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Chemical characterization and anaerobic treatment of bitumen fume condensate using a membrane bioreactor

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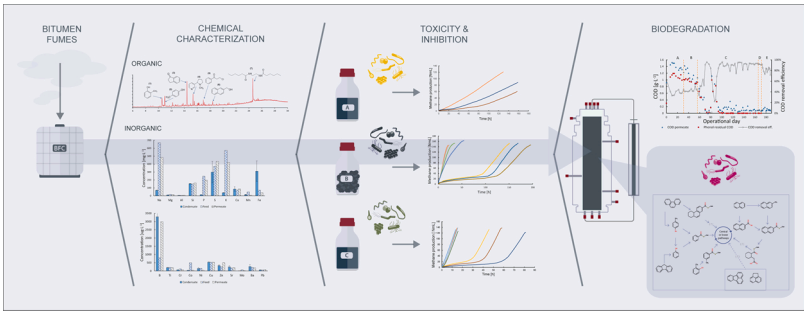
HIGHLIGHTS

- BFC characterized by average COD concentration of 0.58 g COD·L^{-1} and pH = 3.0
- BFC containing more than 900 organic and inorganic compounds
- Phenol-degrading AnMBR-cultivated biomass exerted an IC_{50} of 0.9 gBFC-COD·L^{-1}
- AnMBR sludge load of 97 mgCOD·g$VSS^{-1}d^{-1}$ with 88 % COD removal efficiency
- Presence of the aromatic degrader *Syntrophorhabdus* sp. during reactor operation

GRAPHICAL ABSTRACT



Abbreviations: ABR, Anaerobic baffled reactor; AD, Anaerobic digestion; AnMBR, Anaerobic membrane bioreactor; BFC, Bitumen fume condensate; BTEX, Benzene, toluene, ethylbenzene, xylene; CGWW, Coal gasification wastewater; COD, Chemical oxygen demand; EGSB, Expanded granular sludge bed; FCM, Flow cytometry; GC-FIC, Gas chromatography with flame ionization detector; GC-QTOF, Gas chromatography quadrupole-time-of-flight; GC-TCD, Gas chromatography with a thermal conductivity detector; HRT, Hydraulic retention time; IC_{50}, Half maximal inhibitory concentration; ICP-MS, Inductively coupled plasma mass spectrometry; OLR, Organic loading rate; OCR, Organic conversion rate; MWWTP, Municipal wastewater treatment plant; NIST, National Institute of Standards and Technology; PAH, Polycyclic aromatic compounds; PTA, Purified terephthalic acid; PVDF, Polyvinylidene fluoride; RAP, Reclaimed asphalt pavement; SBBR, Sequencing batch biofilm reactor; SCR, Sludge conversion rate; SLR, Sludge loading rate; SMA, Specific methanogenic activity; SRT, Solids retention time; TOC, Total organic carbon; TSS, Total suspended solids; UASB, Upflow anaerobic sludge blanket; VFA, Volatile fatty acids; VOC, Volatile organic compounds; VSS, Volatile suspended solids.

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ABSTRACT

Bitumen fume condensate (BFC) is a hazardous wastewater generated at asphalt reclamation and production sites. BFC contains a wide variety of potentially toxic organic pollutants that negatively affect anaerobic processes. In this study, we chemically characterized BFC produced at an industrial site and evaluated its degradation under anaerobic conditions. Analyses identified about 900 compounds including acetate, polycyclic aromatic hydrocarbons, phenolic compounds, and metal ions. We estimated the half maximal inhibitory concentrations (IC_{50}) of methanogenesis of 120, 224, and 990 $mgCOD{\cdot}L^{-1}$ for three types of anaerobic biomass, which indicated the enrichment and adaptation potentials of methanogenic biomass to the wastewater constituents. We operated an AnMBR (7.0 L, 35 °C) for 188 days with a mixture of BFC, phenol, acetate, and nutrients. The reactor showed a maximum average COD removal efficiency of 87.7 ± 7.0 %, that corresponded to an organic conversion rate of 286 ± 71 $mgCOD^{-1}{\cdot}L^{-1}d^{-1}$. The microbial characterization of the reactor's biomass showed the acetoclastic methanogen *Methanosaeta* as the most abundant microorganism (43 %), whereas the aromatic and phenol degrader *Syntrophorhabdus* was continuously present with abundances up to 11.5 %. The obtained results offer the possibility for the application of AnMBRs for the treatment of BFC or other petrochemical wastewater.

1. Introduction

Anaerobic digestion (AD) has been successfully applied to treat industrial wastewater since the late 1970s [60]. The first high-rate AD plant for the treatment of wastewater containing purified terephthalic acid (PTA) was installed in 1989 [34]. Since then, the application of AD to petrochemical wastewater has gradually expanded. Further research is nevertheless required to broaden the AD application potentials as a result of the current increase in production and discharge of (petro) chemical wastewater [26,58].

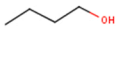
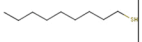
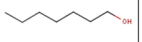
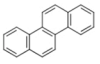
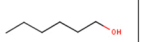
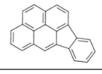
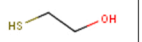
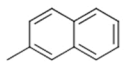
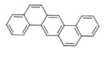
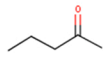
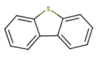
Phenol and phenolics, benzoate, and PTA, are aromatic compounds considered common petrochemical pollutants that are treated under anaerobic conditions [19,27,34]. The treatment of more complex wastewater has, however, received limited attention [5]. Few studies have applied AD for the degradation of non-synthetic industrial petrochemical wastewater [21,25,45,57]. This is especially apparent in the case of petrochemical wastewater from the asphalt and road industry, for example, during the production of reclaimed asphalt pavement (RAP).

The RAP is generated when old asphalt layers or pavement materials are removed, milled, and reused to produce new pavement. In this process, the old asphalt is heated and mixed with new materials such as stones, sand, and bitumen. Such a process releases fumes mainly originated from the bitumen [53,6]. Once the fumes are condensed, the condensate is rich in volatile organic compounds (VOC) [7], polycyclic aromatic hydrocarbons (PAH), and paraffinic, naphthenic, and aromatic (phenol and other phenolics) compounds [53,6].

Several compounds that could be toxic or inhibitory to anaerobic microorganisms have been reported to be present in the bitumen fume condensate (BFC). Among them, the major pollutants reported are naphthalene, acenaphthene, fluorene, phenanthrene, pyrene, benzo[*a*] pyrene, and anthracene (Table 1) [53,6,7]. Remarkably, some of these compounds have been biodegraded under anaerobic conditions [9].

The anaerobic conversion of BTEX (benzene, toluene, ethylbenzene, and xylene), naphthalene, methylnaphthalene, and phenanthrene has been reported [9]. No biodegradation studies have, however, been

Table 1
Major pollutants reported in BFC condensate.

1-Butanol		Acenaphthene		Benzyl alcohol		Fluoranthene		Pentachloro-phenol	
1-Decanethiol		Acetaldehyde		Benzene		Fluorene		Phenanthrene	
1-Heptanol		Acetophenone		Chrysene		Furfural		Phenol	
1-Hexanol		Anthracene		Coronene		Indenol[1,2,3-c,d]Pyrene		Pyrene	
2,6-Dimethylphenol		Benzo[a]anthracene		Cyclohexanol		m-Cresol		Resorcinol	
2-Mercaptoethanol		Benzo[a]pyrene		Cyclohexanone		MTBE		Tetrachloro-ethylene	
2-Methylnaphthalene		Benzo[b]fluoranthene		Dibenz[a,h]-anthracene		Naphthalene		Thioanisole	
2-Pentanone		Benzo[k]fluoranthene		Dibenzothiophene		o-Cresol		Thiophenol	
4-Ethylphenol		Benzoate		Ethylbenzene		Pentachloro-biphenyl		Para-xylene	

conducted for most of the compounds present in the BFC. Similarly, their toxic and inhibitory effects on anaerobic biomass are unknown. Hence, it is uncertain whether the treatment of this type of wastewater is feasible under anaerobic conditions, or if the BFC constituents could negatively affect the operation of anaerobic reactors, for example, by the display of toxic or inhibitory effects on the methanogenic biomass.

The anaerobic treatment of industrial wastewater requires systems that guarantee a robust operation, such as the well-known sludge bed reactors. These types of reactors rely on biomass granulation to immobilize key bacterial populations such as methanogenic archaea and specialized acetogens [60]. Due to its toxic or inhibitory characteristics, industrial wastewater can hamper the granulation process or even promote degranulation which results in biomass washout from the reactor with the subsequent reactor failure [13].

Anaerobic membrane bioreactors (AnMBR) appear as an attractive option to treat (industrial) wastewater that negatively affects the performance of granular-sludge-bed reactors [13]. AnMBRs offer the possibility of full biomass retention which allows for the growth and manifestation of the microorganisms required for the degradation of the (toxic/inhibitory) pollutants and the development of a robust methanogenic consortium [20]. Furthermore, the effluent (or permeate) is free of suspended solids, which enables the possibility for water reclamation when water loop closure is targeted.

AnMBRs have been used for the treatment of various types of industrial wastewater [13]. Nevertheless, most of the reactors have been applied to treat wastewater coming from the food, slaughterhouse and dairy, or paper mill industries [15]. Bhattacharyya et al., [5] reported the use of AnMBR for the treatment of synthetic high-strength petrochemical wastewater that contained formaldehyde and acrylic and acetic acids. The reactor achieved high removal efficiencies (>99 %) of chemical oxygen demand (COD) and total organic carbon (TOC). Garcia Rea et al., [19] reported the simultaneous degradation of phenol/*p*-cresol and phenol/resorcinol in AnMBRs under saline conditions. The study reported high removal efficiencies (≈ 100%) of the phenolics. To the best of the authors' knowledge, however, no research studies on the usage of AnMBRs or other anaerobic technology are reported for the treatment of BFC. Moreover, a better understanding of the microbial community dynamics involved in this process is needed to possibly improve the reactor performance.

This study characterized the BFC produced from RAP, assessed its negative effects on methanogenic biomass, and determined its biodegradation potential when an AnMBR was used. We analyzed the BFC through targeted and non-targeted screening techniques such as gas chromatography and mass spectrometry. Similarly, we determined the BFC's potential inhibitory or toxic effects on three types of anaerobic biomass. Furthermore, we characterized molecularly each of the biomass sources by the 16S rRNA gene analysis. Also, we operated and evaluated the performance of an AnMBR which treated BFC wastewater amended with phenol, acetate, and nutrients. Lastly, we studied the microbial community profiles in the AnMBR's biomass during the different operational stages.

2. Materials and methods

2.1. Chemical oxygen demand and volatile suspended solids

The COD was measured using COD determination kits (Lange Hach COD cuvette test LCK314 and LCK514) and was assessed spectrophotometrically using a Lange Hach DR3900 spectrophotometer. Volatile suspended solids (VSS) concentration was measured following Standard Methods [51].

