BIOCONVERSION OF CARBON MONOXIDE GAS TO ACETIC ACID USING CLOSTRIDIUM ACETICUM IN BATCH AND CONTINUOUS FERMENTATIONS

by

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LIST OF SYMBOLS

A or B	Coded factors
C^*	Equilibrium concentration of CO (g/L)
C_L	Dissolved CO concentration (g/L)
D	Dilution rate (hr^{-1})
F	Flowrate of nutrient solution (L/hr)
k_d	Death rate constant (hr^{-1})
K_p	Saturation constant (atm)
K_p'	Monod saturation constant for substrate uptake (atm)
$\frac{K_L a}{H}$	Volumetric mass transfer coefficient (mmoles/L.hr.atm)
N^G_{CO}	Moles of CO in gas phase (mmole)
P_{CO} or P^L_{CO}	CO partial pressure in the liquid phase (atm)
P^G_{CO}	CO partial pressure in gas phase (atm)
q	Specific substrate uptake rate ($g/g_{cell}.hr$)
q_{CO}	Specific CO uptake rate ($mmoles/g_{cell}.hr$)
q_m	Maximum specific substrate uptake rate ($mmoles/g_{cell}.hr$)
Q_{CO}	Rate of CO transport through gas-liquid interphase (mmoles/L.hr)
R^2	Determination coefficient
S	Limiting substrate concentration (g/L)
S_e	Limiting substrate concentration in liquid outlet (g/L)
t	Fermentation time (hrs)
V_R or V_L	Liquid volume (L)
W	Substrate inhibition to the specific growth rate (atm)

W’	Substrate inhibition to the specific CO uptake rate (atm)
x	Independent variables
X	Cell concentration (g/L)
X_o	Initial cell concentration (g/L)
X_e	Cell concentration in liquid outlet (g/L)
y	Predicted response
$Y_{P/S}$	Product yield coefficient (g product formed per g substrate consumed)
$Y_{P/x}$	Product yield coefficient (g product formed per g cell dry weight)
β_0	Intercept of the plane
$\beta_1,...\beta_n$	Partial regression coefficients
ε	Random error term
μ	Specific growth rate (hr^{-1})
μ_m	Maximum specific growth rate (hr^{-1})
ν	Specific acetic acid production rate ($g_{acetic\ acid}/g_{cell}.hr$)
ν_m	Maximum specific acetic acid production rate ($g_{acetic\ acid}/g_{cell}.hr$)

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LIST OF ABBREVIATIONS

2-D	two-dimensional
2FI	2-factor interaction model
3-D	three-dimensional
A. woodii	*Acetobacterium woodii*
Ar	Argon
atm	atmosphere
C. aceticum	*Clostridium aceticum*
C_4H_9OH	Butanol
CH_3CH_2OH	Ethanol
CH_3COOH	Acetic acid
CH_4	Methane gas
cm	centimetre
CO	Carbon monoxide gas
CO/H_2	Carbon monoxide to hydrogen ratio
CO_2	Carbon dioxide gas
CSTR	Continuous stirred tank reactor
Cysteine-$HCl.H_2O$	L-cysteine monohydrochloride anhydrous
DSMZ	Germany’s Culture Collection
Eh	oxidation-reduction
F/V	Liquid flowrate to liquid volume ratio
FID	Flame Ionization Detector
ft	feet
g	gram
g	gram
GC	Gas chromatography
H_2	Hydrogen gas

H_2/CO_2	Hydrogen to carbon dioxide ratio
H_2O	Water
H_2S	Hydrogen sulfide
HCl	Hydrochloride acid
HPLC	High Pressure Liquid Chromatography
hr	hour
I.D.	Inner diameter
i.e.	example
in	inche
K_a	Acid dissociation constant
KPa	kiloPascal
L	Litre
μl	microlitre
m	metre
μm	micrometre
M	molar
M. thermoautotrophica	*Moorella thermoautotrophica*
mg	milligram
min	minute
ml	millilitre
mV	microVolt
N_2	Nitrogen gas
Na_2S	Sodium sulfide
$Na_2S.9H_2O$	Sodium sulfide 9-hydrate
NaOH	Sodium hydroxide
NH_4Cl	Ammonium chloride
nm	nanometre

O_2	Oxygen gas
OD	Optical density
P. productus	*Peptostreptococcus productus*
psig	pound per square inch gauge
rpm	rotation per minute
RSM	Response surface methodology
sp.	species
TCD	Thermal Conductivity Detector
UK	United Kingdom
US	United States of America
USD	United States of America Dollar
VAM	Vinyl acetate monomer

PENUKARANBIO GAS KARBON MONOKSIDA KEPADA ASID ASETIK DENGAN MENGGUNAKAN CLOSTRIDIUM ACETICUM DI DALAM FERMENTASI KELOMPOK DAN SELANJAR

ABSTRAK

Asid asetik merupakan sumber penting kepada industri kimia dan ia dihasilkan dari takungan bahan api fosil yang semakin merosot seperti minyak galian dan gas asli. Jalan alternatif untuk menghasilkan produk kimia organik berasaskan sumber biologi diperbaharui contohnya gas karbon monoksida (CO) adalah penting untuk mengurangkan pergantungan kepada takungan petroleum. CO yang murah dan bernilai rendah dapat ditukarkan kepada sumber kimia yang berharga melalui proses fermentasi CO. CO ialah gas toksik yang dihasilkan daripada gas ekzos kenderaan, gasifikasi pada biojisim dan kumbahan-enapcemar. *C. aceticum* telah terbukti mampu mensintesiskan asid asetik daripada sumber organik yang berharga dan gas H_2/CO_2. Walaubagaimanapun, sehingga kini kajian tentang penggunaan CO sebagai substratum dalam proses fermentasi asid asetik belum dilaksanakan lagi.

Kebolehlaksanaan penghasilan asid asetik daripada 4% H_2: 18% Argon: 78% CO dengan menggunakan *C. aceticum* telah ditunjukkan. Apabila proses dijalankan pada keadaan optimum iaitu separa tekanan CO pada 1.40 atm dan masa penapaian selama 48 jam, 2.11 g/L asid asetik akan terhasil. Komponen-komponen media yang dipilih untuk diminimumkan ialah NH_4Cl, yis esktrak, cysteine-HCl.H_2O dan $Na_2S.9H_2O$ dengan kepekatan masing-masing sebanyak 0.20 g/L, 1.5 g/L, 0.30 g/L dan 0.30 g/L. Takrif media berjaya diminimumkan dengan pencapaian kepekatan sel yang tertinggi sebanyak 0.80 g/L, kadar penggunaan CO yang tertinggi sebanyak 5.14 mmol/L.jam dan penghasilan asid asetik sebanyak 1.89 g/L dalam media tertakrif yang minima dimana ia adalah sebanding dengan fermentasi dalam takrif media. Di samping itu, fermentasi dalam media takrif yang minima dapat meningkatkan kadar pengangkutan

CO antara fasa disebabkan pencapaian pekali jisim pindah ($\frac{K_L a}{H}$) yang lebih tinggi, 4.54 mmol/L.atm.jam berbanding dengan 4.12 mmol/L.atm.jam yang didapati dalam takrif media.

