Water Research 100 (2016) 296–305

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Community shifts within anaerobic digestion microbiota facing phenol inhibition: Towards early warning microbial indicators?

Simon Poirier*, Ariane Bize, Chrystelle Bureau, Théodore Bouchez, Olivier Chapleur

Irstea, Hydrosystems and Bioprocesses Research Unit, 1 rue Pierre-Gilles de Gennes, F-92761 Antony, France

ARTICLE INFO

Article history:
Received 25 February 2016
Received in revised form
27 April 2016
Accepted 12 May 2016
Available online 13 May 2016

Key words:
Phenol
ARISA
16S rRNA gene sequencing
Microbial indicators
Methanization

ABSTRACT

Performance stability is a key operational issue for anaerobic digestion (AD) and phenolic compounds are regularly mentioned as a major cause of digester failures. To get more insights into AD microbiota response to a wide range of inhibition levels, anaerobic batch toxicity assays were conducted with ten phenol concentrations up to 5.00 g/L. Final AD performance was not impaired up to 1.00 g/L. However, progressive shifts in microbial community structure were detected from 0.50 g/L. The methanogenic function was maintained along with increasing initial phenol concentrations up to 2.00 g/L thanks to the emergence of genus *Methanoculleus* at the expense of *Methanosarcina*. Within syntrophic populations, family *Syntrophomonadaceae* proportion was gradually reduced by phenol while *Synergistaceae* gained in importance in the microbiome. Moreover, at 2.00 g/L, the relative abundance of families belonging to order *Clostridiales* dropped, leading to the predominance of populations assigned to order *Bacteroidales* even though it did not prevent final AD performance deterioration. It illustrates the high level of adaptability of archaeal and bacterial communities and suggests the possibility of determining early warning microbial indicators associated with phenol inhibition.

1. Introduction

Anaerobic digestion (AD) is a sustainable, economically and environmentally attractive bioprocess simultaneously enabling organic waste stabilization and valorization. It provides methane-rich biogas that can be converted into energy and heat, digestion sludge used as an organic amendment for soil as well as other valuable compounds such as alcohols and volatile fatty acids. Nevertheless, this microbially driven process still remains vulnerable to a wide variety of inhibitory substances that induce poor operational stability, preventing AD wide commercialization (Chen et al., 2008). These compounds can be either contained in organic waste, or formed during their degradation within industrial digesters.

Among AD inhibitors, various natural or anthropogenic phenolic compounds are mentioned as the primary cause of digester failure. They are regularly detected at concentrations reaching up to several grams per liter in different types of effluents from coal gasification, coking, petroleum refining, petrochemical manufacturing and paper industry (Rosenkranz et al., 2013; Veeresh et al., 2005). Phenols can also be produced from biodegradation of naturally occurring aromatic polymers such as humic acids and tannins or from degradation of xenobiotic compounds such as pesticides (Fang et al., 2006). Some pre-treatments applied to increase biogas production efficiency from lignocellulosic materials are also known to result in the production of phenolic compounds (Monlau et al., 2014). Their detrimental effects on anaerobic micro-organisms have been observed in anaerobic systems treating municipal solid waste, olive mill wastes and wine distillery wastewater (Busca et al., 2008). Among phenolic compounds, phenol is an essential component widespread in many industries producing plastics, resins and pharmaceuticals (Busca et al., 2008). Moreover, phenol concentrations up to 4288 mg/kg were measured in digestion sludge of anaerobic digesters degrading different types of organic waste such as pig manure, biowaste and plant materials (Levén et al., 2012). Although phenol can be biodegraded to harmless compounds under methanogenic conditions, high concentrations are toxic to different groups of microorganisms involved in methane production (Chapleur et al., 2015; Olguin-Lora et al., 2003; Rosenkranz et al., 2013). Unexpected phenol shock loadings within unacclimated anaerobic digesters can induce major disruptions in

* Corresponding author.
E-mail addresses: simon.poirier@irstea.fr (S. Poirier), ariane.bize@irstea.fr (A. Bize), chrystelle.bureau@irstea.fr (C. Bureau), theodore.bouchez@irstea.fr (T. Bouchez), olivier.chapleur@irstea.fr (O. Chapleur).

http://dx.doi.org/10.1016/j.watres.2016.05.041
0043-1354/

AD bioprocess, leading to the decrease of biogas production rate and longer treatment durations (Veeresh et al., 2005). The functioning of methanogenic bioreactors degrading phenol as the sole carbon source and operated under various conditions is abundantly described in the literature (Hoyos-Hernandez et al., 2014; Veeresh et al., 2005). Metabolic pathways are also well documented, as well as the impact of phenol on AD performances such as degradation rates, lag times, cumulated biogas production, VFA accumulation and limiting concentrations of anaerobic phenol degradation (Hernandez and Edyvean, 2008; Schink et al., 2000). Indeed, many studies used batch toxicity assays to determine inhibition thresholds such as half maximal inhibitory concentration (IC_{50}) (Fang et al., 2004; Fang and Chan, 1997; Fedorak and Hrudey, 1984).

However, few studies investigated the microbial response of an unacclimated methanogenic microbiota exposed to different levels of phenol during sudden shock loadings. Some of them characterized pre-adapted microbial consortia within digesters degrading phenol (Chen et al., 2008; Levén and Schnürer, 2010; Zhang et al., 2005). A recent study also described the acclimatization of a microbial consortium up to 2.0 g/L of phenol (Wirth et al., 2015). Chapleur et al. (2015) qualitatively monitored by Automated Ribosomal Intergenic Spacer Analysis (ARISA) the microbial community dynamics in batch bioreactors facing increasing phenol concentrations. They reported major shifts in both archaeal and bacterial populations occurring at different inhibition thresholds. Our study combines an extended ecological approach linking ARISA fingerprinting and 16S rRNA gene sequencing in order to provide further information to overcome some bottlenecks and bring into focus potential key phylotypes involved in phenol resistance within a methanogenic mixed culture.

In this framework, the present study aims at studying the effect of a wide range of phenol concentrations on an unadapted anaerobic microbiota. The objective is to give more insights into the microbial community shifts occurring in response to increasing levels of inhibition. The possibility of identifying early warning microbial indicators correlated with the decrease of AD performance on the basis of key microbial phylotypes is discussed.

2. Materials and methods

2.1. Experimental set-up

A total of 30 anaerobic batch digesters (glass plasma bottles, total working volume of 500 mL) were seeded with 20 g of sludge pellets obtained by centrifugation of samples from a 50 L stable laboratory bioreactor incubated at 35 °C. This bioreactor had previously been inoculated with sludge collected in an industrial mesophilic digester treating both primary and excess biological sludge on a municipal wastewater treatment plant (Achères, France). The reactor was fed with mashed biowaste provided by an industrial food waste deconditioning unit (Chemaudin, France) in order to acclimatize the microbial consortium to this substrate. This industrial unit is supplied with residual food waste from restaurants, supermarkets, and food industries. Batch digesters were supplemented with 50 g of the same mashed biowaste (C [wt% dry solids] = 49.5%, N [wt% dry solids] = 3.6%; K^+ = 2.48 mg/g; $NH4^+$ = 2.18 mg/g; Mg^{2+} = 3.3 mg/g; Ca^{2+} = 1.11 mg/g) corresponding to an initial substrate/inoculum ratio of 10 g COD/g COD (12 g COD substrate/1.2 g COD inoculum). Phenol (99%, ACROS Organics) was added in order to reach 10 different concentrations (0.00, 0.10, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 2.00, 5.00 g/L) corresponding respectively from 0.00 up to 10.20 g COD per incubation. Biochemical methane potential buffer (International Standard ISO 11734 (1995)) was introduced in all bioreactors to reach a final working volume of 500 mL. All incubations were performed in triplicates. The 30 bioreactors were subsequently hermetically sealed with a screw cap and a rubber septum and headspaces were flushed with N_2 (purity > 99.99%, Linde gas SA) in order to ensure anaerobic conditions. Time zero samples were collected before starting incubation of the reactors under anaerobic conditions at 35 °C, without agitation, in the dark. Liquid samples (8 mL) were periodically collected through the septum and centrifuged at 10,000 × *g* for 10 min. The pellets and supernatants thus obtained were stored separately at −20 °C for analysis of biomass and chemical indicators, respectively. Digestion tests were run for 203 days until the daily biogas productions of each of the reactors decreased below 10 mL.

