Title: Lifeless *Clostridia* stimulate methanogenesis on Fe^0 in an urban lake corrosive community

Authors: *Paola Andrea Palacios*[1] and *Amelia-Elena Rotaru* [1*]

Affiliation: [1]Nordcee, University of Southern Denmark, Odense, Denmark

* **Correspondence**: arotaru@biology.sdu.dk

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Abstract

Urban environments are webbed with iron-steel structures above and belowground. Underground, in non-sulfidic environments, it has been suggested that interspecies interactions cause Fe^0 corrosion. Particularly, *Methanosarcinales* were assumed to interact syntrophically with acetogenic bacteria during Fe^0 corrosion. Here we challenge this assumption and show that a community of methanogens (38% *Methanosarcinales*) prospers on Fe^0 especially after the demise of the acetogens. Acetogens were mainly represented by *Clostridium* (81% of *Bacteria*). Methanogens were however more diverse including *Methanosarcina* (22% of *Archaea*), *Methanosaeta* (17% of *Archaea*) and *Methanothermobacter* (22% of *Archaea*) as key groups. Surprisingly, acetogens started using electrons from Fe^0 immediately, unchallenged by competing methanogens. Acetogens were expected to be outcompeted by energy efficient methanogens with comparatively lower H_2-uptake thresholds. However, acetogens prevailed, perhaps because in contrast to methanogens they contain [FeFe]-hydrogenases (encoded in the lake-*Clostridia* metagenome). [FeFe]-hydrogenases from *Clostridium* were previously shown effective at retrieving electrons from Fe^0 for proton reduction. When acetogens thrived, methanogenic rates were low (25.2±8 µM/day) but increased appreciably (62.5±5 µM/day) when acetogens collapsed. Acetate could not explain more than a third of the observed methanogenic rates. The remaining methane could be explained by methanogens reducing their energy expenses while exploiting *Clostridium* remains such as readily fixed nitrogen and/or exuded [FeFe]-hydrogenases producing H_2 on Fe^0. This has implications on our understanding of viable interactions between autotrophic species retrieving electrons from Fe^0 or other insoluble electron donors.

Implications

Corrosion damages to underground iron-structures distress both the local environment and the economy. Here we studied an urban lake community corroding Fe^0. The corrosive community was dominated by *Clostridium* and *Methanosarcinales*, which sequentially produced first more acetate and later methane. Here we bring evidence for an unusual interaction mechanism on Fe^0, showing that methanogenesis is enhanced by inactive acetogens. Alone, the *Clostridium*-acetogens discovered in this study, could retrieve electrons from Fe^0 effectively, which makes them a good candidate for electrosynthesis of fossil-fuels' alternatives - a future biotechnological application.

INTRODUCTION

Steel infrastructure extends for billions of kilometers below ground enabling transport and storage of clean water, chemicals, fuels, sewage, but also protection for telecommunication and electricity cables. Deep underground, under anoxic, non-sulfidic conditions, steel was expected to persist unharmed for centuries (1-3). And yet, under such conditions, certain groups of anaerobes (methanogens and acetogens) strip electrons off Fe^0 leading to microbial induced corrosion (MIC) (4-8). Damages induced by MIC in the underground are often discovered too late, leading to environmental and economic devastation. Thus, it is important to be able to predict the lifespan of the material if exposed to microbial communities native to the site where steel structures are located. This would lead to effective replacement strategies and recuperation of the metal prior to accidental spills that may be detrimental to the surrounding environment (1-3).

MIC in non-sulfidic environments is often linked to the presence of acetogens like *Clostridium* and methanogens like *Methanosarcinales* on the surface of the corroded steel structure (4-9). It has been suggested that *Methanosarcinales* were growing in a mutualistic relationship with the acetogens, and allegedly both groups were gaining from the interaction (7, 8). This assumption was based on acetogens producing acetate, which would be then consumed by acetotrophic *Methanosarcinales* methanogens. Concurrently, acetogens were expected to be favored by the removal of their metabolic product - acetate. However, apart from establishing a mutualistic interaction on Fe^0, acetogens and methanogens may be interacting in two other ways: 1) by competing and 2) by establishing a succession to maximize access to electrons from Fe^0 for reduction of CO_2 to acetate (acetogens; reaction 1) or methane (methanogens; reaction 2).

$$4Fe^0 + 2CO_2 + 4HCO_3^- + 4H^+ \rightarrow 4FeCO_3 + CH_3COOH + 2H_2O \quad (\Delta G^{0\prime} = -388 \text{ kJ/mol; Reaction 1})$$

$$4Fe^0 + CO_2 + 4HCO_3^- + 4H^+ \rightarrow 4FeCO_3 + CH_4 + 2H_2O \quad (\Delta G^{0\prime} = -446 \text{ kJ/mol; Reaction 2})$$

Theoretically, under standard thermodynamic conditions, methanogens should have an advantage over acetogens when provided with Fe^0 as sole electron donor (Reactions 1 & 2). Especially, since methanogens, unlike acetogens, are more effective at retrieving abiotic H_2 (formed on Fe^0) due to their low H_2-uptake thresholds (10, 11). Several groups of methanogens could corrode Fe^0 independent of acetogenic bacteria, including species of *Methanosarcina* (12-14), *Methanobacterium* (14-16) and *Methanococcus* (6, 13, 14, 17). The mechanism by which methanogens corrode Fe^0, has been debated and includes reports which suggest they retrieve abiotic-H_2 off the Fe^0 surface (12), retrieve electrons directly using an unknown electron-uptake mechanism (15, 18) or use extracellular enzymes, which stimulate enzymatic H_2-evolution on the Fe^0-surface (17). The later mechanism was especially relevant for *Methanococcus* species which harbored an unstable

genomic island encoding [NiFe]-hydrogenases and formate dehydrogenases enzymes additional to those already encoded by their standard genome (19).

