American-Eurasian Journal of Scientific Research 10 (5): 307-315, 2015
ISSN 1818-6785

DOI: 10.5829/idosi.aejsr.2015.10.5.8476

Production of Ethanol by *Zymomonas mobilis* and *Saccharomyces cerevisiae* Using Sunflower Head Wastes-A Comparative Study

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Abstract: Ethanol is one of the good sources of liquid energy for automobiles and industries. Ethanol is used as universal solvent. It is also used as fuel. In future, ethanol is going to blend with petrol in high proportion. Among the liquid fuels, ethanol is used as an alternative to petroleum (gasohol) by blending with petrol at the rate of 20%. To reach the future demand of ethanol, it should be produced in high quantity from the agricultural raw material. Although, there are enormous saccharine and starchy materials, their use as raw material for ethanol production is prohibitive because of their use as a food material for feeding the billions of our population. The present investigation was undertaken to optimize various pretreatment methods for maximum reducing sugar, selection of suitable efficient yeast and bacterial cultures, optimization of fermentation parameters and finally to study the ethanol production from the hydrolysate of deseeded sunflower head waste. Among the different pretreatments, combination of acid + steam pretreatment was found to give more reducing sugar than the other pretreatment for both micro organisms. The maximum ethanol production (18.59 g/L) was obtained at a substrate concentration of 5% (v/v) at pH 6.5 for *Zymomonas mobilis* and (16.46 g/L) was obtained at 3% (v/v) of substrate concentration at pH 5.5 for *Saccharomyces cerevisiae*.

Key words: Ethanol · Sunflower Head Waste · Fermentation · *Zymomonas mobilis* and *Saccharomyces cerevisiae*

INTRODUCTION

Energy requirement and environmental pollution are the two major challenges today. Energy has been always in demand not only in the past, but is a continuing crisis due to the advance technologies and increase in population. Therefore, it is necessary to search for alternate energy sources, which are non-petroleum based and renewable such as solar, wind and biomass. Among these, biomass (Dedicated energy crops) is the most important and excellent energy source from which fuels of solid, liquid and gaseous nature can be produced. Among the liquid fuels, ethanol is used as an alternative to petroleum (Gasohol) by blending with petrol at the rate of 20% [1]. The biological conversion technology for production of ethanol can be divided into four simple processes: Pretreatment, Saccharification, Fermentation and Distillation.

Ethanol is a clear, colourless, flammable, oxygenated hydrocarbon with the chemical formula C_2H_5OH. Ethanol has been made since ancient times by fermenting sugars. All the ethanol used for fuel and alcoholic drinks and most industrial ethanol, is made by this process. Fuel ethanol is also known as bioethanol, since it is produced from plant materials by biological processes. Fuel ethanol is the largest market by far, accounting for 60% of total ethanol production worldwide. The term biofuel is attributed to any alternative fuel that derives from organic material, such as energy crops (Corn, wheat, sugar cane, sugar beet, cassava, among others), crop residues (e.g. rice straw, rice husk, corn stover, corn cobs) or waste biomass (For instance, food waste, livestock waste, paper waste, construction-derived wood residues and others).

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Ethanol was first produced thousands of years ago by fermentation of carbohydrates and in some countries large volumes are still produced by this method. Synthetic alcohol was first produced industrially in the 1930s by indirect catalytic hydration of ethylene but suffered from disadvantages such as corrosion from the large volume of sulphuric acid handled and the energy required for concentration. Fermentation ethanol process based on starch or sugar based feedstock such as corn, potato and sugarcane was being used to meet the demand for ethanol as a fuel. Ethanol fermented from renewable sources for fuel or fuel additives are known as bioethanol. Additionally, the ethanol from biomass based waste materials was considered as bioethanol. Currently, there is a growing interest for ecologically sustainable bio-fuels. The target in the European Union is to increase bioenergy contributions in total energy consumptions from 3 to 12% by the year 2010 [2].

Industrial ethanol accounts for 20% of the market and beverages for about 15%; both these markets are growing comparatively slowly. Ethanol can be used as a transport fuel in at least four forms: anhydrous ethanol (100% ethanol), hydrous ethanol (95% ethanol and 5% water), anhydrous ethanol-gasoline blends (10–20% ethanol in gasoline) and as raw material for ethyl tert-butyl ether (ETBE) [3]. The term biofuel is referred to as liquid or gaseous fuels for the transport sector that are produced from biomass. Bioethanol is one of the most famous biofuels. Ethanol fermented from renewable sources for fuel or fuel additives are known as bioethanol. Additionally, the ethanol from biomass-based waste materials is considered as bioethanol. The use of bioethanol as an alternative motor fuel has been steadily increasing around the world for the number of reasons. The production of ethanol from biomass is progressing in many countries worldwide however the production costs are still relatively high when compared to petrol. The environmental benefits coupled with the social benefits and economic benefits can be seen to have a dollar value offsetting the higher relative cost compared to petrol.

