

Influence of Organic Matrix and Cations on Bio- Methane Yield with Anthracite Methanogenic Consortium

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11 Abstract

12 Organic compounds fermentation of coal has been used to generate secondary
13 biogenic gas and enhance gas reservoirs in coalbed. To enhance the bio-degradation
14 process, culture nutrition plays an important role in remediating the nutritional deficiency
15 of the coal seam. The influence of bio-methane yield with organic inputs and cations
16 concentrations was examined. Research of organic matrix influence revealed that the
17 traditional organic material except yeast extract should forbid, and the input of yeast extract
18 should limit at 1.00g/L also. Further, the study demonstrated that the ion concentration of

19 sodium, potassium, magnesium, calcium and ammonia nitrogen also influenced methane
20 and carbon dioxide yields. And the optimize concentrations for Ca^{2+} , K^+ , Na^+ , Mg^{2+} were
21 5.1, 1.7, 23 and 1.3 mmol/L. The Mg^{2+} was particularly sensitive in inhibiting CH_4
22 metabolism processes largely for gas-coal methanogenic consortium.

23 **Keywords:** Anaerobic bacteria; Culture media; Organic matrix; Coal; Bio-methane.

24

1. INTRODUCTION

25 The demand for gas has seen a tremendous increase in the last 10 years [1]. In
26 particular, coal-bed methane enhance with biotechnology represents an ideal method to
27 increase methane productivity from oil deposits and coal seams using underground
28 anaerobic digestion processing [2,3]. With the addition of adequate nutrient supplements,
29 methanogenic consortium has the potential to degrade organic compounds in coal into
30 methane. In this way, gas reserves and productivity could be enhanced along with
31 secondary biogenic gas generation [4–6].

32 Previous studies involved with the methanogenic microbial traits and fermentation
33 characteristics for bio-methane generation have focused on the processes of bio-
34 degradation by microbial communities in coal seam [2,7,8] Anion research identified that
35 methane biosynthesis process could active where coalbed waters exhibit relatively low
36 salinities (<2 mol/L Cl^-) and low SO_4^{2-} concentrations (<10 mmol/L) [9]. Nutrients
37 supply by meteoric water plays an important role in methanogenic microbial metabolism

38 [10,11]. However, nutritional deficiency exists in most coal seam. Most organic
39 compounds in coal cannot be readily used as nutrients for methanogenic microbes. In
40 addition to the inherent elements present in coal beds, supplemental nutrients are
41 important to remediate the nutritional deficiency and promote microbial growth [12,13].
42 If concentrations of supplemental nutrients are excessive, the methanogenic consortium
43 will be adversely influenced [14]. The goal of this report is to identify the optimal
44 nutrient dose for microbial community culture. Methane and carbon dioxide yield rate
45 were the indicators used to assess the results of experiments.

46 **2. Materials and methods**

47 **2.1 Materials**

48 The coal and coalbed water samples used in this study were collected from Laohutai
49 Fm 103[#] coalbed located in Shenyang province of China (GPS coordinates 41.830256,
50 123.957639). The depth of the Fm 103[#] gas-coal sample was 550 meters and the
51 thickness of the coal seam was 6-18 meters. Laohutai Mine, in the east part of Fushun
52 near Shenyang, Liaoning province, opened in 1901. The mine has 55.6 million tons of
53 recoverable coal reserves until 2004[15]. The samples were collected by the State Key
54 Laboratory of Coal Resources and Safety Mining. The field studies did not involve
55 endangered or protected species, and only small sample quantities were used. And no
56 specific permissions were required for these locations sample collection and research.

57 The coal samples were obtained from a newly exposed coal seam in the heading face
58 by the channel sampling method following ASTM D 4596-86 with nitrogen protection.
59 Two sample points were selected from the Fm 103[#] coal seam. These samples were
60 sealed in gas-tight steel canisters and set with gas-tight valves (manufactured by J&D
61 Technologies) which enabled inert gas to flush the canister and protected with nitrogen
62 immediately. The coal to be used for the microbe community source was crushed into
63 small pieces (10-15 mm in diameter) at an aseptic bench (manufactured by JingXue
64 Technologies). Nitrogen protection was used to maintain the coal in a continuous
65 anaerobic environment. The sample was sealed in a sterilized gas desorption canister
66 (manufactured by J&D Technologies) to desorb the absorbed gas until no gas desorbed in
67 atmospheric pressure at 25 °C[16].

