International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor (2018): 7.426

Involvement of Microorganism in Bioethanol Production: A Review

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Abstract: ***Ethanol production from biomass is an economically feasible process that requires microorganisms which produces ethanol with a high yield from all sugars. Different fermentation organisms among bacteria, yeasts, and fungi (natural as well as recombinant) have been reviewed with emphasis on their performance in fermentation of biomass. Depending on the type of biomass, the process of pre-treatment, fermentation and selection of microorganisms have shown to differ.***

Keywords: Bioethanol, Submerged fermentation, Feed batch fermentation, Solid state fermentation, Alcoholic fermentation

1. Introduction

The microorganism producing bioethanol through fermentation should possess the following characteristics: (a) fermentation of carbohydrate, (b) characteristics of flocculation and sedimentation, (c) genetic stability, (d) osmotolerance (i.e. capacity to ferment concentrated carbohydrate solution), (e) ethanol tolerance and the capacity to generate highly concentrated bio alcohol, (f) high call activity to repeated recycling and (g) temperature tolerance.

The microorganisms (yeast/bacteria) are used to produce significant amount of bioethanol as listed in Tables 1 and 2.

Table 1: Ethanol producing yeast and their substrate

Microorganism	Substrate
Saccharomyces species	
S. cerevisiae & S. uvarum (Carlsbergenesis)	Glucose, fructose, galactose, Sucrose, maltose, maltotriose and xylulose
S. diastaticus	Glucose, maltose, dextrin and starch (glucoamylase)
S. rouxii	Glucose, fructose, maltose and sucrose (osmophilic)
Kluyveromyces fragilis and lactis	Glucose, galactose and lactose
Candida species	
C. pseudotropicalis	Glucose. galactose and lactose
C. tropicalis	Glucose, xylose and xylulose
Pachysolen tannophilus	Glucose and xylose
Schwanniommyces species	
S. alluvius	Dextrin and starch (glucoamylase and α-amylase)
Castellii	Dextrin and starch (glucoamylase and α-amylase)
Endomycopsis fibuligera	Dextrin and starch (glucoamylase and α-amylase)

Table 2: Ethanol producing bacteria and their substrate

Microorganism	Substrate
Zymomonas mobilis	Glucose, fructose, and sucrose
Clostridium Species	
C. thermocellum	Glucose, cellobiose, and cellulose (thermophilic
C. thermohydrosulfuricum	Glucose, xylose, cellobiose, sucrose, and starch (thermophilic)
Thermobacterioides brockii	Glucose, sucrose, cellobiose, and starch (thermophilic)
Thermobacterioides acetoethylicus	Glucose, sucrose and cellobiose (thermophilic)

The production of bioethanol through fermentation involves the following three phases (Figure 1).

- Phase-I: biochemical phase or pretreatment phase.
- Phase II: fermentation phase, i.e. the production of bioethanol occurs from fermentation of glucose or another fermentable substrate.
- Phase III: post fermentation phase that involves distillation, dilution, etc.

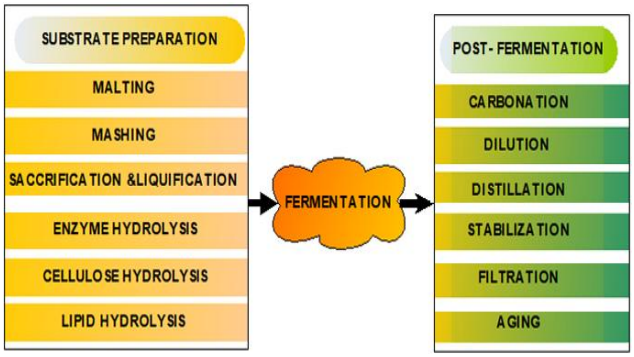

Figure 1: Steps involved in production of bioethanol through fermentation process

There are a number of well explained metabolic pathways to elucidate the production of bioethanol but the selection of pathway depends on the selection of microorganisms.

Vitality change by living cells is a crucial property. Living cells create valuable vitality ATP, which is viewed as the cell's vitality cash. Yeast has the property of keeping up a load of ATP, which is conceivable because of the utilization of sugars like glucose and fructose. Sucrose is the principle segment of sugarcane juice and every particle of sucrose comprises of one glucose atom appended to one atom of fructose [1-5].

