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

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Abstract

Organic compounds fermentation of coal has been used to generate secondary biogenic gas and enhance gas reservoirs in coal bed. To enhance the bio-degradation process, culture nutrition plays an important role in remediating the nutritional deficiency of the coal seam. The influence of bio-methane yield with organic inputs and cation concentrations was examined. Research of organic matrix influence revealed that the traditional organic material except yeast extract should forbid, and the input of yeast extract should limit at 1.00g/L also. Further, the study demonstrated that the ion concentration of sodium, potassium, magnesium, calcium and ammonia nitrogen also influenced methane and carbon dioxide yields. And the optimize concentrations for Ca^{2+}, K^+, Na^+, Mg^{2+} were 5.1, 1.7, 23 and 1.3 mmol/L. The Mg^{2+} was particularly sensitive in inhibiting CH_4 metabolism processes largely for gas-coal methanogenic consortium.

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

Introduction

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

Previous studies involved with the methanogenic microbial traits and fermentation characteristics for bio-methane generation have focused on the processes of bio-degradation by microbial communities in coal seam [2,7,8]. Anion research identified that methane biosynthesis process could active where coal bed waters exhibit relatively low salinities (<2 mol/L Cl) and low SO_4^{2-} concentrations (<10 mmol/L) [9]. Nutrients supply by meteoric water plays an important role inmethanogenic microbial metabolism [10,11]. However, nutritional deficiency exists in most coal seams. Most organic compounds in coal cannot be readily used as nutrients for methanogenic microbes. In addition to the inherent elements present in coal beds, supplemental nutrients are important to remediate the nutritional deficiency and promote microbial growth [12,13]. If concentrations of supplemental nutrients are excessive, the methanogenic consortium will be adversely influenced [14]. The goal of this report to identify the optimal nutrient dose for microbial community culture. Methane and carbon dioxide yield rate were the indicators used to assess the results of experiments.

Materials and methods

Materials

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

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

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

Culture

Five different nutrition culture media were tested in these experiments: MAC-1, MAC-2, MAC-3, MAC-4 and MAC-5 (MAC is the abbreviation of "the influence test of organic matrix and cautions") [4,17]. The final concentrations of the compounds (g/L) were as list in table 1 and 0.1 mL/L of resazurin was added as an oxygen indicator. Yeast extract was produced by Fisher BioReagents, and other chemicals were supplied by Acros Organics.

Culture Media	Sodium Acetate	Glucose	Beef Extract	Yeast Extract	Common Concentrations
MAC-1	2.00	3.00	3.00	2.00	NH_4Cl, 1.00;
MAC-2	2.00	1.00	1.50	1.00	$MgCl_2 \cdot 7H_2O$, 1.00;
MAC-3	0.00	1.00	1.50	1.00	KH_2PO_4, 0.40; KCl, 0.50;
MAC-4	0.00	0.00	0.00	1.00	$NaHCO_3$, 1.00; L-Cysteine Hydrochloride
MAC-5	0.00	0.00	0.00	0.00	Monohydrate, 0.45

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

The distilled water was autoclaved at 121°C for 45 minutes with dissolved oxygen removed. Nutrition medium was prepared in a 500 mL flask with distilled water. The medium was mixed using a magnetic stirrer for 2 hours at 60°C at an aseptic bench and then combined with an equal volume of coal bed water (volume ratio1:1) for another hour at room temperature. Nitrogen protection was used to maintain nutrition in a continuous anaerobic environment throughout the entire experiment. The final pH was maintained at 6.0 for all nutrition cultures. Control samples of 100 mlMAC-1, MAC-2, MAC-3, MAC-4 and MAC-5 were sealed in separate sterile glass bottles and stored at -40°C Argon was infused to seal the top space of bottles.

Anaerobic conditions were ensured in flasks using a gas-replacement method. This gas replacing process was monitored in real time with a carbon dioxide monitor system (manufactured by E2V). For each experiment 50.00±1 g of the coal sample and 500.00 mL of medium were used. Nitrogen was used to seal the upper space of the flask at the beginning of the experiment. The flasks were placed in an incubation shaker at 35°C and were agitated at 80 rpm to maximize the coal-liquid mass transfer rates. Twelve parallel tests were designed for each nutrition group which were cultured for40 days under identical conditions.