Kadar pertumbuhan sel dan kadar pengambilan CO untuk kedua-dua media takrif yang minima dan media takrif dapat dikaitkan dengan persamaan Monod dan persamaan Andrew's dengan pekali regrasi (R^2) dalam julat 0.98 - 1. Pengaplikasian media tertakrif yang minima dalam operasi selanjar terbukti dapat dijalankan kerana tiada pengurangan mendadak dalam kepekatan sel-sel, peratus penukaran CO dan penghasilan asid asetik semasa perubahan dilakukan pada kadar pengaliran gas dan kadar pencairan selama 1020 jam. Melalui analisis statistik, penghasilan asid asetik secara selanjar adalah paling ideal beroperasi pada kadar pengaliran gas 10 ml/min dan kadar pencairan 0.0273 jam^{-1} untuk mencapai penukaran CO sebanyak 93.46% dan kadar penghasilan asid asetik sebanyak 0.14 g/L.jam.

BIOCONVERSION OF CARBON MONOXIDE GAS TO ACETIC ACID USING CLOSTRIDIUM ACETICUM IN BATCH AND CONTINUOUS FERMENTATIONS

ABSTRACT

Acetic acid, an important industrial feedstock for chemicals is mainly produced from depleting fossil fuels resources either from mineral oil or natural gas. An alternative route to produce organic chemicals based on renewable biological resources like carbon monoxide (CO) gas is foremost important to relieve the heavily dependency on petroleum reserves and convert the relatively cheap and low value waste CO to valuable chemical feedstock. Carbon monoxide is a poisonous gas that results from the automobile emission, gasification of biomass and sewage sludge. *C. aceticum* has been proven to synthesize acetic acid from valuable organic sources and H_2/CO_2, but to date no study has been carried out on utilizing CO as gaseous substrate in the acetic acid fermentation.

The production of acetic acid by *C. aceticum* under 4% H_2: 18% Argon: 78% CO was shown to be feasible. When fermentation was operated under optimum CO partial pressure of 1.40 atm and incubated for 48 hrs, 2.11 g/L acetic acid was produced. NH_4Cl, yeast extract, cysteine-HCl.H_2O and $Na_2S.9H_2O$ were the chosen media components to be minimized and the respective desirable concentration were 0.20 g/L, 1.5 g/L, 0.30 g/L and 0.30 g/L. The minimization of the defined medium was successfully implemented with the highest cell concentration of 0.80 g/L, the highest CO consumption rate of 5.14 mmoles/L.hr and comparable acetic acid production of 1.89 g/L were achieved in minimized medium compared to defined medium. In addition, fermentation in minimized medium accelerated the interphase CO transport rate prior to reaction due to higher mass transfer coefficient ($\frac{K_L a}{H}$), 4.54 mmoles/L.atm.hr compared to 4.12 mmoles/L.atm.hr observed in defined medium.

In minimized and defined medium, the cell growth rate and CO uptake rate with CO as gaseous substrate were well correlated by Monod equation and Andrew's equation with regression coefficient (R^2) ranging between 0.98 - 1. The minimized defined medium was feasible in continuous operation because the process was able to retain satisfactory performances without drastic drop in cell concentration, CO conversion and acetic acid production corresponding to alterations made in gas flowrate and liquid flowrate during 1020 hrs fermentation. Through statistical analysis, continuous acetic acid production was best operated at 10 ml/min gas flowrate and 0.0273 hr^{-1} dilution rate in order to achieve 93.46% of CO conversion and 0.14 g/L.hr acetic acid production rate.

CHAPTER 1

INTRODUCTION

1.1 RENEWABLE FEEDSTOCK

Fuel and chemical production in the chemical industries heavily rely on the fossil fuel feedstock for more than six decades. Pusat Tenaga Malaysia (1999) reported that 27.6% of the petroleum products were consumed by the industrial sector. In recent years, the dramatic depletion of petroleum reservoir has encouraged the development of new technologies that are based on other alternatives (Dale, 2003). The potential of producing organic chemicals like acetic acid, methane, acetone and etc. based on the renewable resources is fairly important to sustain the viability and the continuous production of these organic chemicals for future generation (Zeikus, 1980). Therefore, biomass has become the primary renewable resource as world biomass production is estimated to exceed 110 billion tons per year (Dale, 2003).

Malaysia is known as an agricultural based country in nationwide and possesses a wide variety of agricultural biomass resources which include oil palm, rice straw and sugar cane. The amount of biomass waste supply from oil palm are forecasted to be 13 million tones in the year 2020 (Hassan *et al.*, 1997). Therefore, Malaysia's economic may receive a major boost resulting from the utilization of waste biomass as an industrial resource.

The waste biomass can be utilized in gasification process to produce synthesis gas which is rich in carbon monoxide. This toxic carbon monoxide compound is favorable for acetogenic bacteria as sole energy and carbon source in producing acetic acid as primary metabolite. The utilization of these waste biomass as feedstock for synthesis gas production followed by gaseous substrate fermentation allow the

transformation of large disposal problem into a potentially profitable industry (Zeikus, 1980). The purpose of this study is to utilize the carbon monoxide which is a renewable source and convert it to valuable organic chemicals like acetic acid by *Clostridium aceticum*.

1.2 CARBON MONOXIDE SOURCE

A schematic diagram that shows the ways to obtain the carbon monoxide gas from variety of sources is presented in Figure 1.1. Carbon monoxide is a toxic gas and is a major constituent in industrial gas emission. The off-gases from steel industries contain mostly CO which would then be burnt to produce a huge amount of CO_2 and causes a greenhouse effect (Chang *et al*., 2001). In addition, CO is also produced in large quantity from mobile and stationary sources such as automobiles, electric power plants and ion furnaces (Jung *et al.,* 2002).

Synthesis gas, mixtures of primarily carbon monoxide (CO), hydrogen (H_2), and carbon dioxide (CO_2) with contaminants such as nitrogen and hydrogen sulfide is formed by partial oxidation of biomass, sewage sludge and municipal solid (Phillips *et al.,* 1994). Gasification technology converts the complex carbon structure of biomass to simple synthesis gas which can later become the renewable feedstock for the numerous valuable chemicals production (Zeikus, 1980). Carbon monoxide is the main component of synthesis gas which can be converted to multi-carbon compounds for the production of organic chemicals like acetic acid. Gasification process is preferable than direct microbial conversion on biomass into fermentable sugars because a variety of raw materials can be utilized by the gasifier technology and all of the organic components of the feed for gasification process may be converted to synthesis gas (Najafpour *et al.*, 1995). Therefore, the development of an efficient process like CO

fermentation by acetogenic bacteria is important to convert the abundantly cheap CO in atmosphere into value-added products.