68 The formation of water samples from the Fm 103[#] coalbed were collected in the
69 same coal-bed in sterile glass bottles (manufactured by Fisher Scientific) which were
70 filled to overflow to prevent oxygen ingress. Coalbed water (\leq 24 hours after collection)
71 was autoclaved at 121 °C for 45 minutes and sealed in sterile glass bottles. Argon was
72 infused to seal the top space of the bottles. The coalbed water samples were stored at 4 °C
73 for no more than 3 days.

74 **2.2 Culture**

75 Five different nutrition culture media were tested in these experiments: MAC-1,

76 MAC-2, MAC-3, MAC-4, MAC-5 [4,17] (MAC is the abbreviation of “the influence test
77 of organic matrix and cations”). The final concentrations of the compounds (g/L) were as
78 list in table 1, and 0.1 mL/L of resazurin was added as an oxygen indicator. Yeast
79 extract was produced by Fisher BioReagents, and other chemicals were supplied by
80 Acros Organics.

81 **Table 1.** The MAC1-MAC5 nutrition culture media concentrations (g/L)

Culture Media	Sodium Acetate	Glucose	Beef Extract	Yeast Extract	Common Concentrations
MAC-1	2.00	3.00	3.00	2.00	
MAC-2	2.00	1.00	1.50	1.00	NH ₄ Cl, 1.00; MgCl ₂ •7H ₂ O, 1.00;
MAC-3	0.00	1.00	1.50	1.00	KH ₂ PO ₄ , 0.40; KCl, 0.50;
MAC-4	0.00	0.00	0.00	1.00	NaHCO ₃ , 1.00; L-Cysteine Hydrochloride Monohydrate, 0.45
MAC-5	0.00	0.00	0.00	0.00	

82 The distilled water was autoclaved at 121 °C for 45 minutes with dissolved oxygen
83 removed. Nutrition medium was prepared in a 500 mL flask with distilled water. The
84 medium was mixed using a magnetic stirrer for 2 hours at 60 °C at an aseptic bench and
85 then combined with an equal volume of coalbed water (volume ratio 1:1) for another hour
86 at room temperature. Nitrogen protection was used to maintain nutrition in a continuous
87 anaerobic environment throughout the entire experiment. The final pH was maintained at
88 6.0 for all nutrition cultures. Control samples of 100 ml MAC-1, MAC-2, MAC-3, MAC-

89 4, and MAC-5 were sealed in separate sterile glass bottles and stored at -40 °C. Argon
90 was infused to seal the top space of bottles.

91 Anaerobic conditions were ensured in flasks using a gas-replacement method. This
92 gas replacing process was monitored in real time with a carbon dioxide monitor system
93 (manufactured by E2V). For each experiment 50.00±1 g of the coal sample and 500.00
94 mL of medium were used. Nitrogen was used to seal the upper space of the flask at the
95 beginning of the experiment. The flasks were placed in an incubation shaker at 35 °C and
96 were agitated at 80 rpm to maximize the coal-liquid mass transfer rates. Twelve parallel
97 tests were designed for each nutrition group which were cultured for 40 days under
98 identical conditions.

99 The control experiments were performed without coal sample, which named MAC-
100 1*, MAC-2*, MAC-3*, MAC-4*, MAC-5*, to identify whether exogenous organic has
101 the possibility to supply extra carbon to enhance the bio-methane yield. For each control
102 experiment of each nutrition group 100 mL of cultured medium and 400 mL new medium
103 was used. And cultured for 40 days in the same condition as above.