And yet, oftentimes acetogens dominate corrosive communities, outcompeting methanogens when concentrations of H_2 are high and temperatures are low, presumably due to the higher kinetics (V_{max}) of their hydrogenases (10). Moreover, unlike methanogens, acetogens contain [FeFe]-hydrogenases (20), which could retrieve electrons directly from Fe^0 for proton reduction to H_2 possibly (21-23).

In this study, we were interested to understand the dynamics of acetogens and methanogens in an urban area where pipelines for infrastructure are buried underground. We studied corrosion of Fe^0 by an urban lake community from nearby a construction site, on the island of Fyn, Denmark. Alike our previous findings in a coastal-sea environment (24), the corroding microbial community was dominated by *Clostridium* and *Methanosarcinales*. We used a combination of physiological experiments, process inhibition and whole metagenomic analyses to study the interactions of acetogens and methanogens during Fe^0 corrosion. In contrast to our previous report on a corrosive coastal community (24), the acetogens and methanogens in this lake did not compete for retrieval of electrons from Fe^0, instead methanogens appeared to take advantage of metabolites and enzymes released by lifeless acetogens.

RESULTS AND DISCUSSION

Methanogens, attached to corroded structures, were assumed to be indirectly involved in corrosion of Fe^0 (7, 8). Here we show that methanogens from an urban lake may corrode Fe^0 alone, as effectively as a complex community of acetogens and methanogens together. However, within a complex community methanogenesis was stimulated by deactivated, but abundant co-occurring *Clostridium*. We therefore propose an unusual interaction, where methanogens may take advantage of acetogenic proton-reducing enzymes, which are set free during the collapse of the acetogenic community.

Corrosion under electron acceptor limited conditions

From the anoxic sediments of an urban lake near the university of Southern Denmark (**Fig. 1**), we enriched a methanogenic community for four successive transfers, strictly using Fe^0 as electron donor and CO_2 as sole electron acceptor. Under these conditions, the community was highly corrosive as confirmed by gravimetric and product accumulation analyses. Thus, the community utilized 9.5 ± 0.6 mg Fe^0, and induced 41% more weight loss than cell-free controls (6.7 ± 0.05 mg Fe^0; n=3; p=0.01) (**Fig. 2**). Since CO_2 is the only electron acceptor available, two metabolisms are possible: methane production via CO_2-reductive methanogenesis (reaction 3) and acetate production via acetogenesis (reaction 4).

$CO_2 + 8e^- + 8H^+ \rightarrow CH_4 + 2H_2O$ (reaction 3)

$2CO_2 + 8e^- + 8H^+ \rightarrow CH_3COOH + 2H_2O$ (reaction 4)

We determined if either of these processes, methanogenesis or acetogenesis, could be corrosive in isolation. Bacteria (acetogens) were inhibited with a cocktail of antibiotics (kanamycin and ampicillin), in order to favor only methanogens. Alone, methanogens were as corrosive (9.1±0.4 mg Fe^0) as the whole community (n=3, p=0.35) (**Fig. 2**). Methanogens were inhibited with 2-bromoethanesulfonate (a coenzyme A analogue) in order to favor only acetogens. To our surprise acetogens were significantly more corrosive alone (10.6 ± 0.4 mg Fe^0; n=3; p=0.04) than within a mixed community (**Fig. 2**).

To better understand how methanogens and acetogens corroded Fe^0 we corroborated gravimetric determinations with electron recoveries in metabolic products and functional metagenomics of the corrosive community.

Electron recoveries exceeded estimated recoveries from abiotic H_2

Electron recoveries revealed that this lake corrosive community does not rely on the abiotic H_2 chemically generated at the Fe^0 surface ($2e^- + 2H^+ \rightarrow H_2$). As such, the community produced 3.3 times more methane (3.5 ± 0.1 mM) than expected (1.1±0.2 mM) from abiotic H_2 (**Fig. 3**). Interestingly, within the community, methanogens started slowly (27±5.7 µM/day), not rivaling highly productive acetogens (68±1.8 µM/day) for the first month (**Fig. 3**). At the end of the incubation, acetogenesis ceased, whereas methanogens sped up accomplishing rates of methanogenesis (62.5±5.1 µM/day), twofold above those predicted via acetoclastic methanogenesis (28±7.3 µM/day): $CH_3COOH \rightarrow CO_2 + CH_4$ (**Fig. 3**). Perhaps, methanogens were non-competitively accessing electrons from Fe^0 in the absence of the acetogens, during the last month of incubation. To further address this, we verified the effectiveness of methanogenesis after the eradication of bacteria with the help of antibiotics. Bearing in mind that methanogens were as corrosive as the mixed community (**Fig. 2**), we anticipated this would translate in a highly productive methanogenic community once bacteria were rendered ineffective by antibiotics. To our surprise, methanogens unaccompanied by bacteria produced significantly less methane (1.3±0.1 mM) than within the mixed community (3.5±0.1 mM) (**Fig. 3**). On the other hand, acetogens accumulated significantly more acetate (2.5±0.05 mM), than they did within the mixed community (2.0±0.03 mM) (**Fig. 3**). However, the mixed community showed overall better electron recoveries than the summed electron recoveries of unaccompanied acetogens and methanogens put together (**Fig. 3**). We foresee three different scenarios to why the community was 15% more effective in utilizing Fe^0 as electron donor than adding up the corrosive activities of solitary acetogens and solitary methanogens.

First, the collapse of the acetogens may release useful enzymes for the methanogens. During the last month of incubation, when the acetogenic population collapsed, we hypothesize that dying acetogens released enzymes (e.g. [FeFe]-hydrogenase), which boost H_2-production and consequently H_2-dependent methanogenesis. This supposition is backed by previous reports, which showed that acetogenic [FeFe]-hydrogenases in isolation reduce protons to hydrogen when provided with Fe^0 as electron donor (21-23).

