Influences of pH Conditions on Syngas Fermentation using *Clostridium ljungdahlii*

pH 조건이 *Clostridium ljungdahlii*를 이용한 합성가스 발효공정에 미치는 영향

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ABSTRACT

바이오에탄올 생산공정은 당 (Sugar)을 기반으로 하는 공정과 합성가스를 이용하는 공정으로 분류할 수 있다. 이 가운데 합성가스를 이용하는 공정은 촉매를 이용한 화학적 공정과 혐기성 발효에 의한 생물학적 공정의 두 가지로 나뉜다. *Clostridium ljungdahlii*는 일산화탄소와 수소가 주요 성분으로 구성되는 합성가스를 이용하여 에탄올과 아세트산을 생산할 수 있는 균주 중의 하나로 알려져 있다. 합성가스 발효공정에서 pH는 미생물의 증식과 에탄올 등의 생산에 아주 중요한 요인 중의 하나이다. 본 연구에서는 pH 조건이 미생물의 성장과 에탄올 생산성에 미치는 영향을 조사하였다. *C. ljungdahlii* 배양은 엄격한 혐기성 조건에서 100 ml의 serum bottle과 pH 제어가 가능한 반응기를 이용한 실험결과, 회분식 배양 조건에서는 미생물의 성장과 에탄올 생산을 위한 최적 초기 pH는 7.0로 나타났다. 미생물 농도는 0.57 g/L, 에탄올 농도 0.91 g/L로 나타났다. pH 4.5 이하에서는 미생물의 생장이 멈추는 것으로 나타났다. pH 제어가 가능한 생물반응기에서는 pH 6.0 일때 에탄올 생산량이 pH 7.0 일때 보다 높게 나타났다. 일정 수준의 미생물 농도를 유지한 조건에서 합성가스를 기포식으로 주입하고 pH 5.9에서 5.4까지 제어하였을 때 미생물량과 에탄올 농도가 증가하였다. 60 시간이 지난 후에 미생물의 농도는 0.498 g/L, 에탄올은 1.056 g/L까지 이르렀다.

Keywords: Bioethanol; syngas fermentation; syngas; *Clostridium ljungdahlii*

I. INTRODUCTION

There are two pathways in producing fuel ethanol from lignocelluloses biomass. One is sugar-based and the other is syngas-based. As a relatively new approach, bio-ethanol can be produced by syngas fermentation. Syngas is produced by biomass gasification process, which is composed of carbon monoxide, carbon dioxide, hydrogen and methane. The process of ethanol production from syngas fermentation using bacterium could be recognized as conversing biomass such as lignin, celluloses and hemicelluloses to ethanol indirectly through gasification and fermentation processes. The ethanol production would not dependent on crops'

supplies by syngas fermentation process. The research interest of this fermentation process is growing up as it was a new technology to produce ethanol with syngas consumption. The first patent about *Clostridium ljungdahlii* was publicized in 1992 (Gaddy, 1992). It was reported the ethanol production reached 48 g/L at most with a Continuous Stirred-Tank Reactor (CSTR) with cell recycle system according this bacterium (Klasson, Ackerson et al., 1993). And later a bio-ethanol plant was built to produce ethanol from wood, corn stover, tires, RDF (Refuse-Derived Fuel) and material source separated biodegradable waste using thermal and biological-gasification fermentation in 1993 Arkansas, United States. The plant can process a maximum of 1.5 tons per day of various feedstocks. Anhydrous ethanol can be produced 50,000 gallons per year (Henstra, Sipma et al., 2007). More recently, a team at the University of Oklahoma has demonstrated the production of ethanol from clean syngas derived from a biomass gasifier with a new bacterium named P7 (Phillips, Klasson et al., 1993; Dhurjati, 2008). This bacterium has the similar characteristics as *Clostridium ljungdahlii*. Then more bac-

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terium were discovered they can converse syngas to ethanol and acetate including *Clostridium autoethanogenum* (Abrini, Naveau et al., 1994), *Clostridium ragsdalei* (P11) (Kundiya, Huhnke et al., 2010), *Butyribacterium methylotrophicum* (Grethlein, Worden et al., 1991) and *Alkalibaculum bacchi* (Allen, Caldwell et al., 2010). These bacterium require similar pH and temperature environments.

