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Data Article

A comprehensive dataset for assessing the impact of ammonium salts and zeolite on anaerobic digestion performance, microbial dynamics, and metabolomic profiles

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A R T I C L E I N F O

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Biogas
Energy production

A B S T R A C T

This article presents comprehensive data derived from lab-scale batch anaerobic digesters that were subjected to inhibition by various sources of ammonia. To counter this inhibition, zeolite was introduced into selected digesters. The provided dataset offers a detailed depiction of degradation performance dynamics over time, as well as insights into both microbial and metabolic changes during the inhibition. In detail, 10 conditions were tested in triplicate. In a first series of 15 bioreactors ammonia was introduced to achieve a TAN concentration of 8 g/L, utilizing NH3 solution, NH_4Cl salt, $(NH_4)_2CO_3$ salt, or $(NH_4)_2PO_4$ salt as inhibitors. A control condition without ammonia was also set up. A second series of 15 bioreactors was set up exactly as the first one, with the addition of zeolite at a concentration of 15 g/L. The data provided includes information on operational conditions, degradation performance measurements throughout the entire process (using biogas production and composition, dissolved organic and inorganic carbon, volatile fatty acids, pH, free and total ammonia nitrogen, apparent isotopic fractionation of biogas as indicators), microbial community analysis us-

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ing 16S rRNA gene sequencing (50 samples analysed), and metabolomic analysis through liquid chromatography-mass spectrometry (LC-MS) (108 samples analysed). Sequencing data were generated by using IonTorrent PGM sequencer. The sequencing data have been deposited with links to project PRJEB52324, in ENA database from EBI (https://www.ebi.ac.uk/ena/browser/view/PRJEB52324). Sample accession numbers go from SAMEA14277573 to SAMEA14277621. The metabolomic data were generated using an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, MA, US). The metabolomic data have been deposited to the EMBL-EBI MetaboLights database with the identifier MTBLS7859 (https://www.ebi.ac.uk/metabolights/MTBLS7859). This data can be used as a source for comparisons with other studies focusing on the inhibition of anaerobic digestion by ammonia, particularly in the context of exploring microbial or metabolomic dynamics during inhibition. Additionally it provides a multi-omic dataset (metataxonomic and metabolomic) with detailed associated metadata describing anaerobic digesters. The dataset is directly is associated to the research article titled “Inhibition of anaerobic digestion by various ammonia sources resulted in subtle differences in metabolite dynamics.” [1].

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Specifications Table

Subject	*Biological sciences / metabolomics.*
Specific subject area	*Microbial ecology of the anaerobic digestion*
Data format	Raw performance parameters (.csv) *Raw sequencing data (.fastq)* *mzXML-format data converted (.mzxml) from the raw data acquired with LC-MS*
Type of data	Tables, Figures, sequencing data, mass spectrometry data
Data collection	30 anaerobic batch digesters were conducted in triplicate to assess the impact of four distinct sources of ammonia on both anaerobic digestion performance and microbial dynamics. The study also investigated the efficacy of zeolite addition in mitigating inhibition, while control setups without ammonia were included for comparison. The digesters were monitored across time regularly. Gas production and composition were measured directly in the digesters. Gas samples (7 mL) were collected weekly for carbon isotopic composition analysis. Liquid samples (10 mL) were taken weekly and centrifuged at 10,000 X g for 10 minutes. Subsequently, the resulting pellets were promptly snap-frozen in liquid nitrogen. These pellets, along with the supernatants, were stored separately at -80°C before respectively DNA and metabolites extraction and dilution for VFA, DOC/DIC, pH, TAN and FAN analysis. DNA sequencing was carried out with the Ion Torrent Personal Genome Machine. Metabolomic data was acquired with LC-MS composed of Accela 1250 pump system connected to an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, MA, US).
Data source location	*Antony, France*
Data accessibility	*Performance data are available in the article.* The sequencing data have been deposited with links to project PRJEB52324, in ENA database from EBI (https://www.ebi.ac.uk/ena/browser/view/PRJEB52324). Sample accession numbers go from SAMEA14277573 to SAMEA14277621. The metabolomic data have been deposited to the EMBL-EBI MetaboLights database with the identifier MTBLS7859 (https://www.ebi.ac.uk/metabolights/MTBLS7859).
Related research article	Wang, X., et al., *Inhibition of anaerobic digestion by various ammonia sources resulted in subtle differences in metabolite dynamics.* Chemosphere, 2024: p. 141157. [1]

