OPTIMIZATION OF BIOETHANOL PRODUCTION USING OIL PALM FROND JUICE

LAWANYA A/P K.RAMU

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ABSTRACT

This paper presents the optimum conditions for producing bioethanol using oil palm frond juice. Bioethanol has been gaining much interest recently in terms of research and development. Since there are various factors such as rising oil price, environmental issues and high rate consumption of fossil fuel, the global demand for bioethanol has shown a remarkable increase. Oil palm frond is known to be the most generated oil palm biomass annually. OPF is obtained during pruning for harvesting fresh fruit bunch, and the juice can be produced by pressing the fresh OPF using the conventional sugarcane pressing machine. The OPF juice contains higher glucose content, which is about 70% of the total free sugar. Hence, it has a high potential to be the carbon source for producing bioethanol. The parameters investigated in this research work are temperature, pH and agitation speed. Bioethanol was produced from oil palm frond juice by fermentation with *Saccharomyces cerevisiae*. The ethanol concentration from the fermentation sample was analysed using HPLC. Using the ethanol concentration obtained from HPLC analysis, the optimum condition for the production of bioethanol was determined using the Response Surface Methodology with the aid of Design Expert software. The results revealed that, a maximum bioethanol concentration of 22.93 g/L can be obtained at the following optimum conditions : temperature of 32^{o}C, pH 6 and agitation speed of 80 rpm. The validation result revealed that at this optimum condition, a bioethanol yield of 30.1% , which is close to the predicted value, can be obtained.

Key words: oil palm frond juice, bioethanol, *Saccharomyces cerevisae,* HPLC, Design Expert

ABSTRAK

Kertas kerja ini membentangkan keadaan optimum untuk menghasilkan bioetanol menggunakan jus pelepah kelapa sawit. Bioetanol telah mendapat perhatian yang lebih baru-baru ini dari segi penyelidikan dan pembangunan. Oleh kerana terdapat pelbagai faktor seperti kenaikan harga minyak, isu-isu alam sekitar dan penggunaan kadar tinggi bahan api fosil, permintaan global untuk bioetanol telah menunjukkan peningkatan yang luar biasa. Pelepah kelapa sawit dipilih, kerana ia merupakan biojisim kelapa sawit yang paling banyak dihasilkan setiap tahun. Pelepah kelapa sawit (OPF) diperolehi semasa pemangkasan untuk penuaian buah tandan segar, dan jus yang boleh dihasilkan dengan menekan OPF segar menggunakan mesin tebu konvensional. Jus OPF mengandungi kandungan glukosa yang lebih tinggi, iaitu kira-kira 70% daripada jumlah gula bebas. Oleh itu, ia mempunyai potensi yang tinggi untuk menjadi sumber karbon untuk menghasilkan bioetanol. Kajian penyelidikan ini bertujuan untuk mengoptimumkan penghasilan bioethanol menggunakan jus pelepah kelapa sawit berasaskan kepada tiga parameter penting iaitu suhu, pH dan kelajuan kelalang goncang. Bioetanol dihasilkan dari jus pelepah kelapa sawit oleh penapaian dengan Saccharomyces cerevisiae. Kepekatan etanol daripada sampel penapaian dianalisis menggunakan HPLC. Menggunakan kepekatan etanol yang diperolehi daripada analisis HPLC, parameter optimum untuk penghasilan bioetanol ditentukan menggunakan Kaedah Respon Permukaan dengan bantuan perisian Design Expert. Hasil kajian menunjukkan, kepekatan bioetanol maksimum 22.93 g / L boleh didapati pada keadaan optimum berikut: suhu 32^{o}C, pH 6 dan kelajuan kelalang goncang 80 rpm. Keputusan pengesahan mendedahkan bahawa pada keadaan optimum ini, hasil bioetanol sebanyak 30.1%, yang berhampiran dengan nilai yang diramalkan, boleh diperolehi.

