Liquid fuel production from synthesis gas via fermentation process in a continuous tank bioreactor (CSTBR) using *Clostridium ljungdahlii*

Habibollah Younesi[1], Ghasem Najafpour[2]*, Abdul Rahman Mohamed[3]

[1]*Department of Environment, Faculty of Natural Resources and Marine Sceinces, Tarbiat Modares University, Noor, I.R. Iran*
[2]*Faculty of Chemical Engineering, Noshirvani Institute of Technology, University of Mazandaran, Babol, I.R.Iran,* [3]*School of Chemical Engineering, Engineering Campus, Universiti Sains Malaysia, Seri Ampangan, Nibong Tebal, 14300 Penang, Malaysia*

Abstract
The potential bioconversion of synthesis gas (syngas) to fuels and chemicals by microbial cell has attracted considerable attention in past decade. The feasibility of enhancing syngas bioconversion to ethanol and acetate using Clostridium ljungdahlii in a continuous tank bioreactor (CSTBR), kinetics and mass transfer coefficient of carbon monoxide (CO) utilization were evaluated. Two different types of syngas and pure CO were used for CO limitation, in order to achieve high bioconversion of syngas in a single bioreactor. The CO conversion increased with an increase in gas flow rate and agitation speed. For a gas flow rate of 14 ml/min and an agitation rate of 550 rpm, the cell concentration and conversion of pure CO were 1.92 g/l and 80%, respectively. The cell concentration also increased with an increase in CO percentage in the gas phase. Maximum cell dry weight of 2 g/l and CO conversion of 93% were achieved with 70% CO blended syngas at a gas flow rate of 14 ml/min and agitation rate of 500 rpm. The total amounts of ethanol and acetate concentrations were approximately 11 g/l. The mass transfer coefficient was calculated for the culture of C. ljungdahlii with pure CO in the CSTBR. The maximum mass transfer coefficient ($K_L a$) was 135 h^{-1} at an agitation speed of 550 rpm. The $K_L a$ correlation based on various gas flow rates and agitation speeds was fitted with experimental data and the predicted model was in good conformity with the obtained data.
Keywords: Biofuels, Clostridium ljungdahlii, mass transfer coefficient, synthesis gas

Correspondence to:* **Ghasem Najafpour, Ph.D.
Tel: +98111-3234204, Fax: +98111-3240700
E-mail: najafpour8@yahoo.com, najafpoyr@nit.ac.ir

INTRODUCTION

Since pre-World War II, ethanol has been considered as a useful fuel. Inexpensive petroleum and new petrochemical processes developed after 1945 provided a feedstock which then displaced fermentation-derived products. The dramatic rise of petroleum prices over the last decade has caused reconsideration of fermentation routes (Bailey and Ollis, 1986). Thus an increase in biological production of fuels and chemicals from syngas via fermentation became major interests (Najafpour *et al.*, 1995; Gajdos, 1998; Dale, 2003). In fact syngas is obtained from gasification of coal, biomass and solid wastes, is enriched with CO and lacks sufficient H_2 (Hotchkiss, 2003; Jiang *et al.*, 2003). The H_2/CO ratio must be upgraded before any further reaction take place. A suitable ratio of H_2/CO= 2 may yield ethanol as a result of Fischer-Tropsch synthesis (Probstein and Hicks, 1982). In a biological route, a process can be provided by anaerobic bacteria for the production of fuels and chemicals from syngas (Najafpour *et al.*, 1995; Najafpour *et al.*, 2003; Najafpour *et al.*, 2004c). Anaerobic bacteria can be used to convert syngas compounds to useful products, such as ethanol and acetate.

The bioconversion of organic substrates such as glucose and fructose are well demonstrated by anaerobic bacteria (Lundie and Drake, 1984; Schwartz and Keller, 1982a,1982b; Diekert and Thauer, 1978; Najafpour *et al.*, 2004a). Anaerobic fermentation of glucose leading to acetate production was studied by four strains of *Clostridium thermoaceticum* (ljungdahl,

wood, S3 and 1745) (Schwartz and Keller, 1982a). An investigation of *C. thermoaceticum* and *C. formicoaceticum* has demonstrated their suitable growth on hexoses and pyruvate. They have found that these microorganisms simultaneously oxidized CO to acetate and CO_2. It was also reported that acetate was the only fermentation product in both the absence and presence of CO (Schwartz and Keller, 1982b). The electron was supplied from the energy source for the transformation of CO_2 to acetate. In addition, it has been declared that the carbon monoxide oxidation was 10-fold more than pyruvate oxidation. However, Shen and his co-workers (1999) reported that ethanol and buthanol production by *Butyribacterium methylothrophicum* utilizing glucose coupled with CO, were the main products of anaerobic fermentation. Batch experiments were conducted with *C. ljungdahlii* using various sugars and starch concentration (Shen *et al.*, 1999). Ethanol and acetate production were the products of anaerobic fermentation of single inorganic carbon sources, such as CO/H_2 and CO_2/H_2 (Bailey and Ollis, 1986; Klasson *et al.*, 1992; Braun *et al.*, 1981; Kerby and Zeikus, 1983; Vega *et al.*, 1989a, b). Among these studies, Vega and his co-workers (1989a) used a strictly anaerobic bacterium, i.e. *Peptostreptococcus productus* strain U-1. The bacterium was grown on CO to produce acetate in a batch and continuous operation. Acetate production in a batch bioreactor was also conducted by *C. thermoaceticum* on H_2/CO_2 or CO (Braun *et al.*, 1981; Pezacka and Wood, 1984; Phillips

