IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 14, Issue 5 Ser. II (Sep – Oct 2019), PP 42-48
www.Iosrjournals.Org

Review on Production of Bioethanol and Its Future Prospects

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Abstract: *Due to the health andenvironment impacts of fossil fuels utilization, biofuels have beeninvestigated as a potential alternative renewable source of energy. Bioethanol is presently the mostproduced biofuel, mainly of first group, resulting in food-fuel competition. Secondary bioethanol is produced from lignocellulosic biomass, but a costly and difficult pre-treatment isrequired.The pulp and paper industry has the biggest income of biomass for non-food-chain production and concurrently generates a high amount of residues. According to the round economy model these rests, rich in monosaccharaides, or even in polysaccharides besides lignin, can be utilizedas a proper feedstock for second generation bioethanol production. Bio refineries can be integratedin the existing pulp and paper industrial plants by exploiting the high level of technology andalso the infrastructures and logistics that are required to fractionate and handle woody biomass.This would contribute to the diversification of products and the increase of profitability of pulp andpaper industry with additional environmental benefits. This work appraisals the literature supportingthe feasibility of producing ethanol from Kraft pulp, spent sulphite liquor, and pulp and paper sludge,presenting and discussing the practical attempt of bio refineries implementation in pulp and papermills for bioethanol production.*

Keywords: *Bioethanol, pulp and paper industry, lignocellulosic biomass, Kraft pulp, spent sulphite, pulp and paper sludge.*

Date of Submission: 19-09-2019 Date of Acceptance: 07-10-2019

I. Introduction

Raise in the population over the last century lead to the increase of the energy spending worldwide. To meet the increased energy demand crude oil has been used as the major resource. The global oil production would failure to 5 billion barrels from 25 billion barrels approximately. Due to this inevitable reduction of the world petroleum resources in the coming years the worldwide interested aroused in seeking an alternative non-petroleum based energy source. One of the best alternative fuels in order to beat severely the energy crises is from Biofuel. From biologically carbon fixation the energy is derived from Biomass. The various factors like need for increasing energy security and hikes and gaining the scientific and public attention the biomass are driven. The main contents of ethanol are sugar, starch or cellulose. The Bioethanol is one of the environment friendly fuels, the effects on atmosphere is less because the Ethanol contains oxygen. With comparison to the conformist gasoline the blends of E10 resulted in 12-25% less emission of carbon monoxide[1].The sugarcane and corn are the first generation bio-fuels. Due to vast increase in the ethanol production using these crops they cause immoderate pressure on the global food supply. The second generation biofuels can be produced by means of different sources like waste chicken feathers, cellulosic biomass food and organic waste. The cellulosic biomass, such as agricultural residue and industrial waste are the most plentiful and cheap source of renewable energy in the world.

The second generation biofuels may also embrace the fuels produced from diverse paper waste which is separated from the municipal solid waste, cash crops Jatropha, Honge, Cotton, Maize etc. can be utilized to produce bioethanol. The third generation biofuels can be produced from micro-organisms mainly Algae. The fourth generation biofuels produced from vegetable oil, biodiesel.

II. Materials And Methods

COLLECTION OF SUBSTRATE

Newspaper, whichwas used as a substrate for the production of bioethanol,was collected from the households. The substrate was collected in a dust free and fungus-free state and was dried in sunlight and was made into small pieces and stored in sealed plastic bags.

CHEMICAL ANALYSIS OF THE SUBSTRATE

Composition of the substrate and its properties were analysed before pre-treatment. The cellulose content and total carbohydrate in the substrate was estimated by anthrone method[2], Moisture content and ash content of the substrate were also estimated using standard methods[3].

OPTIMIZATION OF THE PRETRETEMENT SUBSTRATE

The pre-treatment optimization for the substrate was carried out by using different combination dilute sulphuric acid ranging from 0 to 6% and heating period of 30, 45 and 60 minutes at 121^0C and 15lb pressure. 1gm of substrate was added with 10 ml of dilute sulphuric acid (1:10). Cellulose released during this optimization process was analysed by anthrone method[4]. After the release of maximum amount of cellulose during pre-treatment process, the solution was taken for hydrolysis.

