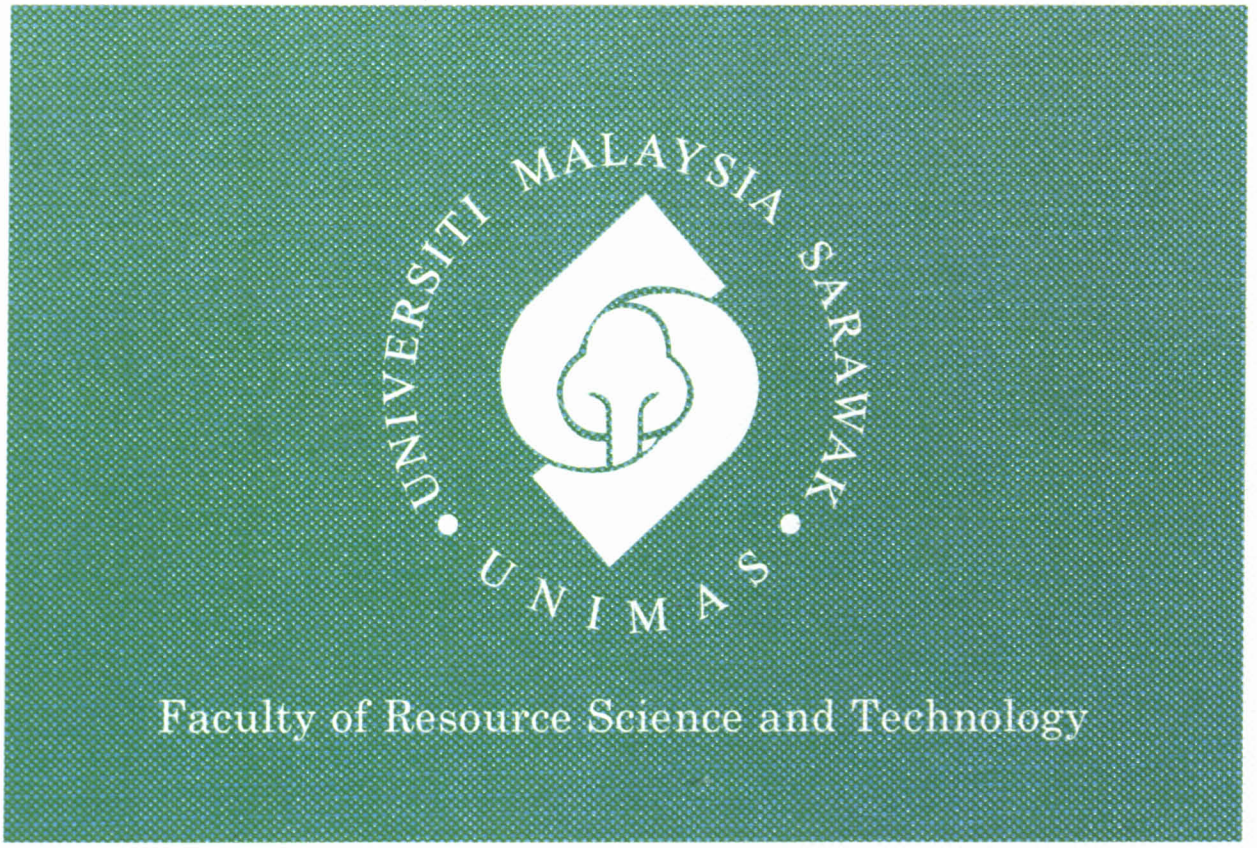

EFFECTS OF CASTOR OIL ON *ESCHERICHIA COLI* K011 GROWTH AND ACTIVITIES DURING BATCH ANAEROBIC FERMENTATION

Nur Hanani Binti Hailmy
(32130)

Bachelor of Science with Honours
(Resource Biotechnology)
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Effects of Castor Oil on *Escherichia coli* K011 Growth and Activities during Batch Anaerobic Fermentation

NUR HANANI BINTI HAILMY

A thesis submitted
in fulfillment of the requirement for the degree of
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LIST OF ABBREVIATIONS

CFU	Colony Forming Unit
E. coli K011	*Escherichia coli* K011
HPLC	High Performance Liquid Chromatography
LB	Luria Broth
rpm	Revolutions per minute
YP Broth	Yeast-Peptone Broth

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Effects of Castor Oil on *Escherichia coli* K011 Growth and Activities during Batch Anaerobic Fermentation

Nur Hanani binti Hailmy

Department of Molecular Biology
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

