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Data Article

Dataset of response characteristics of H₂-producing bacteria consortium to β -lactams, aminoglycosides, macrolides, quinolones antibiotics



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ABSTRACT

Antibiotics on H₂ producing bacteria shall be considered as being one of the critical elements in biological H₂ production utilizing livestock manure as raw resources. Despite the fact that the manure stands a significance role in bio-fermentation, the possibility of antibiotics being contained in excreta shall not be eliminated. Findings of whether the above saying might threaten the safety of bio-H₂

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Keywords:

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production needs to be further studied. The experiment subjects include: six single and three combined antibiotics were tested and analyzed by the application of the gradient experiment method. Along with the H_2 production rate, CHO content, pH and OD_{600} were used to analyze the effects of various antibiotics introduction on the hydrolysis, fermentation and H_2 production. To a further extent, four typical representative samples were selected for biodiversity analysis from the single antibiotic experiment groups. Amounting more than 6000 pieces of data were obtained in a series of experiments. Data suggested that remarkable measure of antibiotics have various degrees of H_2 production inhibition, while some antibiotics, Penicillin G, Streptomycin Sulfate, and their compound antibiotics, could promote the growth of *Ethanoligenens* sp. and improve H_2 yield in the contrary. Correspondent to the transition of key metabolic intermediates and end products, the mechanism of each antibiotic type and dose on H_2 production were summarized as follows: the main inhibitory mechanisms were: (1) board-spectrum inhibition, (2) partial inhibition, (3) H_2 consumption enhancement; and the enhancement mechanisms were: (1) enhance the growth of H_2 -producing bacteria, (2) enhanced starch hydrolysis, (3) inhibitory H_2 consumption or release of acid inhibition. Meanwhile, data analysis found that the effect of antibiotics on H_2 producing was not only related to type, but also to dosage. Even one kind of antibiotic may have completely opposite effects on H_2 -producing bacteria under different dosage conditions. Inhibition of H_2 yield was highest with Levofloxacin at 6.15 mg/L, gas production was reduced by 88.77%; and enhancement of H_2 yield was highest with Penicillin G at 7.20 mg/L, the gas production increased by 72.90%.

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Specifications Table

Subject	Renewable Energy, Sustainability and the Environment
Specific subject area	Response of anthracite H_2 -producing bacteria consortium to Penicillin G and Cefaclor
Type of data	Table and Figure
How the data were acquired	H_2 yield data via gas needle and gas chromatography (7890A, Agilent, America); CHO and OD_{600} data via spectrophotometer (BioMate 3S, Thermo Scientific, America); pH value via electronic pH meter (Star A211, Orion, America). H_2 -producing bacteria community structure data via high-pass sequencing Raw fastq files were demultiplexed, quality-filtered by Trimmomatic and Merged by FLASH Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA database using confidence threshold of 70%. Bacterial Galanz-stained photographs were taken through BX43 microscope (BX43, Olympus, Japan).

(continued on next page)

	Community bar chart: R language (version 3.3.1) tool. Evolutionary tree: Mega (version 10.0). Heatmap: R language (version 3.3.1) vegan package. Ternary analysis: GGTERN. RDA-CCA analysis: R language (version 3.3.1) rda or cca analysis and graphing in the vegan package. Spearman correlation heatmap: R (version 3.3.1) (pheatmap package). Circos chart: Circos-0.67-7.
Data format	Raw Analyzed
Description of data collection	The effects of different type and dosage of antibiotics on H ₂ -producing bacteria were determined by gradient experiment.
Data source location	Anthraxite H ₂ -producing bacteria consortium were collected from Zhaozhuang Mining (GPS coordinates is 35°34'10"N,112°53'55"E).
Data accessibility	Repository name: Mendeley Data Data identification number: 10.17632/vgb4rcsspf.3 Direct URL to data: https://data.mendeley.com/datasets/vgb4rcsspf/draft?a=56945b77-bb3c-4871-aa6f-f3afb5fc972e The 16S rRNA gene sequences were deposited in the NCBI Sequence Read Archive under accession number PRJNA784035.
Related research article	D. Xiao, H. He, X. Yan, N.D. Diaz, D. Chen, J. Ma, Y. Zhang, J. Li, M. Keita, E.O. Julien, X. Yan, The response regularity of biohydrogen production by anthracite H ₂ -producing bacteria consortium to six conventional veterinary antibiotics, J. Environ. Manage. 315 (2022) 115088. https://doi.org/10.1016/j.jenvman.2022.115088 .

Value of the Data

- Datasets proven various types of antibiotic effects and dosage, mainly reflecting on the key intermediate metabolites and production capacity of the H₂-producing bacteria consortium.
- Clearly showing the types and doses of antibiotics that can promote and inhibit hydrogen production.
- It provides basic data for revealing the changes of microbial community diversity and the mechanism of metabolic cooperation among bacteria under the influence of antibiotics.
- It can be used in many different types of studies focusing on bio-hydrogen production from agricultural waste.
- The data can provide support for researchers to study the industrialization of anaerobic digestion and the metabolic pathway of microbial H₂ fermentation process.

1. Data Description

Datasets could be referred as supplementary data, consist of 10 tables and 9 figures. **Table 1** contains two types of key experimental design information. (1) Single antibiotics and combinations of combination antibiotics were used in the experiments. The former includes 6 kinds of 4 categories; the latter includes 3 groups of compound antibiotics. (2) Dosage design of gradient experiment for each antibiotic or compound antibiotics. Dosage gradient gradually decreased from 100.00 % to 1.56 % by dichotomy.

Raw and analytical data of H₂ yield changes due to the application of treatment on various dosage of single antibiotics and compound antibiotics were recorded in **Tables 2** and **3** respectively. Each treatment consists of 5 parallel samples. With reference to the sub-table "Gas yield Raw data" of **Tables 2** and **3**, T_Gas could be defined as the total gas yield amount and the unit generally referred as mL/Sample. Furthermore, C_H₂ is construed as the H₂ concentration of each sample in terms of terminology written as %VOL.; V_H₂ refer to the H₂ yield amount and interpret as the mL/Sample. In terms of ST_H₂, it is the average H₂ production rate and the unit is mL/Treatment; Mm_H₂ is the substrate molar H₂ production rate the unit is mM/g. "ST" sub-table is the statistics of the substrate molar H₂ production rate in the "Gas yield Raw data"

Table 1
Experiment design of the corresponding relationship between each antibiotic gradient and dosage

Group	Category	Antibiotic	Abbreviation	Amount Dosage Corresponding to GP (mg/L)							
				100.00%	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.00%
Signal	β -lactams	Penicillin G	P	55.38	27.69	13.85	6.92	3.46	1.73	0.86	0.00
		Cefaclor	C	15.38	7.69	3.85	1.92	0.96	0.48	0.24	0.00
	Aminogly-cosides	Streptomycin Sulfate	S	30.77	15.39	7.69	3.85	1.92	0.96	0.48	0.00
		Amikacin Sulfate	A	15.00	7.50	3.75	1.88	0.94	0.47	0.23	0.00
	Macrolides	Erythromycin	E	123.08	61.54	30.77	15.39	7.69	3.85	1.92	0.00
	Quinolones	Levofloxacin	L	6.15	3.08	1.54	0.77	0.38	0.19	0.10	0.00
Compound	Penicillin G + Streptomycin Sulfate	P-S	✓	◦	✓	✓	✓	◦	◦	✓	
	Amikacin Sulfate + Streptomycin Sulfate	A-S	✓	◦	✓	✓	✓	◦	◦	✓	
	Levofloxacin + Cefaclor	L-C	✓	◦	✓	✓	✓	◦	◦	✓	

✓: select; ◦: not select.

Table 2

Raw and analytical data of H₂ yield changes due to the application of treatment on various dosage of single antibiotics.

Table 2-1 Raw data of H₂ yield changes due to the application of treatment on various dosage of single antibiotics.

	Antibiotic	Penicillin G Sodium Salt							
		GP	100.0%	50.0%	25.0%	12.5%	6.25%	3.13%	1.56%
T _{Gas}	Dosage	55.38	27.69	13.85	6.92	3.46	1.73	0.86	0.00
	S1	1115.00	1157.00	1267.00	1390.00	859.00	840.00	923.00	699.00
	S2	1224.00	834.00	1376.00	1231.00	847.00	825.00	891.00	814.00
	S3	841.00	1205.00	1102.00	1258.00	863.00	816.00	801.00	837.00
	S4	1141.00	1192.00	841.00	1299.00	797.00	816.00	958.00	687.00
	S5	1140.00	1205.00	1298.00	1004.00	782.00	657.00	725.00	676.00
C _{H₂}	S1	60.72%	58.64%	59.66%	60.26%	58.96%	58.59%	59.59%	58.69%
	S2	59.39%	60.95%	58.48%	60.37%	59.04%	61.19%	60.18%	59.94%
	S3	59.47%	60.79%	59.49%	61.89%	59.11%	61.90%	61.38%	61.36%
	S4	61.73%	60.23%	60.97%	61.78%	58.85%	61.31%	58.93%	60.45%
	S5	61.84%	59.69%	61.25%	61.55%	61.04%	59.81%	60.52%	61.06%
V _{H₂}	S1	677.03	678.46	755.89	837.61	506.47	492.16	550.02	410.24
	S2	726.93	508.32	804.68	743.15	500.07	504.82	536.20	487.91
	S3	500.14	732.52	655.58	778.58	510.12	505.10	491.65	513.58
	S4	704.34	717.94	512.76	802.52	469.03	500.29	564.55	415.29
	S5	704.98	719.26	795.03	617.96	477.33	392.95	438.77	412.77
ST _{H₂} (ml/Sample)	Average	703.41	711.81	752.76	790.55	498.43	500.79	535.43	457.22
	max	23.62	20.47	51.44	46.72	11.81	4.72	28.87	56.17
	min	203.15	203.67	239.90	172.70	29.66	108.14	96.59	46.72
Mm _{H₂} (mM/g)	Average	7.85	7.94	8.40	8.82	5.56	5.59	5.98	5.10
	max	0.26	0.23	0.57	0.52	0.13	0.05	0.32	0.63
	min	2.27	2.27	2.68	1.93	0.33	1.21	1.08	0.52
	Antibiotic	Cefactor							
		GP	100.0%	50.0%	25.0%	12.5%	6.25%	3.13%	1.56%
T _{Gas}	Dosage	15.38	7.69	3.85	1.92	0.96	0.48	0.24	0.00
	S1	291.00	356.00	467.00	732.00	827.00	443.00	447.00	875.00
	S2	238.00	453.00	489.00	802.00	800.00	517.00	433.00	760.00
	S3	291.00	393.00	576.00	891.00	760.00	477.00	345.00	833.00
	S4	296.00	541.00	463.00	888.00	727.00	439.00	314.00	739.00
	S5	243.00	351.00	493.00	882.00	862.00	442.00	300.00	845.00
C _{H₂}	S1	56.28%	56.29%	57.22%	58.19%	56.31%	58.86%	56.13%	56.69%
	S2	56.20%	57.96%	58.97%	58.04%	57.88%	58.84%	57.28%	56.25%
	S3	57.69%	57.19%	56.24%	58.01%	55.63%	57.50%	57.48%	55.34%
	S4	55.04%	55.76%	56.76%	57.63%	58.37%	56.88%	55.29%	55.79%
	S5	58.34%	56.50%	55.66%	56.88%	56.56%	57.27%	57.02%	57.18%
V _{H₂}	S1	163.77	200.39	267.22	425.95	465.68	260.75	250.90	496.04
	S2	133.76	262.56	288.36	465.48	463.04	304.20	248.02	427.50
	S3	167.88	224.76	323.94	516.87	422.79	274.28	198.31	460.98
	S4	162.92	301.66	262.80	511.75	424.35	249.70	173.61	412.29
	S5	141.77	198.32	274.40	501.68	487.55	253.13	171.06	483.17
ST _{H₂} (ml/Sample)	Average	159.06	247.24	288.71	498.95	460.19	259.32	197.90	467.19
	max	8.92	54.59	35.70	18.11	27.47	45.14	53.02	28.87
	min	25.20	49.34	26.25	72.70	37.10	9.97	26.77	54.59
Mm _{H₂} (mM/g)	Average	1.78	2.76	3.22	5.57	5.14	2.89	2.21	5.21
	max	0.10	0.61	0.40	0.20	0.31	0.50	0.59	0.32
	min	0.28	0.55	0.29	0.81	0.41	0.11	0.30	0.61
	Antibiotic	Streptomycin Sulfate							
		GP	100.0%	50.0%	25.0%	12.5%	6.25%	3.13%	1.56%
T _{Gas}	Dosage	30.77	15.38	7.69	3.85	1.92	0.96	0.48	0.00
	S1	775.00	712.00	716.00	1123.00	1053.00	1043.00	976.00	734.00
	S2	792.00	796.00	547.00	918.00	1040.00	1012.00	770.00	886.00
	S3	754.00	824.00	845.00	994.00	947.00	1008.00	944.00	847.00
	S4	755.00	801.00	869.00	1061.00	1067.00	967.00	976.00	741.00
	S5	695.00	624.00	834.00	929.00	975.00	764.00	968.00	838.00
C _{H₂}	S1	57.71%	56.84%	55.55%	55.94%	56.17%	55.22%	56.86%	57.18%
	S2	56.96%	55.84%	56.54%	58.08%	55.28%	56.50%	55.09%	56.00%
	S3	57.44%	58.58%	55.69%	57.30%	55.18%	56.52%	57.32%	58.06%
	S4	55.00%	57.87%	55.94%	56.29%	55.54%	56.74%	57.27%	55.71%
	S5	58.04%	58.42%	55.98%	58.19%	57.63%	58.22%	57.91%	55.10%
V _{H₂}	S1	447.25	404.70	397.74	628.21	591.47	575.94	554.95	419.70
	S2	451.12	444.49	309.27	533.17	574.91	571.78	424.19	496.16
	S3	433.10	482.70	470.58	569.56	522.55	569.72	541.10	491.77
	S4	415.25	463.54	486.12	597.24	592.61	548.68	558.96	412.81
	S5	403.38	364.54	466.87	540.59	561.89	444.80	560.57	461.74
ST _{H₂} (ml/Sample)	Average	436.75	448.82	455.38	582.15	580.32	566.67	553.81	467.19
	max	14.70	34.12	30.71	46.19	12.34	9.19	6.82	28.87
	min	33.07	84.51	145.67	41.47	58.01	122.05	129.66	54.59
Mm _{H₂}	Average	4.87	5.01	5.08	6.50	6.48	6.32	6.18	5.21
	max	0.16	0.38	0.34	0.52	0.14	0.10	0.08	0.32

