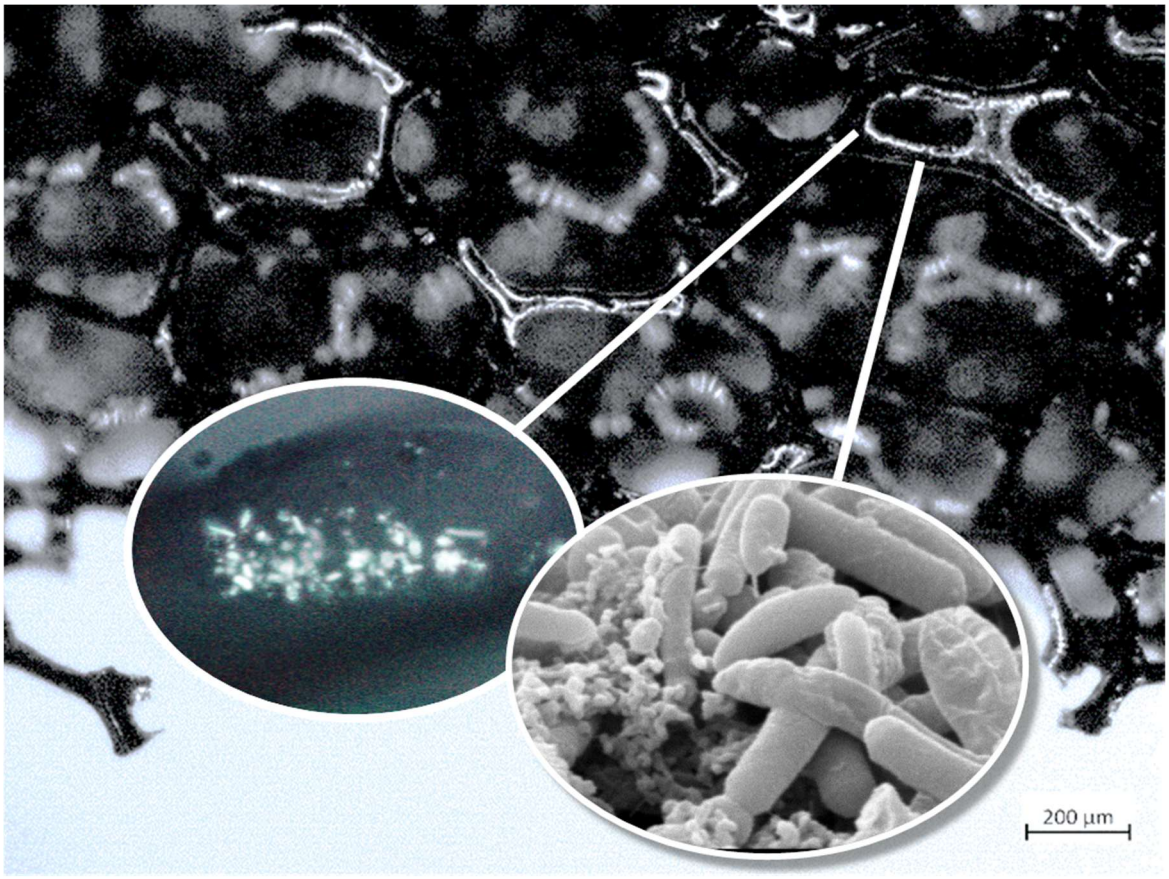
Final report dated 10.03.2026

ACME Anaerobic digestion with Conductive Material and Electrogenic microorganisms



Source: ©supsi 2021-2023

University of Applied Sciences and Arts
of Southern Switzerland

SUPSI

Date: 10.03.2026

Location: Mendrisio

Publisher:

Swiss Federal Office of Energy SFOE
Energy Research and Cleantech
CH-3003 Bern
www.bfe.admin.ch

Subsidy recipients:

SUPSI University of Applied Sciences and Arts of Southern Switzerland

Department for Environment Constructions and Design (DACD)

Institute of Microbiology (IM)

BET lab.

Campus Mendrisio, Via Flora Ruchat Roncati 15

CH-6850 Mendrisio

HES-SO

Institute of Life Technologies
Rte du Rawil 64
1950 Sion 2

Authors:
Pamela Principi, SUPSI DACD, pamela.principi@supsi.ch
Roger König, SUPSI DACD, roger.koenig@supsi.ch
Fabian Fischer, HES-SO, fabian.fischer@hevs.ch

Other partners:

Ing. Paolo Foa, TBF + Partner AG, Via Besso 42, 6900 Lugano, fop@tbf.ch

Ing. Stefano Airaghi, Consorzio Depurazione Acque Chiasso e Dintorni (CDA, CD), Via del Breggia 7, 6833 Vacallo, s.airaghi@cdacd.ch

SFOE project coordinators:

'sandra.hermle@bfe.admin.ch'

SFOE contract number: SI-502026-01_ACME

Zusammenfassung

Bei der anaeroben Vergärung interagieren Mikroorganismen metabolisch, um organische Stoffe in Biogas umzuwandeln eine Gas-mischung aus Methan, Kohlendioxid und Spurenstoffen. Ein Schlüsselfaktor zur Prozessoptimierung ist die Menge des produzierten Methans sowie dessen Produktionsrate pro eingesetzter Substratmenge.

Die mikrobiellen Interaktionen im Prozess können über den Elektronenaustausch zwischen verschiedenen mikrobiellen Taxa erfolgen. Unter den verschiedenen beschriebenen Mechanismen kann der DIET (Direct Interspecies Electron Transfer) durch das Vorhandensein leitfähiger Materialien im Reaktor unterstützt werden. Zahlreiche Studien haben dieses Thema untersucht, und das frühere FOEN-Projekt DIET SI/501716-01 hat es spezifisch adressiert.

Im ACME-Projekt möchten wir den tatsächlichen Vorteil des Elektronenaustauschmechanismus über längere Zeiträume und mit dem für den Vergärer üblichen Substrat bewerten. Um dieses Ziel zu erreichen, wurden zwei in der Literatur als elektroaktiv bekannte syntrophe elektrogene Mikroorganismen Methanosarcina barkerii und Shewanella oneidensis als Inokulum in Laborvergärern eingesetzt. Die Mikroorganismen wurden auf leitfähigen Trägern kultiviert und sowohl einzeln als auch in Kombination mit der indigenen mikrobiellen Gemeinschaft eines realen Vergärers (Vergärer der ARA Chiasso) inoculiert.

Die zeitliche Überwachung der Biogas und Methanentwicklung ermöglichte die Bewertung des tatsächlichen Nutzens der Zugabe eines DIET-fähigen Inokulums mit leitfähigem Material.

Unter denselben Bedingungen wie in der realen Anlage brachte die Zugabe von leitfähigem Material zusammen mit syntrophen Mikroorganismen keine eindeutigen Ergebnisse: Der in einigen Experimenten bei den ersten Fütterungen beobachtete positive Effekt wird von der im Vergärer natürlicherweise vorkommenden mikrobiellen Gemeinschaft überlagert.

Im Testvergärer jedoch also bei gleichzeitiger Anwesenheit von leitfähigem Material und syntrophen Mikroorganismen wurde eine frühere Methanproduktion beobachtet. Die mikrobielle Gemeinschaft unterscheidet sich vom Kontrollansatz und zeigt eine erhöhte relative Häufigkeit methanogener Mikroorganismen, die zum Taxon Methanosarcinae gehören. Zudem wurde eine weitere interessante Differenz festgestellt: Die Produktion von Schwefelwasserstoff war in den ersten Fütterungen des Testvergärers höher.

Riassunto

Nella digestione anaerobica i microrganismi interagiscono metabolicamente per convertire la sostanza organica in biogas, una miscela gassosa composta da metano, anidride carbonica e composti in tracce. Un fattore chiave per l'ottimizzazione del processo è la quantità di metano prodotta e la sua velocità di produzione per unità di substrato utilizzato. L'interazione microbica nel processo può avvenire tramite lo scambio di elettroni tra differenti taxa microbici. Tra i vari meccanismi caratterizzati, il DIET (Direct Interspecies Electron Transfer) può essere favorito dalla presenza di materiali conduttivi nel reattore. Numerosi studi hanno trattato questo argomento e un precedente progetto sempre finanziato dal BFE, DIET SI/501716-01, lo ha affrontato in modo specifico.

Nel progetto ACME vogliamo valutare il reale vantaggio del meccanismo di scambio elettronico su tempi più lunghi e utilizzando come substrato l'alimentazione standard di un digestore. Per raggiungere questo obiettivo, due microrganismi elettrogenici sintrofici, noti in letteratura per essere elettroattivi (capaci cioè di scambiare elettroni attraverso la membrana cellulare), sono stati utilizzati come inoculo in digestori

da laboratorio: Methanosarcina barkerii e Shewanella oneidensis, cresciuti su supporti conduttivi, sono stati inoculati sia singolarmente sia in presenza della comunità microbica indigena di un digestore reale (digestore dell'impianto di depurazione di Chiasso). Il monitoraggio nel tempo dell'evoluzione del biogas e del metano ha permesso di valutare il reale vantaggio dell'aggiunta di un inoculo in grado di attivare il DIET con materiale conduttivo. Nelle stesse condizioni dell'impianto reale, l'aggiunta di materiale conduttivo insieme a microrganismi sintrofici non ha portato a risultati definitivi: l'effetto positivo osservato in alcuni esperimenti nelle prime alimentazioni viene superato dalla comunità microbica normalmente presente nel digestore. Tuttavia, nel digestore sperimentale cioè con la presenza simultanea di materiale conduttivo e microrganismi sintrofici, si osserva una produzione anticipata di metano. La comunità microbica si modifica rispetto al controllo, con un aumento dell'abbondanza percentuale dei microrganismi metanogeni appartenenti al taxon Methanosarcinae. Inoltre, un'altra differenza interessante riguarda la produzione di idrogeno solforato, che risulta più elevata nelle prime alimentazioni del digestore sperimentale.

Summary

In Anaerobic Digestion microorganisms interact metabolically to convert organics in biogas that is the gas mixture made of methane, carbon dioxide, and trace compounds. A key factor in process optimization is the mass of methane produced and its production rate per unit of substrate used. The microbial interaction in the process can occur by the exchange of electrons among different microbial taxa. Among the different mechanisms of electron exchange characterized DIET (Direct Interspecies Electron Transfer) can be supported by the presence of conductive material in the reactor. Many studies have addressed the topic in the literature and past FOEN project DIET SI/501716-01 addressed this topic. these results are mostly related to short term tests (generally fourteen days) and with stable and often synthetic feeding. Therefore, the lack of long-term experiments with real condition results is impacting on the acceptance toward industrial application.

