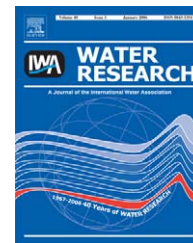


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Development of microbial community structure and activity in a high-rate anaerobic bioreactor at 18 °C

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ABSTRACT

Anaerobic digestion in the psychrophilic ($< 20^{\circ}\text{C}$) or sub-mesophilic temperature range has recently been proven as an effective treatment option for the mineralization of a wide variety of problematic wastewaters. In this study, an expanded granular sludge bed-anaerobic filter (EGSB-AF) bioreactor was seeded with a full-scale, mesophilic sludge and employed to evaluate the long-term operational potential, and underlying microbial ecology, of this approach for the treatment of a medium-strength (5 g chemical oxygen demand [COD] l^{-1}), synthetic, volatile fatty acid-based wastewater. Throughout the trial period of 625 days, extended intervals of consistently stable and efficient wastewater treatment were sustained. These results were highlighted by a short start-up period (21 d), low hydraulic retention times (4.88 h), high organic (up to $24.64 \text{ kg COD m}^{-3} \text{ d}^{-1}$), and volumetric loading rates (up to $4.92 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$). A stable, well-settling granular sludge bed was maintained in the bioreactor for the majority of the trial; however, reduced treatment efficiency and biomass washout were observed at an imposed OLR of $36.96 \text{ kg COD m}^{-3} \text{ d}^{-1}$. The microbial biomass in the bioreactor was investigated using maximum specific methanogenic activity assays and polymerase chain reaction-denaturing gradient gel electrophoresis. A temporal succession of both the bacterial and archaeal populations was noted during the trial, compared to the seed sludge, in response to bioreactor operation at lower temperatures, loading rate increases and to VFA accumulation in the bioreactor. During the trial, an increased contribution of hydrogenotrophic methanogenesis as a pathway of methane production was observed, along with the overall emergence of a highly active psychrotolerant—though still mesophilic biomass.

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1. Introduction

The application of anaerobic digestion (AD) as a full-scale treatment technology for industrial wastes is almost three decades old. AD of wastewaters is now an established and proven technology for the effective treatment of a multitude of wastewater categories (Rebac et al., 1998; Driessen et al., 1999; Macarie et al., 2000). However, the majority of full-scale

applications and research effort, until recently, has been concentrated on AD within the mesophilic temperature range ($25\text{--}45^{\circ}\text{C}$). This has largely been due to the fact that thermophilic ($>45^{\circ}\text{C}$) AD was too expensive (except in necessary cases such as high-temperature effluent discharges) and the belief that sub-ambient or psychrophilic ($>20^{\circ}\text{C}$) AD was not viable because of low microbial activity under low-temperature conditions (Lin et al., 1987; Lettinga

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et al., 2001). Despite this, the majority of industrial effluents are discharged at low ambient temperatures, (c. 18 °C; Lettinga et al., 2001). It follows that one of the main advantages of low-temperature anaerobic wastewater treatment would be increased cost-efficiency, as the need to heat influent wastewaters and to direct AD-produced energy back into system maintenance (e.g. bioreactor heating) is reduced or eliminated. The use of new or modified bioreactor designs, such as various hybrid versions of the upflow anaerobic sludge bed (UASB), internal circulation (IC) and expanded granular sludge bed (EGSB) bioreactors, has, in part, facilitated the successful demonstration of psychrophilic anaerobic digestion (PAD) at laboratory-scale for the treatment of a wide variety of wastewater categories (Rebac et al., 1995; Lettinga, 1999; Collins et al., 2003, 2005a; McHugh et al., 2004). Indeed, the potential applicability of PAD has generated significant scientific and engineering interest. Although intense experimental effort has been dedicated to broadening the demonstrable range of waste categories suitable for treatment using PAD, virtually nothing has been reported on the limits of these new systems with respect to organic loading rates (OLR) or hydraulic retention times (HRT).

