

## DATA NOTE

# Deeply sequenced metagenome and metatranscriptome of a biogas-producing microbial community from an agricultural production-scale biogas plant

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## Abstract

**Background:** The production of biogas takes place under anaerobic conditions and involves microbial decomposition of organic matter. Most of the participating microbes still have to be considered as unknown and non-cultivable. Accordingly, shotgun metagenome sequencing currently is the method of choice to obtain insights into community composition and the genetic repertoire.

**Findings:** Here, we report the first deeply sequenced metagenome and metatranscriptome of a complex biogas-producing microbial community from an agricultural production-scale biogas plant. We assembled the metagenome and reconstructed most genes involved in the methane metabolism, a key pathway involving methanogenesis performed by methanogenic *Archaea*. This exemplary result indicates sufficient sequencing coverage for most downstream analyses.

**Conclusions:** Sequenced at least one order of magnitude deeper than previous studies, our metagenome data will enable novel insights into community composition and the genetic potential of important community members. Moreover, mapping of transcripts to reconstructed genome sequences will enable the identification of active metabolic pathways in target organisms.

**Keywords:** Biogas; Metagenome; Metatranscriptome; Sequencing; Assembly

## Data description

### Background

Production of biogas by means of anaerobic digestion of biomass is becoming increasingly important as biogas is regarded a clean, renewable and environmentally compatible energy source [1]. Moreover, generation of energy from biogas relies on a balanced carbon dioxide cycle.

The process of biogas production takes place under anaerobic conditions and involves microbial decomposition of organic matter, yielding methane as the main final product of the fermentation process. Complex consortia of microorganisms are responsible for biomass decomposition and biogas production. The majority of the participating microbes are still unknown, as is their influence on reactor performance. Since most of the organisms within biogas communities are non-cultivable by today's conventional microbiological techniques, sequencing of metagenomic to-

tal community DNA currently is the best way to obtain unbiased insights into community composition and the metabolic potential of key community members.

Here, we report the first deeply sequenced metagenome of an agricultural production-scale biogas plant on the Illumina platform [2]. We sequenced  $27.3\times$  and  $19.3\times$  deeper, respectively, than previous studies applying 454 or SOLiD sequencing [3, 4]. Metatranscriptomic sequencing of total community RNA complements the metagenome. Combined, these data will enable a deeper exploration of the biogas-producing microbial community, with the objective to develop rational strategies for process optimization.

#### Digester management and process characterization

The biogas plant, located in North Rhine Westphalia, Germany, features a mesophilic continuous wet fermentation technology characterized recently [5]. It was designed for a capacity of  $537\text{ kW}_{el}$  combined heat and power (CHP) generation. The process comprises three digesters: a primary and secondary digester, where the main proportion of biogas is produced, and a storage tank, where the digestate is fermented thereafter.

The primary digester is fed hourly with a mixture of 72 % maize silage and 28 % liquid pig manure. The biogas and methane yields at the time of sampling were at 810.5 and 417.8 liters per kg organic dry matter ( $l/kg\text{ oDM}$ ), respectively. After a theoretical retention time of 55 days, the digestate is stored in the closed, non-heated final storage tank. Further metadata are summarized in Table 1.

#### Sampling and library construction

Samples from the primary digester of the aforementioned biogas plant were taken in November 2010. Prior to the sampling process, approximately 15 l of the fermenter substrate were discarded before aliquots of 1 l were transferred into clean gastight sampling vessels and transported directly to the laboratory.

For the metagenome, aliquots of 20 g of the fermentation sample were used for total community DNA preparation as described previously [6].

For the metatranscriptome, a random-primed cDNA library was prepared by an external vendor (Vertis Biotechnologie AG). Briefly, total RNA was first treated with 5'-P dependent Terminator exonuclease (Epicentre) to enrich for full-length mRNA carrying 5' CAP or triphosphate structures. Then, first-strand cDNA was synthesized using a N6 random primer and M-MLV-RNase H reverse transcriptase, and second-strand cDNA synthesis was performed according to the Gubler-Hoffman protocol [7].

