Design of a System for Lab-scale Production of Ethanol from Artificial Syngas

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EXECUTIVE SUMMARY

Due to the rising demand in ethanol as a fuel additive and as an alternative and environment friendly fuel, there has been interest in developing new and renewable techniques for production of ethanol. Syngas fermentation is another way to produce ethanol which uses some anaerobic autotrophs like *Clostridium ljungdahlii*, and *Clostridium autoethanogenum* in acidic medium (pH 4.5). Low mass transfer and low product yield are the main challenges of syngas fermentation which are inhibiting this technology to commercialize. The most commonly used fermenters are Stir Tank Reactor (STR) type which varies from lab scale (7L total capacity) to pilot scale (100L total capacity). Another lab scale fermenter can be designed using flasks as reactors and are economically much cheaper than STR techniques. In this study, four designs of the flask type bioreactors are proposed which has potential to introduce a new area of research at the Department of Agricultural and Biosystems Engineering, at North Dakota State University.

Four proposed designs for lab scale fermentation are based on two techniques (TYPE I and TYPE II). The design factors studied were temperature controlling device, pH of the solution, agitation speed, agitator type, syngas composition, gas flow rate, shape, size of bioreactor, bacteria, media selection, time of reaction, mass transfer techniques, and condenser within the bioreactor. However, the factors choose to be implemented are temperature controlling device, agitator type, syngas composition, shape and size of fermenter and mass transfer techniques. The designs use 250 ml flasks as a bioreactor with working volume of 160ml which run at 37^0C and 150 rpm. The flasks used in the designs were erlenmeyer flask, serum bottle, 3-neck spinner flask and 3-neck round bottom flask. The main components of the liquid content are bacterial cells, basal media, growth media and water. The yields in both proposed techniques are expected to be different because the mass transfer rate is different. However, mass transfer can be implemented via micro-spargers, increasing syngas flow rate or using hollow fiber membrane reactor.

CHAPTER I
INTRODUCTION

1.1 GENERAL

Increasing consumption in fossil fuels has made the U.S. to depend on the foreign oil rather than country's own production. Renewable fuels such as biofuels especially the use of ethanol as an energy source has the potential to decrease the U.S. dependency on foreign oil. Also, it is more environment friendly than other petroleum based transportation fuels. Currently, ethanol is produced by fermentation of biomass-derived sugars, starch for e.g. cane sugar in Brazil and corn starch in the United States. Most of these crops are either a food crop or, are being used in food industry production. So for sustainable ethanol production, non-food feedstock should be used.

Ligno-cellulosic biomass is the most abundant carbohydrate on earth i.e. about 60-65% of total carbohydrate available. Ligno-cellulosic biomass such as agri-residues (e.g. corn stover, wheat and barley straws), agri-processing byproducts (e.g. corn fiber, sugarcane bagasse, seed cake, etc.) and energy crops (e.g. switch grass, poplar etc.) do not compete with food and feed, and has the potential to be considered as a renewable feedstock. Some techniques can convert cellulosic biomass into ethanol but these techniques are still in the process of development and are not being used at a large scale. High pretreatment and enzymatic costs, low fermentability of 5-carbon sugars and generation of inhibitory soluble compounds are among the major challenges which cellulosic ethanol research teams are trying to overcome. On the other hand, any biomass (starch, cellulose, hemicelluloses, lignin etc.) can be gasified into synthesis gas or syngas (a mixture of CO and H_2) which can be converted to biofuels by using two pathways; thermochemical pathway (Fischer-Tropsch Synthesis) and fermentation pathway (syngas fermentation).

Synthesis gas or more commonly known as syngas is primarily a mixture of CO, H_2 and CO_2. It can be used as a feedstock in production of different kinds of fuels and chemicals and including electricity. The Fischer-Tropsch Synthesis (FTS) process involves the activity of metal catalysts at

different temperatures and pressures range to convert syngas into products like gasoline, diesel and alcohols. However, the FTS process has various limitations like temperature control to control the specificity of the product, specific requirements with H_2: CO ratio, specific use of biocatalyst and issues with metal sulfur poisoning (Bredwell et al., 1999; Munasinghe et al, 2010). The syngas fermentation into ethanol is considered to be more attractive over FTS process because of lower specific requirements with the gas composition and independency upon catalyst poisoning etc. Composition of syngas depends upon the biomass feedstock and the combustion process like whether oxygen or air is used to facilitate the combustion process inside the gasifier. Low sulfur is produced in the gasifier unless tires are used as biomass feedstock, and nitrogen dioxide is produced if air is used for combustion. Gases can be mixed together manually in the form of compressed cylinders to produce an artificial form of syngas. The compressed syngas is used as a source of gas in designing the lab scale system for syngas fermentation. The process of the syngas fermentation has not grown up to commercial scale because of several limitations such as low productivity and poor solubility of gaseous substrates in the liquid phase. The process of syngas fermentation was studied at bench scale and lab scale by reviewing the existing literature on biomass-derived syngas fermentation into ethanol.

1.2 RATIONALE

Until now, not many facilities have been setup for Syngas fermentation. A design proposal for lab scale setup for syngas fermentation can introduce an area of research at Department of Agricultural and Biosystems Engineering, at North Dakota State University. Studying the described process for designing a lab scale system will enable the concerned faculties and students to get familiar with the process of syngas fermentation and it can be an area of research at NDSU in the near future.

1.3 OBJECTIVES

The main objective of the project was to develop syngas fermentation facility to produce ethanol. The specific objectives of this project are to design lab scale syngas fermenter/bioreactors by reviewing the existing literature.

CHAPTER II
LITERATURE REVIEW

2.1 SYNGAS AT INDUSTRIAL-SCALE

Some of the prominent industries in ethanol production studied under this project are- ***Zeachem Inc.*** *Colorado U.S****., Syntec Inc.,*** *Vancouver, Canada,* ***Coskata industries, Warrenville*** *IL,* ***Ze-gen Inc.,*** *Massachusetts,* etc. For instance ***Ze-gen Inc. industry*** has a technology that turns scrap metal into a 2800 degree metal bath and injects construction debris deep into the bubbling cauldron, but this process is only for the production of syngas. Also, ***Inentec Industry*** (based in Bend, Oregon) uses municipal solid waste as feed stock and plasma gasification. The Plasma Enhanced Melter (PEM^{TM}) gasification system is a relatively new technology which essentially creates an artificial bolt of lightning that vaporizes materials which can be applied to solid waste and thus products like ethanol, methanol, etc are produced. They use channel induction furnaces for the production of syngas. Syntec Inc. has developed a certain catalyst for the production of ethanol from syngas which has been named as syntec catalyst. While ***Zeachem Inc industry*** process for the production of ethanol from syngas combines the output of the two traditional ethanol production pathways (fermentation of sugars and gasification of biomass) into a third catalytically-driven step- hydrogenation. Biomass is chemically fractioned to produce a sugar stream containing both xylose and glucose sugars and a lignin residue stream. The sugars are fermented by a carbon-efficient acetogen called as *Clostridium thermoaceticum* (found in termites) to acetic acid without CO_2 as a by-product. The acetic acid is converted to an ester which is then reacted with hydrogen to produce ethanol. The *Zeachem industry* has successfully produced acetogens at the lab scale for more than 1000 fermentation trials of syngas as well as hydrozylate derived from cellulosic biomass.

Process of fermentation takes place inside a fermentor, also called a bioreactor. Inside this equipment the bacteria must be able to convert syngas in to ethanol. Trickle bed reactors are very common in industry. The figure 1 shows the process occurring inside the reactor.

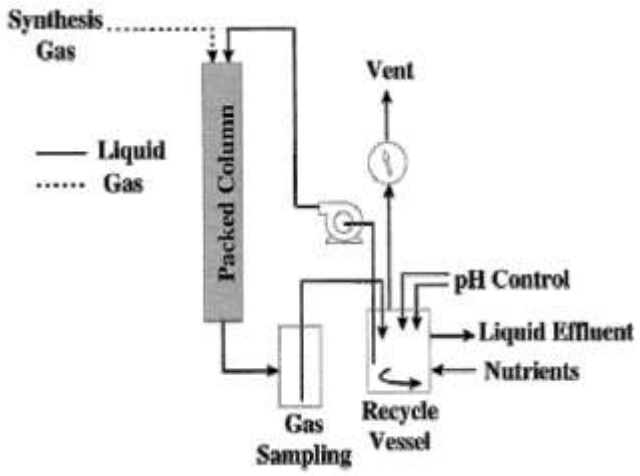

Figure 1 line diagram of the process occurring inside the trickle bed reactor (Kasteren et al., 2005)

Experimental studies have shown that the rate limiting step in syngas fermentation is typically *gas-to-liquid mass transfer* (Kasteren et al., 2005). This means that if ways can be found out to increase the gas-liquid mixing process, the reaction rate will increase.

2.2 CHEMICAL CATALYSIS OF SYNGAS

A burner designed to efficiently and cleanly burn biomass-based gas along with a new catalyst converts the synthesis gas directly into ethanol. Here it is discussed that the carbon-based nano-particles made from graphite, carry a transition metal which produces a chemical reaction. That reaction will be helpful to convert synthesis gas to ethanol. Although, the researchers working on this project already have an existing chemical catalyst that can convert synthesis gas to ethanol. But that catalyst yields very low concentrations of ethanol, whereby producing green house gases. The new catalyst should be able to work at lower temperatures and pressures while capable of delivering higher concentrations of ethanol.

