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Sustainable bioremediation of antibacterials, metals and pathogenic DNA in water

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ABSTRACT

The global antibacterial resistance requires urgent attention from different fields of engineering. Here, several unit operations were assessed in a novel water treatment train capable of remediating antibacterials, metals and pathogenic DNA to generate clean water. The analyses used ^{14}C-respirometry, spectrometry, and a set of molecular analyses. Multiresistant bacteria hold antibacterial resistance genes (ARGs), which were harnessed for bioremediation of pollutant mixtures. Treatment efficiencies were 25–71% for 8-days with aerobic Cr(VI) reduction and removal of Cd and Pb; and 34.8% erythromycin (ERY) was biodegraded aerobically in 20 days. The anaerobic digestion (AD) bioremediated 65–73% mixed antibacterials ERY and sulfamethoxazol (SMX) in 60 days. However, high concentrations of mixed antibacterials induced inhibition of bacteria and methanogens and higher diversity of ARGs. ARGs were eliminated at 60 °C and 5.8 kPa for 10 min. The suggested coupling sequence of operations was metal, then antibacterial aerobic bioremediation, AD (yielding biomethane as energy source), recirculation of ARGs *in situ*, and thermo-pressure pathogenic DNA degradation.

1. Introduction

Failure of antibacterials to cut short bacterial infections is already a prominent global health issue (The Lancet, 2015). The issue is complicated by multiresistance, the ability of bacteria to survive or thrive in the presence of different pollutants, owing to ARG transmission. These mechanisms have been described in several studies; transference of ARGs has been widely documented and is probably the chief mechanism for resistance gene dispersion (Perry et al., 2014). Multiresistance expresses the resistance of bacteria to compounds as different as aromatic-ring pollutants (antibacterials, hydrocarbons) and metals.

Of particular concern are the most extensively used antibacterials. These are often veterinary drugs also listed as essential human medicines, including sulfamethazine (SMZ) and sulfamethoxazole (SMX), and natural bacteriostatics like tetracycline (TET), erythromycin (ERY), or chloramphenicol (CHLO) (World Health Organization, 2013). SMZ, SMX, TET, ERY and CHLO are persistent in the environment due to their aromatic-ring structure, which makes them difficult to degrade. As to metals, they act as gene transcription inducers, increasing expression of regulatory genes associated with antibiotic resistance (Aydin et al., 2015b).

A set of human technologies are hotspots and mixing vessels of pollutants, which force bacteria to maintain ARGs in water (Fig. 1). ARGs can be dispersed between sylvatic reservoirs (Arnold et al., 2016), domestic animals, cultivated vegetables, and human populations, owing to water runoff and roads. Engine fuels and industries contribute metals (Islas-Espinoza and de las Heras, 2015) which washout from the atmosphere or are transported by water. Fuel (aromatic) hydrocarbons, aromatic antibacterials and some metals seem to be resisted by the same bacterial species (Máthé et al., 2012).

Technologies responsible for outstanding gains in human survival are now being implicated in the multiresistance health crisis: sewerage-sanitation, antibiotics, animal husbandry and crop production (Ferriman, 2007; Laxminarayan et al., 2013; Roca et al., 2015). Wastewater treatment plants (WWTPs) are a chief release point of antibiotics and ARGs (Giannakis et al., 2016; Michael et al., 2013). Humans and domestic animals are amplifying hosts for a pathogen, are often co-infected by several pathogens, and add to waterborne ARG transfers (De las Heras et al., 2016). When WWTPs or ranch releases meet drinking water sources, or are reused in crop irrigation, the fecal-oral infection cycle is closed.

New technologies are not devoid of a role in ARG retention by bacteria: SMX is now used as anticonvulsant, anti-diabetic and diuretic (Reis et al., 2014). Food preservation, ethanol production, horticulture, aquaculture and apiculture are expanding the point sources of antibacterial or ARG pollution (Meek et al., 2015). Antimicrobial nanoparticles in textiles, toys and food packaging are propagating metals (Ma et al., 2016).

Removal of antibiotics by chlorination has been explored (Michael et al., 2013), but chlorination and currently used potabilization compounds and processes generate disinfection byproducts (DBPs) which are human carcinogens. Alternatives to chlorination

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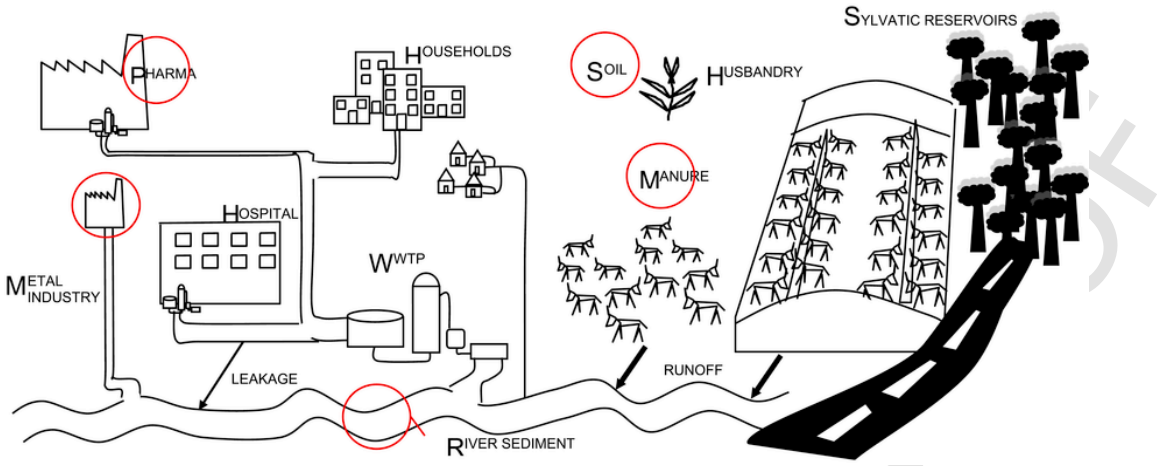

