**Finding cost-effective nutrient solutions and evaluating environmental conditions
for biogasifying bituminous coal to methane ex situ**

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Abstract

Biostimulation through providing nutrients to microorganisms has been proven to be highly effective in enhancing methane yield from bituminous coals in the Illinois basin. In light of the extremely cheap natural gas price at present, it is critically important to decrease the cost of nutrient solutions used to stimulate microbial gasification of coal and improve the cost-effectiveness of the biostimulation approach. To lower cost of a nutrient solution reported in previous studies, the three most expensive components, yeast extract, peptone and mercaptoethanesulfonic acid (Coenzyme M, CoM) were evaluated. Regarding the first two, decreasing their concentrations resulted in decreased methane production. In terms of CoM, its concentration could be reduced to at least half without affecting methane yield negatively. Complete elimination of CoM led to a methane yield of 85% of those with the presence of this

compound in the original medium. Two complex media, trypticase soy broth (TSB) and corn steep liquor (CSL) were intended to replace yeast extract and peptone. TSB had increasingly positive effect with increasing its concentration while CSL had opposite trend. TSB at 100% (v/v) was then investigated under different cultivation conditions. Among the four parameters tested, TSB concentration and agitation were found to exert positive effect while coal loading and temperature had negative impact on methane production. The highest methane yield of 828.9 ft³/ton in 30 days was obtained under temperature of 28°C with a coal loading of 200 g/L and agitation at 100 rpm. This yield is four-time higher than those observed with the original medium. These results clearly demonstrated that biostimulation is a viable method for enhancing methane production from coal and the ingredients of nutrient solutions need to be evaluated in detail in order to make biostimulation cost-effectively at commercial scales.

Keywords

Biogasification; microbial community; bituminous coal; nutrient solution; methane yield; ex situ

1. Introduction

Research in the area of microbially enhanced coalbed methane (MECBM) or coal biogasification has accelerated in the last few years due to several reasons. First, natural gas is the cleanest burning fossil fuel, friendly to the environment, rarely producing toxic gases and has been identified as a strong candidate for energy resource compared to oil and coal [1, 2]. Thus, converting coal to methane, if it could be done successfully, would be an optimal use of coal, an abundant natural resource. Second, this technology is applicable to unmineable coals and abandoned coal mines with large amounts of coals left behind [3]. Third, this technology is applicable to coal waste in the form of fines or of poor quality [4]. Fourth, several regions with significant coalbed methane (CBM) activity are maturing, thus providing the wells and supporting infrastructure required for MECBM [5]. Fifth, the technology is applicable to CBM wells that are abandoned due to poor production rates and recovery [5]. Finally, compared to coal gasification which converts coal to syngas at over 700°C and with the presence of expensive and easily poisoned catalysts [6, 7], biogasification can be operated at temperatures between 25°C and 80°C and necessitates no use of chemical catalysts.

The key challenge for making MECBM a commercial reality is to stimulate microbial activities so that coal can be degraded and act as a real-time methane bioreactor [8] and natural gas can be released faster than those without microbial enhancement. To achieve this purpose, at least four approaches need to be explored in-depth [5, 9]: 1) biostimulation. Through supplying whatever is required for the target microorganisms, their cellular activities can be stimulated to fulfill desired functions; 2) bioaugmentation. For sites that lack microbial communities for gasifying coals to methane, which is rare in the environment, an external microbial consortium or “superbugs” can be added. For the latter, even genetically or metabolically engineered microbes

are available, injecting them to deep underground may face problems in obtaining permits and obstacles dealing with public perception; 3) modifying coal structure physically so that coal is easily accessed by microbes; and 4) increasing coal bioavailability through chemical means. The last two approaches aim to decrease coal's recalcitrance, increase its porosity and surface area considering the facts that pores in coals are typically < 50 nm in diameter, which is too small for microbes (0.5-3 μm) to enter [10].

