Degradation of p-cresol, resorcinol, and phenol in anaerobic membrane bioreactors under saline conditions

Garcia Rea, Victor S.; Egerland Bueno, Beatriz; Cerqueda-Garcia, Daniel; Muñoz Sierra, Julian D.; Spanjers, Henri; van Lier, Jules B.

DOI
10.1016/j.cej.2021.132672

Publication date
2022

Document Version
Final published version

Published in
Chemical Engineering Journal

Citation (APA)
Garcia Rea, V. S., Egerland Bueno, B., Cerqueda-Garcia, D., Muñoz Sierra, J. D., Spanjers, H., & van Lier, J. B. (2022). Degradation of p-cresol, resorcinol, and phenol in anaerobic membrane bioreactors under saline conditions. *Chemical Engineering Journal*, *430*, 1-14. Article 132672. https://doi.org/10.1016/j.cej.2021.132672

Chemical Engineering Journal 430 (2022) 132672

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Degradation of *p*-cresol, resorcinol, and phenol in anaerobic membrane bioreactors under saline conditions

Victor S. Garcia Rea [a], Beatriz Egerland Bueno [a,b], Daniel Cerqueda-García [c], Julian D. Muñoz Sierra [a,d], Henri Spanjers [a], Jules B. van Lier [a]

[a] *Sanitary Engineering Section, Department of Water Management, Delft University of Technology, Stevinweg 1, 2628 CN, Delft, The Netherlands*
[b] *Laboratory of Environmental Biotechnology, Department of Food Engineering, University of Sao Paulo, 225, Duque de Caxias Norte, Pirassununga, Sao Paulo 13635-900, Brazil*
[c] *Unidad de Genómica de Poblaciones Aplicada a la Salud, Chemistry Faculty, National Autonomous University of Mexico-National Institute of Genomic Medicine, Periferico Sur 4809, Arenal Tepepan, Tlalpan, 14610, Mexico City, Mexico*
[d] *KWR Water Research Institute, Groningenhaven 7, 3430 BB, Nieuwegein, The Netherlands*

ARTICLE INFO

Keywords:
Anaerobic
AnMBR
Phenol
p-Cresol
Resorcinol
Salinity

ABSTRACT

Treating petrochemical wastewater is a challenge for conventional anaerobic reactors. One example is coal gasification wastewater that, besides its salinity, is rich in toxic and inhibitory aromatics, such as phenol, cresols, and resorcinol. Studies have shown that phenol and *p*-cresol share the same degradation intermediates, whereas resorcinol is degraded via another route. This study investigated the simultaneous degradation of *p*-cresol or resorcinol with phenol under anaerobic saline conditions. Batch experiments with anaerobic phenol-degrading biomass were conducted to assess the feasibility of the degradation of *p*-cresol and resorcinol. Volumetric uptake rates of 11.4 ± 2.4 $mg_{p\text{-cresol}}\cdot L^{-1}d^{-1}$ and 4.2 ± 1.9 $mg_{resorcinol}\cdot L^{-1}d^{-1}$ were determined. The effect of *p*-cresol and resorcinol on the specific methanogenic activity and the cell viability in phenol-degrading and non-adapted biomass was assessed. Half maximal inhibitory concentration (IC_{50}) values of 0.73 $g_{p\text{-cresol}}\cdot L^{-1}$ and 3.00 $g_{resorcinol}\cdot L^{-1}$ were estimated for phenol-degrading biomass, whereas IC_{50} values of 0.60 $g_{p\text{-cresol}}\cdot L^{-1}$ and 0.25 $g_{resorcinol}\cdot L^{-1}$ were estimated for the non-adapted biomass. *p*-Cresol caused a higher decrease in the non-damaged cell counts in comparison to resorcinol. Two anaerobic membrane bioreactors under saline conditions [8 g $Na^{+}\cdot L^{-1}$] were fed with mixtures of either phenol-*p*-cresol or phenol-resorcinol. At an influent phenol concentration of 2 $g\cdot L^{-1}$, maximum conversion rates of 22 $mg_{p\text{-cresol}}\cdot gVSS^{-1}d^{-1}$ and 16 $mg_{resorcinol}\cdot gVSS^{-1}d^{-1}$ were found. In both AnMBRs, *Syntrophorhabdus* sp. and *Methanosaeta* sp. were the most abundant bacteria and methanogen, respectively. The feasibility of simultaneous conversion of phenolic compounds under saline conditions in AnMBRs opens novel perspectives for the high-rate anaerobic treatment of chemical wastewater.

1. Introduction

Development of industrial activity has led to a considerable increase in the generation of chemical wastewater [1]. For example, coal gasification wastewater (CGWW) from the petrochemical industry is of particular interest due to its high concentration of toxic or inhibitory organic pullutants [2–4]. CGWW comprises a complex matrix with phenolic compounds being the main contaminants, representing 20 to 60% of the total COD [2,5], and consists mainly of monohydric (e.g., phenol and *p*-cresol) and dihydric (resorcinol) phenolic compounds [4]. In addition, CGWW is also characterized by high salinity [6].

During the past four to five decades, anaerobic digestion (AD) has been increasingly applied for the treatment of industrial wastewater [7,8], amongst which petrochemical wastewater (including CGWW) has

Abbreviations: vOLR, Volumetric organic loading rate.; CGWW, Coal gasification wastewater.; COD, Chemical oxygen demand.; UASB, Upflow anaerobic sludge blanket reactor.; AnMBR, Anaerobic membrane bioreactor.; HPLC, High-performance liquid chromatography.; VFAs, Volatile fatty acids.; GC-FIC, Gas chromatography with flame ionization detector.; GC-TCD, Gas chromatography with thermal conductivity detector.; CES, Carbon and energy source.; SMA, Specific methanogenic activity.; FCM, Flow cytometry.; PVDF, Polyvinylidene fluoride.; $r_{s,v}$, Volumetric substrate uptake rate.; $r_{s,s}$, Specific substrate uptake rate.; IC_{50}, Half maximal inhibitory concentration; $v_{p\text{-cresol}}LR$, Volumetric *p*-Cresol loading rate.; $s_{p\text{-cresol}}CR$, Specific *p*-Cresol conversion rate.; $s_{phenol}CR$, Specific phenol conversion rate.; $v_{resorcinol}LR$, Volumetric resorcinol loading rates; $s_{resorcinol}CR$, Specific resorcinol conversion rate.; PCoA, Principal coordinate analysis.; ΔG_R^0, Standard Gibbs free energy change.; $\Delta G_R^{1,TStandard}$, Gibbs free energy change with corrections for temperature and concentrations/partial pressures.; γ, Degree of reduction..

https://doi.org/10.1016/j.cej.2021.132672
Received 29 June 2021; Received in revised form 22 September 2021; Accepted 23 September 2021
Available online 30 September 2021

a specific interest [3,9,10]. To achieve a proper treatment of petrochemical effluents, the different phenolic compounds should be biodegraded simultaneously during the anaerobic conversion[11].

Several studies have investigated the treatment of phenolic compounds mixtures by using up-flow anaerobic sludge blanket (UASB) reactors [12]. According to Fang and Zhou (2000), the UASB reactor effectively removes phenol and *p*-cresol from wastewater at a combined concentration of over 1.0 $g{\cdot}L^{-1}$, corresponding to 2.6 g $COD{\cdot}L^{-1}$ (72% phenol, 28% *p*-cresol). Results indicate that phenol has a better biodegradability than *p*-cresol [13]. Also, using UASB technology to study the biodegradation of phenol and *p*-cresol mixtures, Razo-Flores et al. (2003) reported 90% of COD removal at a volumetric organic loading rate (vOLR) of 7 kg $COD{\cdot}m^{-3}d^{-1}$ [11]. It was concluded that cresols concentration should not exceed 600 $mg{\cdot}L^{-1}$ (1500 mg $COD{\cdot}L^{-1}$)[11]. Wu et al. (2020) obtained high COD removal efficiencies (80–100%) and complete removal of phenolic compounds operating a UASB reactor treating a synthetic CGWW composed of phenol, catechol, resorcinol hydroquinone, acetate, yeast extract, and micro- and macro-nutrients at a vORL of 2.6 g $COD{\cdot}L^{-1}d^{-1}$ [14]. The influent concentration of phenolic compounds was 1.0 $g{\cdot}L^{-1}$, corresponding to 1.5 g $COD{\cdot}L^{-1}$ of phenol and 0.7 g $COD{\cdot}L^{-1}$ of the dihydrophenol isomers [14].

High concentrations of phenol and other phenolic compounds such as *p*-cresol and resorcinol can hamper the proper performance of the treatment process [15]. Furthermore, the high concentration of dissolved salts makes petrochemical wastewater, such as CGWW, a challenge for conventional anaerobic treatment, mainly because the granulation process can be affected, leading to biomass wash-out [16–18].

In anaerobic membrane bioreactors (AnMBRs), the reactor performance is independent of the biomass settling properties or granulation because of the presence of an absolute barrier: the membrane, which guarantees the separation of active methanogenic biomass from the bulk liquid by filtration [19]. Muñoz Sierra et al. (2019) compared the performance of UASB and AnMBR reactors treating highly saline phenolic wastewater [18]. They found that the AnMBR exhibited higher stability, which was related to its enhanced biomass retention, thereby keeping specialized microorganisms (e.g., phenol degraders and methanogens) under increasing influent phenol concentrations of up to 5 $g_{phenol}{\cdot}L^{-1}$ and salinity up to 26 $g\ Na^{+}{\cdot}L^{-1}$. In contrast, when the UASB reached this high influent phenol and sodium concentrations, de-flocculation of sludge was observed, leading to a severe conversion capacity loss. High salt concentrations, in this case mediated by Na^{+}, promote microbial dehydration due to differences in the osmotic pressure [18]. In suspended biomass, such as the one in AnMBRs, the deterioration of the methanogenic activity under saline conditions is suspected to be related to cells plasmolysis [20]. In granular biomass, besides the osmotic effect, Na^{+} may promote the leach of divalent ions such as Ca^{+2}, resulting in a decrease of the granular biomass size, or biomass washout [17,18]. Currently, there are no reports or studies dealing with the simultaneous degradation of mixtures of phenolic compounds under saline conditions using suspended biomass in an enhanced biomass-retention system.

The presence of phenol may affect different microorganisms, such as phenol degraders or methanogens [21], where inhibitory or toxic effects on the methanogens would impede the entire degradation process. Inhibition, is defined as a biostatic effect resulting in the impairment of the biomass function or activity, whereas toxicity is defined as a biocidal effect on microorganisms being mainly irreversible [22]. Compared with the hydrogenotrophic, acetoclastic methanogens may be affected more severely by toxic or inhibitory compounds [21,22]. Therefore, accumulating phenol concentrations might affect mesophilic phenol-degrading reactors since acetoclastic methanogens were previously observed as the predominant methanogenic subpopulation in phenol-degrading AnMBRs under saline conditions [18,23].

Even when the degradation pathways of phenol, *p*-cresol, and resorcinol under anoxic (nitrate-reducing conditions) or sulfate-reducing conditions and by defined cultures have been widely reported [24–26], the degradation of these compounds under strict anaerobic (methanogenic) conditions by mixed cultures is not fully understood. Studies on microbial communities in bioreactors may contribute to a better understanding of the degradation process of these substrates. For example, it has been shown that *Syntrophorhabdus*, a phenol degrader, also has the capability of *p*-cresol degradation under strictly anaerobic conditions [27,28]. However, thus far, it has not been identified which are the microorganisms responsible for resorcinol degradation under anaerobic conditions. In addition, it is not known whether phenol and *p*-cresol degraders also have the capacity to convert resorcinol synergistically.

Through a thermodynamic state analysis of environmental systems, a better understanding of the microbial metabolism in a defined system is achieved [29]. By such analysis, it is possible to better describe the biochemical pathways by determining the overall reaction stoichiometry of microbial growth on a specific substrate and the involved redox reactions, which could be used to better interpret the observed phenomena in batch tests and continuous flow reactors.