2.2. Analytical methods

Biogas accumulation was measured using a differential manometer (Digitron 2082P) in order to calculate biogas production at standard temperature and pressure. Headspace gas composition was analyzed using a micro GC (CP4900, Varian) as described in Chapleur et al. (2015). Grofit package of R CRAN software (version 3.1.2) was used to fit the cumulative CH_4 and CO_2 production data to a modified Gompertz three-parameter model (Eq. (1)) where M(t) is the cumulative methane production (mL) at time t (days); P is the ultimate methane yield (mL); R_{max} is the maximum methane production rate (mL/days); λ is the lag phase (days); e is the exponential constant.

$$M(t) = P \times exp\left\{ - exp\left[\frac{R_{max} \times e}{P} \times (\lambda - t) + 1\right]\right\} \quad (1)$$

Phenol half maximal inhibitory concentration (IC_{50}) was determined by plotting R_{max} values as a function of initial phenol concentrations. This curve was fitted to the Hill model by non-linear regression using the algorithm of Marquard and a non-linear regression program as described by Chapleur et al. (2015).

Acetate, propionate, butyrate, lactate, formate and valerate were quantified by conductometric detection, using a Dionex 120 equipped with IonPAc ICE-AS1 column (9 mm × 250 mm). The mobile phases were heptafluorobutyric acid (0.4 mmol/L) and tetrabutylammonium hydroxide (5 mmol/L). Dissolved organic carbon (DOC) was measured in the samples' supernatant (Bioritech Model 700) as described in Chapleur et al. (2015). Phenol concentrations were measured with LCK346 kit (Hach Lange) according to the manufacturer's instructions.

2.3. Molecular analyses of microbial communities

Total DNA was extracted from the pellets using Powersoil™ DNA isolation kit (Mobio Laboratories Inc. Carlsbad) according to the manufacturer's instructions. Extracted DNA concentrations were quantified using Qubit 2.0 Fluorometer (dsDNA HS or BR Assay Kit, Invitrogen, Life Technologies, Eugene) and DNA integrity was checked by EtBr staining and electrophoresis on a 1% agarose gel.

ARISA method was applied to analyze *Bacteria* and *Archaea* members from the extracted DNA. PCR procedures are described in Chapleur et al. (2015). Bacterial primer set ITSF/ITSReub (5′-GTC GTA ACA AGG TAG CCG TA-3′/5′-GCC AAG GCA TCC ACC-3′) was used to amplify the bacterial 16S–23S ITS region, and primer set 71R/1389F (5′-TCG CAG CTT RSC ACG YCC TTC-3′/5′-CTT GCA CAC ACC GCC CGT C-3′) was used for the archaeal 16S–23S ITS region. To ensure a fast processing of samples, ARISA was performed with a capillary electrophoresis bioanalyzer (2100 Electrophoresis Bioanalyzer, Agilent Technologies, Santa Clara) using the Agilent DNA 1000 Kit (Agilent Technologies).

Extracted DNAs were also used to sequence bacterial and

archaeal hyper variable region V4–V5 of the 16S rRNA gene with the primer 515F and 928R as described in Poirier et al. (2016). Fusion method was employed (IonAmplicon Library Preparation (FusionMethod) Protocol, Revision C). The forward primer was modified by the addition of a PGM sequencing adaptor (adaptor A: 5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG-3′) and a barcode (5′-adaptor A-Barcode-515F-3′). The reverse primer was modified by the addition of a PGM sequencing adaptor (adaptor trP1: 5′-CCTCTCTATGGGCAGTCGGTGAT-3′) (5′-adaptor trP1-928R-3′). V4–V5 region was amplified according to Platinum Pfx Supermix protocol (Life Technologies). PCR cycle characteristics are detailed in Poirier et al. (2016). PCR products were cleaned using the Agencourt AMPure XP magnetic beads purification system (Beckman Coulter) according to the manufacturer's instruction. Quantification was realized with a capillary electrophoresis bioanalyzer (2100 Electrophoresis Bioanalyzer, Agilent Technologies, Santa Clara) using the Agilent DNA 1000 Kit (Agilent Technologies). Purified libraries were diluted and combined in an equimolar concentration of 100 pM for sequencing. Emulsion PCR was carried out to prepare template-positive Ion Sphere Particles (ISPs) containing clonally amplified DNA, using the Ion PGM™ Template OT2 400 Kit with the Ion OneTouch™ 2 Instrument. The template-positive ISPs were enriched with the Ion OneTouch™ ES according to the manufacturer's instructions. Sequencing was performed on an Ion Torrent Personal Genome Machine using Ion 316 chip and the Ion PGM Sequencing 400 Kit according to the manufacturer's instructions. The PGM software filtered out low quality and polyclonal sequence reads, and quality filtered data were exported as a FastQ files. 10,000 to 50,000 high quality reads were generated for each sample.

2.4. Data processing

ARISA profiles were imported into the StatFingerprints R package in order to be aligned as described in Chapleur et al. (2015) before statistical analysis.

Sequencing data were processed with the same pipelines as described previously (Poirier et al., 2016). 16S rRNA tag reads were quality checked and filtered by UPARSE as implemented in USEARCH v8.0.1623. Raw sequence reads were filtered before subsequent analyses to minimize the effect of random sequencing errors. Short sequences with less than 200 bp were discarded; the remaining ones were arbitrarily truncated to 200 bp and filtered for quality, keeping only those with expected errors below 1. Sequences were then dereplicated, and sorted by abundance. Sequences occurring only once (singletons) were removed from the dataset. Reads detected as chimeric artifacts were identified and discarded, using UCHIME against the "gold" database (http://drive5.com/uchime/gold.fa). Resulting sequences were clustered into Operational Taxonomic Units (OTUs) at 97% similarity levels using furthest neighbor approach. OTU sequences were assigned to a taxonomic lineage using mothur v.1.25.0 against Silva database release 119 with a minimum confidence of 0.8, in the QIIME 1.8.0 environment. OTU tables and taxonomy summary files were also generated with QIIME.

2.5. Statistical analysis

R CRAN software (version 3.1.2) was used to statistically examine molecular data. Centered not scaled principal component analyses (PCA) were implemented to separately analyze archaeal and bacterial diversities by using ADE4 R package both for ARISA profiles and 16S rRNA tags datasets. The effect of phenol on microbial community dynamics was investigated by coloring and clustering the individuals with a color scale corresponding to initial phenol concentrations. Correlation circles were drawn in order to examine the influence of different OTUs on the distribution of the samples within the factorial plane. Figures were produced with Adegraphics R package.

3. Results and discussion

3.1. Effect of increasing phenol concentrations on anaerobic digestion performances

3.1.1. Biogas production

Fig. 1A presents the average cumulated CH_4 production of each triplicate of reactors. CO_2 cumulated productions are presented in supplementary material (Figure S1). As phenol concentration increased, rates and levels of total CH_4 and CO_2 production overall decreased progressively. Methanogenesis occurred up to 2.00 g/L of initial phenol concentration, whereas at 5.00 g/L, no CH_4 was produced. Other studies indicated that unadapted inocula produced CH_4 up to 1.50–2.00 g/L of initial phenol (Chapleur et al., 2015; Wang et al., 1991) before being totally inhibited at higher concentrations. Cumulative production curves were analytically compared by fitting experimental data with Eq. (1) (Gompertz equation, see Materials and Methods) in order to calculate kinetic constants (ultimate production, maximum production rate and lag time) presented in supplementary material (Table S1).

The inhibitory effect of phenol on ultimate biogas production was very limited up to initial concentrations of 1.00 g/L. From 0.00 g/L up to 1.00 g/L, final CH_4 production increased from 5067 mL up to 5733 mL respectively. CO_2 ultimate production increased from 2312 mL in the control assays up to 2464 mL at 1.50 g/L of phenol. Increases of ultimate biogas production compared to the control suggested that phenol conversion to biogas occurred along with biowaste degradation, as already observed in previous studies (Chapleur et al., 2015; Fedorak and Hrudey, 1984; Wang et al., 1991) with other substrates. Nevertheless, at 1.25 g/L and 1.50 g/L, average ultimate CH_4 productions respectively declined down to 4729 mL and 4626 mL before dropping more significantly down to 2763 mL at 2.00 g/L. Contrary to CH_4, 333 mL of CO_2 were still produced at 5.00 g/L indicating that methanogenic communities were more sensitive to phenol than bacterial populations involved in fermentative steps. Recent studies corroborate this result (Chapleur et al., 2015; Wirth et al., 2015).

