PRODUCTION OF BIOETHANOL FROM POTATO PEEL USING AMYLASE-PRODUCING *BACILLUS THURINGIENSIS*

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Abstract: Bioethanol production from agricultural waste is a promising approach to sustainable energy generation. This study investigates the potential of potato peel as a substrate for bioethanol production using *Bacillus thuringiensis*. Potato peel, a common byproduct of the food industry, is rich in carbohydrates, making it an ideal candidate for microbial fermentation. *Bacillus thuringiensis* was selected due to its known ability to ferment a variety of sugars into ethanol. While *Bacillus thuringiensis* is primarily recognized for its role as a biopesticide, there is potential for its use in bioethanol production, though this is not its primary function.

The process involved the hydrolysis of potato peel to release fermentable sugars, followed by fermentation using *Bacillus thuringiensis* under optimized conditions. Key parameters such as pH, temperature, and fermentation time were systematically varied to maximize ethanol yield. The results demonstrated that *Bacillus thuringiensis* efficiently converted the potato peel into bioethanol, with a significant yield under optimal conditions.

This study highlights the viability of using for bioethanol production from potato peel, providing a sustainable alternative to conventional feedstocks. The findings suggest that potato peel, often considered waste, can be valorized into a valuable biofuel, contributing to waste reduction and energy sustainability. Further research is recommended to scale up the process and explore the economic feasibility of commercial bioethanol production from this substrate.

Key words : Bioethanol , Amylase , *Bacillus thuringiensis*, Fermentation, Agricultural Waste, Renewable Energy

INTRODUCTION

Biofuel is a renewable and sustainable energy source derived from biological materials such as plants, crops, or organic waste. Unlike fossil fuels, which are finite and pose environmental challenges, biofuels present a cleaner alternative as they are produced from recently living organisms. The two main types of biofuels are bioethanol and biodiesel. Bioethanol is typically made by fermenting sugars and starches found in crops like corn, sugarcane, and even from news paper(Nebiyu Chali Yadeta et al. 2019).In this process, yeasts or

bacteria convert these sugars into ethanol, which is then distilled to produce bioethanol. Biodiesel, on the other hand, is created through the transesterification of vegetable oils, animal fats, or recycled cooking oil, converting these feedstocks into biodiesel and glycerol.

The synthetic production of bioethanol using microbes aims to improve the efficiency and sustainability of biofuel production. By utilizing engineered or naturally efficient microorganisms, this approach can convert a broader range of feedstocks, including agricultural residues and waste materials, into bioethanol more efficiently and at lower costs than traditional methods. This process reduces the dependence on food crops, alleviates competition with food supplies, and supports environmental sustainability by lowering greenhouse gas emissions. Moreover, microbial fermentation provides flexibility and adaptability in production, allowing for optimization based on different feedstocks and conditions. Ultimately, this advancement promotes both technological innovation and sustainable energy solutions.

Amylases is an enzymes that produced by different organisms which help to convert starch into simple sugar such as glucose, maltose, and dextrin (My, T. T. A. et al. 2021). Microbial amylases are the most common and widely preferred in industrial applications than that of other sources because of their advantages, such as cost effectiveness, productivity, thermos stability, and simple optimization process (Ashwini K. et al. 2011). Many bacteria have been isolated and documented as amylase producing bacteria, including genera *Arthrobacter*, *Escherichia*, *Micrococcus, Proteus, Pseudomonas, Serratia* and *Streptomyces* (Joshi, N. et al. 2021). Many bacteria in the genus Bacillus are also capable of producing a high amount of amylase (Gopinath, K. P. et al. 2017). Some Bacillus and Vibrio bacteria were isolated from mangrove soil samples in Brazilian mangrove sediment and reported as amylase-producing bacteria (Klinfoong, R. et al. 2022). The interest in *Bacillus thuringiensis* for ethanol production is driven by its adaptability to diverse and often harsh environmental conditions, as well as its ability to efficiently hydrolyze complex polysaccharides. This capability is particularly significant in the context of utilizing non-food biomass, such as agricultural residues and waste materials, as feedstocks for ethanol production. By harnessing the enzymatic potential of *B. thuringiensis*, researchers aim to develop more sustainable and cost-effective methods for producing ethanol, thereby reducing dependence on food-based crops and minimizing the environmental impact of biofuel production.

