Bioethanol Production from Flowers of *Quisqualis indica*

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Abstract **—**Growth in population and industrialization, worldwide ethanol demand is increasing continuously. India has the largest Floriculture (flowers) industry in the world. A traditional method of ethanol Due to rapid production requires mainly fruits, sugarcane, honey etc. all which are major food products. There is a growing interest to find alternate bioresources for production of ethanol. Flowers are a rich source of fermentable sugars, which can potentially converted into bioethanol. Nectar is a sugar-rich liquid produced by plants. Flowers of Q*uisqualis indica* (Rangoon Creeper) contain 13.2 gm % fermentable sugars. Fermentation of fresh Rangoon creeper flowers with *Saccharomyces cerevisiae* was carried out by submerged fermentation method. Estimation of sugar was done by Cole's Ferricyanide method. The initial sugar content (before fermentation) of *Quisqualis indica* flower was 13.2 gm % and was decreased after fermentation to 5.6 gm %**.** The ethanol estimation was done by dichromate method and the ethanol yield was found to be 1.41 gm % with fermentation efficiency was found to be 36.29 %. Presence of ethanol was confirmed by Gas Chromatography Mass spectroscopy (GCMS). GCMS shows that along with ethanol; dimethyl ether, diethylene glycol mononitrate, formaldehyde oxime trimer, oxalic acid are also present in the distillate.

Index Terms — Bioethanol, fermentation, *Quisqualis indica* Flowers, *Saccharomyces cerevisiae*.

I. INTRODUCTION

Bioethanol is currently produced primarily from sugar and starch sourced from crops (first-generation biomass) such as sugarcane, wheat and corn, which have a high concentration of sugar. However, because these crops are also important food sources, bioethanol produced from them can have a significant impact on food prices and food security. (Seung *et.al.,* 2013)

Source of flowers

Quisqualis indica, also known as Rangoon Creeper, , goes by various names - Chinese honeysuckle, Quiscual in Spanish, Niyog - niyogan in Filipino and in Sanskrit it is called Madhumalati. The flowers are attractant for butterflies and bees and are rich in honey. The growth rate is generally fast, and the plant does not make heavy fertilizer demands. It blooms profusely and, all year round, in the tropics.

Floral nectar is widely known as the key reward offered by animal-pollinated plants to their pollen vectors. This exudate is secreted by nectaries, i.e. glandular tissues located on various floral parts whose features are significant in plant taxonomy and phylogeny. Sugars dominate the total solutes in floral nectar; these are mainly sucrose, fructose and glucose in varying proportions according to the species (Leonardo *et.al.*,2004)

Current status of demand and supply of ethanol in India

In January 2003, the Government of India launched the Ethanol Blended Petrol Programme (EBPP) promoting the use of ethanol for blending with gasoline and the use of biodiesel derived from non-edible oils for blending with diesel at 5%. Due to ethanol shortage during 2004-05, the blending mandate was made optional in October 2004, and resumed in October 2006 in the second phase of EBPP with a gradual rise to 10% blending. These ad-hoc policy changes continued until December 2009, when the Government came out with a comprehensive National Policy on Biofuels formulated by the Ministry of New and Renewable Energy (MNRE), calling for blending at least 20% biofuels with diesel and petrol by 2017. Given that the mandatory blending requirements will be met in phases, the demand projections for ethanol blending are estimated at 5, 10 and 20% blending mandates. Based on the projections 4, it is estimated that bio-ethanol requirement would be 3.46 billion liters by 2020 at the rate of 10% blending. (Basavaraj 2013)

Microorganisms used for ethanol production

Various ethanol producing yeast belonging to *S. cerevisiae* have been used most commonly in fermentation, where the yeast was found to be more ethanol tolerant and produced more ethanol at sugar concentration above 15 % (v/v). Although many fungi can carry out fermentation, species of *Saccharomyces* are generally used because they are comparatively efficient at alcohol production and can tolerate higher levels of ethanol than most fungi. Also during fermentation they produce compounds other than alcohol that are believed to influence the final flavor of the fermented liquid. The species of *Saccharomyces* that are used for alcohol production, primarily *S. cerevisiae* and *S. uuvarum* are able to ferment sugar into ethanol under anaerobic (oxygen-free) conditions, usually in a solution. (Moawad, 2012).

Bioethanol, a long considered suitable alternative to fossil fuel, has shown a sharp increase in production since 2006.
S. cerevisiae strains are the main microbes used for ethanol production because of their capability to efficiently convert hexose to ethanol and carbon dioxide. Traditionally, yeast cells are cultured and propagated until the cell number desired for ethanol fermentation is achieved. This process begins with slant cultivation followed by gradual expansion of the cultivation scale in liquid medium to increase the volume. As an alternative to this hassle, many factories use commercial active dry yeast (ADY) to initiate ethanol fermentation. The application of ADY in fuel ethanol production cuts down initial lag time for fermentation and the risk of bacterial contamination (Daoqiong

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et.al., 2013). This paper will deal with the investigative works on bioethanol production from *Quisqualis indica* flower juice along with fermentation technology, microorganisms used, and the influencing parameters on the process.

