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UTILIZATION OF APPLE POMACE (CELLULOSIC BIOMASS) FOR THE PRODUCTION OF BIOETHANOL

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ABSTRACT

Different microbial strains were used individually as well as in consortia for ethanol production from apple pomace in solid state fermentation (SSF) systems. Saccharification of apple pomace was done, presence of glucose was determined and saccharified apple pomace was fermented under similar conditions of temperature and time for ethanol production in four flasks by using *Saccharomyces cerevisiae* individually and in combination with *Aspergillus foetidus* and *Fuzarium oxysporum*. After distillation, the average ethanol yield was in the range of 31.56 - 47.34g/l. Maximum ethanol yield and alcohol content (47.34g/l and 6% respectively) were obtained using poly microbial culture of *S. cerevisiae*, *A. foetidus* and *F. oxysporum* followed by co-culture of *S. cerevisiae* and *A. foetidus* (44.06 g/l and 5.6% respectively), co-culture of *S. cerevisiae* and *F. oxysporum* (37.75g/l and 4.8% respectively) and *S. cerevisiae* individually (31.56g/l and 4% respectively). The results show that combination of two or more strains of microorganisms gave high yield of ethanol than using a single strain. *S. cerevisiae* produced more ethanol when used in combination with *A. foetidus* and *F. oxysporum* as compared to when used individually. After distillation, the left over apple pomace was dried, weighed and maximum amount (80.31g) of dried apple pomace was obtained after SSF with *S. cerevisiae* individually and minimum (62.28g) with co-culture of *S. cerevisiae*, *A. foetidus* and *F. oxysporum*. In future by optimizing process parameters, the ethanol production can be increased. Fat, proteins, vitamins and mineral contents of dried apple pomace can be determined and can be used as animal feed.

Keywords: Bioethanol, Distillation, Saccharification, Pomace, Solid state fermentation

INTRODUCTION

Due to stricter environmental regulations and the increasing environmental sensitivity of the society, various waste disposal options are becoming more and more important, particularly where these can serve as raw material for new products. After processing apple into juice or juice concentrate, the left

over material is pomace which is discarded causing environmental pollution. It has light yellow colour when fresh with 70-90 % moisture content. This heterogeneous material contains mainly apple flesh, stem particles and seeds. Since, apple pomace is a part of the fruit; it has potential for

conversion into edible products. Being rich source of carbohydrate, pectin, crude fiber and minerals, it is a good source of nutrients. Efforts have been made in the past to utilize pomace in one or the other form but the problem of its utilization still persists [1].

Fruit industry generates a large quantity of waste. Alternative means of handling fruit waste focus more on utilization rather than disposal. The trend in the world today is to convert fruit waste into useful products through the manipulation of microbes and to recycle waste product as much as possible. Waste utilization is an ecologically safe and economically efficient method of waste management. Bioconversion i.e., conversion of biomass to ethanol by yeast or bacteria could be used for waste management. Biotechnological developments are increasingly improving the efficiencies of production processes. Therefore, biotechnology occupies a very important position in the socio-economic development of the country and the world at large [2].

In recent years, growing attention has been devoted to the conversion of biomass into fuel ethanol, considered the cleanest liquid fuel alternative to fossil fuels. Bioethanol produced by such method is completely composed of biological products which results in neutral ecological balance and combustion of this fuel results in completely clean emissions. The apple pomace has shown to be a good substrate in the conversion process from cellulosic biomass into fermentable sugars for the production of second generation biofuels such as cellulosic ethanol. This fuel can then be used to replace fossil fuel in the transportation sector. Cellulose based

materials can be broken down into individual sugars. These sugars can be broken down and fermented into ethanol [3].

Bioethanol is the fuel used as a petrol substitute for road and transport vehicles. Production and use of ethanol for fuel can decrease dependence on foreign oil, reduce trade deficits, create jobs in rural areas, reduce air pollution, global climate change and carbon dioxide buildup [4]. Many medicines, food products, flavorings and cosmetics could not be produced without it. Ethanol proves to be effective against most bacteria and fungi, and many viruses. It is used to process vaccines, compound tonics, syrups, tinctures, liniments and antiseptics as well as being vital in the manufacture of pharmaceuticals such as chloroform, arabrine and barbiturates. Ethanol can be used as an antiseptic to disinfect the skin before injections are given, often along with iodine. Ethanol-based soaps are becoming common in restaurants and are convenient because they do not require drying due to the volatility of the compound. Alcohol based gels have become common as hand sanitizers [5].

