# POTENTIAL FOR CONTAMINATION IN FUEL ETHANOL PRODUCTION WITH PROPOSED SPECIFIC GUIDELINE CRITERIA AND EXPERIMENTAL ON ACIDITY REMOVAL

By

MR. Peerawat WONGSURAKUL

A Thesis Submitted in Partial Fulfillment of the Requirements

for Master of Engineering (CHEMICAL ENGINEERING)

Department of CHEMICAL ENGINEERING

Graduate School, Silpakorn University

Academic Year 2021

# โอกาสการปนเปื้อนในการผลิตเชื้อเพลิงเอทานอลและการเสนอแนวปฏิบัติในการควบคุมและการทดลองกำจัดความเป็นกรด

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Ethanol is a promising biofuel that can replace fossil fuel, mitigate greenhouse gas (GHG) emissions, and represent a renewable building block for biochemical production. However, the lack of collective information about quality control of anhydrous ethanol from up-stream to downstream process brings about the first aim of this research is to create understanding about the causes of impurities formation throughout the whole production process (starting from feedstock acquisition) and their effects on subsequent processes (fermentation, ethanol recovery and storage) and on final ethanol properties.

Ethanol can be produced from various feedstocks. First generation ethanol is mainly produced from sugar- and starch-containing feedstocks. For second-generation ethanol, lignocellulosic biomass is used as a feedstock. Typically, ethanol production contains four major steps, including the conversion of feedstock, fermentation, ethanol recovery, and ethanol storage. Each feedstock requires different procedures for its conversion to fermentable sugar. Lignocellulosic biomass requires extra pretreatment compared to sugar and starch feedstocks to disrupt the structure and improve enzymatic hydrolysis efficiency. Many pretreatment methods are available such as physical, chemical, physicochemical, and biological methods. However, the greatest concern regarding the pretreatment process is inhibitor formation, which might retard enzymatic hydrolysis and fermentation. The main inhibitors are furan derivatives, aromatic compounds, and organic acids. Actions to minimize the effects of inhibitors, detoxification, changing fermentation strategies, and metabolic engineering can subsequently be conducted. In addition to the inhibitors from pretreatment, chemicals used during the pretreatment and

fermentation of byproducts may remain in the final product if they are not removed by ethanol distillation and dehydration. Maintaining the quality of ethanol during storage is another concerning issue. Initial impurities of ethanol being stored and its nature, including hygroscopic, high oxygen and carbon dioxide solubility, influence chemical reactions during the storage period and change ethanol's characteristics (e.g., water content, ethanol content, acidity, pH, and electrical conductivity). During ethanol storage periods, nitrogen blanketing and corrosion inhibitors can be applied to reduce the quality degradation rate, the selection of which depends on several factors, such as cost and storage duration. This comprehensive review part sheds light on the techniques of control used in ethanol fuel production, and also includes specific guidelines to control ethanol quality during production and the storage period in order to preserve ethanol production from first generation to second-generation feedstock. Moreover, the understanding of impurity/inhibitor formation and controlled strategies is crucial. These need to be considered when driving higher ethanol blending mandates in the short term, utilizing ethanol as a renewable building block for chemicals, or adopting ethanol as a hydrogen carrier for the long-term future, as has been recommended.

In the case study of Fakwantip Co. LTD, Thailand, off-spec ethanol can be treated with anion resin exchange to remove excess acidity. The static and dynamic adsorption capacity show maximum values of 91.01 and 87.84 mg acidity/g resin, respectively. Thomas model offer the highest correlation coefficient ($R^2$ between 0.9826 - 0.9915) indicating that the model is appropriate for predicting the breakthrough curve. The obtained important adsorption parameters were further employed for the design calculations of large scale.

# ACKNOWLEDGEMENTS

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MR. Peerawat WONGSURAKUL

# TABLE OF CONTENTS

# LIST OF TABLES

**Page**

# LIST OF FIGURES

Page

# Chapter I

# Introduction

## 1.1 Motivation

Climate change is a major problem that all countries in the world have been facing. The concern about climate change leading to the establishment of the Paris agreement aims to resolve and deal with climate change effects. Other important objectives are to limit the rising of global temperature and reduce greenhouse gas emissions [1]. Thailand is one of many countries that have signed to cooperate in agreement. In order to follow the agreement, Thailand intends to reduce GHG emission by 110-140 million tons of carbon equivalent, or 25 percent of emissions in 2015 by 2030. This results in promoting biofuels production and utilization, increasing energy efficiency in power generation, transportation, construction, and industries [2].

Ethanol is biofuel that is one of the solutions to reduce GHG emission in the transportation sector. Thai government intends to support production and utilization under the Alternative Energy Development Plan (AEDP). In 2015, ethanol utilization target was set to 4.1 million liters per year by 2037. However, the new revision of AEDP of ethanol utilization target has been reduced to 2.4 million liters/year since 2018.

The vehicle engine composes of various materials such as plastic, metallic, and polymeric materials in the fuel tank, fuel pump, engine, and exhaust system [3] [4]. Ethanol contamination can cause the fuel to become very corrosive and swell certain elastomers [5]. Thus, ethanol for gasoline blending must meet the anhydrous ethanol specification to ensure sufficient quality when it is used in vehicles, not harmful, and environmentally friendly because of low emission of pollutants from fuel combustion [6] [7] [8]. Impurities influence ethanol characteristics such as acidity, pHe, water content, and electrical conductivity. They are from the production feedstock or occur during ethanol production and storage. In countries that produce or use ethanol, ethanol quality is set by the organization of each country [9]. Table 1

compares the anhydrous and hydrated ethanol specifications of some countries, including United states, Brazil, Thailand, and the European Union, which consists of 28 countries. It can be noticed that fuel ethanol specifications used to control ethanol quality are different due to the market, climatic conditions, and raw material used in ethanol production [8]. In 2007, Tripartite Task Force was established by cooperation between Brazil, the European Union, and the United States to harmonize the specification among their countries [10] [8]. There is a difference in water content specification between different countries which relies on ethanol-gasoline blending ratio and the method of gasoline transportation. Only EU has a phosphorus specification, based on ethanol producers. USA and Brazil agreed to collect phosphorous levels in their products to consider the adoption of phosphorus specification. There are differences in the inorganic chloride standard. US and EU will review this specification to lower the limit closer to the Brazil limit. Brazil's ethanol standard provides criteria for electrical conductivity, while US and EU standards do not. However, US and EU will soon consider introducing conductivity criteria. In Thailand, anhydrous ethanol specification can be categorized into 3 major applications: denatured ethanol for gasohol production (TIS 2324), ethanol for pharmaceutical use (TIS 640-1) and ethanol for industrial use (TIS 640-2). When compared to EU, USA, and Brazil, Thailand does not include sulfate limitation in anhydrous ethanol for blending with gasoline. The maximum permitted sulfate in the USA, Brazil, and EU specification are 4, 4, and 3 ppm, respectively. For USA, 4 ppm is sulfate limitation for E10 fuel which is agreement between refining, automotive, and ethanol industries. Thus, this limitation may be updated in the future due to the increasing ethanol concentration in ethanol-blended gasoline [11] [12]. Hence, Thailand should include sulfate specification in the future when ethanol demand increases.

There has been more attention in 2$^{nd}$ generation ethanol owing to the conflict between food and fuel. However, it contains higher impurities than 1$^{st}$ generation ethanol. Some scientific confirmation is needed to prove that which

impurities in lignocellulosic ethanol can cause an adverse effect on vehicle engine performance. This finding could lead to the adoption of new specifications or the revision of existing ones to make them more compatible with 2nd generation ethanol. According to the literature review, phosphorus should be limited in fuel ethanol to protect automotive catalyst systems from deactivation if ethanol is produced from non-traditional feedstocks. Since sources of phosphorus in ethanol include fertilizers, nutrients used in the fermentation process, and the feedstock itself if it is non-traditional [10] [13]. Acetic acid in ethanol has the most impact on ethanol acidity, causing more corrosive to automobile engines. Since the acetic content of lignocellulosic ethanol is more than that of 1st generation ethanol [14], it is challenging for ethanol producers to meet the required standards. However, separation by ion exchange resin proposed by Lv, Sun [15] can be applied to remove acetic acid from 2nd generation ethanol. Furthermore, lignocellulosic ethanol contains a significant amount furanic substance. The remaining of furanic compound in ethanol-gasoline blending fuel can lead to lower oxidative stability and the possibility for the formation of dangerous organic peroxides [16].

For anhydrous ethanol for pharmaceutical purposes, the limitations of non-volatile materials, benzene, acetaldehyde, acetal, and any other volatile impurities are included in the specification. If lignocellulosic ethanol will be used for pharmaceutical purposes, the separation technique should be improved to remove these impurities especially acetaldehyde and acetal [17].

Habe, *et al*. [14] reported impurities in 17 different types of bioethanol samples. They concluded that lignocellulosic-derived ethanol contains the highest impurities than sugar- and starch-derived ethanol because lignocellulosic feedstock requires a pretreatment to modify lignocellulose structure and improve the accessibility of enzymes and chemicals. Lignocellulosic ethanol has high concentrations of acetic acid, acetaldehyde, methanol, and furan. On the other hand, these contaminants are lower in sugar- or starch-derived ethanol. Considering

sulfur-containing compounds, dimethyl disulfide and thiazole are only found in lignocellulosic derived ethanol. In contrast, dimethyl sulfide and dimethyl sulfoxide are sulfur-containing compounds in sugar- and starch-derived ethanol.

In addition to the type of feedstock and production process, storage procedure also has an influence on ethanol quality. Naegeli, Lacey [18] concluded that decreasing fuel ethanol pH over storage periods correlates to ethyl sulfate formation, which also increases ethanol conductivity. During ethanol distillation, sulfite, a fermentation byproduct, is carried over with ethanol vapors. Then sulfite can be oxidized to sulfate along storage periods. Recently, the sulfate contamination issue has gained interest due to its effect on the vehicle engine. Many studies have reported that the contamination of sulfate causes deposit formation on inlet valves in combustion chambers and on injector tips [18] [12] [19] [11].

Although the investigation on the impurities in the final fuel product has been received much attention [14] [20] [21] [22], there are a few researches focusing on impurities occurring throughout the production process, and only some previous published documents attempting to set the guideline to control blended gasoline quality during storage periods [23] [24]. The lack of collective information about quality control of anhydrous ethanol from upstream to downstream process brings about the first aim of this thesis is to create understanding about the causes of impurities formation throughout the whole production process (starting from feedstock acquisition) and their effects on subsequent processes (fermentation, ethanol recovery and storage) and on final ethanol properties. Finally, specific guideline to control ethanol quality which covers anhydrous ethanol production till storage periods can be proposed.

Among the impurities in ethanol, weak acid is very important contaminant in ethanol. Ethanol has limits for weak acidity (as acetic acid) and strong acidity (pHe). Weak acidity may affect long-term durability, whereas strong acidity may generate rapid corrosion. Electrical conductivity reflects metallic ions, such as chloride, sulfate,

sodium, and iron. Inorganic chlorides are corrosive towards metals [25] [26]. To determine the causes of changing in these parameters could bring the solution for solving the problem related to acidity in Fakwantip ethanol plant.

**1.2 The objective of this study**

- To propose specific guideline to control ethanol quality which covers anhydrous ethanol production till storage periods.
- To determine the cause of increasing of ethanol acidity at Fakwantip ethanol storage tank.
- To develop the method for acidity removal from anhydrous ethanol for using at the industrial level.

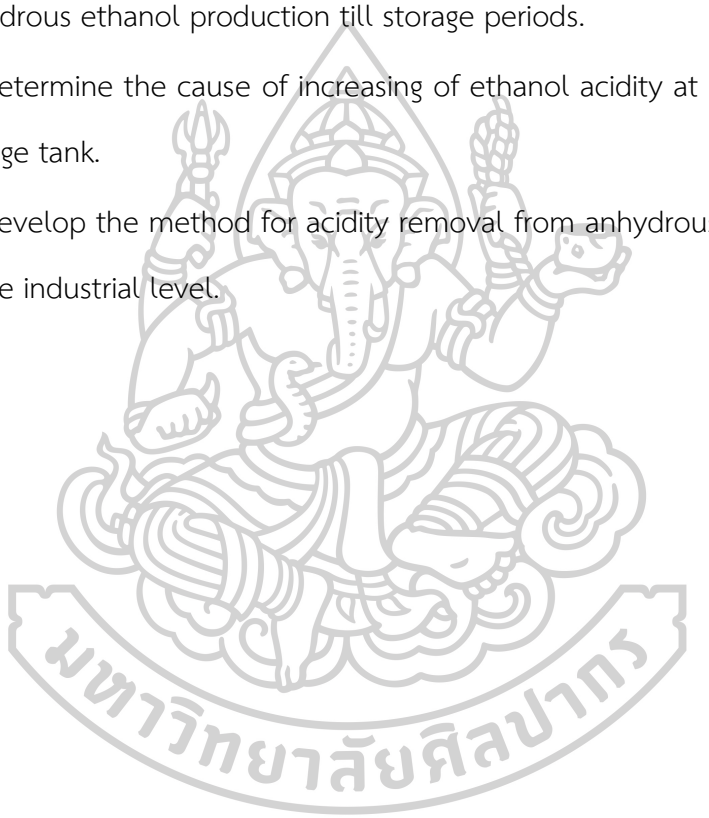

**Table 1** Comparison of anhydrous and hydrated ethanol specification [27] [28] [29] [30]

| Specification | Unit | | European Union | USA | Brazil | Thailand | | |
|---|---|---|---|---|---|---|---|---|
| | | | prEN 15376 | ASTM D-4806-16a | ANP Resolution n° 19 | TIS 2324 | TIS 640-1 | TIS 640-2 |
| **Ethanol type** | - | - | Anhydrous | Denatured anhydrous | Anhydrous | Denatured anhydrous | Anhydrous | Anhydrous |
| **Ethanol** | %(v/v) | Min. | - | - | 98 | - | - | - |
| **Ethanol and higher saturated alcohols** | % by volume, (% by mass) | Min. | (98.7) | 92.1 | (99.3) | 99 | 99.5 | 99.5 |
| **Higher saturated mono-alcohols-C3-C5** | % by volume, (% by mass), [ml/kl] | Max. | (2) | - | 3 | 2 | - | - |
| **Methanol** | % by volume, (% by mass), [ml/kl] | Max. | (1) | 0.5 | 0.5 | 0.5 | [200] | 0.05 |
| **Water content** | % by volume, (% by mass) | Max. | (0.3) | 1 | (0.7) | 0.3 | - | - |
| **Density at 20°C** | kg/m3 | Max. | - | - | 791.5 | - | 790-793 | - |
| **Total acidity (as acetic acid)** | mg/L, (% by mass), [mg/kg] | Max. | (0.007) | 56 (0.0070) [70] | 30 | 30 | 30 | (0.005) |
| **Electrical conductivity** | µS/m | Max. | - | - | 300 | 500 | - | - |

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| Specification | Unit | | European Union | USA | Brazil | Thailand | | |
|---|---|---|---|---|---|---|---|---|
| | | | prEN 15376 | ASTM D-4806-16a | ANP Resolution n° 19 | TIS 2324 | TIS 640-1 | TIS 640-2 |
| pHe | - | | - | 6.5 ~ 9.0 | - | 6.5 ~ 9.0 | - | - |
| Copper | mg/kg, (mg/l) | Max. | 0.1 | 0.1 | 0.07 | 0.07 | - | - |
| Inorganic chloride | mg/kg, (mg/L) | Max. | 1.5 | 6.7 (5) | 1 | (20) | - | - |
| Solvent-washed gum | mg/100 mL | Max. | - | 5 | - | 5 | - | - |
| Sulfur | mg/kg, (ppm) | Max. | 10 | (30) | Report | - | - | - |
| Total sulfate | mg/kg | Max. | 3 | 4 | 4 | - | - | - |
| Phosphorus content | mg/L | Max. | 0.15 | - | - | - | - | - |
| Non-volatile material | mg/100 mL, (mg/l), [% by mass] | Max. | 10 | - | 5 | - | (25) | [0.005] |
| Denaturant content | vol. % | Max. | - | 1.96 ~ 2.5 | - | - | - | - |
| Iron | mg/kg | Max. | - | - | 5 | - | - | - |
| Benzene | ml/kl | Max. | - | - | - | - | 2 | - |
| Acetaldehyde and acetal (as acetaldehyde) | ml/kl, (% by mass) | Max. | - | - | - | - | 10 | (0.10) |
| Any other volatile impurity (as | ml/kl | Max. | - | - | - | - | 300 | - |

**Table 1** Comparison of anhydrous and hydrated ethanol specification [27] [28] [29] [30]

| Specification | Unit | | European Union | USA | Brazil | Thailand | | |
|---|---|---|---|---|---|---|---|---|
| | | | prEN 15376 | ASTM D-4806-16a | ANP Resolution n° 19 | TIS 2324 | TIS 640-1 | TIS 640-2 |
| 4-methylpentan-2-ol) | | | | | | | | |
| Absorbance<br>- Lower than 240 nm<br>- 250 to 260 nm<br>- 270 to 340 nm | | Max. | - | - | - | - | 0.4<br>0.3<br>0.1 | - |
| Sodium | mg/kg | Max. | - | - | 2 | - | - | - |
| Permanganate time | Minute | Min. | - | - | - | - | - | 15 |
| Aspect | - | | Clear and colorless | Clear and colorless | Clear and no impurities | Clear, colorless and no visible suspended solids | Clear and colorless | Corresponding to ISO 2211 |

# Chapter II

# Literature review

This chapter provides information from the integration of various research data to illustrate 5 main topics: 1. Contamination in ethanol in ethanol production processes, 2. Potential cause for *Fakwantip* ethanol plant, 3. Sulfate contamination in ethanol-blended gasoline, 4. Removal of sulfate from ethanol, and 5. Feasibility study of sulfate removal by anion exchange resin

## 2.1 Ethanol production from different feedstocks

In Thailand, ethanol is produced from 2 types of agricultural plants. There are cassava and sugarcane (in the form of sugarcane juice and molasses). The amount of ethanol products from molasses, cassava, and sugarcane juice accounts for 65%, 30%, and 5%, respectively. Sugar- and starch-containing feedstock can be considered as 1st generation ethanol production feedstock. Later, an increase in fuel demand and concern on potential negative risks of using food feedstock leads to the utilization of lignocellulosic feedstock for fuel ethanol production in the 2nd generation technology. Ethanol production processes from any feedstocks can be divided into three main steps, which are: (1) converting feedstock into fermentable sugar, (2) fermentation process to convert fermentable sugar to ethanol, and (3) ethanol recovery process as shown in

. Although the production feedstocks are different, the fermentation and ethanol recovery processes are significantly similar. Hence, when considering the different feedstocks, the difference in contaminations is mainly affected by the feedstock conversion stage to fermentable sugar [31].