Even though phenol did not negatively affect final carbon conversion efficiency up to initial concentration of 1.00 g/L, its inhibitory effect was clearly visible on biogas production kinetics at higher concentrations. From 0.25 g/L to 2.00 g/L, CH_4 and CO_2 maximum production rate values (R_{max}) gradually decreased along with increasing phenol levels from 217 mL/day and 70 mL/day respectively down to 36 mL/day and 16 mL/day. Furthermore, CH_4 lag times (λ) gradually increased from 7.2 days at 0.00 g/L up to 32.4 days at 2.00 g/L of phenol. Consequently, these results indicated that, up to 1.00 g/L, the inhibitory effect of phenol on AD was mainly characterized by slower CO_2 production kinetics and both delayed and slower CH_4 production kinetics. Similar general patterns of inhibition of CH_4 production by phenol have been reported by other authors on different substrates (Chapleur et al., 2015; Fedorak and Hrudey, 1984; Olguin-Lora et al., 2003).

In order to compare the resistance of this inoculum to phenol with other studies, AD inhibition was more precisely quantified by calculating an IC_{50} value. Fig. 1B revealed that an initial phenol concentration of 1.25 g/L induced a 50% reduction of the control assays CH_4 R_{max} value. This IC_{50} was consistent with values reported by other authors. Acetate conversion to CH_4 was indeed reported to be half inhibited by phenol at 1.25 g/L according to Wang et al. (1991). Chapleur et al. (2015) indicated that maximum

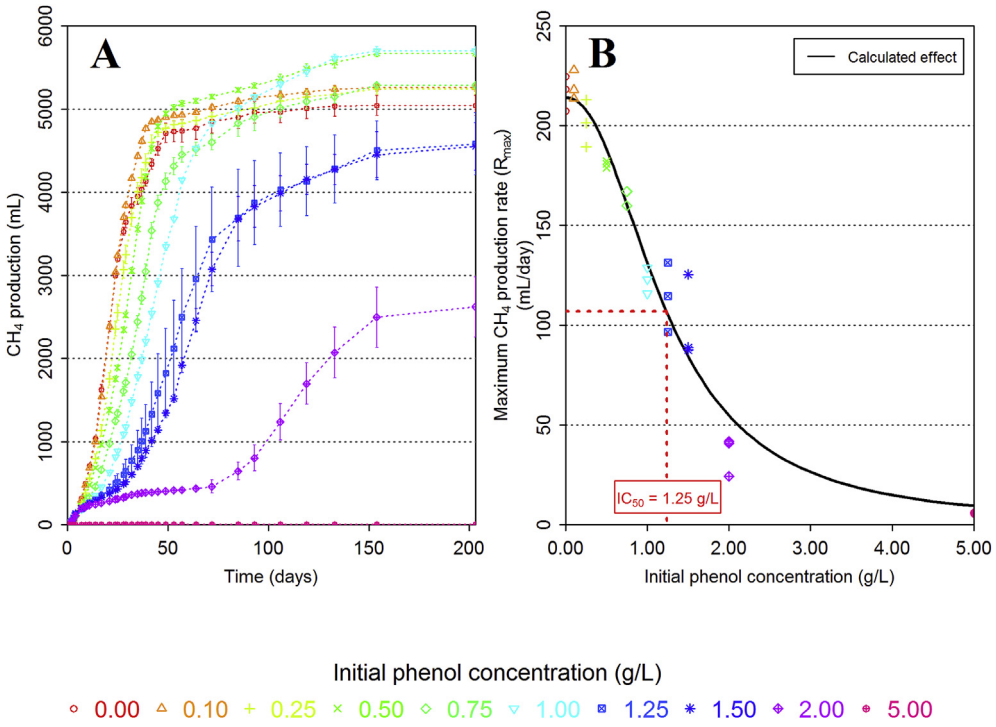

Fig. 1. (A) Cumulated CH_4 production (mL) over time (number of days) and (B) maximum CH_4 production rate for the different initial concentrations of phenol. Mean values of triplicate bioreactors are presented for CH_4 production and error bars represent standard deviation within triplicates. All experimental maximum CH_4 production rates are shown for each triplicate. Calculated fitting of experimental values to the Hill model is shown as well as IC_{50}.

CH_4 rate during cellulose AD was half inhibited by phenol at 1.40 g/L. Various inhibiting phenol concentrations can be attributed to the differences in nature of substrates, environmental conditions (pH, temperature) and acclimation of inoculum.

3.1.2. Phenol degradation

Phenol degradation dynamics are presented in Fig. 2. Phenol was completely degraded in all bioreactors up to 1.50 g/L (except in the 2nd replicate of 1.25 g/L assays). Other studies using unacclimated inoculum reported comparable results (Chapleur et al., 2015; Wang et al., 1991). At higher concentrations (2.00 g/L and 5.00 g/L) phenol degradation did not occur suggesting that specific bacterial populations involved in phenol degradation were completely inhibited. However, regarding biogas production results, methanogenesis still occurred at 2.00 g/L. Moreover, it was interesting to notice that methanogenesis started in all bioreactors before phenol started to be degraded. Both observations tended to corroborate other results indicating that archaeal methanogenic populations were less susceptible to phenol than specific bacterial populations involved in phenol degradation (Fedorak and Hrudey, 1984).

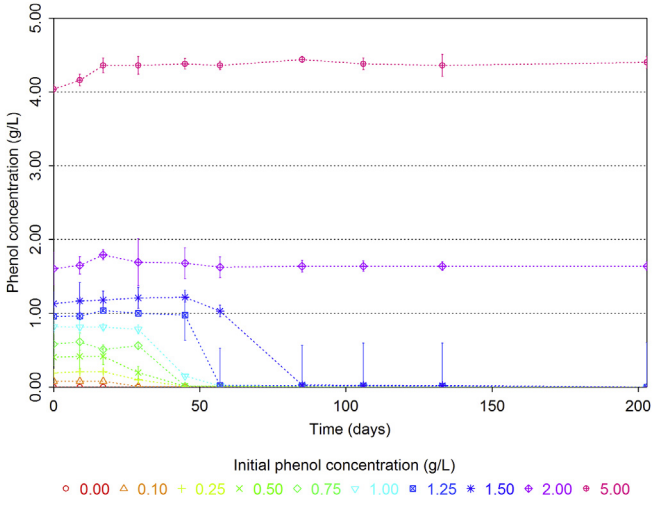

Fig. 2. Phenol concentration dynamics over time. Phenol concentration (g/L) over time (number of days) for the different groups of triplicate bioreactors (mean values are shown and error bars represent standard deviation within triplicates).

3.1.3. VFA accumulation and DOC dynamics

Acetic, propionic and butyric acids were detected in all the incubations. VFA accumulation dynamics are presented in supplementary material (Figure S2). At lowest phenol concentrations (up to 0.25 g/L), cumulated VFA concentration dynamics were virtually similar to the control. However, for all concentrations between 0.50 g/L and 2.00 g/L, maximum acetate concentrations were higher than in the control assays. Same results were also observed for butyrate up to 1.50 g/L of phenol. From 0.00 g/L up to 1.50 g/L, acetate and butyrate accumulation peaks occurred before phenol degradation. Therefore, their increases were attributed to the inhibition of acetate and butyrate degraders rather than to phenol degradation. Moreover, at 2.00 g/L, phenol degradation was completely inhibited throughout the incubation. Thus, acetate accumulation was probably also due to the inhibition of acetate degraders, probably methanogens. By contrast, propionate maximum concentration was lowered when initial phenol concentration was superior to 0.75 g/L, probably because propionate producers were partially inhibited by phenol. Nonetheless, a second significant accumulation phase of propionic acid, starting after 29 days of incubation, was noticed when phenol concentration varied between 1.25 g/L and 2.00 g/L. Since no phenol was degraded at 2.00 g/L, this production was not attributed to phenol degradation but rather to process instability as reported by other authors

(Chapleur et al., 2015).

DOC concentrations, presented in supplementary material (Figure S3) validated hypothesis laid out above. Up to phenol concentrations of 2.00 g/L, DOC values increased after 17 days of incubation, indicating that biowaste hydrolysis still occurred and was thus not totally inhibited by phenol. By contrast, in 5.00 g/L incubations, DOC never increased, confirming that anaerobic digestion was inhibited and that biowaste was not degraded. DOC dynamics were consistent with VFA and CH_4 kinetics. From 0.00 g/L up to 2.00 g/L, DOC decrease which corresponds to the conversion of organic substrate into CH_4 was also progressively delayed with increasing phenol concentration. After 85 days of incubation, DOC concentrations of batch digesters for which initial phenol concentration was between 0.00 and to 0.75 g/L was inferior to 0.10 g/L. By contrast, higher phenol concentrations led to delayed DOC consumption which remained over 0.25 g/L at the same sampling date. Residual DOC at higher phenol concentrations could be either attributed to phenol degradation metabolites which were not completely converted into CH_4, or to phenol itself in 2.00 g/L assays. But it could also reveal that phenol induced a less efficient degradation of the biowaste.

