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Extraction and Quantitative Estimation of Bio-ethanol Production from *Citrus sinensis* (L) Osbeck. peels.

Botany

KEYWORDS : Orange rind, lignocelluloses, fermentation, bio-ethanol and GCMS.

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ABSTRACT

Bio-ethanol is a liquid bio-fuel which can be produced from different ligno-cellulosic waste material like fruit waste, agricultural wastes, wheat, sugarcane and molasses by fermenting the sugars present in them. Bio-ethanol produced from cellulosic materials is identical to ethanol produced from other sources and it is also economical and environmental safe as compare to the ethanol produced from starch and molasses. Cellulose molecules are similar to starch molecules but their structural configuration and encapsulation by lignin makes cellulosic material more difficult to hydrolyze then starch. Cellulosic material requires hydrolysis to release cellulose and hemicelluloses from lignin. This release can be done by chemical and enzymatic hydrolysis of this waste by submerged batch fermentation. Along with the quantitative estimation of bio-ethanol produced from orange rinds a Gas Chromatography (GCMS) Mass spectroscopy is also carried out.

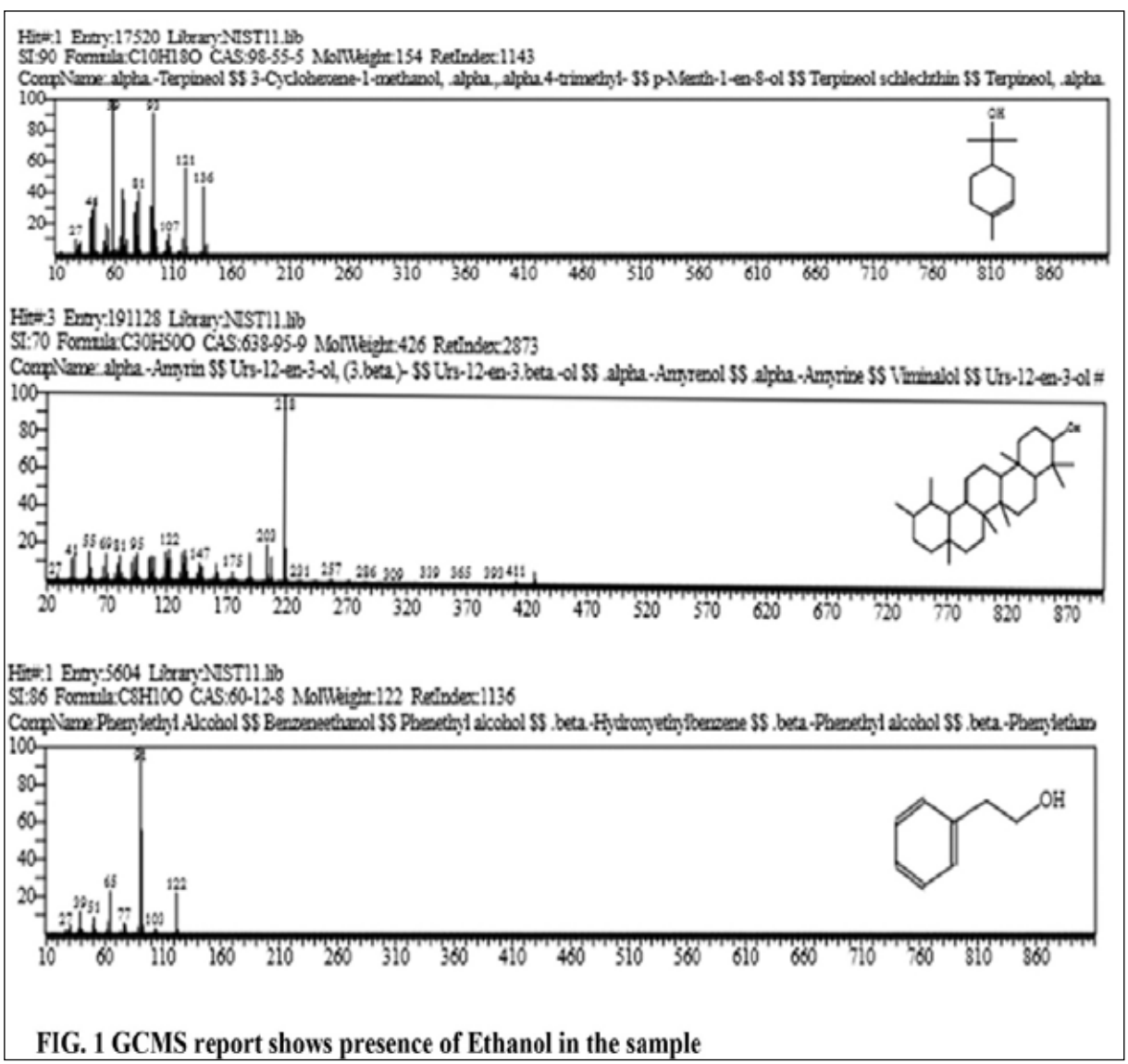

FIG. 1 GCMS report shows presence of Ethanol in the sample

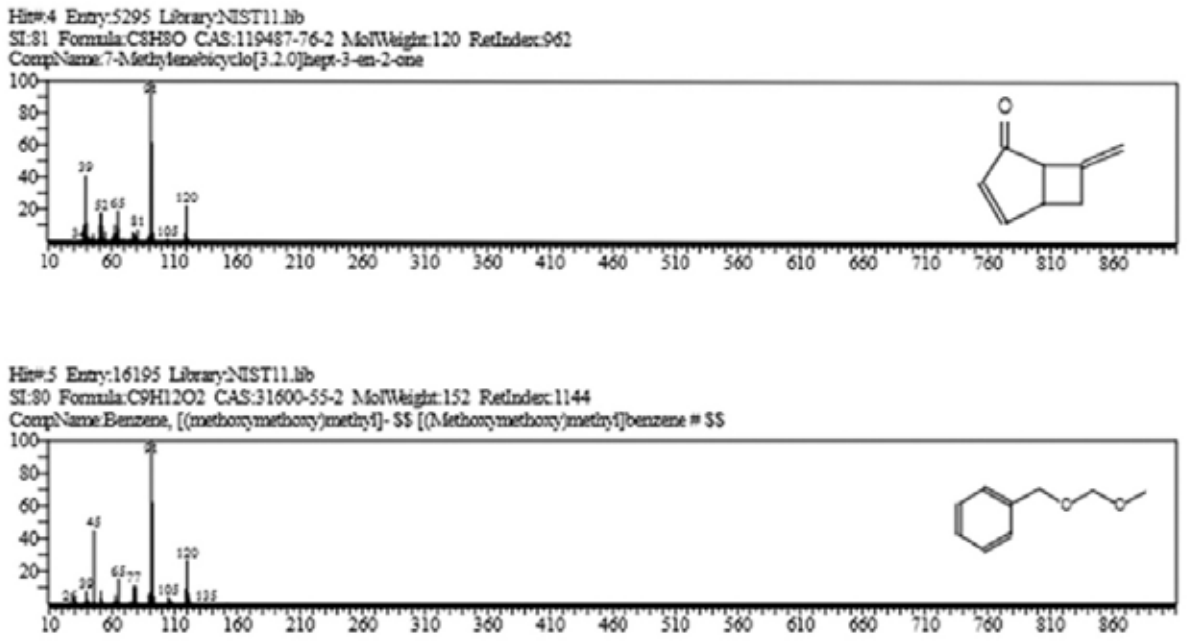

FIG. 2. GCMS report shows presence of other compounds like Benzene in the sample