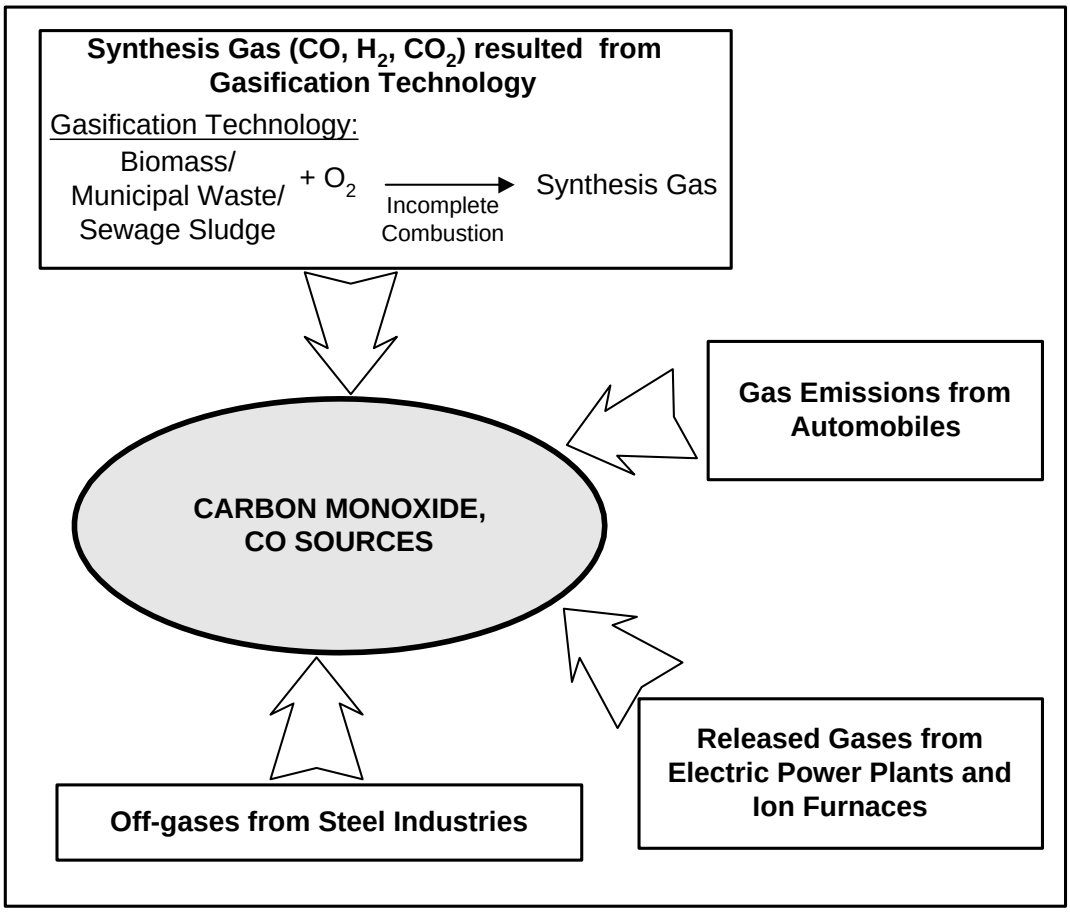

Figure 1.1. Schematic diagram presents the various sources for carbon monoxide gas.

1.3 BIOLOGICAL PROCESS OVER CATALYTIC PROCESS

Synthesis gas that comprises primarily of CO can be converted into a variety of fuels and chemicals such as methane, methanol, formaldehyde and acetic acid either by biological CO conversion process (Klasson *et al*., 1992) or catalytic process (Anderson *et al*., 1984). Figure 1.2 presents the conventional processes being applied in converting synthesis gas to valuable industrial products. Catalytic processing of synthesis gas can either utilize direct route such as Fischer-Tropsch process or indirect route like methanol/CO/H_2 reaction. Although catalytic process may be an instant

reaction but the process is restricted by catalyst selectivity, very intensive operating cost due to the high energy requirement, wide product distributions and the potential risk of catalyst poisoning by the sulfur gases present in the synthesis gas (Chatterjee *et al*., 1996).

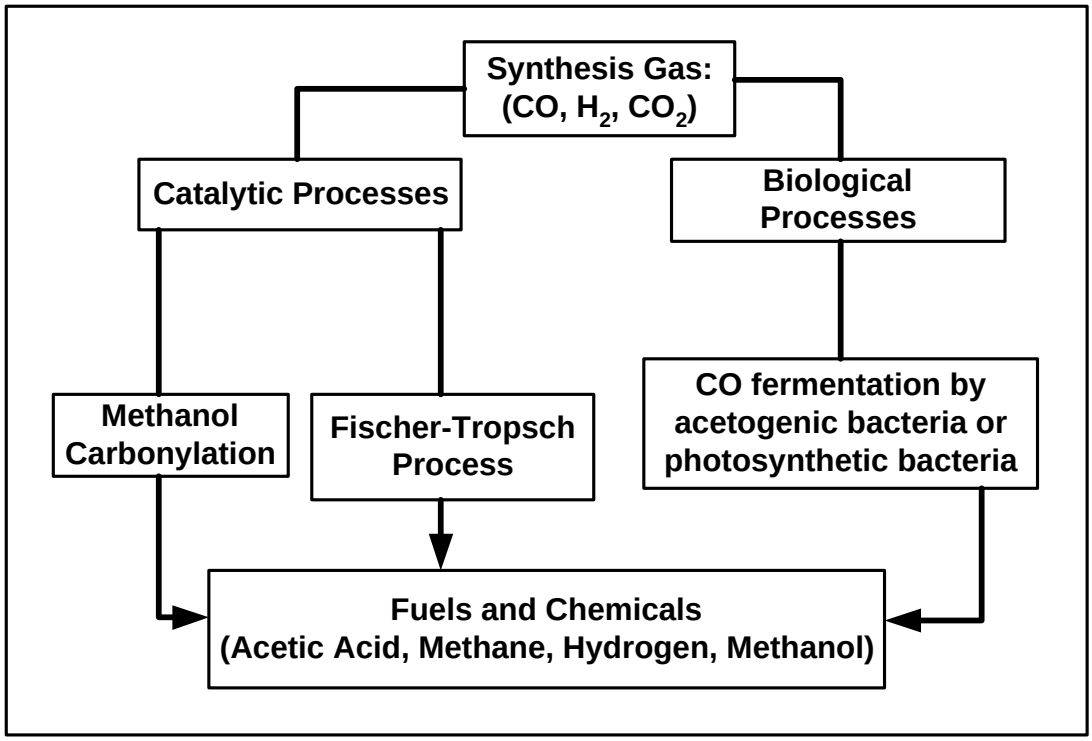

Figure1.2. The utilization of synthesis gas in producing valuable products.