Secondly, the collapse of the acetogens results in methanogens co-feeding on Fe^0 and acetate. Alternatively, when acetogens collapse, acetoclastic methanogens may start utilizing the Fe^0 directly, but can only do so after they were provided with enough acetate for biomass synthesis. This supposition is backed by reports that acetoclastic methanogens retrieve electrons from electrogenic bacteria directly or via conductive particles, while also requiring acetate for incorporation into biomass (25, 26). Moreover, many of the strict H_2-utilizing methanogens, require or are stimulated by acetate (27, 28), likely because it alleviates the need to endogenously produce acetate for biomass synthesis.

Thirdly, the collapse of the acetogens may lead to fixed N_2 accessible to methanogens corroding Fe^0. Methanogens were more effective after the collapse of the acetogenic community, and yet they did require the collapse of the acetogens to achieve the highest methanogenic rates. Some acetogens, including several *Clostridium* species, are effective N_2 fixers (29). N_2 fixation catalyzed by nitrogenase enzymes is an energy demanding process requiring 16 ATPs for one fixed N_2 (30, 31). We hypothesize that the collapse of the acetogens leads to significant release of fixed-dinitrogen, now easily accessible to the methanogens. This supposition was further supported by functional community analyses, which showed a high N_2 fixation capacity within the bacterial community but not the archaea (see below).

Thus, to better understand how acetogens and methanogens interact with each other inducing higher electron recoveries as a community than alone (**Fig. 3**), we examined the functional metagenome of a community residing on Fe^0 for four successive transfers.

Acetogens

Clostridium species (especially 'Lachnoclostridium' *saccharolyticum*) dominated the corrosive community (81.3% of all bacteria; 81.2% of all prokaryotes; **Fig. 4**) according to whole genome sequence analyses. Most *Clostridium* have never been tested for autotrophic acetogenesis including '*L.' sachharolyticum* (32, 33). Nevertheless, we could find the entire Wood Ljungdahl pathway for acetogenesis in its readily available genome (NC_014376.1). Using metagenome analyses we also reconstructed the Wood Ljungdahl pathway almost in its entirety for our corrosive-*Clostridium*, which carried acetogenesis from Fe^0 and CO_2 (**Fig. 5**). This acetogenic pathway has been intensively studied for biotechnological applications such as microbial

158 electrosynthesis, where electrodes are provided as electron donor instead of Fe^0 (34, 35). Our corrosive
159 *Clostridium* was highly effective at generating acetate from Fe^0 at room temperature with rates (ca. 84 µM/day;
160 **Fig. 3**) comparable to those observed for various acetogens incubated at higher temperatures on poised
161 electrodes (36) or Fe^0 (5, 37). Moreover, *Clostridium* acetogens, often associated with corrosion, have been
162 recently reported to corrode Fe^0 as effectively as sulfate reducers (38).

163 In our enrichments on Fe^0, *Clostridium*-acetogens were faster than the methanogens, initiating and completing
164 their activity two months before the methanogens (**Fig. 2**). Acetogens are faster not because the Wood-
165 Ljungdahl pathway is more energy effective than methanogenesis, as acetogenesis generates less ATPs (0.3
166 ATPs/acetate; (39)), than CO_2-reductive methanogenesis (0.5 ATPs/methane; (40)). Instead, these *Clostridium*
167 acetogens may be quicker at accessing electrons from Fe^0 because they contain [FeFe] proton-reducing
168 hydrogenases, which are absent in methanogens (20). For example, the proton-reducing [FeFe] hydrogenases
169 from *C. acetobutylicum* corrodes Fe^0 effectively by drawing electrons for the reaction: $2H^+ + 2e^- \rightarrow H_2$ (21-
170 23). *Clostridial* [FeFe] hydrogenases are operative at H^+-reduction compared to methanogenic [NiFe]-
171 hydrogenases which are rather operative in the opposite direction doing H_2-oxidation, but less effective doing
172 proton reduction (20). In the environmental metagenome of these lake-*Clostridia* we did find [FeFe]-
173 hydrogenases (EC. 1.12.7.2 ferredoxin hydrogenase), indicating they have the genetic potential to effectively
174 carry proton-reduction. However, we still do not understand the exact mechanism how this lake *Clostridium*
175 establishes contact with Fe^0 for successful electron retrieval by the [FeFe]-hydrogenase enzymes.

176 One possibility is that they retrieve electrons (i) directly via membrane-bound proteins as it has been proposed
177 for *Clostridium ljungdahlii* grown on electrodes (34, 35), or (ii) indirectly by exuding exocellular enzymes
178 like hydrogenases or formate dehydrogenases for effective electron uptake for enzymatic H_2 production (17,
179 21-23). However, if enzymes were exocellular they would deliver H_2 non-specifically for both the *Clostridium*-
180 acetogens and hydrogenotrophic methanogens, resulting in competition for enzymatically-released H_2. Then
181 the microorganisms with highest affinity for H_2 would be favored. Generally, *Clostridium* species have a lower
182 H_2-threshold (minimum H_2-concentration for growth) compared to most methanogens (41). Thus, if
183 *Clostridium* releases enzymes extracellularly it would not benefit. We therefore propose that during the first
184 phase *Clostridium* is most likely to directly retrieve electrons possibly using membrane bound [FeFe]-
185 hydrogenases rather than expelling these enzymes extracellularly to be used by their competitors - the
186 methanogens (**Fig. 6a**).

