INVESTIGATION ON THE HYBRIDOMA CELL REQUIREMENT OF GLUCOSE AND GLUTAMINE CONCENTRATION FOR GROWTH

SAIFUDDIN BIN BAHARUDDIN

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

JULY 2013

ABSTRACT

A type of monoclonal antibody (MAb) has been developed to diagnose Congenital Adrenal Hyperplasia (CAH). This disease is due to gene effect that causes overproducing of androgen and can cause high mortality of new-born. In order to reduce the death rate of new-born's due to this disease, large amount of research have been done to produce antibody that can helping the detection of this disease. This research will like to investigate on the hybridoma cells requirement of glucose and glutamine for growth rate and also the effect of the by-products to the growth rate of the cell. Previous study showed that the researcher uses a glucose and glutamine concentration from 2.7 – 24 mM and 4 – 12 mM batch culture. The glucose and glutamine concentration used in this research were varied from 4 – 6 mM and 4 – 5.5 mM respectively. Growth, metabolites consumption (glucose and glutamine) and waste production (lactate and ammonia) were monitored. At the end of this research, the cell growth curve and the specific growth (μ) of each cell culture was determined with the effect of glucose and glutamine as well as lactate and ammonia to the growth of this hybridoma cell. From the results, the maximum cell density achieved at the lowest concentration of glucose and glutamine (4mM) was 7.85×10^5 cells/mL, whereas for the highest was 13.15×10^5 cells/mL. From these results, it shows that low glucose and glutamine concentration could also be sufficient enough to achieve a good and stable growth of hybridoma cell. The maximum μ of 0.2026 $h^{-1}$was achieved in a medium containing 6mM glucose and 5.5mM glutamine. The glutamine was found to play a major role in the cell growth where it also affects the specific growth rate of the hybridoma cell. The metabolites were determined using YSL biochemical analyser, where it detects glucose, glutamine, lactate and ammonia. Due to equipment breakdown and insufficient time, the ammonia needs to be skipped. The glucose concentration of 6mM in the culture produces maximum concentration of lactate at 10.1mM. The data obtained for $q_{glucose}$, $q_{glutamine}$ and $q_{lactate}$ was highest at the highest glucose and glutamine concentration (6mM and 5.5mM) where $q_{glucose}$ and $q_{glutamine}$ were consumed and $q_{lactate}$ was produced the most. The glucose ($q_{glucose}$) consumed was -0.0020 nmol/10^6 cells.hr, glutamine ($q_{glutamine}$) consumed was at -0.0018 nmol/10^6cells.hr and lactate produced of 0.58 nmol/10^6 cells.hr. This shows that the feeding of high glucose was not suitable due to high lactate was produced. Thus, the best low concentration to be used for feeding the hybridoma cell was for glucose concentration of 4mM and glutamine concentration of 5.5mM. The result from the experiment shows that at lower glucose concentration, the specific production rate of lactate will decrease as the glutamine concentration increases. It shows that glutamine can act as a carbon source (energy) for the hybridoma cell.