The present study aims to fill the research gap studying first the possibility of using an electroactive inoculum and then presenting the result of a six-months monitoring campaign of the anaerobic digestion of thickened sludge weekly sampled in 60L reactors amended with Powdered Activated Carbon (PAC) at 20g/L as conductive material (CM). To reach this goal, two syntrophic electrogenic microorganisms known by literature to be electroactive (able to exchange electrons cross membrane), were used as inoculum in lab-scale digesters: Methanosarcina barkerii and Shewanella oneidensis grown on conductive carriers were inoculated both alone and in the presence of the indigenous microorganisms of a real digester (Chiasso WWTP digester).

Monitoring the evolution of biogas and methane over time has made it possible to evaluate the effective advantage of the addition of a DIET-able inoculum with conductive material. Under the same conditions as the real plant, the addition of conductive material with syntrophic microorganisms did not bring definitive results: the positive effect present in some experiments in the first feedings is over-come by the microbial community normally present in the digester.

However, in the test digester, i.e. with the simultaneous presence of conductive material and syntrophic microorganisms, an anticipated production of methane is observed, the microbial community changes compared to the control, increasing the percentage abundance of methanogenic microorganisms belonging to the Methanosarcinae taxon. Furthermore, another interesting difference concerns the production of hydrogen sulphide which is higher in the first feedings of the test digester.

Main findings

- The hypothesis to use electrogenic inoculum to foster the DIET mechanism together with Reticulated Vitreous Carbon as conductive material at 8.6 g/l did not completely succeed: Shewanella sp. did not survive the competitive environment.
- The methanogen DIET-able microorganism was still present at the end of the experiments and the corresponding taxa increased its abundance in presence of conductive material.
- On long-term experiments in 60L reactors the presence of Conductive Material at the given conditions increased biogas and methane production rate compared to the reference reactor with no CM addition. The microbial community dynamics assessed by DNA metabarcoding evidenced an enrichment of hydrogenotrophic methanogens by the CM presence.
- The results suggest that the conductive material addition influenced the methanogenesis pathway by producing methane by the reduction of carbon dioxide, reducing as consequence the release into atmosphere. Further research is needed to understand the condition for CM addition and to quantify the advantage in terms of methane production.

Contents

Abbreviations

AD – Anaerobic Digestion

BMP – BioMethane Potential

CH_4 – Methane

CM – Conductive Material

CO_2 – Carbon Dioxide

COD / sCOD – (Soluble) Chemical Oxygen Demand

DB-RDA / dbRDA – Distance-based Redundancy Analysis

DIET – Direct Interspecies Electron Transfer

DNA – Deoxyribonucleic Acid

EPS – Extracellular Polymeric Substances

FISH – Fluorescent in Situ Hybridization

H_2S – Hydrogen Sulphide

HRT – Hydraulic Retention Time

IR – Infrared

LoD – Limit of Detection

LOESS – Locally Estimated Scatterplot Smoothing

MOs – Microorganisms

OTU / OTUs – Operational Taxonomic Unit(s)

PAC – Powdered Activated Carbon

PBS – Phosphate Buffered Saline

PCR – Polymerase Chain Reaction

qPCR – quantitative Polymerase Chain Reaction

RVC – Reticulated Vitreous Carbon

SEM – Scanning Electron Microscopy

SRT – Sludge Retention Time

TS – Total Solids

VFA / VFAs – Volatile Fatty Acids

VS – Volatile Solids

WP – Work Package

WWTP – WasteWater Treatment Plant

1 Introduction

1.1 Background information and current situation

Anaerobic digestion (AD) is a biological process wherein microorganisms break down biodegradable materials in the absence of oxygen, producing biogas primarily composed of methane and carbon dioxide (Appels *et al.* 2008). AD has been widely implemented in waste treatment and is increasingly recognized for its role in renewable energy production (Wang *et al.* 2023),(Q. Zhang *et al.* 2016). The AD process involves four stages of biochemical reactions: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Bhujbal *et al.* 2021). Process performance entirely relies on operating strategies, such as inoculum selection, substrate preprocessing or hydraulic retention time and process parameters monitoring, such as pH, Volatile Fatty Acids (VFAs) concentrations or temperature (Subbarao *et al.* 2023). Despite the growing interest in AD as a renewable energy source, significant research gaps remain, particularly in optimizing feedstock pre-treatment processes to enhance biogas yield and system efficiency (Ampese *et al.* 2022).

However, challenges such as process instability, substrate variability, and high capital costs still limit widespread AD adoption (Li *et al.* 2018). Therefore, ongoing research is essential to enhance process efficiency, reduce operational costs, and expand the role of AD in sustainable waste management and energy systems (Khalid *et al.* 2011). Over the past decade, to improve methane yield and reduce CO_2 emissions, significant efforts have focused on the treatment of recalcitrant organic matter through pre-treatment technologies (König *et al.* 2022; Wei *et al.* 2022; Harris and McCabe 2015; Principi *et al.* 2019) and optimization of process management (Subbarao *et al.* 2023; Liu *et al.* 2025).

Aiming to optimize the process by increasing methane production, several scientific papers have reported that Direct Interspecies Electron Transfer (DIET) has a positive effect in the process of AD (Park *et al.* 2018) (Perego *et al.* 2025) (He *et al.* 2021). The positive impact of conductive materials (CM), such as biochar, magnetite, and Powdered Activated Carbon (PAC), on microbial community and methane production has been demonstrated in numerous laboratory-scale studies (Wu *et al.* 2018, J. Zhang *et al.* 2018). However, these findings are primarily based on short-term experiments and under controlled conditions. The scarcity of data from pilot scale applications influenced by real feedstocks highlights that DIET-based AD optimization remains largely at the research stage. The lack of long-term studies under realistic operational conditions significantly limits, therefore, the industrial application.

In this context, the present research investigates the impact of PAC as CM on the performance of a 60 L anaerobic digester. The results present process parameter and microbial community analysis data obtained from a long-term monitoring campaign, aiming to provide insights into DIET-driven AD under more realistic and scalable conditions. Many authors have reported in literature the existence of electron exchange mechanisms in anaerobic digestion microbial consortia in lab scale settings. This mutualistic metabolism is supported by different electrons exchange mechanisms that are defined as Interspecies Electron Transfer (Gahlot *et al.* 2020).

1.2 Purpose of the project

The project aims to grow specific selected electrogenic microorganisms and methanogens to assess their ability to colonize conductive surface in a biofilm mode, and to evaluate biogas production in lab scale conditions. This microbial consortium has the potential ability to use the DIET mechanism and the performance compared to a control without conductive material, is monitored by biogas production.

The ability to apply the DIET-able microorganisms to anaerobic digestion processes is based on their behavior in real scale conditions: the resistance toward the microorganisms present in the anaerobic biomass in normal condition will be evaluated.

The biodiversity of the digestate poses a risk to the maintenance of the cultures used as starters, on the other hand the selective pressure and the advantages of DIET exploiting cultures against the indigenous microflora could be the edge guaranteeing their survival and proliferation.

It is foreseen that electrogenic microorganisms, by electron exchange advantage, improve the overall anaerobic digestion process. The benefit would be expressed as shortened lag phase and/or increased steepness and/or maximum methane yield.

The impact of PAC as CM on the performance of a 60 L anaerobic digester is then investigated to provide insights into DIET-driven AD under more realistic and scalable conditions.

1.3 Objectives

To obtain clear data, the experiments and the reactors need to be carefully designed. The main points to be researched in the project are structured as deliverables in each work package. In the following table (Table 1) the deliverable list is reported.

Table 1: activities and deliverable list as planned in the project.

Activity	Deliverable	Details
WP1 requirement analysis	D1.1 requirement table for microorganisms	Choice of the microorganisms and growing conditions
	D1.2 Growing chamber	System to grow *Shewanella oneidensis* on electrogenic carriers
	D1.3 Reactor design	Specifics for the lab scale reactors
WP2_growing chambers and reactor realization and wet test	D2.1 Growing chamber	
	D2.2 Testing reactors	Manufacturing and testing
WP3: MOs selection and culturing	D3.1 electrodes with attached specific microorganisms,	
	D3.2 specifics of the attached grown system	
	D3.4 Proofs of the presence of ElectroActive Biofilms.	Microbial community analysis
M1 Milestone 1 GO NO GO	Proof of the culturing the selected microorganisms on the conductive material	
WP4: Lab scale tests with synthetic media	Short term BMP tests and long-term BM tests results	
WP5: Lab scale tests with real AD conditions	Investigation on the effect of the addition of conductive materials with selected microorganisms in real scale conditions.	
WP6: MO characterization/identification	Sampling and analysis of the microbial community composition of the different reactor setups.	

2 Description of facility

AD Reactors.

To carry out the experiments as planned we designed and manufactured six bench-scale inox reactors pressure-tight and equipped with manometers to monitor the biogas evolution. The top cover has been specifically designed to hold the carriers and a connection valve has been modified to feed and withdraw samples without contaminating the microbial consortium.

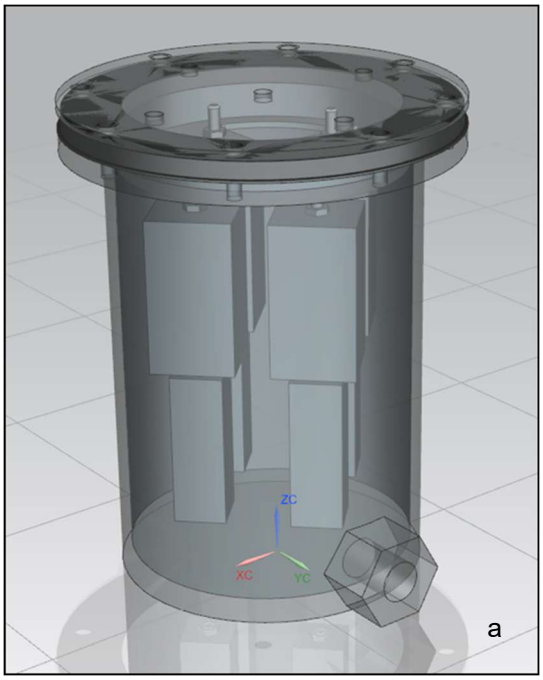


Figure 1: on left (a) the technical drawing for the reactor showing the flange for the carrier holder; on the right (b) the insertion of the carriers during the setup of the experiment.

3 Procedures and methodology

Analytical techniques. The total solids (TS) and volatile solids (VS) content were determined in accordance with Standard Methods .(American Public Health Association APHA 2016)

The biogas production was measured by manometer measuring the overpressure daily on the small-scale set and automatically every hour for the higher scale reactors. The pressure values were converted to Liter of biogas in normal conditions (15°C, 1 bar) applying the ideal gas law PV=nRT.

Methane concentration. The methane concentration in the biogas has been measured by IR through the Gas Analyzer (Biogas5000 Geotech, Lauper Instruments AG, Murten, CH) and expressed as %.