et al., 1994). A continuous system of immobilized-cell and membrane-filtration type reactor was applied for acetate production. It was stated that *C. thermoaceticum* and *C. formicoaceticum* required pyruvate as an electron donor for bioconversion of CO_2 to acetate (Vega, *et al.*, 1989a). The bioconversion of three different gases for the purpose of acetate and ethanol production and mass transfer correlations under various gas flow rates and agitation speeds are reported in the present study.

MATERIALS AND METHODS

A pure culture of *C. ljungdahlii* was obtained from American Type Culture Collection (ATCC), 55383, University Boulevard, Manassas, Virginia, 20110-2209 USA. It was anaerobically grown in rich ATCC media at 37ºC in an incubator shaker (Barnstead/Lab-Line, MaxQ 4000, USA). The growth media used for propagation of the organisms were based on previous studies (Najafpour and Younesi, 2005).

Figure 1 shows the schematic representation of the fermentation vessel and the associated experimental set-up. Temperature and pH control was associated with the fermenter vessel. A gas controller was used to measure the gas flow rate of the bioreactor. Two 20 l carboys were used for fresh media preparation and holding effluent from the outlet of the bioreactor. The anaerobic techniques for the start-up of the system and
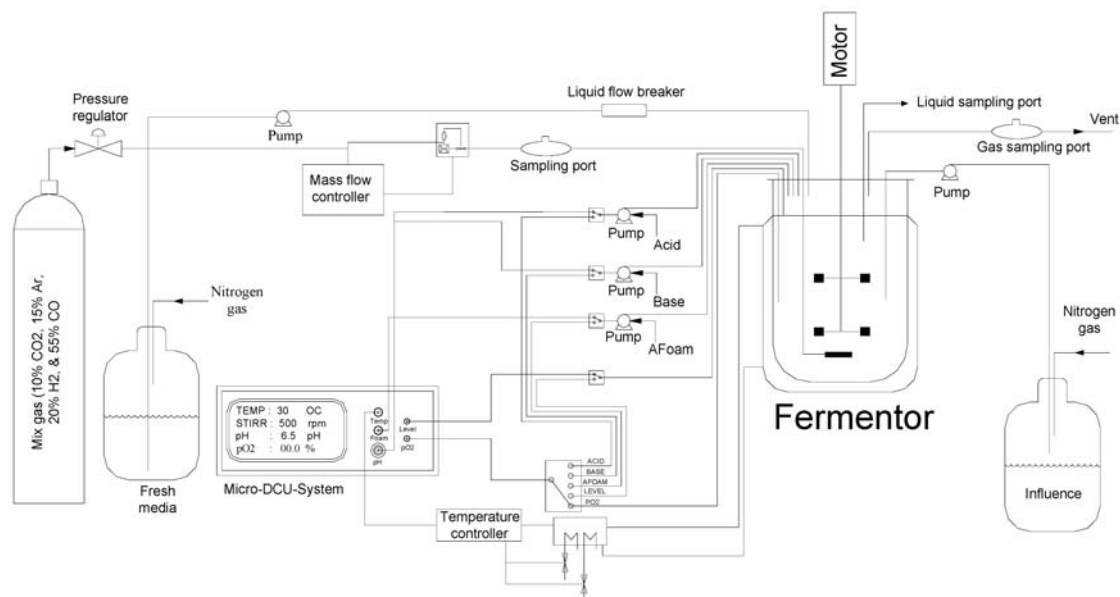