To further elucidate the anaerobic conversion potential of phenolic-rich wastewater by suspended (non-granular) biomass in a full-biomass-retention system (AnMBR) in which a more specific microbial consortium is expected to be cultivated, the maximum conversion rates of *p*–cresol and resorcinol when simultaneously degraded with phenol in a matrix with a sodium concentration of 8 $g{\cdot}L^{-1}$ were determined. Also, the inhibitory effect of *p*-cresol and resorcinol on the acetoclastic methanogenic activity and the toxic effect, as cellular membrane damage, of both phenolic compounds on the phenol degrading biomass were assessed. By analyzing the thermodynamics of the anaerobic degradation of *p*-cresol and resorcinol, a theoretical backup to the results observed during the reactor operation was provided. Microbial community analyses revealed which microorganisms may be responsible for the degradation of phenol, *p*-cresol, and resorcinol in mixed culture systems under anaerobic conditions such as those of the AnMBRs. As well, these molecular analyses allowed to compare the phylogenetic similarity between microbial communities of the AnMBRs degrading either phenol-*p*-cresol or phenol-resorcinol under saline conditions. The results obtained in this research may be of considerable practical importance, because granular-sludge reactors are prone to suffer from biomass degranulation and wash out when treating this type of toxic or inhibitory wastewater under saline conditions.

2. Materials and methods

2.1. Analytical techniques

2.1.1. Phenol, p-cresol, and resorcinol concentrations

Phenol and *p*-cresol concentrations were measured by gas chromatography (GC) as reported in Garcia Rea et al. (2020) using an Agilent 7890A (Agilent Technologies, California, USA) chromatographer [23]. The runtime was 15 min with a flow of 67 $mL{\cdot}min^{-1}$. The sample injection volume was 1 μL. Sample preparation was done as previously reported [23]. Phenol concentration was double-checked by spectrophotometry using a Hach DR 3900 spectrophotometer (Colorado, USA) and LCK346 phenol cuvette tests (Hach, Colorado, USA) following the manufacturers' instructions.

Resorcinol was measured by high-performance liquid chromatography (HPLC). The column used was a C18 Kinetex Core-Shell (Phenomenex, California, USA). The column temperature was 40 °C. As eluent, a mixture of ultrapure water (60%) and acetonitrile (40%) was used, with a flow of 0.6 $mL{\cdot}min^{-1}$. A UV detector was used, with wavelengths of 270 nm for channel 1 and 280 nm for channel 2.

2.1.2. COD, volatile fatty acids, and biogas composition determination

COD was measured by spectrophotometry using a Hach DR3900 (Hach, Colorado, USA) spectrophotometer. Depending on the COD concentration, LCK314 or LCK514 COD cuvette tests (Hach, Colorado,

USA) were used following the manufacturers' instructions. To prepare the sample, ≈1 mL of permeate was filtered through a 0.45 μm filter (Chromafil Xtra PES-45/25). Proper dilutions were made to keep the COD concentration in the measurable range and to avoid interference due to Cl^-.

Volatile fatty acids (VFAs) were measured by GC-FID using the same sample preparation and chromatography protocols as described in section 2.1.1.

Biogas composition was determined by GC. The chromatograph was an Agilent 7890 (Agilent Technologies, California, USA) coupled to a thermal conductivity detector (TCD) and a column HP-PLOT Molesieve (19095P-MS6) with dimensions of 60 m × 530 μm × 20 μm. Helium was used as a carrier gas with a flow rate of 10 mL·min^{-1} and a split ratio of 1:1. The oven temperature was 40 °C for 6 min and then increased by 25 °C·min^{-1} to 100 °C, the total run time was 10 min. The temperature of the TCD was 200 °C. For the samples, 5 to 10 mL of biogas were taken from the reactor's biogas line and injected into the GC-TCD.

2.2. Batch tests

2.2.1. Anaerobic biodegradability tests for p-cresol and resorcinol by phenol-degrading biomass

A series of batch tests with initial concentrations of 50, 100, 250, or 500 mg·L^{-1} with either *p*-cresol or resorcinol as sole carbon and energy source (CES) were carried out. The tests were performed in triplicates in 250 mL Schott glass reactors (working volume 200 mL) (Schott, Germany), using a substrate-biomass ratio of 2 g COD·gVSS^{-1}. The biomass was taken from a phenol-degrading AnMBR. To ensure anaerobic conditions, the glass reactors were flushed with N_2 for one minute before starting the test. Per gram of substrate COD, 0.75 mL of micronutrient solution, 1.53 mL of macronutrient solution, and 50 mg of yeast extract (Sigma Aldrich) were added [30]. Per each mL of medium, 30.5 mL of phosphate solution A and 19.5 mL of phosphate solution B were added. The composition of the different solutions is reported in our previous work [23].

The bottles were incubated at 35 °C in a temperature-controlled shaker (New Brunswick Innova, Germany) at 130 rpm. Samples (0.5 to 1.0 mL) were periodically taken from the bottles and filtered through a 0.45 μm filter (Chromafil Xtra PES-45/25). *p*-Cresol, resorcinol, VFAs, and COD were determined in the filtrate as specified in Section 2.1.

2.2.2. Specific methanogenic activity inhibition and cellular membrane damage

For assessing the specific methanogenic activity (SMA), the protocol proposed by Spanjers and Vanrolleghem was used, except for the micronutrient dosage which was 0.6 mL·L^{-1} instead of 6 mL·L^{-1} [31]. Tests were carried out in triplicates in 250 mL Schott glass bottles (Schott, Germany) at 35° C and a constant mixing (130 rpm). N_2 was flushed for one min to ensure anaerobic conditions. Sodium acetate-trihydrate (Sigma Aldrich, USA) was used as the substrate at a concentration of 2.0 $gCOD_{acetate}$·L^{-1}. The biomass was taken from a phenol-degrading AnMBR [23]. The batch reactors kept a substrate-inoculum ratio of 2 (gCOD·gVSS^{-1}). Macro and micro-nutrient solutions were added as described in Section 2.2.

Accumulated biogas production was recorded by an AMPTS II system (Bioprocess Control, Sweden). Initial and final COD and acetate concentrations and pH values were measured as described in section 2.1. Different *p*–cresol and resorcinol concentrations were used.

Membrane cell damage was assessed by flow cytometry (FCM). Samples of the biomass were taken before starting the SMA tests and once the tests were finished. For sample preparation, 5 mL of biomass were taken and diluted with 0.22 μm-filtered phosphate-buffered saline (PBS) 1:500 [32]. The mixture was sonicated three times for 45 s at 4 °C and 100 W. To determine the total cell count, 495 μL of the sonicated mixture were stained with 5 μL of SYBR Green I dilution (S7563, Thermo Fisher, Massachusetts, USA). For the damaged-wall-cells measurement, the cells in the sonicated mixture were stained with 5 μL of SYBR Green I and propidium iodide (P4170, Merk, Germany) [30 mM] solutions. After the staining addition, the samples were gently vortexed and incubated in the dark at 37 °C for 10 min. The FCM was done in a BD Accuri C6 flow cytometer (Becton Dickinson, New Jersey, USA). The concentration of the samples was adjusted to have<2000 events μl^{-1}. The results are reported as the percentage of non-damaged cells in comparison to a control bottle.

Once the assays with the AnMBR biomass were completed, the SMA inhibition and membrane cell damage tests for *p*-cresol and resorcinol were repeated using anaerobically digested sewage sludge coming from a municipal wastewater treatment plant (Harnaschpolder, Den Hoorn, The Netherlands).

2.3. Simultaneous degradation of phenol with p-cresol or resorcinol

2.3.1. Anaerobic membrane bioreactors operation

Two reactors (6.5 L working volume, 7.0 total volume) equipped with a membrane module (approximately 130 cm^3), with a 64 cm length and 5.5 mm diameter, inside-out ultrafiltration (30 nm nominal pore size) tubular PVDF membrane (Pentair, The Netherlands), were used for the continuous experiment for the simultaneous degradation of phenol with either *p*-cresol or resorcinol. Both reactors shared the same configuration (Fig. 1). R1 was used for the degradation of phenol-*p*-cresol and R2 for phenol-resorcinol.

Each setup was fully automated and was equipped with influent and effluent pumps (Watson Marlow 120 U, Watson Marlow, England) and a recirculation pump (Watson Marlow 160 U, Watson Marlow, England). To determine the TMP, two pressure sensors (AE Sensors, The Netherlands) with a range from −800 to 600 mbar measured the pressure of the bulk sludge at the entrance and the exit of the membrane, and a third sensor of the same characteristics measured the pressure at the permeate side. The reactor volume was kept constant using two pressure sensors (AE Sensors, The Netherlands), ranging from 0 to 100 mbar, located at the top and the bottom of the reactor. Temperature and pH were measured online using pH/temperature sensors (Endress & Hauser, Memosens and Mettler Toledo). To keep temperature constant (35 °C), a water bath (Tamson Instruments, The Netherlands) was used to recirculate water through the reactor's jacketed double wall.

The biomass was mixed by internal recirculation at a rate of approx. 300 d^{-1} with a flow of approx. 1830 L·d^{-1}. Therefore, the membrane was operated with a cross-flow velocity of 1.0 m·s^{-1}.

The reactors were fed with synthetic wastewater containing phenol [2 g·L^{-1}], acetate [2 g COD·L^{-1}], and either *p*-cresol (R1) or resorcinol (R2) at different concentrations (Table 1). Micro- and macronutrient solutions, phosphate buffers, and yeast extract were added to the feeding medium as specified in section 2.2.1. Sodium chloride was added to keep the Na^+ concentration at 8 g·L^{-1}.

2.3.2. DNA extraction for microbial community composition analysis

Samples from the reactors were taken at specified days of each reactor operation. A total volume of 1.5 – 2.0 mL of biomass were transferred to microcentrifuge tubes (Eppendorf, Germany). The tubes were centrifuged for 5 min at 10.000 g. The supernatant was discarded and the biomass pellet was frozen at −80 °C. For the extraction, the cell pellets were melted and DNA was recovered using a DNA extraction kit (DNeasy UltraClean Microbial Kit, Qiagen, Germany) following the instructions provided by the manufacturer.

2.3.3. 16S rRNA gene amplification, sequencing, and data processing

DNA (16S rRNA gene) amplification was done using Illumina Novaseq 6000 platform by Novogene. The hypervariable regions V3-V4 were amplified using the primer set 341F [(5′- 3′) CCTAYGGGRBG-CASCAG] and 806R [(5′- 3′) GGACTACNNGGGTATCTAAT]. The PCR reactions were carried out with Phusion ® High- Fidelity PCR Master Mix (New England Biolabs). The DNA sequencing data was processed as

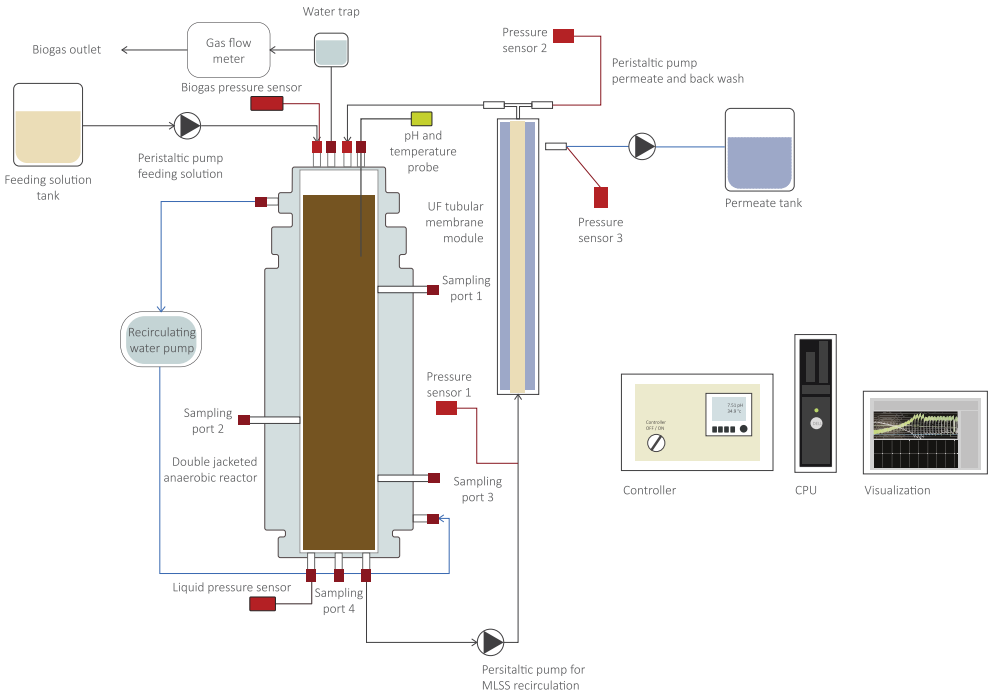

Fig. 1. Scheme of the AnMBR set-up.

Table 1
Influent concentrations and resulting volumetric loading rates of the phenolic compounds during the operation of R1 and R2.