Fig. 1. The anthropogenic multiresistance cycle. Humans disperse antibiotics and pollutants that maintain the need for ARGs in bacteria. The circles denote the point sources where aromatic-ring antibacterials and metals were sampled for this study; pharma wastewater was artificially prepared.

such as ozone, UV or bromide/iodide compounds may still release many toxic DBPs (Richardson et al., 2007). Moreover, DBPs might enhance cross-resistance to antibiotics (Ma et al., 2016). Contrariwise, thermal disinfection appears to be DBP-free (Loo et al., 2012).

An additional problem is that ARGs are more difficult to destroy than pathogens (Giannakis et al., 2016). ARGs are found in plasmids (as well as integrons and transposons) which commonly exist in water and facilitate the transmission of ARGs (Karni et al., 2013). Antibacterial, Metal and ARGs (AMA) pollution should be treated *in situ* (by industries, hospitals and households) to separate AMA pollutants, unlike WWTP that mix and disperse pollutants.

The hypothesis is that a combination of natural bacterial degradation capabilities can fulfill the depuration functions in wastewaters polluted with antibacterials, metals and DNA (ARGs and potential pathogens). We aimed at measuring the efficiency of unit operations in a treatment train capable of remediating water polluted with antibacterials, metals and DNA.

2. Materials and methods

Here, four unit operations (Fig. 2) were tested individually, a common practice to assess in the laboratory the viability of coupling and scaling them up in a prototype. In particular, efficiency and rate of biodegradation, initial pollutant concentration, and volumetric flows were obtained to determine a better sequence of operations and size of the apparatus.

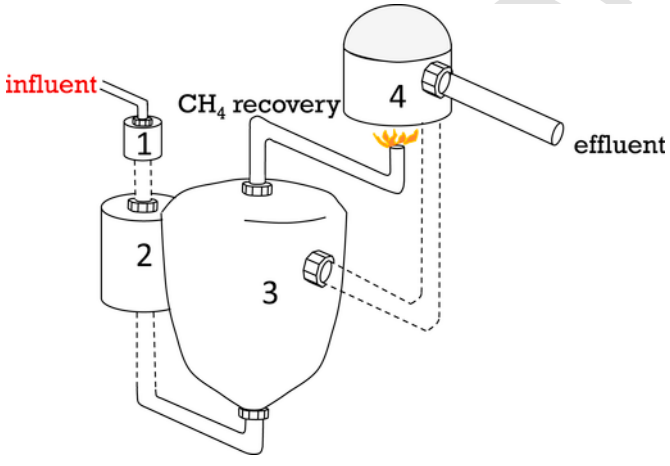

Fig. 2. AMA wastewater treatment train, conceptual design. 1–2: Aerobic Metal and Antibacterial biofilters. 3: Anaerobic Antibacterial and Metal digester. 4: Thermo-pressure DNA Degradation using AD CH_4 as fuel.

2.1. Aerobic treatment of antibacterial pollution

The faster catabolism of aerobic antibacterial-resistant bacteria was used to mitigate antibacterial peaks that jeopardize optimal operation of AD. Aerobic catabolic activity of the aromatic-ring antibacterials ^{14}C-labeled Sulfamethazine (SMZ) and ^{14}C-labeled Erythromycin (ERY) was determined by respirometry assays (Islas-Espinoza et al., 2012); the bacteria were isolated from soil and/or manure from pigs treated with antibacterials. A clone library was built to identify the soil-manure bacterial communities from which the most able antibacterial-degraders were determined and isolated. Bacterial 16S rRNA genes from isolated bacteria were amplified by PCR for identification, and known bacterial pathogens were excluded. The antibacterial doses to be treated were based on minimum inhibitory concentrations (MICs) as well as bacterial growth measured by optical density spectrophotometry (OD_{600}). Four bacteria that showed multidrug resistance (ERY, SMZ, Chloramphenicol (CHLO), Tetracycline (TET) and Kanamycin (KAN)), high MICs and grew in presence of the antibacterials, were selected and pooled into a consortium.

The effect of cometabolism (i.e. the use in the culture medium of a cosubstrate providing complementary mineral, vitamin or carbon source) was ascertained. Cometabolism was used to initiate the degradation, because to break the aromatic-ring structure of the antibacterials high energy levels are required. They were provided by an available carbon source (yeast extract) different from the recalcitrant carbons of the aromatic-ring (Islas-Espinoza et al., 2012).

