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Research Article

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Synthesis of bioethanol from tamarind seeds using marine strain of *Saccharomyces cerevisiae*

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ABSTRACT

Bioethanol can be used as a second generation advanced biofuels. Currently it is mainly produced from starch but bioethanol production from starch leads to competition for food, land and price. Therefore, ligno-cellulosic agricultural residues are potentially used for bioethanol production to solve such challenges. In the present work acid pretreated tamarind kernel powder is used as a ligno-cellulosic biomass for bioethanol production using marine yeast. Greater osmosis tolerance, greater special chemical productivity and production of industrial enzymes are the unique characteristics of marine yeast over terrestrial strains. Hence, marine yeasts have great potential to be applied in various industries. Therefore, the marine strain of saccharomyces cerevisiaewas isolated from marine water and was used for bioethanol production and the bioethanol yield was optimized using the full factorial design methodology. The amount of Bioethanol yield on day 2 was found to be 2.3g/l and the interaction effects were also studied using Minitab 17 software.

Keywords: Tamarind kernel powder, Pretreatment, Marine yeast, Fermentation, Full factorial design

INTRODUCTION

The depletion of oil reserves which supplies conventional fuels has created an increasing demand for alternative fuels to sustain the requirement of the world'senergy. More importantly, the carbon dioxide emitted from burning conventional fossil fuels affects adversely towards the climate change. Bioethanol is found as an important alternative to conventional fuels posing many advantages over the conventional fuels. Maize, sugarcane and tapioca starch are the most commonly used raw materials for the bioethanol production.In 2004, nearly 32 million tons of maize that was produced was used for bioethanol production, amongwhich accounts to about 12% of the total maize cultivation and is expected to increase in the next decade by several folds[1].However this poses a challenge for developing countries like India where the food crops like sugarcane, maize are not available in excess to convert them to fuel. This requires a need for other alternatives as raw material. Lignocellulosic wastes serves as one of the alternative substrates for bioethanol production.

Tamarindusindicus is a widely grown tree in tropical regions like India and is a seed kernel could be used as a lignocellulosic material needed for the fermentation process. Nearly 2,30,000 tonnes of fruits are harvested annually and contains 55% pulp , 33.9 % seed, 11.1 % shell and fibres[2]. In India about 20,000 tonnes of Tamarind Kernel Powder (TKP) is produced[2] .The TKP comprises of 73.68 % of carbohydrates, proteins (14.38%), ash (3.28%) and moisture (8.67%) [2]. Xyloglucan is the major polysaccharide found in the tamarind seeds. Transforming of the lignocellulosic biomass into bioethanol requires microbial fermentation of the sugars and other carbohydrates present. The lignocellulosic biomass is in the form of a matrix which has to be broken down to release the carbohydrates, so that it is available for the fermentation process. Energy consumption and in turn the cost of production of bioethanol is crucially dependent on the pretreatment method. There are various pretreatment available such as with acid pretreatment, ionic liquid pretreatment, steam explosion, basic treatment and ammonia fibre explosion. By using these pretreatment methods it is possible to breakdown the matrix and increase the

fermentable carbohydrates, hence increase the yield of bioethanol. Saccharomyces cerevisiae has unique feature of producing ethanol by fermentation of wide range of reducing sugars. It has been reported that the marine strain produced 17.31 % higher bioethanol yield over terrestrial and fresh water strains[3].

The goal of our work is to investigate the yield of bioethanol using marine strain of Saccharomyces cerevisiae isolated from sea water and carry out the fermentation process with TKP as the raw material. Also we would investigate the interactions between various parameters that are said to affect the fermentation process by implementing the concept of design of experiments.

EXPERIMENTAL SECTION

Yeast culture

Sea water samples were collected from Mangalore sea coast, India at different locations. The sample collected were serially diluted in BSCP solution(Buffered Sodium chloride-peptone solution) in order of 10^{-10} and Sabouraud Chloramphenicol Agar(SCA) medium was used for the isolation of yeast. Sterile media were allowed to solidify after pouring it into the petri dishes. The serially diluted sample in quantity of one milliliter was pipetted out intoPetri dishes and was uniformly distributed in the plate first by rotating it in clockwise and then in anti-clockwise direction and then spread with the help of a ''L'' rod. Further the plates were incubated at 20^0C to 25^0Cin an inverted position. After 7 days of incubation the colonies were observed on the plates. The observed colonies were sent for species identification by carrying out the streaking of the isolated colonies. After the species identification the yeast colony obtained was further enriched in a sterile yeast malt broth media for its further use to carry out the fermentation.

Tamarind kernel powder

Tamarind kernel powder used was obtained from Sri Balasanka Mill, Theni, Tamil Nadu, India.