R1				R2			
Phase	*Operation days*	*p-Cresol [mg·L^{-1}]*	*p-Cresol vOLR [mg·L^{-1}d^{-1}]*	*Phase*	*Operation days*	*Resorcinol [mg·L^{-1}]*	*Resorcinol vOLR [mg·L^{-1}d^{-1}]*
I	0–8	25	4	I	0–15	50	8
II	9–21	50	8	II	16–25	100	17
III	22–33	100	17	III	26–31	200	30
IV	34–47	200	30	IV	32–43	400	65
V	48–58	300	50	V	44–53	600	100
VI	59–67	400	65	VI	54–63	800	130
VII	68–94	800	130	VII	64–77	1200	200
VIII	95–101	1200	200	–	–	–	–
IX	102–112	1600	267	–	–	–	–

described in Garcia Rea et. al 2020 [23]. The sequences were deposited in the SRA (NCBI) database under the accession number PRJNA739995.

2.3.4. Thermodynamic state analysis

For the thermodynamic analysis of the *p*-cresol and resorcinol degradation, the Gibbs Energy Dissipation method [29] was used. Through the coupling of catabolism and anabolism, the overall metabolic equations of anaerobic growth on *p*-cresol and resorcinol were derived. To perform the required calculations, the following foreseen concentrations and partial pressures expected in the AnMBR were used: *p*-cresol and resorcinol 0.1 mmol (10 mg·L^{-1}), acetate 0.02 mmol (1.2 mg·L^{-1}), carbon dioxide 0.4 atm, hydrogen 1×10^{-4} atm, ammonium 5×10^{-8} mmol, and H^+ 1×10^{-7}M.

3. Results

3.1. Anaerobic biodegradability of p-cresol and resorcinol by phenol-degrading biomass

To assess whether *p*-cresol and resorcinol could be anaerobically converted by using biomass from a phenol degrading AnMBR under saline conditions, and if so, to have an insight into the degradation kinetics, either *p*-cresol or resorcinol were fed as sole CES to the biomass and the concentration of the compounds was continuously measured. The anaerobic conversion at different initial concentrations of *p*-cresol and resorcinol is presented in Fig. 2 A and B, respectively.

A zero-order kinetic model was fitted to the data to estimate the volumetric ($r_{s,v}$) and specific ($r_{s,s}$) substrate uptake rates (Table 2).

Fig. 2 shows that, even without previous exposure of the biomass to *p*-cresol or resorcinol, both phenolic compounds were degraded. The degradation in the batch reactors seemed to follow a linear relationship, with conversion of *p*-cresol (average $r_{s,v} = 11 \pm 3.3$ mg·L^{-1}d^{-1}) faster than resorcinol (average $r_{s,v} = 6.6 \pm 2.1$ mg·L^{-1}d^{-1}). However, even when the volumetric uptake (conversion) rates were similar between the different initial concentrations, the biomass specific uptake (conversion) rates ($r_{s,s}$) for both compounds showed a decrease with higher (initial) concentrations (Table 2).

3.2. Specific methanogenic activity inhibition and cell viability

To determine if *p*-cresol and resorcinol inhibited the acetoclastic methanogenesis, the SMA inhibition of the phenol-degrading AnMBR biomass in the presence of both phenolic compounds (Fig. 3 A & B) was assessed. In addition, to determine whether *p*-cresol and resorcinol had a toxic effect on the AnMBR biomass, we analzyed the cell membrane integrity by the live–death cell staining protocol [33](Fig. 3 C & D) once

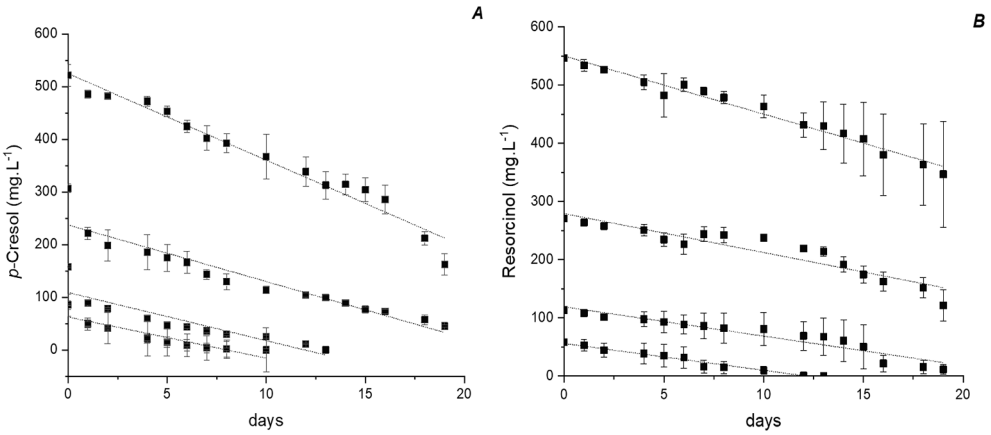

Fig. 2. Zero order-kinetic model fitting to the batch test data for *p*-cresol (A) and resorcinol (B).

Table 2
Volumetric ($r_{s,v}$) and specific ($r_{s,s}$) substrate uptake rates for *p*-cresol and resorcinol in batch tests.

	p-Cresol			Resorcinol		
Initial concentration [mg·L^{-1}]	$r_{s,v}$[mg·L^{-1}d^{-1}]	R^2	$r_{s,s}$[mg·gVSS^{-1}d^{-1}]	$r_{s,v}$[mg·L^{-1}d^{-1}]	R^2	$r_{s,s}$[mg·gVSS^{-1}d^{-1}]
50	7.8 ± 1.3	0.84	44.0 ± 7.8	4.6 ± 0.3	0.97	26.6 ± 6.4
100	9.1 ± 1.45	0.81	19.3 ± 0.6	5 ± 0.4	0.91	8.8 ± 1.5
250	10.8 ± 0.9	0.89	8.5 ± 1.2	6.7 ± 0.7	0.87	5 ± 0.8
500	16.4 ± 0.8	0.96	6.5 ± 1.7	10 ± 0.4	0.97	3.5 ± 1.6

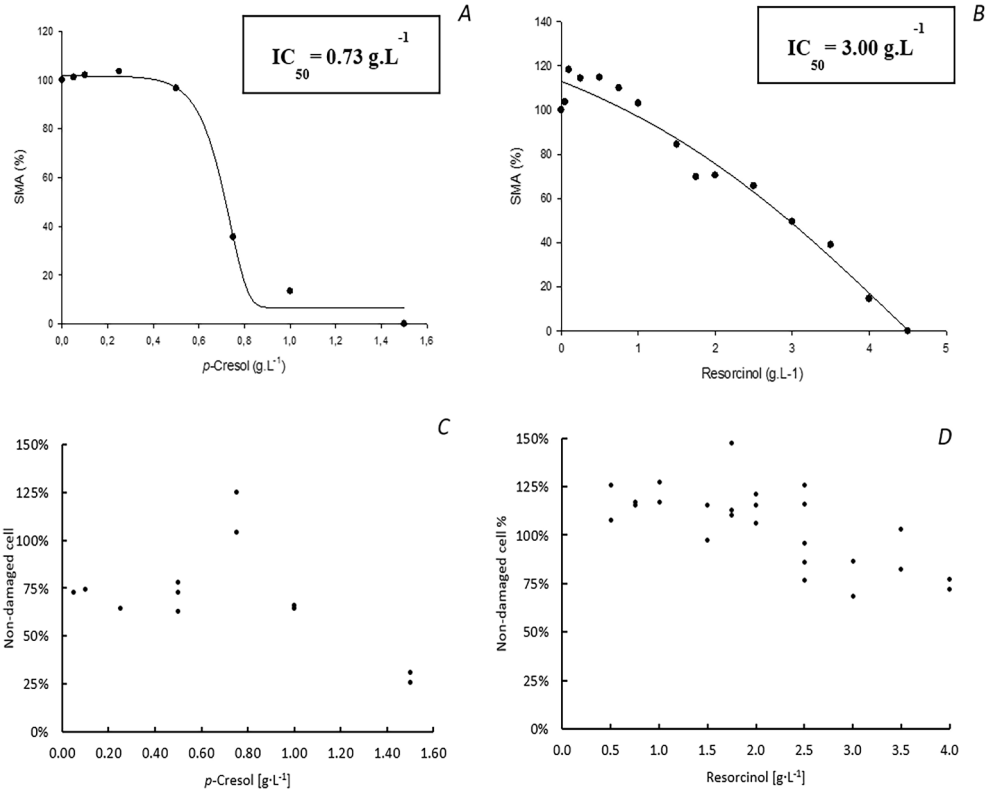

Fig. 3. Acetoclastic SMA inhibition percentage in the phenol-degrading sludge in the presence of *p*–cresol (A) and resorcinol (B). Percentage of non-damaged cells, in respect of the control, in the anaerobic biomass after the SMA inhibition tests with *p*-cresol (C) and resorcinol (D).

the SMA was finished. Finally, to assess and compare the inhibitory and toxic effects of *p*-cresol and resorcinol on non–adapted biomass, the tests were repeated with biomass coming from a municipal anaerobic sludge digester (Fig. 4 A-D).

By fitting a four parameter logistic model [34], the half maximal inhibitory concentrations (IC_{50}) for both compounds in the phenol-degrading biomass were estimated as $IC_{50p\text{-}cresol} = 0.73\ g{\cdot}L^{-1}$, and $IC_{50resorcinol} = 3.00\ g{\cdot}L^{-1}$, which implies that *p*-cresol has a higher

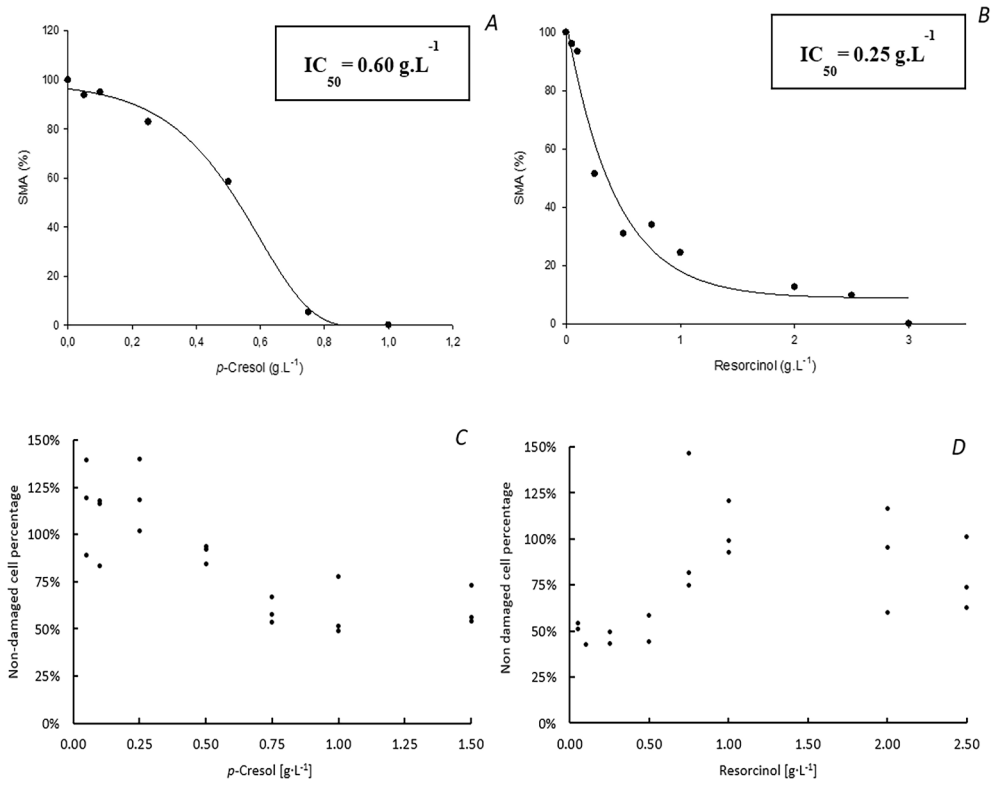

Fig. 4. Acetoclastic SMA inhibition percentage in the MWWTP sludge in the presence of *p*-cresol (A) and resorcinol (B). Percentage of non-damaged cells, in respect of the control, in the anaerobic biomass after the SMA inhibition tests with *p*-cresol (C) and resorcinol (D).

inhibitory effect on the acetoclastic methanogens in comparison to resorcinol. Fig. 3 (C & D) shows the results of the FCM performed once the SMA inhibition tests were finished. It can be observed that at *p*-cresol concentrations of up to 1.0 g·L^{-1} there was not a marked decrease in non-damaged cells. At 0.75 g *p*-cresol·L^{-1}, an over-estimation of non-damaged cells was observed, which is further considered as an outlier. However, at a concentration of 1.5 g·L^{-1}, just 25% (approx.) of the cells remained as non-damaged; nevertheless, the variability of the data should be considered. These results imply that at concentrations below 1.0 g·L^{-1} the inhibition was mainly biostatic, whereas at 1.5 g·L^{-1}, in addition to the biostatic inhibition, there was a higher percentage of cell-membrane-damaged cells, which may be associated with toxicity (a biocidal inhibitory effect) [22]. With respect to resorcinol, results show that even at the highest concentrations, the percentages of non-damaged cells hardly decreased. At a concentration of 4 $g_{resorcinol}$·L^{-1} there was a 25% decrease in non-damaged cells in comparison with the control. Implying that resorcinol had mainly biostatic and not toxic (biocidal) effects on the biomass.