Yeast extract is the nutriment as the nitrogen source. It is known that this bacterium grow well on sugar substrates at higher growth rates and with more predictable growth curves than on syngas substrates (Cotter, Chinn et al., 2009). High yeast extract concentrations promoted growth and acetate, low concentration of yeast extract suppressed yielding increased ethanol concentration (Klasson, Ackerson et al., 1991). The ethanol production was reported as 0.062 to 11 g/L and acetate production was reported as 0.0094 to 28 g/L through syngas fermentation using *Clostridium ljungdahlii* (Phillips et al., 1994; Younesi et al., 2005; Najafpour and Younesi, 2006).

Nowadays researches were conducted in various fields such as optimum medium conditions for producing ethanol (Zhu, 2003; Cotter, Chinn et al., 2009), pathway modification in the syngas transfer process (Ragsdale, 1997), genetic transformation (Tanner, Miller et al., 1993) and effects of syngas composition on fermentation process (Ahmed and Lewis 2007; Heiskanen, Virkajärvi et al., 2007) and pressure effects on microbial cell population and ethanol production (Younesi, Najafpour et al., 2005; Gaddy, Arora et al., 2007; Heiskanen, Virkajärvi et al., 2007). Since carbon monoxide has low solubility in water, it was found that the mass transfer of the syngas feed to the liquid culture is very important for syngas fermentation. However, too much gas injection may affect microbial cell growth (Gaddy, Arora et al., 2007). The optimum ranges of gas pressure should be determined for cell growth and product concentration. Effect of initial pressure on ethanol production was conducted by Younesi et al. (Younesi, Najafpour et al., 2005). In Younesi and his fellow's research it was shown that the maximum product ratio of ethanol to acetate was 0.54 g at the pressure of 1.8 atm. High ethanol production and consumption of H₂ and CO₂ were observed under pressures in batch reactors at 1.6 atm and 1.8 atm. The highest

ethanol yield was 0.41 mg with per mg CO consumption at 1.6 atm.

Syngas fermentation also has several limitations such as low productivity and poor solubility of gaseous substrates in the liquid phase. These limitations are thus preventing the commercialization of the syngas fermentation technology. PH is an important paramant for bacterium growth. Cells performance different growing ability under different pH ranges. It will be necessary to investigate ethanol production and cell growth of *Clostridium ljungdahlii* in different pH conditions. And the research will help to investigate methods of promoting ethanol production through changing pH conditions.

In this study a series of experiments of syngas fermentation were conducted for different initial pH conditions to assess ethanol productivity. The objective of this study was to investigate the effect of pH in medium on cell growth and the production of ethanol and acetate.

II . MATERIALS AND METHODS

1. Syngas Fermentation

Clostridium ljungdahlii was used in this fermentation process. It was discovered and isolated from chicken manure in Arkansas University in 1987 (Gaddy, 1992). It has ability to transform syngas or mixture gas of carbon monoxide, carbon dioxide and hydrogen to ethanol and acetate according anaerobic fermentation process (Munasinghe and Khanal, 2010). An electron micrograph of cells from a young culture (16 hours, fructose-grown) of *Clostridium ljungdahlii* is shown in Fig. 1. The bacteria is gram-positive with motile rods (0.6 by 2 to 3 um) and occur mostly alone. Cells rarely sporulate. Growth occurs only under anoxic conditions. The optimum temperature is 37 °C and the optimum pH is between 5.8 and 6.0 (Tanner, Miller et al., 1993). The characteristics of *Clostridium ljungdahlii* are shown in Table 1. The reaction for the syngas fermentation is as follows (Gaddy, 1992) :