Table 2. (Continued)

(mM/g)	Antibiotic	Penicillin G Sodium Salt								
	min	0.37	0.94	1.63	0.46	0.65	1.36	1.45	0.61	
T _{Gas}	Antibiotic	Amikacin Sulfate								
	GP	100.00%	50.0%	25.0%	12.5%	6.25%	3.13%	1.56%	0.00%	
	Dosage	15.00	7.50	3.75	1.88	0.94	0.47	0.23	0.00	
	S1	643.00	768.00	846.00	868.00	755.00	705.00	706.00	804.00	
	S2	768.00	834.00	716.00	683.00	833.00	608.00	680.00	927.00	
	S3	868.00	723.00	753.00	895.00	893.00	524.00	804.00	708.00	
	S4	948.00	828.00	780.00	636.00	804.00	587.00	670.00	807.00	
	S5	643.00	723.00	758.00	801.00	690.00	638.00	676.00	752.00	
	S1	52.47%	54.90%	55.58%	52.29%	54.00%	52.63%	54.22%	55.52%	
	S2	52.86%	52.46%	54.85%	54.21%	52.60%	54.20%	52.91%	55.41%	
C _{H₂}	S3	54.78%	54.94%	52.61%	52.12%	52.48%	55.97%	53.90%	55.19%	
	S4	54.03%	55.05%	55.44%	53.37%	55.39%	54.91%	53.08%	53.46%	
	S5	52.61%	52.95%	54.52%	53.06%	55.58%	53.39%	54.04%	55.67%	
	S1	337.38	421.63	470.21	453.88	407.70	371.04	382.79	446.38	
	S2	405.96	437.52	392.73	370.25	438.16	329.54	359.79	513.65	
V _{H₂}	S3	475.49	397.22	396.15	466.47	468.65	293.28	433.36	390.75	
	S4	512.20	455.81	432.43	339.43	445.34	322.32	355.64	431.42	
	S5	338.28	382.83	413.26	425.01	383.50	340.63	365.31	418.64	
	Average	433.07	428.08	427.82	428.87	439.89	321.52	365.88	452.49	
	max	78.74	27.56	41.99	37.80	28.87	49.61	67.19	60.89	
(ml/Sample)	min	95.54	44.88	35.17	89.76	56.69	28.08	10.50	61.42	
	Average	4.83	4.78	4.77	4.79	4.91	3.59	4.08	5.05	
(mM/g)	max	0.88	0.31	0.47	0.42	0.32	0.55	0.75	0.68	
	min	1.07	0.50	0.39	1.00	0.63	0.31	0.12	0.69	
T _{Gas}	Antibiotic	Erythromycin								
	GP	100.00%	50.0%	25.0%	12.5%	6.25%	3.13%	1.56%	0.00%	
	Dosage	123.08	61.54	30.77	15.38	7.69	3.85	1.92	0.00	
	S1	398.00	435.00	430.00	1067.00	1074.00	955.00	1036.00	895.00	
	S2	404.00	406.00	516.00	913.00	928.00	830.00	991.00	879.00	
	S3	395.00	434.00	461.00	780.00	916.00	1018.00	1016.00	823.00	
	S4	418.00	419.00	721.00	828.00	1049.00	1047.00	1062.00	857.00	
	S5	405.00	391.00	482.00	851.00	1088.00	996.00	935.00	974.00	
	S1	49.62%	49.72%	48.09%	48.39%	48.01%	50.80%	49.44%	51.46%	
	S2	49.24%	51.00%	49.35%	49.19%	49.38%	50.72%	50.37%	50.53%	
C _{H₂}	S3	49.88%	51.91%	49.54%	50.02%	51.76%	49.57%	50.29%	50.44%	
	S4	51.72%	48.00%	48.37%	49.85%	50.80%	48.33%	48.68%	48.11%	
	S5	48.79%	51.62%	48.60%	51.05%	49.35%	51.83%	50.17%	48.26%	
	S1	197.49	216.28	206.79	516.32	515.63	485.14	512.20	460.57	
	S2	198.93	207.06	254.65	449.10	458.25	420.98	499.17	444.16	
V _{H₂}	S3	197.03	225.29	228.38	390.16	474.12	504.62	510.95	415.12	
	S4	216.19	201.12	348.75	412.76	532.89	506.02	516.98	412.30	
	S5	197.60	201.83	234.25	434.44	536.93	516.23	469.09	470.05	
	Average	202.63	206.56	230.97	453.02	514.96	502.89	509.71	447.24	
	max	13.65	18.64	117.59	62.99	22.05	13.12	7.35	22.57	
(ml/Sample)	min	5.77	4.46	2.62	62.99	56.69	81.89	40.94	35.17	
	Average	2.26	2.31	2.58	5.06	5.75	5.61	5.69	4.99	
(mM/g)	max	0.15	0.21	1.31	0.70	0.25	0.15	0.08	0.25	
	min	0.06	0.05	0.03	0.70	0.63	0.91	0.46	0.39	
T _{Gas}	Antibiotic	Levofloxacin								
	GP	100.00%	50.0%	25.0%	12.5%	6.25%	3.13%	1.56%	0.00%	
	Dosage	6.15	3.08	1.54	0.77	0.38	0.19	0.10	0.00	
	S1	108.00	294.00	626.00	1257.00	835.00	1006.00	944.00	818.00	
	S2	113.00	279.00	584.00	1106.00	682.00	1007.00	1017.00	949.00	
	S3	95.00	323.00	687.00	1231.00	793.00	1052.00	884.00	822.00	
	S4	113.00	261.00	610.00	789.00	1077.00	951.00	925.00	989.00	
	S5	111.00	270.00	578.00	1029.00	1219.00	914.00	1061.00	937.00	
	S1	49.35%	49.56%	49.41%	50.30%	52.20%	50.65%	49.78%	50.34%	
	S2	50.95%	50.35%	52.86%	49.29%	51.54%	49.69%	50.96%	49.89%	
C _{H₂}	S3	50.64%	50.42%	51.15%	51.53%	51.36%	49.52%	50.28%	49.58%	
	S4	49.66%	51.86%	49.76%	50.19%	52.32%	49.73%	51.71%	49.62%	
	S5	51.15%	51.16%	49.97%	49.94%	51.38%	49.66%	49.57%	49.27%	
	S1	53.30	145.71	309.31	632.27	435.87	509.54	469.92	411.78	
	S2	57.57	140.48	308.70	545.15	351.50	500.38	518.26	473.46	
V _{H₂}	S3	48.11	162.86	351.40	634.33	407.28	520.95	444.48	407.55	
	S4	56.12	135.35	303.54	396.00	563.49	472.93	478.32	490.74	
	S5	56.78	138.13	288.83	513.88	626.32	453.89	525.94	461.66	
	Average	56.17	146.81	318.46	581.37	508.14	501.05	498.16	459.32	
	max	1.58	15.92	33.25	52.76	118.11	20.21	27.82	31.50	
(ml/Sample)	min	7.87	11.90	29.75	185.04	156.43	46.98	53.54	51.97	
	Average	0.63	1.64	3.55	6.49	5.67	5.59	5.56	5.13	
(mM/g)	max	0.02	0.18	0.37	0.59	1.32	0.23	0.31	0.35	
	min	0.09	0.13	0.33	2.07	1.75	0.52	0.60	0.58	

Table 2-2ST data of H₂ yield changes due to the application of treatment on various dosage of single antibiotics

GP	Penicillin G Sodium Salt			Cefaclor			Streptomycin Sulfate		
	average	max	min	average	max	min	average	max	min
0.00%	5.10288	0.62687	0.52142	5.21419	0.32223	0.6093	5.21419	0.32223	0.6093
1.56%	5.97582	0.32223	1.07799	2.20871	0.59172	0.29879	6.18087	0.07616	1.44708
3.13%	5.58915	0.05273	1.20688	2.89417	0.50384	0.11131	6.3244	0.10253	1.36213
6.25%	5.56278	0.13182	0.33101	5.13608	0.3066	0.41401	6.47673	0.13768	0.64738
12.5%	8.82312	0.52142	1.92749	5.56864	0.20212	0.81142	6.49723	0.51556	0.46283
25.0%	8.40129	0.57415	2.6774	3.22225	0.39839	0.29293	5.08237	0.34273	1.62577
50.0%	7.94432	0.22849	2.27315	2.75942	0.6093	0.55071	5.00914	0.38081	0.94324
100.00%	7.85058	0.26364	2.26729	1.77517	0.0996	0.28121	4.87439	0.16404	0.36909
GP	Amikacin Sulfate			Erythromycin			Levofloxacin		
	average	max	min	average	max	min	average	max	min
0.00%	5.05015	0.6796	0.68546	4.99156	0.25192	0.39253	5.12631	0.35152	0.58001
1.56%	4.08347	0.74991	0.11717	5.68874	0.08202	0.45697	5.55985	0.31051	0.59758
3.13%	3.58842	0.55364	0.31344	5.61258	0.14647	0.91395	5.59207	0.22556	0.52435
6.25%	4.90954	0.32223	0.63273	5.74733	0.24606	0.63273	5.67117	1.31819	1.74588
12.5%	4.78651	0.42182	1.00183	5.05601	0.70304	0.70304	6.48845	0.58879	2.06517
25.0%	4.77479	0.46869	0.39253	2.5778	1.31234	0.02929	3.55424	0.37105	0.33199
50.0%	4.77772	0.30758	0.50091	2.30538	0.20798	0.0498	1.63847	0.17771	0.1328
100.00%	4.83338	0.8788	1.06627	2.26144	0.15232	0.06445	0.62687	0.01758	0.08788

sub-table. The above table is being prepared for the intention of graphing. The “ST-analysis” sub-table records the analysis data of inhibition or enhancement of H₂ production by individual antibiotic.