3.2. Investigation of microbiota response to increasing levels of phenol by ARISA fingerprinting

Microbial community dynamics were firstly studied with ARISA. Since low standard deviation was observed within each triplicate for all previous physicochemical indicators, one replicate per initial phenol concentration was selected for ARISA analysis apart for the 1.25 g/L assays for which the three replicates (R1, R2 and R3) were used. PCAs performed on archaeal and bacterial ARISA profiles are presented on Fig. 3. As CH_4 production was gradually delayed along with increasing initial phenol concentrations, a first approach consisted in computing independent PCAs for each sampling date. The objective was to identify potential patterns in microbial responses to different inhibition levels. To avoid any bias linked to microbial reorganizations caused by substrate depletion, samples collected after 9, 29 and 45 days of incubation were selected. A color scale depending on the initial phenol concentration in the bioreactors was used. The PCA axes that best separated these groups were PCA Axes 1 and 2 for all PCAs.

Archaeal and bacterial responses to increasing initial levels of phenol clearly suggested two distinct inhibition patterns. After 9, 29 and 45 days of incubation, archaeal profiles were regularly distributed along Axes 2 of the PCAs from 0.00 g/L up to 2.00 g/L, indicating the gradual influence of phenol on these communities (Fig. 3A–C). Nevertheless, beyond this threshold concentration, profiles recovered in the 5.00 g/L assay remained closer to the inoculum than the other profiles up to 29 days of incubation, suggesting that *Archaea* were strongly inhibited at this concentration. This corroborated CH_4 production kinetics which showed that methanogenesis was inhibited at this concentration. However, although they remained inactive throughout the experiment at this concentration, archaeal populations seemed to evolve after 45 days of incubation (Fig. 3C). This was mainly attributed to an analytic artefact induced by biomass decay.

By contrast, bacterial ARISA profiles were clustered into two groups, suggesting that phenol initially shaped bacterial communities at an initial concentration threshold varying around 1.00 g/L according to the sampling date (Fig. 3D–F). Phenol had a limited effect on bacterial communities throughout the incubation as long as its initial concentration did not exceed 0.50 g/L, since the corresponding profiles were close to the profile obtained in absence of phenol (0.00 g/L on Fig. 3) at each of the three time points. Above 0.75–1.25 g/L, bacterial communities evolved, from the earliest days of incubation, toward a distinct stable composition and they were probably more resistant to phenol. Moreover, since the composition remained relatively constant within each group throughout the experiment, it suggested that phenol degradation over time did not induce major bacterial reorganizations. Consequently, it could be hypothesized that phenol initial inhibitory effect towards *Bacteria* was possibly irreversible.

ARISA results tended to indicate that phenol induced microbial reorganizations from the lowest concentrations for *Archaea*, and from 0.50 g/L for *Bacteria* whereas AD final performances were not impaired up to 1.00 g/L of phenol. In order to confirm previous hypotheses and to verify if these adaptation patterns were permanent throughout the experiment, two additional PCAs, respectively presented in supplementary material (Figure S4A and S4B), were computed. Instead of comparing all profiles corresponding to the same sampling date, PCAs were computed on all the ARISA profiles for which CH_4 production was active. Hence, profiles for which CH_4 cumulated production had reached more than 90% of its ultimate yield were not considered representative of the active microbiota and were thus excluded from the analysis. It implied that additional sampling dates were included in these analyses, corresponding to the highest initial phenol concentrations for which ultimate CH_4 production was delayed. So as to evidence potential patterns in samples distribution during time, symbols' size was correlated with the progression of the incubations.

These additional PCAs confirmed both the distinct inhibition patterns previously observed for *Archaea* and *Bacteria*. Figure S4A (*Archaea*) revealed that within PCA factorial plane, samples were regularly distributed along with increasing phenol concentrations, confirming that the composition of active archaeal communities was gradually influenced by phenol up to 2.00 g/L. Nevertheless, samples collected in the ultimate days of incubation tended to cluster together, implying that archaeal communities tended to evolve toward a less differentiated composition. This pattern could be related to phenol degradation that allowed, once phenol concentration declined below a certain threshold, the progressive emergence of initially inhibited methanogens. Figure S4B showed that bacterial populations were modified by phenol at an initial concentration threshold comprised between 1.00 g/L and 1.25 g/L which seemed to remain constant throughout the experiment. These results confirmed that inhibitory effect of phenol on bacterial communities was probably irreversible. Level-dependent inhibition of phenol was already observed by Chapleur et al. (2015) who described different thresholds from 0.50 g/L up to 2.00 g/L, between which great modifications of archaeal and bacterial communities were evidenced while methanogenesis still occurred.

3.3. Identification of key phylotypes involved in microbial responses to different levels of inhibition by 16S rRNA sequencing

In order to further investigate patterns revealed by ARISA in microbial community dynamics and to identify key microorganisms potentially involved in community structure changes along increasing phenol inhibition levels, 16S rRNA gene high-throughput sequencing was performed. A total of 44 out the 70 samples used for ARISA analysis were processed. Several thousands of sequences were identified. They were assembled into 1693 and 42 OTUs for *Bacteria* and *Archaea*, respectively. Relative abundance of archaeal and bacterial OTUs were used for the analysis. In histogram representations, samples were aggregated by initial phenol concentration and by sampling date. Two PCAs were performed to statistically analyze *Archaea* and *Bacteria* 16S rRNA gene tags. For the sake of clarify of the PCA representation, T_0 sample, which was collected just before the beginning of the incubation and thus considered as an image of the initial inoculum, was not included in