Figure 1. Schematic representation of the fermenter vessel and associated setup.
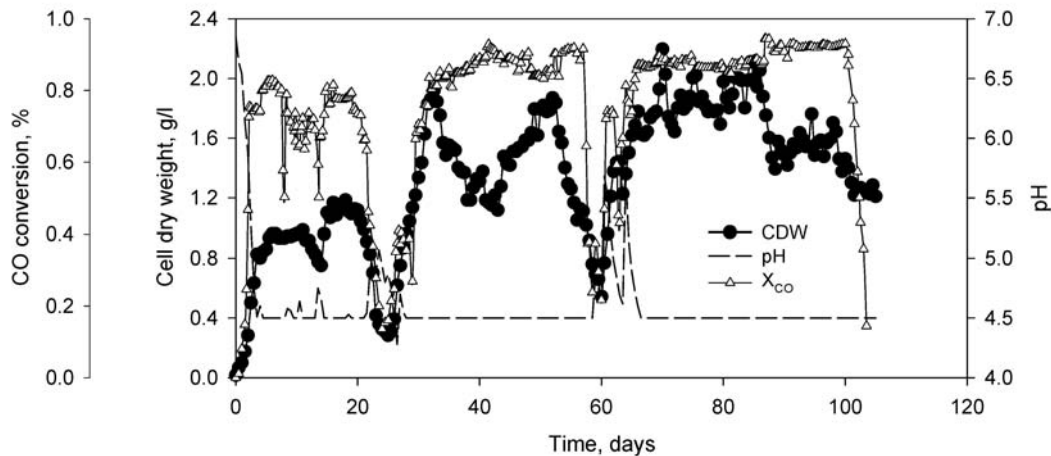
Figure 2. Cell dry weight concentration and CO conversion at a constant liquid flow rate of 0.55 ml/min.
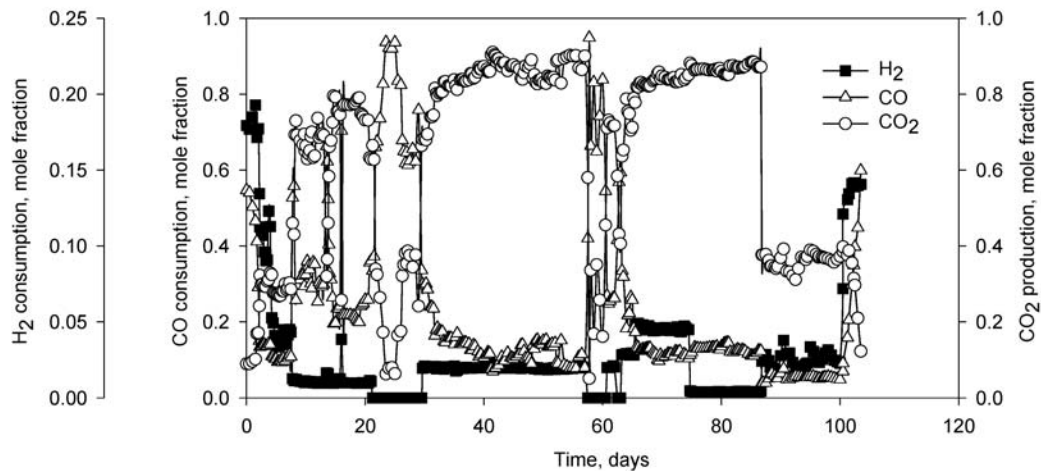

Figure 3. Three different types of synthesis gas at a constant liquid flow rate of 0.55 ml/min.

the preparation of media used in the continuous studies were slightly different from those in batch culture (Najafpour and Younesi, 2005). The media were clear solutions before and after autoclave. Fresh media was fed at a flow rate of 0.55 ml/min (0.4 day^{-1} dilution rate) as the liquid flow rate into the bioreactor. The syngas consisted of CO_2 (10 vol%), Ar (15 vol%), H_2 (20 vol%) and CO (55 vol%). Further, a syngas rich in CO consisted of Ar (15 vol%), H_2 (15 vol%) and CO (70 vol%) blended for industrial use (Air Products, Malaysia). The feed gas flow rates were 8, 10, 12, 14 ml/min for pure CO at atmospheric pressure. The temperature of the bioreactor was fixed at 37ºC. For gas analysis by Gas chromatography (GC), argon was used as an internal standard. The media of the bioreactor

were reduced by addition of 10 ml of $Na_2S \cdot 9\ H_2O$ solution (10% w/v). Gas and liquid samples were analysed based on previous studies (Younesi *et al.*, 2005).