Table 1 Description of *Clostridium ljungdahlii*

	Descriptions	References
Bacterium name	<i>Clostridium ljungdahlii</i>	
Source	Named after Dr. Lars G. Ljungdahl, discovered in 1987, and Isolated from chicken yard waste, identified characteristics of ATCC No. 49587.	(Munasinghe and Khanal, 2010)
Cell characters	Gram-positive, motile rods (0.6 by 2 to 3 μ m), occur mostly singly, rarely sporulate and show motility.	(Tanner, Miller et al., 1993)
Growth Requirements	Growth occurs only under anoxic conditions, optimum temperature is 37 °C, initial pH for growth is 6.0, requires a minimum level of yeast extract (0.01 %) or, in the alternative, an enriched medium containing minerals, trace minerals, B-vitamins, and an amino acid solution.	(Gaddy, 1992)
Fermentation conditions	Under optimum growth pH (5.0-7.0), ferments synthesis gas to mixture of ethanol (minor) and acetic acid (major). Under non-growth pH (4.0-4.5) and without yeast extract as a nutrient source, ethanol is the major production.	(Gaddy, 1992)

Fig. 1 Electron micrograph of cells from a culture (fructose-grown) of *Clostridium ljungdahlii*

Tanner (1993) (Tanner, Miller et al., 1993) pointed out that under the optimal pH and temperature conditions, a doubling time of 0.26 h^{-1} was observed for growth with either fructose or $\text{H}_2\text{-CO}_2$. It was found that 4 mmol of hydrogen and 2 mmol of dioxide were converted into 1 mmol of acetic acid or 1 mmol of fructose into 2.44 mmol of acetic acid. The fermentation balance demonstrates that *Clostridium ljungdahlii* can synthesis acetate from one-carbon compounds (Tanner, Miller et al., 1993).

2. Bacteria

The microorganism *Clostridium ljungdahlii* was obtained

from the ATCC® (American Type Culture Collection, USA). The stock was stored in deep freezing condition (-80°C). The cell was grown in 100 ml serum bottles with strictly anaerobic conditions, at 37°C , 200 rpm in a sharking incubator with a modified medium named carbonate buffered basal medium.

3. Culture Mediums

Growth of *Clostridium ljungdahlii* requires a minimum level of yeast extract (0.01 %) or, in the alternative, an enriched medium containing minerals, trace minerals, B-vitamins, and an amino acid solution (Gaddy, 1992). The growth medium is modified from ATCC® 1754 PETC medium. The composition of growth medium per liter is given as below: basal medium was prepared by mixing the following chemicals (50 ml): NaCl (18 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (6.4 g/L), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (4 g/L), NH_4Cl (20 g/L). Trace Mineral Solution (10 ml): nitrilotriacetic acid (1.5 g/L), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1 g/L), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.1 g/L), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (0.17 g/L), ZnCl_2 (0.1 g/L), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.1 g/L), $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.02 g/L), H_3BO_4 (0.01 g/L), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.015 g/L), Na_2SeO_4 (0.017 g/L), $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ (0.026 g/L), $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ (0.0002 g/L), $\text{Al}_2(\text{SO}_4)_3 \cdot 12\text{H}_2\text{O}$ (0.014 g/L), NaCl (1.0 g/L), KCl (0.1 g/L). And 0.20 % resazurin 0.1 ml and 1 g yeast extract. These chemicals prepared in 1.0 L distilled water. This liquid media degassed and dispensed anaerobically under 100 % carbon dioxide gas, sterilized at 121°C in an autoclave for 15 minutes. Then the cooled media solution added to vitamin solution (10 ml), the chemicals of vitamin solution was reported in the patent (Gaddy, 1992). At last add 3.69 g/L L-cysteine · HCl (10.84 ml), 2.5 % $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$

(5.26 ml), fructose (5 g/L) were added in the last step. The final pH of medium is 5.9. All mediums were autoclaved or filtered before culture process. In the growth medium, fructose was added as sugar substrate for promoting cell growth. 100 % N₂ gas was injected in the serum bottles to make anaerobic condition. The composition of syngas used is H₂ 13.1 %, CO 19.9 %, CH₄ 4.99 %, CO₂ 19.9 %, N₂ 42.2 % (Dongmin Gas Co. Ltd., Korea). Syngas was sterilized by filter before injected to the reactor.

4. Bioreactor

Batch experiments were conducted in 100 ml serum bottles with 50 ml liquid media. The medium was boiled and degased with nitrogen for 5 minutes to remove oxygen. The bottles were allowed under strictly anaerobic conditions with nitrogen headspace in serum bottles before autoclaved. After inoculation (4 %), medium was placed at 37 °C in shaking incubator. A 3-liters bioreactor (Fermentec, Korea) was used for cell growth and ethanol production. The pH is adjusted with 0.1 N HCl and 0.1 N NaOH. Syngas was injected with a circle bubbling system. Fig. 2 show the 3 L pH controlling bioreactor system. Temperature, pH and RPM were controlled and data was record by a computer.