In addition to knowledge of the operational range of PAD, much more information is required on the nature, identity and capacity of the microbial consortia involved in the process. Although information is available on both the functioning of anaerobic microbial consortia and their immobilisation in particulate bioreactor environments (e.g. O'Flaherty and Lens, 2003; McHugh et al., 2003), there remains a lack of integration of the fundamental processes occurring at microorganism level (scale c. 1 µm–1 mm) into the processes occurring within bioreactors (scale >1 m). Moreover, wastewater treatment processes are still mostly operated as black boxes, taking the effluent concentration as an output value that cannot be improved and the process control strategy, if applied at all, does not generally take into account processes occurring at microorganism level. The result is that wastewater treatment bioreactors are mainly designed using empirical design criteria, which can lead to over-dimensioning of bioreactor volumes and sub-optimal or unstable treatment. Culture-independent, biomolecular methods have the potential to make a valuable contribution to resolving this knowledge gap and new information has recently been obtained, using molecular approaches such as PCR-DGGE (Pereira et al., 2002; Roest et al., 2005), on the nature of anaerobic bioreactor communities and on the limitations and potential of the microbial consortia involved (McHugh et al., 2003; Collins et al., 2003, 2005b,c; Pender et al., 2004; Keyser et al., 2006; Akarsubasi et al., 2006).

In light of the above, this study focussed on the potential of PAD for high-rate treatment of a typical industrial wastewater. A modified EGSB-based design was used to determine loading rates and hydraulic retention time (HRT) thresholds under low-temperature conditions, while the microbial population structure and specific methanogenic activity profiles of the biomass were investigated throughout the course of the trial. The results are discussed with regard to overall process efficiency and feasibility while also considering the ecology of the bioreactor biomass.

2. Materials and methods

2.1. Source of biomass

A mesophilic anaerobic granular sludge (VSS, 63.17 g l⁻¹) was obtained from a full-scale treatment plant operated at 37 °C in the Netherlands (Paques B.V.).

2.2. Bioreactor design and operation

A 3.7 l (active liquid volume, 3.2 l) glass laboratory-scale expanded granular sludge bed-anaerobic filter (EGSB-AF) bioreactor, as described by Collins et al. (2005a), was inoculated with 1 l of the granular sludge. A medium-strength, synthetic volatile fatty acid (VFA)-based wastewater containing ethanol, butyrate, acetate and propionate, in the chemical oxygen demand (COD) ratio of 1:1:1:1, to a total of 5 g COD l⁻¹, was used as influent feed. The influent was buffered with NaHCO₃ (8 g l⁻¹) and fortified with macro- (10 ml l⁻¹) and micro- (1 ml l⁻¹) nutrients, as described by Shelton and Tiedje (1984). The trial was divided into 8 different operational periods, P1–P8. Each period was characterised by a change in either the applied organic loading rate (OLR), HRT or applied

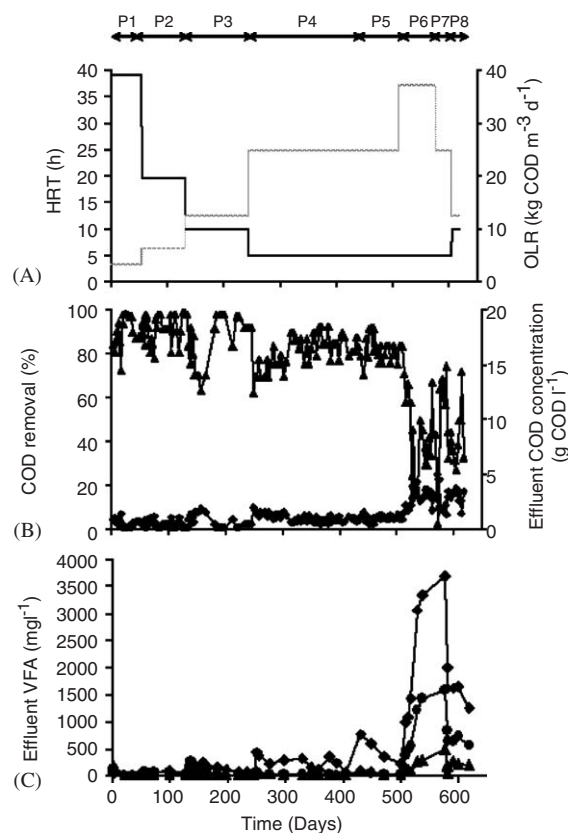


Fig. 1 – (A) HRT decreases (-) and OLR (- -) during the eight operational phases (P1–P8), (B) COD removal efficiency (▲), effluent COD concentration (●) (C) Concentration of volatile fatty acids in bioreactor effluent during the eight operational phases (P1–P8): acetate (◆), propionate (●), butyrate (▲).

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