The initial step of yeast's action is to break and separate the glucose and fructose unit which enters the vitality

metabolization apparatus to give vitality. In the event that yeast develops in oxygenated medium, the sugar will be separated well ordered into littler and littler atoms toward the end carbon dioxide is freed. If there is little or no oxygen accessible to the yeast, then the arrangement of compound separate procedures cannot be finished and the sugar is separated into ethanol, a fuel.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad ,\Delta G^0 = 56 \text{ Kcal}$$

Glucose is broken down into two particles of pyruvic acid by means of the responses of glycolysis. Alcoholic maturation and vigorous corruption take after a similar response succession up to this point [6-16]. While aging, pyruvic acid is debased enzymatically to ethanol and carbon dioxide.

For the yeast like saccharomyces and other yeasts, the formation of bioethanol can be explained with help of Embden -Meyerhof-Parnas pathway (Figure-2 and 3).

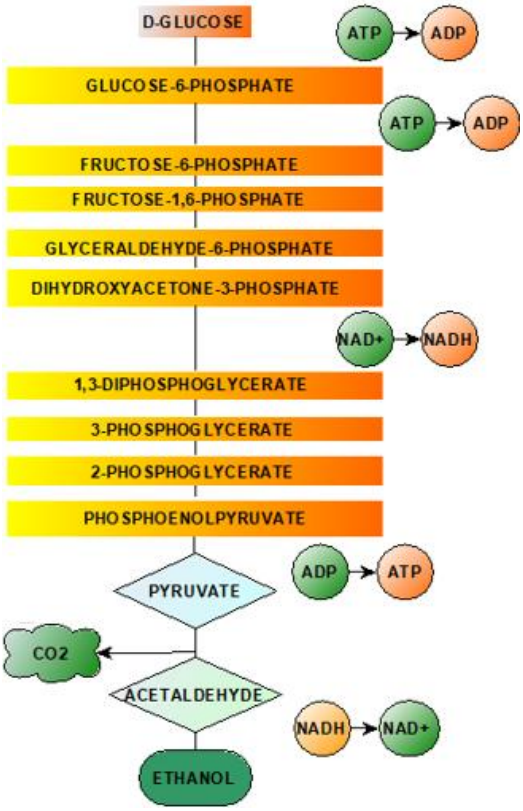

Figure 2: Formation of ethanol from glucose through Embden-Meyerhof-Parnas pathway

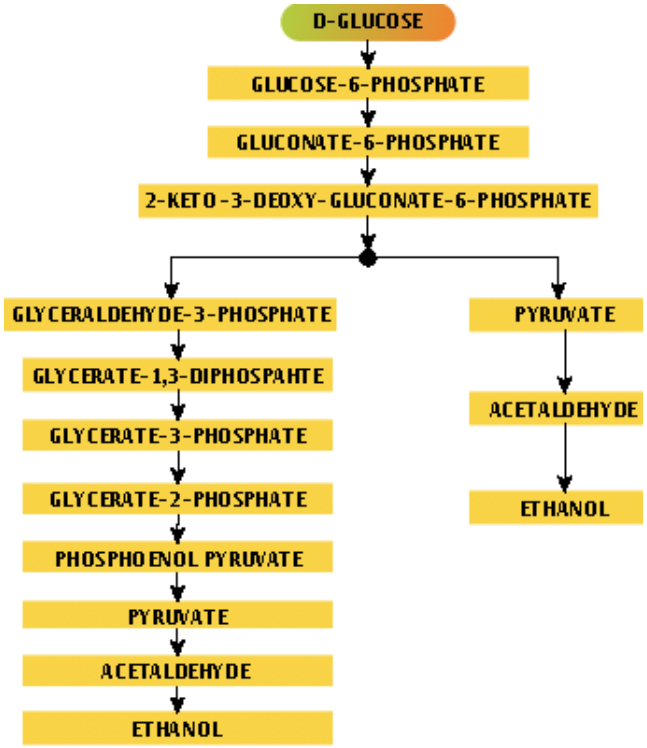

Figure 3: Formation of ethanol from glucose by inducible Entner-Doudoroff pathways

Life forms, for example, yeast, which is involved in alcoholic fermentation, contains the protein pyruvate decarboxylase (pyruvate decarboxylase-2-oxoacid carboxylase) that catalyses the decarboxylation of pyruvate to acetaldehyde by an irreversible response. The protein has been discovered in plant tissues till now. In the last reaction of alcoholic maturation, acetaldehyde is converted to ethanol by NADH within the sight of alcohol dehydrogenase. The chemical is generally conveyed and found in the liver, retina and serum of the organisms, in seeds and leaves of higher plants and numerous microorganisms including yeasts. Clearly the catalyst is not confined to tissues which deliver a lot of ethanol [17-24].

