On-Line Measurement of Dissolved Methane Concentration During Methane Fermentation in a Loop Bioreactor

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ABSTRACT: *A dissolved methane sensor based on silicone tube was designed, constructed and optimized. The silicone tube diameter, silicone tube length and helium flow rate (as the carrier gas) were considered as process parameters to be optimized. A continuous stream of helium (50 mL/min) was directed through the tubing, sweeping out the dissolved methane which diffused through the walls of the tubing from the fermentation broth. The probe was made of silicone rubber tubing, 10 cm in length with inner and outer diameters of 0.25 cm and 0.35 cm, respectively (Detakta Company; NO. 02502). A semi-conductor methane gas sensor (Figaro TGS 2611) - which is highly sensitive and selective to methane gas - was used to measure the dissolved methane continuously. Henry's law along with a special circuit experimental method was applied for calibration. The output concentration was displayed in mg/l (or ppm) of dissolved gas. The accuracy and response time of this system are ± 2 % and 2 minutes, respectively. Moreover, a control system was installed for recycling methane gas during fermentation.*

KEY WORDS: *Dissolved methane, Semi-conductor sensor, On-line measurement, Loop bioreactor, Calibration.*

INTRODUCTION

Methanotrophic bacteria are a group of aerobic bacteria that can use methane as their source of carbon and energy [1-3]. The processed biomass extracted from these species of microorganisms could be utilized as a source of potential protein either for food supplement or as fodder [4-6]. Therefore, measurement of dissolved methane as a sole source of carbon in a methane fermentation bioreactor can be done to optimize biomass growth rate, while minimizing methane supply [7].

This is why understanding the aqueous phase methane concentration is critical to control process kinetics in such bioreactors. In spite of the efforts made by some researchers [8-11] since no market-existing dissolved methane probe is commercially available, relevant studies in this area have relied on analyzing dissolved methane by gas chromatography or measurement of volatile concentration from the outlet gases, which is less accurate, time consuming and very difficult to perform on

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line [7, 10, 11]. This is the reason why a dissolved gas probe for direct extraction of volatile compounds from the liquid phase is now a needed tool in modern fermentation technology [11]. Sensor systems evaluate the dissolved volatiles, usually having the advantage of the permeability of silicone to gases but not to liquids [7,10-12]. This property permits the extraction of volatiles from the liquid without affecting the sterile environment of the closed fermenter. Typically, the permeability of rubbery materials has been studied at low pressure and it is usually found to be independent of pressure for most gas penetrates [12]. Permeation through a thin, flat and dense film (like the wall of the silicone tube) at low pressures can be characterized in terms of a simple one-dimensional diffusion model [13]. The local flux through a film is given by the following expression (Fick law) Eq. (1):

$$N = -D_{eff}(C).\frac{dC}{dx} \quad (1)$$

where the diffusion coefficient (D_{eff}) may be a function of the local concentration (C) and temperature. The permeability (P) can be defined as shown in Eq. (2) in terms of the steady state permeation flux through a membrane of thickness (l).

$$P = \frac{N}{\Delta p/1} \quad (2)$$

where N is the steady state flux ($mL/cm^2.s$) and Δp is the pressure difference between the upstream and downstream membrane faces (cm Hg) [13].

In the present study, we have made a low pressure sensor based on a thin silicone tube suitable for on-line measurement of dissolved methane during its fermentation in a loop bioreactor. Variables including silicone tube diameter, silicone tube length and helium flow rate (as the carrier gas) were selected to be studied as challen-geable parameters affecting optimal response time. Also, we have evaluated the possibility of continuous on-line dissolved methane analysis by a Programmable Logic Controller (PLC).

MATERIALS AND METHODS

Dissolved methane sensor

The dissolved methane concentration in the fermenter was continuously monitored during air and CH_4 feed gas

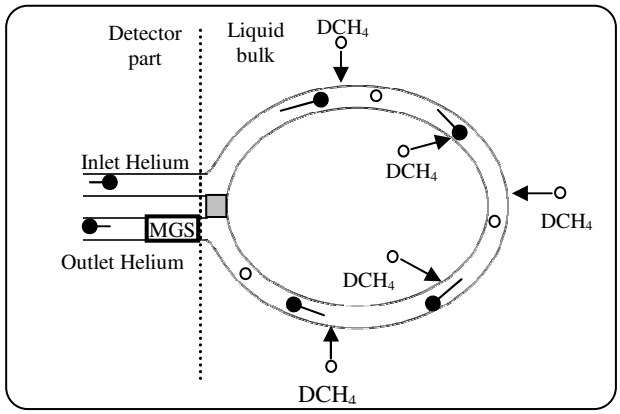

Fig. 1: A schematic diagram of dissolved methane sensor based on silicone tube (DCH_4: dissolved methane; MGS: methane gas sensor).