2.2. Phenol and volatile fatty acids

Ethanol, propanol, butanol, cyclohexanol, cyclohexanone, phenol, *p*-cresol, volatile fatty acids (VFA) (acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, and hexanoic acids), and benzoate concentrations were measured in a gas chromatographer coupled to a flame ionization detector (GC-FID). The machine used was a GC Agilent 7890 A chromatograph (Agilent, USA) equipped with an Agilent 19091F-112 column of 25 m x 320 μm x 0.5 μm. Helium was used as carrier gas with a flow rate of 2.46 mL·min^{-1}. The GC oven temperature was programmed to be 80 °C for 1 min; then it was increased to 120 °C and finally to 180 °C in 4.5 min. The run time was set to 25 min. The temperature of the detector was set at 240 °C. The phenol concentration was double-checked spectrophotometrically (DR 3900 Hach) using Merck – Spectroquant® Phenol cell tests (Merck, Germany).

2.3. Characterization of the organic compounds in the BFC

BFC from a road and asphalt industry (Royal BAM, The Netherlands) was continuously withdrawn from an industrial plant (Bergen op Zoom, The Netherlands) for 180 days. A short description of the bitumen fume condensate generation method is provided in the Supplementary material S1 section. Various analytical techniques were applied to characterize the composition of the BFC. These included gas chromatography-tandem mass spectrometry (GC-MS/MS), gas chromatography-mass spectrometry quadrupole time of flight (GC/MS-QTOF), and inductively coupled plasma mass spectrometry (ICP-MS). A volatilization test was performed to verify the possible volatilization of the compounds present in the BFC (Supplementary material S2).

2.3.1. Targeted analysis: gas chromatography-tandem mass spectrometry

A targeted (68 compounds) GC-MS/MS analysis was performed by Het Waterlaboratorium (Haarlem, The Netherlands) using a Thermo Scientific TSQ 8000 triple quadrupole GC-MS/MS equipment with a high-volume programmable temperature vaporizing injector (Thermo Scientific, Massachusetts, USA). The column used was a Rxi-5-Sil-MS (fused silica) capillary column (Restek, Pennsylvania, USA) with a size of 25 m x 0.32 mm. Samples were prepared by filtering 10 mL of the BFC [1.12 gCOD·L^{-1}] through a 0.45 μm filter (Spartan, Whatman). The organic components of the BFC were then extracted using an in-vial extraction with pentane as the organic phase. For the injection, the inlet had a temperature of 40 ºC and had a split flow of 40 mL·min^{-1}. The program method utilized a carrier flow of 2.0 mL·min^{-1}. The oven temperature program was as follows: initial temperature 60 ºC held for 5 min, then ramped with a rate of 10 ºC·min^{-1} to 150 ºC. Afterward, a second ramp was performed with a rate of 15 ºC·min^{-1} until a temperature of 300 ºC was reached and subsequently held for 14 min. The quantification of the different compounds was performed in the single reaction monitoring mode and identified according to the Dutch standard NTA 8379:2014 en: Water quality – Guidelines for the identification of target compounds by gas and liquid chromatography and mass spectrometry.

2.3.2. Non-targeted analysis: gas chromatography-mass spectrometry quadrupole time of flight

A full scan analysis in electronic impact and positive chemical ionization mode was conducted using a GC/MS-QTOF on an Agilent 7200 Accurate mass GC/MS-QTOF. The column used was a DB-5 30 m x 0.25 mm × 0.25 μm (capillary column). The program method utilized a column flow of 1.2 mL·min^{-1} with an oven temperature program as follows: initial temperature 40 ºC held for 5 min, then ramped with 10 ºC·min^{-1} to 300 ºC and held for 5 min. The inlet temperature was 250 ºC and the transfer line temperature was 280 ºC. Liquid-liquid extraction was used to extract the volatile organic compounds. The procedure was as follows: 100 mL of BFC sample were added into a 125 mL separation funnel. Then 5 mL of methylene chloride were added and shaken for about 3 min. The methylene chloride was collected into conical extraction vials. This procedure was repeated twice to obtain approximately 15 mL of the organic phase. The extractions were dried through 1 g of sodium sulfate set on glass Pasteur pipets. After that, the

extractions were re-concentrated through nitrogen evaporation at a flow rate of 0.8 mL·min^{-1}. The final volume was approximately 2 mL. Before injection, the extractions were stored for 24 h at −20 °C in 2 mL amber glass vials.

Mass Hunter unknown analysis version B.08.00 was used for identifying unknown compounds. The mass spectral similarity search was performed by using NIST MS search 2.0 (NIST/EPA/NIH Mass Spectral Library, NIST 08, National Institute of Standards and Technology, 2008, Gaithersburg, MD).

2.4. Elemental analysis by inductively coupled plasma mass spectrometry

Various elements and their concentrations were analyzed in six samples of BFC, two of the feeding solution, and two of the AnMBRs permeate using ICP-MS. Before the analysis was performed, the samples were acidified (1 % v/v) with HNO_3 (69 %). Proper dilutions were prepared to keep the concentration of the analytes below 5 mg·L^{-1}. An ICP-MS model PlasmaQuant MS (Analytik Jena, Germany) was used for the analysis. 10 mL samples were injected into the equipment. The argon flow was 0.9 L·min^{-1} and the nebulizer flow was 1.1 L·min^{-1}.

2.5. Precipitation of the metals in the BFC

Per each L of BFC (pH = 3) 30.5 mL of phosphate solution A and 19.5 mL of phosphate solution B were added (pH = 7.4). The composition of the solutions is reported in [20]. The precipitate was separated from the solution by centrifugation (14 min, 10,000 g) and the supernatant was filtered through a glass-fiber filter of 0.7 μm. The concentration of the metals was measured in the BFC, in the filtrate, and reactor's permeate as specified in Section 2.4.

2.6. Effect of the BFC on the specific methanogenic activity and cell membrane integrity of anaerobic biomass

2.6.1. Specific methanogenic activity inhibition

Batch tests with various BFC concentrations (Table 2) were performed in 250 mL Schott (Schott Germany) glass bottles using three distinct inocula: a) phenol-degrading AnMBR-cultivated suspended biomass [20], b) granular biomass coming from a UASB treating petrochemical wastewater (Shell Moerdijk, The Netherlands), and c) anaerobic suspended biomass coming from a municipal wastewater treatment plant (MWWTP) (Harnashpolder, The Netherlands). Enough biomass inoculum was taken to have a final reactor volume of 200 mL. The VSS concentration in the batch reactor was adjusted to 4 g·L^{-1}. Acetate at a concentration of 2 gCOD·L^{-1} was used as substrate. Per each gram of COD, 0.3 mL of micronutrients and 3 mL of macronutrients solution were added [24]. The composition of the solutions is reported in [20].

The reactors were incubated at 130 rpm under mesophilic conditions (35 °C) in a temperature-controlled rotary shaker (New Brunswick Scientific, Innova 44). Methane production was measured online using an AMPTS II system (Bioprocess Control, Sweden). Each experimental condition was performed in triplicates. The specific methanogenic activity (SMA) value was calculated according to Spanjers and Vanrolleghem [56]. For the estimation of the half-maximum inhibitory concentration (IC_{50}) of the BFC on the SMA, a four-parameter logistic model was fit to the data using Sigma Plot software 12 [31].

Table 2
BFC concentration used for the SMA/cell membrane integrity batch tests.

Inoculum	Bitumen fume condensate concentration [mgCOD·L^{-1}]
Municipal WTTP	0, 75, 125, 250.
Petrochemical WTTP	0, 75, 125, 250, 500, 750, 1000.
AnMBR-cultivated	0, 75, 125, 250, 500, 750, 1000.

2.6.2. Cell membrane integrity determination

The cell membrane integrity of the anaerobic biomass used for the SMA assays was measured by flow cytometry (FCM) before and after the exposure to the various concentrations of BFC (section 2.5.1). Approximately, 1 mL sample of inoculum and medium were taken before the start of the SMA test and after the experiments were finished. The samples were diluted with PBS 1:500 (0.22 μm-filtered) [16] and prepared and measured as reported by Garcia Rea et al. [19].

2.7. AnMBR operation

2.7.1. AnMBR setup

An AnMBR was operated for 188 days. The setup comprised a fully-automated 7 L AnMBR with a working volume of 5 L (Fig. 1). The reactor was coupled to an ultrafiltration polyvinylidene fluoride (PVDF) membrane (Pentair, The Netherlands) with a nominal pore size of 30 nm. The reactor volume was controlled using two pressure sensors (AE Sensors, The Netherlands). The sensor located at the bottom of the reactor, measured the hydrostatic and gas pressures and had a range of 0–70 mbar. The sensor located at the top of the reactor was used for the gas pressure measurement and measured a range of 0–20 mbar. The temperature was kept constant at 35 °C with a water bath (Tamson Instruments, The Netherlands) that recirculated water through the reactor double-jacketed wall. To improve the mixing in the reactor, the biogas produced was recirculated in the reactor using a gas pump (KNF, The Netherlands). The total biogas production was measured by a gas meter (Ritter, Germany).

Anaerobic granular biomass from a UASB reactor treating petrochemical wastewater (Shell Moerdijk, The Netherlands) was used as seed biomass. The initial VSS concentration was 6 g·L^{-1}.

2.7.2. AnMBR feeding medium and operational conditions

Table 3 shows the composition of the feeding medium of the AnMBR within the different stages of the reactor operation. Micro- & macronutrient and phosphate buffer solutions were added to the feeding medium as stated in Section 2.6.1. Based on Hendriks et al. [24] and García Rea et al. [19], 50 mg of yeast extract were added per gram of COD in the feeding medium. Table 3 also shows the corresponding organic loading rates (OLR) and hydraulic retention time (HRT).

2.8. Microbial community dynamics

2.8.1. Biomass sampling, DNA extraction, 16S rRNA gene amplification, and DNA data processing

Approximately, 1 mL samples were taken from each of the three biomass sources used for the batch assays before the tests were started (Section 2.6). Similarly, 0.5–1.0 mL of biomass samples were taken from the reactor during several days of reactor operation. All the samples were processed and stored as reported in [20]. The DNA was extracted with the DNeasy UltraClean Microbial Kit (Qiagen, Germany). The DNA quantity and quality were checked using the Qubit 3.0 DNA detection system (Qubit dsDNA HS assay kit, Life Technologies, United States). The amplification of the V3-V4 hypervariable regions of the 16 S rRNA gene was conducted by Novogene as reported in [20]. The sequences were deposited in the SRA (NCBI) database under the accession number PRJNA748451.