One of the vital processes to acquire ethanol from cellulose and hemicellulose is the enzymatic hydrolysis or the chemical hydrolysis of polysaccharides into disaccharides and monosaccharides for further fermentation. However, the recalcitrance of this lignocellulosic material requires pretreatment to facilitate enzymatic reaction [25, 26]. Several approaches of plant biomass pretreatment have been studied, for e.g., milling [27], acid treatment [28, 29], irradiation [30], hydrothermal treatment [31, 32], hydrothermal alkaline [33], pyrolysis [34], steam explosion [35, 36], catalyzed steam explosion [37-39], carbon dioxide [40], chlorine dioxide, nitrogen and sulfuric acid [41], organo solvation [42], microwave and alkaline therapies [43] and organic remedies [28, 44-48]. The lists different substrates, microorganisms, pretreatments and fermentation processes for ethanol production discussed by various researchers are given in Table 3.

Table 3: Production of cellulosic ethanol from different pre-treatments, microorganisms and bioprocesses

Microorganisms	Substrate	Pre-treatments	Bioprocess	Alcohol yield(g/g)	Ref
Escherichia coli	Wheat straw	Acid digestion, over liming	Batch/SHF	0.21	[47]
Escherichia coli	Wheat straw	Acid digestion	Batch/SHF	0.24	[47]
Escherichia coli	Wheat straw	Acid digestion, over liming	Batch/SSF	0.21	[47]
Escherichia coli	Wheat straw	Acid digestion	Batch/SSF	0.17	[47]
Escherichia coli	Rice husks	Alkaline digestion	Batch/SHF	0.21	[48]
Escherichia coli	Rice husks	Alkaline digestion	Batch/SSF	0.20	[48]
Kluyveromyces marxianus	Wheat straw	Steam explosion	Batch/SSF	0.32	[49]
Kluyveromyces marxianus	Wheat straw	Steam explosion	Batch/SSF	0.27	[50]
Kluyveromyces marxianus	Waved old paper	Acid digestion	Batch/SSF	0.31	[51]
Mucor indicus	Rice straw	Acid digestion, steam	Batch/SHF	0.43	[30]
Pachysolen tannophilus	Sugarcane bagasse	Acid digestion, steam, electrodialysis	Batch	0.53	[52]
Pichia stipitis	Wild sugarcane bagasse	Acid digestion, steam	Batch/SSF	0.35	[53]
Pichia stipitis	Sugarcane bagasse	Acid digestion, steam explosion	Batch/SSF	0.39	[54]
Pichia stipitis	Sugarcane bagasse	Milled	Batch/SSF	0.29	[55]
Pichia stipitis	Sugarcane bagasse	Milled	Batch/SHF	0.27	[55]
Pichia stipitis	Corn cobs	Acid digestion, steam	Batch/ SHF	0.44	[56]
Pichia stipitis	Algarroba	Acid digestion, steam, delignification	Batch/SHF	0.39	[57]
Rhizopus oryzae	Rice straw	Acid digestion, steam	Batch/SHF	0.41	[58]
Saccharomyces cerevisiae	Cotton stalks	None	Batch/SSB/SHF	0.004	[59]
Saccharomyces cerevisiae	Cotton stalks	None	Batch/SHF	0.027	[59]
Saccharomyces cerevisiae	Cotton husks and straw	Alkaline digestion	Batch/SSF	0.48	[60]
Saccharomyces cerevisiae	Canola straw	Acid digestion, hydrothermal	Batch/SHF	0.21	[61]
Saccharomyces cerevisiae	Wheat straw	Acid digestion, steam explosion	Batch/SSF	0.13	[62]