Among the four strategies, the first one of biostimulation has been investigated the most. At laboratory level, coals from different coal basins/countries have been tested, such as: the Powder River Basin in US [11-13], the San Juan Basin in US [14], the Illinois basin in US [15-17], the Indio formation in Texas, US [18], the south Sydney Basin in Australia [19], the eastern Surat basin in Australia [20, 21], the Jitpur mine in India [22], the Alberta coal beds in Canada [23], and a thermogenic coal mine in China [24]. At the industrial level, different companies, for example, Luca Technologies, Inc., Next Fuel, Inc., Ciris Energy, Synthetic Genomics, and ExxonMobil have invested on this approach to different extents [5].

The heart of the biostimulation approach is nutrient recipes which have been kept secret by different companies or investigators. But, as the microbial community and coal structure are different at different sites, the nutrient solutions are expected to be specific for each location. In another word, one nutrient solution that is the best for one site may not be the optimal cocktail for another. Typically, the nutrient recipes include ingredients in four categories: 1) organic nutrients. Yeast extract and peptone are the most commonly used nitrogen sources. In addition, casein hydrolysates, soy proteins and even milk have been tested; 2) major minerals, such as: Mg, Ca, Na, K, etc. Some of these chemicals are phosphate salts which provide buffering capacity besides serving as sources of phosphorous; 3) trace elements, such as: B, Zn, Co, Mo,

Fe, etc. These elements are added in the forms of salts at ppb or ppm levels; and 4) vitamin solutions: vitamin B12, thiotic acid, nicotinic acid, etc. which are supplemented at below ppm concentrations, too.

If all of these ingredients are included, which is the case for most nutrient solutions reported in the literature, then the final nutrient solution is highly complex and is certainly not cheap if used at commercial scales. Considering the unstable and currently low price of natural gas, it is extremely important that the nutrient solution be as simple and inexpensive as possible while maintaining its stimulating effect. Specific to coals in the Illinois basin, we have studied different nutrient solutions and identified one that has demonstrated significantly positive impact on coal biogasification [15]. Here we report our very recent effort on evaluating the effects of the three most expensive ingredients in the nutrient solution. Specifically, we aimed to investigate whether the three components could be used at lower concentrations, eliminated or substituted by other compounds without impacting methane yield negatively. Additionally, cultivation conditions, such as temperature, agitation speed and coal loading were studied at different levels to understand their effects on methane release ex situ. To the best of our knowledge, this is the first study on developing cost-effective nutrient solutions for MECBM and it is no doubt the first of its kinds targeting bituminous coal in the Illinois basin. The methodology reported in this work can be used elsewhere for generating methane from coal economically at industrial scales.

2. Materials and methods

2.1. Coal samples

The coal samples used for the current study were the same as what were investigated and reported before [15, 16]. Blocks of bituminous coal were obtained from the Herrin Seam, #6 in

the Illinois basin. Immediately before use, the coal block was broken into lumps approximately 1.3 cm in size. The coal lumps were subsequently ground and sieved to obtain a desired sample size of less than 40 mesh (< 0.42 mm). At this particle size, the coal (dry weight basis) contained 70.1% of carbon, 1.4% of nitrogen, 5.2% of hydrogen, 0.6% of sulfur, and 15.4% of oxygen. The proximate analysis revealed that the coal had 7.5%, 49.9%, and 42.6% of ash, volatile matter and fixed carbon, respectively. Ground coal samples were stored in re-sealable plastic bags at room temperature in order to prevent moisture loss.

2.2. MS medium and reactor setup

The cultivation media used in the following series of experiments were based on a MS medium which has shown significantly stimulating effect on methane production from coal [15]. This medium contained (per L of distilled and deionized water (DDW)) 0.1 mol of NaHCO_3 , 2.0 g of yeast extract, 2.0 g of trypticase peptones, 0.5 g of mercaptoethanesulfonic acid (Coenzyme M, CoM), 0.25 g of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, 1.0 g of NH_4Cl , 0.4 g of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 1.0 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.4 g of CaCl_2 , 1.0 mg of resazurin, and 10 ml of trace mineral solution [25]. The trace mineral solution contained (per L of DDW) 500 mg of $\text{NaEDTA} \cdot 2\text{H}_2\text{O}$, 150 mg of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 100 mg of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 100 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 100 mg of ZnCl_2 , 40 mg of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, 30 mg of $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$, 20 mg of CuCl , 20 mg of $\text{Ni}_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$, 10 mg of H_3BO_3 , 10 mg of H_2SeO_3 , and 10 mg of $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$.