ABSTRACT

Escherichia coli K011 has been one of the organism of choice in the study of bioethanol production since it is one of the facultative anaerobes that has excellent growth and researchers are all familiar with its fermentation capabilities under anaerobic environment. In this study, the growth and activities of *E. coli* K011 monitored along the process of batch anaerobic fermentation. Both of the carbon sources, xylose and glucose were consumed during fermentation. The addition of castor oil showed different results in the growth and fermenting activities of *E. coli* K011 because castor oil has been known to have the ability reduce the bioethanol concentration in the fermentation broth. The viable cell count and High Performance Liquid Chromatography (HPLC) results explained that the presence of castor oil affected the ethanol production and growth of *E. coli* K011 and the best growth were in 10% of glucose-xylose in the fermentation broth without castor oil. *E. coli* K011 has higher tendency to consume glucose first compared to xylose hence producing bioethanol but both of the carbon sources are favoured. When castor oil was present, the bioethanol concentration was lowered so it did not disrupt the enzymes for its metabolic activities. The highest ethanol production recorded was in 10% glucose-xylose in fermentation broth without castor oil, 47.77% of theoretical yield. Hence, either xylose or glucose alone was preferred to be consumed by *E. coli* K011 for a better production of bioethanol so that tendency factor can be omitted.

Keywords: Bioethanol, Castor oil, *Escherichia coli* K011, fermentation, glucose, xylose

ABSTRAK

Escherichia coli *K011 merupakan organisma yang menjadi pilihan untuk mengkaji penghasilan bioetanol kerana ia merupakan organisma anaerobik yang mempunyai kadar pertumbuhan yang sangat baik dan para penyelidik mengetahui keupayaan penapaian organisma ini dalam persekitaran anaerobik. Dalam kajian ini, pertumbuhan dan aktiviti* E. coli *K011 dipantau sepanjang proses penapaian anaerobik. Kedua-dua sumber karbon, glukosa dan xylosa digunakan sepanjang proses penapaian. Penambahan minyak kastor menunjukkan hasil yang berbeza terhadap pertumbuhan dan aktiviti penapaian oleh* E. coli *K011 kerana minyak kastor telah diketahui mempunyai kebolehan untuk mengurangkan kepekatan bioetanol dalam cecair pekat penapaian. Kiraan sel hidup dan kromatografi menunjukkan menyatakan bahawa minyak kastor telah memberi kesan terhadap penghasilan etanol dan pertumbuhan* E. coli *K011.* Kadar *pertumbuhan maksimum ialah pada 10 % kandungan glukosa-xylosa dalam cecair penapaian tanpa minyak kastor.* E. coli *K011 mempunyai kecenderungan yang lebih tinggi untuk menggunakan glukosa terlebih dahulu berbanding dengan xylosa untuk menghasilkan bioetanol tetapi kedua-duanya digunakan oleh organisma ini. Minyak kastor menyebabkan kepekatan bioetanol menjadi rendah maka ia tidak mengganggu enzim untuk aktiviti metabolisme bagi* E. coli *K011. Penghasilan etanol yang paling tinggi* berlaku pada 10 % *kandungan glukosa-xylosa dalam cecair penapaian tanpa minyak kastor.Oleh itu, hanya satu antara glukosa atau xylosa harus digunakan oleh* E. coli *K011 bagi penghasilan bioetanol yang lebih tinggi agar faktor kecenderungan dapat diabaikan.*

Kata kunci: Bioetanol, *Escherichia coli* K011, glukosa, minyak kastor, penapaian, xylosa

CHAPTER 1

INTRODUCTION

1.1 Introduction

Bioethanol is one of the renewable energy sources produced from sugar crops by fermentation process and it is environmentally friendly besides having a potential to become the alternative fuel in the future (Farrell et al., 2006). It is mainly produced from by raw materials of agriculture and forestry origins (Hill et al., 2006). The main advantage for utilizing bioethanol is that it is in the liquid form at atmospheric pressure. This means it is easier to be distributed, stored and utilized as a fuel than biogas which requires new distribution accommodations (Antoni et al., 2007). According to Antoni et al. (2007), two moles of carbon dioxide and two moles of ethanol is obtained from one mole of glucose as in the following equation:

$$C_6H_{12}O_6 \rightarrow 2\ CO_2 + 2\ C_2H_5OH$$

Ethanol production is relative with the growth of the fermenting organism. If the ATPs are not consumed by the growth of bacterium cells, the intracellular increase of ATP will inhibit the glycolysis process and no more ethanol will be produced (Bai et al., 2008). The high concentration of ethanol produced which is the waste product of *Escherichia coli* K011 is then toxic to the bacterium.