HYDROLYSIS OF THE PRETRETED SUBSTRATE

Maximum cellulose unrestricted during the pre-treatment was hydrolysed by the isolated cellulose degrading bacteria. The pre-treated substrate was washed with distilled water several times to neutralise the acid concentration. The substrate was oven dried till constant weight and the pH was adjusted to 7.0. Pure culture Cytophagahutchisonni (CH) (NCIM 2338) was procured from National Collection of Industrial Microorganisms (NCIM), Pune and the isolated organism is taken from Department of Biotechnology, BEC Bagalkot. Comparison study between isolated cellulose demeaning bacteria and the pure culture, CH was performed. A 24hr grown inoculum of isolated cellulose degrading bacteria and pure culture, CH were added to the pretreated substrate. Reducing sugars release during substrate hydrolysis were analysed by Dinitrosalicylic Acid (DNS) method every 24hr from zero hour, for both the organisms[4]. Maximum sugars released during this period were additional taken for fermentation to produce bioethanol.

FERMENTATION OF HYDROLYSED BROTH

Fermentation was carried out using commercially available yeast, *Saccharomyces cerevisiae*. The pH of hydrolysed broth was adjusted to 4.6 and an inoculum of active yeast (in log phase) was added to the hydrolysed broth. The fermentation was carried out at 360C until maximum sugars are converted into bioethanol. The reducing sugar utilization during fermentation was analysed by DNS method[4], and the bioethanol production was analysed by using specific gravity method.

CALCULATION OF SPECIFIC GRAVITY

$$W2 - W1 = \text{Specific Gravity} \qquad W3 - W1$$

Where, W1 = empty weight of specific gravity bottle W2 = Weight of sample + specific gravity bottle W3 = Weight of distilled water + specific gravity bottle.

SACCHARIFICATION

Hydrolysis, also known as Saccharification, is a crucial step as it changes the cellulose and hemicelluloses in their monomers, *i.e.*, fermentable sugars. This can be achieved either biologically (enzymatic hydrolysis) or chemically (acidic hydrolysis) [21,22].

ACIDIC HYDROLYSIS

Acidic hydrolysis commonly involves the use of sulphuric or hydrochloric acids to break down cellulose and hemicelluloses. Concentrated acidic hydrolysis can be performed at low temperatures and a high sugar yield is obtained (i.e., 90% of the theoretical glucose yield) [23]. However, it requires high acid concentrations, usually in the range of 30–70%, which leads to equipment corrosion. Therefore, concentrated acidic hydrolysis entails economic and environmental problems [24]. Conversely, diluted acidic hydrolysis requires a much lower amount of acid, 2–5%, and it is more commonly applied in industry [23,25]. However, it needs a temperature around 200^oC, which can lead to the formation of different inhibitory compounds, such as acetic acid, furfural, HMF, and phenols. These compounds not only negatively affect the following fermentation step but also decrease the sugar yield [21,24].

ENZYMATIC HYDROLYSIS

Hydrolysis can be catalysed by highly specific enzymes that are able to convert the complex carbohydrates of LCB to simple monomers. Enzymatic hydrolysis requires mild temperature and pH conditions (i.e., 50–60 ◦C and pH 4.5–5.5). These conditions require less energy and they do not lead to the formation of inhibitory compounds or to equipment corrosion [25,26]. Most importantly, enzymatic hydrolysis attains high yields of sugars, 80–95%, and has a reduced environmental impact [27]. The cost of enzymes, which is estimated to account for about 20% of the ethanol production costs, is still a major limitation of enzymatic

hydrolysis [26]. Another disadvantage of using enzymes is the slowness of reactions, which results in long hydrolysis times (e.g., 1.5 days) [28]. Cellulases and hemicelluloses are the enzymes that are usually employed for the hydrolysis of the LCB [29]. Cellulases, for cellulose hydrolysis, usually comprise three complementary groups of enzymes that are able to hydrolyse the β-(1,4)-glycosidic bonds: endoglucanases (EG), cellobiohydrolases (CBH), and β- glucosidases (BG) [30]. EG (endo-1,4-β-D-glucanases, EC 3.2.1.4) cleave the formless regions of cellulose. CBH (exo-1,4-β-D-glucanases, EC 3.2.1.91) hydrolyse the free ends of cellulose chain into the disaccharide cellobiose and are divided in CBHI and CBHII that act on the reducing and non-reducing ends, respectively. BG (EC 3.1.1.21) hydrolyzecellobiose to produce glucose [26,31]. These groups of enzymes are usually obtained at the industrial level from fungus *Trichodermarese*i [32].

FERMENTATION

The fermentable sugars coming from saccharification are the preferred substrate for bioethanol production by a diversity of microorganisms [34]. The anaerobic reaction of hexoses and pentoses conversion to ethanol can be expressed by Equations (1) and (2), respectively [35]:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (1)$$

$$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2 \quad (2)$$

The maximum theoretical yield of the fermentation process is 0.511 kg of ethanol, produced with 0.489 kg of CO2, per kg of hexose or pentose [36].

