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Effect of Inhibitors on Ethanol Production: A Mini Review

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Mini Review Article

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ABSTRACT

The interest in biofuels is caused by the drawbacks of using fossil fuels: high oil prices, their role in global warming, and their non-renewable nature as an energy source. Production of fuel ethanol from biomass is an interesting alternative to traditional fossil fuel. In the process of pretreating and hydrolyzing the materials to get sugars, along with sugars for fermentation some toxic compounds are formed which affect microorganisms during fermentation. To overcome these effects, encapsulation of yeast is one possible method.

Keywords: Biomass; Fossil fuels; Lignocellulosic materials; Hydrolysis; Fermentation

Introduction

Earlier ethanol was used as a transportation fuel; it was used in the internal combustion engine of the car invented by Nikolaus Otto in 1897. Later there was not much interest in ethanol due to the usage of fossil fuels. As a result of the oil crisis and climate change, developed countries decided to decrease the CO₂ emissions hence alternative fuels like ethanol drew attention. Ethanol can be blended with gasoline at up to 30% without any necessary changes in the engines of the vehicles. When added to gasoline, ethanol improves the fuel combustion and reduces the emission of CO₂ and unburned hydrocarbon that form smog [1-5].

Ethanol as a fuel

Ethanol has higher octane number than gasoline, by adding it to gasoline it boosts the octane number and thereby reduces the use of toxic additives like benzene. Ethanol has some fine properties over the conventional fossil fuels such as high heat of vaporization, low flame temperature, greater gas volume changes and high specific energy. So, with modified engines that facilitate the use of pure ethanol as a fuel in the near future, it will be advantageous and better than fossil fuels. For instance, in Brazil vehicles equipped with ethanol compatible materials and with on board electronic engine managements are running on pure ethanol [6-11].

The trade of fuel ethanol is comparatively less than that of the usage in alcoholic beverages, for solvent purpose and many other industrial applications. The cost of ethanol varies based on production, raw materials and policy [12-16] (Table 1).

Table: 1 World fuel ethanol production (2012) (www.ethanolrfa.org/pages/world-fuel-ethanol-production)

Continent	Millions of Gallons
North & Central America	13,768
South America	5,800
Brazil	5,577
Europe	1,139
Asia	952
China	555
Canada	449
Australia	71
Africa	42

Properties of ethanol

Ethanol is also known as ethyl alcohol which is a flammable and colorless liquid. It is mostly found in alcoholic beverages. The molecular formula of ethanol is C_2H_6O and it is often symbolized as EtOH [17-19] (Table 2).

Table: 2 Properties of ethanol

Density	0.789g/cm ³ , liquid
Solubility in water	Fullly miscible
Melting point	-114.3°C (158.8K)
Boiling point	78.4°C (351.6)
Acidity (pKa)	15.9 (H ⁺ from the OH group)
Viscosity	1.200 cP at 20°C
Dipole moment	1.69 D (gas)
Melting point	-114.3°C (158.8K)
Boiling point	78.4°C (351.6)
Acidity (pKa)	15.9 (H ⁺ from the OH group)

Sweden towards ethanol

Research in Sweden on ethanol is being reinforced by the Swedish Energy Agency or a Swedish national board of Technical and Industrial development, since 30 years. Along with these institutions, many companies in Sweden knowing the importance of ethanol as an alternative fuel, team up with universities providing technical and financial support for the exploration [20-26]. The first research program based on lignocellulosic ethanol was designed in the year 1998 and it was completed in 2004. Swedish pilot plant units were established in the same year in some universities which were one of the defining moments in the history of ethanol research. In 2010, according to Swedish law, at least one renewable fuel should be available at filling stations distributing more than 1000 m³/yr. nearly 1500 stations are offerings E85 as fuel in Sweden (<http://www.sekab.com/biofuel>).