Isolated bacteria were incubated and agitated at 150 rpm (digital oscillator, Thermolyne, USA). MICs of antibiotics in bacteria were determined by optical density (OD_{600}) on a (Bio-Rad SmartSpec Plus, USA) spectrophotometer. The equipment used to measure bacterial $^{14}CO_2$ respiration was a (Canberra Packard Tri-carb 2250CA, USA) liquid scintillation counter. More information on the process parameters can be found in the online Supplementary Material.

2.2. Aerobic treatment of metal pollution

Bioremediation of metal mixtures in chromium-plating wastewater prior to anaerobic digestion (AD) was conducted with the bacteria *Brevundimonas diminuta*, which was isolated from a Cr-plating workshop. Bioreduction of CrVI to CrIII was assessed as well as biotransformations of Pb, Cd and the mixture of CrVI, Pb and Cd. Total Cr, Cd and Pb were determined using atomic absorption, and UV–Vis spectrophotometry for CrVI. Because Pb is only soluble in water at acidic pH, its remediation was probed at pH = 2. Cr and Cd were probed at pH 2 and 6. Growth of the bacteria in the presence of metals was measured via optical density spectrophotometry (OD_{600}). Cellular sorption or accumulation of CrVI, Pb and Cd in the bacteria were ascertained via freezing-thawing thermal shock to induce cell lysis, release and resuspend the metals in water.

The equipment used to determine total Cr, Cd and Pb was a (Varian SpectrAA-600, USA) atomic absorption spectrophotometer. To conduct CrVI reduction measurements, a (Cary 1E UV–Vis, Agilent Technologies, Inc., Australia) spectrophotometer was used at $\lambda = 543$ nm. Growth of isolates in presence of metals was measured via OD_{600} using a (Hach DR 2800, USA) spectrophotometer.

2.3. Anaerobic treatment of antibacterial pollution

Three anaerobic sequencing batch reactors (ASBRs) were inoculated with anaerobic granular sludge. A synthetic substrate (see Startup and operation in the online Supplementary Material) was used to resemble wastewater from a pharmaceutical industry. After acclimation, SMX and ERY were spiked at increasing concentrations in the influent until CH_4 production collapsed (irreversibly initiated decline until total absence of this gas), at which point antibacterial spiking stopped to observe possible recovery. The control ASBR was fed synthetic wastewater, another ASBR was spiked with SMX (50 mg L^{-1} on days 1 and 31 and 100 mg L^{-1} on days 61 and 91) and the other ASBR with ERY+SMX (25 + 25 mg L^{-1} on days 1 and 31 and 50 + 50 mg L^{-1} on days 61 and 91).

During anaerobiosis, mRNA copies of genes encoding formyltetrahydrofolate synthetase (FTHFS), methyl-coenzyme M reductase (mcrA) and acetyl-coA synthetase (ACAS) were targeted to measure enzymatic activity inhibition in homoacetogens, methanogens and acetoclastic methanogens, respectively. Homoacetogenic bacteria use H_2 and CO_2 and oxidize volatile fatty acids to acetate (Town et al., 2014), while acetoclastic methanogenic archaea only use acetate and are responsible for 70% of the CH_4 produced.

Total RNA was extracted and cDNAs were synthesized from isolated RNAs by reverse transcription PCR (RT-PCR). Biogas production and volatile fatty acids (VFA) were measured by gas chromatography. Biodiversity changes were evaluated by sequencing the whole 16S rRNA using genomic DNA. Determination of the active microbial community used synthesized cDNA templates and quantitative real-time PCR targeting bacteria, archaea and methanogens. Abundances of ERY (*ere*A, *ere*B, *erm*B, *erm*F, *erm*X, *mef*A, *erm*A), sulfonamide (*sul*1, *sul*2, *sul*3) and *mex*B, *mdt*F resistance genes were quantified with qPCR from the effluent and sludge of the reactors using Illumina sequencing (Aydin et al., 2015b). SMX and ERY degradation was measured using HPLC with a UV detector (Aydin et al., 2015a).

Daily biogas production was measured using a (Ritter Digital Counter, USA) milligas counter. Methane content in biogas was measured using a (Perichrom, France) gas chromatograph. VFA concentrations were measured using gas chromatography with a flame ionization detector (Agilent Technologies 6890N, USA) and an Elite-FFAP column (30 m × 0.32 mm). The ERY, SMX and TET assays were performed using HPLC (Shimadzu LC-10 AD, Japan) equipped with a (SPD 10-A) UV–Vis Detector, and a C18 analytical column.

Genomic DNAs were extracted using PureLink Genomic DNA Kits (Invitrogen, UK). Extracted gDNA concentrations were determined using a Qubit 2.0 Fluorometer (Invitrogen, UK). Absolute quantification analysis of cDNA was carried out with a LightCycler 480 (Roche Applied Science, Switzerland). PCR products were sequenced using an ABI prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit on an ABI Prism 377 DNA sequencer (Applied Biosystems, USA). Purified PCR products were used as calibration standards for real time PCR quantification after their DNA concentrations were determined by fluorometry (Qubit, Invitrogen, USA).

