

Inhibition of the methanogenic fermentation of *p*-toluic acid (4-methylbenzoic acid) by acetate

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Abstract. The potential inhibitory effect of acetate on *p*-toluic acid methanogenic fermentation was studied during the continuous operation at 5.3 days hydraulic retention time of an upflow anaerobic sludge blanket reactor fed with a synthetic waste-water containing 3.67 mM *p*-toluic acid as sole carbon and energy source. In the absence of acetate, a chemical oxygen demand removal efficiency of 56.8% and an estimated *p*-toluic acid removal efficiency of 62.8% were achieved. Immediately after the addition of 58.3 mM acetate into the reactor influent, *p*-toluic acid degradation stopped while most of the acetate was consumed. The inhibition is explained by thermodynamic considerations. It is emphasized that such phenomena could occur during the treatment of waste-waters containing high concentrations of acetate and aromatic compounds that require a syntrophic association to be degraded to acetate and H₂.

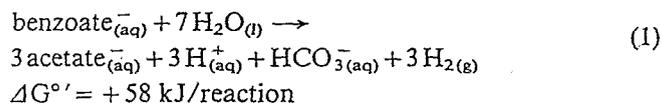
Introduction

Recently, anaerobic treatment has been applied successfully to chemical industrial waste-waters containing phenol or benzoate (Borghans and van Driel 1988; Frankin et al. 1991) as well as to petrochemical effluents resulting from the production of terephthalic acid (1,4-benzenedicarboxylic acid) (Guyot et al. 1990a; Noyola et al. 1990; Macarie et al. 1992) and dimethylterephthalate (DMT) (Reule 1990; Frankin and Koevoets 1991). Beside mono-substituted benzenes and benzoic acids, the major pollutant in these wastes is acetic acid. For instance, the chemical oxygen demand (COD) of DMT waste-water (20–50 g O₂/l) is 49–57% due to acetic acid and only 16–19% to aromatic compounds (terephthalic, phthalic, *p*-toluic, benzoic acids, etc.) (Leenheer et al. 1976; Reule 1990).

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It is generally admitted that the methanogenic fermentation of mono- or bi-substituted but mono-hydroxylated benzenes depends on a syntrophic association between (1) acetogenic bacteria that convert the aromatic compound to acetate and H₂ and (2) hydrogenophilic methanogens that convert H₂/CO₂ to methane (Holliger et al. 1988). The cleavage of these aromatic compounds to acetate is highly endergonic under standard conditions, as illustrated for benzoic acid (Eq. 1) [the variation of Gibbs free energy (ΔG) was calculated from $\Delta G_{r^{\circ}}$ tabulated by Thauer et al. (1977) except for benzoate (Thauer and Morris 1984)].



Hydrogen (H₂) is the key compound that controls the thermodynamics of these reactions. It must be continuously removed by a hydrogenotroph in order to maintain an H₂ partial pressure for which the $\Delta G'$ of the reaction becomes exergonic (i.e. pH₂ < 10⁻⁴ atm for benzoate, pH₂ < 10⁻² atm for phenol). Quantitatively, acetate is, after H₂, the second product of the metabolism of acetogenic aromatic degraders. Its role in the thermodynamics of the reactions that these bacteria catalyse has been generally neglected. However, Dolfing and Tiedje (1988), using a batch syntrophic culture of a benzoate degrader and a hydrogenophilic methanogen (*Methanospirillum hungatei*), showed that Eq. 1 was inhibited by addition of high concentrations of acetate, whereas the H₂ partial pressure was adequate. They concluded that acetate was thermodynamically equivalent to H₂. The effect of acetate concentration on the ΔG of Eq. 1 calculated with the in situ conditions used by Dolfing and Tiedje (1988) confirms that the energy available for the growth of the benzoate-degrader partner decreases when the acetate concentration increases (Fig. 1).