104 **2.3 The cation orthogonal analysis**

105 The cation orthogonal analysis was based on consumption of cation elements in bio-
106 degradation processes, according to the $L_{16}(4^5)$ orthogonal table which includes Na^+ , K^+ ,
107 Ca^{2+} , Mg^{2+} . The lowest ion level was based on the MAC-4 medium while the highest ion

108 level was based upon that of the East China Sea ion concentration. A quantity of 50.00 ± 1
109 g of Fm 103[#] gas-coal sample was used in each experiment. The upper space of the flasks
110 was sealed with nitrogen. The flasks were placed on an incubation shaker at 35 °C and
111 agitated at 80 rpm to maximize coal-liquid mass transfer rates. Cation orthogonal
112 experiments were of 40 days of culture.

113 **2.4 Gas analysis**

114 Gas samples were obtained with a 50- μ L gas syringe. Methane and carbon dioxide
115 analyses were performed using an Agilent 7890A gas chromatograph (manufactured by
116 Agilent). The nitrogen (carrier gas) flow rate was set at 1.00 mL/min. The injection port
117 was maintained at 150°C with the oven temperature set at 25°C and the Thermal
118 Conductivity Detector (TCD) at 200°C. Retention times for methane were 3.76 minutes
119 and 5.0 minutes for carbon dioxide. Calibration standards consisted of 40% methane,
120 20% carbon dioxide, 10% hydrogen and 30% nitrogen, which were injected at
121 atmospheric pressure to generate the calibration plot.

122 **2.5 Nutrient Metabolism Analysis**

123 Nutrient ion concentrations were analyzed using an HC-800 ionization analyzer
124 (manufactured by Histrong Technologies). The main indicators included fluoride,
125 chloride, nitrate and nitrite nitrogen, phosphate, sulfate, carbonate, bicarbonate, ammonia
126 nitrogen, sodium, potassium, magnesium, calcium, pH, water hardness and total

127 alkalinity.

128 **3. Results**

129 **3.1 The nutrient abundance influence for bio-degradation of coal**

130 Five different organic compound nutrient concentrations designated as MAC-1,
131 MAC-2, MAC-3, MAC-4, and MAC-5 were assessed in this study. In these medium,
132 Sodium Acetate is a collective medium, as carbon resource, for *methanogenic* normally
133 [18]. Glucose is the medium for *hydrogen-producing acetogens*. Beef Extract is a mixture
134 of peptides and amino acids, nucleotide fractions, organic acids, minerals, and some
135 vitamins. It is often used to supply carbon and nitrogen sources. Yeast extract contains a
136 mixture of amino acids, peptides, water soluble vitamins, and carbohydrates. And it is
137 often used in culture media [19]. The rank order of organic concentrations were MAC-1 >
138 MAC-2 > MAC-3 > MAC-4 > MAC-5.

139 Coalbed methanogenic groups are comprised of a variety of microbial types. With
140 regard to *methanogens*, only a limited number of simple carbon compounds such as CO₂
141 or acetate can serve as substrates. For the conversion of complex organic compounds to
142 methane, fermentative and *acetogenic bacteria* are required. Thus they group an
143 interactive methanogenic consortium [20]. In the process of gas-coal bio-degradation, the
144 capacity for fermentative bacteria to hydrolyze and ferment the organic compounds of
145 coal plays an important role, and CO₂ is the main compound of gas productions. Beef

146 extract in medium supplied the extra carbon and nitrogen for bacteria. For the *acetogenic*
147 *bacteria* fermentation process, the long chain fatty acids and sugar degrade to form
148 acetate, CO₂ and H₂ [18]. Glucose is the medium to enhance the *acetogenic bacteria*
149 fermentation. And *methanogens* yield CH₄ with CO₂ and H₂ or acetate. Sodium Acetate
150 was introduced to raise the acetate supply for *methanogens* [21].