The bioinformatics for the analysis of the 16 S rRNA gene sequences was performed as reported in [20]. The QIIME2 pipeline [8] and the R environment with the phyloseq library were employed to perform the statistical analysis. A principal coordinate analysis (PCoA) for the three biomass sources used for the batch assays (Section 2.6) was conducted using R and applying the weighted UniFrac distance matrix. An association analysis with the MaAsLin2 (Microbiome Multivariable Associations with Linear Models) approach was performed in R using the MaAsLin 2 package [35]. The genera with a relative abundance over 1 % and 25 % of prevalence were correlated with the COD removal efficiency

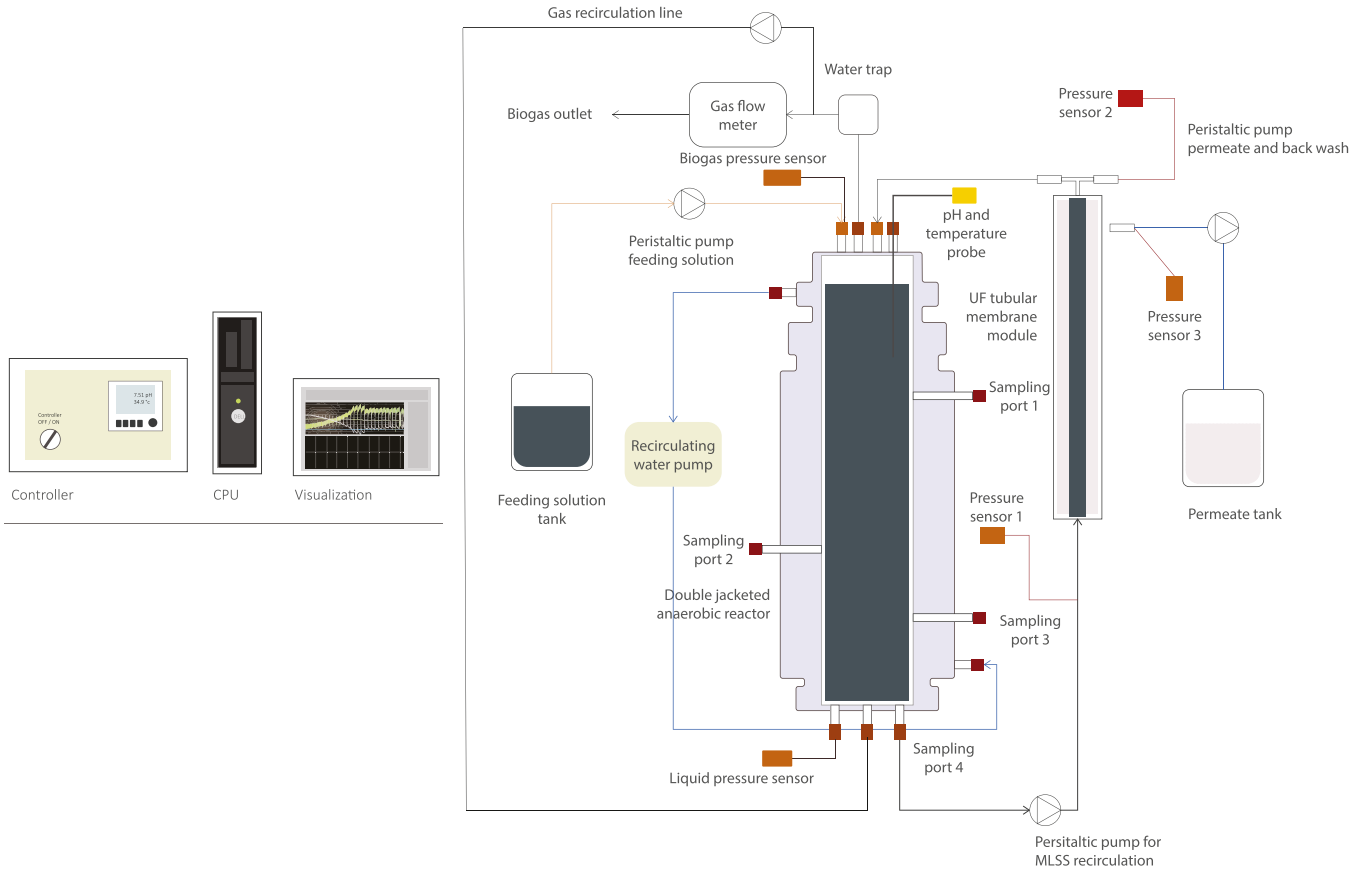


Fig. 1. Scheme of the AnMBR setup used during the continuous experiment.

Table 3
AnMBR feeding medium composition and operational conditions.

Stage	Day	BFC [gCOD·L^{-1}]	Acetate [gCOD·L^{-1}]	Phenol [gCOD·L-1]	OLR* [gCOD·L^{-1}d^{-1}]	HRT [d]
A	0–30	1.2	0.5	1.2	0.82 ± 0.19	2.9 ± 0.7
B	31–57	1.2–0.4	0.5	1.2	0.59 ± 0.23	3.7 ± 1.2
C	58–165	0.4–0.2	0.5	0.5	0.33 ± 0.09	4.1 ± 1.0
D	166–172	0.3	0.5	0.2	0.29 ± 0.07	3.7 ± 0.4
E	173–188	0.3	0.25	0	0.31 ± 0.06	2.1 ± 0.7

* The OLR and the HRT are reported with ± standard deviation (SD).

of the reactor during the different reactor operation stages. The associations with a corrected p-value < 0.05 were considered significant.

3. Results and discussion

3.1. Bitumen fume condensate characterization

3.1.1. COD

The COD was measured in the different samples to have an initial characterization of the BFC. The COD showed a range from 0.24 to 1.20 gCOD·L^{-1} (average: 0.58 gCOD·L^{-1} ± 0.39, n = 33), most likely because the BFC collection method at the industrial site was not standardized (Supplementary material, S1). Boczkaj et al., [7] reported COD concentrations between 18 and 22 g·L^{-1} in post oxidative effluents from a bitumen production plant; however, to the best of the authors' knowledge, there are no reports on COD values of BFC. The COD-BFC could cause toxic or inhibitory effects on the anaerobic biomass because of the nature of its pollutants, even when such COD concentration is low in comparison to other industrial effluents (e.g. food industry) [34,41].

3.1.2. Phenol and volatile fatty acids

Based on the GC-FID analysis, acetic acid at a concentration of 13 mg·L^{-1} (13.9 mgCOD·L^{-1}) and phenol at a concentration of 8.3 mg·L^{-1} (19.7 mgCOD·L^{-1}) were found in a BFC sample of 500 mgCOD·L^{-1}. Phenol and VFA can be found as pollutants in petrochemical wastewater [26,55] and can be used as carbon and energy sources by anaerobic microorganisms. Other VFAs were not detected by the GC-FID; nor were compounds such as ethanol, propanol, butanol, cyclohexanol, cyclohexanone, *p*-cresol, or benzoate.

3.1.3. Organic compounds targeted analysis: GC-MS/MS

The GC-MS/MS targeted analysis showed different concentrations of aromatic hydrocarbon compounds such as naphthalene [0.70 µg·L^{-1}], acenaphthylene [0.27 µg·L^{-1}], propyzamide [0.18 µg·L^{-1}], p,p-DDT [0.07 µg·L^{-1}], fluorene [0.04 µg·L^{-1}], phenanthrene [0.04 µg·L^{-1}], acenaphthene [0.03 µg·L^{-1}], and fluoranthene [0.02 µg·L^{-1}]. Table S1 (Supplementary material S3) shows the list of the 67 analyzed organic compounds that are commonly reported to be present in the BFC and their measured concentrations. The aromatic compounds and PAH determined in the analysis agree with what is reported in the literature [53,6,7] (Table 1).

3.1.4. Organic compound non-targeted analysis: GC-MS QTOF

The GC-MS QTOF analysis detected several peaks corresponding to compounds such as p-cresol, o-cresol (phenol, 2-methyl), and 2-naphtalene methanol when the unknown organic compounds in BFC were analyzed. Similarly, the compounds 1-indanone (1H-inden-1-one,2,3-dihydro), nicotine (pyridine, 3-(1-methyl-2-pyrrolidinyl)), β-chloropropiophenone (1-propanone, 3-chloro-1-1phenyl) and hexanoic acid, (2-hexanoylaminoethyl)-amide exhibited high peak areas. We proposed the structures of these compounds based on their accurate mass obtained from the matching NIST data library. Fig. 2 shows the chromatogram of the BFC where the compounds with the largest peak areas are depicted. A full scan detected 933 compounds, but only 430 matched the NIST's database. Compounds shown in Table S2 (Supplementary material S4) are tentative compounds that corresponded to an 80 % of chemical structure match with those found in the library. Surrogate organic parameters analyses (Supplementary material S5) confirmed the high content of aromatic compounds.

3.1.5. Elemental analysis by inductively coupled plasma mass spectrometry

Our results showed the presence of 20 elements (metals and several non-metals) in the BFC, the reactor's feeding, and permeate (Fig. 3 A & B). The highest concentrations in the BFC corresponded to iron [310.9 ± 129.8 mg·L^{-1}], sulphur [299.0 ± 135.3 mg·L^{-1}], silicon [153.2 ± 2.6 mg·L^{-1}], calcium [85.9 ± 27.2 mg·L^{-1}], and sodium [69.8 ± 1.4 mg·L^{-1}]. Due to the nature of the BFC, the presence of metals was not expected. However, the presence of the ions may be related to the leaching of these metals from the chimney and piping systems as the BFC was acidic (pH = 3). The metal precipitation and filtration steps were conducted prior to the batch biochemical tests or to the reactor feeding to avoid a possible inhibition of the methanogenic biomass due to a relatively high metal concentration, for example, iron; or an increase in the clogging and fouling of the membrane [1,3]. The iron concentration was decreased from 310 ± 130 mg·L^{-1} in the BFC to 70 mg·L^{-1} in the feeding solution. Iodine and bromine ions were detected in low concentrations (Supplementary material S6).

3.2. Biochemical tests

3.2.1. Specific methanogenic activity inhibition

The results showed a completely inhibited biogas production at an initial BFC concentration of 250 mgCOD-BFC·L^{-1} for the MWWTP's anaerobic suspended biomass (Fig. 4A). We estimated an IC_{50} value of 120 mgCOD-BFC·L^{-1} (Fig. 4 B) by applying the four-parameter model equation [31]. A decrease in the SMA values was observed when the COD-BFC's concentrations were increased (Fig. 4 B). Such a decrease corresponded to 22.1 ± 9.4 % and 54.4 ± 12.5 % less methane production rate than the control (acetate 2 gCOD·L^{-1}) for the concentrations of 75 and 125 mgCOD-BFC·L^{-1}, respectively.

The granular biomass coming from the UASB treating petrochemical wastewater was less sensitive to the BFC in comparison to the MWWTP's biomass. For example, no complete SMA inhibition was observed (Fig. 5 A), even at the highest BFC-COD concentration (1000 mgCOD·L^{-1}). An IC_{50} value of 224 mgCOD·L^{-1} was estimated for this biomass (Fig. 5 B). Although a considerable lag phase was observed for BFC concentrations higher than 250 mgCOD·L^{-1}, all COD was ultimately converted to methane.

The inhibitory effect of the BFC-COD on the SMA of the phenol-degrading AnMBR-cultivated suspended biomass was even lower than on the other two biomass sources (Fig. 6A), having an estimated IC_{50} value of 930 mgCOD·L^{-1} (Fig. 6 B). The extent of the methane production was not affected even at the highest BFC-COD concentrations (Fig. 6 A), which was similar to the results observed for the granular biomass treating petrochemical wastewater. The lag phase was only present for concentrations higher than 500 mgCOD·L^{-1}, and it was shorter in comparison to the lag phases found for the petrochemical biomass.

