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Comparative Study of Bioethanol Production from Agricultural Waste Materials Using *Saccharomyces cerevisiae* (MTCC 173) and *Zymomonas mobilis* (MTCC 2427) by Enzymatic Hydrolysis Process

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Abstract: Ethanol is an important solvent and chemical feed stock in the chemical, food and Pharmaceutical industries. The use of renewable materials would be more economical, since they are cheaper and easily available and the large quantities of agro waste are available from plantations, their disposal can be a problem. Attempts made here to process Agricultural waste into alcohol, which will have industrial applications, using ability of *Saccharomyces cerevisiae* and *Zymomonas mobilis* to produce ethanol. In the present investigation the production of ethanol by *Saccharomyces cerevisiae* and *Zymomonas mobilis* using Pine apple waste, Barley waste, Copra cake and Corn Stalk substrates was compared. The ethanol was produced from agricultural wastes by using two enzymes namely Amylase from *Aspergillus niger* and Cellulase from *Trichoderma viridae* to hydrolyse the starch and cellulose present in the raw materials. The hydrolysed and filtered extracts were fermented using *Saccharomyces cerevisiae* and *Zymomonas mobilis*. The fermented product was purified by distillation process and the presence of ethanol was determined by alcoholmeter method. Results indicated that the Cellulase containing *Zymomonas mobilis* organism yielded maximum ethanol where as minimum ethanol yield was recorded with Amylase containing *Saccharomyces cerevisiae* organism.

Key words: Bioethanol · *Saccharomyces cerevisiae* · *Zymomonas mobilis* · Enzymes · Fermentation

INTRODUCTION

Historically the most important sugar by product is ethanol and it is produced by yeast as a result of carbohydrate fermentation. Ethanol from sugar distillery is also used for IMFL and from April 2004 it will be mixed with petrol and diesel for automobiles and ethanol is a desirable fuel additive because it allows fuel to burn more cleanly and lower green house gas emissions. It is cost-effective to blend ethanol into gasoline in view of high crude oil prices in recent years [1]. Bioethanol can be synthesized from cellulose and hemicellulose that originates from the many sources of biomass [2].

Cellulosic materials are cheaper and available in plenty but their conversion to ethanol involves many steps and is expensive. Starchy crops like Pineapple waste, Barley waste, Copra cake and Corn Stalk substrates are being exploited for the production of bio ethanol worldwide [3, 4]. The most efficient microorganisms for converting glucose into ethanol are industrial yeast strains of *Saccharomyces cerevisiae* and bacterial strains of *Zymomonas mobilis*. Alcoholic fermentation is the main activity of yeasts, while *Saccharomyces cerevisiae* is the major species used in wine making [5]. It utilizes sucrose, glucose, fructose, maltose and maltotriose as carbon sources to produce alcohol under anaerobic conditions. *Zymomonas mobilis* has several advantages over yeast like higher rates of glucose uptake and ethanol production, higher ethanol yields and ethanol tolerance [6, 7]. In comparing the presently used yeast *Saccharomyces cerevisiae* with *Zymomonas mobilis,* it was realized that the bacterium may have a greater potential for future industrial ethanol production.

Pineapple waste contains valuable components which are mainly sucrose, glucose, fructose and other nutrients [8, 9]. The conversion of these nutrients into useful products of higher value added products like ethanol or even as raw material for other industries or for

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use as food or feed after its biological treatment. A 0.6 Tg of barley waste can efficiently produce 0.21 GL of bioethanol [10]. Barley is first germinated, dried (baked on pan) and crushed for the whole process [11]. The resulting sugars are then by help of yeast (*Saccharomyces cerevisiae*.) converted into ethanol [12]. Generally the yeast attack on hexose sugars, but carbohydrates containing pentose subunits could also be digested by specific yeast into ethanol [13].

Copra cake is used for the production of bioethanol by the process of coconut oil extraction is done by crushing copra to produce coconut oil (70%) the by-product is known as copra cake or copra meal (30%), through enzymatic hydrolysis process and at present about 30-40% ofthe coconut oil extracted from copra is used as a source of biofuel replacing fuel of fossil origin. In 2005, the oilseed produced from copra meal in South-East Asia was estimated to be 4.39 million metric tons, with approxiately1.67 million metric tons obtained from Thailand [14]. Corn stalk is a potential raw material for the production of various products, including fuel ethanol, because it is available in countries in which corn grains are processed [15, 16]. It is obtained in the process of wet milling of corn. Corn stalk, similar to other lignocellulosic materials is the complex of polysaccharides (35% hemicelluloses, 20% cellulose, up to 20% starch) and lignin. The main component of corn fibre is the outer corn grain layer-pericarp and residual part of starchy endosperm [17]. Conversion of starch along with the lignocellulosic components in the corn fibre would increase ethanol yields from corn wet mill by 13% [18].