151 If consortia in a good banlance, most CO₂ and H₂ will be used to format CH₄. And
152 the concentration of CH₄ should be high, meanwhile, the concentration of CO₂ and H₂
153 should keep in low. So the yield gas concentration with microbial fermentation process
154 could reflect the bacteria balance conditions. The presence of sufficient organic nutrients
155 in the medium could promote fermentative and *acetogenic bacteria* flourish and improve
156 methanogen nutrient generation. However, if excessive amounts of organic nutrients are
157 contained within the medium, which like beef extract and glucose, the high propagation
158 of fermentative or *acetogenic bacteria* would break the microbial balance, and carbon
159 dioxide yield rate could be enhanced, meanwhile, methanogenesis could be inhibited.
160 This phenomenon showed in MAC-1, MAC-2 and MAC-3 culture experiments (Figure
161 1).

162 The results confirmed that MAC-4 was the most effective medium for enhancing the
163 bio-methane generation rate. 1 g/L yeast extract provided the best concentration of
164 organics amino acids, peptides, and water-soluble vitamins to optimize the microbial
165 fermentation. The maximal methane concentrations of the MAC-4 culture group achieved

166 23.62% (Figure 1). It was 4 times higher than that of the MAC-2, MAC-3, MAC-5
167 groups on average. The MAC-4* control experiment and MAC-5 group verified that the
168 anthracite is important to provide carbon for bio-methane yield (Figure 1).

169 **Figure 1 here**

170 The high carbon dioxide and low methane concentration data obtained from MAC-1
171 and MAC-2 media demonstrate that hydrolytic bacterial had enhanced with an abundance
172 of organic substrate supplement, however, methane biosynthesis tended to inhibit in
173 culture even with additional sodium acetate. In MAC-1 and MAC-2 experiments, the
174 *acetogenic bacteria* inhibited also with sodium acetate influence in the medium.

175 The MAC-3 medium, which follows the same medium concentration of MAC-2
176 except sodium acetate, enhanced *acetogenic bacteria*, and inhibited the *methanogens*.

177 After 40 days of culture, the average H₂ volume concentration for the MAC-3 and MAC-
178 3* group achieved 67.2% and 59.15% as indicated by gas analysis. This amount was 33-
179 40 times greater than that of all other experimental groups. The exogenous organic
180 material played an important role in carbon supply.

181 However, organic nutrition plays an important to supply the vitamin and other
182 microelements besides organic nutrients. Without the vitamin and microelements besides
183 organic nutrients supply, the consortia would grow at a slower rate, such as that observed
184 for MAC-5.

185 **3.2 The nutritional metabolism analysis of methanogenic consortium**

186 Changes in MAC-4 ion concentrations were analyzed in the initial and final media.

187 The data from this analysis revealed that the concentrations of sodium, potassium,

188 ammonia nitrogen and magnesium were consumed microbially. In particular, 85.78% of

189 the sodium was utilized with microbial fermentation. The medium pH changed from 6.65

190 to 7.32 over this period. In contrast, the concentrations of sulfate and bicarbonate

191 increased 1.5-7 fold (Table 2). These data indicated that sodium, nitrogen, potassium, and

192 magnesium are the key elements for methanogenic consortium fermentation. However,

193 the sulfate should be maintained at a low level in the medium.

194 **Table 2.** The MAC-4 medium ion concentration changes with methanogenic consortium

195 bio-degradation.

	pH	Fluoride	Chloride	Nitrate Nitrogen	Nitrite Nitrogen	Phosphate	Sulfate	Carbonate
Initial	6.65	3.70	81.58	-	-	44.67	195.80	-
final	7.32	5.18	95.90	-	-	38.60	478.40	-
	Bicarbonate	Sodium	Ammonia Nitrogen	Potassium	Magnesium	Calcium	Water Hardness	Total Alkalinity
Initial	40.66	811.90	193.60	159.00	38.89	21.06	212.56	40.66
final	328.77	115.40	39.23	35.30	14.30	22.76	115.62	328.77

196 **3.3 The cations orthogonal analysis**

197 From the analysis of nutrient element concentrations, it is clear that sodium,

198 ammonia, nitrogen, potassium, and magnesium play important roles in the methanogenic

199 microbial metabolism. The orthogonal analysis experiment was designed to identify the
200 influence of cations in bio-methane yield. The orthogonal module introduced 4 cations,
201 (Na^+ , K^+ , Ca^{2+} , Mg^{2+}) as tested with 4 different concentrations. The lowest ion
202 concentration was set by MAC-4 and the highest reference level for cation analysis was
203 that of the East China Sea ion concentration. The $L_{16}(4^5)$ orthogonal table was used as an
204 orthogonal analysis module (Table 3).