The AD process can be inhibited by most of the organic compounds present in the BFC such as PAH, (alkyl-) benzenes, phenolics, alkanes, etc. [11,12]. Olguin-Lora et al. [46] reported IC_{50} values of 0.43 and 0.58 g·L^{-1} for *o*-cresol and 0.39 and 1.03 g·L^{-1} for *p*-cresol on non-adapted and adapted anaerobic granular biomass, respectively.

The suspended biomass coming from the phenol-degrading AnMBR was the most active and resistant to the inhibitory effects of the BFC in comparison to the other two biomass sources. Garcia Rea et al., [19] showed that anaerobic biomass coming from a phenol-degrading AnMBR is less inhibited by other phenolic compounds, such as *p*-cresol and resorcinol, compared to anaerobic suspended biomass harvested from a MWWTP. The lower inhibition was attributed to the (full) biomass retention offered by the membrane. Garcia Rea et al., [19] states that the resulting methanogenic consortia was more resistant to phenolics, likely due to the continuous exposure to phenol that combined with the membrane filtration ensured that all the exposed biomass

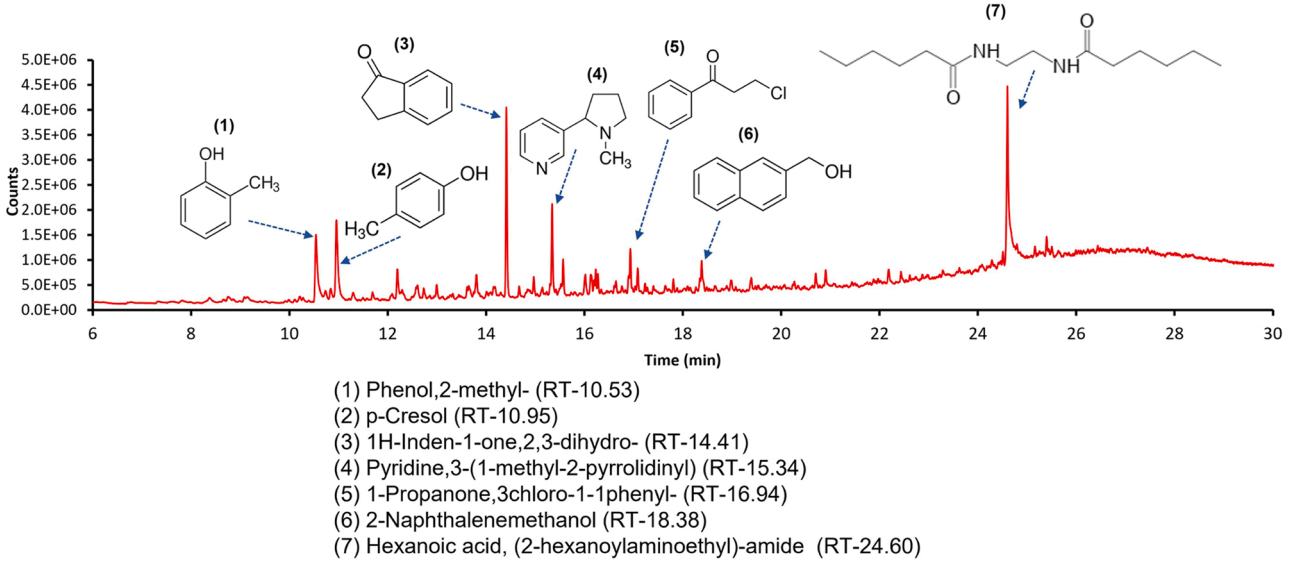


Fig. 2. GC-MS/QTOF chromatogram of the bitumen fume condensate with the proposed molecular structures and names corresponding to the major peaks found. The most common names for the next compounds are provided in brackets: phenol, 2-methyl (o-cresol), 1H-inden-1-one,2,3-dihydro (1-indanone), pyridine, 3-(1-methyl-2-pyrrolidinyl) (nicotine), and 1-propanone, 3-chloro-1-1phenyl (β-chloropropiophenone).

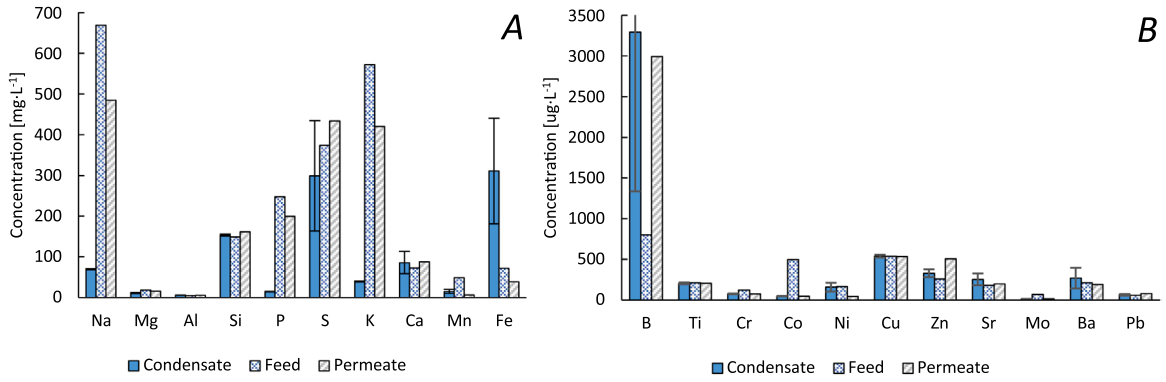


Fig. 3. Concentrations of the major (*A*) and minor (*B*) elements found in the bitumen fume condensate and the reactor's feed & permeate. Error bars = SD, n = 6 for BFC and 2 for feeding solution and permeate.

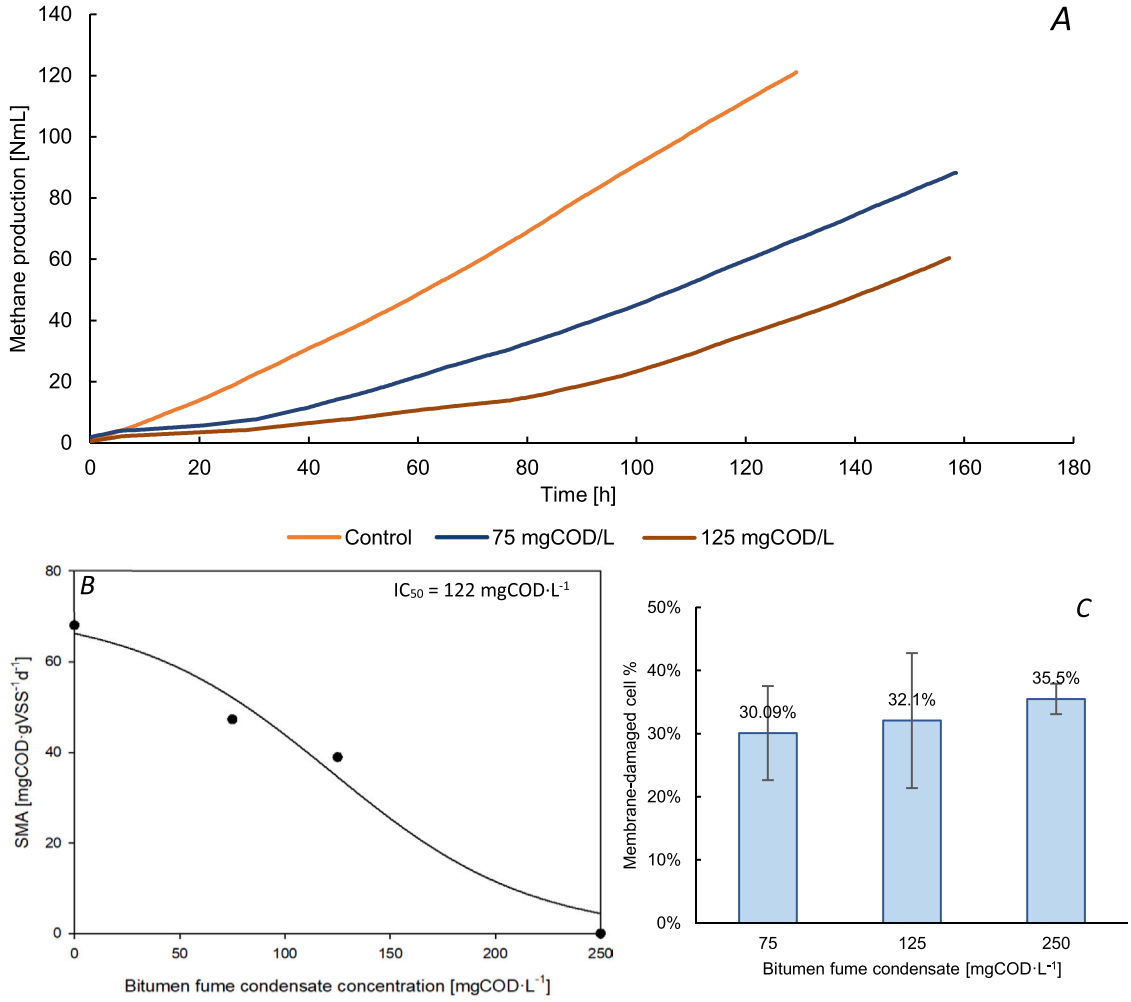


Fig. 4. Accumulated methane production for the different BFC concentrations used. The assays were performed in triplicates, the most representative curves are shown (*A*). In the assay corresponing to 250 mg BFC-COD·L^{-1} there was a total inhibtion of the methanogenic activity, meaning no biogas production (*A*). SMA inhibition in anaerobic municipal sludge due to the dosage of BFC, each point represents the average of three assays (*B*). Membrane-damaged-cell percentage after finalizing the SMA test (*C*), n = 3, bars = SD.

was retained inside the reactor. Our present results subscribe to this hypothesis, as we saw that the phenol-degrading AnMBR biomass was less prone to inhibition by the aromatic compounds-rich BFC (Fig. 6 A and B) and had the most abundant methanogenic population (Section 3.2.3).