Analysis of biogas production. Biogas production was analysed using a modified Gompertz equation (Lay *et al.* 1997), which can estimate ultimate biogas volume, maximum biogas production rate, and lag time based on the following equation.

$$Y = Ym * \exp\left(-\exp\left(Rm * \frac{2.7186}{Ym} * (l - x) + 1\right)\right)$$

where Y (t) is the accumulative biogas production (mLg-1VS) at an anaerobic digestion time t (d), Ym is the biogas production potential (mLg-1-VS), Rm is the maximum biogas production rate (mLg-1VSd-1), l is the duration of lag-phase time (d), and e = 2.7183. Kinetic parameters were obtained by nonlinear regression fitting using GraphPad Prism (version 8.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com) and least square regression method, goodness of fit quantified by R-squared.

Microscopic observations. Samples collected from the different reactors are observed under microscopy (Axio Scope, Zeiss) in phase contrast or epifluorescence at 40x and 100x magnification (oil immersion) and images are taken by Axiocam (Zeiss) camera. **FISH (fluorescent in situ hybridization) and molecular quantification of**

Methanosarcina barkeri* and *Shewanella oneidensis. For the microscope analyses, we performed FISH on fixed samples (paraformaldehyde 4%) collected from the reactors. We used specific probe for *Methanosarcina barkeri* and *Shewanella oneidensis* labelled with two different fluorescent dyes (see Table 2)Table 2: Primer and probe for FISH of *Methanosarcina barkeri* and *Shewanella oneidensis*

Table 2: Primer and probe for FISH of *Methanosarcina barkeri* and *Shewanella oneidensis*

Probe name	Fluorescent dye	Sequence 5'-3'	Reference
SHEW227_FAM	FAM	AGCTAATCCCACCTAGGTWCATC	(Dolch *et al.* 2014)
MS821	Cy3	CGCCATGCCTGACACCTAGCGAGC	(Narihiro and Sekiguchi 2011)

DNA of *Methanosarcina barkeri* and *Shewanella oneidensis* was quantified by quantitative PCR (qPCR) using specific primers (and probes) for 16S rDNA (Table 3). For *Shewanella oneidensis* we used the sybr green technology whereas for *Methanosarcina barkeri* we used TaqMan technology.

Table 3: Primer and probe for qPCR of *Methanosarcina barkeri* and *Shewanella oneidensis*

Primer/probe name	Sequence 5'-3'	Reference
640F *Shewanella*	RACTAGAGTCTTGTAGAGG	(Ancona *et al.* 2020)
815R *Shewanella*	AAGDYACCAAAYTCCGAGTA	
MSL812F	GTAAACGATRYTCGCTAGGT	(Narihiro and Sekiguchi 2011)
MSL1159R	GGTCCCCACAGWGTACC	
MSL860F probe	AGGGAAGCCGTGAAGCGARCC	

For both microorganisms, we defined the standard curves with known concentrations of amplified 16SrDNA, to define the limit of detection (LoD). The Ct (cycle threshold) of known standards will be used to quantify our samples.

DNA extraction from samples, taken from the different reactors, was performed using DNeasy PowerSoil Pro Kits (Qiagen) following the manufacturer's instructions (Mang *et al.* 2022; Fernandez-Bayo *et al.* 2020).

Microbial community analysis. From selected samples DNA was extracted with DNeasy PowerSoil kit (Qiagen) and then was quantified with Qubit 4 (Thermo Fisher Scientific). Two different PCR, using Taq PCR Master Mix (Qi-agen), were performed for each sample collected. Bacteria sequences were amplified with the primer set 27F and 1492R while for Archaea the primer set was 21F and 1492R.

27F	5′-TTT CTG TTG GTG CTG ATA TTG CAG AGT TTG ATC CTG GCT CAG-3′
1492R	5′-ACT TGC CTG TCG CTC TAT CTT CGG TTA CCT TGT TAC GAC TT-3′
21F	5′- TTT CTG TTG GTG CTG ATA TTG CTC CGG TTG ATC CYG CCG G-3′

Table 4: 16S Primer set sequences.

All the primers were linked to a flag sequence (provided by Oxford Nanopore Technologies) to allow the compatibility of the amplificates with the MinION™ kit (SQK-PBK004, Oxford Na-nopore). The amplicons

were purified with CleanNGS kit (Labgene scientific) and were quantified with Qubit 4 (Thermo Fisher Scientific). Afterwards, bacterial, and archaeal amplicons of the same sample were combined and barecoded, as explained in the Nanopore protocol. All barcoded DNA were subjected to a second step of purification and quantification, using the same methods as before, and were combined in equal ratios to generate a pool. According to the manufacturer's protocol, the pool was quantified and was diluted (if necessary), the RAP adaptor was added. Pool was prepared for loading using the Flow Cell Priming Kit (EXP-FLP002), was loaded into the R9.4 flow cell (FLO-MIN106; Oxford Nanopore Technologies) and was sequenced on the MinION™ Mk1B for 72 hours.

Bioinformatic data analysis. Libraries were sequenced on the Oxford Nanpore MinION sequencer. Generated reads were basecalled and demultiplexed using Guppy version 5.0.7+2332e8d65. Using NanoClust pipeline, reads from each sample were de novo clustered, consensus sequences generated for each cluster and taxonomic classification performed. NanoClust was run with adjusted parameter umap_set_size = 50000 (v1.0dev, commit: eb6a2c822088500cede4e7fc1463bd72a9692094).

Consensus fasta sequences of each cluster and read counts on the OTU species level were extracted from NanoClust results and combined into a single multifasta file and OTU count table, respectively.

To assess microbial community ecology, the R package microeco (microeco_0.12.1) was used. Relative abundances were calculated and compositon information of communities were shown in barplots.

Flow cytometry cells count. Samples were fixed as for FISH analysis and was diluted ten times with pbs and then filtered with a thirty µm porosity on a polycarbonate filter. 10 µL of the filtrate was analysed with BD Accuri C6 plus cytometer with the following settings: capillary 10 µm flow 14 µL/min, gate set on FL1 + for *Methanosarcina barkerii* and *Shewanella oneidensis*. The analysis was repeated by changing the capillary settings to 22µm and 66µl/min and acquiring variable volumes for testing aggregate.

Long term effect of CM addition. For the scaled-up experiments with real conditions, two biogas production processes, a control and a test, were carried out in 60 L-capacity reactors (BTP 2, Umwelt- und Ingenieurtechnik GmbH, Germany), the headspace was replaced with pure nitrogen to assure anaerobic conditions. Inocula were collected from the anaerobic digestor of the Wastewater Treatment Plant (WWTP) of Chiasso (CDA,CD, Vacallo, Switzerland). Reactors were run semi-continuously with daily feeding regime on working days. Sludge Retention Time (SRT) was set at 35 days according to the reference WWTP AD system, feedstock consisted of primary thickened sludge from the same plant. A test experiment was set to evaluate the addition of CM in terms of process performance and microbial community dynamic with real feedstock influence. According to König *et al.* Powdered Activated Carbon (PAC) (Norit Nederland B.V., Amersfoort, The Netherlands) was added in a fix concentration of 20g/L. Start-up phase was preliminarily carried out to adjust the pilot settings according to the full-scale plant. Once the steady state was reached (approximately three times the SRT), the experiment was run and monitored for three months

4 Results and discussion

The use of conductive materials to promote DIET and enhance anaerobic digestion and methane production is widely reported in the literature (Ahuja *et al.* 2024; Sun *et al.* 2023). This approach is undoubtedly promising, but it requires careful evaluation before being implemented at full scale. Indeed, most recent studies on this topic have been conducted under laboratory-scale conditions only (Nguyen *et al.* 2021).

Several critical factors may negatively affect the full-scale application of the DIET-based approach, including reactor volume, hydraulic retention time, feedstock characteristics, the use of electrogenic inocula, and the composition of the indigenous microbial community in the digester.

Initially, the ability of the two pure cultures to produce methane syntrophically in the presence of conductive materials was assessed in short-term experiments. Subsequently, using a real feedstock and conductive materials, the fate of the bioaugmentation inoculum, process performance, and microbial community dynamics were evaluated.

The biogas production of the consortium composed of *Shewanella oneidensis* and *Methanosarcina barkerii* evaluated in batch mode with RVC as CM. In Figure 2 are reported the non-linear fitted curves obtained for the three experimental settings: SM RVCbio stands for the consortium inoculated as biofilm and SM RVC stands for the consortium inoculated as planktonic cells both with RVC as CM. SM is the control without the CM.

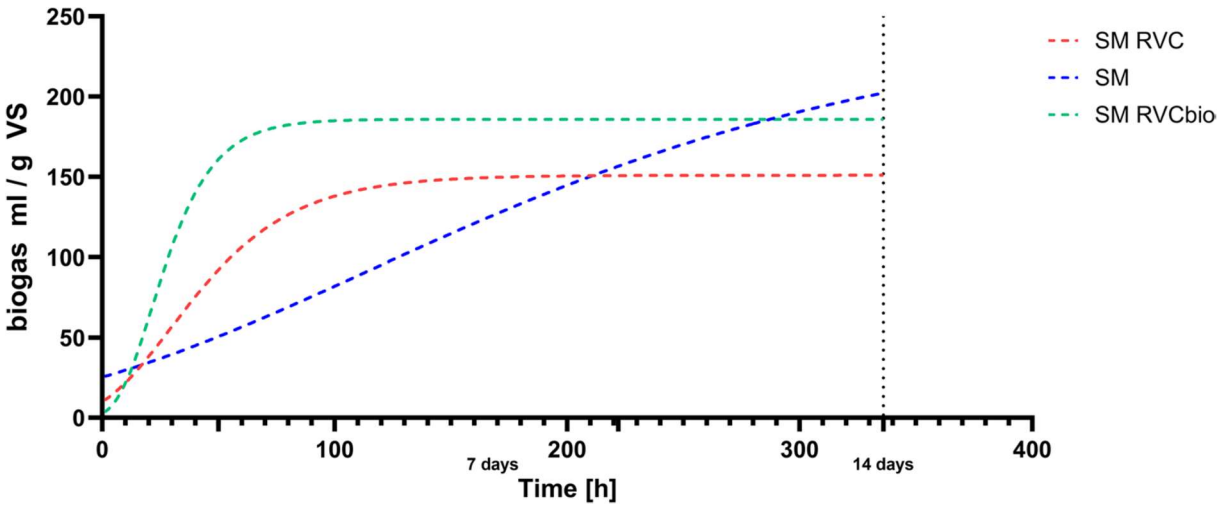


Figure 2: Non-linear fitting data for the three experimental setups with three replicates for each test, Gompertz mod. equation, and least square

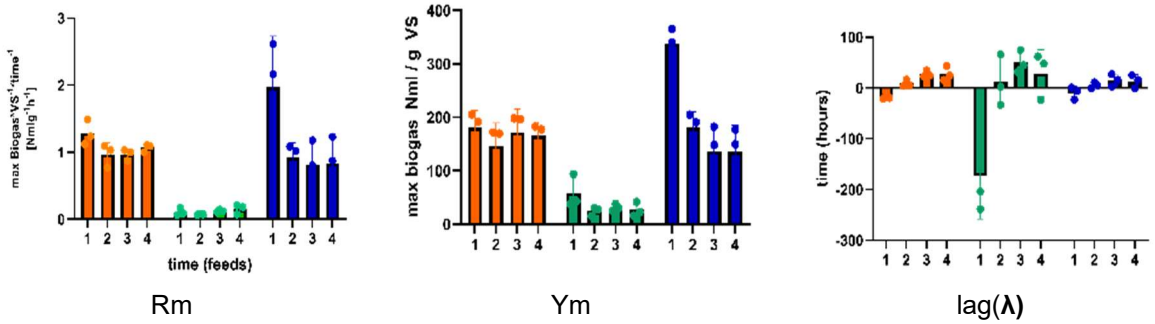
The biogas production for the tests with CM addition (SM RVCbio and SM RVC) showed a rapid increase in biogas production reaching the plateau around 100 h, while the reactor with just the syntrophic inoculum (SM) showed a less steep slope reaching the plateau at around 2 weeks (336 h).

