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# Low-temperature (7 °C) anaerobic treatment of a trichloroethylene-contaminated wastewater: Microbial community development

*Alma Siggins, Anne-Marie Enright, Vincent O'Flaherty**

*Microbial Ecology Laboratory, Microbiology, School of Natural Sciences and Ryan Institute, National University of Ireland, Galway (NUI, Galway), University Road, Galway, Ireland*

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ABSTRACT

The feasibility of low-temperature (7 °C) anaerobic digestion for the treatment of a trichloroethylene (TCE) contaminated wastewater was investigated. Two expanded granular sludge bed (EGSB) bioreactors (R1 and R2) were employed for the mineralisation of a synthetic volatile fatty acid based wastewater at an initial organic loading rate (OLR) of 3 kg COD $m^{-3}$ $d^{-1}$, and an operating temperature of 15 °C. Successive reductions in OLR to 0.75 kg COD $m^{-3}$ $d^{-1}$, and operational temperature to 7 °C, resulted in stable bioreactor operation by day 417, with COD removal efficiency and biogas $CH_4$ content ≥74%, for both bioreactors. Subsequently, the influent to R1 was supplemented with increasing concentrations (10, 20, 30 mg $l^{-1}$) of TCE, while R2 acted as a control. At an influent TCE concentration of 30 mg $l^{-1}$, although phase average TCE removal rates of 79% were recorded, a sustained decrease in R1 performance was observed, with COD removal of 6%, and % biogas $CH_4$ of 3% recorded on days 595 and 607, respectively. Specific methanogenic activity (SMA) assays identified a general shift from acetate- to hydrogen-mediated methanogenesis in both R1 and R2 biomass, while toxicity assays confirmed an increased sensitivity of the acetoclastic community in R1 to TCE and dichloroethylene (DCE), which contributed to acetate accumulation. Quantitative Polymerase Chain Reaction (qPCR) analysis of the methanogenic community confirmed the dominance of hydrogenotrophic methanogens in both R1 and R2, representing 71–89% of the total methanogenic population, however acetoclastic *Methanosaeta* were the dominant organisms, based on 16S rRNA gene clone library analysis of reactor biomass. The greatest change in the bacterial community, as demonstrated by UPGMA analysis of DGGE banding profiles, was observed in R1 biomass between days 417 and 609, although 88% similarity was retained between these sampling points.



## 1. Introduction

Trichloroethylene (TCE) is a chlorinated aliphatic compound that is widely used in industry, and is particularly associated with the vapour degreasing of metals (Hansen et al., 2004). Recently, the treatment of TCE-contaminated wastewaters via anaerobic digestion is emerging, as complete conversion of TCE to ethylene can be accomplished under anaerobic conditions by reductive dechlorination, due to symbiotic interactions between phylogenetic groups (Gu et al., 2004; Richardson et al., 2002).

* *Corresponding author*. Tel.: +353 (0) 91 493734; fax: +353 (0) 91 494598.
E-mail address: vincent.oflaherty@nuigalway.ie (V. O'Flaherty).
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Although both laboratory- and full-scale anaerobic digestion bioreactor trials have been traditionally implemented at mesophilic or thermophilic conditions, the potential for low-temperature adaptation of the anaerobic digestion process provides an attractive alternative. Significantly, previous arguments that low-temperature anaerobic digestion proceeds too slowly and inefficiently to be economically viable have now been addressed, with efficient degradation comparable to that from mesophilic trials demonstrated for a range of wastewater types (Collins et al., 2003; Connaughton et al., 2006; Enright et al., 2009; Lettinga et al., 1999; Madden et al., 2010; Nozhevnikova et al., 2000; Rebac et al., 1995). The development of bioreactor designs, such as the expanded granular sludge bed (EGSB) bioreactor (Zoutberg and de Been, 1997), was unquestionably accountable for advances towards successful anaerobic digestion trials at reduced operational temperatures. In addition, reducing the process temperature allows direct treatment of industrial wastewater at ambient temperature (average Irish temperature 2010, 7.9 °C; Met Eireann, 2011), removing the costly energy requirement for heating of the system. Moreover, the treatment of volatile organic chemicals by low-temperature anaerobic digestion could decrease the rate of evaporation of these solvents during the treatment process; increased solubility of gaseous lower chlorinated compounds should allow for increased solvent:biomass contact, thereby maximising the opportunity for complete dechlorination to innocuous end products.

In recent years, recognition of the importance of analysis of microbial community structure and function has allowed a greater understanding of the process, and optimisation of the technology (Enright et al., 2009; Madden et al., 2010). This is of particular importance when employing bioreactor trials for the treatment of a toxicant, and can allow for the identification of factors contributing to the failure of a system (Siggins et al., 2011). To date, nucleic acid based molecular techniques have been extensively employed for the analysis of the archaeal and bacterial communities present in anaerobic bioreactors at low temperatures (Chachkhiani et al., 2004; Enright et al., 2009; Madden et al., 2010), aiding the successful implementation of this technology.