Agriculture wastes contain a high proportion of cellulosic matter which is easily decomposed by a combination of physical, chemical and biological processes. The bunch consists of 70 moisture and 30% solid; of which holocellulose accounts for 65.5, lignin 21.2, ash 3.5, hot water-soluble substances 5.6 and alcohol-benzene soluble 4%. Lignin is an integral cell wall constituent, which provides plant strength and resistance to microbial degradation [4]. The recognition that environmental pollution is a worldwide threat to public health has given rise to a new massive industry for environmental restoration. Biological degradation, for both economic and ecological reasons, has become an increasingly popular alternative for the treatment of agricultural, industrial, organic as well as toxic waste.

Sunflower is an important oilseed crop and it ranks third after soya bean and groundnut as a source of edible oil in the world. In India, it is cultivated in an area of 1.33 million hectares with an annual production of 1.00 million tonnes. In Tamil Nadu, Sunflower is gaining importance as second major oil seed crop after groundnut. Sunflower is grown in an area of 20000 hectares with the production of 20000 tonnes. Looking to the potentiality of the substrate that could be made available, the present investigation was taken up to exploit suitable bacterial and yeast strains and to optimize the parameters for maximum ethanol production.

The most common way of bioethanol production today is by fermentation using the yeast *Saccharomyces cerevisiae* with high ethanol yields from starch based substrates. In the past decades, thermophilic bacteria have gained more attention because of fast growth rates and their ability to degrade a broad variety of both hexoses and pentoses [5-7]. Although, ethanol tolerance of thermophiles is generally less than those of *Saccharomyces cerevisiae* and the well known mesophilic bacterium *Zymomonas mobilis*, they have several advantages like lower risk of contamination, increased bioconversion rates and product recovery. The present investigation was undertaken to optimize various pretreatment methods for maximum reducing sugar, selection of suitable efficient yeast and bacterial cultures, optimization of fermentation parameters and finally to study the ethanol production from the hydrolysate of deseeded sunflower head waste.

MATERIALS AND METHODS

Microorganism: *Zymomonas mobilis* (MTCC 2427) and *Saccharomyces cerevisiae* (MTCC 182) was selected in this studies. They microbial cultures were obtained from Institute of Microbial Technology (MTCC), Chandigarh.

Preparation of Medium

For *Zymomonas mobilis*: The bacteria fermentation medium had the following ingredients in distilled water (In grams per liter). Glucose – 20 g/L, Yeast extract - 10 g/L and KH_2PO_4 – 1 g/L. The pH was adjusted to 6 and autoclaved at 121°C to maintain 15 psi for 20 minutes.

For *Saccharomyces cerevisiae*: The yeast fermentation medium had the following ingredients in distilled water (in grams per liter). Dextrose – 20 g/L, Yeast extract – 3 g/L and Peptone – 10 g/L. The pH was adjusted to 5 and autoclaved at 121°C to maintain 15 psi for 20 minutes.

Substrate for Fermentation: The Sunflower head wastes were cut into small bits. These bits were then sun- dried for 3 - 4 days. The dried sunflower head bits were finely ground in an electric grinder and the flour was collected.

Pretreatment of the Substrate

Physical Pretreatment Method

Steam Explosion: The powdered substrate was autoclaved at 121°C for 20 min; the contents were cooled and incubated at room temperature.

Chemical Pretreatment Methods

Acid Hydrolysis: Twenty five grams of Sunflower head flour was slurried with 100 ml water in 250 ml conical flask. The flour suspension was treated with sulphuric acid at an acid concentration of 0.05, 0.1 and 0.2 N acidity. The acid flour mixture was centrifuged at 10,000 rpm for 15 min. The clear supernatant of the hydrolysate was taken for the estimation of reducing sugars.

Alkali Hydrolysis: Twenty five grams of Sunflower head flour was slurried with 100 ml water in 250 ml conical flask. The flour suspension was treated with sodium hydroxide at an alkali concentration of 0.05, 0.1 and 0.2 N alkalinity. The alkali flour mixture was centrifuged at 10,000 rpm for 15 min. The clear supernatant of the hydrolysate was taken for estimation of reducing sugars.

Treatment Combination (Physical + Chemical Method): Twenty five gram of Sunflower head flour was slurried with 100 ml water in 250 ml conical flask. The flour suspension was treated with sulphuric acid at an acid concentration of 0.05, 0.1, 0.2 N acidity. The acid flour mixture was autoclaved at 121°C for 30 min. The acid hydrolyzed slurry was cooled and the filtrate centrifuged at 10,000 rpm for 15 min. The clear supernatant of the hydrolysate was taken for the estimation of reducing sugars.

Determination of Glucose and Ethanol Concentration: Glucose concentration was determined by DNS method. Ethanol was determined using Gas chromatograph. The column used was Poropak column by which all the hydrocarbons could be determined.

RESULTS AND DISCUSSION

Many opportunities may be explored using different costless renewable waste materials with a lot of usable substrate for microorganisms to grow upon and then produce useful products (For example, agricultural food waste, wood chips, molasses, whey, permeate, rice straw and newspaper waste). Most renewable energy source can be used in a fermentation process with microorganisms to produce bioethanol. Ethanol production benefits the society and the environment and may be easily produce from thermotolerant yeast strains with a capacity to withstand high temperature and different types of fermentation process. There are a lot of ways to maximize ethanol production, such as through continuous SSF process with either two stages in sequence or with a two stage anaerobic fermentation process with cell recycling [8].