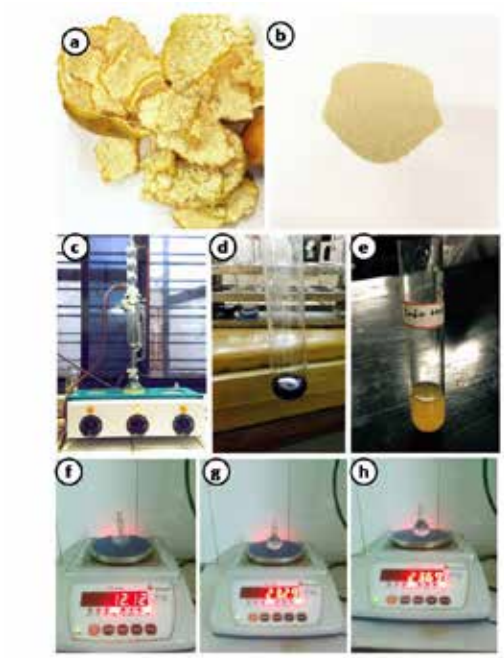

PLATE: I a) Plant Material- Citrus Peels. b) *Saccharomyces cerevisiae*. c) Soxhlet apparatus. d) Test for Phenol. e) Test for Iodine. f) Specific gravity showing weight of empty bottle. g) Weight of bottle with distilled water. h) Weight of bottle with ethanol.

INTRODUCTION:

Bio-fuel is produced through contemporary biological processes such as agriculture and anaerobic digestion rather than a fuel produced by geological processes like those involved in the formation of fossil fuels like coal and petroleum. Bio-fuels can be directly derived from plants or indirectly from agricultural, commercial, domestic and industrial wastes (Bindu *et al*., 2014)

Bio-ethanol is an alcohol made by fermentation of carbohydrates produced in sugar and starch crops like sugarcane, corn and sorghum. This can be used as fuel for vehicles in its pure form but it is usually used as gasoline additives. Now a days anhydrous ethanol (ethanol with 1 % water) can be blended with gasoline in varying quantities to reduce air pollution. Bio-ethanol can be mass produced by fermentation of sugar or by hydration of ethylene from petroleum. The current interest in ethanol lies in production derived from lingo-cellulosic wastes and also whether it is a sustainable energy resource that may offer environmental and economic advantages over fossil fuels like gasoline and diesel. It is readily obtained from the starch or sugar in a wide variety of fruits, crops and agricultural wastes.