2.4. Thermo-pressure degradation of DNA

Thermal DNA degradation under dry conditions occurs above 190 °C, while complete DNA degradation occurs at 100–110 °C when water temperature and pressure are combined (Karni et al., 2013). Temperature and pressure conditions can be obtained more sustainably by combustion of biomethane obtained from AD treatment of wastewater.

Escherichia coli DNA was extracted with an UltraClean Water DNA kit (Mo Bio Laboratories, Inc.). Tick and chicken kidney DNA were extracted by phenol-chloroform extraction and ethanol precipitation (Girish et al., 2005). About 0.75 μg DNA was used for each reaction (n = 3).

Low-iron borosilicate glass tubes (20 mL, Kimax) were used to heat the DNA contained in (1 mL) distilled water, and closed with rubber caps. Temperatures tested were 20, 40, 60, 80, 90, 100 and 150 °C and pressures were observed in the 1.4–16 kPa gauge pressure range. Additionally, *E. coli* colonies (resistant to 100 mg L^{-1} ERY, SMX and CHLO, separately) were tested for inactivation in the same tubes (n = 3). Temperatures were controlled with a digital sensor coupled to a hot plate (MS400 Magnetic Stirrer, Bante Instruments, China). The tubes were placed on a pan with sand to keep them in place, and heated on the hot plate, covered with aluminium foil. Pressure was measured with a data logger equipped with a needle (Almemo 2690-8, Ahlborn, Germany).

The breaking of the covalent bonds of DNA was determined by gel electrophoresis using 1% agarose, for 60 min at 70 V. Staining was done with ethidium bromide (Invitrogen, UK) and DNA was visualized under UV light (Karni et al., 2013).

3. Results

3.1. Aerobic antibacterial and metal bioremediation

Bacterial growth simultaneous with biodegradation of antibacterials or metals demonstrated the possibility of sustained biodegradation (Figs. 3 and 4), which was enhanced by yeast extract addition (Supplementary Material Tables 6 and 7).

ERY was used as carbon source by the bacteria: they produced $^{14}CO_2$ while ^{14}C-ERY concentrations decreased. Exposure to ^{14}C-ERY in water (750 mg L^{-1}) led to 34.8% biodegradation by an aerobic consortium of four bacteria in a 20-day assay (Fig. 3). Bacteria of the consortium adapted differently to antibacterials: *A. defluvium* and *P. putida* grew better with CHLO, *B. licheniformis* and *Alcaligenes* sp. with TET or ERY (all differences $p < 0.05$) (GenBank accession numbers in Supplementary Material Table 5). Higher biodiversity en-

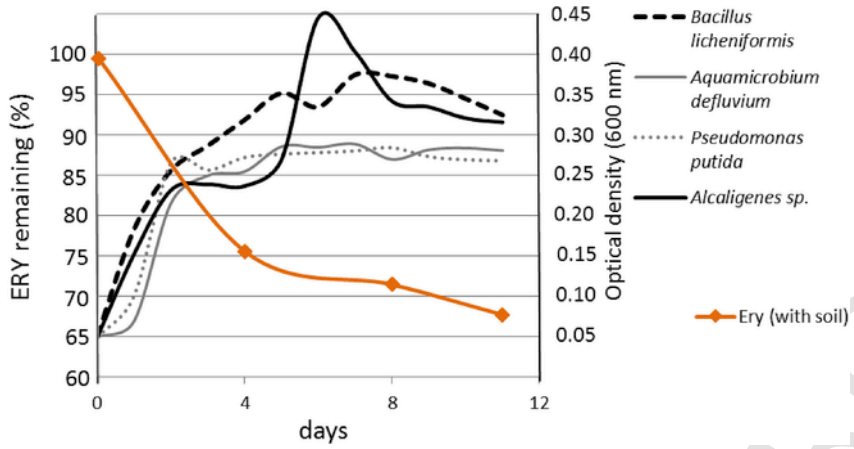

Fig. 3. Erythromycin aerobic bioremediation by a consortium which grew in presence of the antimicrobial and used it as carbon source.

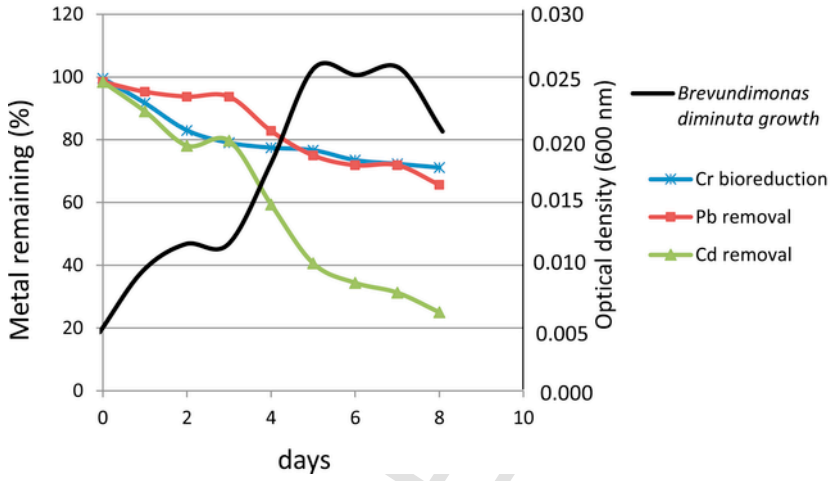

Fig. 4. Metal mixture bioremediation by aerobic metal-resistant bacteria. *B. diminuta* grew in presence of and remediated the mixture.

hanced antimicrobial biodegradation (Supplementary Material Table 6).