As a consequence of the industrial development and population growth, there is an increase of energy consumption in the world. The world-wide energy consumption has increased 17 fold in the last century. However, conventional energy resources, like fossil fuels, cannot meet the increasing energy demand. The quantities of non-renewable (Conventional) energy resources are limited and they have a considerable negative environment impact. Therefore, one of the challenges for the society is to meet the growing demand for energy for transportation, heating and industrial processes; also to provide raw materials for the industry in a sustainable way and to reduce green house gas emissions. Our energy systems will need to be renewable and sustainable, efficient and cost-effective, convenient and safe.

Bioethanol produced from renewable biomass such as starch, sugar materials, is believed to be one of these alternatives. It is expected to be one of the dominating renewable biofuels in the transport sector within the twenty years to come. The transport sector itself is considered as one of the largest energy consumers as well as environmental pollutant. The face of ethanol production technology is old and ever changing. It is widely noted that centuries ago man discovered and began employing fermentation technology to produce alcohol; today ethanol is produced from a variety of materials for use as an industrial chemical and fuel. Through, decades of research and development, the production of fuel ethanol has been developing throughout the world. Conventional processes have been maximized while advances continue to be made in starch

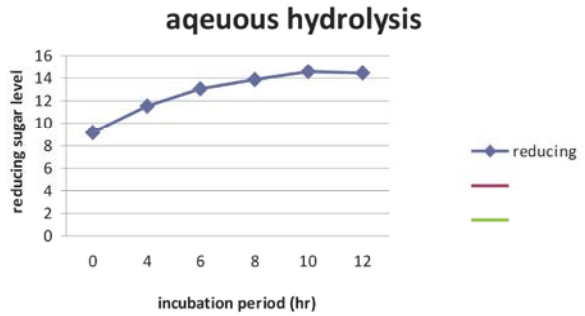

Fig. 1: Effect of aqueous hydrolysis on the release of reducing sugar content from sunflower head waste

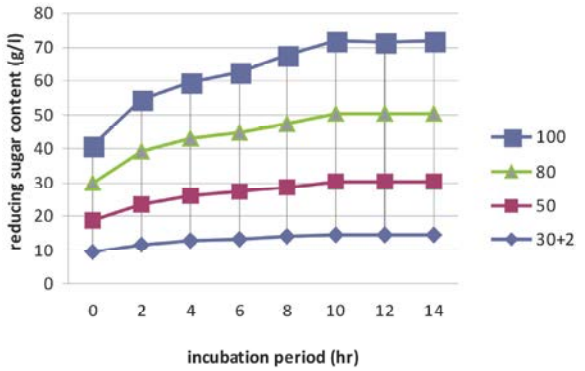

Fig. 2: Effect of temperature on the release of reducing sugar content from sunflower head waste

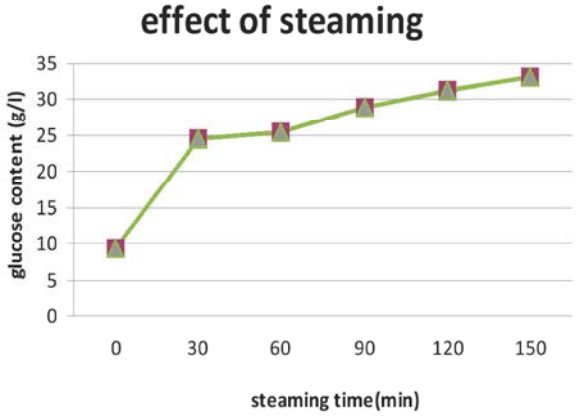

Fig. 3: Effect of Steaming on the release of reducing sugar content from Sunflower head waste

biomass conversion. In general, aqueous hydrolysis had positive correlation with the release of reducing sugars from sunflower head waste at different incubation periods. The residual sugar released from 14.22 to 19.53 g/L. The reducing sugar content in sunflower head waste increased significantly with increase in incubation periods upto 10 hrs and 10th hrs was significantly superior as compared to other incubation periods (Figure - 1).

The reducing sugar content increased with increase in temperature significantly, which ranged from 14.22 g/L to 26.73 g/L, when incubated for 10 hrs. The highest reducing sugar content in sunflower head waste recorded was 26.73 g/L at 100°C followed by 80°C, which showed 23.06 g/L. At ambient temperature at 50°C was 19.53 g/L and 20.76 g/L respectively (Figure - 2). However, the reducing sugar content proportionately increased upto 10 hrs. Thus, an incubation period of 10 hrs was optimum for maximum release of reducing sugars. The highest reducing sugar content (26.73 g/L) was recorded at 100°C at 10 hrs of incubation period and further release of sugar almost remain constant after 10 hrs of incubation.