2.3 SYNGAS AT PILOT-SCALE

2.3.1 Syngas fermentation facility

The main research for the project started from the broad view of the syngas fermentation facility. One of the reports studied was “Bio-ethanol from bio-syngas” (Kasteren et al., 2005) which describes the economical and technical feasibility of a process for the conversion of biomass (wood) into syngas with subsequent biological conversion to ethanol. Some of important facts from the report were the advantage of *syngas ethanol* over *cellulosic ethanol* in which all kinds of biomass can be treated, even waste streams and lignin content of wood which is practically impossible in cellulosic ethanol. The production of ethanol from cellulose is another prominent method in which the lignocellulose, consisting cellulose, hemicelluloses and lignin, is converted to ethanol although it is difficult to break the sugar molecules present in cellulose to ferment into ethanol (NREL, 2007).

It was also found that in overall design of the plant, most of costs were related to the costs of the fermentor unit. Here overall plant design includes a gasification unit, gas purification unit, fermentation unit, and distillation unit and that is due to the low solubility of syngas in water. The conversion of biomass to ethanol depends strongly on the mass transfer of the syngas to water in order for the bacteria to convert it to ethanol. Meanwhile, using the same condition it was also found that there are bacteria which can produce butanol besides ethanol. However for research, lab scale production of the ethanol from syngas is also in use.

In another research (Rajagopalan et al., 2002) at Oklahoma State University, *Clostridium Ljungdahlii* bacteria (isolated from an agriculture lagoon) has been used as a catalyst for the production of ethanol from syngas which showed yields of 0.062 for ethanol and 0.094 for acetic acid and cell yield of 1.378 g/mol.

The process of acid hydrolysis, used for the research (Rajagopalan et al., 2002), consists of steps in which the feedstock is pretreated to remove the lignin from the lignocelluloses. The cellulose and hemicelluloses are treated with dilute sulfuric acid (0.2%-2%) at 120-200^{0}C to produce fermentable syrup containing five or six carbon sugars. Microbial catalysts such as E. Coli have been used to convert into ethanol (NREL, 2007). Also, experimentation was done for the production of ethanol by using new bacteria P7 as a catalyst which was provided by Ralph Tanner from the University of Oklahoma. For the growth of the bacteria strict anoxic technology was used. The growth media had some trace metal and vitamin solutions whereas bioreactor media, which contained Pfennig's minerals and trace metals along with vitamins and reducing agents, was used to cultivate cells. Additionally, some experimentation was done where a column is generated to enhance the gas-liquid mass transfer.

2.3.2 Bubble Column Design and Operation

The experiments were performed in a bubble column bioreactor made from plexiglass (Klasson et al, 1992). A fritted glass disc was attached to the base so that the feed gas could be dispersed as bubbles. Liquid was re-circulated at 200/300 ml/min for enhancing the mixing. The liquid feed was kept in a nitrogen atmosphere and was taken through the bioreactor by a 0.5 µm pore size sterilization filter at 2 ml/min by using a peristaltic pump. While media was being added and deoxygenated in other tank for future use, the feed gas flow rate was 200 cm^3 at 5 psig and 25^0 C going inside the bioreactor. The pH was initially around 5.75 but was then controlled around 5.3 using a ph controller and the temperature was maintained at 37^0 C using a warm water jacket. The bubble column was kept under sterile conditions and inoculum was transferred to the bioreactor using sterilized disposable syringes. Then the cells were grown for at least 3 days in batch mode and afterwards liquid feed and products were withdrawn at 2ml/min.

2.4 SYNGAS AT LAB SCALE

Lab scale ethanol production from syngas provides a good opportunity for research in this field. A paper on "the production of ethanol from synthesis gas using the bacteria *clostridium ljungdahlii*" from the *Universiti Sains Malaysia* was found useful. It was found that the researchers grew the bacteria in ATCC (American Type Culture Collection) media at 37^0 C in an incubator shaker. The microbial culture was maintained in Wheaton serum bottles. Gas impermeable butyl rubber septum-type stoppers and aluminum crimp seals were used to seal the bottles (2). The bottles were sterilized at 121^0 C in an autoclave. An orbital shaker incubator was used for providing agitation. A seed culture was prepared which was used for inoculation. That inoculation culture was grown under syngas. The argon was used as an internal standard. Liquid samples were directly withdrawn from the bioreactor with the help of a sterile syringe. It was concluded that microbial cell concentration increased with the uptake of CO in the presence of the bacteria and it was found that hydrogen as an electron acceptor may promote ethanol production. Also, it is speculated that higher concentration of ethanol and acetate was the result of CO_2/H_2 utilization occurring at a total pressure of 1.6 and 1.8 atm. It was also found out that under batch operations *C .ljungdahlii* produced acetate as a major product which may be due to the acetyl-CoA pathway which showed that the production of acetate is balanced with ATP unlike ethanol production that resulted in net consumption of ATP. The maximum acetate and ethanol concentration were found to be 1.4 and 0.6 gL^{-1} in the batch bioreactor.

For lab scale idea a thesis titled "Production of ethanol from the fermentation of synthesis gas" (Kasteren et al., 2005) was also studied. The objective of this thesis was to test and optimize a two-step process (cell growth and fermentation) for ethanol production from synthesis gas. The bacterium was grown in a fructose-rich medium then concentrated in ethanol production medium for synthesis gas fermentation. While the known ethanol-producing bacterium *clostridium ljungdahlii* was used to provide baseline values for syngas utilization and ethanol production, syngas fermentation was conducted with a culture discovered at Mississippi State University.

2.4.1 Bacterium as *Biocatalyst:*

Bio-catalysis has some advantages over the metal catalysis. Bacterium requires less specificity in gas composition for the conversion. They are more tolerant to sulfur and contaminants in the medium, and result a higher yield. Additionally, bacteria metabolism follow complicated enzymatic pathways, which are more difficult to reverse, which allows for the stable and complete conversion of the synthesis gas to the desired product.

The bacteria used in this process are anaerobic microorganisms which produce compounds like ethanol, acetate, n-butanol, butyrate, and methanol in various metabolic pathways. For this project, microorganisms studied are *Clostridium autoethanogenum* and *Clostridium ljungdahlii*. *Clostridium autoethanogenum* is a gram-positive, motile, rod-shaped bacterium. The optimum growth condition occurs at 37^0C and pH 5.8-6.0. It converts carbon monoxide and carbon dioxide to ethanol and acetate. Ethanol concentrations range from 1.56mmol/l to 7.71mmol/l and acetate concentrations in the 5.62mmol/l to 7.96mmol/l range were detected under growth conditions. Conversion of CO to ethanol and acetate by *C. autoethanogenum* follows the stochiometry of equations as shown below:

$$4\ CO + 2\ H_2O \rightarrow CH_3COOH + CO_2 \qquad \text{---Eq (1)}$$

$$6\ CO + 3\ H_2O \rightarrow CH_3CH_2OH + 4\ CO_2 \qquad \text{--- Eq(2)}$$

Clostridium ljungdahlii is a gram-positive, rod shaped, motile anaerobic bacterium capable of producing ethanol and acetate from CO, CO_2, and H_2.The end product composition from the synthesis gas fermentation is highly dependent on pH. Growth, ethanol production, and acetate production occur at an optimal temperature of 37ºC (Gaddy and Clausen, 1992). A pH range of 4.0 to pH 4.5 favored production of ethanol while pH 5.0 to pH 7.0 favored acetate production. The stochiometry of synthesis gas fermentation to ethanol and acetate is as follows (Klasson et al., 1992a):

$$6\ CO + 3\ H_2O \rightarrow CH_3CH_2OH + 4\ CO_2 \qquad \text{Eq (3)}$$

$$2\ CO_2 + 6\ H_2 \rightarrow CH_3CH_2OH + 3\ H_2O \qquad \text{Eq (4)}$$

$$4\ CO + 2\ H_2O \rightarrow CH_3COOH + 2\ CO_2 \qquad \text{Eq (5)}$$

$$2\ CO_2 + 4\ H_2 \rightarrow CH_3COOH + 2\ H_2O \qquad \text{Eq (6)}$$

Ethanol concentrations were found to be less than 1 g/l with an ethanol to acetate molar ratio of 0.05 at pH 5.0 under batch conditions (Gaddy and Clausen, 1992). Lowering the medium pH in a batch reactor to pH 4.5 yielded an increase in ethanol production to a concentration of 7g/l, and a molar ratio of 9 moles ethanol per mole acetate produced.

