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Production of Itaconic Acid from Carbohydrate-Rich Material by using Fungi

Anand pol[1], Ajay Wadekar[2] Sanket Khobragade[3], Dr. V. R. Diware[4]
[1, 2, 3]*B.E. Student, Department of Chemical Engineering, SSBT's COET, Bambhori, NMU Jalgaon, India.*
[4]*Head of Department of Chemical Engineering, SSBT's COET, Bambhori, NMU Jalgaon, India.*

Abstract: ***Itaconic acid is dicarboxylic white crystalline acid which is soluble in water, ethyl alcohol and acetone, 2-Methylidenebutanedioic acid is that the IUPAC name of itaconic acid. In industries, itaconic acid is produced by fermentation of glucose using fungi because of the high cost of glucose commercial itaconic acid production became relatively expensive. Thus, there is a requirement to use cheaper substrate such as carbohydrate- rich material to bring down the price of itaconic acid. In the present study was made to produce Itaconic acid from carbohydrate- rich material (sweet potato, sugarcane, beetroot) by using Pencillium fellutanum with NCIM no.1227 and employing submerged fermentation. The acid production was characterized by FTIR, this project adds value to the environment by utilizing carbohydrate rich waste as a substrate thereby contributing to cleaner society.***

Keyword: Pencillium fellutanum with NCIM no.1227, Carbohydrate-rich Material, FTIR Analysis

I. INTRODUCTION

Itaconic acid is often referred as methylene succinic acid and has the chemical formula $C_5H_6O_4$. Currently, *A. terreus* used for the production of itaconic acid by fermentation at temperature around 37◦C, with glucose. Itaconic acid gives higher yield by fungal species than bacterial species [1]. Itaconic acid was historically produced by various chemical methods like Destructive distillation of citric acid was first described by Baup in (1836)[2] and this was the main method of producing itaconic acid, the demand of itaconic acid had been increasing year after year, due to high distillation cost also the citric acid leads to low itaconic acid yields, therefore, the product became relatively expensive, due increasing demand there is a need to bring down the cost of itaconic acid. Then the first biosynthesis by fungi from carbohydrates was established by Kinoshita in (1932) and bioprocesses are more environmentally friendly provide yield at a lower cost. later many attempts were additionally created to develop a biotechnical process for itaconic acid production, The fermentation process for the production of itaconic acid was carried out using *Aspergillus terreus* strain and *ustilago maydis strain* by using rice, corn, and potato starch waste as substrate[3] Itaconic acid is commercially produced by the cultivation of *Aspergillus terreus* using starch hydrolysate as a carbon source[4]itaconic acid production was carried out using *Ustilago maydis* from numerous agro waste like ground nut shells, rice bran, rice husk, orange pulp, peanut oil cake, orange pulp, and sugarcane bagasse as a carbon substrate[5]. Itaconic acid synthesis is the first confirmation of a highly specific mechanism disrupting microbial metabolism by immune cells. [6]. The demand for biologically fermented itaconic acid is increasing due to its applications in textile, chemical, and pharmaceutical industries, by using carbohydrate-containing waste helps to reduce the cost of itaconic acid. The present study was made for the production of itaconic acid by *Pencillium fellutanum* with *NCIM* no.*1227* and The waste that we are utilizing in this project is readily available in the market which is not in use, utilization of cheaper substrates to make the process even more economical and it adds value to the environment by utilizing waste as a substrate.

II. MATERIALS AND METHODS

Pencillium fellutanum with *NCIM* no.1227 was obtained from CSIR-National Chemical Laboratory, Pune.
We did subculture of that fungal strain to increase the quantity of that strain by providing the required medium for their growth.

III. PREPARATION OF MEDIUM (POTATO DEXTROSE AGAR)

200 g of peeled potatoes are slashed into small pieces and mixed in 1000ml of distilled water and steamed for 30min. pour the extract or filter through strainer and create the entire volume to 1000ml. add 20g of dextrose 0.1g yeast extract and twenty g of agar. This medium helps for the expansion of fungus strain because it acts like nutrients to fungus. *Pencillium fellutanum* with *NCIM* no.*1227* was cultivated on Petri dishes and slants by providing PDA as a nutrition but before this, we have to sterilize the required equipment in the autoclave for 20min during this experiment Make sure the surrounding is clean and fresh and fungus culture was cultivated by streaking on petri dishes and slants and this experiment is carried out in laminar flow ultra clean air unit. The

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inoculated agar plate is covered and the tube slant is sealed with non- absorbent cotton wool. And moved in the incubator at 28°C for 4 for days. After 4 days we observed that round, white, cottony colonies of fungus grew out of the indicator medium agar plate.

IV. PREPARATION OF SUBSTRATE

The substrates utilized in the present study are carbohydrate-rich waste like beetroot, sugarcane, sweet potato was collected from the fruit market, Jalgaon So before fermentation needs pre-treatment of substrate for submerged fermentation. A high amount of oxygen is required for this process. The bioactive compounds are secreted into the fermentation broth, in an additional advantage of this Submerged Fermentation technique is that the purification of products became easier.[7] we required all material in liquid form for this process.

