A STEP INTO FUTURE FUEL INDUSTRIAL EFFLUENTS INTO BIOETHANOL: A REVIEW

ABSTRACT:

Industrial revolution in agro based industries with improved technologies, machineries, etc. has resulted in the ease of operation, availability of increased number of value added products in the field of agriculture, effective labour management, etc. Agro industries are one of the major contributors for environmental pollution and accounts for about 10-15% for pollution. As the wastes generated from agro-industries are of biological origin, they can be effectively reused; if their alternate uses are explored. As these industrial effluents contain lignocellulose, cellulose, and hemicellulose, which are rich sources of lignin, and cellulose, it is possible to use these materials as a substrate to produce bioethanol. Our current review focuses on bioethanol production from different industrial effluents and Bio-microbial based fermentation.

Keywords: Bioethanol, fossil fuel, industrial effluent, fermentation

1.Introduction

The world population is moving away from fossil fuel usage, due to increasing demand and population. However, the usage of fossil fuels is replaced by the usage of biofuel. Biofuels are gaining popularity as an alternative energy source because they are non-polluting and cost-effective (Sekoai et al. 2019). In an epoch marked by environmental concerns, climate change, and the need for sustainable energy solutions, bioethanol has emerged as a promising biofuel that is obtained from different biomass substrates such as lignocellulose, starch, and cellulosic substances on the global stage.(Bhadana and Chauhan 2016). In terms of the application, a combination of bioethanol and gasoline increases the efficiency of vehicles. Globally bioethanol production has rapidly increased from 18 billion Liters at the turn of the century to 110 billion Liters in 2019(Hoang and Nghiem 2021). In recent days, bioethanol production also has been industrialized with the effective utilization of industrial effluents. The industrialization process also produces waste, which is primarily disposed of in landfills or thrown into rivers. As of 2011, there were 9.2 million tons of industrial waste generated worldwide (Vignesh et al. 2021), with an annual industrial waste generation rate of 1.74 tons per capita. Industrial effluents are wastewater streams produced by industrial activities with higher organic contaminants. Various types of industrial effluents are produced, such as those produced by palm oil, sugar, paper mills, and dairy farms. These effluents from industrial sources contain a large number of organic pollutants that are rich in starchy and lignocellulosic compounds, which can be used to produce biofuels. They are less expensive to purchase and require less land for cultivation than other feedstocks. This review elucidates the current understanding of industrial effluents around the world with emphasis on their contribution to bioethanol as an alternative fuel resource through their use in Palm oil, paper mills, dairy, sugar, and sago industries.

2.PALM OIL INDUSTRY:

Oil palm is one of the world's most productive oil-producing crops, producing over 10 to 35 tons of fresh fruit bunches annually per hectare. Typically, the fruit is the part that is harvested for oil extraction. Though the production process is efficient more than 70% of fresh fruit is wasted during the production process. Crude palm oil contains fatty acid esters of glycerol, often known as triglycerides, which contributes to the world's requirement for edible oil and fats. The oil's unusual colour is attributable to fat-soluble carotenoids (pigment), which are also responsible for its vitamin E concentration. Worldwide, over 90% of palm oil production comes from Indonesia and Malaysia, which makes them second-largest exporters. Based on 2017 statistics, Malaysia alone produced 19.92 million metric tons of palm oil, and exported 16.56 million metric tons, and there are currently 454 palm oil mills (POMs) operating (Tan and Lim 2019). During this production process several wastes are generated one of the most common wastes is Palm oil mill effluent.