Banerjee *et al*. [9] lowered the operating temperatures and pressures during the concentrated acid hydrolysis process. The concentrate acid – hydrolysis involves longer retention times and results in higher ethanol yields than the dilute acid – hydrolysis process. Concentrated acid hydrolysis requires high amount of acid and hence becomes uneconomical acid recycling also entails considerable lost. Alkaline pretreatment consists of two different alkaline reagents were used, aqueous ammonia and dilute NaOH, were used in pretreatment of biomass for delignification and enhancement of digestibility. Two pretreatment process with aqueous ammonia (SSA) and ammonia recycle percolation (ARP). SSA was a batch process whereas ARP is a flow through semi batch process.

The results of hydrolysis of sunflower head waste with steaming at different intervals (0, 30, 60, 90, 120, 150 min) are given in Figure - 3. The reducing sugar content increased with increase in steaming time significantly and ranged 27.51 g/L to 35.42 g/L. The highest reducing sugar content recorded in sunflower head waste was 35.42 g/L at 121°C for 150, 180 and 210 min followed by 120 min, which showed 33.87 g/L, beyond a period of 150 min did not improve the yield of reducing sugars.

During the acid hydrolysis, diluted H_2SO_4 was used for reducing the pH of the mixture which was shaken and heated by steam upto 100°C [10]. After 6 hours, about 95% of the starch chains are transformed into glucose. The hydrolysis of starch from fresh potato tubers by HCl and H_2SO_4 at different ratios of plant material to acid solution was investigated. The final reducing sugars concentration in the hydrolyzates depended on the type and concentration of acid and the ratio of plant material to acid solution but not on the type of potato. There was a growing interest in ecologically sustainable biofuels such as ethanol from starchy materials. The industrial processes mainly use grain, tuber and root starches.

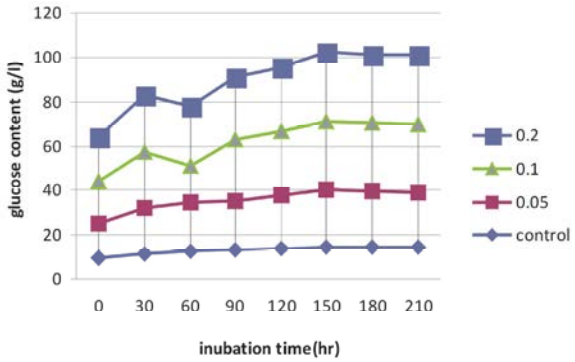

Fig. 4: Effect of Acid hydrolysis on the release of reducing sugar content from sunflower head waste

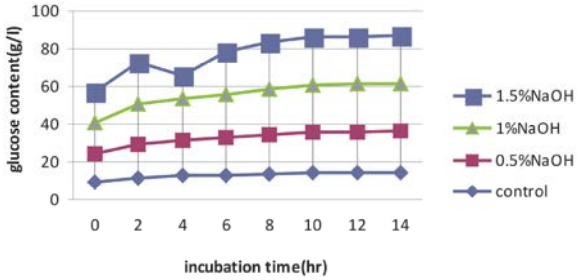

Fig. 5: Effect of Alkali hydrolysis on the release of reducing sugar content from sunflower head waste

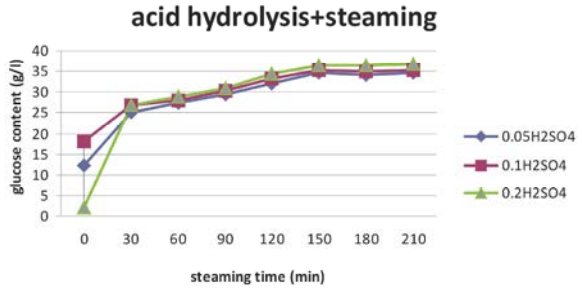

Fig. 6: Effect of combination of acid hydrolysis and steaming on the release of reducing sugar content from sunflower head waste

These materials must be first hydrolyzed to fermentable sugars by enzyme catalysis or inorganic acids. Currently, enzyme - catalyze starch hydrolysis was preferred, as it offers a number of advantages. However, the high cost of the initial investment and enzymes, as well as the requirements for specialized labor and sophisticated laboratories are factors limiting the use of enzymes [11].

Acid treatment showed increase in reducing sugars with both increase in concentration and period of incubation (Figure – 4). The reducing sugar content of sunflower head waste was found to be significantly increased with increase in concentration, which ranged from 16.10 - 36.11 g/L. The results of acid hydrolysis of sunflower head waste with sulphuric acid at different concentrations. (0.05, 0.1 & 0.2 N). The data indicated that out of different concentrations of acid 0.2N sulphuric acid gave better yield of reducing sugar 36.11 g/L than other.

The sugary substrate available are comparatively expensive than molasses but can be easily used for ethanol production with some modification in the process. The starchy substrates are promising due to their economic viability and availability starch crops like corn, barely, wheat, rice, potato, sweet potato, are being exploited for the production of bioethanol worldwide [12]. The world over production of potatoes in 2007 was 325.3 million tons while in India it was approximately 26 million tons [13] showing this as a promising crop but is being used for production of ethanol in some countries [14].

