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Evaluation of Produced Volumes of Carbon Dioxide from the Concentration of the Gas Absorbed in the Media during Microbial Fermentation for Enhanced Oil Recovery Purposes

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Carbon dioxide produced by microbes during microbial enhanced oil recovery process (MEOR) promotes oil displacement and productivity through re-pressurization of the oil field and dissolution of the rock matrix. In the laboratory studies conducted using an adapted strain of *Clostridium tyrobutyricum* (DSMZ 663*)* in fermentation media of 500 mL, the volume of the produced gas, the concentration of the dissolved gas determined using titrimetric method and pH of the media as a result of microbial metabolic activities at different salt concentrations (0, 30, 60, 90 and 100 g/L) were measured after 24, 72 and 120 h. The volume of produced gas decreased from about 3000 mL at 0 g/L to 250 mL at 100 g/L. The rate of absorption, volumetric mass transfer coefficient and partial pressure were then related quantitatively as a function of salinity and were compared. The result shows that the rate of absorption decreases exponentially with salinity suggesting a strong correlation with R^2 value of 0.75-0.98 at constant coefficient of 0.0002. The volumetric mass transfer coefficient for carbon dioxide at 0 g/L is approximately 6 times greater than at 100 g/L suggesting the influence of the salinity of the media. The correlation of the gas concentration in the solution with the gas bulk volumes produced at different salinities after 120 h (R^2 = 0.97) suggests an accurate tool for the estimation of the amount of gas produced by microbes.

1. Introduction

Microbial enhanced oil recovery (MEOR) involves the use of specific bacteria capable of producing useful metabolites in-situ such as gases, acids, surfactants, solvents and polymers in order that their presence will aid further reduction of residual oil left in the reservoir after secondary recovery (Lazar et al., 2007). The production of biogenic gases as earlier mentioned creates a free gas phase that can account for incremental oil recovery in MEOR processes either by reduction of the oil viscosity by solution of the gas in the oil, or by repressurization of the reservoir causing displacement from trapped capillaries and enhancing mobilization of the oil to the producing wells (Sen, 2008). The most important gas-producing bacteria are *Clostridium, Desulfovibrio, Pseudomonas*, and some methanogens (Behlülgil and Mehmetoğlu, 2002). The composition of the biogenic gas from bacteria metabolism can include carbon dioxide, hydrogen, methane and nitrogen.
However, certain factors can affect gas production by either reducing the volume of gas produced or the amount that can go into solution. One of such factor is salinity of the media. Salinity is known to affect the solubility of gas in liquid because of the salting out effect (Duan and Sun, 2006). Oilfield

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brines can have a wide range of concentrations of a large variety of cations and anions with concentrations ranging from less than 100 mg/L to more than 200,000 mg/L (Donaldson et al. 1989).
During fermentation process under anaerobic condition, gases are produced by conversion of carbon source such as molasses which is a cheap and universally available nutrient. The effectiveness of the gas produced to enhance oil recovery will depend on how much of it can be transferred into the system. Evaluation of the amount of gas that can dissolve into the solution during microbial process is thus important. This can give an idea of how much of the gas produced goes into solution for displacement of oil at the oil-water-rock interface. However, all these processes happen inside the reservoir which can be very difficult to determine and monitor. Therefore, there is a need for simple system to determine measurement to handle fluid samples from reservoirs. Measurement of concentration of gas produced in situ can give an indication of effectiveness and limit of bacteria metabolism. For example, a fixed amount of bacteria inoculum under laboratory conditions when grown in a medium produces gas, of which the concentration in the solution can be determined by chemical methods and correlated with the measured volume of the produced gas. If the concentration of the gas in the fluid sample from a reservoir is known, then it will be possible to predict the volume of gas produced. Correspondingly, this information would be crucial in simulation of MEOR performance as the salinity level is different for many reservoirs.
For this reason, a simple experimental procedure was used to assess the gas production and evaluate the concentration of carbon dioxide in media of different salinity in order to estimate how much of such can go into solution. Another objective is to find correlation between gas concentration in the media and volume of gas produced.

