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Microbial population changes in anaerobic membrane bioreactor treating landfill leachate monitored by single-strand conformation polymorphism analysis of 16S rDNA gene fragments

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ABSTRACT

The Jebel Chakir discharge area, a sanitary landfill, is nowadays the common way to eliminate municipal waste in Tunis (Tunisia). In this study, we developed an anaerobic membrane bioreactor (AnMBR) process for the treatment of Jebel Chakir landfill leachate (LFL). The theoretical organic loading rate (^{T}OLR) in the reactor was gradually increased from 4 g COD/l/d to 10 g COD/l/d. The composition, diversity, and changes in microbial communities were analysed using the polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) fingerprint method during OLR increasing. The positive effect of the AnMBR system to maintain stability of the microbial diversity present at the treatment startup during OLR increasing was demonstrated. High loading rate decreased bacterial diversity, but the archaeal one remained stable. At the optimal OLR (7 g COD/l/d), small-subunit (16S) rDNA clone libraries of *Bacteria* and *Archaea* populations were performed. The results show that, within the domain *Archaea*, all phylotypes were related to the CA11 group and one sequence belonged to cultivate genus, the acetoclastic *Methanosaeta*. Within the domain *Bacteria*, rDNA genes were affiliated with six phyla: *Bacteroidetes*, *Firmicutes*, *Synergistetes*, *Proteobacteria*, unclassified bacteria, and *Verrucomicrobia*. This study related the performance of the AnMBR treating leachate to the change of microbial communities in order to better understand the process and monitor this anaerobic digestion.

1. Introduction

Solid waste landfills may cause severe environmental impacts if leachate and gas emissions are not controlled. Leachates generated in municipal landfills contain large amounts of organic and inorganic contaminants (Kettunen and Rintala, 1998; Li et al., 2009), a high concentration of metals, and some hazardous organic chemicals. The removal of organic material based on chemical oxygen demand (COD), biological oxygen demand (BOD), and ammonium from leachate is the usual prerequisite before discharging the leachate into natural waters (Kettunen et al., 1996; Kettunen and Rintala, 1998). Anaerobic treatment methods are more suitable for the treatment of concentrated leachate streams, and offer lower operating costs, as well as the production of a usable biogas product and of a pathogen-free solids residue that can be used as cover material (Im et al., 2001). The main objective of anaerobic digestion is the degradation and destruction of organic substances, with consequent reduction of the odorous emissions and pathogens. This conversion is catalyzed by a large number of *Bacteria* and *Archaea* that operate in synergy, catalyzing different chemical reactions.

The anaerobic digestion process follows four major steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Among various biological technologies, it is attractive to use an anaerobic cross-flow membrane bioreactor for the removal of COD and ammonia, especially for the treatment of landfill leachate (Chen et al., 2008).

In Tunisia, more than 6000 tons of domestic garbage are collected daily (ANGed, 2007). Landfilling is a fundamental step in any waste management strategy. The site of Jebel Chakir is the first controlled discharge area in Tunisia, located at 10 km at the

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Western South of Tunis. Since 1999 it has received, daily, 2000 tons of domestic and assimilated waste coming from greater Tunis (Trabelsi et al., 2011). Unfortunately, Tunisian landfill leachate (LFL) is typically heavily loaded with organics, ammonia, nitrogen, and even harmful compounds such as heavy metals. These substances can be a major source of contamination to groundwater, surface water, fauna, and flora (Ellouze et al., 2008). In previous studies, Tunisian landfill leachate was treated by the use of an anaerobic batch reactor (Sellami et al., 2009) or by combining a biological aerobic membrane reactor and electro-oxidation processes (Aloui et al., 2009; Feki et al., 2009). In addition, the use of an anaerobic membrane bioreactor for the removal of organic micropollutants of landfill leachate has been reported (Xu et al., 2008). The COD removal exceed 95% with leachate addition of 10% and 20% (v/v) when treating landfill leachate using an anaerobic submerged membrane bioreactor (Bohdziewicz et al., 2008). Moreover, it was reported that membrane bioreactors (MBR) can be operated at very long sludge times and can greatly extend the field of application of biological processes for concentrated streams, such as leachate (Bohdziewicz et al., 2001), particularly for the biological removal of nitrogen and recalcitrant COD. Biomass can be kept in the system regardless of its ability to settle. Under these conditions, slow-growing bacterial species can develop and much time is also left to degrade refractory compounds (Canziani et al., 2006).