Biomass for ethanol production

Ethanol can be produced from every sort of carbohydrate material that has the typical formula of $(CH_2O)_N$. These can be divided into starchy, sugary and lignocellulosic raw materials. High percentage of sugar is present in sugar beets and sugar cane, which are easily fermentable to ethanol. In Brazil ethanol is regularly produced from sugar cane [27-32]. Wheat, corn, barley, rye and other cereals are starch containing feed stocks. In USA, ethanol is mainly produced from corn and in Europe (main producers are France, Germany and Spain) produces mainly from cereals [33-39]. Ethanol can also be produced from potatoes and waste potatoes from the food industry. Ethanol produced from parts of the plant like starch or sugar is known as first generation fuels. Ethanol produced from integral parts of plants such as hemicelluloses, celluloses are known as second generation fuel. In order to reach large scale production, second generation fuels such as ethanol from lignocellulosic materials have to be further developed [40-43]. It is more difficult to convert hemicelluloses and cellulose into sugars compared to that of starch feed stocks. Lignocellulosic feed stocks include agricultural wastes, forest residues, municipal solid wastes (MSW), waste from pulp/paper processes and energy crops. Cellulosic agricultural wastes for ethanol include crop waste such as wheat straw, corn stover (leaves, stalk and cobs), rice straw and bagasses (sugar cane waste) and forest waste is mostly wood chips from forest residues which is left in the forest.

Environmental and Social aspects

Sustainable development of ethanol comprises Environmental, Economic and Social aspects. Also known as three Ps (People, Plant and Profit) or three Es (Environment, Economy and Equity). Life cycle assessment of ethanol includes from raw materials production to consumption of ethanol by vehicles [44-49].

In the raw material production stage, fertilizers, herbicides, equipment and fuels are involved. Due to external factors at production level, such as change in price of fertilizers, herbicides, equipment and fuels which leads to changes in cost of production and also some are produced from petrochemicals which are nonsustainable. Water usage for ethanol feed stock is questionable as it affects water availability for humans and due to uncleanliness of water from fields, waste water from field need to be treated prior to disposal.

Transport of feed stocks from fields to production need heavy vehicles which leads to depreciation of roads, change in traffic flows and may lead to accidents (threat to children's safety) are possible negative social impacts [50-56].

In the ethanol production plant, during conversion of feed stock to ethanol, along with ethanol co-products are produced which increases the plant profits. In order to have positive impacts on society, plants management need to take care of workers safety, health and providing education to their children. Maintenance of schools and hospitals for local communities will also have positive impact [57-59]. During ethanol production, plants releases liquid and air pollutants which leads to health problems for local people. So ethanol industries need to address the ethical and environmental issues such as climate change (reducing of greenhouse gases GHG), sustainability, energy and water conservation, co-products (generation and utilization), and waste water treatment.

Ethanol from lignocellulosic materials

The lignocellulosic materials contain cellulose, hemicelluloses, lignin and extractives. Cellulose is a polymer of β -D-glucose. Cellobiose, a dimer of glucose units, is the basic building block of cellulose [60-66] (Figure 1).

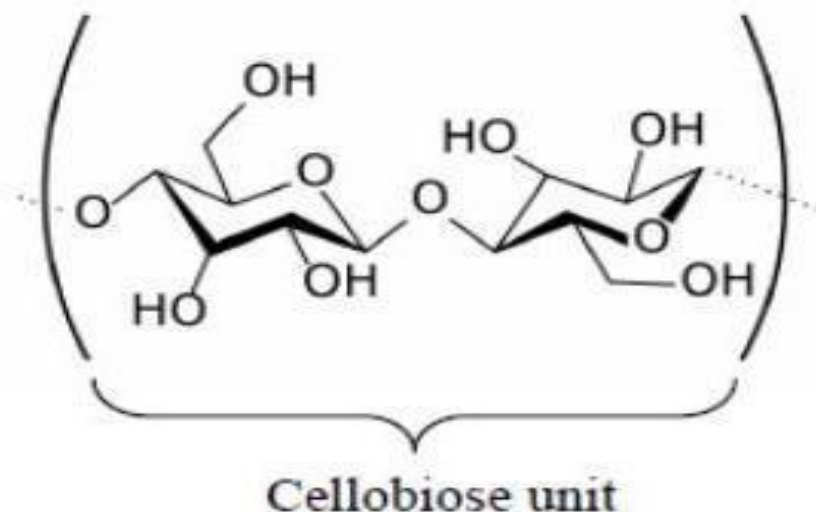


Figure 1: Chemical structure of Cellulose [18].