1. Value of the Data

- This dataset establishes correlations between anaerobic digesters performance metrics (biogas production, levels of dissolved organic and inorganic carbon, accumulation of volatile fatty acids, and methanogenesis pathways), the source of ammonia causing inhibition, the presence or absence of zeolite as an inhibition mitigator, and the microbial community composition along with metabolomic profiles at different time points.
- The comprehensive data presented in this paper serves as a valuable resource for comparative studies related to ammonia-induced inhibition in anaerobic digestion. Researchers interested in co-inhibition effects or inhibition mitigation will find this information particularly relevant for their investigations.
- Under similar experimental conditions, ten different conditions were examined in triplicates, utilizing identical inoculum, feeding protocols, and sampling times. A substantial number of samples from selected conditions were sequenced and analyzed via LC-MS at various time points, enhancing the robustness and depth of the study.
- The sequencing data provides insights into the distinctive microbial characteristics associated with different types of inhibition, while metabolomics data elucidates the metabolic alterations within digesters due to varying ammonia sources and the addition of zeolite. Access to detailed 16S rRNA sequence data, LC-MS data, and associated metadata empowers researchers to conduct innovative analyses, including the identification of bioindicators of inhibition. Furthermore, it could foster the development of novel methodologies for integrating and visualizing multi-omics data.

2. Background

Ammonia is recognized as a significant inhibitor of anaerobic digestion (AD). Numerous studies have extensively explored its impact on the performance of the process, as well as on the associated microbial dynamics. They also evaluated different strategies to mitigate the inhibition, such as the addition of support materials. Among them, zeolite, a mineral medium, is known for its potential to reduce ammonia stress during anaerobic digestion [2] and inhibitory stress in general [3]. It was found to have multiple application for biological treatment processes such as such as anaerobic digestion, nitrification, denitrification and composting [3].

While ammonia in anaerobic digesters primarily arises from the decomposition of nitrogen-rich compounds such as proteins, urea, or nucleic acids, at lab-scale and for practical reasons researchers often opt for the direct addition of ammonium salts to simulate its accumulation [4–6]. This approach involves adding an anion along with the ammonium, potentially causing an additional effect on the process—a facet that we wanted to examine in this dataset. Furthermore, our objective was to assess the zeolite's capacity to alleviate the inhibition caused by different types of salt.

In addition to the original research article [7], this data paper also provides data on microbial dynamics during the experiment, details the performance and metabolomics data and present data of the digesters with zeolite.

3. Data Description

Fig. 1 illustrates the global experimental design. Briefly, 30 anaerobic batch digesters were set up to evaluate in triplicate the influence of 4 sources of ammonia (NH_3 solution or NH_4Cl salt or $(NH_4)_2CO_3$ salt or $(NH_4)_2PO_4$ salt) on anaerobic digestion performances and microbial dynamics, in presence, or not, of zeolite. Non-inhibited controls were also set up. Data presented in Table 1 details the nomenclature of the digesters, the source of ammonia added in each digester, the presence or absence of zeolite, as well as the samples which were selected to sequence the