3.2.2. *Cell membrane integrity determination*

To determine whether the inhibitory effect of the BFC on the different biomass sources was biostatic or biocidal [4], we analyzed the cell membrane integrity by the live-dead cell protocol [16] once the SMA inhibition tests were concluded. For the suspended biomass coming from the MWWTP, we observed a maximum of 35 % increase in

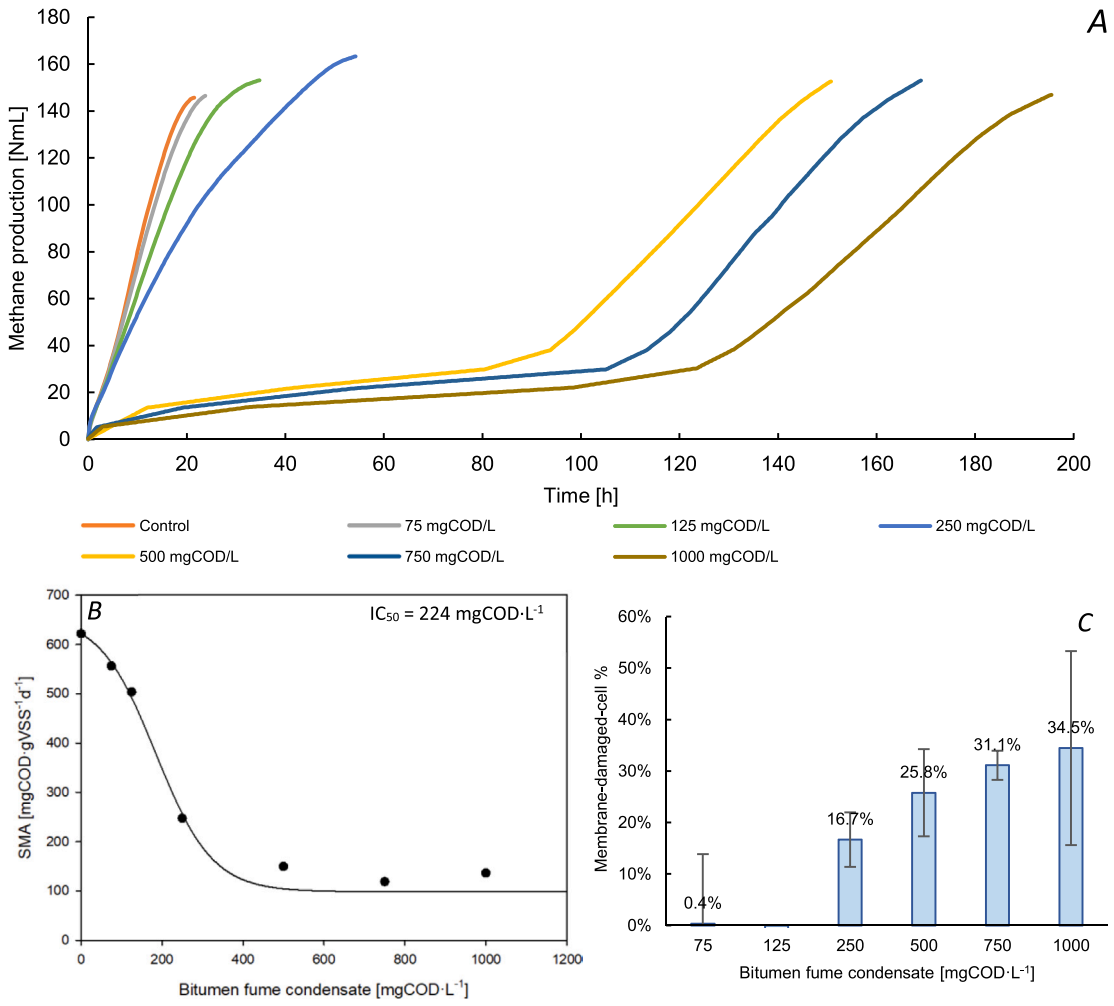


Fig. 5. Accumulated methane production for the different BFC concentrations used. The assays were performed in triplicates, the most representative curves are shown (*A*). SMA inhibition in anaerobic sludge from a petrochemical WWTP due to the dosage of BFC, each point represents the average of three assays (*B*). Membrane-damaged-cell percentage after finalizing the SMA test (*C*), n = 3, bars = SD.

damaged cells with the highest BFC concentration of 250 mgCOD·L^{-1} in comparison to the control assays (Fig. 4C). For the granular biomass that treated the petrochemical wastewater, we observed a maximum of 34.5 % increase in damaged cells at a concentration of 1000 mgCOD·L^{-1} (Fig. 5 C). In contrast, for the suspended AnMBR-cultivated biomass there was a 16.5 % increase in damaged cells with a BFC concentration of 1000 mgCOD·L^{-1} (Fig. 6 C).

The SMA inhibition observed in the anaerobic suspended biomass coming from the MWWTP (Section 3.2.1) could have also been related to an increase in biomass decay. The granular biomass coming from the UASB reactor had higher percentages of membrane-damaged cells for the three highest COD-BFC concentrations in comparison to the AnMBR-cultivated biomass, but suffered less damage than the municipal biomass. The kinetic and morphologic performance of the UASB reactor's granular biomass might be attributed to: 1) the granular biomass was acclimated to (petrochemical) aromatic compounds, mainly benzoate and other hydrocarbons, and 2) the granular morphology provided extra protection to the core anaerobic microorganisms due to a) the possible degradation of toxic organic compounds at the outer layers of the granules, and b) steric and/or electrostatic hindrance of the more hydrophobic aromatic compounds which may have access to the inner parts of the granules. The latter reasons might result in a lower exposure of the methanogens to inhibitory or toxic concentrations of specific pollutants. Although the analysis was targeted to all the prokaryotes in the biomass, and not solely to the methanogenic population, it could be possible that the methanogens were most affected, as (acetoclastic) methanogens are reported as one of the most sensitive groups in the AD process [12,2].

From the obtained results, it can be concluded that biomass grown in an AnMBR with a previous (chronic) exposure to phenol was less prone to biocidal inhibition caused by the BFC in comparison to the other two biomass sources.

3.2.3. *Microbial community structure of the three biomass sources*

The molecular analysis performed in the three different biomass sources used in the batch tests showed that the biomass coming from the phenol-degrading AnMBR had the highest relative abundance of the acetoclastic methanogenic microorganism *Methanosaeta* sp. (36.7 ± 8.9 %), namely 7.0 and 20.6 times higher in comparison to the granular (5.3 ± 1.3 %) and suspended anaerobic biomass coming from the MWWTP (1.8 ± 0.3 %), respectively (Fig. 7). It should be noticed, however, that this reactor was fed with an acetate-phenol mixture [20], which explains the high abundance of the (acetoclastic) methanogens. *Thermovirga* sp. was found in high abundance as well (14.6 ± 1.7 %). This microorganism has been reported in phenol-degrading AnMBRs and UASB reactors [20,40,61]; although, its role is still unknown. *Syntrophus* sp. had a relative abundance of 4.0 ± 1.1 %. This microorganism is a reported syntrophic anaerobic degrader of aromatic compounds such as benzoate [36] and has been reported as a possible degrader of aromatic compounds present in various (petro)chemical wastewater

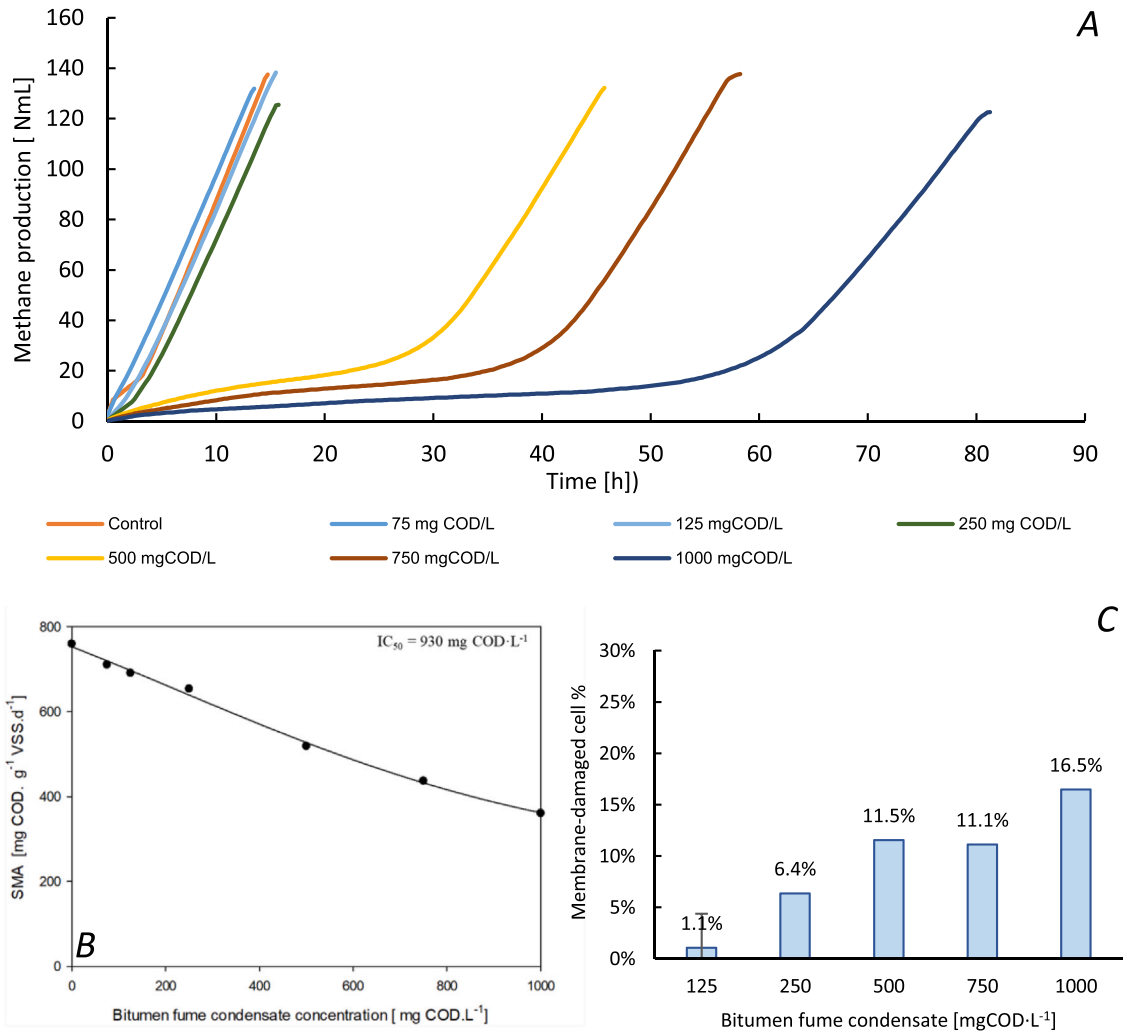


Fig. 6. Accumulated methane production for the different BFC concentrations used. The assays were performed in triplicates, the most representative curves are shown (A). SMA inhibition in an AnMBR-cultivated phenol-degrading anaerobic sludge due to the dosage of BFC, each point represents the average of three assays (*B*). Membrane-damaged-cell percentage after finalizing the SMA test (*C*), n = 3 for [125 mgCOD·L^{-1}] and n = 2 for [250 − 1000 mgCOD·L^{-1}], bars = SD.

[48,54]. *Syntrophorhabdus sp.,* a reported phenol degrader [44] presented a relative abundance of 2.5 ± 0.8 %.