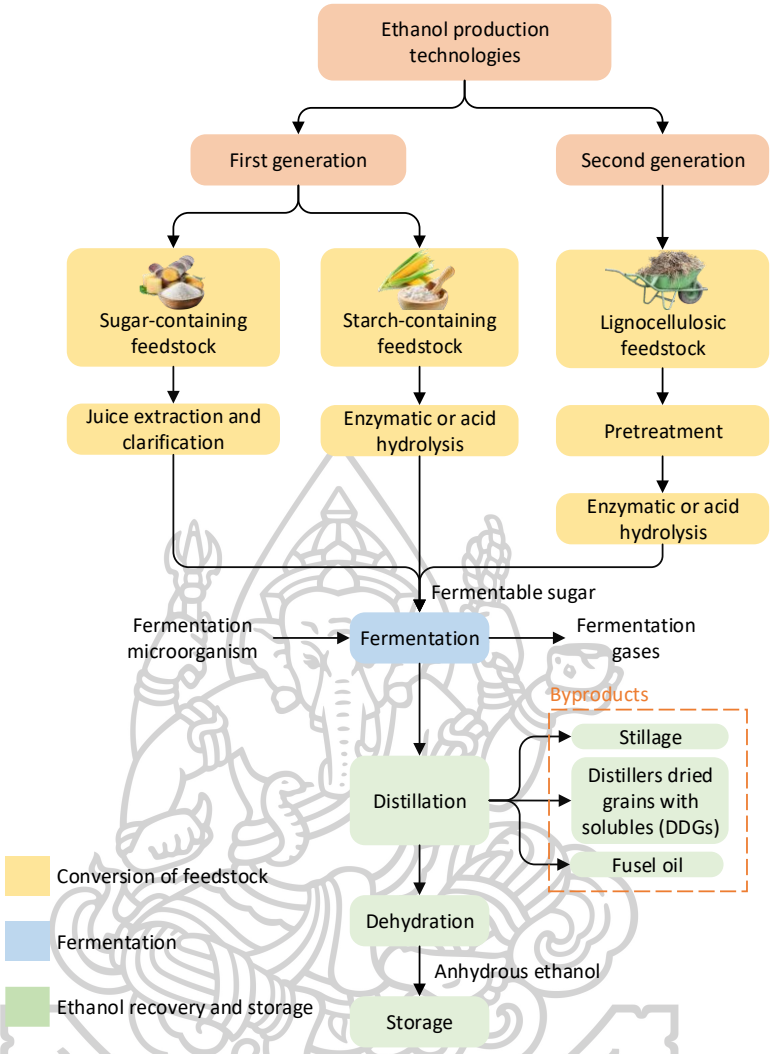



**Figure 1** Ethanol production routes from different feedstocks

### 2.2 Impact of different feedstocks on impurities in fuel ethanol

As mentioned previously, the ethanol production process from each type of feedstock includes three major steps: conversion of feedstock, fermentation, and ethanol recovery. This section separately describes the conversion of each feedstock. The key process is to release sugar molecules from the feedstock structure. The difficulties in releasing sugar molecules depend on feedstock type, which involves different required steps to convert feedstock, and consequently results in various contamination profiles in the ethanol product.

### 2.3 Conversion of sugar-containing feedstock

In many countries, such as Thailand, Brazil, India, and Colombia, sugarcane is cultivated for sugar production [32] [33]. The valuable byproduct from sugar production is molasses, which is used in ethanol production. Besides, sugarcane juice is also utilized to produce ethanol in some countries such as Thailand [32] [34] [35]. Therefore, the sugar production process needs to be considered as it determines the quality and impurities of the feedstock for the ethanol production.

Attached and autonomous distillery are two types of sugarcane-derived ethanol production plants, classified by ethanol feedstocks. The overall production process and chemical addition in each step for these two categorized sugarcane-derived ethanol production plants are shown in Figure 2. In the case of autonomous distillery, the process section in the dashed blue box can be excluded.

#### 2.3.1 Attached distillery

The attached distillery mainly produces sugar from sugarcane juice, while molasses appears as a byproduct. In the case of the attached distillery, molasses can be considered as the primary feedstock for ethanol production. However, sugarcane juice can be allocated between sugar and ethanol production, depending on the product demand [36] [37] [32]. The production process of the attached distillery is illustrated in a schematic diagram shown in Figure 2.

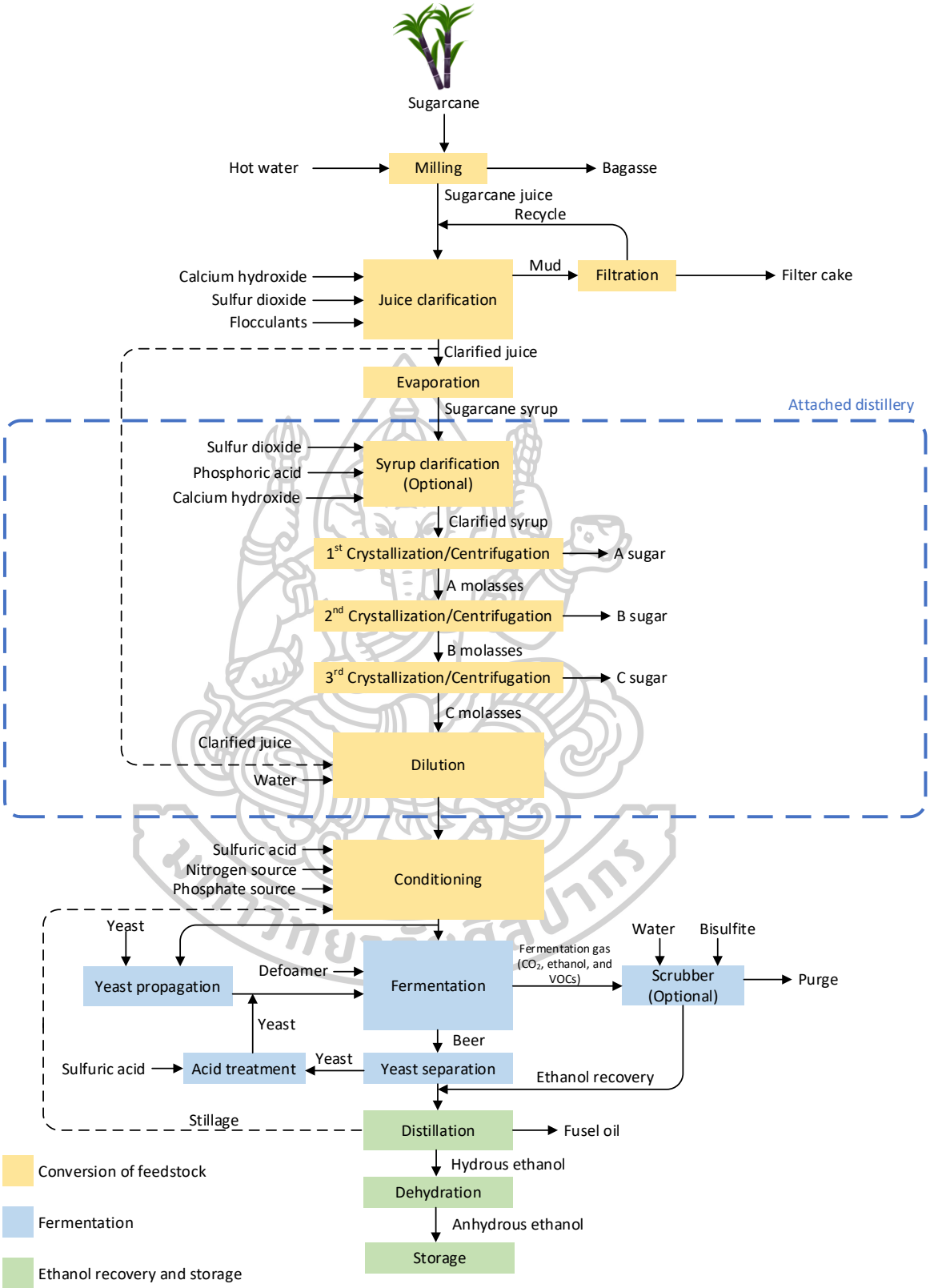



**Figure 2** Type of sugarcane derived ethanol production plant

### 1. Sugarcane plantation and harvesting

Sugar production from sugarcane begins with plantation. In this stage, Gilbert, Shine Jr [38] reported that the main climatic factors influencing cane crops are rainfall, temperature, and sunlight. Besides, Cardona, Sanchez [33] also described that the composition of sugarcane depends on the cultivated condition. Most variations in sugarcane composition are based on the difference in moisture content, sugar, and ash.

Harvesting of sugarcane can be done by two methods, including manual harvesting and mechanical harvesting. Thai and Doherty [39] found that the sugarcane harvesting method influences the chemical composition of cane juice. Almost all manually cultivated sugarcane fields are burnt before harvesting. The composition of the burnt cane differs significantly from the non-burnt cane. Non-burnt cane juice contains a higher proportion of soluble inorganic ions and ionizable organic acids than burnt cane juice. In addition to the harvesting method, harvesting age is another factor affecting the juice extraction method, which will be discussed in the further section.

After harvesting, sugarcane must be processed into ethanol production quickly because sucrose losing has been reported relating to invertase activity and proliferation of acid, ethanol, and polysaccharides (dextran) producing microbes. Besides, biodeterioration can occur related to delays between harvesting and milling. Biodeterioration also relates to other factors such as ambient temperature, humidity, cane variety, storage period, invertases activities, and maturity status [40].

As shown in Figure 3, the average composition of sugarcane can be simply classified into 86.7% broth and 13.3% fiber. Generally, most fibers are separated priorly in the juice extraction process for electricity generation. Broth consists of 69.7% water and 17% soluble solids. Mostly, soluble solid contains 15.35% sugar and some non-sugar, which is removed in the juice clarification step. Sugar comprises

non-fermentable sugar and fermentable sugars necessary for fermentation such as sucrose, glucose, and fructose [33] [41].

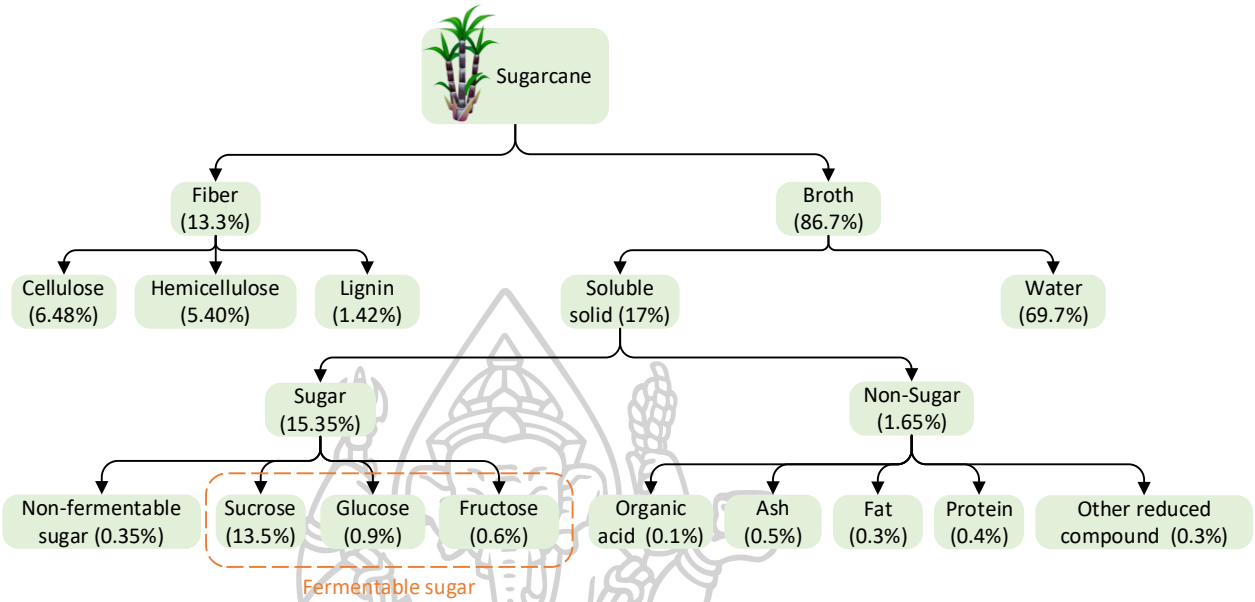



**Figure 3** Average sugarcane composition, modified from: [33] [41]

**2. Juice clarification**

Raw juice is obtained from the extraction. It contains various impurities such as minerals, salts, organic acids, dirt, and fiber particles [42]. In this step, raw juice is fed through the clarification process with sulfur dioxide addition to eliminate bacteria. The clarification process includes 3 steps: coagulation, flocculation, and precipitation [43]. In the first step, coagulation, lime (calcium hydroxide) is added to neutralize and alleviate the loss of sucrose content due to sucrose inversion. Then limed juice is heated to coagulate colloids particles. Proteins and polysaccharides are adsorbed on colloidal particles. In the flocculation step, calcium from lime reacts with phosphate in sugarcane into calcium phosphate. Calcium phosphate particles are involved in the formation of flocs which are responsible for the removal of impurities. In the precipitation step, flocs are precipitated in the clarifier tank as mud [44]. Mud is separated from clarified juice as a filter cake by vacuum rotary filters. The sucrose concentration in clarified juice is approximately 10-15% [45].

**3. Evaporation**

The primary purpose of evaporation is to remove water from clarified juice. However, there are some differences between the case of autonomous distillery and attached distillery. In the autonomous distillery, the evaporation step is carried out before the fermentation process to adjust juice concentration to achieve an appropriate concentration and diminish the required energy for distillation [46] [42]. A multiple-effect evaporator is employed to increase clarified juice concentration. As a result, the obtained sugarcane syrup concentration is approximately 60-70 °Bx [46] [47].

However, in the attached distillery, evaporation is performed before the crystallization and centrifugation steps. Since the clarified juice contains large amounts of water, 75% of water is removed with the multiple-effect evaporator. The achieved steam or condensate from this step could be reapplied in other process steps. After the water has been removed, sugarcane syrup with 60 °Bx concentration is fed to a vacuum evaporator and centrifuged to produce sugar and yield a byproduct as molasses in the further step [42] [33].

**4. Crystallization and centrifugation (For attached distillery only)**

During the crystallization step, excess water in sugarcane syrup is removed by the vacuum pan. Seeding with sucrose crystal is necessary to form sugar crystals in the mother liquor. The mixture of sugar crystals and mother liquor is called Massecuite [48] [33]. Then, sugar crystals are separated from the mother liquor by centrifugation. After crystallization and centrifugation, the raw sugar and C-molasses (final molasses) are yielded as feedstock for ethanol production.

**5. Dilution (For attached distillery only)**

In the attached distillery, molasses is needed to be diluted before fermentation. It is not appropriate to use as the fermentation medium directly because of high osmotic pressure on yeast cells. In the attached distillery, molasses should be diluted by clarified juice or water below 25 °Bx because high osmotic

pressure effect yeast metabolism or decrease yeast viability [33] [49] [50]. Adjusting pH and elimination of bacteria by using sulfuric acid are also significantly needed [33] [48] [51]. The obtained molasses from sugar production is a dark-brown viscous liquid. When considering molasses composition, it contains up to 50% of soluble carbohydrates such as sucrose, D-glucose, and D-fructose. The major components excluding carbohydrates are calcium, potassium, and magnesium salts, such as magnesium chloride and magnesium sulfate. The minor constituents include cuticle wax, sugarcane fats and sterols, plant phenolics, polysaccharides, aconitic, plant pigments, amino acids and proteins, inorganic ions (such as sodium-ion, iron, aluminum), silicon compounds, and trace metals [48].

- **Water used in the dilution step**

For ethanol production, water quality is a crucial factor in the production process since water is the main component of fermentation media for yeast [52]. So, the dissolved constituents in added water can significantly affect the ethanol production process and ethanol quality.

Dissolved constituents, usually found in surface water and groundwater, can be divided into major, minor constituents, and trace constituents. The major constituents with concentrations higher than 1.0 - 1000 mg/L are Ca, Mg, Na, Cl, Si, $SO_4^{2-}$, $H_2CO_3$, $HCO_3^-$, while other minor constituents with a concentration between 0.01 - 10 mg/L are B, K, F, Sr, Fe, $CO_3^{2-}$, $NO_3^-$. Whereas Al, As, Ba, Br, Cd, Co, Cu, Pb, Mn, Ni, Se, Ag, Zn, and others are dissolved with the trace amount lower than 0.1 mg/L [53] [54].

Iowa State is the highest ethanol production state in the United States. There was research which quote that ethanol production relies on water quality in municipal wells pumped from Cambrian – Ordovician groundwater sources. Water samples contain high amounts of chloride and sulfate: at concentration of 160 – 230 mg/L and 560 – 720 mg/L, respectively. Besides chloride and sulfate, it also contains

other dissolved constituents (such as Ca, Na, K, $HCO_3^-$, $CO_3^{2-}$, Cl, $SO_4^{2-}$, F, $SiO_2$, Fe) [55].

High concentration of dissolved constituents can cause osmotic stress which negatively affects the function of yeast cells in the production process. Variation in water quality can have a significant impact on yeast's growth rate and consequently conversion efficiency. To avoid this problem, the water quality utilized in the fermentation must be carefully monitored. The common parameters for testing of water are pH, nitrate, nitrite, and trace elements. The indication of polluted water by sewage or animal waste can be determined from the concentration of nitrate and nitrite salts. When they are higher than 50 ppm, it would be advisable to avoid using this water in fermentation process.

However, fermentation medium property after dilution with process water is more important and straightforward for yeast growth. For example, many types of yeast can grow in a pH range of 4 to 6.5. The minimum and marginal concentration of dissolved in fermentation medium will be summarized and discussed in Section 4.1.