Table 3: Composition (%) of hard wood and soft wood.

Material	Glucan	Mannan	Galactan	Xylan
Hard woods :				
Alder	40.5	1.5	0.8	16.1
Aspen	43.2	2.2	0.5	15.1
Birch	40.7	1.7	0.7	20.0
Soft wood:				
Pine	42.4	11.8	1.9	4.7
Spruce	41.6	11.5	2.0	4.7

Hemicelluloses are heterogeneous polysaccharides, that when hydrolyzed by acids degrades into the monomeric compounds glucose, mannose, galactose, xylose and arabinose. Hemicellulose of softwood and hardwood varies in composition, mentioned in table 5. Sugar analysis of some woods in percentage of dry wood weight is pointed out in the above table 3 [67-73]. Lignin provides the mechanical strength to plants and trees. Its structure is complex, hydrophobic, cross linked, with the building blocks of aromatic polymers of phenolpropane. Lignin is derived from glucose through the development of precursor alcohols such as P-coumaryl alcohol (I), coniferyl alcohol (II) and sinapyl alcohol (III) which are building blocks of lignin. As a result of its complex structure it is resistant to biological and chemical degradation. Biological degradation can be done mostly by fungi and some actinomycetes (Figure 2).

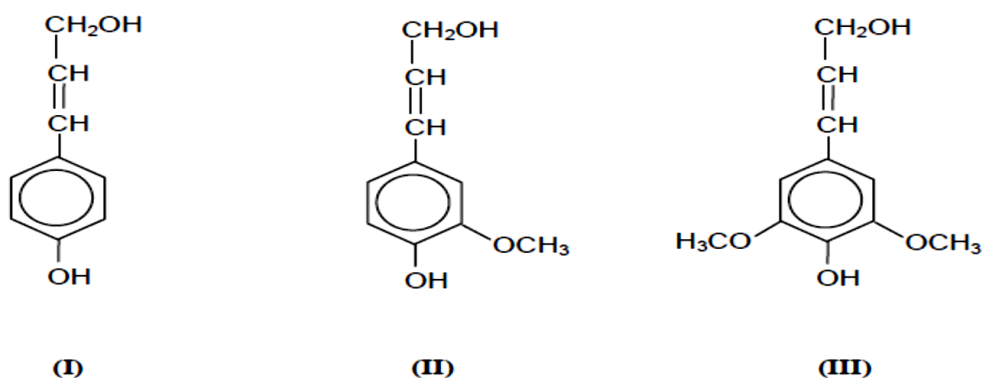


Figure 2: Precursor alcohols structure in lignin.

Extractives of lignocellulosic materials are terpenoids, steroids, fats, waxes, phenolic constituents and inorganic components. Terpenoids and steroids are soluble in nonpolar organic solvents. Fats are saturated and unsaturated fatty acids. Metal components are calcium, potassium and magnesium.

Table 4: Composition (%) of agricultural residues and waste.

Agricultural residue	Cellulose	Hemicellulose	Lignin
Hardwood	40-50	24-40	18-25
Softwood	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Grasses	25-40	35-40	10-30
Wheat straw	33-40	20-25	15-20
Rice straw	40	18	5.5
Leaves	15-20	80-85	0
Switch grass	30-50	10-40	5-20
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Primary wastewater solids	8-15	NA	24-29
Paper	85-99	0	0-15
Newspaper	40-55	25-40	18-30
Hardwood stem	40-50	24-40	18-25

In order to produce ethanol from lignocellulosic materials, steps involved are pretreatment, hydrolysis, fermentation and distillation. During the pretreatment and hydrolysis steps along with the sugars, many toxic compounds are derived such as acetic acid, furfural, HMF and phenolic compounds, which affect the fermentation step [74-79] (Table 4 and Figure 3).

