BIOBUTANOL PRODUCTION FROM GLYCEROL USING ANAEROBIC FERMENTATION PROCESS

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by

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I Dedicate This Thesis To

My Parents, Mrs. Sheulee Dey and Mr. Dilip Kumar Dey,

and My Sister, Ms. Rituparna Dey

Certificate

This is to certify that the thesis entitled "**Butanol Production from Glycerol Using Anaerobic Fermentation Process**" submitted by **Ms. Nituparna Dey** to the Indian Institute of Technology Delhi, for the award of the degree of **Doctor of Philosophy**, is a bonafide record of original research work carried out by her. She has worked under my supervision and has fulfilled the requirements, which to my knowledge, has reached the requisite standard for the submission of this thesis. The results contained in this thesis have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

Prof. Ashok N. Bhaskarwar

Thesis Supervisor

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Nituparna Dey

Abstract

There is an increasing demand growing worldwide for alternative sources of energy and the biodiesel industry is one such which is on the rise. Glycerol (10 wt.%) is produced as a by-product along with biodiesel production and it remains an underutilized product. Only a small fraction of the glycerol produced goes into the glycerol markets since there are higher costs involved with glycerol purification. It presents an opportunity for value-addition to the surplus glycerol so produced. The glycerol can be used for biobutanol production, a potential alternative fuel, which has a very high energy content of 29.2 MJ/L. Biobutanol production is conventionally carried out using an anaerobic fermentation process using *Clostridium* species which involves biphasic stages which are acidogenic (acid-producing) and solventogenic (solvent-producing).

The present study explored biobutanol production from glycerol with the objective to improve the overall butanol yield using three strains of *Clostridium pasteurianum*: ATCC 6013, NCIM 2880, and NCIM 2882. Notably, existing literature typically report low overall butanol yields from glycerol for wild-type *Clostridium pasteurianum* strains, often around 0.3 g/g (g of butanol/g of glycerol). By employing a co-substrate fermentation approach with butyric acid along with glycerol in the fermentation medium, the strain ATCC 6013 achieved a significant 30% increase in overall butanol yield, reaching 0.39 g/g. This work also documented the first-time use of the strains NCIM 2880 and NCIM 2882 for glycerol-based butanol production and highlighted strain-specific differences in their performance towards butanol production.

Furthermore, mathematical analysis was carried out describing the kinetics of glycerol consumption and cell growth. The kinetics of glycerol consumption was based on an integral equation development and a growth invariance function was used to study the kinetics of cell

growth. The development of integral equation was done with the assumption that glycerol consumption and cell growth are linked through the kinetics of the process. Two different approaches were adopted for calculating the rates of glycerol consumption and cell growth respectively.

The glycerol consumption rate, $\left(\frac{\mu_m k_1}{k_S}\right)$ (h^{-1}); range $1.30*10^{-3}$ to $29.7*10^{-3}$, was estimated from the integral equation through the experimental values of glycerol consumption.

The cell growth rate, r (h^{-1}); range $3.06*10^{-3}$ to $32.4*10^{-3}$, was estimated from the growth invariance function through the calculated values of cell mass concentrations.

It was observed from the evaluated values that the parameters, r (h^{-1}) and $\left(\frac{\mu_m k_1}{k_S}\right)$ (h^{-1}) are related. Therefore, using two different approaches (i) integral equation, and (ii) growth invariance function, the calculated rates were observed to be similar that validated the development of the integral equation and the basis that glycerol consumption and cell growth are interrelated. Additionally, the growth invariance function was used to represent the clostridial growth data, corresponding to different experimental conditions, in terms of a narrow band of curves of dimensionless population density versus dimensionless time. It depicted invariance that is represented graphically by the consistent clostridial growth profiles under different experimental conditions, with quantitative support from the range obtained for the cell growth rate $\{r\ (h^{-1})$: $3.06*10^{-3}$ to $32.4*10^{-3}\}$.