fermentations with a probe based on the tubing concept of Phillips and Johnson [8]. A silicone rubber with supports was immersed in the fermentation. The probe was made of silicone rubber tubing, 10 cm in length with inner and outer diameters of 0.25 cm and 0.35 cm, respectively (Detakta Company; NO. 02502). A stream of helium (50 mL/min) was directed through the tubing, sweeping out the gases which diffused through the walls of the tubing from the fermentation broth. A schematic diagram of dissolved methane sensor based on silicone tube is illustrated in Fig. 1. The helium from a gas cylinder was directed through a two-stage pressure regulator.

The gas within the probe contained helium, oxygen, methane, carbon dioxide and water vapor.

The analysis was performed by a semi-conductor gas detector (Figaro TGS 2611). This approach provides simple and continuous analysis in which the resistance of the sensor changes according to the in-liquid concentration of the measured compound. The sensing element is comprised of a metal oxide semiconductor layer formed on an alumina substrate of a sensing chip together with an integrated heater. In the presence of a detectable gas, the sensor's conductivity increases depending on the gas concentration in the air. A simple electrical circuit can convert the changes in conductivity to an output signal which corresponds to the gas concentration. The TGS 2611 has high sensitivity and selectivity to methane gas. Due to miniaturization of the sensing chip, TGS 2611 requires a heater current of 56 mA and the device is housed in a standard TO-5 package. Fig. 2 is a schematic diagram of a typical

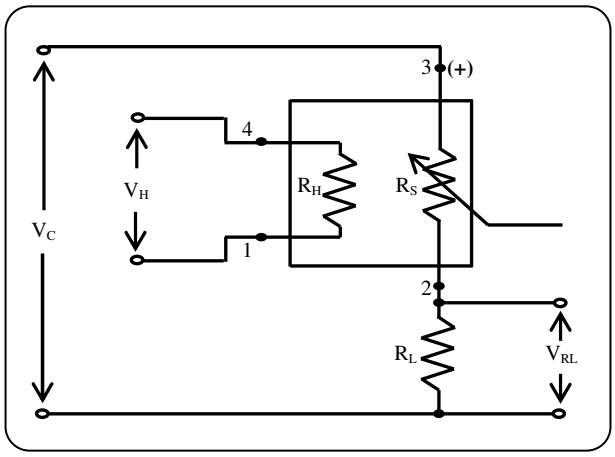

Fig. 2: A schematic diagram of a typical measuring circuit in gas sensor (methane).

measuring circuit. The sensor requires two voltage inputs: heater voltage (V_H) and circuit voltage (V_C). The heater voltage is applied to the integrated heater in order to maintain the sensing element at a specific temperature which is optimal for sensing. Circuit voltage is applied to allow measurement of voltage (V_{RL}) across a load resistor (R_L) which is connected in series with the sensor. A common power supply circuit can be used for both V_C and V_H to fulfill the sensor's electrical requirements. The value of the load resistor should be chosen in a way that optimizes the alarm threshold value, keeping power dissipation (P_S) of the semiconductor below a limit of 15 mW. Power dissipation will reach its peak when the value of sensor resistance (R_s) is equal to R_L on its exposure to gas. The value of power dissipation can be calculated by utilizing the following Eq. (3):

$$P_s = \frac{(V_c - V_{RL})^2}{R_s} \tag{3}$$

Also, sensor resistance is calculated via a measured value of V_{RL} by using the following formula:

$$R_s = \frac{(V_c - V_{RL})^2}{V_{RL}} R_L \tag{4}$$

The gas probe may be hooked-up to a PLC or to a computer and may be used for real-time measurement of various dissolved volatile organic compounds, such as methane. A diagram of the bioreactor and probe assembly is given in Fig. 3. The gas recycle system for controlling of methane concentration in gas phase during fermentation is also shown in Fig. 3.