RESULTS

The cell dry weight, CO bioconversion and pH profile in a continuous process with an operation period of more than 3 months are shown in Figure 2. The CO and CO_2/H_2 bioconversion was conducted using microbial catalysts. Figure 3 shows the microbial consumption and dynamic uptake of gaseous substrates using CO and CO_2/H_2 with various compositions of
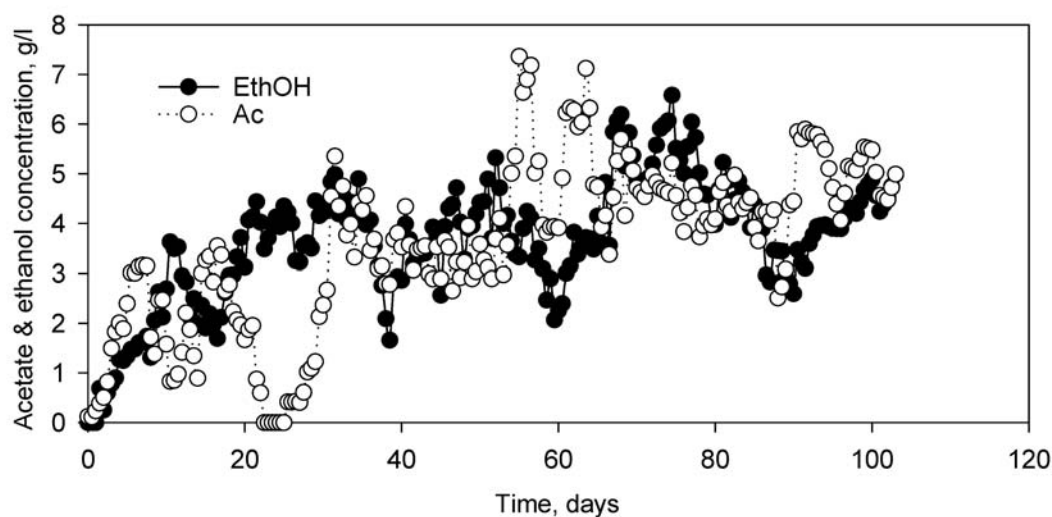
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Figure 4. Formation of acetate (Ac) and ethanol (EthOH) from synthesis gas composition and pure CO at a constant liquid flow rate of 0.55 ml/min.

syngas and pure carbon monoxide at atmospheric pressure. Results obtained for the products concentration profiles such as ethanol and acetate are shown in Figure 4. The microorganisms as biocatalysts (*C. ljungdahlii*) were implemented to produce acetate and ethanol. The steady operation and continuous CO fermentation were successfully accomplished in the bioreactor at the higher gas flow rates. The results related to CO consumption and uptake rate, productivity and yield are presented in Table 1. Results related

to the effect of gas flow rate and agitation speed upon CO bioconversion in a CSTBR are shown in Figure 5 and 6. Variations in cell dry weight concentrations with respect to changes in the volumetric liquid flow rate, gas flow rate and agitation speed in the bioreactor were monitored.

The mathematical presentation for correlation of overall mass transfer coefficient is discussed (Najafpour *et al.*, 2004b). The results were fitted with the project correlation. The concept of the overall mass

Table 1. Synthesis gas and CO fermentation by *C. ljundahlii* in a CSTBR.

Type of gas	Agitation speed (rpm)	Gas flow rate (ml·min⁻¹)	Cell growth status	CO consumption (mmol CO·h⁻¹)	CO uptake rate and productivity (mmol CO·g⁻¹ cell·h⁻¹)			Cell and product yield (g·mmol⁻¹ CO)		
					CO	Ac	EthOH	$Y_{cell/CO}$	$Y_{Ac/CO}$	$Y_{EthOH/CO}$
Syngas[a]	400	14	Steady	30.5	30.7	0.282	0.155	0.031	0.067	0.072
Syngas[b]	500	14	Decreased/Steady	29.8	22.7	0.222	0.282	0.051	0.143	0.141
Pure CO	400	8	Decreased	14.2	114.2	0.153	0.117	0.04	0.088	0.067
		10	Increased	16.6	16.5	0.218	0.233	0.035	0.092	0.091
		12	Increased	18.5	19.3	0.074	0.320	0.037	0.090	0.107
		14	Decreased/Steady	22.7	23.4	0.274	0.274	0.037	0.110	0.114
	450	8	Decreased	15.3	13.1	0.247	0.150	0.032	0.078	0.072
		10	Increased/Steady	19.1	18.8	0.196	0.222	0.037	0.090	0.082
		12	Steady	21.5	21.5	0.157	0.275	0.038	0.068	0.112
		14	Decreased/Increased	25.7	24.2	0.164	0.414	0.040	0.125	0.124
	500	8	Decreased	17.4	12.2	0.190	0.117	0.038	0.098	0.085
		10	Steady	20.5	12.8	0.248	0.275	0.046	0.122	0.133
		12	Increased	24.3	12.8	0.210	0.198	0.056	0.134	0.136
		14	Increased	27.6	20.3	0.155	0.254	0.044	0.140	0.150
	550	8	Steady	17.8	12.8	0.224	0.184	0.037	0.098	0.085
		10	Steady	21.6	17.5	0.262	0.285	0.040	0.096	0.105
		12	Increased	25.7	16.4	0.221	0.210	0.044	0.105	0.117
		14	Increased	29.3	17.8	0.201	0.255	0.047	0.120	0.142