To decrease cost of the MS medium, three ingredients, yeast extract, peptone and CoM that account for 94% of the total medium cost were evaluated. For the first two, concentration of each at 1 g/L, 0.5 g/L or 0 g/L were considered. For CoM, concentration of 0.25 g/L, 0.05 g/L and 0 g/L were tested. Each microcosm (120-mL serum bottle) contained 10 g of ground coal, 45 mL of different medium and 5 mL of inoculum. The inoculum was developed from a frozen

stock of cells originally collected from formation water in an Illinois coal mine. Microbial composition of the inoculum was reported previously [15]. In short, this microbial community comprises 185 bacterial and nine archaea species that can convert coal to methane in a collaborative and synergistic fashion. All tested conditions were evaluated by setting up three replicates and all microcosms were maintained at 28°C in an incubator. At day 5, 10, 20 and 30, the headspace gas was withdrawn for analyzing concentrations of methane and CO₂ as described below.

Besides testing effects of these three components at reduced concentrations, nutrient solutions, such as trypticase soy broth (TSB) or corn steep liquor (CSL) were investigated. Each of these two nutrient supplements was studied at concentrations of 10 - 50% (v/v) of the MS medium without yeast extract and peptone. For each tested condition, three replicate microcosms were established. Each one contained 10 g of ground coal, 45 mL of TSB or CSL at different strengths and 5 mL inoculum which was the same as discussed above. Additionally, control microcosms which contained no coal but the inoculum and TSB or CSL medium at 50% of concentration were set up. These control reactors were aimed to evaluate methane yield from the nutrient solution itself. All microcosms were kept in an incubator at 28°C. At different time intervals, the headspace gas was withdrawn for analyzing concentrations of methane and CO₂ as described below.

2.3. TSB as the nutrient solution: evaluating effects of different parameters on coal bioconversion

As discussed below, with increasing concentration of TSB, higher methane yield was observed. To study the effects of TSB concentration, temperature, agitation speed, and coal loading on methane yield, a 2³-factorial design was used to establish eight microcosms (Table 1).

Each parameter was evaluated at the low and high levels. In terms of TSB concentration, the levels were either zero or 100%. For temperature, it was 28 or 40°C. The agitation speed was studied at 0 or 100 rpm. The coal loading was evaluated at 200 or 700 g/L. It needs to be noted that the 2-level factorial design does not allow evaluating different parameters at duplicates or triplicates. Considering the fact that TSB can be converted to methane by our microbial community, another eight microcosms were set up at different temperatures and shaking speeds to monitor methane yield from just TSB. These eight reactors represented four different testing conditions, each of which was studied by setting up two duplicate microcosms. Except temperature and agitation difference, all of these eight microcosms contained TSB at 100%, but with no coal loading. All of the 16 microcosms received the same inoculum at 10% of the final volume in each reactor.

At different time intervals, the headspace gas in each reactor was sampled and measured for content of methane in addition to total gas volume. Net methane production (mL) from coal was calculated at each time point by subtracting methane volumes from those observed in control reactors where no coal was present. Methane release (ft³/ton) was then computed based on net methane volume and coal loading. The final day (day 30) methane yields (ft³/ton) were then analyzed by the Design Expert software (version 8.0.6, Stat-Ease Inc., Minneapolis, M, USA).