Regarding the non-adapted biomass, the estimated IC_{50} values were 18% ($IC_{50p\text{-}cresol}$ = 0.60 g·L^{-1}) and 92% ($IC_{50resorcinol}$ = 0.25 g·L^{-1}) lower than the ones for the phenol-degrading AnMBR biomass (Fig. 4 A & B). Opposite to what was observed for the phenol-degrading AnMBR biomass, *p*-cresol at a concentration of 1.5 $g_{p\text{–}cresol}$·L^{-1} caused a decrease of approx. 50% of the non-damaged cells, which was lower in comparison with what was observed with the AnMBR-adapted biomass (Fig. 4 C & D). However, even at the concentration of 0.75 $g_{p\text{–}cresol}$·L^{-1} the methanogenic activity was almost completely inhibited, giving an activity of only 5.2% in respect of the control. On the other hand, the IC_{50} value for resorcinol in the non-adapted biomass was 92% lower than the obtained IC_{50} value for the phenol-degrading biomass. The FCM, showed that there was not a decrease in the non-damaged cells even at concentrations that caused high inhibition of methanogenesis. However, and opposite to what was observed with the phenol degrading AnMBR biomass, there was no negative trend in the non-damaged cell percentage with increasing concentration of resorcinol.

3.3. Anaerobic membrane bioreactor operation for the simultaneous degradation of phenol with p-cresol or resorcinol

Two AnMBRs seeded with phenol-degrading and salt-adapted biomass were operated to assess the maximum conversion rates of *p*-cresol or resorcinol, together with phenol, under continuous-flow reactor conditions with low to moderate bulk concentrations of the phenolic compounds. Periodically, biomass samples were taken to study the dynamics of the microbial communities. Reactor 1 (R1) was fed with a mixture of *p*-cresol and phenol, and Reactor 2 (R2) with a mixture of resorcinol and phenol (Table 1).

3.3.1. Simultaneous degradation of phenol and p-cresol

Nine different volumetric *p*-cresol loading rates ($v_{p\text{-}cresol}$LR) were applied (Table 1). Up to a load of 130 $mg_{p\text{–}cresol}$·L^{-1}d^{-1}, 100% removal of phenol and *p*-cresol was achieved, corresponding to a specific *p*-cresol conversion rate ($s_{p\text{-}cresol}$CR) of 22 $mg_{p\text{–}cresol}$·gVSS^{-1}d^{-1} and a specific phenol conversion rate of (s_{phenol}CR) of 56 mg_{ph}·gVSS^{-1}d^{-1}. However, on day 102, when the $v_{p\text{-}cresol}$LR reached 200 $mg_{p\text{-}cresol}$·L^{-1}d^{-1}, the removal efficiencies of both phenol and *p*-cresol decreased practically at the same time, most likely implying an intoxication of the reactor biomass caused by accumulating *p*-cresol (Fig. 5 A).

Concerning the microbial community structure (Fig. 5 B), the 4 most abundant microorganisms comprising 62.2 ± 4.7% of the entire community during the whole reactor operation included the strict acetoclastic methanogen *Methanosaeta* sp. (20.4 ± 4.8%), *Syntrophorhabdus* sp. (18.0 ± 6.9%) a reported phenol and *p*-cresol degrader, *Thermovirga* sp. (15.8 ± 5.6%), and *Desulfatiglans* sp. (11.0 ± 6.3%). After the increase in the $v_{p\text{-}cresol}$LR and therefore in the *p*-cresol concentration in the bulk of the reactor, there was a decrease in the relative abundance of *Syntrophorhabdus* sp from 17.8 ± 1.0% (day 106), corresponding to a 100% removal efficiency of *p*-cresol and phenol, to 9.9 ± 0.5% on day

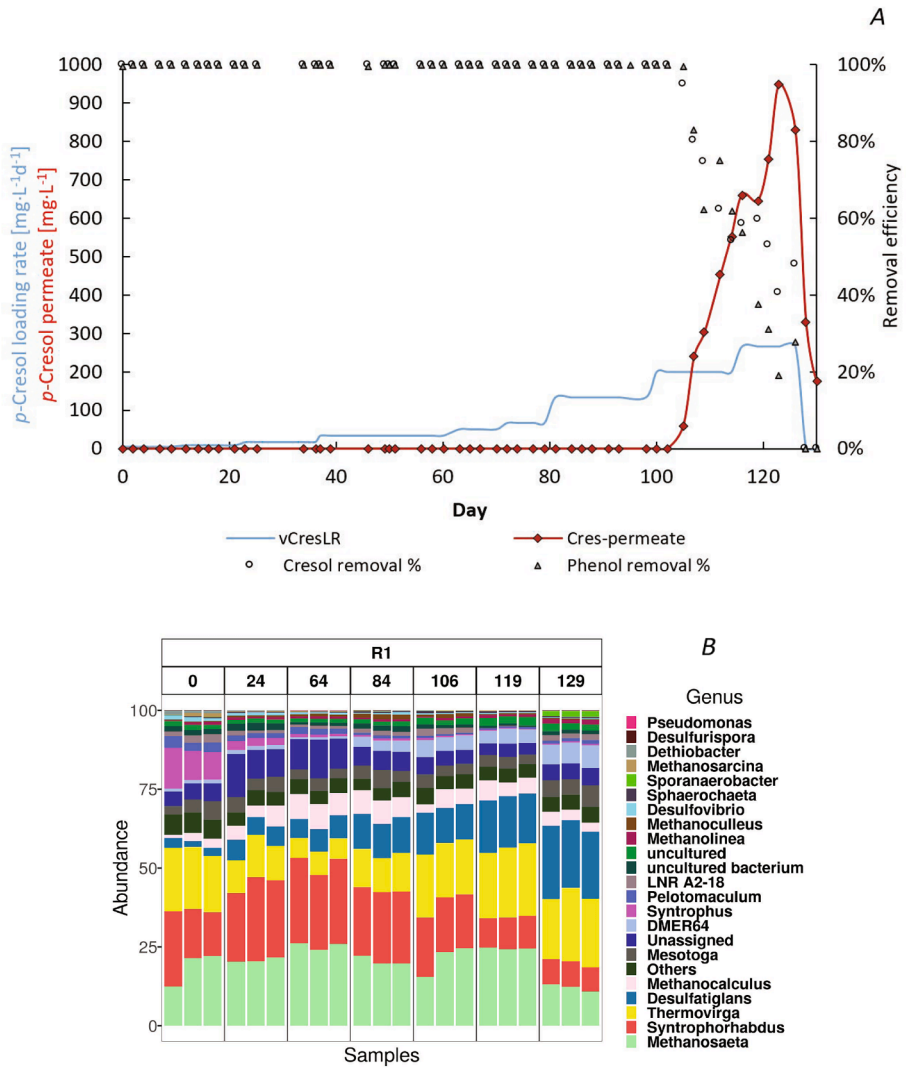

Fig. 5. Operation of R1 for the simultaneous degradation of *p*-cresol and phenol (A), and relative abundance of microorganisms (genus) during different operation days (B). In (A), the left y axis represents either the *p*-cresol volumetric loading rate [$mg_{p-cresol}·gVSS^{-1}d^{-1}$] or the concentration of *p*–cresol in the permeate [$mg_{p-cresol}·L^{-1}$].

119, and then to 7.9 ± 0.3% on day 129. *Methanosaeta* had a decrease in its relative abundance as well, from 24.5 ± 0.3% on day 119 to 12.1 ± 1.1% on day 129. The other two most abundant genera did not show a clear decrease in their relative abundances.

3.3.2. Simultaneous degradation of phenol and resorcinol

Seven different volumetric resorcinol loading rates ($v_{resorcinol}LR$) were applied to R2. Each time the $v_{resorcinol}LR$ was increased, there was a decrease in the removal efficiency of resorcinol (Fig. 6 A). The phenol removal efficiency was affected when the resorcinol concentration in the reactor started to increase, e.g., days 29–48. However, even when resorcinol concentrations were higher than 200 $mg·L^{-1}$ (day 43 and 69, Fig. 6 A), the conversion rates, and therefore, the removal efficiencies of phenol and resorcinol were recovered. On day 59, at a $v_{resorcinol}LR$ of 100 $mg_{resorcinol}·L^{-1}d^{-1}$, removal efficiencies of 98% and 100% were found for resorcinol and phenol respectively, corresponding to specific conversion rates ($s_{resorcinol}CR$) of 16 $mg_{resorcinol}·gVSS^{-1}d^{-1}$ and 54 $mg_{phenol}·gVSS^{-1}d^{-1}$. However, on day 66, there was a decrease to 52% and 73% in the resorcinol and phenol removal efficiencies, respectively. On day 69, when the $v_{resorcinol}LR$ was increased to 130 $mg_{resorcinol}·L^{-1}d^{-1}$, the resorcinol concentration in the permeate started to increase, resulting in a reduced resorcinol and phenol removal efficiencies of 52% and 66%, respectively. Nonetheless, on day 73, and with a $v_{resorcinol}LR$ = 200 $mg_{res}·L^{-1}d^{-1}$, a recovery in the removal efficiency was observed, reaching 90% and 99% for resorcinol and phenol, respectively. The subsequent increase in the $v_{resorcinol}LR$ to 250 $mg_{resorcinol}·L^{-1}d^{-1}$ on day 87 caused the resorcinol and phenol conversion failure. In contrast to what was observed in R1, the phenol removal efficiency decreased after two days (day 87) of the last drop in the resorcinol removal efficiency.

For the microbial community structure (Fig. 6 B), three main microorganisms comprised 66.5 ± 7.9% of the entire community during the whole reactor operation. These microorganisms were the phenol degrader *Syntrophorhabdus* sp. (24.2 ± 5.6%), *Thermovirga* sp. (21.8 ± 4.3%), and the acetoclastic methanogen *Methanosaeta* sp. (20.6 ± 5.0%). Opposite to what was observed in R1, *Methanosaeta* sp. was not the most abundant microorganism. In contrast, *Syntrophorhabdus* sp. dominated the community, which suggested that *Syntrophorhabdus* sp. could have contributed to the degradation, not just of phenol but of resorcinol as well. Another observed difference concerning R1 is that even in the periods when the removal efficiency of resorcinol decreased, there was no noticeable impact on the main groups of microorganisms, especially *Syntrophorhabdus* sp. and *Methanosaeta* sp. As well, *Thermovirga* sp. remained constant during all the stages and, similar to what was observed for the operation with *p*-cresol, *Desulfatiglans* sp. was the fourth most abundant microorganism; though, on average, its relative abundance was 3.9 ± 1.5% in comparison to the 11.0 ± 6.3% found for R1.