2. MATERIAL AND METHODS

Substrate

Quisqualis indica flowers were obtained from local market of Goregaon, Mumbai, Maharashtra , India. Flowers were washed with tap water.
Commercial active dry yeast (ADY) was used as a source of yeast *S.cerevisiae*.

Fungi and culture conditions

S.cerevisiae was maintained on Yeast Extract-Peptone-Dextrose (YPD) media (Yeast Extract, 10 g/L; Peptone, 20 g/L; Dextrose, 20 g/L; pH 6.5 ± 0.2). Culture was stored at 4±0.5°C till further use.

Optimization of growth condition

Standardization of media

Four different media were selected which are generally used for growth of *Saccharomyces cerevisiae.*
Media used for standardization:

1. Sabouraud's broth (Dextrose,b20 g/L; Peptone, 10 g/L; pH 5.4)
2. Yeast Extract-Peptone-Dextrose (YPD) Broth (Yeast Extract, 10 g/L; Peptone, 20 g/L; Dextrose, 20 g/L; pH 6.5 ± 0.2).
3. Malt Extract Broth (Malt Extract, 17 g/L; Peptone, 3 g/L; pH 5.4±0.2.)
4. Potato sucrose broth with bromocresol green (Potatoes, 300 g/L; Sucrose, 10 g/L; Bromocresol Green (~1mL of 1% solution)

Saccharomyces cerevisiae was inoculated in sterile media and incubated at RT for 48 hrs. The O.D. was determined at 530 nm using Equiptronics colorimeter.

Temperature optimization

The *S. cerevisiae* was inoculated in the optimized medium (St. YPD broth) in different flasks which were kept at different temperatures (0°C, 25°C, 37°C, 50°C) and O.D. was observed at 530 nm.

Tolerence level of *S. cerevisiae* to glucose

Various concentrations of glucose using suitable stock and diluents (as mentioned Table-1) were prepared. Then 0.1 ml of respective culture was added and the tubes were incubated at appropriate temperatures.

Table-1-Standard conditions for tolerance level of *S. cerevisiae* to glucose

Stock	St. YPD Broth containing 60% glucose
Diluent	St. YPD Broth without any sugar
Range	10-60% glucose at intervals of 10%
Total volume	4 ml
Culture	0.1 ml of 48 hrs old *S. cerevisiae* culture at 530 nm
Incubation period and time	25 °C /48 hrs

Minimum inhibitory concentration (MIC) of ethanol for *S. cerevisiae*

Various concentrations of ethanol using suitable stock and diluents (as mentioned Table-2) were prepared. Then 0.1 ml of respective culture was added and the tubes were incubated at appropriate temperatures.

Table-2-Standard conditions for MIC of ethanol

Stock	St. YPD Broth containing 20% ethanol
Diluent	St. YPD Broth without ethanol
Range	2-20% ethanol at intervals of 2%
Total volume	5 ml
Culture	0.1 ml of 48 hrs old *S. cerevisiae* culture at 530 nm
Incubation period and time	25°C /48 hrs

Estimation of Sugar

Total sugar of *Quisqualis indica* flower was estimated by Cole's Ferricyanide method .

Inoculum development

To prepare the starter culture, 50 ml of the growth medium (YPD Broth) was taken in 250ml capacity conical flask. The medium was sterilized at 121°C and 15 psi pressure for 20 minutes and was inoculated with approximately 1 to 2 ml of dense culture of *S. cerevisiae.* The density was adjusted to 1×10^6 cells/ml. The flask was incubated at R.T. for 48 hrs. The broth was centrifuged. Supernatant was discarded and the pellet was resuspended in 5ml of saline and used as inoculums.

Media preparation

Quisqualis indica flowers were ground in mixer-grinder to make slurry. Then slurry was sterilized at 121°C and 15 psi pressure for 20 min. After cooling $(NH_4)_2SO_4$ was added to

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slurry as nitrogen source and pH was adjusted to 5.5 for fermentation by yeast *S. cerevisiae.* Add the pellet suspension to 100 ml of sterile fermentation media in a 150 ml flask to provide anaerobic condition.

Distillation

In the present study, the batch distillation method was adopted. The distillation unit consisted of three components: a reboiler, condenser pipe and a distillate or receiving flask. The filtered sample was transferred into the reboiler and heated to boil. The vapors started to rise into the still head and passed through condenser pipe. The continuous circulation of cold water around the condenser pipe assisted in cooling the ethanol rich vapors back to liquid state. The condensed liquid enters the still receiver and then collected in the distillate. (Raikar, 2012)

Estimation of Ethanol

Ethanol was estimated by potassium dichromate oxidation method and followed by confirmation with Gas chromatography-mass spectroscopy.
Distillate contains 1.41 gm % of ethanol and fermentation efficiency was found to be 36.29 %.