2.4.2 Media for *Clostridium ljungdahlii:*

Media plays an important role in the production process. It is required by the bacteria as a nutrient for cell growth. Meanwhile, constituents of media can also be helpful to achieve the desired product. No effect was observed by yeast extract on the observed ethanol to acetate ratios (Klasson et al., 1992). Phillips et al. (1993) attempted to develop an ideal medium for cell growth on synthesis gas and ethanol and acetate production from synthesis gas. Pfennig's Basal Medium table was used initially to culture *C. ljungdahlii* by Phillips et al. Changing component concentrations resulted some important facts. Doubling medium concentrations created a hypertonic solution that inhibited growth. Trace metal concentrations were determined to be the growth-limiting factor. The formulation for the final designer medium developed by Phillips et al. is outlined in Table 1. This medium was used in efforts to culture *C. ljungdahlii* at Mississippi State University.

Table 1 List of elements required for original basal media, defined media and designed media. Designed media was the media designed for maximum concentration of ethanol/acetate

Basal Media Comparison (in mg/L)			
Pfenning's minerals	**Original medium**	**Defined medium**	**Designed medium**
KH2PO4	500	500	0
MgCl2.6H2O	330	330	500
NaCl	400	440	200
NH4Cl	400	400	0
CaCl2.2H2O	50	50	200
Pfenning trace metals			
ZnSO4.7H2O	0.1	0.1	1
MnCl2.4H20	0.03	0.03	0.3
H3BO3	0.3	0.3	3
CoCl2.6H2O	0.2	0.2	2
CuCl2.H2O	0.01	0.01	0.1
NiCl2.6H2O	0.02	0.02	0.2
FeCl2.4H2O	0.03	0.03	0.3
Na2SeO3	1.5	1.5	15
B-vitamins			
Biotin	0.1	0.05	0.106
Folic acid	0.1	0.05	0.005
Pyridoxal-HCl	0.05	0.025	0.0025
Lipoic acid	0.3	0.15	0.015
Riboflavin	0.25	0.125	0.0125
Thiamine-HCl	0.25	0.125	0.266
Ca-D-panththenate	0.25	0.125	0.413
Cyanocobalamin	0.25	0.125	0.0125
P-aminobenzoic acid	0.25	0.125	0.0125
Nicotinic acid	0.25	0.125	0.0125
Supplements			
Yeast extract	1000	0	0
(NH4)2HPO4	0	0	2000
H3PO3	0	0	1.5
NaH2CO3	2500	0	0
KCl	0	0	150

Courtesy of Phillips et al. 1993

Several laboratory test methods for evaluating ethanol production from syngas gas fermentation have been studied. These bench scale methods vary by reactor type, reactor size, media components, bacterial source, and gas composition.

1st Method

Bredwell et al. (1999) reported the best ethanol concentration produced by *C. ljungdahlii* in a batch reactor which came out as 1 g/l. Vega et al. run experiments on batch process with Clostridium ljungdahlii. They prepared Pfennig Basal Medium under an 80% N_2 and 20% CO_2 atmosphere and adjusted pH to 5.0. Serum bottles, each with a total volume of 1216 ml, were filled with 200 ml of medium and sealed with butyl rubber stoppers. The medium and bottles were autoclaved for 20 minutes at 121ºC. Before the culture was added, reducing agents L-cysteine and sodium sulfide were added. A concentration of 50ppm of reducing agent when added to the production medium could increase ethanol to acetate ratio from 0.12mol ethanol/mol acetate to 0.20 mol ethanol/mol acetate. The bottles were sparged with synthesis gas and pressurized. Argon was added as an inert gas. The reactors were incubated at 37ºC on a shaker incubator at 100 rpm. These batch fermentations were reported to be "highly irreproducible" (Vega et al., 1989).

2nd Method

Philips et al. (1994) conducted further batch studies on *C. ljungdahlii* in a basal medium. The basal medium (see Table 1) was prepared under a nitrogen atmosphere and adjusted to a pH of 4.5 with 0.5 M NH_4OH. Total volume of 158 ml serum bottles were used with 50ml of medium. The bottles were sealed with butyl rubber stoppers and autoclaved. L-cysteine was added to the medium as a reducing agent before *C. ljungdahlii* was added to it. The vials were flushed with synthesis gas composed of 24% H_2, 65% CO, and 11% CO_2. Methane was used as an inert gas with concentration of 50ml and the bottles were incubated on a shaker incubator at 37ºC and 130 rpm. Ethanol production was not reported for these experiments.

3rd Method

Batch experiments were performed by Klasson et al. (1991) using the designed basal medium with *C. ljungdahlii*. Total volume of 157.5 ml serum bottles were used to perform the experiments. The prepared medium was dispensed into serum bottles and sealed with butyl rubber stoppers. Reducing agents (methyl viologen and benzyl viologen) were added to alter electron flow for ethanol production. The bottles were incubated on a shaker incubator in the dark at 100 rpm and 37ºC. The bottles were only removed from the dark incubator for a maximum of 3 minutes each time when samples were taken. Without the addition of benzyl viologen, the highest achieved ratio of ethanol to acetate was 0.658 while adding 30ppm benzyl viologen caused an increase in ethanol to acetate production ratio to 1.1(Phillips et al., 1994).

CHAPTER III
MATERIAL AND METHODOLOGY

3.1 MATERIALS

An itemized list of the materials that were required in the design was prepared. The diameter of the cylinder (K-size) used was 9 inch with height being 51 inch. The composition of the syngas in the cylinder was 50%CO + 50% H_2 which has CGA 350 pressure regulator available from Praxair Inc., Fargo. The vessels used in the design of experiment are 250ml spinner flask, 250 ml of round bottom flask, 250 ml of Erlenmeyer flask and 250 ml of serum bottle, a PYREX® fritted cylinder gas dispersion tube which are available from VWR scientific, Philadelphia. The flasks used have a common working volume of 160 ml. Other essential elements of use are butyl rubber stopper, incubator shaker (Bench top water bath), aglass-body AccuTupH pH probe (AccuStandard Inc, New Haven, Connecticut), Hamilton 100 microliter Gas Tight Syringes. Chemicals used in basal media are available at the Sigma-Aldrich, St. Louis, Missouri or VWR-scientific, Arlington Heights, Illinois. The bacteria strains, nutrients for growth media and basal media are available at (American Type Culture Collection) ATCC. Itemized list in Appendix I may be referred for a detailed list of materials.

3.2 METHODOLOGY

Process for inoculation of bacterial cell culture was studied and developed. Number of different methods has been developed for designing the system unit for the production of ethanol from Syngas. A continued feed system i.e. continuous supply of syngas is adopted for all the design methods. Mainly two different techniques were described in the design of the experimental setup. Depending upon the source of agitation (shaking incubator or stir bar) two techniques (TYPE I, TYPE II) were developed and reaction

system were designed. TYPE I has erlenmeyer flask and serum bottle as bioreactors while TYPE II has spinner flask and round bottom flask as bioreactor.

3.3 ORGANISM AND INOCULUM PREPARATIONS

3.3.**1. Clostridium Ljungdahlii**

Although several bacteria have been found to produce ethanol under strict anaerobic conditions but for the purpose of designing system at bench scale for ethanol production from syngas the bacteria *Clostridium Ljungdahlii* was found to be most suitable. For that fact, research was done on how to extract and grow this bacterium and use for the purpose of producing ethanol from syngas. More information regarding obtaining the bacteria *Clostridium Ljungdahlii* strain was found through the official website of ATCC. The ATCC 55383 was found as the redeposit of the original strain (ATCC 49587) which was extracted from the chicken waste in the university of Arkansas laboratories. The bacterium is available online from the ATCC website for the experiment.

3.3.**2. Media**

As only strain of bacteria is available in the market so, a growth media is required to grow the strain. For this purpose a growth media was also found to be available on the website of ATCC which has specific contents since the bacteria require strict anoxic conditions to grow. Also, contents of basal media used for the purpose of extracting ethanol were found. It required pfenning's minerals, some particular trace materials and some supplements such as potassium chloride and yeast extract. More information regarding the contents of the media were found from some of the research studies. In one of the study conducted at University of Arkansas (Phillips et al, 1993) two different media were used, other than the specific Basal media, which were prepared while working on ethanol production from syngas just to increase the productivity of ethanol. In these medium some of the quantity of the minerals and trace metals were changed which had an effect on the overall production of the ethanol from the fermentation process using syngas. In the paper the actual concentration of each metal and mineral is also defined so it is easy to prepare even those media if required (refer table 2, 3 and 4) for each element concentrations).