The control experiments were performed without coal sample, which named MAC-1*, MAC-2*, MAC-3*, MAC-4* and MAC-5*, to identify whether exogenous organic has the possibility to supply extra carbon to enhance the bio-methane yield. For each control experiment of each nutrition group100 mL of cultured medium and 400 mL new medium was used. And cultured for 40 days in the same condition as above.

The cation orthogonal analysis

The caution orthogonal analysis was based on consumption of cation elements in bio-degradation processes, according to the $L_{16}(4^5)$ orthogonal table which includes Na^+, K^+, Ca^{2+} and Mg^{2+}. The lowest ion level was based on the MAC-4 medium while the highest ion level was based upon that of the East China Sea ion concentration. A quantity of 50.00±1 g of Fm 103[#] gas-coal sample was used in each experiment. The upper space of the flasks was sealed with nitrogen. The flasks were placed on an incubation shaker at 35°C and agitated at 80 rpm to maximize coal-liquid mass transfer rates. Caution orthogonal experiments were of 40 days of culture.

Gas analysis

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

Nutrient metabolism analysis

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

Results

The nutrient abundance influence for bio-degradation of coal

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

Coal bed methanogenic groups are comprised of a variety of microbial types. With regard to methanogens, only a limited number of simple carbon compounds such as CO_2 or acetate can serve as substrates. For the conversion of complex organic compounds to methane, fermentative and acetogenic bacteria are required. Thus they group an interactive methanogenic consortium [20]. In the process of gas-coal bio-degradation, the capacity for fermentative bacteria to hydrolyze and ferment the organic compounds of coal plays an important role, and CO_2 is the main compound of gas productions. Beef extract in medium supplied the extra carbon and nitrogen for bacteria. For the acetogenic bacteria fermentation process, the long chain fatty

acids and sugar degrade to form acetate, CO_2 and H_2 [18]. Glucose is the medium to enhance the acetogenic bacteria fermentation. And methanogens yield CH_4 with CO_2 and H_2 or acetate. Sodium Acetate was introduced to raise the acetate supply for methanogens [21].

If consortia in a good banlance, most CO_2 and H_2 will be used to format CH_4. And the concentration of CH_4 should be high; meanwhile, the concentration of CO_2 and H_2 should keep in low. So the yield gas concentration with microbial fermentation process could reflect the bacteria balance conditions. The presence of sufficient organic nutrients in the medium could promote fermentative and acetogenic bacteria flourish and improve methanogen nutrient generation. However, if excessive amounts of organic nutrients are contained within the medium, which like beef extract and glucose, the high propagation of fermentative or acetogenic bacteria would break the microbial balance, and carbon dioxide yield rate could be enhanced, meanwhile, methanogenesis could be inhibited. This phenomenon showed in MAC-1, MAC-2 and MAC-3 culture experiments (Figure 1).
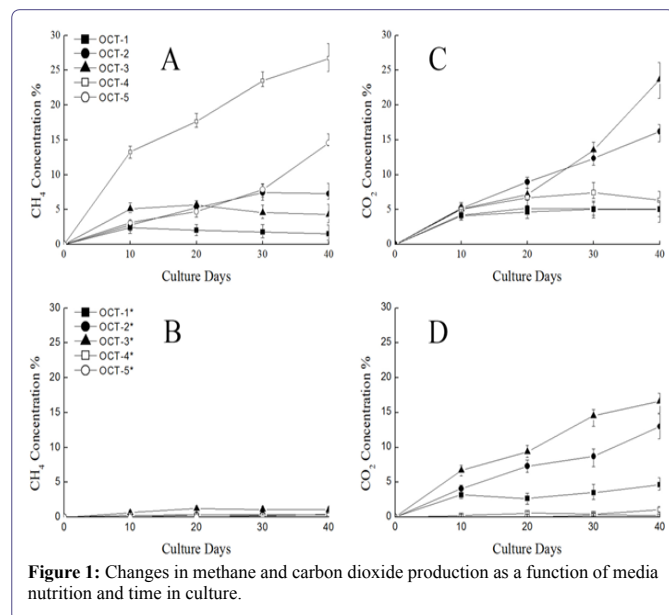

Figure 1: Changes in methane and carbon dioxide production as a function of media nutrition and time in culture.