Hydrolysis of Tamarind kernel powder

Tamarind kernel powder containing cellulose, hemicelluloses and polysugars, was used as a substrate for the bioethanol production after pretreatment. The TKP was pre-hydrolyzed by using 0.3N sulphuric acid. The pretreated TKP was assimilated at different concentrations into the inoculum based on the experimental design given in Table 1.

After the acid pretreatment the mixture was centrifuged at 10000rpm and the supernatant obtained was used for carrying out fermentation. The pH of the hydrolysate obtained was adjusted using 0.5N sulphuric acid. By using gas chromatography the percentage of ethanol in the filtrate was estimated in periodic intervals.

Table 1: Full factorial experimental design for bioethanol production

Trial no.	pH	Amount of substrate(% by weight)	Amount of inoculum(ml)	Bioethanol produced(g/l)	Yield obtained (%)
1	3	10	25	1.2	1.939394
2	3	10	100	1.6	2.585859
3	3	14	25	1.3	1.386386
4	3	14	100	1.8	1.919611
5	7	10	25	2.3	3.717172
6	7	10	100	1.6	2.585859
7	7	14	25	1.2	1.279741
8	7	14	100	1.8	1.919611

Determination of Ethanol

Perkin Elmer Clarus 680 gas chromatography containing ZB-624 column along with nitrogen as carrier gas was used to estimate the ethanol. The temperature of the detection port, injection port and oven was 250^0C, 220^0C and 240^0C, respectively. 200 micro liters of liquid samples was taken for analysis and was injected into the gas chromatography headspace. A standard ethanol plot was made and was used to determine the concentration of ethanol and was reported in percentage.

RESULTS AND DISCUSSION

The colonies obtained on Sabouraud Chloramphenicol Agar (SCA) medium were further checked for species identification using VITEK 2 systems version 06.01 using YST card. It was found that the isolated species was *Saccharomyces cerevisiae* with 98% probability. The colonies of the isolated *Saccharomyces cerevisiae*on SCA plate were obtained as shown in Figure 1.

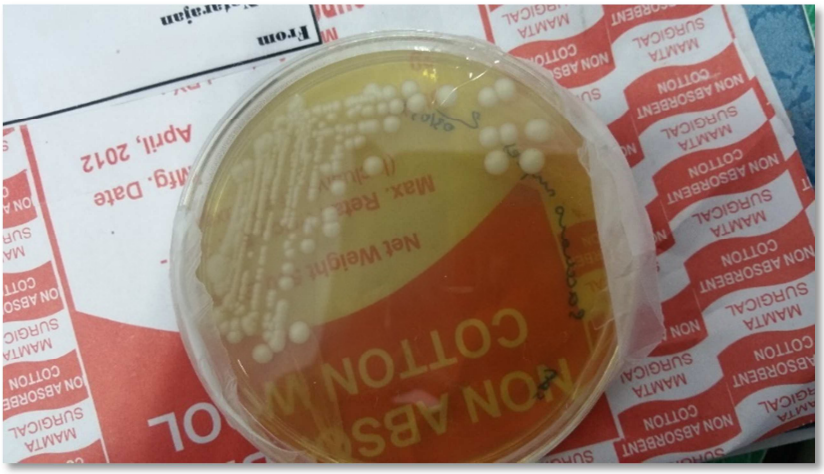

Figure 1: Isolated Saccharomyces Cerevisiae colonies on SCA Petri plate

The gas chromatography analysis of all the 8 trials was done every day in a 5 day's period. It was found that the 5th trial produced the maximum amount of bioethanol among all the trials on 2nd day of fermentation. To analyze the effect of the selected parameters on the yield the main effect plots and the interaction effect plots were created using Minitab software. From the main effects plot for pH it can be clearly concluded that the bioethanol production increased with the increase in pH. From the main effects plot as shown in Figure 2 for the amount of substrate it can be clearly concluded that the bioethanol production decreased with the increase in amount of substrate. From the main effects plot for amount of inoculum it can be clearly concluded that the bioethanol production increased with the increase in amount of inoculum. From the interaction effect plots as shown in Figure 3-5 of the three factors it can be concluded that the interaction effect of amount of substrate-amount of inoculum on the bioethanol production is maximum among the selected parameters. The main effect plots and the interaction effect plots are shown in figure 2-5.