2. Materials and methods

2.1 Fermentation set up

Solutions of different salinity were prepared in fermentation bottles of 1 L volume by accurately weighing specific amount of sodium chloride and dissolving in 500 mL of demineralized water to make solution of salinity 0, 30 60, 90 and 100 g/L. The fermentation bottles and media were initially purged of air by passing pure nitrogen for about 5 min. This was to create anaerobic conditions in the bottles. The nutrient consists of 50 mL of molasses and was added into already prepared 500 mL of salt solution of different salinity; the molasses acted as the substrate for the bacteria. Each set up was inoculated with 10 mL inoculum of an adapted strain of *Clostridium tyrobutyricum* (DSMZ 663) cultivated on reinforced clostridia medium after growth of 48 h indicated by change in turbidity. The full process of adaptation for this strain is described in (Rudyk and Søgaard, 2009). The set up was completed with water displacement apparatus for gas collection and measurement of volume produced. The whole process was maintained in water bath at 37 ^{o}C. Cumulative gas production was calculated as total sum of gas produce during the period of 120 h. Liquid samples were carefully taken out from the fermentation bottles every 24 h for pH measurement using a pH meter (Model: PH 2000 Radiometer Analytical).

2.2 Gas analysis

The gas composition analysis was carried out using gas chromatography equipment (Model: Clarus 500 Perkin Elmer). The sample amount was 1.0 mL with a dilution factor of 1.0. Each sample was run for 3 cycles. The gas samples analyzed were collected after a fermentation period of 48 h.

2.3 Titration

The determination of concentration of dissolved CO_2 in the liquid sample was carried out using the method of end point pH with the titration manager (Model: ABU 901 Radiometer Analytical). 10 mL of samples were carefully taken from fermentation bottle at intervals of 48 h. 5 mL of each sample was titrated with a standard sodium hydroxide solution. Free CO_2 reacts with the sodium hydroxide to form sodium bicarbonate. The completion of the reaction is indicated automatically at end point pH of 8.3. The equivalent concentration of CO_2 in each sample is indicated after the completion of the reaction. All the samples were corrected for any dissolved CO_2 in the molasses by subtracting the background value in molasses from the measured values of dissolved CO_2 at different time.

The concentration of the dissolved CO_2 determined by the titration method as described above can be expressed by Henry's equation (Yagi and Yoshida, 1977) as given in equation 1 below.

$$[CO_2] = K_H pCO_2 \quad (1)$$

where K_H is Henry's law constant (mol/L.atm) and pCO_2 is the partial pressure of CO_2 in the gas phase (atm). The value of Henry's constant used for calculation is 3.4 x 10^{-2} (mol/L.atm) at temperature of 37 ^{o}C.

As shown by (Dixon and Kell, 1989), due to stepwise increase of the pCO_2 in the gas phase, the rate of gas absorption (R_s) can be written as:

$$R_s = dC / dT \quad (2)$$

C is the concentration of the gas in the bulk liquid, and *T* is the time. It follows that the rate of absorption R_s of CO_2 can be calculated from the slope of the graph of CO_2 concentration and time and this is the basis of the work to be described.

The volumetric mass transfer coefficient $K_L a$ of CO_2 in the fermentation bottle is calculated from equation 3 below.

$$K_L a = R_s / Hp_g \quad (3)$$

H is the Henry constant already described above, and p_g is the partial pressure of CO_2 in the gas phase (assumed to be 1 bar). The transfer rate of the gas produced is dependent upon the volumetric transfer coefficient $K_L a$.

3. Result

During fermentation process of molasses by *Clostridia tyrobutyricum* in solutions of different salinity, gas was produced. Figure 1 illustrates the cumulative gas production at different salinity per 50 mL of molasses in each fermentation bottle. At salt concentration of 0 g/L, the cumulative gas production reached 2900 mL. With increasing salinity, the cumulative gas decreases and is about 250 mL at the highest salt concentration of 100 g/L measured in the experiment within 120 h. The cumulative gas production at 30, 60 and 90 g/L are in between 500-1200 mL. It can be inferred that there is an influence of salinity on the total amount of gas produced as indicated by volume reduction with increase salinity.