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LIST OF ABBREVIATIONS

CCD	Central Composite Design
CO_2	Carbon Dioxide
$C_6H_{12}O_6$	Glucose
C_2H_5OH	Ethanol
Elaeis guineensis Jacq.	Oil palm
EFB	Empty fruit bunch
OPF	Oil palm frond
OPT	Oil palm trunk
RSM	Response Surface Methodology

CHAPTER 1

INTRODUCTION

1.1 Background of Study

During the last few decades, the demand for alternative fuel resources has increased. Among the key factors driving a strong interest in these renewable energy sources are the ever rising fossil fuel prices, along with the growing demand for energy, and environmental concern due to excessive consumption of fossil fuels (Chin and H'ng,2013). It has been widely known that fossil fuels trigger environmental issues, such as greenhouse gas emissions. This tends to elevate the atmospheric temperature excessively (Hashem et al.,2013). Consequently, the renewable energy sources are sought after, to be utilized as biofuels. Biofuel refers to fuel energy which is derived from agricultural materials. Biofuels are divided into two groups based on the production technology. First generation biofuels are already being produced on a commercial scale, using plant materials as raw materials. However, this process may pose threat to food chain and biodiversity. Whereas, second-generation biofuels are still in R&D, pilot or lab scale, and utilize lignocellulosic biomass as raw materials. One such biofuel is bioethanol. Bioethanol is, by far, the most commercialized technology on the global market. To date, Brazil and the United States of America (USA) have already implemented mass production of bioethanol using sugarcane and corn as raw materials respectively.

1.2 Motivation and Problem Statement

Bioethanol is chemically known as ethyl alcohol with the chemical formula C_2H_5OH. It is produced from fermentation of simple sugars from plant sources using microbes. Bioethanol is biodegradable, low in toxicity and less likely to affect the environment. Among the advantageous properties of bioethanol as fuel energy include higher octane number (108), evaporation enthalpy, and flame speed and wider range of flammability. Other than that, it gives higher compression ratio (CR) with shorter burning time (Zabed et al.,2014). Bioethanol produces carbon dioxide (CO_2) and water when burned . This CO_2 is absorbed by the plant and at the same time, oxygen is released in the same volume. This proves to be advantageous over fossil fuels which emit CO_2 along with other toxic gases. Some bioprocesses have recommended possible routes to produce bioethanol in large volumes using low cost substrates (Gunasekaran and Raj, 1999). Late 1990, the concept of waste to wealth has been implemented. As for Malaysia, oil palm plantation and the palm oil industries contribute to the generation of most of the agricultural waste (Zahari et al., 2012). In 2008, Malaysia generated about 51 million tons of OPF, which accounts for 53% of the total palm biomass (Goh et al.,2010; MPOB,2009). Hence, OPF is a solid agrowaste which is available abundantly on oil palm plantations and usually disposed by directly left to decay or by burning on site, with only a small amount being composted. Bioethanol is obtained through the batch fermentation process using *Saccharomyces cerevisae.* Research by Zahari et al. (2012) suggests that OPF juice, which contains renewable sugars, could be a potential carbon source for bioethanol production using *S. cerevisea.* Hence, the optimum condition to produce bioethanol using OPF juice needs to be determined.

1.3 Objective

The objective of this research work is to determine the optimum temperature, pH and speed of agitation for bioethanol production from oil palm frond juice by yeast *S.cerevisae* with the use of Design Expert software.

1.4 Scope of the Research

The scope of this research is to produce bioethanol from oil palm frond juice and to optimize the production of bioethanol using Response Surface Methodology with the aid of Design Expert software. First of all, we need to obtain fermentation profile before measuring the final product concentration. Following that, we need to screen the effect of the parameters: temperature, pH and speed of agitation and finally, determine the optimum parameters in obtaining a high yield of bioethanol.

1.5 Main Contribution of This Work

The main contribution of this research is to determine the optimum condition for bioethanol production using OPF juice by using Response Surface Methodology with the aid of Design Expert software. The optimum condition obtained through this method may be applied in large-scale for mass bioethanol production.

1.6 Organisation of This Thesis

The structure of the reminder of the thesis is outlined as follows:

Chapter 1 provides the information on introduction of bioethanol. Findings that are related to optimum conditions to produce bioethanol using OPF juice are further discussed that gives rise to the motivation and problem statement of this research. This chapter also covers the objectives and scope of this research.

Chapter 2 gives a review on previous studies related to the research. This chapter provides the information about oil palm fronds, bioethanol and the factors affecting bioethanol production. The effects of all three factors are also discussed in this chapter.

Chapter 3 is a comprehensive description of the methodology for the research. This chapter covers explanations on the fermentation process, analysis method, and optimization method.