Inhibition by acetate can be expected for all aromatic compounds the methanogenic fermentation of which involves a syntrophic association. As acetate is often pres-



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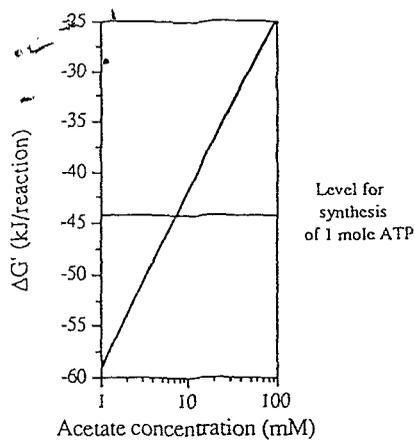


Fig. 1. Effect of acetate concentration on the Gibbs free energy of benzoate degradation to H_2 and acetate (see Eq. 1). The curve was calculated via the equation $\Delta G' = \Delta G^\circ + RT \ln \left(\frac{[\text{acetate}]^3 [\text{H}^+]^3 [\text{HCO}_3^-] [\text{P}_{H_2}]^3}{[\text{benzoate}]} \right)$ with the assumption

dustrial grade; all the other chemicals were of analytical grade. *p*-Toluic acid was dissolved in boiling water to obtain, after cooling, a concentration of $1 \text{ g} \cdot \text{l}^{-1}$. This solution was then diluted twice with the concentrated solution containing all the mineral salts. When acetate was added to the medium, NaHCO_3 was omitted because the medium maintained a convenient pH and alkalinity.

Reactor operation. Both reactors were inoculated with an anaerobically adapted activated sludge from the conventional waste-water treatment plant of the National University campus at Mexico city. They were fed for 9 months with *p*-toluic acid synthetic medium before starting the study. The sludge of the reactors presented high counts of *p*-toluic-acid degraders, hydrogenophilic, and acetoclastic methanogens.

During the whole experiment (day 285 to day 474 of operation), reactor 1 was operated at 5.3 days ($\text{SD} = 0.8$) of hydraulic retention time (HRT), with a feeding stop between days 380 to 420. On day 321, $4.78 \text{ g} \cdot \text{l}^{-1}$ of industrial anhydrous sodium acetate (58.3 mM acetate) was added to the *p*-toluic acid synthetic medium. This concentration was maintained till the end.

Reactor 2 was operated at three different HRTs: 1 day

Table 1. Characterization of the steady-state periods for reactor 2 and the difference between chemical oxygen demand (COD) removal and estimated *p*-toluic acid removal efficiencies

Days of operation	280-295	296-358	403-455
Influent COD ($\text{mg}\cdot\text{l}^{-1}$)	1020	1014	1010
HRT (days)	1.0	7.5	5.1
Organic Load ($\text{kg COD}\cdot\text{m}^3\cdot\text{day}$)	1.02	0.14	0.20
E% COD	18.2	67.8	62.6
E% <i>p</i> -toluic acid	29.3	74.0	71.6
E% <i>p</i> -toluic acid - E% COD	11.1	6.2	9.0

HRT, hydraulic retention time; E, Removal efficiency

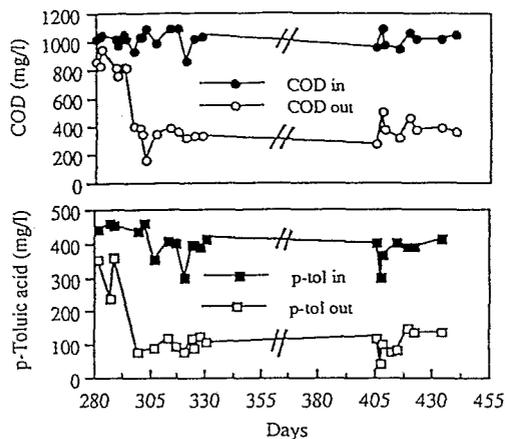
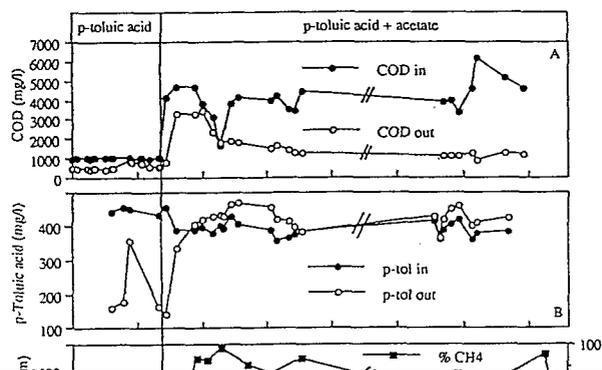