205 **Table 3.** The cation orthogonal analysis table for CH_4 and CO_2 . The results represent
206 volume percent units for both gases. The table shows the analysis of CH_4 and CO_2
207 ranges.

Serial Number	Factors (mmol/L)				Results	
	Na	Mg	Ca	K	CH_4	CO_2
1	23	1.3	0.5	10.7	6.65	4.71
2	23	6.7	1.7	1.7	3.92	4.27
3	23	13.0	3.4	3.4	0.92	4.03
4	23	20.0	5.1	5.1	0.46	3.23
5	130	1.3	1.7	3.4	6.59	4.84
6	130	6.7	0.5	5.1	3.85	3.56
7	130	13.0	5.1	10.7	0.99	3.31
8	130	20.0	3.4	1.7	0.38	2.94
9	260	1.3	3.4	5.1	6.49	4.47
10	260	6.7	5.1	3.4	3.76	4.73
11	260	13.0	0.5	1.7	0.57	2.47
12	260	20.0	1.7	10.7	0.34	2.84
13	390	1.3	5.1	1.7	7.01	4.86
14	390	6.7	3.4	10.7	0.30	3.90
15	390	13.0	1.7	5.1	0.47	2.28
16	390	20.0	0.5	3.4	0.30	2.67
CH_4	k_{1j}	2.99	6.69	2.84	2.97	
	k_{2j}	2.95	2.96	2.83	2.89	
	k_{3j}	2.79	0.74	2.02	2.82	

	k _{4j}	2.02	0.37	3.06	2.07
	R _j	0.97	6.32	1.03	0.90
	k _{1j}	4.06	4.72	3.35	3.64
	k _{2j}	3.66	4.12	3.56	4.07
CO ₂	k _{3j}	3.63	3.02	3.84	3.39
	k _{4j}	3.43	2.92	4.03	3.69
	R _j	0.63	1.80	0.68	0.68

208 Range analysis was used to indicate the affected order among factors. Range R_j was
209 calculated with module 1.

210

$$R_j = Max_{k_{jm}} - Min_{k_{jm}} \quad (1)$$

211 Where k_{jm} is the average result of the m level j factor (module 2), (j, m =1, 2, 3, 4).

212

$$k_{jm} = \sum_{i=1}^4 y_{jmi} / 4 \quad (2)$$

213 Where y_{jmi} is the result of the m level j factor number i data, (j, m, i =1, 2, 3, 4).

214 Range analysis confirmed that for methanogenic activity, the best ion concentrations
215 for enhancement CH₄ yield were Na₁, Mg₁, Ca₄, and K₁, with the rank order of element
216 effectiveness being Mg > Ca > Na > K. The most effective ion concentration for control
217 CO₂ generation were Na₄, Mg₄, Ca₁, and K₃, with its ran order being Mg > Ca = K > Na.

218 Notable differences exist regarding the influence of CH₄ and CO₂ on yield rates as a
219 function of cation concentrations. The CH₄ biosynthesis is substantially more sensitive to
220 element content. Maximal Mg²⁺ concentrations to enhance methanogen metabolism
221 processes were 1.3 mmol/L, while maximal cation concentrations for Ca²⁺, K⁺, Na⁺ were
222 5.1, 1.7 and 23 mmol/L, respectively.

223 Variance analysis has been used to analyze the Factor Significance (F) in this
224 system. F was calculated with module 3.

225

$$F_j = \frac{4 \sum_{i=1}^4 K_{ij}^2 - (\sum_{i=1}^{16} x_i)^2}{16 \sum_{i=1}^{16} x_i^2 - (\sum_{i=1}^{16} x_i)^2} \times 5 \quad (3)$$

226

$$K_{ij} = \sum_{i=1}^4 y_{jmi} \quad (4)$$

227 Where x_i is the experiment results for every factor and level.

228 **Table 4.** The variance analysis table for 4 factors with CH₄ and CO₂ data.