ABSTRAK

Sejenis antibodi monoklonal (MAb) telah di produksikan untuk mendiagnosis Hyperplasia adrenal kongenital (HAK). Penyakit ini adalah disebabkan oleh kesan gen yang menyebabkan overproducing androgen dan boleh menyebabkan kematian yang tinggi daripada bayi yang baru lahir. Dalam usaha untuk mengurangkan kadar kematian bayi yang baru lahir kerana penyakit ini, sejumlah besar penyelidikan telah dilakukan untuk menghasilkan antibodi yang boleh membantu mengesan penyakit ini. Kajian ini akan diuji untuk menyiasat keperluan sel-sel hybridoma terhadap glukosa dan glutamin untuk kadar pertumbuhan dan juga kesan pembuangan untuk kadar pertumbuhan sel. Kajian sebelum ini menunjukkan bahawa penyelidik menggunakan glukosa dan penumpuan glutamin 2.7 - 24 mM dan 4 - 12 mM budaya kumpulan. Glukosa dan penumpuan glutamin digunakan dalam kajian ini adalah berbeza-beza 4 - 6 mM dan 4 - 5.5 mM masing-masing. Pertumbuhan, penggunaan metabolit (glukosa dan glutamin) dan sisa pengeluaran (laktat dan ammonia) telah dipantau. Pada akhir kajian ini, keluk pertumbuhan sel dan pertumbuhan tertentu (μ) setiap budaya sel telah ditentukan dengan kesan glukosa dan glutamin serta laktat dan ammonia kepada pertumbuhan sel hybridoma ini. Penentuan kinetik juga telah dibangunkan untuk strategi suapan. Daripada keputusan, ketumpatan sel maksimum dicapai pada kepekatan yang rendah glukosa dan glutamin (4mM) adalah 7.85×10^5 sel / mL, manakala yang tertinggi adalah 13.15×10^5 sel / mL. Daripada keputusan ini, ia menunjukkan bahawa glukosa yang rendah dan kepekatan glutamin juga boleh menjadi cukup untuk mencapai pertumbuhan yang baik dan stabil sel hybridoma. μ maksimum telah dicapai iaitu 0.2026 h^{-1} dalam medium yang mengandungi glukosa 6mM dan glutamin 5.5mM. Glutamin itu didapati memainkan peranan penting dalam pertumbuhan sel di mana ia juga memberi kesan kepada kadar pertumbuhan spesifik sel hybridoma itu. Metabolit telah ditentukan dengan menggunakan penganalisis biokimia YSL, di mana ia mengesan glukosa, glutamin, laktat dan ammonia. Oleh kerana kerosakan peralatan dan masa tidak mencukupi, ammonia perlu dilangkau. Kepekatan glukosa 6mM dalam budaya menghasilkan kepekatan maksimum laktat 10.1mM. Data yang diperolehi untuk $q_{glukosa}$, $q_{glutamin}$ dan $q_{Jactate}$ adalah tertinggi pada glukosa yang tinggi dan kepekatan glutamin (6mM dan 5.5mM) di mana $q_{glukosa}$ dan $q_{glutamin}$ dimakan dan $q_{Jactate}$ dihasilkan yang paling banyak. Glukosa ($q_{glukosa}$) yang digunakan adalah -0.0020 nmol/10^6 sel.jam, glutamin ($q_{glutamin}$) yang digunakan adalah pada -0.0018 nmol/10^6 sel.jam dan laktat dihasilkan daripada 0.58 nmol/10^6 sel.jam. Ini menunjukkan bahawa suapan glukosa yang tinggi tidak sesuai kerana laktat tinggi telah dihasilkan. Oleh itu, kepekatan rendah terbaik untuk digunakan untuk memberi makan sel hybridoma adalah dengan kepekatan glukosa 4mM dan penumpuan glutamin yang 5.5mM. Hasil daripada eksperimen menunjukkan bahawa pada kepekatan glukosa yang lebih rendah, kadar pengeluaran tertentu laktat akan berkurangan dengan pertambahan kepekatan glutamin. Ia menunjukkan glutamin yang boleh bertindak sebagai sumber karbon (tenaga) untuk sel hybridoma itu.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

mg/L	concentration (milligram per litre)
µm	Size of filter (micrometre)
mM	concentration (milliMolar)
mL	volume of water (millilitre)
L	volume of water (litre)
v/v	Volume per volume
%	Percentage
^{o}C	Temperature (degree Celsius)
CO_2	Carbon Dioxide
µL	volume of water (microliter)
N_T	Total no of cell
N_V	no of viable cell
$q_{glucose}$	Consumption rate of glucose concentration
$q_{glutamine}$	Consumption rate of glutamine concentration
$q_{lactate}$	Production rate of lactate concentration

Subscripts

G	gravity
min	minutes
hr/ h	hour
d	days
<	less then

LIST OF ABBREVIATIONS

ACTH	Adrenocortocotropic
21OHD	21-hydroxylase deficiency
CRH	Corticotropin-releasing hormone
CAH	Congenital Adrenal Hyperplasia
ERK	Extracellular signal-regulated kinase
JNK	c-Jun-terminal kinase
PKA	cAMP-activated protein kinase
AHA	Alpha hydroxyl acid
LDH	Enzyme lactate dehydrogenase
MAb	Monoclonal Antibody
DMEM	Dulbecco's Modified Eagle's Medium
ATP	Adenosine 5'-triphosphate

