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Research Article

BIOETHANOL PRODUCTION FROM IXORA COCCINEA AND QUISQUALIS INDICA FLOWERS

Mrunalini Appe *, Ramya Margam and Sridevi Boddireddy

Department of microbiology, Chaithanya degree and postgraduate college (Autonomous), kishanpura, Hanamkonda -506 001, Warangal, Telangana, India.

ABSTRACT

Biofuels , the fuels that are derived from living matter, usually plants, have emerged as a highly promising source of alternative energy, and have drawn global Research and Development for their production using biomass which is defined as the organic matter used for producing fuel such as lignocelluloses wastes, municipal solid wastes, flowers, weeds etc . A traditional method of bioethanol production mainly requires fruits, sugarcane, honey etc. all which are major food products. There is an increased interest in finding an alternative, cheaper biomass for production of bioethanol. Nectar is a sugar-rich liquid produced by flowers. Flowers of *Ixora coccinea* (the West Indian jasmine) and *Quisqualis indica* (Rangoon creeper) contain fermentable sugars. Fermentation of these two fresh flowers with two different strains of *Saccharomyces cerevisiae* was carried out by submerged fermentation method. Estimation of reducing sugar was done by DNS and glucose by GOD-POD method. The initial reducing sugar content of *Ixora coccinea* was 1350µg/ml and was decreased after the fermentation to 740µg/ml. Whereas in the flower sample of *Quisqualis indica*, the initial sugar content was 1900µg/ml and was decreased to 570µg/ml and the glucose concentration was estimated to be 620µg/ml in *ixora coccinea* and 340µg/ml in *quisqualis indica* by GOD-POD method. The bioethanol estimation was done by dichromate method and the bioethanol yield was found to be 1.34gm% in *Quisqualis indica* and 1.46gm% in *Ixora coccinea.*

Keywords: Biofuel, Biomass, Bioethanol, *Ixora coccinea*, *Quisqualis indica*

INTRODUCTION

Bioethanol production from renewable sources to be used in transportation is on increasing demand worldwide due to continuous depletion of fossil fuels . Because of the dependence on petroleum-based fossil fuel which is exhausted very fast to meet the continuously increasing demands, the need to produce biofuels has risen. It has been realized that fossil energy causes greenhouse gas emissions that have adverse effects on the environment. Burning of petroleum-based fuels causes the increase of CO_2 level in the environment which is directly responsible for global warming. Hence, it is an ongoing interest to find out a renewable and environmentally friendly source of energy for our industrial economies and consumer societies. Bioethanol in this aspect is an attractive option for renewable and sustainable energy source.

Bioethanol is primarily produced by sugar fermentation process, although it can be produced by the chemical process of reacting ethylene with steam. Bioethanol is reduced from biomass mostly via a fermentation process using glucose derived sugars, starch, cellulose etc., as raw materials extracted from plants. However, these crops can have a significant impact on food security. Hence an alternative method of producing alcohol from flowers and weed is studied in present day's work.

Production of bio-ethanol from maize, agro waste has been attempted with enzymes from different sources for hydrolysis of lignocelluloses and with different organisms for fermentation (oh green et al., 2006; Eken Saracoglu and Arslan, 2000; Cao et al., 1996 and

Wyman et al., 1992). Currently, the Second generation bio-products such as bioethanol, biodiesel, biohydrogen and methane wastes are increasingly been produced from lignocellulosic biomass rather than from energy crops like sugarcane, sorghum, jatropha, switch grass, etc. Because the latter competes for land and water with food crops that are already in high demand, the use of food crops such as corn and sugarcane to produce biofuels is increasingly being discouraged due to the current worldwide rise in food prices. In order to minimize food-feed-fuel conflicts, it is necessary to integrate all kinds of biowaste into a biomass economy (Mahro and Timm, 2007). The lignocellulosic biomass, which represent the largest renewable reservoir of potentially fermentable
carbohydrates on earth (mtui and Nakamura, 2005), is mostly wasted in the form of pre-harvest and post-harvest agricultural losses and wastes of food processing industries. Due to their abundance and renewability, there has been a great deal of interest in utilizing LCW for the production and recovery of many value-added products (Pandey et al., 2000; Das and Singh, 2004; Foyle et al., 2007). Among the main recovery products include enzymes, reducing sugars, furfural, bioethanol, protein and amino acids, carbohydrates, lipids, organic acids, phenols, activated carbon, degradable plastic composites,
Cosmetics, biosorbent, resins, medicines, foods and feeds, methane, biopesticides, biopromoters, secondary-metabolites, surfactants, fertilizer and other miscellaneous products (Tengerdy and Szakacs, 2003; Mtui, 2007; Ubalua, 2007; Galbe and Zacchi, 2007; Demirbas, 2008.
Bioethanol is considered as an important form of biofuel and has a wide range of worldwide applications. 84% of the world's bioethanol production is used in fuel sector for internal combustion engines as a high performance fuel component.
In Europe, direct blending of bioethanol to petrol has led to ethyl tertiary butyl ether (an octane booster), less important. Around 47% ethanol and 53% isobutylene is used as a petrol additive to enhance its anti-knock properties. Bioethanol is used as an additive in petroleum in most of the countries.

MATERIALS AND METHODS

Fresh flowers of *Ixora coccinea* and Q*uisqualis indica* were collected from the campus research garden of Chaithanya Degree College, kishanpura, hanmakonda, Warangal district, telangana state, India, as the bioresource, and washed under running tap water and sun-dried for a day to reduce the moisture.