[a] Synthesis gas consists of CO_2 (10 vol%), Ar (15 vol%), H_2 (20 vol%), CO (55 vol%). [b] Synthesis gas consists of Ar (15 vol%), H_2 15 vol%), CO (70 vol%).
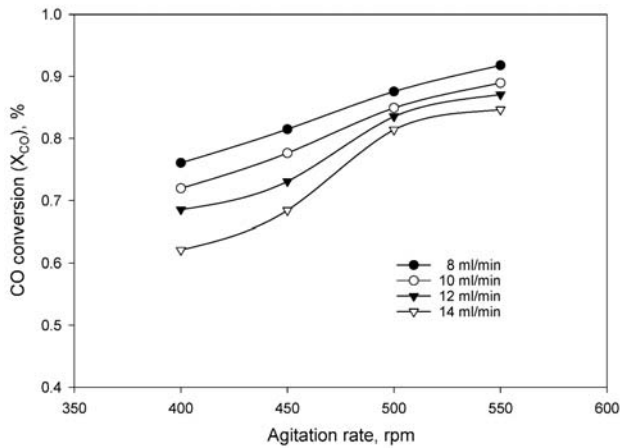
Figure 5. Effect of agitation speed on CO conversion at a constant liquid flow rate of 0.55 ml/min.
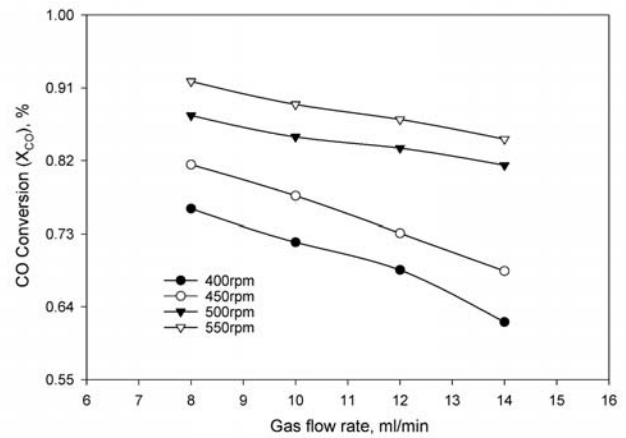

Figure 6. Effect of gas flow rate on CO conversion at various agitation speeds and a constant liquid flow rate of 0.55 ml/min.
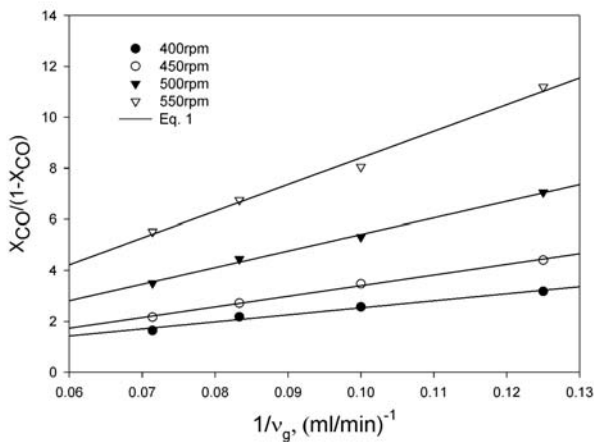

Figure 7. Overall volumetric mass transfer coefficient resulted from Equation 1.
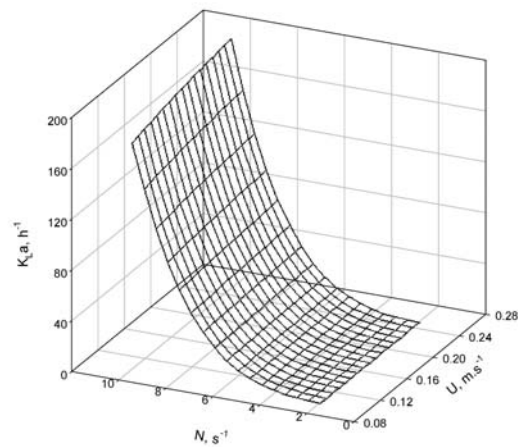

Figure 8. Effect of gas flow rate and agitation speed on mass transfer coefficient.

transfer coefficient experimentally determined for CO conversion with respect to gas flow rate, is shown in Figure 6. The empirical correlation for mass transfer in a stirred tank bioreactor was projected. The value of K_La was determined. The masstransfer coefficient was obtained by Figure 7. Figure 8 shows the effect of agitation speed and gas flow rate on K_La. SigmaPlot5 was used to examine the empirical correlation of value for the syngas fermentation media in a stirred tank bioreactor. The effect of gas flow rate and agitation speed on K_La is presented by SigmaPlot5.

DISCUSSION

The present study was conducted to evaluate *C. ljungdahlii* as an active biocatalyst for bioconversion of CO-rich gases such as syngas and off-gas (from steel mills) to biofuel using a continuous fermentation (Najafpour and Younesi, 2005; Morinaga and Kawada, 1990). In our previous study, the experiments were focused on tolerance of *C. ljungdahlii* to different CO pressures in the batch process (Bouaifi *et al.*, 2001). The cell concentration was increased with the decrease in CO percentage in the gas phase. This may be due to the low availability of CO in blended gas at low gas flow rate. The syngas was initially supplied into the bioreactor by continuously sparging at a flow rate of 14 ml/min (0.007 vvm). The agitation speed was at 400 rpm. The gas flow rate was decreased to 10 ml/min in the second week.