At present, only a small portion of apple pomace is used as an ingredient in livestock rations, and the remainder is discarded as solid waste. Disposal of this waste material presents a serious environmental problem. The most promising method for complete utilization of apple pomace may be through solid state fermentation with yeasts. So the present study was planned to convert apple pomace into valuable product bioethanol and animal feed using different microbial strains *Saccharomyces cerevisiae*, *Aspergillus*

foetidus and *Fuzarium oxysporum* individually as well as in consortia in solid state fermentation systems.

MATERIALS AND METHODS

Collection of Sample

In this study, apple pomace used as a substrate for bioethanol production was collected from fruit juice shop at Banur, Patiala, Punjab. Microbial strains such as *Saccharomyces cerevisiae* (MTCC 179), *Aspergillus foetidus* (MTCC 281) and *Fuzarium oxysporum* (MTCC 1755) used for fermentation were procured from Institute of Microbial Technology, Chandigarh, India.

Saccharification and Ethanol Production

Saccharification (Hyrolysis) is a process utilized for breakdown of cellulose, starch, fat molecule into small fermentable molecule such as amino acids, fatty acids and simple sugars. For the saccharification of cellulose present in apple pomace 20 ml dilute sulphuric acid was added to 100 grams of apple pomac and saccharification was monitored by dinitrosalicyclic acid (DNS) method. Ethanol production was carried out under solid state fermentation (SSF). SSF is the cultivation of microorganisms under controlled conditions in the absence of free water. Industrial enzymes, fuel and nutrient enriched animal feeds are the main products of SSF. For fermentation of saccharified apple pomace individual as well as consortia of microorganisms were used. SSF of saccharified apple pomace was carried out in four flasks using *S. cerevisiae* individually in first flask, co-culture of *S. cerevisiae* and *A. foetidus* in second flask, co-culture of *S. cerevisiae* and *F. oxysporum* in third flask

and consortia of *S. cerevisiae*, *A. foetidus* and *F. oxysporum* in fourth flask. 100 gm apple pomace was taken in each conical flask and fermentation was carried out at 30°C for six days.

Qualitative Estimation of Bioethanol

After 6 days of fermentation, bioethanol production was examined by Jones reagent [$K_2Cr_2O_7+H_2SO_4$] 1ml of $K_2Cr_2O_7$ (2%), 5ml H_2SO_4 and 3ml of sample was added after incubation. It was reported that ethanol oxidized to acetic acid with an excess of potassium dichromate in the presence of sulfuric acid, giving off a blue-green colour [6]. The presence of green colour indicates that the used carbon source was able to produce bioethanol after confirmation.

Quantitative Estimation of Bioethanol

Quantitative estimation of bioethanol was done by specific gravity method. Specific gravity refers to the density of any liquid [7]. 25 ml of fermented sample and 150 ml distilled water were mixed and this mixture was distilled on distillation unit. Distillation is the process of heating a solution to its boiling point, passing the vapours through a cooling device called condenser and collecting the liquid which condenses. Because the boiling point of alcohol is lower than that of water, almost all the alcohol will boil off first followed by the water. After distillation of sample specific gravity was taken and percentage of bioethanol was calculated using gravity method [8]. Apple pomace left after distillation was dried and weighed. This dried apple pomace can be used as animal feed.

