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Bioethanol Production from organic kitchen waste using Saccharomyces cerevisiae

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Abstract

A continuous depletion of non renewable resources especially the fossil fuels, economic crises in the country and a constant elevation in the environmental pollution demands a need for the development of alternative fuels that can not only satisfy the increasing need but also environment friendly causing no add on to the pollution. One of the best alliterative and eco friendly protocol is the use of bioethanol produced from the organic wastes. Use of this kind of fuel for transportation can enhance the quality of atmosphere by reducing the environmental burden from poisonous gases. Here is an organic based approach for the laboratory scale production of bioetahnol from some major resources which include a sugar source, carbohydrate specifically starch source and a lignocellulostic source. Kitchen waste gather for a small period of time is used as a source of ethanol production that serves all the above three purposes. Fermentation of these sources with a suitable microbe serves the principle for ethanol production. The common fermentation friendly organism, the bakers yeast *Saccharomyces cerevisiae* has been used in the current research. In spite of the several choices available in the organism selection, bakers yeast serves the better option due to its ease in commercial availability, low economy involved and non sensitive nature. Apart from the nutritional media and the organism maintenance of optimal conditions for microbial growth play a key role in the production quality and quantity, which can be studied in the research.

Keywords: Bioethanol, Organic Waste, Kitchen waste, Feedstock, Global warming, Fermentation, Saccharomyes, Chlorofluoro carbons, Green house gases.

Introduction

The complete dependence on petroleum based fossil fuels, and incensing technology with a constant raise in population had created a heavy global energy crisis which needs to be answered with an utmost urgency. Apart from the depletion of the fuels an additional concern in using these fossil fuels is its direct influence in global warming and disturbance to the biological habitats [15]. Chlorofluoro carbons are the main byproducts released into the environment due to the combustion of fossil fuels which are the key agents in global warming. A constant raise in the CO2 concentration in the atmosphere is recorded with an increasing use of fossil fuels creating a havoc due to global warming [14]. Apart from the environmental and resource issues political crisis is an add on in the hazardous effects of fossil fuel usage. One such example is the disruption in the oil supply by Middle east countries in the year 1970 created an unrest in the sector [4, 10]. These circumstances lays the foundation for a need of alternative eco friendly technology to substitute fossil fuels that can satisfy the consumer needs and industry demands [16]. Bioethanol is one of the best options for environmental friendly and sustainable energy resource.

Chemically bio ethanol has certain properties like a high rate of octane number being 108, higher rate of evaporation enthalpy, a high speed of flame and the flammability of it among a wide range can be of additional advantages in using it. A higher compression ration is exhibited by fuel ethanol with a reduced burning time summarizing its efficacy higher than the gasoline used in integrated circuit [7]. The combination of ethanol with gasoline termed as gasohol is a blend that can be used in transportation machinery [6] E-10 is the commonly used gasohol n USA contains 10% ethanol and 90% gasoline. Incase of Brazil commonly used fuel may be direct ethanol or a blend of 76% gasonilne with 24% ethanol [9]. In cases of some specialized combinations like E-85 containing a high concentration of 85% ethanol needs a modification of engine to become compatable [1]. Ethanol possess a low ambient photochemical reactivity which accounts for its less interference of ozone layer [5] .

Kitchen is one of the huge resources with high organic and carbohydrate rich waste which can cause contamination and growth of un required microbes leading to adverse environment impact and foul smell. An inefficient processing of food and left over management flaws leads an immense wastage of carbohydrate rich food stocks. Proper utilization of these waste material by converting it to bio ethanol production can cause both eco friendly development with an alternative fuel resource [2]. There are several types of kitchen wastes which may include homogeneous and heterogeneous wastes [13] which need pre treatments like physical, chemical, biological and physicochemical for their conversion to bioethanol. Once developed and proved to be efficient these conversion systems for food waste into bioethanol needs the establishment of mini outlets in several areas for production of bioethanol and utilization of organic waste .

Materials and Methods:

Collection of organic Kitchen waste

In one of the articles by THE HINDU, states that the urban Indian citizens produce 700gms of solid waste per person approximately. This accounts for 250kgs of waste in an Year [11]. For the current work approximately 100 to 150 gms of fresh one day kitchen organic waste was collected into a sterile container which was bought to the lab for direct usage in media perpetration. However in case a need for huge quantities of fresh kitchen waste several options like Hostels, Temple Kitchens, Kitchens of old age or orphan age homes, college hostels, Function halls etc can be opted. For the current study simple kitchen waste was obtained form the home kitchen containing Vegetable peels, Fruit pulp and peel, waste food material etc which can be processed further.