Peeled of the collected material and prepared a juice of raw material and transferred for sterilization, Sterilization was done at 121°C pressure of 15 psi for twenty min in an autoclave. After sterilization centrifuged that juice at 4000rpm for 7min supernatant was used to assay itaconic acid production, supernatant collected in 500ml conical flask and *Pencillium fellutanum* was inoculated to the production medium, and transferred to orbital shaking incubator for 5days because It was discovered that there is a rise within the itaconic acid production with an rise in time of incubation showing most at fifth day where itaconic acid production was maximum[5] the production method needs continuous aeration and high-power input with high stirring intensities, resulting in increased mechanical stress that damages the mycelia. To beat this disadvantage, we should have carried out itaconic acid production process in air-lift bioreactors or orbital shaking incubator containing draft tubes instead of stirrers were developed to increase production rates.

It is reported that the itaconic acid production was increased with temperature up to 35°C[1] so the fermentation was carried out at 35°C and rotation speed of orbital shaking incubator was maintained at 200rpm.The fermented liquor was decolorized with activated carbon and send for filtration then the collected sample was analyse by FTIR.

V. RESULT

A. Characterization of Itaconic Acid by FTIR

The filtrate sample was characterized by the functional groups using FTIR technique. The IR spectrum was presented in **Figure 1**. The sample O-H stretch appears in the region 3000 - 2500 cm−1, cantered at about 2902 cm−1. An intense band at 1685 cm−1 is associated with the carbonyl C = O stretch of a carboxylic acid. It indicates that the sample is conjugated unsaturated carboxylic acid. The carboxylic acid C-O stretch appears at 1163 cm−1, and the O - H stretch is at 1390cm−1. The band at approximately 1623 cm−1 is the C = C stretch. The outcomes show that structure of sample is a carboxylic acid with conjugated double bonds, which is consistent with the structure of Itaconic acid.