Strain growth media
ATCC medium: 1754 PETC medium

Table 2 List of growth media required for bacterial culture

NH_4Cl	1.0 g
KCl	0.1 g
$MgSo_4 . 7H_2O$	0.2 g
NaCl	0.8 g
KH_2PO_4	0.1 g
$CaCl_2 . 2H_2O$	20.0 mg
Yeast extract	1.0 g
Trace Elements (see below)	10.0 ml
Wolfe's Vitamin Solution (see below)	10.0 ml
$NaHCO_3$	2.0 g
Fructose	5.0 g
Reducing Agent (see below)	10.0 ml
Distilled water	980.0 ml

(Courtesy: ATCC official website)

Table 3 List of trace elements required for culture media

Trace Elements:	
Nitrilotriacetic acid	2.0 g
$MnSO_4 . H_2O$	1.0 g
$Fe(SO_4)_2(NH_4)_2 . 6H_2O$	0.8 g
$CoCl_2 . 6H_2O$	0.2 g
$ZnSO_4 . 7H_2O$	0.2 mg
$CuCl_2 . 2H_2O$	20.0 mg
$NiCl_2 . 6H_2O$	20.0 mg
$Na_2MoO_4 . 2H_2O$	20.0 mg
Na_2SeO_4	20.0 mg
Na_2WO_4	20.0 mg
Distilled water	1.0 L

(Courtesy: ATCC official website)

Table 4 List of wolf's vitamin solution

Wolfe's Vitamin Solution:	
Biotin	2.0 mg
Folic acid	2.0 mg
Pyridoxine hydrochloride	10.0 mg
Thiamine . HCl	5.0 mg
Riboflavin	5.0 mg
Nicotinic acid	5.0 mg
Calcium D-(+)- pantothenate	5.0 mg
Vitamin B12	0.1 mg
p-Aminobenzoic acid	5.0 mg
Thioctic acid	5.0 mg
Distilled water	1.0 L
Reducing Agent:	
NaOH	0.9 g
L-Cysteine. HCl	4.0 g
$Na_2S.9H_2O$	4.0 g
distilled water	100.0 ml

(Courtesy: ATCC official website)

CLOSTRIDIAL GROWTH MEDIA PREPARATION

ATCC recommended medium PETC 1754 is used for growth of the bacteria clostridium ljungdahlii. Also, the method is modified for the proper growth of the bacterial strain which includes addition of 10 g beef extract, 10g proteose peptose, .5 g cysteine-HCl and .5 ml 0.1% resazurin to the PETC 154 medium. Firstly, all the ingredients including 10 ml wolfe's vitamins solution and 10 ml trace elements solution are added together and mixed with 1 L distilled water. The pH is adjusted at 5.5. Then $NaHCO_3$ is added and the solution is boiled, degassed and dispensed anaerobically under pure CO_2. Then fructose and reducing agents are added from sterile anaerobic stock solutions prepared under pure N_2.Then other ingredients other than PETC 1754 are added to the medium such as beef extract. Autoclaving is done at 121^o C for 15 minutes.

For preparing trace elements solution nitrolotriacetic acid is added to water and the pH is then adjusted to 6.0 with KOH. Then, remaining ingredients are added to it. For preparing reducing agent NaOH and water both are boiled under pure N_2. Then cysteine is added to the solution. Thereafter Na_2S is added.

The Clostridium ljungdahlii strain is transferred anaerobically from the frozen stock at -80^o C (Cotter et al, 2009) into balch tubes containing the 10 ml clostridial growth media. Incubate the culture at 37^o C for 48 hours.

BASAL MEDIA PREPARATION FOR ETHANOL PRODUCTION:

The basal medium has to be either prepared with the required materials given in the table 1 (section 2) or can be purchased accordingly. The bacterial culture is stored on the basal medium at ph 5 and at 37^o C. The medium preparation has to be carried out under nitrogen atmosphere (Phillips et al, 1993). The basal medium basically contains Pfenning minerals, trace metals, B-vitamins and supplements such as yeast extract. In one of the experiments done in University of Arkansas (Phillips et al, 1993) the original basal medium was defined accordingly by changing the quantity of some of the trace metals and

vitamins. Also, another media was prepared called as designed media which when used provided 23g/L of ethanol concentration as compared to the original basal medium which provided 1.5 g/L. This designed media has some more components added to it such as KCl, H_3PO_4 and $(NH_4)_2HPO_4$ whereas the yeast extract was completely removed from both the defined and designed media (Phillips et al, 1993). Either of the basal media can be used. But for initial experimentation purposes the original basal media is used for the growth of clostridium Ljungdahlii.

In the experiment of basal medium is stored in the experimental flask. The flask is flushed with syngas (50% CO_2 and 50% H_2). 15 % inoculums are transferred into the basal medium and the growth curve is analyzed as the dense growth is achieved over the period of time. Culture is transferred weekly and the fresh gas is supplied every day.

The maximum cell mass is calculated by assuming that each element present in the basal medium behaves as a limiting element when its turn comes. Then the lowest calculated mass gives the predicted growth potential and defines the expected limiting nutrient (Phillips et al, 1993).

The flow chart (figure 2) shows the whole procedure from production of the inoculum to the process taking place inside the fermenter. Firstly, the frozen bacterial is grown on a clostridium growth media (ATCC 1754) in serum bottles and then incubated for 48 hours. After the inoculum has been prepared the preparation of basal media takes place (see table 5). Thereafter the whole setup is sterilized. The setup is then flushed through nitrogen to ensure anoxic conditions inside the setup. Then the basal media is poured in the flask and syngas is flushed inside the flask. Soon the inoculum is added to the mixture at a rate of 10 % vv^{-1}. The growth of the bacterial cell bimass takes place over a period of time. After the product growth takes place the product cell and gas concentrations are analyzed using UV Spectrometer and Gas Chromatograph Analyser.

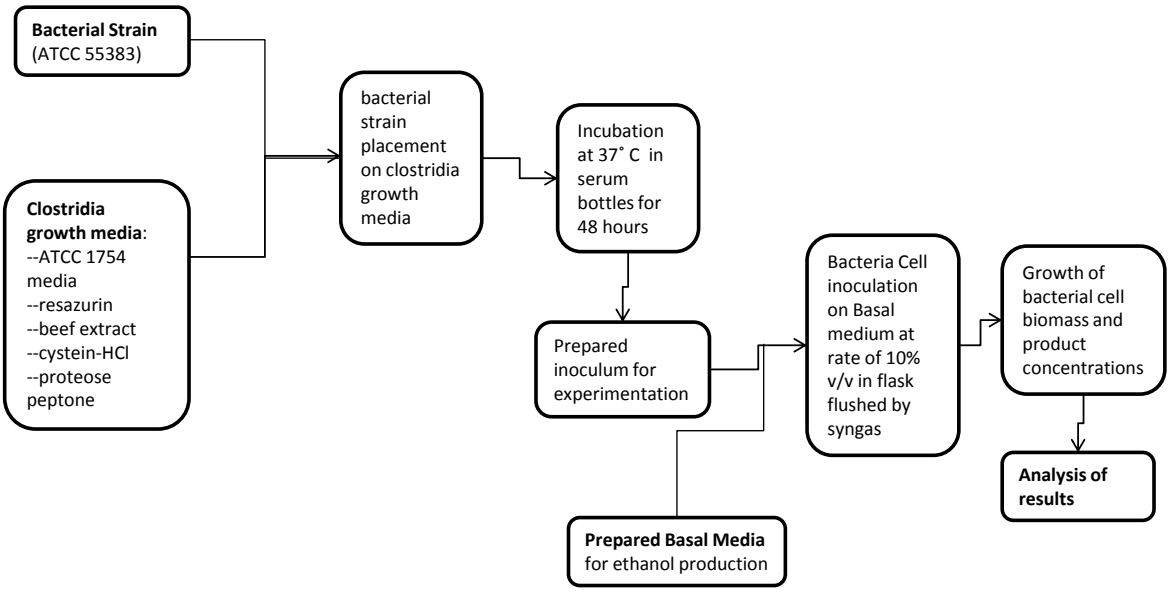

Figure 2 a flow chart of growth of bacterial strain with the used of clostridia growth media and ethanol production using basal media

For preparation of clostridium growth media (section 3.3.2) all the ingredients consisting of Pfennig minerals, trace metals along with B-vitamins and supplements are mixed with 1 liter of distilled water. After the pH is adjusted at 5.5 autoclaving is done for 20 minutes at 121^{o} C.

Table 5 the list of chemicals required for the preparation of basal medium

Pfennig minerals	**KH_2PO_4** **$MgCl_2.6H_2O$** **NaCl** **NH_4Cl** **$CaCl_2.2H_2O$**
Pfennig trace metals	**$ZnSO_4.7H_2O$ $MnCl_2.4H_20$ H_3BO_3 $CoCl_2.6H_2O$ $CuCl_2.H_2O$ $NiCl_2.6H_2O$ $FeCl_2.4H_2O$ Na_2SeO_3**
B-vitamins	**Biotin** **Folic acid** **Pyridoxal-HCl** **Lipoic acid** **Riboflavin** **Thiamine-HCl** **Ca-D-panththenate** **Cyanocobalamin** **P-aminobenzoic acid** **Nicotinic acid**
Supplements	**Yeast extract** **$(NH_4)_2HPO_4$** **H_3PO_3** **KCl**

CHAPTER IV
PROCEDURE AND EQUIPMENTS

4.1 THEORY

All the proposed designs follow same theoretical concept. As shown in figure 3 compressed syngas from cylinder is sparged to the vessel via plastic hose. Gas regulator controls the pressure of the gas while the gas flow meter controls the flow rate of the gas. The heating and agitation is controlled by a stir and hot plate E. The time of the reaction can vary depending upon the desired ethanol yield. The time range used in this design is 30-50 hours. After an optimum time of 30 hours, ethanol is expected to produce at the bottom of the flask.