सार

दुनिया भर में ऊर्जा के वैकल्पिक स्रोतों की मांग बढ़ रही है और बायोडीजल उद्योग ऐसा ही एक उद्योग है जो तेजी से बढ़ रहा है। ग्लिसरॉल (10 wt.%) बायोडीजल उत्पादन के साथ-साथ एक उप-उत्पाद के रूप में उत्पादित किया जाता है और यह एक कम उपयोग वाला उत्पाद बना हुआ है। उत्पादित ग्लिसरॉल का केवल एक छोटा सा हिस्सा ही ग्लिसरॉल बाज़ारों में जाता है क्योंकि ग्लिसरॉल शुद्धिकरण में उच्च लागत शामिल होती है। यह उत्पादित अधिशेष ग्लिसरॉल में मूल्य-संवर्धन का अवसर प्रस्तुत करता है। ग्लिसरॉल का उपयोग बायोब्यूटेनॉल उत्पादन के लिए किया जा सकता है, जो एक संभावित वैकल्पिक ईंधन है, जिसमें 29.2 MJ/L की बहुत उच्च ऊर्जा सामग्री होती है। बायोब्यूटेनॉल उत्पादन पारंपरिक रूप से क्लॉस्ट्रिडियम प्रजातियों का उपयोग करके अवायवीय किण्वन प्रक्रिया का उपयोग करके किया जाता है जिसमें द्वि-चरणीय चरण शामिल होते हैं जो एसिडोजेनिक (एसिड-उत्पादक) और सॉल्वेंटोजेनिक (विलायक-उत्पादक) होते हैं। वर्तमान अध्ययन ने क्लॉस्ट्रिडियम पेस्ट्यूरियनम के तीन उपभेदों का उपयोग करके समग्र ब्यूटेनॉल उपज में सुधार करने के उद्देश्य से ग्लिसरॉल से बायोब्यूटेनॉल उत्पादन की खोज की: ATCC 6013, NCIM 2880, और NCIM 2882। उल्लेखनीय रूप से, मौजूदा साहित्य में आमतौर पर जंगली-प्रकार के क्लॉस्ट्रिडियम पेस्ट्यूरियनम उपभेदों के लिए ग्लिसरॉल से कम समग्र ब्यूटेनॉल उपज की रिपोर्ट की जाती है, जो अक्सर लगभग 0.3 ग्राम/ग्राम (ब्यूटेनॉल का ग्राम/ग्लिसरॉल का ग्राम) होता है। किण्वन माध्यम में ग्लिसरॉल के साथ ब्यूटिरिक एसिड के साथ सह-सब्सट्रेट किण्वन दृष्टिकोण को नियोजित करके, स्ट्रेन ATCC 6013 ने समग्र ब्यूटेनॉल उपज में 30% की महत्वपूर्ण वृद्धि हासिल की, जो 0.39 ग्राम/ग्राम तक पहुंच गई। इस कार्य ने ग्लिसरॉल-आधारित ब्यूटेनॉल उत्पादन के लिए उपभेदों NCIM 2880 और NCIM 2882 के पहली बार उपयोग को भी प्रलेखित किया और ब्यूटेनॉल उत्पादन के प्रति उनके प्रदर्शन में उपभेद-विशिष्ट अंतरों को उजागर किया। इसके अलावा, ग्लिसरॉल की खपत और कोशिका वृद्धि की गतिकी का वर्णन करते हुए गणितीय विश्लेषण किया गया। ग्लिसरॉल की खपत की गतिकी एक अभिन्न समीकरण विकास पर आधारित थी और कोशिका वृद्धि की