2.4. Sample analysis

As a result of microbial activities, the reactor headspace became over-pressured with time. To release this overpressure, a stainless steel needle connected to a 50-mL gas tight syringe was inserted to each microcosm headspace at different sampling points. Gas volume in the syringe was recorded and used for calculation of methane yield. The molar contents of methane and CO₂ in the reactor headspace were analyzed through a 17A gas chromatograph (GC, Shimadzu,

Columbia, MD, USA). This GC was equipped with a 60 m × 0.53 mm RT-Msieve 5A porous layer molecular sieve (Restek, Bellefonte, PA, USA) and a flame ionization detector with argon being the carrier gas with a flow rate of 10.1 mL/min. The isothermal zone temperatures for the injector and detector were set at 75°C and 310°C, respectively. The retention time for methane was 4.73 min and that for CO₂ was 6.71 min. Calibration curves for methane and CO₂ were established using standard gases (Air Liquide, Plumsteadville, PA, USA).

3. Results and Discussion

3.1. Evaluating the three components in the MS medium

Our previous studies have demonstrated that: 1) adding nutrients to the same microbial community resulted in much higher methane yield compared to those without; and 2) the MS medium was a better nutrient solution than another commonly used medium for anaerobic microorganisms [15, 16]. In this study, we aimed to understand whether the most expensive ingredients of the MS medium could be used at decreased concentrations, eliminated or substituted for the purpose of decreasing its cost while not compromise methane production. This is to consider the future application of this nutrient solution at industrial scales.

As shown in Fig. 1a, the use of MS medium as a nutrient solution for the microbial consortium led to $200.9 \pm 2.0 \text{ ft}^3 \text{ CH}_4/\text{ton coal}$ produced over the span of 30 days. The methane production rate of $6.7 \text{ ft}^3/\text{ton-day}$ was greater than others reported for bituminous coals. Studies on low volatile bituminous (Pocahontas No. 3, USA) and high volatile bituminous (Pittsburgh No. 8, USA) coals demonstrated methane production rate of 2.1 and $1.2 \text{ ft}^3/\text{ton-day}$, respectively [26]. These data were a result of bioconverting coal at a loading of 50 g/L at 22°C for 60 days. Compared with methane yield from coals with different ranks, our results were higher than 2.7

ft³/ton-day reported for coals from the Powder River Basin [27] but far less than the 30.0 ft³/ton-day for Surat Basin coals in Australia [21]. For the former, the studied coals were subbituminous B and a Tanner medium was adopted [28]. Coal (250-600 µm size) bioconversion was conducted at 22°C with shaking at 100 rpm and a coal loading of 25 g/L. Regarding the latter, Wallon coal that has the same rank was studied with the same medium and same loading of coal (300-600 µm). The temperature was 37°C and the agitation rate was 50 rpm. The reported methane production of 200 ft³/ton was derived from 260 µmol methane/g coal which was observed after 15 days. If this time period was correct, then it is unclear how the authors came up with the 30 ft³/ton-day methane production rate. Comparing all of these studies, it is obvious that even though samples of coals and culturing conditions are similar, the methane yield can vary significantly.

Since methane release from our targeted bituminous coal was significant, we then evaluated the effects of the three most expensive components of the MS medium on methane production. As shown in Fig. 1b, modification of the original MS medium recipe did not lead to change of CO₂ in microcosms' headspace. However, changing the nutrient solution by decreasing the concentrations of or eliminating yeast extract and trypticase peptone, both are conventional organic nitrogen sources, did impact methane productivity negatively (Fig. 1a). For microcosms with modified MS media, methane productivity at day 30 were all lower than those with the original MS medium. Thus, both yeast extract and peptone are critical for methane production and the impact of the former is greater than that of the latter. Considering the fact that the initial methane production rates (ft³/ton-day) during the first 20 days were similar among the three sets: with MS medium, with MS medium but without peptone and with MS medium with yeast extract and peptone each at 1 g/L, a fed-batch cultivation scheme could be developed to

sustain methane production for the latter two nutrient solution compositions by feeding either one or both nutrients intermittently. Our results are consistent with the observation that the presence of yeast extract and trypticase peptone is not essential to the process [29], but the decrease of the concentrations of these components may impact the population of heterotrophic communities and subsequently have a negative effect on CH₄ yield [25].

