UTILIZATION OF BIOCHAR AND STABILIZED MICROBIAL CONSORTIUM FOR ENHANCED BIOMETHANATION OF RICE STRAW

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by

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Dedicated to my beloved Parents

“**Sh. Krushna Ambadas Bhujbal**

&

Smt. Godavari Krushna Bhujbal”

CERTIFICATE

This is to certify that the thesis entitled **"Utilization of Biochar and Stabilized Microbial Consortium for Enhanced Biomethanation of Rice Straw"** being submitted by **Mr. Sachin Krushna Bhujbal** to the Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy**, is a record of bona fide research work carried out by him. He has worked under our supervision and has fulfilled the requirements for the submission of this thesis, which has attained the standard required for a Ph. D. degree of the Institute. The research results presented in this thesis have not been submitted elsewhere for any degree or diploma award.

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ABSTRACT

Anaerobic digestion of lignocellulosic biomass faces the challenges of lower biodegradability and biogas yield due to its complex recalcitrant chemical structure. To improve the biodegradability and biomethanation of lignocellulosic biomass, the development of an efficient lignocellulolytic microbial consortium is necessary. Rumen microorganisms are recognized as efficient degraders of lignocellulosic biomass than conventional anaerobic digester microorganisms due to their inherent capacity to break down substrates rich in cellulosic fibers. Biochar has emerged as a promising additive in anaerobic digestion due to its inherent properties, such as hydrolysis promotion, immobilization capability, facilitation of direct interspecies electron transfer, and buffering capacity. This Ph.D. thesis aimed to enhance the biomethanation of rice straw by employing a combination of strategies: utilizing a robust rumen fluid microbial consortium and incorporating lignocellulosic biomass-derived biochar as an additive. The rumen fluid microbial consortium was pre-acclimatized to rice straw through a series of batch subcultures to enrich and stabilize the microbial consortium. Subsequently, a biomethane potential assay was conducted using this enriched consortium, resulting in promising biogas and methane yields of 321.4 ± 31.2 mL/g VS and 158.9 ± 20.8 mL/g VS without any physical, chemical, or biological pre-treatment of the rice straw.

The influence of biomass type and pyrolysis temperatures on the physicochemical properties of biochar was studied. Three biomass feedstocks – rice straw, sugarcane bagasse, and corn stover were pyrolyzed at temperatures ranging from 400 °C to 600 °C at a heating rate of 10 °C/min for 1 hour of residence time. Pyrolysis temperature significantly influenced the physicochemical properties of the biochars. Increasing pyrolysis temperature from 400 °C to 600 °C resulted in an increase in ash content, fixed carbon content, pH, BET surface area, and total pore volume of biochar. Conversely, moisture content, volatile matter, hydrogen, nitrogen, oxygen content, mean pore diameter and the abundance of acidic and polar surface

functional groups decreased with increasing pyrolysis temperature. A batch anaerobic digestion experiment was conducted to investigate the impact of the substrate-to-inoculum ratio (1:1 and 1:2), biochar types (rice straw biochar, corn stover biochar, and sugarcane bagasse biochar), and dosages (1%, 2% and 3% w/v) on biogas and methane production from rice straw. The highest cumulative methane yield of 245.3 ± 3.9 mL/g VS was obtained at the substrate-to-inoculum ratio of 1:1 with 2% (w/v) corn stover biochar addition, followed by 2% sugarcane bagasse biochar addition and substrate to inoculum ratio of 1:2, which yielded 232.9 ± 9.8 mL/g VS of methane yield. The Pearson Correlation analysis revealed the correlations between the physicochemical properties of biochar and cumulative methane yield. The O/C ratio, H/C ratio, BET surface area, and total pore volume of the biochar were positively correlated with cumulative methane yield. Contrarily, the mean pore diameter and pH of the biochar were negatively correlated with cumulative methane yield.

Optimization of anaerobic digestion process parameters and biochar dosage is necessary to improve methane production. Therefore, two independent optimization experiments were conducted. These experiments employed an artificial neural network-genetic algorithm and response surface methodology. Different dosages of biochar, along with varying loading rates of rice straw and inoculum, were added for these experiments. In the first optimization experiment, the optimal operational conditions predicted by response surface methodology yielded cumulative biogas yield, cumulative methane yield, and volatile solids reduction values of 533.1 ± 22.3 mL/g VS, 269.7 ± 11.3 mL/g VS, and 80.3 ± 2.9%, respectively. The optimal operational conditions predicted by response surface methodology showed higher cumulative biogas yield (7.8%), cumulative methane yield (6.7%), and volatile solids reduction (8%) than the genetic algorithm. In the second optimization experiment, the optimal operational conditions predicted by genetic algorithm yielded cumulative methane yield of 293.7 ± 7.3

mL/g VS. The methane yield obtained at optimal conditions of the genetic algorithm was 8.6% higher than the response surface methodology.