2.1. PALM OIL MILL EFFLUENT(POME):

Palm-oil mill effluent (POME), is a plentiful organic residue, that is produced during oil extraction from fresh palm fruit. POME contains lignin and phenolic chemicals, which makes this adverse from environmental disposal. For every tonne of fresh fruit from, 0.5-0.75 t of POME is discarded into the environment. The effluent from palm oil mills is an acidic, thick, brownish colloidal suspension that contains 95–96% water, 0.7–0.7% oil, and 2–4% total suspended solids (TSS) (Wakil et al. 2013). The general characteristics of POME are given (Table.1) It has a temperature range of 80°C to 90°C and is made up of a variety of components, such as cell walls, organelles, short fibres, a range of carbohydrates, from hemicelluloses to simple sugar, and a variety of nitrogenous compounds, including proteins, amino acids, and free organic acids. These wastes are generally discharged into the environment or dumped in a landfill. This POME is a good source for biofuel production, especially bioethanol. Limited research has been done on bioethanol production from POME.

2.2 **FERMENTATION**:

POME bioethanol production utilizes statistically optimized conditions such as oxygen saturation level (pO2%), pH, and temperature in conjunction with the co-culture of lignocellulolytic fungus and Saccharomyces cerevisiae. The direct bioconversion of POME into ethanol occurs in three phases in this process. Delignification of lignocellulosic materials from their complicated structure by lignocellulolytic fungus (*Trichoderma harzianum* and/or *Phanerocheate chrysosporium*) is the initial stage. The second step is to depolymerize carbohydrate polymers (cellulose and hemicellulose) into reducing sugars (glucose, fructose, xylose, etc.) using cellulolytic enzymes produced by cellulolytic fungi (*T. harzianum*/*Mucor hiemalis*), which is followed by the third step, sugar fermentation by yeast (*S. cerevisiae*) for bioethanol production (Alam et al. 2009) . Bioethanol production from POME with different pH and have been finalized with a maximum yield of 6.5% or 51.3g/l on the 4th day of fermentation. pH 6 has the greatest bioethanol production of 44 mg/L and in pH 7 conditions highest concentration of bioethanol production was observed to be 88,85 mg/L.(Anggamulia et al. 2020a)

3.**PAPER INDUSTRY**:

The main source of renewable biomass is lignocellulosic biomass, which has been historically utilized as a feedstock for the manufacturing of biofuels. Large quantities of main and secondary sludge from the wood digestion process are discarded annually in the USA; the majority of 26% of waste which are dumped in landfills or burned(Turner et al. 2022). Depending on the percentage of solids in the sludge, the facility's cost to dispose of sludge will vary. In Wisconsin, a reasonable figure for the cost, of disposing of sludge in a landfill is roughly US $30 per dry ton. Assuming 350 working days per year and sludge that comprises 50% solids, this cost for disposal for a large mill that produces 100 tons of sludge per day may be as high as $1 million per year. The generation of PMS is anticipated to climb by 48% to 86% globally throughout the next 50 years. Increased PMS has detrimental implications on landfill capacity and incinerator capacity overload. Sludge disposal solutions are becoming more limited as more onerous environmental rules increase the expense of landfilling or incinerating sludge. The development of gas and leachate during the decomposition process accounts for most landfilling sludge's negative environmental effects, with liquid leachate runoff possibly contaminating land, groundwater, and surface water bodies. Incineration of paper mill sludge is not a viable solution because it poses harm to the environment's air, water, and land.

4.1 CHARACTERISTICS OF PAPERMILL SLUDGE:

Paper Mill Sludge (PMS) from pulp and paper mills contains lignin and lignin derivatives which are harmful to aquatic life. After mixing with water bodies, it reduces photosynthesis, raises the water temperature, and lowers the dissolved oxygen content. Depending on the amount of lignin and lignin derivatives present in the PMS, the level of wastewater toxicity varies. PMS has a carbohydrate content of 25-75%, which provides several benefits for the development of biofuels, including (1) Low feedstock cost (2) No need for high-temperature chemical and mechanical pretreatment. Because the biomass's structure and composition have already been altered and removed during the pulp-making process, further unit operations are not required to remove inhibiting substances. In this way, pollution is reduced and a sustainable environment is created. Furthermore, PMS can be used for a variety of applications such as the production of bioethanol and biodiesel, etc.