The high level of ethanol present in the living environment of *E. coli* reduces the respiration rate of the bacterium due to intoxication (Ingram et al., 2002). Concentrated ethanol interrupts the cell membranes of *E. coli*, causing the pH of the surroundings to decrease and starts to denature the metabolic enzymes of *E. coli*. Hence, the bacterium starts to have conformational changes and this will alter the shape of the active site of the

enzyme, and become ineffective since the enzymes are highly specific (Choudry et al., 2012).

In this study, castor oil was used to assist in the batch fermentation system in reducing ethanol toxicity in the broth. The growth and activities of *E. coli* K011 was observed by viable cell counts on agar plates and the glucose consumption by using High Performance Liquid Chromatography (HPLC) method.

1.2 Objectives

The objectives of this study are:

a) To study the growth of *Escherichia coli* K011 in batch anaerobic fermentation.

b) To study the activities of *E. coli* K011 in batch anaerobic fermentation.

c) To measure the ethanol production by *E. coli* K011 in batch anaerobic fermentation.

CHAPTER 2

LITERATURE REVIEW

2.1 Castor oil

Castor oil, also known as ricinus oil is a pale amber viscous liquid derived from the seeds of the plant *Ricinus communis* (Marter, 1981). As one of the vegetable oils, it is a triglyceride that basically consists of a glycerol molecule and three hydroxyl groups. The fatty acid in this oil is unsaturated, hydroxylated 1-hydroxy, 9-octadeconic acid is also known as ricinoleic acid (Akpan et al., 2006). According to Weise (1983), the castor plants grow naturally over a wide range of geographical regions under a variety of physical and climatic regimes including temperate regions. The castor beans contain about 30-35% oil which can be extracted by variety of processes such as pressing and solvent extraction. In terms of acidity, crude castor oil is more acidic then the refined castor oil because during the refinement process, the castor oil undergo neutralization (Akpan et al., 2006).

Figure 1: Castor oil plant and seeds (Source: Wolf, 2010).

Udeozo et al. (2013) reported that the derivatives of castor oil that have been processed to give benefits in different industrial applications such as biodiesel production and paints production. Hence, the demand for this oil increases. The castor meat or cake is mainly used as fertilizer because it is unsuitable as an animal feed because of the presence of toxic protein called ricin and toxic allergen often referred to as castor bean allergen (Ogunniyi, 2005). This makes the castor oil valuable chemical feed stocks with higher price than other seed oils.

The castor seeds of *R. communis*, are usually heated and cage pressed to acquire the best quality of oil. Ogunniyi (2005) also stated that the residual pulp then goes through a solvent extraction for the remaining oil. The importance of castor oil in this study can be seen as it also acts as ethanol absorber, reducing the toxicity of broth during fermentation as stated by Walton (2009).

2.2 *Escherichia coli* K011

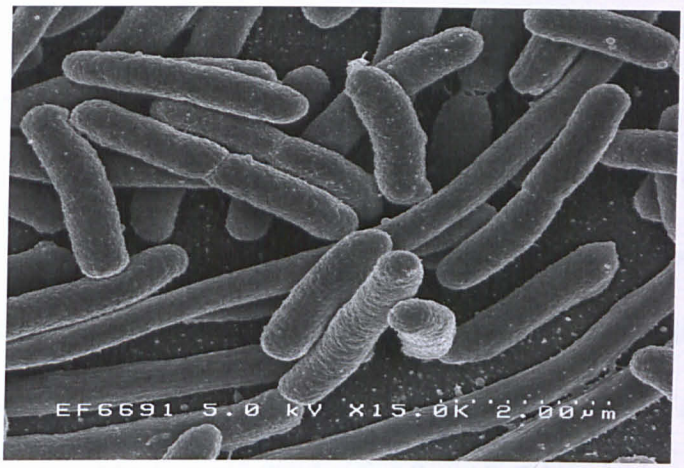

Figure 2: The cell structure of *E. coli* (Source: Wilcox, 2012).

Escherichia coli is commonly found in the gut of endotherms, or warm blooded organisms (Taylor, 2007). A German pediatrician and bacteriologist, Theodor Escherich discovered the bacterium in 1885 after isolating it from the feces of a newborn and it is now classified as part of the Enterobacteriaceae family of gamma-proteobacteria (Shulman et al., 2007).