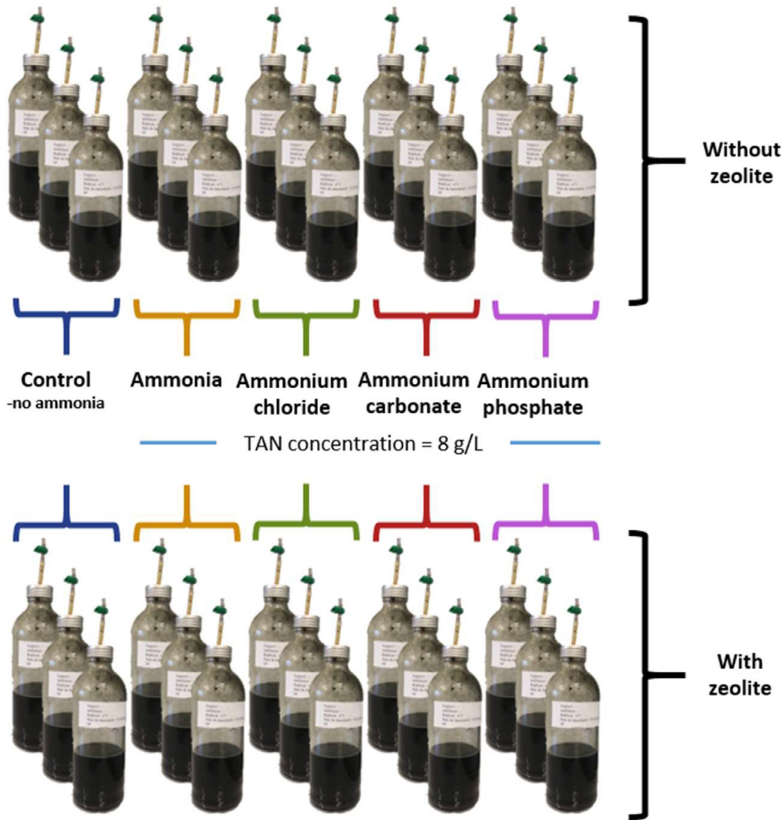

Fig. 1. Experimental design.

V4-V5 region of 16S rRNA and for LCMS metabolomic analysis. Table 2 and Table 3 present cumulated CH_4 and CO_2 production over time of each digester while Fig. 2 illustrates this data (mean per triplicate of digesters). Table 4, Table 5 and Table 6 present the volatile fatty acids (acetic acid, propionic acid, butyric acid) accumulation over time of each digester while Fig. 3 illustrates the same datasets (mean per triplicate of digesters). Table 7 and Table 8 show the dissolved organic and inorganic carbon (DOC and DIC) accumulation over time of each digester while Fig. 4 illustrates the same datasets (mean per triplicate of digesters). Table 9, Table 10 and Table 11 show the pH, total ammonia nitrogen (TAN) and free ammonia nitrogen (FAN) concentration over time of each digester while Fig. 5 illustrates the same datasets (mean per triplicate of digesters). Table 12 presents the apparent isotope fractionation (α_{app}) of the biogas over time (number of days) for the different conditions tested. It is shown in Fig. 6. It was not measured for the condition with ammonia solution as no biogas was produced.

4. Experimental Design, Materials and Methods

4.1. Experimental design and sampling

30 anaerobic batch bioreactors were initially seeded with 6.3 g of centrifuged methanogenic sludge as inoculum and fed with 52.2 g of mashed biowaste as substrate corresponding to an