The most expensive chemical in the MS medium, which accounts for 75% of the total medium cost, is mercaptoethanesulfonic acid, or CoM. This compound is required by an enzyme: methyl-coenzyme M reductase in the final step of methane formation from various substrates in anaerobic environments [30]. Based on our results (Fig. 2), concentration of this compound could be at least decreased to half of its original without compromising methane yield. Further decrease to 10% or 0% of the original led to approximately 85% of methane yield observed from the original MS medium. Given the high cost of this CoM, we conclude that the MS medium without this compound will be more cost-effective than those with.

3.2. Evaluating TSB and CSL as substitutes of yeast extract and peptone

Since yeast extract and peptone cannot be eliminated and their concentrations cannot be decreased without compromising methane production, we then tested whether these two could be substituted by TSB or CSL. According to Becton, Dickinson and Company (New Jersey, US), the supplier of this product, TSB is enzymatic digests of casein and soybean meal, which provide amino acids and other complex nitrogenous substances. In addition, glucose is an energy source, sodium chloride maintains the osmotic equilibrium and dibasic potassium phosphate acts as a buffer to control pH. CSL purchased from Sigma-Aldrich (St. Louis, US) was described as a

viscous concentrate of corn solubles. It is rich in vitamins, amino acids, minerals and other growth stimulants.

As shown in Fig. 3, the highest headspace methane content was 58.5% by day 22 from those containing 50% of TSB. This methane content was similar to 57% detected in microcosms containing the original MS medium at day 30. A trend of increasing TSB concentration resulting in increasing methane content was obvious. But for reactors comprising CSL, the trend was just opposite. Regarding accumulated methane release, the nutrient solution containing 50% TSB resulted in a methane production of $231.9 \pm 12.8 \text{ ft}^3/\text{ton coal}$ (Fig. 4a). When subtracted by those from the controls, the methane yield was $199.6 \pm 16.4 \text{ ft}^3/\text{ton coal}$, which was $9.98 \text{ ft}^3/\text{ton-day}$ during the first 20 days. This rate is higher than $6.7 \text{ ft}^3/\text{ton-day}$ achieved through using the original MS medium. Thus, nutrient solution containing 50% TSB performed better than the MS medium in terms of methane release rate. In contrast, all reactors containing CSL underperformed when compared to the MS medium (Fig. 4b). Drastic decreases in pH from the initial 7.0 to as low as 5.5 were observed in the CSL-treated reactors, indicating the possibility of excessive fermentation and superfluous accumulation of fatty acids that may inhibit methanogenesis. Therefore, TSB at 50% was identified as a suitable substitute of yeast extract and peptone in the original MS medium.

3.3. Effects of different parameters on methane release from coal

Considering the positive correlation between TSB concentration and methane production within the range of 10-50% (v/v), we then investigated whether a 100% (v/v) of TSB solution was even better than the MS medium. In addition, we sought to study effects from other parameters, such as temperature, agitation speed and coal loading which are related to coal bioconversion ex situ. As aforementioned, the 16 microcosms established for this purpose were

monitored over time by measuring gas volume and methane content in the reactor headspace. The product of these two parameters gave pure methane volume (mL) at different time points (Table 1). Briefly, the microbial community we used could convert TSB to methane (#9 - #16), but the accumulated methane volume was much lower than the majority of those with the presence of coal. Among those (#1 - # 8) that contained coals at different loadings, methane volume varied significantly among different cultivation conditions. For these eight microcosms resulted from the two-level factorial design, the final day methane volume after subtraction by those of the corresponding controls, was converted to methane yield in the unit of ft^3/ton .

Regarding the four microcosms (#1 - # 4) that comprised both TSB and coal, accumulated methane release increased with time (Fig. 5a). This is especially true for microcosm #3 which yielded methane at $828.9 \text{ ft}^3/\text{ton}$ at day 30. Methane content, however, did not have this consistent increasing trend (Fig. 5b). For reactor #3, methane content increased between day 0 and day 15, but stayed basically the same thereafter. In terms of reactor #2, methane content increased during the first 20 days and then decreased with time. While some researches on coal bioconversion only measured methane content at one time point [4], this phenomenon of methane content being increasing but then decreasing with time has been reported by other researchers [21]. The possible explanations could be: 1) exhaustion of nutrients in the medium and coal used; 2) product inhibition. CO_2 and methane in the headspace may inhibit microbial activities; 3) accumulation of toxic or inhibitory degradation products; 4) the residual coal was not available to microbial cells anymore; and 5) methane in the headspace was consumed by some of the microbes in the community. The exact reasons for this observation deserves further investigation for the purpose of sustaining methane release from coal.