Saccharomyces cerevisiae	Wheat straw	Acid digestion, steam	Batch/SSCF	0.35	[63]
Saccharomyces cerevisiae	Sugarcane bagasse	Acid digestion, steam explosion	Batch/SSF	0.44	[54]
Saccharomyces cerevisiae	Sugarcane bagasse	Acid digestion, steam explosion, delignification	Batch/SSF	0.32	[64]
Saccharomyces cerevisiae	Sugarcane bagasse	Acid digestion, steam explosion	Batch/SSF	0.29	[64]
Saccharomyces cerevisiae	Sugarcane bagasse	Acid digestion, steam explosion	Batch/SHF	0.30	[80]
Saccharomyces cerevisiae	Sugarcane bagasse	Acid digestion, steam explosion, delignification	Batch/SHF	0.35	[80]
Saccharomyces cerevisiae	Corn cobs	Acid digestion, steam	Batch/SSCF	0.39	[65]
Saccharomyces cerevisiae	Algarroba	Acid digestion, steam, delignification	Batch/SHF	0.49	[66]
Saccharomyces cerevisiae	Rice straw	Acid digestion, steam	Batch/SHF	0.45	[67]
Saccharomyces cerevisiae	Rice straw	Acid digestion, steam	Batch/SSF	0.09	[68]
Saccharomyces cerevisiae	Spruce	Acid digestion, steam explosion	Batch/SSF	0.44	[69]
Saccharomyces cerevisiae	Spruce	Acid digestion, steam explosion	Feed batch/SSF	0.43	[69]
Saccharomyces cerevisiae	Waved old paper	Acid digestion	Batch/SSF	0.32	[70]
A. ellipticus, A. fumigatus, S. cerevisiae NCIM 3570	Banana pseudo stem	Enzymatic hydrolysis	SSF	0.171	[71]
S. diastaticus	Liquefied cassava starch	Enzymatic hydrolysis	Monoculture or mixed culture fermentation with yeasts	0.34	[72]
Trichoderma reesei,	Sugar cane leaves	Alkaline digestion.	SSF	0.35	[73]
S. cerevisiae NRRL-Y-132, Kluyveromyces fragilis NCIM 3358,.	Alfalfa fibers	hot water treatment,	SSF/SHF	0.64	[74]
Candida shehatae FPL-702	Alfalfa fibers	Without LHW	SSF/SHF	0.96-1.8	[74]
Clostridium thermocellum &thermo saccharolyticum	Banana leaves	Alkaline digestion/acid digestion/hot water digestion	Co-culture fermentation	2.2	[75]
Saccharomyces cerevisiae	Apple waste	Enzymatic hydrolysis.	SSF/SmF	0.615	[76]
Saccharomyces cerevisiae	Grapes waste	Acidic hydrolysis/enzymatic hydrolysis	SSF/SmF	0.804	[77]
Saccharomyces cerevisiae	Papaya waste	Hot water treatment	SSF/SmF	0.818	[78]
Saccharomyces cerevisiae	Water melon (Citrullus canatus)	Acidic hydrolysis	SmF	1.010	[80]
Saccharomyces cerevisiae	Mosambi (Citrus cimetta	Acidic hydrolysis	SmF	0.623	[80]

The thermophilic pentose-aging anaerobe, *Clostridium thermo saccharolyticum*, is developed in mix with *C. thermocellum*. This blend culture has appeared to age both solkafloc and cornstover to ethanol, and furthermore,

deliver expansive amounts of acidic corrosive agents like lactic acid [49, 50, 52-54, 56-58].

Petite yeasts yield up to twice as much alcohol as their typical relatives. An ordinary yeast strain (IZ-1904) produced 41% of alcohol, and the petite vemon of the yeast Z. mobilis was utilized for agave juice fermenter in Central America. It ferments sugar all the more proficiently to alcohol [59-65].