The characteristics of the waste should be freshly collected, wet but not too watery, no foul smell etc.

Pre treatment of kitchen waste

The material bought to the lab was first subjected for a gentle water wash followed by a simple air dry to remove excess water. Chopping, pulverizing and blending was performed with sterile distilled water (1 litre) to bring the waste to the required consistency. This material is used as a major solid media substituent in fermentation. To 250ml of the above medium 2ml of HCl was added which would convert the calcium present in the medium to calcium sulphate salt [3]. This is essential as the presence of calcium causes a high rate of inhibition in fermentation process. Further additional of acid regulates the pH of the medium thereby inhibiting the bacterial contamination. This also causes an enhancement in the chemical hydrolyses of the plant material. This was than allowed to boil for a duration of 1.5 hours resulting in the simplification of carbohydrate to individual units of cellulose and starch. Further enhancement in the degradation causes the production of monomers or sine sugars of amylose, amylopectin and sugar. Urea was added to the media at a rate of 1% which acts as a nitrogenous source. The pH of the medium was adjusted to 6.0.

Preparation of Yeast culture for inoculation

In order to obtain an active yeast culture to be used for inoculating the medium the source used was store bought bakers yeast granules. YEPD broth was prepared with the following composition: Yeast extract: 0.3%, peptone : 1%, Dextrose: 2%. The media was sterilized and cooled. The sterile cool media was inoculated to 6 to 8 granules of yest and was incubated at 30^0C for a period of 48hrs

Preparation of active yeast plate

For obtaining active yeast cells in the form of isolated colonies the preferable technique to be used is streak plate onto YEPD media plate. The plate was sterilized by dry air heating technique followed by UV exposure. The media with the above composition (and an extra 30%agar) was pre sterilized and cooled which needs to be poured into the late. The process must be performed under laminar airflow conditions. Once the media is cooled and solidified the plate was inoculated with the culture from the previous YEPD broth using streaking technique. The plates were incubated at 30^0C for a period of 48hrs.

Fermentation media inoculation

The media from 250 ml conical flask is transferred to a 500ml erlenmeyer flask and a homogenous suspension of YEPD broth was inoculated under aseptic conditions. Rotatory incubator was used for incubating the media at 30^0C for a period of 48hrs and n RPM of 120rpm. A control was also used in which all the treatment remains constant except the inoculation of organism. Ethanol production in the

media was recorded at a interval of 24 and 48hrs. This can enable the identification of average rate of ethanol production. The ethanol thus produced was analyzed using Conway method [12]. Before the isolation of usable ethanol downstream processing is performed.

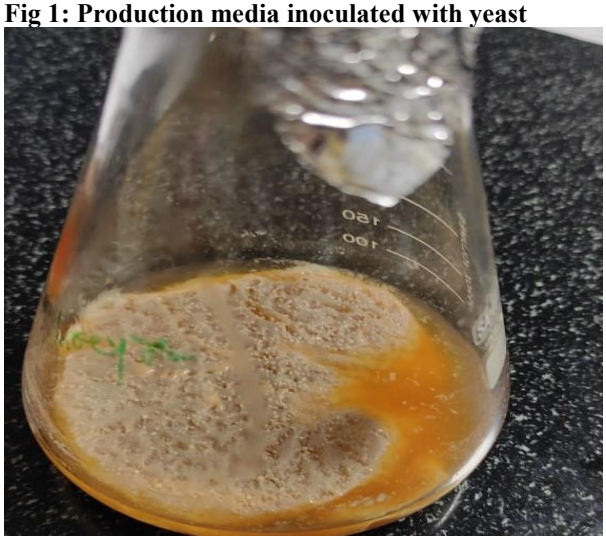
Determination of various parameters affecting ethanol production:
The major criteria behind the selection of topic is to optimize the conditions required for ethanol production. The tested conditions for ethanol production include effect of fermentation temperature, Effect of pH. The same fermentation media were prepared and incubated at various temperatures and pH. After the prescribed period of incubation the ethanol content was measured after downstream processing using Conway method [8]. The results are furnished below.