187 **Methanogens**

188 Methanogens were highly diverse comprising both acetoclastic *Methanosarcinales* (ca. 38% of all archaea;
189 including 22% *Methanosarcina* and 16% *Methanosaeta*), as well as hydrogenotrophic groups – mostly

represented by *Methanothermobacter* (ca. 22% of all Archaea) (**Fig. 4**). *Methanothermobacter* species are rarely associated with corroded structures (42) and were shown to be especially corrosive when provided with a partner bacterium (43). On the other hand, *Methanosarcinales* have been often associated with corroded structures (4-9) were capable of utilizing Fe^0 as electron donor in pure cultures (12-14), but assumed to be indirectly involved in corrosion feeding on acetate delivered by acetogens (7, 8). Here we showed that methanogens alone could indeed corrode Fe^0 as effectively as a mixed community of acetogens and methanogens (**Fig. 2**).

During the acetogenic period (**Fig. 6**), when *Clostridium* proliferates on Fe^0 and produces acetate, we expected *Methanosarcinales* to feed on the acetate produced by *Clostridium*. Our data confirmed that during this period acetate explained all the methane produced. Accordingly, when acetogens collapsed, the rate of acetate consumption (40.1 ± 6 µM/day) surpassed that of methanogenesis (25.2 ± 8 µM/day) (**Fig. 3**) likely due to incorporation in the biomass of the methanogens. During this acetogenic period, the survival of hydrogenotrophic groups like *Methanothermobacter* may depend on retrieval of abiotic-H_2 as well as H_2-produced by acetoclastic methanogens (44, 45).

During the methanogenic period (**Fig. 6**), when acetogens collapsed and methanogenic rates rose steeply from ca. 25 µM/day to ca. 63 µM/day, methanogens appear to take advantage of inactivated acetogens. Methanogens alone did not produce methane with the same rates (max. 17 µM/day) as they did together with acetogens (**Fig. 3**). Thus, methanogens appear to require the preceding occurrence of the acetogens. We propose this is due to a combination of factors provided by 'dying' *Clostridia* such as: (i) leaked [FeFe]-proton reducing hydrogenases; (ii) available acetate for methanogenic biomass production; and (iii) readily fixed nitrogen. Our proposition was supported by metagenome analyses, which showed the presence of [FeFe]-ferredoxin hydrogenases in the acetogens, and an abundance of *Firmicutes*-nitrogenase genes (86% of the nifH, within the entire corrosive community), which were far less abundant in the metagenome of methanogens (0.2% nifH from *Methanosarcina*; 0.9% from *Methanosaeta*; and 1.6% in *Methanomicrobia*).

In conclusion, we describe here a novel form of interspecies interaction between acetogenic *Clostridia* and *Methanosarcinales*-methanogens during Fe^0 corrosion. We observed the interaction between acetogens and methanogens was not limited to acetate-transfer and required the collapse of the acetogens for an advantageous succession of the methanogens.

Thus, during Fe^0 corrosion, methanogenesis was stimulated by lysed co-occurring *Clostridium* and we provide support for three different scenarios: (i) the collapse of the acetogens releasing enzymes that boost methanogenesis, (ii) acetate contributing to increased biomass production and (iii) the collapse of the acetogens leading to more fixed N_2 accessible to the methanogens corroding Fe^0. Moreover, when acetogens collapse,

methanogens don't have to compete anymore for electrons from Fe^0. Thus, the interaction is a form of opportunistic scavenging with methanogens prospering due to the demise of the *Clostridium*.

MATERIALS AND METHODS

Sample collection and enrichment culture conditions

Sediment cores were sampled during the month of July 2016 from a small lake located near a construction site on the campus of the University of Southern Denmark (SDU), Odense (**Fig. 1**). The salinity of the lake was 0.6 psu, and gas bubbles (including methane) were continuously released to the water surface while sampling. Sediment cores were sliced in the laboratory, and the depth horizon 15- 20 cm was used for downstream enrichments in a DSM modified 120 media (modifications: 0.6g/L NaCl, without casitone, without sodium acetate, without methanol, and without $Na_2S \times 9H_2O$). The enrichment cultures were prepared in 50 mL blue butyl-rubber-stoppered glass vials with an anoxic headspace of a CO_2: N_2 gas mix (20:80, v/v). Iron granules (99.98% Thermo Fisher, Germany) or iron coupons (3cm × 1cm × 1mm) were the only source of electrons over the course of five successive transfers. All incubations were performed in triplicate.

All enrichments were transferred when methane production reached stationary phase. DNA extractions, SEM analyses, and further experiments were performed at the fourth transfer, after 2 years of enrichment on Fe^0. In addition, methanogen-specific coenzyme F_{420} auto-fluorescence was monitored via routine microscopy to confirm the presence or absence of methanogens. To evaluate the solitary corrosive potential of methanogens, we blocked all bacteria with an antibiotic cocktail 200 µg/mL of kanamycin and 100 µg/mL of ampicillin as done before (46). To evaluate the solitary corrosive potential of the acetogens, we inhibited all methanogens with 2 mM 2-bromoethanesulfonate (BES) as done before (47).

Chemical analyses

Methane concentrations were analyzed on a Thermo Scientific Trace 1300 gas chromatograph system coupled to a thermal conductivity detector (TCD). The injector was operated at 150°C and the detector at 200°C with 1.0 mL/min argon as reference gas. The oven temperature was constant at 70°C. Separation was done on a TG-BOND Msieve 5A column (Thermo Scientific; 30-m length, 0.53-mm i.d., and 20-µm film thickness) with argon as carrier gas at a flow of 25 mL/min. The GC was controlled and automated by a Chromeleon software (Dionex, Version 7). On our set-up the limit of detection for H_2 and CH_4 was 5 µM.

Acetate production was measured using the Dionex ICS-1500 Ion Chromatography System (ICS-1500) equipped with the AS50 autosampler, and an IonPac AS22 column coupled to a conductivity detector (31 mA).

For separation of volatile fatty acids, we used 4.5 mM Na_2CO_3 with 1.4 mM $NaHCO_3$ as eluent. The run was isothermic at 30°C with a flow rate of 1.2mL/min. The limit of detection for acetate was 0.1 mM.