E. coli have been genetically engineered to produce many types of strains for the usage in studying its mechanism of growth and activities because they can be grown and cultured easily (Leite et al., 2000). Ethanologenic *E. coli* K011 strains require simpler fermentation conditions, produce higher concentrations of ethanol, and are more efficient than pentose-fermenting yeasts for ethanol production from xylose and arabinose (Beck, 1989).

2.3 Bioethanol

Bioethanol is ethanol produced by fermentation of raw material from agriculture and forestry and the production of bioethanol today is the largest industrial microbial process (Bai et al., 2008). Ethanol can be used as a fuel and is by being both renewable and environmentally friendly making it one of the best alternatives to fossil fuels (Bai et al., 2008). One advantage for bioethanol compared to biogas is that it is a liquid at atmospheric pressure so it is easier to allocate, store and exploit as a fuel (Antoni et al., 2007).

Table 1: Fuel Ethanol Production in Ten Leading Countries, 2010 (Source: Licht, 2010).

Country	**Production (Million Gallons)**
United States	11,993
Brazil	7,270
China	555
Canada	304
France	277
Germany	238
Spain	159
Thailand	139
Belgium	85
Columbia	85
World Total	**21,926**

Distillation is a process where a mixture is separated into various components based on their individual volatility and it can produce a higher concentration of ethanol (Archalya, 2012). The Greek alchemists working in Alexandria during the first century A.D. carried out distillation, and the medieval Arabs learned from the Alexandrians (Ahman, 2010). Orning (2010) stated that the disadvantages of bioethanol is its corrosiveness and the low vapour pressure which makes cold starts difficult and its lower energy density than gasoline since bioethanol has 66% the energy that gasoline has. The availability of the raw material differs from season to season and is dependent on geographic locations (Orning, 2010). These are some of the causes to why the price of the raw material is highly volatile and therefore strongly affect the production costs of bioethanol (Balat et al., 2008).

2.4 Batch Anaerobic Fermentation

Batch fermentation process is defined as a process that starts with the inoculation and finish with the retrieval of the product happens inside a single fermenter with no intermediary steps (Ramaswami, 2009). The ethanol production from fermentation is affected by a few conditions such as temperature, pH and sugar concentration (Ohta et al., 1991). Otha et al. (1991) also reported that natural factors also have great influence for example culture medium, dissolved oxygen, immobilization and other micronutrients. Since *E. coli* K011 is a facultative anaerobe, the overall proportion of lactic acid production increases under acidic conditions, while the ratio of ethanol to acetate remains approximately 1:1 (Blackwood et al., 1956).

Dombek and Ingram (1986) reported that the viability remains at or above 90% meanwhile the internal pH remains near neutrality and the specific activities of the glycolytic and alcohologenic enzymes remains at high rate throughout batch fermentation. Magnesium is an essential cofactor for many of the glycolytic enzymes and it has been identified as a limiting nutrient in fermentation broth containing peptone and yeast extract (Dombek and Ingram, 1986).

According to Dombek and Ingram (1986), the supply of these nutritional needs only reduce the decline in fermentative activity during batch fermentation, not eliminating the declination. The replacement of fermentative broth containing ethanol with fresh medium containing less ethanol does not restore fermentative activity (Dombek and Ingram, 1986).

2.5 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is a separation technique that involves the injection of a small volume of a liquid sample into a tube packed with porous particles that known as stationary phase where individual components of the sample are transported along the packed column by a liquid called mobile phase moved down by the force of high pressure delivered by a pump (Clark, 2007). The modern form of liquid chromatography is now referred as flash chromatography. This type of chromatography has been used widely for about 35 years. The HPLC works better than typical liquid chromatography in terms of efficiency, speed, sensitivity and ease of operation (Al-Nema, 2006).

