OPTIMIZATION OF BIOBUTANOL PRODUCTION BY *Clostridium* species UPM-A1 WITH LIQUID PINEAPPLE WASTE USING CENTRAL COMPOSITE DESIGN (CCD)

AHMED, IBRAHIM GALADIMA

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Universiti Teknologi Malaysia

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DEDICATION

This research work is specially dedicated to my late grandparents, both paternal (Alhaji Umar Galadima and Hajiya Aishatu Umar Galadima) and maternal (Mallam Aliyu Kwadon and Kaka Adda).

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ABSTRACT

Optimization of biobutanol production by *Clostridium* species UPM-A1 was conducted using Central Composite Design (CCD) with liquid pineapple waste as carbon source. The Design Expert® Software (State-Ease Inc., 2021 Ease Hennepin Ave, Suite 191, Minneapolis, MN, 55413) Version 6.0.4 was employed for generating the experimental design which consisted of three significant parameters, including temperature, yeast extract, and pH. The design consisted of twenty different experiments including six replicates at central points. The experiments were conducted in accordance with the conditions provided by the software, during which highest biobutanol production was attained at run three with temperature 42.50°C, yeast extract 2.14g/L, pH 5.5, and a total biobutanol concentration value of 0.441 g/L. Then, a model was generated to see the effects of the variables towards the biobutanol produced. Furthermore, the software suggested optimum value for each parameter as temperature 49.35°C, yeast extract 1.80 g/L, and pH 4 with a biobutanol concentration of 0.405 g/L. A laboratory experiment was conducted to test the predicted variables and eventually 0.417 g/L of biobutanol concentrations was achieved, which is slightly higher than the predicted values. Moreover, growth kinetics of the bacteria towards both solvents (biobutanol, acetone, and ethanol) and acids (acetic acid and butyric acid) production under the optimized conditions was conducted and found acetic acid with the highest yield (Y p/s) value of 15.26 g/g with maximum productivity of 0.23g/L/h, while biobutanol has the lowest yield (Y p/s) value of 1.29 g/g with maximum productivity of 0.01g/L/h. The specific growth rate (μ) and doubling time (t_d) were found as $0.021h^{-1}$ and 33h respectively. The research concluded that Central Composite Design (CCD) is a handy and promising approach for variable optimization towards biobutanol production. However, due to the kinetics evaluated results, the bacterial strain is not apposite for biobutanol production. Nevertheless, it could be used for acetic acid production.

ABSTRAK

Pengoptimuman penghasilan biobutanol oleh kumpulan spesies *Clostridium* UPM-A1 telah dijalankan menggunakan Rekabentuk Pusat Komposit (CCD) dengan sisa cecair nanas sebagai sumber karbon. Perisian Design Expert®, State-Ease Inc., (2021 Ease Hennepin Ave, Suite 191, Minneapolis, MN, 55413) Versi 6.0.4 telah digunakan untuk menghasilkan reka bentuk eksperimen yang terdiri daripada tiga parameter penting, termasuk suhu, ekstrak yis, dan pH. Reka bentuk terdiri daripada dua puluh eksperimen yang berbeza termasuk enam replikasi pada titik pusat. Eksperimen telah dijalankan selaras dengan syarat-syarat yang disediakan oleh perisian pada ketika penghasilan biobutanol tertinggi dicapai pada kendalian tiga, yang terdiri daripada suhu 42.50°C, ekstrak yis 2.14g/L, dan pH 5,5, dengan jumlah nilai kepekatan biobutanol 0.441 g/L. Setelah itu, satu model telah dibina untuk melihat kesan pembolehubah terhadap biobutanol yang dihasilkan. Tambahan pula, perisian telah mencadangkan nilai optimum bagi setiap parameter seperti suhu 49.35°C, ekstrak yis 1.80 g/L, dan pH 4 dengan kepekatan biobutanol 0.405 g/L. Satu eksperimen makmal telah dijalankan untuk menguji pembolehubah yang diramalkan dan akhirnya biobutanol berkepekatan 0.417 g/L telah dicapai, yang mana sedikit lebih tinggi daripada yang diramalkan. Selain itu, kinetik pertumbuhan bakteria terhadap kedua-dua penghasilan pelarut (biobutanol, aseton dan etanol) dan asid (asid asetik dan asid butyric) di bawah keadaan optimum telah dijalankan dan didapati bahawa asid asetik dengan nilai hasil tertinggi (Y p/s) iaitu 15.26 g/g dengan produktiviti maksimum 0.23g/L/h, manakala biobutanol dengan nilai hasil terendah (Y p/s) iaitu 1.29 g/g dengan produktiviti maksimum 0.01g/L/h. Kadar pertumbuhan tertentu (μ) dan masa penggandaan (t_d) telah ditemui masing-masing sebagai $0.021h^{-1}$ dan 33h. Penyelidikan ini pada akhirnya, membuat kesimpulan bahawa Rekabentuk Pusat Komposit (CCD) adalah satu pendekatan yang berguna dan menjanjikan harapan baik untuk pengoptimuman pembolehubah dalam penghasilan biobutanol. Walau bagaimanapun, kumpulan bakteria tersebut tidak sesuai untuk penghasilan biobutanol, namun ia boleh digunakan untuk penghasilan asid asetik.