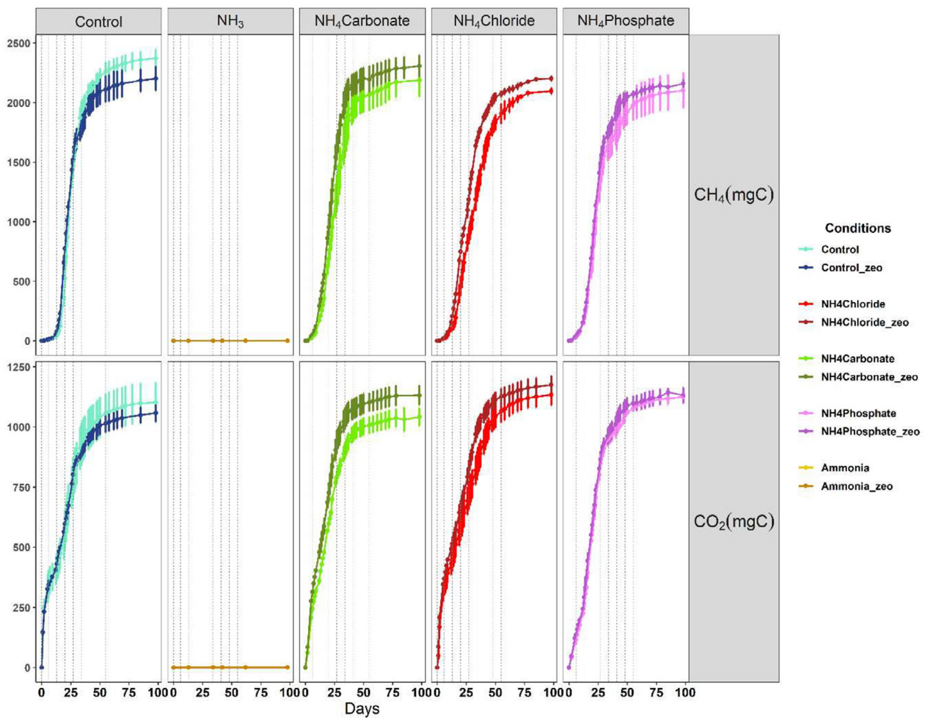

Fig. 2. Cumulated CH_4 and CO_2 production (mg of C) over time (number of days) for the different conditions tested. The data are the mean values for the triplicate bioreactors, standard deviations are indicated with error bars. Vertical lines show the dates of sampling.

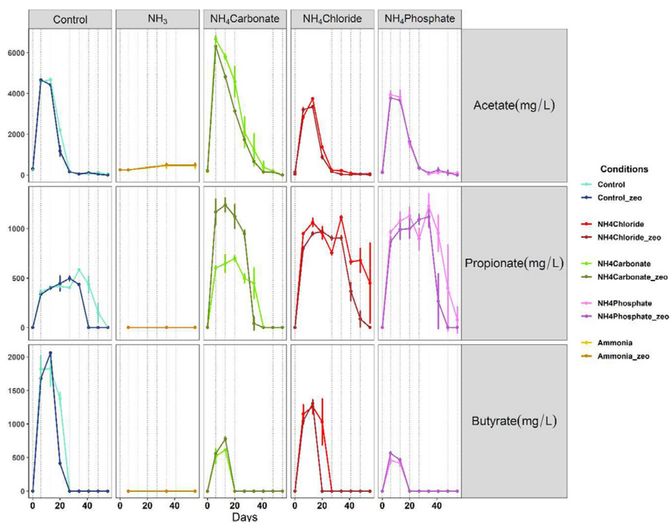

Fig. 3. Volatile fatty acids (acetic acid, propionic acid, butyric acid) concentrations (mg/L) over time (number of days) for the different conditions tested.
The data are the mean values for the triplicate bioreactors, standard deviations are indicated with error bars. Vertical lines show the dates of sampling.

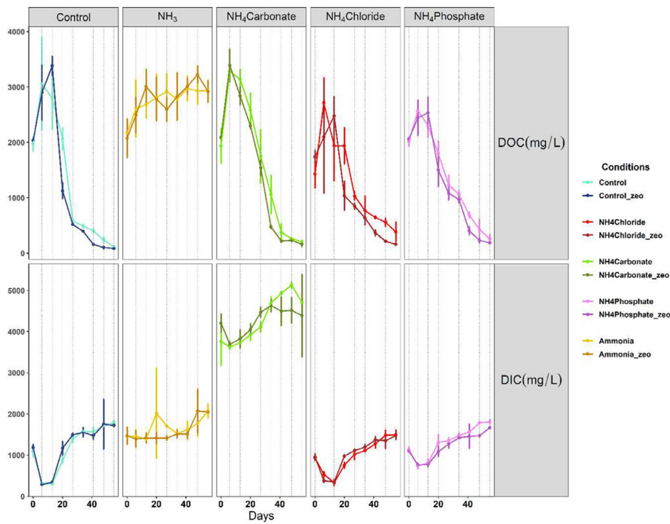

Fig. 4. Dissolved organic and inorganic carbon (DOC and DIC) concentrations (mg of C/L) over time (number of days) for the different conditions tested.
The data are the mean values for the triplicate bioreactors, standard deviations are indicated with error bars. Vertical lines show the dates of sampling.