Hence in our present study Pineapple waste, Barley waste, Copra cake and Corn stalk substrates were used as raw material for the production of bioethanol using *Saccharomyces cerevisiae* and *Zymomonas mobilis*.

MATERIALS AND METHODS

Collection of Substrates: Agricultural raw materials such as Pineapple waste, Barley waste, Copra cake and Corn stalk substrates were used for ethanol production. The sliced pineapple waste and its peel off were collected and crushed, Barley waste was collected from Beverages industry, Palakkad, Kerala, it was further crushed to get fine extract.The copra cake was obtained from the local oil mill of Palakkad district, Kerala and it was made into powder form using mortar and pestle. The substrate, Corn stalk was collected from vegetable shops, Coimbatore, then it was made into small pieces.

Pre-Treatment of Substrates: Peeled pineapple (1kg) was thoroughly washed and cooked in a pressure cooker in one litre water containing 0.5% potassium metabisulfite. It was further washed with distilled water. Barley waste was Pre-treated with 1N NaOH for 1 hour at 100^0c. It was further washed thoroughly with distilled water, dried at room temperature and stored in desiccators. The Copra cake was treated with 3% NaOH solution and it was heated at the water bath at 100^0c for 25 minutes. The Corn stalk was heated at 120^0c for 20 minutes. After cooling to room temperature, it was treated with 5% sodium hydroxide solution for 20 minutes.The purpose of the pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity and increase the porosity of the materials. Pretreatment must meet the following requirements: i) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis; (ii) avoid the degradation or loss of carbohydrate; and (iii) avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes [19].

Source of Organisms: *Saccharomyces cerevisiae* (MTCC 173), *Trichoderma viridae* (MTCC 800) and *Zymomonas mobilis* (MTCC 2427) were obtained from IMTECH, Chandigarh. *Aspergillus niger* was isolated from spoiled bread sample and identified. The stock cultures of *Saccharomyces cerevisiae*, *Trichoderma viridae and Zymomonas mobilis were* maintained on Yeast Extract Peptone Dextrose Agar (YEPD), Malt Extract Agar (MA) and Rich medium (RM) respectively. Isolation and identification of the organisms were done on the basis of morphological, cultural and biochemical characteristics [20].

Enzyme Production and Assay: *Aspergillus niger* and *Trichoderma viridae* were inoculated in Potato dextrose broth and incubated at room temperature for 7 days. After incubation, fermented media was filtered by Whattman No-1 filter paper and centrifuged and the supernatant was collected for enzyme assay. Amylase and cellulase enzymes were determined by Starch agar plate method [20] and Dinitro Salicylic acid (DNS) method [21, 22].

Enzymatic Hydrolysis: Twenty g of Pineapple waste, Barley waste, Copra cake and corn stalk samples were crushed and dissolved in 200ml of distilled water. The content was boiled and filtered through Whatman 1NO - 1 filter paper. Then extract was sterilized and after

sterilization 5% of enzymes were added to the extracts and incubated at 37°C for 3 hours for hydrolysis process [23].

Determination of Total Sugar and Residual Sugars: The total sugar content of the samples were determined by Phenol sulphuric acid method [24] and the residual sugar content of samples were determined by Nelson Somogyi [25] method with glucose as standard. The Reducing sugar (sugar utilized by the organism during fermentation) percentage was calculated by subtracting the residual sugar% from total sugar%.

Fermentation and Distillation: The Hydrolyzed and filtered extracts were fermented using *Saccharomyces cerevisiae* and *Zymomonas mobilis* for 7 days of incubation at room temperature under anaerobic condition. After fermentation it was filtered and distillation was carried out in round bottom flask at 80°C [23]. After that percentage of ethanol was estimated by using Alcoholmeter.

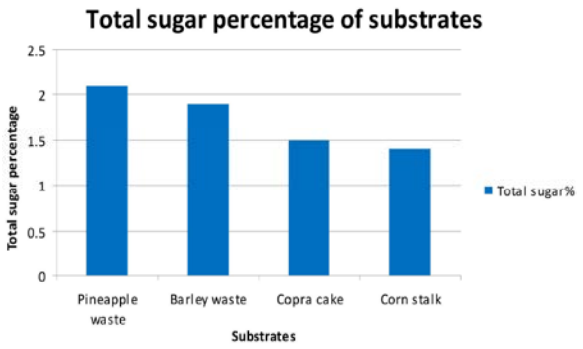

Fig. 1: Total sugar percentage of substrates

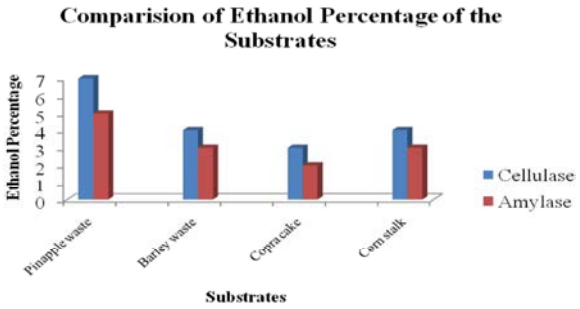

Fig. 2: Shows Ethanol production from the substrates.