Toxic CrVI was reduced to the micronutrient CrIII while Cd^{2+} and Pb^{2+} concentrations decreased from the water, as shown by atomic absorption following 8 days of bioremediation (Fig. 4). Bacteria grew in water at pH = 2 with mixed metals. In general, *Brevundimonas diminuta* reduced more CrVI and accumulated less CrIII at lower pH and in the metal mixture (Supplementary Material Tables 8–10). Metal reduction and removal efficiencies were 25–71% in 8-days assays (CrVI: 255, Cd: 0.65, and Pb: 0.65 mg L^{-1}, respectively).

3.2. High doses and mixtures of antibacterials curtail methanogenesis

The anaerobic reactors monthly degraded 73% SMX and 65% ERY (after a 25:25 mg L^{-1} SMX:ERY mixture was applied twice), for 60 days before CH_4 production waned. They subsequently degraded 39% SMX and 24% ERY (after 50:50 mg L^{-1} SMX:ERY applied twice) (Fig. 5). These data confirmed the ability of AD to biodegrade antibacterials but waning called for pre-treatments to reduce chronic and acute exposure to mixed antibacterials.

In presence of ERY+SMX, COD biodegradation stabilized in a few days (Fig. 5), but a second (low) dose of ERY+SMX drove the reactor to a lower COD degradation efficiency, after a 20-day lag.

Early on, CH_4 production in the SMX+ERY reactor initiated a slow descent by stages, indicative of independent water bioremediation and gas production processes. CH_4 production collapse followed a gradual pH decrease suggestive of inhibition of volatile fatty acid utilization pathways at higher SMX+ERY concentrations, particularly acetic acid (by the SMX and SMX+ERY reactors) and propionic or butyric acids (by SMX and SMX+ERY reactors, respectively) (Supplementary Material Fig. 1).

Methanogens decreased 100 times compared to a 10 thousand-fold decrease of bacteria in general (Supplementary Material Fig. 2). Concurrently, ACAS expression (by acetoclastic methanogens) resisted more (slower and shallower inhibition) than the other metabolic enzymes (Supplementary Material Fig. 2). Nevertheless, a second ERY+SMX low dose simultaneously tipped all enzymatic gene expressions and microbial taxa downwards. Simultaneous onset and decline of FTHFS and mcrA probably occurred because the former is expressed at an earlier step of AD and feeds methanogenesis. In turn, depressed FTHFS (just before CH_4 production demise) may have obeyed pH decrease and VFA accumulation (Supplementary Material Figs. 1 and 2).

As to ARGs, their abundance increased in long term AD operation, and the mixed antibacterials had a synergistic effect increasing ARG diversity and changing the dominance from SMX to ERY resistance genes (Fig. 5). Abundance of SMX resistance genes was

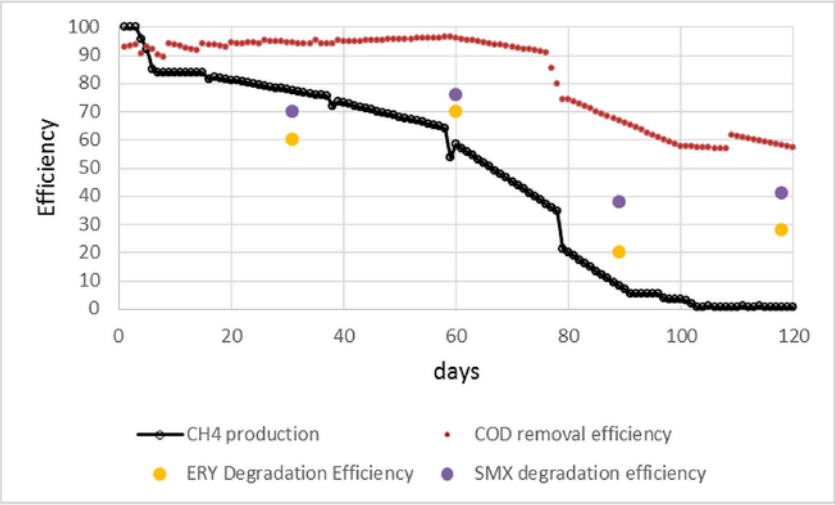

Fig. 5. Organic matter (COD) degradation, antibacterial multidegradation efficiencies, and CH_4 production index [day 1 = 100] in the ERY+SMX anaerobic reactor.

sul1>sul2>sul3 in effluent wastewater and sludge of the SMX reactor. ERY resistance genes were more diverse with *ereA>ereB>ermB>ermX>ermF>mefA>ermA* abundances in the SMX+ERY reactor (Fig. 6).