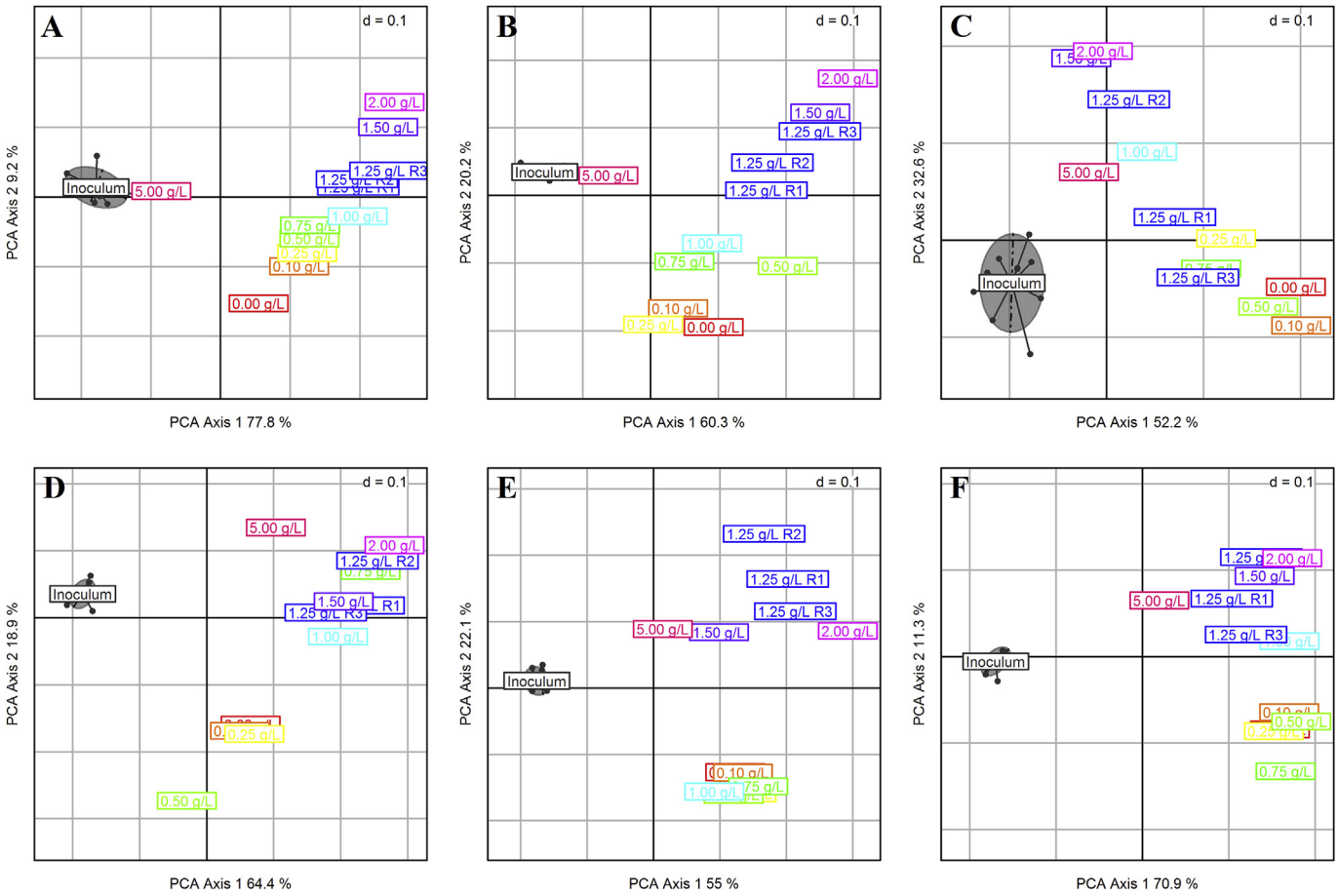

Fig. 3. Principal Component Analysis (PCA) of archaeal (A, B, C) and bacterial (D, E; F) diversity profiles generated by ARISA respectively after 9, 29 and 45 days of incubation. The color scale represents the initial concentration of phenol. The first and second axes of the PCA provided the clearest distribution of ARISA profiles with respectively 77.8–9.2% (A), 60.3–20.2% (B), 52.2–32.6% (C), 64.4–18.9% (D), 55.0–22.1% (E) and 70.9–11.3% (F) of the total variance.

analysis. In order to evidence potential patterns in sample distribution during time, symbols' size was correlated with the number of days of incubation. Associated correlation circles were used to identify principal representative OTUs differentiating samples within the factorial plane. Key phylotypes' phylogenetic assignations were abbreviated. Archaeal and bacterial OTU tables are presented in supplementary material (Tables S2 and S3).

3.3.1. *Archaea*

Archaeal OTU dynamics are presented on Fig. 4A. Methanogenic *Archaea* detected in the inoculum mainly belonged to an OTU assigned to genus *Methanolinea* with a relative abundance of 42% (ML1). Genera *Methanosarcina* and *Methanosaeta* as well as order *WCHA1-57* were also relatively abundant with relative abundances of 23%, 7% and 11%, respectively. Fig. 4B and C respectively showed associated PCA and correlation circle. According to the correlation circle, archaeal composition dynamics in all the samples appeared to be highly influenced by a core consisting of three OTUs: *Methanosarcina* OTU 2 (MS2), *Methanoculleus* OTU 1 (MC1) and *Methanolinea* OTU 1 (ML1). Therefore, the minor effect of all the other archaeal OTUs throughout the incubation is not discussed hereafter.

As long as archaeal communities were not able to overpass phenol inhibition and thus produce CH_4, their composition remained relatively close to the inoculum. Hence, ML1 was predominant in all samples for which methanogenesis remained inactive, indicating that members of this OTU were not involved in active CH_4 production. In particular, complete inhibition of methanogenic communities at 5.00 g/L was associated with the great dominance of this OTU (65%) throughout the incubation. In 2.00 g/L assays, ML1 abundance remained circa 40–53% until 57 days of incubation whereas it dropped under 20% as early as between 9 and 29 days in lower phenol-concentrated bioreactors. However, when methanogenesis became active after 133 days, ML1 proportion eventually decreased down to 20%. These observations were confirmed by PCA analysis. Indeed, from 0.75 g/L up to 2.00 g/L, the distribution of the samples collected during the early days of incubation seemed to be highly correlated with the relative abundance of ML1.

Phenol had a great influence on shaping archaeal communities throughout the incubation. In particular, a gradual shift between MS2 and MC1 seemed to occur along with increasing phenol concentrations from 0.50 g/L up to 2.00 g/L, corroborating the gradual inhibition pattern observed with ARISA.

Up to 0.50 g/L, phenol did not seem to influence archaeal composition since the relative abundance of these two OTUs remained respectively circa 43–47% and 6–11% all along the incubation. By contrast, from 0.75 g/L up to 1.50 g/L, MC1 proportion gradually increased from 19% to 51% at the expense of MS2 which dropped from 36% down to 12%. Since MS2 became predominant over time in the microbiota at lower initial phenol concentrations (up to 1.00 g/L), it suggested that this OTU played a key role in AD performance. Moreover, a recent study reported *Methanosarcina* as a sign of process stability (De Vrieze et al., 2012; Moset et al., 2015).

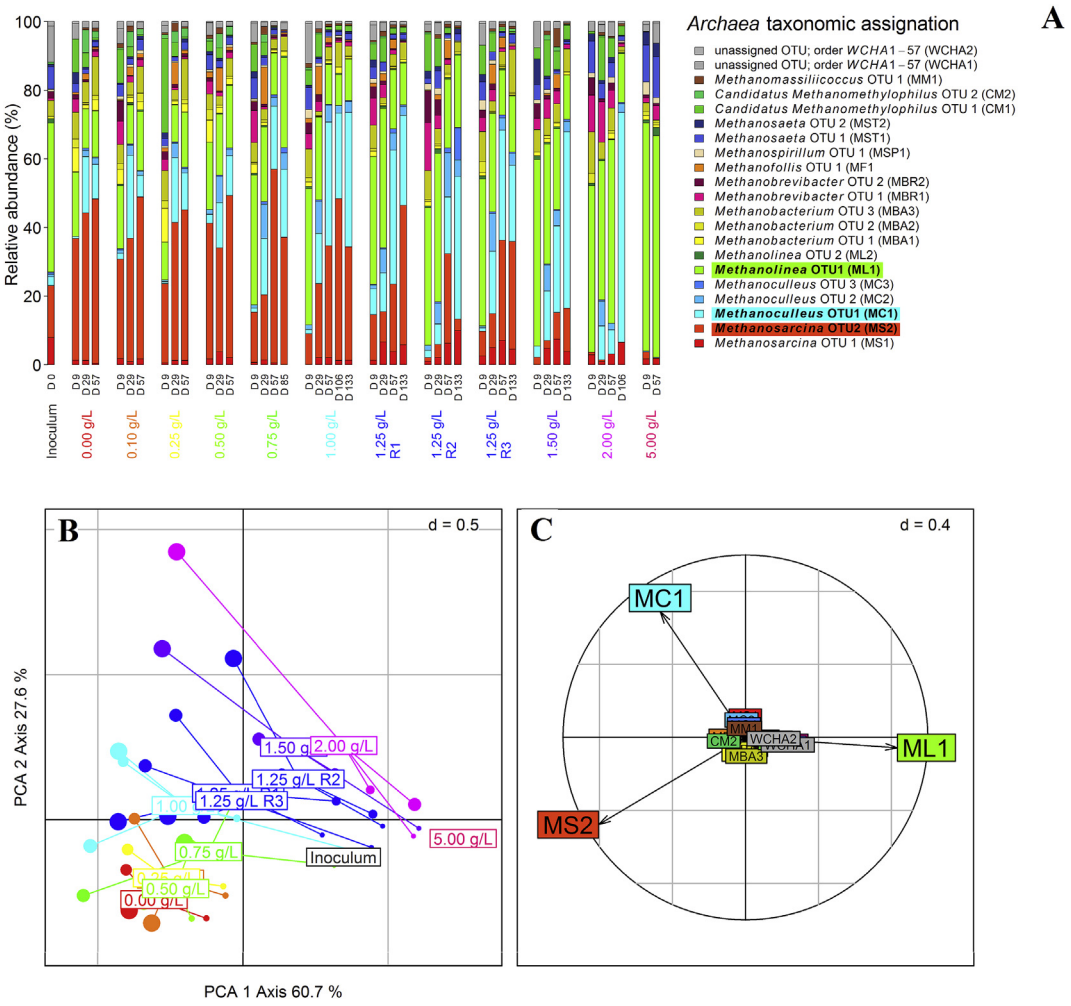

Fig. 4. Relative abundances of the archaeal OTUs generated by 16S rRNA gene sequencing (A) statistically analyzed by Principal Component Analysis (B). For the histogram representation (A), samples were clustered by initial phenol concentration (g/L) and by collection date (number of days). Different shades of the same color were used to represent archaeal OTUs belonging to the same genus. The influence of the principal archaeal OTUs on the PCA is shown with the arrows in the correlation circle (C). Within PCA, the color scale represents the initial concentration of phenol. The size scale of the symbols represents the percent of total cumulative methane production. OTUs belonging to the same genera were represented with the same color palette. The first and second axes of the PCA provided the clearest distribution with respectively 60.7% and 27.6% of the total variance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

