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A REVIEW ARTICLE ON DIFFERENT CULTURE AND DOWNSTREAM PROCESSES USED IN ETHANOL PRODUCTION FROM AGRICULTURE WASTE

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ABSTRACT

Fossil fuels like coal and crude oil is a vast energy source but these traditional sources of energy cannot continue to power humanity's growth into the future. Ethanol a fuel obtained from cheap agriculture waste is sustainable and Ethanol generally burns cleaner than petroleum fuel. This paper is a review about different substrates used for bio ethanol production and their yield obtained .The selection of the best culture for the ethanol production and different recovery techniques of it i.e. the down streaming processes are discussed here. Different areas in which ethanol can be used are discussed in brief.

Keywords: Molasses, barley, cassava, pervaporation, Ethanol, Extractive Distillation

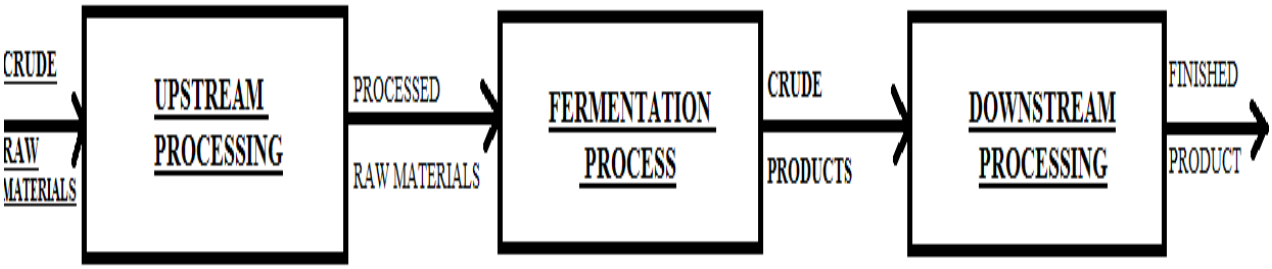
A. INTRODUCTION

Ethanol can be produced from different agricultural waste by fermentation.Ethanol fermentation, also called alcoholic fermentation, is a biological process which converts sugars such as glucose, fructose, and sucrose into cellular energy, producing ethanol and carbon dioxide as a side effect. In other words fermentation means a process in which microorganisms that are cultured on a large-scale under aerobic or anaerobic conditions, convert a substrate into a product which is useful to man. Because yeasts perform this conversion in the absence of oxygen, alcoholic fermentation is considered an anaerobic process.

Any industrial fermentation operation can be broken down into three main stages, viz, upstream processing, the fermentation process and downstream processing which is shown in fig: 1

Upstream processing includes formulation of the fermentation medium, sterilisation of air, fermentation medium and the fermenter, inoculum preparation and inoculation of the medium.

Fig 1:Schematic Diagram of Fermentation process



The fermentation medium should contain an energy source, a carbon source, a nitrogen source and micronutrients required for the growth of the microorganism along with water and oxygen, if necessary Sterilisation is essential for preventing the contamination with any undesired microorganisms. Air is sterilised by membrane filtration while the medium is usually heat sterilised. Any nutrient component which is heat labile is filter-sterilised and later added to the sterilised medium. The fermenter may be sterilised together with the medium or separately.

Inoculum build up is the preparation of the seed culture in amounts sufficient to be used in the large fermenter vessel. This involves growing the microorganisms obtained from the pure stock culture in several consecutive fermenters. This process cuts down the time required for the growth of microorganisms in the fermenter, thereby increasing the rate of productivity. Then the seed culture obtained through this process is used to inoculate the fermentation medium.

The fermentation process involves the propagation of the microorganism and production of the desired product. The fermentation process can be categorised depending on various parameters.

It can be either aerobic fermentation, carried out in the presence of oxygen or anaerobic fermentation, carried out in the absence of oxygen. Many industrial fermentation are carried out under aerobic conditions where a few processes such as ethanol production by yeast require strictly anaerobic environments.

Down streaming processes the components in the effluent from the fermentation unit are separated and purified in downstream processing .The feed to the downstream processing system is the fermentation broth.
This broth comprises an aqueous phase in which some solids are suspended.

B. DIFFERENT TYPE OF SUBSTRATES USED FOR BIO-ETHANOL PRODUCTION

Substrates are mainly classified into two they are :(I) sucrose-containing feedstocks (e.g. Sugar beet, sweet sorghum and sugar cane), (ii) starchy materials (e.g. wheat, corn, and barley), and (iii) lingo cellulosic biomass (e.g. wood, straw, and grasses).