	Factor	Square of Deviance	Degree of Freedom	F Value	Significant
CH ₄	Na ⁺	2.465	3	0.892	
	Mg ²⁺	100.905	3	36.533	*
	Ca ²⁺	2.486	3	0.900	
	K ⁺	2.080	3	0.753	
CO ₂	Error	2.762	3		
	Na ⁺	0.842	3	8.592	
	Mg ²⁺	9.120	3	93.061	**
	Ca ²⁺	1.079	3	11.010	*
	K ⁺	0.954	3	9.735	
	Error	0.098	3		

229 Based upon the F calculation, Mg^{2+} was the significant cation factor for CH_4 and
230 CO_2 (Table 4). This ion is particularly sensitive in inhibiting CH_4 metabolism processes
231 largely for gas-coal methanogenic consortium.

232 4. Discussion

233 Microbial bio-degradation of some organic compounds of coal represents a current
234 technology available to enhance coal-bed methane. Microbial cooperation via anaerobic
235 digestion processes results in the implementation of secondary biogenic methane
236 generation and gas reservoirs enhancement in mining. Based on the experiments
237 performed in this report, the following conclusions can be garnered: (1) The organic
238 nutrient dose should be adjusted to methanogenic consortium requirements. Excessive or
239 deficient organic nutrient support doses adversely affecting biomethane yield; (2) MAC-4
240 was the most effective medium in enhancing anaerobic digestion in Fushun gas-coal
241 seam; (3) The sodium, nitrogen, potassium, and magnesium are the key elements for
242 methanogenic consortium fermentation; (4) The cation rank order of influence Fushun
243 methanogenic consortium metabolism was $Mg > Ca > Na > K$, and maximal cation
244 concentrations of Mg^{2+} , Ca^{2+} , K^+ , Na^+ were 1.3, 5.1, 1.7, and 23 mmol/L, respectively;
245 (5) Mg^{2+} was a particularly sensitive factor, which could inhibit methanogenic bacteria.

246 5. Conclusion

247 5.1 The nutrient concentration for gas-coal methanogenic consortium culture

248 The biomethane generation from coal involves a complex interaction between
249 environmental factors and biotic communities. Hydrolytic fermentative bacteria,
250 syntrophic *acetogenic bacteria*, methanogenic bacteria, and many other bacteria comprise
251 the biotic community. The environment includes not only the physical factors but also the
252 coal, coalbed water, coal-bed gas and other complex formations which we call the
253 environment.

254 The medium injected into coal seam enriching nutrients of the coal seam. The series
255 of experiments of this report confirm that nutrient media require a strict concentration of
256 control to be effective, especially for those involving organic materials. If the organic
257 compounds are too rich, like that modeled with our formulations of MAC-1, MAC-2, and
258 MAC-3, the excessive levels of nutrients will adversely affect the microbial community
259 structure. High rates growth of hydrolytic fermentative bacteria or *acetogenic bacteria*
260 could inhibit methane biosynthesis. In contrast, if lack organic nutrition, such as that
261 modeled in MAC-5, the potential for enhancing methane biosynthesis is low and the yield
262 proceeds at a slow rate. Concentrations of nutrition required should differ as a function
263 of: (1) the methane biosynthesis type, such as carbon dioxide reduction or acetate
264 fermentation; (2) coal maturity grade, such as gas-coal, flame coal, bituminous coal or
265 lignite. The exact organic nutrition required for different coal ranks needs to be identified
266 to maximize bio-methane yield rates.