In the experimental timeframe a positive effect of CM on biogas was observed. The biogas production of the SM control test proved that the syntrophic cooperation between *Shewanella oneidensis* and *Methanosarcina barkerii* in methanogenesis did occur. The methane content obtained is reported in Table 5.

Table 5: Methane percentages measured in the three experiments

Time [h]	SM	SM RVC	SM RVCbio
Beginning	–	–	–
45–55	32.5	45.8	54.4
210–220	55.7	60.8	55.7

The kinetic parameters calculated by fitting the data with the Gompertz model equation, are reported in Figure 3.



Rm Ym lag(**λ**)

Figure 3: kinetic parameters for the SM RVC (orange) SM (green) and SM RVCbio (blue) calculated for each feeding.

In the first figure from the left is reported the maximum yield rate (Rm) for each reactor during the four feeds time periods. For both SM RVC and SM RVCbio the maximum rate decreased after the first feed and then remained quite stable, the same is registered also for SM even if at much lower values. The figure in the middle represents the trend of the maximum biogas production (Ym): for SM RVC there is a decrease after the first feed, and then the values increased again, the same is observable for SM; for the and SM RVCbio the decrease after the first feed continues during the experiment. The last figure on the right represents the changes in the lag phase parameters i.e. the time needed to reach the exponential activity phase.

The change of the kinetic parameters during the feeding is important to evaluate the effect of a change in the process useful for a starting up. A comparison among the different tests, on the other hand, can give insights into the efficiency of the process in the three conditions. In Figure 4 are reported the kinetic parameters expressed as mean and error and the comparison among the three tests. Negative values as reported for SM mean that the consortium was already active before the start of the experiment and at the time of inoculation was in active growth.

The addition of the syntrophic has a good effect on the test biogas production as can be seen in the first feed. This advantage is not however maintained over the next feedings.

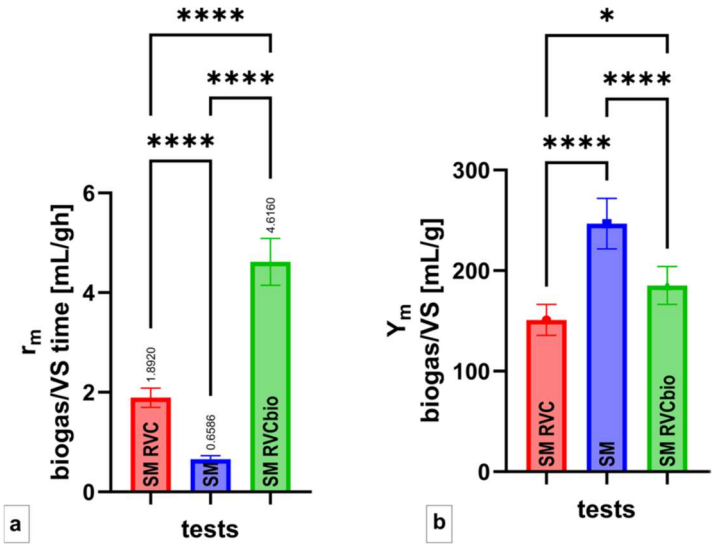


Figure 4: comparison of a max rate of biogas production (rm) and b max cumulative biogas production. One-way ANOVA, Tukey's post hoc, one asterisk (*) identifies adjusted *P* values between 0.01 and 0.05, four asterisks (****) identify adjusted *P* < 0.0001

The comparison of the kinetic data for biogas production showed that the test SM RVCbio had the highest rate (rm) compared to both the SM and the SM RVC, considering then the maximum cumulative production (Ym), the highest value was for the two taxa in suspension (SM). Analyzing the biogas production rate (rm) the three tests gave different results: test SM with the two microbial taxa in suspension gave the lowest value, higher was SM RVC with the conductive carrier and the highest value was recorded for SM RVCbio the test with microorganisms, conductive carrier and *Shewanella* sp. biofilm.

Therefore, considering the material characteristics of RVC and the experimental settings, the data showed that the addition of 8.78 gRVC/L of RVC as CM with an available surface of 1.79 m2 resulted in 86% higher maximum velocity comparing SMRVCbio (2.57 mL/gVS*h) with SM (0.37 mL/gVS*h).

Real conditions tests. In a second set of experiments the effect of the use of the syntrophic inoculum (DIET-able microorganisms) in real conditions meaning the presence of indigenous microbial community that is the sludge and the long-term advantage of CM addition were evaluated.

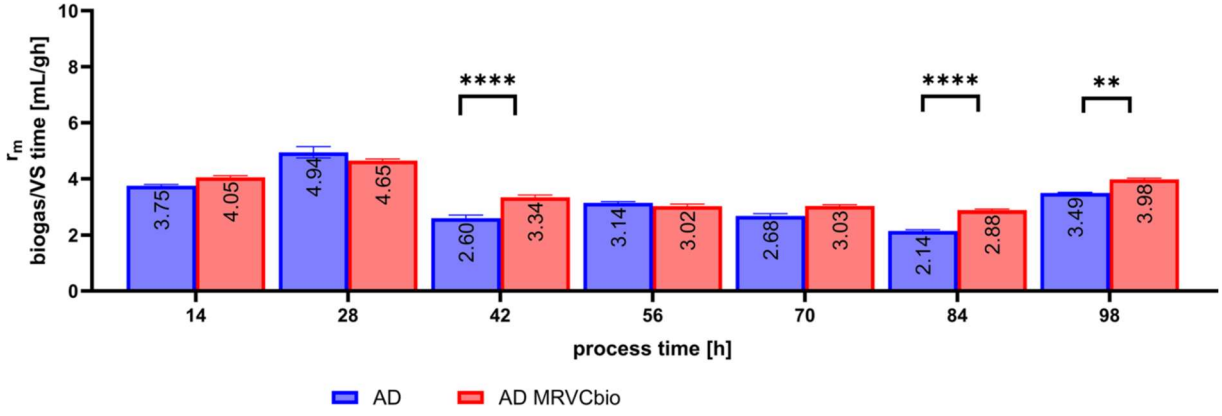


Figure 5: Rate of biogas production compared among feeds asterisks indicates significance (****P < 0.0001 and **P = 0.0032) one-way ANOVA

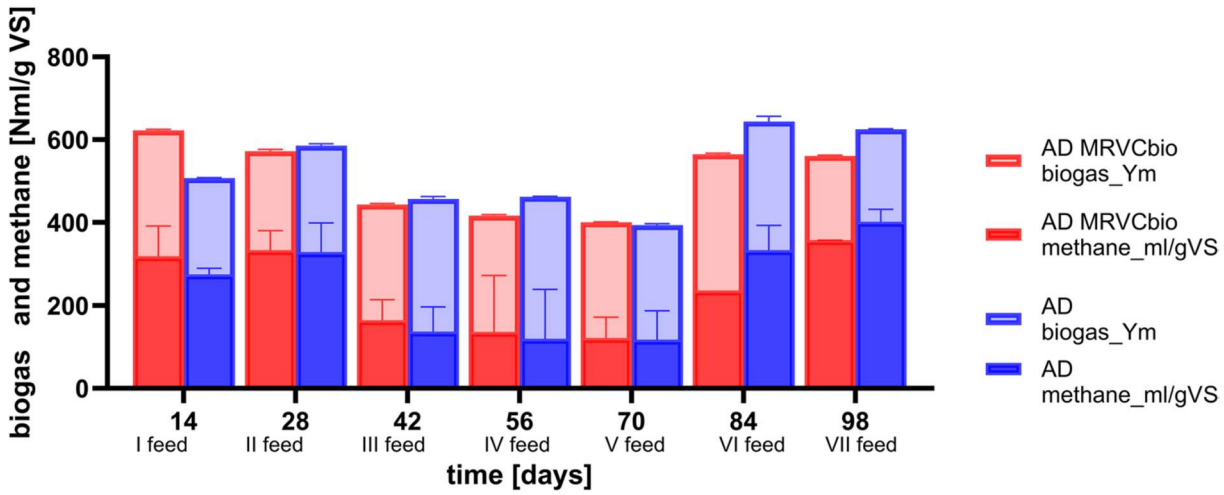


Figure 6: Biogas and methane production measured for each feed

Also, in these set of experiments biogas and methane production measured at each feed were analyzed and the kinetic parameters calculated by nonlinear fitting (Gompertz mod). Results are reported in Figure 5 and Figure 6.

The maximum biogas production rate (rm) differed significantly between the two conditions at 1004 h and during the last two feedings. In these phases, the AD MRVCbio reactor exhibited a faster biogas production, as shown by the one-way ANOVA with Tukey's post hoc test (adjusted $P < 0.0001$).

Regarding the maximum cumulative biogas production (Ym), at the beginning of the experiment specifically after the first feeding the AD MRVCbio produced higher cumulative biogas. However, this trend later reversed, with the reference reactor (AD) generating statistically greater cumulative biogas over time.

Another key factor influencing the energetic value of biogas is its methane content, measured as a percentage and subsequently converted to volume. For the AD reactor, the lowest methane yield was 137.2 mL CH_4/g VS, while for the AD RVCbio reactor it was 164.5 mL CH_4/g VS; both minimum values were recorded at 1341 h. In both reactors, the highest methane yields 401.7 mL CH_4/g VS for AD and 357.1 mL CH_4/g VS for AD RVCbio were observed at the end of the monitoring period (2354 h).

In the assays, 8.78 gRVC/L of conductive material (CM) were added, corresponding to an available surface area of 1.79 m² for biofilm growth. SEM analysis confirmed the presence of microorganisms on the RVC carrier sampled from the AD MRVCbio reactor. Cells putatively belonging to *Methanosarcina* sp. were clearly identifiable due to their characteristic sarcina-like morphology (see Figure 7).