Confirmation of ethanol production
Gas chromatography Mass spectroscopy (GCMS) is an analytical technique for volatile and semi-volatile compounds. GCMS was done at Dr. P.S.Ramanathan Advanced Instrumentation Centre Ramnarain Ruia College, Matunga, Mumbai - 400019.

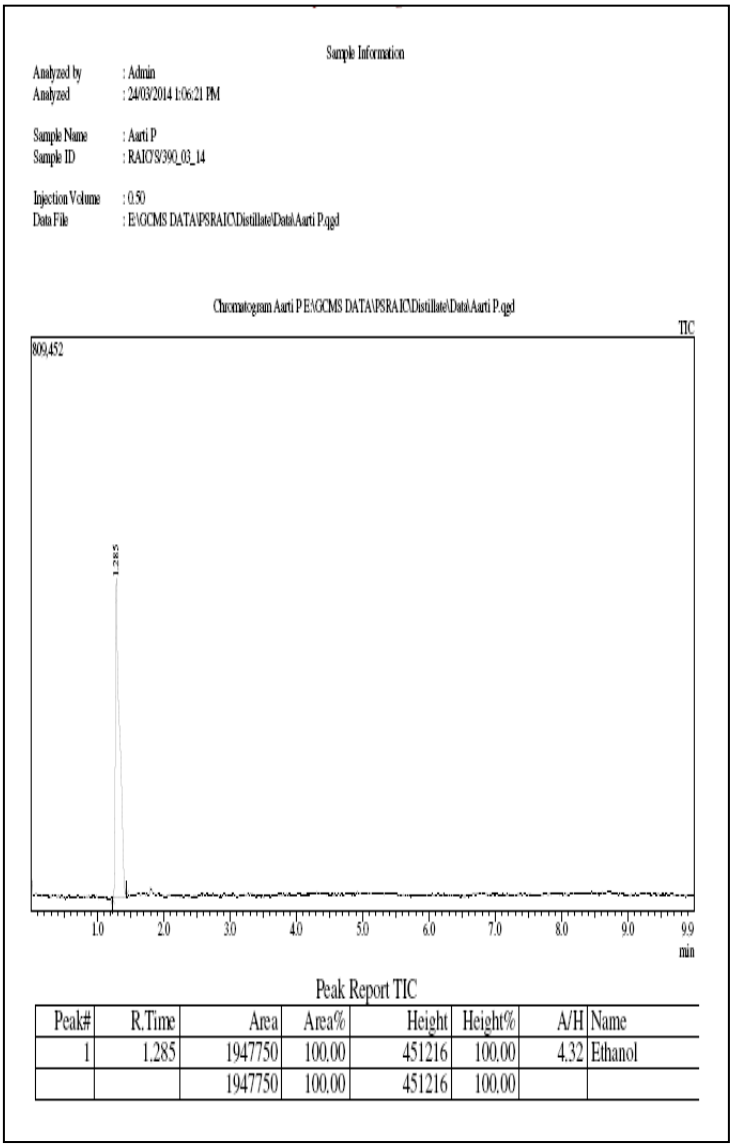
3. RESULT AND DISCUSSION

Fuel ethanol is one of the most important clean fuels and renewable energy resources, which would play an important role in effectively solving the problem of the forthcoming oil shortage. (Zhang and Feng, 2012)

The establishment of ethanol industry requires sufficient and cheap feedstock in order to reduce the costs of production that has been recognized as a critical point. However, the transformation of some conventional raw materials (like corn, wheat and rice, etc) is not feasible, especially in developing countries, because they are mainly used for food. For the sake of the win-win prospect between energy and food security, the development of fuel ethanol industry should be based on the non-grain crops and biomass (Ragauskas *et al.,* 2006; Lin and Tanaka, 2006).

In this investigation attempt was made to fermentation of sugars with low cost substrate like Rangoon Creeper flowers by *S. cerevisiae. S. cerevisiae* was subjected to varying concentration of sugar and ethanol to check the tolerance by organism. The higher the tolerance of the organism towards higher concentration of sugar and ethanol the greater is the ability of the organism to produce higher yield of ethanol. The *S. cerevisiae* can tolerate 40% sugar and 12% ethanol. The optimum growth conditions were standardized in this study. Optical density obtained for Sabouraud's broth, YPD broth, Malt extract broth, Potato sucrose broth with bromocresol green, was 1.03 nm, 1.24 nm, 0.6 nm, 0.88 nm respectively.

