Supplementary Material

S2 Materials and Methods

S2.1. Liquid Chromatography Instrument Detection Conditions

The liquid chromatography instrument used is the Waters 1525, equipped with the Waters 2489 UV detector and Waters 1525 liquid chromatography pump. The chromatographic column is Waters C18 (Atlantis® T3 C18, 4.6×250 mm, 5 μm particle size). The detector wavelength is set at 286 nm, determined after performing full-spectrum scanning using a UV spectrophotometer.

Methanol and 2.5% (v/v) ice acetic acid were used as the mobile phase, with flow rates of 0.8 mL/min and 0.15 mL/min, respectively. The column temperature was maintained at 35°C. The retention time for 2,4,6-Trichlorophenol was approximately 2.27 minutes. Automatic integration mode was employed, and the integrated area exhibits a strong linear relationship with the concentration of 2,4,6-Trichlorophenol (R^2>0.999). The chromatographic peak for 2,4,6-Trichlorophenol was depicted in Figure S1.

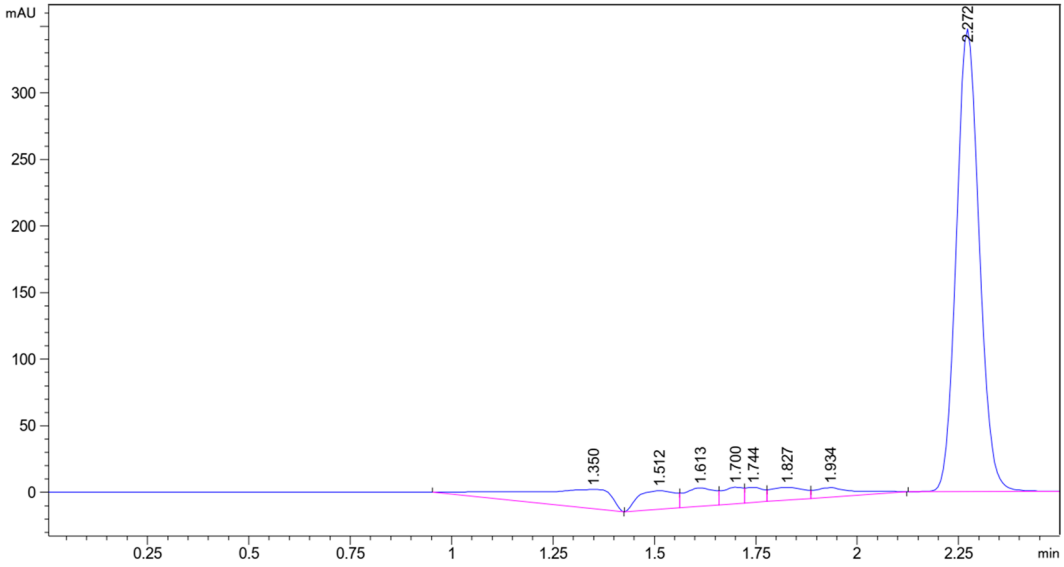

Figure S1. 2-4 Liquid chromatographic peak of 2,4,6-TCP

S2.2. Chloride Ion Detection Conditions

Chloride ions were determined using an ion chromatograph (883 Basic IC plus) with a dedicated anion exchange column (Metrosep A Supp 5-150/4.0). The ion chromatography eluent consists of 3.2 mmol/L sodium carbonate and 1 mmol/L sodium bicarbonate. The regenerating solution was a 0.5% volume fraction sulfuric acid solution. Ultra-pure water was used throughout to prevent chloride ion interference. The instrument was configured in an anion mode with the following operating conditions: stable operating pressure at around 11 mbar, eluent conductivity between 16-18 μS/cm, and detection temperature at room temperature.

Sample Handling: 10 mL of mixed sludge samples are collected, then centrifuged at 8000 rpm, filtered through a 0.45 μm membrane, and subsequently loaded into a specific sample introduction tube for analysis.

S2.3. Volatile Fatty Acids (VFAs) Detection Conditions

VFAs were detected using gas chromatography. The mixed fermentation liquid was first subjected to high-speed centrifugation (8000 rpm) and then filtered through a 0.45 μm needle filter. The filtered liquid was acidified to pH 4.0 using 10% phosphoric acid. The gas chromatograph (7890A) operates with detector and injector temperatures set at 250°C and 220°C, respectively. The injection volume was 2 μL.

The chromatographic column used was Agilent DB-WAXetr (30 mm × 530 μm × 1 μm), with nitrogen serving as the carrier gas at a flow rate of 3.8 mL/min. For detailed instrument conditions, please refer to the references [1,2].

S2.4. Sludge Microbial Community Analysis

Microbial diversity analysis, utilizing 16S rRNA sequencing, is primarily employed for species composition, inter-species evolutionary relationships, and the analysis of community diversity. The specific analysis process is as follows:

(1) Extraction of Activated Sludge Sample DNA and PCR Amplification

Total DNA Extraction: Total DNA from the sludge samples is extracted using a DNA extraction kit (E.Z.N.A.® soil). DNA quality is assessed by measuring DNA concentration with the Picogreen method. The purity of the extracted DNA is determined using NanoDrop 2000, and DNA integrity is assessed by performing 2% agarose gel electrophoresis. If the quality meets the requirements, the next step of testing can proceed.