Recording the raw and statistical data of Aldehyde Group (CHO) modification with the application on differ dosage of single and compound antibiotics respectively in Tables 4 and 5. Each treatment contains of 5 parallel samples. C_CHO is the CHO concentration and the unit is mM/L; ST_CHO is the average CHO concentration for reach treatment and the unit is mM/L.

Tables 6 and 7 documented the raw and statistical data of pH by utilising diverse treatment on distinct dosage of single and compound antibiotics respectively. Each treatment consist of 5 parallel samples. V_pH is the pH value of samples solution and ST_pH is the average pH for each treatment.

Tables 8 and 9 record the raw and statistical data of OD₆₀₀ with the approach on various dosage of single and compound antibiotics in correspondingly Respective treatment accommodated 5 parallel samples. V_OD₆₀₀ specified the absorbance value of samples solution, it could be represented in the form of A. ST_OD₆₀₀ is the average absorbance value for each treatment and the unit is A.

Table 2-3ST-analysis of H₂ yield changes due to the application of treatment on various dosage of single antibiotics.

GP	Penicillin	Cefaclor	Streptomycin	Amikacin	Erythromycin	Levofloxacin
100.00%	7.850581	1.775169	4.874391	4.83338	2.261436	0.626875
50.0%	7.944319	2.759421	5.009139	4.777723	2.305376	1.638467
25.0%	8.401294	3.222253	5.082373	4.774794	2.577803	3.554243
12.5%	8.823116	5.56864	6.497235	4.786511	5.056009	6.488447
6.25%	5.562781	5.136077	6.476729	4.909543	5.747328	5.671166
3.13%	5.589145	2.894169	6.324405	3.588419	5.61258	5.592074
1.56%	5.975816	2.208708	6.180868	4.083474	5.688742	5.559852
0.00%	5.102878	5.214192	5.214192	5.05015	4.991564	5.126312
Inhibit Rate		-65.96%	-6.52%	-28.94%	-54.69%	-87.77%
Enhancement Rate	72.90%	6.80%	24.61%		13.85%	26.57%

Table 3Raw and analytical data of H₂ yield changes due to the application of treatment on various dosage of compound antibiotics**Table 3-1** Raw data of H₂ yield changes due to the application of treatment on various dosage of compound antibiotics

	Group	P-S				
	GP	100.00%	25.00%	12.50%	6.25%	0.00%
T-Gas	S1	884	648	610	457	697
	S2	888	626	538	460	714
	S3	938	654	558	434	700
	S4	899	681	551	490	726
	S5	876	616	575	437	679
C-H₂	S1	61.81%	61.95%	61.79%	61.11%	58.13%
	S2	60.89%	61.99%	60.47%	58.72%	58.58%
	S3	59.75%	61.78%	60.62%	58.83%	58.20%
	S4	61.55%	60.55%	61.93%	58.57%	58.25%
	S5	61.80%	61.03%	60.44%	60.82%	58.84%
V-H₂	S1	546.40	401.44	376.92	279.27	405.17
	S2	540.70	388.06	325.33	270.11	418.26
	S3	560.46	404.04	338.26	255.32	407.40
	S4	553.33	412.35	341.23	286.99	422.90
	S5	541.37	375.94	347.53	265.78	399.52
ST-H₂ (ml/sample)	avg	550.22	401.47	345.44	272.92	413.43
	max	10.23	10.88	31.48	14.07	9.46
	min	9.52	25.53	20.11	17.60	13.91
Mm-H₂ (mM/g)	avg	6.14	4.38	3.92	3.08	4.62
	max	0.11	0.22	0.29	0.13	0.11
	min	0.11	0.05	0.29	0.22	0.16
	Group	A-S				
	GP	100.00%	25.00%	12.50%	6.25%	0.00%
T-Gas	S1	823	736	724	771	750
	S2	888	771	686	728	733
	S3	828	730	736	764	743
	S4	886	738	720	788	755
	S5	908	704	721	709	760
C-H₂	S1	57.94%	56.96%	57.89%	52.59%	54.34%
	S2	53.73%	55.93%	56.97%	53.98%	53.80%
	S3	54.38%	56.55%	56.89%	53.10%	52.85%
	S4	52.89%	56.58%	56.86%	52.96%	53.66%
	S5	54.71%	57.83%	56.89%	54.47%	54.15%
V-H₂	S1	476.85	419.23	419.12	405.47	407.55
	S2	477.12	431.22	390.81	392.97	394.35
	S3	450.27	412.82	418.71	405.68	392.68
	S4	468.61	417.56	409.39	417.32	405.13

Table 2. (Continued)

	Group	P-S				
	S5	496.77	407.12	410.18	386.19	411.54
ST _{H₂} (ml/Sample)	avg	468.21	420.21	409.51	405.36	399.93
	max	28.56	11.01	9.61	11.96	11.61
	min	17.94	13.08	18.70	19.17	7.25
Mm _{H₂} (mM/g)	avg	5.23	4.62	4.63	4.44	4.46
	max	0.32	0.19	0.05	0.22	0.13
	min	0.20	0.08	0.26	0.13	0.08
	Group	L-C				
	GP	100.00%	25.00%	12.50%	6.25%	0.00%
T-Gas	S1	374	434	664	862	795
	S2	398	440	660	872	770
	S3	395	461	638	848	751
	S4	375	407	647	861	802
	S5	371	479	700	862	768
C-H ₂	S1	49.52%	49.21%	49.82%	49.08%	52.24%
	S2	49.14%	49.62%	49.09%	49.39%	51.99%
	S3	50.00%	51.88%	49.32%	49.84%	52.51%
	S4	50.88%	49.29%	49.34%	49.31%	52.37%
	S5	50.71%	49.60%	49.13%	49.33%	52.39%
V-H ₂	S1	185.20	213.57	330.80	423.07	415.31
	S2	195.58	218.33	323.99	430.68	400.32
	S3	197.50	239.17	314.66	422.64	394.35
	S4	190.80	200.61	319.23	424.56	420.01
	S5	188.13	237.58	343.91	425.22	402.36
ST _{H₂} (ml/Sample)	avg	192.27	217.92	322.17	425.24	407.50
	max	5.23	21.25	21.74	5.44	12.51
	min	7.07	17.31	7.51	2.59	13.15
Mm _{H₂} (mM/g)	avg	2.15	2.54	3.60	4.75	4.57
	max	0.03	0.11	0.24	0.05	0.12
	min	0.09	0.30	0.03	0.04	0.17

Table 3-2ST data of H₂ yield changes due to the application of treatment on various dosage of compound antibiotics

GP	P-S			A-S			L-C		
	average	max	min	average	max	min	average	max	min
100.00%	6.14	0.11	0.11	5.23	0.32	0.20	2.15	0.03	0.09
25.00%	4.38	0.22	0.05	4.62	0.19	0.08	2.54	0.11	0.30
12.50%	3.92	0.29	0.29	4.63	0.05	0.26	3.60	0.24	0.03
6.25%	3.08	0.13	0.22	4.44	0.22	0.13	4.75	0.05	0.04
0.00%	4.62	0.11	0.16	4.46	0.13	0.08	4.57	0.12	0.17

Table 3-3ST-analysis of H₂ yield changes due to the application of treatment on various dosage of compound antibiotics.

GP	P-S	A-S	L-C
100.00%	6.14	5.23	2.15
25.00%	4.38	4.62	2.54
12.50%	3.92	4.63	3.60
6.25%	3.08	4.44	4.75
0.00%	4.62	4.46	4.57
Inhibit Rate	-33.38%		-52.86%
Enhancement Rate	33.09%		

Table 4

Data of Aldehyde Group (CHO) modification with the application on differ dosage of single antibiotics.

Category	Antibiotic	GP	Dosage	C_CHO					ST_CHO				
			mg/L	S1	S2	S3	S4	S5	average	max	min		
β-Lactams	Penicillin G Sodium Salt	100.00%	55.38	0.10455	0.09830	0.07149	0.08310	0.10187	0.08936	0.01519	0.01787		
		50.0%	27.69	0.06486	0.07277	0.08068	0.09809	0.07040	0.0791	0.01899	0.01424		
		25.0%	13.85	0.04178	0.05830	0.04955	0.04469	0.05247	0.04858	0.00972	0.0068		
		12.5%	6.92	0.01795	0.01993	0.01736	0.02368	0.01756	0.01973	0.00395	0.00237		
		6.25%	3.46	0.05441	0.04818	0.04648	0.07765	0.06065	0.05668	0.02097	0.0102		
		3.13%	1.73	0.05047	0.05280	0.04673	0.03692	0.05280	0.04673	0.00607	0.00981		
		1.56%	0.86	0.05981	0.04322	0.04875	0.04928	0.04373	0.05026	0.00955	0.00704		
		0.00%	0.00	0.05716	0.05091	0.05928	0.04447	0.04975	0.05293	0.00635	0.00846		
	Cefaclor	100.00%	15.38	0.04791	0.06539	0.05299	0.05919	0.06370	0.05637	0.00902	0.00846		
		50.0%	7.69	0.06767	0.06377	0.05206	0.07678	0.06897	0.06507	0.01171	0.01301		
		25.0%	3.85	0.10972	0.13029	0.11543	0.10172	0.12458	0.11429	0.016	0.01257		
		12.5%	1.92	0.05222	0.05831	0.05476	0.03751	0.04360	0.0507	0.00761	0.01319		
		6.25%	0.96	0.04759	0.06849	0.06733	0.04875	0.06094	0.05804	0.01045	0.01045		
		3.13%	0.48	0.05019	0.06084	0.04918	0.04259	0.04462	0.0507	0.01014	0.00811		
Aminoglycosides	Streptomycin Sulfate	100.00%	30.77	0.05766	0.03973	0.05814	0.03827	0.05523	0.04845	0.00969	0.01018		
		50.0%	15.38	0.04594	0.04884	0.03917	0.05949	0.05513	0.04836	0.01113	0.00919		
		25.0%	7.69	0.04417	0.04272	0.05194	0.05533	0.04320	0.04854	0.00679	0.00582		
		12.5%	3.85	0.04012	0.04280	0.03923	0.05617	0.04948	0.04458	0.01159	0.00535		
		6.25%	1.92	0.04772	0.04955	0.03670	0.04955	0.04175	0.04588	0.00367	0.00918		
		3.13%	0.96	0.04751	0.05284	0.04751	0.02974	0.04218	0.0444	0.00844	0.01466		
		1.56%	0.48	0.04618	0.05797	0.05404	0.03833	0.05060	0.04913	0.00884	0.0108		
		0.00%	0.00	0.04922	0.05187	0.05928	0.05135	0.05134	0.05293	0.00635	0.00371		
	Amikacin Sulfate	100.00%	15.00	0.06034	0.06221	0.06221	0.06408	0.06408	0.06221	0.00187	0.00187		
		50.0%	7.50	0.06345	0.07121	0.08319	0.06415	0.05852	0.0705	0.01269	0.00705		
		25.0%	3.75	0.08534	0.09571	0.07816	0.05983	0.09252	0.07976	0.01595	0.01993		
		12.5%	1.88	0.08680	0.08998	0.06848	0.07326	0.07963	0.07963	0.01035	0.01115		
		6.25%	0.94	0.05998	0.06700	0.06062	0.06764	0.06381	0.06381	0.00383	0.00383		
		3.13%	0.47	0.05927	0.06043	0.06741	0.04533	0.05579	0.05811	0.0093	0.01278		
1.56%		0.23	0.07715	0.07517	0.07385	0.03759	0.06660	0.06594	0.01121	0.02835			
0.00%		0.00	0.03930	0.04300	0.05549	0.04717	0.05096	0.04624	0.00925	0.00694			
Macrolides	Erythromycin	100.00%	123.08	0.05283	0.03805	0.05104	0.03716	0.03985	0.04477	0.00806	0.00761		
		50.0%	61.54	0.03869	0.03599	0.03869	0.06659	0.04049	0.04499	0.0216	0.009		
		25.0%	30.77	0.03788	0.04389	0.04897	0.05406	0.03927	0.0462	0.00786	0.00832		
		12.5%	15.38	0.04444	0.03878	0.04706	0.04400	0.04967	0.04357	0.00349	0.00479		
		6.25%	7.69	0.07008	0.07075	0.07816	0.05053	0.06334	0.06738	0.01078	0.01685		
		3.13%	3.85	0.07062	0.06297	0.05120	0.05061	0.05355	0.05885	0.01177	0.00824		
		1.56%	1.92	0.04807	0.05756	0.06112	0.07061	0.06231	0.05934	0.01127	0.01127		
		0.00%	0.00	0.05349	0.03929	0.03977	0.05681	0.04166	0.04734	0.00947	0.00805		
		Quinolones	Levofloxacin	100.00%	6.15	0.04367	0.04856	0.04081	0.03020	0.03306	0.04081	0.00775	0.01061
				50.0%	3.08	0.04763	0.04344	0.06176	0.05653	0.05967	0.05234	0.00942	0.0089
25.0%	1.54			0.07749	0.09606	0.08476	0.06457	0.09444	0.08072	0.01534	0.01615		
12.5%	0.77			0.05913	0.05913	0.07603	0.06563	0.06888	0.06498	0.01105	0.00585		
6.25%	0.38			0.05631	0.08105	0.04979	0.06993	0.05690	0.05927	0.01066	0.00948		
3.13%	0.19			0.04540	0.05844	0.04279	0.06209	0.04696	0.05218	0.00991	0.00939		
1.56%	0.10			0.04549	0.04971	0.05534	0.03706	0.04878	0.0469	0.00844	0.00984		
0.00%	0.00			0.04464	0.05208	0.04414	0.05754	0.05654	0.0496	0.00794	0.00546		

