

CO-CULTURE STUDY ON GLUCOSE AND
XYLOSE MIXTURE IN BATCH AND CHEMOSTAT
MODE FOR BIOETHANOL PRODUCTION

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INDIAN INSTITUTE OF TECHNOLOGY DELHI

DECEMBER 2021

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CO-CULTURE STUDY ON GLUCOSE AND
XYLOSE MIXTURE IN BATCH AND CHEMOSTAT
MODE FOR BIOETHANOL PRODUCTION

by

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Submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

to the



Indian Institute of Technology Delhi

December 2021

Dedicated

to

my family

CERTIFICATE

This is to certify that the thesis entitled "Co-culture study on glucose and xylose mixture in batch and chemostat mode for bioethanol production" being submitted by **Mr. Shashi Kumar** is worthy of consideration for the award of the degree of Doctor of Philosophy. The thesis has been prepared by him under my supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology Delhi and is a record of the original bonafide research work. The results presented in this thesis have not been submitted in part or full to any other universities or institutes for the award of any other degree or diploma.

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ACKNOWLEDGEMENTS

I want to express my deepest gratitude to my supervisor, **Prof. T. R. Sreekrishnan**, and **Prof. G. P. Agarwal** for his exemplary guidance, monitoring, and constant encouragement throughout this thesis. The blessings, help, and guidance given by him from time to time shall carry me a long way in the journey of life on which I am about to embark.

I would also like to thank my student research committee members, Prof. Atul Narang, Dr. Ashish Misra, and Prof. Vivek V. Buwa, for their valuable suggestions and review that helped me make my work better and presentable.

I also take this opportunity to express a deep sense of gratitude to the Indian Institute of Technology Delhi and the Ministry of Human Resource and Development, Government of India, to grant me financial assistance during my Ph.D. Research work.

I am also thankful to Mr. Anish Raju, Dr. Himanshu Verma, Dr. Ziauddin Ansari, and Mr. Rajeev Kumar Dahiya for their support during my research work in the Bioprocess lab and department instrumentation lab. Their support made my workflow very smooth and easy.

I would express special thanks to Dr. Anita Srivastava and Dr. Sunil Kumar for their valuable suggestions, kind help, and guidance, which helped me keep myself motivated.

It would be a great pleasure to thank my friends for making this long and challenging journey always joyful and easy.

I would like to thank my parents, brother, and sister for their regular support and blessings that helped me to carry out my work very smoothly and stress-free.

Shashi Kumar

ABSTRACT

The present study was designed to study the behavior of co-culture of *Saccharomyces cerevisiae* NCIM 3219 and *Pichia stipitis* NCIM 3507 for bioethanol production in batch mode followed by a chemostat and chemostat with cell recirculation mode from a mixture of glucose and xylose. The study was divided into three objectives involving strategy development to maximize ethanol concentration and its volumetric productivity. The monoculture of *S. cerevisiae* has reported an increase in ethanol concentration upon increasing the glucose content. A maximum ethanol concentration of 38.3 ± 0.66 g/L, corresponding to an ethanol yield and volumetric productivity of 0.48 g/g, and 1.07 g/L/h, respectively, resulted from 80.0 g/L glucose with *S. cerevisiae*. A similar pattern was observed with *P. stipitis* fermentation. The *P. stipitis* resulted in a maximum ethanol concentration of 23.5 ± 0.44 g/L, corresponds to 0.39 g/g ethanol yield and 0.32 g/L/h volumetric productivity from 60.0 g/L xylose. However, 50 g/L xylose resulted in 22.6 g/L ethanol, which corresponds to 0.45 g/g ethanol yield and 0.61 g/L/h volumetric productivity. The obtained results in the case of *P. stipitis* have shown that 50.0 g/L xylose was the maximum sugar utilized efficiently. In contrast, 60.0 g/L initial xylose resulted in a 13.7% decrease in ethanol yield, which might be due to the toxic effect of ethanol on *P. stipitis*. In context to the optimization of the essential process parameter for co-culture fermentation using mixed sugar substrate, the maximum ethanol concentration and yield were achieved at 0.05 vvm aeration rate and 2:1 glucose/xylose ratio. The co-culture resulted in maximum ethanol concentration, ethanol yield, and volumetric productivity of 12.3 ± 0.10 g/L, 0.43 g/g, and 0.26 g/L/h, respectively. In contrast, the monoculture of *P. stipitis* resulted in 8.96 ± 0.13 g/L, 0.36 g/g, and 0.19 g/L/h, respectively. The fermentation carried out in microaerobic mode delivered 10.7% more ethanol concentration and 10.6% more ethanol yield than the combination of anaerobic and microaerobic modes from glucose. Also, the