Different possibilities to integrate hydrolysis and fermentation bioprocesses are possible. These configurations include separate hydrolysis and fermentation (SHF), simultaneoussaccharification and fermentation (SSF), and consolidated bioprocessing (CBP) [37].

ETHANOLOGENIC MICROORGANISM

An ethanologenic microorganism should bear the following characteristics: (i) robust growth with simple requirements allowing for the use of inexpensive media; (ii) tolerance to acidic pH or high temperatures in order to retard contamination; (iii) high ethanol yield, above 90.0% of theoretical value; (iv) tolerance to ethanol concentration higher than 40.0 g·L L 1 ; (v) ethanol productivity above 1.0 g·L L 1 ·h h 1 ; and, (vi) resistance to inhibitors, being able to grow in undiluted hydrolysates [38]. *Saccharomyces cerevisiae* is the most commonly used microorganism for bioethanol production, being robust and well-suited yeast for fermentation of lignocellulosic hydrolysates. S. cerevisiae has high ethanol yield, high ethanol and inhibitors tolerance, and can use a wide range of hexoses (glucose, mannose, and galactose) and disaccharides (sucrose and maltose) [39,40]. Among bacteria, *Zymomonasmobilis*, a gram-negative that is able to ferment glucose, sucrose, and fructose, is the most studied. When compared to *S. cerevisiae*, *Z. mobilis* has a higher ethanol yield and a much higher ethanol specific productivity, since it produces less biomass [41,42]. It also presents a higher ethanol tolerance. However, *Z. mobilis* is able to utilize a narrower range of sugars and has lower tolerance to inhibitors, like acetic acid [43]. Besides, *Z. mobilis* requires a neutral pH range, a common feature to most bacterial species [44,45].

Although *Z. mobilis* and *S. cerevisiae* are the most commonly utilized microorganisms, they are incapable of fermenting pentoses [41]. Hydrolysates obtained from LCB present a high content in pentoses, mainly xylose, which can reach about 25% of sugars, followed by arabinose [46]. *Scheffersomycesstipitis*, *Candida shehatae*, and *Pachysolantannophilus* are the most efficient yeasts to use pentoses, in addition to hexoses [47]. However, these yeasts require micro-aerophilic conditions and are sensitive to low pH, inhibitors and high ethanol concentration. *S. stipitis* is the most promising pentose-fermenting organism for industrial applications, presenting the best performance in xylose fermentation with a higher ethanol yield [41,42]. Besides yeasts and bacteria, filamentous fungi, such as *Mucorindicus*, *Neurosporaintermedia*, *Peniophoracinereal*, and *Trametessuaveolens*, were also tested for ethanol production [43,44]. Some of these microorganisms are capable of fermenting both hexoses and pentoses [45,46], and since several of these microorganism have the ability of producing both lignocellulolytic enzymes and ethanol, they could be applied for CBP [47,48]. Nevertheless, low ethanol yields, due to the formation of significant amounts of by-products (e.g., acetic acid), low productivity and low growth rates are associated to ethanol production by filamentous fungi [47,49,50,51].

RECOVERY AND DEHYDRATION

Usually, at the end of the fermentative step, the broth contains only about 5 wt % of bioethanol, a low value when compared with first generation bioethanol, which can reach 12 wt %. The fermentation broth is first distilled in a stripper column that concentrates ethanol to above 20 wt %, and, then the ethanol stream is further concentrated in a rectifier column to no more than 95.6 wt % ethanol in water, due to the formation of a minimum boiling azeotrope at 78.15 ◦C and 1 atm. Distillation is energybookkeeping for 600 80% of total

separation cost of bioethanol from water, particularly due to low ethanol concentration in the broth. In order to be mixed with gasoline, anhydrous ethanol (>99.5 wt % ethanol) should be obtained and a dehydration step after distillation is required [52,53]. In the past, dryness was usually achieved by azeotropic distillation [52]. Due to the high energy demand, azeotropic distillation was replaced by adsorption with zeolite molecular sieves [52].

BIOETHANOL PRODUCTION

Different studies previously proved the viability of Kraft pulping as a LCB pretreatment by obtaining hydrolysates with sugar profiles suitable for fermentation through enzymatic hydrolysis of Kraft pulp of different origins, including sweet sorghum bagasse [54], pine, poplar, birch, beech, and wheat straw [56], hemp [57], eucalyptus [58], moso bamboo [59], spruce, and birch-aspen mixture [60]. Several studies attentive on the fermentation configuration to produce ethanol from Kraft pulp. Additionally, the possibility of finding other products from Kraft pulp by applying simultaneous saccharification and fermentation was also assessed. These products comprisedcaffeic acid, a building block for thermoplastics and precursor for biologically active compounds [62], phenyllactic acid, a precursor for pharmaceutical and bio-based polymers [63], and D-lactic acid, a raw material for the synthesis of polylactic acid [23]. . Monrroy*et al.* (2012) inspected the SSF of Eucalyptus globulus Kraft pulps that were pretreated under different conditions. The ethanol concentrations varied between 30–38 g·L L^{-1} and a maximum ethanol yield of 0.202 g·(g dw)) 1 was obtained [64]. Ko et al. (2012) also calculated SSF of unbleached Kraft pulps of E. globulus for ethanol production using a different S. cerevisiae strain and testified lower ethanol concentration and yield.