In order to better understand the functions and development of the microbial community, a full description of the microbial ecosystem is required. However, cultivation-dependent approaches do not necessarily provide reliable information on the composition of entire microbial communities. The use of molecular biological techniques, especially those that take advantage of the small subunit (SSU) rRNA molecule, has eliminated the dependence on isolation of pure cultures as a means of studying the diversity and structure of microbial communities (Godon et al., 1997). Molecular tools based on a polymerase chain reaction, and a single strand conformation polymorphism (PCR-SSCP), were also used for the study of the microbial community and its change over time (Khelifi et al., 2009a,b). This approach has been used to analyze a large variety of microbial consortia, such as soil, blanket bog peat, mine galleries, marine sediments, hydrothermal vents, human colonic biota, termite guts, biodegraded wall paintings, activated sludge, olive fermentation brine, and anaerobic digesters (Godon et al., 1997; Delbès et al., 2001; Chamkha et al., 2008). Moreover, the characterization of the microbial community structure in anaerobic digesters has attracted interest from engineers because understanding microbial community structure in any type of configuration of an anaerobic process can provide valuable information that can be used to optimize reactor conditions in order to favour efficient breakdown of wastes (Shin et al., 2010).

The aim of this work was to carry out an evaluation of the influence of the OLR changes on the performance and stability of a membrane bioreactor treating landfill leachate. Simultaneously, the microbial diversity was analysed using the single strand conformation polymorphism (SSCP) technique. Partial clone libraries of bacterial and archaeal 16S rDNA were constructed at the optimal OLR (7 g COD/l/d) and an analysis of the retrieved sequences was performed to obtain a description of the bacterial and the archaeal diversity.

2. Materials and methods

2.1. Experimental design

The experimental setup was constructed within the framework of the Inco-med project "ICA3-CT 1999-00013 MBR recycling" and it was installed in the Centre of Biotechnology of Sfax, Tunisia. The schematic diagram of the experimental setup has already been presented in previous studies (Saddoud and Sayadi, 2007; Zayen et al., 2010). The jet flow anaerobic bioreactor was constructed with Plexiglas having a working volume of 50 l. The temperature was maintained constant at 37 °C by circulating water through the water jacket of the reactor. Sludge wasting was avoided throughout all the treatment process. The capillary ultra-filtration module used in this study has a nominal membranes pore size of 0.1 μm and the effective filtration area is 1 m^2. The influent was introduced into the reactor without pH correction as it was close to the neutral (Table 1). The HRT was kept constant (7 days) and the OLR was increased by the decrease of the dilution of the feed solution.

2.2. Characterization of LFL

The LFL was obtained from the controlled discharge of Jebel Chakir. Leachate was collected in 20-l tanks and transported to the laboratory to be stored at 4 °C in order to avoid its acidification and modification of its chemical composition. The chemical composition of LFL is shown in Table 1. The notable characteristics of this effluent are the high COD (more than 80 g l^{-1}); its high biodegradability, measured by the ratio BOD_5/COD ($\approx$0.58); its low level of suspended materials; and the high concentration of volatile fatty acids (VFA).

Standards methods (APHA, 1992) were used for measurement of total suspended solids (TSS), volatile suspended solids (VSS), and volatile fatty acids (VFA). Total Kjeldahl nitrogen (TKN) and ammonia nitrogen (NH_4^+) were measured according to Kjeldahl (1883). The pH level was measured with a pH meter model Istek-NeoMet. Chemical oxidation demand (COD) was determined as described by Knechtel (1978). Five-day biological oxygen demand (BOD_5) was determined by the manometric method with a respirometer (BSB-controller Model 620 T [WTW]). The volatile fatty acids VFA were analysed by a gas chromatograph (SHIMADZU GC-9A, Japan) equipped with a flame ionisation detector, as previously described (Saddoud and Sayadi, 2007). Biogas was quantified daily by the use of a biogas flow meter apparatus (Gallus 2000, Germany).

2.3. Total DNA extraction

DNA extractions were performed on samples collected from the bioreactor sludge at the middle of the period of each applied OLR

Table 1
Physico-chemical characteristics of the samples of landfill leachate.

Characteristics	Average	Discharge standards of rejects
pH (25 °C)	6.84	6.5–6.8
Conductivity (mS/cm)	24.9	–
Salinity (g/l)	21.16	–
COD (g/l)	84.29	1
BOD_5 (g/l)	46.1	0.4
BOD_5/COD	0.58	–
TSS (mg/l)	1970	0.03
VSS (mg/l)	1460	–
TKN (mg/l)	3177	100
NH_4^+ (mg/l)	2800	100
Ca (mg/l)	975.5	1
Cu (mg/l)	533.3	1
Fe (mg/l)	67.75	5
K (mg/l)	8.02	50
Mg (mg/l)	14.37	300
Na (mg/l)	20	1000
Volatile fatty acids (g/l)	22.79	–
Acetic acid (g/l)	3.57	–
Propionic acid (g/l)	3.23	–
Butyric acid (g/l)	1.02	–
Isobutyric acid (g/l)	10.44	–
Valeric acid (g/l)	4.52	–