An alternative route is the biological conversion by utilizing the capability of acetogenic bacteria in synthesizing organic chemicals such as acetic acid from CO. The acetogens grow chemolithotrohically under CO atmosphere and utilize CO as the sole carbon and energy source while producing acetic acid. The CO fermentation had successfully overcome several weaknesses of the catalytic conversion. Biological acetic acid production can be carried out at ambient temperature and pressure, which result in substantial energy and equipment savings (Vega *et al*., 1988b).

In addition, these biocatalysts are resistant to poisoning by trace contaminants, sulphur compounds than the chemical catalysts. The sulphur compounds are beneficial to acetic acid fermentation by lowering the redox potential of the culture medium to stimulate the growth of anaerobic bacteria (Vega *et al*., 1990). Other advantages include higher specificity and complete conversion due to the irreversible character of biological reactions (Klasson *et al*., 1992). Therefore, biological process would be better than a chemical process to utilize CO-containing gas as a feedstock. It was reported that U.S. Department of Energy has a seven years plan starting from year 2003 to evaluate the economic and energy feasibility used in acetic acid production and intend to implement the bioconversion of one-carbon compound to acetic acid into the process by 2010. The advantages offered by biological process over catalytic process are summarized in Table 1.1.

Table 1.1. Comparison between biological process over catalytic process.

Catalytic Process	Biological Process
• fast reaction rate	• slow reaction rate
• operated at high temperature and pressure	• reactions happened at ambient temperature and pressure
• catalyst poisoning by sulphur gases	• biocatalyst resistance to trace contaminants poisoning
• wide variety of products	• product specificity

1.4 THE MARKET PROSPECT FOR ACETIC ACID

Today, global acetic acid is manufactured either through the industrial production from petrochemical feedstocks or by the biological means production via fermentation process. The statistical data in Table 1.2 shows the world's demand for acetic acid each year together with the corresponding selling price starting from year 1997 to year 2002. Acetic acid is an important industrial product as could be clearly seen from the worldwide demand. In 2001, acetic acid demand was as high as 5,526 million pounds. It is interesting to note that the demand of acetic acid will be expected

to reach 6,095 million pounds in 2006 (Wikipedia, the free encyclopedia, 2006). From Table 1.2, the statistical values for acetic acid demand and selling price, justifies that acetic acid is a valuable organic chemical which has promising great market value and can be profitable when produced in large scale.

Table 1.2. The demands and average selling prices for acetic acid from year 1997- year 2002 (Wikipedia, free encyclopedia, 2006).

Year	Demand, (millions of pounds)	Average Selling Price, (USD per pound)
1997	5,324	0.250
1998	5,286	0.265
1999	5,398	0.200
2000	5,628	0.215
2001	5,526	0.280
2002	5,659	0.370

Although acetic acid is a mature product but its manufacturer is only restricted to a limited number of large producers (Table 1.3). World demand growth for acetic acid is in the range of 3 to 4% per year. Nowadays, the fastest growing region of acetic acid production is in Asia, particularly in China where the demand for this chemical is forecasted to increase at 8 to 10% per year. Therefore, most of the new plans are targeted for Asia with a number of projects and expansions are planned in China and Taiwan. Asia has now overtaken North America to become the largest region for acetic acid production in terms of acetic acid capacity. The world prime acetic acid chemical companies are listed in Table 1.3.

Table 1.3. The world leading acetic acid producers (Smejkal *et al*., 2005).

Company	Global Capacity, (thousand of millions tones per year)
Celanese	2065
BP Chemicals	1175
Millennium Chemicals	450
Acetex	400

1.5 PROBLEM STATEMENT

Carbon monoxide, CO is a toxic gas that has been emitted into our environment in huge quantities from automobiles and industry. Moreover, CO as the main component of the off-gases from the steel industry attributed to the green-house effect when off-gases are burnt. CO can be converted into multi-carbon compounds either through catalytic processes (Fischer-Tropsch process) or biological processes (fermentation process). Fischer-Tropsch process are very expensive process as the reaction needs to be operated at high operating temperature and pressure to supply high energy requirements for the system. Besides, the CO should be purified and concentrated prior to reaction to prevent sulfur compounds in the gases from poisoning the catalyst (Klasson *et al*., 1992; Anderson *et al*., 1984).

CO has appeared as the important primary feedstock for biological acetic acid production as CO can also be obtained renewably through the gasification of biomass or municipal wastes. Gasification is a technology that converts all the complex carbon structures in the biomass or municipal wastes to single carbon structure like CO, CO_2 and H_2 in the synthesis gas through incomplete combustion process.

Acetogenic bacteria is able to grow chemolithotrophically on CO and convert CO into multi-carbon compounds such as acetic acid under ambient temperature and ambient pressure. These bacteria are resistant to the sulphur compounds present in the gases that poison the chemical catalysts (Vega *et al*., 1990). Several acetogens includes *Eubacterium limosum* KIST 612 (Chang *et al*., 2001) and *Peptosteptococcus productus* U-1 (Vega *et al*., 1988b) have been tested on using CO as the gaseous substrate to produce acetic acid. These bacteria are less favorable to be used as biocatalysts due to a considerably low CO tolerance by both bacteria (less than 2.0 atm CO partial pressure) (Chang *et al*., 2001). Therefore, studies have been conducted on

searching a potential biocatalyst that has higher CO tolerance with higher acetic acid productivities.

Clostridium aceticum was selected as the biocatalyst because the bacteria is able to grow in alkaline medium (pH: 8.5) and this is an added advantage especially for acetic acid producer. The fermentation medium which sustains the cells growth and maintains the bacteria contributes partly to the production costs. Therefore, the efforts in minimizing the nutrients requirements into minimum levels were carried out in the experiment.

1.6 RESEARCH APPROACH

Clostridium aceticum is either a chemolithotrophic or a chemoorganotrophic bacteria that can consume synthesis gas components as sole carbon and energy source while producing acetic acid. This bacteria have been proven to produce acetic acid from carbon dioxide and gas hydrogen (Wieringa, 1940). However no efforts have been developed to employ different inorganic gaseous substrates for acetic acid fermentation by *C. aceticum*. In this research, the project is targeted at exploring the capability of *C. aceticum* in converting carbon monoxide (CO) to acetic acid at ambient temperature (30^{o}C) and ambient pressure (1.0 atm) in batch and continuous system.