Analysis of the final methane yield (ft³/ton) through use of the Design of Expert software revealed that temperature and coal loading had negative but TSB concentration and agitation had positive effects. This conclusion was based on a half-normal probability plot for methane production (ft³/ton) (Fig. 6) where insignificant effects have values close to zero and manifest themselves in a linear fashion, while parameters with significant effects are observed far from the plotted line. Among the four parameters, TSB concentration and coal loading had larger positive or negative effect than the other two as reflected from Table 2. This demonstrated that higher TSB concentration would result in higher methane yield, which is consistent with our results discussed above. On the contrary, higher coal loading led to lower methane yield. This is due to the fact that at high solid content, such as 700 g/L, not all coals were immersed in the medium and some of these coals may not be accessible to the microorganisms.

The significance of each parameter was also confirmed by the analysis of variance (ANOVA) (Table 3). The *p*-value for each parameter and AC (temperature * agitation speed) was all lower than 0.05, which implied that all of the parameters tested had a significant effect on methane yield. The software-generated model is significant, too with an F-value of 1059.78 and a *p* value of 0.0009. This indicated that there is only a 0.09% chance that a "Model F-Value" this large could occur due to noise. In terms of actual factors, the equation for predicting methane yield is the following. Unit for each parameter is described in Table 1.

$$\begin{aligned} \text{Ln (Methane yield)} = & 10.28805 - 0.16011 \times \text{Temperature} + 0.017080 \times \text{TSB conc.} - \\ & 0.037384 \times \text{Agitation} - 3.06285\text{E-}003 \times \text{Coal loading} + 1.25820\text{E-}003 \times \text{Temperature} \times \\ & \text{Agitation} \end{aligned}$$

Similar analyses were also conducted for the second response: methane content (%). As demonstrated by Fig. 7, temperature and coal loading had negative while the other two had positive effects. Among the four parameters, TSB concentration had the most positive effect followed by temperature possessing the most negative impact (Table 4). The ANONA analysis indicated that except coal loading and the interaction between temperature and TSB concentration, all of the other parameters had significant effects on methane content with p values less than 0.05 (Table 5). For the developed model, the F-value of 385.37 implies the model is significant. There is only a 3.90% chance that a "Model F-Value" this large could occur due to noise. The developed model in terms of actual parameters is:

$$\begin{aligned} \text{Ln (Methane content)} = & 6.50994 - 0.10246 \times \text{Temperature} + 1.78235\text{E-}003 \times \text{TSB conc.} - \\ & 0.021962 \times \text{Agitation} - 4.92702\text{E-}004 \times \text{Coal loading} + 1.94973\text{E-}004 \times \text{Temperature} \times \text{TSB} \\ & \text{conc.} + 7.46827\text{E-}004 \times \text{Temperature} \times \text{Agitation} \end{aligned}$$

The highest methane yield of 828.9 ft³/ton in 30 days with a methane content of 65% was accomplished when the temperature, TSB content, agitation speed, and coal loading was 28°C, 100% (v/v), 100 rpm, and 200 g/L, respectively. These parameters are in agreement with those reported. For example, optimal methanogenic activity has been observed at 25°C for archaea in the order of *Methanobacteriales* [31], which coincides with the results with the current study, as 89.8% of the archaea in this study was categorized as *Methanobacteriales* [15]. The positive effect of TSB input implied that TSB contained an abundance of nutrients that had additional benefits to the effects of trypticase peptones and yeast extract in the original MS medium. Compared to the effects of other parameters, the positive effect of agitation was not as

significant. Although mixing typically enhances mass transfer, some studies have reported little effect of the treatment on methanogenic activity [32].