**6. Conditioning**

Sucrose-containing feedstocks, such as sugarcane juice and molasses, can contain substances which can inhibit microorganisms for converting sugar to alcohol. However, there is a difficulty in predicting the composition of sucrose-containing feedstocks because of several related factors such as cultivation techniques, sunlight, weather conditions, fertilizers, water availability, and harvesting method [33]. The concentration of inhibitors in feedstock is difficult to control. To improve fermentability of feedstocks, inhibitors in feedstock should be removed or diluted before fermentation.

- **Synthetic zeolites**

Synthetic zeolites are conventionally applied for eliminating inhibitory substances [56] by their ionic exchange and adsorption properties. When zeolites are added to the fermentation system, $Na^+$ is mostly found in the fermentation medium as an inhibitor that can be removed through ion exchange resin by replacing $K^+$ - containing zeolite [57] [56]. Potassium salt was found less inhibitory than sodium salts [58]. Besides, zeolites also serve as a pH regulator during fermentation and maintain cellular viability and metabolic activities [59].

- **Antiscalant**

Sucrose-containing feedstocks can contain ash. In particular, sugarcane molasses feedstock consists of 10-16% ash [60]. Cardona, Sanchez [33] claimed that more than 10% of ash content can cause scale problems which occur in pipelines and distillation towers. Antiscalant or scale inhibitor is chelating compounds. It can be applied to water or molasses beer to reduce scale formation in heat exchangers or distillation columns by preventing calcium sulfate formation [33] [49].

- **Nitrogen source**

Nitrogen source plays a vital role in fermentation because inadequate nitrogen can slow down sugar utilization because nitrogen functions in protein synthesis and sugar transport. [61]. Thus, starting feedstocks for ethanol production should contain not only sufficient carbon sources but also other nutrients, such as free amino nitrogen (FAN), mineral, vitamin, and other growth factors [62], which are essential components for yeast health and efficiency.

High nitrogenous materials may be present in the fermentation medium, but they occur in a complex form that yeast cannot consume unless being hydrolyzed into amino acids, dipeptides, or tripeptides. Nitrogen that can be used as a nutrient source for yeast during the fermentation process is called free amino nitrogen (FAN) [63] [64] [65] [66]. Depending on feedstock, fermentation media sometimes contains

a small amount of FAN, which is insufficient and needs to provide additional amino nitrogen source. An insufficiency of FAN decreases yeast growth and reduces fermentation efficiency, leading to prolonged fermentation time [64] [67] and generation of hydrogen sulfide [68]. To release more FAN from soluble protein, protease is also added into the fermentation medium. [33].

In addition to FAN, ammonium sulfate can be a nitrogen source for yeast [69]. However, the addition of ammonium sulfate may lead to sulfate salt precipitation in automotive fuel injector.

Urea is a more preferable nitrogen source for ethanol fuel fermentation [11] [19] [33]. In terms of economics and yield, urea is the best option. Urea does not only improve the ethanol yield and decrease the formation of byproducts, but it also increases the specific growth rate and capacity to tolerate ethanol [70]. In contrast, urea is unsuitable for alcohol fermentation in beverage production because of carcinogenic ethyl carbamate formation [62].

- **Phosphate source**

In the fermentation process, phosphate insufficiency leads to decreased cell growth rate. Typically, phosphate is necessary for nucleotide, phospholipid, and metabolite biosynthesis. Addition of di-ammonium phosphate as a phosphorous source could reduce the requirement of urea [33] [71].

### 2.3.2 Autonomous distillery

The autonomous distillery usually feeds all sugarcane to produce ethanol [32], which is different from the attached distillery in that this plant does not produce sugar. Therefore, this type of distillery employs sugarcane juice as the primary feedstock. The feedstock conversion process for autonomous distillery can be described in Figure 2 with the exception of the dashed blue box step.

### 2.4 Comparison of contamination between an attached distillery and autonomous distillery in sugarcane-based feedstock

Brazil is the world's largest ethanol producer. Most of the ethanol production plants are attached distillery. This type of distillery allows the producer to take advantage of the synergy between sugar and ethanol [72]. In terms of production feedstocks in each type of ethanol distilleries, the autonomous distillery uses only sugarcane juice as a feedstock for ethanol production. In an attached distillery, molasses is considered as primary feedstock. Sugarcane juice is sometimes used in parallel with molasses.

Considering impurities in feedstocks, molasses has higher impurities, such as inorganic salts, unfermentable sugars, sulfated ash, and pigment, than sugarcane juice as it is further contaminated during the sugar production process [33] [73]. Molasses composition depends on the sugarcane juice extracting process. Sulfur dioxide is usually added as a preservative when extracting cane juice from young sugarcane. which possibly remains as sulfite in ethanol product because of difficulty to remove in the distillation stage [74] [75]. Due to high impurities in molasses, Khoja, Ali [76] have studied the effect of impurities in sugarcane molasses on fermentation. They reported that impurities in molasses may influence enzymatic activity. Ethanol yield can be improved by using some enzyme stabilizers or some agents/additives, which alleviate the effects of impurities.

### 2.5 Conversion of starch-containing feedstock

Ethanol production from starch-containing feedstocks, such as corn kernels and cassava, can be classified into two processes: (1) wet milling process and (2) dry milling process, as presented in Figure 4. The major difference between these two methods is that the wet milling process has been developed to separate high-value products from the starchy feedstock, while the latter does not.

The wet milling process is applied for corn grain feedstock because it provides high-value products, such as corn gluten meal, corn gluten feed, and corn

germ meal, which are usually applied as poultry feed. However, the drawbacks of wet milling process include high capital cost, high energy consumption, and less ethanol yield. Dry milling process is then chosen as an alternative way for corn grain feedstock.

The dry milling process is appropriate for cassava chips feedstock in ethanol production because cassava chips do not provide high-value components [6].

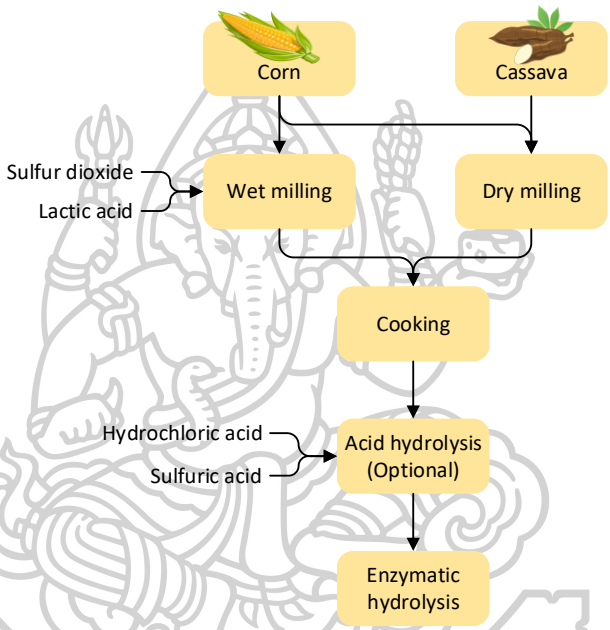



**Figure 4** Conversion of starch-containing feedstock

### 2.5.1 Wet milling distillery

In general, wet milling process is applied for corn grain because it contains high-value components. Corn grain contains around 70-73% starch, 9-10% protein, 9-10% crude fiber, 4-5% fat, 1-2% ash, 2% sugar [77].

As shown in Figure 5, wet milling process begins with cleaning and soaking corn kernel in a steeping solution consisting of sulfur dioxide and lactic acid [78]. The role of steeping is to soften corn kernel, break down protein coating starch particles, and remove some soluble constituents. Then soft corn kernel is milled with a corn degerminator, and then corn germ is separated by the liquid cyclone. The rest from the separation process, the degermed ground kernels, is washed, ground, and

screened to remove fiber. The centrifuge separates protein as a corn gluten meal (CGM) from the free fiber starch slurry. Steep liquor obtained from evaporated steep water is mixed with corn fiber or together with condensate soluble to achieve corn gluten feed (CGF) [79] [80]. After completing the component fractionation, the starchy slurry is finally delivered to cooking and enzyme hydrolysis processes [6].

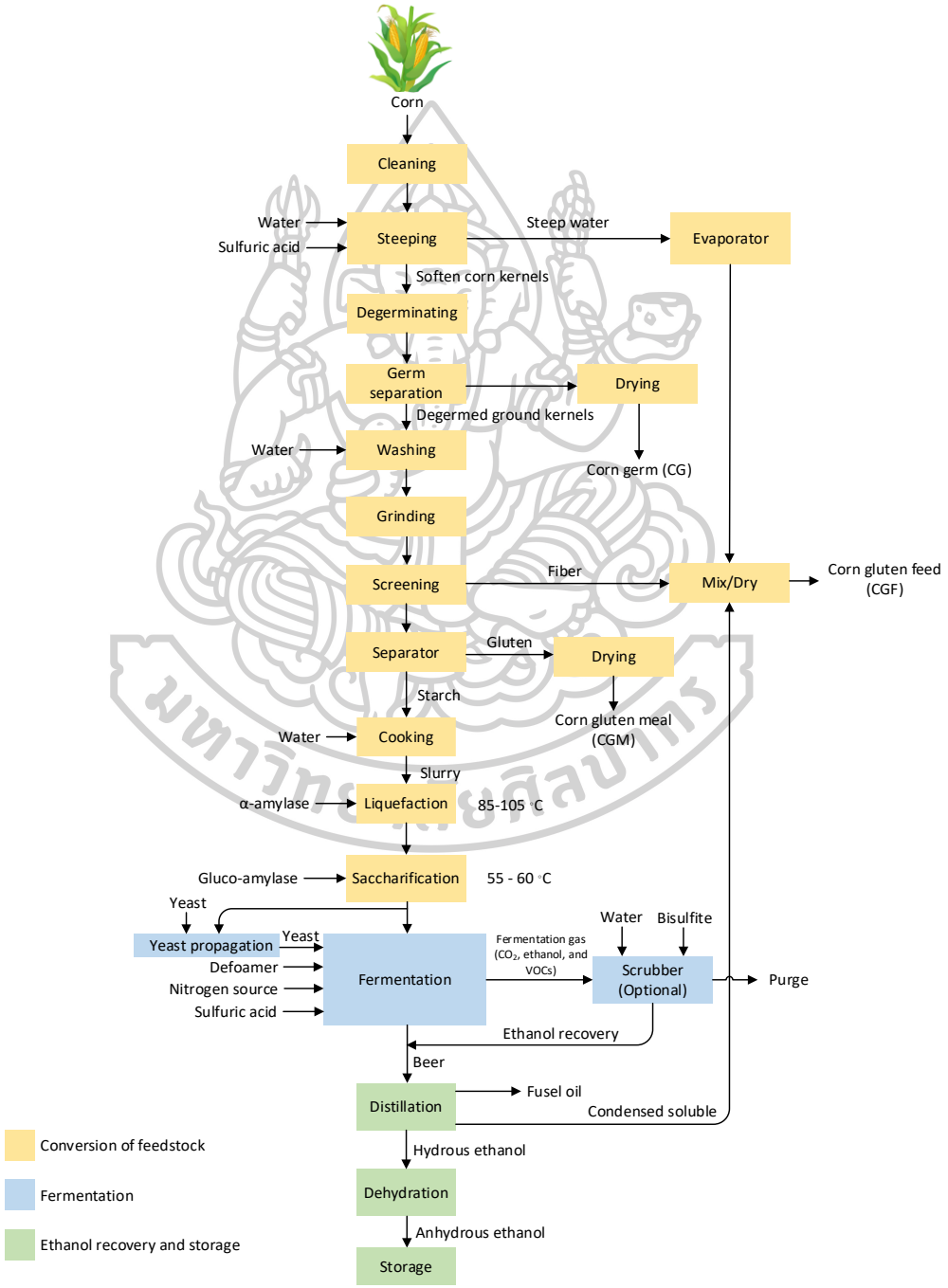



**Figure 5** Schematic diagram for corn wet milling process

### 2.5.2 Dry milling distillery

In Thailand and China, ethanol production from cassava usually operates through the dry milling process mainly carried out in the batch regime, requiring less capital and energy costs because there is no need to fractionate the valuable products. The steps of this process are shown in Figure 6.

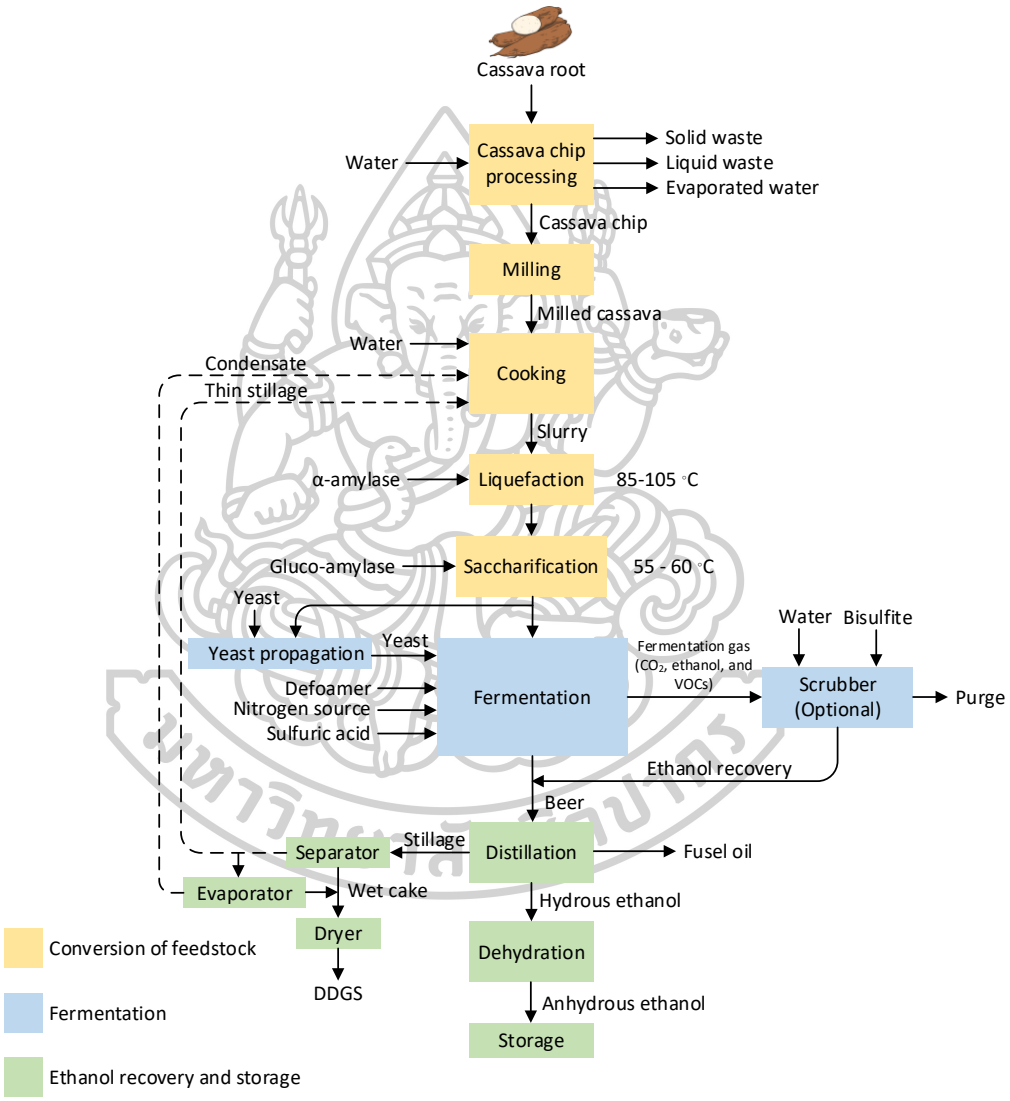



**Figure 6** Conventional process for ethanol production from cassava

1. **Cassava chip processing**

Cassava is a starch-containing plant that has low cost and offers high potential for ethanol production. Under appropriate conditions, cassava is one of the highest

ethanol yields per unit land area crop when compared with other ethanol crops such as sugarcane, carrot, sweet sorghum, corn, wheat and rice [81]. Furthermore, ethanol production from cassava requires a non-complex process with less equipment costs [82].

Various cassava forms, such as fresh root, cassava chip, and cassava starch, can be fed to ethanol production. Fresh cassava has high moisture content at approximately 60-70% based on a wet basis. This moisture in fresh cassava affects deterioration.

Cassava contains sulfur compounds in amino acid forms such as cysteine and methionine. Then, this sulfur concentration increases by a factor of 2-3 times during the ethanol-production process. However, nearly all are removed from the ethanol product stream by separating into distiller's dried grains with soluble (DDGs) fraction as shown in Figure 6 after the dryer step [83] [19].

Cassava fresh roots are used as raw materials after harvesting through cleaning, washing, peeling, and chopping into cassava chips [84] [33]. Later, chips are distributed on the cement floor and exposed to sunlight for 2–3 days to reduce moisture content. For safe storage, moisture content should be less than ca. 14 wt.% [85]. Cassava chips with low moisture content have a longer shelf life and lower volume, making them easier to transport [86]. Finally, obtained cassava chips are then processed to the feedstock preparation step [6] [82] [84] [33] [87].

**2. Milling**

In the dry milling step, cassava chips are sent to the hopper and metal detector and then crushed and sieved to obtain fine flour [6] [82] [33].

**3. Cooking**

Cassava starch is a polysaccharide that requires degradation to glucose. Initially, it is necessary to gelatinize starch via the cooking process in excess boiled water above the gelatinization temperature [88] [6]. Cooking with excess water assists starch granule destruction, yielding more soluble and susceptible glucose polymers

to enzyme hydrolysis in the next procedure [6]. However, α-amylase enzyme can be added for liquefaction at above 85 ℃ simultaneously with gelatinization [88].

**4. Starch hydrolysis process**

During the hydrolysis process, water and enzyme break down the polymer chain into fermentable sugar. This can be carried out via two techniques: enzyme hydrolysis and acidic hydrolysis [89] [90].

- **Enzyme hydrolysis**

Enzyme hydrolysis has two following steps. It starts with liquefaction, followed by saccharification.