4.2 PRE-TREATMENT:

(i)**Enzyme hydrolysis**:

In PMS, cellulose and lignin are the most abundant ingredients, which cannot be used in fermentation without being converted into simple monomers. Therefore, enzyme hydrolysis precedes fermentation. PMS hydrolysis was done by cellulase and hemicellulose enzyme over 72 hrs(Gurram et al. 2015). Sugar content was determined and compared with the initial concentration. Usually, these enzymes break down complex carbohydrates into simple sugars by cleaving the β(1,4)-glycosidic bonds. Apart from cellulases and hemicelluloses, other non-hydrolytic accessory proteins play a significant role in Lignin degradation, such as lytic polysaccharide monooxygenases and carbohydrate-binding modules. In some cases, microbial contamination may occur during the hydrolysis process, which is why sodium azide is not added (Branco et al. 2018).

(ii) **Acid hydrolysis**:

To break down cellulose and hemicellulose, acidic hydrolysis is usually carried out with the aid of sulfuric or hydrochloric acids which is generally a cheaper method. Concentrated acidic hydrolysis can also be performed at low temperatures which produces a high sugar yield. However, this method requires high acid concentrations, often between 30 to 70%, which may cause corrosion to equipment. As a result, intense acidic hydrolysis causes economic and environmental issues also raises. On the other hand, diluted acidic hydrolysis requires a significantly smaller amount of acid i.e., 2-5 percent, and is more widely used. But it also requires a temperature of roughly 200℃, which can result in the creation of several inhibitory chemicals such as acetic acid, furfural, HMF and phenols. These chemicals not only have a deleterious impact on the subsequent fermentation process, but they can also reduce sugar yield.

4.3 Fermentation:

The fermentable sugars coming from saccharification is the preferred substrate for bioethanol production by a diversity of microorganisms. There are some different possibilities to integrate hydrolysis and fermentation bioprocesses are conceivable. These configurations include distinct hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and consolidated bioprocessing (CBP). The fundamental advantage of this configuration is that both processes can take place under their Optimal conditions. Alternating, the temperature could elevate the production, but when exposed to temperatures higher than their optimal temperature, yeasts morphology and physiology changes and cell damage can occur, decreasing cell viability and yeast metabolism. Hence, high temperature leads to a reduction of ethanol concentration, yield, and productivity. However, the optimized condition would be about 28 to 37^{o}C. (Branco et al. 2018)

The use of SSF in the synthesis of ethanol from primary sludge. *S. cerevisiae* outperformed the thermotolerant *Kluyveromyces marxianus* in batch fermentation experiments with a carbohydrate content of 50 g/L (Mendes et al. 2017). Batch-fed mode allowed for larger solid loading and lower enzymatic load, resulting in more ethanol produced but lower ethanol yields. Fed-batch SSF of two different PPMS reported that sludge from virgin pulp manufacturing was viscous, resulting in lower ethanol concentration and yield than sludge from corrugated recycled paper (Boshoff et al. 2016). Batch SHF of primary sludge without any pretreatment, an optimized strategy based on statistical experimental design experiments to improve enzymatic hydrolysis performance and to get a successful subsequent fermentation by *S. cerevisiae*. (Peng and Chen 2011)

4. DAIRY INDUSTRY EFFLUENT:

The dairy industry is one of the most polluting industries, not only in terms of effluent volume but also in terms of its characteristics. It yields between 0.2-10 Liters of effluent per liter of processed milk, with an average output of approximately 2.5 liters of wastewater per liter of processed milk.(Raghunath et al. 2016) Dairy processing effluents are created on an intermittent basis, and their discharge rates vary greatly.

Whey is a waste product produced in substantial quantities by the dairy industry (about 10 l/kg of cheese output). It is an organic waste that is high in proteins (6-8 g/l), peptides, lipids, minerals, vitamins, and, most importantly, lactose (4-6%). Whey is produced in vast quantities all around the world, with over 108 tons produced each year. It contains a higher amount of lactose, which makes ethanol production a sophisticated effluent treatment method. This process is further aided by the growing global demand for bioethanol.