PCR Amplification: PCR amplification is performed targeting the V3-V4 hypervariable regions of DNA fragments. The PCR primers used are 338F/806R, as detailed in reference [2]. Primers with barcodes are synthesized. The PCR amplification program is in accordance with the procedure reported in reference [3]. To ensure the accuracy and representativeness of the results, the amplification process needs to satisfy two basic conditions: minimize the number of cycles as much as possible within permissible limits and strive to ensure the same number of cycles for each sample. The ABI GeneAmp® 9700 PCR amplifier is used for the amplification process. TransGen AP221-02: TransStart Fastpfu DNA Polymerase is used for the PCR.

(2) Construction of MiSeq Library

The library is constructed following the Illumina MiSeq standard process, which includes adding Illumina official adapter sequences to the outer ends of the V3-V4 region through PCR. Gel recovery reagents are used to cut and recover the PCR products. The recovered PCR products are eluted using Tris-HCl buffer, and the quality of the products is assessed using 2% agarose gel electrophoresis to ensure they meet the sequencing requirements. NaOH is then used to denature the amplification products, forming single-stranded DNA fragments. The reagents used in the library construction process are from the TruSeqTM DNA Sample Prep Kit.

(3) Illumina MiSeq Sequencing

Sequencing is conducted by cycling modified DNA polymerase with four fluorescently labeled dNTPs to form bases. Laser scanning is used to obtain the sequence on each template, all of which are nucleotides polymerized on the template in the first round. Chemical cleavage is then performed on the "termination group" and "fluorescent group" to restore the 3' end, enabling the synthesis of the second nucleotide. The fluorescent signal results from each round of reactions are counted and collected to obtain the sequence of DNA fragments. The sequencing results are deposited in the National Coalition Building Institute (NCBI) database, and the detailed data sequence numbers can be found in the Materials and Methods summary of each chapter.

(4) Data Processing

The sequences are subjected to the final OTU clustering analysis at 97% similarity to obtain information about the microbial community abundance and structural diversity in the activated sludge samples.

S2.5. Genetic Analysis

The general steps for metagenomic sequencing are illustrated in Figure S2.

Figure S2. General process of high-throughput sequencing

(1) Total DNA Extraction from Activated Sludge and PCR Amplification

DNA Extraction: DNA extraction is performed following the same procedure as described in 2.4(1), including quality checks for total DNA.

PCR Amplification: In contrast to diversity amplification, metagenomic PCR amplification targets all DNA fragments without specific differentiation of gene regions.

(2) Construction of Paired-End (PE) Libraries

The construction of PE libraries is similar to that of diversity analysis. This process ultimately yields single-stranded DNA fragments with an approximate length of 400 bp.

(3) Bridge PCR

The purpose of bridge PCR is to further amplify single-stranded, fragmented DNA, forming DNA clusters, which enhances the signal response during sequencing and is a fundamental step in the sequencing process. The specific steps are as follows: The DNA library, after denaturation, yields single-stranded DNA. One end of the single strand is complementary to and anchored by a primer on the chip; The other end of the single-stranded DNA randomly pairs with another primer, creating a "bridge-like" connection; Using the first strand as a template, PCR amplification is performed, resulting in the formation of DNA clusters; The amplified DNA clusters are linearized to prepare them for subsequent sequencing. The reagents used for this step are typically from the HiSeq 3000/4000 PE Cluster Kit.

(4) Illumina Sequencing

The specific steps for sequencing are the same as for diversity analysis. The raw sequencing data generated is deposited in the NCBI database, and the database access sequence numbers are provided in the relevant section of the Materials and Methods summary.

(5) Data Analysis

Metagenomic data analysis differs from diversity testing and mainly includes sequence assembly and quality control, gene prediction, and the construction of a non-redundant gene set, taxonomic classification annotation, and KEGG functional annotation. For detailed principles and methodological steps in the analysis, please refer to the literature [4].

References

1. Bardi M.J.;Oliaee M.A. Impacts of different operational temperatures and organic loads in anaerobic co-digestion of food waste and sewage sludge on the fate of SARS-CoV-2. *Process Safety and Environmental Protection* **2021**, 146, 464-472.
2. Wang B.; Wang S.; Li B.; Peng C.;Peng Y. Integrating waste activated sludge (WAS) acidification with denitrification by adding nitrite (NO2-). *Biomass and Bioenergy* **2014**, 67, 460-465.
3. Czaplicka M. Sources and transformations of chlorophenols in the natural environment. *Science of the Total Environment* **2004**, 322, 21-39.
4. Cui B.; Yang Q.; Liu X.; Wu W.; Liu Z.;Gu P. Achieving partial denitrification-anammox in biofilter for advanced wastewater treatment. *Environment International* **2020**, 138, 105612.