Carrier from HESSO Microbial Fuel Cell inoculated with *Shewanella oneidensis*	Carrier from HESSO Microbial Fuel Cell inoculated with *Shewanella oneidensis* and inoculated in ADMRVCbio with a suspension of *Methanosarcina barkerii* in the reactor ADMRVCbio for 96 days.

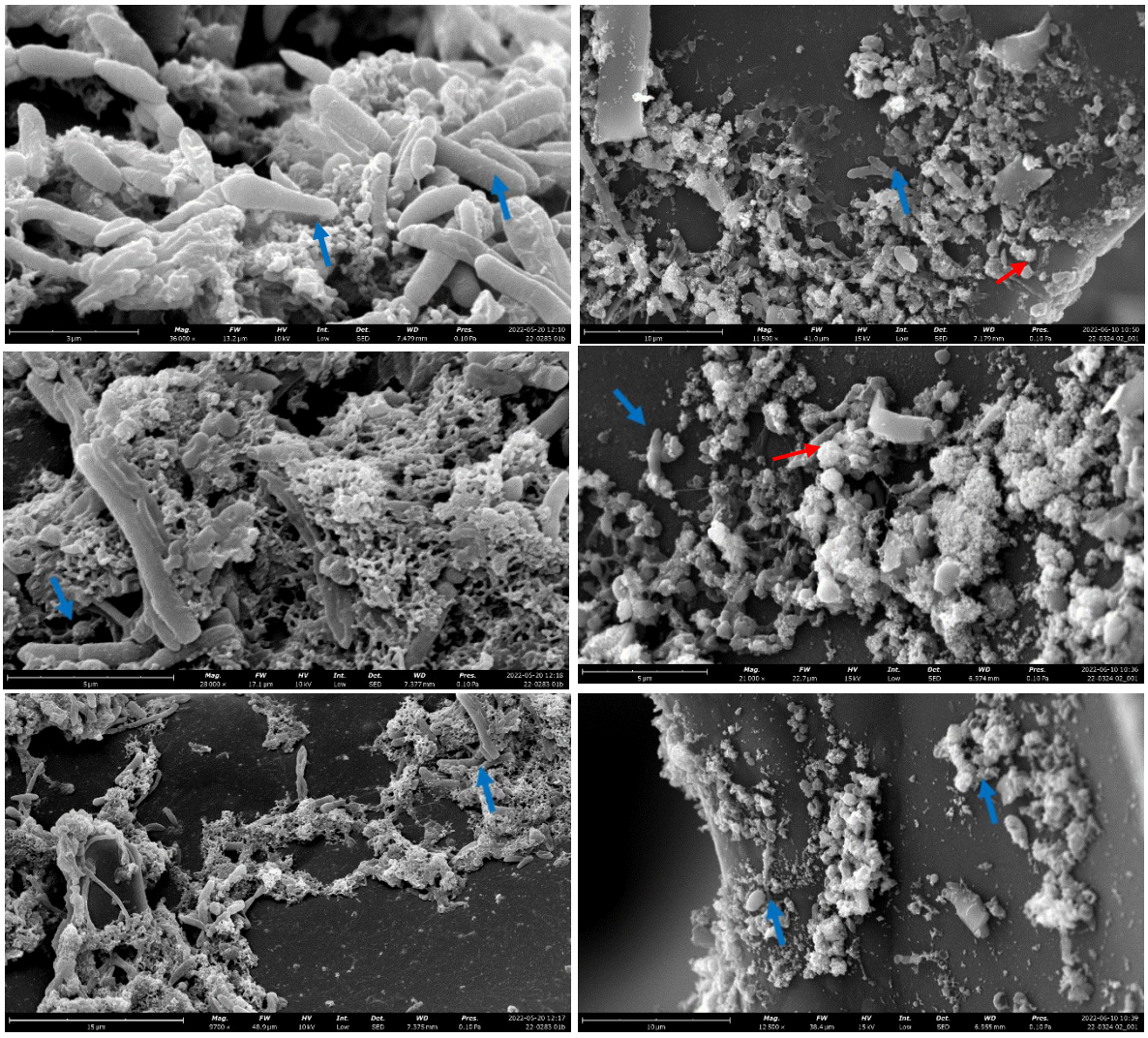


Figure 7: carriers with fresh culture of *Shewanella oneidensis* (left column, and with biofilm after 96 days in the reactor (right column)

The addition of 1.77 (S/m)*m^2 RVC as carrier, after 100 days of process resulted in a faster methane production: 13% higher maximum velocity comparing AD MRVCbio (2.39 mL/gVS*h) with AD (2.08 mL/gVS*h). This advantage can be translated for process engineering applications in shorter retention time with possible smaller reactors volume.

To determine how the syntrophic inoculum behaved when introduced into the complex digestate community, we performed a statistical analysis of the sequencing data. Comparisons of alpha diversity using Wilcoxon rank-sum tests indicated that the two experimental systems maintained similar levels of microbial complexity throughout the assay. In contrast, beta-diversity analyses revealed that the community compositions diverged significantly over time. This divergence confirmed by both Wilcoxon Rank Sum and Signed Rank Tests is illustrated in Figure 8. By 669 h, the microbial profiles had clearly separated into two distinct clusters, demonstrating that compositional differentiation had emerged, even though overall community complexity (alpha diversity) remained stable.

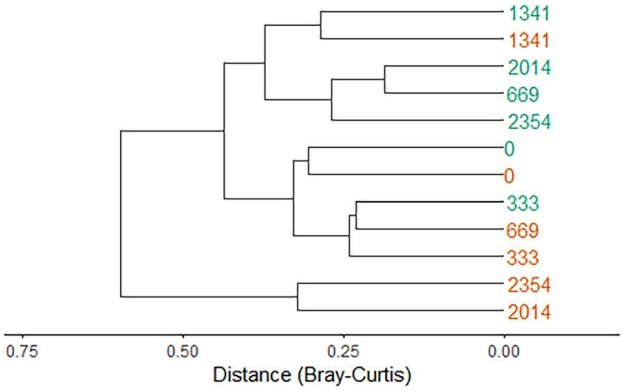


Figure 8: Cluster distance of the two communities over time. In red the OTUs sampled at different times for the AD MRVCbio and in green the OTUs sampled from AD reactors

The relative abundance of microbial taxa was assessed across multiple taxonomic ranks, with the order level selected as the most informative for interpreting community dynamics. The temporal variation of the 20 most abundant orders is shown in Figure 9. The heatmap highlights that both experimental conditions followed broadly similar trends in community structure. The dominant taxa included archaeal groups belonging to *Methanotrichales, Methanomicrobiales*, and *Methanobacteriales*, as well as bacterial orders such as *Eubacteriales, Marinilabiliales, Anaerolineales, Syntrophales*, and *Burkholderiales.* Over the course of the experiment, several bacterial taxa (*Marinilabiliales, Anaerolineales, Syntrophales, Burkholderiales*) and the archaeal *Methanobacteriales* decreased in abundance, whereas the archaeal *Methanosarcinales* became increasingly prominent. In parallel, the bacterial orders *Thermolithobacterales, Thiotrichales,* and *Synergistales* showed a notable increase.

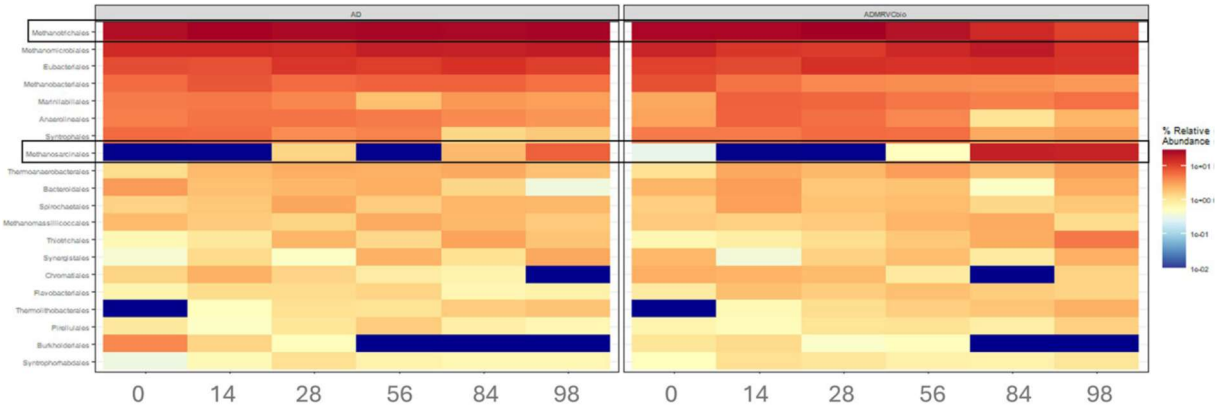


Figure 9: Heatmap of the most abundant 20 taxa reported at order level

A comparison of the two experimental setups revealed distinct patterns in the archaeal community dynamics. In the reference reactor (AD), there was an overall increase in the relative abundance of *Methanotrichales, Methanomicrobiales*, and *Methanosarcinales*, accompanied by a decline in *Methanobacteriales*. Conversely, in the AD MRVCbio reactor, *Methanotrichales* and *Methanomicrobiales* progressively declined, while *Methanosarcinales* expanded sharply, eventually becoming the most abundant among the 20 monitored orders.

Figure 10 presents the distance-based Redundancy Analysis (db-RDA), illustrating how different process parameters acted as predictors of microbial community composition. Among the variables

examined process time, biogas volume, feeding characteristics (TS, VS, COD, total N), and biogas composition (CH_4, CO_2, H_2S) only process time and H_2S significantly contributed to shaping the taxonomic profiles. In the AD MRVCbio system, the late-stage communities (sampled at 2014 and 2354 h), characterized by a strong representation of Methanosarcinales, were primarily influenced by time and were positioned along gradients associated with methane and CO_2 concentrations.