1 INTRODUCTION

1.1 Background of study

Congenital Adrenal Hyperplasia (CAH) is a disease that can occur in adults or newborn babies. It can be caused by either family inheritance or by the over secretion of the corticotropin-releasing hormone (CRH), adrenocortocotropic hormone (ACTH) and hyperplasia of adrenal glands. The main reasons is the defect of enzyme 21-hydroxylase (21-hydroxylase deficiency [21OHD]). It has been reported that more than 90% (Antal and Zhou, 2009) up to 95% (National Institute of Health Clinic Center (NIHCC), 2004) of CAH disease was caused by this defect. This disease can happen in both male and female, but for female the disease can detect easily. This is due to the formation of penis at their clitoris because of excessive excretion of androgen. For the male, the newborn have no obvious visual symptoms but as they grow older mainly 2 to 3 years old, they start to become muscular, experience penis growth, pubic hair development and deepening voice occurred (Stresing, 2011). The most severe CAH can lead to death. This is due to the dehydration and low blood pressure of patient that affected by this disease (National Institute of Health Clinic Center (NIHCC), 2004). To solve this disease and death problem, an antibody was created in order to detect the CAH disease. This antibody is produced from a merge between mouse myeloma cell and mouse spleen cell in which to develop hybridoma cell. This hybridoma cell is a long lasting cell that can help in the production of the required antibodies. In order to improve the antibody production, the nutrients requirement must be known for this hybridoma cell to achieve a good growth rate and to know the byproduct (wastes) build up in the cell medium. These nutrients (glucose and glutamine) will ensure the growth of cell is at good condition whereas the byproducts (lactate and ammonia) will show the disturbance to the cell growth.

1.2 Problem Statement

One type of cell can produce this antibody for the detection of 17α-hydroxyprogesterone, which is overproducing due to deficiency of 21OHD. This cell is known as hybridoma cell. In order to know the cell growth, the glucose and glutamine concentration has been determined to achieve the high yield of this antibody. It is known that the byproduct being produced also effect the cell growth. The problem with the hybridoma cell is that it cannot grow well under low and high concentration of glucose and glutamine due to the production of byproducts of the cell. This cell

growth can be affected by the byproducts production in a fed batch scale method due to the medium containing the byproducts cannot be removed. Therefore, the cell growth of the hybridoma cell can be improved by using batch method. However, the right time and right amount of nutrient must be fed to sustain growth and increase growth rate. This can only be done if the metabolites of this hybridoma cells are determined.

1.3 Objective

The objective of this study is to investigate the hybridoma cells requirement of glucose and glutamine for cell growth and the effects of byproducts to the growth metabolism.

1.4 Research Scope

In order to achieve the sole objective of this research, few scopes have been determined:

1. To identify the concentration of glucose and glutamine needed for hybridoma in order to maintain cell growth at optimum condition.
2. To identify the effects of byproducts to the growth of the hybridoma cell

1.5 Significant of Studies

This study is significant in determining the hybridoma cell characteristics. By knowing the profiles of the metabolites such as glucose, glutamine, lactate and ammonia for this hybridoma cell, the kinetic model can be developed. These metabolites typical consumption or production trends are presented in the Figure 1.1 (Ozturk and Palsson, 1990).