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glucose uptake rate was improved by 50.1%, suggesting that the lower microaerophilic condition was supportive for glucose conversion faster by yeast cells in the co-culture system. Hence, co-culture cultivation in a microaerobic mode would be a better condition to achieve maximum ethanol and productivity. At relatively higher concentration of mixed sugar substrate (i.e., 40.0 g/L glucose and 20.0 g/L xylose), the sequential co-culture (combination of anaerobic and microaerobic mode) resulted in a maximum ethanol concentration, yield, and its volumetric productivity of 22.5 ± 1.15 g/L, 0.38 g/g, and 0.23 g/L/h. The sequential co-cultivation was found to give 1.32-fold more ethanol and more xylose conversion, from 33% to almost 100%, than the simultaneous co-culture. Despite achieving a higher ethanol concentration in sequential co-culture, volumetric productivity and ethanol yield were still low. Therefore, continuous cultivation was used. The continuous cultivation resulted in the ethanol volumetric productivity of 0.29 g/L/h, 0.58 g/L/h, and 0.71 g/L/h, and yield coefficient of 0.33 g/g, 0.45 g/g, and 0.47 g/g from 30.0 g/L, 45.0 g/L, and 60.0 g/L mixed sugar substrate, containing glucose to xylose ratio of 2:1 at 0.03 h^{-1} dilution rate. Continuous mode improved the ethanol productivity and yield by 3.08-fold and 1.24-fold, respectively, compared to sequential co-culture, which was further increased using a high dilution rate with cell recycling mode. The maximum volumetric productivity of 3.28 g/L/h was attended at a 0.13 h^{-1} dilution rate, corresponds to 69.5% xylose consumption and 100% glucose consumption through continuous mode with a cell recirculation system. The lower dilution rate, 0.08 h^{-1} resulted in 93.1% xylose consumption and 1.96 g/L/h volumetric productivity. The continuous mode with cell recycles at 0.13 h^{-1} dilution rate improved productivity by 4.60-fold compared to continuous mode without cell recycling from 40.0 g/L glucose and 20.0 g/L xylose. The ethanol concentration and yield resulting from this condition were 25.2 g/L and 0.47 g/g, respectively. These findings represented the membrane bioreactor as a potential tool to make the bioethanol production process economic from mixed sugar substrate.