Source of biomass

Ixora coccinea (jungle geranium, west Indian jasmine) and the other variety *Quisqualis indica* commonly known as Rangoon creeper were collected as the bio-resource because they were observed to be attractive to butterflies and bees and are rich in nectar. The growth rate of these plants is very fast and they do not make heavy fertilizer demand. These plants bloom throughout the year. Floral nectar is mainly composed of sugars like sucrose, fructose, and glucose in different proportions according to species (Leonardo et .al s., 2004).

MICROORGANISM USED FOR ETHANOL PRODUCTION

Mostly yeast are being used for producing bioethanol worldwide . Yeast belonging to *S.cerevisiae* has been used most commonly in fermentation. A number of factors related to the fermentation greatly influence the process and their optimization is the key point for efficient ethanol production from these feedstocks.
In this method, two types of yeast were used which include *S.cerevisiae* isolated from fermenting grapes and the other yeast isolated from toddy collected from local market, to study the comparative production by both strains on same substrates.

MEDIA PREPERATION

Flowers of *Ixora coccinea* and *Quisqualis indica* were ground in mixer grinder to make slurry. This slurry was sterilized at 121°C and 15 psi pressure for 20 minutes. After cooling, the pH was adjusted to 5.5 for fermentation by yeast (*S.cerevisiae)*. Add 2ml of inoculum to the fermentation media in conical flasks and provide anaerobic conditions. No other carbon or nitrogen sources were added to the media in this study.

Estimation of reducing sugars

Estimation was done both before and after fermentation to determine the reducing concentration of sugar in different extracts by DNS and GOD-POD methods respectively for all the samples taken.

1. Estimation of reducing sugar by DNS method

To 1ml of each sample , DNS reagent is added and incubated in hot water bath for 10-20 minutes to develop the reddish brown color. After cooling the tubes, the absorbance was measured at 540nm with a spectrophotometer.

A standard graph was plotted using glucose solution (100µg/ml of working standard). The sugar content of the selected flowers was calculated by comparing their optical density values at 540nm with the standard graph.

2. Estimation of glucose by GOD-POD (Glucose oxidase-peroxidase) method.

To 1ml of each sample along with glucose standard, add 1ml of the GOD-POD reagent and mix thoroughly. Incubate these tubes at room temperature for 5-10 minutes. Observe the color change and measure the absorbance of these tubes at 500nm against blank and compare the readings.

FERMENTATION

Starter culture was inoculated into sterile media (hydrolysate of selected flowers) under sterile conditions and the submerged state fermentation was allowed to take place for 5 days at room temperature.

DISTILLATION

The distillation unit was set up using components like: a boiler, a condenser pipe and a distillate collecting flask. The filtered sample was transferred into the reboiler and heated to boil. The vapors started to rise into the head and passed through the condenser. The continuous circulation of cold water around the condenser pipe assisted in cooling the ethanol rich vapors back to liquid and entered the receiver and then got collected as distillate. (Raiker, 2012).

ESTIMATION OF BIOETHANOL

Bioethanol in the distillates of all the taken samples is estimated by acid dichromate volumetric titration method and then compared.

RESULTS AND DISCUSSION

Initially, the reducing sugar concentration in *Ixora coccinea* was found to be 1456µg/ml before fermentation (on day zero) and was reduced *to 740 µg/ml* after fermentation (on day 5) whereas in *Quisqualis indica*, the initial concentration of reducing sugar (on day zero) was calculated to be 1245µg/ml before fermentation and was reduced to 570µg/ml after fermentation (on day 5). Results obtained for reducing sugar before and after fermentation shows that the reducing concentration decreased as it was utilized in the fermentation process (**Table-1 and Table-2).** In the present study, production of bioethanol from two different flowers using two different strains of *S.cerevaciae* on both of them is studied and the results are compared. It was observed that the hydrolysate of both *Ixora coccinea* and *Quisqualis indica* inoculated with *S.cerevisiae* isolated from toddy showed comparatively faster and effective yield of bioethanol than those of the samples inoculated with *S.cerevisiae* isolated from fermenting grapes is shown in **Table-3** and **Table-4**.

Table 1: Estimation of glucose by GOD-POD method

SAMPLE	ESTIMATED GLUCOSE CONCENTRATION
Ixora coccinea	620µg/ml
Quisqualis indica	340µg/ml

Table 2: Concentration of reducing sugar estimated by DNS method

SAMPLE	REDUCING SUGAR CONCENTRATION	
	Before fermentation	After fermentation
Ixora coccinea	1350µg/ml	740µg/ml
Quisqualis indica	1900µg/ml	570µg/ml

Table 3: Bioethanol estimated in the samples inoculated with Saccharomyces cerevisiae isolated from fermenting grapes.

SAMPLE	ESTIMATED BIOETHANOL
Ixora coccinea	1.20gm%
Quisqualis indica	1.00gm%

Table 4: Bioethanol estimated in the samples inoculated with *S. cerevisiae* isolated from toddy

SAMPLE	ESTIMATED BIOETHANOL CONCENTRATION
Ixora coccinea	1.46 gm%
Quisqualis indica	1.34 gm%

CONCLUSION

The results in this study reveal that the chosen biomass of flowers can be used as a source for production of bioethanol. This study has also revealed that different strains of brewer's yeast which are easily isolated from different sources like toddy and surface of fermenting grapes show an effective ability of fermentation in the substrates other than those from which they are isolated.

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