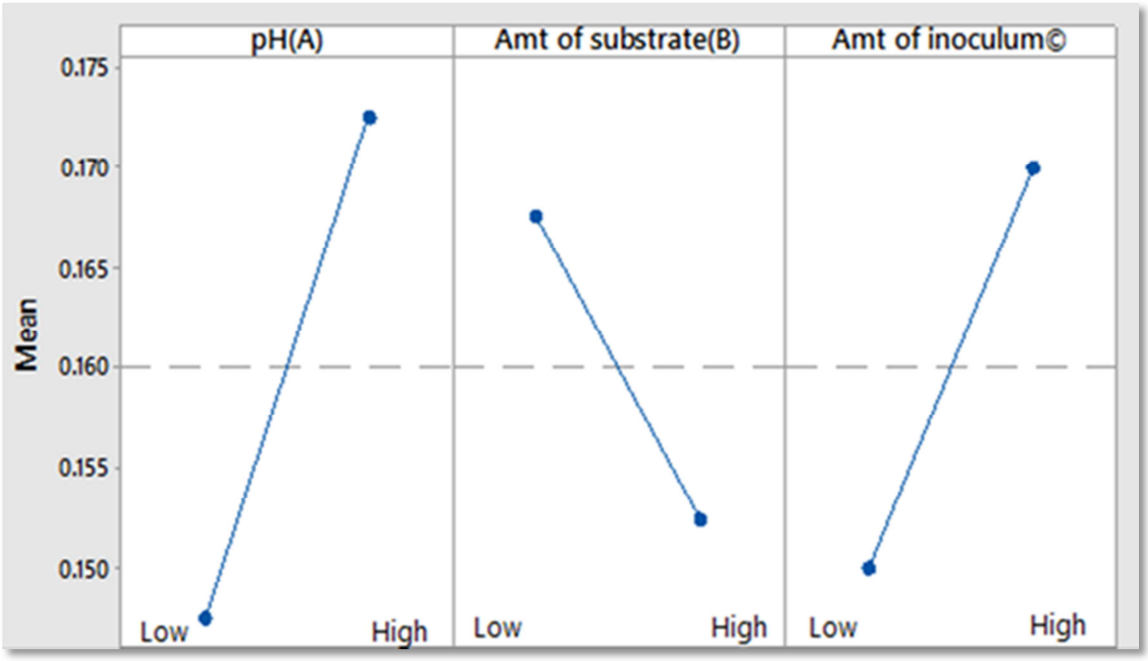

Figure 2:Main effect of pH,amount of substrate and amount of Inoculum on bioethanol production

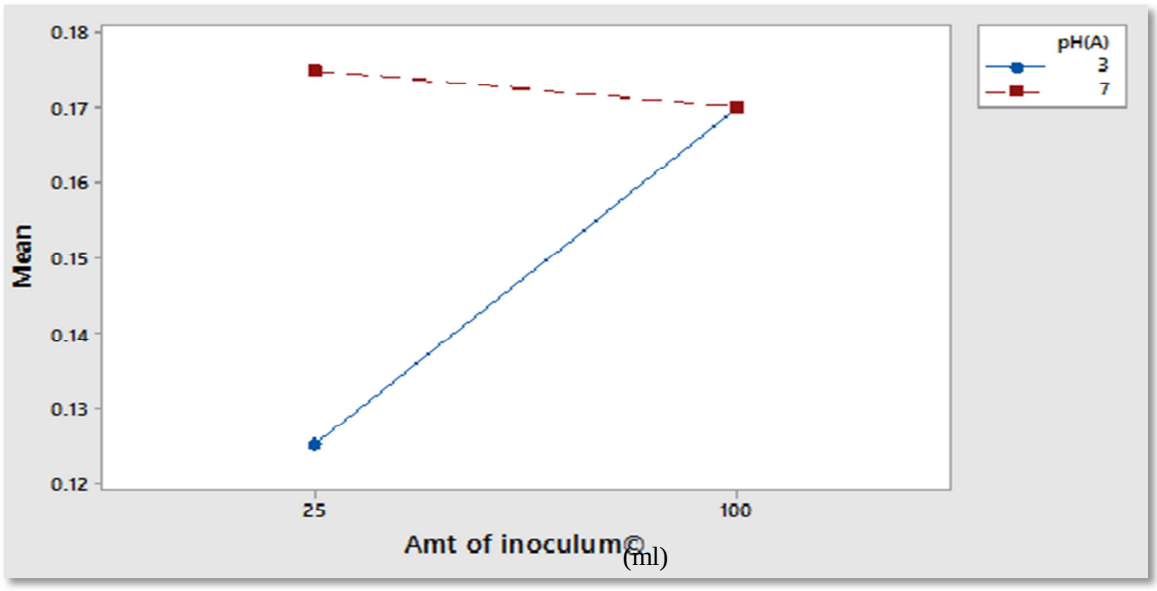

Figure 3:Interaction effect of pH and amount of inoculum on bioethanol production