#### Metagenomic and metatranscriptomic sequencing

We sequenced one metatranscriptome and two metagenome shotgun libraries on Illumina's Genome Analyzer IIx system, applying the Paired-End DNA Sample Preparation Kit (Illumina Inc.) as described by the manufacturer and generating  $2\times 161\text{ bp}$  paired-end reads. On Illumina's MiSeq system, we sequenced three further metagenome shotgun libraries, applying the Nextera DNA Sample Preparation Kit (Illumina Inc.) as described by the manufacturer and generating  $2\times 155\text{ bp}$  paired-end reads. Our sequencing efforts, yielding 35 gigabases in total, are summarized in Table 2.

### Metagenome assembly

Prior to assembly, we used Trimmomatic [8], version 0.32, for adapter removal and moderate quality trimming. After adapter clipping, using Trimmomatic's *Truseq2-PE* and *Nextera-PE* templates, we removed leading and trailing ambiguous or low quality bases (below Phred quality scores of 3). Table 3 summarizes the effect on sequencing depth, more than 25 gigabases of sequence data passed quality control.

We assembled the metagenome with Ray Meta [9], version 2.3.1, trying a range of *k*-mer sizes from 21 to 61 in steps of 10. To estimate the inclusivity of the set of assemblies, we aligned the post-QC sequencing reads to the assembled contigs with bowtie2 [10], version 2.2.4. We then used samtools [11], version 1.1, to convert SAM to BAM, sort the alignment file, and calculate the mapping statistics. Based on total assembly size, contiguity, and the percentage of mapped back metagenomic reads, we selected the assembly produced with a *k*-mer size of 31. Here, we assembled approximately 228 megabases in 54,489 contigs greater than 1,000 bp, with an N50 value of 9,796 bp. 77 % (79 %) of metagenomic (metatranscriptomic) reads mapped back to the assembly.

### Gene prediction and annotation

We used MetaProdigal [12], version 2.6.1, to predict 250,596 protein-coding genes on the assembled contigs. We blasted the protein sequences of all predicted genes against the KEGG database [13], release 72.0, using Protein-Protein BLAST [14], version 2.2.29+. Of the 250,596 predicted genes, 191,766 (76.5 %) had a match in the KEGG database, using an Evalue cutoff of  $10^{-6}$ . We determined the KEGG Orthology (KO) for each gene by mapping the top-scoring BLAST hit to its orthologous gene in KEGG, resulting in 109,501 genes with an assigned KEGG Orthology. Table 4 summarizes our results.

### Relating the metagenome and the metatranscriptome

To illustrate potential use cases, we counted the number of reads within genes using BEDTools [15], version 2.22.0, and highlighted metagenomic and metatranscriptomic coverage of the methane metabolism pathway in Figure 1. We assembled the majority of genes involved in the methane metabolism from our metagenomic data, with accompanying metatranscriptomic data suggesting active gene expression for many.

For a second example, we calculated the reads per kilobase per million mapped reads (RPKM) for each gene as a crude measure for abundance (metagenome) or expression (metatranscriptome). Figure 2 relates the two. Here, we accentuated all genes assigned to either of the three known types of methanogenic pathways. Hydrogenotrophic methanogenesis, i.e. the reduction of CO<sub>2</sub> with hydrogen, appears to be highly expressed in the reactor analyzed, which is in agreement with previous work [16].

## Discussion

We report extensive metagenomic and metatranscriptomic profiling of the microbial community from a production-scale biogas plant. Given the unprecedented sequencing depth and established bioinformatics, our data are of great interest to the biogas research community in general and microbiologists working on biogas-producing

microbial communities in particular. In a first applied study, our metagenome assembly was used to improve the characterization of a metaproteome generated from biogas plant fermentation samples and to investigate the metabolic activity of the microbial community [17].