The pH variation in the fermentation medium over time at different salinity is shown in Figure 2. The initial pH values fall significantly in the first 24 h at all measured salinity. The decrease of pH values was probably due to acid formation and production of gas. At salinity 0 g/L the pH appeared to increase slightly from 72 h after the process started, while at other salinity ranges it appeared lowered or remain constant after 72 h.

The composition of the gas phase was determined and main constituents are shown in Table 1 as percentage composition. The other gases in Table 1 are assumed to be mixture of hydrogen and methane. With the result of gas composition supporting that main constituent is carbon dioxide, further analysis was done using equations 2 - 3 to evaluate those parameters described.

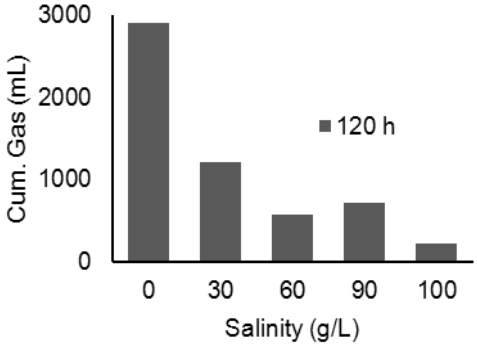

Figure: 1 Cum. gas production at 120 h

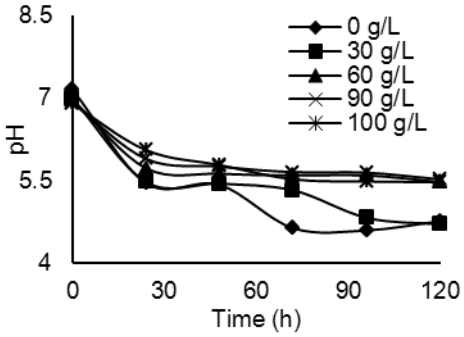

Figure 2: pH variation with time at different salinity

Figure 3 shows the measured amount of dissolved CO_2 in the fermentation medium at different time and salinity. The highest concentrations were found at 120 h. If the salt concentration of the media is considered, irrespective of time, concentration of dissolved CO_2 in the medium is in decreasing order of 0, 30, 60, 90 and 100 g/L respectively. The measured values for 90 g/L and 100 g/L are similar due to the closeness of their salinity range. This result further suggests the effect of salinity on dissolution of CO_2 as well. From the slope of the graph in Figure 3, the rate of absorption was calculated using equation 2 and the result is shown as function of salinity in Figure 4.

Table 1: Average gas composition for gas sample from fermentation of molasses

Component	% Composition
Carbon dioxide	83.66
Others	16.23
Nitrogen	0.11
Total	100.0

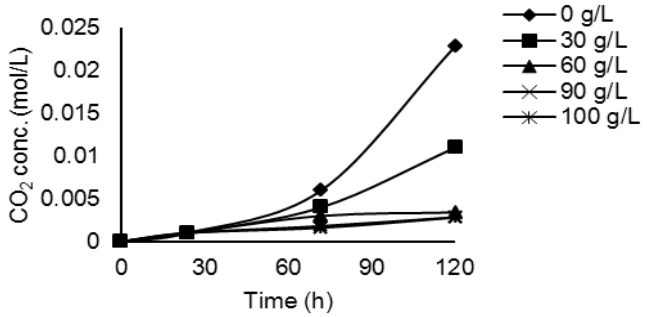

Figure 3: Concentration of dissolved carbon dioxide with time at different salinity

The rate of absorption (R_s) decreases exponentially with salinity with R^2 value of 0.88 - 0.98. This suggests a strong correlation between rate of absorption and medium salinity. However the highest rate occurred at 24 h probably because the largest volume contributing to cumulative gas production

was produced in the first 24 h. The concentration of dissolved carbon dioxide is plotted against the cumulative gas production after 120 h and is shown in Figure 5.