- **Ethanol can be produced from different raw materials such as:**

Molasses of sugar cane**,** Bio mass, corn, wheat, Barley ,sugar from corn and wheat 's starch**,** Cellulosic feed stock, Corn fiber, sweet potato, potatoes**,** Cassava , Maize, rice, sweet sorghum**,** Bagasse, other cellulose biomass ,Mixture of wheat straw & meat**,** Bio ethanol feed stocks Different substrates(feed stock) for bio-ethanol production and their comparative production potential are shown in Table 1.

C. SELECTION OF THE BEST CULTURE FOR THE PRODUCTION OF ETHANOL

As studied by (Tahir et.al, 2010), the ethanol fermentation from sugarcane molasses by yeast strain was carried out by isolating ten yeast strain (Bio-01 to Bio-10) from soil and cultured in 15% molasses medium. *Saccharomyces cerevisiae*Bio-07 gave maximum productivity (52.0g/L).Fermentation conditions were optimized for maximum production of ethanol. Maximum yield of ethanol (76.8 g/L) was obtained with 15% molasses concentration, 3% inoculum size, pH4.5 and temperature 30 ° C. Potassium

TABLE 1: (Ethanol yield obtained with different type of substrates used) (Ref [6], Kumar et.al, 2006)

SR. NO.	RAW MATERIAL (AGRICULTURAL WASTE)	YIELD
1	Corn grain	124.4 (gallons/ton of dry stock)
2	Corn stower	133.0(gallons/ton of dry stock)
3	Rice straw	109.9(gallons/ton of dry stock)
4	Cotton grain starch	56.8(gallons/ton of dry stock)
5	Forrest thinning	81.5(gallons/ton of dry stock)
6	Hard wood saw dust	100.8(gallons/ton of dry stock)
7	Bagasse	45.5(gallons/ton of dry stock)
8	Mixed paper	116.2(gallons/ton of dry stock)
9	Switch grass	96.7(gallons/ton of dry stock)
10	Sugar cane	70 lt/ton
11	Sugar beet	110 lt/ton
12	Sweet potato	125 lt/ton
13	Potato	110 lt/ton
14	Cassava	150 lt/ton 110 lt/ton
15	Maize	360 lt/ton

16	Sweet sorghum	80 lt/ton 340lt/ton
17	Rice	430 lt/ton
18	Barley	250 lt/ton
19	Wheat	390 lt/ton 250 lt/ton
20	Corn	410 lt/ton
21	Sweet bagasse & cellulose bio-mass	280/ton

Ferrocyanide (150ppm) was used to control the trace metals present in the molasses medium. *Saccharomyces cerevisiae*is the cheapest strain used for bio-ethanol production from sugar molasses. As per the studies *S.cerevisiae*is capable of very rapid rates of ethanol production under optimal conditions. (Dombek and Ingram, 1986)

Tahir et.al, 2010 in their work optimized some factors affecting the ethanol productivity of yeast in molasses such as sugar concentration, inoculum size, pH, temperature and time of fermentation. The cultures of *Saccharomyces cerevisiae*were isolated from soil by pour plate method. Dry powdered yeast was also used. The samples were streaked on nutrient agar medium and incubated at 30ºC for 24h. All cultures were stored in refrigerator at 4 ºC.

TABLE 2: Screening of *Saccharomyces cerevisiae*strains for ethanol production in sugarcane molasses medium. (Tahir et.al, year 2010)

SERIAL NO.	ISOLATED YEAST STRAINS	ETHANOL PRODUCTION (G/L) MEAN ± S.E
01	Bio-01	49.1± 0.01
02	Bio-02	51.5± 0.03
03	Bio-03	49.1± 0.04
04	Bio-04	51.9± 0.01
05	Bio-05	51.3± 0.02
06	Bio-06	49.7± 0.01
07	Bio-07	52.0± 0.03

08	Bio-08	50.5± 0.05
09	Bio-09	49.5± 0.04
10	Bio-10	49.7± 0.01

D. FERMENTATION TECHNIQUE USED IN TABLE 2:

Batch fermentation was carried out in 250ml conical flasks. Sugarcane molasses was obtained from Pattokisugar mill. Sugar concentration in sugarcane molasses was 40% (w/v). Sulphuric acid was added to adjust the pH. Inoculum was prepared by inoculating cells from 24h old slant culture into yeast extract agarmedium and incubated at 30 ºC for 24 hr. inoculum (2%) was added into molasses for ethanol production.(Tahir et.al, year 2010).