Currently, commercial ethanol production relies on the fermentation of sucrose from cane sugar and molasses or glucose derived from starch –based crops such as corn, wheat and cassava and there is a growing need for the industry to improve technology and expand production [15]. Efficient bioethanol production requires a rapid fermentation leading to high ethanol concentrations [16]. An important issue for the efficient ethanol production is to optimize the fermentation step regarding following main parameters temperature, pH, media composition, mixing aeration, elimination of infection etc.

The reducing sugar content in sunflower head waste increased significantly with the concentration of alkali used. The highest reducing sugar content was recorded at 1.0 and 1.5 percent alkali concentrations, which accounts to 30.12 g/L and 36.62 g/L respectively and they were significantly superior to 0.5 per cent alkali treatment and control which recorded only 26.01 g/L and 19.53 g/L respectively (Figure - 5). Similarly, the reducing sugar content also increased significantly with increase in incubation period (1 - 10 hrs), which ranged from 16.10 to 30.62 g/L. The reducing sugar content increased significantly upto 10 hrs. However, the reducing sugar at 8 and 10 hrs of incubation was significantly higher as compared to other period of incubation.

The results on the hydrolysis of sunflower head waste with acid (H_2SO_4) at different concentrations (0.05, 0.1 and 0.2 N) plus steaming for different intervals of time (0, 30, 60, 90, 120 and 150 min) were given in Figure - 6. The data indicated that out of different concentrations of acids, 0.2 N sulphuric acid plus steaming at 121°C for 150 minutes gave the higher yield of

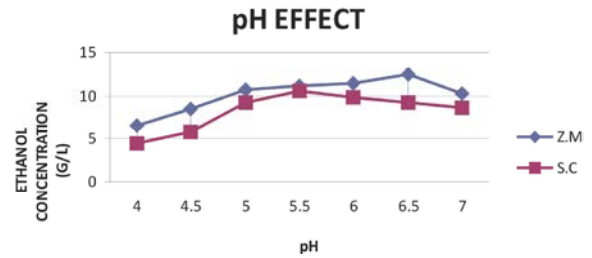

Fig. 7: Effect of different levels of pH on Ethanol yield of Sunflower head waste

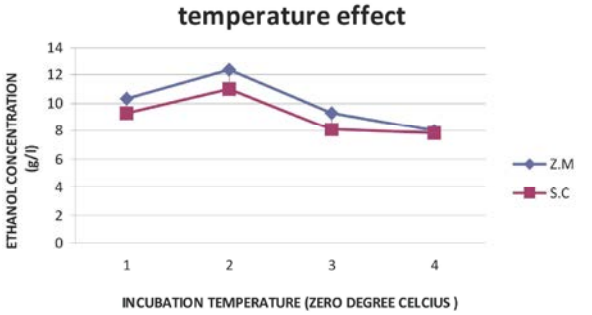

Fig. 8: Effect of different levels of incubation temperature on ethanol yield of sunflower head waste

the reducing sugars (41.63 g/L). The hydrolysis was poor, when 0.05N H_2SO_4 was obtained 39.80 g/L, compared to 40.26 and 41.63g/L of reducing sugars at 0.1 N and 0.2 N sulphuric acids respectively at 121°C.

The ethanol yield significantly influenced by different pH levels as indicated in Figure - 7. The ethanol yield increased significantly from pH 4 to 6.5. Beyond this level the ethanol yield slightly increased due to inoculation of *Zymomonas mobilis* (17.42 g/L). The ethanol yield was also increased from pH 4 to 5.5 due to inoculation of *Saccharomyces cerevisiae*. Beyond this level, ethanol yield decrease slightly (14.81 g/L) at pH 6. The maximum ethanol concentration was obtained at pH 6.5 for *Zymomonas mobilis* and 5.5 for *Saccharomyces cerevisiae*. The same optimum pH was used for further study.

Tasic *et al*. [17] used the cold, concentrated hydrochloric acid to produce glucose contaminated with 6% of isomaltose within 50 hours. In another process of hydrolysis, the concentrated acid was deposited on an inert mineral sorbent, which was then blended and heated with starch or cereals for potato tuber starch hydrolysis, the optimal reaction conditions such as 1M hydrochloric acid, the ratio of plant material to acid solution of 1:2(10/v) 1 hour and 98°C, were established. During the acid hydrolysis, diluted H_2SO_4 was used for reducing the pH of the mixture which was shaken and heated by steam upto 100°C. After 6 hours, about 95% of the starch chains are transformed into glucose [18].

The ethanol yield increased due to the increasing temperature from 25 to 30°C with the inoculation of both *Zymomonas mobilis* and *Saccharomyces cerevisiae* (Figure - 8). Beyond this temperature, the ethanol content decreased significantly for 35 and 40°C. When compare to the other temperature the room temperature gave high ethanol yield. The *Zymomonas mobilis* gave high ethanol yield 17.39 g/L than *Saccharomyces cerevisiae* 16.03 g/L and the room temperature was used for further study.