In the preliminary study, the potential of *C. aceticum* in catalyzing CO to acetic acid was recognized. This was done by attempting to grow the *C. aceticum* autotrophically under mixed gas (4% H_2: 18% Argon: 78% CO) and later verifying the production of acetic acid using gas chromatography. Then the CO tolerance for *C. aceticum* was determined by applying different CO partial pressure in the serum bottle ranging from 1.40 atm to 2.02 atm. The toxicity of carbon monoxide gas to *C. aceticum* was evaluated based on the cell density, CO concentration and acetic acid concentration.

The main focus of this research is to develop a minimally defined medium for acetic acid fermentation by *C. aceticum* which attributes to part of the acetic acid production cost. Ammonium chloride, yeast extract, L-cysteine hydrochloric monohydrate and sodium sulfide that contain in the culture media of *C. aceticum* provided by Germany's Culture Collection (DSMZ) are the selected chemical compositions to be minimized.

The predetermined minimally defined medium during batch fermentation was employed in continuous stirred tank reactor (CSTR) study to confirm the viability of the microbes in a continuous fermentation. In continuous fermentation, the optimum values for gas flowrate together with dilution rate of fresh medium will be determined by applying a four-levels full factorial design and response surface methodology (RSM).

1.7 OBJECTIVES

1.7.1 Main Objective

To investigate the bioconversion activities of *Clostridium aceticum* which is grown chemolithotrophically on carbon monoxide (CO) as sole carbon source and to produce acetic acid from CO in batch and continuous fermentation processes.

1.7.2 Measurable Objectives

1. To induce the chemolithotrophic growth of *C. aceticum* in 4% H_2: 18% Argon: 78% CO and produce acetic acid at ambient temperature and pressure.
2. To identify the CO tolerance of the *C. aceticum*'s and develop a minimally defined medium in batch fermentation. The reaction kinetics will be developed based on the growth and CO uptake.

3. To evaluate the applicability of minimally defined medium in continuous system and to optimize liquid dilution rate together with gas flowrate using statistical approach.

CHAPTER 2

LITERATURE REVIEW

2.1 CARBON MONOXIDE CONVERSION IN BIOLOGICAL ROUTE

The bacteria that are capable of oxidizing CO was discovered almost 95 years ago (Schlegel & Meyer, 1981). A wide variety of anaerobic bacteria has been examined for their ability to transform one-carbon compounds such as carbon monoxide to fuels and chemical products. Although CO is toxic but it is a valuable source since it can be the renewable feedstock for biological production of valuable fuels and chemicals such as hydrogen, acetic acid or ethanol (Tanner *et al*., 1993; Klasson *et al*., 1993a). The feasibility of biological H_2 production from CO depends mainly on the performance of the microorganism that catalyzes the reaction. The microbial species that are able to convert CO in synthesis gas and water to hydrogen and CO_2 are photosynthetic bacteria, such as *Rhodobacter* sp., *Rhodopseudomonas gelatinosa*, *Rhodospirillum rubrum* and anaerobic bacterium such as *Methanosarcina barkeri, Citrobacter* sp. Y19 (Klasson *et al*., 1993b; Uffen, 1976; Bhatnagar *et al*., 1987; Jung *et al*., 2002). *Rhodospirillum rubrum* has been shown to have high specific CO uptake rate and high conversion yield close to the theoretical value (Klasson *et al*., 1993b).

Clostridium ljungdahlii was isolated and was able to convert CO to a mixture of acetic acid and ethanol. C. *ljunddahlii* produce acetic acid as the major product, with ethanol to acetic acid ratio of only 0.05 (Klasson *et al*., 1992). *Butyribacterium methylotrophicum* can withstand growth tolerance up to 100% CO headspace in cultures besides producing butanol and ethanol from CO (Lynd *et al*., 1982). Bacterium P7 provided by Ralph tanner, University of Oklahoma converts components of synthesis gas (CO) into liquid products such as ethanol, butanol and acetic acid (Rajagopalan *et al*., 2002). The acetogenic bacteria that includes *Clostridium*

thermoaceticum (Fontaine *et al*., 1942), *Clostridium formicoaceticum* (Andreesen *et al*., 1970), *Acetobacterium woodii* (Balch *et al*., 1977), *Clostridium thermoautotrophicum* (Wiegel *et al*., 1981) and *Acetogenium kivuii* (Leigh *et al*., 1981) have shown their ability to convert CO to acetic acid. The potential microorganisms that are able to convert toxic gas, CO to variety of fuels and chemicals products are summarized into Table 2.1.

2.2 ACETIC ACID

Acetic acid or also known as ethanoic acid is the simplest carboxylic acid that is responsible for the vinegar sour taste and its pungent smell. Typically, vinegar which is popular as a food additive contains 4 to 8% acetic acid. The physical and chemical properties of acetic acid are shown in Table 2.2. Acetic acid's empirical formula is CH_3COOH with molecular weight of 60.05 g/mol and its chemical structure is shown in Figure 2.1. Pure acetic acid is colorless and a corrosive liquid with an irritating odor of vinegar. The boiling point and melting point for acetic acid is 118.1^{o}C and 16.7^{o}C respectively. Acetic acid is classified as a weak acid that partially dissociate into component ions in aqueous solution. The acid dissociation constant (K_a value) for acetic acid is 1.8 x 10^{-5} at 25^{o}C. Acetic acid is a hydrophilic (polar) protic solvent which makes it miscible in all proportions with all polar and nonpolar compounds such as water, ethyl alcohol and diethyl ether. Commonly, acetic acid undergoes a series of chemical reactions of carboxylic acid.

Table 2.1. Potential microorganism in CO conversion.

Microorganism	Fermentative Products	References
Rhodobacter sp., *Rhodopseudomonas gelatinosa*, *Rhodospirillum rubrum, Methanosarcina barkeri, Citrobacter* sp. Y19	Hydrogen $CO + H_2O \rightarrow CO_2 + H_2$	Klasson *et al*., 1993; Uffen, 1976; Bhatnagar *et al*., 1987; Jung *et al*., 2002
Clostridium ljungdahlii	mixture of acetic acid and ethanol. $6CO + 3H_2O \rightarrow CH_3CH_2OH + 4CO_2$ $4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$	Klasson *et al*., 1992
Butyribacterium methylotrophicum	mixture of butanol and ethanol. $12CO + 5H_2O \rightarrow C_4H_9OH + 8CO_2$	Lynd *et al*., 1982
Bacterium P7	mixture of ethanol, butanol and acetic acid. $6CO + 3H_2O \rightarrow CH_3CH_2OH + 4CO_2$ $4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$ $12CO + 5H_2O \rightarrow C_4H_9OH + 8CO_2$	Rajagopalan *et al*., 2002
Clostridium thermoaceticum, Clostridium formicoaceticum, *Acetobacterium woodii*, *Clostridium thermoautotrophicum, Acetogenium kivuii*	Acetic acid $4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$	Fontaine *et al*., 1942; Andreesen *et al*., 1970; Balch *et al*., 1977; Wiegel *et al*., 1981; Leigh *et al*., 1981

Table 2.2. Physical and chemical properties of acetic acid.