E. ANALYTICAL METHODS THAT ARE USED FOR ESTIMATION OF IMPORTANT PARAMETERS:

- **Sugar estimation:**
  Sugar concentration in sugar cane molasses was estimated using 3, 5-dinitrosalicylic acid and glucose as standard (Miller, 1959).
- **Ethanol estimation:**
  The levels of ethanol was measured by gas chromatography (GCMS QP 2010, Germany) with a flame ionization detector.

F. IMPORTANT RESULTS DERIVED IN REFERENCE TO BIOETHANOL PRODUCTION:

- **Isolation and screening of *Saccharomyces cerevisiae***

Ten cultures of *Saccharomyces cerevisiae*were isolated from soil and screened for the production ofethanol. The locally isolated *Saccharomyces cerevisiae*Bio-07 gave better (52.0g/L) ethanol production after 48h of inoculation. *Saccharomyces cerevisiae*Bio-07 was selected for further studies. (Tahir et.al, year 2010).

- **Effect of sugar concentration**

They varied Molasses concentration (5, 10, 15, and 20 %) to study their effect on ethanol fermentation by *Saccharomyces cerevisiae*. They observed that ethanol production was maximum (61.5 g/L) after 48h of inoculation when sugar concentration was 15 %, they observed that with increase in sugar concentration ethanol production decreased. Hexose sugar is the primary reactant in yeast metabolism. Under fermentative condition, the rate of ethanol production is related to the available sugar concentration (Park and Sato, 1982; Atiyeh and Duvnjak, 2001). At very low substrate concentration, the yeast starved and productivity decreases (Levenspiel, 1980). An important secondary effect of higher sugar content is catabolite repression of the oxidative pathways. (Tahir et.al, year 2010)

- **Effect of inoculum size**

The size of inoculum in ethanol fermentation is of great importance in completing the fermentation process. Different sizes of inoculum 1-5 %(v/v) were used to inoculate the production flasks. The amount of ethanol produced gradually increased with the increase in the inoculum size. However, it was found that maximum ethanol production (65.0g/L) was achieved at 3.0% (v/v) inoculum. Further increase in inoculums size did not result in the considerable enhancement of ethanol production. [(Bajaj *et al*., 2001; Nowak, 2001; Kordowska-Wiater*et al*., 2001 and Alegre*et al*., 2003)

- **Effect of initial pH value on ethanol fermentation**

Initial pH of the fermentation media was maintained in the range of 2.5 - 6.0. The maximum ethanolproduction (65g/L) was achieved at pH 4.5. With a further increase in pH decrease in ethanol production was observed (Mollison, 1993 and Maltby, 1953.)Control of pH during ethanolfermentation is important for two reasons:

For the growth of harmful bacteria is retarded by acidic solution.

Yeast grows well in acidic conditions (Mathewson, 1980). With increase in pH yeast produces acidrather than alcohol. Molasses has naturally alkaline pH and must be acidified prior to fermentation.

- **Effect of temperature on ethanol fermentation**

It was found that temperature has profound effect on ethanol fermentation. Ethanol production was optimum at 30°C and ethanol production decrease to 0.50g/L at 40°C. Temperature between 30-35°C has been usually employed for culturing of yeast and temperature above 30°C has been found inhibitory to ethanol fermentation due to yeast growth inhibition at higher temperatures. (Tahir et.al, 2010)

- **Time Course of ethanol fermentation**

When fermentation was carried at different time period 24, 48, 72 and 96h under optimum conditions. They observed after 72h (76.78 g/L) after inoculation maximum ethanol production. Initially, the rate of alcohol production was quite low, but as the number of yeast cells increases the overall production rate increased. The effect of reduced sugar concentration and ethanol inhibition became important after optimum fermentation time. The fermentation continued at a decreasing rate until 94%of the sugar was utilized. Fermentation time also varied with yeast strains and substrates being used as source of sugar. (Tahir et.al, 2010)

Thus from their studies it has been observed that *Saccharomyces cerevisiae* Bio- 07 has great potential for the production of ethanol from sugarcane molasses. The results indicated that the optimization of cultural conditions, such as sugar concentration, inoculum size, pH, temperature and time of fermentation.