Table 5

Data of Aldehyde Group (CHO) modification with the application on differ dosage of compound antibiotics.

Group	GP	C_CHO					ST_CHO		
	%	S1	S2	S3	S4	S5	average	max	min
P-S	100.00%	0.03618	0.04106	0.04187	0.04349	0.04106	0.04065	0.00284	0.00447
	25.00%	0.03774	0.03298	0.03920	0.03664	0.03408	0.03664	0.00256	0.00366
	12.50%	0.03128	0.04007	0.03023	0.03902	0.03023	0.03515	0.00492	0.00492
	6.25%	0.03820	0.04135	0.03899	0.03898	0.03780	0.03938	0.00197	0.00118
	0.00%	0.03805	0.03288	0.03953	0.03730	0.04063	0.03694	0.00259	0.00406
A-S	100.00%	0.03894	0.04463	0.04113	0.05030	0.03981	0.04375	0.00655	0.00481
	25.00%	0.07106	0.07724	0.07261	0.08805	0.08110	0.07724	0.01081	0.00618
	12.50%	0.05840	0.06693	0.07153	0.06562	0.07153	0.06562	0.00591	0.00722
	6.25%	0.06608	0.05401	0.05909	0.07498	0.06926	0.06354	0.01144	0.00953
	0.00%	0.04217	0.03753	0.03946	0.03560	0.04295	0.03869	0.00348	0.00309
L-C	100.00%	0.04394	0.04684	0.04062	0.03440	0.04518	0.04145	0.00539	0.00705
	25.00%	0.03352	0.04200	0.04200	0.03660	0.04392	0.03853	0.00347	0.00501
	12.50%	0.04184	0.03994	0.04184	0.02854	0.03728	0.03804	0.00380	0.00950
	6.25%	0.04217	0.03974	0.04339	0.03690	0.03974	0.04055	0.00284	0.00365
	0.00%	0.04883	0.04232	0.04557	0.04928	0.04976	0.04650	0.00278	0.00418

Table 6
Data of pH by utilising diverse treatment on distinct dosage of single antibiotics.

Category	Antibiotic	GP	Dosage	V_pH					ST_pH				
			mg/L	S1	S2	S3	S4	S5	average	max	min		
β-lactams	Penicillin G Sodium Salt	100.00%	55.38	4.71	4.05	4.19	5.89	4.38	4.71	1.18	0.66		
		50.0%	27.69	5.16	5.16	4.59	4.01	4.49	4.73	0.43	0.72		
		25.0%	13.85	4.09	4.21	3.88	4.50	4.67	4.17	0.33	0.29		
		12.5%	6.92	4.75	3.88	3.80	4.09	3.55	4.13	0.62	0.33		
		6.25%	3.46	5.31	6.36	5.09	5.36	5.97	5.53	0.83	0.44		
		3.13%	1.73	5.68	6.01	5.34	5.01	5.51	5.51	0.5	0.5		
		1.56%	0.86	5.80	5.85	5.16	4.47	4.63	5.32	0.53	0.85		
		0.00%	0.00	4.77	4.97	4.53	5.41	4.43	4.92	0.49	0.39		
	Cefaclor	100.00%	15.38	4.92	5.27	5.33	7.64	5.27	5.79	1.85	0.87		
		50.0%	7.69	5.13	5.19	5.30	7.18	5.87	5.7	1.48	0.57		
			25.0%	3.85	6.25	6.08	4.93	4.86	5.81	5.48	0.77	0.82	
			12.5%	1.92	5.25	4.41	4.46	4.64	4.22	4.69	0.56	0.28	
			6.25%	0.96	4.33	5.28	4.14	5.29	4.28	4.76	0.53	0.62	
			3.13%	0.48	4.55	4.46	4.21	6.58	4.46	4.95	1.63	0.74	
1.56%			0.24	4.97	4.31	3.99	5.49	4.03	4.69	0.8	0.7		
0.00%			0.00	4.44	4.89	4.08	4.71	4.26	4.53	0.36	0.45		
Aminoglycosides			Streptomycin Sulfate	100.00%	30.77	5.87	6.64	6.29	4.92	5.34	5.93	0.71	1.01
	50.0%	15.38		4.66	5.85	4.82	6.35	4.82	5.42	0.93	0.76		
	25.0%	7.69		5.07	4.80	5.77	5.92	4.90	5.39	0.53	0.59		
	12.5%	3.85		4.89	5.09	5.04	5.34	5.60	5.09	0.25	0.2		
	6.25%	1.92		4.58	5.45	4.77	4.68	4.24	4.87	0.58	0.29		
	3.13%	0.96		4.53	4.78	5.33	5.28	4.88	4.98	0.35	0.45		
	1.56%	0.48		5.18	5.03	4.55	4.80	4.79	4.84	0.34	0.29		
	Amikacin Sulfate	0.00%	0.00	4.97	4.97	4.72	5.02	5.51	4.92	0.1	0.2		
		100.00%	15.00	4.46	4.15	4.06	5.37	5.10	4.51	0.86	0.45		
		50.0%	7.50	5.01	5.15	5.01	4.27	5.30	4.86	0.29	0.59		
		25.0%	3.75	4.60	5.13	4.12	5.31	4.55	4.79	0.52	0.67		
		12.5%	1.88	4.62	4.38	4.71	5.53	4.38	4.81	0.72	0.43		
		6.25%	0.94	5.24	4.00	4.05	5.11	4.23	4.6	0.64	0.6		
		3.13%	0.47	4.00	4.99	4.63	4.54	4.04	4.54	0.45	0.54		
		1.56%	0.23	5.15	5.20	4.14	3.91	3.91	4.6	0.6	0.69		
		0.00%	0.00	5.22	5.13	4.02	4.11	4.90	4.62	0.6	0.6		
		Macrolides	Erythromycin	100.00%	123.08	5.02	5.50	4.38	4.58	4.29	4.87	0.63	0.49
50.0%	61.54			4.20	5.26	4.88	4.98	4.35	4.83	0.43	0.63		
25.0%	30.77			4.72	4.46	4.17	3.49	4.13	4.21	0.51	0.72		
12.5%	15.38			4.00	4.31	4.54	5.31	4.13	4.54	0.77	0.54		
6.25%	7.69			4.16	4.11	4.07	5.94	4.57	4.57	1.37	0.5		
3.13%	3.85			5.14	4.87	4.82	3.53	4.50	4.59	0.55	1.06		
		1.56%	1.92	4.82	4.19	4.87	4.32	4.19	4.55	0.32	0.36		
		0.00%	0.00	4.94	5.27	4.90	3.37	4.44	4.62	0.65	1.25		
		Quinolones	Levofloxacin	100.00%	6.15	6.97	6.90	6.16	4.85	6.84	6.22	0.75	1.37
				50.0%	3.08	5.04	6.34	5.49	5.77	6.51	5.66	0.68	0.62
				25.0%	1.54	4.85	5.05	4.55	5.75	4.70	5.05	0.7	0.5
				12.5%	0.77	4.51	3.97	4.60	4.96	4.19	4.51	0.45	0.54
6.25%	0.38			4.72	4.63	5.24	4.29	4.30	4.72	0.52	0.43		
3.13%	0.19			4.20	4.29	4.77	5.82	4.96	4.77	1.05	0.57		
1.56%	0.10			4.96	5.51	4.96	4.41	4.76	4.96	0.55	0.55		
0.00%	0.00			3.90	4.89	4.35	4.98	4.26	4.53	0.45	0.63		

Table 7

Data of pH by utilising diverse treatment on distinct dosage of compound antibiotics.

Group	GP	V_pH					ST_pH		
	%	S1	S2	S3	S4	S5	average	max	min
P-S	100.00%	4.32	4.73	4.64	4.87	4.18	4.64	0.23	0.32
	25.00%	5.18	4.81	5.08	6.08	4.76	5.29	0.79	0.48
	12.50%	4.61	4.61	4.56	6.05	5.06	4.96	1.09	0.40
	6.25%	4.56	4.32	4.51	5.61	5.13	4.75	0.86	0.43
	0.00%	4.92	4.28	4.33	4.51	4.83	4.51	0.41	0.23
A-S	100.00%	4.51	5.06	4.88	3.96	4.83	4.60	0.46	0.64
	25.00%	4.54	4.49	4.64	5.45	4.68	4.78	0.67	0.29
	12.50%	4.44	4.44	4.53	5.28	4.39	4.67	0.61	0.23
	6.25%	5.15	4.87	5.25	3.82	4.29	4.77	0.48	0.95
	0.00%	4.68	4.48	5.11	5.21	4.77	4.87	0.34	0.39
L-C	100.00%	4.76	4.95	4.72	4.25	4.48	4.67	0.28	0.42
	25.00%	4.10	4.50	4.54	4.50	4.15	4.41	0.13	0.31
	12.50%	4.67	4.86	5.24	4.47	4.81	4.81	0.43	0.34
	6.25%	5.11	4.85	5.37	5.31	5.37	5.16	0.21	0.31
	0.00%	5.64	6.16	5.69	5.99	6.05	5.87	0.29	0.23

Table 8
Data of OD₆₀₀ with the approach on various dosage of single antibiotics.