In the liquefaction step, an α-amylase enzyme is used for hydrolysis of α-1,4 glycosidic linkage in amylose and amylopectin of gelatinized starch into dextrin, maltose, and maltotriose [91]. The optimum liquefaction temperature depends on feedstock type. For example, cassava is ca. 85 ℃ [92]. After the liquefaction step, the temperature of the liquefied slurry is decreased before entering the saccharification process, ca. 60 ℃ in the case of cassava feedstock [92].

In the saccharification, glucoamylase enzyme is used for hydrolysis α-1, 4 and α-1, 6 glycosidic linkages of dextrin into glucose [93] [94].

- **Acidic hydrolysis**

Though the enzyme hydrolysis is typically employed for starch-containing feedstock, acidic hydrolysis can be performed to break down starch molecules into fermentable sugar [95]. One study carried out by Candra, Kasma [96] conducted hydrolysis of grated cassava by employing 0–1.0 M sulfuric acid and hydrochloric acid at 100 ℃, 1 bar for 30 min. The result showed that sulfuric acid offers higher hydrolysis efficiency than hydrochloric acid. The optimum concentration of sulfuric acid and hydrochloric acid resulted in a yield of reducing sugar of 28.20 and 25.60%,

respectively. However, the addition of hydrochloric acid during pretreatment could lead to high chloride remaining in fuel ethanol [97].

## 2.6 Comparison of ethanol production between dry milling and wet milling

Even though ethanol has been produced by wet and dry milling for long period, the comparison of impurities in ethanol obtained from the different techniques is scarce. However, the difference in these two processes is that they generate the different impurities in ethanol product. Generally, wet milling is suitable for food grade ethanol production. In corn wet milling, corn components are firstly separated, which results in lower impurities. In case of corn dry milling, the cyclic and heterocyclic compounds are generated from lignin in the corn hull. Some of these volatile byproducts are still remained in the distillate, causing unpleasant flavors and harmful ethanol [98].

Due to unconverted starch and cellulose fraction in dry milling process, Ramirez-Cadavid, Kozyuk [99] improved commercial-scale corn dry milling for ethanol production using controlled flow cavitation (CFC) and cellulose hydrolysis. This improvement resulted in a significant increase in ethanol yield.

## 2.7 Conversion of lignocellulosic feedstock

The 1$^{st}$ generation of ethanol production uses sugar and starch as feedstocks because they are easily converted into ethanol. However, the 2$^{nd}$ generation allows producing ethanol from lignocellulosic biomass. Its abundance and ability to grow in several areas drive lignocellulose to be the promising feedstock for ethanol production [100].

Lignocellulosic biomass can be divided into many categories: agricultural residues, agro-industrial residues, hardwood, softwood, herbaceous biomass, cellulosic wastes, and municipal solid waste [33]. Lignocellulosic biomass comprises cellulose (40-60% of total dry weight), hemicellulose (20-40%), and lignin (10-25%) [31] [101] with some acids, various minerals, and extractives [33] [102].

### 2.7.1 Lignocellulose composition

Lignocellulosic biomass can be divided into many categories: agricultural residues, agro-industrial residues, hardwood, softwood, herbaceous biomass, cellulosic wastes, and municipal solid waste [33]. Lignocellulosic biomass comprises cellulose (40-60% of total dry weight), hemicellulose (20-40%), and lignin (10-25%) [31] [101] with some acids, various minerals, and extractives [33] [102]. Different types of lignocellulosic biomass have different chemical compositions affecting the yield and the amount of substrate produced during the pretreatment stage, the size of the equipment, and the energy requirements [103]. Lignocellulosic biomass with heterogeneous structure requires more complicated processes than uniform raw materials [104].

1. **Cellulose**

Based on different crystallinity order, cellulose has two regions: amorphous and crystalline. Amorphous cellulose nano-fibrils arranging disorderly is a linear polymer chain of beta glucose monomers connected by β(1,4) glycosidic linkage [102] [101] [33]. However, cellulose chains linked by hydrogen bonds between repeating chains or different chains leads to high crystallinity cellulose nano-fibrils regions, which is more difficult to hydrolyze, as seen in Figure 7. The amount of crystalline regions in cellulose nano-fibrils relies on source of cellulose and can be referred as crystallinity index (CI).

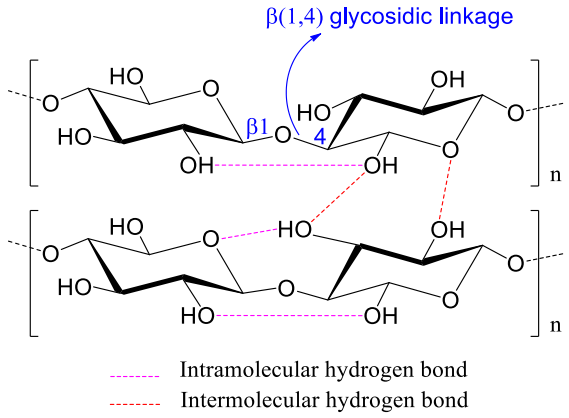



**Figure 7** High crystallinity cellulose structure due to H-bonding

## 2. Hemicellulose

Hemicellulose is a branched-chain polymer that consists of 200 different types of sugar, mainly pentose and hexose. Pentose sugars include xylose, arabinose, whereas hexose sugars include galactose, glucose, and mannose. The rest are other carbohydrate-related compounds such as glucuronic, methyl glucuronic, and galacturonic acids [33] [105].

Hemicellulose backbones consist of a similar type of sugar (Homo-polymer) or different sugar types (Hetero-polymer). They are considered as amorphous region, which is easy to be hydrolyzed [102].

Hemicellulose compositions are depended upon the type of plants. In hardwood, hemicellulose compositions mainly contain xylans [106]. Xylans backbones consist of many xylose molecules. Each xylose molecule is connected by β(1,4) glycosidic linkage. It can also be linked to the methyl gluconic acid and arabino furanose via α(1,2) glycosidic linkage and α(1,3) glycosidic linkage, respectively [33, 107].

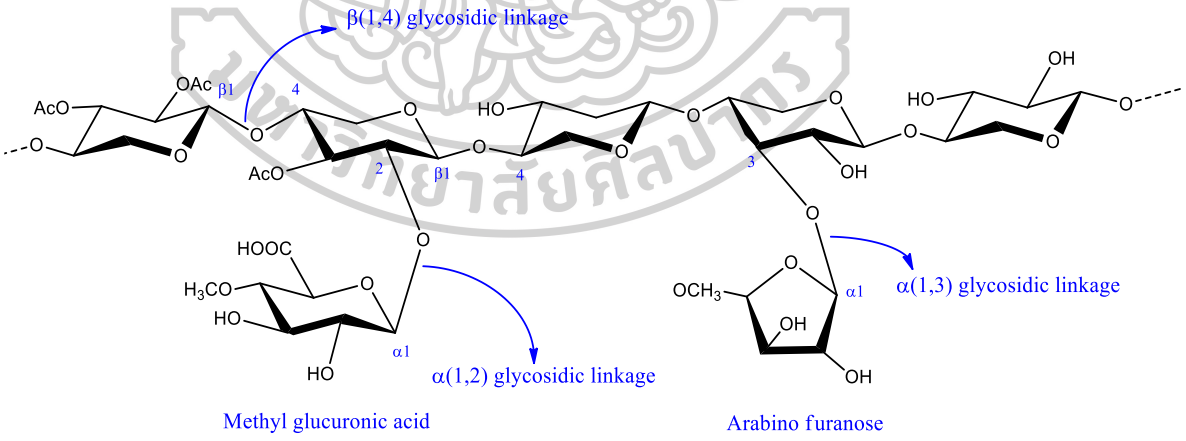



**Figure 8** Xylans structure

However, in softwood, hemicellulose compositions mainly consist of glucomannans [106] [108], which most of their structure are linear polymers and a minor part of branched-chain. Glucomannan linear backbones structure is comprised

of D-mannose and D-glucose connected by β(1,4) linkage with the additional acetyl groups randomly attached to the 6th carbon position [109] [110] [111]. However, in branched polymers, they are connected to glycosyl and mannosyl via β(1,3) linkage.

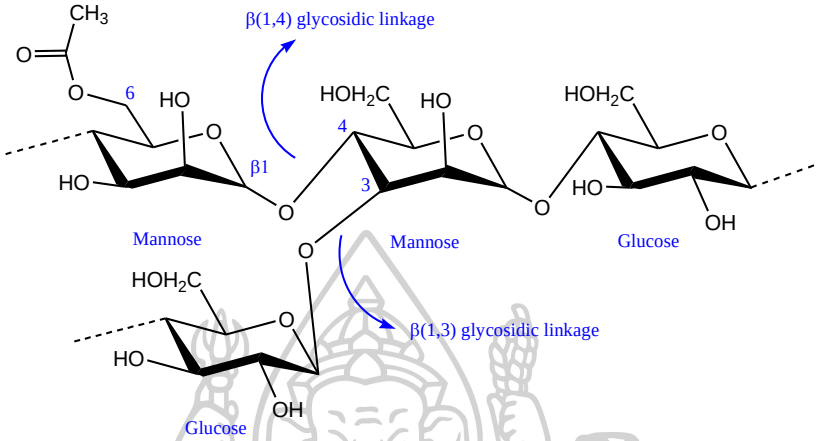



**Figure 9** Glucomannan structure

### 3. Lignin

Lignin is a complex phenolic polymer initiated by the polymerization reaction of monolignols, including coniferyl alcohol, sinapyl alcohol, and P-coumaryl alcohol [101]. These three acetyl alcohols are derived from units of guaicyl (G), syringyl (S), and p-hydroxyphenyl (H), respectively [33] [112]. The difference in the proportion of guaicyl, syringyl, and p-hydroxyphenyl are based on plant types [113] [101].

- In hardwood, lignin is mainly composed of guaicyl, syringyl, and a small amount of p-hydroxyphenyl.
- In softwood, lignin contains mainly guaicyl with little p-hydroxyphenyl.
- In grasses, the proportion of guaicyl is close to syringyl, whereas p-hydroxyphenyl proportion is higher than in hardwood.

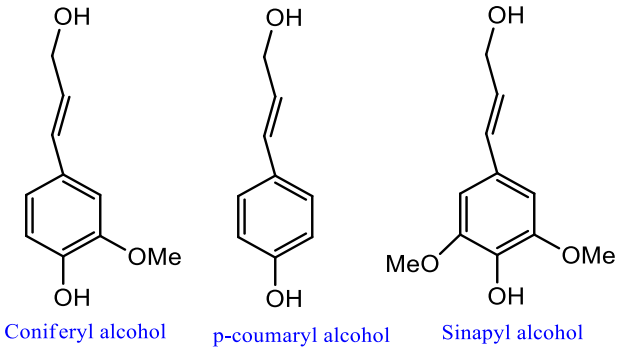



**Figure 10** Structure of monolignol composed lignin

**4. Extractives**

Extractives are natural compounds in biomass that can be extracted by polar or non-polar solvents (e.g., ethanol, water, acetone, benzene, toluene, dichloromethane, and hexane). The major compositions of extractives are phenolics, fats, waxes, and terpenes. However, the minority are some proteins, gums, resins, simple sugars, starches, essential oils, pectin, mucilage, glycosides and saponins, fatty acids, sterols, and flavonoids [114] [115].

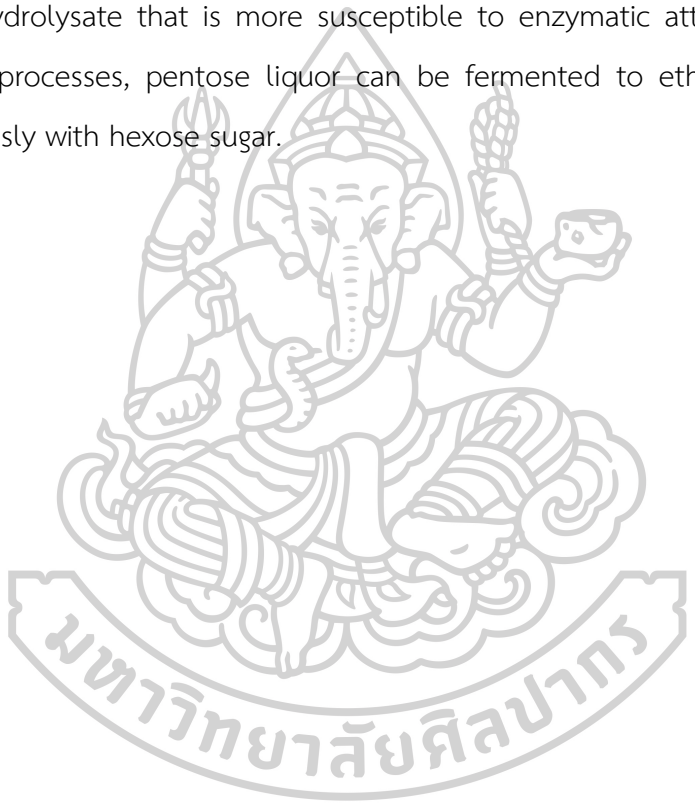
**5. Ash**

Ash is usually considered as a residual after lignocellulosic biomass has been incinerated. Its content in biomass is depended lignocellulosic on the types of lignocellulosic biomass. Major elements in the range of concentration between 1,500 - 280,000 ppm are found in woody biomass ash and aligned in order Ca > K > P > Mn > Fe > S. While minor elements which concentration less than 400 ppm are aligned in order Zn > Cu > Ni > Cr > Pb > As [116].

### 2.7.2 Ethanol production from lignocellulosic biomass

Figure 11 shows ethanol production from lignocellulosic biomass using steam explosion pretreatment. Steam explosion pretreatment is a majority of the pretreatment used in commercial lignocellulosic ethanol production [117]. Sulfuric acid is widely used as a catalyst to improve the rate of hydrolysis and reduce sugar degradation [118]. Steam explosion solubilizes hemicellulose fraction into pentose

sugar and inhibitors. The solid fraction contains mainly lignin and cellulose as called cellulignin. The separation of lignin can be done in two different ways. First, lignin is removed after the fermentation process. Thus solid fraction subjected to enzymatic hydrolysis process containing cellulose and lignin, which possibly create a toxic effect on yeasts [119]. In this case, enzymatic hydrolysis produces relatively low yields of sugar. To improve enzymatic hydrolysis efficiency, in a second way, an alkaline delignification step is introduced to remove most of the lignin. It produces high purity cellulose hydrolysate that is more susceptible to enzymatic attack [120]. In some production processes, pentose liquor can be fermented to ethanol separately or simultaneously with hexose sugar.



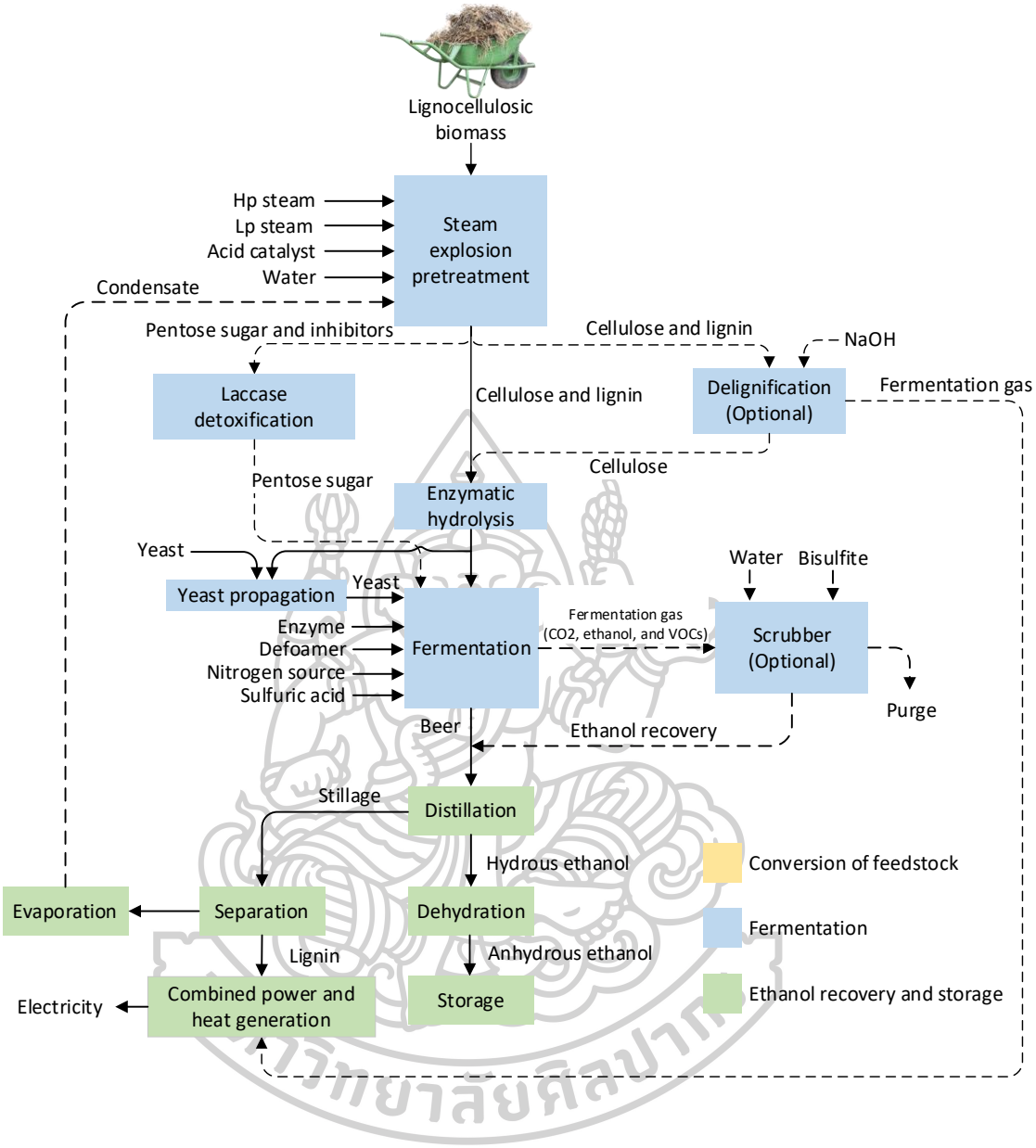



**Figure 11** Ethanol production process from lignocellulosic biomass using steam explosion pretreatment

### 2.7.3 Pretreatment methods

The cellulose part of lignocellulosic biomass is in the form of a microfibril structure surrounded by hemicellulose. In contrast, the lignin part locates in the void between the cell wall, cellulose, and hemicellulose [121] [33] [110]. Lignin in lignocellulosic biomass causes difficulties in bond-breaking and chemical/enzyme

access. Therefore, the pretreatment is essential to separate lignin for improving digestibility and suitability for dissolving cellulose and hemicellulose [110].