Chapter 4 provides the overall findings of the study with detailed description for each parameters studied and each analysis made. This chapter also covers the comparison of optimum conditions for bioethanol production using various raw materials.

CHAPTER 2

LITERATURE REVIEW

2.1 *Oil Palm Frond Juice as Renewable Energy Source*

Malaysia is the world's second largest palm oil producer, next to Indonesia. Since oil palm trees (*Elaeis guineensis* Jacq.) were introduced as a major crop from West Africa to Southeast Asian countries such as Malaysia, Indonesia and India, the increasing land area of oil palm plantation has been generating huge amounts of oil palm waste including trunks, fronds and empty fruit bunches (EFB) (Sun et al.,1999). In Malaysia alone, the quantity of oil palm waste production is estimated to be 115 million tons per annum. The oil palm fronds (OPF) represent approximately 83 million tons per year in available biomass from Malaysian plantations. This quantity, according to MPOC (2010), is 5.5 and 4.7 times larger than those of trunks and EFB in Malaysia, respectively.

OPF is a solid agricultural waste which is available abundantly on oil palm plantations (Goh et al.,2010). Currently, OPF is disposed by left to decay in the natural environment or by burning on site, with only a small amount being composted. This practice creates environmental problems, and better alternatives are needed to utilize or dispose OPF (Tan et al.,2011). Figure 2.1 shows the OPF that has been pruned.

Figure 2.1: Oil palm fronds after being pruned

OPF is currently under-utilized, since the plantation owners are having a misconception that all the OPF is necessary for nutrient recycling and soil conservation purposes (Hasan et al.,1994). Hence, the pruned fronds are just left in the plantation. However, a study by Zahari et al.(2012) proved that OPF does not contain high metal contents, but contain high carbohydrates in the form of simple sugars. Table 2.1 shows the metals composition in OPF, while Table 2.2 shows the sugars concentration in OPF juice.

Table 2.1: Nutrient and metallic elements in OPF and OPF juice

Analysis	Fresh OPF	OPF Juice
Nitrogen (%)	0.9	0.8
Carbon (%)	49	39
Organic Carbon (%)	37	29
Composition of nutrients and metal elements		
Sulphur (%)	0.2	0.4
Phosphorus (%)	0.02	0.02
Potassium (%)	0.2	2.3
Cadmium (%)	1.4	2.9
Magnesium(%)	0.2	0.5
Boron (ppm)	4	2
Manganese (ppm)	61	2
Copper (ppm)	2	2
Ferrum(ppm)	100	66

Table 2.2: Amount of sugars in OPF juice

Sugar(g/L)	
Fructose	1.68(±0.75)
Glucose	53.95(±2.86)
Sucrose	20.46(±1.56)
Total sugar	76.09(±2.85)

Production of sugars from dried OPF fibres has been reported by Fazilah et al.(2009) and Goh et al.(2010), which involves the conversion of cellulose and hemicellulose into glucose and xylose through hydrothermal treatment, followed by enzymatic hydrolysis. Other than that, research by Jung et al.(2012) suggested soaking in aqueous ammonia pretreatment. However, Zahari et al. (2012) recently found that renewable sugars can be obtained from OPF by simply pressing the fresh OPF using conventional sugarcane press machine to obtain the juice.

2.2 *Bioethanol*

The rising fossil fuel prices associated with growing demand for energy, and environmental concerns are some of the key factors driving a strong interest in utilizing renewable energy sources, particularly in biofuel. Bioethanol is one such biofuel, with the structural formula C_2H_5OH. Bioethanol is chemically known as ethyl alcohol and produced from fermentation of simple sugars from plant sources using microorganisms. Equation 2.1 shows the fermentation equation of glucose to ethanol.

$$C_6H_{12}O_6 \longrightarrow 2\,C_2H_5OH + 2\,CO_2 \qquad \text{(Equation 2.1)}$$

Bioethanol is a colourless liquid. It is biodegradable, low in toxicity and causes little environmental issues. In the 1970s, Brazil and the United States of America (USA) began their mass production of bioethanol from sugarcane and corn respectively. However, this is not applicable in long term, as the food chain could be affected due to decreasing food supply. Therefore, current interest lies in producing bioethanol from lignocellulosic materials.