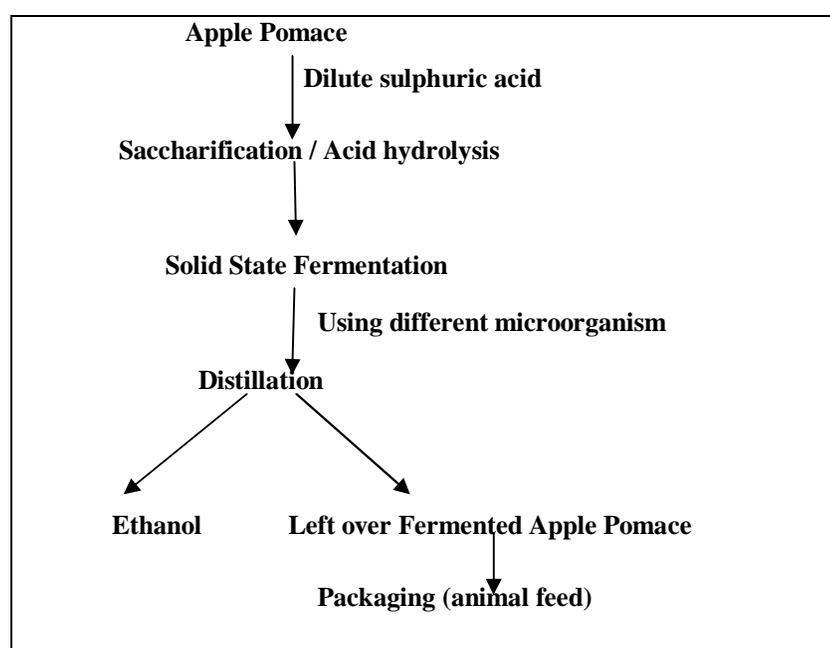


Figure 1: Ethanol Production from Apple Pomace after Distillation

RESULTS AND DISCUSSION

This study was planned to prepare bioethanol from apple pomace by using individual as well as combination of microorganisms such as *S. cerevisiae*, *A. foetidus* and *F. oxysporum*. Saccharification or hydrolysis was carried out to convert cellulose to glucose with dilute sulphuric acid and saccharification was determined by DNS method. A graph was plotted showing absorbance with respect to sample concentration which confirmed the presence of glucose. Absorbance was increased with increase in sample concentration indicating the presence of glucose in the sample. Straight line graph also confirmed the same (Figure 2).

After saccharification fermentation was carried out. Crude ethanol obtained after six days of fermentation contained high amount of impurities like acetic acid, acetaldehyde etc. formed during the process. For most industrial and fuel purposes, ethanol needs to be purified. For purification of bioethanol distillation was carried out for all the flasks. Distillation is the method to separate two liquids utilizing their different boiling points.

Alcohol Content and Ethanol Yield

Direct distillation method was used in the present study for purification of ethanol. Five fractions of distillates were collected via distillation for determination of alcohol contents for all the flasks. The density method ($d=m/v$) was used to for determination of alcohol content. In all the flasks maximum ethanol yield was calculated in distillate 3 (63.12 g/l, 78.30 g/l, 78.30 g/l and 94.68 g/l respectively), followed by distillate 2, distillate 1 and distillate 4. Last distillate (5th) indicated 0% alcohol content. Average alcohol content and ethanol yield were found maximum (6% and 47.34 g/l respectively) in fourth flask where consortia of *S. cerevisiae*, *A. foetidus* and *F. oxysporum* were used for fermentation followed by second flask, third flask and first flask (Table 1 & Figure 3).

Results shows that the maximum ethanol yield (47.34 g/l) was obtained when polymicrobial culture of *S. cerevisiae*, *A. foetidus* and *F. oxysporum* was used in SSF of apple pomace, followed by co-culture of *S. cerevisiae* and *A. foetidus* (44.06g/l), co-culture of *S. cerevisiae* and *F. oxysporum*

(37.75g/l) and *S. cerevisiae* (31.56 g/l) (Figure 3).

These results were correlated with the past findings where the researchers used different fungi for the production of ethanol. *S. cerevisiae*, *A. foetidus* and *F. oxysporum* were used individually as well as in consortia for ethanol production from apple pomace in solid state fermentation (SSF) systems and indicated that the alcoholic fermentation of apple pomace might be an efficient method for alleviating waste disposal [9].

The results of present study were found to be in accordance with results of previous studies

that ethanol yield was increased using co-culture of microorganisms in SSF of apple pomace as compared to using single microorganism. It was found in previous study that a combination of *Saccharomyces cerevisiae*, *Aspergillus foetidus* and *Fusarium oxysporum* has given highest percentage of ethanol (1.3702 gm%) followed by *Saccharomyces cerevisiae* (1.326 gm%) and that of combination of *Aspergillus foetidus* and *Fusarium oxysporum* (1.292%) [10].