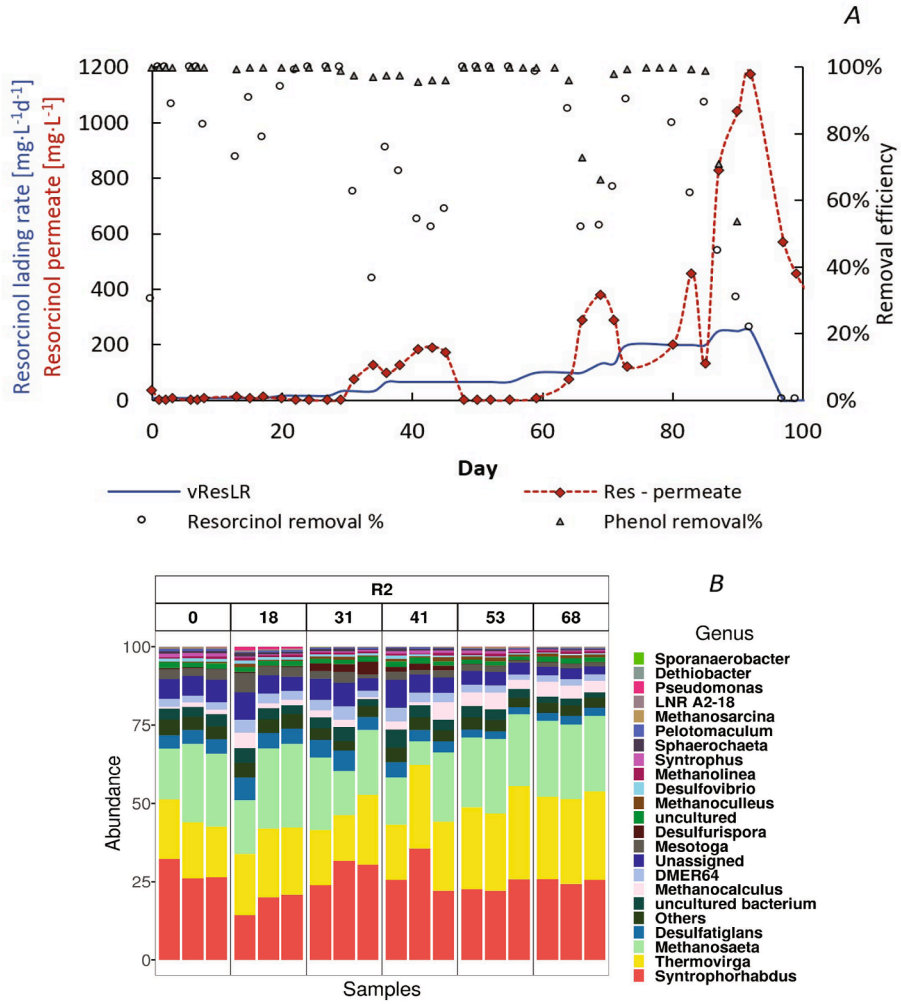

Fig. 6. Operation of R2 for the simultaneous degradation of resorcinol and phenol (A), and relative abundance of microorganisms (genus) during different operation days (B). In (A), the left y axis represents either the resorcinol volumetric loading rate [$mg_{resorcinol} gVSS^{-1} d^{-1}$] or the concentration of resorcinol [$mg_{resorcinol} \cdot L^{-1}$] in the permeate.

3.3.3. *Analysis of the communities of the anaerobic membrane bioreactors*

To compare the similarities between the microbial communities of the two reactors, a principal coordinate analysis (PCoA) was performed (Fig. 7A). By comparing the microbial communities of both reactors, it is possible to conclude that there was no statistical ($p < 0.05$) difference between the communities as there is no clear separation, neither any clustering between the samples (Fig. 7B). This suggests that the microbial communities were phylogenetically similar, and the mixtures of phenol-*p*-cresol and phenol-resorcinol might be degraded either by the same microorganism or by phylogenetically closely related microorganisms.

3.4. *Thermodynamic state analysis*

To theoretically support the observed results obtained during the batch and continuous experiments and to better understand the (bio) chemical reactions of the anaerobic growth on *p*-cresol and resorcinol, a thermodynamic analysis was performed.

3.4.1. *p-Cresol*

Degradation of *p*-cresol under strict anaerobic (methanogenic) conditions has been reported elsewhere [28,48]. Specifically, one microorganism species, *Syntrophorhabdus (aromaticivorans)* has been identified to convert *p*-cresol into acetate in a syntrophic association with a hydrogenotrophic methanogen [27,37]. For the anaerobic growth on *p*-cresol, the complete oxidation of *p*–cresol into acetate was considered for the catabolic reaction (Table 3, Eq. 1A). For the reduction reaction, the proton (H^+) respiration was considered (Table 3 Eq. 2B), as this is a common electron acceptor under methanogenic conditions [29]. For the anabolism, the oxidation reaction was considered to be the conγersion of *p*–cresol (degree of reduction $\gamma = 4.86$) into biomass ($C_1N_{0.2}O_{0.5}H_{1.8}$;$\gamma = 4.20$) [29], whereas the reduction reaction was considered the same as in the catabolism. Table 3 presents the values for standard Gibbs free energy change (ΔG_R^0) of the reactions and the values corrected for the expected concentrations and temperature ($\Delta G_R^{1,T}$) in the reactor.

3.4.2. *Resorcinol*

For the catabolism, the complete conversion of resorcinol into acetate was considered as the oxidation reaction (Table 4. Eq. 1.B). As the process will occur under methanogenic conditions, H^+ respiration was considered for the reduction reaction (Table 4. Eq. 2B). For the anabolism, the conversion of resorcinol ($\gamma = 4.33$) into biomass ($\gamma = 4.2$)[38] was considered as the oxidation reaction, and H^+ respiration as the reduction reaction. Table 4 shows the values of ΔG_R^0, and $\Delta G_R^{1,T}$ (corrections for reactor conditions).

As can be observed in Tables 3 and 4, both catabolic oxidation reactions (Eq. 1A & 1B) are thermodynamically non-favorable under standard conditions (T = 298 K, P = 1 atm, concentrations = 1 M). However, under physiological conditions, correction for temperature,

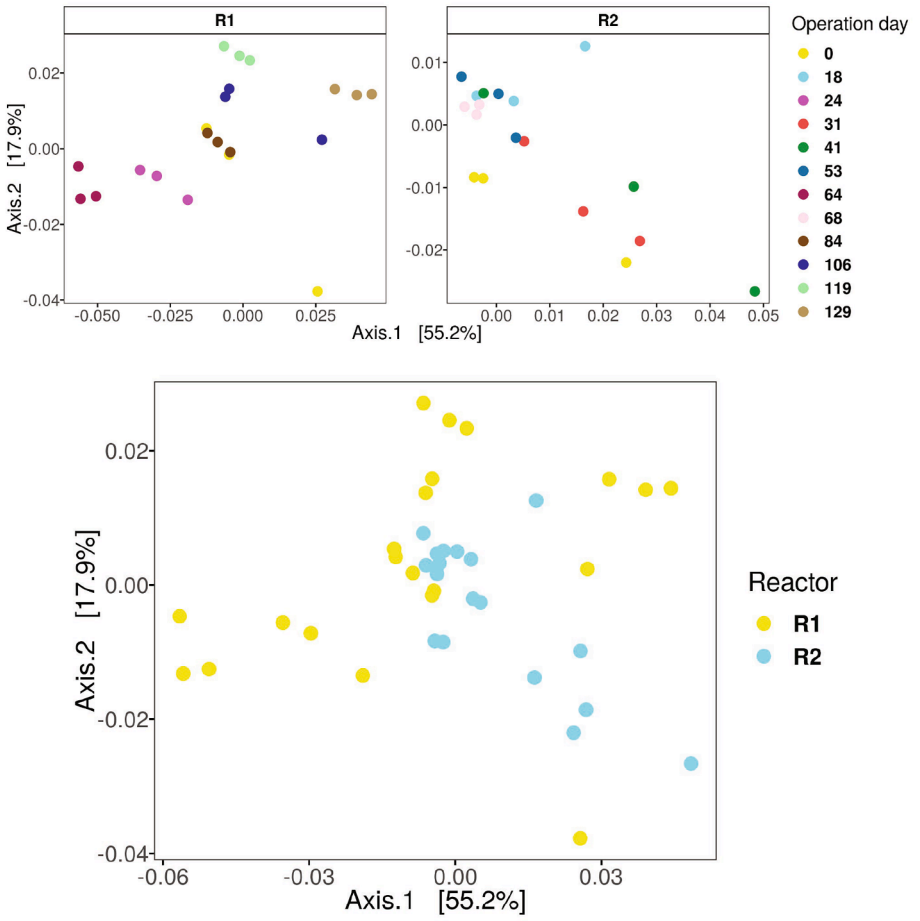

Fig. 7. PCoA of the microbial communities from R1 and R2 during the reactor operation (A). PCoA of the combined samples of the reactors (B). Each dot represents a different sample, and community structure, in a specific moment of the operation of R1 (yellow) or R2 (blue).

Table 3
Stoichiometry and thermodynamic values for the degradation of *p*-cresol under methanogenic conditions. Units of all ΔG_R are in kJ·mol^{-1}.

	Reaction	Stoichiometry	ΔG_R^0	$\Delta G_R^{1,T}$	Eq. No
p-Cresol Catabolism	Oxidation: *p*-cresol to acetate	$-C_7H_8O_1 - 6H_2O + 3.5C_2H_3O_2^- + 9.5H^+ + 6e^-$	163.1	−275.5	Eq. 1A
	Reduction: H^+ respiration	$-e^- - H^+ + 0.5H_2$	0	27.7	Eq. 2A
	Overall catabolic reaction	$-C_7H_8O_1 - 6H_2O + 3.5C_2H_3O_2^- + 3H_2 + 3.5H^+$	163.1	−108.9	Eq. 3A
p-Cresol Anabolism	Oxidation: *p*-cresol to biomass	$-0.14C_7H_8O_1 - 0.36H_2O - 0.2NH_4 + C_1N_{0.2}O_{0.5}H_{1.8} + 0.86H^+ + 0.66e^-$	39.0	12.0	Eq. 4A
	Reduction: H^+ respiration	$-e^- - H^+ + 0.5H_2$	0	27.7	Eq. 5A
	Overall anabolic reaction	$-0.14C_7H_8O_1 - 0.36H_2O - 0.2NH_4 - 0.08CO_2 + C_1N_{0.2}O_{0.5}H_{1.8} + 0.33H_2 + 0.2H^+$	39.0	30.32	Eq. 6A
	Metabolism of anaerobic growth on *p*-cresol	$-2.41C_7H_8O_1 - 13.96H_2O - 0.2NH_4 - 0.08CO_2 + C_1N_{0.2}O_{0.5}H_{1.8} + 7.94C_2H_3O_2^- + 7.13H_2 + 8.14H^+$	408.7	−216.6	Eq. 7A

and (expected) concentrations and partial pressures, the $\Delta G_R^{(01,T)}$ becomes more negative.

4. Discussion

Our present results show that the degradation of *p*-cresol and resorcinol started without any lag phase using AnMBR phenol-fed suspended biomass that was never exposed to these compounds (Fig. 2 A & B). The estimated $r_{s,s}$ showed a high variability in results with maximum values of 44.0 ± 7.8 $mg_{p-cresol}$·gVSS^{-1}d^{-1} and 26.6 ± 6.4 $mg_{resorcinol}$·gVSS^{-1}d^{-1} (average$_{p\text{-cresol}}$ = 11.3 ± 15.8 mg·gVSS^{-1}d^{-1}; average$_{resorcinol}$ = 8.2 ± 7.5 mg·gVSS^{-1}d^{-1}) (Table 2), in which increasing initial concentrations of the phenolic compounds yielded lower $r_{s,s}$ values. Results indicate that substrate inhibition was limiting the conversion rates. For *p*-cresol, the calculated $r_{s,v}$ were higher than those reported in a recent study (3.7 – 7.8 mg·L^{-1}d^{-1}, VSS = 4.0 g·L^{-1}) [28]. The immediate start of *p*-cresol conversion might be attributed to the fact that phenol and *p*-cresol share the same degradation route via 4-hydroxybenzoyl-CoA [24,25,39], and at least one of the identified species of microorganisms, *Syntrophorhabdus* sp., has been reported to have

Table 4
Stoichiometry and thermodynamic values for the degradation of resorcinol under methanogenic conditions. Units of all ΔG_R are kJ·mol^{-1}.

	Reaction	Stoichiometry	ΔG_R^0	$\Delta G_R^{1,T}$	Eq. No
Resorcinol Catabolism	Oxidation: resorcinol to acetate	$-C_6H_6O_2 - 4H_2O + 3C_2H_3O_2^- + 5H^+ + 2e^-$	66.5	−186.4	Eq. 1B
	Reduction: H^+ respiration	$-e^- - H^+ + 0.5H_2$	0	27.7	Eq. 2B
	Overall catabolic reaction	$-C_6H_6O_2 - 4H_2O + 3C_2H_3O_2^- + 1H_2 + 3H^+$	66.5	−130.9	Eq. 3B
Resorcinol Anabolism	Oxidation: resorcinol to biomass	$-0.17C_6H_6O_2 - 0.17H_2O - 0.2NH_4 + C_1N_{0.2}O_{0.5}H_{1.8} + 0.34H^+ + 0.14e^-$	26.9	24.1	Eq. 4B
	Reduction: H^+ respiration	$-e^- - H^+ + 0.5H_2$	0	27.7	Eq. 5B
	Overall anabolic reaction	$-0.17C_6H_6O_2 - 0.17H_2O - 0.2NH_4 + C_1N_{0.2}O_{0.5}H_{1.8} + 0.07H_2 + 0.2H^+$	26.9	24.1	Eq. 6B
	Metabolism of anaerobic growth on resorcinol	$-1.98C_6H_6O_2 - 7.43H_2O - 0.2NH_4 + C_1N_{0.2}O_{0.5}H_{1.8} + 5.45C_2H_3O_2^- + 1.89H_2 + 5.65H^+$	147.7	−199.3	Eq. 7B

the capability to degrade both compounds under methanogenic conditions [27]. Under anoxic (nitrate-reducing) conditions or sulfate-reducing conditions, phenol is carboxylated in the *para*-position yielding 4-hydroxybenzoate, whereas *p*-cresol is hydroxylated at the methyl group by an oxygen-independent reaction; in both cases, the benzoyl-CoA is the intermediate from which the subsequent steps in the degradation pathway are shared [25,40].