The most common usage of bioethanol is to power automobiles. It can be combined with gasoline in any concentration up to pure ethanol (E100). Ethanol fuel blends are now widely available in the United States of America, Brazil, Europe and China. Today, bioethanol contributes around 3% of total road transport fuel globally on an energy basis (IEA,2010).

In Malaysia, the National Biomass Strategy 2020 was launched on year 2011 by the Malaysian government to promote the use of biofuel (AIM,2011) . Aiming to create higher value-added biomass economic activities that contribute towards Malaysia's gross national income (GNI), the Strategy outlined the production of bioethanol from lignocellulosic biomass, particularly oil palm biomass.

The cost of bioethanol depends on the raw materials that has been utilized. The cost of one litre of bioethanol produced from oil palm trunk is estimated to be at about RM 1.25/litre. The production cost from lignocellulosic materials is estimated at RM 0.26 .

2.3 *Factors Affecting Bioethanol Production*

2.3.1 *Temperature*

Temperature increases the rate of a reaction. The fermentation of ethanol at relatively high temperature is reported to be important for effective production in tropical countries, where average day-time temperatures are usually high throughout the year (Hashem et al.,2013) . The advantages of rapid fermentation at high temperatures are not only to reduce contamination, but also to reduce the cooling cost. However, yeast are greatly affected by temperature. Temperature affects yeast metabolism.

2.3.2 *pH*

The pH of a solution can have several effects of the enzymatic structure and activity. Changes in pH affects the shape of an enzyme, structure of an enzyme, and properties of the substrate, so that either the substrate cannot bind to the active site or it cannot undergo catalysis.

2.3.3 *Speed of Agitation*

Agitation speed is one of the factors which will affect the amount of dissolved oxygen in the cultivation medium along the fermentation process. Agitation rate is important for adequate mixing, mass transfer and heat transfer because it assists mass transfer between different phases present in the culture.

CHAPTER 3

METHODOLOGY

3.1 *Medium and Reagent Preparation*

3.1.1 *Medium Preparation*

Three types of mediums such as nutrient agar (NA) , nutrient broth (NB) and oil palm frond juice are used in this research work.

The nutrient agar (NA) that is used for this research work is the Yeast Extract Peptone Dextrose (YPD) agar. The agar can be prepared by using 20 g peptone, 20 g dextrose, 10 g yeast extract and 15 g agar. First of all, the agar powder is mixed with 900 mL of purified water in 1 Liter Schott bottle. Next, 5 g of glucose is mixed with 100 mL of distilled water in a 250 ml flask and covered with aluminium foil, before being autoclaved for 20 minutes at 121^oC. After autoclave, glucose is added to prevent Maillard reaction from occurring. After temperature has dropped to below 90^oC, agar is poured into Petri plates and left to solidify. All the plates are closed and sealed before stored in refrigerator at 4^oC.

The nutrient broth (NB) is prepared in a similar way, except that the agar powder not to be added. After autoclave, glucose is added in the broth and the solution is mixed well before being refrigerated at 4^oC.

3.1.2 *Preparation of OPF Juice*

The oil palm fronds are obtained from Felda Lepar Hilir, Kuantan, Pahang. The OPF juice is extracted by pressing the oil palm frond using the conventional sugarcane pressing machine. The OPF juice was then stored at -20^oC before use.

3.2 *Microorganism and Medium*

3.2.1 *Pure Culture Preparation*

The yeast that is used for this research work is *Saccharomyces cerevisiae* Kyokai no.7 (ATCC 26422). The yeast was dissolved in YPD broth and incubated for 1 day at 100 rpm and 30^{o}C to form yeast suspension. This suspension was used to streak on new agar plate and incubate for 2-3 days at 30^{o}C. The strain was stored at 4^{o}C.

3.2.2 *Inoculum Preparation*

About 3-4 loops were taken from the pure culture and transferred into YPD broth in shake flask. The strains were incubated for 12-18 hours at 30^{o}C and 150 rpm until reach standard initial concentration (0.2-0.4 g/L).