Category	Antibiotic	GP		V_OD ₆₀₀					ST_OD ₆₀₀				
		%	mg/L	S1	S2	S3	S4	S5	average	max	min		
β-lactams	Penicillin G Sodium Salt	100.00%	55.38	0.89170	1.07410	0.86131	1.22609	1.03357	1.0133	0.21279	0.15199		
		50.0%	27.69	1.01453	0.91942	1.10964	1.18361	1.21532	1.0568	0.12681	0.13738		
		25.0%	13.85	1.35087	1.38738	1.22917	0.90058	1.14398	1.217	0.17038	0.31642		
		12.5%	6.92	1.21321	1.41541	1.22766	1.92092	1.44430	1.4443	0.47662	0.23109		
		6.25%	3.46	1.07576	1.12823	1.12823	1.91538	1.27254	1.3119	0.60348	0.23614		
		3.13%	1.73	1.20063	1.30391	1.25227	1.40719	1.31682	1.291	0.11619	0.09037		
		1.56%	0.86	1.08963	1.30756	1.15017	1.29544	1.19859	1.2107	0.09686	0.12107		
		0.00%	0.00	1.11362	1.16101	1.30317	1.16100	1.29132	1.1847	0.11847	0.07108		
	Cefidrol	100.00%	15.38	0.66477	0.58167	0.54971	0.76065	0.63281	0.6392	0.12145	0.08949		
		50.0%	7.69	0.97048	0.95346	0.97048	0.51078	0.92792	0.8513	0.11918	0.34052		
			25.0%	3.85	1.42267	1.17067	1.43526	1.00720	1.18346	1.259	0.17626	0.2518	
			12.5%	1.92	1.45176	1.62534	1.62534	1.60956	1.57800	1.578	0.04734	0.12624	
			6.25%	0.96	1.54103	1.40094	1.74339	1.54104	1.54103	1.5566	0.18679	0.15566	
			3.13%	0.48	1.55788	1.24925	1.39622	1.67545	1.26394	1.4697	0.20575	0.22045	
1.56%			0.24	1.36719	1.00422	1.28249	1.18570	1.34299	1.2099	0.15729	0.20568		
0.00%			0.00	1.14556	1.23368	1.04643	0.98033	1.21165	1.1015	0.13218	0.12117		
Aminoglycosides			Streptomycin Sulfate	100.00%	30.77	0.89900	0.84560	0.81889	0.99691	0.72988	0.8901	0.10681	0.07121
	50.0%	15.38		1.04515	1.00848	0.94430	0.66927	0.90763	0.9168	0.12835	0.24753		
	25.0%	7.69		1.06517	1.02420	1.07541	0.93202	1.00372	1.0242	0.05121	0.09218		
	12.5%	3.85		1.07644	1.20656	1.06461	1.38399	1.19473	1.1829	0.20109	0.11829		
	6.25%	1.92		1.14958	0.95609	1.12682	1.32031	1.19511	1.1382	0.18211	0.18211		
	3.13%	0.96		1.01060	1.12415	1.14666	1.26039	1.10144	1.1355	0.12489	0.1249		
	1.56%	0.48		1.21715	1.11080	1.34714	1.05171	1.03990	1.1817	0.16544	0.12999		
	0.00%	0.00		1.29282	1.03658	1.08317	1.24623	1.18799	1.1647	0.12812	0.12812		
	Amikacin Sulfate	100.00%	15.00	1.38871	1.33630	1.46731	1.04808	1.42801	1.3101	0.15721	0.26202		
		50.0%	7.50	1.36257	1.42451	1.32541	0.84231	1.42451	1.2387	0.18581	0.39639		
		25.0%	3.75	1.22024	1.19845	1.24203	0.69728	1.01324	1.0895	0.15253	0.39222		
		12.5%	1.88	1.28232	1.00997	1.22558	1.02133	1.08941	1.1348	0.14752	0.12483		
		6.25%	0.94	1.19624	1.33837	1.07780	1.12519	1.21993	1.1844	0.15397	0.1066		
		3.13%	0.47	1.14041	1.29812	1.39518	1.01909	1.20107	1.2132	0.18189	0.19411		
		1.56%	0.23	1.06675	1.06675	1.03342	1.27788	1.14454	1.1112	0.16668	0.07778		
		0.00%	0.00	1.34007	1.35193	1.20962	0.84198	1.28077	1.1859	0.16603	0.34392		
Macrolides	Erythromycin	100.00%	123.08	0.84378	0.95571	0.74907	0.89544	0.91266	0.861	0.09471	0.11193		
		50.0%	61.54	0.97157	1.07433	0.76604	0.92486	1.05565	0.9342	0.14013	0.16816		
		25.0%	30.77	1.50980	1.64302	1.24337	1.52461	1.39139	1.4802	0.16282	0.23663		
		12.5%	15.38	1.37385	1.05205	1.18819	1.33671	1.34909	1.2377	0.13615	0.18555		
		6.25%	7.69	1.65198	1.65198	1.17793	1.26411	1.26412	1.4365	0.21548	0.25857		
		3.13%	3.85	1.11009	1.43279	1.16172	1.45860	1.09718	1.2908	0.1678	0.18071		
		1.56%	1.92	1.06184	1.19609	1.07404	1.55003	1.07404	1.2205	0.32953	0.15866		
		0.00%	0.00	1.12661	1.16218	1.34007	1.11474	1.20962	1.1859	0.15417	0.07116		
		Quinolones	Levofloxacin	100.00%	6.15	0.59770	0.50012	0.54891	0.79287	0.60990	0.6099	0.18297	0.10978
				50.0%	3.08	0.88326	0.89167	0.89167	0.69820	0.73184	0.8412	0.05047	0.143
				25.0%	1.54	1.08646	1.27378	1.09894	1.53602	1.37368	1.2488	0.28722	0.16234
				12.5%	0.77	1.70432	1.71996	1.39160	1.43852	1.37597	1.5636	0.15636	0.172
				6.25%	0.38	1.54430	1.35126	1.64824	1.39580	1.54430	1.4849	0.16334	0.13364
				3.13%	0.19	1.38976	1.52142	1.52142	1.41900	1.27272	1.4629	0.05852	0.07314
1.56%	0.10			1.16040	1.11992	1.29533	1.82155	1.24136	1.3493	0.47225	0.22938		
0.00%	0.00	0.94729	1.25571	0.96932	1.23368	1.22267	1.1015	0.15421	0.15421				

Table 9Data of OD₆₀₀ with the approach on various dosage of compound antibiotics.

Group	GP	V_OD ₆₀₀					ST_OD ₆₀₀		
	%	S1	S2	S3	S4	S5	average	max	min
P-S	100.00%	0.85115	0.70929	0.84327	0.74869	0.71717	0.78810	0.06305	0.07881
	25.00%	1.04841	0.88638	0.86732	1.01029	0.87685	0.95310	0.09531	0.08578
	12.50%	0.89496	0.89496	0.94468	1.24300	0.99440	0.99440	0.24860	0.09944
	6.25%	1.05111	0.98928	1.04081	1.04080	1.04081	1.03050	0.02061	0.04122
	0.00%	1.22816	1.18854	1.36022	1.50548	1.18854	1.32060	0.18488	0.13206
A-S	100.00%	0.88305	0.79054	0.83259	0.85782	0.88305	0.84100	0.04205	0.05046
	25.00%	1.00631	1.01608	0.89884	0.98677	0.89884	0.97700	0.03908	0.07816
	12.50%	1.11553	1.01224	1.05356	0.95027	0.92961	1.03290	0.08263	0.08263
	6.25%	0.97546	0.89819	0.92717	1.06238	1.04306	0.96580	0.09658	0.06761
	0.00%	0.97722	1.08344	1.11531	1.07283	0.95598	1.06220	0.05311	0.08498
L-C	100.00%	0.79680	0.91300	0.79680	0.81340	0.77190	0.83000	0.08300	0.03320
	25.00%	1.00501	1.05427	1.01486	0.86706	0.89662	0.98530	0.06897	0.11824
	12.50%	1.17593	1.12098	0.98910	1.10999	1.00009	1.09900	0.07693	0.10990
	6.25%	1.18524	1.32695	1.28830	1.35271	1.28830	1.28830	0.06441	0.10306
	0.00%	1.22858	1.07352	1.21666	1.25244	1.20473	1.19280	0.05964	0.11928

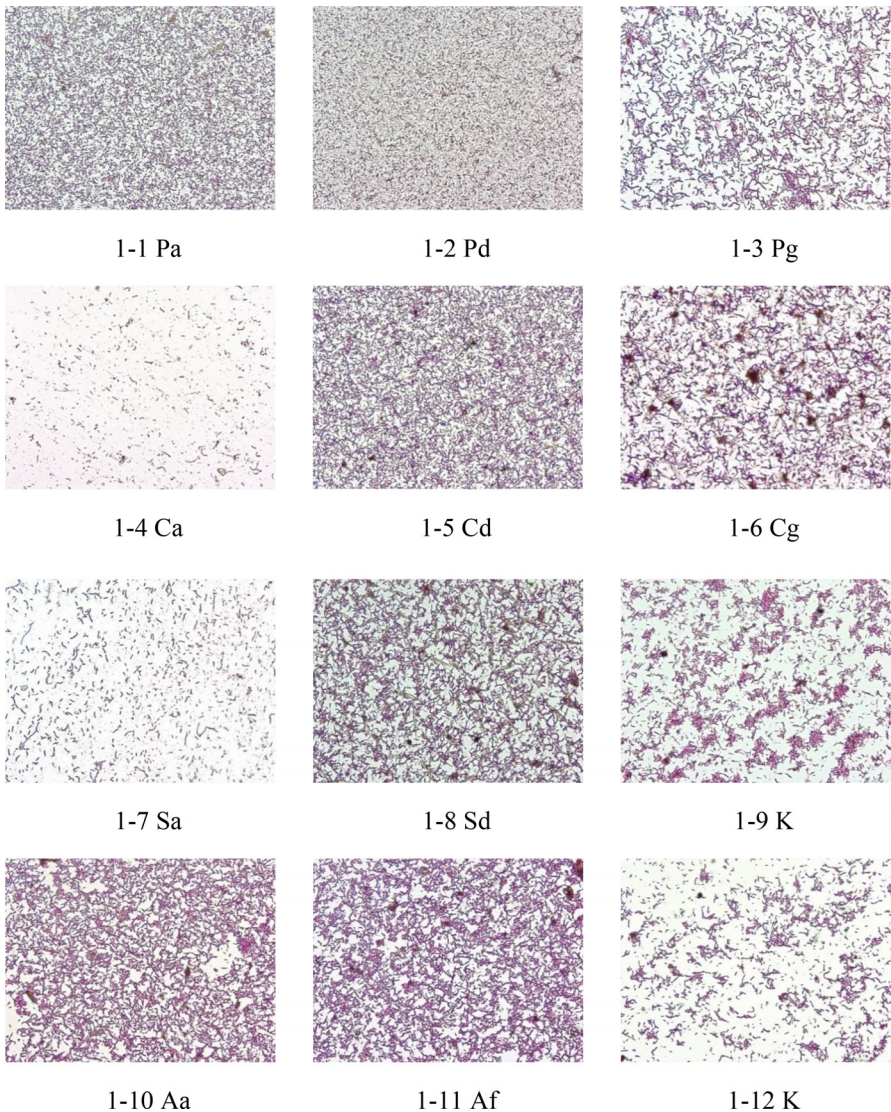


Fig. 1. Gram staining of typical samples of single antibiotics and compound antibiotics with $40 \times$ objective lens.

Images in [Fig. 1](#) are gram staining of typical samples of single and compound antibiotics. In the below microscopy image gallery, Fig. 1-1 to 1-18 refers to the gram test photo of single antibiotic treatment samples; Fig. 1-19 to 1-21 refers to the gram test photo of compound antibiotic treatment samples. The objective lens magnification used for all images was $40 \times$.

Images in [Fig. 2](#) are gram staining of typical samples of Penicillin G, Amikacin Sulfate, Levofloxacin treatment groups. The objective lens magnification used for all images was $100 \times$. The correspondence between photos and samples is shown in [Table 10](#).

The correspondence between photos ([Fig. 1](#) and [Fig. 2](#)) and sample numbers is shown in [Table 10](#).