Fig. 2 Bioreactor with pH controller for the experiment

5. Analytical Method

Cell growth was determined by the measurement of optical density at the wave length of 660 nm using a UV-spectrophotometer (Shimadzu UV-1201, Japan). 2.0 ml sample was taken every 24 hours by syringe during the fermentation process and centrifuged at 13,500 rpm for 10 min before analyzed. The liquid samples were analyzed for ethanol and acetic acid concentration using a Gas Chromatography (Agilent®7890A, USA), equipped with a FID (flame ionization detector) and a column with 30 meter×0.250 mm ID×0.25 um film (Agilent®DB 122-3232, USA). Cell pellets were re-suspended with de-ionized water then dispensed into a pre-weighed tin and dried in 80 °C for 24 hours. The dried cell pellets and tin were weighed and calculated, then corresponded the weights with a linear relationship between measured optical density and culture densities (g dry cell/L). All the samples were performed three times.

III. RESULTS AND DISCUSSIONS

1. Changes in pH

Initial pHs were set to 5.0, 6.0, 7.0 and 8.0 in batch cultures. Fig. 3 shows pH was reduced to around 4.5 in 24 hours in initial pH was 5.0, 6.0 and 7.0. It appeared pH change was slower in initial pH 8.0 than others but all

Fig. 3 pH changes of batch culture in different initial pH

the pH of culture reached about 4.5 after longer period of time. And then nearly no pH changes occurred. One reason for pH drop was after acetic acid was produced in the growth process. It was reported that ability of acetate generation was stopped when pH about 4.5. Gaddy pointed out that the ethanol production increased when pH was changed to about 4.0 while yeast extract was limited (Gaddy, 1992).

2. Effects of Different Initial pH Conditions

Fig. 4 shows that ethanol was observed in the growth stage. It was shown that all the maximum ethanol concentration was reached in 48 hours with different initial pH. Ethanol concentration from the largest to the smallest in the order was pH 7.0, pH 6.0, pH 5.0 and pH 8.0. The maximum of ethanol concentration was 0.92 g/L in 48 hours when initial pH was 7.0. The maximum of ethanol concentration was 0.86 g/L in 216 hours when initial pH was 6.0. The maximum of ethanol concentration was 0.56 g/L when initial pH was 5.0. Ethanol concentration was low as 0.17 g/L when initial pH was 8.0.

It was observed that pH reduced with acetic acid production, when pH under 5.0, cell comes to non-growing stage. Ethanol concentration was not increased when pH decreased to 4.5. Ethanol nearly not produced in 24 hours while pH was decreased because acetic acid was produced first and then ethanol was produced. Cell grew better in

Fig. 4 Ethanol production of batch culture in different initial pH

pH 7.0 and pH 6.0 than in pH 5.0 and pH 8.0 according Fig. 5. In a typically cell growth process, cell growing occurred in optimum growth pH 5.0 to 7.0, carbon source from syngas and fructose was transferred to cell, ethanol and acetic acid or other organic acid as butyric acid was observed in this process. It presented that growing cell tend to use liquid carbon source (such as fructose) than gas phase carbon source such as syngas or CO, CO₂. In addition H₂ and CO₂ production was observed in cell growing process with fructose addition.

It was presented that initial medium pH had significant effects on metabolism growing cultures of *Clostridium ljungdahlii*. More acidic pH level lead to slower growth rates and lower ethanol yields (Cotter, Chinn et al., 2009). Fructose is used to promote cell growing in growth medium. It was observed that OD₆₆₀ (optical density) can increased to 1.0 in 48 hours with 5.0 g/L fructose addition. Yeast extract is complex nutrients for cell growing. It was pointed that removal nutrient sources promote ethanol production while reduce acetate production (Gaddy, 1992). It suggests that pH is the important factor in ethanol production. When pH was under 5.0, a non-growing stage started. The major production is ethanol in this stage. In the pH shift process, the pH is adjusted with 0.1 N HCl or 0.1 N NaOH. The medium pH can also be reduced by fructose addition considering strong acid such as HCl maybe prohibit cell from growing.