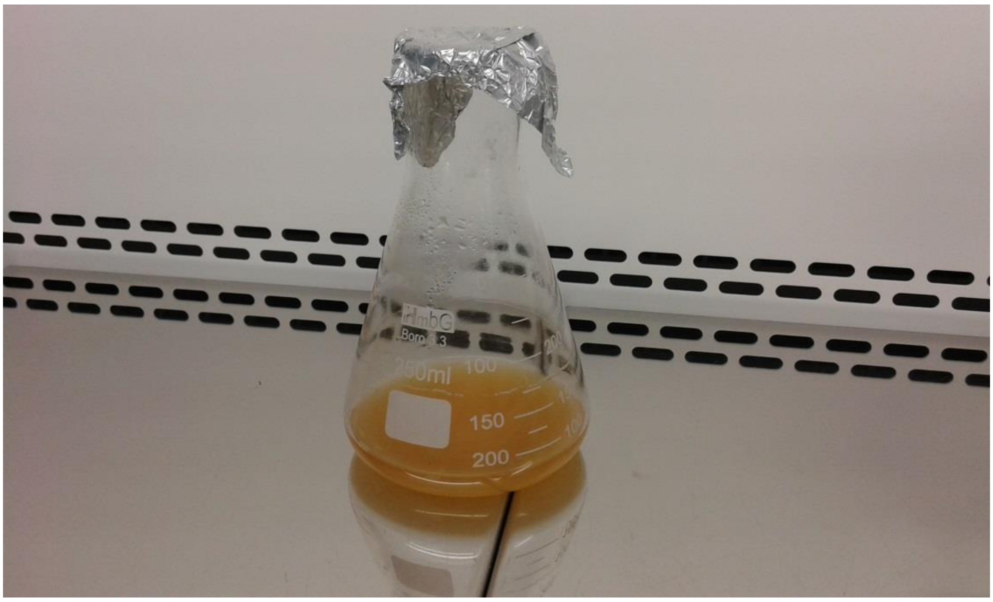

Figure 3.1 : Preparation of inoculum for fermentation

3.3 *Fermentation*

3.3.1 *Preparation of OPF Juice for Fermentation*

OPF juice was filtered and centrifuged for 30 minutes at 5000 rpm and 4^{o}C. The OPF juice was then transferred to a shake flask and pH adjustment was carried out by the addition of H_2SO_4 and NaOH using pH meter. Following that, the shake flask was covered and autoclaved for 15 minutes at 121^{o}C.

3.3.2 *Preparation of Fermentation Profile*

10% (v/v) of inoculums suspension from activated yeast flask was transferred into the sterilized OPF juice. Total working volume for each flask was kept constant at 100 mL for every run. Then, the shakeflask was purged with nitrogen gas, before being placed in incubator shaker at preferred setting. After 24 hours, 5 mL sample was collected and centrifuged at 10,000xg for 30 minutes at 4^{o}C (Thermo Fisher Scientific, NC, USA). The supernatant was then filtered using Whatman 0.22µm syringe filters into vial for HPLC analysis.

Figure 3.2 : Fermentation set-up

3.3.3 *Optimization of Parameter using Central Composite Design (CCD)*

Batch fermentation was used for the optimization of bioethanol production. The investigated parameters were temperature, pH and speed of agitation. The variable and the selected levels for the fermentation process were: pH (6-8); temperature (25-35^{o}C); agitation speed (50-100 rpm) as shown in Table 1. The range selected for the bioethanol fermentation from the OPF juice was determined according to the previous research by Nasarudin (2013).

Table 3.1: The experimental region for optimization of parameter

Independent variable	**Symbol**	**Variation levels**				
		-α	-1	0	+1	+α
Initial pH	A	5.5	6.0	7.0	8.0	8.5
Temperature (°C)	B	22	25	30	35	38
Agitation speed (rpm)	C	35	50	75	100	120

The experimental plan was generated using the Design Expert Version 7.1.6 software as shown in Table 3.2. The best combination condition suggested by the design program was validated by performing fermentation in triplicate according to the suggested parameters.

Table 3.2: Fermentation conditions for the fermentation parameter optimization

Standard Run	**Factor 1 A (Initial pH)**	**Factor 2 B (Temperature)**	**Factor 3 C (Agitation speed)**
1	6	25	50
2	8	25	50
3	6	35	50
4	8	35	50
5	6	25	100
6	8	25	100
7	6	35	100
8	8	35	100
9	5.5	30	75

10	8.5	30	75
11	7	22	75
12	7	38	75
13	7	30	35
14	7	30	120
15	7	30	75
16	7	30	75
17	7	30	75
18	7	30	75
19	7	30	75
20	7	30	75

3.4 *Methods of Analysis*

3.4.1 *High Performance Liquid Chromatography (HPLC)*

HPLC (Agilent Series 1200, USA) was used to analyse the concentration of sugar in OPF juice and ethanol content in fermentation samples. The mobile phase was acetonitrile: water (75%:25%) at a flow rate of 1.0 mL/min. The loop of injection was optimized for 10 μL injection volume. The specification for HPLC analysis for product analysis is shown in Table 3.3.