Calibration

The calibration of dissolved methane sensor was evaluated based on Beland's method [7]. The relationship between sensor resistance and the concentration of the gas can be expressed by the following Eq. (5) over a certain range of gas concentration.

$$R_s = A \cdot [C]^{\alpha} \tag{5}$$

where R_s: sensor resistance (Ω), C: gas concentration, α: slope of R_s curve and A: a constant dependent on the sensor type. The final objective is to find the actual concentration by measuring the sensor resistance. The Eq. (6) may be written as:

$$[C] = \left(\frac{A}{R_s}\right)^{\frac{1}{\alpha}} \tag{6}$$

The circuit of Fig. 2 can be equivalently considered as a simple voltage divider as shown in Fig. 4. As it is obvious, this voltage divider gives an output voltage (see Eq. (7)):

$$V_{RL} = V_{CC} \times \frac{R_L}{(R_S + R_L)} \tag{7}$$

which can be re-written as:

$$R_S = R_L \times \frac{V_{CC}}{(V_{RL} - 1)} \tag{8}$$

and as a result of combining Eq. (6) and (8), we get:

$$[C] = \left[-V_{RL} \cdot A / R_L (V_{RL} - V_{CC})\right]^{\frac{1}{\alpha}} \tag{9}$$

In Eq. (9) A, V_{CC} and R_L are known and therefore C can be uniquely determined by V_{RL}. As the sensor has a Log versus Log type relationship, it is advisable to change R_L in order to maximize the sensor sensitivity to the gas to be monitored. The sensor resistance is given by Eq. (10).

$$R_s = \frac{V}{I} \tag{10}$$

where V: voltage on the sensor (Volts) and I: current generated by the source (Ampere). Meanwhile, Eq. (5) defines the behavior of the sensor resistance to the gas concentration. By applying concentration C=1 to the sensor a, the sensor will react by modifying its resistance and therefore the voltage $V_{[1]}$ will be read. Thus, R_S is calculated as follows:

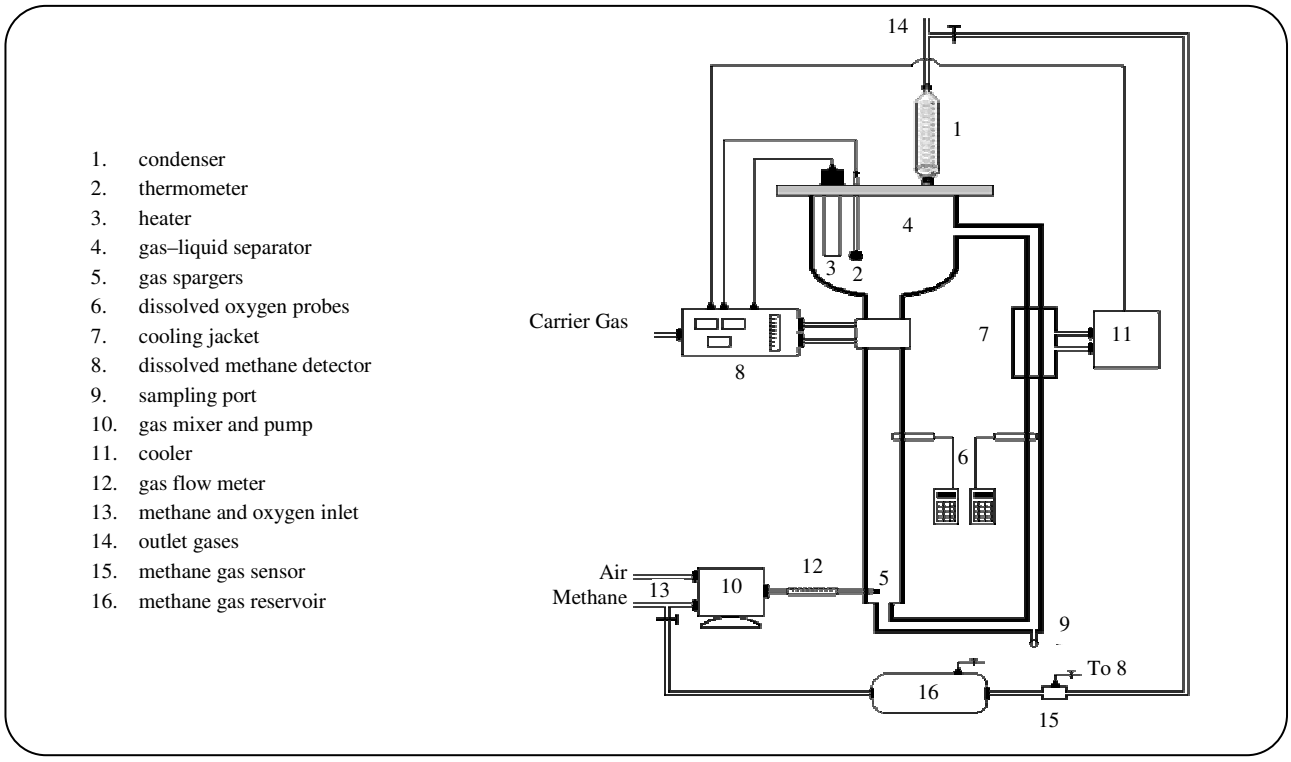

Fig. 3: A diagram of the loop bioreactor with dissolve methane sensor and gas recycle system.

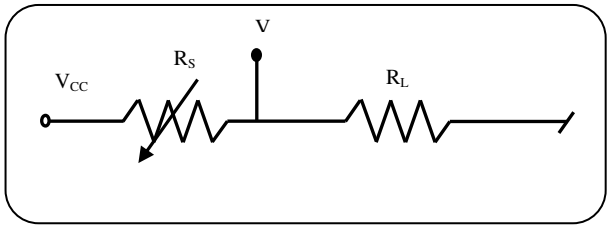

Fig. 4: A schematic diagram of an equivalent circuit of Fig. 2.

$$R_S = A \cdot [C]^{-\alpha} = \frac{V_{[1]}}{I} \quad (11)$$

By arranging the sensor's equation, we obtain:

$$C = (A \cdot I)^{\frac{1}{\alpha}} \cdot V^{\frac{-1}{\alpha}} \quad (12)$$

and by combining the former equations we obtain:

$$C = \left(V_{[1]}\right)^{\frac{1}{\alpha}} \cdot V^{\frac{-1}{\alpha}} \quad (13)$$

All the resistance and current elements have been removed from Eq. (13) as well as the A parameter. This will facilitate the identification of the parameters. Therefore, if $V_{[1]}$, the voltage at a concentration C=1, and α parameter are known, the measured sensor voltage (V), once introduced in Eq. (13), will indicate the actual concentration of the gas interest. $V_{[1]}$ and α parameter are obtained for each gas sensor by calibration. In order to obtain $V_{[1]}$ and α parameter, the sensor is calibrated making use of different methanol dilutions and recording the corresponding produced voltage (Figaro TGS 2611 is sensitive to iso-butane, hydrogen and alcohol such as ethanol, too). Then, Eq. (13) is transformed into Log terms as bellows:

$$\mathrm{Log}(C) = \frac{-1}{\alpha} \cdot \mathrm{Log}V + \frac{1}{\alpha} \mathrm{Log}V_{[1]} \quad (14)$$

The slope and the intercept is $-1/\alpha$ and $1/\alpha \cdot \log V_{[1]}$ respectively. Now, $V_{[1]}$ and α parameter are obtained and therefore all the parameters in the Eq. (13) are identified. Using Eq. (13), the concentration of a gas or gases of interest is obtained by V which is being measured.

In addition, Henry's law is used in order to determine the real concentration of dissolved methane. Henry's law states that the partial pressure of a gas over an aqueous solution in which the gas is dissolved is proportional, i.e. C = H.P. P is the partial pressure of the gas, C,

the concentration of the gas in the solution and H, Henry's constant. Furthermore, the sorption of low-molecular-weight volatile components in rubbery materials is typically described by Henry's law for cases where the sorbed concentrations are low [12].

Response time

The other important parameter of the sensor is the response time. It can be measured by making a step-by-step change in methane partial pressure in the measurement medium on a time-serial basis and then measuring the sensor response [14].

The sensor can be approximated as a first order system (Eq. (15)):

$$C - C_p = \tau_p \cdot \left(\frac{dC_p}{dt} \right) \quad (15)$$

where C is the methane concentration in the measurement sample, C_p, the methane concentration measured by the sensor and τ_p is the sensor time constant. Once a change is made in C (by transferring the sensor from methane into nitrogen saturated, stirred water), the sensor output decreases roughly exponentially (not exactly exponentially because the sensor may not be a true first order system). The time constant (τ_p) is the time when the sensor response reaches 63.7 % of the ultimate response. The solution to Eq. (15) with the following boundary condition is an exponential function:

$$\frac{C}{C_p} = 1 - \exp\left(\frac{-t}{\tau_p} \right) \quad (16)$$

Eq. (16) indicates that when $t = \tau_p$, C/C_p will be 0.64. Also, the time constant (τ_p) can be determined conveniently by using an integral method, the area above the response curve being equal to τ_p. This method is especially useful when there is a lot of noise in the measured signal.