Based on the biodiversity test of Pd, Aa, La and K samples (The 16S rRNA gene sequences were deposited in the NCBI Sequence Read Archive under accession number PRJNA784035), a se-

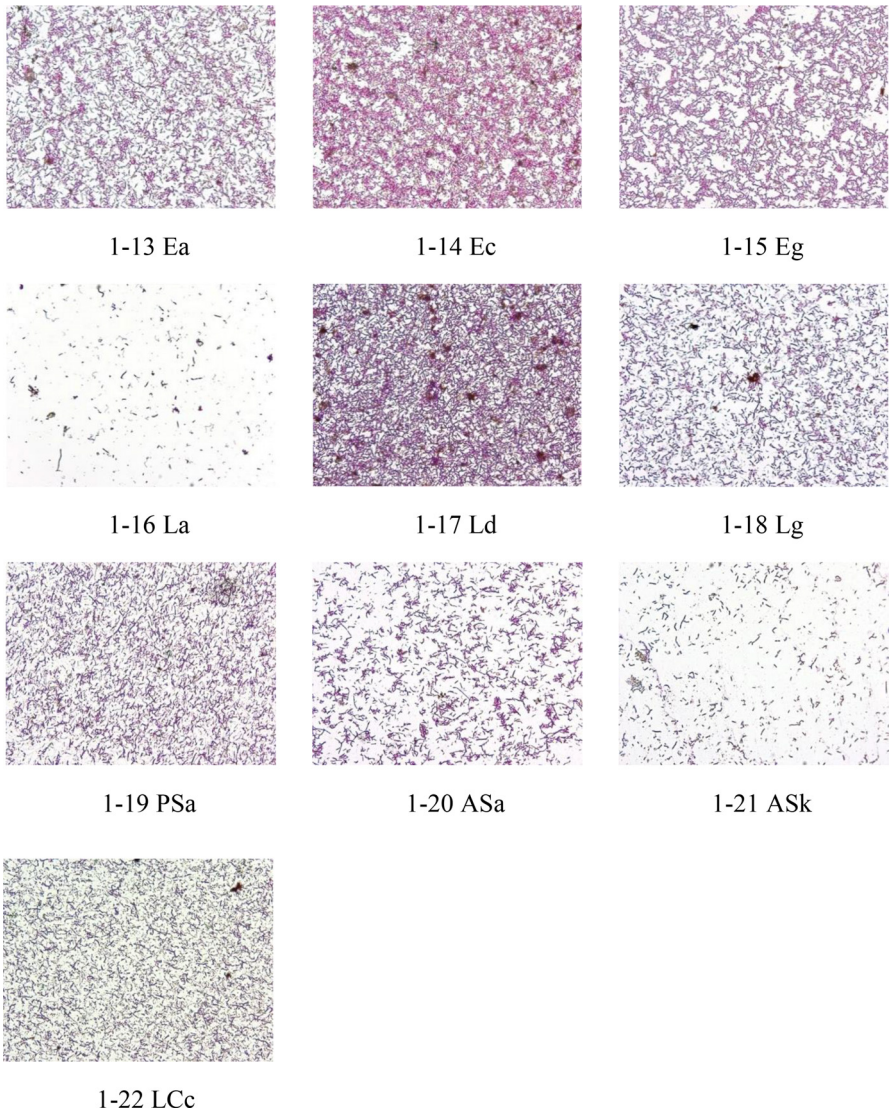


Fig. 1. Continued

ries of species composition analysis and correlation analysis were carried out and data mapping were performed. Analysis and mapping include: bar chart of the distribution of microbial diversity (Fig. 3), evolutionary tree on Genus level (Fig. 4), community heatmap analysis on Genus level (Fig. 5), Ternary analysis (Fig. 6), spearman correlation heatmap of bacterial on Genus level (Fig. 7), circo graph of the correspondence between samples and species (Fig. 8), PDA-CCA analysis of the correlation between Penicillin G, Amikacin Sulfate, Levofloxacin treatment and CHO, pH, H₂ yields (Fig. 9).

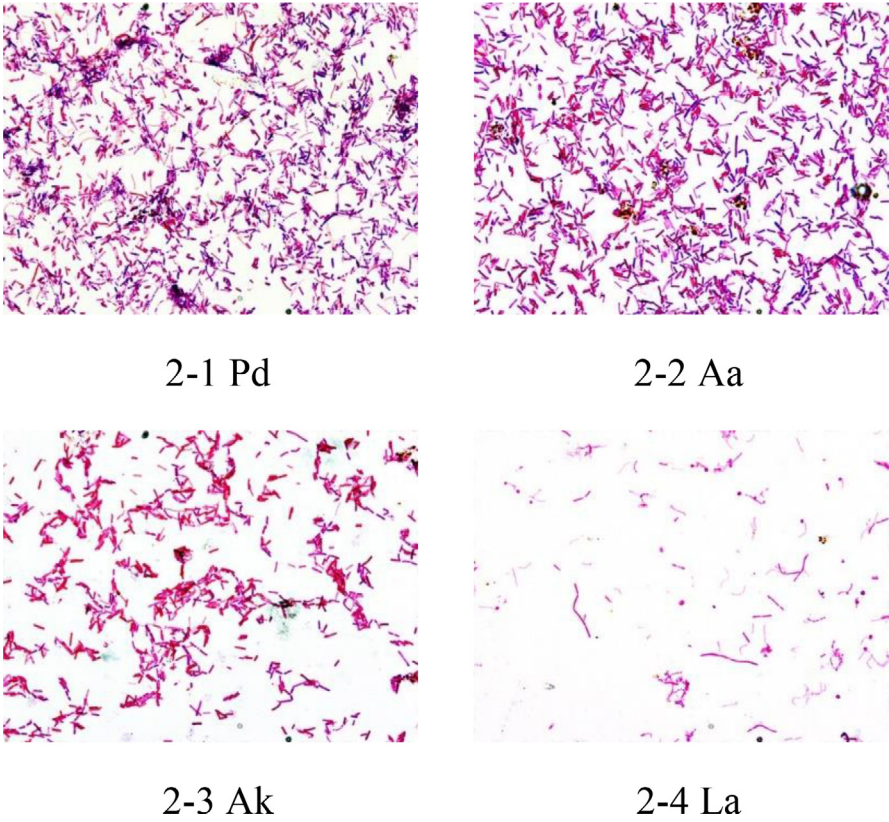


Fig. 2. Gram staining of typical samples with 100 × objective lens.

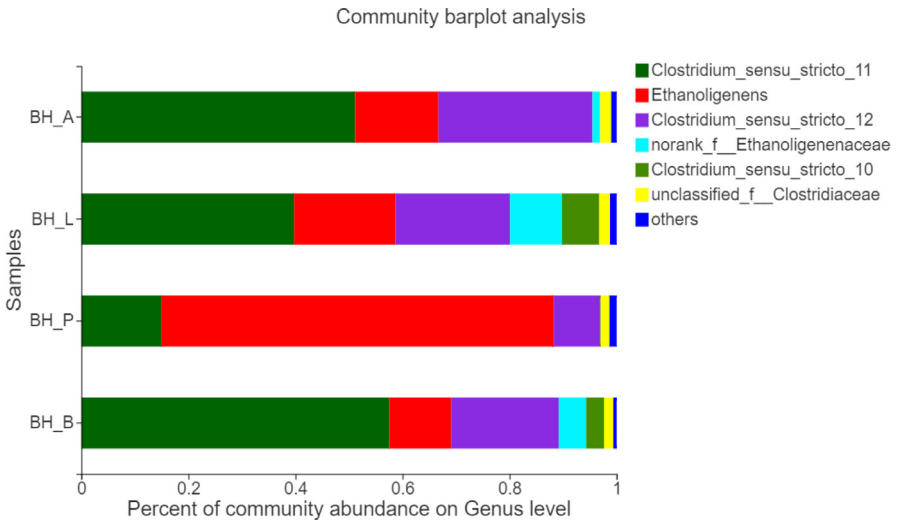


Fig. 3. Community bar chart.

Table 10
Sample table of gram test photographs.

Group	Antibiotic	Abbreviation	Amount Dosage Corresponding to GP (mg/L)							
			100.00 %	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.00%
Signal	Penicillin G	P	Pa	Pb	Pc	Pd*	Pe	Pf	Pg	K
	Cefaclor	C	Ca	Cb	Cc	Cd	Ce	Cf	Cg	K
	Streptomycin Sulfate	S	Sa	Sb	Sc	Sd	Se	Sf	Sg	K
	Amikacin Sulfate	A	Aa*	Ab	Ac	Ad	Ae	Af	Ag	K*
	Erythromycin	E	Ea	Eb	Ec	Ed	Ee	Ef	Eg	K
	Levofloxacin	L	La*	Lb	Lc	Ld	Le	Lf	Lg	K
Com-pound	Penicillin G + Streptomycin Sulfate	P-S	PSa	o	PSc	PSd	PSe	o	o	K
	Amikacin Sulfate + Streptomycin Sulfate	A-S	ASa	o	ASc	ASd	ASe	o	o	K
	Levofloxacin + Cefaclor	L-C	LCa	o	LCc	LCd	LCE	o	o	K

XX: Red and bold numbers are photographed samples

XX*: one photo each was taken under the 40× and the 100× objective.

o: not sample.

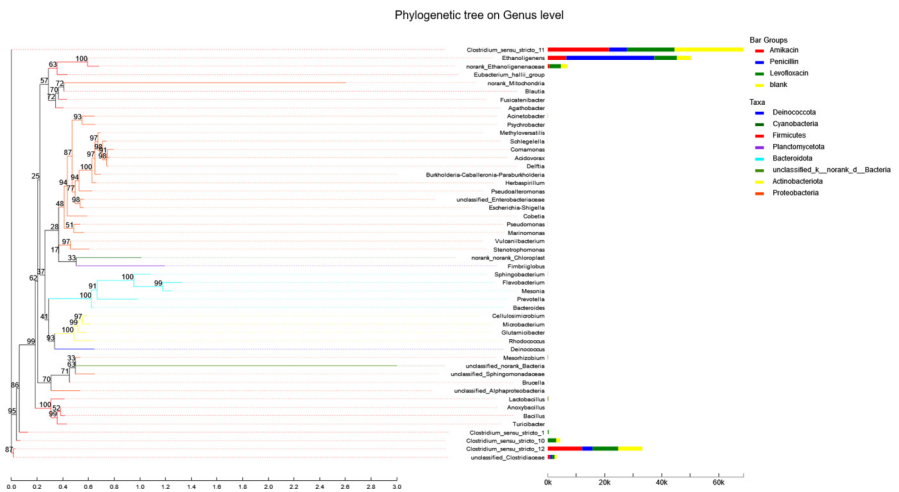


Fig. 4. The evolutionary tree.

Community heatmap analysis on Genus level

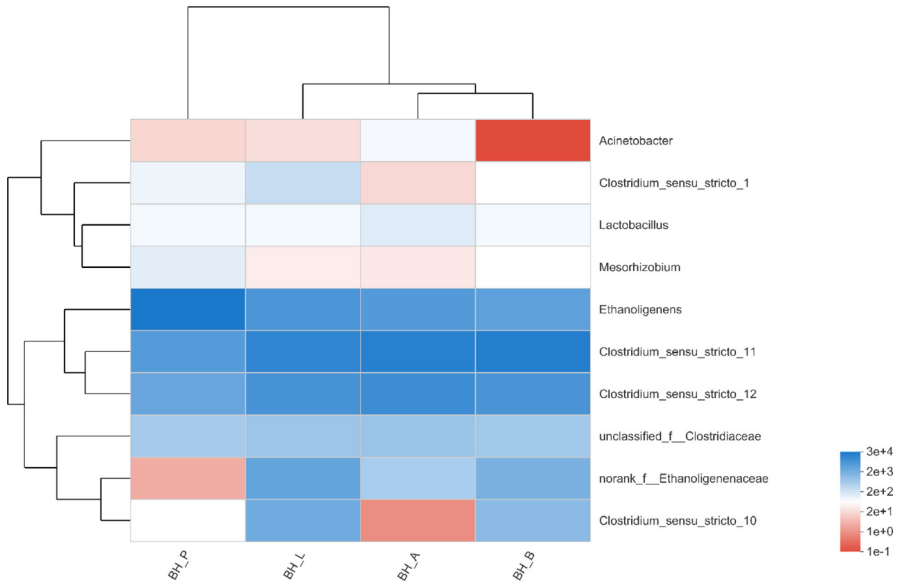


Fig. 5. Top 10 species heatmap.