The results confirmed that MAC-4 was the most effective medium for enhancing the bio-methane generation rate. 1 g/L yeast extract provided the best concentration of organics amino acids, peptides, and water-soluble vitamins to optimize the microbial fermentation. The maximal methane concentrations of the MAC-4 culture group achieved 23.62% (Figure 1). It was 4 times higher than that of the MAC-2, MAC-3, MAC-5 groups on average. The MAC-4* control experiment and MAC-5 group verified that the anthracite is important to provide carbon for bio-methane yield (Figure 1).

Figure A is the methane concentration changes with culture days. And figure B is the control experiment without coal sample supply in experiment. Figure C is the carbon dioxide concentration changes with culture days. And figure D is the control experiment without coal sample supply in experiment. Methane is the main factor to identify the methanogens activity. And carbon dioxide is an important factor to identify the fermentative and acetogenic bacteria activity. The methane yield rate would high, only if the fermentative and

acetogenic bacteria activity in a limited condition. Organic material could enhance the fermentative and acetogenic bacteria. However, if the activity of fermentative and acetogenic bacteria is too high, it would inhibit the methanogens. The microbial group will in a good balance when achieved the highest methane yield and the best ratio of methane and carbon dioxide. Thus the MAC-4 medium culture group fit for the requirements.

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

The MAC-3 medium, which follows the same medium concentration of MAC-2 except sodium acetate, enhanced acetogenic bacteria and inhibited the methanogens. After 40 days of culture, the average H_2 volume concentration for the MAC-3 and MAC-3* group achieved 67.2% and 59.15% as indicated by gas analysis. This amount was 33-40 times greater than that of all other experimental groups. The exogenous organic material played an important role in carbon supply.

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

The nutritional metabolism analysis of methanogenic consortium

Changes in MAC-4 ion concentrations were analyzed in the initial and final media. The data from this analysis revealed that the concentrations of sodium, potassium, ammonia nitrogen and magnesium were consumed microbial. In particular, 85.78% of the sodium was utilized with microbial fermentation. The medium pH changed from 6.65 to 7.32 over this period. In contrast, the concentrations of sulfate and bicarbonate increased 1.5-7 fold (Table 2). These data indicated that sodium, nitrogen, potassium and magnesium are the key elements for methanogenic consortium fermentation. However, the sulfate should be maintained at a low level in the medium.

The cautions orthogonal analysis

From the analysis of nutrient element concentrations, it is clear that sodium, ammonia, nitrogen, potassium and magnesium play important roles in the methanogenic microbial metabolism. The orthogonal analysis experiment was designed to identify the influence of cations in bio-methane yield. The orthogonal module introduced 4 cations, (Na^+, K^+, Ca^{2+}, Mg^{2+}) as tested with 4 different concentrations. The lowest ion concentration was set by MAC-4 and the highest reference level for cation analysis was that of the East China Sea ion concentration. The $L_{16}(4^5)$ orthogonal table was used as an orthogonal analysis module (Table 3).

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

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

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

	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 Aalkalinity		
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

Table 2: The MAC-4 medium ion concentration changes with methanogenic consortium bio-degradation.

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	
CO$_2$	k$_{1j}$	4.06	4.72	3.35	3.64	
	k$_{2j}$	3.66	4.12	3.56	4.07	
	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	

Table 3: The caution orthogonal analysis table for CH$_4$ and CO$_2$. The results represent volume percent units for both gases. The table shows the analysis of CH$_4$ and CO$_2$ ranges.