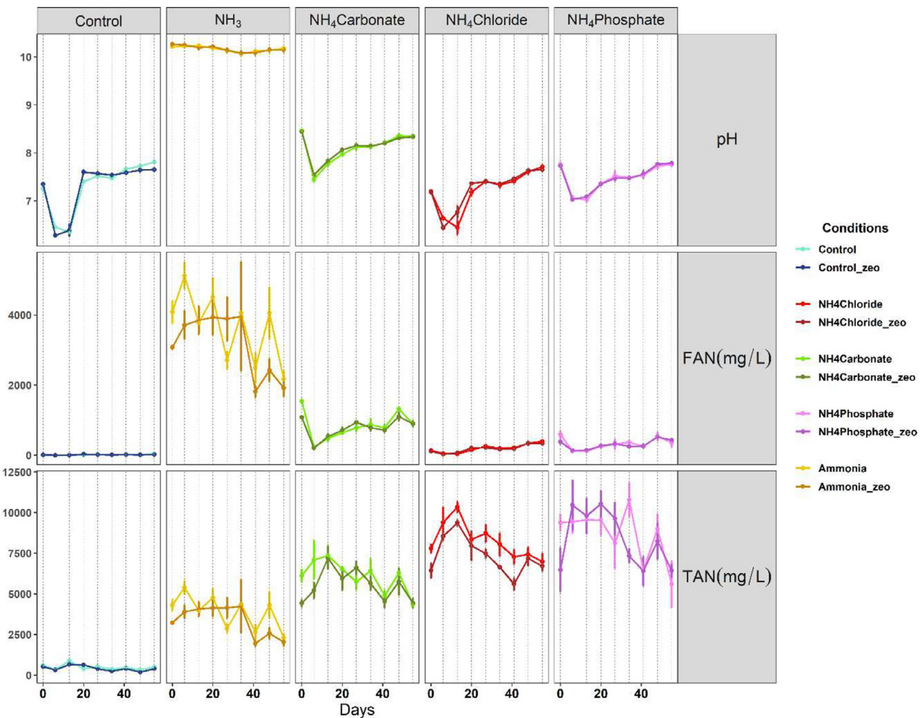

Fig. 5. pH, total ammonia concentration (TAN) (mg per L) and free ammonia concentration (FAN) (mg per L) over time (number of days) for the different conditions tested.
The data are the mean values for the triplicate bioreactors, standard deviations are indicated with error bars. Vertical lines show the dates of sampling.