By sharing our data, we want to actively encourage its reuse. This will hopefully result in novel biological and biotechnological insights, eventually enabling a more efficient biogas production.

## Availability

### Data accession

The datasets supporting the results of this article are available in the European Nucleotide Archive (ENA) under study accession [PRJEB8813](#).

*Intermediate results for the review process are deposited in the project's GitHub repository for now [18], and will be uploaded to GigaDB [2] upon acceptance.*

### Reproducibility

The complete workflow is organized in a single GNU Makefile and available on GitHub [18]. All data and results can be reproduced by a simple invocation of *make*. To further support reproducibility, we bundled all tools and dependencies into one Docker container available on DockerHub [19]. *docker run* executes the aforementioned Makefile inside the container. Reproduction requires roughly 74 GiB of memory for the metagenome assembly.

Excluding the KEGG analysis, which relies on a commercial license of the KEGG database, all steps are performed using free and open-source software.

### Competing interests

The authors declare that they have no competing interests.

### Author's contributions

AB conceived and performed all bioinformatic analyses and wrote the paper. IM investigated all metadata and drafted part of the data description. PB implemented the accompanying Docker container. FE collected the study material. AW and AA provided the sequencing service. AP acquired funding and revised the paper. ASch and ASch jointly directed the project and extensively revised the paper. All authors read and approved the final manuscript.

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### Author details

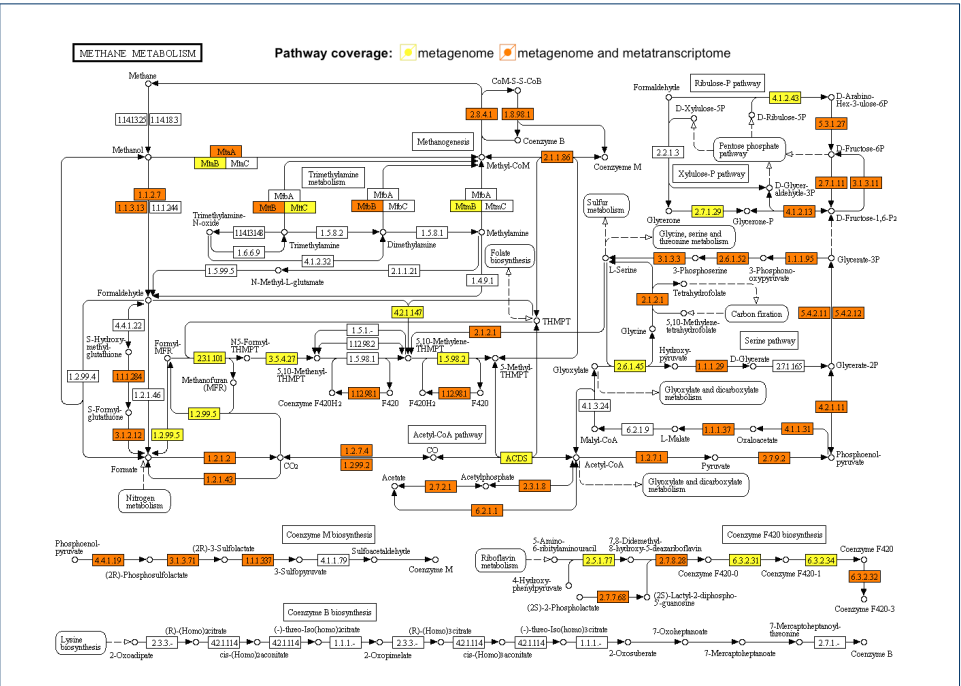
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### References