Bacillus thuringiensis (Bt), traditionally recognized for its role in biological pest control due to its insecticidal properties, has recently attracted attention for its potential application in bioethanol production. This bacterium is capable of producing a variety of enzymes, such as cellulases and amylases, which are essential for breaking down complex carbohydrates into fermentable sugars. In bioethanol production, starch materials are first converted into simple sugars through the addition of enzymes like alpha-amylase, a process known as liquefaction. However, the high cost of commercially available amylase can be a challenge. To address this, *Bacillus thuringiensis* can be utilized as a cost-effective alternative, replacing the need for commercial amylase by producing the necessary enzymes naturally. These sugars serve as the primary substrates for ethanol fermentation, making *B. thuringiensis* a viable candidate for bioethanol production from

lignocellulosic biomass. This research explores the optimization of *Bacillus thuringiensis* for ethanol production, focusing on enhancing its enzymatic activity, fermentation efficiency, and overall ethanol yield. The findings from this study could contribute to advancing bioethanol production technologies, offering new insights into the utilization of microbial systems in renewable energy production.

Potato peel, a by-product of the potato processing industry, represents a considerable source of starch-rich waste. Studies have shown that potato peel can be an effective substrate for bioethanol production due to its high carbohydrate content (Kumar, P. et al. 2017). Utilizing such agro-industrial waste aligns with the principles of a circular economy, enhancing resource efficiency and sustainability (Galanakis, C. M. 2018). Potato peel, a by-product of the potato processing industry, represents a considerable source of starch-rich waste. Studies have shown that potato peel can be an effective substrate for bioethanol production due to its high carbohydrate content . Utilizing such agro-industrial waste aligns with the principles of a circular economy, enhancing resource efficiency and sustainability (Galanakis, C. M. 2018).

The enzymatic hydrolysis of starch into fermentable sugars is a critical step in the bioethanol production process. Amylases, which catalyze the breakdown of starch, are pivotal in this process. Research indicates that certain bacterial strains, such as *Bacillus* species, are proficient in amylase production, making them suitable for industrial applications (Gupta, R. et al. 2003). Specifically, *Bacillus thuringiensis*, traditionally known for its insecticidal properties, has shown potential in producing amylases that can efficiently hydrolyze starch (Saxena, R. K. et al. 2007).

Bioethanol is often viewed as more environmentally friendly than fossil fuels because it reduces greenhouse gas emissions when burned. Its production also supports agricultural development and can foster rural economic growth (Iqbal, et al. 1997). However, concerns have been raised about the sustainability of bioethanol, particularly regarding land use, food security, and the energy required for its production.

This study investigates the utilization of amylase-producing *Bacillus thuringiensis* for the hydrolysis of potato peel starch and subsequent fermentation to produce bioethanol. By integrating findings from various research efforts, this work aims to contribute to the growing body of knowledge on the use of agricultural waste for renewable energy production and highlight the potential of *Bacillus thuringiensis* as a bioethanol production agent.

MATERIALS AND METHODS

Isolation of Amylase producing organisms

A soil sample was collected from nutrient-rich soil and suspended in 90 ml of sterile 0.85% NaCl solution. A 0.1 ml aliquot of the soil suspension was then spread onto starch agar plates after serial dilution. The plates were incubated at 37°C for 3 days. Following incubation, the plates were flooded with iodine solution, and clear zones around the colonies were observed.

Identification of Isolates

Microscopic Examination of Isolates

The Gram staining technique was performed to classify the bacterial isolates based on their cell morphology. Additionally, capsule and endospore staining were conducted to identify any special structures.