In the case of the lower dose of a single antibacterial, anaerobic biodegradation of organic matter was a robust process: 94–98% COD (2500 mg L^{-1} initial concentration) was degraded in the 60 days following SMX spiking, and organic matter was feedstock for biomethane production. Metabolic enzyme gene expression was also several orders of magnitude higher than in the mix.

3.3. Thermo-pressure degradation of DNA

The DNA from *E. coli*, chicken, and tick showed that complete thermo-pressure degradation of DNA in water was effective after 10 min at 60 °C and 5868 Pa gauge pressure (Fig. 7). No *E. coli* colony grew above this temperature and pressure in the tubes, nor grew in solid medium.

3.4. Sequence of operations

Although same orders of magnitude were achieved in aerobic and anaerobic antibacterial biodegradation rates (Table 1), initial concentrations in aerobic concentrations were one order of magnitude higher, justifying them as pre-treatments to the AD. Acclimation of the biomass was in the order of 100 days prior to any of the bioremediation unit operations. This strongly suggested recirculation of ARGs in the AMA treatment train.

Since the metal treatment was faster, it should be the first operation. Bioremediation-capable biomass, pollutant concentration and contact time determine this operation's size. It should be followed by a larger aerobic antibacterial treatment. Size could be traded for a set of parallel antibacterial biofilters. As to the anaerobic digester, it allowed for water bioremediation and CH_4 production. Finally, a fast CH_4-fed DNA-elimination and pathogen-disinfection would be able to treat any effluent volumetric flow from the other operations.

4. Discussion

This study analyzed the bioremediation mechanisms underlying "improved water and sanitation" technologies as suggested by the Global Antibiotic Resistance Partnership's Strategy number 1 on Sustainable antibacterial use (The Center for Disease Dynamics Economics and Policy - Global Antibiotic Resistance Partnership, 2015). The study used similar antibacterial concentrations to pharmaceutical and other industrial wastewaters which are in the 100–500 mg L^{-1} range (Aydin, 2016).

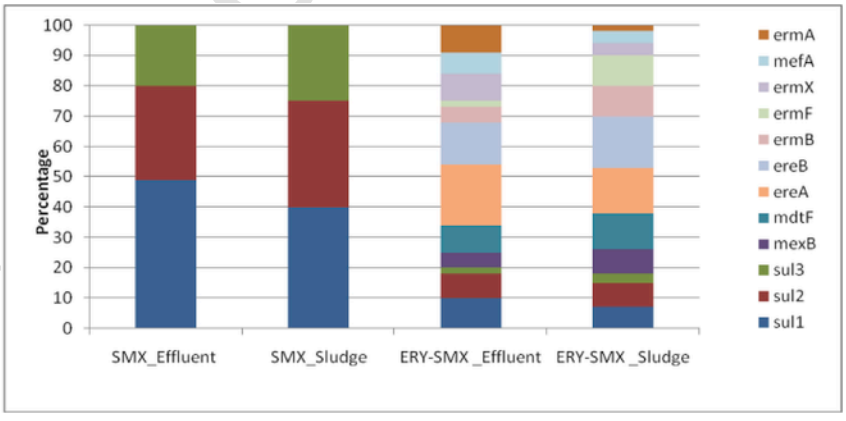

Fig. 6. Distribution of antibacterial resistance genes in the SMX and ERY+SMX anaerobic reactors.

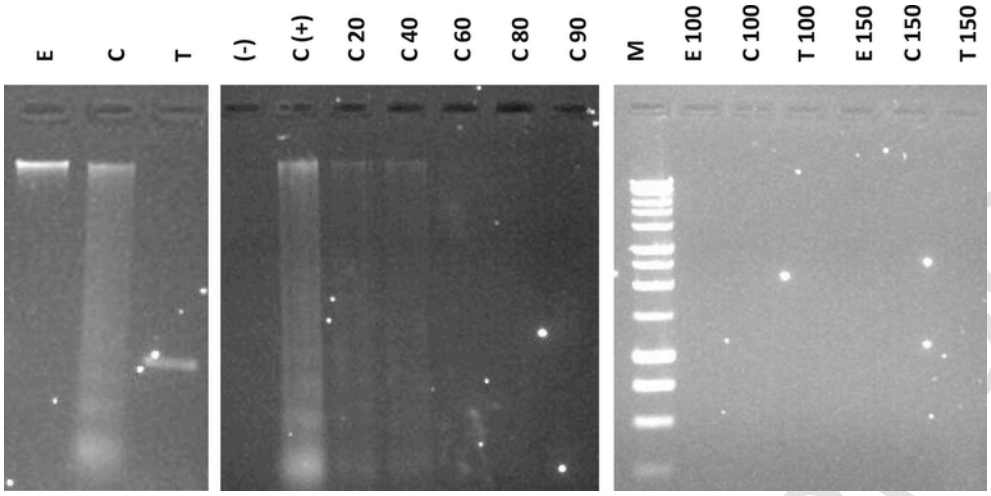

Fig. 7. Gel images of DNA. E: *E. coli*, C: chicken, T: tick, (−): Negative control, (+): Positive control, and M: Molecular weight marker (1000 kb), following 20, 40, 60, 80, 90, 100 and 150 °C, for 10 min.