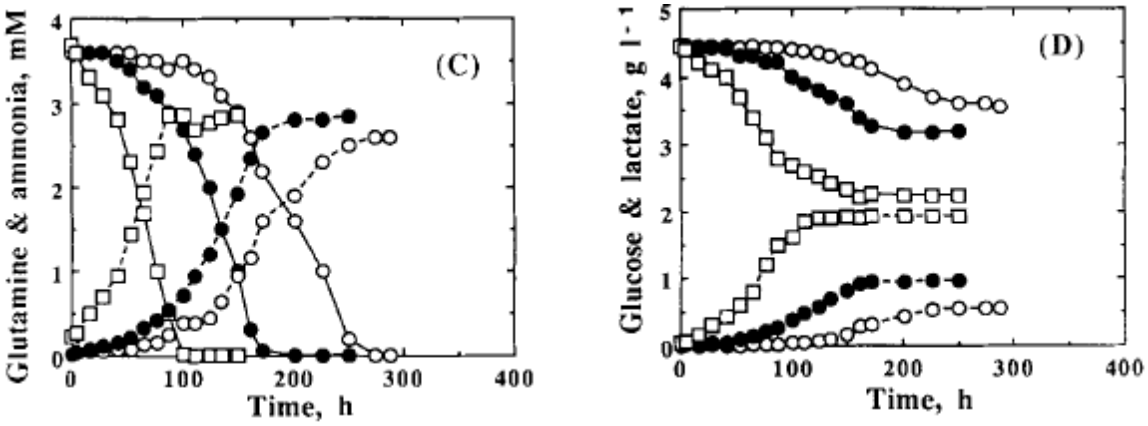

Figure 1-1: The metabolites profile of hybridoma cell (mouse spleen and mouse myeloma cell) growth and production of monoclonal antibody of a researcher (Ozturk and Palsson, 1990)

This kinetic model can be used to develop a feeding strategies for fed-batch or continuous culture in the hybridoma cell in order to achieve the highest cell growth in order to produce the antibody at maximum condition.

2 LITERATURE REVIEW

2.1 What is Hybridoma Cell?

Hybridoma cell is a hybrid cell line that was formed by combining two kinds of cells that are spleen cell (B-cell) of a mammalian and myeloma cell. Myeloma cell is a cell that was taken from a tumor at the bone marrow where cancerous cell were formed (Tyagi et al., 2011), whereas the B-cell is a B-lymphocyte which secretes antibody. The fusion of both these cells will achieve an antibody production cell which it will be long lasting (immortality) than without the hybrid (Hayter, 1989). Figures 2.1, 2.2, and 2.3 show the example of spleen cells used for hybrid, myeloma cells used for hybrid and the combination of myeloma cell and spleen cell to form hybridoma cell.

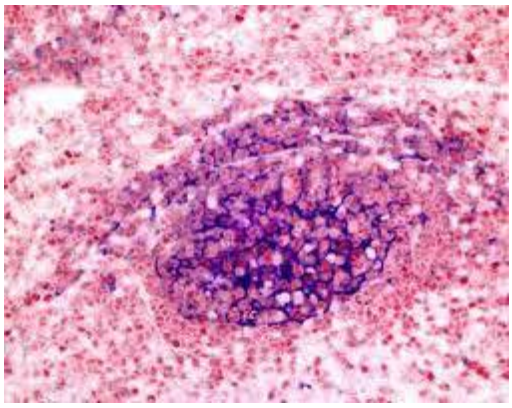

Figure 2-1: Spleen cells used for hybrid (Tyagi et al., 2011)

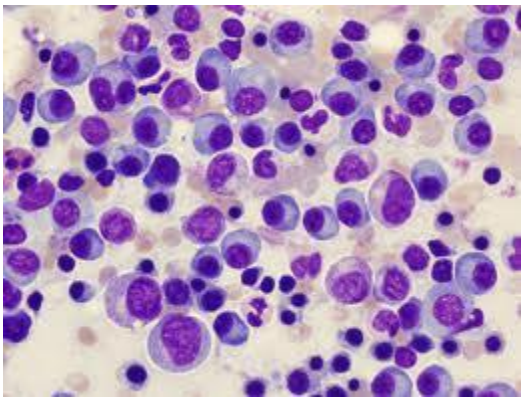

Figure 2-2: Myeloma cells used for hybrid (Tyagi et al., 2011)