Accuracy

Seven streams of mixed gases were used for evaluation of the accuracy of dissolved methane sensor. Inlet gas flow rates of air and methane were adjusted in a way that mixtures from 94 v % to 100 v % methane (with 1 v % interval) were supplied. The oxygen flow rate was 0.5 m/s. All data was monitored continuously.

RESULTS AND DISCUSSION

An improved tube probe for measuring methane in a liquid has been constructed. In particular, the probe was designed in such a way that substantially only the gas permeable tube is immersed into the fermenter. Also, a supporting circular metal has been inserted into the silicone tube. This prevents any mechanical stress to the tube due to agitation or aeration. The electrical circuit was also used with such gas permeable tubing probe.

In this study, the silicone diameter size, silicone tube length quantity and helium flow rate were selected as effective factors to be optimized. In order to investigate the interactions among all parameters mentioned, full factorial design was applied. Table 1 illustrates these variables in three levels. The criterion for measurement in all experiments was the response time. Table 2 shows the response times in all assessments. The optimum full factorial test, as mentioned before, selected 0.25 cm, 0.1 m and 50 mL/min as the most appropriate values of silicone internal diameter, silicone tube length and helium flow rate, respectively. The response time was 2 minutes in these conditions. According to table 2, helium flow rate and silicone diameter were determined as important parameters. However, the response time did not change significantly for two different lengths of silicone tubes (0.1 m and 0.15 m). This observation was shown proved by examining four values of silicone tube length (0.07, 0.09, 0.16 and 0.18 m). The response time extended more than 2 minutes for 0.07 and 0.09 m of the tube lengths. The tube with lengths of 0.16 and 0.18 m resulted in a response time very similar to the silicone tube with length of 0.1 cm. Therefore, the optimum value of silicone tube length was chosen to be 0.1 cm.

Henry's law along with a special circuit experimental method mentioned above was used for calibration of dissolved methane sensor. Table 3 illustrates Henry's constants and the solubilities of oxygen and methane at different temperatures. Table 3, also, shows that the solubility of methane is more than oxygen since the pressure fraction of methane (o.85) was higher compared to oxygen pressure fraction in air (0.21) although the Henry's constant of methane is lower than that of oxygen at 30 °C. The output of gas sensor was calibrated based on the solubility of methane (see table 3). In Fig. 5, the Y-axis (sensor output) is calibrated based on Henry's law. As it is apparent, the dissolved methane concentration increased up to saturation point (Fig. 6; a and b).

Table 1: The variables and its levels for optimization of dissolved methane sensor's parameters.

Variables	Unit	Levels		
		1	2	3
Silicone diameter*	(cm)	0.15	0.25	0.35
Silicone tube length	(m)	0.05	0.10	0.15
Helium flow rate	(mL/min)	40	50	60

** Internal diameter*

Table 2: The response time results under different conditions.

Elements of Full Factorial test used in this work				Response time (min)
NO.	Diameter (cm)	Tube length (m)	Flow rate (mL/min)	
1	0.15	0.05	40	11
2	0.15	0.05	50	10
3	0.15	0.05	60	12
4	0.15	0.10	40	9
5	0.15	0.10	50	8
6	0.15	0.10	60	10
7	0.15	0.15	40	9
8	0.15	0.15	50	8
9	0.15	0.15	60	10
10	0.25	0.05	40	7
11	0.25	0.05	50	6
12	0.25	0.05	60	8
13	0.25	0.10	40	5
14	0.25	0.10	50	2
15	0.25	0.10	60	4
16	0.25	0.15	40	5
17	0.25	0.15	50	2
18	0.25	0.15	60	4
19	0.35	0.05	40	12
20	0.35	0.05	50	11
21	0.35	0.05	60	13
22	0.35	0.10	40	11
23	0.35	0.10	50	10
24	0.35	0.10	60	12
25	0.35	0.15	40	11
26	0.35	0.15	50	10
27	0.35	0.15	60	12

Table 3: Henry's constants and solubility for air and methane at different temperatures.