Subject	Description
Formula Molecule	$C_2H_4O_2$
Molecular Weight	60.05 g/mol
Appearance	Colourless liquid or crystals
Odor	Irritating odor of vinegar
Boiling Point	118.1^oC
Melting Point	16.7^oC
Acidity (pK_a)	4.76
Solubility in water	Fully miscible
Density	1.049 g/cm^3, liquid

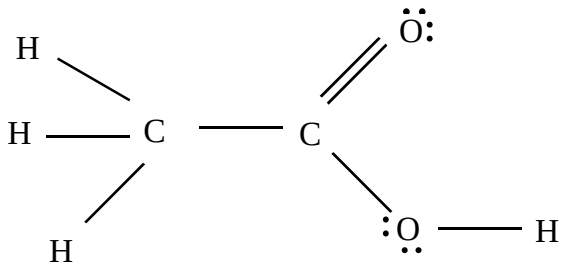

Figure 2.1. Chemical structure of acetic acid.

Acetic acid has been used as food additive for decades. Nowadays, acetic acid has become an important feedstock for various chemicals and pharmaceuticals industries. The major usage of acetic acid in industries is to produce vinyl acetate monomer (VAM), followed by the production of acetic anhydride and the third usage is for ester production. Comparatively, only a small amount of acetic acid has been used in vinegar. The percentage of worldwide acetic acid usage is shown in Figure 2.2.

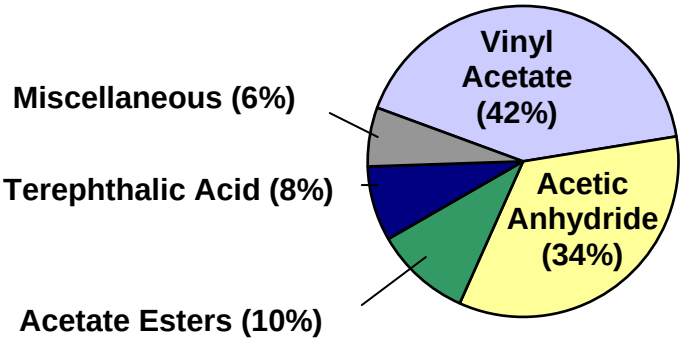

Figure 2.2. Uses of world production acetic acid (Kirschner, 2003).

The VAM when polymerized can be applied in latex emulsion resins for paints, adhesives, paper coatings and textile finishing agents. Acetic anhydride is primarily used in the manufacture of cellulose acetate for films and plastic goods. The esters produced are ethyl acetate, n-butyl acetate, isobutyl acetate where they are commonly used as solvents for inks, paints and coatings.

2.2.1 Conventional Processes for Acetic Acid Production

Nowadays, various approaches have been used to produce acetic acid either synthetically using catalysis or by biological route. Approximately half of the acetic acid production in the world today originated from methanol carbonylation process while one-third of the acetic acid is manufactured from acetaldehyde oxidation reaction (Wagner, 2002). The summary of the conventional ways to produce acetic acid is shown in Figure 2.3.

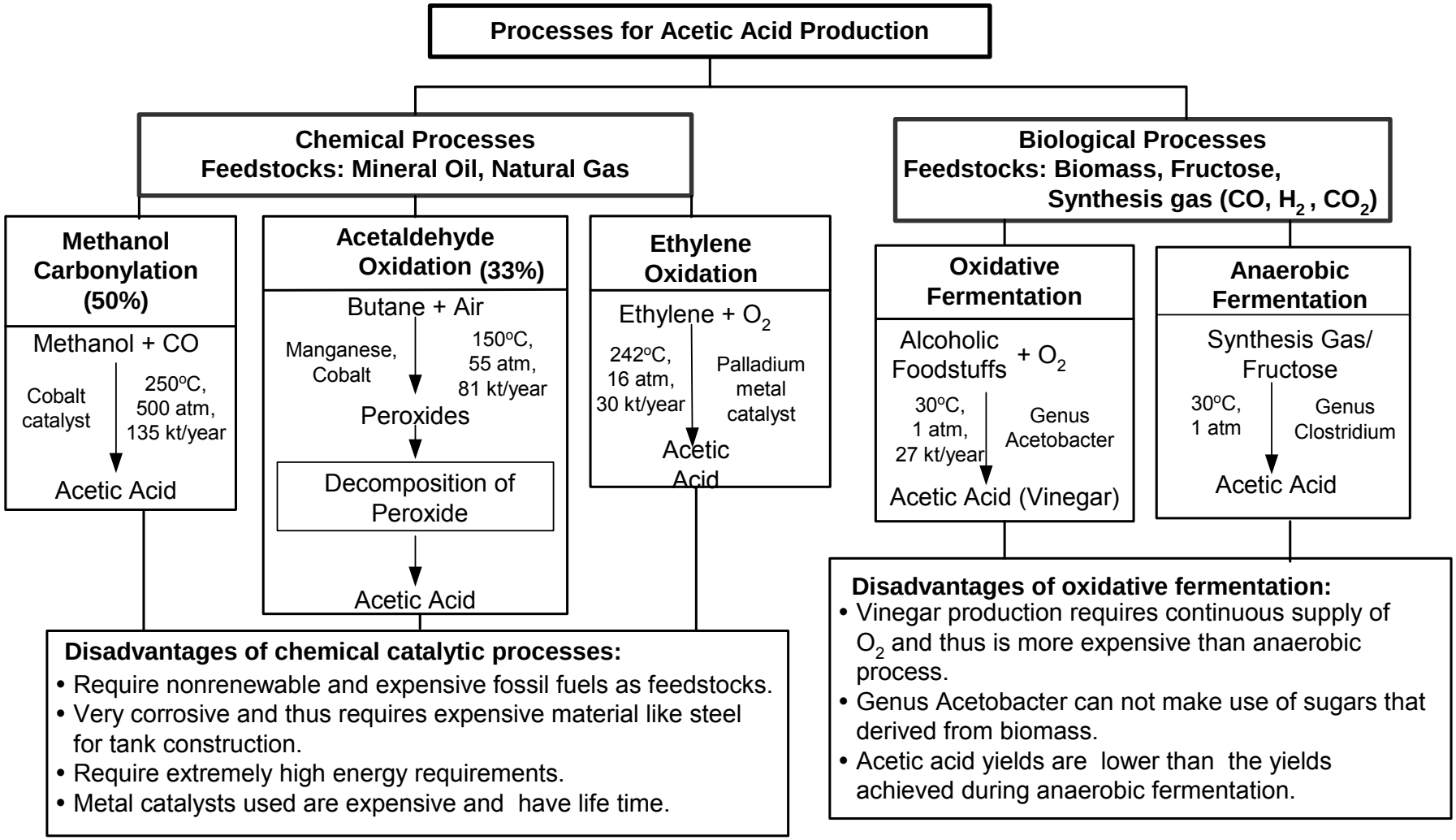

Figure 2.3. Chart shows the conventional routes used for acetic acid production.