Cultural and biochemical Identification of isolates

The isolates were inoculated on Nutrient Agar and incubated at 37°C for 3 days. The cultures were identified by observing colony morphology, including characteristics such as color, shape, size, colony nature, and pigmentation. Additionally, the cultures were inoculated into various biochemical media, and the isolates were identified based on the resulting outcomes.

Molecular identification of isolates

The bacterial isolates were sent to the Rajiv Gandhi Centre for Biotechnology (RGCB) for molecular identification. The resulting nucleotide sequences have been submitted to the NCBI GenBank database.

Enzyme Extraction

A 10 mL inoculum was transferred into 90 mL of sterile starch broth medium and incubated at 37°C for 24 hours. After incubation, the culture broth was centrifuged at 13,000 rpm for 10 minutes at 4°C to remove the cells. The resulting supernatant, labeled as crude enzyme, was collected and stored at −20°C for further studies.

Enzyme assay

A standard starch solution was prepared by dissolving 2 g of starch in 100 mL of distilled water. From this solution, 1 mL was pipetted into each tube. Increasing concentrations of the extracted amylase enzyme (0.25, 0.50, 0.75, 1.00, and 1.25 mL) were added to the tubes. Then, 1 mL of DNS reagent was added to each tube, mixed well, and all tubes were placed in a water bath at 50°C for 5 minutes. After cooling to room temperature, the absorbance was measured at 540 nm against a blank. The concentration of reducing sugar was measured, and a standard graph was plotted.

Bioethanol Production

Potato peels were cut into small pieces, dried, and stored at room temperature (25-5°C) until use. The dried potato peels were then added to a beaker containing nutrient broth with the organism and kept at room temperature for 1 day. After 24 hours, yeast was added to the beaker, and the mixture was left for 3 days.

Estimation of Bioethanol

Potassium Dichromate Method: Approximately 34 grams of potassium dichromate is dissolved in 500 mL of distilled water in a 1-liter volumetric flask. The flask is then placed in an ice container, and 325 mL of concentrated H_2SO_4 is added drop by drop to minimize heat generation. The solution is thoroughly mixed, cooled, and diluted to a final volume of 1 liter with distilled water. A 1 mL sample is pipetted into a volumetric flask, followed by the addition of 10 mL of dichromate reagent. The flask is incubated in a water bath at 60°C for 20 minutes, after which the mixture is cooled and the volume is adjusted to 50 mL with distilled water. A linearity curve is plotted using ethanol concentrations ranging from 1% to 10% (v/v), with the blank solution prepared using distilled water. The ethanol content in the test sample is determined by UV spectrophotometry, using the linearity curve at 620 nm.

RESULT AND DISCUSSION

Isolation of organisms

Upon the addition of iodine solution, several colonies (labeled A1-A7) displayed clear zones of clearance around them on starch agar, indicating starch degradation. These selected colonies were identified as Gram-positive and rod-shaped following Gram staining (fig 2). Further special staining revealed that these organisms are capsulated (fig 3) and capable of forming endospores (fig 4).

Fig : 1 zone of clearance on starch agar plate

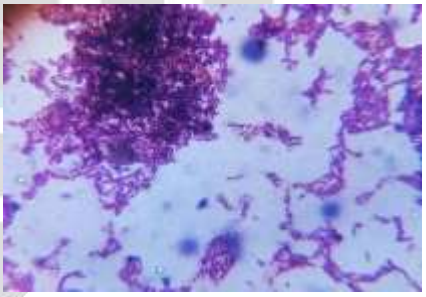


Fig : 2 Gram positive rod shape

Fig : 3 presence of endospores

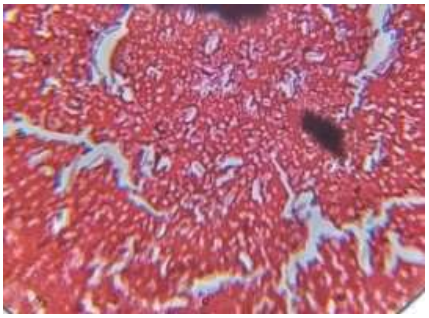


Fig : 4 Presence of capsule

Identification of organism

The isolated colonies were sub cultured onto nutrient agar plates to establish pure cultures and then tested on various biochemical media. Their characteristics were documented and summarized in **Table 1.**