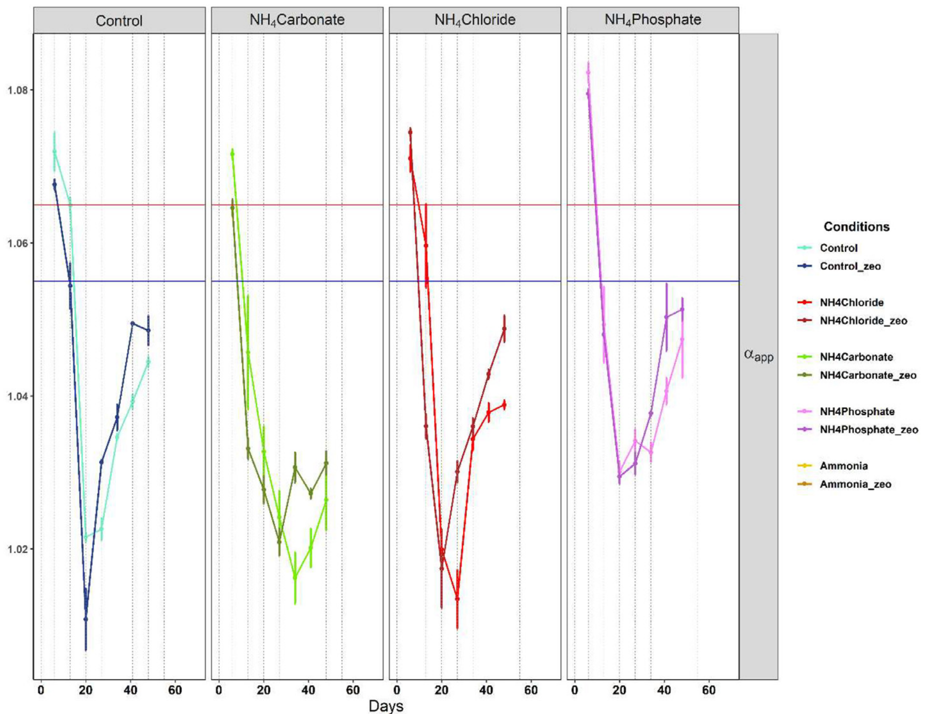

Fig. 6. Apparent isotope fractionation (α_{app}) of the biogas over time (number of days) for the different conditions tested. The horizontal blue line shows the limit for acetoclastic methanogenesis and the horizontal red line shows the limit for hydrogenotrophic methanogenesis. The data are the mean values for the triplicate bioreactors, standard deviations are indicated with error bars. Vertical lines show the dates of sampling.

initial organic loading of 11.7 g COD/1.4 g COD. Methanogenic sludge was sampled from a 60 L laboratory anaerobic bioreactor fed with the same biowaste and centrifuged at 10,000 g during 10 min. Mashed biowaste was provided by an industrial food waste collector (Valdis Energie, Issé). All the digesters were complemented with a biochemical potential buffer (International Standard ISO 11734 (1995)) to reach a final working volume of 700 mL. In a first series of 15 bioreactors, ammonia was added in the bioreactors to reach a TAN concentration of 8 g/L in the form of 40.8 ml of NH_3 solution (13%, Onyx) or 16.6 g of NH_4Cl salt (Acros Organics) or 14.9 g of $(NH_4)_2CO_3$ salt (Alfa Aeser) or 20.5 g of $(NH_4)_2PO_4$ salt (Acros Organics). A control condition without ammonia was also set up. A second series of 15 bioreactors was set up exactly as the first one, except that zeolite (Siliz 24® provided by Somez company - France) was also added at a concentration of 15 g/L (10.5 g of zeolite per bioreactor). The 30 bioreactors thus set up were sealed with a screw cap and a rubber septum and headspaces were flushed with N2 (purity>99.99%, Linde gas SA). Time zero (T0) samples were taken and all reactors were incubated without agitation, in the dark, at 35°C. However, reactors were mixed before each sampling and after each gas measurement (3 times per week). All batch digesters were prepared and started on the same day. Experimental design is illustrated in Fig. 1. Liquid samples (10 mL) were taken weekly through the septum and centrifuged at 10,000 x g for 10 min. Pellets were separated from the supernatant, snap-frozen in liquid nitrogen and stored at -80°C. One bottle was broken during manipulation (control with zeolite) and associated data are not presented in this dataset.

4.2. Biodegradation performance monitoring

Gas production and composition were measured respectively by using a differential manometer (Digitron 2082P, Margam, UK) and a micro gas chromatography (CP4900, Varian, Palo Alto, USA) as described in [8]. Volatile fatty acids concentrations were quantified by ionic chromatography (ICS 5000+, Thermo Fisher Scientific) equipped with IonPAC ICE-AS1 column as described in [9]. Dissolved organic and inorganic carbon (DOC and DIC) were measured following the French standard NF EN 1484 using a TOC-L CPN analyzer (Shimadzu). Isotopic fractionation of the methane and the carbon dioxide ($\delta^{13}CH_4$ and $\delta^{13}CO_2$) was measured with a Trace Gas Chromatograph Ultra (Thermo Scientific) attached to an isotope ratio mass spectrometer Delta V Plus (Thermo Scientific) via a combustion machine GC III (Thermo Scientific) to calculate apparent isotopic fractionation as described in [9]. Apparent isotope fractionation (α_{app}) is an indicator of the methanogenic pathway. It is usually assumed that if the α_{app} is superior to 1.065, the hydrogenotrophic way is the most important. On the contrary if the α_{app} is inferior to 1.055, the methanogenesis is dominated by the acetoclastic way [10]. TAN concentration was measured using Nessler's colorimetric assay following the French standard (NF T 90-105) and a spectrophotometer (DR-3900, Hach). pH was also measured and used to calculate FAN values [11].