G. DOWNSTREAM PROCESSING

Downstream processing includes the recovery of the products in a pure state and the effluent treatment. Product recovery is carried out through a series of operations including cell separation by settling, centrifugation or filtration; product recovery by disruption of cells (if the product is produced intracellular); extraction and purification of the product. Finally, the effluents are treated

by chemical, physical or biological methods. Downstream process groups: Distillation, Rectification, Ethanol dewatering (molecular sieve, membranes, or entrainer distillation), Decantation, Evaporation, Drying.

Fermentation Products: Commercially important products of fermentation can be described in five major groups as follows: Biomass (Baker's yeast, SCP, Starter cultures, animal feed, etc.), Primary metabolites (amino acids, organic acids, vitamins, polysaccharides, ethanol, etc.) and secondary metabolites (antibiotics, etc.),Bioconversion or biotransformation products (steroid biotransformation, L-sorbitol etc.), Enzymes (amylase, lipase, cellulase, etc.), Recombinant products (some vaccines, hormones such as insulin and growth hormones etc.)(Ref: bio technology forums,MalithiWeerakkody)

The separation of ethanol from water is difficult because of the existence of an azeotrope in the mixture. The two traditional methods of high purity ethanol separation are: Extractive distillation, Azeotropic distillation

Other three emerging techniques are: Salt distillation, Pressure swing distillation, Pervaporation

- **Extractive Distillation**

In extractive distillation, salt is added as the third component and pervaporation is the direct separation, without salt addition. Extractive distillation was considered as a separation technique for ethanol-water system using a dissolved salt as a separating agent. The salt used was calcium chloride which has a high boiling point. Extractive distillation is the distillation in the presence of a component/solvent which is not volatile when compared to the separated components. The solvent is charged continuously near the top of the distilling column so that the concentration is maintained on all plates of the column (Hilmen, 2000). The main characteristic of extractive distillation is that the solvent with a high boiling point is charged to the components to be separated so as to increase the relative volatility of one component.

Ethanol forms minimum-boiling azeotropes with water at about 90 mol% ethanol. Extractive distillation is a technique used for the separation of minimum boiling binary 4 azeotropes by the use of a solvent/salt that has the heaviest species in the mixture and does not form any azeotropes with the original components. The solvent should be completely miscible with the original components. A small concentration of salt is capable of increasing the relative volatility of the more volatile component of the solvent mixture to be distilled (Mario and Jamie, 2003)

- **Pervaporation membrane**

Pervaporation is a membrane separation process for liquid mixtures in which the initial solution comes in contact with the internal surface of a membrane module, permeate in the form of vapours with a low partial pressure was removed from its outer surface (Belyaev *et al.*, 2003). Pervaporation membrane separation can be considered as one effective method and energy-saving

process for the separation of the ethanol/water azeotropic system (Huang *et al.*, 2008; Huang, 1991; Shao and Huang, 2006 and Wang *et al.*, 2007

- **Salting out method**

Water and ethanol are miscible due to their existing intermolecular forces, the strongest of those being hydrogen bonding. As an electrolyte (in this case K2CO3) is added to water, the solvation of the electrolyte makes water unavailable to hydrogen bond with ethanol. The solubility of ethanol decreases as a result of the lack of hydrogen bonding interactions with water. As a result, the organic dye which was initially yellow due to the acidic solution is absorbed into the organic phase with ethanol and becomes blue. (Shakhashiri, B. Z.)

H. APPLICATION OF BIO-ETHANOL

Ethanol can be used as a transport fuel to replace gasoline the most cost effective aid is the blending of ethanol with a small proportion of a volatile fuel such as gasoline. Thus, various mixture of bio ethanol with gasoline or diesel fuels have been used , as a fuel for power generation by thermal combustion, as a fuel for fuel cells by thermo chemical reaction, as a fuel in cogeneration systems and as a feed stock in the chemical industry.

- **Environmental impacts of ethanol versus gasoline**.

Ethanol generally burns cleaner than petroleum fuel because it is made up of organic compounds which are less complex chemically than gasoline and diesel feed, using ethanol blended fuel can reduce co_2 emissions

In comparison to other alternative fuels, ethanol may not provide the largest reduction in emissions or the best mileage. But it is renewable, produced domestically, and provides economic benefits to farmers. Although there are many questions still unanswered regarding ethanol's future, studies like the one undertaken here will hopefully guide us in a constructive direction.

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