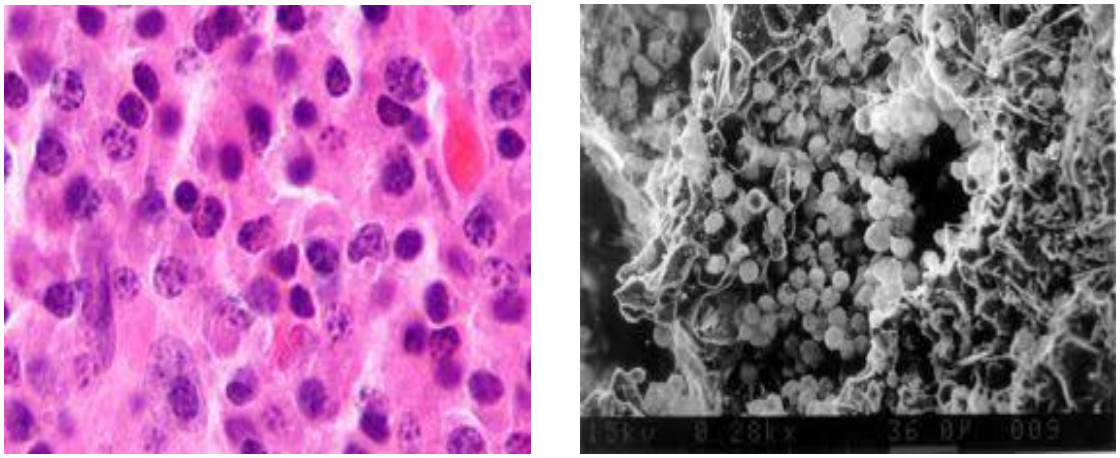

Figure 2-3: Combination of myeloma cell and mammalian cell (hybridoma cell) (Tyagi et al., 2011)

In order to achieve the hybridoma that secretes the desired antibody, an animal (usually a mouse) is being immunized with the required antigen by using in vitro immunization technique. The spleen of the animal is then being harvested in order to get the B-lymphocytes which are then fused with the myeloma cell which we obtained from a cancer cell. Based on Hayter (1989), the B-cell obtained from the mouse spleen is then merging with the cultured myeloma cell to get the hybrid cell. Figure 2.4 shows the principles of hybridoma production via mouse spleen and myeloma cell (Hayter, 1989).

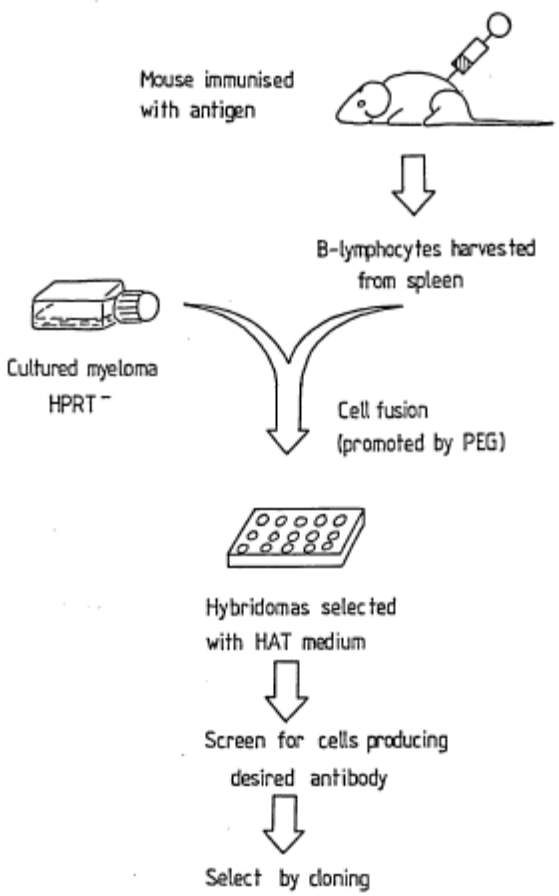

Figure 2-4: The principles of hybridoma production via mouse spleen and myeloma cell (Hayter, 1989)

2.2 Monoclonal Antibody

The term monoclonal means a single clone cells. The antibodies that produced by a single clone cells are called monoclonal antibodies. The content in the system of producing antibodies is B cells. The B cells follows an antigenic stimulation proliferate that gives clone cells the capability to produce homologous antibodies with a paratope that can bind with the epitope of antigen responsible for its creation. Since a single antigen possesses number of epitopes, it can initiate proliferation and differentiation of a variety of B cell clones in the system. In return, the production of heterogenous group of antibodies in normal conditions was achieved.

Since monoclonal antibody is produced by identical immune cells or clones, they have monovalent affinity that they bind to the same epitope. The epitope is known as antigenic determinant, which the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells. Monoclonal antibody can function to bind with other substances in which the purposes are to detect or purify the substances. Figure 2.5 shows the sequence of production of monoclonal antibody.