Oxygen	Temp (°C)	5	10	15	20	25	30	35
	$H\times10^{-4}$ (atm/m_g*)	2.91	3.27	3.64	4.01	4.38	4.75	5.07
	x (m_g/m_t**)	12.80	11.40	10.20	9.30	8.50	7.80	7.30
Methane	$H\times10^{-4}$ (atm/m_g)	2.59	2.97	3.37	3.76	4.13	4.49	4.48
	x (m_g/m_t)	29.14	25.41	22.39	20.07	18.27	16.81	16.53

**) m_g: moles of dissolved gas. **) m_t: total moles of dissolved gas and liquid*

Table 4: The solubility of methane in different gas mixtures at 30 °C.

Methane[2]	Gas ratio[1] (v%)	0.94	0.95	0.96	0.97	0.98	0.99	100
	x (ppm)	11.28	11.40	11.52	11.65	11.76	11.88	12.01
Methane[3]	x (ppm)	11.05	11.16	11.28	11.41	11.51	11.64	11.77

1) methane/air ratio. 2) solubility of methane based on Henry's law. 3) solubility of methane based on dissolved methane sensor.

The air flow rate decreased methane concentration due to the reduction of pressure fraction of methane molecules (Fig. 6; c). There was an equilibrium region between oxygen and methane molecules (Fig. 6; d). The dissolved methane concentration decreased while the methane flow rate was stopped (Fig. 8; e).

The accuracy was determined in different ratios of methane and oxygen. Table 4 shows the solubility of methane in different gas mixtures based on Henry's law and dissolved methane sensor. The air flow rate was constant. The accuracy of system was calculated to be approximately ± 2 % (of dissolve methane).

Fig. 6 represents the typical sensitivity characteristics of TGS 2611: the Y-axis is indicated as sensor resistance ratio (R_s/R_o) where R_s is the sensor resistance in displayed gases at various concentrations and R_o, sensor resistance in 5000 ppm of methane. Based on Fig. 6, the response time analysis is not affected by the fermenter air flow rate. Therefore, TGS 2611 is sensitive to methane gas that passes through silicone tube. The probe is sensitive to variations in temperature and reactor pressure as well as to the carrier gas humidity. Fig. 7 represents typical temperature and humidity dependency characteristics.

The Y-axis is indicated as sensor resistance ratio (R_s/R_o), defined as R_s, sensor resistance in 5000 ppm of methane at various temperatures/humidities and R_o, sensor resistance in 5000 ppm of methane at 20 °C and 65 % R.H. The semi conductor methane is not extremely sensitive to humidity. To balance the temperature, all It is necessary to decrease the response time by varying the voltage response of the sensor in the further studies. In addition, the thickness of silicone tube should be optimized for better accuracy.

CONCLUSIONS

A dissolved methane sensor based on a thin silicone tube was designed and constructed. In particular, the probe was designed in such a way that substantially only the gas permeable tube is immersed into the fermenter. The dissolved methane concentration in the fermenter was continuously monitored by a PLC during air and CH_4 feed gas fermentations. Variables including: silicone tube diameter, silicone tube length and helium (as the carrier gas) flow rate were selected to be studied as challengeable parameters affecting optimal response time. In order to experiments were carried out at 30±0.5 °C. This was done by a temperature loop controller (T.L.C.) placed inside the detector and connected to an electrical heater positioned at the top of the bioreactor.

The capability of dissolved methane sensor was illustrated in Fig 8. The scenario of biomass growth (based on the results of optical density at 600 nm) is demonstrated in the same figure. The methane flow rate and oxygen flow rate were 0.47 m/s and 0.5 m/s, respectively. The experiments were started by 7 volume percent inoculum of active *Methylomonas* culture [8] and carried out in triplicate. The dissolved methane concentration decreased sharply in logarithmic phase. Also, its concentration became constant in stationary phase to some extent.