4.3. DNA extraction, amplification and sequencing

Total DNA was extracted from the pellet using DNeasy PowerSoil Isolation Kit (QIAGEN) according to the manufacturer's instructions. DNA extracts were used for the amplification of the bacterial and archaeal hypervariable region V4–V5 of the 16S rRNA genes with the primers 515F (5'-GTGYCAGCMGCCGCGGTA-3') and 928R (5'-CCCCGYCAATTCMTTTRAGT-3') as described in [12]. Sequencing was performed on the Ion Torrent Personal Genome Machine using Ion 316 chip and the Ion PGM Sequencing 400 Kit.

4.4. Sequence read processing

PGM software filtered out low-quality and polyclonal sequence reads, and quality filtered data was exported as a FastQ file.

4.5. Samples preparation for metabolomic analysis

Each sample underwent a filtration process using a 0.45 μm Nylon filter, following which it was freeze-dried overnight. The resulting freeze-dried powder was dissolved in 580 μL of MilliQ water and subsequently centrifuged at 15500 x g for 3 minutes at 4°C. Following centrifugation, 530 μL of the supernatant was carefully transferred to HPLC vials. To ensure quality control (QC), 30 μL of supernatant from each vial was collected and pooled together. Additionally, for each vial, 500 μL of MilliQ water was added. Furthermore, a blank sample was prepared by combining equal volumes of MilliQ water and acetonitrile (Optima LC/MS grade, Fisher Chemical).

4.6. LC-MS analysis of samples

The instrument setup consisted of an Accela 1250 pump system coupled with an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, MA, US). The HPLC analysis employed an EC 100/2 NUCLEODUR HILIC analytical column (Macherey Nagel) with dimensions of 100 mm length, 2 mm inner diameter, and a particle size of 1.8 µm. Procedure is as described in [13]. Two mobile phases were utilized: solvent A, consisting of 50 mM ammonium acetate at pH 5.0,

and solvent B, which was acetonitrile. The chromatographic gradient program employed a linear gradient of solvents A and B, operating at a flow rate of 200 μL/min. The gradient transitioned from a 20:80 ratio to 10:90, with each ratio maintained for a minimum of 10 minutes. This was followed by a 10-minute period at a flow rate of 400 μL/min, maintaining a ratio of 10:90. Subsequently, the ratio was changed to 5:95, and samples were injected and analyzed under this condition. During analysis, a volume of 10 μL from each sample was injected into the analytical system at a flow rate of 400 μL/min. To mitigate potential batch effects, the samples were injected in random order. The sequencing began with two blank samples, followed by a QC (quality control) sample. Additionally, a blank and a QC sample were injected after every nine experimental samples. The Orbitrap mass spectrometer operated in positive electrospray ionization mode (ESI+), performing detection in full scan mode across an m/z range from 50 to 800. A resolution of 60,000 was utilized during the acquisition, which had a total duration of 25 minutes.

The generated RAW HPLC-MS data were converted into mzXML-format files using MSConvert (ProteoWizard 3.0).

Limitations

The outcomes are influenced by the choice of sludge used to inoculate the digesters, and may exhibit variations if a different inoculum is employed. Similarly, the results are also affected by the composition of the waste introduced into the digesters. Conducting additional tests with a comparable ammonia level induced by protein degradation would have enhanced the experiment, but proved to be complex to set up.

Ethics Statement

We confirm that the authors have read and follow the ethical requirements for publication in Data in Brief and confirm that the current work does not involve human subjects, animal experiments, or any data collected from social media platforms.

Data Availability

metabolomics data (Original data) (EMBL-EBI MetaboLights database).
sequencing data (Original data) (ENA database).

CRediT Author Statement

Stephany Damaris Campuzano Zagal: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing; **Xiaoqing Wang:** Validation, Investigation, Data curation, Writing – review & editing; **Nadine Derlet:** Investigation, Data curation; **Angéline Guenne:** Investigation, Data curation; **Chrystelle Bureau:** Investigation, Data curation; **Sophie Thibault:** Investigation; **Olivier Chapleur:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Visualization, Supervision, Project administration, Funding acquisition.

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Declaration of Competing Interest

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

Supplementary Data

Supplementary data related to this article can be found online at https://doi.org/10.1016/j.dib.2024.110357.

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