वर्तमान अध्ययन को बैच मोड में बायोथेनॉल उत्पादन के लिए *सैक्रोमाइस सेर्विसे* एनसीआईएम ३२१९ और *पिचिया स्टिपिटिस* एनसीआईएम ३५०७ की सह-संस्कृति के व्यवहार का अध्ययन करने के लिए डिजाइन किया गया था जिसके बाद ग्लूकोज और जाइलोज के मिश्रण से किण्वन के सेल रिसर्चर मोड के साथ कीमोस्टेट और कीमोस्टेट किया गया था। पूरे अध्ययन को तीन उद्देश्यों में विभाजित किया गया था, जिसमें इथेनॉल एकाग्रता और इसकी मात्रात्मक उत्पादकता को अधिकतम करने के लिए रणनीति विकास शामिल था। *एस सेरेविसिया* की मोनोकल्चर ने माध्यम में ग्लूकोज की मात्रा बढ़ाने पर इथेनॉल एकाग्रता में वृद्धि दिखाई है। इसके परिणामस्वरूप ग्लूकोज की ८० ग्राम प्रति लीटर से ३८.३३ ± ०.६६ ग्राम प्रति लीटर की अधिकतम इथेनॉल सांद्रता हुई, जो क्रमशः ०.४८ ग्राम प्रति ग्राम और १.०७ ग्राम प्रति लीटर प्रति ऑवर की इथेनॉल उपज और मात्रात्मक उत्पादकता के अनुरूप है। *पी स्टिपाइटिस* किण्वन के साथ इसी तरह के पैटर्न देखे गए। *पी स्टिपाइटिस* के परिणामस्वरूप अधिकतम इथेनॉल सांद्रता २३.५० ± ०.४४ ग्राम प्रति लीटर, ०.३९ ग्राम प्रति ग्राम इथेनॉल उपज और ०.३२ ग्राम प्रति लीटर प्रति ऑवर उत्पादकता से ६० ग्राम प्रति लीटर जाइलोज से मेल खाती है। हालांकि, ५० ग्राम प्रति लीटर जाइलोज के परिणामस्वरूप २२.६१ ग्राम प्रति लीटर इथेनॉल, ०.४५ ग्राम प्रति ग्राम इथेनॉल यील्ड और ०.६१ ग्राम प्रति लीटर प्रति ऑवर वॉल्यूमेट्रिक उत्पादकता से मेल खाती है। *पी स्टिपाइटिस* के मामले में प्राप्त परिणामों से पता चला है कि ५० ग्राम प्रति लीटर जाइलोज अधिकतम चीनी थी जिसका कुशलतापूर्वक उपयोग किया जाता था। इसके विपरीत, ६० ग्राम प्रति लीटर प्रारंभिक जाइलोज के परिणामस्वरूप इथेनॉल उपज में १३.६८% की कमी आई, जिसके परिणामस्वरूप *पी स्टिपाइटिस* पर इथेनॉल के जहरीले प्रभाव के कारण हो सकता है। मिश्रित चीनी सबस्ट्रेट का उपयोग करके सह-संस्कृति किण्वन के लिए आवश्यक प्रक्रिया पैरामीटर के अनुकूलन के संदर्भ में, टीवह अधिकतम इथेनॉल एकाग्रता और उपज ०.०५ वॉल्यूम प्रति वॉल्यूम प्रति मिनट वातारण दर और २:१ ग्लूकोज/जाइलोज अनुपात पर प्राप्त किए गए थे। सह-संस्कृति के परिणामस्वरूप अधिकतम इथेनॉल एकाग्रता, इथेनॉल यील्ड और वॉल्यूमेट्रिक उत्पादकता क्रमशः १२.३३ ± ०.१ ग्राम प्रति लीटर, ०.४३ ग्राम प्रति ग्राम, और ०.२६ ग्राम प्रति लीटर प्रति ऑवर हुई जबकि, *पी स्टिपाइटिस* की मोनोकल्चर क्रमशः ८.९ ६ ± ०.१३ ग्राम प्रति लीटर, ०.३६ ग्राम प्रति ग्राम, और ०.१९ ग्राम प्रति लीटर प्रति ऑवर हुई। माइक्रोएरोबिक मोड में किए गए किण्वन ने एनारोबिक और माइक्रोएरोबिक मोड के संयोजन की तुलना में ग्लूकोज से क्रमशः १०.६८% और १०.५६% अधिक इथेनॉल एकाग्रता और उपज प्रदान की। इसके अलावा, ग्लूकोज तेज दर ५०.१६% से सुधार किया गया था, सुझाव है कि कम मिक्रोएरोबिक हालत ग्लूकोज रूपांतरण के लिए तेजी से सह संस्कृति प्रणाली में खमीर कोशिकाओं द्वारा सहायक था। इसलिए, माइक्रोएरोबिक मोड में सह-संस्कृति खेती अधिकतम इथेनॉल और उत्पादकता प्राप्त करने के लिए बेहतर स्थिति होगी। मिश्रित चीनी सबस्ट्रेट की अपेक्षाकृत अधिक एकाग्रता पर (यानी, ४० ग्राम प्रति लीटर ग्लूकोज और २० ग्राम प्रति लीटर जाइलोस) अनुक्रमिक सह-संस्कृति (एनारोबिक और माइक्रोएरोबिक