Table 1
Pollutant removal rates.

Treatment	Initial concentration (mg/L)		Treatment duration (days)	Pollutant removal (%)	Removal rate (%/day)
^{14}C-ERY	750		20	34.77	1.74
^{14}C-SMZ	100		20	7.83	0.39
CHLO	428		10	31.00	3.10
TET	36		10	20.00	2.00
Pb + Cd + CrVI mixture	Pb	0.65	8	32.81	4.10
	Cd	0.65		71.88	8.99
	CrVI	255		25.28	3.16
ERY + SMX mixture	ERY	day 1 + day 31 25 + 25	60	65.00	1.08
	SMX	25 + 25		73.00	1.22
DNA degradation	0.75 µg		1/6	100.00	600.00

As regards aerobic pre-treatments of antibacterials, literature is still scarce, but SMX at initial concentration of 100 mg L^{-1} was biodegraded aerobically in 8 days (Jiang et al., 2014), similar to 10–20 days here for several antibacterials. SMX 84–94% biodegradation at 16 h with 50 mg L^{-1} initial concentration was enabled by vitamin cometabolism (Zhang et al., 2016). SMX removal at 48 h by a consortium including *Alcaligenes faecalis* (also used here), and vitamins cometabolism, outperformed single species. SMX was biodegraded rather than adsorbed by the cell (Li et al., 2016), similar to what occurred here.

Biodegraders found here were also reported elsewhere: *Pseudomonas putida* reduced CrVI in a pollutant mix, using MSM cometabolism (Mahmood et al., 2013). *Pseudomonas putida* 5-day ±80% biodegradation from 10 mg ERY L^{-1} initial concentration, using yeast extract (as done here), outperformed other treatments (Gao et al., 2015). *Microbacterium* bioremediated antibacterials (Supplementary Material Table 4) and metals (Supplementary Material Table 8). *Microbacterium* with previous exposure (as done here) helps accelerate biodegradation of SMZ and a tetracycline in soil (Topp et al., 2013). *Pseudomonas* and *Microbacterium* seem capable of bioremediation in a variety of environments. Multiresistance acquisition required acclimation time, so recirculation of ARGs in the treatment train is recommended.

As regards the anaerobic treatment, sustainability hinges on it: wastewater treatments are now required to be more energy-efficient (Christgen et al., 2015), or generate useful byproducts. AD generates biomethane, usable as fuel rather than dispersed in the atmosphere. The combustion of CH_4 produces one molecule of CO_2 (as does respiration), unlike other hydrocarbons (e.g. fossil fuels). AD operation costs are routinely cut by CH_4 recovery, and using waste feedstocks. Worthy of notice is the CH_4 production potential using agricultural waste and the mixed microbiome of different domestic animals (Islas-Espinoza et al., 2017). This water reclamation train may in future recover metal during AD-acidogenesis (Meulepas et al., 2015), residues for green chemistry (such as CO_2 from both AD and CH_4 combustion), and sludge as soil amender.

The AD used synthetic pharma wastewater. It differed from the widely varied mixtures of process compounds and sanitizing agents found in the pharma industry discharges; these make any wastewater characterization and treatment difficult, and any one study difficult to extrapolate. But industrial processes are often standardized which allows for specific wastewater pollutants to be targeted.

As to the thermo-pressure post-treatment, it appears justified by ARG-pathogens co-occurrence in AD, relative AD inefficiency in ARG removal compared to pathogen removal (Ju et al., 2016), and by the 27% ARG subtypes still detected in effluents of sulfonamide and CHLO anaerobic-aerobic coupled treatments (Christgen et al., 2015).

The exposure of DNA to temperature and pressure affected irreversibly the DNA structure of different organisms (Bacteria and Eukarya). Since ARGs are encoded in genomic or plasmid-DNA, the complete degradation of DNA destroyed ARGs effectively. It must be noted that polyextremophile archaea can survive up to 106 °C and 70 MPa, so further research on ARG elimination might be required.

Large networked sewerage and centralized wastewater treatments currently act as mixing vessels of pollutants and magnifying vessels of ARGs. The pharma and metal industries, hospitals, husbandry operations, wastewater treatment plants, but also groups of households, should consider adapting AMA operations to their wastewater installations.

4.1. Techno-economic viability and scaling up potential to commercial practice

The treatment train considered here would use mass-produced fluid containers and appurtenances worth 2000 US dollars and ex-

pected to work for 15 years (0.37$/m^3). Alongside a liquid nitrogen container used as bacterial strain reservoir (0.033$/m^3 per plant, intended to hold a reserve for ten 1 m^3/day plants), an electrophoresis chamber and UV camera for DNA analyses (0.115$/m^3) to monitor disinfection, and replacement biofilters ($0.044/m^3), the capital cost would be 0.562$/m^3.