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

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

Range analysis confirmed that for methanogenic activity, the best ion concentrations for enhancement CH$_4$ yield were Na$_1$, Mg$_1$, Ca$_4$ and K$_1$, with the rank order of element effectiveness being Mg > Ca > Na > K. The most effective ion concentration for control CO$_2$ generation were Na$_4$, Mg$_4$, Ca$_1$, and K$_3$, with its ran order being Mg > Ca = K > Na.

Notable differences exist regarding the influence of CH$_4$ and CO$_2$ on yield rates as a function of cation concentrations. The CH$_4$ bio-synthesis is substantially more sensitive to element content. Maximal Mg^{2+} concentrations to enhance methanogen metabolism processes were 1.3mmol/L, while maximal cation concentrations for Ca^{2+}, K$^+$, Na$^+$ were5.1, 1.7 and 23 mmol/L, respectively.

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

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

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

Where x$_i$ is the experiment results for every factor and level.

	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	
	Error	2.762	3		
CO$_2$	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		

Table 4: The variance analysis table for 4 factors with CH$_4$ and CO$_2$ data.

Based upon the F calculation, Mg^{2+} was the significant caution factor for CH$_4$ and CO$_2$ (Table 4). This ion is particularly sensitive in inhibiting CH$_4$ metabolism processes largely for gas-coal methanogenic consortium.

Discussion

Microbial bio-degradation of some organic compounds of coal represents a current technology available to enhance coal-bed methane. Microbial cooperation via anaerobic digestion processes results in the implementation of secondary biogenic methane generation and gas reservoirs enhancement in mining. Based on the experiments performed in this report, the following conclusions can be garnered: (1) The organic nutrient dose should be adjusted to methanogenic consortium requirements. Excessive or deficient organic nutrient support

doses adversely affecting biome thane yield; (2) MAC-4 was the most effective medium in enhancing anaerobic digestion in Fushun gas-coal seam; (3) The sodium, nitrogen, potassium and magnesium are the key elements for methanogenic consortium fermentation; (4) The caution rank order of influence Fushun methanogenic consortium metabolism was Mg > Ca > Na > K, and maximal caution concentrations of Mg^{2+}, Ca^{2+}, K^+, Na^+ were 1.3, 5.1, 1.7 and 23 mmol/L, respectively; (5) Mg^{2+} was a particularly sensitive factor, which could inhibit methanogenic bacteria.

Conclusion

The nutrient concentration for gas-coal methanogenic consortium culture

The biome thane generation from coal involves a complex interaction between environmental factors and biotic communities. Hydrolytic fermentative bacteria, syntrophic acetogenic bacteria, methanogenic bacteria and many other bacteria comprise the biotic community. The environment includes not only the physical factors but also the coal, coalbed water; coal-bed gas and other complex formations which we call the environment.

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

Ion concentration for bio-methane yield enhancement

Organic biological degradation to methane is a microbial cooperation process. Fermentative bacteria initially hydrolyze complex organic compounds to acetate, longer chained fatty acids, carbon dioxide, hydrogen, NH_4^+, and HS^-. Syntrophic hydrogen-producing (proton-reducing) acetogenic bacteria reduce intermediary metabolites to acetate, carbon dioxide, and hydrogen. Hydrogen-utilizing acetogenic bacteria demethoxylate low molecular weight ligneous compounds and ferment some hydroxylated aromatic compounds. Carbon dioxide reduction methanogenic bacteria are dependent on hydrogen, produced by other bacteria, to reduce carbon dioxide or bicarbonate to methane. And acetate fermentation methanogen yields methane via acetate bio-degradation.

Different nutrients are required for different microbial activity in the community. Results from the caution orthogonal experiments revealed that caution concentrations critically influence the metabolism process of the microbial. Except for the examination of sodium, potassium, magnesium, calcium which analyzed in the cautions orthogonal analysis, the nitrogen, yeast extract, salinity and pH could have

the potential to influence the biomethane synthesis. More researches to identify the effects of ions upon the coal seam biotic community are needed to reveal the microbial activity in gas-coal beds.

Competing Interests

The authors declare that they have no competing financial interests.

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