Continuous pilot-scale anaerobic digestion was conducted in 307 L digesters (working volume: 192 L) for a study period of 64 days. The corn stover biochar supplementation exhibited the daily average specific biogas and methane yield of 368.6 ± 81.6 L/kg VS and 230 ± 54.4 L/kg VS, which were 35% and 37% higher than the control. Principle component analysis indicated that the volatile solids reduction positively correlated with the daily average specific biogas and methane yield. Metagenomic analysis revealed that the corn stover biochar supplementation facilitated microbial colonization with the enrichment of unclassified genera from families Planococcaceae, Clostridiaceae, and Ruminococcaceae, and genus *Clostridium* and methanogenic archaea (*Methanosarcina* and *Methanobacterium*). The co-occurrence network revealed a notable shift in microbial interactions following the supplementation of corn stover biochar as an additive in anaerobic digestion.

सार

लिग्नोसेल्युलोसिक अपशिष्टों की जटिल संरचना के कारण उनका अवायवीय पाचन चुनौतीपूर्ण होता है। इन अपशिष्टों में जैव-अपघटन की दर कम होती है और परिणामस्वरूप बायोगैस का उत्पादन भी कम होता है। लिग्नोसेल्युलोसिक बायोमास के जैव-अपघटन और बायोमेथेनेशन को बेहतर बनाने के लिए, एक कुशल लिग्नोसेल्युलोसिक सूक्ष्मजीवीय समुदाय का विकास आवश्यक है। जुगाली करने वाले जानवरों के पेट में रहने वाले सूक्ष्मजीवों को पारंपरिक सूक्ष्मजीवों की तुलना में लिग्नोसेल्युलोसिक बायोमास के अत्यधिक कुशल अपघटक के रूप में मान्यता प्राप्त है, क्योंकि इनमें सेल्युलोजिक रेशा से भरपूर सब्सट्रेट को तोड़ने की आंतरिक क्षमता होती है। बायोचार अपने अंतर्निहित गुणों, जैसे हाइड्रोलिसिस प्रमोशन, स्थिरीकरण क्षमता, प्रत्यक्ष अंतर-प्रजाति इलेक्ट्रॉन हस्तांतरण की सुविधा और बफरिंग क्षमता के कारण अवायवीय पाचन में एक आशाजनक योजक के रूप में उभरा है। इस पीएचडी शोध का उद्देश्य चावल के भूसे के बायोमेथनीकरण को बढ़ाने के लिए जुगाली करने वाले जानवरों के पेट के द्रव से प्राप्त सूक्ष्मजीवों के समुदाय और लिग्नोसेल्युलोसिक अपशिष्ट से प्राप्त बायोचार का संयुक्त उपयोग करके एक प्रभावी रणनीति विकसित करना है। जुगाली करने वाले जानवरों के पेट के द्रव में पाए जाने वाले सूक्ष्मजीवों को बैच सबकल्चर विधि से चावल के भूसे के लिए अनुकूलित किया गया। इस प्रक्रिया के माध्यम से सूक्ष्मजीवों का एक मजबूत और स्थिर समुदाय विकसित किया गया। इसके बाद, इस समृद्ध समुदाय का उपयोग करके एक बायोमेथेन क्षमता परीक्षण किया गया, जिसके परिणामस्वरूप बिना किसी पूर्व-उपचार के 321.4 ± 31.2 mL/g VS और 158.9 ± 20.8 mL/g VS की आशाजनक बायोगैस और मीथेन उपज प्राप्त हुई।

बायोचार के भौतिक-रासायनिक गुणों पर बायोमास के प्रकार और पायरोलिसिस तापमान के प्रभाव का अध्ययन किया गया। तीन बायोमास फीडस्टॉक्स - चावल का भूसा, गन्ना छिलका और मक्का का डंठल को