Fig. 5 Optical density (OD₆₆₀) changes of batch culture in different initial pH

Fig. 6 Ethanol and acetic acid production in the process of cell growth (pH 7.0)

Fig. 6 shows Initial pH was set to 7.0 in the bioreactor. pH did not increase again after it was adjusted to 7.0. Cell concentration increased from 0.07 g/L to 0.243 g/L in 4 hours and then increased from 0.243 g/L to 0.26 g/L in 2.5 hours. Cell concentration did not increase after pH was adjusted to 7.0. It was found that cell concentration increased fast when pH between 5.6 to 5.9, the cell grow rate was 0.0425 g/L·h. It was found that ethanol produced later than cell concentration increased. Acetic acid was reduced while ethanol was increased. Ethanol concentration was not increased after pH was adjusted to 7.0. The maximum ethanol concentration was 0.1 g/L.

Fig. 7 shows cell growth and ethanol production in the process of pH was hold in 6.0. Cell grew fast after 24 hours later. The maximum cell concentration is 0.4 g/L after 40 hours. It was also observed that acetic acid was reduced when ethanol was increased. Ethanol and acetate acid increased in the cell growth process and they remained when cell concentration reached the maximum. The maximum ethanol concentration was 0.34 g/L and maximum acetate acid concentration was 0.556 g/L.

Fig. 8 shows a pH shift process in pH controlling reactor. Initial pH was set to 5.9 and decreased to 5.4 after 36 hours with HCl addition. Syngas was injected and bubbled in 44 hours when cell density was not increased. It was observed that ethanol production started from pH reducing and acetate acid appearance. Cell concentration increased fast when pH between 5.6 and 5.9. It was presented that

Fig. 7 Ethanol and acetic acid production in the process of cell growth (pH 6.0)

Fig. 8 Ethanol and acetic acid production in the process of cell growth (pH shift)

the maximum cell concentration was reached to 0.498 g/L in 60 hours when pH to 5.6. The ethanol production was 1.056 g/L at the same time. In a typically cell growing process, cell growing occurred in optimum growth pH 5.0 to 7.0, carbon source such as syngas and fructose was transferred to cell, ethanol and acetic acid or other organic acid in this process.

IV. CONCLUSIONS

Gasification of lignocellulosic biomass produces synthesis gas which mainly consists of H_2 , CO_2 and CO . Syngas fermentation has significant advantages over the metal

catalytic conversions. PH is an important parament for bacterium growth. It will be necessary to investigate ethanol production and cell growth of *Clostridium ljungdahlii* in different pH conditions. A series of experiments were conducted under strictly anaerobic conditions in 100 ml serum bottles and a pH-controlling bioreactor. The pH was conducted from 5.0 to 8.0. It was found that in batch culture the optimum initial pH for cell growth and ethanol production is 7.0 as the cell concentration was 0.57 g/L and ethanol concentration was 0.91 g/L. Cell growth was stopped bellow pH was 4.5. pH at 7.0, 6.0 and pH shift from 5.4 to 5.9 in a pH-controlling bioreactor. It was observed that ethanol production started when pH was decreased and acetate acid was produced. Cell concentration increased rapidly when pH between 5.6 and 5.9. In optimum pH ranges were between 5.0 and 7.0, showing greater cell density and product concentrations. The result of this study will provide information required to improve ethanol productivity in syngas fermentation.