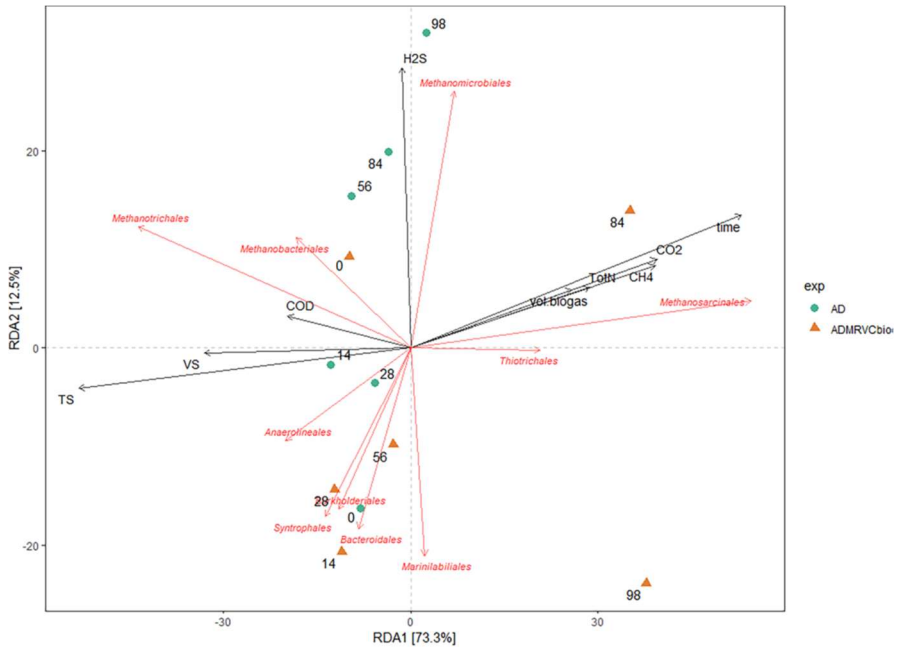


Figure 10:dbRDA describing the correlations between environmental variables and taxa. Pearson correlation method, 999 permutations and Mantel

In short-term AD tests, the addition of ceramic material (RVC) led to an 84% increase in the methane conversion rate. The co-culture of *Shewanella oneidensis*, acting as an endoelectrogen, and *Methanosarcina barkerii*, acting as an exoelectrogen, further confirmed their ability to syntrophically convert organic substrates into methane through electron-transfer mechanisms. In the 100-day experiment using real feedstock, the enhancement in methane production rate was also observed, although to a lesser extent than in the short-term assay, reaching a 13% increase. The presence of RVC appears to promote the metabolic versatility typical of *Methanosarcinales*, ultimately supporting the development of a more stable microbial community capable of coping with the variability of feed composition typically encountered in full-scale anaerobic digestion systems.

Long-term semicontinuous tests with real feed. A pilot-scale experiment was established to evaluate the effect of adding Powdered Activated Carbon (PAC, 20 g/L) as a conductive material on anaerobic digestion performance and on the dynamics of the microbial community under real feedstock conditions. An initial start-up phase was conducted to align the operational parameters of the pilot system with those of the full-scale plant. Once steady-state conditions was reached corresponding to approximately three solids retention times (SRTs) the experiment was initiated and monitored for a total duration of three months.

Biogas production tests were performed using 60L anaerobic digesters installed at the Wastewater Treatment Plant (WWTP) of Chiasso (Switzerland). The reactors were operated following the WWTP standard conditions: a hydraulic retention time of 35 days, a sludge temperature of 37 °C, and

intermittent mixing. The systems ran in semi-continuous mode, with daily feeding of thickened sludge sampled weekly from the WWTP. This configuration allowed for the assessment of PAC addition under realistic operational variability typical of full-scale anaerobic digestion.
Key-process performance parameters have been monitored for the test (R1) and control reactor (R2) for more than 70 days after CM addition and reported in Table 6: reactors performance and process parameters. Data shown are average values and standard deviation in brackets (minimum N=3)Table 6.

Table 6: reactors performance and process parameters. Data shown are average values and standard deviation in brackets (minimum N=3)

	SRT	FOS/TAC		TAC		FOS		pH		sCOD conversion		SO_3^{--}		SO_4^{--}	
	days	- (SD)		mg/L as $CaCO_3$ (SD)		mg/L CH_3COOH (SD)		- (SD)		% (SD)		mg/L (SD)		mg/L (SD)	
R2 (Ctrl)	35	0.141	(0.011)	4379	(159.8)	667.6	(107.8)	7.07	(0.03)	81.25	(4.95)	4.136	(1.022)	94.07	(25
R1 (Test)	35	0.149	(0.023)	3959	(392.8)	612.3	(58.55)	7.23	(0.04)	85.26	(5.45)	4.513	(2.634)	103.5	(21

Comparing biogas production, the test reactor (R1) with Conductive Material (CM) and the reference (R2) performed similarly until the PAC addition. Once the PAC was added as (CM), the two reactors performed similarly for three weeks; after that, the test reactor started to produce more biogas. At the same time as the peak, the maximum methane content was observed (figure 1).

Reactor performance, evaluated through soluble COD (sCOD) removal, consistently exceeded 80% in both the test and control systems. Nevertheless, the reactor supplemented with conductive material exhibited a noticeably higher sCOD conversion efficiency, indicating an improved degradation of soluble organic matter under CM-amended conditions. The FOS/TAC ratios remained within comparable and acceptable ranges for both reactors, suggesting that no substantial acidification events occurred; however, the control reactor showed a lower TAC value, reflecting a reduced buffering capacity. Throughout the monitoring period, pH values remained stable and close to neutrality in both the CM-amended reactor (R1) and the control reactor (R2), confirming that the operational stability of the anaerobic digestion process was maintained across both setups.

The primary indicator for assessing anaerobic digestion (AD) performance is the temporal evolution of biogas and methane production(He *et al.* 2021). In semi-continuous operation, however, the direct comparison of hourly biogas output between experimental conditions is challenging due to the inherent variability of the process. As expected, each feeding event produced a distinct increase in the biogas production rate, generating a recurrent pattern in the dataset. This cyclic behavior can be modelled and quantitatively isolated. For this reason, the biogas production time series was decomposed into three components: the cyclic effect associated with daily feeding (seasonal), the long-term evolution of the process (trend), and the residual unexplained variability (noise) ('Time Series Analysis: With Applications in R | Springer Nature Link', n.d.) (Figure 11: timeseries decomposition of hourly biogas production over 170 days in the test reactor, r1 (left), and the control reactor, r2 (right).Figure 11).

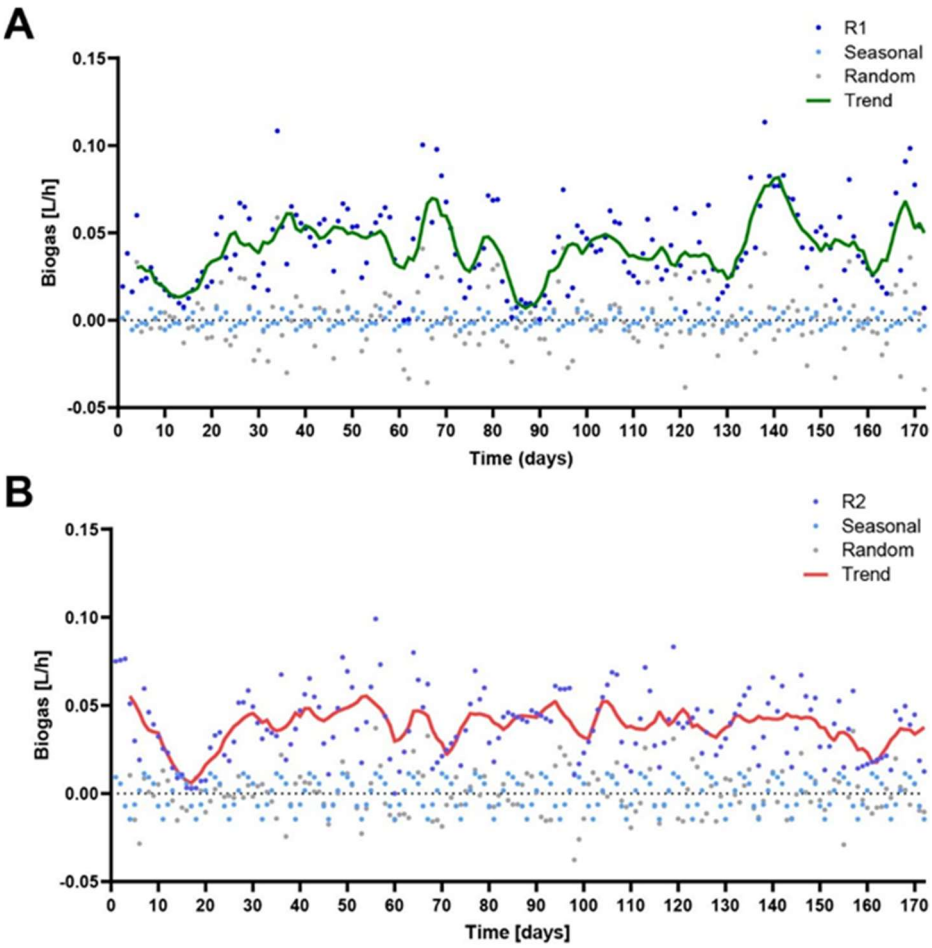


Figure 11: timeseries decomposition of hourly biogas production over 170 days in the test reactor, r1 (left), and the control reactor, r2 (right).

Since the same decomposition algorithm was applied to both the control and the test reactors, the resulting components are directly comparable. Over the entire operational period, the seasonal component displayed similar patterns in both systems, reflecting the identical feeding regime imposed on the reactors.

To better highlight the effect of the conductive material, the analysis focused on the trend component from day 104 onward, corresponding to the period following CM addition (Figure 12). To emphasize differences between the two reactors, the trend curves were further smoothed using a LOESS regression based on least-squares estimation (Gijbels and Prosdocimi 2010), and the resulting smoothed profiles were superimposed.

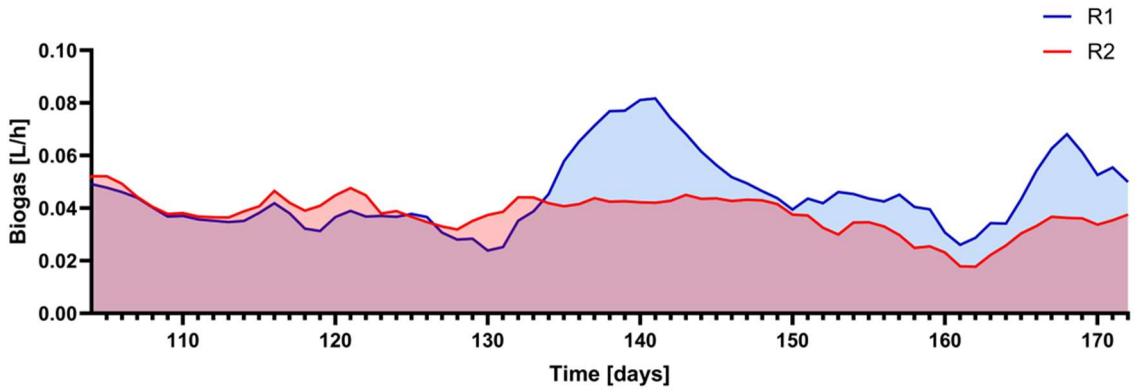


Figure 12:trends for test (R1, blue) and control (R2, red) test with shaded area in blue for R1>R2 and red for R2>R1. The production is expressed as L/h g VS feedstock

Figure 12 highlights the differences in hourly biogas production between the two reactors, visualized as shaded areas. During the first hydraulic retention time (HRT, 35 days), the control reactor (red line) and the CM-amended reactor (blue line) exhibited comparable trends, indicating similar baseline performance.