Although hemicellulose encapsulating cellulose can be converted into sugar, sometimes approximately 50% of hemicellulose is essential to be removed for increasing cellulose digestibility [122]. However, hemicellulose can be degraded to undesired products, such as furfurals and hydroxymethyl furfurals, which might inhibit ethanol production during fermentation [110].

In the further step, cellulose structure modification is also required to reduce crystallinity and increase chemical accessibility by the pretreatment because it is mostly crystalline which is unable to directly hydrolyze by enzyme [33] [110] [123] [124].

Three purposes of the pretreatment stage are (1) to break down cellulignin [110], (2) to increase amorphous regions of cellulose, making it to be easily hydrolyzed, and (3) to increase porosity which could enhance chemicals and enzymes accessibility. Afterward, cellulose was separated from hemicellulose and lignin [102] [110]. Pretreatment can be classified as physical, chemical, physical-chemical, and biological types [33].

- **Physical pretreatment**

Physical pretreatment is usually operated before other pretreatments. This pretreatment provides a high potential for further hydrolysis process because it focuses on diminishing the particle size, leading to an increase in the contact area, decreasing the degree of polymerization, and reducing crystallization [125] [126] [127] [128].

Physical pretreatment consists of several methods such as milling, microwave radiation, extrusion, ultra-sonication, and pyrolysis. Among these methods, milling is the most frequently applied because it significantly reduces particle size and degree of crystallinity, improving enzymatic hydrolysis efficiency [129].

- **Chemical pretreatment**

Chemical pretreatment is a method to disrupt lignocellulosic biomass structure by chemical reactions. It promotes lignin removal (delignification) to reduce crystallinity and enhance enzyme accessibility [123] [130]. Chemical pretreatments can be classified as acid pretreatment, alkali pretreatment, organosolv pretreatment, ozonolysis, and ionic liquid (ILs) [123].

Acid pretreatment assists in dissolving the hemicellulose part and making cellulose easier accessible for the enzyme. Various acids are used in the acid pretreatment, for example, sulfuric acid, hydrochloric acid, phosphoric acid, and nitric acid. Among these acids, sulfuric acid is the most commonly used [131].

Alkali pretreatment requires an alkali substance. The suitable alkali reagent is sodium hydroxide and lime. However, sodium hydroxide is preferable because of its inexpensive cost and high potential. Using lime causes poor pretreatment performance and also sedimentation [132] [102]. Alkali pretreatment possesses more advantages than acid pretreatment due to less sugar degradation [123] [102]. Alkali compounds inhibit furfural formation and eliminate the acetyl group, which can be hydrolyzed to be acetic acid [102] [133] [115] [134]. However, alkali pretreatment can cause inhibitors in ethanol fermentation. Therefore, the removal step of inhibitors is necessary [123] [41].

- **Physiochemical pretreatment**

Physiochemical pretreatment combines physical and chemical methods for controlling conditions and compounds. This pretreatment method affects the physical and chemical properties of lignocellulosic biomass. Examples of these pretreatments include steam explosion, liquid hot water, ammonia fiber explosion, ammonia recycling percolation, soaking aqueous ammonia, microwave, ultrasound, and carbon dioxide explosion [130] [131].

- **Biological pretreatment**

The principle of biological pretreatment is the usage of microorganisms to dissociate biomass structure. It is an environmentally friendly pretreatment due to its absence of chemicals, less corrosive, less energy consumption, low pretreatment cost, and less possibility to generate inhibitors. However, it provides a slower degradation rate when compared with the other methods [123] [135] [136] [137] [138].

### 2.7.4 Inhibitors formation during lignocellulosic pretreatment

During pretreatment, the more significant and most often appeared inhibitors have been reported due to the dissolution and degradation of hemicellulose and lignin. Additionally, cellulose and extractives can also be converted into inhibitors [115]. The possible inhibitors generated during lignocellulosic pretreatment are visually summarized in Figure 12. Moreover, other details include the reaction/pretreatment types that yield the inhibitors, and the effect of inhibitors are shown in Table 2. This table also presents detoxification methods for each type of inhibitor, which will be further discussed in Section 3.3.5.

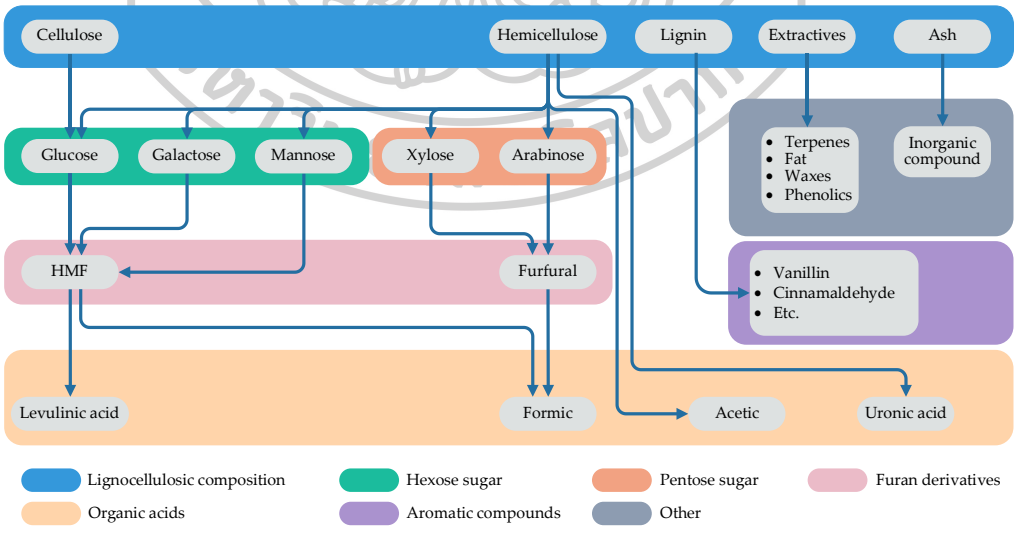



**Figure 12** Inhibitors generated during lignocellulosic pretreatment

**Table 2** Possible generated inhibitors during lignocellulosic pretreatment

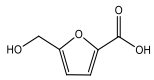
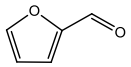
| Compound Type | Compound | Structure | Reaction | Possible methods originated | Effects | Some detoxification methods |
|---|---|---|---|---|---|---|
| Furans derivatives | Hydroxymethyl furfural (HMF) |  | Degradation of hexose sugar [139] [33] | Dilute acid [140] [115] [141], Concentrated acid [115], Steam explosion [142], Liquid hot water [143], Hydrothermal processing [115] | 1. HMF reduces enzymatic and biological activities [144].<br>2. HMF breaks down DNA and inhibits protein and RNA synthesis [144].<br>3. Furfural and HMF synergistically suppressed cell growth [144] [145]. | Adsorption with activated coal [146], pyrochar [147], PEI polymer [148] Nanofiltration [149], Anion exchange resin [150] |
| | Furfural |  | Degradation of pentose sugar [139] [33] | Dilute acid [115] [141], Concentrated acid, Steam explosion [142], Liquid hot water [143], Hydrothermal processing [115] | 1. Furfural reduces enzymatic and biological activities [144].<br>2. HMF breaks down DNA, inhibiting protein and RNA synthesis [144].<br>3. Furfural and HMF synergistically suppressed cell growth [144] [145].<br>4. At the same | Adsorption with activated coal [146], pyrochar [147], PEI polymer [148], Nanofiltration [149], Anion exchange resin [150], Sodium borohydride [151] |

**Table 2** Possible generated inhibitors during lignocellulosic pretreatment

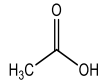
| Compound Type | Compound | Structure | Reaction | Possible methods originated | Effects | Some detoxification methods |
|---|---|---|---|---|---|---|
| | | | | | concentration between HMF and furfural, furfural cause higher inhibitory effect on cell growth than HMF [144]. | |
| Organic acids | Acetic |  | Hemicellulose hydrolysis [139] [33] | Mild alkaline [115], Diluted acid [152] [141], Concentrated acid, Liquid hot water [143], Hydrothermal processing [115], Oxidative [115], Steam explosion [115] [142] | 1. Acetic acid severely inhibits yeast cell growth [153].<br>2. Acetic acid diffuses through yeast. Therefore intracellular pH is reduced [134].<br>3. It decreases ethanol yield [139]. | Nanofiltration [149], Adsorption with PEI polymer [148], Anion exchange resin [150] |
| | Formic |  | Degradation of HMF and furfural [139] [33] | Dilute acid [141], Steam explosion [142], Liquid hot water [143] | 1. Formic acid diffuse through yeast cell leads to a decrease in the intracellular pH [134]. | Adsorption with PEI polymer [148], Anion exchange resin [150] |

**Table 2** Possible generated inhibitors during lignocellulosic pretreatment

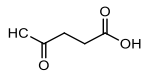
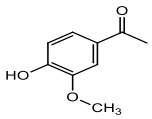
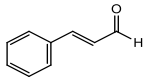
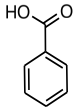
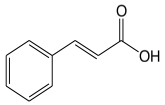
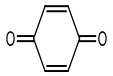
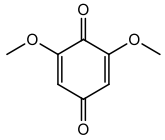
| Compound Type | Compound | Structure | Reaction | Possible methods originated | Effects | Some detoxification methods |
|---|---|---|---|---|---|---|
| | | | | | 2. It decreases ethanol yield [139]. | |
| | Levulinic acid |  | Degradation of HMF [139] [33] | Acid, Steam explosion [142], Dilute acid [141] | 1. The Defusion of levulinic acid through yeast cells leads to a decrease in the intracellular pH [134].<br>2. It decreases ethanol yield [139]. | Adsorption with activated coal [146], Anion exchange resin [150] |
| Aromatic compounds | Vanillin |  | Depolymerization of lignin [139] [115] | Mild alkaline [115], Steam explosion [142] [126] | 1. It may cause negative impact on enzymatic saccharification [115] [154].<br>2. Phenolic compounds damage cell membrane and DNA repair mechanisms [139].<br>3. Yeast growth rate and ethanol productivity are reduced [139]. | Laccase enzyme [155], Peroxidase enzyme [156], Nanofiltration [149], Anion exchange resin [150], Adsorption with activated coal [157] |
| | Cinnamaldehyde |  | Depolymerization of lignin [139] [115] | Mild alkaline [115], Steam explosion [126] | | Laccase enzyme [155], Anion exchange resin [150] |

**Table 2** Possible generated inhibitors during lignocellulosic pretreatment

| Compound Type | Compound | Structure | Reaction | Possible methods originated | Effects | Some detoxification methods |
|---|---|---|---|---|---|---|
| | Benzoic acid |  | Degradation of lignin [158] | Acid [115] [134], Steam explosion [126] | Benzoic acid reduces growth rate and biomass yield [159]. | Not available |
| | Cinnamic acid |  | Degradation of lignin [160] | Acid [115] [134], Steam explosion [126] | Cinnamic acid hindered yeast growth in ethanol fermentation [161]. | Not available |
| | p-benzoquinone (BQ) |  | Oxidation of lignin and lignin-derived compounds [162] | Acid [115] [134], Steam explosion [126] | BQ at 20 to 200 ppm severely inhibited microorganism's cell growth and fermentability [163]. | Sodium borohydride [151] |
| | 2,6-Dimethoxy-1,4-benzoquinone (DMBQ) |  | Oxidation of lignin and syringyl-type compounds [162] | Acid [115] [134], Steam explosion [126] | DMBQ had negative impact on balanced ethanol yield and productivity than on glucose consumption [162]. | Sodium borohydride [151] |

In this work, the major inhibitors during lignocellulosic pretreatment are categorized into furans derivatives, organic acid, and aromatic compounds.

- **Furan derivatives**

Main furans derivatives in lignocellulosic hydrolysate are furfural and HMF. The part of hemicellulose can be hydrolyzed to pentose sugar. Further, pentose can decompose to furfural. Hydrolysis of hemicellulose can be presented in Equation (1) [164].

$$\text{Hemicellulose} \rightarrow \text{Xylan} \rightarrow \text{Xylose} \rightarrow \text{Furfural} \quad (1)$$

Besides, hexose can be dehydrated into HMF [165].

- **Organic acids**

The organic acids are derived from hemicellulose and lignin parts [115]. Acetic acid is a significant hydrolysis product of the acetyl group that can be found in lignin and hemicellulose [166] [167]. Hydrolysis of hemicelluloses backbone also leads to uronic acid formation [115]. Under severe pretreatment conditions, formic and levulinic acid can be obtained as HMF degradation products [115] [166].

- **Aromatic compounds**

Aromatic compounds are classified into 3 groups including 1) phenolic compounds, 2) non-phenolic compounds, and 3) benzoquinone. The aromatic compound is mainly caused by lignin degradation [166].

The first group of aromatic compounds, phenolic aromatic compounds, can be formed mainly during lignocellulosic pretreatment via partial lignin degradation, depending on the pretreatment method. Under alkaline wet oxidation pretreatment cause lignin and carbohydrate degradation to produce some phenolic compounds and furan aldehydes, which can be oxidized into a carboxylic acid (acetic, propionic, formic, etc.) and non-carboxylic, i.e. furoic acid, respectively. The consequence of this oxidation leads to the formation of phenolic acids such as 4-hydroxy phenolic,

vanillic and syringic acids [115] [168] [169]. Moreover, the quantities and types of phenolic compounds also depend on the type of lignocellulosic biomass. In wood acid pretreatment hydrolysate, phenolic compounds are mostly found include 4-hydroxy benzoic acid, 4-hydroxy benzaldehyde, vanillin, dihydroconiferyl alcohol, coniferyl aldehyde, syringaldehyde, syringic acid, and Hibbert's ketones [170] [171] [115]. *p*-coumaric acid and ferulic acid are often found in the pretreated hydrolysate of annual plants e.g. sugarcane bagasse, wheat straw, and switchgrass [169] [172] [173].

The second group of aromatic compounds, non-phenolic aromatic compounds are the phenylic constituents of the lignocellulosic hydrolysates e.g. benzoic acid, benzyl alcohol, cinnamic acid, cinnamaldehyde, 3,4-dimethoxy-cinnamic acid, and para- and ortho-toluic acid [115] [162].

The last group of aromatic compounds is benzoquinone, such as p-benzoquinone and 2,6-dimethoxybenzoquinone, which normally appear during lignin and lignin-derived compounds oxidation [174] [175] [162].

### 2.7.5 Lignocellulosic hydrolysate detoxification

Since the main problem in lignocellulosic pretreatment is the formation of many inhibitors which hinder enzymatic hydrolysis and fermentation yeast, the detoxification can be applied to improve the fermentability of lignocellulosic hydrolysates [151]. There are several categories of detoxification methods such as physical detoxification, chemical detoxification, and biological detoxification [176]. To choose the suitable methods to detoxify in each type of inhibitors, the important key is to identify the potential inhibitors present in the hemicellulose hydrolysates, as provided in Table 2.

- **Physical detoxification**

One example of physical detoxification strategies is vacuum evaporation which can reduce the concentration of inhibitors in lignocellulosic hydrolysates by diminishing the volatile compounds, including acetic acid, furfural, and vanillin [177]. However, this treatment has some drawbacks: (1) increasing of nonvolatile poisonous compounds such as extractives and lignin derivatives, (2) less effective removal of phenolic chemicals, (3) requiring large amount of energy [41] [178].

Membrane utilization is superior to evaporation in that it is easy to scale up because of standard unit composition. The membrane can eliminate metabolic inhibitors such as acetic acid, 5-hydroxymethylfurfural, furfural, formic, levulinic, and sulfuric acid. Membrane adsorption avoids poisonous mixing to microorganisms between hydrolysate and the organic phase (solvent) [41] [176].

Physical detoxification methods are less time-consuming than other detoxification methods, but they provide high sugar loss, time consumption, high capital cost and operation cost, and environmental concerns [179].

- **Chemical detoxification**

In chemical detoxification, adding the chemical to precipitate and ionize some inhibitors can change the toxicity degree of lignocellulosic hydrolysate. Many adsorbents, such as activated charcoal, ion exchange resin, polyethyleneimine, pyro char, and fly ash, can be applied to reduce toxicity in hydrolysate [166].

Overliming treatment with $Ca(OH)_2$ is the most common among chemical detoxification methods. This method partially removes the phenolic compounds, furfural, and HMF [180]. Compared with NaOH treatment, the overliming method showed better results in increasing fermentability.

- **Biological detoxification**

Biological detoxification is the utilization of enzymes or microorganisms to increase the fermentability of hydrolysate. For enzymatic detoxification, oxidative polymerization is involved in detoxifying low molecular weight phenolic compounds [179]. For example, laccase and peroxidases are useful in removing phenolics from lignocellulosic hydrolysates. In the case of microorganisms, each microorganism can remove specific inhibitors. *T. versicolor* is white-rot fungi that can release laccase and peroxidase enzymes to detoxify acid and phenolic compounds [189], whereas *C. ligniaria* can remove furfural and 5-HMF from corn stover hydrolysate [181].

### 2.7.6 Hydrolysis of cellulose

The microorganism used in cellulose hydrolysis is called *Zymomonas mobilis* bacteria. It functions in converting lignocellulosic biomass to ethanol. As described before, it can be categorized as enzymatic hydrolysis and acidic hydrolysis.

1. **Enzymatic hydrolysis**

In enzymatic hydrolysis, cellulase and hemicellulase enzyme are used to depolymerize cellulose and hemicellulose into hexose and pentose sugar. It is preferable more than acidic hydrolysis due to no chemical addition, greater yields and selectivity, less energy consumption, mild reaction conditions, non-toxic, and less corrosive. However, an expensive enzymatic cost and long retention time are still its drawbacks. The high retention time of enzymatic hydrolysis is due to substrate structure and enzyme mechanism [182] [110].