On the other hand, resorcinol follows a 4-hydroxybenzoyl-CoA independent pathway [25,39]. Resorcinol can be anaerobically degraded either oxidatively or reductively by conversion routes that do not involve benzoyl-CoA [24,26]. The reduction of resorcinol was found in fermenting *Clostridium* sp., which possesses resorcinol reductase, catalyzing the reduction of resorcinol to 1,3-cyclohexadione. The reduced intermediate is then hydrolytically cleaved to 5-oxocaproic acid that is broken down into acetate and butyrate. The second possible pathway, which is energetically favorable, but thus far only identified for denitrifying bacteria, is an oxidative reaction releasing hydroxyhydroquinone [25,39]. Therefore, resorcinol conversion by anaerobic phenol-degrading biomass was not anticipated, implying that the biomass had a wider catabolic diversity than expected. However, and in comparison with phenol and *p*-cresol, resorcinol can be reduced more easily because it carries hydroxyl groups in meta position, which allows tautomerization to the enol form generating an isolated double bond that can act as electron acceptor [41].

The anaerobic biomass coming from the phenol-degrading AnMBR under saline conditions showed a higher inhibition on the SMA due to *p*-cresol compared with resorcinol addition (Fig. 3 A & B). The results obtained during the inhibition tests agree with the observations of Liang and Fang (2010), who attributed the increased inhibitory effect of *p*-cresol to the higher hydrophobicity of the molecule in comparison to resorcinol [42].

Different IC_{50} values for methanogenesis when using *p*-cresol and resorcinol have been reported by other researchers [35,36] (Table 5). However, most of the values were obtained using anaerobic granular biomass as inoculum, mainly coming from UASB reactors. On the other hand, although *p*-cresol and resorcinol are toxic compounds, to the best of our knowledge no studies report the effect of these compounds on the cell viability.

The phenol-degrading AnMBR biomass had a 29% lower IC_{50} value for *p*-cresol than the one reported for phenol-adapted granular biomass, but 18% higher than the one from a distillery wastewater (Table 5). In the case of resorcinol, the IC_{50} value was 66% higher than the reported value for the granular biomass from the distillery wastewater. Opposite to the reported studies, the AnMBR biomass used in the present study was not granular but suspended, so more susceptible to toxicants. Additionally, the tests were performed under saline conditions, which might add additional stress to the biomass [43].

Cell membrane damage related to toxicity can be considered as biocidal inhibition [44]. Therefore, the decrease in the percentage of non-damaged cells after the SMA tests was related to whether the decrease in the methanogenic activity was due to toxicity (biocidal inhibition) or to an impairment of the methanogenic function (biostatic inhibition). Our results support the hypothesis that the effect of *p*-cresol on the methanogens is mainly biostatic at low concentrations and toxic, or biocidal, at high concentrations. In contrast, the inhibition by resorcinol is mainly owing to a biostatic inhibition. As well, combining the results from the batch tests (biodegradability kinetics, inhibition, and toxicity tests) and the reactor operation, it may be possible to conclude that the activity of the *p*-cresol and resorcinol degraders was affected before the methanogenic activity.

Table 5
IC_{50} values reported for *p*-cresol and resorcinol.

References	*Compound*	*IC_{50}*	*Biomass*
Olguin-Lora et al. 2003	*p*-Cresol	0.39 g·L^{-1}	Non-acclimated granular sludge
Olguin-Lora et al. 2003	*p*-Cresol	1.03 g·L^{-1}	Phenol-acclimated granular sludge
Sierra-Alvarez and Lettinga, 1991	*p*-Cresol	0.62 g·L^{-1}	Granular sludge from a full-scale UASB treating distillery wastewater
Sierra-Alvarez and Lettinga, 1991	Resorcinol	1.81 g·L^{-1}	Granular sludge from a full-scale UASB treating distillery wastewater
This study	*p*-Cresol	0.73 g·L^{-1}	Suspended biomass from a phenol-degrading AnMBR (8 gNa·L^{-1})
This study	*p*-Cresol	0.60 g·L^{-1}	Anaerobically digested biomass from an MWWTP
This study	Resorcinol	3.00 g·L^{-1}	Suspended biomass from a phenol-degrading AnMBR (8 gNa·L^{-1})
This study	Resorcinol	0.25 g·L^{-1}	Anaerobically digested biomass from an MWWTP

The results from R1 showed a rapid adaptation of the biomass to the degradation of the new substrate *p*-cresol, which might be attributed to the shared microbial metabolic pathway during phenol and *p*-cresol conversion. Additionally, full biomass retention likely led to in-situ bioaugmentation of the proper bacteria [45]. As previously reported, an adapted, abundant, and active methanogenic population is required for the proper degradation of phenol [23], which appeared to be the case for the combined degradation of phenol along with *p*-cresol (R1) (or resorcinol in R2). The thermodynamic analysis showed that the catabolic conversion of *p*–cresol has a positive ΔG_R^0 (standard conditions), but becomes negative under the prevailing reactor conditions, mainly because of the neutral pH and the decrease in the concentration of produced intermediates, i.e., hydrogen and acetate. The anabolic metabolism of one mol of *p*-cresol produces 7.13 mol of hydrogen; therefore, and similar to the degradation of phenol, *p*-cresol requires a syntrophic association, meaning that there should be a continuous removal of the (produced) hydrogen by, e.g., hydrogenotrophic

methanogens, so both anabolic and catabolic reactions can proceed (Eq. 3A & 7A). In a recent study, our group reported the enhancement of anaerobic phenol degradation during the simultaneous conversion with acetate, which correlated with the presence of an abundant acetoclastic methanogenic population [23]. For the combined degradation of phenol and *p*-cresol, the same effect could be expected as 3.3 mol of acetate per mol of *p*-cresol are produced during its anaerobic conversion (Eq. 7A), implying that a strong acetoclastic methanogenic population may allow a better degradation of both compounds.

The inhibition of the reactor's biomass, and thus the simultaneous decrease in the conversion of the phenolic compounds, confirmed that the same microorganism or microbial group might perform phenol and *p*-cresol degradation.

About R2 and in agreement with the batch tests, it was demonstrated that anaerobic resorcinol degradation occurred without any lag phase, which confirmed the hypothesis of the suspected broader metabolic capacity of the biomass. Even at the beginning of the reactor operation, and with a $v_{resorcinol}$LR of 30 mg·L^{-1}d^{-1}, the resorcinol removal efficiency remained about 100%. As previously discussed, this result was not expected; in fact, resorcinol was chosen because of 1) its abundancy in GCWW and 2) itis a substrate with a different degradation pathway than phenol.

Opposite to what was observed for R1, the degradation of resorcinol was not as stable in comparison to *p*-cresol degradation, as the reactor's removal efficiency decreased each time a higher $v_{resorcinol}$LR was implemented. Nevertheless, even when resorcinol concentration in the reactor was as high as 450 mg·L^{-1} (day 83, Fig. 6), the biomass recovered the resorcinol (and phenol) degradation activity. This is in line with the data gathered in the toxicity analysis (Fig. 3 D), that showed no lethal (toxic) effects of resorcinol on the biomass, especially at the concentrations prevailing in the reactor; therefore, in a case of an acute inhibition by resorcinol, as what was observed druing R2 operation, it could be expected a rapid recovery of the removal efficiency, mainly because all the biomass is retained inside of the reactor due to the membrane filtration process.

Similar to *p*-cresol degradation, the anaerobic conversion of resorcinol produces hydrogen and acetate. Under standard conditions, the catabolic ($\Delta G_R^0 = 66.5$ kJ·mol^{-1}) and the metabolic reactions ($\Delta G_R^0 = 147.0$ kJ·mol^{-1}) are non-spontaneous; however, under the prevailing reactor conditions, the reactions become thermodynamically favorable. Parallel to *p*-cresol conversion, this improvement in the reactions' thermodynamics is attributed, partly, to the effective consumption of intermediate products by syntrophic microbial associations of the phenolic-compounds-degradraders with acetoclastic and hydrogenotrophic methanogens [27,46].

Although it has been reported that resorcinol can be fermented into acetate and malate [39], or butyrate and acetate [47], the complete oxidation into acetate in syntrophic cooperation with a hydrogen scavenger in a mixed culture was reported as well [47]. In this respect, Tschech and Schink, 1985, mentioned that the complete oxidation into acetate and hydrogen might offer energetic advantages. Even though resorcinol fermentation into butyrate and acetate is thermodynamically more advantageous than the complete oxidation into acetate and H_2, the acetate-producers would be able to participate in the interspecies hydrogen transfer, which would benefit them over the butyrate-forming resorcinol-degraders if the reducing equivalents are indeed effectively transferred [47].

Most of the studies on the anaerobic degradation of *p*-cresol and resorcinol were based on granular sludge bed technology, mainly UASB (Table 6). In some of these studies, the authors did not report the VSS concentration in the reactor(s); therefore, it is not feasible to compare the biomass performance just based on the vOLRs. As well, just a couple studies were performed under saline conditions [14,43]. Wang et al. 2017, working with UASB reactors at a [Na^+] of 10 g·L^{-1}, reported sCR of approx. 16 mg_{phenol}·$gVSS^{-1}d^{-1}$, 15 $mg_{catechol}$·$gVSS^{-1}d^{-1}$, 8 $mg_{resorcinol}$·$gVSS^{-1}d^{-1}$, and 7 $mg_{hydroquinone}$·$gVSS^{-1}d^{-1}$, whereas average sCR of 54.6±9.7 mg_{phenol}·$gVSS^{-1}d^{-1}$ and 95.4±10.4 mg_{phenol}·$gVSS^{-1}d^{-1}$ were found for R1 for R2, respectively, and maximum sCRs of 22 $mg_{p\text{-}cresol}$·$gVSS^{-1}d^{-1}$ (R1) and 16 $mg_{resorcinol}$·$gVSS^{-1}d^{-1}$ (R2) were found in the here presented study. Thus, to the best of the authors' knowledge, the results presented in this article are the only results reported for AnMBRs, operating with suspended biomass.

For the microbial community study, the combined results from the molecular analysis and reactor perfomance suggested, as reported in the literature, that the degradation of phenol and *p*-cresol was performed by *Syntrophorhabdus* sp. [27,46]. However, this hypothesis should be further tested because studies based on the 16S rRNA gene give information regarding the presence and not the (metabolic) activities of the microorganisms. Nonetheless, clearly *Syntrophorhabdus* sp. was the second most abundant microorganism during the operation of R1. For R1, the conversion capacities of phenol and *p*-cresol were lost simultaneously with the increase in the organic load to 200 and 260

Table 6
Reactors treating phenolic compound mixtures.