Fig 5: Colony morphology on nutrient agar plate

SIZE	MEDIUM
Surface Appearance	1.Rough 2.Mucoid 3.Opaque
Color	White
Pigmentation	Non pigmented
Shape	Round
Margin	Undulate

Table 1 : cultural Characteristics of isolates

No.	Biochemical test	Result
1.	Indole test	Negative
2.	Methyl red test	Positive
3	Citrate test	Positive
4.	Triple Sugar Iron Agar test	Positive
5.	Urease test	Positive
6.	Nitrate test	Positive
7.	Catalase test	Negative
8.	Oxidase test	Positive

Table :2 Biochemical characteristics of isolate

Molecular identification of isolate

After molecular sequencing the sequence of organism is

ATCTTATGAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATA
ACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGC
TCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGG
GAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAA
GTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAG
CGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTSTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAAA

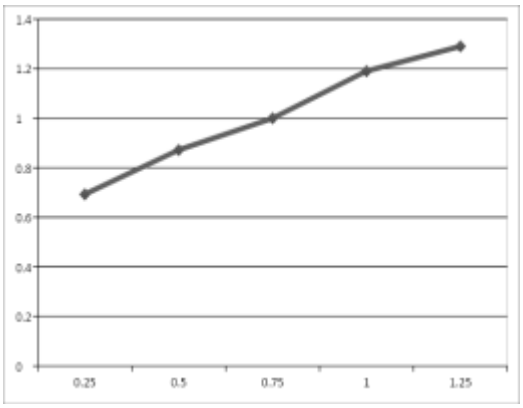
CTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGAC
TTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAA
GTGTTAGAGGGTTTCCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTG
ACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGA
TAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA
CCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGC
CCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTT
CGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAA.

The molecular sequence provided was analyzed using NCBI, and it was identified that the isolated organism is ***Bacillus thuringiensis.***

Enzyme extraction and its assay

The enzyme amylase was extracted using centrifugation from a selected strain (fig 6) and its optical density was measured to assess starch-degrading activity using the DNS method. The results, as illustrated in the graph, indicate that the isolate exhibited starch-degrading activity. Furthermore, the rate of starch hydrolysis increased with higher concentrations of amylase.

Fig : 6 Extracted enzyme from isolate



NO	Concentration of Enzyme (ml)	OD value
1.	0.25	0.693
2.	0.50	0.872
3.	0.75	1
4.	1	1.19
5.	1.25	1.29

Table 3: starch degrading activity of amylase isolates and plotted graph against the values

The graph clearly showed that concentration degrading activity increases with concentration of amylase enzyme from isolates

Analysis of alcohol fermentation and its estimation

To evaluate the potential of the isolate to convert starch into glucose as a substrate for biofuel production, fermentation was carried out. After fermentation, a preliminary analysis was conducted based on aroma, color, turbidity, and acidity. The pH was found to be acidic, and a clear solution with a pleasant fruity smell was observed. The level of fermentation was measured using the potassium dichromate method, revealing an alcohol content of 7.02% in 1 ml of the sample.