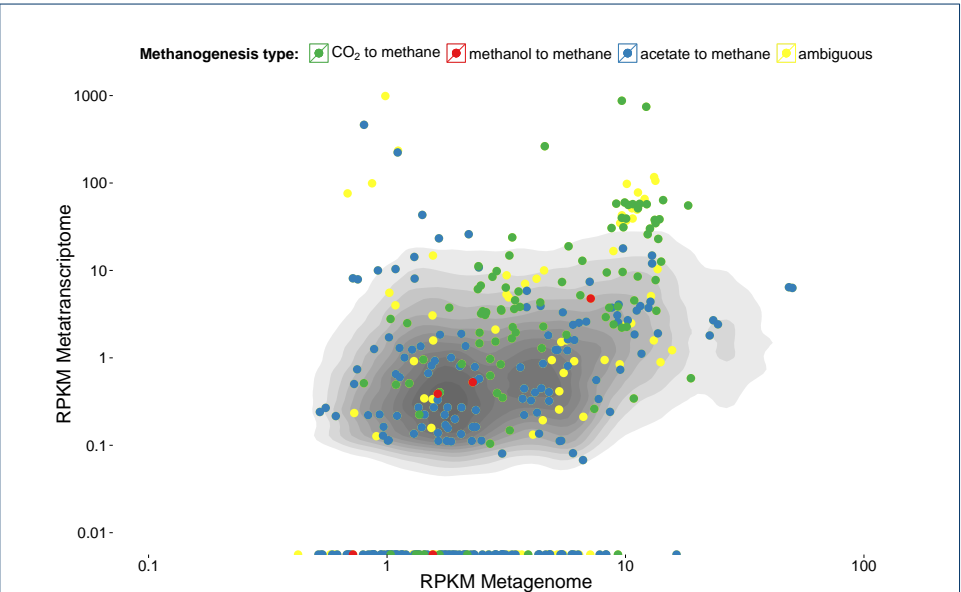
1. Weiland, P.: Biogas production: current state and perspectives. *Appl. Microbiol. Biotechnol.* **85**(4), 849–860 (2010). doi:[10.1007/s00253-009-2246-7](#)
2. Bremges, A., Maus, I., Belmann, P., Eikmeyer, F., Winkler, A., Albersmeier, A., Pühler, A., Schlüter, A., Szczyrba, A.: Supporting data and materials for “Deeply sequenced metagenome and metatranscriptome of a biogas-producing microbial community from an agricultural production-scale biogas plant”. *GigaScience Database* (2015). doi:[http://dx.doi.org/foo/bar](#)
3. Jaenicke, S., Ander, C., Bekel, T., Bisdorf, R., Dröge, M., Gartemann, K.H., Jünemann, S., Kaiser, O., Krause, L., Tille, F., Zakrzewski, M., Pühler, A., Schlüter, A., Goesmann, A.: Comparative and joint analysis of two metagenomic datasets from a biogas fermenter obtained by 454-pyrosequencing. *PLoS ONE* **6**(1), 14519 (2011). doi:[10.1371/journal.pone.0014519](#)