The ethanol produced by fermentation ranges in concentration from a few percent up to about14 %. Ethanol is normally concentrated by distillation of aqueous solution but the composition of the vapour from aqueous ethanol is 96 % ethanol and 4 % water. Therefore pure ethanol cannot be obtained by distillation. Dehydrating agents can be used to remove the remaining water and produce absolute alcohol (Potphode Arati & Agarwaal Seema, 2015).

MATERIALS AND METHODS:

Substrate:

The Sweet orange is the fruit of citrus species which is botanically known as *Citrus sinensis* belonging to the family Rutaceae. The fruits were collected from the fruit market, Market Yard, Pune, Maharashtra, India. The rinds of these fruits were used as a main substrate for the bio-ethanol production. The commercial dry yeast was used as a source of yeast (*Saccharomyces cerevisiae*). (Plate 1).

Inoculum preparation:

For the growth of *Saccharomyces cerevisiae* Yeast extract peptone – dextrose broth was used. Yeast was inoculated in a sterile media and incubated at RT for 48 hrs. The broth was centrifuged & the supernatant was discarded and the pellet was re-suspended in 5 ml saline & used as inoculum.

Media preparation:

The fruit rinds were ground in mixer to make slurry. This slurry

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was sterilized at 121°C for 15 psi pressure for 20 minutes. After cooling $(NH_4)_2SO_4$ was added as nitrogen source. To the 100 ml slurry the pellet suspension was added and maintained in anaerobic conditions.

Distillation:
In the present study the Soxhlet apparatus is used for the extraction of bio-ethanol. (Plate 1). The apparatus consist of thimble loaded into the main chamber of the soxhlet extractor. The extraction solvent to be used is placed in a distillation flask. This flask is placed on the heating mantle. The sample was transferred in to the extractor & heated to boil. The vapours started to rise in to the thimble passed through the condenser pipe. This pipe is conducted with inlet & outlet pipe for continuous cooling of vapours. This helps in cooling the ethanol rich vapours back to liquid state. The condensed liquid enters the extractor chamber (Raikar, 2012)

Confirmatory tests: Number of confirmatory tests was conducted to confirm the bio-ethanol production.

Iodine test: (Using Iodine & Sodium Hydroxide solution) Iodine solution was added to a small amount of an alcohol, followed by just enough sodium hydroxide solution to remove the colour of the iodine. If nothing happens in the cold, it may be necessary to warm the mixture very gently. A positive result was seen as a very pale yellow precipitate of tri-iodo methane (previously known as iodoform) - CHI_3. (Plate 1).

Estimation of Total Amount of Bio-ethanol: It was carried out by Pipetting out different volumes of ethanol in test tubes (0.2-2ml). Making up the volume in each tube to 3ml with distilled water. Then 0.5 ml of Folin – Ciocalteu reagent was added. After 3 min, 2ml 20% Sodium Carbonate solution was added to each tube & place in boiling water bath for 1 minute. After cooling the absorbance was read at 650nm. (Plate 1) (Sadasivam & Manickam, 2015)

Calculations:
From the standard curve, Conc. of bio-ethanol in test sample is 111mg/ml (0.1gm/ml).

Determination of specific gravity of given bio-ethanol:
Specific gravity is the ratio of the density of a substance to the density of a reference substance equivalently; it is the ratio of the mass of the substance to the mass of a reference substance (distilled water) for the same given volume. Density of liquid is determined by density weighing certain volume of liquid taken, & same volume of distilled water at same temperature taken in a specific gravity bottle. By using AOAC table the percentage of alcohol present in ethanol sample was determined by using formula (W2-W1/W3-W1) were W1- weight of empty bottle, W2-weight of bottle containing ethanol, W3-weight of bottle containing water. Therefore according to the AOAC table the specific gravity is 1.035, percentage of sugar 8 & alcohol value is 4.5. (Plate 1)

Boiling Point: Boiling point of the given sample is 78 which were similar to ethanol.

Estimation of ethanol: Bio-ethanol was estimated by Folin – Ciocalteu reagent, iodine test using iodine solution & sodium hydroxide solution and specific gravity. This is followed by confirmation with Gas chromatography mass spectroscopy (GCMS). The distillate contains 10 gm % of ethanol. (Plate 1)

Confirmation of Ethanol production: The GCMS is done mainly for volatile & semi-volatile compounds which is an analytical technique. GCMS for the above sample was done at the Department of Chemistry, Savitribai Phule Pune University, Pune 411007 (Fig 1 & 2).

Result & Discussion: The chemical & enzymatic hydrolysis of lingo-cellulosic waste like peels of orange shown positive result for the production of reducing sugars. The released sugars were fermented for 48 hrs. for production bio-ethanol. The estimation of bio-ethanol was done preliminary by taking boiling point, specific gravity, Folin Ciocalteu test & it is confirmed by gas chromatography and mass spectroscopy.

The generation of bio-fuel from the waste like fruit rinds is an attractive solution towards both waste management & energy generation. The fruit peels can be used as a possible source of cellulosic bio-ethanol production because fruit peels consist of high amount of glucose which can be converted to bio ethanol. Use of bio-ethanol as a fuel can reduce greenhouse gas emission thus reduces air pollution.

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