Figure 2 shows the cell dry weight, CO bioconversion and pH profile in a continuous process for more than 3 months. At the early stage of the opera-

tion, the cell dry weight dropped to 0.75 g/l due to an increase in liquid flow rate of 0.65 ml/min (D = 0.0195 h^{-1}). However, the cell concentration was decreased from 0.98 to 0.75 g/l under the same conditions. Then, the liquid flow rate was adjusted to 0.55 ml/min in subsequence experiments. The decrease in cell concentration was also observed at the lower input gas flow rate. The same situation was observed for all agitation speeds through fluctuation of gas flow rate. The decrease in cell concentration caused the CO bioconversion to decrease from 83 to 50%. In order to reactivate *C. ljungdahlii*, the pH of the bioreactor was adjusted to 4.75 using 2 ml of bicarbonate solution (2.5 N NaHCO$_3$). However, a maximum cell concentration and CO bioconversion of 1.2 g/l and 83% were obtained, respectively. Pure CO gas at atmospheric pressure was switched when the bioreactor was operated at an agitation rate of 400 rpm, gas flow rate of 14 ml/min and liquid flow rate of 0.55 ml/min (D = 0.4 day^{-1}). At this stage, it was decided to monitor the dynamics of the fermentation process during one cycle of CO replenishment.

Figure 3 shows the dynamics of CO and CO$_2$/H$_2$ utilization with variable syngas compositions and pure carbon monoxide at atmospheric pressure. The syngas initially consists of CO/H$_2$/CO$_2$/Ar (55/20/10/15 vol%). The utilization of mix syngas is presented by *C. ljundahlii* in the early part of Figure 4 (0-7 days of operation). The bioreactor was set with agitation speed of 300 rpm and a gas flow rate of 14 ml/min. The utilization of CO was observed at defined operating conditions. An increase in CO utilization was observed during the course of investigation of process conditions. The outlet gas composition of the bioreactor at the early stage was analyzed by GC equipped with thermal conductivity detector (TCD), showing only 14% (by volume) of the CO was utilized by *C. ljundahlii*. The present data showed that the CO uptake rate at low agitation speed (300 rpm) caused insufficient mass transfer that resulted from insufficient cell growth. In an attempt to overcome CO mass transfer limitation and to supply the maintenance energy, the experiment was conducted with a higher agitation speeds. Mole fraction of CO in the outlet gas phase was reduced to 10% (vol%) when the agitation speed was kept constant at 400 rpm for duration of a week. The H$_2$/CO$_2$ utilization was monitored during this period of fermentation. The mole fraction of CO$_2$ in the gas phase was reached 33% (vol%) after 10 days of fermentation. The CO$_2$ initiated to be utilized when H$_2$ was consumed by *C. ljundahlii*. The maximum value of 26% (vol%) CO$_2$ was obtained, while mole fraction of H$_2$ was reduced to 2% (vol%). On the other hand,

about 18% of H$_2$ was consumed and 6% of CO$_2$ which is in good agreement based on the stoichiometric coefficient proposed by other researchers (Shen *et al.*, 1999; Rajagopalaan, *et al.*, 2002; Morinaga and Kawada, 1990).

It has been stated by many researchers that the acetogenic bacterium *C. ljundahlii* was involved in the synthesis of active enzymes such as hydrogenase, CO dehydrogenase, methyltransferase and corrinoid enzymes. Hydrogenase catalyzes the reduction of ferredoxin by H$_2$ and carbon monoxide dehydrogenase (CODH) uses the reduced ferredoxin to CO$_2$ then to acetate (Kellum and Drake, 1984; Lundie and Drake, 1984; Diekert and Thauer, 1978; Rajagopalaan *et al.*, 2002; Cheng *et al.*, 1999, 2001).

Pure CO fermentation is shown in the middle part of Figure 3. The difference between pure CO and syngas was in the cell dry weight, CO bioconversion and products formation in the culture media. The cell concentration was increased about 28% when pure CO was used as a sole carbon source. In order to examine pure CO fermentation, pure CO bioconversion was prolonged for 80 days in continuous operation. The rate of CO mass transfer in the bioreactor was conducted at various gas flow rates and agitation speeds. The culture was checked for possible contaminations under the microscope using gram stain method. There was no contamination presented in the bioreactor. The CO bioconversion of syngas rich in CO was about 96%. A cell dry weight of 2.1 g/l was obtained for syngas rich in CO that was about the same cell concentration achieved with pure CO as the incoming carbon source.