Operation costs would include firstly, maintenance labor of all unit operations including unclogging pre-treatment filters (8.6$/m^3), weekly monitoring of metals in the influent and effluent, especially while the biofilters initiate, with absorption and UV–Vis spectrophotometry ($2.13/m^3 including reagents). Similar antibacterial monitoring with HPLC ($31.43/m^3) and DNA disinfection monitoring (reagents: $0.71/m^3) would complete the monitoring process. Disinfection electricity in a lossless world (perfectly isolated vessel) would amount to 3.73$/m^3, but more realistically, a 30% efficiency would mean 12.43$/m^3. The total costs would be 55.86$/m^3 (1% capital and 99% operation).

Lower costs would stem from lessening HPLC antibacterial-biodegradation monitoring costs; this may include sporadic monitoring allowed by stable production processes, and avoiding mixtures of wastewaters. Monitoring improvements could also correct for underestimates of antimicrobial degradation (Polesel et al., 2016). But wise use of sustainable energy sources should be a chief concern too. This includes use of gravity rather than pumps; *in situ* treatments are more adapted to the use of gravity. Reuse of heat waste from biomethane combustion can be used to heat the AD. This would further avert CO_2 emissions. The system's resilience to intermittent availability of energy could also improve via solar thermal/biomethane hybridization of disinfection. And, since solid waste is a vast biomethane resource (Islas-Espinoza et al., 2017) as well as a reservoir of ARGs and potential pathogens, additional use of solid waste feed for biomethane is warranted. Finally, small-size and modularity make for easier upgradings.

In the absence of alternative coupled treatments for antibacterial, metal and genetic pollution, comparison hinges on several unit operations: Assuming that wastewaters are similar, the cost of metal treatments varies from 1.19 to 1.81 $/m^3 (electrocoagulation of metal compounds with Al and Fe electrodes, respectively) (Demirbas and Kobya, 2017) to 31.31$/m^3 (flocculation and chemical treatment of CrVI) (Boer and Blaga, 2016). Much more difficult to come by are costs of biological treatments of antibacterials and metals. Disinfection ranges 0.02–0.14 $/m^3 (thermal pasteurization and high pressure processing, respectively, with intermediate costs for pulse electric fields). Capital costs range from 5% to 26% of total costs (thermal pasteurization and pulse electric fields, respectively) (Sampedro et al., 2014). As to generalist treatments, such as ceramic and polymer membranes, their total costs are 1087.82 and 934.02 $/m^3, respectively, with capital shares of the cost higher than 99.9% (Park et al., 2014).

Techno-economic assessments should also factor in irredeemable drawbacks in several existing technologies. For lack of space, only general criteria are outlined here: Firstly, conventional treatments in wastewater plants both contribute ARG spread (Pruden et al., 2013) and are ineffectual in removing compounds like antibacterials (Homem and Santos, 2011). Secondly, sludge-producing treatments have low efficiencies in degrading pharmaceuticals (Ahmed et al., 2017) and produce vast quantities of biosolids that spread ARGs (Pruden et al., 2013); pyrolysis of biosolids will remain expensive (Marousek et al., 2017) and may release toxic polycyclic aromatic hydrocarbons, depending on temperature and oxygen content (Islas-Espinoza and de las Heras, 2015). Thirdly, membranes greatly enrich the xenobiotic content of the obtained concentrate (Dainelli et al., 2017; Prasse et al., 2015). Fourthly, electrochemical treatments might produce toxic disinfection byproducts (Prasse et al., 2015). Finally, the fate of titanium dioxide used in photocatalysis (Homem and Santos, 2011), and similar hazardous materials, require more information. Contrariwise, progress in biological treatments is expected from combining (an)aerobic treatments targeted to specific pollutants (Falås et al., 2016); combined processes using renewable energy and producing useful byproducts seem most adequate when treating antibiotics (Homem and Santos, 2011). Failing to combine temperature and pressure leads to incomplete disinfection (Sampedro et al., 2014).

5. Conclusions

This was the first proof-of-concept of a sustainable bioremediation train aimed at modifying wastewater technologies implicated in multiresistance. Bacterial resistance was harnessed for aromatic-ring antibacterial and metal bioremediation, water organic pollution, and production of biomethane.

Biodegradation was initiated and enhanced by biodiverse communities. Their growth media can be complemented by a diversity of wastes. And conversely, mixed, chronic, frequent or highly-dosed pollutants inhibit methanogenic gene expression and augment the diversity of ARGs. ARGs must be treated in a controlled environment to avoid dispersion.

The hypothesis set forth in this study seems to have been demonstrated: A combination of bacterial capabilities (found in one species, a selected consortium or a whole community) may be organized in specifically targeted pre-treatments to smooth out pollution peaks in the influent of the treatment. The disinfection post-treatment avoids releases of the pollutants. The sustainability of the system hinges on the use of wastewater organic matter as energy and nutrient source for the biodegraders, and for the production of an energy carrier (CH_4) useful in disinfection.

Biomedical advances such as discovery of new antibacterials, and policies such as reduced antibacterial use, need to be complemented by sustainable technologies that prevent resistance genes, antibacterial and metal accumulations in the environment.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jclepro.2018.02.068.

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