267 **5.2 Ion concentration for bio-methane yield enhancement**

268 Organic biological degradation to methane is a microbial cooperation process.

269 Fermentative bacteria initially hydrolyze complex organic compounds to acetate, longer

270 chained fatty acids, carbon dioxide, hydrogen, NH_4^+ , and HS^- . Syntrophic hydrogen-

271 producing (proton-reducing) *acetogenic bacteria* reduce intermediary metabolites to

272 acetate, carbon dioxide, and hydrogen. Hydrogen-utilizing *acetogenic bacteria*

273 demethoxylate low molecular weight ligneous compounds and ferment some

274 hydroxylated aromatic compounds. Carbon dioxide reduction methanogenic bacteria are

275 dependent on hydrogen, produced by other bacteria, to reduce carbon dioxide or

276 bicarbonate to methane. And acetate fermentation methanogen yields methane via acetate

277 bio-degradation.

278 Different nutrients are required for different microbial activity in the community.

279 Results from the cation orthogonal experiments revealed that cation concentrations

280 critically influence the metabolism process of the microbial. Except for the examination

281 of sodium, potassium, magnesium, calcium which analyzed in the cations orthogonal

282 analysis, the nitrogen, yeast extract, salinity and pH could have the potential to influence

283 the biomethane synthesis. More researches to identify the effects of ions upon the coal

284 seam biotic community are needed to reveal the microbial activity in gas-coal beds.

285 **6. Competing interests**

286 The authors declare that they have no competing financial interests.

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294 8. References

- 295 1. Lv Y, Tang D, Xu H, Luo H. Production characteristics and the key factors in high-rank
296 coalbed methane fields: A case study on the Fanzhuang Block, Southern Qinshui Basin,
297 China. *Int J Coal Geol.* 2012; doi:10.1016/j.coal.2012.03.009
- 298 2. Park SY, Liang Y. Biogenic methane production from coal: A review on recent research
299 and development on microbially enhanced coalbed methane (MECBM). *Fuel.* 2016.
300 doi:10.1016/j.fuel.2015.10.121
- 301 3. Scott AR. Improving Coal Gas Recovery with Microbially Enhanced Coalbed Methane.
302 Coalbed Methane: Scientific, Environmental and Economic Evaluation. 1999.
303 doi:10.1007/978-94-017-1062-6_7
- 304 4. Green MS, Flanagan KC, Gilcrease PC. Characterization of a methanogenic consortium
305 enriched from a coalbed methane well in the Powder River Basin, U.S.A. *Int J Coal Geol.*
306 2008; doi:10.1016/j.coal.2008.05.001
- 307 5. Penner TJ, Foght JM, Budwill K. Microbial diversity of western Canadian subsurface coal

308 beds and methanogenic coal enrichment cultures. *Int J Coal Geol.* 2010;
309 doi:10.1016/j.coal.2010.02.002

310 6. Strąpoć D, Picardal FW, Turich C, Schaperdorth I, Macalady JL, Lipp JS, et al. Methane-
311 producing microbial community in a coal bed of the Illinois Basin. *Appl Environ
312 Microbiol.* 2008;74: 2424–2432. doi:10.1128/AEM.02341-07

313 7. Li D, Hendry P, Faiz M. A survey of the microbial populations in some Australian
314 coalbed methane reservoirs. *Int J Coal Geol.* 2008; doi:10.1016/j.coal.2008.04.007

315 8. McIntosh J, Martini A, Petsch S, Huang R, Nüsslein K. Biogeochemistry of the Forest
316 City Basin coalbed methane play. *Int J Coal Geol.* 2008; doi:10.1016/j.coal.2008.03.004

317 9. Hall RO, Tank JL, Baker MA, Rosi-Marshall EJ, Hotchkiss ER. Metabolism, Gas
318 Exchange, and Carbon Spiraling in Rivers. *Ecosystems.* 2016; doi:10.1007/s10021-015-
319 9918-1

320 10. Beckmann S, Luk AWS, Gutierrez-Zamora ML, Chong NHH, Thomas T, Lee M, et al.
321 Long-term succession in a coal seam microbiome during in situ biostimulation of coalbed-
322 methane generation. *ISME Journal.* 2018. doi:10.1038/s41396-018-0296-5

323 11. Chen T, Zheng H, Hamilton S, Rodrigues S, Golding SD, Rudolph V. Characterisation of
324 bioavailability of Surat Basin Walloon coals for biogenic methane production using
325 environmental microbial consortia. *Int J Coal Geol.* 2017; doi:10.1016/j.coal.2017.05.017