DNA purification and metagenomic analyses

DNA was isolated as previously described before (24), using a combination of two commercially available kits: MasterPure™ Complete DNA and RNA Purification Kit (Epicenter, Madison, Wi, USA), and the Fast Prep spin MP_{tm} kit for soil (Mobio/Quiagen, Hildesheim, Germany). DNA quality was verified on an agarose gel, and DNA was quantified on a mySPEC spectrophotometer (VWR®/ Germany). Whole metagenome sequencing was performed on a NovaSeq 6000 system, using an Illumina TrueSeq PCR-free approach via a commercially available service (Macrogen/ Europe). Unassembled DNA sequences were merged, quality checked, and annotated using the Metagenomics Rapid Annotation (MG-RAST) server (v4.03) with default parameters (48). Illumina True Seq sequencing resulted in 3,723,388 high-quality reads of a total of 4,032,354 with an average length of 250 bp. For taxonomic analyses, the metagenomic data was compared with the RefSeq (49) database available on the MG-RAST platform. Of a total of 2,664,384 OTUs, only 391 were not classified in a domain. The rarefaction curve indicated that most of the prokaryotic diversity was covered in our sample. To investigate genes involved in carbon and nitrogen fixation, sequencing reads were annotated against the KEGG Orthology (KO) reference database. Both taxonomic and functional analyses were performed with the following cutoff parameters: *e*-value of 1e–5, a minimum identity of 80%, and a maximum alignment length of 15 bp.

Removal of corrosion crust and corrosion rates

The corrosion crust from the iron coupons was removed with inactivated acid (10% hexamine in 2M HCl) (50). Then, the iron coupons were dried with N_2 gas stream, weighted and anaerobically stored.

Scanning electron microscopy

Fixation of cells on tron coupons was performed anaerobically by adding 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.3) and incubating at 4°C for 12 h. The corroded coupons were then washed three times with 0.1 M phosphate buffer at 4°C for 10 min each. Dehydration was accomplished by a series of anoxic pure ethanol steps (each step 10 min; 35%, 50%, 70%, 80%, 90%, 95% and 100% v/v) (51). The coupons were chemical dried with hexamethyldisilazane under a gentle N_2 gas stream. Specimens were stored under N_2 atmosphere and analyzed within 18-24 h at the UMASS electron microcopy facility using the FEI Magellan 400 XHR-SEM with a resolution of 5kV.

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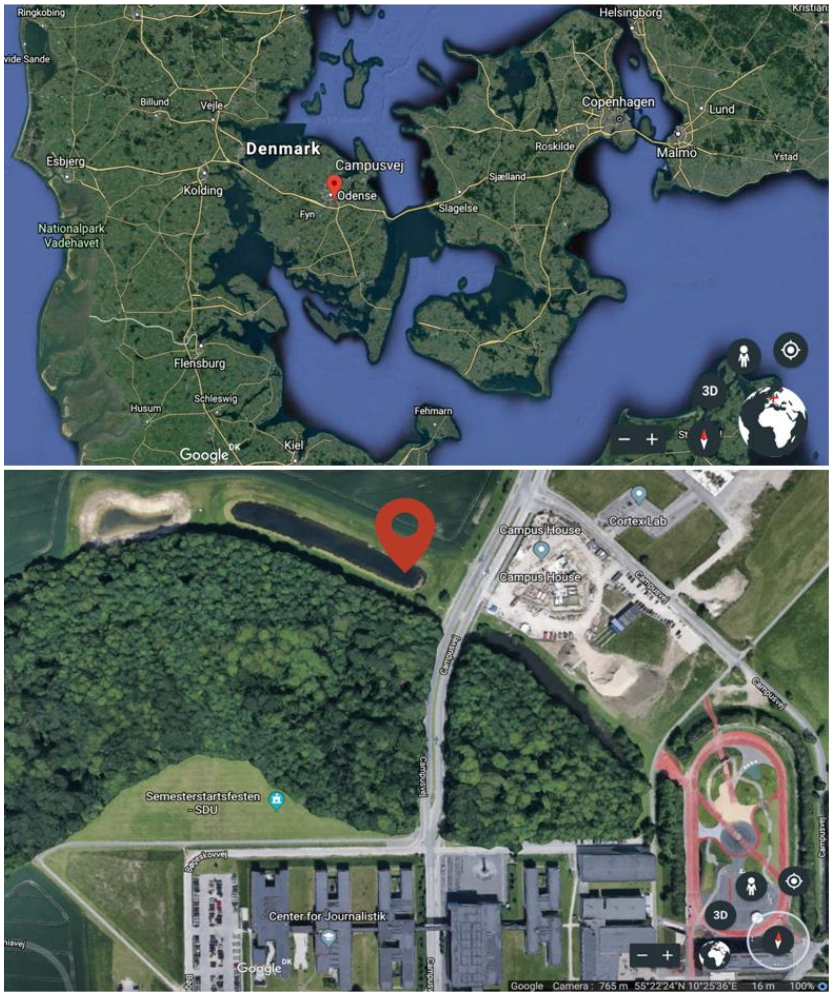
Figures