Rikhvanov *et al*. [19] described some successful attempts to adapt yeast to high temperature. *Saccharomyces cerevisiae* yeasts, capable of fermenting at 40°C and 45°C, have been obtained using progressive cultures. Higher cell mass was produced in monoculture than in co-culture, suggesting that substantially more carbon was used for cell production in monoculture, whereas in the co-culture most of the substrate carbon is utilized for ethanol production. *Saccharomyces cerevisiae* was the dominant organism in co-culture. Time course of starch utilization, reducing sugar, ethanol concentration and amylolytic activity in co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae* in 5% starch medium were then determined. Concomitant with starch hydrolysis there was a rapid increase in reducing sugar concentration initially. During the rest of fermentation, rapid fermentation of sugar to ethanol by *Saccharomyces cerevisiae* kept the sugar concentration low enough to prevent feedback inhibition of amylolytic activity previously observed in monocultures of *Aspergillus niger*.

Araquue *et al*. [20] studied the bioethanol production at higher temperatures, yeast cells die resulting in a decrease in alcohol yield when the pulp was concentrated, while optimal temperatures for maximal productivity occurs at 32°C. It was therefore, necessary to select the optimal temperature at which yeast strains can ferment the sugars from lignocellulosic material. It can also be that between 25°C and 30°C, the sugars were used up faster than at 20°C and 40°C. Among these technologies, ethanol production under non- sterilized condition could save the energy in cooking starch and sterilization. It could make process simpler than even before, thus has gained interest of many researches [21].

Banerjee *et al*. [22] lowered the operating temperatures and pressures during the concentrated acid hydrolysis process. The concentrate acid – hydrolysis involves longer retention times and results in higher

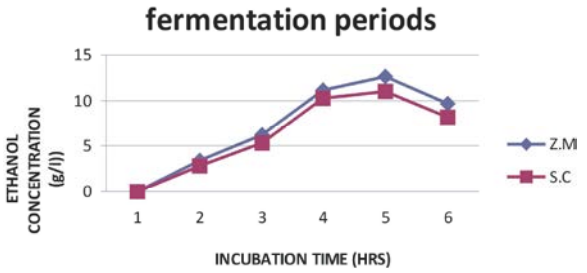

Fig. 9: Effect of different levels of incubation period on ethanol yield of sunflower head waste

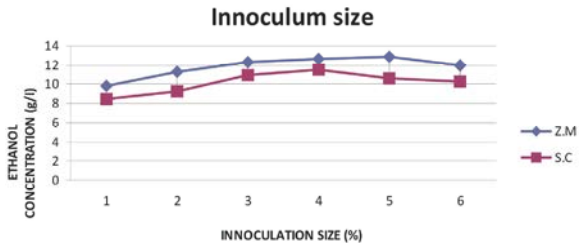

Fig. 10: Effect of different levels of inoculum size on ethanol yield of sunflower head waste

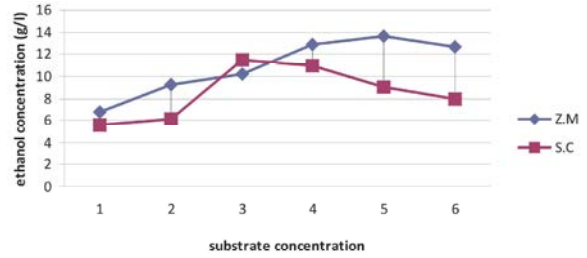

Fig. 11: Effect of substrate concentration on ethanol yield of sunflower head waste

ethanol yields than the dilute acid – hydrolysis process. Concentrated acid hydrolysis requires high amount of acid and hence becomes uneconomical acid recycling also entails considerable lost. Alkaline pretreatment consists of two different alkaline reagents were used, aqueous ammonia and dilute NaOH, were used in pretreatment of biomass for delignification and enhancement of digestibility. The ethanol yield from sunflower head waste increased with the period of incubation as observed in Figure - 9. The ethanol yield increased significantly with increasing inoculation period 0 to 48 hrs for both *Zymomonas mobilis* (17.59 g/L) and *Saccharomyces cerevisiae* (16.04 g/L). However, it decreased significantly upto 60 hrs of fermentation.

Verma *et al*. [23] studied that the effect of four different fermentation periods *viz*., 24, 48, 72 and 96 hours on ethanol production from starch medium. A maximum ethanol concentration of 24.8 g L^{-1} at 48 hours was achieved as compared to 13.7 and 21.6 g L^{-1} at 24 and 96 hours respectively. Marakis and Marakis [24] studied that the effect of 6 different fermentation period *viz*., 0, 24, 48, 72, 90 and 100 hours on ethanol production from aqueous carob extract and achieved maximum alcohol concentration of 4.75% (v/v) at 100 hours of fermentation period.

For different inoculum size (2%, 4%, 6%, 8%, 10% and 12% v/v) the experiment was carried out for a period of 2 days at the optimum pH (6.5 for *Zymomonas mobilis* and 5.5 for *Saccharomyces cerevisiae*) and temperature (30°C). The other study medium compositions are same as used in the previous study. The maximum ethanol concentration (17.89 g/L for *Zymomonas mobilis* and 15.92 g/L for *Saccharomyces cerevisiae*) were obtained at 10% inoculum size for *Zymomonas mobilis* and 8% inoculum size for *Saccharomyces cerevisiae* on 2nd day (Figure – 10).