Using unbleached Kraft pulps of *Acacia confusa*, the authors obtained an ethanol concentration of 5.88 g·L L 1 and ethanol yield of 0.045 g·(g dw)) 1 Alternatively, the authors applied acid steam-explosion as pretreatment for both woods, obtaining better results [106]. Buzała*et al.* (2017) confirmed the production of ethanol from Kraft pulps of different origins through SHF. Ethanol crop (per dry weight of wood) from the five hardwood unbleached pulps used ranged from 0.11–0.14 g·(g dw)) 1 . For the long (i.e., softwood) unbleached and bleached pulps, ethanol yields of 0.02 g·(g dw)) 1 and 0.20 g·(g dw)) 1 were obtained, respectively. The low ethanol yield from the hydrolysate of unbleached pine pulp was attributed to the high content of extractives in the pulp [47]. Wistara*et al.* (2016) investigated the SSF of Kraft pulps of Jabon wood with different lignin content and freeness. Ethanol yield (per dry weight of pulp) varied between 0.022 and 0.129 g·(g dw)) 1 , and pulps with lower lignin contents and higher pulp freeness resulted in higher yields of ethanol [71].

Concerning bioethanol production, the most motivating feedstock coming from sulfite pulping is the liquor, SSL, a side product that is obtained at end of the process. The main workings of SSL are LS and carbohydrates resulting from hemicelluloses, being mostly fermentable sugars, such as arabinose, xylose, mannose, galactose, and glucose. SSL also contains sugar degradation products, like furfural and HMF, besides acetic acid, uronic acids, methyl glyoxal, formaldehyde, methyl alcohol, and extractives [64]. Concerning the black liquor from Kraft pulping, it is unsuitable for bioethanol production since it contains no sugars but mainly lignin and carbohydrate degradation products (i.e., hydroxycarboxylic acids, acetic acid, and formic acid), and also contains small amounts of extractives (e.g., turpentine and talloil) and other miscellaneous product Due to the differences between softwood and hardwood hemicelluloses, the sugar composition of SSL depends on the type of wood that is used for pulping.

FUTURE PROSPECTS

The studies described throughout this review demonstrate the feasibility of producing ethanol from different pulp and paper industry wastes. However, in many studies using Kraft pulp, SSL, or PPMS, the concentrations of ethanol obtained in the fermentation broth were much lower than the recommended minimum of 4 wt % that is required to have a lower energy demand in the recovery step [52]. The most used method in the recovery of ethanol, distillation, is energy-intensive, accounting for 60 - 80% of the total separation cost of bioethanol from water, particularly due to low ethanol concentrations in the fermented broth [52]. The ethanol concentrations that were reported so far would significantly increase the recovery costs. In some cases, ethanol yields and productivities are also low. Hence, it is important to further study new process strategies to improve bioethanol production from the wastes of pulp and paper industry to obtain more efficient bioprocesses with higher yields and productivities.

In most studies regarding ethanol production from Kraft pulp, *S. cerevisiae* was the microorganism chosen for fermentation. Alternatively, using a hexose- and pentose-fermenting microorganism or a co-culture strategy would allow for the consumption of both hexose and pentose sugars that are present in the hydrolysate, increasing the amount of ethanol produced, as well as the ethanol yield. To the best of our knowledge, the production of ethanol from low-quality Kraft pulp or Kraft pulp obtained from bark and other rejects has not been studied yet. Hence, this seems to be the next step in order to convert Kraft pulp mills into integrated biorefineries.

Production of ethanol from SSSL is being applied since the last century, while using HSSL as a feedstock for ethanol production still faces several challenges. Some technical bottlenecks must be eliminated by developing a microorganism that is able to ferment pentose in the presence of inhibitors. Also, detoxification techniques that efficiently decrease inhibitors concentrations of HSSL should be developed to be applied at industrial scale.

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N. Prabhu ." Review on Production of Bioethanol and Its Future Prospects. " IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 14.5 (2019): 42-48.