramakrishna, C., Desai, J. D., Bhatt, N. M. (1994). Anaerobic biodegradation of a petrochemical waste-water using biomass support particles. *Appl. Microbiol. Biotechnol.*, **40**, 768-771.

Sierra-Alvarez, R. and Lettinga, G. (1991). The effect of aromatic structure on the inhibition of acetoclastic methanogenesis in granular Sludge. *Appl. Microbiol. Biotechnol.*, **34**, 544-550.

Thauer, R. K., Jungermann, K. and Decker K. (1977). Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev.*, **41** (1), 10-180.