Cellulase enzymes can be categorized into endoglucanase, exoglucanase, and β-glucosidase. Due to hemicellulose complexity, many enzymes can be applied for hemicellulose hydrolysis, for instance, endo-1,4-β-xylanase, β-1,4-xylosidases, β-mannosidase, and α-glucuronidase [9] [122]. The appearance of inhibitors during pretreatment and hydrolysis stages, which are 5-HMF and phenolic compounds derived lignin- i.e., trans-cinnamic acid, 4-hydroxybenzoic acid, syringaldehyde, and

vanillin - could strongly affect enzymatic hydrolysis efficiency by inhibiting cellulase activity [183] [122].

**2. Acidic hydrolysis**

Concentrated acids or diluted acids can hydrolyze lignocellulosic materials.

- **Diluted acid hydrolysis**

In diluted acidic hydrolysis, sulfuric acid is often used at concentrations below 4% to generate monosaccharides by hydrolyzing glycosidic linkages. Dilute hydrolysis can be performed in one (single) or two stages [9] [184].

The single-stage acidic hydrolysis can be conducted using 1.5% acid under 200 - 240 °C, in which the hydrolysis of crystalline cellulose region occurs. This hydrolysis step can generate inhibitors, such as HMF, from glucose degradation. In contrast, furfural and other derivatives compound form by xylose degradation [33]. These chemical compounds inhibit ethanol fermentation and reduce sugar yield [123] [184] [177].

The two-stage hydrolysis is another option of the single stage. It has less possibility to generate inhibitors and sugar degradation [184]. It is initially operated under mild conditions at a temperature of 190 °C with 0.7% acid for 3 min, where the amorphous region of hemicellulose can be degraded to the xylose monomer. Afterward, the cellulose is degraded to glucose under harsh conditions at the temperature of 215 °C with 0.4% acid for 3 min, yielding 50% glucose [33, 124].

- **Concentrated acidic hydrolysis**

Concentrated acidic hydrolysis yields nearly 90% of glucose. According to the economic concern, acid recovery is significantly considered leading to much effort to separate the obtained glucose from acid. There are several techniques to recover acid from acid and sugar mixture solution. Ion exclusion chromatography, solvent extraction, and electrodialysis are the three most studied and best performing methods [185]. In concentrated acidic hydrolysis, 30 to 70% of sulfuric acid is applied

to achieve 90% glucose. The process residence time is between 10 and 12 hours. In this type of acidic hydrolysis, the high-cost reactor with acid resistance and the high energy cost are critically concerned [33].

However, concentrated acid hydrolysis can cause decomposition products: HMF ($C_6H_6O_3$), levulinic ($C_5H_8O_3$), formic acid ($CH_2O_2$), and levoglucosan ($C_6H_{10}O_5$). HMF can occur when three molecules of water dehydrate one molecule of glucose. Levulinic acid and formic acid are formed when HMF re-hydrates with two water molecules. Intense severity acid treatment results in dehydration of glucose to levoglucosan. Forming inhibitors including HMF, levulinic, formic acid, and levoglucosan should be considered since these decomposition products can inhibit yeast activity in the fermentation process [186].

$$C_6H_{12}O_6 \rightarrow C_6H_6O_3 + 3H_2O \quad (2)$$

$$C_6H_6O_3 + 2H_2O \rightarrow C_5H_8O_3 + CH_2O_2 \quad (3)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow C_6H_{10}O_5 + H_2O \quad (4)$$

The distinctive advantage of biological detoxification is its mild operating conditions. Some microorganisms can effectively break down lignin, while cellulose and hemicellulose remain in the substrate. Therefore, the lignocellulosic substrate is easily hydrolyzed to fermentable sugars [41]. Currently, the biological method is gaining interest because of its simplicity, high effectivity, economics, and environmental friendliness [187]. However, prolonged incubation time and high costs of enzymes are still its drawbacks.

### 2.7.7 Other options to mitigate the effect of lignocellulosic inhibitors

- **Changing fermentation strategies**

Fermentation strategies can be changed to reduce the impact of inhibitors on fermentation yeast. In fermentation with lignocellulosic hydrolysate, ethanol productivity is determined by cell-specific productivity and cell mass concentration.

The conversion of inhibitors to less toxic compounds is more efficient at high yeast cell concentration [179] [188].

The mode of fermentation operation is also essential in terms of yeast inhibitory effects. Ethanol fermentation can be carried out in batch, fed-batch, or continuous modes. Fermentation in batch mode results in high inhibitor concentrations since all fermentation materials are fed at the beginning. In contrast, inhibitors can be kept at a low level in fed-batch and continuous modes [188].

- **Microorganism modification**

Metabolic engineering can be applied to increase inhibitory resistance for microorganism. Engineered microorganism has high tolerance to inhibitors by overexpressing genes encoding enzymes such as laccase, furfural reductase, phenyl acrylic acid decarboxylase. Engineered strain can tolerate ethanol and inhibitors [189]. Some engineered microorganism can convert sugar mixture to ethanol [190]. Adaption of microorganism can increase inhibitor tolerance. For example, UV-C mutagenesis has been applied to increase the ability of *Scheffersomyces shehatae* yeast in both glucose and xylose conversion, resulting in improving fermentation efficiency and obtain higher ethanol yield. The adapted strain of *S. cerevisiae* obtained from cell recycle batch fermentation (CRBF) shows higher tolerance with inhibitors and higher ethanol yield than non-adapted strain [191].

## 2.8 Comparative contamination between each type of pretreatments and concerning issues in ethanol production from lignocellulosic biomass

Depending on appropriateness, several pretreatments have been applied to produce ethanol from lignocellulosic biomass. However, the use of different pretreatment leads to different ethanol product characteristics. For example, acid pretreatment bringing about 10,000 - 20,000 ppm of residual acetic acid and 10,000 - 30,000 ppm of furan-related compounds (hydroxymethylfurfural and furfural) can be generated as contamination in pretreated hydrolysate due to sugar degradation [14]

[192]. Table 3 shows a comparison and the concerned issues among different pretreatments.

**Table 3** Concerning issues on different pretreatment methods

| Type of pretreatments | Methods | Chemical/Enzyme addition | Concerns |
|---|---|---|---|
| Physical | Mechanical | No additives | - |
| | Pyrolysis | No additives | • Possible to cause the formation of volatile products (Aldehydes, Phenol, benzene, furan, furfuryl derivatives, and other oxygenated compounds) and char residuals through mild dilute acidic hydrolysis [33] [193] [194] [164]. |
| Physical-chemical | Acid-catalyzed steam explosion | Sulfuric acid, sulfur dioxide, or carbon dioxide | • In severe conditions, cellulose can be depolymerized to form cello-oligomers or oligosaccharides [195] [196].<br>• Possible to cause the formation of HMF from hexose dehydration (glucose) and furfural from pentose dehydration (xylose) [197] [122] [164] [110] [143].<br>• Incomplete destruction of lignin-carbohydrate complex [143] |
| | Uncatalyzed steam explosion | No additives | • Cause sugar decomposition [131].<br>• Inhibitors concentration depends on pretreatment condition severity [198].<br>• Hemicellulose degradation results in the generation of aliphatic acids (acetic acid and formic acid), as well as furans [198].<br>• Lignin is also partially degraded to phenolics [198]. |
| | Liquid hot water (LHW) | Hot water | • Cellulose depolymerization can occur at a certain degree [33].<br>• In high temperatures, pentose can be degraded to form Furfural. Acetyl groups in hemicellulosic polymers can be hydrolyzed to form acetic acid. Hexoses can be decomposed to form 5-hydroxymethyl furfural [199].<br>• High energy and water consumption [143]<br>• Long residence times [143] |
| | Ammonium fiber explosion | Ammonia | • Low or no formation of inhibitors [122] [131].<br>• Cellulose depolymerization can occur at a certain degree [14].<br>• Not suitable for high lignin content materials. |
| | Carbon dioxide explosion | Carbon dioxide | • Low or no formation of inhibitors [33] [131]. |
| Chemical | Ozonolysis | Ozone | • Low formation of inhibitors and xylitol, lactic, formic, and acetic acid were only found in |

**Table 3** Concerning issues on different pretreatment methods

| Type of pretreatments | Methods | Chemical/Enzyme addition | Concerns |
|---|---|---|---|
| | | | hydrolysate [200] [33] [131].<br>● There is no formation of furan derivatives [200]. |
| | Dilute acidic hydrolysis | Sulfuric acid, Hydrochloric acid, Nitric acid, Phosphoric acid | ● Generate inhibitors, such as furfural and phenolic components, and cause gypsum formation [33] [199].<br>● Other inhibitors, such as chloric, phosphoric, or nitrous acids, are formed with the increasing temperature, depending on the hydrolyzing agent [199].<br>● It can increase material and equipment corrosion risk [143]. |
| | Concentrated-acid hydrolysis | Sulfuric acid, Peracetic acid | ● Cause formation of inhibitors such as furfurals, 5-hydroxy methyl furfural, phenolic acids, and aldehydes [131] [126]. |
| | Alkaline hydrolysis | Sodium hydroxide, Calcium hydroxide, Hydrogen peroxide | ● It results in low inhibitors formation [110] [33].<br>● High cost of alkaline catalyst [143]<br>● Long residence times [143] |
| | Oxidative delignification | An oxidizing agent such as hydrogen peroxide, ozone, oxygen, or air | ● Lignin polymer will be converted into carboxylic acids [201]. |
| | Wet oxidation | Water, Sodium carbonate, Sulfuric acid | ● Wet oxidation cause lignin degradation to $CO_2$, $H_2O$, and carboxylic acids [33] [202].<br>● During wet oxidation process, phenolic compounds are degraded to carboxylic acids [184] [203].<br>● Lower production of furfural and HMF compared to steam explosion or Liquid hot water method [204] [202]. |
| | Organosolv process | Organic solvents (Methanol, Ethanol, Acetone, Ethylene glycol, Triethylene glycol), Sulfuric acid, Hydrochloric acid, Ethyl acetate | ● Require removal of solvent [183] [110] [33] [129] [143].<br>● High inhibitor formation [131] [183]. |
| | Ionic liquid (ILs) | 1-Ethyl-3-methylimidazolium acetate, 1-Butyl-3-methylimidazolium chloride | ● The ionic liquid remaining in pretreated materials is toxic to the enzyme and fermentative microorganism [115].<br>● Ionic liquid may produce impurities, including water, halides, and other volatile substances [123] [123] [205].<br>● High solvent cost and require solvent recovery [143] |
| Biological | Fungal | Cellulases, Hemicellulase, Ligninases, Laccase, and quinone-reducing enzymes | ● Low or no inhibitor formation [123] [135] [136]<br>● Long residence times [143] |

**Table 3** Concerning issues on different pretreatment methods

| Type of pretreatments | Methods | Chemical/Enzyme addition | Concerns |
|---|---|---|---|
| | Bio-Organosolv | Ethanol | • Hemicellulose hydrolysis |

### 2.9 Fermentation

In general, sugar conversion to ethanol takes place in a fed-batch fermentation process with a cell recycling system, which recovers yeast cells from the previous batch into the next batch. After adding sugarcane juice into the fermenter, yeasts convert fermentable sugar into ethanol and other fermented byproducts such as carbon dioxide, other alcohols, organic acids. The yeast mostly employed to produce ethanol is saccharomyces cerevisiae [33]. Typically, the fermentation temperature is 30 - 37 °C [206].

#### 2.9.1 Fermentation media

Fermentation media contains a carbon source, water, nitrogen source, micronutrients, and salts [207]. The carbon source in ethanol production is sugar derived from the sac-clarification of different feedstock. Water is the major component of fermentation media [208]. In industrial ethanol production, urea or ammonium sulfate can be added as nitro-gen source. Yeasts require several micronutrients for optimum growth and fermentation performance at quantities typically between 0.1 to 100 mM depending on the yeast strain, fermentation conditions, and interactions with other components [209]. However, salts in the medium can cause osmotic stress on fermentation yeast. In Table 4, the impact of micronutrients and salts on ethanol production are provided along with their minimum concentration required and marginal concentration in fermentation medium that increase osmotic stress to yeast cells at high concentration and induce other adverse effects.

**Table 4** Micronutrients and salts in fermentation medium and their impacts on ethanol production

| Element | Impact on ethanol production | | Concentration in the fermentation medium | |
|---|---|---|---|---|
| | Positive effect | Negative effect | Minimum required | Marginal |
| Potassium ($K^+$) | • Potassium is a major cation involved in the yeast fermentation process [210]. Potassium plays a vital role in divalent cation transport and $H_2PO_4^-$ assimilation [49].<br>• Potassium is typically required at 160 ppm [209]. | • At above 4 – 10 mM of potassium, the fermentation rate could be decreased [49] [209].<br>• Above 10 mM concentration, it show growth inhibition [49] [209].<br>• Total inhibition was observed at about 2 M [49] [209].<br>• Increase osmotic stress to yeast cells at high concentration [211]. | 160 ppm | 400 ppm |
| Magnesium ($Mg^{2+}$) | • Magnesium is a major cation involved in the yeast fermentation process [210]. Magnesium regulates the metabolic enzyme of the fermentation pathway [212].<br>• Magnesium is necessary for the synthesis of DNA and ATP. It also stimulates essential fatty acids synthesizing [209].<br>• Magnesium concentrations of 300 ppm are required for good yeast activity [213].<br>• Magnesium concentrations of 500 ppm can increase yeast tolerance on temperature, ethanol, and osmotic pressure stress [214] [209].<br>• Magnesium concentration in the fermentation medium should be controlled via adjusting the Mg:Ca ratio [215]. Increasing Mg to Ca ratio can | • It can inhibit yeast growth at 1 M [49] [209].<br>• Increase osmotic stress to yeast cells at high concentration [211]. | 300 ppm | 24,000 ppm |

**Table 4** Micronutrients and salts in fermentation medium and their impacts on ethanol production

| Element | Impact on ethanol production | | Concentration in the fermentation medium | |
|---|---|---|---|---|
| | Positive effect | Negative effect | Minimum required | Marginal |
| | increase fermentation performance in terms of the rate and yield of ethanol produced [215]. Anthony and Nwabueze [216] concluded that 2:1 Mg to Ca ratio with Zn supplemented results in maximum ethanol yield at 12.53% v/v. | | | |
| Zinc ($Zn^{2+}$) | • Zinc ions positively affect the respiratory activity and the growth rate of yeast [217].<br>• Zinc is recognized as a major cation involved in yeast fermentation [210]. Zinc is an essential cofactor rapidly assimilated by yeast [49].<br>• At an appropriate concentration, it can increase yeast activity. De Nicola, Hall [218] have reported the optimum $Zn^{2+}$ concentration at 1.5 - 2.5 ppm, depending on yeast strain. | • Excess $Zn^{2+}$ can inhibit yeast growth.<br>• When Mn concentration is below 7 µM, growth inhibition occurs above ~30 µM [49].<br>• When Mn concentration is higher than 7 µM, Zn concentration can be as high as 1 mM before growth inhibition occurs. | 1.5 ppm | 2 or 60 ppm depending on Zn concentration |
| Calcium ($Ca^{2+}$) | • Calcium may not be required, but some evidence may stimulate cell growth. It can also protect membrane structure and help maintain membrane permeability under adverse conditions [49] [209].<br>• The concentration of $Ca^{2+}$ of 4.5 mM is optimum for cell growth [49]. | • Calcium inhibits the transphosphorylases enzyme of the glycolysis pathway, stimulated by magnesium [212].<br>• When $Ca^{2+}$ amount is over 1 mM, it can inhibit amino acid uptake [49] [209].<br>• When $Ca^{2+}$ concentration is over 25 mM, it can inhibit yeast growth depending on yeast strain [49].<br>• Calcium can react with carbonate to form calcium | 150 ppm | 1,000 ppm |

**Table 4** Micronutrients and salts in fermentation medium and their impacts on ethanol production

| Element | Impact on ethanol production | | Concentration in the fermentation medium | |
|---|---|---|---|---|
| | Positive effect | Negative effect | Minimum required | Marginal |
| | • Calcium concentration in the fermentation medium should be controlled by adjusting the Mg:Ca ratio. Increasing the Mg to Ca ratio can increase fermentation performance in terms of the rate and yield of ethanol produced [215]. Anthony and Nwabueze [216] concluded that 2:1 Mg to Ca ratio with Zn supplemented results in maximum ethanol yield at 12.53% v/v. | carbonate scale [49].<br>• Increase osmotic stress to yeast cells at high concentration [211]. | | |
| Manganese ($Mn^{+}$) | • Manganese ions positively affect the respiratory activity and the growth rate of yeast [217].<br>• Yeast cells require manganese as an essential trace element at a concentration of 2–10 μM for optimal yeast growth [219]. | • $Mn^{2+}$ can inhibit cell growth at concentration more than 10 mM [209]. | 0.11 ppm | 550 ppm |
| Iron ($Fe^{2+}$) | • Iron is required as an essential nutrient for yeast, enzyme cofactor [209]. Iron cations are involved in ribosome synthesis, protein translation, replication, and repair [220] [221].<br>• Yeast typically requires 0.17 ppm of Fe2+, which is usually abundant in mash [209]. | • Iron concentration higher than 10-15 mM can inhibit yeast growth [49].<br>• Excess Fe can decrease malate, pyruvate, and succinate dehydrogenase activity [49]. | 0.2 ppm | 500 ppm |

**Table 4** Micronutrients and salts in fermentation medium and their impacts on ethanol production

| Element | Impact on ethanol production | | Concentration in the fermentation medium | |
|---|---|---|---|---|
| | Positive effect | Negative effect | Minimum required | Marginal |
| | • At all levels up to 500 ppm, iron is considered non-toxic [213]. | | | |
| Copper ($Cu^{2+}$) | • Copper ions have a positive effect on the respiratory activity and the growth rate of yeast [217]. Trace amount of copper is an essential enzyme cofactor [49].<br>• The optimal concentrations of $Cu^{2+}$ ions in the nutritive medium for the yeast growth and fermentation activity are in the range of 1–10 μM [219]. | • Copper concentration higher than 1 ppm can inhibit yeast growth, and at 15,000 ppm cell growth completely ceases [209] [213].<br>• Copper affects the changing yeast plasma membrane, leading to low molecular weight compounds' leakage and disturbing nutrients assimilation [49]. | 0.06 ppm | 1 ppm |
| Sodium ($Na^{+}$) | | • High sodium concentration reflects high osmotic stress on the yeast. The specific growth rate is reduced because yeast cell produces intracellular compatible solutes, such as glycerol and arabitol, against $Na^{+}$ diffusion into the cell [49].<br>• At acidic pH, sodium concentration of 5-100 mM can inhibit the enzymatic activity of yeast 38 to 44% [222].<br>• Sodium levels could increase flocs formation during the clarification process [221]. | - | 115 ppm at acidic pH |
| Chloride ($Cl^{-}$) | | • Some nutrients for fermentation yeast can be added in the form of chloride salts such as sodium chloride, potassium chloride, and ammonium chloride [213]. The addition of nutrients in the form of salt shows inhibitory effects on yeast depending on the type of cation (sodium, potassium, and ammonium) [58]. | - | 500 ppm |