After this initial phase, the positive effect of the conductive material on biogas generation became evident. Between days 135 and 172, the reactor supplemented with CM (R1) showed a clear increase in biogas production, illustrated by the blue-shaded region in Figure 2. This enhancement emerged after one additional HRT, corresponding to the time required for the microbial community to adapt to the amended conditions. Overall, the reactor with CM achieved a higher specific biogas production rate 4.52 mL h^{-1} gVS^{-1} compared with 3.79 mL h^{-1} gVS^{-1} measured in the control system, confirming the beneficial impact of conductive material addition on process performance.

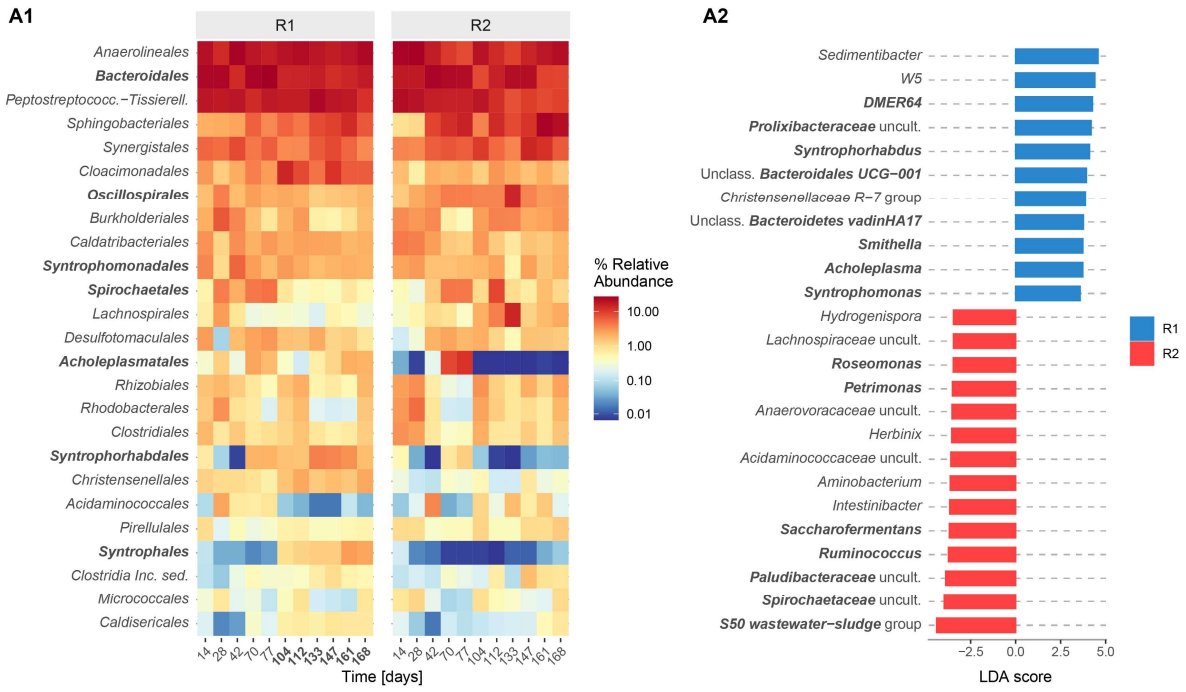


Figure 13: Relative abundance heatmap for the 25 most represented bacterial orders across timepoints in test (R1) and control (R2) reactors

To assess the impact of PAC addition on the microbial community, 16S rDNA metabarcoding was performed on all sampling points using long-read sequencing. Bacterial and archaeal communities were analyzed separately. The results are presented as heatmaps showing the relative abundance of the 20 most represented taxa at the order level (Figure 13).
Alpha diversity, computed using the Shannon index, did not differ significantly between the two reactors ($p > 0.05$), with mean values of 3.14 ± 0.15 s.d. for the PAC-amended reactor (R1) and 3.19 ± 0.18 s.d. for the control (R2). This indicates that community richness and evenness remained broadly comparable across conditions.

5 Conclusions

The present project aimed to evaluate the feasibility of introducing syntrophic microorganisms into a real-scale anaerobic digestion (AD) environment. Previous studies, including work from our research group, have demonstrated that the addition of conductive materials can enhance AD performance by supporting direct interspecies electron transfer (DIET) among electrogenic syntrophic microorganisms. Based on this evidence, two microorganisms known for their electrogenic activity within AD systems *Shewanella oneidensis* and *Methanosarcina barkerii* were selected, cultivated at laboratory scale, and subsequently inoculated into 3.5 L reactors. A key challenge considered in this study was the competitive interaction between the introduced syntrophic consortium and the indigenous microbial community naturally present in the digestate. To capture this ecological dynamic, three long-term semi-continuous feeding experiments were conducted.
In short-term AD tests, the addition of reticulated vitreous carbon (RVC) as a conductive material resulted in an 84% increase in methane conversion rate. The co-culture of *S. oneidensis* (acting as an endoelectrogen) and *M. barkerii* (functioning as an exoelectrogen) confirmed their syntrophic cooperation in converting organic substrates to methane through electron-transfer mechanisms. In a subsequent 100-day experiment using real feedstock, the positive effect of conductive material persisted, although to a lesser degree: methane production increased by 13%. The presence of RVC appeared to promote the metabolic versatility characteristic of *Methanosarcinales* an archaeal group capable of performing hydrogenotrophic, acetoclastic, and methylotrophic methanogenesis resulting in a more stable and resilient microbial community, better suited to the fluctuating feed composition typical of full-scale digesters.
A complementary pilot-scale trial further investigated the influence of conductive materials using powdered activated carbon (PAC, 20 g L^{-1}) added to reactors fed daily with thickened sludge. After approximately one hydraulic retention time (35 days), the PAC-supplemented reactor exhibited a higher specific biogas production rate (4.52 mL h^{-1} gVS^{-1}) than the control (3.79 mL h^{-1} gVS^{-1}). These results are consistent with previous findings from our group and align with literature reports, such as (Wang *et al.* 2023), who observed enhanced biogas production even at lower PAC concentrations (10 g L^{-1}).
Microbial community analysis provides insight into the mechanisms underlying this enhanced performance. In the PAC-amended reactor (R1), an increase in syntrophic bacteria and hydrogenotrophic methanogens was observed, suggesting that PAC fosters closer metabolic interactions potentially through DIET between syntrophic partners and hydrogenotrophic archaea. Despite shifts in several functional groups, *Methanosarcinales* remained the dominant archaeal order. Due to its broad metabolic capabilities, an increase in *Methanosarcinales* abundance does not, by itself, indicate a preferential methanogenic pathway. However, *Methanosarcina* is commonly enriched in digesters with elevated acetate levels (> 0.2 mM) (Grotenhuis 1992), and the absence of increased syntrophic taxa in the control reactor (R2) suggests that acetoclastic methanogenesis may play a greater role in the reference system.

These observations align with recent literature indicating that hydrogenotrophic methanogens often dominate when conductive materials such as PAC or GAC are applied (Nabi *et al.* 2024).
The mechanisms by which PAC stimulates AD performance remain the subject of active investigation. (Yan *et al.* 2020) proposed that PAC may enhance hydrolysis, thereby improving substrate availability. Other authors(Nabi *et al.* 2024) have suggested that conductive materials may influence the composition or structure of extracellular polymeric substances (EPS), facilitating more efficient microbial interactions. The present results particularly the enrichment of syntrophic and hydrogenotrophic taxa point toward improved interspecies electron transfer as a plausible contributing mechanism, while synergistic effects involving hydrolysis and EPS modulation cannot be excluded.

6 Outlook and next steps

The integration of conductive materials and syntrophic microorganisms into anaerobic digestion systems offers promising opportunities for improving the efficiency, robustness, and adaptability of full-scale biogas production. Although the present findings support the beneficial role of conductive materials in enhancing interspecies electron transfer and stabilizing microbial communities, several aspects remain to be explored before these strategies can be fully optimized for operational deployment. Future research should focus on understanding the long-term ecological interactions within complex digestate communities, including potential competition, resilience to perturbations, and the stability of electrogen-assisted syntrophic networks.

Advances in sequencing technologies, multi-omics approaches, and real-time process monitoring will be essential to unravel the mechanistic basis of DIET-mediated enhancement at real scale. Moreover, determining the optimal type, concentration, and lifetime of conductive materials including their economic and environmental impacts will be critical for translating laboratory successes into practical full-scale applications. Investigating alternative conductive materials, engineered consortia, and adaptive operational strategies may further expand the potential of this technology.

Ultimately, integrating microbial-electrochemical concepts into traditional anaerobic digestion could contribute to more efficient resource recovery, increased methane yields, and a more resilient circular bioeconomy. Continued interdisciplinary collaboration between microbiologists, process engineers, and material scientists will be crucial to fully harness the potential of these emerging strategies.

7 National and international cooperation

The project partnership is a diverse group of researchers affiliated at SUPSI and HES-SO and industrial partners.

8 Communication

Part of the results have presented at the SFOE-Conference "Bioenergieforschung in der Schweiz" on the 25.4.2023.

9 Publications

König, R., Cuomo, M., Pianta, E., Buetti, A., Mauri, F., Tanadini, M., & Principi, P. (2022). Addition of conductive materials to support syntrophic microorganisms in anaerobic digestion. Fermentation, 8(8), 354.

Perego, Camilla, Margherita Cappa, Simone Olivo, Juan Diaz-Miyar, Fabian Fischer, e Pamela Principi. *Conductive material addition to optimize semicontinuous biogas production*. 2025. https://aris.supsi.ch/handle/123456789/345347.

Perego, Camilla, Roger König, Maurizio Cuomo, *et al.* «Shewanella Oneidensis and Methanosarcina Barkerii Augmentation and Conductive Material Effects on Long-Term Anaerobic Digestion Performance». *Biotechnology for Biofuels and Bioproducts* 18, fasc. 1 (2025): 10. https://doi.org/10.1186/s13068-025-02609-6.

10 References

Ahuja, Vishal, Chhavi Sharma, Debarati Paul, *et al.* 2024. 'Unlocking the Power of Synergy: Cosubstrate and Coculture Fermentation for Enhanced Biomethane Production'. *Biomass and Bioenergy* 180 (January): 106996. https://doi.org/10.1016/j.biombioe.2023.106996.

American Public Health Association APHA. 2016. 'Standard Methods For the Examination of Water and Wastewater'. In *Standard Methods For the Examination of Water and Wastewater*, 22st edn. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. https://doi.org/doi:10.2105/SMWW.2882.013.