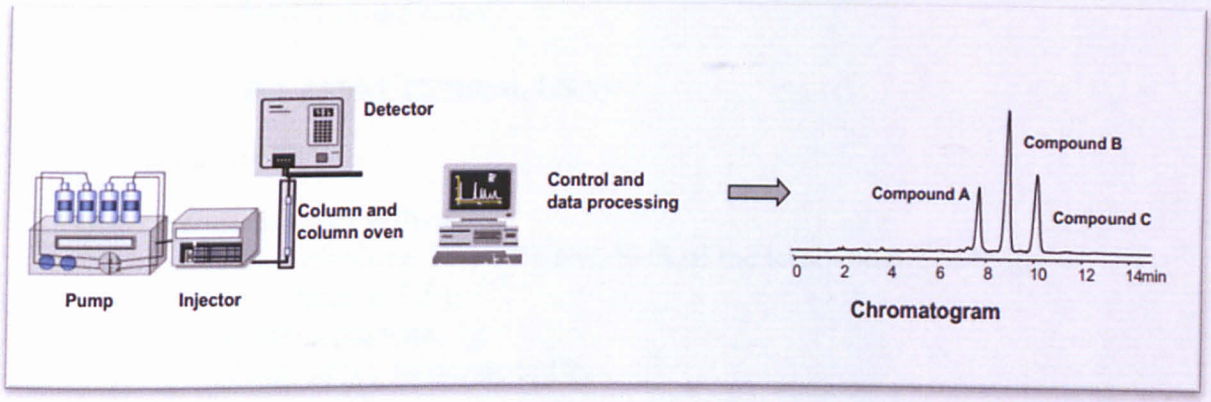

Figure 3: The principle of HPLC (Source: Clark, 2007).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Apparatus

The materials and apparatus used for this study are:

1) 1 M Citrate Buffer
   i. Citric acid, 44 g
   ii. Sodium citrate, 86 g
   iii. Distilled water, 500 ml

2) PBS Buffer
   i. Sodium chloride, 4 g
   ii. Potassium chloride, 0.1 g
   iii. Sodium hydrogen phosphate, 0.72 g
   iv. Potassium dihydrogen phosphate, 0.12 g
   v. 500ml distilled water

3) Castor oil (SAFC, Aldrich chemical, USA)

4) *Escherichia coli* K011

5) Yeast-Peptone Broth (YP Broth)
   i. Glucose-xylose, 5%, 10% and 20 % of the total volume of broth
   ii. Yeast extract, 1.5 g
   iii. Bacterial peptone, 3g
   iv. 150 ml of 0.5 M citrate buffer

6) 100 ml of 50 g/l glucose

7) Glycerol Stock

8) Luria Broth (LB)
   i. Distilled water, 100 ml
   ii. Luria Broth powder, 2.5 g

9) High Performance Light Chromatography (HPLC) system (LC-20A, Shimadzu, Japan)

10) Mobile Phase
   i. 56 µL of 0.005 M Sulphuric acid, H_2SO_4
   ii. 2 L of ultrapure water

11) Autoclave (Model 25X-2, Foundry inc., USA)

12) Plate Count Agar
   i. Plate count agar powder, 5.6 g
   ii. Distilled water,250 ml

12) Incubator shaker (Innova 4000, New Brunswick Scientific Co. Inc., USA)

3.2 Methods

3.2.1 Raw Materials Preparation

Castor oil, which was extracted from castor bean seeds and obtained from the supplier, was kept under room temperature until upon usage.

3.2.2 Culture and Inoculum Preparation

Escherichia coli K011 culture and inoculum preparation was done as described in the Figure 4 shown below.