The granular biomass coming from the UASB reactor had a higher abundance of *Syntrophus* sp. (24.7 ± 2.4 %) which may explain the decrease in the inhibitory effects caused by the BFC. Other abundant bacteria included *Pseudomonas* sp. (5.6 ± 0.8 %), *Thermovirga* sp. (3.2 ± 0.3 %), and *Syntrophorhabdus* sp. (1.6 ± 0.1 %). The archaeal population was mainly represented by *Methanosaeta* sp. (5.3 ± 1.3 %) and the hydrogenotrophic methanogen *Methanolinea* sp. (5.3 ± 0.1 %).

The anaerobic biomass coming from the MWWTP had a low relative abundance of methanogens, e.g., *Methanosaeta* sp. (1.8 ± 0.3 %), and syntrophic aromatic degraders such as *Syntrophus* sp. (2.6 ± 0.2 %) and *Syntrophorhabdus* sp. (2.3 ± 0.3 %). The highest abundance (39.4 ± 2.1 %) corresponded to non-identified microorganisms.

A principal coordinate analysis performed on the three biomass sources showed that the communities were significantly different ($p < 0.05$) (Supplementary material S7, Fig S4). The results observed in the microbial community analysis confirmed that the AnMBR-cultivated biomass had an abundant methanogenic population.

The continuous exposure of (anaerobic) biomass to a certain type of stress, for example a toxic substrate or wastewater, will likely result in the adaptation of the biomass to the new conditions. Most commonly, adaptation entails a shift of the microbial community towards a more resistant, and in principle, less diverse community [28]. The new community will comprise microorganisms that are capable of withstanding the stressful conditions to which the biomass has been exposed. Regarding the phenol-fed AnMBR biomass, a microbial community with a large fraction of phenol degraders was expected. Similarly, the petrochemical granular biomass was continuously exposed to benzoate, which would results in enrichment of benzoate degraders. Therefore, and very likely, a low percentage of membrane-damaged microorganisms was observed in the biomass sources adapted to aromatics after exposure to BFC, compared to the non-adapted biomass. A second adaptation mechanism is the induction, activation or up-regulation of gene expression which results in the production of enzymes [28]. Newly or highly expressed enzymes may provide a higher resistance to the microorganisms after the exposure to the BFC. A third and last mechanism considered is the development or acquisition of mutations that will offer the microorganisms a higher resistance to the stress conditions.

3.3. BFC biodegradation in AnMBR

The AnMBR was seeded with biomass coming from the UASB reactor treating petrochemical wastewater, characterized by a microbial community rich in aromatic-degrading microorganisms. The BFC influent was enriched with phenol and acetate to enhance the phenol-degrading and methanogenic activity of the biomass [20,62]. After the reactor's start-up, the phenol addition was gradually reduced and eliminated

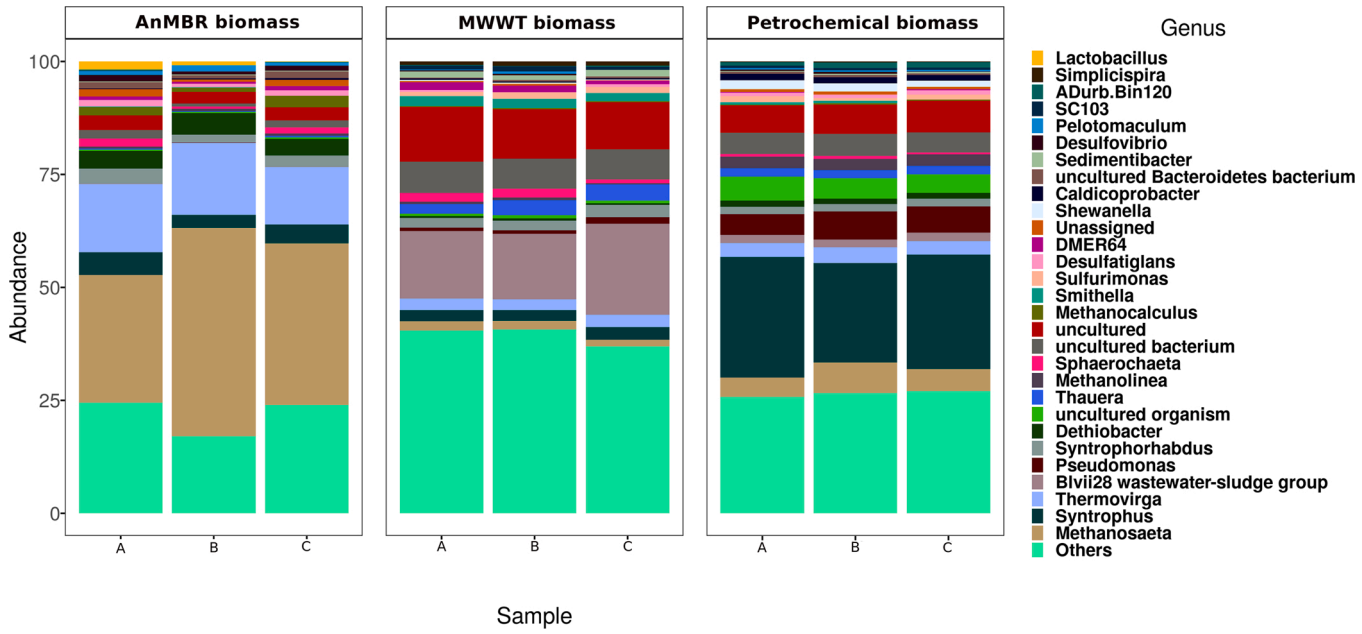


Fig. 7. Microbial community structure at genus level of the different sludge sources. A, B, and C represent each of the triplicates of the samples.

from the feeding solution (Table 3).

3.3.1. *Conversion rates and COD and phenol removal efficiencies*

Stages A and B (Fig. 8A) were distinguished by relatively high OLRs (Table 3). The corresponding average organic conversion rates (OCR) were 330 ± 110 mgCOD·L^{-1}d^{-1} and 254 ± 103 mgCOD·L^{-1}d^{-1}, respectively. Average sludge conversion rates (SCR) of 45 ± 15 mgCOD·gVSS^{-1}d^{-1} and 45 ± 14 mgCOD·gVSS^{-1}d^{-1} were determined

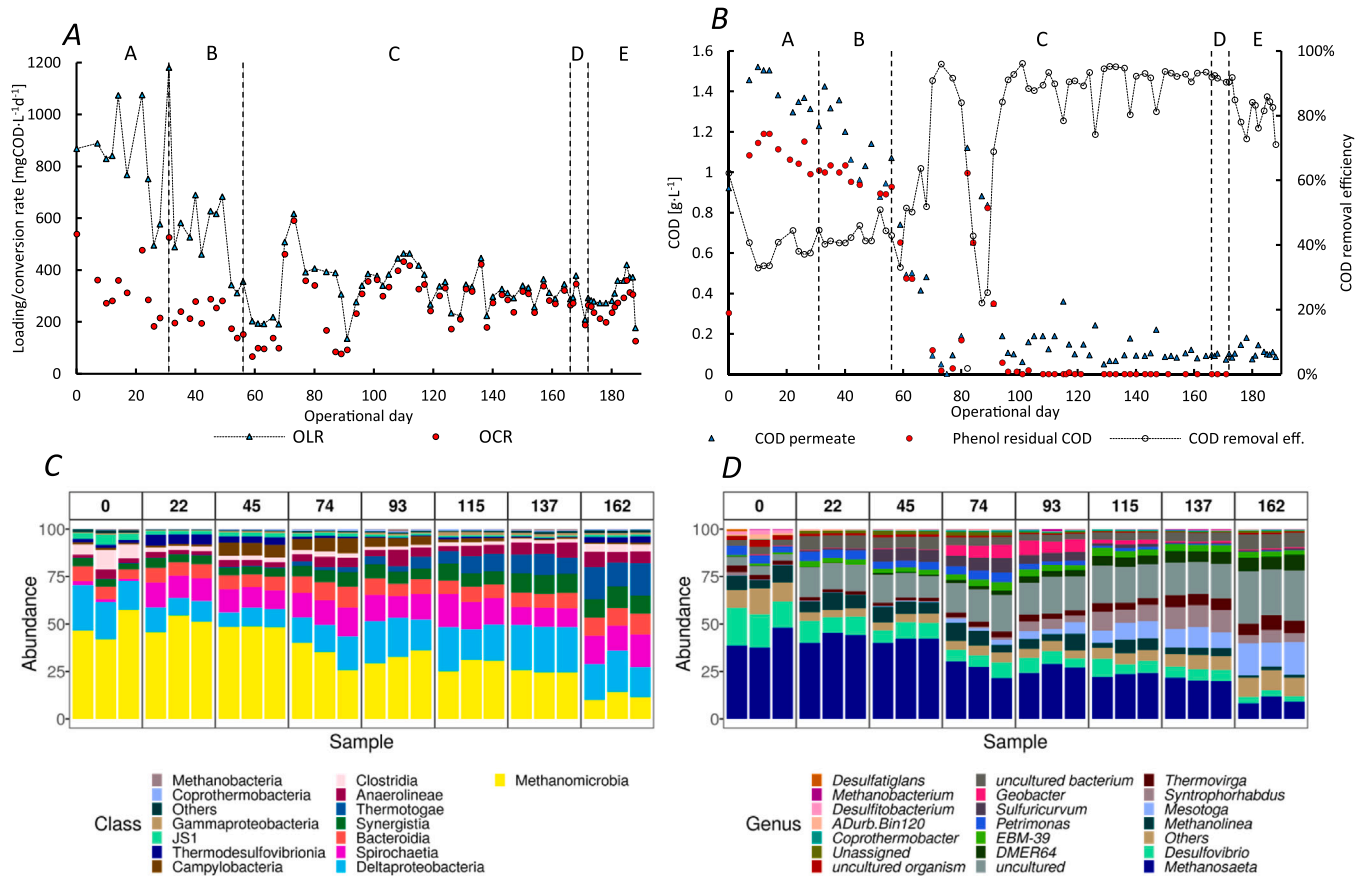


Fig. 8. Volumetric organic loading and conversion rates in the AnMBR (*A*) and total COD & phenol COD in the influent, and COD removal efficiency (*B*). Microbial community profile during the reactor operation in Class (*C*) or Genus (*D*), the triplicates of each of the samples are shown.

for stages A and B, respectively, after the consideration of the VSS concentration.

The corresponding average OCRs remained at a similar level in the next three stages, i.e., 274 ± 116 mgCOD·L^{-1}d^{-1} (C), 269 ± 65 mgCOD·L^{-1}d^{-1} (D), and 257 ± 59 mgCOD·L^{-1}d^{-1} (E), respectively. However, the average SCR increased to 77 ± 41 mgCOD·gVSS^{-1}d^{-1} (Stage C), 120 ± 39 mgCOD·gVSS^{-1}d^{-1} (Stage D), and 97 ± 29 mgCOD·gVSS^{-1}d^{-1} (Stage E) because of a decreased VSS concentration in the reactor.