400°C से 600°C के तापमान पर 10°C/मिनट की ताप दर पर 1 घंटे के लिए पायरोलाइज़ किया गया। पायरोलिसिस तापमान ने बायोचार के भौतिक-रासायनिक गुणों को महत्वपूर्ण रूप से प्रभावित किया। पायरोलिसिस तापमान को 400°C से 600°C तक बढ़ाने से राख की मात्रा, स्थिर कार्बन की मात्रा, पीएच, बीईटी सतह क्षेत्र और बायोचार के कुल छिद्र आयतन में वृद्धि हुई। इसके विपरीत, बढ़ते पायरोलिसिस तापमान के साथ नमी की मात्रा, वाष्पशील पदार्थ, हाइड्रोजन, नाइट्रोजन, ऑक्सीजन की मात्रा, औसत छिद्र व्यास और अम्लीय और ध्रुवीय सतह कार्यात्मक समूहों की प्रचुरता में कमी आई। चावल के भूसे से बायोगैस उत्पादन को अधिकतम करने के लिए विभिन्न सब्सट्रेट-टू-इनोकुलम अनुपातों (1:1 और 1:2), विभिन्न प्रकार के बायोचार (चावल के भूसे, मक्का के डंठल और गन्ना छिलके से बने) और विभिन्न मात्रा में बायोचार (1%, 2% और 3% w/v) के प्रभाव का अध्ययन करने के लिए बैच अवायवीय पाचन प्रयोग किया गया। परिणामों से पता चला कि 1:1 के सब्सट्रेट-टू-इनोकुलम अनुपात और 2% (w/v) मक्का के डंठल के बायोचार को मिलाने पर सबसे अधिक मीथेन (245.3 ± 3.9 mL/g VS) प्राप्त हुआ। इसके बाद 2% गन्ना छिलका बायोचार और 1:2 के सब्सट्रेट-टू-इनोकुलम अनुपात पर 232.9 ± 9.8 mL/g VS मीथेन प्राप्त हुआ। पियर्सन सहसंबंध विश्लेषण से पता चला है कि बायोचार के भौतिक-रासायनिक गुणों और मीथेन उत्पादन के बीच एक गहरा संबंध है। बायोचार में कार्बन-ऑक्सीजन अनुपात, कार्बन-हाइड्रोजन अनुपात, बीईटी सतह क्षेत्र और कुल छिद्र आयतन का मीथेन उत्पादन के साथ सीधा संबंध पाया गया। इसके विपरीत, बायोचार के औसत छिद्र व्यास और पीएच का मीथेन उत्पादन के साथ विपरीत संबंध पाया गया।

अवायवीय पाचन प्रक्रिया के मापदंडों और बायोचार की खुराक का अनुकूलन मीथेन उत्पादन में सुधार के लिए आवश्यक है। इसलिए, दो स्वतंत्र अनुकूलन प्रयोग किए गए। इन प्रयोगों में एक कृत्रिम न्यूरल नेटवर्क-आनुवंशिक एल्गोरिदम और प्रतिक्रिया सतह पद्धति का उपयोग किया गया। इन प्रयोगों के लिए बायोचार की

विभिन्न खुराक, साथ ही चावल के भूसे और इनोकुलम की अलग-अलग लोडिंग शामिल थी। पहले अनुकूलन प्रयोग में, प्रतिक्रिया सतह पद्धति द्वारा अनुमानित इष्टतम परिचालन स्थितियों से संचयी बायोगैस उपज, संचयी मीथेन उपज और वाष्पशील ठोस कमी के मान क्रमशः 533.1 ± 22.3 mL/g VS, 269.7 ± 11.3 mL/g VS और 80.3 ± 2.9% प्राप्त हुए। प्रतिक्रिया सतह पद्धति द्वारा अनुमानित इष्टतम परिचालन स्थितियों ने आनुवंशिक एल्गोरिथम की तुलना में उच्च संचयी बायोगैस उपज (7.8%), संचयी मीथेन उपज (6.7%), और वाष्पशील ठोस कमी (8%) दिखाई। दूसरे अनुकूलन प्रयोग में, आनुवंशिक एल्गोरिथम द्वारा अनुमानित इष्टतम परिचालन स्थितियों से 293.7 ± 7.3 mL/g VS की संचयी मीथेन उपज प्राप्त हुई। आनुवंशिक एल्गोरिथम की इष्टतम स्थितियों पर प्राप्त मीथेन उपज प्रतिक्रिया सतह पद्धति की तुलना में 8.6% अधिक थी।