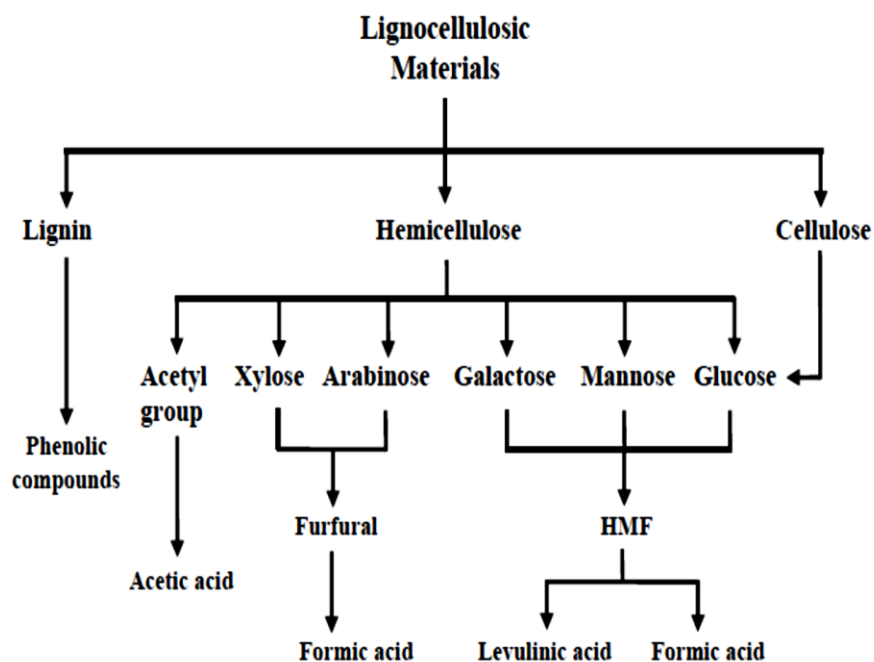


Figure 3: Inhibitory compounds formed during dilute-acid hydrolysis.

Pretreatment

The process of breaking down complex cellulose structures into simpler material is known as pretreatment. In bio processing, pretreatment has a major role to play in the final production rate and the difference in quantity and quality is always noticed in the process. There are several possible methods to be used in the pretreatment process, based on the material being treated and depending upon the conditions provided the treatment methods can be classified as physical, chemical and biological [80-83].

In physical processing, methods such as ball-milling, colloid milling, hammer milling, high pressure steam, extrusion, expansion, and pyrolysis are principally used. At present, as a result of advancements in Biotechnology, several enzymatic pretreatments are used followed by physical and chemical pretreatment process if required (Table 5).

Table 5: Common pretreatment methods.

Method	Effects
Physical Mechanical comminution (milling and grinding), Pyrolysis	Increase in specific surface size of pores, decrease of the degrees of polymerization of cellulose and its crystallinity, decomposition of cellulose
Physico-chemical Steam explosion, ammonia fiber explosion (AFEX), CO ₂ explosion	Partial degradation of hemicellulose, increase of the specific surface area and size of pores, lignin transformation
Chemical Ozonolysis, Acid hydrolysis, Alkaline hydrolysis, oxidative delignification, organic solvents	Hemicellulose degradation, lignin removal, increase in internal surface area due to swelling decrease in degree of polymerization and crystallinity, increase in porosity
Biological Brown, white and soft-rot fungi	Hemicellulose and lignin degradation, increase of the specific surface area and size of pores

The aim of pretreatment and hydrolysis is to produce fermentable sugars such as pentose and hexoses from lignocellulosic material, leaving lignin as by-product which can be used to produce fuel or electricity [84-89].

Hydrolysis

After pre-treatment, the cellulose and hemicellulose are separated into glucose, mannose, galactose, arabinose and xylose, sugars used later in the fermentation process to produce ethanol. The hemicelluloses of soft wood largely comprise mannose and that of hardwood contains xylose. Hydrolysis can be carried out by chemical hydrolysis or enzymatic hydrolysis.