On the other hand, *Methanoculleus* was revealed to be the predominant group of methanogens in digesters with syntrophic acetate oxidation (SAO) (Schnürer et al., 1999). It has been reported to be highly tolerant to environmental pressures such as ammonia and high VFA concentrations (Franke-Whittle et al., 2014; Poirier et al., 2016). The emergence of *Methanoculleus* has also been previously proposed as a potential warning indicator of acidosis (Goux et al., 2015). Hence, its increasing abundance along phenol concentrations suggested that MC1 played a key role in the maintenance of active methanogenesis through the establishment of specific syntrophic interactions probably with bacterial syntrophic acetate oxidizers. However, ultimate CH_4 yields began to decrease from an initial phenol concentration of 1.25 g/L indicating that beyond a certain threshold comprised between 1.00 and 1.25 g/L, despite MC1 members were in turn affected by the presence of phenol. At 2.00 g/L, MS2 proportion remained below 2% while MC1 became highly predominant (66%). It could thus be hypothesized that MC1 seemed to be the only OTU able to grow despite this inhibitory level and thus produce CH_4. However, ultimate CH_4 production was greatly impaired at 2.00 g/L, which could be related to the complete inhibition of phenol degradation inducing a constant pressure on the microbiota. This archaeal shift was clearly visible on PCA factorial plane within which bioreactors with increasing phenol concentrations were gradually positioned along PCA Axis 2. Furthermore, the correlation circle confirmed the antagonistic influences of MC1 and MS2 on this distribution.

Wirth et al. (2015) observed a strong shift from a *Methanosarcinales* dominated composition at low concentrations of phenol (up to 0.10 g/L) to an equal distribution of *Methanosarcinales*, and *Methanobacteriales* during the progressive adaptation of an inoculum up to 2.00 g/L of phenol corroborating the sensitivity of *Methanosarcina* to this inhibitor. These reorganizations within archaeal communities could potentially be associated with a shift in methanogenesis metabolic pathway. Indeed, several studies described an inhibition of acetoclastic methanogens by phenolic compounds (Olguin-Lora et al., 2003; Wang et al., 1991). Since *Methanoculleus* is an exclusive hydrogenotrophic methanogen, its gradual predominance along with increasing phenol concentrations tended to indicate that CH_4 was only produced via the hydrogenotrophic pathway at high inhibitory levels.

3.3.2. *Bacteria*

In total, *Bacteria* sequences were affiliated to 216 families. Among them, 189 represented individually less than 2% of the total sequences. The remaining core of 27 families, accounting together for more than 70% of the total number of bacterial sequences in each of the samples, was kept for the histogram representation on Fig. 5A as well as for PCA analysis. Fig. 5B and C respectively show PCA and associated correlation circle.

Most of the OTUs recovered in the inoculum belonged to family *Lactobacillaceae* (LACT) (51%) and to several families affiliated to order *Bacteroidales* (32%). Throughout the experiment, *Bacteria* recovered in every sample for which biogas was produced, were dominated by families belonging to three major orders: *Synergistales*, *Clostridiales* and *Bacteroidales*. Nevertheless, within these orders, bacterial populations revealed distinct adaptation patterns towards increasing phenol concentrations. Correlation circle associated to PCA evidenced six bacterial families which had the greatest influence on sample distribution: *Synergistaceae* (SYNE) affiliated to order *Synergistales*; *Lachnospiraceae* (LACN), *Ruminococcaceae* (RMNC) and *Syntrophomonadaceae* (SYNT) belonging to order *Clostridiales*; *Bacteroidaceae* (BCDA) and *Porphyromonadaceae* (PORP) assigned to order *Bacteroidales*. Therefore, the minor impact of other families is not discussed hereafter.

Similarly to *Archaea*, from 0.00 g/L up to 0.50 g/L, it appeared that bacterial composition dynamics was not influenced by phenol. Although *Synergistaceae* (SYNE) proportion in inoculum was recovered at only circa 2%, OTUs affiliated to this family significantly emerged from the earliest days of incubation in all bioreactors within this range of concentrations and relative abundances varying between 15% and 22% were reached. Moreover, the total proportion of families belonging to orders *Bacteroidales* and *Clostridiales* remained relatively stable circa 36%–42% and 23–39% respectively. Within order *Clostridiales*, an important shift seemed to occur over time. In the early days of incubation, it was dominated by families *Lachnospiraceae* (LACN) and *Ruminococcaceae* (RMNC) before *Syntrophomonadaceae* (SYNT) became predominant. This shift was clearly visible on PCA factorial plane and associated correlation circle. Order *Clostridiales* consists of fermentative organisms thought to use hydrocarbon intermediates to generate organic acid precursors. Due to their capacity to process a wide range of substrates, their importance has been stood out by many authors (Carballa et al., 2015). Moreover, they were also identified in many

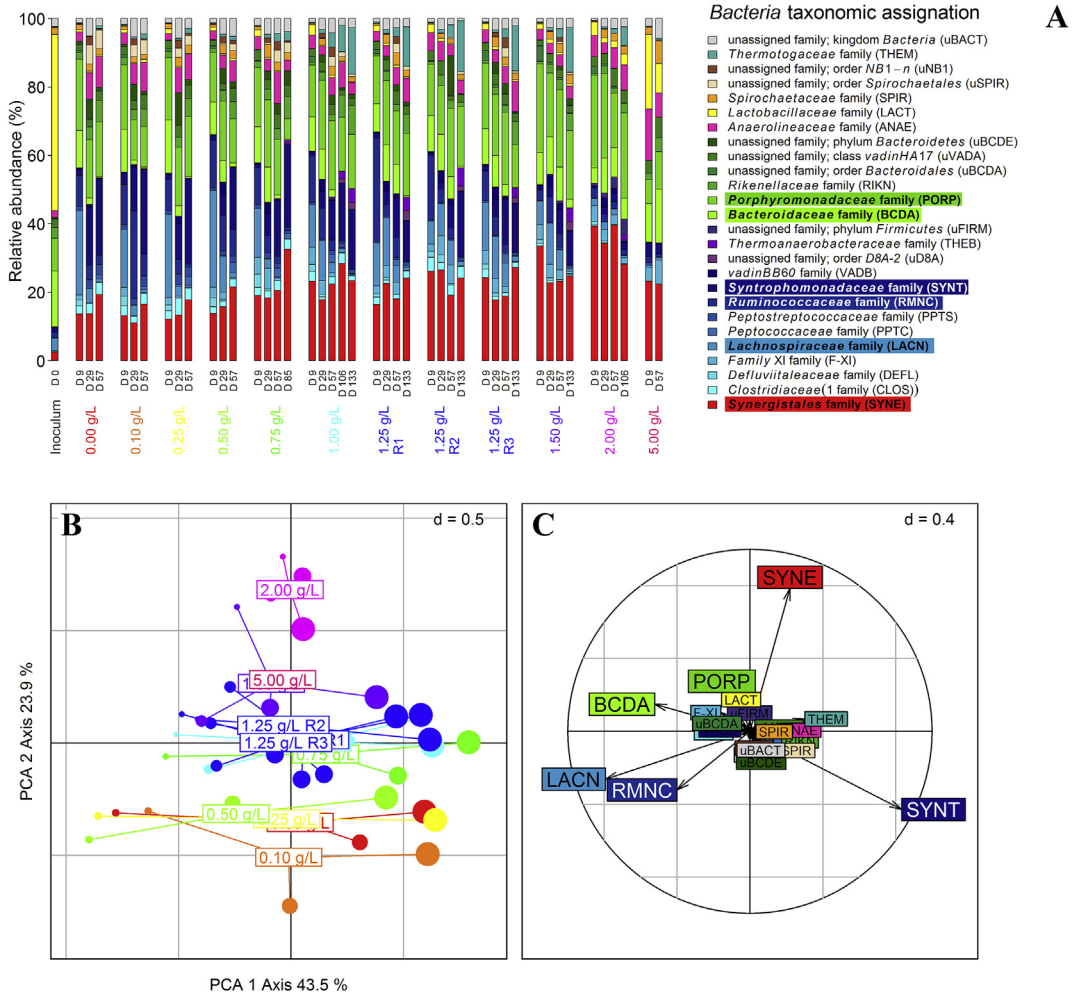

Fig. 5. Relative abundances of the bacterial families generated by 16S rRNA gene sequencing (A) statistically analyzed by Principal Component Analysis (B). For the histogram representation (A), samples were clustered by initial phenol concentration (g/L) and by collection date (number of days). Different shades of the same color were used to represent archaeal OTUs belonging to the same genus. The influence of the principal archaeal OTUs on the PCA is shown with the arrows in the correlation circle (C). Within PCA, the color scale represents the initial concentration of phenol. The size scale of the symbols represents the percent of total cumulative methane production. OTUs belonging to the same genera were represented with the same color palette. The first and second axes of the PCA provided the clearest distribution with respectively 43.5% and 23.9% of the total variance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