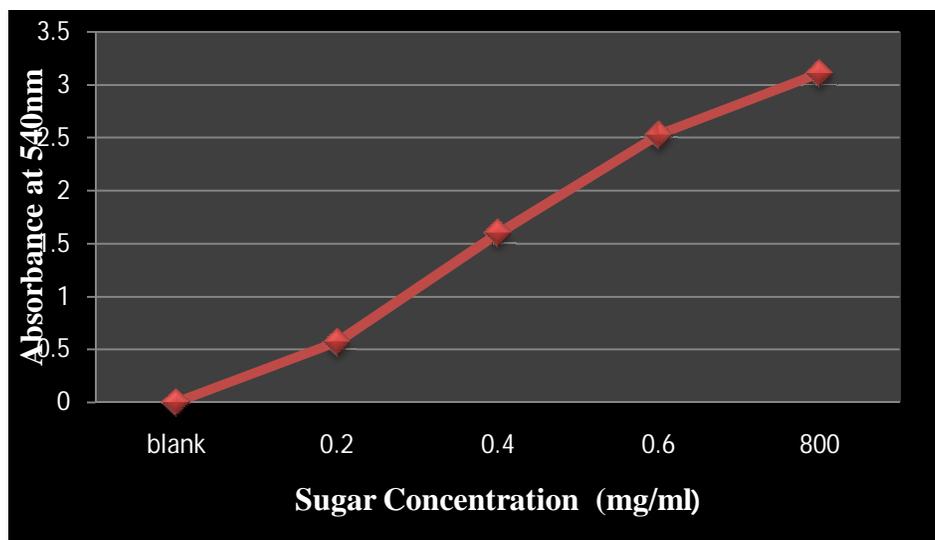


Figure 2: Saccharification of Apple Pomace

Table 1: Alcohol Content and Ethanol Yield From Fermented Apple Pomace

| Distillate (5ml) | First Flask | | Second Flask | | Third Flask | | Fourth Flask | |
|------------------|---------------------|------------------|---------------------|------------------|---------------------|------------------|---------------------|------------------|
| | Alcohol content (%) | Eth. Yield (g/l) |
| 1 | 4 | 31.56 | 6 | 47.34 | 4 | 31.56 | 6 | 47.34 |
| 2 | 6 | 47.34 | 8 | 63.12 | 8 | 63.10 | 8 | 63.10 |
| 3 | 8 | 63.12 | 10 | 78.30 | 10 | 78.30 | 12 | 94.68 |
| 4 | 2 | 15.78 | 4 | 31.56 | 2 | 15.78 | 4 | 31.56 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Average → | 4 | 31.56 | 5.6 | 44.06 | 4.8 | 37.75 | 6 | 47.34 |

** First Flask: Fermentation with *S. cerevisiae*, Second Flask: Fermentation with *S. cerevisiae* and *A. foetidus* Third Flask: Fermentation with *S. cerevisiae* and *F. oxysporum*, Fourth Flask: Fermentation with *S. cerevisiae*, *A. foetidus* and *F. oxysporum*