The maximum methane yield corresponds to a 27.6 ft³/ton-day of methane release rate. This rate is higher than: 1) 6.7 ft³/ton-day when the original MS medium served as the nutrient solution as discussed above although optimal conditions for using this medium has not been identified yet; and 2) 2.7 ft³/ton-day observed for India coals under optimized conditions [22]. However, it is lower than 73.1 ft³/ton-day reported for bituminous coals [4]. Specific to this study, TSB could have been used as the medium, but the exact medium composition was not disclosed. Although the reported methane yield is exceptionally high, the methane content in the highest performing reactors was only 200,000 ppm, which is 20%. It is unknown what were the other gases in the headspace besides CO₂ (5%) and methane.

Compared with other nutrient solutions revealed by scientific labs and companies, the 100% TSB solution did not contain any other organic ingredients, CoM, and vitamins. It is much simpler and easier to make. The cost of a 100% of TSB solution is similar to that of the original MS medium without the CoM. It needs to be noted that we used chemical prices from the same source for calculating costs of different media. However, since different suppliers have dramatically different pricings which keep changing over time, the exact prices of the studied nutrient solutions are not reported here. But the greatest contribution of this study is that we initiated the discussion and raised the issue on cost of nutrient solutions that are to be used at large field scales under the climate of extremely low and volatile natural gas price. While almost all investigators and companies have only targeted methane yield without serious consideration of costs of their nutrient solutions, this manuscript provided an example on how to evaluate individual ingredients that contribute to majority of the cost. The simplified and less expensive

nutrient solutions can certainly be applied to Illinois basin for the purpose of enhancing methane yield from coal. The methodology discussed in this work can be adopted elsewhere for promoting MECBM at a cost-effective fashion.

Conclusion

In this study, a nutrient solution, the MS medium which demonstrated enhancing effect on methane release from coal was further investigated. In particular, we sought to understand whether the three most expensive components, yeast extract, peptone and CoM can be used at decreased concentrations, eliminated or substituted by TSB or CSL for the purpose of reducing the medium's cost. Our results revealed that: (1) yeast extract and peptone could not be eliminated or used at decreased concentrations without comprising methane yield; (2) CoM can be used at 50% of its original concentration without affecting methane production negatively. Elimination of this chemical from the MS medium recipe resulted in a 15% of reduction in methane release. But considering its high cost, the MS medium without this component should be more cost-effective; (3) positive correlation between TSB concentration and methane release was observed. TSB concentration at 50% led to a methane yield that was similar to those from the original MS medium; (4) at 100%, a methane yield of 828.9 ft³/ton in 30 days was observed. This yield was four times of those with the unmodified MS medium; (5) while TSB concentration and agitation had statistically significant positive effects on methane release, temperature and coal loading were just opposite; and (6) models developed from this study could predict methane yield and content accurately when parameters were within the ranges tested.

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414 **Figure legends**

415 Fig. 1: Yield of gas on bituminous coal under different conditions. A: Methane, B: CO₂.

416 Fig. 2: Methane yield from coal in reactors with different concentrations of CoM.

417 Fig. 3: Volumetric methane contents in the reactors that substituted trypticase peptone and yeast
418 extract in the MS medium with TSB or CSL.

419 Fig. 4: Methane yield in (a) TSB- and (b) CSL-containing reactors.

420 Fig. 5: Performances of the microcosms containing both 100% TSB and coal. A: Accumulated
421 methane yield; B: methane content.

422 Fig. 6: Half-normal probability plot for methane yield.

423 Fig. 7: Half-normal probability plot for methane content.

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426 **Table captions**

427 Table 1: Volume of methane from different microcosms over time.

428 Table 2: Percent contributions from different parameters for methane yield.

429 Table 3: Results of analysis of variance (ANOVA) for methane yield.

430 Table 4: Percent contributions from different parameters for methane content.

431 Table 5: Results of analysis of variance (ANOVA) for methane content.

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Fig. 1: Yield of gas on bituminous coal under different conditions. A: Methane, B: CO₂.

Fig. 1b:

Fig. 2: Methane yield from coal in reactors with different concentrations of CoM.