Figure 4 shows the products concentration profiles for ethanol and acetate. The ability of *C. ljungdahlii* to produce acetate and ethanol was observed in the bioreactor experiments. The process has achieved stability by reaching steady state condition. The corresponding acetate concentration reached zero due to the low gas flow rate into the bioreactor. That was justified due to the high productivity of *C. ljungdahlii* at high cell concentrations to convert CO/H$_2$ or CO$_2$/H$_2$ into ethanol. The cell population was inhibited at high ethanol concentration. However, the ethanol production showed a similar trend with acetate formation at higher gas flow rates. The ethanol concentration decreased from 4.44 to 3.5 g/l in a situation of low gas flow rate. A maximum acetate concentration of 7 g/l was achieved.

Table 1 presents data obtained for three months of continuous CO fermentation in the bioreactor at the higher gas flow rate (14 ml/min), CO consumption and uptake rate, productivity and yield were higher than those of continuous culture that operated at a smaller CO gas flow rate (8 ml/min). The cell production rate

decreased at the low gas flow rate of 8 ml/min for all agitation speeds. However, a steady cell growth was observed at the high gas flow rate of 14 ml/min and agitation speed of 500 rpm, as stated in Table 1. For syngas with 55% CO, the maximum CO consumption and uptake rate of 30.5 mmol CO/h and 30.7 mmol CO·g^{-1} cell·h^{-1} were observed together with a low cell concentration up to 1.1 g/l. The minimum and maximum CO consumption rates were 14.2 mmol CO/h (gas flow rate of 8 ml/min) and 29.3 mmol CO/h (gas flow rate 14 ml/min) with syngas rich in CO (70% CO and 15% H$_2$). However, the maximum CO uptake rate was obtained at 24.2 mmol CO·g^{-1} cell·h^{-1}, gas flow rate of 14 ml/min and agitation speed of 450 rpm, while an increase of the cell growth was observed. These values were slightly higher for *C. ljungdahlii* when the bioreactor was operated with pure CO and syngas rich in CO, but cultures with higher cell dry weight concentration of apprpximately 2.1 g/l were obtained. These results showed that the operation of the bioreactor with high CO concentration did not inhibit the cultures of *C. ljungdahlii*. Unlike CO consumption and uptake rate, the high products yields were obtained with a high concentration of CO (Table 1). A maximum acetate yield ($Y_{A\ acetate/CO}$) of 0.14 g Ac/mmol CO obtained with syngas rich in CO (70% CO and 15% H$_2$) was higher than that obtained with continuous culture using pure CO. However, the minimum acetate yield was 0.067 g Ac/mmol CO with syngas in the presence of 55% CO, at an agitation speed of 400 rpm. The corresponding ethanol yield ($Y_{Ethanol/CO}$) showed the same trend. The minimum ethanol yield was obtained at 0.067 g Ac/mmol CO with pure CO at 400 rpm. However, maximum ethanol was at 0.142 g EthOH/mmol CO with syngas rich in CO (70% CO and 15% H$_2$), at an agitation speed of 550 rpm. The cell yield ($Y_{cell/CO}$) decreased with the decrease in volumetric gas flow rate supplied into the bioreactor at all the agitation speeds. It can be concluded that the CO supplied at low flow rate was insufficient for growth of the culture.

The CO bioconversion was illustrated as a function of gas flow rate and agitation speed in a CSTBR (see Figs. 5 and 6). At the volumetric liquid flow rate of 0.55 ml/min, the various cell dry weight concentrations (0.3 to 2.2 g/l) were obtained by changing the gas flow rate and agitation speed in the bioreactor. Similar studies were carried out by *Eubacterium limosum* KIST612 in a bubble column bioreactor with a working volume of 0.2 l, total gas volume of 80 ml/min (0.4 vvm) and initial pure CO pressure of 96 kPa. When the cell concentration in the bioreactor reached 5.25 g/l, the maximum CO bioconversion was 60% (Morinaga

and Kawada, 1990). As a comparison, other experiments were performed in a bubble column bioreactor with *C. ljungdahlii* p7 (Morinaga and Kawada, 1990). A 60% CO bioconversion was achieved from the mixture of syngas (25% CO, 15% CO$_2$, and 60% N$_2$). However, in the present study, a 90% CO bioconversion was obtained with syngas consisted of 55% CO at 400 rpm and 14 ml/min of gas flow rate. The bioreactor was also run with syngas consisted of 70% CO. The volumetric gas flow rate and agitation speed were 14 ml/min and 500 rpm, respectively. The volumetric liquid flow rate was kept at 0.55 ml/min (0.4 day^{-1}) during the operation. A 93% bioconversion of CO was achieved. In addition, products yields of 0.141 g EthOH/mmol CO and 0.143 g Ac/mmol CO and a cell yield of 0.051 g cell/mmol CO were achieved. The results of similar studies with *C. ljungdahlii* showed yields of 0.062 g EthOH/mmol CO for ethanol and 0.094 g AC/mmol CO for acetate and a cell yield of 1.38 g/mol CO (Rajagopalaan *et al.,* 2002).