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SYMBOLS AND ABBREVIATION LIST

ABE	-	Acetone-Butanol-Ethanol
ANOVA	-	Analysis of Variance
ATP	-	Standard Temperature and Pressure
CAS	-	Chemical Abstracts Services
CCD	-	Central Composite Design
CO_2	-	Carbon Dioxide
DF	-	Degree of Freedom
DNS	-	Dinitrosalicyclic acid
$Fe_2S0_4.H_20$	-	Iron anhydrous Sulphate
g	-	Gram
g/g	-	Gram per gram
g/L	-	Gram per Liter
g/L/h	-	Gram per Liter per Hour
h^{-1}	-	Per Hour
H_2	-	Hydrogen gas
HCL	-	Hydrochloric acid
Hrs	-	Hours

Hz	-	Hertz
IUPAC	-	International Union of Pure and Applied Chemistry
K_2HP0_4	-	Dipotassium Hydrogen Phosphate
Kcal	-	Kilo Calorie
KH_2P0_4	-	Potassium Dihydrogen Phosphate
KJ	-	Kilo Joule
LPW	-	Liquid Pineapple Waste
M	-	Molar
$MgSO_4.7H_20$	-	Manganessium Pentahydrose Sulphate
mL	-	Milli Liter
$MnSO_4.\ H_20$	-	Manganese anhydrose Sulphate
Nacl	-	Sodium Chloride
NADH	-	Dinucleotide Nicotinamide Adenine
NaOH	-	Sodium hydroxide
NCIMB	-	National Collections of Industrial, Marine and Bacteria
NH_4NO_3	-	Ammonium nitrate
OD	-	Optical Density
R^2	-	R- Squared
RCM	-	Reinforced *Clostridium* Medium
rpm	-	Rotation per minutes
S_{max}	-	Maximum Substrate Concentration
STD	-	Standard Temperature and Pressure
t_d	-	Doubling time
UPM	-	University Putra Malaysia
UV	-	Ultra violet

V	-	Voltage
v/v	-	Volume per volume
w/v	-	weight per volume
X	-	Biomass/Cell mass
$Y_{p/s}$	-	Yield coefficients of products based on substrate Consumption
$Y_{p/x}$	-	Yield coefficients of products relating to biomass concentration
α	-	Alpha
β	-	Beta
%	-	Percentage
μ	-	Specific growth rate
μL	-	Micro Liter
μm	-	Micro meter
μ_{max}	-	Maximum specific growth rate
°C	-	Degree Celsius

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Butanol is an alcohol (C_nH2_n+1 OH), colorless liquid with odor closely similar to that of fossil oil, containing four carbon atoms and has molecular formula C_4H_9OH. Other members of alcohol apart from butanol are methanol with a carbon atom-CH_3OH, ethanol with 2 carbon atoms-C_2H_5OH, and propanol with 3 carbon atoms-C_3H_7OH. Therefore, the term "Biobutanol" is the butyl alcohol produced from plant raw materials by means of microbial processes. Biobutanol obtained via microbial culture is highly fascinating nowadays. Since it has great achievements including automobiles as biofuels and many other applications, particularly in chemical industries which is used as solvent (Durre, 2007).