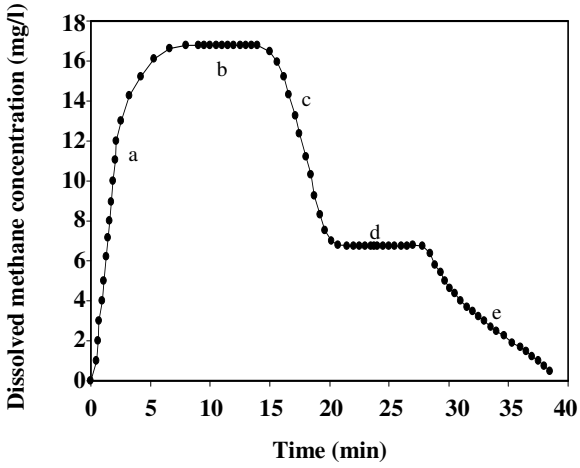

Fig. 5: The variation of dissolved methane concentration versus different air flow rates (a: pure methane; b: saturation zone; c: mixture of methane and air; d: equilibrium zone; e: pure air).

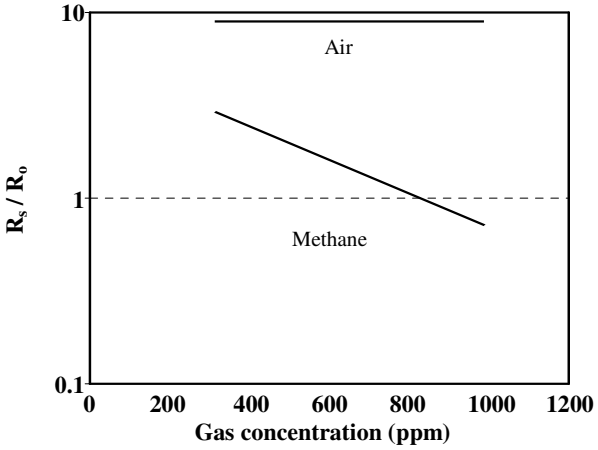

Fig. 6: The sensitivity characteristics for methane gas sensor.

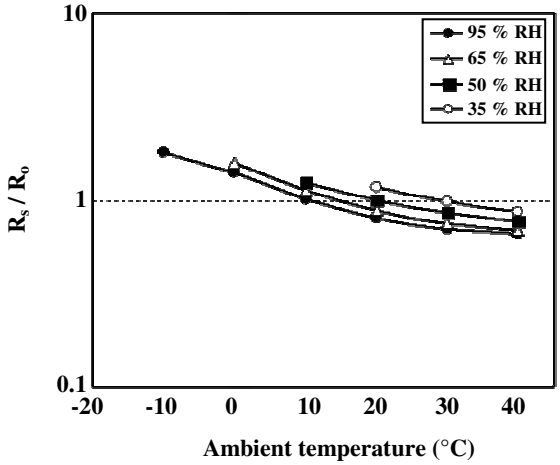

Fig. 7: Temperature/humidity dependency for methane gas sensor.

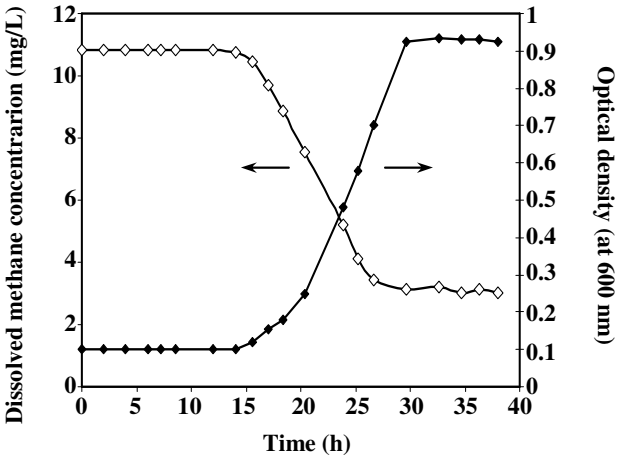

Fig. 8: The variation of dissolved methane concentration versus time at different optical density.

investigate the interactions among all parameters mentioned, full factorial design was applied. A semi-conductor methane gas sensor was used to measure the dissolved methane continuously. This approach provided simple and continuous analysis in which the resistance of the sensor, which is highly sensitive and selective to methane gas, changed according to the in-liquid concentration of dissolved methane. Henry's law along with a special circuit experimental method was applied for calibration. Seven streams of mixed gases (methane and air) were used for evaluation of the accuracy of dissolved methane sensor. The response time was calculated. The accuracy and response time of this system were determined ± 2 % (of dissolved methane concentration) and 2 minutes, respectively. Moreover, a control system was installed for recycling methane gas during fermentation.

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