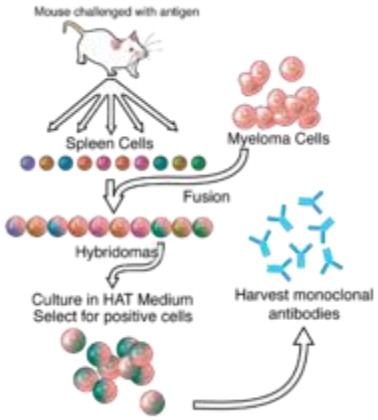

Figure 2-5: The sequence of production of monoclonal antibody (Ethan & Lerner, 1981)

Monoclonal antibody is currently the most demanding product in pharmaceutical industries due to it is the main ingredient in producing the medical treatment. Since this antibody can also serve as detection to certain diseases, this antibody has been used as diagnostic substances to identify the disease. This antibody can be used in treatments such as therapeutic cancer treatment, drug abuse treatment, and others.

For therapeutic treatment, the usage of this antibody is to help in reducing and destroying the disease inside the body. In 1975, a modern era therapy for cancer was launched due to the antibody was discovered by Kohler and Milstein. The antibody is used for therapy because of its ability to bind specifically to primary and metastatic cancer cells with high affinity and create antitumor effect by complement-meditated cytolysis and antibody-dependent or by focused delivery of radiation to the cancer cells (Ross et al., 2003). For cancer treatment, the antibody has the ability to destroy the tumor or cancerous cell. The ability of this antibody enhanced the antitumor effects of chemotherapy and radiotherapy in preclinical models by inhibiting cell proliferation, angiogenesis, and metastasis and by promoting apoptosis (Ross et al., 2003).

For drug abuse treatment, the monoclonal antibody medications have the ability to preselect the affinity and specificity of the drug, and thereby have the medication parameters been constant from one kind of drug to another. It also has the ability to give an immediate protection to the drug abuse person (Peterson et al., 2006). Thus, this MAb can be used for curing drug addiction.

The monoclonal antibody is required in large amount in order to reduce the number of diseases affected people. Large scale production means that the byproducts will be increased. Since byproducts are one of the main causes for cell death, thus the nutrient requirements need to be controlled.

2.3 Major Nutrient

2.3.1 Glucose

Glucose is one of the most important carbohydrates that required by all living cell. Carbohydrate is an organic that consists of the main important molecule inside living things, for example carbon, hydrogen and oxygen. Glucose is a main compound that needed inside the cell in order to give energy and it is important for the metabolism of the cell (Siegwart et al., 1999). It is required in order to make the cell live longer and healthier so it can give a well-developed product. Hybridoma cell is one of the cells that require a lot of glucose in order to grow in stable condition and give high yield of products (Xie and Wang, 1996). Hybridoma cell need glucose for the metabolism and reproduction of the cell but if the glucose is too high, the cell will eventually die due to the inhibition of waste products (lactic acid). If the concentration is too low, the cell cannot reproduce in a good condition. Thus, maintaining the glucose concentration at a sufficient level is required. Based on Siegwart et al (1999), glucose was fed at low level to the hybridoma cell. The concentration used was between 1.1-5.5 mM, where this concentration can redirects the metabolism of the hybridoma cell to a reduction of glucose uptake rate and lactic acid production rate in batch bioreactor (Siegwart et al., 1999). The purpose of study by Siegwart et al. (1999) was to determine the glucose effect towards the growth of hybridoma cell and production of monoclonal antibody. They found that the yield of hybridoma cell density decrease slightly as the glucose supply and consumption increases. In another research, a lower

concentration of glucose supply was being used that was 0.5mM in a fed batch scale (Xie and Wang, 1996). The low concentration supply of glucose is for the condition so that the hybridoma cell can grow in good condition for batch scale. Based on other research from Lee (2002), the glucose concentration used is 2.7mM to 24mM, where good growth curve and antibody production of the cell by using the batch method was obtained. Xie and Wang (1996) found that glucose was not the only nutrient or supplement that can cause the decrement in production rate, other supplement such as glutamine also affects the production.