As a result of global increasing stipulate for the production of renewable fuels, more interests in microbial production of biobutanol are generated. This opened great opportunities for biologists, because anaerobic bacteria particularly *Clostridium* species are capable of converting carbohydrates into a variety of solvents such as acetone, butanol, ethanol and more the like.

The production of solvents by *Clostridium acetobutylicum* is of increasing interest worldwide due to its potential significance commercially. One of the research interests intended for raw materials cost reduction towards solvent production is the use of starch and other related materials due to their availability and can be obtained at reasonable prices (Madihah et al., 2000). Qureshi and Blaschek (2001) reported that "the cost of the substrate (raw materials) is an integral part for the overall cost of biobutanol production". It is equally important to note that biobutanol production by *Clostridium* species had been successful via Ethanol-Acetone-Biobutanol (EAB) culture processes (Durre, 2007).

In earlier generations, the substrates used for biobutanol productions includes sugar cane, raw potatoes, cereal grains etc.. It can be seen that these materials are food related which could cause a competition between utilizing them for food and at the same time for biobutanol production. As time goes on, food shortage became pronounced, because more interest is given to biobutanol production utilizing the food-related materials. This, therefore, necessitated the exploration of crop wastes and agricultural residues, including husks, shells, stovers, hulls, cobs, leaves, molasses, bagasse (Thaddeus et al., 2007), and many other materials that are non-food related, and at the same time, they are cost effective and relatively abundant.

1.2 Statement of Problems

The global demand for energy has been quite up surging exponentially, and fossil fuel reserves keep on dwindling. This could probably be due to the high utilization and turning on to fossil fuels as the foremost source of primary energy, which eventually contributed to global climate change, environmental degradation, and health problems. Nevertheless, biobutanol production from waste (agricultural raw materials, readily available and relatively less expensive) fermentation would enable a clean and environmental friendly technology for energy generation and waste treatment at the same time.

Majority of researches have been conducted to look at the effects of different factors on biobutanol production, but they confined only on conventional ways (one to one factor at a time). As well known that biobutanol production is influenced by two or more factors concurrently, hence the needs to explore the multi-factorial experimental design approach to optimize the production of biobutanol.

1.3 Objectives of the research

The study gives more concern towards the production of biobutanol from liquid pineapple wastes by *Clostridium* species UPM-A1. The study equally investigated Central Composite Design (CCD) to see the effects of the variables towards biobutanol production. Therefore, the main objectives of this work are:

a) To optimize biobutanol production by locally isolated Clostridium species UPM- A1 in batch culture using Central Composite Design (CCD).

b) To study the relationship between kinetics of solvents (biobutanol, acetone and ethanol) and acid (butyric and acetic acid) production in batch culture.

1.4 Research Scope

The research gives more priority on the optimization biobutanol production in batch culture using liquid pineapple waste. The significant factors that influence biobutanol production were equally investigated and optimized. The significant factors were evaluated through the exploration of statistical multi-factorial design method. The research investigated three significant parameters, which were previously screened by Nafizah (2010) influencing biobutanol production. The parameters include; temperature, pH, and yeast extracts. Each independent variable was studied at a high (+1) and a low (-1) level. The whole design, therefore, consisted of 20 experiments including replication at six center points. Moreover, Central Composite Design (CCD) was applied through investigating the effects of the variables for the optimization of biobutanol production.

Kinetic evaluations of products (acids and solvents) during biobutanol production were determined by identifying the value of the specific growth rate (μ), maximum specific growth rate (μ_{max}), doubling time (t_d), products productivity, yields coefficients of product formation ($Y_{p/s}$) based on substrate utilization, and yields of biomass based on substrate utilization ($Y_{p/x}$).

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