studies as aromatic hydrocarbon degraders (Rosenkranz et al., 2013). *Lachnospiraceae* and *Ruminococcaceae* were identified as two of the most abundant families from the order *Clostridiales* found in the mammalian gut environment and have been suggested to play important roles in protein hydrolysis (Biddle et al., 2013). They were reported to hydrolyze a variety of polysaccharides by different mechanisms. Consequently, these families were probably involved in the early steps of biowaste degradation. OTUs affiliated to family *Syntrophomonadaceae* (SYNT) were all assigned to genus *Syntrophomonas*, which has already been associated with efficient acetogenic performance, as well as with SAO (Carballa et al., 2015; Schnürer et al., 1999). Hence, it could be hypothesized that these families played a key role in AD process ranging from hydrolysis to acetogenesis and to SAO. Some of them could also be probably involved in phenol degradation according to Rosenkranz et al. (2013).

From 0.75 g/L up to 1.50 g/L, increasing phenol concentrations seemed to cause important modifications within dominant communities. Since these reorganizations occurred from the early days of incubation and remained relatively unchanged throughout the experiment, it suggested that contrary to *Archaea*, phenol degradation did not influence *Bacteria* response during time. Therefore, within this range of concentration, it could be hypothesized that *Bacteria* were only shaped by the initial selection pressure induced by phenol. In particular, relative abundance of *Synergistaceae* (SYNE) increased along with increasing initial phenol concentrations until reaching 24%–33%. Bacteria affiliated to this family have been described as anaerobic amino-acid degraders and syntrophic acetate oxidizers able to cooperate with *Methanosaeta* (Ito et al., 2011). Their presence is also associated to efficient acetotrophic performance (Carballa et al., 2015). Moreover, this family has also been recovered in hexadecane-degrading culture (Cheng et al., 2013). To the best of our knowledge, their role in phenol resistance or their potential role in SAO with *Methanoculleus*, which was the predominant methanogen at high inhibitory concentrations, has not been described yet. By contrast, total proportions of families belonging to order *Clostridiales* slightly decreased from 31% at 0.75 g/L, down to 21% at 1.50 g/L. This decrease was similar to that of *Syntrophomonadaceae* (SYNT) whose relative abundance dropped from 24% down to 10%. Consequently, the inhibition of these families seemed associated to the gradual emergence of *Synergistaceae* (SYNE). It could be hypothesized that this shift could lead to the implementation of new syntrophic interactions with *Archaea*. It also suggested that SAO could be maintained due to a possible functional redundancy between *Syntrophomonadaceae* and *Synergistaceae*. Within this range of concentrations, final AD performances were only impaired when initial phenol concentration was superior to 1.00 g/L while bacterial shifts occurred from a threshold comprised between 0.50 and 0.75 g/L. It evidenced that *Bacteria* started to adapt at initial phenol concentrations where the final CH_4 production was not deteriorated.

At 2.00 g/L, modifications seemed to be greatly accentuated compared to those observed between 0.75 g/L and 1.50 g/L. *Synergistaceae* (SYNE) relative abundance increased up to 40% evidencing its high phenol resistance, while the total proportion of families belonging to order *Clostridiales* (LACN, RMNC, and SYNT) dropped down to 13%. Nevertheless, resulting AD performances were strongly disturbed. Consequently, despite the emergence of *Synergistaceae*, the decline of families belonging to order *Clostridiales* and especially of *Syntrophomonadaceae* (SYNT) possibly evidenced the limits of microbiota adaptation ability, leading to deteriorated biodegradation. Moreover, since phenol degradation was completely inhibited at this concentration, it suggested that some of the OTUs belonging to *Clostridiales* could potentially be involved in related degradation pathways, as previously suggested (Rosenkranz et al., 2013). By contrast, total relative abundance of order *Bacteroidales*, which remained relatively constant circa 20–30% throughout the experiment up to initial phenol concentration of 1.50 g/L, increased up to 32–41% at 2.00 g/L. This order mainly consisted of OTUs affiliated to families *Bacteroidaceae* (BCDA) and *Porphyromonadaceae* (PORP) which were reported as the dominant families of the phylum *Bacteroidetes* in cattle manure (Moset et al., 2015). Family *Porphyromonadaceae* was suggested to play an important role in the degradation of the accumulated volatile fatty acids (Regueiro et al., 2014) while members of *Bacteroidaceae* have been characterized as important cellulolytic microorganisms (Patel et al., 1980). The maintenance of important percentages of *Bacteroidales* within a digester has already been suggested to be responsible for the ability of the anaerobic microbiota to counteract disturbances such as shock loadings (Regueiro et al., 2015). Since families *Bacteroidaceae* (BCDA) and *Porphyromonadaceae* (PORP) seemed to be resistant to phenol even up to 2.00 g/L, it suggested that they could be part of bacterial key players at high phenol concentrations.

As a result, it could be assumed that while families assigned to *Clostridiales* which are able to perform a wide range of metabolic steps within AD process in uninhibited conditions, were progressively inhibited by phenol, families belonging to *Bacteroidales* and *Synergistales* gained in importance within the microbiota at different thresholds. Increasing phenol concentrations also induced increasing selection pressure that also modified syntrophic populations. Since they are known to have extremely specific functions, their role is crucial to preserve the overall system performance (Carballa et al., 2015). This could be one of the reasons why, syntrophic families such as *Syntrophomonadaceae* (SYNT) were the first to be gradually substituted by *Synergistaceae* (SYNE) from lower initial phenol concentrations, thus contributing to the stability of methanogenesis. On the other hand, when selection pressure was too strong for *Clostridiales* hydrolytic families such as *Lachnospiraceae* (LACN), *Ruminococcaceae* (RMNC), other families from order *Bacteroidales* such as *Bacteroidaceae* (BCDA) and *Porphyromonadaceae* (PORP) supplanted them.

3.4. Towards the possibility of identifying microbial indicators associated with AD inhibition?

Phenol induced level-dependent modifications of the microbial community composition. Results moreover revealed that archaeal and bacterial major shifts occurred at lower concentration thresholds than those for which AD performance was impaired. It evidenced the difficulty to establish a direct correlation between degradation performance and community structure. It also raised the question whether microbial indicators could be used to anticipate anaerobic digester disruptions rather than remediate them.

In particular, genus *Methanosarcina* and family *Syntrophomonadaceae* both appeared as key players in efficient AD performance under uninhibited conditions. We hypothesized that their predominance within the microbiota explained why AD final performance were not deteriorated up to an initial phenol concentration of 1.00 g/L. Recent assumptions corroborated these observations by proposing robust methanogenesis microbiota based on *Syntrophomonas* and *Methanosarcina* for intensive energy production reactor systems (Ali Shah et al., 2014). However, from 0.50 g/L up to 2.00 g/L, the dominant *Methanosarcina* tended to be supplanted by *Methanoculleus* along increasing initial phenol concentrations while among syntrophic populations, *Synergistaceae* progressively emerged at the expense of *Syntrophomonadaceae*. The microbiome ability to adapt to increasing phenol concentrations and avoid the total process disruption was probably allowed by an ecological plasticity that was evidenced by both archaeal and bacterial shifts.

Since these shifts began to occur while final AD performances were still not impaired, we believe that they could be possible early warning microbial indicators portending phenol inhibitory effect.

The perspective of identifying microbial indicators associated with AD performance could produce new management tools for anaerobic digestion. In this objective, other analyses are needed to provide statistical robustness and to explore the different factors that have a strong influence on the microbial community composition (e. g. substrate, inoculum, environmental conditions, type of reactor etc.). A globalized database built with a standardized method of microbial characterization could then allow the determination of benchmark values useful to conduct microbial resource management and apply microbial based control prior to the process deterioration. These insights could pave the way to reach an optimal digestion rate despite the presence of various types of inhibitors, and thus improve waste treatment efficiency.