References	*Reactor*	*Temp (°C)*	*HRT*	*OLR (gCOD·L^{-1}d^{-1})*	*Operation time (d)*	*Substrate*	*Influent Concentration*	*Removal percentage*	*Additional carbon/energy source*
Latkar and Chakrabarti, 1994	UASB	RT	–	9.5	10	Resorcinol, catechol, hydroquinone	50 $mg_{resorcinol}$·L^{-1} + 200 $mg_{catechol}$·L^{-1} / 50 $mg_{resorcinol}$·L^{-1} + 200 $mg_{hydroquinone}$·L^{-1}	95%	
Kennes et al., 1997	UASB	35	0.67 d	<6*	50	*p*-Cresol	650 mg·L^{-1}	80%	VFA
Fang and Zhou, 2000	UASB	37	2–24 h	2.2 – 7.4	440	Phenol and *p*-cresol	800 mg_{phenol}·L^{-1} and 300 $mg_{p-cresol}$·L^{-1}	Up to 95% phenol, up to 65% *p*-cresol, COD 22–98%	Sucrose for startup/No
Razo-Flores et al., 2003	UASB	30	0.5–0.6 d	7.0	200	Phenol, *p*–cresol, *o*-cresol	550 mg_{phenol}·L^{-1}, 132 $mg_{p\text{-}cresol}$·L^{-1} and 132 $mg_{o\text{-}cresol}$·L^{-1}	> 90 %	Acetate for startup
Wang et al., 2017	UASB	35	48 h	2.7	250	Phenol, catechol, resorcinol, hydroquinone	1000 mg·L^{-1}	80–100%	Acetate
Wu et al., 2020	UASB	35	48 h	≈ 2.5–4.1	162	Phenol, catechol, resorcinol, hydroquinone	1000 mg·L^{-1}	80–96%	Acetate
This study	*AnMBR*	*35*	*6 d*	*1.63*	*112*	*p-Cresol and phenol*	*1200 $mg_{p\text{-}cresol}$·L^{-1} and 2000 mg_{phenol}·L^{-1}*	*Up to 100%*	*Acetate*
This study	*AnMBR*	*36*	*6 d*	*1.44*	*77*	*Resorcinol and phenol*	*800 $mg_{resorcinol}$·L^{-1} and 2000 mg_{phenol}·L^{-1}*	*Up to 100%*	*Acetate*

$mg_{p-cresol}·L^{-1}d^{-1}$, which corresponded with the decrease in the relative abundance of *Syntrophorhabdus* sp. Even though some microorganisms, such as *Syntrophorhabdus* sp., have been identified as phenol degraders [27,46], the key microorganisms that perform the anaerobic degradation of other phenolic compounds are still uncertain, and the results found in the literature are diverse. *Syntrophus,* and other genera belonging to *Anaerolineaceae*, were the main bacteria found in an upflow anaerobic sludge blanket reactor treating synthetic CGGW containing phenol, catechol, and resorcinol [14]. Based on their results, the authors indicated that those microorganisms were mainly responsible for the degradation of the phenolic compounds. In other studies, *Syntrophorhabdus* and *Bacillus* appeared as the responsible microorganism for the hydrolysis of phenol and *p*-cresol in a UASB reactor [48]. Franchi et al. (2018), found that *Syntrophorhabdus,* together with hydrogenotrophic archaea, increased their relative abundance after the degradation of phenol and *p*-cresol, indicating the important role of these microorganisms during the degradation of the mentioned aromatics, which agrees with the here obtained results [28].

Regarding the third most abundant genera during R1 operation, *Thermovirga* sp., it has been reported in UASB reactors [14,43] and AnMBRs degrading phenol under saline conditions [23,49]. *Thermovirga* sp. is a halotolerant microorganism; however, no phenol degrading activity has been associated with this microorganism yet; instead, it was related to protein and aminoacids fermentation [50].

Desulfatiglans sp. was the fourth most abundant microorganism during R1 operation. It was found in R2 as well, though in lower abundance. This is a mesophilic and strictly anaerobic microorganism reported to (mainly) use sulfate and other inorganic sulfur compounds as electron acceptors [51,52]. Phenol and benzoate serve as preferable electron donors [52]. However, according to Jochum et al., *Desulfatiglans* related populations have a versatile metabolic potential [53]. They can be sulfate-reducers or conserve energy by acetogenesis or fermentation. Such microorganisms can access a wide range of carbon substrates, including aromatic compounds which are preferred over fatty acids [53]. The best growth occurs with benzoate [52], which is the intermediate product from phenol and *p*-cresol anaerobic degradation, explaining its high relative abundance in R1 and R2.

Because of the additional acetate that was fed in the reactor influent, the high abundance of acetate consuming methanogens, such as *Methanosaeta* sp. was expected. In addition, hydrogenotrophic methanogens, such as *Methanobacterium* sp., *Methanolinea* sp., (reported as halotolerant) [54], and *Methanocalculus* were present; however, they represented only 3 % and 2 % of the all identified methanogens.

For R2, as well as for R1, the results suggest that *Syntrophorhabdus* sp. may also have resorcinol fermentative capacities. This broader substrate diversity would explain the results observed in the batch tests for the degradation of resorcinol (Section 3.2). At the start of the incubation, resorcinol degradation was not expected because 1) the biomass was previously not in contact with resorcinol, 2) *Syntrophorhabdus* sp. was not reported as a resorcinol degrader, and 3) phenol and resorcinol are reported to have different conversion pathways when anaerobically degraded [24,25,39]. However, it is still unclear why phenol conversion was not affected in R2 when the $v_{resorcinol}$LR was increased, while resorcinol conversion was (days 85 – 87). As previously mentioned, metabolic hypotheses cannot be verified using solely ribosomal gene analysis, which implies that a functional or metabolic study using an enriched culture should be performed to conclude the hypothesis's validity. In addition, and because phenol conversion continued in R2 after resorcinol degradation was stopped, it may be that resorcinol was converted by another microorganism or group of microorganisms, which were overloaded at the increased $v_{resorcinol}$LR. Nevertheless, *Syntrophorhabdus* sp. was the most abundant microorganism during all the stages of Reactor 2 operation, and *Thermovirga* sp., the second most abundant genus, has not been reported as a resorcinol degrading microorganism; though, it has been commonly found in AnMBRs degrading phenolic compounds [23].

Finally, the PCoA analysis of R1 and R2 (Fig. 7B) showed no changes in the microbial community that could be related to a specialization of the biomass towards *p*-cresol or resorcinol degradation. Furthermore, there were no statistical ($p < 0.05$) differences in the comparison of the communities of R1 with the communities of R2, supporting the hypothesis that *Syntrophorhabdus* sp. could play a role as well in the resorcinol degradation.

5. Conclusions

Based on the results of this research, the following is concluded:

The degradation of both *p*-cresol and resorcinol in batch tests was described with a zero-order kinetic model. The maximum values for the specific uptake (conversion) rates were 44 ± 8 $mg_{p-cresol}·gVSS^{-1}d^{-1}$ and 26 ± 6.4 $mg_{resorcinol}·gVSS^{-1}d^{-1}$. However, increasing bulk concentrations of these phenolic compounds caused a decrease in the conversion rates.

IC_{50} values for the acetoclastic SMA of 0.73 $g·L^{-1}$ for *p*-cresol and 3.00 $g·L^{-1}$ for resorcinol were estimated for phenol-degrading biomass and 0.60 $g·L^{-1}$ for *p*-cresol and 0.25 $g·L^{-1}$ for resorcinol for non-adapted biomass.

For both phenol-degrading and non-adapted biomass, *p*-cresol caused a higher decrease in the viable cells count than resorcinol.

For R1, 100% removal efficiency at a $v_{p-cresol}$LR of 130 $mg_{p-cresol}·L^{-1}d^{-1}$ and an influent concentration of 800 $mg·L^{-1}$ was found, corresponding to a $s_{p-cresol}$CR of 22 $mg_{p-cresol}·gVSS^{-1}d^{-1}$. For R2, 90% removal efficiency at a $v_{resorcinol}$LR of 200 $mg_{Res}·L^{-1}d^{-1}$ and an influent concentration of 1200 $mg_{resorcinol}·L^{-1}$ was found, corresponding to a $s_{resorcinol}$CR of 16 $mg_{resorcinol}·gVSS^{-1}d^{-1}$.

In both AnMBRs, *Methanosaeta* sp. was the most abundant methanogen, while *Syntrophorhabdus* sp. was the most abundant bacteria, suggesting that *Syntrophorhabdus* sp. was the responsible microorganisms for the degradation of phenol and *p*-cresol. In addition, *Syntrophorhabdus* sp. may have a role in resorcinol degradation.

PCoA analyses showed that the microbial community structures found during the operation of the two reactors had a high phylogenetic similarity.

Funding

This research was supported by the Dutch Technology Foundation (STW, Project No 13348), which is part of the Netherlands Organisation for Scientific Research (NWO), partly funded by the Dutch Ministry of Economic Affairs. This research is co-sponsored by Evides Industriewater and Paques B.V.

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.

Acknowledgments

The authors thank the lab technicians Armand Middeldorp, Mohammed Jaffar, and Thor Reijis for the technical support. Ingrid Pinel for helping to conduct the FCM tests. Alberto Nuñez for the help in the lab work during his internship, and Flor Arminda Garcia Rea for the help in the graphical design. Victor S. Garcia Rea thanks all the Mexican people, who through the Mexican National Council of Science and Technology (CONACyT), granted him the PhD scholarship No. 410669. Beatriz Egerland Bueno is grateful to the Coordination for the Improvement of Higher Education Personnel (CAPES)-Finance Code 001, for the PhD and international internship fellowships (CAPES-PDSE), and the Post-Graduation Program in Food Engineering at FZEA-USP (Brazil).

References

[1] L. Aljerf, Data of thematic analysis of farmer's use behavior of recycled industrial wastewater, Data in Brief 21 (2018) 240–250, https://doi.org/10.1016/j.dib.2018.09.125.

[2] Q. Ji, S. Tabassum, S. Hena, C.G. Silva, G. Yu, Z. Zhang, A review on the coal gasification wastewater treatment technologies: past, present and future outlook, J. Clean. Prod. 126 (2016) 38–55.

[3] Q. Zhao, Y.u. Liu, State of the art of biological processes for coal gasification wastewater treatment, Biotechnol. Adv. 34 (5) (2016) 1064–1072, https://doi.org/10.1016/j.biotechadv.2016.06.005.

[4] P.C. Singer, F.K. Pfaender, J. Chinchilli, A.F. Maciorowski III, G. J.C.L, R., Assessment of Coal Conversion Wastewaters: Characterization and Preliminary Biotreatability. , in: E. EPA (Ed.)U.S. Washington D.C., 1978.

[5] W. Wang, H. Han, M. Yuan, H. Li, Enhanced anaerobic biodegradability of real coal gasification wastewater with methanol addition, J. Environ. Sci. (China) 22 (12) (2010) 1868–1874, https://doi.org/10.1016/S1001-0742(09)60327-2.

[6] D. Zhao, W. Lun, J. Wei, Discussion on Wastewater Treatment Process of Coal Chemical Industry, IOP Conf. Ser.: Earth Environ. Sci. 100 (2017) 012067, https://doi.org/10.1088/1755-1315/100/1/012067.

[7] J. Van Lier, F. Van der Zee, C. Frijters, M. Ersahin, Celebrating 40 years anaerobic sludge bed reactors for industrial wastewater treatment, Rev. Environ. Sci. Biotechnol. 14 (2015) 681–702.

[8] Z. Kong, L. Li, Y. Xue, M. Yang, Y.-Y. Li, Challenges and prospects for the anaerobic treatment of chemical-industrial organic wastewater: a review, J. Clean. Prod. 231 (2019) 913–927, https://doi.org/10.1016/j.jclepro.2019.05.233.

[9] M.T. Suidan, P. Fox, J.T. Pfeffer, Anaerobic Treatment of Coal Gasification Wastewater, Water Sci. Technol. 19 (1987) 229-236 DOI: 10.2166/wst.1987.0204 %J Water Science and Technology.

[10] H. Macarie, Overview of the application of anaerobic treatment to chemical and petrochemical wastewaters, Water Sci. Technol. 42 (2000) 201-214.

[11] E.ı.a. Razo-Flores, M. Iniestra-González, J.A. Field, P. Olgu í n-Lora, L. Puig-Grajales, Biodegradation of mixtures of phenolic compounds in an upward-flow anaerobic sludge blanket reactor, J. Environ. Eng. (N. Y.) 129 (2003) 999-1006.

[12] G.S. Veeresh, P. Kumar, I. Mehrotra, Treatment of phenol and cresols in upflow anaerobic sludge blanket (UASB) process: a review, Water Res. 39 (1) (2005) 154–170, https://doi.org/10.1016/j.watres.2004.07.028.

[13] H.H. Fang, G.-M. Zhou, Degradation of phenol and p-cresol in reactors, Water Sci. Technol. 42 (2000) 237-244.

[14] B. Wu, J. Wang, Z. Hu, S. Yuan, W. Wang, Anaerobic biotransformation and potential impact of quinoline in an anaerobic methanogenic reactor treating synthetic coal gasification wastewater and response of microbial community, J. Hazard. Mater. 384 (2020) 121404, https://doi.org/10.1016/j.jhazmat.2019.121404.

[15] Y. Li, S. Tabassum, C. Chu, Z. Zhang, Inhibitory effect of high phenol concentration in treating coal gasification wastewater in anaerobic biofilter, J. Environ. Sci. (China) 64 (2018) 207–215, https://doi.org/10.1016/j.jes.2017.06.001.