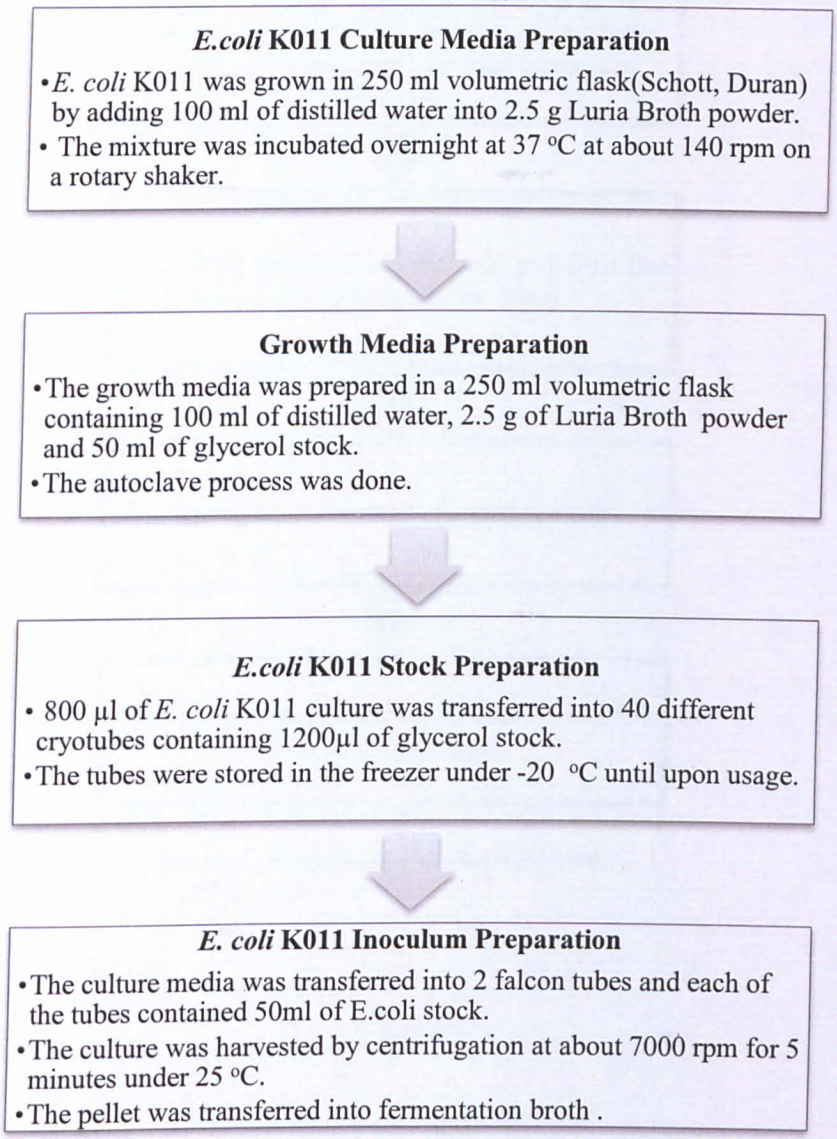

Figure 4: *E. coli* K011 culture and inoculum preparation.

3.3 Fermentation Broth Preparation

The process of fermentation broth preparation is described in the Figure 5 shown below.

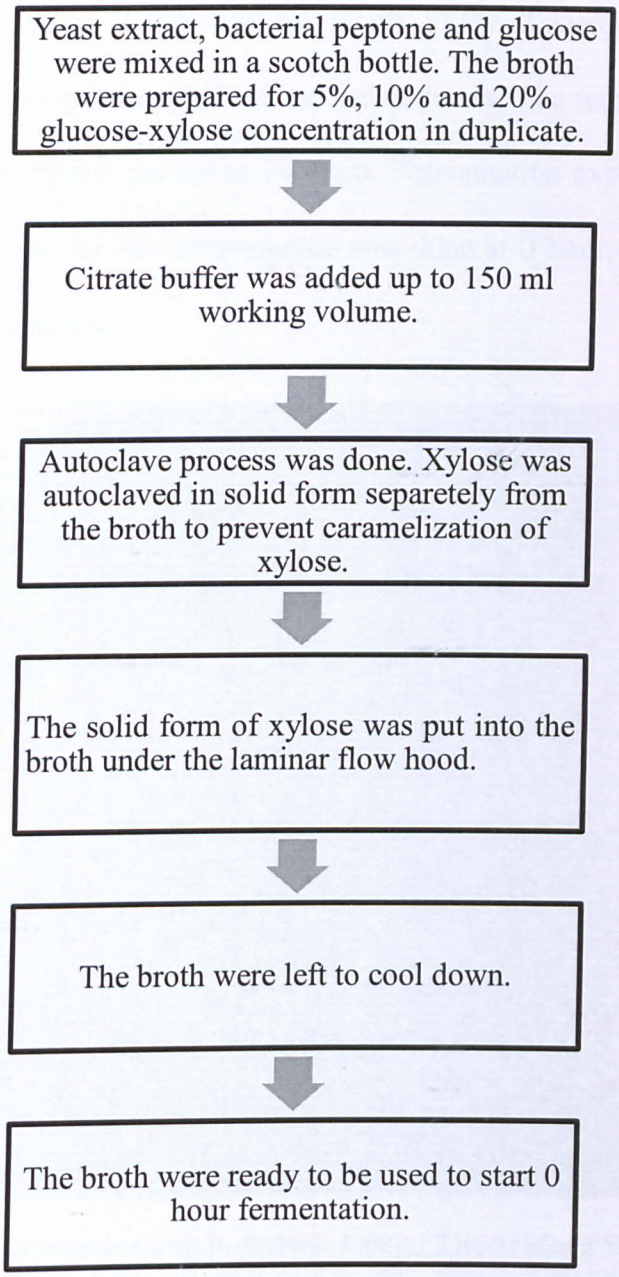

Figure 5: Fermentation broth preparation.