4. Wirth, R., Kovács, E., Maróti, G., Bagi, Z., Rákhely, G., Kovács, K.L.: Characterization of a biogas-producing microbial community by short-read next generation DNA sequencing. *Biotechnol Biofuels* **5**, 41 (2012). doi:[10.1186/1754-6834-5-41](https://doi.org/10.1186/1754-6834-5-41)
5. Stolze, Y., Zakrzewski, M., Maus, I., Eikmeyer, F., Jaenicke, S., Rottmann, N., Siebner, C., Pühler, A., Schlüter, A.: Comparative metagenomics of biogas-producing microbial communities from production-scale biogas plants operating under wet or dry fermentation conditions. *Biotechnol Biofuels* **8**, 14 (2015). doi:[10.1186/s13068-014-0193-8](https://doi.org/10.1186/s13068-014-0193-8)
6. Schlüter, A., Bekel, T., Diaz, N.N., Dondrup, M., Eichenlaub, R., Gartemann, K.H., Krahn, I., Krause, L., Krömeke, H., Kruse, O., Mussnug, J.H., Neuweyer, H., Niehaus, K., Pühler, A., Runte, K.J., Szczepanowski, R., Tauch, A., Tilker, A., Viehöver, P., Goesmann, A.: The metagenome of a biogas-producing microbial community of a production-scale biogas plant fermenter analysed by the 454-pyrosequencing technology. *J. Biotechnol.* **136**(1–2), 77–90 (2008). doi:[10.1016/j.jbiotec.2008.05.008](https://doi.org/10.1016/j.jbiotec.2008.05.008)
7. Gubler, U., Hoffman, B.J.: A simple and very efficient method for generating cdna libraries. *Gene* **25**(2–3), 263–269 (1983). doi:[10.1016/0378-1119\(83\)90230-5](https://doi.org/10.1016/0378-1119(83)90230-5)
8. Bolger, A.M., Lohse, M., Usadel, B.: Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**(15), 2114–2120 (2014). doi:[10.1093/bioinformatics/btu170](https://doi.org/10.1093/bioinformatics/btu170)
9. Boisvert, S., Raymond, F., Godzaridis, E., Laviolette, F., Corbeil, J.: Ray Meta: scalable de novo metagenome assembly and profiling. *Genome Biol.* **13**(12), 122 (2012). doi:[10.1186/gb-2012-13-12-r122](https://doi.org/10.1186/gb-2012-13-12-r122)
10. Langmead, B., Salzberg, S.L.: Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **9**(4), 357–359 (2012). doi:[10.1038/nmeth.1923](https://doi.org/10.1038/nmeth.1923)
11. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R.: The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**(16), 2078–2079 (2009). doi:[10.1093/bioinformatics/btp352](https://doi.org/10.1093/bioinformatics/btp352)
12. Hyatt, D., LoCascio, P.F., Hauser, L.J., Uberbacher, E.C.: Gene and translation initiation site prediction in metagenomic sequences. *Bioinformatics* **28**(17), 2223–2230 (2012). doi:[10.1093/bioinformatics/bts429](https://doi.org/10.1093/bioinformatics/bts429)
13. Kanehisa, M., Goto, S., Sato, Y., Kawashima, M., Furumichi, M., Tanabe, M.: Data, information, knowledge and principle: back to metabolism in KEGG. *Nucleic Acids Res.* **42**(Database issue), 199–205 (2014). doi:[10.1093/nar/gkt1076](https://doi.org/10.1093/nar/gkt1076)
14. Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L.: BLAST+: architecture and applications. *BMC Bioinformatics* **10**, 421 (2009). doi:[10.1186/1471-2105-10-421](https://doi.org/10.1186/1471-2105-10-421)
15. Quinlan, A.R., Hall, I.M.: BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**(6), 841–842 (2010). doi:[10.1093/bioinformatics/btq033](https://doi.org/10.1093/bioinformatics/btq033)
16. Zakrzewski, M., Goesmann, A., Jaenicke, S., Jünemann, S., Eikmeyer, F., Szczepanowski, R., Al-Soud, W.A., Sørensen, S., Pühler, A., Schlüter, A.: Profiling of the metabolically active community from a production-scale biogas plant by means of high-throughput metatranscriptome sequencing. *J. Biotechnol.* **158**(4), 248–258 (2012). doi:[10.1016/j.jbiotec.2012.01.020](https://doi.org/10.1016/j.jbiotec.2012.01.020)
17. Kohrs, F., Wolter, S., Benndorf, D., Heyer, R., Hoffmann, M., Rapp, E., Bremges, A., Sczyrba, A., Schlüter, A., Reichl, U.: Fractionation of biogas plant sludge material improves metaproteomic characterization to investigate metabolic activity of microbial communities. Submitted (2015)
18. GitHub Repository. <https://github.com/metagenomics/2015-biogas-cebitec>
19. DockerHub Registry. <https://registry.hub.docker.com/u/metagenomics/2015-biogas-cebitec>

Figures



**Figure 1 Methane metabolism pathway analysis.** Genes reconstructed in our assembly, that are involved in the methane metabolism [PATH:ko00680], are highlighted: Genes with only metagenomic support in yellow, genes with also metatranscriptomic support, suggesting active gene expression, in orange. KEGG pathway map courtesy of Kanehisa Laboratories.