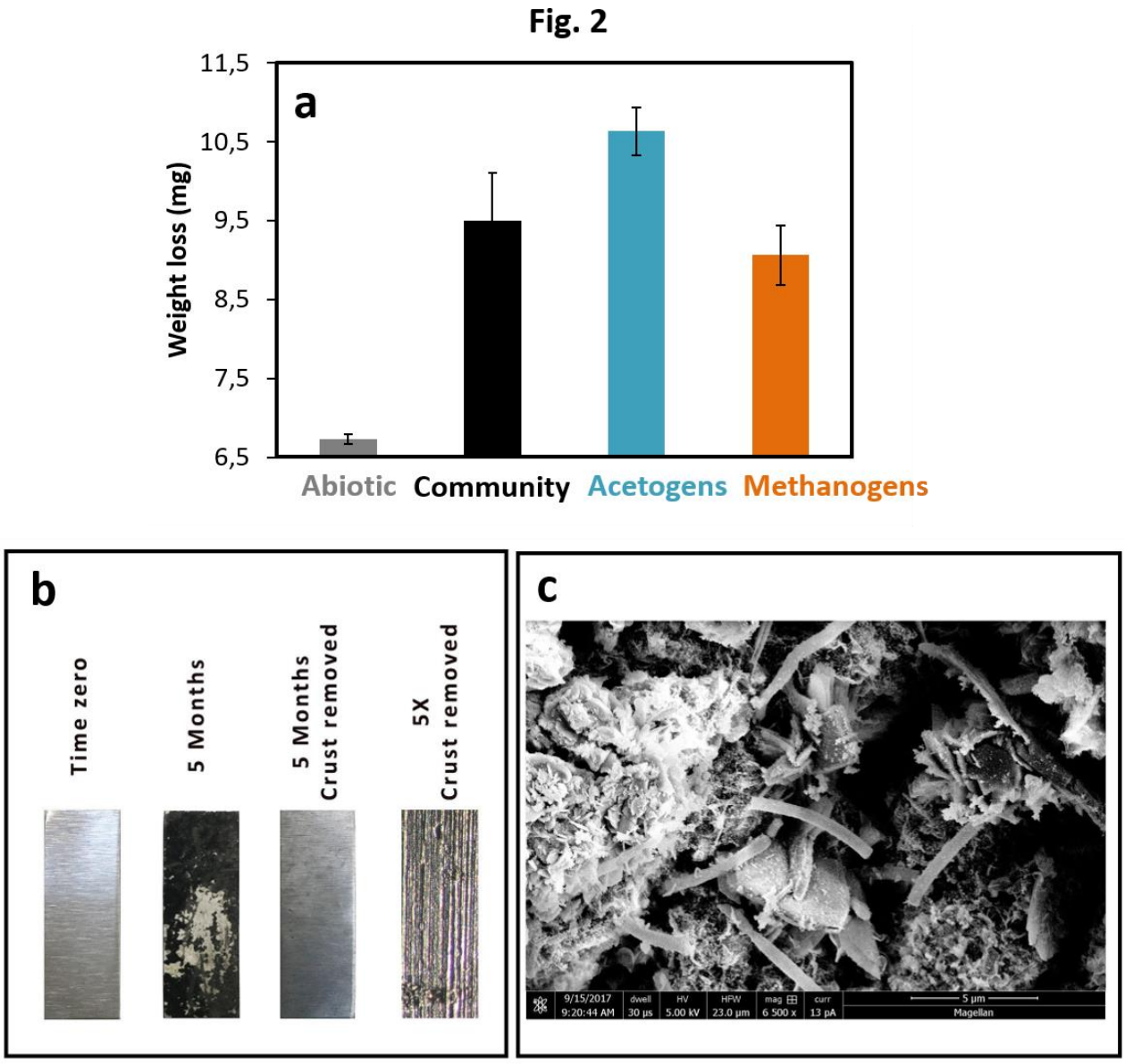
Fig. 1. The sampling site located in an urban construction area on the island of Fyn, Denmark (upper panel) is a small lake near the university of Southern Denmark (lower panel). Anoxic lake sediment was sampled with push cores during the summer of 2017, when ebullition due to increased methanogenesis is commonly observed.

Fig. 1



436 **Fig. 2.** Corrosion of Fe^0 by a microbial community (4^{th} successive transfer on Fe^0) as determined by weight
437 loss determination (a). Inhibition experiments revealed that acetogens were more corrosive than the
438 mixed community, whereas methanogens were as corrosive alone as within the mixed community (a).
439 (b) Visual observations of the Fe^0-surface after exposure to a corrosive community for 5 months
440 Removal of the black crust revealed changes in surface roughness. (c) Scanning electron microscopy
441 of a mixed community after 5 months of incubation reveals cells are attached to the Fe^0-surface.