307 लीटर के पाचक (कार्यशील आयतन: 192 लीटर) में 64 दिनों की अवधि के लिए निरंतर पायलट-स्केल अवायवीय पाचन का संचालन किया गया। मक्का के डंठल के बायोचार के पूरक ने प्रतिदिन औसत विशिष्ट बायोगैस और मीथेन उपज क्रमशः 368.6 ± 81.6 L/kg VS और 230.0 ± 54.4 L/kg VS प्रदर्शित की, जो नियंत्रण की तुलना में क्रमशः 35% और 37% अधिक थी। प्रिंसिपल कंपोनेंट एनालिसिस ने संकेत दिया कि वाष्पशील ठोस पदार्थों की कमी दैनिक औसत विशिष्ट बायोगैस और मीथेन उपज के साथ सकारात्मक रूप से सहसंबद्ध थी। मेटा-जीनोमिक विश्लेषण से पता चला कि मक्का के डंठल के बायोचार के पूरक ने प्लैनोकोकेसी, क्लोस्ट्रिडिएसी और रुमिनोकोकेसी परिवारों से असंवर्गीकृत जेनेरा और क्लोस्ट्रीडियम जीनस और मेथेनोजेनिक आर्किया (मेथेनोसरसिना और मेथेनोबैक्टीरियम) के समृद्धिकरण के साथ सूक्ष्मजीवी उपनिवेशीकरण की सुविधा प्रदान की। सह-घटना नेटवर्क ने अवायवीय पाचन में मक्का के डंठल के बायोचार को योजक के रूप में पूरक करने के बाद सूक्ष्मजीवी अंतःक्रियाओं में एक उल्लेखनीय बदलाव का पता लगाया।

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°C	=	Degree Celsius
>	=	Greater than
<	=	Lower than
%	=	Percent
/	=	Per
&	=	And
L	=	Liter
mL	=	Milliliter
d	=	Day
g	=	Gram
GJ	=	Gigajoule
Kg	=	Kilogram
m^3	=	Cubic meter
mg	=	Milligram
mm	=	Millimeter
µm	=	Micrometer
w/v	=	Weight by volume
R^2	=	Coefficient of determination
ASTM	=	American Society for Testing and Materials
AD	=	Anaerobic digestion
ADF	=	Acid detergent fiber
ADL	=	Acid detergent lignin
AMPTS	=	Automatic methane potential test system

ANN	=	Artificial neural nbetwork
ANOVA	=	Analysis of varience
BBD	=	Box-Behnken design
BET	=	Brunauer-Emmett-Teller
BJH	=	Barrett–Joyner–Halenda
BMP	=	Biomethane potential assay
BP	=	Backpropagation
$CaCO_3$	=	Calcium carbonate
CAZymes	=	Carbohydrate-active enzymes
CBMs	=	Carbohydrate-binding modules
CBY	=	Cumulative biogas yield
CEs	=	Carbohydrate esterases
C/N ratio	=	Carbon to nitrogen ratio
CCD	=	Central campsite design
CMY	=	Cumulative methane yield
COD	=	Chemical oxygen demand
CSB	=	Corn stover biochar
CSB 400	=	Corn stover biochar produced at 400 °C
CSB 500	=	Corn stover biochar produced at 500 °C
CSB 600	=	Corn stover biochar produced at 600 °C
DIET	=	Direct interspecies electron transfer
DTG	=	Differential thermogravimetric analysis
FE-SEM	=	Field emission scanning electron microscopy
FFNNs	=	Feed-forward neural networks
FTIR	=	Fourier Transform Infrared Spectroscopy

GA	=	Genetic algorithm
GHs	=	Glycoside hydrolases
H/C ratio	=	Hydrogen to carbon ratio
HCL	=	Hydrochloric Acid
HRT	=	Hydraulic retention time
LCB	=	Lignocellulosic biomass
LOF	=	Lack of fit
MLP	=	Multilayer perceptron
MSE	=	Mean squared error
MT	=	Million tonnes
NaOH	=	Sodium hydroxide
NDF	=	Neutral detergent fiber
NH_3	=	Ammonia
NH_3-N	=	Ammonia nitrogen
NH_4^+	=	Ammonium ions
O/C ratio	=	Oxygen to carbon ratio
OLR	=	Organic loading rate
PLs	=	Polysaccharide lyases
RMSE	=	Root mean squared error
RS	=	Rice straw
RSB	=	Rice straw biochar
RSB 400	=	Rice straw biochar produced at 400 °C
RSB 500	=	Rice straw biochar produced at 500 °C
RSB 600	=	Rice straw biochar produced at 600 °C

RSM	=	Response surface methodology
SBC	=	Sugarcane bagasse biochar
SBC 400	=	Sugarcane bagasse biochar produced at 400 °C
SBC 500	=	Sugarcane bagasse biochar produced at 500 °C
SBC 600	=	Sugarcane bagasse biochar produced at 600 °C
S/I ratio	=	Substrate to inoculum ratio
TGA	=	Thermogravimetric analysis
TS	=	Total solids
VFAs	=	Volatile fatty acids
VS	=	Volatile solids
VSR	=	Volatile solids reduction