2.3.2 Glutamine

Glutamine is amino acids that are the building blocks of the cell. It is not only for protein production but also a compound that influenced several cellular signaling pathways inside the cell including extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK), the cAMP-activated protein kinase (PKA) (Xia et al., 2003) and the mammalian target of rapamycin(mTOR) pathways (Xia et al., 2003). This compound is also involved in gene expression control (Abcouwer et al., 1999; Huang et al., 1999; Wischmeyer, 2002) where it is needed to modulate the activity of the ubiquitin-proteasome protein degradation pathway (Paquette et al., 2005). This compound is also involved in the proliferation and the survival of hybridoma cell. Since glutamine is also one of the important intake of this cell, the concentration of the glutamine need to be identified. High concentration of glutamine will result in the higher ammonia production. High ammonia concentration causes the cell cannot live healthy since ammonia is toxic to cell. Low concentration of glutamine causes the cell to reduce in growth rate and eventually die. Thus, maintaining the required glutamine is essential. The glutamine concentration of 0.2mM was fed continuously to the cell culture in the study of Xie and Wang (1996). The cell density was found to increase from 6.5-7.5 x 10^6 cells/mL to 1.7 x 10^7 cells/mL (Xie and Wang, 1996). Based on another researcher (Li et al., 2005), two concentration of glutamine, 0.5-4mM and 0.5-2mM were continuously fed to the reactor. This researcher found that glutamine was totally consumed by both NORIg 7.16.2 hybridoma cell lines. The production of ammonia from the first concentration of glutamine (0.5-4mM) was much higher than the second concentration (0.5 – 2mM) of glutamine supply (Li et al., 2005). Other than that, Lee (2002) observed that at these concentrations (4mM to 12mM) of glutamine, a good growth curve can be obtained as well as a good antibody production. This shows that glutamine is essential in the growth of hybridoma cell.

2.4 Waste production

2.4.1 Lactate

Lactate or lactic acid is a chemical compound that plays role in various biochemical processes. Lactic acid is a carboxylic acid that has a hydroxyl group adjacent to carboxyl group making it a alpha hydroxyl acid (AHA). In animal cells, L-lactate is constantly produced from pyruvate via the enzyme lactate dehydrogenase (LDH). The process that produce this lactate is fermentation which during normal metabolism and exercise. The lactate concentration does not increase in concentration until the rate of lactate production exceeds the rate of lactate removal. This process is governed by a number of factors, including monocarboxylate transporters, concentration and isoform of LDH, and oxidative capacity of tissues. The factor that increases the lactate concentration is due to the exceeding of glucose consumption. The higher the glucose consumption will increase the lactate production. The production rate of lactate in a research article of Xie and Wang (1996) was 4.2mM. Since this was considered as low lactate production, the conversion of glucose to lactate was only 3.4% (Xie and Wang, 1996). This is because the consumption of glucose was only 0.5mM. Thus the lactate production was not so high and this can cause only little effect to the hybridoma cell. Lee (2002) stated that the increase of glucose will increase the lactate production, where his research shows that the lactate concentration increases from 0mM to 30mM as the concentration of glucose added into the culture increases from 2.7mM to 24mM. Since the growth metabolism was affected by the high concentration of inhibitor (lactate), higher glucose consumption resultant in faster inhibition to take place. Therefore, lactate is a byproduct from glucose consumption and causes the inhibition towards the cell growth but lactate will not give a toxicity effect. This shows that reduction of initial glucose concentration produce only little lactate byproducts.

2.4.2 Ammonia

Ammonia is a compound of nitrogen and hydrogen. It is a colorless gas with a characteristic pungent odor. Ammonia contributes significantly to the nutritional needs of terrestrial organisms by serving as a precursor to food and fertilizers. Ammonia is a chemical compound that has benefits to living things but if it exceeds the maximum amount, it will become toxic inside living cell. For hybridoma cell, high concentration of ammonia can cause the cell to become toxic and finally the cell will die due to too toxic. Ammonia is the reasons why hybridomas become toxic at the late growth of the cell (Miller et al., 1987). In order to decrease the ammonia production, the glutamine consumption needs to be lower. From a research article, the ammonia production rate was 5.6mM which is 38% lower than the previous studies of ammonia production (Xie and Wang, 1996). This is because the consumption of glutamine was only 0.2mM and this will produce only little ammonia. From another research journal, the production rate of ammonia increases due to the consumption of glutamine was high (4mM) (Li et al., 2005). Other than that, Lee (2002) observed that the ammonia production was from 0mM to 15mM as the glutamine concentration increases from initial concentration of 4mM to 12mM. This researcher stated that the high ammonia concentration can lead to the death of the cell due to the toxicity and it also affect greatly to the cell growth and the antibody production. Thus the concentration of ammonia production increases as the glutamine consumption increases.