गतिकी का अध्ययन करने के लिए एक वृद्धि अपरिवर्तनशीलता फ़ंक्शन का उपयोग किया गया था। अभिन्न समीकरण का विकास इस धारणा के साथ किया गया था कि ग्लिसरॉल की खपत और कोशिका वृद्धि प्रक्रिया की गतिकी के माध्यम से जुड़ी हुई हैं। ग्लिसरॉल की खपत और कोशिका वृद्धि की दरों की गणना के लिए क्रमशः दो अलग-अलग दृष्टिकोण अपनाए गए। ग्लिसरॉल की खपत दर $\left(\frac{\mu_m k_1}{k_S}\right)$ (h^{-1}); रेंज $1.30*10^{-3}$ से $29.7*10^{-3}$, ग्लिसरॉल की खपत के प्रायोगिक मूल्यों के माध्यम से अभिन्न समीकरण से अनुमानित की गई थी। सेल वृद्धि दर, r (h^{-1}); रेंज $3.06*10^{-3}$ से $32.4*10^{-3}$, सेल द्रव्यमान सांद्रता के गणना किए गए मूल्यों के माध्यम से वृद्धि अपरिवर्तनशीलता फ़ंक्शन से अनुमानित की गई थी। मूल्यांकित मानों से यह देखा गया कि पैरामीटर, r (h^{-1}) और $\left(\frac{\mu_m k_1}{k_S}\right)$ (h^{-1}) संबंधित हैं। इसलिए, दो अलग-अलग तरीकों (i) इंटीग्रल समीकरण, और (ii) ग्रोथ इनवेरिएंस फ़ंक्शन का उपयोग करके, गणना की गई दरें समान पाई गईं, जिसने इंटीग्रल समीकरण के विकास और इस आधार को मान्य किया कि ग्लिसरॉल की खपत और सेल वृद्धि आपस में संबंधित हैं। इसके अतिरिक्त, ग्रोथ इनवेरिएंस फ़ंक्शन का उपयोग आयामहीन जनसंख्या घनत्व बनाम आयामहीन समय के वक्रों के एक संकीर्ण बैंड के संदर्भ में, विभिन्न प्रयोगात्मक स्थितियों के अनुरूप, क्लोस्ट्रीडियल वृद्धि डेटा का प्रतिनिधित्व करने के लिए किया गया था। इसने इनवेरिएंस को दर्शाया है जिसे सेल वृद्धि दर r (h^{-1}) के लिए प्राप्त सीमा से मात्रात्मक समर्थन के साथ, विभिन्न प्रयोगात्मक स्थितियों के तहत सुसंगत क्लोस्ट्रीडियल वृद्धि प्रोफाइल द्वारा ग्राफ़िक रूप से दर्शाया गया है: $3.06*10^{-3}$ से $32.4*10^{-3}$}।

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Nomenclature

ABE	Acetone-Butanol-Ethanol
ATCC	American Type Culture Collection
ATP	Adenosine Triphosphate
BA	Butyric Acid
BADH	Butyraldehyde Dehydrogenase
BDH	Butanol Dehydrogenase
BBWD	Biobutanol-Water-Diesel
BWD	Butanol-Water-Diesel
CDH	Central Drug House
COD	Coefficient of Determination
DCW	Dry Cell Weight
DHAP	Dihydroxyacetone Phosphate
DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen
FID	Flame Ionization Detector
GC	Gas Chromatography
HMF	Hydroxymethyl Furfural
MA	Mathematical Analysis
MTCC	Microbial Type Culture Collection and Gene Bank
NA	Nicotinic Acid
NAD	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide Hydrogen
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NCIM	National Collection of Industrial Microorganisms

NCL	National Chemical Laboratory
O.D.	Optical Density
PDO	Propanediol
PEP	Phosphoenolpyruvate
PSIG	Pound-force per Square Inch Gauge
RCM	Reinforced Clostridial Medium
RPM	Revolutions Per Minute
RT	Retention Time
TES	Trace Element Solution
C_g	Glycerol concentration (g/L), single chemical species (growth rate limiting)
k	Specific carrying capacity of the environment (m^{-3})
K_d	Rate of loss of cell mass due to cell death (h^{-1})
k_S	Saturation constant (g/L)
k_1	Arbitrary integration constant (g_{cell}/L)
k_2	Proportionality constant ($g_{cell}/g_{glycerol}$)
r	Reproductive potential of the individual species (h^{-1})
t	Time (h)
X	Cell mass concentration (g/L)
$\bar{X}$	Dimensionless population density
θ	Dimensionless time
μ_g	Gross specific growth rate (h^{-1})
μ_m	Maximum specific growth rate (h^{-1})
μ_{net}	Net specific growth rate (h^{-1})

τ	Time at which cell population attains a chosen degree of approach to the limiting population density (h)
φ	Modified dimensionless population density