4.2 MICROORGANISM:

Lactose is a disaccharide that can be fermented only by some microorganisms, that produce both the membrane transporter lactose permease and the hydrolytic enzyme b-galactosidase. *Kluyveromyces marxianus* is a lactose-fermenting yeast with advantageous physiological characteristics such as (i)thermotolerant, (ii) rapid growth rate, (iii)adapts to a wide range of substrates, and many strains have been identified as GRAS (Generally Recognized As Safe)(Lane and Morrissey 2010). The fermentative metabolism is almost entirely tied to oxygen limitation, but it cannot grow under anaerobic circumstances, hence yields are often lower than in *Saccharomyces cerevisiae*. Thermotolerance is an important quality for alcoholic fermentations since it can contribute to cost savings by eliminating the need for cooling equipment and lowering contamination concerns. *K. marxianus* has been observed to produce ethanol at temperatures even above 40^{o}C.

4.3 FERMENTATION:

The fermentation of whey is optimized by two parameters, namely oxygen availability and temperature with the organism *Kluyveromyces marxianus*. The best performances were reached at low temperatures (28^oC), but high temperatures allow good ethanol yields in short times in whey fermentations in particular, batch anaerobic fermentation carried out at 40^oC with high biomass inoculum has shown the best performances(Zoppellari and Bardi 2013). The optimized condition maximized ethanol concentration is 22.2 g L^{-1} after 72 h (Sampaio et al. 2020). However, if the lactose concentration exceeds 10% the ethanol fermentation is lowered(Kourkoutas et al. 2002)

5. SAGO INDUSTRY:

Tapioca cassava also known as manioc, sago and yucca. The Portuguese brought this tuber crop to India in the 17th century. It is the most significant food and industrial crop for emerging nations. Sago industries are the principal consumers of tapioca, which is valued for its starch content. Cassava is a key alternative source of renewable energy that can be used globally to reduce reliance on fossil fuels because it is 15–30% cheaper to produce per acre than corn starch.

The sago pith waste (SPW) or sago 'hampas' is a fibrous starchy lignocellulosic byproduct generated from the pith of Metroxylon sagu (sago palm) after extraction of starch. Malaysia produces 100 to 110 tonnes of SPW per day of starch production. Bioethanol production from lignocellulosic biomass involves different steps such as pretreatment, hydrolysis, fermentation and ethanol recovery The bioethanol production from lignocellulosic biomass involves different steps such as pretreatment, hydrolysis, fermentation and ethanol recovery. Sago pith waste (SPW) serves as a potential substrate for bioethanol production with the contents of 58% starch, 23% cellulose, 9% hemicellulose and 4% lignin [12].

5.2 PRETREATMENT:

The bioethanol production from sago waste biomass involves different steps of pretreatment, such as physical, chemical, physicochemical and biological, which have been studied in the past decade to enhance the ethanol yield (Alvira et al. 2010). Hydrolysis is a crucial step to produce fermentable sugars. Enzymes such as cellulase amylase are used to break the starch and cellulose content in sago waste. This method of conversion is economical and faster way is the greatest concern for commercial fuel ethanol production. Currently, chemical and enzyme hydrolysis are widely employed to breakdown the starch and cellulose into fermentable sugar

One of the emerging pretreatments, Microwave heating presents a potentially faster, more efficient, and more selective method for the thermal treatment of biomass. Microwave pretreatment was considered an energy-efficient approach for biomass pretreatment under low pressure and temperature(Chen et al. 2011). As water is highly effective in microwave energy absorption, the combination of microwaves and hydrothermal conditions accelerated the conversion of starchy lignocellulosic biomass into simpler compounds