**Table 4** Micronutrients and salts in fermentation medium and their impacts on ethanol production

| Element | Impact on ethanol production | | Concentration in the fermentation medium | |
|---|---|---|---|---|
| | Positive effect | Negative effect | Minimum required | Marginal |
| | | • Chloride is considered nondetrimental at all levels up to 500 ppm [213].<br>• Increase osmotic stress to yeast cells at high concentration [211]. | | |
| Sulfate ($SO_4^{2-}$) | | • Some nutrients for fermentation yeast can be added in the form of sulfate salts such as magnesium sulfate, ammonium sulfate, zinc sulfate, calcium sulfate, and copper sulfate [213]. The addition of nutrients in the form of salt shows inhibitory effects and sugar consumption on yeast depending on the type cation (sodium, potassium, and ammonium). Compared to chloride salt, Casey, Mosier [58] suggest that the addition of sulfate salt shows lower inhibitory than chloride salt.<br>• Increase osmotic stress to yeast cells at high concentration [211]. | - | Depending on the cationic of sulfate |
| Fluoride ($F^-$) | | • Fluoride concentrations higher than 160 ppm can inhibit yeast growth [213]. | - | 160 ppm |
| Nitrates ($NO_3^-$) and Nitrites ($NO_2^-$) | | • When the concentration of these salts is higher than 50 ppm, yeast is harmful in the fermentation process [213]. | - | 50 ppm |
| Tin ($Sn^{2+}$) | | • Tin concentrations higher than 360 ppm can inhibit yeast growth [213]. | - | 360 ppm |
| Tellurium (Te) and beryllium | | • A higher concentration of Te and Be than 350 ppm can inhibit yeast growth [213]. | - | 350 ppm |

**Table 4** Micronutrients and salts in fermentation medium and their impacts on ethanol production

| Element | Impact on ethanol production | | Concentration in the fermentation medium | |
|---|---|---|---|---|
| | Positive effect | Negative effect | Minimum required | Marginal |
| (Be) | | | | |
| Nickel (Ni) | | • Nickel concentration higher than 185 ppm can inhibit yeast growth [213]. | - | 185 m |

### 2.9.2 Contamination during fermentation

#### 1) Bacterial contamination

Bacteria can contaminate the commercial ethanol during the fermentation process under poor sterile and pure-culture conditions through instruments, reactors, feed pipelines, chemicals/minerals, and yeast recycling systems [223] [224]. This contamination brings about the formation of acetic acid and lactic acid. It reduces ethanol yield by inhibiting yeast from sugar and minerals utilization, reducing cell viability, causing foam formation, and yeast cell flocculation.[225] [46] [226].

Most of the bacterial contamination in alcoholic fermentation is lactic acid bacteria. Lactic acid bacteria can be classified according to glucose metabolism into two types: homo-fermentative producing only lactic acid, and hetero-fermentative producing ethanol, lactic acid, acetic acid, and carbon dioxide [227] [228] [73] [46].

*Lactobacillus sp.* is lactic acid bacteria usually found in ethanol fermentation because it can tolerate high ethanol concentrations. They can survive in low pH and low oxygen conditions. *Lactobacillus sp.* can produce both lactic acid and acetic acid. They also compete with other yeast cells for nutrients [229] [226].

The source of bacterial contamination in sugarcane is soil [230]. Another source of bacterial contamination is borer. The sugarcane penetrated by borer leads to the accumulation of organic acid and phenolic compounds that can inhibit fermentation [73].

When bacterial contamination occurs during ethanol fermentation, antibacterial agents or antibiotics are required to reduce contamination. Sodium fluoride (NaF) or hydrogen peroxide ($H_2O_2$) can be used as antibacterial agents. Antibiotics, such as virginiamycin and penicillin, are usually employed [224] [231]. However, these antibacterial agents cannot prevent long-term contamination because they can cause drug-resistant strains, reducing the effectiveness of

antibiotics. Moreover, antibiotic utilization causes antibiotic residuals to be left over in byproducts [224] [231].

The increasing of metabolites (lactic acid and acetic acid) resulting from bacterial contamination leads to pH decreasing and acidity increasing during fermentation [229] [225] [15]. Also, produced metabolites inhibit ethanol production [231] [226]. Lactic acid and acetic acid in undissociated form can diffuse through the cell membrane and dissociate to release the hydrogen ion according to (5) and (6). This mechanism can increase the acidity of the yeast cell's cytoplasm, resulting in inhibition of ethanol production [229].

$$C_2H_4OHCOOH \leftrightarrow C_2H_4OHCOO^- + H^+ \quad (5)$$

$$CH_3COOH \leftrightarrow CH_3COO^- + H^+ \quad (6)$$

Yeast flocculation is usually found when contaminated by bacteria. The flocculation results in poor mass transfer, low cell viability, reducing contact surface area between yeast and culture media, and thus reducing ethanol production yield [224] [226]. In Brazil, yeast flocculation can be resolved by treating saccharomyces cerevisiae with sulfuric acid. [232]. However, the use of sulfuric acid can cause contamination in co-product which will be discussed further in Section 4.2.4.

**2) Byproducts generated by yeast**

In ethanol fermentation, glycerol, lactic acid, acetic acid, and succinic acid are major byproducts [233]. However, other byproducts can be generated. Campbell [234] summarized the main byproducts of the fermentation of sugars to alcohol into four groups: Alcohols (ethanol, propanol, butanol, amyl alcohol, glycerol, phenethyl alcohol), Acids (acetic, caproic, caprylic, lactic, pyruvic, succinic), Ester (Ethyl acetate and any other combination of acids and alcohols), and others ($CO_2$, acetaldehyde, diacetyl, $H_2S$).

Sulfite also can be produced by yeast metabolism via sulfate assimilation pathway which yeast consumes sulfate from fermentation medium to produce sulfur-containing amino acids that can also produce sulfite. The amount of produced sulfite depends on the yeast species, fermentation conditions, and sulfur-containing compounds in the fermentation feedstock. The mechanism of the sulfate assimilation pathway is shown in Figure 13.

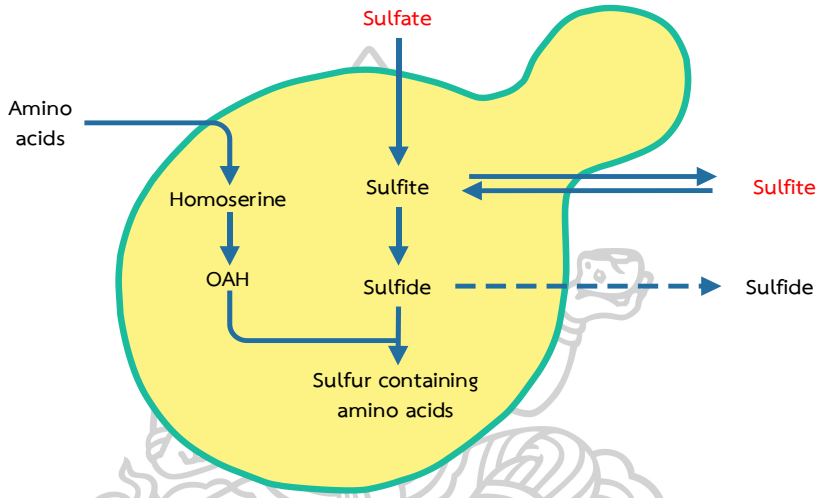



**Figure 13** Sulfate assimilation pathway (modified from [235] [235, 236])

### 3) Sulfur dioxide as an antioxidant

In the ethanol fermentation process, sulfur dioxide is employed as a bactericide and antioxidant [237] [18]. Sulfur dioxide is very reactive and inhibits ethanol fermentation [232]. Sulfur dioxide in dilute aqueous solution can occur in three forms: $SO_2$ (Molecular sulfur dioxide), $HSO_3^-$ (Bisulfite ion), and $SO_3^{2-}$ (Sulfite ion), depending on pH [238] [11]. At low pH, sulfur dioxide is often found in molecular form. While at pH 5.0 - 9.0, bisulfite and sulfite are found [238] [11] [232]. The chemical equilibrium between molecular, bisulfite, and sulfite forms in an aqueous solution is shown in (7) and Figure 14. Sulfite considerably affects ethanol pH in the form of $SO_2$ and $HSO_3^-$ because it can react with carbonyl groups of aldehydes or organic acids to sulfonic acid [239] [232].

$$SO_2 + H_2O \leftrightarrow H^+ + HSO_3^- \leftrightarrow 2H^+ + SO_3^{2-} \quad (7)$$

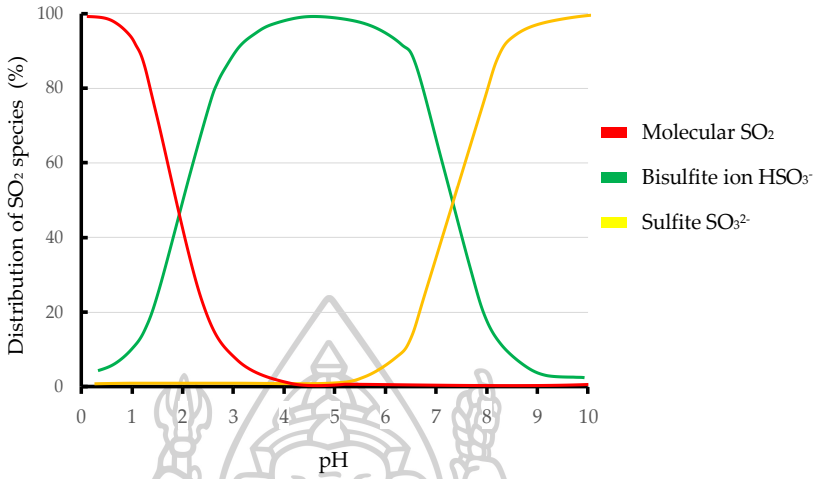



**Figure 14** Effect of pH on $SO_2$ species present in aqueous solution (modified from [240])

**4) Sulfuric acid as pH regulator and antimicrobial agent**

Sulfuric is used in different steps, especially as a pH regulator fermentation. Moreover, it is also used after fermentation to remove bacteria from yeast cells before fermentation in the next batch [241]. Sulfuric acid utilization in these steps results in sulfate formation. Since it can react with ethanol to ethyl sulfate and diethyl sulfate as equations ((8)) and ((9)), respectively [18] [242] [243]. However, these sulfate from sulfuric utilization could remain in co-product. In case of co-product is used for animal feed, these sulfates could be of concern in excessive levels [19] [83] [244].

$$C_2H_5OH + H_2SO_4 \leftrightarrow C_2H_5HSO_4 + H_2O \quad (8)$$

$$2C_2H_5HSO_4 \leftrightarrow (C_2H_5)_2SO_4 + H_2SO_4 \quad (9)$$

**5) Addition of defoamer**

In ethanol production, foam formation normally occurs due to carbon dioxide production as a co-product of ethanol [245] [246]. The foam reduces the fermentation tank's working capacity, resulting in higher production costs and lower productivity [247] [248]. Therefore, employing a defoamer, such as polypropylene glycol-based defoamer and silicone polymer-based defoamer, is necessary. Different defoamers cause different effects on microbial physiology and cell growth rate [248].

However, the use of some defoamers can cause contamination. Silicone polymer-based defoamer can stimulate glycerol production during the fermentation process with *Saccharomyces cerevisiae* at low oxygen and excess glucose conditions [249].

### 2.9.3 Chemical use for fermentation gas removal

The fermentation gas is produced during the fermentation process. This fermentation gas discharged through the vent stream consists of carbon dioxide, vaporous ethanol, and other volatile organic compounds (VOCs) [250]. Presently, more stringent pollutant emission regulations are in most countries. Typically, ethanol distilleries employ scrubbers connected to the fermentation tank to recover vaporous ethanol and control the emission of VOCs into the atmosphere [251]. Since ethanol is a good solvent for VOCs, scrubber bottom contains water, ethanol, and VOCs [251]. Depending on ethanol concentration obtained from different scrubbing techniques, i.e., low ethanol concentration ca. 1-6 wt.%, scrubber bottom would be recycled back to the cooking process to reduce water consumption. However, ethanol in recycle stream will be consumed by bacteria in the cooking step [252] [253]. Presently there are many techniques to recover ethanol in vent stream. With a high concentration of ethanol, the scrubber bottom can be recycled directly to distillation column [254] [250] [255].

VOCs can be divided into soluble and insoluble volatile organic compounds, as shown in

Table **5** [256] [257]. Sometimes, bisulfite may be used as an additive to increase the solubility of insoluble VOCs including acetaldehyde, ethyl acetate, acrolein, and acetone [251]. However, the use of bisulfite to control the VOCs release may cause a remaining acid. Sodium bisulfite ($NaHSO_3$) can either react with acetaldehyde and convert to 1-hydroxy-ethane sulfonic acid salt (10) or with acrolein resulting in sulfonic acid salt (11) [19] [11].

**Table 5** Categories of volatile organic compounds generated during the ethanol fermentation

| Categories of volatile organic compounds (VOCs) | |
|---|---|
| **Soluble** | **Insoluble** |
| Ethanol<br>Formic acid<br>Lactic acid<br>Acetic acid<br>Amyl Alcohol<br>Formaldehyde | Acetone<br>Acrolein<br>Acetaldehyde<br>Ethyl Acetate |

$$NaHSO_3 + CH_3CHO \rightarrow CH_3CH(OH)SO_3^-Na^+ \quad (10)$$

$$NaHSO_3 + CH_2CHCHO \rightarrow CH_2CHCH(OH)SO_3^-Na^+ \quad (11)$$

Moreover, sodium bisulfite is an unstable substance that can decompose into sulfur dioxide. Therefore, acidity is increased, according to (12) [258].

$$2NaHSO_3 \rightarrow Na_2SO_3 + SO_2 + H_2O \quad (12)$$

**2.10 Ethanol recovery**

**2.10.1 Distillation process**

In sugar and starch fermentation, other alcohols, aldehydes, ketones, fatty acids, and esters are produced as volatile byproducts. Whereas cyclic and heterocyclic compounds are volatile byproducts in lignocellulosic ethanol fermentation [98]. After the fermentation process is finished, the centrifuged broth is obtained by separating the yeast from the fermented beer. The centrifuged broth containing ethanol about 5-15 wt.% is passed to the distillation column to remove water. The distillation column consists of 2 columns. The first one is called the

distillation column or beer column. In this column, approximately 50 wt.% ethanol can be achieved. The second column is the rectifying column. Hydrous ethanol (about ethanol 93 wt.%) can be achieved in this column [37] [42].

Distillation can remove some impurity from ethanol simultaneously with increasing ethanol concentration. However, volatile impurities (acetaldehyde, acetone, ester, methanol) still show up in distillate. These contaminants result in lower engine efficiency when ethanol is used as fuel [98] [12] [11] [20] [259] [22].

**2.10.2 Stillage recycles**

The remaining bottom liquid product after distillation of the ethanol from the beer column is called whole stillage. The whole stillage can contain ethanol up to 0.02 wt.%. Not only ethanol, but also solid particles, such as yeast cells, dissolved matter, and minerals, can be found [33] [260]. After removing solid particles through solid-liquid separation unit (e.g. centrifuge or decanter), the obtained liquid product called thin stillage can be recycled back to different process steps, e.g. fermentation or saccharification, to minimize effluent treatment cost. However, $_{thin}$ stillage recycling can possibly cause some drawbacks such as the accumulation of lactic acid, minerals, and unutilized substrates [33] [260] [261].

The difference in the type of feedstock affects the impurities in the stillage. When stillage is recycled, it causes different contaminations. In the case of cane molasses feedstocks, whole stillage (without yeast cell separation) can be recycled to the fermentation step [33]. In the case of starch-containing feedstock, 25-75% thin stillage can be recycled to fermentation or saccharification processes [33]. Other feedstocks such as corn, wheat, and triticale can be recycled at 75%, 60%, and 60% of thin stillage, respectively [260] [262].

In Thailand, produced stillage during ethanol production from molasses or cassava is often treated and converted into methane gas. Stillage can also be distributed to farmers because stillage provides minerals for plants [263] [264].

### 2.10.3 The fate of electrolytes during distillation

During ethanol distillation, sulfite as sulfur dioxide could be distilled into final ethanol product. The presence of sulfite in distilled ethanol appears to be a common experience in the distilled spirits industry [11] [265]. Zhang, Du [266] reported distillate of chardonnay contained 12% ethanol and sulfite as $SO_2$ 176 mg/l. After 2 stages of distillation, the concentration of ethanol and sulfite as $SO_2$ were increased to 69 vol% and 654 ppm, respectively. This phenomenon can be explained with vapor-liquid equilibria for dilute aqueous solutions of $SO_2$ as volatile weak electrolyte [267].

### 2.10.4 Dehydration process

The distillation process produces 95 vol% ethanol approximately because of the azeotropic mixture between ethanol and water (95.6 wt.% at 78.15 degrees Celsius). Before mixing ethanol with gasoline, it is necessary to increase ethanol concentration to 99.3 wt.%, called anhydrous ethanol. Anhydrous ethanol can be obtained by several dehydration methods such as molecular sieves, azeotropic distillation, and pervaporation. Molecular sieve is most commonly used because of lower investment costs than pervaporation and requires lower steam than azeotropic distillation [37] [42].

The most common dehydration methods in Brazil are heterogeneous azeotropic distillation, extractive distillation, and molecular sieves adsorption [42]. The heterogeneous azeotropic distillation method requires an entrainer to increase separation. Many entrainers, such as benzene, toluene, cyclohexane, can be used to separate ethanol from water [42] [268]. However, using an entrainer can cause product contamination [269] [270].