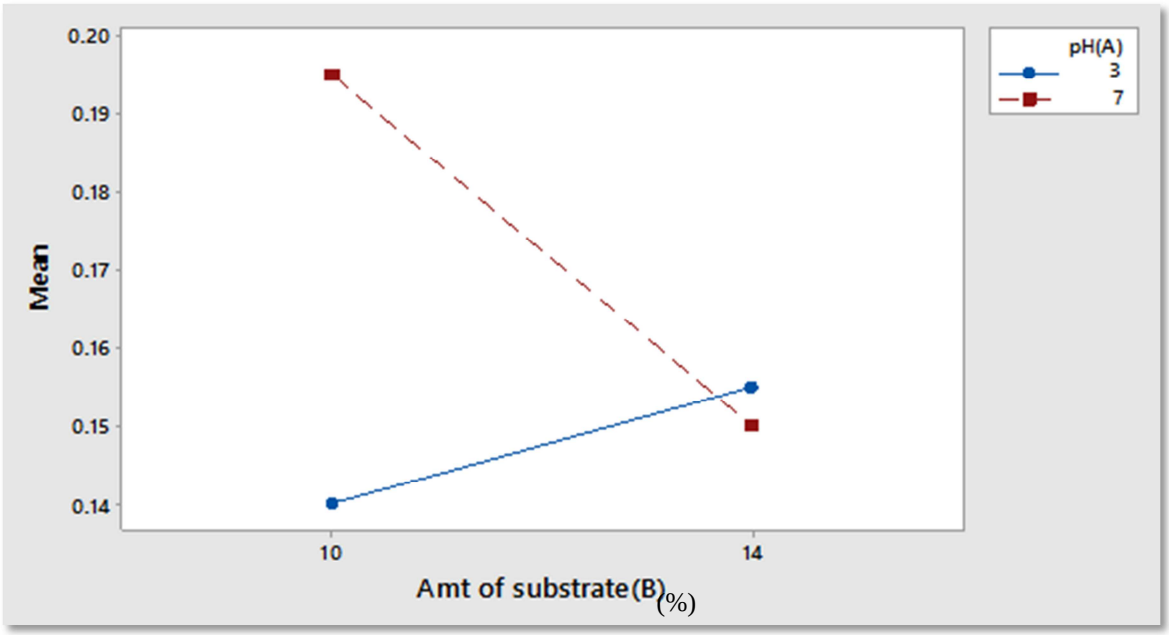

Figure 4:Interaction effect of pH and amount of substrate on bioethanol production

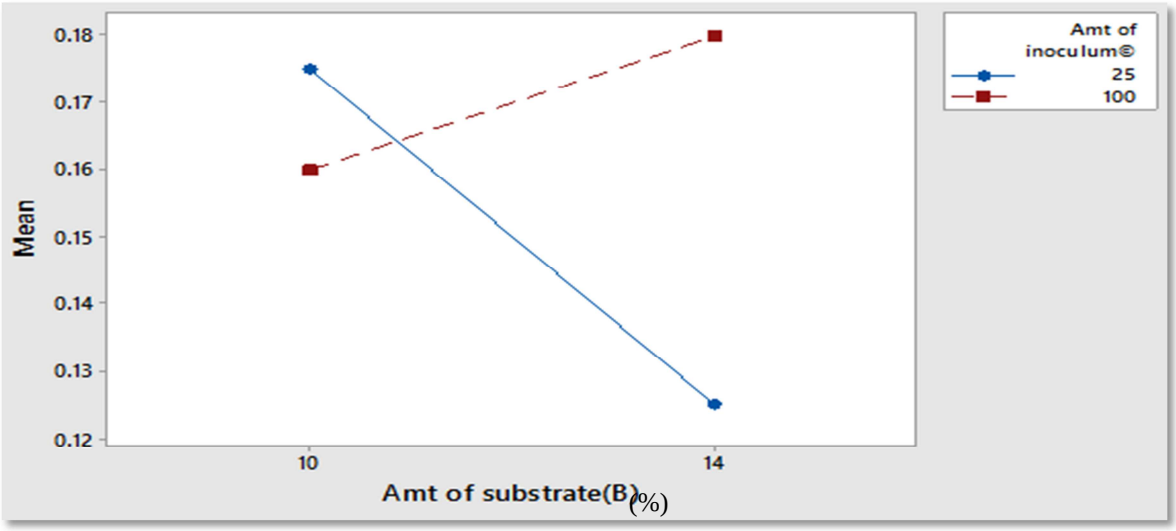

Figure 5:Interaction effect of amount of inoculum and amount of substrate on bioethanol production

CONCLUSION

Tamarind kernel powder serves as a promising lignocellulosic substrate for bioethanol production due to its large carbohydrate content.By seeing the main effect plot of effect of pH on bioethanol yield it can also be concluded that the isolated marine strain of *Saccharomyces Cerevisiae*was more resistant to pH variation.Among the selcted parameters the amount of substrate and amount of inoculum together has a significant impact on the bioethanol yield.The maximum bioethanol which could be produced by considering the factors pH,amount of substrate and amount of inoculum was found to be 2.3g/l.

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