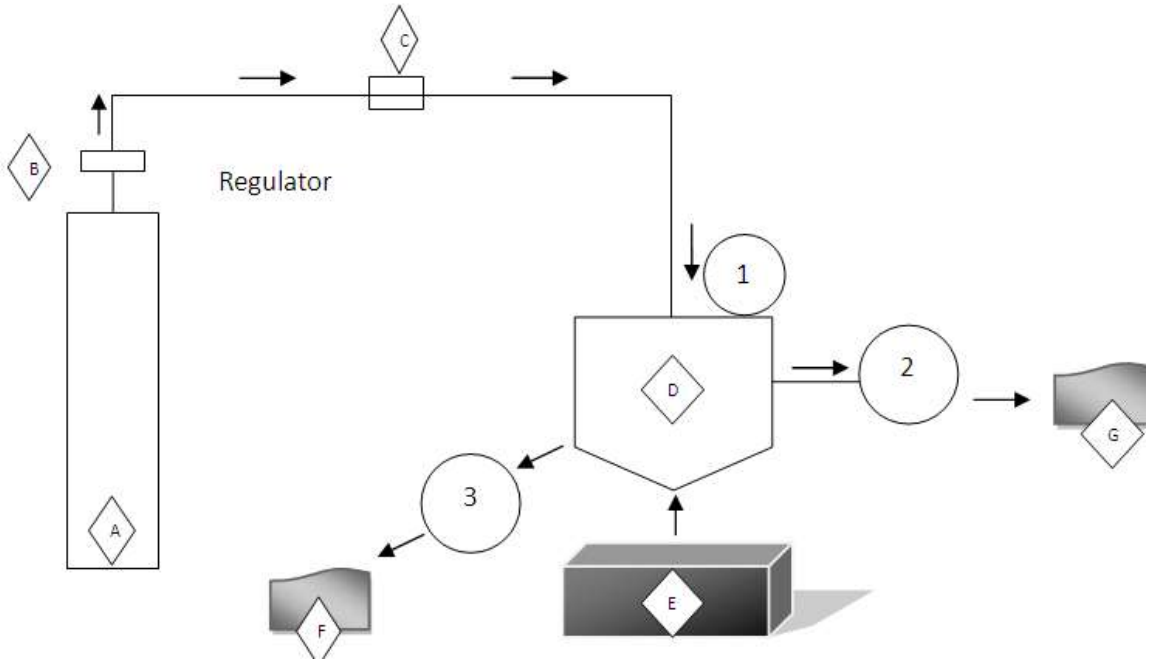

Figure 3 the flow diagram of overall process for lab scale fermentation design

A-Artificial Syngas, B- Gas Regulator(CGA 350), C- Gas flow meter, D- Bioreactor, E- Heating and stir plate (37^0C at 150 rpm), F- HPLC (High Performance Liquid Chromatography), G- GC (Gas Chromatography)

Process 1: Gas Inlet, Process 2: Exhaust gas through syringe filter, Process 3: Sampling via gas tight syringe

Batch production of ethanol takes place inside a reactor (flask) which contains liquid media. It has continues supply of feed of the gaseous substrate to enhance the yield of product. Due to toxic nature of carbon monoxide and flammable behavior of syngas, the reaction setup is placed inside a fume hood which has access to ventilation. Tubing, reactor, water bath, heat and stir plate and stand are kept inside the hood. The gases are regulated at low pressure to prevent overpressure of the reactor.

The flasks used in all designs have working volume of 160ml. The first step of the reaction is to steam sterilize the reaction flask (reactor) at 121^0C for 20 min. (Cotter et al, 2009). The steam sterilized flask is then attached to the setup and syngas is sparged inside the flask for about 1-2 min. Methane, 50ml can also be added as a reference inert. Then, 100 ml of basal medium is added to the reactor. The medium is agitated for 30min. before supplying gas to it. A gas supply is turned on at a pressure (16psi) correspondent to flow rate of 7.5ml/min. Bacterial culture is added through rubber septum with the use of syringe. All the experiments are performed at 37^0C at 125-140 rpm at about 15-20 psi (200kPa). The pH of the reaction medium is controlled by adding either 0.5M NH_4OH or 0.5M NaOH. The optimum time of the reaction is about 30-50 hours depending upon the yield of ethanol desired until a detectable amount of yield is not noticed. It is experimentally considered that volume of ethanol, acetate and cell culture will increases overtime (Cotter et al, 2009). Liquid sample removed from the bottom of the flask is tested with High Performance Liquid Chromatography (HPLC) for the % concentration of ethanol in the sample. Gas sample removed via gas filter is tested for the presence of % of carbon dioxide and hydrogen mixture using GC chromatography.

4.2 SYNGAS SOURCE

The K-size high pressure steel cylinder with internal capacity of 48.2 liters (see Appendix II for details) is used to provide syngas to the bioreactor. A CGA 350 type regulator regulates the outlet pressure of the gas. The ratio of carbon monoxide and hydrogen can be different but for this experiment a ratio of 50:50 of carbon monoxide with hydrogen is used. The cylinder can contain total of 215 ft^3 of gas at a pressure of 1660 psi and 70^0F temperature. To obtain these information two companies were contacted and they provided information on cylinder types and their cost. *The Paraxair* provided information on three cylinders- Q cylinder, K cylinder and T cylinder. While *Airgas* provided with information on 33 A type cylinder with gas amount of 33 ft^3. (see Appendix I for cost)

4.3 DESIGN OF THE SETUP

Two techniques were proposed to design the setup: TYPE I and TYPE II. These were based on the type of flasks and way of agitation in conducting the experiment. Both techniques use 250 ml flask as a bioreactor which has a working volume of 160ml. The main components of the liquid content are bacterial cells, basal media, growth media and water. The yields in both proposed techniques are expected to be different which would depend upon the type of reaction vessel used.

Figure 4 the shaker incubator available at NDSU Pilot Plant to be used in Technique I

4.3.1 TYPE I

This technique facilitates the repetition process of the experiment in which the bioreactor i.e. reaction flask is kept in an agitator bath to aid the gas liquid mass transfer. With this technique, **Temperature and agitation is controlled more accurately as a shaker incubator** is used shown in figure 4. The experiments can be conducted by running more than one flask (maximum 14 250 ml flask) with each flask having its own supply of syngas from the cylinder. The head of the flask have a butyl rubber cork which have passage for the gas inlet and exhaust air. The syngas inlet tube is also connected to a gas dispersion cylinder to enhance the dispersion of the gas into the liquid. The flasks also contain 160 ml of mixture of media (growth and basal) in water solution.

1) Design 1

In this design, the K size cylinder with gas regulator and gas flow meter attached through a hose is connected to the flasks (250ml). As explained earlier (section 4.2), each flask is connected to the cylinder through hoses which are flexible to the movement of the flasks at shaker incubator's shaking radius. When gas is sparged inside the flask through gas dispersion cylinder, it is expected that gas would

come in contact with the liquid media and bacterial cell culture. As shown in equation 3-6 in chapter II, the evolution of carbon dioxide is expected in aid to production of ethanol. Most of this carbon dioxide, usually reacts with hydrogen to produce more ethanol, so it is a auto synthesis reaction. However, carbon dioxide is also expectd in the exhaust air. For adding cell culture to the flask, two methods can be used. It can either be added by a long needle syringe through the rubber cork or by flexible end needle syringe through the side arm of the Erlenmeyer flask.