RESULTS

In the present study raw materials such as Pineapple waste, Barley waste, Copra cake and Corn stalk samples were collected in and around Coimbatore and Kerala, India. The enzymes amylase and cellulase produced by *Aspergillus niger* and *Trichoderma viridae* were determined. Assay of amylase was done in starch agar plate method, results in zone of inhibition which indicates presence of amylase and cellulase activity was determined by DNS method.

In the present investigation, total sugar estimation was carried out by Phenol sulphuric acid method using untreated Pineapple waste, Barley waste, Copra cake and Corn stalk substrates. The maximum total sugar percentage of Pineapple waste, Barley waste, Copra cake and Corn stalk substrates was found to be 2.2%, 1.9 %, 1.5% and 1.4% (Fig 1). The Residual sugar estimation was carried out by the method of Nelson somogyi using fermented Pineapple waste, Barley waste, Copra cake and Corn stalk substrates. The Reducing sugar percentage of amylase and cellulase treated Pineapple waste, Barley waste, Copra cake and Corn stalk substrates were found to be 2.16%, 1.89%, 1.47% and 1.39% respectively (Table 1 and 2).

In the cellulase treated (*Zymomonas mobilis*) Pineapple waste, Barley waste, Copra cake and Corn stalk substrates, the ethanol yields were noticed as 7.0, 4.0, 3.0 and 4.0%, where as in the amylase treated (*Saccharomyces cerevisiae*) Pineapple waste, Barley waste, Copra cake and Corn stalk substrates was found to be 5.0, 3.0, 2.0 and 3.0%. The percentage of ethanol was found to be reduced in the amylase treated substrates when compared with cellulase treated substrates (Table 1 and 2 and Fig. 2).

DISCUSSION

In the recent years, production of ethanol using fermentation on a large scale has attained considerable interest. The economic feasibility, however always has been focused towards high yield of ethanol that is full use of raw material associated with high productivities so as to reduce the cost of production. The crude enzymes like amylase and cellulase were used for the enzymatic hydrolysis of biomass. In the hydrolysis process, few extract of biomass and whole biomass were treated with 5% of the crude enzymes and incubated at 37°C for 3 hrs and reaction was arrested by incubating at 4°C for 15 minutes. Purification of the crude enzymes and optimization parameters may give better result for degradation of starch or hemicelluloses and cellulose present in biomass [23].

Table. 1: Ethanol production from Amylase treated Pine apple waste, Barley waste, Copra cake and Corn stalk substrates using *Saccharomyces cerevisiae*

Sl. No	Substrates	Residual Sugar %	Reducing sugar %	Total sugar %	Ethanol %
1	Pineapple waste	0.04	2.16	2.2	5
2	Barley waste	0.01	1.89	1.9	3
3	Copra cake	0.03	1.47	1.5	2
4	Corn stalk	0.01	1.39	1.4	3

Table 2: Shows the Ethanol production from cellulase treated Pine apple waste, Barley waste, Copra cake and Corn stalk samples using *Zymomonas mobilis*

S.No	Substrates	Residual Sugar%	Reducing sugar%	Total sugar%	Ethanol %
1	Pineapple waste	0.04	2.16	2.2	7
2	Barley waste	0.01	1.89	1.9	4
3	Copra cake	0.03	1.47	1.5	3
4	Corn stalk	0.01	1.39	1.4	4

The Maximum ethanol percentage of Amylase treated (*Saccharomyces cerevisiae*) Pineapple waste, Barley waste, Copra cake and Corn stalk substrates, and cellulase treated (*Zymomonas mobilis*) Pineapple waste, Barley waste, Copra cake and Corn stalk substrates, were found to be 5.0 % and 7.0% respectively. This is due to the fact that *Zymomonas mobilis* organism has several advantages over yeast like higher rates of glucose uptake and ethanol production, higher ethanol yields and ethanol tolerance [6, 7]. Some of the reported advantages of *Zymomonas mobilis* over yeast are high sugar uptake and ethanol productivities on cell recycle systems, no oxygen requirement and genetic manipulation potential [26].

CONCLUSION

Bio ethanol was produced from Pineapple waste, Barley waste, Copra cake and Corn stalk substrates using an enzymatic hydrolysis process, viz. enzyme hydrolysis (amylase and cellulase) followed by fermentation. Result showed that, the Cellulase containing *Zymomonas mobilis* yielded maximum ethanol where as minimum ethanol was recorded with Amylase containing *Saccharomyces cerevisiae*. Further studies by optimizing certain parameters and by proceeding secondary distillation to produce pure form of ethanol using cheap raw materials and other sources.

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