Fig. 3: Volumetric CH₄ contents in the reactors that substituted trypticase peptone and yeast extract in the MS medium with TSB or CSL.

Fig. 4: Methane yield in (a) TSB- and (b) CSL-containing reactors.

Fig. 4b:

Fig. 5: Performances of the microcosms containing both 100% TSB and coal. A: Accumulated methane yield; B: methane content.

Fig. 5b:

Fig. 6: Half-normal probability plot for methane yield.

Fig. 7: Half-normal probability plot for methane content.

Table 1: Volume of methane from different microcosms over time.

Run	Temperature (°C)	TSB conc. (%)	Agitation (rpm)	Coal loading (g/L)	Day 10	Day 15	Day 20	Day 25	Day 30
1	40	100	100	700	2.94	23.50	64.60	89.06	106.98
2	28	100	0	700	0.86	36.91	171.58	194.45	205.80
3	28	100	100	200	8.28	143.10	183.40	202.00	220.20
4	40	100	0	200	10.38	28.08	36.58	39.73	41.42
5	28	0	0	200	30.45	40.20	43.35	44.75	46.15
6	40	0	100	200	15.20	19.13	22.46	24.87	25.31
7	40	0	0	700	0.00	2.82	3.80	5.21	5.37
8	28	0	100	700	12.77	22.26	25.62	27.06	27.54
9	28	100	0	0	0.72	2.96	3.57	4.96	6.84
10	28	100	0	0	1.44	4.76	6.89	11.25	11.71
11	28	100	100	0	0.00	3.44	3.45	4.38	4.63
12	28	100	100	0	0.00	2.48	4.55	6.12	6.51
13	40	100	0	0	1.24	4.83	5.70	5.95	6.01
14	40	100	0	0	2.30	5.17	6.15	6.36	6.42
15	40	100	100	0	2.47	4.38	5.62	6.02	6.07
16	40	100	100	0	0.00	0.83	2.06	2.66	3.54

Table 2: Percent contributions from different parameters

Term	Effect	SumSqr	% Contribution
A-Temperature	-1.17	2.72	18.17
B-TSB conc.	1.71	5.83	38.96
C-Agitation	0.54	0.58	3.89
D-Coal loading	-1.53	4.69	31.33
AB	-0.05	0.00	0.03
AC	0.75	1.14	7.61
AD	0.02	0.00	0.01

Table 3: Results of analysis of variance (ANOVA)

Source	Sum of squares	df	Mean square	F value	p-value Prob > F
Model	14.97	5.00	2.99	1,059.78	0.0009
A-Temperature	2.72	1.00	2.72	963.31	0.0010
B-TSB conc.	5.83	1.00	5.83	2,065.49	0.0005
C-Agitation	0.58	1.00	0.58	206.07	0.0048
D-Coal loading	4.69	1.00	4.69	1,660.54	0.0006
AC	1.14	1.00	1.14	403.51	0.0025
Residual	0.01	2.00	0.00		
Cor Total	14.97	7.00			

Table 4: Percent contributions from different parameters for methane content

Term	Effect	SumSqr	%Contribution
A-Temperature	-0.66	0.88	28.62
B-TSB conc.	0.84	1.42	45.87
C-Agitation	0.34	0.24	7.63
D-Coal loading	-0.25	0.12	3.93
AB	0.12	0.03	0.89
AC	0.45	0.40	13.02

Table 5: Results of analysis of variance (ANOVA) for methane content.

Source	Sum of squares	df	Mean square	F value	p-value Prob > F
Model	3.08	6.00	0.51	385.37	0.039
A-Temperature	0.88	1.00	0.88	662.13	0.0247
B-TSB conc.	1.42	1.00	1.42	1061.02	0.0195
C-Agitation	0.24	1.00	0.24	176.41	0.0478
D-Coal loading	0.12	1.00	0.12	91.01	0.0665
AB	0.03	1.00	0.03	20.52	0.1383
AC	0.40	1.00	0.40	301.11	0.0366
Residual	0.00	1.00	0.00		
Cor Total	3.09	7.00			