मोड का संयोजन) के परिणामस्वरूप अधिकतम इथेनॉल एकाग्रता, उपज, और इसकी मात्रा 22.85 ± 1.15 ग्राम प्रति लीटर, 0.32 ग्राम प्रति ग्राम, और 0.23 ग्राम प्रति लीटर प्रति ऑवर। अनुक्रमिक सह-खेती को 1.32 गुना अधिक इथेनॉल और अधिक जाइलोज रूपांतरण देने के लिए पाया गया, 33% से एक साथ सह-संस्कृति की तुलना में लगभग 100% तक। बावजूद, अनुक्रमिक सह-संस्कृति में उच्च इथेनॉल एकाग्रता प्राप्त करना, वॉल्यूमेट्रिक उत्पादकता और इथेनॉल उपज अभी भी कम थी। इसलिए लगातार खेती की गई। निरंतर खेती के परिणामस्वरूप इथेनॉल की मात्रा 0.29 ग्राम प्रति लीटर प्रति ऑवर, 0.46 ग्राम प्रति लीटर प्रति ऑवर और 0.79 ग्राम प्रति लीटर प्रति ऑवर हुई और 0.33 ग्राम प्रति ग्राम, 0.45 ग्राम प्रति ग्राम, और 0.47 ग्राम प्रति ग्राम से 30 ग्राम प्रति लीटर, 45 ग्राम प्रति लीटर, और 60 ग्राम प्रति लीटर मिश्रित चीनी सबस्ट्रेट का गुणांक प्राप्त करें, जिसमें ग्लूकोज से जाइलोज अनुपात $2:1$, 0.03 प्रति ऑवर डायलूशन दर से होता है। सतत मोड ने अनुक्रमिक सह-संस्कृति की तुलना में इथेनॉल उत्पादकता और उपज में क्रमशः 3.06 गुना और 1.24 गुना सुधार किया, जिसे सेल रीसायकल मोड के साथ उच्च डायलूशन दर का उपयोग करके और बढ़ाया गया था। 3.26 ग्राम प्रति लीटर प्रति ऑवर की अधिकतम मात्रा में 0.13 प्रति ऑवर डायलूशन दर से भाग लिया गया, सेल रिसर्चर प्रणाली के साथ निरंतर एम स्तोत्र के माध्यम से 69.4% जाइलोज खपत और 100% ग्लूकोज खपत से मेल खाती है जबकि, 0.06 प्रति ऑवर की डायलूशन दर 93.1% जाइलोज खपत और 1.96 ग्राम प्रति लीटर प्रति ऑवर वॉल्यूमेट्रिक उत्पादकता के परिणामस्वरूप हुई। 0.13 प्रति ऑवर डायलूशन दर पर सेल रिसाइकिल के साथ सतत मोड 40 ग्राम प्रति लीटर ग्लूकोज और 20 ग्राम प्रति लीटर जाइलोज से सेल रीसायकल के बिना निरंतर मोड की तुलना में उत्पादकता में 4.60 गुना सुधार हुआ। इसके अलावा, इथेनॉल एकाग्रता और उपज इस स्थिति में क्रमशः 25.21 ग्राम प्रति लीटर, और 0.47 ग्राम प्रति ग्राम था। इन निष्कर्षों ने मिश्रित चीनी सबस्ट्रेट से बायोइथेनॉल उत्पादन प्रक्रिया को आर्थिक बनाने के लिए एक संभावित उपकरण के रूप में झिल्ली बायोरिएक्टर का प्रतिनिधित्व किया।