Ampese, Larissa Castro, William Gustavo Sganzerla, Henrique Di Domenico Ziero, Ackmez Mudhoo, Gilberto Martins, and Tânia Forster-Carneiro. 2022. 'Research Progress, Trends, and Updates on Anaerobic Digestion Technology: A Bibliometric Analysis'. *Journal of Cleaner Production* 331 (January): 130004. https://doi.org/10.1016/j.jclepro.2021.130004.

Ancona, Valeria, Claudia Campanale, Marina Tumolo, *et al.* 2020. 'Enhancement of Chromium (VI) Reduction in Microcosms Amended with Lactate or Yeast Extract: A Laboratory-Scale Study'. *International Journal of Environmental Research and Public Health*, ahead of print. https://doi.org/10.3390/ijerph17030704.

Appels, Lise, Jan Baeyens, Jan Degrève, and Raf Dewil. 2008. 'Principles and Potential of the Anaerobic Digestion of Waste-Activated Sludge'. *Progress in Energy and Combustion Science* 34 (6): 755–81. https://doi.org/10.1016/j.pecs.2008.06.002.

Bhujbal, Sachin Krushna, Madan Kumar, Virendra Kumar Vijay, Vivek Kumar, and Pooja Ghosh. 2021. 'Potential of Termite Gut Microbiota for Biomethanation of Lignocellulosic Wastes: Current Status and Future Perspectives'. *Reviews in Environmental Science and Bio/Technology* 20 (2): 419–38. https://doi.org/10.1007/s11157-021-09576-y.

Dolch, Kerstin, Joana Danzer, Tobias Kabbeck, *et al.* 2014. 'Characterization of Microbial Current Production as a Function of Microbe-Electrode-Interaction'. *Bioresource Technology*, ahead of print. https://doi.org/10.1016/j.biortech.2014.01.112.

Fernandez-Bayo, Jesus D., Christopher W. Simmons, and Jean S. VanderGheynst. 2020. 'Characterization of Digestate Microbial Community Structure Following Thermophilic Anaerobic Digestion with Varying Levels of Green and Food Wastes'. *Journal of Industrial Microbiology and Biotechnology* 47 (12): 1031–44. https://doi.org/10.1007/s10295-020-02326-z.

Gahlot, Pallavi, Banafsha Ahmed, Satya Brat Tiwari, *et al.* 2020. 'Conductive Material Engineered Direct Interspecies Electron Transfer (DIET) in Anaerobic Digestion: Mechanism and Application'. *Environmental Technology and Innovation* 20: 101056. https://doi.org/10.1016/j.eti.2020.101056.

Gijbels, Irène, and Ilaria Prosdocimi. 2010. 'Loess'. *Wiley Interdisciplinary Reviews: Computational Statistics* 2 (5): 590–99. https://doi.org/10.1002/wics.104.

Grotenhuis, J. T. C. 1992. *Structure and Stability of Methanogenic Granular Sludge*. June 23. https://doi.org/10.18174/203137.

Harris, Peter W., and Bernadette K. McCabe. 2015. 'Review of Pre-Treatments Used in Anaerobic Digestion and Their Potential Application in High-Fat Cattle Slaughterhouse Wastewater'. *Applied Energy* 155 (October): 560–75. https://doi.org/10.1016/j.apenergy.2015.06.026.

He, Xia, Zhenyu Guo, Jian Lu, and Ping Zhang. 2021. 'Carbon-Based Conductive Materials Accelerated Methane Production in Anaerobic Digestion of Waste Fat, Oil and Grease'. *Bioresource Technology* 329 (June): 124871. https://doi.org/10.1016/j.biortech.2021.124871.

Khalid, Azeem, Muhammad Arshad, Muzammil Anjum, Tariq Mahmood, and Lorna Dawson. 2011. 'The Anaerobic Digestion of Solid Organic Waste'. *Waste Management* 31 (8): 1737–44. https://doi.org/10.1016/j.wasman.2011.03.021.

König, Roger, Maurizio Cuomo, Elisa Pianta, *et al.* 2022. 'Addition of Conductive Materials to Support Syntrophic Microorganisms in Anaerobic Digestion'. *Fermentation* 8 (8). https://doi.org/10.3390/fermentation8080354.

Lay, Jiunn-Jyi, Yu-You Li, and Tatsuya Noike. 1997. 'Influences of pH and Moisture Content on the Methane Production in High-Solids Sludge Digestion'. *Water Research* 31 (6): 1518–24.

Li, Lei, Xuya Peng, Xiaoming Wang, and Di Wu. 2018. 'Anaerobic Digestion of Food Waste: A Review Focusing on Process Stability'. *Bioresource Technology*, Bioconversion of Food Wastes, vol. 248 (January): 20–28. https://doi.org/10.1016/j.biortech.2017.07.012.

Liu, Xiaojun, André Pauss, Laura André, and Thierry Ribeiro. 2025. 'An Upgraded FOS/TAC Titration Model Integrating Phosphate Effects for Accurate Assessments of Volatile Fatty Acids and

Alkalinity in Anaerobic Media'. *ChemEngineering* 9 (3). https://doi.org/10.3390/chemengineering9030053.

Mang, Stefania Mirela, Vincenzo Trotta, Antonio Scopa, and Ippolito Camele. 2022. 'Metagenomic Analysis of Bacterial Community Structure and Dynamics of a Digestate and a More Stabilized Digestate-Derived Compost from Agricultural Waste'. *Processes* 10 (2). https://doi.org/10.3390/pr10020379.

Nabi, Mohammad, Keke Xiao, and Dawen Gao. 2024. 'Elevating Anaerobic Digestion Efficiency: Unveiling the Alchemical Effects of Conductive Materials on Extracellular Polymeric Substances (EPSs) Composition'. *Journal of Environmental Chemical Engineering* 12 (5): 113491. https://doi.org/10.1016/j.jece.2024.113491.

Narihiro, Takashi, and Yuji Sekiguchi. 2011. 'Oligonucleotide Primers, Probes and Molecular Methods for the Environmental Monitoring of Methanogenic Archaea'. In *Microbial Biotechnology*. Preprint. https://doi.org/10.1111/j.1751-7915.2010.00239.x.

Nguyen, Luong N., Minh T. Vu, Md Abu Hasan Johir, *et al.* 2021. 'Promotion of Direct Interspecies Electron Transfer and Potential Impact of Conductive Materials in Anaerobic Digestion and Its Downstream Processing - a Critical Review'. *Bioresource Technology* 341 (December): 125847. https://doi.org/10.1016/j.biortech.2021.125847.

Park, Jeong-Hoon, Hyun-Jin Kang, Kang-Hee Park, and Hee-Deung Park. 2018. 'Direct Interspecies Electron Transfer via Conductive Materials: A Perspective for Anaerobic Digestion Applications'. *Bioresource Technology* 254 (April): 300–311. https://doi.org/10.1016/j.biortech.2018.01.095.

Perego, Camilla, Roger König, Maurizio Cuomo, *et al.* 2025. 'Shewanella Oneidensis and Methanosarcina Barkerii Augmentation and Conductive Material Effects on Long-Term Anaerobic Digestion Performance'. *Biotechnology for Biofuels and Bioproducts* 18 (1): 10. https://doi.org/10.1186/s13068-025-02609-6.

Principi, Pamela, Roger König, and Maurizio Cuomo. 2019. 'Anaerobic Digestion of Lignocellulosic Substrates: Benefits of Pre-Treatments'. *Current Sustainable/Renewable Energy Reports* 6 (3): 61–70. https://doi.org/10.1007/s40518-019-00131-6.

Subbarao, Paruchuri M. V., Tinku Casper D' Silva, Komalkant Adlak, Subodh Kumar, Ram Chandra, and Virendra Kumar Vijay. 2023. 'Anaerobic Digestion as a Sustainable Technology for Efficiently Utilizing Biomass in the Context of Carbon Neutrality and Circular Economy'. *Environmental Research* 234 (October): 116286. https://doi.org/10.1016/j.envres.2023.116286.

Sun, Jiaji, Eldon R. Rene, Yuhe He, Weifang Ma, Qian Hu, and Bin Qiu. 2023. 'Carbon, Iron, and Polymer-Based Conductive Materials for Improving Methane Production in Anaerobic Wastewater Treatment Systems: A Review on Their Direct Interspecific Electron Transfer Mechanism'. *Fuel* 342 (June): 127703. https://doi.org/10.1016/j.fuel.2023.127703.

'Time Series Analysis: With Applications in R | Springer Nature Link'. n.d. Accessed 10 March 2026. https://link.springer.com/book/10.1007/978-0-387-75959-3.

Wang, Panliang, Xunan Li, Ye Li, Yinglong Su, Dong Wu, and Bing Xie. 2023. 'Enhanced Anaerobic Digestion Performance of Food Waste by Zero-Valent Iron and Iron Oxides Nanoparticles: Comparative Analyses of Microbial Community and Metabolism'. *Bioresource Technology* 371 (March): 128633. https://doi.org/10.1016/j.biortech.2023.128633.

Wei, Wei, Xingdong Shi, Lan Wu, Xiaoqing Liu, and Bing-Jie Ni. 2022. 'Calcium Peroxide Pre-Treatment Improved the Anaerobic Digestion of Primary Sludge and Its Co-Digestion with Waste Activated Sludge'. *Science of The Total Environment* 828 (July): 154404. https://doi.org/10.1016/j.scitotenv.2022.154404.

Wu, Li-Jie, Takuro Kobayashi, Hidetoshi Kuramochi, Yu-You Li, Kai-Qin Xu, and Yongkang Lv. 2018. 'High Loading Anaerobic Co-Digestion of Food Waste and Grease Trap Waste: Determination of the Limit and Lipid/Long Chain Fatty Acid Conversion'. *Chemical Engineering Journal* 338 (April): 422–31. https://doi.org/10.1016/j.cej.2018.01.041.

Yan, Wangwang, Liang Zhang, Surya Maitri Wijaya, and Yan Zhou. 2020. 'Unveiling the Role of Activated Carbon on Hydrolysis Process in Anaerobic Digestion'. *Bioresource Technology* 296 (January): 122366. https://doi.org/10.1016/j.biortech.2019.122366.

Zhang, Jishi, Wenqian Zhao, Huiwen Zhang, Zejie Wang, Chuanfang Fan, and Lihua Zang. 2018. 'Recent Achievements in Enhancing Anaerobic Digestion with Carbon- Based Functional Materials'. *Bioresource Technology* 266 (October): 555–67. https://doi.org/10.1016/j.biortech.2018.07.076.

Zhang, Quanguo, Jianjun Hu, and Duu-Jong Lee. 2016. 'Biogas from Anaerobic Digestion Processes: Research Updates'. *Renewable Energy* 98 (December): 108–19. https://doi.org/10.1016/j.renene.2016.02.029.