No	Concentration of alcohol	OD value
1	2	.3
2	4	.6
3	6	.9
4	8	1.1
5	Un known	1

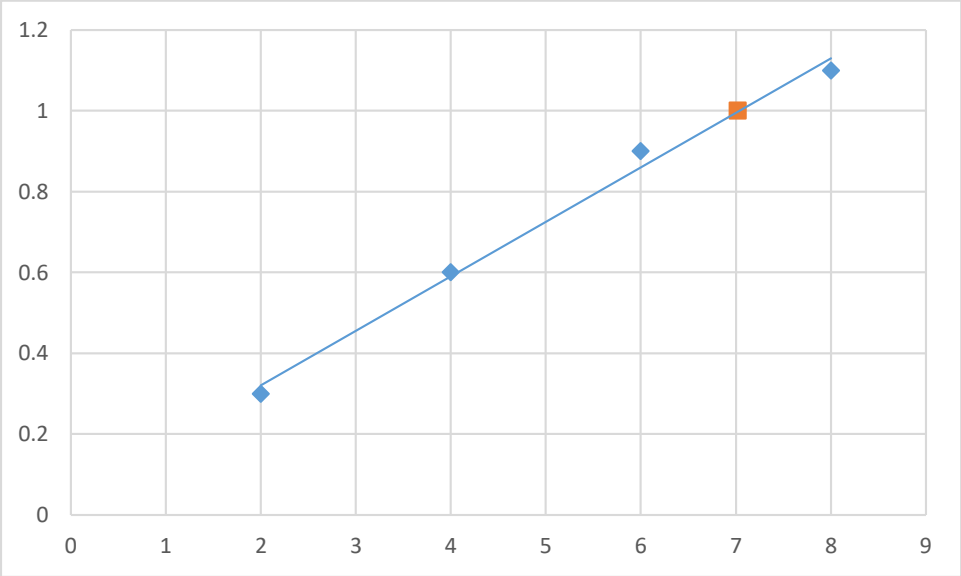


Table 4 Displaying the various alcohol concentrations and the OD values of an unknown sample, plotted on a graph .

DISCUSSION

This study underscores the potential of potato peel as a cost-effective and sustainable substrate for bioethanol production, with *Bacillus thuringiensis* playing a crucial role in the enzymatic hydrolysis of starch. The amylase enzyme produced by *Bacillus thuringiensis* effectively catalyzed the breakdown of starch in potato peel into fermentable sugars, which were then successfully converted into bioethanol through fermentation. The efficiency of this process highlights the bacterium's capability as a reliable source of amylase, which could serve as an economical alternative to commercially available enzymes. Potato peel, being an abundant by-product of the potato processing industry, offers distinct advantages, including its high starch content, ease of access, and the limited need for extensive pretreatment, which is typically required for other biomass

sources such as corn stover or sugarcane bagasse. The bioethanol yield from potato peel was found to be on par with those from traditional feedstocks, suggesting that potato peel could be a valuable resource for bioethanol production. Moreover, the use of *Bacillus thuringiensis* could reduce the overall production costs, making this process more feasible for industrial applications, especially in regions with significant potato processing activities. Nonetheless, further research is necessary to optimize the hydrolysis and fermentation processes to enhance bioethanol yield. Additionally, long-term studies on the stability and performance of *Bacillus thuringiensis* amylase under industrial conditions are essential to confirm its industrial scalability and reliability.

CONCLUSION

Growing environmental concerns, increased population, and the need to meet the diversification of the source of global energy have led to increased demand for biofuels. However, the high cost of raw materials for biofuels production has continued to slow down the acceptability, universal accessibility, and affordability of biofuels. Potato is one of the most popular and essential foods in the world. The huge quantity of potato peel waste (PPW) generated globally has been a source of concern to waste managers and environmentalists over the years. Currently, efforts are being made regarding the conversion of PPW to a form of biofuel including biogas and bioethanol.

In this method we have used as the source of PPW and the organism isolated from the soil .By this method used bulk amount of bio ethanol produced .The production of biofuel is carried out with the hydrolytic enzymes and yeast. So *Bacillus thuringenesis* can be used in the PPW we can obtained bulk amount of bioethanol. The application of PPW for biofuel application will reduce the pump price of biofuels, ensure the appropriate disposal of waste, and contribute towards environmental cleanliness

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