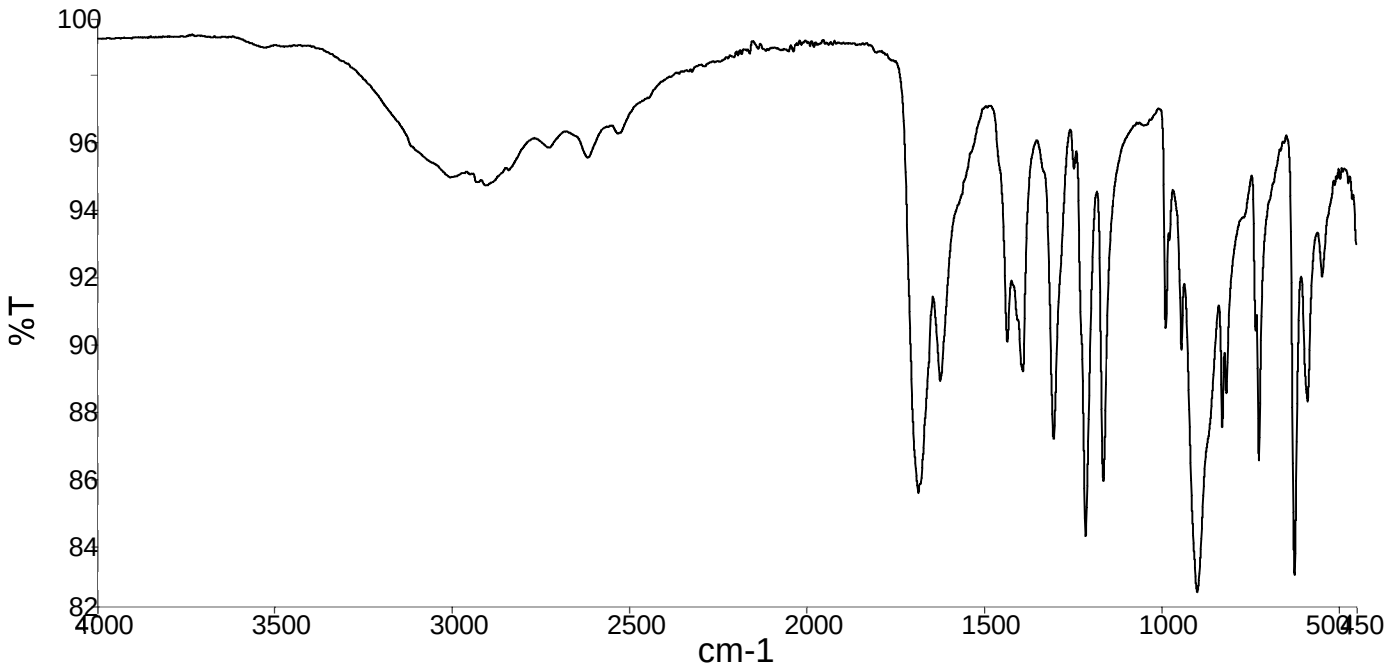


Figure 1

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VI. CONCLUSION

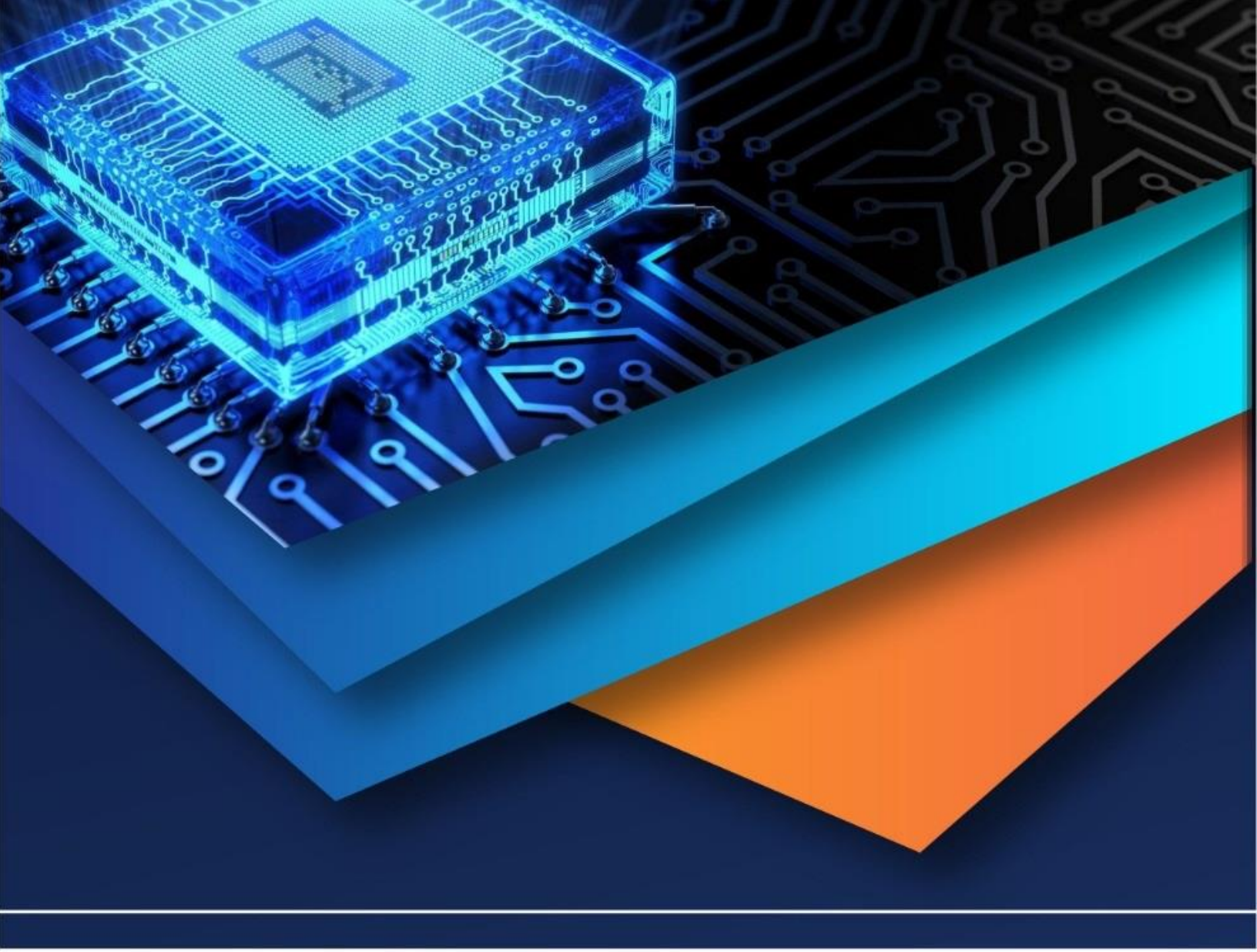
By analysis, it is concluded that the *Pencillium fellutanum* with *NCIM* no.*1227* is an alternative producer of itaconic acid. The fungal strain was selected is *Pencillium fellutanum* with *NCIM* no.*1227* because it has an application of producing itaconic acid but there was no latest study on itaconic acid production by using *Pencillium fellutanum* with *NCIM* no.*1227* and sugar cane, beetroot, sweet potato as substrate. By keeping this in view, the present study was planned to utilize the waste and making useful product. Utilization of cheaper substrates to make the process even more economical and it adds value to the environment by utilizing waste as a substrate. The result of the present study indicates that by utilizing Carbohydrate rich waste as a cheaper substrate we can produce itaconic acid and this is the better way to reduce to cost of itaconic acid by lowering the cost of the substrate.

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