During World War II, mixing of methanol and ethanol in different amount with petrol was carried out in Europe. Thereafter, blending of alcohol with petrol was used as transport fuel, which started after the oil crisis in 1973. The use of ethanol in combination with petrol was made compulsory in Brazil by 1931. This country has been the second highest sugar producer in the world; therefore, large quantity of alcohol is produced from sugarcane and cassava. Amid World War II, blending of methanol and ethanol in various sum with petroleum was completed in Europe. In 1985, Brazil propelled a program of mixing 20% ethanol (produced from sugarcane and cassava) with oil and in this way spared around 40% of its petroleum utilization. In 1990, it delivered around 20.5 billion litres of alcohol from molasses and spared 11 billion dollars of remote trade. This nation promoted around one million autos running on ethanol alone (as extra heat exchanger is required to ignite the engine of an auto, which is higher for alcohol as compared to gas). In 1986, Brazil offered work to 3.5 million individuals from 14 billion litres of sloshed fuel [66-70].

In 1980, U.S.A popularized the 'gasohol' (20% liquor added to petroleum). This work supported liquor generation in the nation even from grains. Additionally, the option of methanol (a wood liquor) in oil has just gotten in the US with the creation of 10,000 alternative fuel vehicles. Methanol fired autos would soon hit the American market. India is sufficiently blessed for having numerous sources of biomaterials to be utilized as a part of ethanol preparations. The legislature is confronting an emergency on account of molasses. Consistently, potatoes have spoiled for absence of purchasers. Cassava is developed on extensive scale in Kerala and a few sections of Tamil Nadu. Countless are in activity and numerous more are to be set up. The usage of sugary and bland materials for the generation of ethanol would be a decent advance to chop down the oil cost and take care of the fuel demand in nation [71-75].

As of late Lubrizol India Limited, Bombay (a joint effort between the govt. of India Corporation, USA) has begun to fabricate the uncommon execution of synthetics and substance added to give the qualities required in oil-based goods, especially in motor oils, equip oils, car transmission liquids, and other mechanical and marine greases. This organization has chosen to guide its task to accomplish two goals: to create concoction added substances to decrease utilization of powers and greases, and to make the modern items ecologically protected [76-79, 81-86].

2. Methods of Culturing

For the most part, there are a few techniques for refined microorganisms on substantial scale. These are: (I) surface culture techniques, (ii) submerged culture strategy and (iii) semisolid or strong state culture strategy.

Surface Culture Method

In this technique, the microbes are allowed to develop on liquid medium's surface with no agitation. Then it is followed by culture filtrate separation for the product recovery. For e.g., alcohol, beer and citric acid production is done via this method. This implemented method is time consuming that also needs more area [87-89].

Submerged Culture Process

In this procedure, the life forms are developed in a fluid medium which has air circulation through it and is unsettled in vast tanks called fermenter, which could be either an open tank or a shut tank that are by and large made of non-destructive sort of metal or glass lined or of wood. In group maturation, the life form is developed in a known measure of culture medium for a characterized timeframe and after that the cell mass is isolated from the fluid before additional handling. In this, the microbes grow in a liquid medium with aeration. Fermentor are used for this process, which are basically large tanks (open or closed types). In case of batch fermentation, the culture is prepared in a known or fixed amount of medium within specified time frame. This is followed by cell mass separation. In case of continuous culture, the medium of culturing is withdrawn with addition of fresh culture medium. Most aging businesses today utilize the submerged procedure for the creation of microbial items [90-93].

Semisolid or Solid Method

In this, the medium is impregnated, for example, bagasse, wheat grain, potato mash, and so forth, and the life form is permitted to develop on this. This technique permits more noteworthy surface zone for development. The creation of the attractive substance and the recuperation is for the mostly less demanding and agreeable.

In the course of fermentation, medium's composition plays an important role in determining the end product characteristics. For instance, a sucrose containing culture medium boosts better production of alcohol by *S. cerevisiae* than any other sugars. However, in addition to this, there are few fermentation governing factors that needs to be optimized, such as the pH, incubation temperature, air circulation and so on. There is raised concern over use of cheap raw materials that would lower the production cost of the whole process and the end product as well [94-100].

Alcoholic Fermentation

In yeast and other microorganisms, the reactions of glycolysis up to pyruvate formation are identical to those described for anaerobic glycolysis and the difference occurs only in its terminal steps. In contrast to animals, which utilize lactate dehydrogenase reaction for the oxidation of NADH to generate NAD^+, the yeast cells utilize two enzymatic reactions for the purpose, as lactate dehydrogenase is not found in them [101-116].

3. Acknowledgement

I would like to thank www.researchedit.org for editing and language improvement of my manuscript.

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