Effect of substrate concentration was studied by conducting the experiment with different substrate concentration values (1, 2, 3, 4 and 5%). The experiments were carried out for the fermentation period of 2 days at the optimum pH (6.5 for *Zymomonas mobilis* and 5.5 for *Saccharomyces cerevisiae*) and temperature (30°C). The maximum ethanol concentrations (18.59 g/L and 16.46 g/L) were obtained at 5% of Substrate concentration 30°C for *Zymomonas mobilis* and 3% for *Saccharomyces cerevisiae* on 2nd day. The results are shown in Figure - 11.

Nimbkar *et al*. [25] studied the effect of different inoculum level *viz*., 2, 4, 6, 8 and 10% on the ethanol production from unspecialized juice of sweet sorghum and obtained maximum alcohol concentration of 12.45 and 12.23% (v/v) at 6 and 2% respectively. They also studied the effect of three different incubation temperatures *viz*., 25, 30 and 35°C, on the ethanol production from unsterilized juice of sweet sorghum with *Saccharomyces cerevisiae* and obtained maximum alcohol of 12.45% (v/v) at 30°C. The effect of different temperatures *viz*., 25, 30 and 40°C, on the ethanol production from starch and observed that maximum ethanol concentration 21.8 gL^{-1} at optimum temperature of 30°C in 48 hours of fermentation period.

Panesar *et al*. [26] optimized the fermentation parameters *viz*., temperature, pH and inoculum levels as 30°C, 6, 10% respectively for ethanol production from molasses medium. The foremost parameter, pH of the substrate was tested at various levels to know the optimum level for ethanol production. The maximum ethanol concentration of 79.1ml/litre from cane molasses

at operating variables of pH 4.5, temperature 32°C in 5 days operation was obtained. Marakis and Marakis [24] reported maximum alcohol concentration of 5.8% (v/v) at pH 4.5 from aqueous carob extract after 120 hours of fermentation. Ethanol production was maximum at pH 6 and it was 30% less in pH 7.7 [27].

CONCLUSIONS

From the study conducted, by varying the pretreatment method, pH, temperature, incubation time, inoculum size and substrate concentration, the above given results were concluded. The combination of acid treatment and steam explosion gave more reducing sugar when compared to other pretreatments. Out of different concentrations of acids, 0.2 N sulphuric acid at 121°C for 150 min gave better yield of reducing sugars (36.63 g/L), followed by acid treatment (31.11 g/L) and steam explosion (35.42 g/L). The optimum pH was observed to be 6.5, temperature was 30°C, inoculum size was 10% and substrate concentration was 5% (v/v) on 48 hours for maximum ethanol production (13.59 g/L) for *Zymomonas mobilis*. The optimum pH was observed to be 5.5, temperature was 30°C, inoculum size was 8% and substrate concentration was 3% (v/v) on 48 hours for maximum ethanol production (11.46 g/L) for *Saccharomyces cerevisiae*. From the results, it was revealed that the yeast and bacterial cultures differed significantly in their ability to ferment the acid – steam hydrolysate sunflower head waste. Among the cultures, *Zymomonas mobilis* recorded highest ethanol yield than the *Saccharomyces cerevisiae*.