In light of the above, the aim of this study was (1) to evaluate the feasibility of treatment of TCE by low-temperature (7 °C) anaerobic digestion; (2) to determine the effect of TCE on the process of anaerobic digestion, by continued monitoring of bioreactor performance and metabolic analysis of the methanogenic activity and toxicity thresholds demonstrated by the granular biomass throughout the trial; (3) to monitor the adaptation of the archaeal and bacterial communities in response to the presence of TCE.

## 2. Materials and methods

### 2.1. Source of biomass

A granular anaerobic sludge was obtained from a mesophilic (37 °C), full-scale bioreactor as described by Siggins et al. (2011).

### 2.2. Design and operation of EGSB bioreactors

Two glass, laboratory scale (3.5 l) EGSB bioreactors, R1 and R2, were utilised for this 609 day study. R1 and R2 were each inoculated with 70 g VSS of biomass and employed for the treatment of a synthetic volatile fatty acid (VFA) based wastewater as described by Siggins et al. (2011). The initial 15 °C operational temperature was decreased by 1 °C on days 74, 81, 88, 95, 102, 109, 143 and 161, until a final temperature of 7 °C was achieved. The initial 3 kg COD $m^{-3}$ $d^{-1}$ organic loading rate (OLR) was decreased to 1.5 kg COD $m^{-3}$ $d^{-1}$ on day 172, and subsequently to 0.75 kg COD $m^{-3}$ $d^{-1}$ on day 231, in response to an accumulation of VFA in both bioreactors. R1 influent was supplemented with TCE at increasing concentrations of 10, 20 and 30 mg $l^{-1}$ on days 418, 500 and 522, respectively, resulting in seven operational phases (Phase 1 – Phase 7; Table 1).

### 2.3. Specific methanogenic activity and toxicity testing

Seed biomass and biomass sampled from the bioreactors on days 342 and 609 were screened for metabolic capability using

**Table 1 – Operational and performance characteristics of R1 and R2 EGSB bioreactors. Values are averages of phases (P1–P7). Standard deviations are given in parenthesis, where applicable. n.a. not applicable. n.d. not determined.**

| Phases | | P1 | P2 | P3 | P4 | P5 | P6 | P7 |
|---|---|---|---|---|---|---|---|---|
| Days | | 0–73 | 74–171 | 172–230 | 231–417 | 418–499 | 500–521 | 522–609 |
| Operational temperature | | 15 °C | 14–7 °C | 7 °C | 7 °C | 7 °C | 7 °C | 7 °C |
| R1 Influent TCE (mg $l^{-1}$) | | 0 | 0 | 0 | 0 | 10 | 20 | 30 |
| % TCE removal | | n.a. | n.a. | n.a. | n.a. | 19 (60) | 86 (8) | 79 (37) |
| Influent COD (mg $l^{-1}$) | | 3000 | 3000 | 1500 | 750 | 750 | 750 | 750 |
| % COD removal | R1 | 57 (17) | 68 (13) | 68 (20) | 76 (16) | 82 (10) | 78 (12) | 56 (15) |
| | R2 | 54 (17) | 62 (16) | 72 (15) | 75 (14) | 83 (10) | 83 (11) | 86 (6) |
| % Biogas $CH_4$ | R1 | 64 (12) | 66 (12) | 72 (6) | 74 (7) | 51 (15) | 68 (5) | 46 (23) |
| | R2 | 68 (6) | 73 (4) | 69 (12) | 75 (6) | 70 (11) | 75 (2) | 73 (7) |
| Effluent Acetic Acid mg COD $l^{-1}$ | R1 | n.d. | 19 (12) | 18 (15) | 10 (3) | 11 (4) | n.d. | 375 (167) |
| | R2 | n.d. | 22 (12) | 18 (13) | 10 (5) | 8 (4) | n.d. | 135 (71) |
| Effluent Propionic Acid mg COD $l^{-1}$ | R1 | n.d. | 142 (45) | 35 (32) | 6 (6) | 4 (2) | n.d. | 0 (0) |
| | R2 | n.d. | 143 (41) | 53 (57) | 5 (5) | 1 (2) | n.d. | 0 (0) |
| Effluent Butyric Acid mg COD $l^{-1}$ | R1 | n.d. | 3 (3) | 2 (2) | 1 (1) | 0 (0) | n.d. | 2 (5) |
| | R2 | n.d. | 5 (4) | 2 (2) | 1 (1) | 0 (0) | n.d. | 0 (0) |