Chemical hydrolysis

In chemical hydrolysis, lignocellulosic materials are treated with chemicals like acids for some time, in order to break down the polymers into monomeric sugars. Dependent on the method different time and concentration of acids is used. Chemical hydrolysis is divided into concentrated-acid hydrolysis and dilute acid hydrolysis [90-92].

In concentrated-acid hydrolysis, sulfuric or hydrochloric acid is used. When compared to dilute acid one gets high sugar yields and a high yield of ethanol can thus be obtained but it has drawbacks such as that it is extremely corrosive and therefore the process needs expensive alloys or non-metallic constructions. This creates high production and maintenance costs.

Dilute acid hydrolysis of lignocellulosic materials is simple and fast, except the generation of high concentrations of toxic inhibitors influencing the fermentation process. Generally low concentrations of acids, 0.5-1% of H₂SO₄, and high temperature are used. In one stage dilute acid hydrolysis step, with 0.5% sulfuric acid at temperature 188-234°C for 7 minutes it was observed that higher glucose yield was obtained at temperatures above 220°C and mannose was obtained at temperatures below 200°C. In two stages dilute-acid hydrolysis, at low temperature mannose is separated in the first stage and in the second stage glucose is separated at high temperature. These two stage dilute acid hydrolysis are more effective than one stage hydrolysis [93-96].

Enzymatic hydrolysis

Before enzymatic hydrolysis, chemical pretreatment of lignocellulosic materials is essential. Hydrolysis of lignocellulose is carried out by enzymes. Different types of enzymes are used to separate the sugars from

cellulose and hemicellulose. Mostly a set of enzymes such as endoglucanases, exoglucanases, β -glucosidases and cellobiohydrolases is applied, where polysaccharides are broken into shorter sugar chains by endoglucanases. Exoglucanases removes cellobiose moieties and β -glucosidases thereafter degrade the cellobiose and oligosaccharides to glucose fermentation.

Fermentation of lignocellulose hydrolyzates varies depending on dissolved oxygen concentration, cell physiological conditions, pH of media and different levels of toxic compounds. Fermentation of sugars from lignocellulosic materials broken down by dilute acid-hydrolysis is difficult due to inhibitors which might affect the fermentation process [97-101].

Batch cultivation

Batch culture is known as system where microorganisms are grown in static or set cultivation medium and fixed conditions such as temperature, pressure and aeration. In situ detoxification in batch cultivation may be improved by increasing the cell concentration, e.g. by cell immobilization or by genetic modifications of the cells to increase their inhibitor tolerance and changing the fermentation parameters such as pH to reduce the inhibitory effect of carboxylic acids.

Continuous cultivation

A microbial population can be maintained in the exponential growth phase and at a constant biomass concentration for extended periods. It is an open system with constant environmental conditions maintained through continual provision of nutrients and removal of wastes and products. Continuous cultivation has some advantages over batch cultivation, cells can be easily separated and high fermentation rate. Disadvantages are low in situ detoxification of inhibitory compounds. In continuous cultivation of lignocellulosic hydrolyzates, encapsulation of yeast provides higher biomass when compared to freely suspended cells in a bioreactor.

Detoxification

Detoxification mainly depends on the type of lignocellulosic material, and technology, General methods are biological, physical and chemical which can reduce the concentration of inhibitors or convert them into nontoxic compounds.

The effects of inhibitory compounds on *S. cerevisiae* during fermentation

The effect of inhibitors mainly depends on the type of microorganism, medium conditions, type of fermentation and number of inhibitors [24]. Many inhibitors are formed along with sugars during pretreatment and hydrolysis of wood, among the formed inhibitors are weak acids, furan aldehydes, and phenolic compounds, which are derived from cellulose, hemicelluloses and lignin. The concentration of inhibitors depends on the type of raw materials and operational conditions of hydrolysis.

There are different ways of minimizing the inhibitors in hemicellulosic hydrolyzates. They are preventing the formation of the inhibitors during hydrolysis and remove the toxic compounds prior to fermentation (detoxification) or develop microorganisms which can withstand the effect of the inhibitors and convert the

toxic compounds into products which will not interfere with the metabolism (Figure 4).