**Figure 2 Relating the metagenome and metatranscriptome.** Highlighted are genes involved in methanogenesis, color coded by pathway type: CO<sub>2</sub> to methane [MD:M00567] green (96 genes), methanol to methane [MD:M00356] red (5 genes), and acetate to methane [MD:M00357] blue (209 genes). Common genes, shared between pathway types, are yellow (80 genes). In the background a two-dimensional density estimation for all 250,596 genes.

## Tables

**Table 1** Characteristics of the studied biogas plant. Primary digester, sampling date: Nov 15, 2010.

Process parameter	Sample
Net volume	2041 m <sup>3</sup>
Dimensions	6.4 m high, diameter of 21 m
Electrical capacity	537 kW <sub>el</sub>
pH	7.83
Temperature	40 °C
Conductivity	22.10 mS/cm
Volatile organic acids (VOA)	5327 mg/l
Total inorganic carbon (TIC)	14397 mg/l
VOA/TIC	0.37
Ammoniacal nitrogen	2.93 g/l
Acetic acid	863 mg/l
Propionic acid	76 mg/l
Fed substrates	72 % maize silage, 28 % pig manure
Organic load	4.0 kg oDM m <sup>-3</sup> d <sup>-1</sup>
Retention time	55 d
Biogas yield	810.5 l/kg oDM
Methane yield	417.8 l/kg oDM

**Table 2** Overview of the different sequencing libraries.

Accession	Library name	Library type	Insert size <sup>1</sup>	Cycles	Reads	Bases
ERS697694	GAllx, Lane 6	RNA, TruSeq	202 ± 49	2 × 161	78,752,308	12,679,121,588
ERS697688	GAllx, Lane 7	DNA, TruSeq	157 ± 19	2 × 161	54,630,090	8,795,444,490
ERS697689	GAllx, Lane 8	DNA, TruSeq	298 ± 32	2 × 161	74,547,252	12,002,107,572
ERS697690	MiSeq, Run A1 <sup>2</sup>	DNA, Nextera	173 ± 53	2 × 155	4,915,698	761,933,190
ERS697691	MiSeq, Run A2 <sup>2</sup>	DNA, Nextera <sup>3</sup>	522 ± 88	2 × 155	1,927,244	298,722,820
ERS697692	MiSeq, Run B1 <sup>2</sup>	DNA, Nextera	249 ± 30	2 × 155	3,840,850	573,901,713
ERS697693	MiSeq, Run B2 <sup>2</sup>	DNA, Nextera <sup>3</sup>	525 ± 90	2 × 155	4,114,304	614,787,564

<sup>1</sup>Insert sizes determined with Picard tools. <sup>2</sup>Partial runs. <sup>3</sup>This Nextera library was sequenced twice.

**Table 3** Metagenomic and metatranscriptomic sequencing and quality control.

Library type	Reads, raw	post-QC	Bases, raw	post-QC
Metagenome (total)	143,975,438	137,365,053	23,046,897,349	17,267,320,221
Metatranscriptome	78,752,308	73,165,986	12,679,121,588	8,455,809,264

**Table 4** Metagenome assembly statistics, minimum contig size of 1,000 bp.

Assembly metric	Our assembly
Total size	228,382,457 bp
Number of contigs	54,489
N50 value	9,796 bp
Largest contig	333,979 bp
Mapped DNA reads	105,461,596 (77 %)
Mapped RNA reads	57,436,058 (79 %)
Predicted genes	250,596
of these, full-length	172,372 (69 %)
Match in KEGG Genes	191,766
of these, assigned KO	109,501
of these, in KEGG pathways	61,100