Table 3.3: Specification for HPLC for product analysis

Column	Supelcosil LC-NH2
Mobile phase	75% acetonitrile: 25% water
Standard preparation	5 g/l, 10 g/l, 15 g/l, 20 g/l and 25 g/l for each sample
Flow rate	1.0 mL/min
Injection volume	10 μL

3.4.2 *Response Surface Methodology (RSM)*

RSM consists of a set of statistical and mathematical methods which can be used to develop, improve or optimize process. RSM uses an experimental design to fit a model by least squares technique. This design must be completed by integrating experimental design with interpolation of equations in a series testing procedure (Han et al., 2011). The main advantage of RSM is the decreased amount of experimental trials required to assess various parameters and also their interactions (Karacan et al., 2007). Furthermore, even in the presence of complex interactions, RSM can be employed to analyze the relative significance of multiple affecting factors. RSM normally consists of three steps; design and experiments, followed by response surface modeling through regressions and finally optimization (Sharma et al., 2009).

Optimization of the fermentation process using RSM has been used to enhance productivity without increasing cost.Among the types of design under RSM are Central Composite Design (CCD), Box-Behnken design (B-B), One Factor design and D-optimal design. In this study, CCD was used as a method for the optimization of parameters on production of bioethanol from OPF juice.

3.4.3 *Central Composite Design (CCD)*

Central composite design (CCD) is a famous second order experimental design. The CCD is known as successful design that is used for any sequential experimentation and supply rational number of information for testing the quality of fit and not necessarily require unusually huge amount of design points thereby reducing the overall cost associated with the experiment (Korbhati et al., 2007).

CCD is an appropriate design for sequential experiments to obtain suitable information regarding lack of fit testing without a huge number of design points (Myers and Montgomery, 1996). The advantages of using CCD are easy to identify the effects of factors, optimum value determination, facilitate system modeling and offer higher precision. The important factors at different levels also can be decided by CCD design by comparing the results between predicted and experimental which can prove the accuracy and applicability of the model. This situation indicates that CCD-RSM is an effective technique to optimize the experiment (Bandaru et al., 2006).

CCD has been known by using three set of experimental runs, firstly, fractional factorial runs in which factors are studied +1, -1. Secondly, center points with all factors at their center points which help in understanding the curvature and replication helps to estimate pure error. It is followed by axial points which is similar to center points but one factor takes values above and below the median of two factorial levels typically both outside their range. The design is rotatable from its axial point (Sharma et al., 2009).

The whole fermentation process for bioethanol production can be summarized in Figure 3.3.

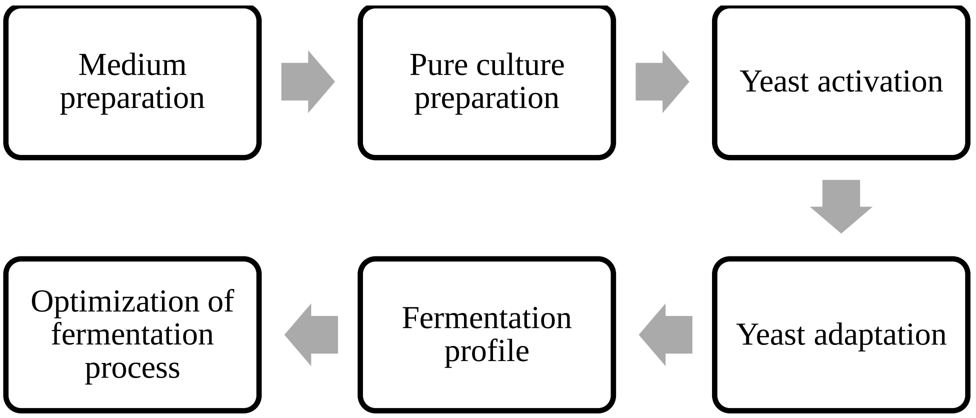

Figure 3.3: The method of fermentation process for bioethanol production