2.5 Congenital Adrenal Hyperplasia (CAH)

Congenital Adrenal Hyperplasia (CAH) refers to a family of inherited disorders of adrenal steroidogenesis. The cause of this disease is due to the impaired cortisol secretion in which the hypersecretion of corticotropin-releasing hormone (CRH), adrenocortocotropic hormone (ACTH) and hyperplasia of adrenal glands. There are two types of CAH disease which the severe form called classic CAH while the mild form is called nonclassic CAH. The most common cases of CAH disease are caused by defect in the enzyme 21-hydroxylase (21-hydroxylase deficiency [21OHD]) and has been reported that more than 90% (Antal and Zhou, 2009) up to 95% (National Institute of Health Clinic Center (NIHCC), 2004) CAH diseases was caused by this defect. This CAH is also a several autosomal recessive diseases resulting from mutations of genes for the biochemical steps in producing cortisol from cholesterol by adrenal glands. Figure 2.6 shows the adrenal glands which causes the CAH disease.

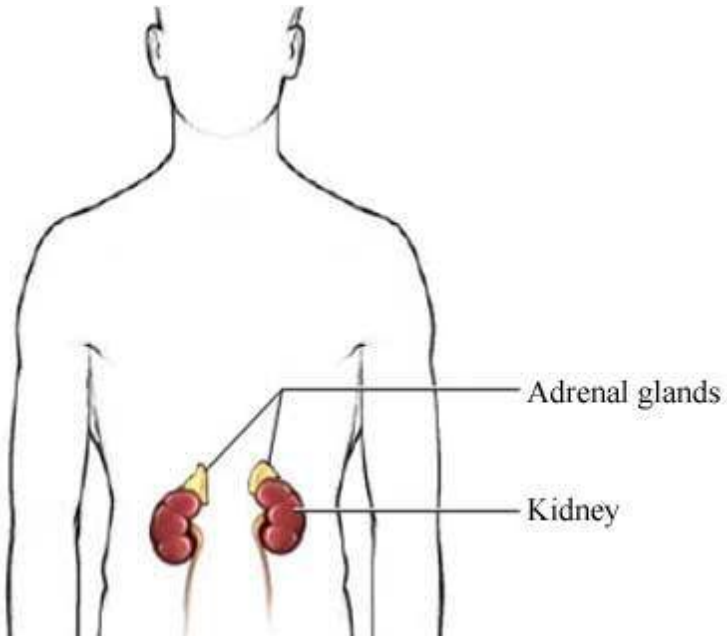

Figure 2-6: The adrenal glands which causes the CAH disease (Stresing, 2011)

A child with this type of CAH disease has adrenal glands that cannot make enough cortisol and may or may not make aldosterone. As a result, the glands over-work trying to make these hormones and end up making too much of what they can make which is androgens. For example, a girl that has this disease tends to be masculinized and the clitoris resembles a penis due to the excretion of excessive androgen by the adrenal gland during prenatal development. In newborn boys, there are no obvious visual symptoms but as they grow older mainly 2 to 3 years old, they start to become muscular, experience penis growth, pubic hair development and deepening voice occurred (Stresing, 2011). The second most common form of CAH is 11-hydroxylase deficiency. A child with this type of CAH has adrenal glands that make too much androgen and not enough cortisol. Children with this type of CAH may also have high blood pressure. These patients do not have aldosterone deficiency (National Institute of Health Clinic Center (NIHCC), 2004). Other than that, the non-classical CAH has one type of deficiency which is 21-hydroxylase deficiency. People with this deficiency make enough cortisol and aldosterone, but they make excess androgens. Symptoms come and go, beginning at any time but typically in late childhood or early adulthood. Boys often do not need treatment. Girls usually need treatment to suppress their excess androgens. This disease is quite dangerous if leave untreated. The cause can be a severe damage to them as they grow older. This is because other diseases can occur such as respiratory illness and infections that are very hard to cure, high blood pressure with low blood

potassium, poor feeding and vomiting, failure to gain weight, short stature and severe acne (Stresing, 2011). Other than that, the CAH causes the pituitary adrenocorticotrophic hormone (ACTH) to overproduce and thus the metabolic abnormalities increases. This effect will increase the salt development inside the infant body causes the body to quickly dehydrate and lastly can lead to death. As such early diagnosing ensures the preventive treatment can be carried out to the patient.