Table 2. Characterization of the steady-state periods for reactor 1

Days of operation	285-320	321-474
Organic load ($\text{kg COD}\cdot\text{m}^3\cdot\text{day}$)	0.19	0.86
Influent COD ($\text{mg}\cdot\text{l}^{-1}$)	1004	4524
Effluent COD ($\text{mg}\cdot\text{l}^{-1}$)	436	1131
Influent pH	7.26	7.30
Sludge pH	7.20	8.05
Sludge E_h (mV)	-124.0	-261.5
E% COD	55.8	73.2
E% <i>p</i> -toluic acid	62.8	0.0
E% <i>p</i> -toluic acid - E% COD	7	-
Methane yield ($\text{N}\cdot\text{m}^3\cdot\text{CH}_4\cdot\text{kg}^{-1}\text{COD}_{\text{rem}}$)	-	0.28

N, Normal temperature and pressure (0°C , 1 atm); E_h , redox potential relative to hydrogen reference



the addition of acetate and indicated that it began to be degraded. In parallel, the COD of the effluent decreased. From day 374 to the end of reactor operation, a steady state with high COD removal and fairly good methane yield was achieved (Fig. 3D, Table 2). This COD removal was only caused by acetate consumption. *p*-Toluic acid remained undegraded, as indicated by Fig. 3E and by the residual COD of the effluent close to the theoretical COD of the *p*-toluic acid initially present in the influent (Fig. 3A, Table 2). Only 2–4 mM acetate was detected in the reactor effluent.

At the end of the experiment, the specific activities of acetate consumption of the sludges from both reactors were determined. The results were $0.184 \text{ mmol} \cdot \text{g}^{-1} \text{ VSS} \cdot \text{h}^{-1}$ for reactor 1 and $0.032 \text{ mmol} \cdot \text{g}^{-1} \text{ VSS} \cdot \text{h}^{-1}$ for reactor 2. The operating conditions applied to reactor 2 during its last feeding period (days 403–455) were similar to that applied to reactor 1 during its first feeding period (days 285–320) (same HRT and composition of the effluent). Therefore the acetate consumption

ic acid has a slow degradation kinetic and its removal decreases with HRT. At 1 day HRT, less than 19–30% *p*-toluic acid was effectively degraded. However, because of its different hydraulic regime pattern and its higher exchange surface, a fluidized bed reactor presents better mass transfer characteristics than a UASB reactor. As a consequence, even at lower HRT than a UASB digester, it generally treats waste-water with a higher COD removal efficiency. Hence, an inhibitory effect of acetate in this case should not be discounted.

No inhibition was observed during the anaerobic treatment with UASB reactors of a chemical waste-water containing $6\text{--}7 \text{ g} \cdot \text{l}^{-1}$ of benzoic acid (49–57 mM; 59–69% of COD) and $4\text{--}6 \text{ g} \cdot \text{l}^{-1}$ of acetic acid (66–100 mM; 21–31% of COD) (Frankin et al. 1991), and of other chemical effluents containing mainly phenol ($0.45 \text{ g} \cdot \text{l}^{-1}$; 4.8 mM; 3.5% of COD) and acetic acid ($19.5 \text{ g} \cdot \text{l}^{-1}$; 325 mM, 68% of COD) (Borghans and van Driel 1988): 50–74% of benzoic acid (estimated from the reported 80–95% COD removal) and 95% of phenol

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