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REFERENCES

1. Abrini, J., H. Naveau, et al., 1994. *Clostridium autoethanogenum* sp. nov., an anaerobic bacterium that produces ethanol from carbon monoxide. *Archives of Microbiology*, 161(4): 345-351.
2. Ahmed, A. and R. S. Lewis, 2007. Fermentation of biomass-generated synthesis gas: Effects of nitric oxide. *Biotechnology and Bioengineering*, 97(5): 1080-1086.
3. Allen, T. D., M. E. Caldwell, et al., 2010. *Alkalibaculum bacchi* gen. nov., sp. nov., a CO-oxidizing, ethanol-producing acetogen isolated from livestock-impacted soil. *International Journal of Systematic and Evolutionary Microbiology*, 60(10): 2483-2489.
4. Cotter, J., M. Chinn, et al., 2009. Ethanol and acetate production by *Clostridium ljungdahli* and *Clostridium autoethanogenum* using resting cells. *Bioprocess and Biosystems Engineering*, 32(3): 369-380.
5. Cotter, J. L., M. S. Chinn, et al., 2009. Influence of process parameters on growth of *Clostridium ljungdahlii* and *Clostridium autoethanogenum* on synthesis gas. *Enzyme and Microbial Technology*, 44(5): 281-288.
6. Dhurjati, P., 2008. Biorefineries - Industrial Processes and Products, Status Quo and Future Directions: Volumes 1 and 2 By Birgit Kamm, Patrick Gruber and Michael Kamm. *AIChE Journal*, 54(11): 3036-3036.
7. Gaddy, J. L., 1992. Biological production of ethanol from coal. Final report. Other Information: PBD: Dec 1992: Medium: ED; Size: 298 p.
8. Gaddy, J. L., D. K. Arora, et al., 2007. Methods for increasing the production of ethanol from microbial fermentation. United States, Emmaus Foundation, Inc. (Fayetteville, AK, US).
9. Gaddy, J. L., Clausen, Edgar C., 1992. *Clostridium ljungdahlii*, an anaerobic ethanol and acetate producing microorganism. United States, The Board of Trustees of the University of Arkansas.
10. Grethlein, A. J., R. M. Worden, et al., 1991. Evidence for production of n-butanol from carbon monoxide by *Butyribacterium methylotrophicum*. *Journal of Fermentation and Bioengineering*, 72(1): 58-60.
11. Heiskanen, H., I. Virkajärvi, et al., 2007. The effect of syngas composition on the growth and product formation of *Butyribacterium methylotrophicum*. *Enzyme and Microbial Technology*, 41(3): 362-367.
12. Henstra, A. M., J. Sipma, et al., 2007. Microbiology of synthesis gas fermentation for biofuel production. *Current Opinion in Biotechnology*, 18(3): 200-206.
13. Klasson, K. T., M. D. Ackerson, et al., 1991. Bioreactors for synthesis gas fermentations. *Resources, Conservation and Recycling*, 5(2-3): 145-165.
14. Klasson, K. T., M. D. Ackerson, et al., 1993. Biological conversion of coal and coal-derived synthesis gas. *Fuel*, 72(12): 1673-1678.
15. Kundiyan, D. K., R. L. Huhnke, et al., 2010. Feasibility of incorporating cotton seed extract in *Clostridium strain P11* fermentation medium during synthesis gas fermentation. *Bioresource Technology*, 101(24): 9673-9680.

16. Liou, J. S.-C., D. L. Balkwill, et al., 2005. *Clostridium carboxidivorans* sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen *Clostridium scatologenes* strain SL1 as *Clostridium drakei* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 55(5): 2085-2091.
17. Munasinghe, P. C. and S. K. Khanal, 2010. Biomass-derived syngas fermentation into biofuels: Opportunities and challenges. *Bioresource Technology*, 101(13): 5013-5022.
18. Phillips, J., K. Klasson, et al., 1993. Biological production of ethanol from coal synthesis gas. *Applied Biochemistry and Biotechnology*, 39-40(1): 559-571.
19. Ragsdale, S. W., 1997. The Eastern and Western branches of the Wood/Ljungdahl pathway: how the East and West were won. *BioFactors*, 6(1): 3-11.
20. Tanner, R. S., L. M. Miller, et al., 1993. *Clostridium ljungdahlii* sp. nov., an Acetogenic Species in Clostridial rRNA Homology Group I. *Int J Syst Bacteriol*, 43(2): 232-236.
21. Younesi, H., G. Najafpour, et al., 2005. Ethanol and acetate production from synthesis gas via fermentation processes using anaerobic bacterium, *Clostridium ljungdahlii*. *Biochemical Engineering Journal*, 27(2): 110-119.
22. Zhu, Y., 2003. Enhanced butyric acid fermentation by *Clostridium tyrobutyricum* immobilized in a fibrous-bed bioreactor. Doctor of Philosophy, Ohio State University.