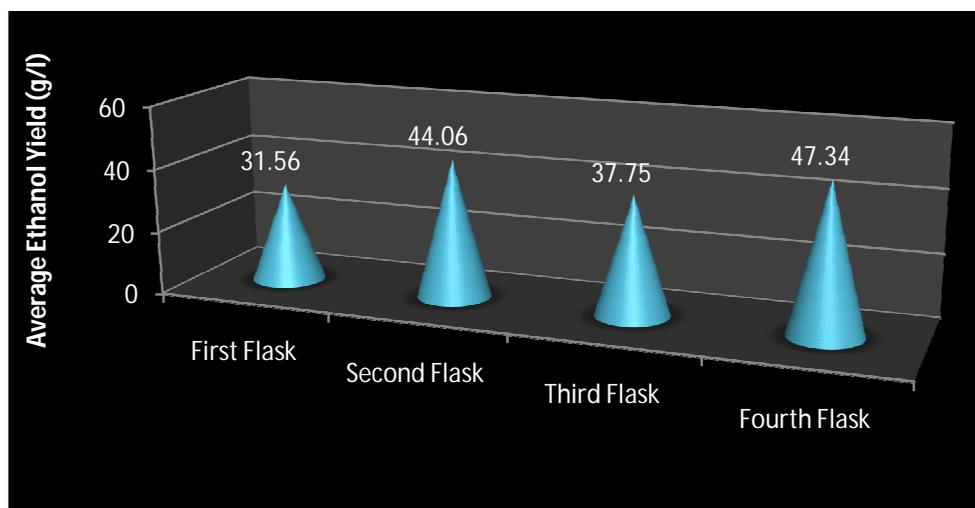


Figure 3: Average Ethanol Yield Calculated After Distillation

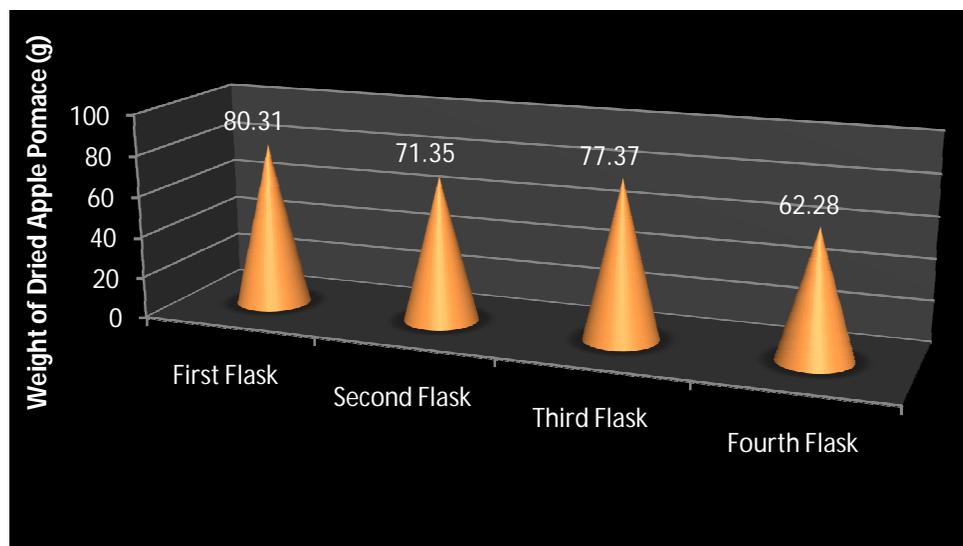


Figure 4: Weight of Dried Apple Pomace Obtained After Distillation

The results of the comparison test between single strain and mixed strain fermentation shows that the mixed strain fermentation was superior to single strain fermentation [11]. The co-culture of *S. cerevisiae* and *A. niger* increased the alcohol output in SSF of apple pomace for production of ethanol [12]. Weight of dried apple pomace left after distillation was found to be highest when only *S. cerevisiae* was used for SSF and this weight was minimum when co-culture of *S. cerevisiae*, *A. foetidus* and *F. oxysporum* was used for SSF (Figure 4). This dried apple pomace can be used as animal feed. The protein content of dried apple pomace after distillation increases by 3 times [13].

In some studies, solid state fermentation had performed for the effective production of ethanol [14], the same process was performed in the present study. Thus in future solid state fermentation could become a potential tool for solid waste management of food processing plant to prevent environment pollution as well. Utilizing apple pomace as a noble substrate for the production of ethanol has shown a promising potential [15]. Thus, apple pomace has a large economic potential for conversion into ethanol.

CONCLUSION

A solid state fermentation process has been reported for the production of ethanol from apple pomace using consortia of cultures viz., *S. cerevisiae*, *A. foetidus* and *F. oxysporum*. This process yielded as high as 47.34g/l bioethanol from apple pomace by solid state fermentation. It was also found that the combination of two and three fungi have given more percentage of ethanol than that of the fungi alone. Apple pomace proved to be the promising substrate for bioethanol production through solid state fermentation, which is an economical technique and does not require any sophisticated instrumentation. This study indicates that the alcoholic fermentation of apple pomace might be an efficient method for alleviating waste disposal with the concomitant production of bioethanol. Research on the conversion of fruit waste into ethanol will contribute substantially to bio-based economy. The method can create a renewable alternative feedstock for fossil fuel production and suggest a feasible solution to multiple environmental problems simultaneously creating a sink for waste utilization.

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