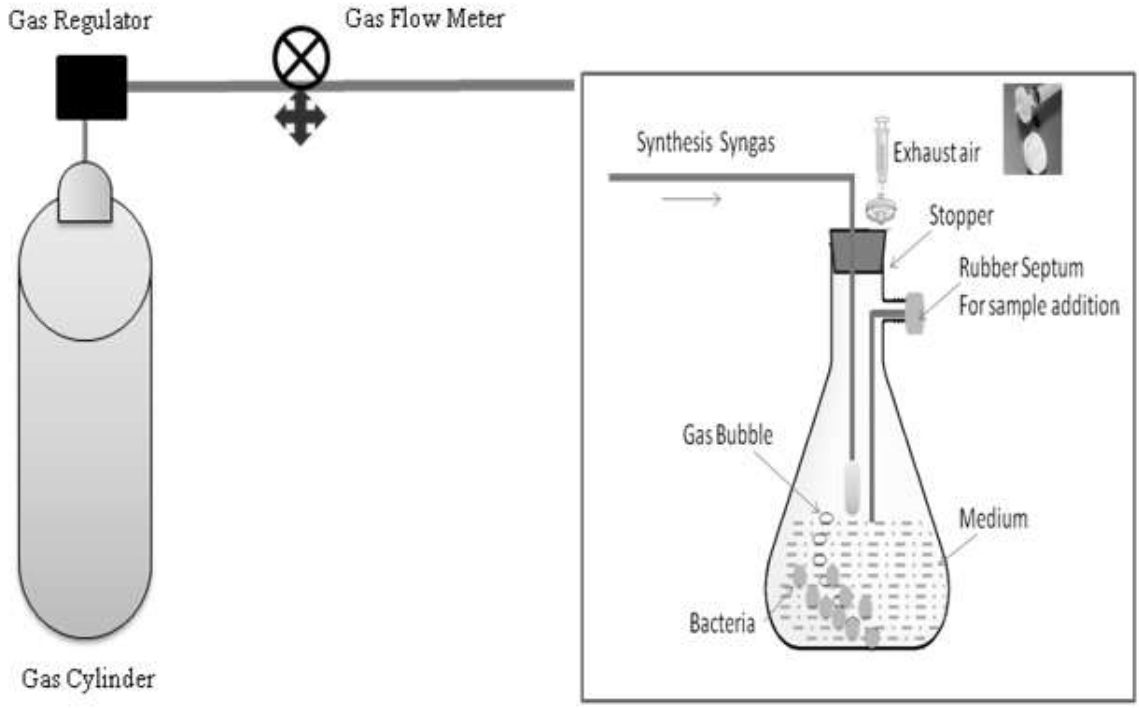

Figure 5 the experimental setup for DESIGN I of technique TYPE I

2) Design 2

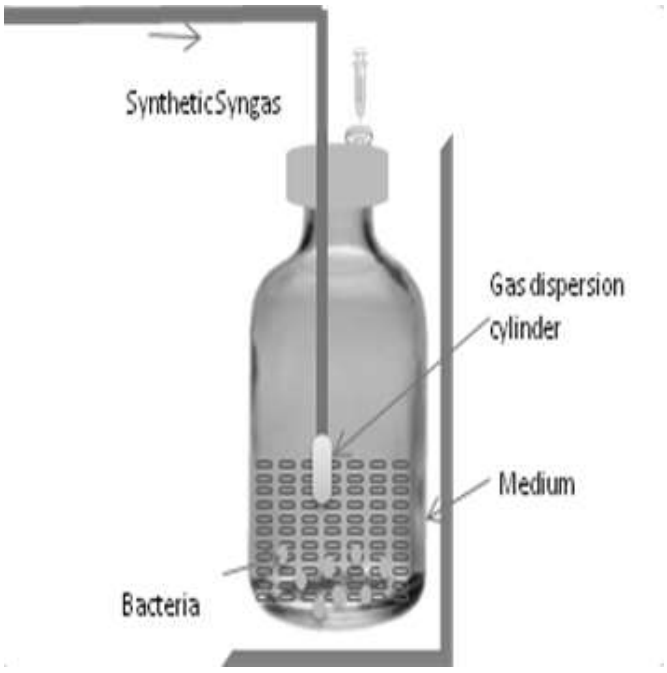

Figure 6 the design of bioreactor for DESIGN II of technique TYPE I

This design is most conventional design, used for experimental setups for syngas fermentation. 250 ml serum bottle is used for this setup which has one opening which is covered by rubber septum as shown in figure 6. The septum has two openings, an inlet for the gas and an outlet for the exhaust. Solution addition and sampling can only be done by using a long neck syringe.

4.3.2 TYPE II

In this technique, a stir bar is used as source of agitation instead of shaking incubator. Unlike TYPE I this technique has less scope of conducting multiflask experiment. As the flasks used in this setup are relatively expensive and are not meant to work inside the shaker incubator, so if the shaker incubator is not available, this technique can be used. One of the main benefits of this process is its ease towards cell culture addition and sampling. Use of fume hood is also optional in this technique.

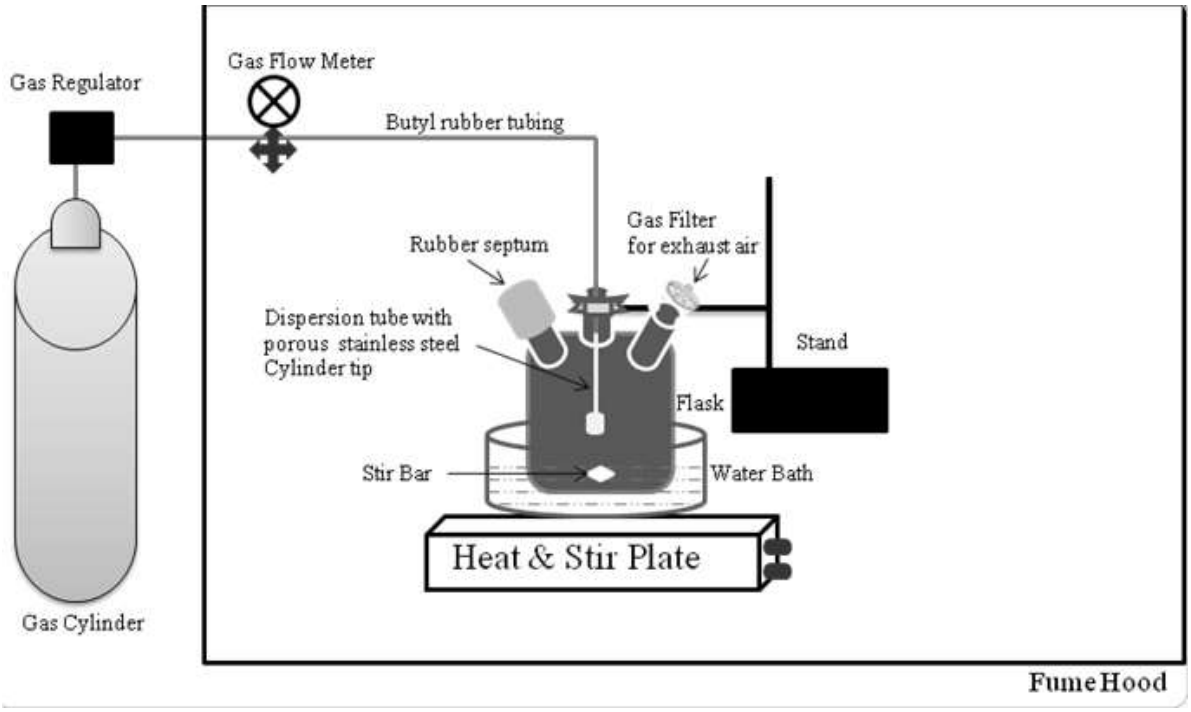

Figure 7 the experimental setup for DESIGN I for technique TYPE II

1) Design 1

A 3-neck spinner flask is used in this setup which has a glass tube connected to a gas dispersion cylinder through one of its neck. Left neck having a rubber septum is open to add cell culture and remove samples for testing. Inoculums addition and sampling are also facilitating by three ports. Use of temperature, pH probes is also an option in this technique.

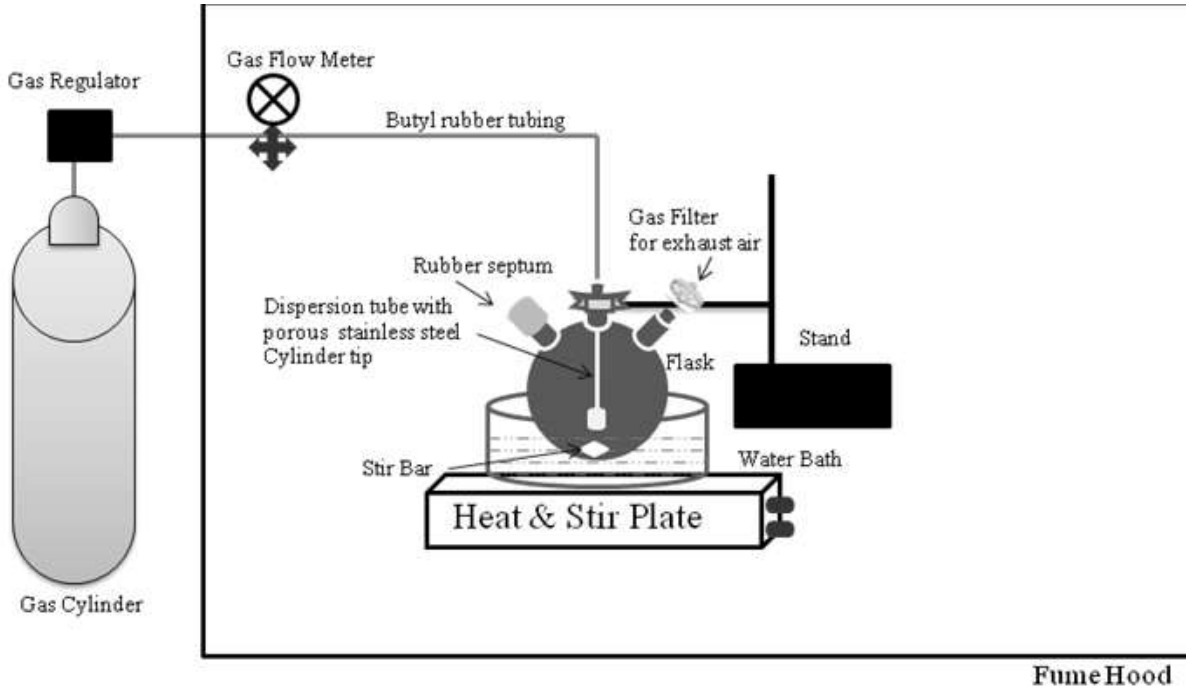

Figure 8 the experimental setup for DESGIN II of TYPE II

2) Design 2

A 3 neck round bottom flask is used in this design replacing the spinner flask. This design is more economical as the round bottom flask is more cheaper than spinner flask. However, this design may affect yield of the ethanol produced as less surface area is available to the gas –liquid phase in round bottom flask than spinner flask.