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LIST OF SYMBOLS AND ABBREVIATIONS

$Y_{P/S}$	Ethanol yield co-efficient (g/g)
P_f	Final ethanol concentration (g/L)
P_0	Initial ethanol concentration (g/L)
S_0	Initial substrate concentration (g/L)
S_f	Final substrate concentration (g/L)
$Y_{X/S}$	Biomass yield co-efficient (g/g)
X_f	Final biomass concentration (g/L)
X_0	Initial biomass concentration (g/L)
$Y_{Z/Xyl}$	Xylitol yield co-efficient (g/g)
Z_f	Final xylitol concentration (g/L)
Z_0	Initial xylitol concentration (g/L)
Xyl_0	Initial xylose concentration (g/L)
Xyl_f	Final xylose concentration (g/L)
$Y_{P/Glu}$	Ethanol yield co-efficient for glucose (g/g)
Glu_0	Initial glucose concentration (g/L)
Glu_f	Final glucose concentration (g/L)
$Y_{P/Xyl}$	Ethanol yield co-efficient for xylose(g/g)
η (%)	Fermentation efficiency

List of Symbols and Abbreviations

$Y_{P/S (E)}$	Experimental observed yield co-efficient (g/g)
$Y_{P/S (M)}$	Maximum theoretical yield co-efficient (g/g)
Q_P	Volumetric ethanol productivity (g/L/h)
T_f	Final time (h)
T_0	Initial time (h)
$r_{s Glu}$	Glucose consumption rate (g/L/h)
$r_{s Xyl}$	Xylose consumption rate (g/L/h)
D	Dilution rate
F	Flow rate
V	Volume
μ	Specific growth rate
μm	Micrometer
$^{\circ}\text{C}$	Degree centigrade
T	Temperature
kDa	Kilodalton
h	Hour
g	Gram
L	Liter
g/L	Gram per liter
g/L/h	Gram per liter per hour

List of Symbols and Abbreviations

g/g	Gram per gram
DCW	Dry cell weight
mM	Millimolar
M	Molar
mL	Milliliter
min	Minute
mL/min	Milliliter per minute
rpm	Revolution per minute
vvm	Volume per volume per minute
psi	Pounds per square inch
~	Approximately
%	Percentage
nm	Nanometer
h ⁻¹	Per hour
v/v	Volume per volume
YPD	Yeast extract peptone dextrose
YPX	Yeast extract peptone xylose
GYP	Glucose yeast extract peptone
XYP	Xylose yeast extract peptone
LGXYP	Low glucose and xylose yeast extract peptone

List of Symbols and Abbreviations

HGXYP	High glucose and xylose yeast extract peptone
CGYP	Chemostat glucose yeast extract peptone
CXYP	Chemostat xylose yeast extract peptone
XYPA	Xylose yeast extract peptone ammonium sulfate
LGXYPA	Low glucose and xylose yeast extract peptone ammonium sulfate
HGXYP	High glucose and xylose yeast extract peptone ammonium sulfate
BGYPA	Batch glucose yeast extract peptone ammonium sulfate
BXYPA	Batch xylose yeast extract peptone ammonium sulfate
HCl	Hydrochloric acid
H ₂ SO ₄	Sulfuric acid
NaOH	Sodium hydroxide
MgSO ₄	Magnesium sulfate
CaCl ₂	Calcium chloride
NaCl	Sodium chloride
(NH ₄) ₂ SO ₄	Ammonium sulfate
KH ₂ PO ₄	Potassium dihydrogen phosphate