Ternary analysis

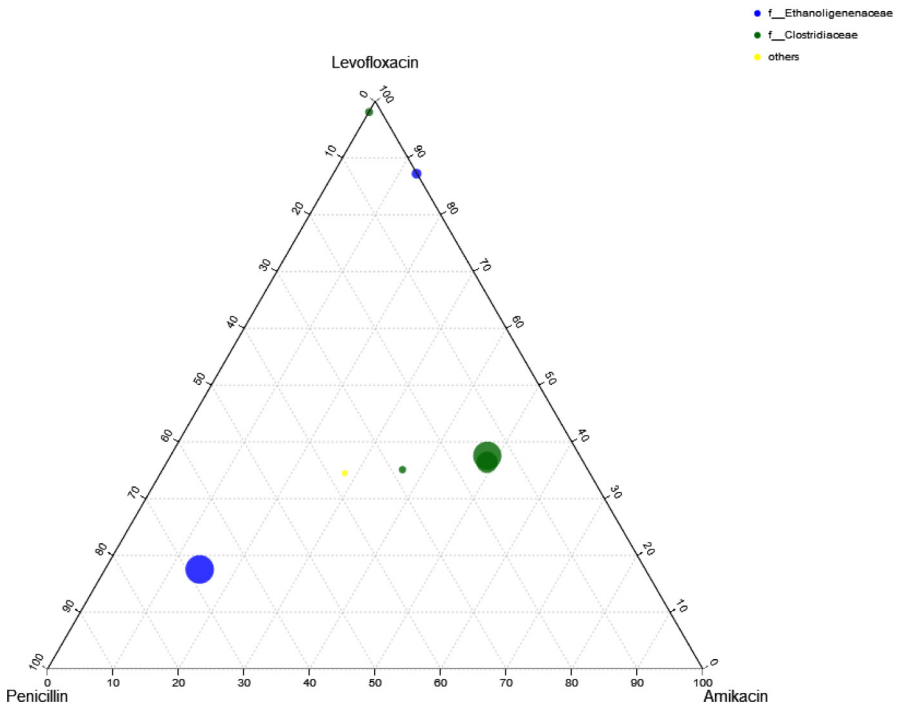


Fig. 6. Ternary analysis.

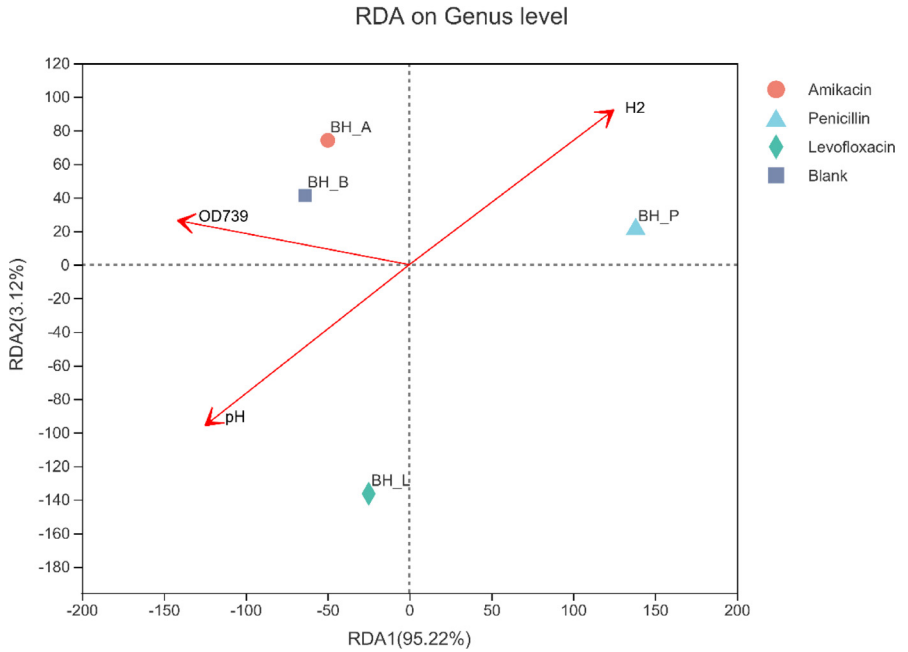


Fig. 7. RDA-CCA analysis.

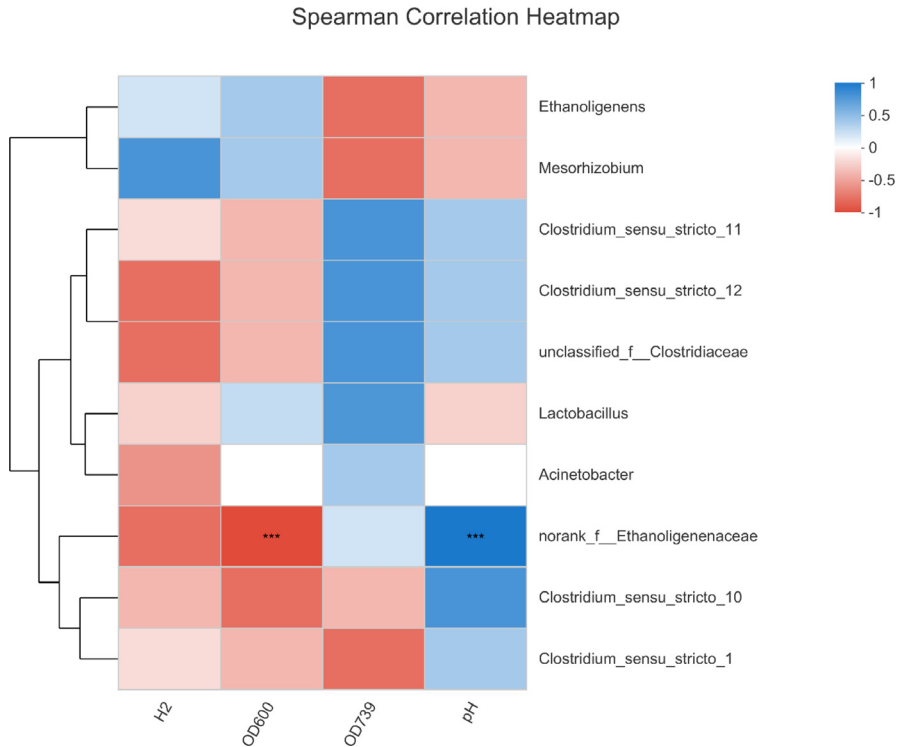


Fig. 8. Spearman correlation heatmap.

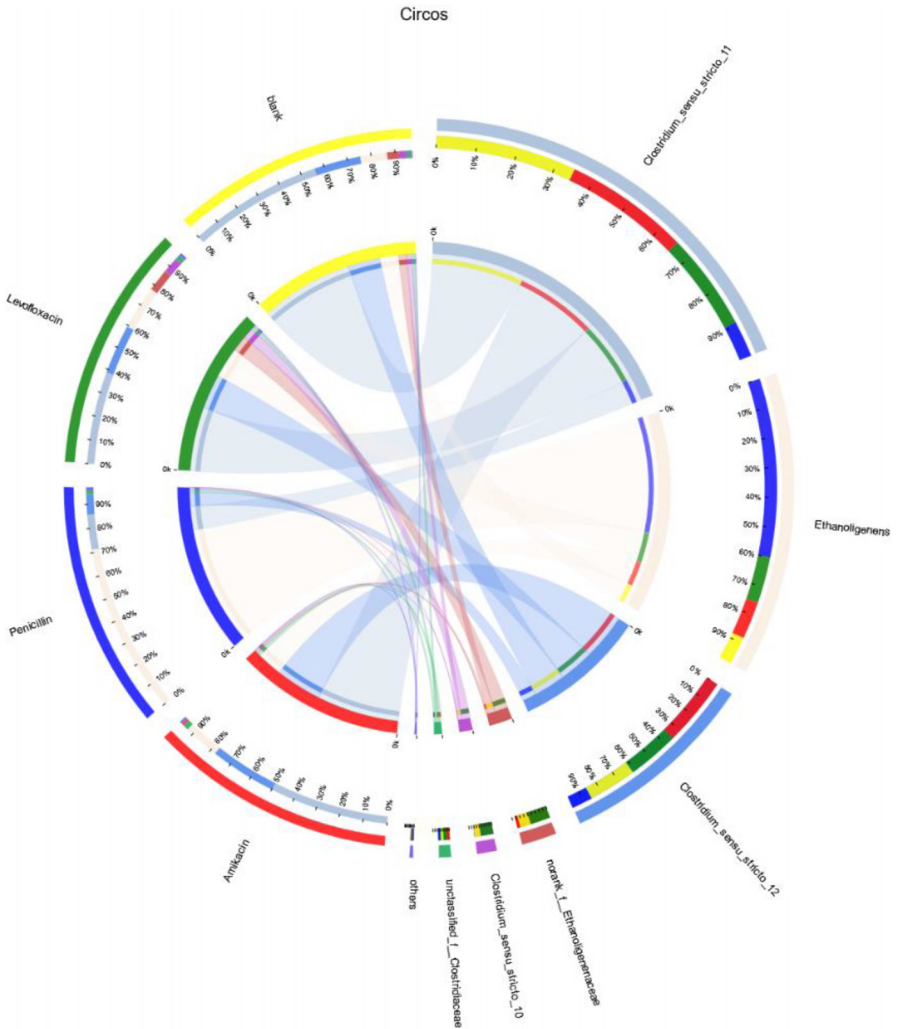


Fig. 9. Circos analysis.

2. Experimental Design, Materials and Methods

2.1. Medium and Culture Conditions

Coal geology H_2 -producing bacteria community was isolated from enrichment samples collected from an anthracite sample extracted in Zhaozhuang coal mining located in Jincheng, Shanxi Province (GPS coordinates is $35^{\circ}34'10''N$, $112^{\circ}53'55''E$). The H_2 -producing bacteria were grown anaerobically on Potato Dextrose medium (abbreviated as PD medium)[1]. The content of the PD medium was (g/L): potato soluble starch, 20.00; dextrose, 20.00; NH_4Cl , 3.50; KCl , 3.20; $NaCl$, 0.70; $MgSO_4 \cdot 7H_2O$, 0.20, $FeCl_3$, 0.05; $CaCl_2$, 0.02; yeast extract, 0.50, and 1.00 mL/L of $C_{12}H_7NO_4$ was added as an oxygen indicator [1]. Final medium pH=6.2. The prepared PD medium was sterilized at $121^{\circ}C$ and 0.105 MPa for 25 minutes. The PD medium was then mixed with the bacterial solution at a ratio of 4:1 in an anaerobic chamber (A 95, WDS, Britain). The

mixed medium was divided into 200 mL aliquots to anaerobic culture flasks, then sealed with butyl rubber stoppers and removed from the chamber. The samples configured in accordance to experimental design and were placed in shakers (JK-LI-15, Jingke, China) with temperature set at 40 °C with a shaking speed set at 60 rpm [2]. Cultivation time: 3 days.

2.2. Selection of Antibiotics and Gradient Experiment Design

Six antibiotics used in experiments comprise Penicillin G, Cefaclor, Streptomycin Sulfate, Amikacin Sulfate, Erythromycin, and Levofloxacin. The maximum dosage (abbreviated as MD) for each antibiotic was referred to the highest concentration in urine which was recorded in the instructions.

The gradient percentage (abbreviated as GP) of single antibiotic was set by dichotomy method from 100% to 1%, which were: 100.00%, 50.00%, 25.00%, 12.50%, 6.25%, 3.13%, 1.56%. The compound antibiotic concentration grade was set as 100.00%, 25.00%, 12.50%, 6.25%. The corresponding relationship between each antibiotic gradient and dosage is provided in Table 2.

Meanwhile, 0.00% comparison group was set for each antibiotic. 5 parallel samples were set for each antibiotic and each concentration.