442

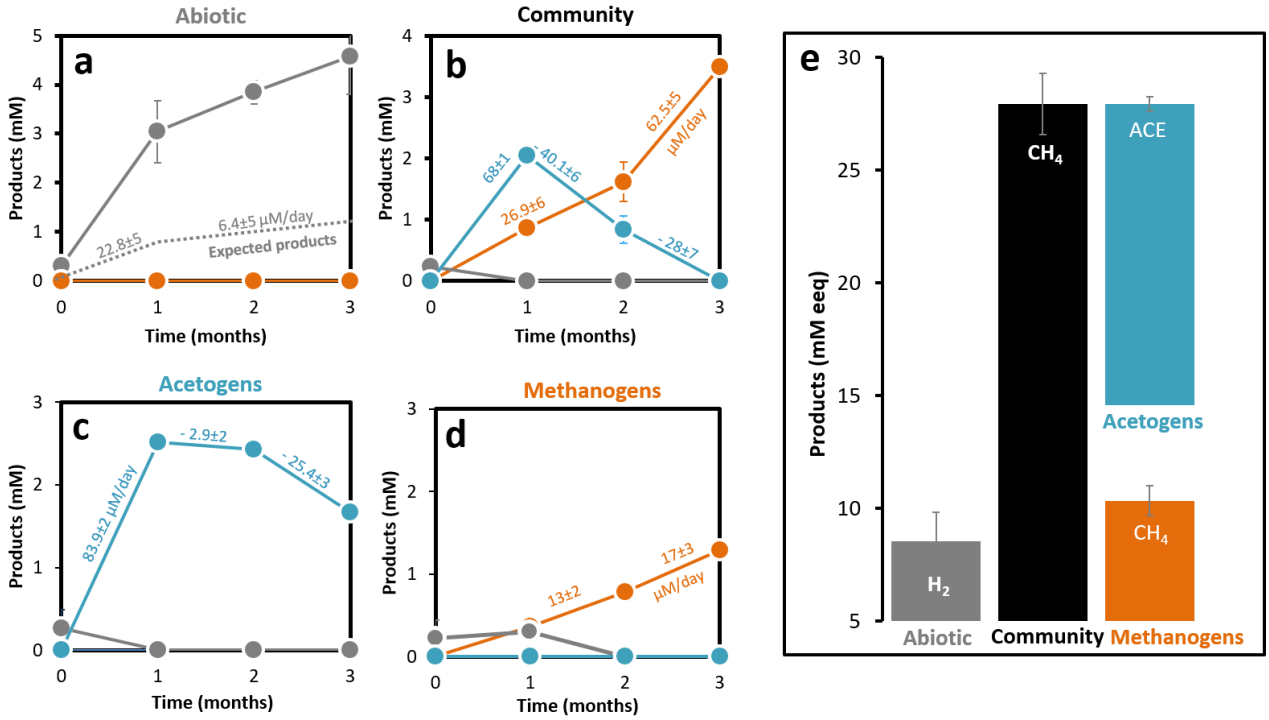


443

444

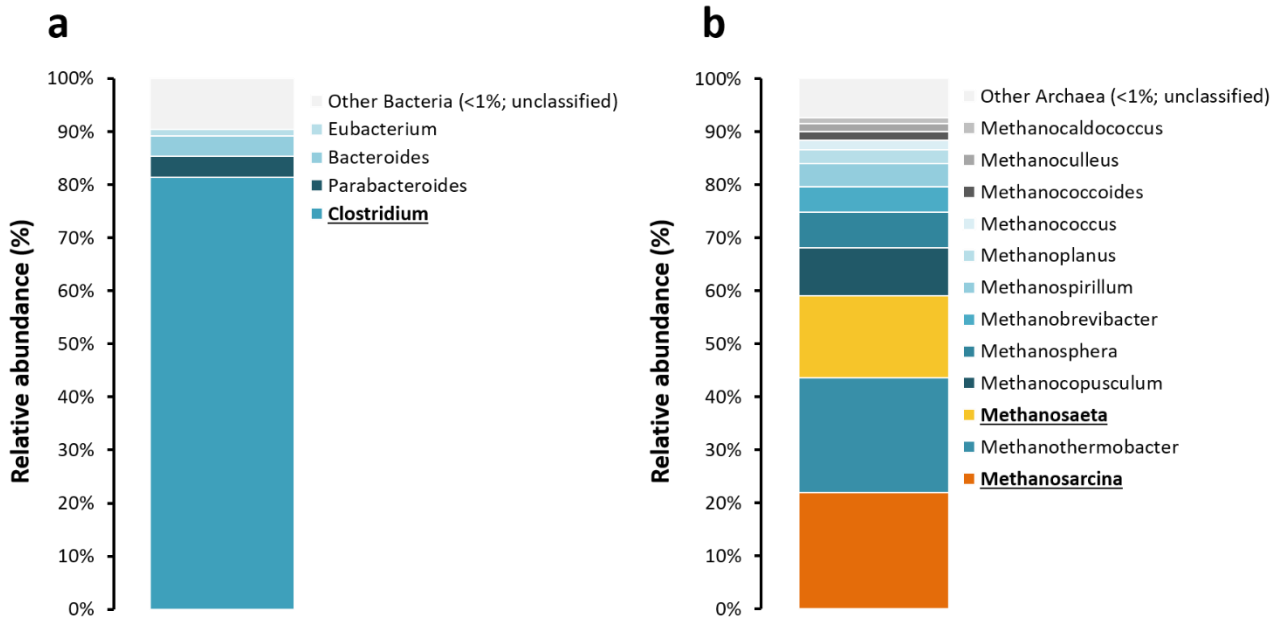
Fig. 3. Product formation using Fe^0 as sole electron donor. (a) An abiotic control which includes Fe0 exposed to the culture medium containing bicarbonate, will build up H_2 in the absence of cells. From abiotic H_2 microorganisms could use four mols H_2 to produce a mol of products (acetate or methane) according to reactions 3 and 4. (b) A mixed community after four successive transfers solely with Fe^0 as electron donor, generated successively acetate with very high rates and later methane with similarly high rates. The products generated could not be explained solely by abiotic-H_2 (c) Acetogens were even more effective alone during the first month of incubation. The amount of acetate they produced could not be explained by abiotic-H_2. (d) Methanogens alone although as corrosive as the mixed community, they were not as effective at producing methane as they were within the mixed community. (e) Total products as mM electron equivalents (eeq) produced on Fe^0 under four different conditions. The following values are considered for each product: 2 mM eeq per mol H2, 8 mM eeq for each mol of methane or acetate (see reactions 3 and 4). All experiments are run in triplicates (n=3). When error bars are not shown they were smaller than the symbols.