Estimation of ethanol by Conway method
This protocol involves the redox titration for the determination of alcohol. In the protocol an oxidation of ethanol to ethanoic acid takes place. The acid Thus produced is allowed to react with potassium dichromate solution (0.05N). The unreacted dichromate is further determined by adding potassium iodide (50%). The dichromate react with iodide to produce iodine. The iodine thus released id titrated against a standard of sodium thiosulphate (0.1N). The reading of the titration is used to calculate the ethanol content in the solution, which is produced as a result of fermentation. The result should be negligible in control which is left un inoculated. The etahnol detected by this protocol is measured in conway unit center.

1. *Add 1 ml 0.05 N potassium dichromate solution in Conway unit center Add 1 ml sample in Conway unit Round*
2. *Reaction complete within 24 hours*
3. *Add 50% KI solution 0.5 ml + 1-2 drop soluble starch in Conway unit center*
4. *Take 0.1N sodium thiosulfate in Microburatte 56*
5. *Titration*
6. *Untill the center becomes Colourless*

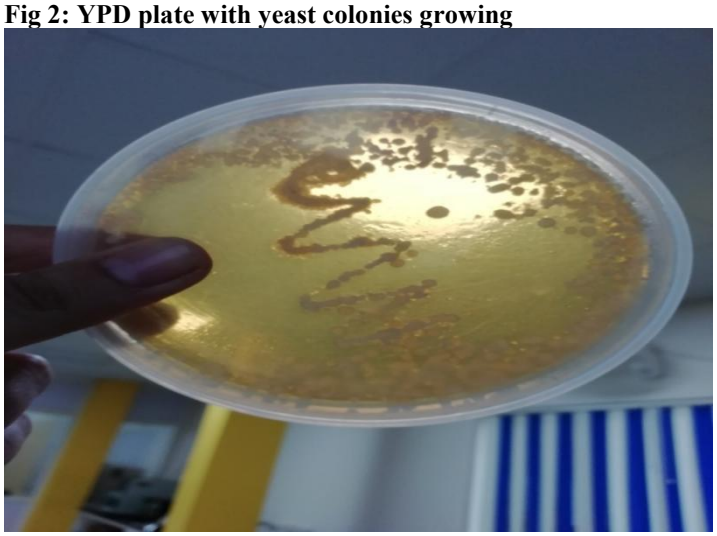
Results and Discussion

Fig 1: Production media inoculated with yeast



The above picture shows the east colonies produced after inoculation and incubation. The can be further used for plating onto the YPD agar plate fr colonies.

Fig 2: YPD plate with yeast colonies growing



The above plate is the YPD media plate which was inoculated with the yeast cells from YPD broth. The colonies showing isolated growth are depicted in the picture.

Table 1: Effect of Temperature on Ethanol production

S.NO	Temperature	Ethanol produced gms/Litre
1	25	18
2	28	24
3	30	38
4	33	36
5	35	30

From the above table it can be calculated that the optimum temperature required or the fermentation media to yield highest ethanol is 30^0C. Further an increase or decrease of temperature may decline the ethanol production due to bacterial growth inhibition.

Table 2: Effect of pH on ethanol production

S.NO	pH	Ethanol produced gms/Litre
1	5.0	20
2	5.4	26
3	6.0	42
4	6.4	37
5	6.8	35

The table 2 show the results of various pH on ethanol production by *Saccharomyces*. The results revel that the optimum pH required for ethanol production by fermentation is 6.0. above and below these values declines the ethanol produced.

Conclusion

The current work aimed to develop a standard protocol for the utilization of kitchen organic waste in the production of bio ethanol, which can not only clean the environment from waste dumping but also satisfactorily replace the needs of fossil fuel. The most interesting and practical part of the research was its economic friendly nature along with environmental protection. The study involved the use of domestic waste with some added chemicals as a nutrient media for the growth of saccharomyces cereviciae followed by the production of ethanol by shaker flask fermentation method. The optimal conditions of temperature and pH were also tested in the study which were found to be 30^0C and 6.0 respectively that could yield a high amount of ethanol. The ethanol thus produced was quantitatively measured using conway method which is based on titration. All the results indicate that the use of kitchen waste as a sole source of carbon for the growth of yeast followed by production of ethanol needs the optimum temperature of 30 and pH of 6. The study can further be extended for the evaluation of other media parameters which could enhance the yield of ethanol.

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