4.3.3 MASS –BALANCE DIAGRAM

A mass balance diagram (figure 10) states the mass input and output of the whole process. Here the inputs are syngas supply and cell culture while the output is ethanol produced. Gas flow rate considered for design was 5ml/min for an optimum time of 50 hours. Total gas supplied was calculated as 15 lit. With the supplied gas ethanol produced was calculated as 60-75 g.

INPUT	OUTPUT
Syngas (a mixture of 50%CO +50%H2)	Ethanol, Acetate, Gas Exhaust
Gas Flow rate: 5ml/min. Optimum time: 30-200hrs depends upon yield required Total gas flow in 50hrs. = 5*60*50=15,000ml= 15lit.	Optimum ethanol produced: 4-5g/lit of solution Then ETOH produced= 15 x optimum= 60-75gm

Figure 9 diagram comparing the input and output of the reaction

CHAPTER V
DISCUSSION

As shown in the theoretical design description, the working volume of the flasks is 160 ml. Working volume stands for the space available for the reaction to occur which is approximately, the volume of the liquid inside the flask, and that volume contains the basal media, cell growth media, cell culture and water. However, the ratio of the liquid to the head space (space available for the gaseous constitutes above the liquid surface) is different in each flask. In design I (TYPE I), the ratio is higher because of the shape of the flask which has less area available for gas constituents above the liquid surface. While in design II (TYPE II), the ratio is lower because the area available for gas vapors is higher. The higher the ratio of liquid to head space, higher the interaction between the molecules of the syngas and bacterial cells is, and the higher the production of ethanol would be. Design I (TYPE II), has a higher liquid to head space ratio which is the same as in design I (TYPE I) while the round bottom flask in design II (TYPE II) has less head space, So an increase in ethanol production is also expected in design I (TYPE II). Also for design II automation system has been employed as there was more room in the vessel system to apply other advanced techniques. Some of the utilized automation includes a temperature probe, ph probe, foam sensor and a dissolved oxygen probe (Kundiyana et al, 2010). The media utilized for all the designs is same i.e. basal media which provides same growing conditions in all the designs but the results depend upon the shape and size of the flask.

Comparisons of the techniques (TYPE I and TYPE II) can be based on the easiness of the replicates of the experiments. In TYPE I, number of flasks (12-14) can be placed inside a shaker incubator with accurate control over temperature and agitation speed. On the other hand, TYPE II has benefits of cell culture addition and sampling.

MASS TRANSFER TECHNIQUES

Mass transfer improvement techniques were aimed to increase the interaction between gases and liquid to increase the ethanol yield. The study (Kundiyana et al, 2010) conducted at Oklahoma State University used various mass transfer improvement techniques. Three mass transfer techniques were reviewed from the same study to use in this paper.

1. Use of Micro-sparger

Micro-spargers increase the gas-liquid mass transfer by increasing the mass transfer coefficient by generating very fine bubbles. The gas dispersion through fine bubbles are more efficient than a biz size bubble as the combined surface area of small bubbles is much greater than the surface area of a big size bubble. The bubble size created by the micro-sparger was found to be a function of the pore size of micro-sparger, syngas exit velocity and viscosity of the liquid in use. The bubbles generated by sparger were found uniform in size and were uniformly distributed in the liquid media inside the bioreactor.

2. Increasing the syngas flow rate

To increase the productivity of the fermentation process, the supply of the substrate can be increased. From the Le Chatelier's principle studied for physical equilibrium in reactions, increasing the concentration of the reactants is expected to increase the product concentration if rests of conditions are kept same. So increasing the syngas flow rate is expected to increase the productivity of ethanol.

3. Use of hollow fiber membrane reactors

In this technique, hollow membrane reactors are used which prevent the loss of cell density and increase the yield of product. A biofilm is formed on the side of membrane in contact with liquid surface which traps the syngas and immobilized the bacterial cell. This facilitates the interaction of the biocatalyst and substrate gas. The rates of mass transfer are much greater in these reactors.

DESIGN SAFETY AND PRECAUTIONS

The cylinder being used in this study is pressurized at 1660 psi, so precautions need to be taken while refilling and moving the cylinders because the pressurized cylinder if dropped on the floor, would act as a projectile and has the potential to harm the facility as well as manpower. Syngas consists of a mixture of 50% CO and 50% H_2. While handling the syngas, proper precautions are taken since the gas CO is highly poisonous and dangerous to the individuals working nearby it. CO is a colorless, odorless and tasteless gas so it is very difficult to detect. Proper precautions are taken while handling the CO gas such as working in a hood ventilator and wearing a protection mask, if necessary. Also, Hydrogen gas is extremely flammable and high concentration of Hydrogen can cause an oxygen deficient environment. It is necessary to store the gas away from any flammable object. Since CO and H_2 are harmful gases, it becomes necessary to handle syngas cylinders properly. Proper care is taken while using the cylinder for the experimental purposes.

Appropriate handling of bacteria is required as the bacteria *Clostridium ljungdahlii* is an anaerobe and requires oxygen deficient conditions to survive and grow. So it is necessary to maintain proper anoxic conditions before and during the experimentation

CASE STUDY

A case study (shown in figure 10) was done to study the theoretical production of ethanol from a fixed amount of gas. If acetate production is not considered, three moles of carbon monoxide reacts with three moles of hydrogen to form one mole of ethanol. A total gas volume of 6088 liters would have 3044 liters of carbon dioxide as the mixture gas has 50% CO. From ideal gas equation, the number of moles of CO was found to be 124.4 moles. Considering the stoichiometry, maximum ethanol produced was roughly 2.4 liters. This case study is helpful to understand the efficiency of the syngas fermentation. Comparing the ethanol yield produced the % conversion of gases into liquid can be calculated. If the ethanol produced is

less than the maximum yield, either acetate is being formed or the mass transfer rate is less than the maximum value.

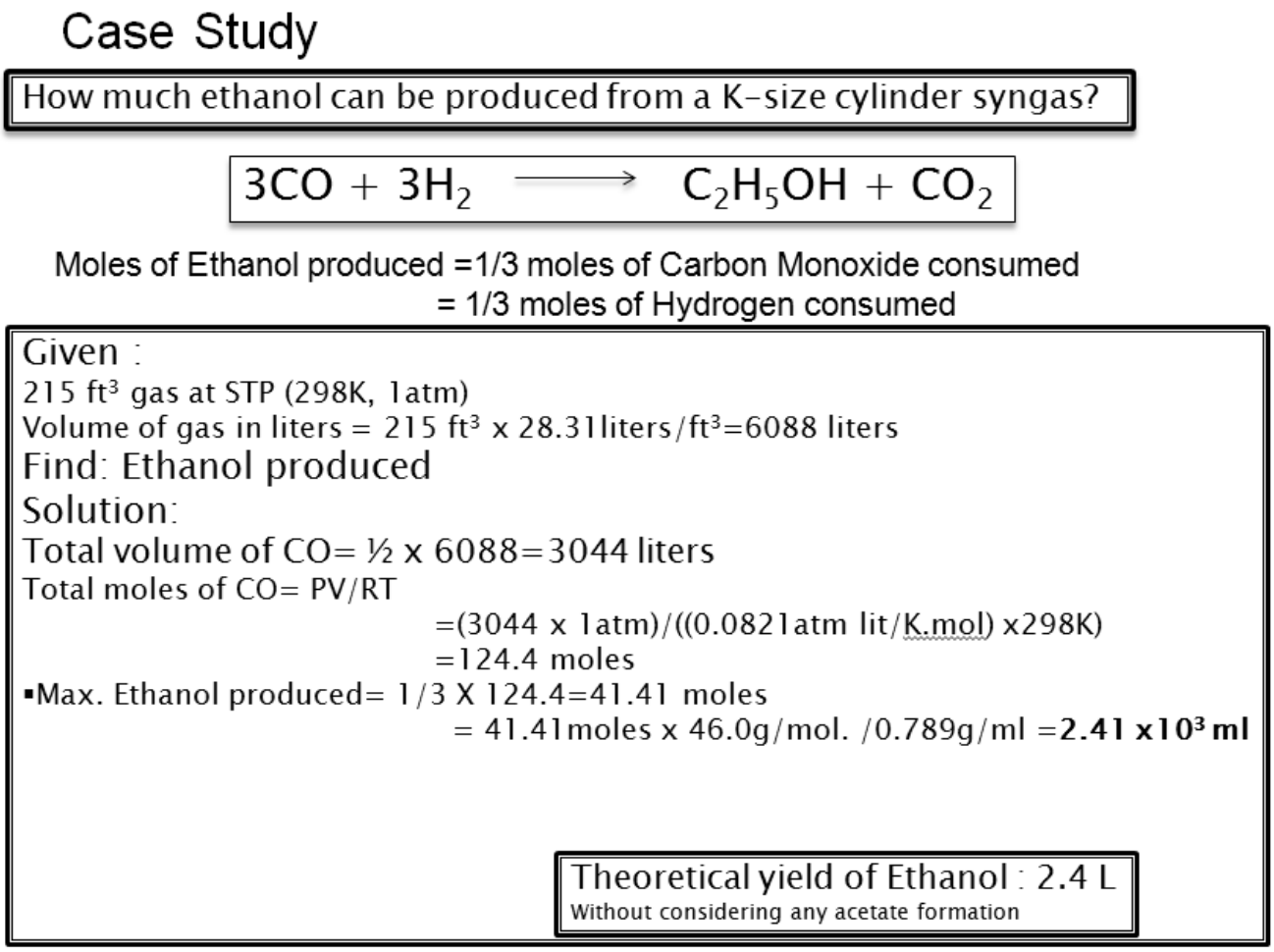

Figure 10 Sample calculations of the case study

CHAPTER VI
CONCLUSION

At this report, we have shown the successful designs of a lab scale fermenter. The variation in the ethanol productivity is expected as the size and shape of each bioreactor design is different. An itemized list also elaborates the equipment used in the design. The round off estimate for the lab scale fermenter designed in this study was $4000-$5000. This amount is much cheaper than the one for STR used in the industries which cost about $40,000. We have also discussed three techniques to improve gas to liquid mass transfer rate: micro-spargers, syngas flow rate and hollow fiber membrane reactors. A direction for future research to install and operate the designs for lab scale fermenter has been indicated.