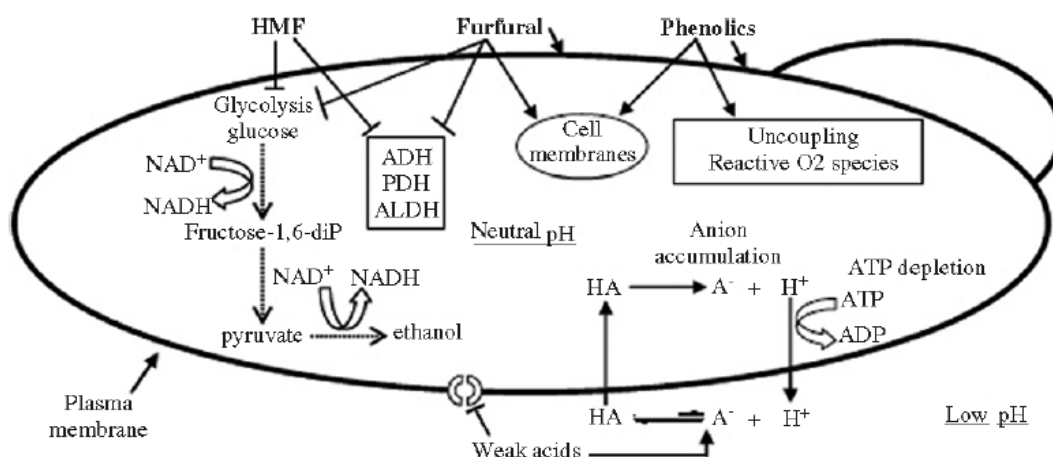


Figure 4: Effect of inhibitors on yeast mechanisms.

Furan aldehydes

Furfural and HMF are formed through the pentose and hexose sugars. High concentration of furfural and HMF will affect growth of yeast.

Furfural can affect the enzymes alcohol dehydrogenase (ADH), pyruvate dehydrogenase (PDH), aldehyde dehydrogenase (ALDH) and damages the cell membrane. Furfural is toxic to batch cultivations. When furfural is present in the medium it is primarily converted to furfuryl alcohol, through the production of acetic acid and hence when it is present in a medium, a high amount of acetate is produced, which leads to the low production of ethanol. Alcohol dehydrogenase (ADH) helps in this conversion. In low concentration furfural has a positive effect on growth of the cell and in high concentrations it can stop the growth or fermentation. In batch cultivations, during the presence of furfural concentrations of more than 1g/l, the CO₂ evolution and viability is decreased [101]. Fermentation of sugars with high concentration of furfural (4g/l) can inhibit growth (80 %) and ethanol production (97 %) of *S. cerevisiae*.

HMF (Hydroxymethylfurfural) is derived from degradation of hexose sugars. HMF will affect the enzymes alcohol dehydrogenase (ADH), pyruvate dehydrogenase (PDH) and aldehyde dehydrogenase (ALDH). In general, the concentration of HMF is low compared to furfural; and it is less toxic due to its high reactivity. Low quantity of hexose in hemicelluloses and hydrolysis conditions doesn't degrade hexose in a large level. At a concentration of 1g/l, hydroxymethylfurfural can inhibit cell growth of *S. cerevisiae* and stop fermentation.

Carboxylic acids

Carboxylic acids inhibit biomass formation and ethanol production. Weak acids will cause ATP depletion, toxic anion accumulation and inhibition of aromatic amino acids uptake [26]. Acetic acid is derived from acetyl groups of hemicellulose. At low pH conditions, acetic acid will be in its undissociated form and can diffuse through the plasma membrane. In the cytoplasm the increased pH (7.4) can lead to dissociation, i.e. release of protons which lead to a decrease in internal pH and inhibition of the cellular activity. Formic acid is inhibitorier than levulinic acid due to its low molecular size [101-104]. The level of inhibition of acetic acid depends upon medium conditions such as pH and oxygen concentration. Ethanol production was accelerated by presence of 10g/l acetic acid in medium free of other inhibitory compounds.

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