[16] A. Hemmelmann, A. Torres, C. Vergara, L. Azocar, D. Jeison, Application of anaerobic membrane bioreactors for the treatment of protein-containing wastewaters under saline conditions, J. Chem. Technol. Biotechnol. 88 (4) (2013) 658–663.

[17] D. Jeison, B. Kremer, J.B. van Lier, Application of membrane enhanced biomass retention to the anaerobic treatment of acidified wastewaters under extreme saline conditions, Sep. Purif. Technol. 64 (2) (2008) 198–205, https://doi.org/10.1016/j.seppur.2008.10.009.

[18] J.D. Muñoz Sierra, M.J. Oosterkamp, W. Wang, H. Spanjers, J.B. van Lier, Comparative performance of upflow anaerobic sludge blanket reactor and anaerobic membrane bioreactor treating phenolic wastewater: Overcoming high salinity, Chem. Eng. J. 366 (2019) 480-490 DOI: https://doi.org/10.1016/j.cej.2019.02.097.

[19] R.K. Dereli, M.E. Ersahin, H. Ozgun, I. Ozturk, D. Jeison, F. van der Zee, J.B. van Lier, Potentials of anaerobic membrane bioreactors to overcome treatment limitations induced by industrial wastewaters, Bioresour. Technol. 122 (2012) 160–170, https://doi.org/10.1016/j.biortech.2012.05.139.

[20] J.D. Muñoz Sierra, C. Lafita, C. Gabaldón, H. Spanjers, J.B. van Lier, Trace metals supplementation in anaerobic membrane bioreactors treating highly saline phenolic wastewater, Bioresour. Technol. 234 (2017) 106-114 DOI: https://doi.org/10.1016/j.biortech.2017.03.032.

[21] J.L. Chen, R. Ortiz, T.W.J. Steele, D.C. Stuckey, Toxicants inhibiting anaerobic digestion: a review, Biotechnol. Adv. 32 (8) (2014) 1523–1534, https://doi.org/10.1016/j.biotechadv.2014.10.005.

[22] S. Astals, D.J. Batstone, S. Tait, P.D. Jensen, Development and validation of a rapid test for anaerobic inhibition and toxicity, Water Res. 81 (2015) 208–215, https://doi.org/10.1016/j.watres.2015.05.063.

[23] V.S. García Rea, J.D. Muñoz Sierra, L.M. Fonseca Aponte, D. Cerqueda-Garcia, K. M. Quchani, H. Spanjers, J.B. van Lier, Enhancing phenol conversion rates in saline anaerobic membrane bioreactor using acetate and butyrate as additional carbon and energy sources, Front. Microbiol. 11 (2020), https://doi.org/10.3389/fmicb.2020.60417310.3389/fmicb.2020.604173.s00110.3389/fmicb.2020.604173.s002.

[24] B. Philipp, B. Schink, Different strategies in anaerobic biodegradation of aromatic compounds: nitrate reducers versus strict anaerobes, Environ. Microbiol. Rep. 4 (2012) 469–478, https://doi.org/10.1111/j.1758-2229.2011.00304.x.

[25] G. Fuchs, M. Boll, J. Heider, Microbial degradation of aromatic compounds - from one strategy to four, Nat. Rev. Microbiol. 9 (11) (2011) 803–816, https://doi.org/10.1038/nrmicro2652.

[26] J. Gibson, C. S. Harwood, Metabolic diversity in aromatic compound utilization by anaerobic microbes, Annu. Rev. Microbiol. 56 (1) (2002) 345–369, https://doi.org/10.1146/annurev.micro.56.012302.160749.

[27] Y.-L. Qiu, S. Hanada, A. Ohashi, H. Harada, Y. Kamagata, Y. Sekiguchi, Syntrophorhabdus aromaticivorans gen. nov., sp. nov., the first cultured anaerobe capable of degrading phenol to acetate in obligate syntrophic associations with a hydrogenotrophic methanogen, Appl. Environ. Microbiol. 74 (7) (2008) 2051–2058, https://doi.org/10.1128/AEM.02378-07.

[28] O. Franchi, F. Rosenkranz, R. Chamy, Key microbial populations involved in anaerobic degradation of phenol and p-cresol using different inocula, Electron. J. Biotechnol. 35 (2018) 33–38, https://doi.org/10.1016/j.ejbt.2018.08.002.

[29] R. Kleerebezem, M.C.M. Van Loosdrecht, A generalized method for thermodynamic state analysis of environmental systems, Crit. Rev. Env. Sci. Tec. 40 (1) (2010) 1–54, https://doi.org/10.1080/10643380802000974.

[30] A.T.W.M. Hendriks, J.B. van Lier, M.K. de Kreuk, Growth media in anaerobic fermentative processes: the underestimated potential of thermophilic fermentation and anaerobic digestion, Biotechnol. Adv. 36 (1) (2018) 1–13, https://doi.org/10.1016/j.biotechadv.2017.08.004.

[31] H. Spanjers, P.A. Vanrolleghem, Respirometry, in: D. Brdjanovic, P.H. Nielsen, C. M. López-Vazquez, M.C.M. van Loosdrecht (Eds.) Experimental Methods in Wastewater Treatment, IWA Publishing2016, DOI: https://doi.org/10.2166/9781780404752.

[32] T. Falcioni, A. Manti, P. Boi, B. Canonico, M. Balsamo, S. Papa, Comparison of disruption procedures for enumeration of activated sludge floc bacteria by flow cytometry, Cytometry. Part B, Clinical cytometry 70B (3) (2006) 149–153, https://doi.org/10.1002/(ISSN)1552-495710.1002/cyto.b.v70b:310.1002/cyto.b.20097.

[33] A. Nescerecka, F. Hammes, T. Juhna, A pipeline for developing and testing staining protocols for flow cytometry, demonstrated with SYBR Green I and propidium iodide viability staining, Journal of microbiological methods 131 (2016) 172–180, https://doi.org/10.1016/j.mimet.2016.10.022.

[34] J. Lee, S. Hwang, Single and combined inhibition of Methanosaeta concilii by ammonia, sodium ion and hydrogen sulfide, Bioresour. Technol. 281 (2019) 401–411, https://doi.org/10.1016/j.biortech.2019.02.106.

[35] R. Sierra-Alvarez, G. Lettinga, The effect of aromatic structure on the inhibition of acetoclastic methanogenesis in granular sludge, Appl. Microbiol. Biotechnol. 34 (1991) 544–550, https://doi.org/10.1007/BF00180586.

[36] P. Olguin-Lora, L. Puig-Grajales, E. Razo-Flores, Inhibition of the acetoclastic methanogenic activity by phenol and alkyl phenols, Environ. Technol. 24 (8) (2003) 999–1006, https://doi.org/10.1080/09593330309385638.

[37] M.K. Nobu, T. Narihiro, T. Hideyuki, Y.-L. Qiu, Y. Sekiguchi, T. Woyke, L. Goodwin, K.W. Davenport, Y. Kamagata, W.-T. Liu, The genome of Syntrophorhabdus aromaticivorans strain UI provides new insights for syntrophic aromatic compound metabolism and electron flow, Environ. Microbiol. 17 (12) (2015) 4861–4872, https://doi.org/10.1111/1462-2920.12444.

[38] J.J. Heijnen, R. Kleerebezem, Bioenergetics of Microbial Growth, Encyclopedia of Industrial Biotechnology2010, pp. 1-66 DOI: 10.1002/9780470054581.eib084.

[39] M. Carmona, M.T. Zamarro, B. Blázquez, G. Durante-Rodríguez, J.F. Juárez, J. A. Valderrama, M.J.L. Barragán, J.L. García, E. Díaz, Anaerobic catabolism of aromatic compounds: a genetic and genomic view, Microbiol. Mol. Biol. Rev. 73 (1) (2009) 71–133, https://doi.org/10.1128/MMBR.00021-08.

[40] G. Fuchs, Anaerobic metabolism of aromatic compounds, Ann. N. Y. Acad. Sci. 1125 (2008) 82–99.

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

[42] D. Liang, H.H.P. Fang, Anaerobic treatment of phenolic wastewaters, Environmental Anaerobic Technology (2010) 185–205, https://doi.org/10.1142/9781848165434_0009.

[43] W. Wang, B. Wu, S. Pan, K. Yang, Z. Hu, S. Yuan, Performance robustness of the UASB reactors treating saline phenolic wastewater and analysis of microbial community structure, J. Hazard. Mater. 331 (2017) 21–27, https://doi.org/10.1016/j.jhazmat.2017.02.025.

[44] D.J. Batstone, J. Keller, I. Angelidaki, S.V. Kalyuzhnyi, S.G. Pavlostathis, A. Rozzi, W.T.M. Sanders, H. Siegrist, V.A. Vavilin, The IWA Anaerobic Digestion Model No 1 (ADM1), Water Sci. Technol. 45 (2002) 65-73 DOI: 10.2166/wst.2002.0292 %J Water Science and Technology.

[45] M. Lebiocka, A. Montusiewicz, A. Cydzik-Kwiatkowska, Effect of bioaugmentation on biogas yields and kinetics in anaerobic digestion of sewage sludge, Int. J. Environ. Res. Public Health 15 (8) (2018) 1717, https://doi.org/10.3390/ijerph15081717.

[46] Y.-L. Qiu, S. Hanada, A. Ohashi, H. Harada, Y. Kamagata, Y. Sekiguchi, Anaerobe capable of degrading phenol to acetate in obligate syntrophic associations with a hydrogenotrophic methanogen, Appl. Environ. Microbiol. 74 (2008) 2051–2058, https://doi.org/10.1128/AEM.02378-07.

[47] A. Tschech, B. Schink, Fermentative degradation of resorcinol and resorcylic acids, Arch. Microbiol. 143 (1) (1985) 52–59.

[48] O. Franchi, P. Bovio, E. Ortega-Martínez, F. Rosenkranz, R. Chamy, Active and total microbial community dynamics and the role of functional genes bamA and mcrA during anaerobic digestion of phenol and p-cresol, Bioresour. Technol. 264 (2018) 290–297, https://doi.org/10.1016/j.biortech.2018.05.060.

[49] J.D. Munoz Sierra, W. Wang, D. Cerqueda-Garcia, M.J. Oosterkamp, H. Spanjers, J. B. van Lier, Temperature susceptibility of a mesophilic anaerobic membrane bioreactor treating saline phenol-containing wastewater, Chemosphere 213 (2018) 92-102 DOI: 10.1016/j.chemosphere.2018.09.023.

[50] H. Dahle, N.-K. Birkeland, Thermovirga lienii gen. nov., sp. nov., a novel moderately thermophilic, anaerobic, amino-acid-degrading bacterium isolated

from a North Sea oil well, Int. J. Syst. Evol. Microbiol. 56 (2006) 1539–1545, https://doi.org/10.1099/ijs.0.63894-0.

[51] R. Rabus, T.A. Hansen, F. Widdel, Dissimilatory Sulfate- and Sulfur-Reducing Prokaryotes, in: M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, E. Stackebrandt (Eds.) The Prokaryotes: Volume 2: Ecophysiology and Biochemistry, Springer New York, New York, NY, 2006, pp. 659-768 DOI: 10.1007/0-387-30742-7_22.

[52] D. Suzuki, Z. Li, X. Cui, C. Zhang, A. Katayama, Reclassification of Desulfobacterium anilini as Desulfatiglans anilini comb. nov. within Desulfatiglans gen. nov., and description of a 4-chlorophenol-degrading sulfate-reducing bacterium, Desulfatiglans parachlorophenolica sp. nov, Int. J. Syst. Evol. Microbiol. 64 (2014) 3081–3086, https://doi.org/10.1099/ijs.0.064360-0.

[53] L.M. Jochum, L. Schreiber, I.P.G. Marshall, B.B. Jørgensen, A. Schramm, K. U. Kjeldsen, Single-cell genomics reveals a diverse metabolic potential of uncultivated desulfatiglans-related deltaproteobacteria widely distributed in marine sediment, Front. Microbiol. 9 (2018), https://doi.org/10.3389/fmicb.2018.0203810.3389/fmicb.2018.02038.s00110.3389/fmicb.2018.02038.s002.

[54] S. Sakai, M. Ehara, I.C. Tseng, T. Yamaguchi, S.L. Bräuer, H. Cadillo-Quiroz, S. H. Zinder, H. Imachi, Methanolinea mesophila sp. nov., a hydrogenotrophic methanogen isolated from rice field soil, and proposal of the archaeal family Methanoregulaceae fam. nov. within the order Methanomicrobiales, Int. J. Syst. Evol. Microbiol. 62 (2012) 1389–1395, https://doi.org/10.1099/ijs.0.035048-0.