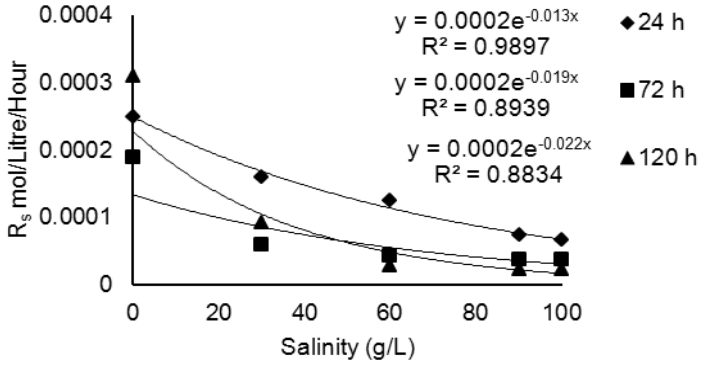

Figure 4: Rate of absorption of carbon dioxide at different salinity and time

The alteration of dissolved CO_2 concentration with salinity correlates with the bulk volumes of the produced gas after 120 h (R^2 = 0.97). The two deviating points are 60 g/L and 90 g/L respectively; however the reason for the deviation is not clear yet. The equation for the relationship is given below in equation (4):

$$[CO_2] = 0.000008 * C_g \quad (4)$$

where C_g is the cumulative gas produced after 120 h.

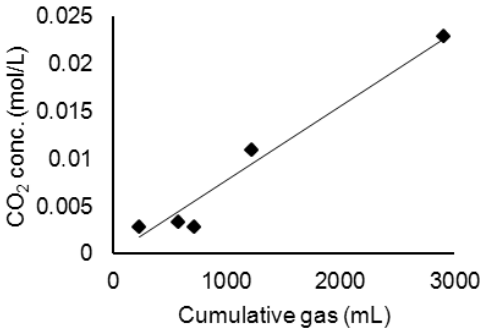

Figure 5: Correlation between concentration of dissolved CO_2 and cumulative gas after 120 h

Table 2 shows the result of transfer coefficient as calculated using equation 3. The result shows that value of volumetric mass transfer coefficient decreases with increase salinity. It was observed that the trend in $K_L a$ followed that of the absorption rate CO_2 quite closely.

Table 2: Result of calculated volumetric mass transfer coefficient at different salinity

Salinity (g/L)	0	30	60	90	100
$K_L a$ (H^{-1})	0.010	0.0042	0.0026	0.0018	0.0017

4. Discussion

In the production of gas by an adapted strain of *Clostridium tyrobutyricum* (DSMZ 663) grown on molasses, we have shown that the main constituent of the gas phase is carbon dioxide (Table 1). The measured pH values also decreases and it is comparable to our earlier result (Jimoh et al., 2011) where we reported a decrease of pH for this strain of bacteria as indication of gas and acid production. The cumulative volume of gas produced decreases with salinity as a result of the inhibition of the bacterial growth. The volume of cumulative gas at salinity 0 g/L is approximately 13 times greater than at 100 g/L. However, the rates of gas production of approximately 300-700 mL from initial volume of media of 500 mL at salinity range of 90-100 g/L showed that this strain underwent adaptation process successfully and can be productive even at such high salt concentrations.

Furthermore, the calculated values of volumetric mass transfer coefficient of carbon dioxide (Table 2) show a significant influence of salinity of the medium. Thus at high salinity (low carbon dioxide production), a low volumetric mass transfer coefficient of carbon dioxide can be expected. The measured concentration of dissolved CO_2 from titration experiment decreases with salinity of the medium also (Figure 1). The concentration of dissolved CO_2 in the media by the end of the experiment at different salinity probably settles at a value that provides the absorption rate which is directly related to the volume of gas produced. When the concentration of dissolved CO_2 is plotted against the cumulative gas produced at 120 h there is a strong correlation between the two parameters with R^2 value of 0.97. If concentration of dissolved CO_2 correlates almost at 100 % with the total produced gas, salting out effect could be considered to be minimal taking into account low gas volumes produced. This probably suggests that a reliable estimate of the amount of gas produced can be made from the measurements of the concentration of dissolved CO_2 in the fluid sample and applied to practical purposes of the monitoring of the microbial gas production during MEOR operations.

5. Conclusion

The adapted strain of *Clostridium tyrobutyricum* (DSMZ 663) is able to produce gas at high salinity. It was shown that about 84 % of the gas produced during fermentation process of molasses is composed of carbon dioxide and the absorption rate decreases with increase salinity. Further evaluation showed that from the concentration of the dissolved gas in microbial media measured from titration method, the estimation of volume of gas produced can be made.

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