2.2.1(a) Methanol Carbonylation

In the first commercial methanol carbonylation process, carbon monoxide in the liquid phase will react with methanol using cobalt catalysis promoted by iodine (Smejkal *et al*., 2005). The reaction is operated at conditions of 250^{o}C under 500 – 700 atm (3,000 - 10,000 psig) with 90% selectivity to acetic acid (Wikipedia, the free encyclopedia, 2006).

2.2.1(b) Acetaldehyde Oxidation

During the process, butane or light naphtha together with various metal ions (manganese, cobalt, chromium) is heated in the stream of air under 55 atm at 150^{o}C to form peroxides. Then, decomposition of the peroxide results in the production of acetic acid (Wikipedia, the free encyclopedia, 2006).

2.2.1(c) Ethylene Oxidation

Acetaldehyde is produced from ethylene and is oxidized via acetaldehyde oxidation process to produce acetic acid. Palladium metal catalyst supported on heteropoly acid is used to catalyze the process (Sano *et al*., 1999).

2.2.1(d) Oxidative Fermentation

Bacteria from the genus *Acetobacter* acts as the biocatalyst by oxidizing alcohol to acetic acid under the stream of oxygen (Yoneda *et al*., 2001).

2.2.1(e) Anaerobic Fermentation

Acetogenic bacteria include genus *Clostridium* are able to convert sugar almost stoichiometrically to acetic acid without using ethanol as an intermediate. In addition, these bacteria can also convert one-carbon compound, including methanol, carbon monoxide, or a mixture of carbon dioxide and hydrogen to acetic acid (Ljungdahl, 1983). The direct acetic acid conversion from sugar by acetogenic bacteria may reduce the input cost and thus the process is more efficient than the oxidative fermentation (Witjitra *et al.*, 1996).

Currently, the acetic acid production is mainly by chemical processes while 10% of acetic acid is manufactured using the biological route. However, the chemical processes require relatively high temperatures and pressures, exotic materials of construction, extensively safety-related equipment besides creating a toxic or corrosive environment (Chatterjee *et al.*, 1996). Therefore, all these factors resulted in high operating cost and low scaling factor of chemical processes. According to U.S. Department of Energy, the economic feasibility of using this route could be possible by operating the acetic acid production in a very large plant (500 – 1000 million pounds per year). Therefore, there are strong market, economic and energy benefits to developing processes for production of acetic acid in scalable, regional-sized plants, such as fermentation. The advantages of producing acetic acid by fermentation include its operational in small-scale production, lower cost for feedstocks, low energy membrane-based purification, lower temperature and pressure requirements (Klasson *et al*., 1992). Potential energy savings of using fermentation are estimated at around 18 trillion Btu as reported by U.S. Department of Energy.

2.3 APPROACHES ON ACETIC ACID FERMENTATION

Acetic acid is an important industrial feedstock that is produced mainly from mineral oil and natural gas either through methanol carbonylation or acetaldehyde oxidation (Spath & Dayton, 2003). At present, high petroleum cost due to substantially depleting of fossil fuel resources has stimulated the development of new technologies based on renewable resources. Consequently, fermentation and catalysis processes that change resource entry from nonrenewable (petroleum) to renewable (biomass) feedstocks have drawn great attention (Klasson *et al*., 1992). In addition, fermentation process is of great interest for researchers because it is economically feasible due to low energy and pressure requirement and high durability of biocatalyst as compared to catalytic processes (Probstein & Hicks, 1985). Thus, the focus of many researchers has changed towards employing acetogenic bacteria as biocatalyst to produce acetic acid almost stoichiometrically by fermentations of renewable resources.

The direct utilization of cheap and abundantly available biomass into fermentation process for acetic acid production is an alternative effort that was based on renewable resources (Slapack *et al*., 1985). The conventional conversion of cellulosic biomass to acetic acid includes acid or enzymatic hydrolysis of the cellulosic biomass to fermentable sugar and followed by bacteria fermentation. Acid hydrolysis is hindered due to low glucose yields and corrosion of the equipment. Enzymatic hydrolysis employs enzymes to break down the lignocellulose to fermentable sugars and subsequently fermented to acetic acid. This process may achieve higher substrate conversion yield but its production is very expensive (Parisi, 1989; Vallender & Eriksson, 1990).

Fermentation of milk permeate to produce acetic acid was made possible when *Clostridium thermolacticum* DSM 2910 was co-cultured with *Moorella thermoautotrophica* DSM 7417. *C. thermolacticum* DSM will first produce lactic acid

from milk permeate and the *M. thermoautotrophica* would convert lactic acid to acetic acid (Talabardon *et al*., 2000). However, the ratio of the two species in the treatment process is critical where sudden shifts in the composition of the population may lead to failure of the process. Besides, the study of these cells interaction with one another is difficult to evaluate (Shuler & Kargi, 1992).

Direct conversion of cellulosic biomass to acetic acid by single fermenting organism is economical but formed a variety of by-products which include ethanol and some lactic acids (Ravinder *et al*., 2001; Florenzano & Poulain 1984). These potential anaerobic bacteria are *Clostridium lentocellum* SG6 (Ravinder *et al.*, 2001) and *Clostridium thermocellum* (Florenzano & Poulain, 1984) for a single step fermentation of cellulose to acetic acid.

The employment of microorganism in fermenting synthesis gas into chemical products like acetic acid is another alternatives and efficient route (Klasson *et al*., 1992). The process can be implemented at ambient temperature and pressure with high yields and specificity (Vega *et al*., 1988b; Klasson *et al*., 1992). Gasification technology can convert all of the biomass, including lignin into synthesis gas that can be fermented by bacteria (Natarajan *et al*., 1998; Reed & Jantzen, 1979). Synthesis gas composes mainly of CO, CO_2, CH_4, N_2 and H_2 (McKendry, 2002). Several anaerobic bacteria have been found to grow autotrophically on synthesis gas. These organisms derived the energy for growth from the conversion of the reduced species, CO or H_2 to acetic acid by acetogenic pathway (Ljungdahlii, 1986). The first isolated acetogenic bacterium, *Clostridium aceticum* was reported to form acetic acid from CO_2 and hydrogen gas as in reaction (2.1) (Wieringa, 1940).