2.3. H₂ Yield Data Collection

The gas yield of each sample was collected through 1500 mL gas sampling bags. The total gas yield (record as V_T) was tested with 100 mL gas needle at the end of each experiment. The H₂ yield was calculated on the base of the total gas yield and H₂ concentration (formula 1).

Gas composed of H₂ was analysed by using an Agilent 7890A gas chromatograph. The column was Agilent Carbonplot (60 m × 320 μm) and the carrier gas is high purity nitrogen (99.999%). The carrier gas flow rate was set at 3 mL/min. The injection port was maintained at 150 °C, the oven temperature was 25 °C, the TCD was operated at 200 °C, reference flow rate 400 mL/min, tail flow rate 8 mL/min. The retention time for H₂ was 3.2 minutes, and CO₂ was 4.4 minutes [1]. Calibration standards consisting of 40% H₂, 20% CO₂, 10% CH₄, and 30% N₂ were injected to generate the calibration plot. Each sample gas composition test was repeated 3 times. The average value of the three test results was recorded as the original data of the H₂ concentration of the sample. The H₂ concentration was recorded as C_{H₂}.

The H₂ yield was calculated as follow (formula 1):

$$M_{H_2} = \frac{V_T \times C_{H_2} \times 273.15}{22.4 \times (W_s \times 0.20) \times (273.15 + T_r)} \quad (1)$$

Where: M_{H₂}: molar amount of H₂ (mM);

V_T: total gas yield for each sample (L);

C_{H₂}: H₂ concentration for each sample (%);

T_r: ambient temperature (°C);

W_s: the content of potato soluble starch in medium (g/L).

The total gas production (T_Gas), H₂ concentration (C_H₂), H₂ production (V_H₂), average H₂ production rate (ST_H₂) and deviation, and substrate molar H₂ production rate (Mm_H₂) and deviation of each experimental group, show in Table 3 and Table 7.

Calculation method of average H₂ production rate: After removing the maximum or minimum deviation from each group, the average H₂ production was calculated with remaining 4 data.

Calculation method of deviation value: Calculated from the average H₂ production value and all 5 data in each group.

2.4. CHO Molarity Data Collection

In the completion stage of each experiment, the samples of every group were re-randomized thus CHO was determined.

The CHO molarity in each sample was measured with Benedict's test method. 2 mL sample was mixed with 0.5 mL Benedict's reagent in a clean test tube. And the solution was heated in a boiling water bath for 5 minutes. Immediately after the solution was ultrasonically diffused, the absorbance was measured at 739 nm by spectrophotometer (defined as OD_{739}) (BioMate 3S, Thermo Scientific, America). OD_{739} is correlated with CHO molarity. Glucose was used as calibration standards consisting of (mM) 5.00, 2.50, 0.50, 0.25, 0.05, 0.025, and 0.010 were measured to generate the calibration plot. Each gas composition test sample was repeated 3 times. The average value of the three test results was recorded as the original data of the CHO concentration of the sample.

The CHO concentration (C_{CHO}) and average CHO concentration (C_{CHO}) and deviation show in table 4 and table 8.

Calculation method of average CHO concentration: the average CHO concentration was calculated with the 4 samples in each group which selected in the calculation of average H_2 production.

Calculation method of deviation value: Calculated from the average CHO concentration value and all 5 data in each group.

2.5. pH Data Collection

The samples of every groups were re-randomized and then pH was measured.

15 mL of culture medium was centrifuged at $12000 \times g$ for 5 minutes (SL 16A, Thermo Scientific, America), and the supernatant used to test pH value. The pH level of each sample has been measured by pH meter (Star A211, Orion, America). Each test sample was repeated 3 times. The average value of the three test results was recorded as the original data of the pH of the sample.

The pH value (pH) and average pH (ST_{Ph}) and deviation show in Table 5 and Table 9.

Calculation method of average pH value: the average pH value was calculated with the 4 samples in each group which is being selected in the calculation of average H_2 production.

Calculation method of deviation value: Calculated from the average pH value and all 5 data in each group.

2.6. OD_{600} Data Collection

The samples of every groups were re-randomized and then OD_{600} was measured.

OD_{600} was measured at 600 nm by spectrophotometer (BioMate 3S, Thermo Scientific, America). OD_{600} test for each sample was repeated 3 times. The average value of the three test results was recorded as the original data of the OD_{600} value of the sample. A blank culture medium containing no starch was used as a blank sample to zero the spectrophotometer.

The OD_{600} value (OD_{600}) and average pH (ST_{OD600}) and deviation show in table 6 and table 10.

Calculation method of average OD_{600} value: the average OD_{600} value was calculated with the 4 samples in each group which selected in the calculation of average H_2 production.

Calculation method of deviation value: Calculated from the average OD_{600} value and all 5 data in each group.

2.7. Gram Stain Test and Bacterial Morphology Observation

The method of gram stain was used to distinguish and classify bacterial species, gram-positive bacteria, and gram-negative bacteria, based on the physical properties of cell walls. The microbial density of the gram stain was observed at 40 × and 100 × objectives (BX43, Olympus, Japan) and photos taken.

According to the variation of H₂ production under different kinds of signal antibiotic treatment, 3 representative sample were selected in each group, and each sample retained 1 representative photograph with 40 × objective lens. For compound antibiotic treatment groups, 1 representative sample were selected in each group, and each sample retained 1 representative photograph with 40 × objective lens (Table 10, Fig 1-1 to 1-20).

In addition, 1 photograph was taken for each the biodiversity analysis samples with 100 × objective lens.

2.8. DNA Extraction and PCR Amplification

- (1) 10 mL of cultured medium in each sample was collected at the end of the experiment. Bacteria was concentrated to 1 mL by centrifugation (SL 16A, Thermo Scientific, America) and stored in cryovials at -80 °C (DW-86L728J, Haier, China). The centrifugal force was set to 13000 × g, and centrifuged for 10 minutes.
- (2) Total genomic DNA was extracted from 1 mL concentrated underground water samples using E.A.N.A. Soil DNA Kit (OMEGA, Georgia, GA, America) according to manufacturer's instructions. The final DNA concentration and purification were determined by spectrophotometer (NanoDrop 2000 UV-vis, Thermo Scientific, America), and DNA quality was checked by 1% agarose gel electrophoresis.
- (3) The V3-V4 hypervariable regions of bacteria 16S rRNA gene was amplified with primers 338F (5'- ACT CCT ACG GGA GGC AGC AG - 3') and 806R (5'- GGA CTA CHV GGG TWT CTA AT - 3') by thermocycler polymerase chain reaction (PCR) (GeneAmp 9700, ABI, America) [3]. The DNA amplification was performed using the following program: 3 min of denaturation at 95°C, 27cycles of 30 s at 95°C, 30 s for annealing at 55°C, and 45 s for elongation at 72°C, and a final extension at 72°C for 10 min [4]. PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA.
- (4) The result PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, America) and quantified using QuantiFluorTM-ST (Promega, America).
- (5) Illumina MiSeq sequencing

Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq sequencing (Illumina, San Diego, America) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd (Shanghai, China). The 16S rRNA gene sequences were deposited in the NCBI Sequence Read Archive under accession number PRJNA784035.

- (6) Process of sequencing data

Raw fastq files were demultiplexed, quality-filtered by Trimmomatic and Merged by FLASH with the following criteria:

- 1) The reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window;
- 2) Primers were exactly matched allowing 2 nucleotide mismatching, and reads containing ambiguous bases were removed;
- 3) Sequences with overlap longer than 10 bp were merged according to their overlap sequence.

- (7) Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 <http://drive5.com/uparse/>) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (<http://rdp.cme.msu.edu/>) against the Silva (SSU123) 16S rRNA database using confidence threshold of 70%.

2.9. Microbial Diversity and Correlation Analysis with Environmental Factors

Community column chart, with respect to the results of taxonomic analysis, the species composition at the genus level of the four samples was calculated. Software: Based on the data table in the tax_summary_a folder, use the R language (version 3.3.1) tool (Fig. 3).

The evolutionary tree selects the top 50 species in the total abundance of the species taxonomic level, uses ML (Maximum likelihood) for construction, presents the phylogenetic relationship of the species in the form of a ring diagram. Software: Mega (version 10.0 <https://www.megasoftware.net/>) (Fig. 4).

Heatmap mapping adopted the top 10 species of Species level, the second classification level: Phylum, and the species hierarchical clustering method: average. Software and algorithms: R language (version 3.3.1) vegan package (Fig. 5).

Ternary phase diagram for comparative analysis of the species composition of the three samples based on taxonomic information. Taxonomy level: genus; Combined calculation method of samples within a group: average value; Color level: family. Software: GGTERN (<http://www.ggtern.com/>) (Fig. 6).

RDA analysis is a PCA analysis constrained by pH, CHO (OD₇₃₉) and H₂ yield rate factors, which combines corresponding analysis with multiple regression analysis, each step of the calculation is regressed with environmental factors. RDA based on a linear model and CCA based on a unimodal model (Fig. 7).

- 1) Selection principle of RDA or CCA model: initially employing species-sample data (sample OTU table with 97% similarity) to undertake DCA analysis, examine the size of the first axis of Lengths of gradient in the analysis result, hypothetically assuming that it is greater than or equal to 3.5, it could be assumed as CCA, granted that it is less than 3.5, the result of RDA is better than that of CCA.
- 2) Determine the maximum Pearson correlation coefficient of the distribution difference between environmental factors and sample communities through the bioenv function, obtain a subset of environmental factors through the maximum correlation coefficient.
- 3) Perform CCA or RDA analysis on the sample species distribution table and environmental factors or environmental factor subsets respectively.
- 4) Judging the significance of CCA or RDA analysis by permutest analysis similar to ANOVA.

Software: R language (version 3.3.1) RDA or CCA analysis and graphing in the vegan package.

Spearman correlation heatmap, calculate the Spearman rank correlation coefficient between H₂ yield rate, OD₆₀₀, CHO (OD₇₃₉), pH with the top 10 species of Genus level, and the obtained numerical matrix displayed by Heatmap. Software: R (version 3.3.1) (pheatmap package) (Fig. 8).

The Circos chart was drawn using the Genus taxonomy level, and the abundance of the samples in the group is calculated by summing up, and the relative abundance >0.01. Software: Circos-0.67-7 (<http://circos.ca/>) (Fig. 9).

Ethics Statements

This work involves the research based on the response law of H₂ yield capacity of H₂-producing bacteria to different types and dosages of antibiotics. This manuscript presents datasets that are the authors' original work and co-submitted with the manuscript "The response regularity of bio-hydrogen production by anthracite H₂-producing bacteria consortium

to six conventional veterinary antibiotics” (<https://doi.org/10.1016/j.jenvman.2022.115088>) and is not currently being considered for publication elsewhere. The paper reflects the authors' own research and analysis in a truthful and complete manner. In addition, the paper properly credits the meaningful contributions of co-authors and co-researchers. All sources used are adequately disclosed. All authors have been personally and actively involved in substantial work leading to the paper and will take public responsibility for its content.

CRediT Author Statement

Dong Xiao: Conceptualization, Writing – Original draft preparation, Funding acquisition; **Hailun He:** Writing – review & editing, Funding acquisition; **Xiaoxin Yan:** Conceptualization, Writing – review & editing; **Norberto Daniel Diaz:** Methodology, Data Curation, Funding acquisition; **Dayong Chen:** Data Curation; **Jing Ma:** Data Curation; **Yidong Zhang:** Visualization, Data Curation; **Jin Li:** Visualization; **Mohamed Keita:** Data Curation, Writing – review & editing; **Es-sono Oyono Julien:** Data Curation, Writing – review & editing; **Xiaotao Yan:** Data Curation.

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.

Data Availability

Antibiotic sensitivity test of hydrogen-producing bacteria (Original data) (National Library of Medicine).

Dataset of response characteristics of H₂-producing bacteria consortium to β -Lactams, Aminoglycosides, Macrolides, Quinolones antibiotics (Original data) (Mendeley Data).

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