The CO bioconversion was affected by the cell dry weight concentration, agitation speed and volumetric gas flow rate. This fact is due to kinetic limitations, the bioconversion of CO in the outlet of the bioreactor changes with the cell concentration. The following equation represents by CO bioconversion (X_{CO}) and the volumetric mass transfer coefficient:

$$\frac{X_{CO}}{1 - X_{CO}} = \frac{RTV_L K_L a}{\pi H v_g} \qquad (1)$$

The above equation was plotted as a function of inverse of the volumetric gas flow rate (Fig. 7). The slopes of the straight lines are proportional to mass transfer coefficients ($K_L a$). The Henry's constant for CO is 1.226 l·atm·mmol^{-1} CO (Vavilin, *et al.*, 2000). The operation temperature of the bioreactor was 38 ^{O}C. Furthermore, the values of $K_L a$ were found to be 36, 54.3, 84.2 and 135.2 h^{-1} at agitation speeds of 400, 450, 500 and 550rpm, respectively.

Generally, solubility of the gaseous substrate at ambient temperature and pressure are very low. The gaseous substrate is utilized by the biocatalysts only if the gaseous substrate can be maintained at a desired level in a utilizable form (Kerby and Zeikus, 1983). To improve transfer of gas into the culture media, mechanically agitated CSTBR is the preferred reactor configuration as it allows an efficient contact between the two immiscible phases (Martin *et al.*, 1983). The laboratory scale fermentor was used in these studies. Microsparger was used to make fine bubbles with a size of 20 μm. The bubble size distribution also

depends on the operating conditions, the configuration type, agitation speed and gas flow rate (Vega *et al.*, 1989b).

Derivation of empirical correlation of K_La was examined for gas liquid mass transfer in a stirred tank bioreactor. As the superficial gas velocity increases, the mean bubble size decreases. Thus, the superficial area can be increased as a result of higher gas hold up at high gas velocity. An increase in gas velocity also enhances the turbulence induced by the gas flow, which increases mass transfer coefficient (Schneeberger *et al.*, 1999). Increase in interfacial area and K_L at the high gas velocities predominates over the decrease in the mass transfer coefficient because of the shorter gas-liquid contact time, which resulted in an increase in K_La. A number of researchers have characterized the volumetric mass transfer coefficient (Vega *et al.*, 1989a,b; Schneeberger *et al.*, 1999; Geankoplis, 2003; Arjunwadkar, *et al.* 1998), the empirical correlation followed by the power law $K_La=AN^B$. Also it has been recommended that to use the empirical equation in order to estimate the K_La of a laboratory-scale bioreactor (Lau *et al.*, 2004).

Combining the effect of agitation speed and gas flow rate on K_La is shown in Figure 8. SigmaPlot5 was very beneficial for analysis of empirical correlation defined for the K_La value using syngas-fermentation media in a stirred tank bioreactor. The illustrated results in SigmaPlot5 showed that the correlation for the effect of gas flow rate and agitation speed on K_La are given by the following equation;

$$K_L a = 131 \times 10^{-4} N^{4.24} U^{0.11} \qquad (2)$$

The results of this investigation showed that at an agitation speed below the critical speed for gas dispersion ($N < 6 \ S^{-1}$), K_La increased slowly. Then, the K_La increased with increasing gas flow rate (V_g) and agitation speed (N), as shown in Figure 8.

CONCLUSION

The present study was conducted to evaluate *C. ljundahlii* as an active biocatalyst for bioconversion of CO-rich gases such as synthesis gas and off-gas (from steel mills) to bio-fuel using a continuous fermentation process. *C. ljundahlii* was also used for the bioconversion of syngas to liquid fuels, and to understand the mass transfer phenomena process and kinetics of CO utilization in a 2 litter CSTBR bioreactor. The opera-

tion temperature was 37ºC. The pH was automatically adjusted to 4.5 during continuous ethanol and acetate production. A maximum CO consumption and CO uptake rate 30.5 mmol CO/h and 30.7 mmol $CO \cdot g^{-1}$ cell·h^{-1} were obtained, respectively. However, a maximum cell concentration and CO bioconversion of 2.1 g/l and 93% were obtained, respectively. The total amount of ethanol and acetate concentrations were approximately 11 g/l with gas flow rate at 14 ml/min and agitation speed at 500 rpm. An acetate yield ($Y_{A\ acetate/CO}$) of 0.14 g Ac/mmol CO with syngas rich in CO (70% CO) was obtained. The maximum mass transfer coefficient (K_La) of 135 h^{-1} at an agitation speed of 550 rpm was achieved. The results of the present study provided important implications of the bioconversion of various CO concentrations as raw syngas and also off-gas from steel mills for the industrial scale of the CO fermentation processes.

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