326 12. Xiao D, Wang E-Y, Peng S-P, Wu J-Y. Responses of coal anaerobic fermentation
327 fractures development. *Meitan Xuebao/Journal China Coal Soc.* 2017;42.
328 doi:10.13225/j.cnki.jccs.2016.1109

329 13. Meng J, Nie B, Zhao B, Ma Y. Study on law of raw coal seepage during loading process
330 at different gas pressures. *Int J Min Sci Technol.* 2015; doi:10.1016/j.ijmst.2014.12.005

331 14. Galagan JE, Nusbaum C, Roy A, Endrizzi MG, Macdonald P, Fitzhugh W, et al. The

332 genome of *M. acetivorans* reveals extensive metabolic and physiological diversity.

333 *Genome Res.* 2002; doi:10.1101/gr.223902

334 15. Wang E, Chen P, Li Z, Shen R, Xu J, Zhu Y. Resistivity response in complete stress-strain
335 process of loaded coal. *Meitan Xuebao/Journal China Coal Soc.* 2014;39.
336 doi:10.13225/j.cnki.jccs.2013.1070

337 16. Ali Shah F, Mahmood Q, Maroof Shah M, Pervez A, Ahmad Asad S. Microbial ecology
338 of anaerobic digesters: The key players of anaerobiosis. *The Scientific World Journal.*
339 2014. doi:10.1155/2014/183752

340 17. Parks DH, Chuvochina M, Waite DW, Rinke C, Skarszewski A, Chaumeil P-A, et al. A
341 standardized bacterial taxonomy based on genome phylogeny substantially revises the tree
342 of life. *Nat Biotechnol.* 2018; doi:10.1038/nbt.4229

343 18. Zinder SH. *Physiological Ecology of Methanogens.* Methanogenesis. 1993.
344 doi:10.1007/978-1-4615-2391-8_4

345 19. Bellou S, Baeshen MN, Elazzazy AM, Aggeli D, Sayegh F, Aggelis G. Microalgal lipids
346 biochemistry and biotechnological perspectives. *Biotechnology Advances.* 2014.
347 doi:10.1016/j.biotechadv.2014.10.003

348 20. Gerritsen J, Fuentes S, Grievink W, van Niftrik L, Tindall BJ, Timmerman HM, et al.
349 Characterization of *Romboutsia ilealis* gen. nov., sp. nov., isolated from the gastro-
350 intestinal tract of a rat, and proposal for the reclassification of five closely related
351 members of the genus *Clostridium* into the genera *Romboutsia* gen. nov., *Intestinib.* *Int J*
352 *Syst Evol Microbiol.* 2014; doi:10.1099/ijsm.0.059543-0

353 21. Kwietniewska E, Tys J. Process characteristics, inhibition factors and methane yields of
354 anaerobic digestion process, with particular focus on microalgal biomass fermentation.
355 *Renew Sustain Energy Rev.* 2014; doi:10.1016/j.rser.2014.03.041

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357 **Figure Legends**

358 **Figure 1. Changes in methane and carbon dioxide production as a function of media**

359 **nutrition and time in culture.** Figure A is the methane concentration changes with

360 culture days. And figure B is the control experiment without coal sample supply in

361 experiment. Figure C is the carbon dioxide concentration changes with culture days. And

362 figure D is the control experiment without coal sample supply in experiment. Methane is

363 the main factor to identify the *methanogens* activity. And carbon dioxide is an important

364 factor to identify the fermentative and *acetogenic bacteria* activity. The methane yield

365 rate would high, only if the fermentative and *acetogenic bacteria* activity in a limited

366 condition. Organic material could enhance the fermentative and *acetogenic bacteria*.

367 However, if the activity of fermentative and *acetogenic bacteria* is too high, it would

368 inhibit the *methanogens*. The microbial group will in a good balance when achieved the

369 highest methane yield and the best ratio of methane and carbon dioxide. Thus the MAC-4

370 medium culture group fit for the requirements.