Applied volumetric loading and conversion rates were relatively low, mainly attributable to the low COD-BFC concentration (Table 3 and Supplementary material S8, Fig. S5). Unfortunately, we did not have access to higher volumes of BFC to increase the loading rate. Therefore, the biomass concentration in the reactor was kept below the average AnMBR reported values to allow us to observe faster responses in the sludge loading rate (SLR) when the influent COD was varied [13,15]. Hence, employing higher biomass concentrations can easily increase the AnMBR removal efficiency.

Fig. 8 B shows the COD concentration in the permeate, the COD which corresponded to the residual phenol in the permeate, and the COD removal efficiency during the reactor operation. There was a significant difference $t(82) = 24.27$, $p < 0.001$ for the COD concentration in the feeding of the reactor and the permeate during the whole reactor operation, similarly the significant difference ($p < 0.01$) was kept among each of the different stages (Supplementary material 2). In the first 30 days of operation (stage A), the COD removal efficiency was 37.9 ± 3.9 %, mostly related to the fact that phenol was not biodegraded (average removal $= 5.1 \pm 4.8$ %); however, all the acetate was converted. The HRT was increased to 4 days on day 31 (stage B) with a concomitant increase to 43.0 ± 3.2 % in the COD removal efficiency; nonetheless, phenol degradation remained low (average $= 13.1 \pm 2.4$ %). The phenol concentration in the influent was reduced from 1.2 to 0.5 $gCOD_{Phenol}·L^{-1}$ (stage C) after 57 days of the exposure of the biomass to the phenol which caused an increase in the COD removal efficiency. On day 73, the removal efficiency decreased due to a technical failure in the pumping system of the reactor. The minimum removal efficiency of 22 % was reached on day 87. Nevertheless, the COD removal efficiency was recovered (84 %) on day 94. The average COD and phenol removal efficiencies in stage C were 80.2 ± 20.7 % and 87.0 ± 22.4 %, respectively. In stage D (day 166), the phenol concentration was further decreased from 0.50 to 0.20 $gCOD_{Phenol}·L^{-1}$. The COD and phenol removal efficiencies were 91.6 ± 0.9 % and a 100% respectively. For stage E, an average COD removal efficiency of 82.1 ± 6.2 % was found. Therefore, considering the last 100 days of operation, the reactor had an average COD removal efficiency of 87.7 ± 7.0 % corresponding to an OLR of 286 ± 71 mgCOD·L^{-1}d^{-1} and an SCR of 97 ± 33 mgCOD·gVSS^{-1}d^{-1}. It is suggested that the dosage of acetate to an AnMBR enhances the phenol conversion due to an increase in the (acetoclastic) methanogenic population that allowed higher acetate consumption, likely because of the improvement in the conversion of phenol to acetate [20]. Wang et al., [62] suggested that the dosage of phenol as an additional carbon and energy source may enhance the degradation of toxic and refractory compounds, such as those present in coal gasification wastewater (CGWW). The phenol was completely removed from the feeding on day 172 (Stage E), which resulted only in a slight decrease in the COD removal efficiency to 82.1 ± 6.2 %.

We estimated the removed COD that corresponded to the BFC by performing a COD balance based on the known COD concentrations of phenol and VFAs in the feeding solution and the reactor's permeate. Fig. S5 (Supplementary Material S8) shows the COD-BFC concentration in the influent and the calculated COD-BFC removal efficiency.

The initial anaerobic conversion of aromatic hydrocarbons, such as phenolics and PAHs that were found in the BFC, is started by an enzymatic process known as activation [17]. For this process, five main reactions are suggested: a) fumarate addition, b) methylation of unsubstituted aromatics, c) hydroxylation via dehydrogenases, d) direct carboxylation, and e) phosphorylation [17,30]. After the activation, the molecules can be directed via pathways yielding central metabolites such as the benzoyl-CoA, which can be then further metabolized via ring saturation, ring cleavage, and β-oxidation in the so-called central or lower pathways [30,9].

Various compounds found in the BFC analysis are known to be biodegradable under anaerobic conditions [29,30,9]. Carmona et al. [9, 44] reported the detailed anaerobic degradation pathway for compounds such as phenol, p-cresol, and o-cresol. Also for fluoranthene, its methanogenic degradation has been reported [18], but the degradation pathway is unknown. For compounds such as naphthalene, fluorene, acenaphthene, anthracene, and phenanthrene the degradation by mixed cultures under strict anaerobic conditions is either reported [10] or strongly suggested [14] but has not yet been described [17]. Nevertheless, insights into the degradation routes have been obtained under sulfate and/or nitrate reducing conditions (Fig. 9), for example, for naphthalene and methylnaphthalene [37], phenanthrene [29], acenaphthene [38], fluorene [59]. Nonetheless, the detailed degradation routes for the majority of the compounds contained in the BFC are unknown. Fig. 9 shows a summary scheme of the reported degradation routes for the major organic compounds found in the BFC.

Previous research studies conducted in the past decades investigated the anaerobic treatment of (petro)chemical wastewater (Supplementary material S9, Table S5), but most of them focused on the degradation of specific compounds such as purified terephthalic acid (PTA) [23,27,45], and benzene, toluene, ethylbenzene, and xylenes (BTEX) [52]. AD showed to be a feasible option for the treatment of those types of wastewater, with reactors that achieved COD removal efficiencies exceeding 80 %. However, poor reactor performance with low COD removal efficiency (45 %) was also reported [23].

Fewer studies have dealt with more complex and non-synthetic petrochemical wastewater (Supplementary material S9, Table S5). Coal gasification wastewater (CGWW) is a typical toxic/inhibitory matrix with similar compounds as those reported in BFC, such as phenolics and PAH. Wang et al., [62] reported the application of AD to treat this wastewater, either with the dosage of methanol (500 mg·L^{-1}) as an additional carbon and energy source or by using a step-feed strategy [64]. These studies reported COD and phenol removal efficiencies between 55 % and 75 %, applying an OLR of 3.5 kgCOD·m^{-3}d^{-1} for the former study [62], and influent concentrations of 2500 mgCOD·L^{-1} and 500 mgPhenol·L^{-1} for the latter [64]. Gasim et al. [22] reported the degradation of petroleum refinery wastewater using a UASB reactor under six OLRs between 0.6 and 4.1 kgCOD·m^{-3}d^{-1}. The conclusion of their study was that AD in UASB reactors is suitable to treat the petroleum refinery wastewater. The UASB reactor achieved a COD removal efficiency of 83 % (effluent COD = 350 mg·L^{-1}) at an OLR of 1.2 kgCOD·m^{-3}d^{-1}. Jafarzadeh et al. [25] reported the treatment of petrochemical wastewaters with pollutants such as ethylene, propylene, benzene, among others. The hybrid reactor (UASB/filter) treated OLRs between 0.5 and 24 kg_{total}COD·m^{-3}d^{-1}, with an HRT of 4–48 h and removal efficiencies between 42 % and 86 %.

Most of the studies conducted for the anaerobic treatment of petrochemical wastewater have reported the usage of sludge bed reactors, such as UASB and EGSB reactors, even when petrochemical wastewater may hamper the anaerobic granulation process (Supplementary material S9, Table S5). Only a few studies were conducted with AnMBRs. Wang et al., [63] reported the treatment of bamboo industry wastewater, rich in alkenes, benzenes, esters, and phenolics, using AnMBR. Different OLRs from 2.2 to 11.0 kgCOD·m^{-3}d^{-1} were applied with HRTs of 2–10 d. The COD removal efficiency reported was 91 %, higher than that of an EGSB under the same conditions. Bhattacharyya et al., [5] used a 25 L submerged AnMBR for the treatment of high-COD (85 gCOD·L^{-1}) synthetic petrochemical wastewater containing formaldehyde besides acrylic and acetic acids as the main pollutants. At an OLR of 3.4 kgCOD·m^{-3}d^{-1}, the COD and acrylic acid removal efficiencies were higher than 99 %.

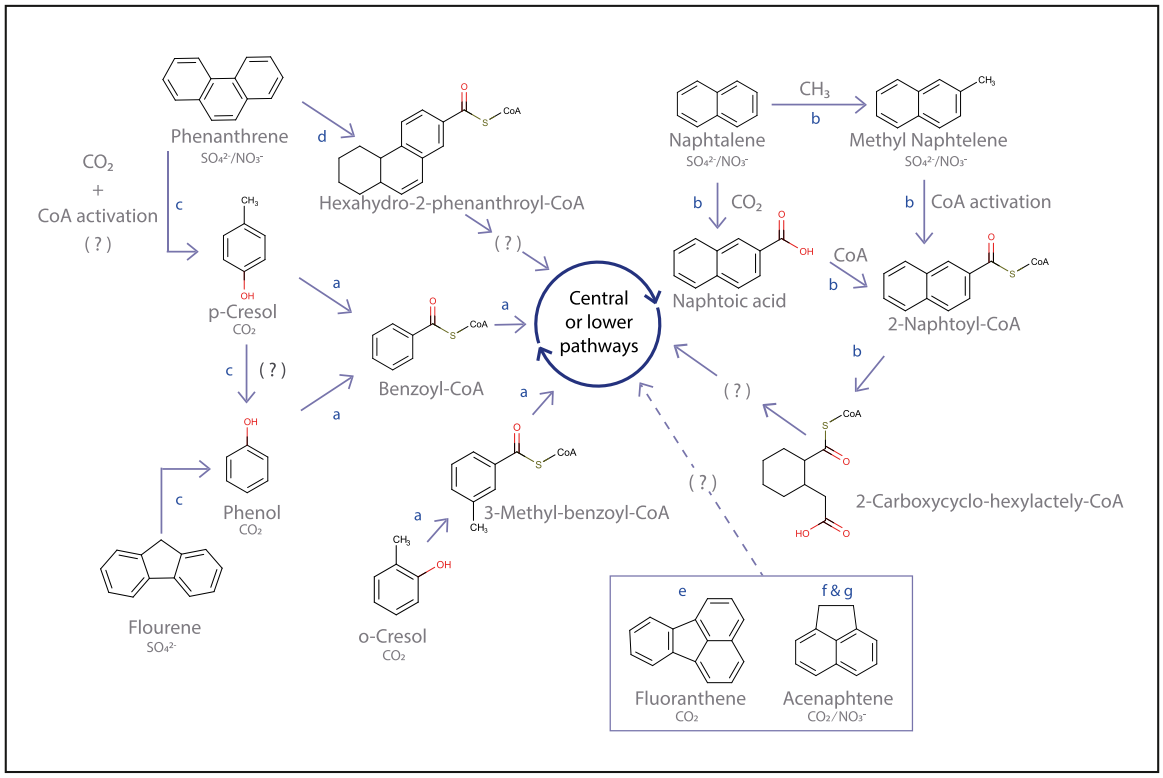


Fig. 9. Summary scheme of the reported degradation pathways of the major organic compounds found in the BFC. The redox conditions for which the routes have been elucidated or proposed are indicated. References: a [9], b [37], c [59], d [29], e [18], f [38], and g [10].