Fig. 3



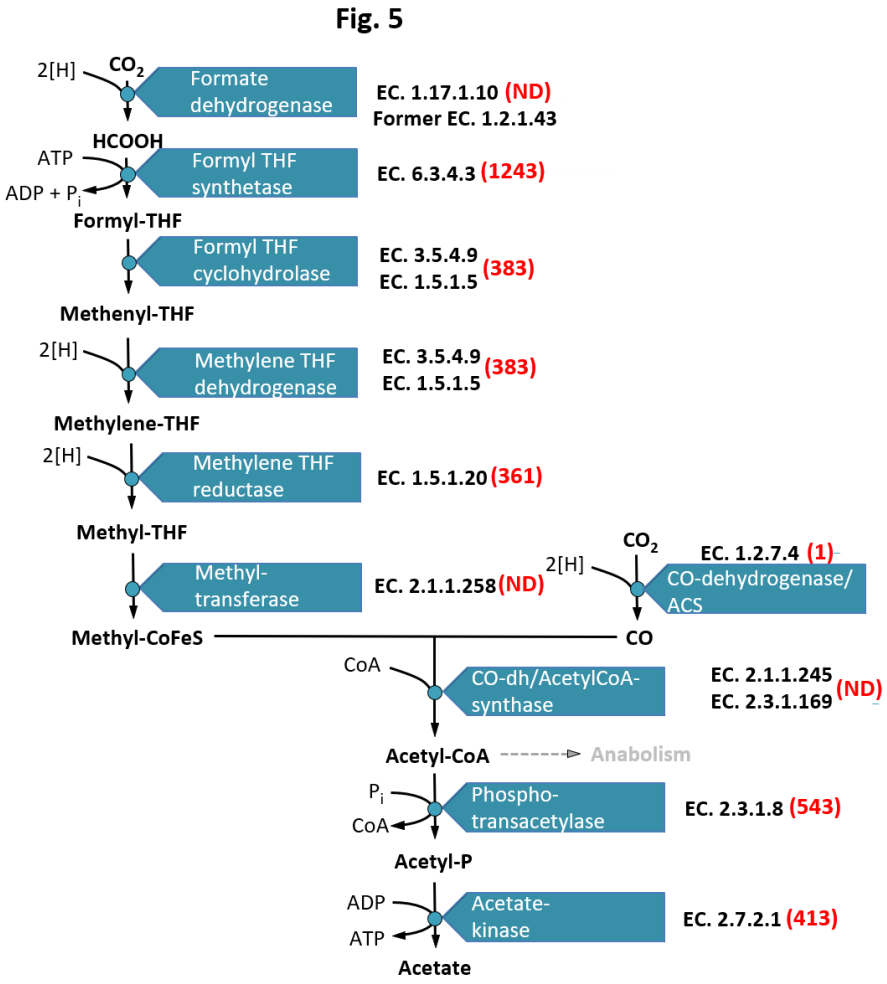
460 **Fig. 4.** Whole genome sequence distribution for (a) bacterial and (b) archaeal genera representing a corrosive
461 community enriched on Fe^0 after four subsequent transfers.

Fig. 4



462

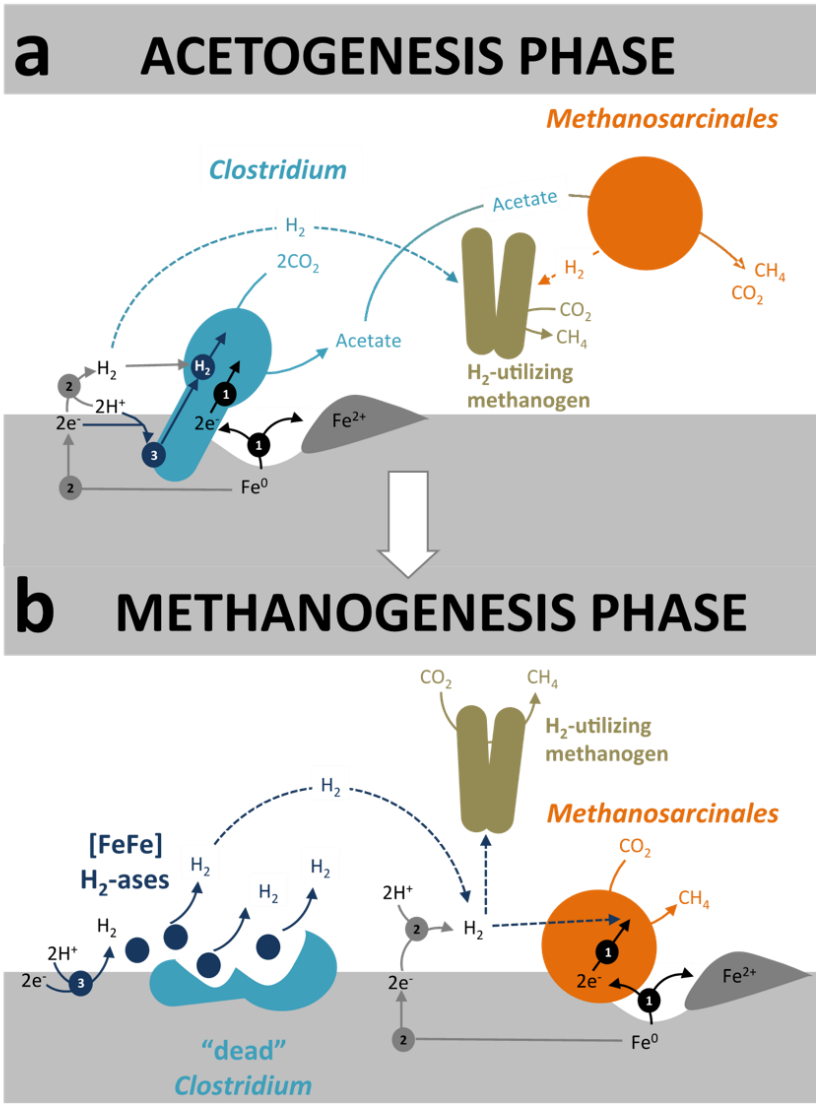
463 **Fig. 5.** Representative enzymes of the Wood-Ljungdahl pathway discovered in the metagenome of *Clostridia*
464 from an Fe^0-corrosive community enriched from an urban lake. The numbers are shown in red. ND
465 means not detected.



466

467 **Fig. 6.** Modeled interactions between *Clostridium*-acetogens with acetoclastic *Methanosarcinales* and
468 hydrogenotrophic methanogens during the acetogenic phase when acetate production was highest (a)
469 and (b) during the methanogenesis phase when methane production was highest.

Fig. 6



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