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \qquad (2.1)$$

Acetobacterium woodii (Balch *et al*., 1977), *Acetogenium kivuii* (Leigh *et al*., 1981), *Clostridium thermoautotrophicum* (Wiegel *et al*., 1981) and *Clostridium thermoaceticum* (Zeikus, 1980) are additional bacteria proven to convert CO to acetic acid according to mechanism described in reaction (2.1). *Clostridium aceticum* (Braun *et al.*, 1981) and *A. woodii* (Balch *et al*., 1977) were able to catalyze formate to acetic acid whereas *C. formicoaceticum* (Braun *et al*., 1981) and *A. woodii* (Bache & Pfennig, 1981) were able to produce acetic acid from methanol. *C. thermoautotrophicum* (Wiegel *et al*., 1981) and *C. thermoaceticum* (Lynd *et al*., 1982) can synthesize acetic acid from carbon monoxide as shown in reaction (2.2).

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \qquad (2.2)$$

However, it might be possible to manufacture acetic acid from CO with H_2 as an electron donor (Ljungdahl, 1983). The alternatives to reaction (2.2) would be reaction (2.3).

$$2CO + 2H_2 \rightarrow CH_3COOH \qquad (2.3)$$

These acetogenic bacteria appear to be effective biocatalyst especially in synthesis gas fermentation as the bacteria could grow unicarbonotrophically and synthesize acetic acid as the sole fermentation end product (Braun *et al*., 1981). A detail review on acetogenic bacteria from previous researches are discussed in section 2.4. Previous researches that have used different types of substrates for acetic acid fermentation are summarized in Table 2.3.

Table 2.3. Compilation of microorganisms capable of utilizing various substrates to produce acetic acid.

Type	Microorganism	Substrate	Reference
Chemolithotrophically Bacteria	*Butyribacterium methylotrophicum*	CO	Lynd *et al.* (1982)
	Acetobacterium woodii	$H_2 + CO_2$	Balch *et al.* (1977)
		CO	Brown (1987)
	Clostridium thermoaceticum	CO	Kerby and Zeikus (1983)
	Eubacterium Limosum KIST612	CO	Chang *et al*., (2001)
	Peptostreptococcus productus	CO	Lorowitz and Bryant (1984)
	Clostridium ljungdahlii	CO	Najafpour & Younesi (2006)
Chemoorganotrophically Bacteria	*Clostridium formicoaceticum*	Fructose/ pyruvate	Andreesen *et al.* (1970)
	Clostridium lentocellum SG6	Cellulose	Ravinder *et al.* (2001)
	Moorella thermoautotrophica DSM 7417; Clostridium thermoacetica DSM 2955	Milk Permeate	Talabardon *et al.* (2000)
	Moorella thermoacetica	Glucose	Shah & Cheryan (1995)

2.4 REVIEW ON ACETOGENIC BACTERIA

The acetogenic bacteria to be reviewed in this section are *C. thermoaceticum* (Fontaine *et al*., 1942), *C. ljungdahlii* (Gaddy & Clausen, 1992), *Eubacterium limosum* (Chang *et al*., 1999) and *Peptostreptococcus productus* (Grethlein & Mahendra, 1992).

2.4.1 *Clostridium thermoaceticum*

C. thermoaceticum is thermophilic and grows optimally at 55^{o}C and at pH 7 - 8 under an atmosphere of 100% CO_2 (Fontaine *et al*., 1942). It ferments fructose, glucose and xylose to acetic acid and pyruvate to acetic acid and CO_2. This organism can be adapted to grow on CO as an energy source with a doubling time of 18 hours (Grethlein and Mahendra, 1992).

2.4.2 *Clostridium ljungdahlii*

C. Ljungdahlii (strain PETC) is a Gram-positive, motile, rod-shaped anaerobic bacterium which sporulates infrequently. It is capable of growing on xylose and fructose. In 1987, *C. ljungdahlii* was reported to convert CO, H_2 and CO_2 to a mixture of acetic acid and ethanol (Klasson *et al*., 1992). The produced ethanol to acetic acid ratio is only 0.05 under anaerobic conditions. Previous study discovered that non-growth conditions for *C. Ljungdahlii* is favored for ethanol production while growth conditions favored for acetic acid production (Klasson *et al*., 1992).

2.4.3 *Peptostreptococcus productus*

P. productus, strain U-1, obtained from M.P. Bryant was reported to grow rapidly with CO as the energy source at mesophilic temperature (Lorowitz & Bryant, 1984). The doubling time for *P. productus* was 1.5 hr with acetic acid and CO_2 as the major products under 50% CO (Grethlain and Mahendra, 1992). A product yield of 0.25 mol acetic acid/ mol CO and a cell yield of 0.034 g cells/ g CO were obtained. Cell replication was inhibited at acetic acid concentration of 30 g/L (Vega *et al*., 1989).

2.4.4 *Eubacterium limosum*

E. limosum KIST 612 has been isolated from an anaerobic digester and able to grow rapidly under high CO concentrations. The growth was not inhibited up to CO

partial pressure of 101.3 kPa (1.0 atm) but was slightly inhibited at 202.6 kPa (2.0 atm) (Chang *et al*., 1999). Although *P. productus* U-1 can grow fast as *E. limosum* KIST 612 but *P. productus* was inhibited when P_{co} was higher than 60 kPa (Vega *et al*., 1989). *E limosum* exhibited a doubling time of 7 hours under P_{co} of 101.3 kPa and increased by 2 - 6 times under P_{co} of 151.9 kPa (Genthner & Bryant, 1982). The physiology and metabolism of *Clostridium aceticum* that is used as the biocatalyst in the study are discussed extensively in section 2.5.

2.5 *Clostridium aceticum*

Clostridium aceticum was the first acetogenic bacteria that have been demonstrated to produce acetic acid from H_2 and CO_2 (Wieringa 1936, 1940). Then, the organism was lost from 1948 till 1981, where it was recovered from a spore preparation of the original strain (Ljungdahlii, 1983). *Clostridium thermoaceticum* had replaced *C. aceticum* as the acetogenic bacteria in the research studies for nearly 40 years (Daniel *et al*., 1990). Therefore, only few researches have been carried on the strain of *C. aceticum* to produce acetic acid and the gaseous substrates used as carbon sources were only limited to H_2/CO_2 and methanol. Hence, there is a necessity to explore the potential of *C. aceticum* to convert other inorganic substrates like CO to acetic acid at ambient temperature and pressure. The aim of this study is to exploit *C. aceticum* as the biocatalyst in acetic acid fermentation.

2.5.1 Physiology and Biochemistry of *Clostridium aceticum*

Clostridium aceticum is a Gram-negative bacteria. The cells were rod-shaped with 0.8 - 1.0 μm wide and 5 μm long when grown chemolithotrophically. They are motile by means of peritrichously inserted flagella and round spores are formed on the polar region cell. The optimal growth conditions for *C. aceticum* is in mineral medium containing 0.2% yeast extract under an atmosphere of H_2/CO_2 at 30^{o}C and pH 8.5. *C.*