3.3.2. *Microbial community dynamics in the AnMBR*

The molecular analysis of the reactor's biomass showed a dynamic microbial community during the different stages. Fig. 8 (C & D) shows the relative abundance of microorganisms in the biomass either at class (A) or genus (B) level. During the start of reactor operation (day 0) and the first two stages (day 22, Stage A; day 45, Stage B), the highest relative abundance corresponded to the (acetoclastic) methanogen *Methanosaeta* sp. with 42.3 ± 4.2 % and 41.5 ± 1.3 % respectively. This was in part expected as the BFC wastewater was amended with acetate. Furthermore, methanogenesis occurs mainly via the acetoclastic pathway when phenolic wastewater is degraded under mesophilic conditions [20]. The samples of the stage C and beginning the stage D (days 74, 93, 115, 137, and 162), showed a decrease in the relative abundance of methanogens, which may be related to the decrease in the influent acetate concentration. However, this decline did not affect the COD conversion rates and COD removal efficiencies.

The mesophilic hydrogenotrophic archaea *Methanolinea* sp. was the second most abundant methanogen during the AnMBR operation. According to Narihiro et al. [43], the *Methanolinea* genus was found in microbial communities enriched with substrates that require syntrophic conversion. Likewise, *Methanolinea* preferentially partner in syntrophic consortia that scavenges the reducing equivalents. *Desulfovibrio* sp. was identified during the entire AnMBR operation, and its relative abundance decreased together with the methanogenic microorganisms. The *Desulfovibrio* genus includes sulfate-reducing bacteria that contributes in the metabolism of fermentative systems [66]. For example, it is reported that sulfate reducers can obtain energy via fermentative pathways at low (or none) sulfate concentrations. Under these conditions, sulfate reducers act as syntrophic partners through H_2 production, but they switch to sulfate respiration once SO_4^{-2} is available [50]. *Desulfovibrio* sp. can degrade nitroaromatic compounds [67] and hydroxyhydroquinone [47]. This microorganism has also been found in a UASB reactor that treated saline phenolic wastewater [65], horizontal anaerobic immobilized biomass reactors for BTEX treatment [42], and in the anaerobic degradation of CGWW [32].

In stage C, we found a noticeable presence of the phenol (and other aromatic compounds) degrader *Syntrophorhabdus* sp. [49]. *Syntrophorhabdus* sp. can convert some aromatic compounds into easily biodegradable substrates, such as acetate, which could eventually be used by other microorganisms as a carbon source. At this stage (C), *Syntrophorhabdus'* relative abundance increased from 0.9 ± 0.3 % on day 74 to 11.6 ± 0.1 % on day 137. However, on day 162 there was a decrease to 5.3 ± 0.2 % which coincided with the decrease in the COD removal efficiency (Fig. 8).

Although 16S rRNA analysis does not allow to conclude about the activity of microorganisms, it might be possible that *Syntrophorhabdus* sp. was a key microorganism in the degradation of aromatic compounds present in the BFC due to its metabolic capabilities [20,44,49].

Geobacter genus was found with an abundance of up to 6.8 ± 0.7 % in stage C (day 93). This microorganism is reported to be capable of metabolizing aromatics via the Benzoyl-CoA pathway with the help of iron reducers [17,47]. In addition, it also has been identified in anaerobic biomass which treated coal gasification wastewater [68] and wastewater containing phenanthrene [33].

The genera *Mesotoga* and *Thermovirga* were identified in increasing abundance from day 74–162. The genus *Mesotoga* includes bacteria with a potential role in the degradation of halogenated aromatic compounds. For example, *Mesotoga* has been reported in the anaerobic degradation of phenanthrene [33], a compound found in the BFC. *Thermovirga* sp. is a fermentative bacteria genus with a strong hydrolysis ability and it has been reported in phenol-degrading [20,39] and in a UASB reactor which treated CGWW. Its role in the degradation of phenol, however, has not yet been identified.

The results from the MaAsLin 2 approach showed the significant correlation ($p < 0.05$) of the twelve most important genera found in the reactor's biomass and the COD removal efficiency during the different reactor operation stages (Fig. 10). For microorganisms such as *Desulfovibrio*, *Methanolinea*, or *Methanosaeta*, there was a negative correlation.

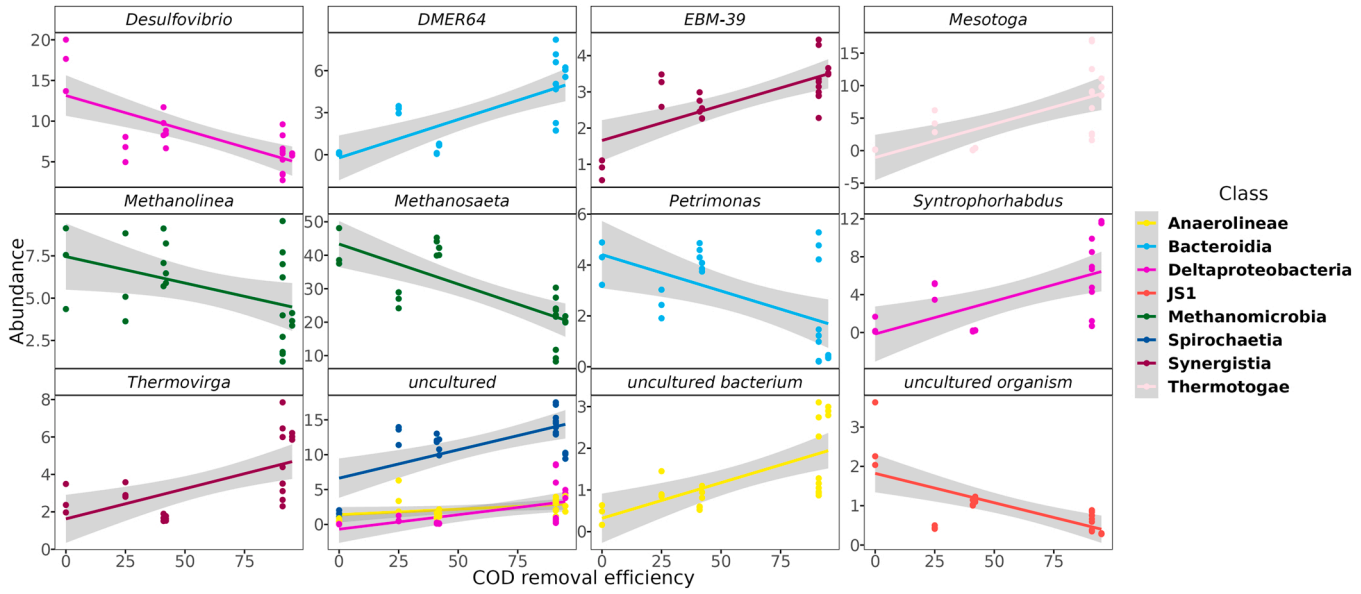


Fig. 10. MaAsLin 2 analysis performed for the twelve most abundant genus in the reactor's biomass and the COD removal efficiency during the different reactor operation stages.

Increased COD removal efficiencies were measured when the relative abundances of the aforementioned microorganisms decreased. Nevertheless, *Syntrophorhabdus* (a reported phenol and aromatic compound degrader) [44,49], *Thermovirga*, and other microorganisms such as DMER64, EBM-39, and a fraction of uncultured bacteria, had an increase in their relative abundance. As previously stated, it must be noted that genomic analysis based on the 16S rRNA gene cannot be directly linked to microbial activity. These results allow us, however, to have a better understanding of the process(es) that might be occurring in the reactor biomass. Based on our results, we hypothesized that the increase in the COD removal efficiency observed during the reactor operation was related to the increase in the aforementioned genera instead of to the decrease of microorganisms such as the methanogens. Methanogenesis can be considered the key step in the AD process. Thus, a decrease or impairment in the methanogenic subpopulations (acetoclastic or hydrogenotrophic) will likely result in the a decrease or the complete cessation of the AD biochemical processes.

4. Conclusions

The BFC was characterized by an average of 0.58 ± 0.39 mgCOD·L^{-1} ($n = 33$), and a maximum value of 1.20 gCOD·L^{-1}. From the 993 compounds detected in the GC-QTOF analysis, only 72 compounds corresponded with an 80 % match in relation to the compounds described in the reference library.

The major organic compounds identified were acetate, phenol, naphthalene, acenaphthylene, propyzamide, fluorene, phenanthrene, acenaphthene, and fluoranthene. The BFC content of iron, sulfur, silicon, and calcium was high (85 – 310 mg/L). After the precipitation step, iron concentration was decreased by 76.9 % in the feeding of the AnMBR. BFC concentrations of 120 mgCOD·L^{-1} for anaerobic biomass coming from a MWWTP, 224 mgCOD·L^{-1} for granular biomass coming from a petrochemical WWTP, and 900 mgCOD·L^{-1} for the biomass coming from a saline phenol-degrading AnMBR decreased the methanogenic activity to half of the maximum value and were regarded as the 50 % inhibition concentration (IC_{50}). Accordingly, when exposed to BFC [1000 mgCOD·L^{-1}], the biomass coming from the saline phenol-degrading AnMBR had less damaged (membrane) cells in comparison to the other two biomass sources. Furthermore, the biomass coming from the phenol degrading AnMBR had the most abundant acetoclastic methanogenic population (*Methanosaeta* sp.), but the granular biomass treating petrochemical wastewater showed a higher relative abundance of syntrophic degraders of aromatic compounds (*Syntrophus* sp.).

We operated for 188 days an AnMBR fed with a mixture of BFC, phenol, acetate, and nutrients. During the last 100 days, the reactor had an average COD removal efficiency of 87.7 ± 7.0 % which corresponded to an OLR of 286 ± 71 mgCOD·L^{-1}d^{-1} and an SCR of 97 ± 33 mgCOD·gVSS^{-1}d^{-1}. The microbial community dynamics in the reactor's biomass indicated that the most abundant microorganisms were the methanogens. The syntrophic degrader of aromatic compounds, *Syntrophorhabdus* sp., remained relatively constant during the reactor operation.

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CRediT authorship contribution statement

Victor S. Garcia Rea: Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original, Visualization. **Beatriz Egerland Bueno**: Methodology, Formal analysis, Investigation. **Julian D. Muñoz Sierra**: Methodology, Writing – Review and Editing. **Athira Nair**: Methodology, Formal analysis, Investigation. **Israel J. Lopez Prieto**: Methodology, Formal analysis, Investigation. **Daniel Cerqueda-García**: Software, Data curation, Formal analysis. **Jules. B. van Lier**: Resources, Writing – Review and Editing, Supervision. **Henri Spanjers**: Resources, Writing – Review and Editing, Supervision.

Environmental statement

Bitumen fume is released during the production of asphalt in a process known as asphalt reclamation. However, due to legislative changes, bitumen fumes are not allowed anymore to be released into the atmosphere implying the formation of bitumen fume condensate (BFC). Due to its composition, rich in aromatic hydrocarbons and other toxic, mutagenic, and teratogenic compounds, BFC poses a threat to the environment and the health of people. This research presents the first

study on the anaerobic treatment of BFC, broadening the application of anaerobic digestion using membrane bioreactors and offering a treatment solution for this hazardous material.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2022.130709.

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