4. Conclusions

Up to 0.50 g/L, optimized AD performance was preserved regardless initial phenol concentration and AD microbiota composition remained unchanged. OTUs belonging to archaeal genus *Methanosarcina* and to bacterial orders *Clostridiales*, *Bacteroidales* and *Synergistales* were predominant within the microbiota.

From 0.50 g/L up to 1.00 g/L, microbial reorganizations started to occur without AD final performances being impaired. These changes were mainly evidenced by an increasing proportion of genus *Methanoculleus* and family *Synergistaceae*. These increases were suggested as early warning indicators portending phenol inhibition towards AD microbiota.

From 1.00 g/L up to 2.00 g/L, AD performance was gradually impaired. Community structural changes observed at lower initial phenol levels were more pronounced: *Methanoculleus* and *Synergistaceae* progressively became predominant. By contrast, *Methanosarcina* and *Syntrophomonadaceae* proportions decreased. Moreover, the proportion of OTUs affiliated to order *Bacteroidales* gained in importance at 2.00 g/L at the expense of *Clostridiales*.

At an initial phenol concentration of 5.00 g/L, methanogenesis was completely inhibited. Genera *Methanosarcina* and *Methanoculleus* were not detected. Orders *Bacteroidales* and *Synergistales* were predominant whereas *Clostridiales* was inhibited.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.05.041.

References

Ali Shah, F., Mahmood, Q., Maroof Shah, M., Pervez, A., Ahmad Asad, S., 2014. Microbial ecology of anaerobic digesters: the key players of anaerobiosis. Sci. World J. 2014, 21.

Biddle, A., Stewart, L., Blanchard, J., Leschine, S., 2013. Untangling the genetic basis of fibrolytic specialization by Lachnospiraceae and Ruminococcaceae in diverse gut communities. Diversity 5 (3), 627.

Busca, G., Berardinelli, S., Resini, C., Arrighi, L., 2008. Technologies for the removal of phenol from fluid streams: a short review of recent developments. J. Hazard Mater 160 (2–3), 265–288.

Carballa, M., Regueiro, L., Lema, J.M., 2015. Microbial management of anaerobic digestion: exploiting the microbiome-functionality nexus. Curr. Opin. Biotechnol. 33, 103–111.

Chapleur, O., Madigou, C., Civade, R., Rodolphe, Y., Mazeas, L., Bouchez, T., 2015. Increasing concentrations of phenol progressively affect anaerobic digestion of cellulose and associated microbial communities. Biodegradation 27 (1), 15–27.

Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. Bioresour. Technol. 99 (10), 4044–4064.

Cheng, L., Rui, J., Li, Q., Zhang, H., Lu, Y., 2013. Enrichment and dynamics of novel syntrophs in a methanogenic hexadecane-degrading culture from a Chinese oilfield. FEMS Microbiol. Ecol. 83 (3), 757–766.

De Vrieze, J., Hennebel, T., Boon, N., Verstraete, W., 2012. Methanosarcina: the rediscovered methanogen for heavy duty biomethanation. Bioresour. Technol. 112, 1–9.

Fang, H.H., Liu, Y., Ke, S.Z., Zhang, T., 2004. Anaerobic degradation of phenol in wastewater at ambient temperature. Water Sci. Technol. 49 (1), 95–102.

Fang, H.H.P., Chan, O.-C., 1997. Toxicity of phenol towards anaerobic biogranules. Water Res. 31 (9), 2229–2242.

Fang, H.H.P., Liang, D.W., Zhang, T., Liu, Y., 2006. Anaerobic treatment of phenol in wastewater under thermophilic condition. Water Res. 40 (3), 427–434.

Fedorak, P.M., Hrudey, S.E., 1984. The effects of phenol and some alkyl phenolics on batch anaerobic methanogenesis. Water Res. 18 (3), 361–367.

Franke-Whittle, I.H., Walter, A., Ebner, C., Insam, H., 2014. Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities. Waste Manag. 34.

Goux, X., Calusinska, M., Lemaigre, S., Marynowska, M., Klocke, M., Udelhoven, T., Benizri, E., Delfosse, P., 2015. Microbial community dynamics in replicate anaerobic digesters exposed sequentially to increasing organic loading rate, acidosis, and process recovery. Biotechnol. Biofuels 8 (1), 1–18.

Hernandez, J.E., Edyvean, R.G., 2008. Inhibition of biogas production and biodegradability by substituted phenolic compounds in anaerobic sludge. J. Hazard Mater. 160 (1), 20–28.

Hoyos-Hernandez, C., Hoffmann, M., Guenne, A., Mazeas, L., 2014. Elucidation of the thermophilic phenol biodegradation pathway via benzoate during the anaerobic digestion of municipal solid waste. Chemosphere 97, 115–119.

Ito, T., Yoshiguchi, K., Ariesyady, H.D., Okabe, S., 2011. Identification of a novel acetate-utilizing bacterium belonging to Synergistes group 4 in anaerobic digester sludge. ISME J. 5 (12), 1844–1856.

Levén, L., Nyberg, K., Schnurer, A., 2012. Conversion of phenols during anaerobic digestion of organic solid waste – a review of important microorganisms and impact of temperature. J. Environ. Manage 95 (Suppl. l), S99–S103.

Levén, L., Schnürer, A., 2010. Molecular characterisation of two anaerobic phenol-degrading enrichment cultures. Int. Biodeterior. Biodegrad. 64 (6), 427–433.

Monlau, F., Sambusiti, C., Barakat, A., Quemeneur, M., Trably, E., Steyer, J.P., Carrere, H., 2014. Do furanic and phenolic compounds of lignocellulosic and algae biomass hydrolyzate inhibit anaerobic mixed cultures? A comprehensive review. Biotechnol. Adv. 32 (5), 934–951.

Moset, V., Poulsen, M., Wahid, R., Højberg, O., Møller, H.B., 2015. Mesophilic versus thermophilic anaerobic digestion of cattle manure: methane productivity and microbial ecology. Microb. Biotechnol. 8 (5), 787–800.

Olguin-Lora, P., Puig-Grajales, L., Razo-Flores, E., 2003. Inhibition of the acetoclastic methanogenic activity by phenol and alkyl phenols. Environ. Technol. 24 (8), 999–1006.

Patel, G.B., Khan, A.W., Agnew, B.J., Colvin, J.R., 1980. Isolation and characterization of an anaerobic, cellulolytic microorganism, Acetivibrio cellulolyticus gen. nov., sp. nov. Int. J. Syst. Evol. Microbiol. 30 (1), 179–185.

Poirier, S., Desmond-Le Quéméner, E., Madigou, C., Bouchez, T., Chapleur, O., 2016. Anaerobic digestion of biowaste under extreme ammonia concentration: identification of key microbial phylotypes. Bioresour. Technol. 207, 92–101.

Regueiro, L., Carballa, M., Lema, J.M., 2014. Outlining microbial community dynamics during temperature drop and subsequent recovery period in anaerobic co-digestion systems. J. Biotechnol. 192 Pt A, 179–186.

Regueiro, L., Lema, J.M., Carballa, M., 2015. Key microbial communities steering the functioning of anaerobic digesters during hydraulic and organic overloading shocks. Bioresour. Technol. 197, 208–216.

Rosenkranz, F., Cabrol, L., Carballa, M., Donoso-Bravo, A., Cruz, L., Ruiz-Filippi, G., Chamy, R., Lema, J.M., 2013. Relationship between phenol degradation efficiency and microbial community structure in an anaerobic SBR. Water Res. 47 (17), 6739–6749.

Schink, B., Philipp, B., Muller, J., 2000. Anaerobic degradation of phenolic compounds. Naturwissenschaften 87 (1), 12–23.

Schnürer, A., Zellner, G., Svensson, B.H., 1999. Mesophilic syntrophic acetate oxidation during methane formation in biogas reactors. FEMS Microbiol. Ecol. 29 (3), 249–261.

Veeresh, G.S., Kumar, P., Mehrotra, I., 2005. Treatment of phenol and cresols in upflow anaerobic sludge blanket (UASB) process: a review. Water Res. 39 (1), 154–170.

Wang, Y., David Gabbard, H., Pai, P., 1991. Inhibition of acetate methanogenesis by phenols. J. Environ. Eng. 117 (4), 487–500.

Wirth, B., Krebs, M., Andert, J., 2015. Anaerobic degradation of increased phenol concentrations in batch assays. Environ. Sci. Pollut. Res. Int. 22 (23), 19048–19059.

Zhang, T., Ke, S.Z., Liu, Y., Fang, H.P., 2005. Microbial characteristics of a methanogenic phenol-degrading sludge. Water Sci. Technol. 52 (1–2), 73–78.