REFERENCES

1. Ramanathan, M., 2000. Biochemical conversion - ethanol production from root crops. In: Biomass conversion Technologies for Agriculture and Allied Industries. Short course manual, organized by Department of Bioenergy, Tamil Nadu Agricultural University, Coimbatore, pp:157-162.
2. Grassi, G., 1999. Modern Bioenergy in the European Union, Renewable Energy, 16: 985-990.
3. Prasad, S., A. Singh and H.C. Joshi, 2007. Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resources Conservation and Recycling, 50(1): 1.
4. Shibata, M.G., R.S. Singhal and P.R. Kulkarni, 2006. Resistant starch: A review. Comprehensive Reviews in Food Science and Food Safety, 5: 1-17.
5. Georgieva, T.I. and B.K. Ahring, 2007. Evaluation of the continuous fermentation of dilute-acid corn stover hydrolysate using the thermophilic anaerobic bacterium Thermoanaerobacter BG1L1. Applied Microbiology and Biotechnology, 77: 61-68.
6. Koskinen, P.E.P., S.R. Beck, J.O. Orlygsson and J. Puhakka, 2008. Ethanol and hydrogen production by two thermophilic, anaerobic bacteria isolated from Icelandic geothermal areas. Biotechnology and Bioengineering, 101: 679-690.
7. Orlygsson, J. and S.R.B. Baldursson, 2007. Phylogenetic and physiological studies of four hydrogen-producing thermoanaerobes from the Icelandic and geothermal areas. Icelandic Agricultural Sciences, 20: 93-106.
8. Banat, I.M. Singh and D.R. Marchant, 1996. The use of thermotolerant fermentative Kluyveromyces marxianus IMB3 Yeast strain for ethanol production. Acta Biotechnology, 16: 215-223.
9. Banerjee, S., S. Mudliar, B. Giri, D. Satpute, T. Chakrabarti and R.A. Pandey, 2010. Commercializing lignocellulosic Bioethanol; Technology bottlenecks and possible remedies. Biofuels, Bioproducts and Biorefining, 4: 77-93.
10. Bohorquez, C. and S. Herrera, 2005. Determination de las mejoriescondicions de hihrolisis del banana verde rechazo, Tesis (Pregrado), Facultal de Minas, Universidal Nacional de Colombia, Sede Medellin, Medellin. Brettanomyces custersii. Journal of Microbiology and Biotechnology, 16: 135-1461.
11. Surmely, R., H. Alvarez, M.P. Cereda and O.F. Vilpoux., 2004. Hydrolysis of starch, in collections, in: Cereda, M.P; Vilpoux,O.F.(Eds), Latin American starchy tubers, Book 3: Technology, use and potentialities of Latin American starchy tubers. NGO Raizes and Cargill Foundation, Sao Paulo, Brazil, pp: 389-496.
12. Shigechi, H., J. Koh, Y. Fujita, T. Matsumoto and Y. Bito, 2004. Direct production of ethanol from raw starch via fermentation by use of a novel surface engineered yeast strain co-displaying glucoamylase and α –amylase. Applied Environmental Microbiology, 70: 5037-5040.
13. Rani, P., F.C. Garg, S.S. Sharma and L. Wati, 2009. Ethanol production from potato starch. Bioresource Technology, 63(4): 63-68.
14. Kobayashi, F., T. Sawada, Y. Nakamuraa, M. Ohnaga, M. Gogliving and T. Ushiyama, 1998. Saccharification and alcohol fermentation in starch of steam exploded potato. Applied Biochemistry Biotechnology, 69: 177-189.

15. Wyman, C.E., 1999. Biomass ethanol: technical progress, opportunities and commercial challenges, Annual Reviews in Energy and Environment, 24: 189-226.
16. Nikolic, S., L. Mojovic, D. Pejin, M. Rakin and V. Vucurovic, 2009. Bioethanol and fermentation. Food Technology Biotechnology, 47: 83-89.
17. Tasic, M.B., B.V. Konstantinovic, M.L. Lazic and V.B. Veljkovic, 2009. The acid hydrolysis of potato tuber mash in Bioethanol production, Biochemical Engineering Journals, pp: 43.
18. Bohorquez, C. and S. Herrera, 2005, Determination de las mejoriescondicions de hihrolisis del banana verde rechazo, Tesis (pregrado), Facultal de Minas, Universidal Nacional de Colombia, Sede Medellin, Medellin. Brettanomyces custersii. Journal of Microbiology and Biotechnology, 16: 135-1461.
19. Rikhvanov, E., N. Varakina, T. Rusaleva, E. Rachenko, V. Kiseleva and V. Voinikov, 2001. Heat shock- induced changes in the respiration of the Yeast Saccharomyces cerevisiae. Microbiology, 70: 462-465.
20. Araque, E., C. Parra, M. Rodriguez, J. Freer and J. Baeza, 2008. Selection of thermotolerant yeast strains Saccharomyces cerevisiae for bioethanol production. Enzyme Microbiology and Technology, 43: 120-123.
21. Tao, F., J.Y. Miao, G.Y. Shi and K.C. Zhang, 2005. Ethanol fermentation by an acid- tolerant Zymomonas mobilis under non-sterilized condition, Process Biochemistry, 40: 183-187.
22. Banerjee, S., S. Mudliar, B. Giri, D. Satpute, T. Chakrabarti and R.A. Pandey, 2010. Commercializing lignocellulosic Bioethanol; Technology bottlenecks and possible remedies. Biofuels, Bioproducts and Biorefining, 4: 77-93.
23. Verma, G., P. Nigam, D. Singh and K. Chaudhary, 2000. Bioconversion of starch to ethanol in a single step process by co-culture of amylolytic yeasts and Saccharomyces cerevisiae. Bioresource Technology, 72: 261-266.
24. Marakis, S.G. and G.S. Marakis, 1996. Fructose syrup and ethanol from deseeded carob pod. Journal of Food Science and Technology, 33: 108-111.
25. Nimbkar, N., A.R. Ghanekar and R.D. Joseph, 1989. Development of improved cultivars and management practices in sweet sorghum as a source of ethanol. In: Technology and Application for Alternative use of sorghum, National seminar held at Marathwada Agriculture University, Parbhani, February 2-3. pp: 180-188.
26. Panesar, P.S., S.S. Marwaha, S.S. Gill and R. Rai, 2001. Screening of Zymomonas mobilis strains for ethanol production from molasses. Indian Journal of Microbiology, 41: 187-189.
27. Sreenath, H.K. and T.W. Jeffries, 2000. Production of ethanol from wood hydrolysate by yeast. Bioresource Technology, 72: 253-260.