For temperature 0°C, 25°C, 37°C, 50°C, O.D. obtained were 0.25nm, 1.27nm, 0.63 nm, 0.13 nm respectively. The organism showed maximum growth in Yeast Extract-Peptone-Dextrose (YPD) Broth at R.T. with these standard conditions of growth, organism was further enriched for fermentation.
Results obtained for reducing sugar before and after fermentation shows that the reducing sugar concentrating decreased as fermentation progressed. The initial value of 13.2 gm % was obtained on the zero day and 5.6 gm % at the end of the fermentation. The decrease in sugar values may be attributed to utilization of the sugars for growth and metabolic activities by the organism. The estimation of ethanol was done for distillate obtained after distillation by dichromate method. Ethanol concentration was found to be 1.41 gm %. Therefore, fermentation efficiency was 36.29 %. Further confirmation of presence of ethanol in the distillate obtained was done by GC-MS. The GC analysis confirms the presence of ethanol in distillate (Fig-1) and result for MS shows that along with ethanol; Dimethyl ether, Diethylene glycol mononitrate, Formaldehyde oxime trimer, Oxalic acid are also present in the distillate. (Fig -2)



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	1.285	1947750	100.00	451216	100.00	4.32	Ethanol
		1947750	100.00	451216	100.00		

Fig-1- GCMS reports showing presence of ethanol in distillate

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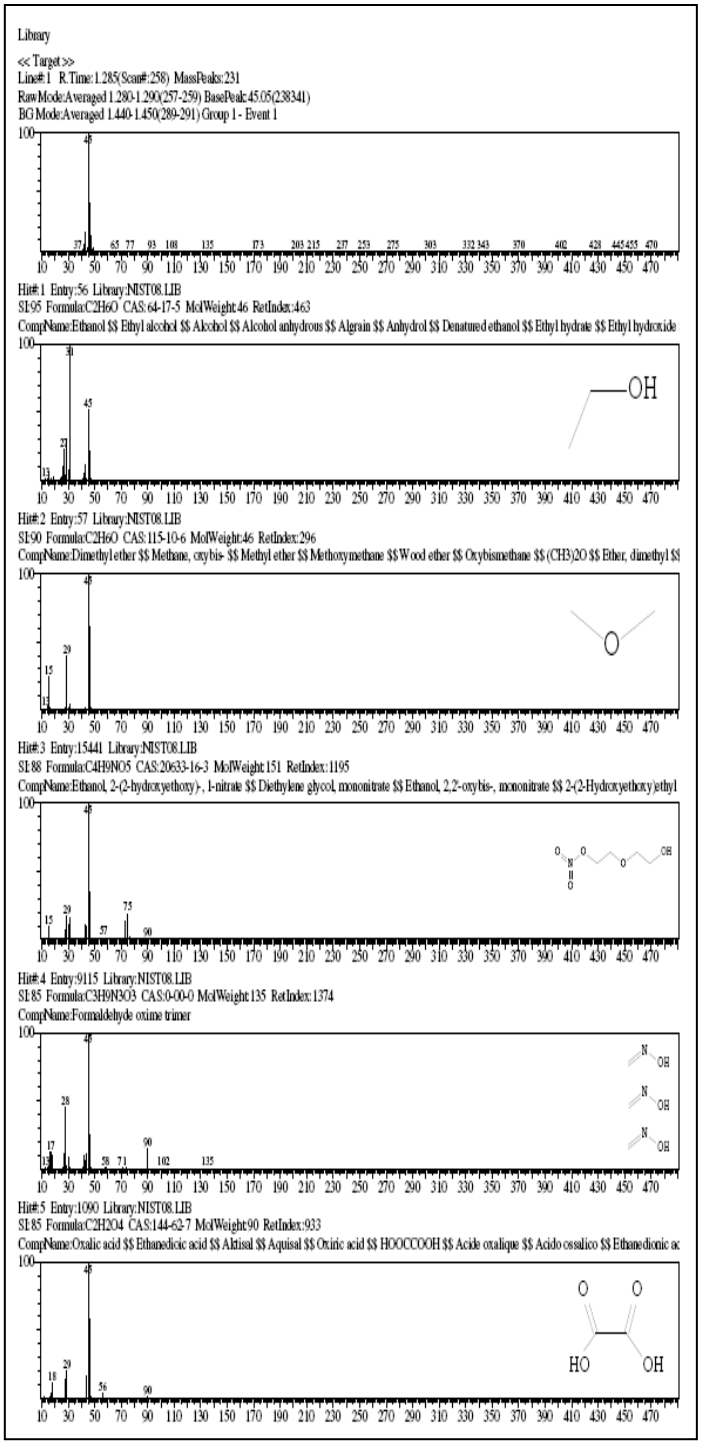

Fig-2- GCMS reports showing presence of other chemicals in distillate.

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