5.3 FERMENTATION:

The fermentation of sago waste is mostly batch fermentation under lab-scale studies. The highest ethanol concentration of 15.6 g/L was produced at 48 h fermentation.(Thangavelu et al. 2014) Hydrolysate produced an ethanol concentration of only 3.1 g/L at 60 h fermentation with 72% fermentation efficiency and 0.05 g/L/h productivity. In microwave-assisted pretreated sago waste, the ethanol concentration would be more of 3.9g/l .(Thangavelu et al. 2019)

6. SUGAR INDUSTRY:

India is the second-largest country in terms of sugar production and one of the world's largest sugar-consuming countries(Kushwaha 2015). Sugar, a by-product of sugarcane most important substrate in the human diet. Over 309.9 lakh tons of sugar are produced per year in India(ISMA,2021.). The sugar industry is a seasonal industry that basically works 150 to 210 days per year. However, this sugar industry also generates a significant amount of waste which causes environmental pollution(Poddar and Sahu 2017).

Molasses is a waste product generated during sugar production which has a sugar content of 40 to 50%. Molasses is produced in over 14 million tons per year in India. This molasses is disposed as wastewater into the environment affecting human health and aquatic life. However, this molasses can also be used as a renewable resource for bioethanol production.

6.1 MICROORGANISM:

Over the last decade, intense research has been held on the fermentation of sugarcane molasses with different microorganisms. The most common organism used for commercial-scale fermentation is *Saccharomyces cerevisiae*, which is known as baker's yeast or brewer's yeast. *Zymomonas mobilis,* a gram-negative bacterium is also used in molasses fermentation but this *Zymomonas mobilis* is not used in commercial-scale fermentation since the ethanol yield is lower compared to *Saccharomyces cerevisiae*. Diverse studies were conducted to sort out the issues in fermentation process by using yeast and bacteria(Asif et al. 2015).

6.2 FERMENTATION:

Ethanol production from the sugarcane molasses is optimized with two factors pH and temperature. Molasses fermentation is carried out by both batch and continuous fermentation. Studies were held with pH ranging from 4 to 6 and temperature ranging from 28 to 40^{o}C. *Saccharomyces cerevisiae* has shown more ethanol yield in pH 5.5 and 30^{o}C. If the pH and temperature are altered, the ethanol yield also lowered(El-Gendy et al. 2013). A maximum yield of 225 g/l of ethanol has been achieved with Saccharomyces cerevisiae during the optimization of ethanol production(Asif et al. 2015). While *Zymomonas mobilis* has also shown good ethanol production in pH 4.5(Doelle and Doelle 1990) and 28^{o}C, with a maximum ethanol yield of 7.9 (v/v).

Conclusion:

Pollution of natural, environmental and water resources caused through industrial wastes is becoming a major concern nowadays. Agro-industries contribute 10-15% for the cause of pollution. The major advantage of wastes generated from agro industries is that they can be effectively reused and recycled. Future research priorities would focus on the development of biofuel generation such as biodiesel, economic feasibility, and industrial adaptation of treatment plants. Despite the feasibility, researchers and industries also develop new technologies in the treatment of effluent-like native microorganisms degrading the toxic compounds and more attention in favour of environmental concern

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Table.1 General characteristics of palm oil effluents

Parameter	Values (mg/l)	References

Biochemical Oxygen Demand BOD 3 days- 30°C	25 000	(Chin et al. 2013)
Chemical Oxygen Demand (COD)	44,300–102,696	(Chin et al. 2013)
Total Solids	40,500 – 72,058	(A. Aziz and Hanafiah 2017)
Suspended Solids	400	(Rupani et al.)
Total Nitrogen	224	(Anggamulia et al. 2020b)
pH	5-9	(Rupani et al.)
Temperature	45	(Rupani et al.)

Table.2 Characteristics of PMS source:(Vaez and Zilouei 2020)

Characteristics	**Values (mg/L)**
Chemical oxygen demand (COD)	30000
Biological oxygen demand (BOD)	20500
Total suspended solids (TSS)	4540
Total dissolved solids (TDS)	15808
Total nitrogen	1032.3