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Appendix 1

List of required materials and their sources along with their quantity required, catalogue no and prices:

Items	Types	Quantity required	Source	Catalogue no.	Price (in $) per unit
Flask	1. Spinner flask (500 ml)	1). 1 flask	VWR	1). 89089-814	1). $1020.78
	2. 3-neck round (500 ml)bottom flask	2). 1 flask		2). 60002-810	2). $ 63.75
	3. Erlenmeyer flask(500 ml)	3). 1 flask		3). 89000-366	3). $ 7.05
	4. Serum bottles (500 ml, wide mouth)	4). 1 bottle		4). 83014-014	4). $ 11.25
Gas regulator	1. CGA 350	1		1). 55850-225	1). $ 195.02
Gas cylinder	1). Q cylinder (67 ft^3) 2). K cylinder (215 ft^3) 3). T cylinder 2. 33 A(7X16 aluminum)	1	1. Paraxair company 2. Airgas company	--	1. 1). $ 190 2). $ 210 3). $ 210 2. $ 108.11
Strain growth medium*			ATCC	ATCC medium 1754	
Bacteria	Clostridium Ljungdahlii	1	ATCC	ATCC - **55383**	1). $ 205
Water bath	Digital water bath	1	VWR	89032-212	1). $ 751.53
Stir bar	Dimension: 6 x 10 ($^7/_{32}$ x $^3/_8$)	1	VWR	89030-550	1). $ 5.30
Heat plate			Pilot plant	N/A	
Stand			Pilot plant	N/A	
Rubber septum	1). PTFE/Silicone Septum, 8 mm 2). PTFE/Silicone Septum, 13 mm			1). 60005-060 2). 60005-062	1). $ 0.52 2). $ 0.58
Butyl rubber tubing	1). 50 in. reel length	2	VWR	62996-350	1). $ .90
Dispersion tube	Diameter: 20 mm, extra coarse		VWR	60003-636	1). $ 33.42
Syringe exhaust filter	Syringe Filters with Polypropylene Housing, 25 mm diameter	1	VWR	28145-487	1). $ 1.83
Syringe	Sterile Combi-Syringes 1). Up to 5 µL 2). Up to 250 µL	1	VWR	1). 40000-056 2). 40000-016	1). $ 2.61 2). $ 1.74
Basal media**	1). Pfennig minerals 2). Pfennig trace metals 3). B-vitamins		http://www.bio-world.com/		

	4). Supplements such as yeast extracts		
Fume hood		N/A	N/A
Incubator		Van Es Hall Laboratory	
Gas Chromatography			
Spectrophoto-meter			

*requirement: A login account in ATCC for making a purchase

** List of chemicals in table

Appendix 2

Chart of the syngas cylinder supply received from *Praxair*

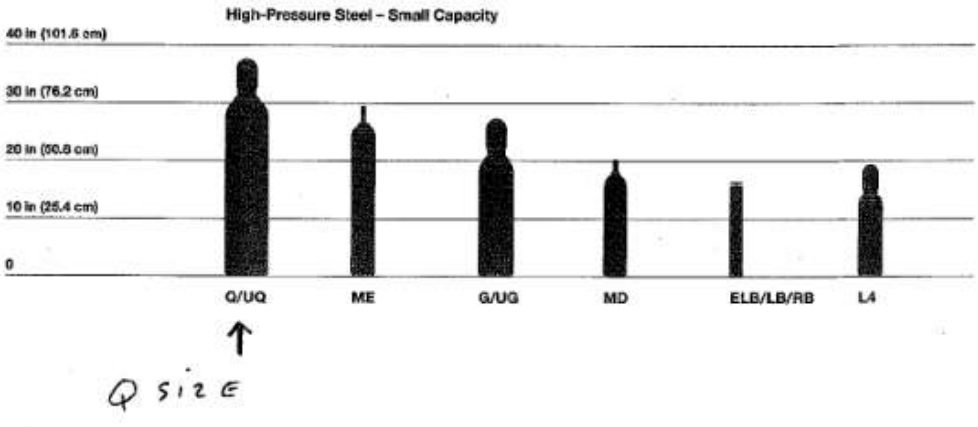
Cylinders and Containers

PRAXAIR

Compressed Gas Cylinders

Part Number Code	Q/UQ	ME	G/UG	MD	ELB/LB/RB	L4
	High-Pressure Steel – Small Capacity					
Transport Canada Specifications	3AAM(153)	3AAM(153)	3AAM(153)	3AAM(153)	3AAM(153)	3AAM(153)
DOT Specifications	3AA-2015	3AA-2015	3AA-2015	3AA-2015	3AA-2015	3AA-2015
Internal Volume						
ft³	0.22	0.17	0.25	0.10	0.033	0.075
L	14.72	4.81	7.08	2.83	0.92	2.12
Water Capacity						
lb	32.46	10.61	15.61	6.24	1.94	4.68
kg	14.73	4.81	7.08	2.83	0.92	2.12
Nominal Dimensions						
Diameter (in)	7.00	4.00	6.00	4.00	2.00	4.00
Diameter (cm)	17.78	10.16	15.24	10.16	5.08	10.16
Height (in)	31.00	20.00	20.00	13.00	16.00	13.00
Height (cm)	78.74	50.80	50.80	33.02	40.64	33.02
Average Tare Weight						
lb	65	29	29	9	4	9
kg	29.48	13.15	13.15	4.08	1.81	4.08

Note: Dimensions and capacities are approximate and may vary slightly.



Q SIZE

B 2

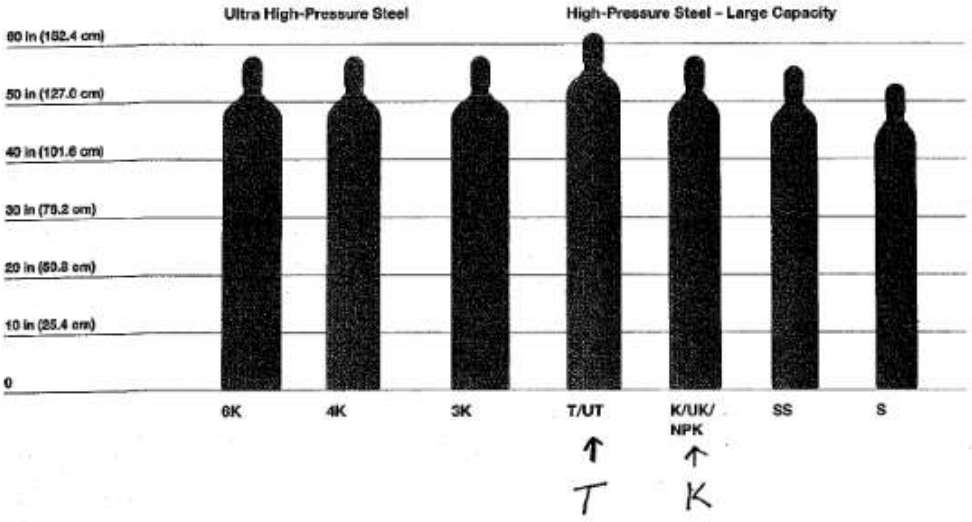
1-877-PRAXAIR www.praxair.com/specialtygases

PRAXAIR

Compressed Gas Cylinders

	Ultra High-Pressure Steel			High-Pressure Steel – Large Capacity			
Part Number Code	6K	4K	3K	T/UT	K/UK/ NPK	SS	S
Transport Canada Specifications					3AM(153)		3AAM(153)
DOT Specifications		E9421-4500			3A-2015		3AA-2015
Internal Volume							
ft³		1.61			1.69		0.99
L		45.59			48.01		28.00
Water Capacity							
lb							
kg						27.4	
Nominal Dimensions							
Diameter (in)	10.00		10.00	9.25		7.50	
Diameter (cm)	25.40		25.40	23.50		19.05	
Height (in)	51.00		51.00	55.00		49.00	
Height (cm)	129.54		129.54	139.70		124.46	
Average Tare Weight							
lb	303		189	143		110	
kg	137.44		85.73	64.86		49.90	

Note: Dimensions and capacities are approximate and may vary slightly.



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