Extractive distillation, as an alternative method, requires the third component's addition to change the relative volatility of ethanol and water. The

third component acts as a separating agent, such as ethylene glycol, glycerol, 1,3 diamino pentane, diethylenetriamine, and hexachlorobutadiene. The separating agent and water mixture is obtained at the bottom of the column, which is fed to the second column to recover the separating agent. Anhydrous ethanol is obtained at the top of the extractive column. Compared to azeotropic distillation, this method provides less energy consumption and less ethanol contamination [42].

In case of molecular sieve adsorption, there is no requirement to add solvent. Ethanol vapor is fed to zeolite beds. When hydrated ethanol contacts zeolite, water molecules are absorbed. When compared to azeotropic distillation and extractive distillation, molecular sieve adsorption offers lower energy consumption and no chemical contamination [42].

Pervaporation, a membrane dehydration, is a relatively new alternative of the dehydration process. While adsorbents need regeneration, membrane separation offers a continuous operation and energy saving. Industrial applications of zeolite membranes are reported [271].

**2.11 Ethanol storage**

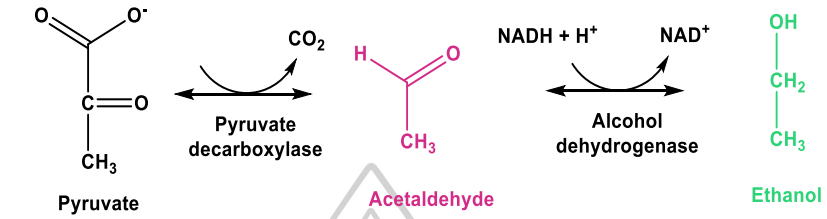
Of course, ethanol derived from different biomass feedstock may have contributed to the inconsistency composition which can cause storage stability issues. Besides, ethanol characteristics also change during storage due to its nature.

**2.11.1 Oxidative degradation**

Normally, ethanol acidity increases along with storage periods due to oxidative degradation [272]. The oxidation reaction in ethanol relates to oxygen solubility in ethanol. Oxygen solubility in ethanol is approximately 44 $cm^3/L$ at 25 °C, compared to 6.4 $cm^3/L$ for distilled water [273] [274].

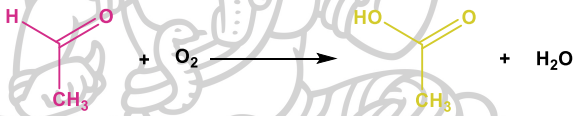
Acetic acid is the main component affecting acidity [15]. During storage periods, acetic acid is produced from the oxidation reaction of acetaldehyde. Ethanol contains acetaldehyde as impurities from pyruvate decarboxylation in the

fermentation stage [275]. Another source of acetaldehyde is the product of ethanol oxidation. Acetaldehyde can be oxidized to acetic acid during storage periods [273] [98] [276]. Additionally, ethyl acetate can form by the esterification reaction between acetic acid and ethanol [277] [98].




(13)


(14)

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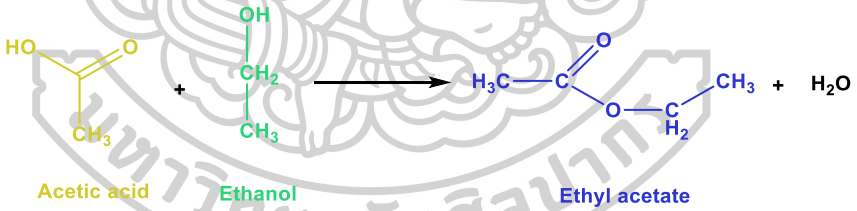



(16)

Acetic acid is a monoprotic molecule. As illustrated in the equation, the

$$CH_3COOH + H_2O \rightarrow H_3O^+ + CH_3COO^- \quad (17)$$

hydrogen atoms attached to acetic acid can detach and form hydronium ions [278].

When moisture is present, acetic acid tends to corrode metals by donating hydrogen ions to the exposed material.

### 2.11.2 Increasing water content

The hygroscopic nature of ethanol causes ethanol to absorb water well from the surrounded environment even being stored in a controlled environment such as in the laboratory. Kane, Eden [279] reported that when ethanol is exposed to the atmosphere during storage and transportation, the water content in ethanol tends to increase. Cummings [3] has reported that controlling water content of ethanol product can maintain storage stability. Ethanol surface area in tank, headspace volume, tank type, type of tank layer material in contact with ethanol, and tank breathing system affect water intake through the tank [280]. According to the experiment conducted by Nakajima and Yahagi [281], E0 (Pure gasoline), E10, and E100 ethanol were exposed to a humid environment. After 30 days, it was found that the higher the ethanol content, the higher moisture is absorbed from the environment as arranged in the order of E100, E10 and E0, respectively.

### 2.11.3 Sulfite oxidation

Sulfite is generally converted from sulfur dioxide added during the wet milling process, juice clarification, and fermentation process [282] [238]. The addition of sulfuric acid to adjust the pH during fermentation can also increase residual sulfite. Yeast metabolism is another issue that can result in the contamination of sulfite during fermentation. The amount of sulfite generated by yeast depends on fermentation conditions, yeast strains, and sulfur content in raw materials [11].

In the distillation step, sulfite in ethanol is distilled with ethanol simultaneously because sulfite in the form of sulfur dioxide vaporizes with ethanol during distillation easily. When storing ethanol for an extended period, sulfite can be oxidized to sulfate by oxygen, as shown in equation ((18)). However, there is no evidence of the oxidation of sulfite to sulfate in fuel ethanol, but the related evidence was found in the study on reducing sulfur dioxide in beer due to oxidation that showed the rate of $SO_2$ reduction is pseudo-first-order. The rate of $SO_2$ loss increase with increasing storage temperature [283].

$$2SO_3^{-2} + O_2 \rightarrow 2SO_4^{2-} \quad (18)$$

### 2.11.4 Carbon dioxide

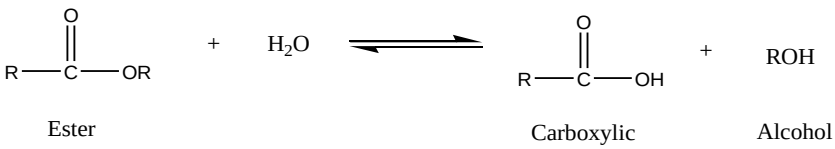
Carbon dioxide can dissolve in ethanol better than water as there was an order of magnitude difference in Henry's constants [284]. A study by General Motors (GM) concluded that ethanol contains high dissolved carbon dioxide gas because carbon dioxide is a fermentation byproduct. The presence of water can cause the formation of carbonic acid during storage time [285].

Typically, the dissolution of carbon dioxide in ethanol fuel causes the value of measured pHe to be biased, showing acidity higher than reality. Hence, acidity measurement should be determined with the ASTM D1613 (Standard Test Method for Acidity in Volatile Solvents and Chemical Intermediates Used in Paint, Varnish, Lacquer, and Related Products) because this method allows carbon dioxide to be removed [23].

### 2.11.5 Ester hydrolysis

Ester is mainly yielded from yeast fermentation [286]. Volatile esters can form as fermentation byproducts during ethanol fermentation via biosynthesis of 2 enzymes: acyl-CoA synthetase and alcohol acetyltransferase. The most abundant ester is ethyl acetate. Other esters comprise isoamyl acetate, isobutyl acetate, ethyl caproate, and 2-phenyl ethyl acetate.

According to Ramey and Ough [287] research, they studied the factors that affect the hydrolysis reaction of volatility ester in wine (when the concentration of ethanol is 10-14%) and found that the rate of hydrolysis mainly depends on ester types, temperature, and pH. Similarly, esters in ethanol fuel are possibly hydrolyzed during storage ethanol fuel. This can yield carboxylic which increases acid content.




(19)

**2.12 Ethanol quality control strategies during storage**

During storage periods, nitrogen blanketing should be applied. It can be performed for a wide range of functions.

- It reduces the water intake rate to the tank (maintain water content).
- Maintaining water content can minimize the cause of carboxylic formation from ester hydrolysis.
- Oxygen concentration which is the cause of oxidation reaction can be minimized, as a result, the formation of acetaldehyde, acetic, and ethyl acetate is reduced.

In order to maintain ethanol storage stability, corrosion inhibitor can be applied. Many available commercial corrosion inhibitors can control acidity and buffer pHe [3]. In addition to corrosion inhibitor and nitrogen blanketing, storage tank characteristics also play a significant role in maintaining ethanol quality during storage. American Petroleum Institute (API) [24] recommended a suitable storage tank for storing ethanol, a fixed roof tank with an internal floating cover. Compatible materials for tank construction can be carbon steel, stainless steel, aluminium, or bronze. However, carbon steel is mostly used. A suitable internal storage tank liner should be selected. For example, the specific type of epoxy compound can be used because of its most compatibility with ethanol [23].

**2.13 Comparison study of contamination in ethanol derived from different feedstocks**

Bioethanol can contain up to 300 different organic compounds depending on feedstock type, process type, operating conditions [14]. Moreover, ethanol contamination is also a result of the storage process. Considering the contamination in ethanol is necessary to improve fuel ethanol quality when used in the vehicle engine. Bioethanol usually contains organic impurities, water, and organic acid. Many

contaminants, such as water, methanol, volatile acidity, copper, chloride, and sulfate, are listed in specifications of fuel ethanol [20] as they can cause corrosion on engine components, fuel storage, and fuel transportation systems. To ensure fuel ethanol quality, Monteiro, Ambrozin [288] concluded that the amount of water and various contaminants (sulphate, chloride, acetate, etc.) must be monitored.

Habe, *et al*. [14] investigated the different amounts of organic impurities, organic acid, sulfur compound, cationic, and anionic in diverse ethanol samples. The ethanol sample derived from lignocellulosic ethanol has a higher number of organic impurities than sugar and starch-derived ethanol. Twenty-nine types of organic impurity were found in lignocellulosic ethanol but in sugar and starch derived ethanol, only 16 types were detected. Commonly in sugar and starch-based ethanol, methanol, acetaldehyde, 1-propanol, ethyl acetate, 2-methyl-1-propanol, and acetal were found to be more significant among other impurities.

Lignocellulosic ethanol, the quantity of 2-methyl-1-butanol, and 3-methyl-1-butanol are greater than in sugar- and starch-based ethanol. Other important impurities found in lignocellulosic ethanol are furans-related compounds due to acid pretreatment, leading to acetic acid and furans-related compound formation. The types of organic impurities and organic acids found in different derived feedstock ethanol are shown in Figure 15.

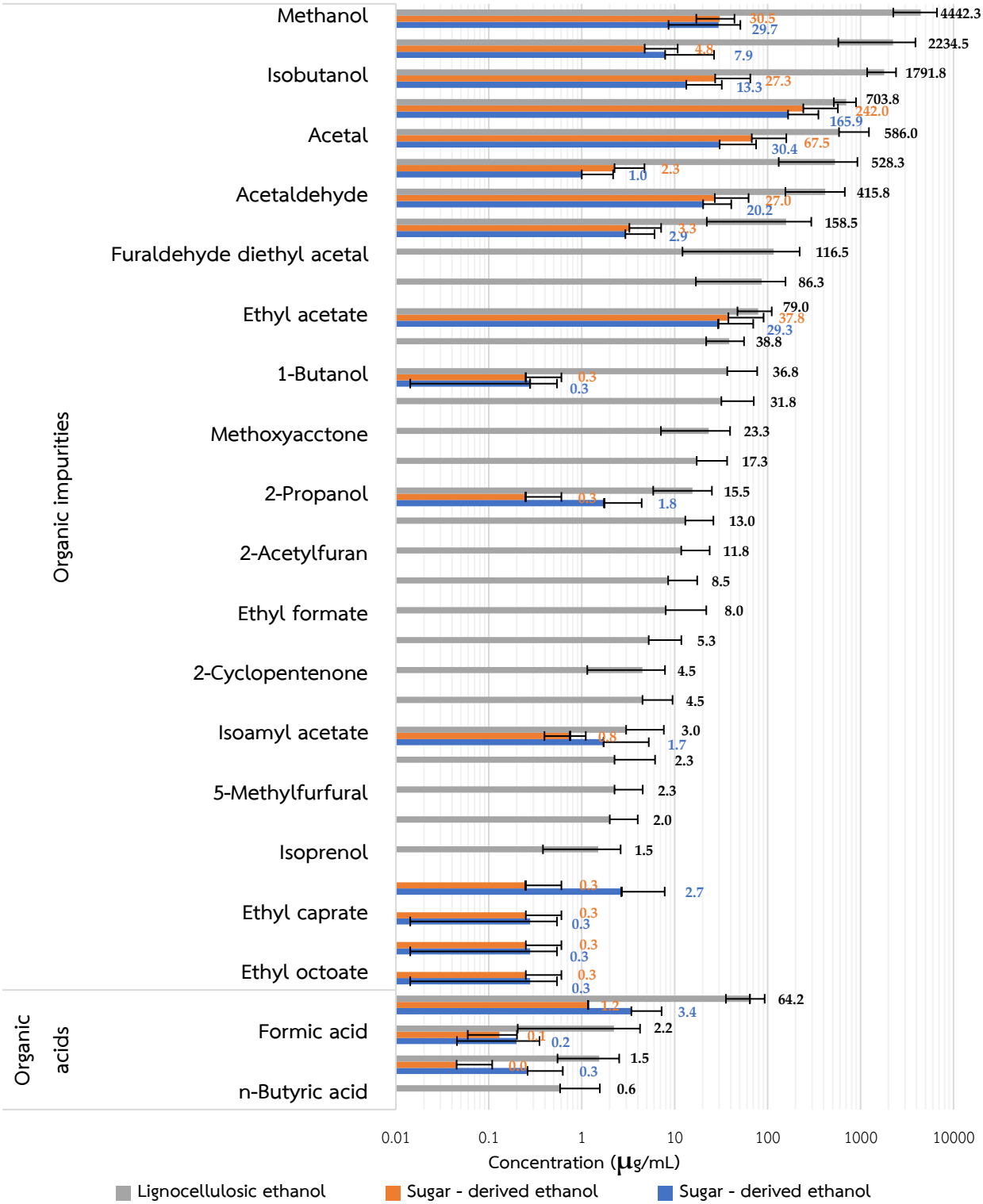



**Figure 15** Organic impurities and organic acids found in ethanol derived from different feedstocks, data taken from [14].

The most organic acids found in ethanol are formic, acetic, propionic, and n-butyric acid. For lignocellulosic ethanol, the amount of acetic acid is high due to the lignocellulosic pretreatment and the autohydrolysis process. Generated residual acetic acid in the fermentation broth can remain in final ethanol after the distillation and dehydration process.

Other impurities found in ethanol are sulfur compounds. In sugar- and starch-based ethanol, only dimethyl sulfide (DMS) and dimethyl sulfoxide (DMSO) were found as organosulfur compounds, but these organosulfur were scarcely found in lignocellulosic ethanol. In lignocellulose ethanol, Dimethyl disulfide (DMDS) and Thiazole were found as the sulfur compound.

Significant cationic and anionic impurity found in lignocellulosic ethanol is silicon as wood and herbaceous plant feedstock contain ash around 0.5-5%. Thus, the amount of Si detected in lignocellulosic ethanol is higher than in sugar- and starch-derived ethanol.

After reviewing the inorganic impurities in Brazilian ethanol [289], sugarcane ethanol has a higher amount of inorganic impurities than corn ethanol. These inorganic impurities include sulfate, sodium, potassium, calcium, magnesium, and sulfur.

Starch-based ethanol can be produced by two methods. There are wet milling and dry milling method. Weaver, Skaggs [290] compared the ethanol compositions between corn wet milling and dry milling. Ethyl acetate and 1,1-Diethoxyethane were detected in wet milling ethanol. Thus, not only does feedstock affect impurities in ethanol, but the production process also affects too.

Besides impurities in the form of compounds, ethanol also found trace elemental. Sánchez, Sánchez [21] have analyzed metal and metalloid content in ethanol fuel. Trace elements in ethanol fuel can be summarized in Table 6. However, the source of these metals in ethanol fuel is difficult to identify. Some

studies report that metal content in ethanol depends on soil for growing feedstock and environmental conditions [291]. Furthermore, metals can contaminate ethanol fuel during production. Various metals can be contaminated during storage and transport due to contacting the metallic container.

**Table 6** Main elements found in ethanol fuel

| Concentration | Elements |
|---|---|
| **> 1 mg/L** | Na |
| **10 µg/L - 1 mg/L** | Mg, Cr, Fe, Ni, Cu, Zn, Al, Si |
| **< 10 µg/L** | Ba, V, Mo, Mn, Co, Ag, Cd, Ga, Tl, Sn, Pb, As, Bi, Se |

# Chapter III

# Theory

## 3.1 Ion exchange resin

Ion exchange resin (IER) is a spherical bed, insoluble with water, approximately 0.5-1.2 mm in diameter. The color of the ion exchange resin is different, but most are often opaque yellow. Some types of IER can swell up to 2-3 times compared to their normal weight. The ion exchange resin consists of two parts. Part one is polymer matrix. Another part is functional groups that can bind to counter ion. The type of function group can be acid or base. The role of the IER functional group can determine the type of resin and resin behavior, such as ion-exchange capacity [292].

Ion exchange resin can exchange ion reversibly between the solid phase and the liquid phase. Charged functional groups need to be neutralized. The opposite charges from free ion (Counterion) temporarily bind to charged functional groups and are ready to exchange with other ions [3].

An ion exchange resin with an acidic function group is called a cation exchange resin, which can capably exchange positive ions such as calcium, and sodium. Ion-exchange resins with base functional groups are called anion exchange resin. This type of resin can be used for exchanging anion such as Chloride, and Sulfate [293].

### 3.1.1 Strong acid cation exchange resin (SAC)

Strong acid cation exchange resin is one type of ion exchange that has sulphonic acid (-SO3H) functional group. The function group of this resin binds to cation ion in 2 forms. The first form is hydrogen form (-SO3-H +) and another one is sodium form (-SO3- Na +). SAC can exchange cation at low pH conditions. Low selective is the limitation of this type of IER [294].

- **Adsorption reaction**

  - Sodium form

$$Na_2R + \begin{bmatrix} Ca \\ Mg \\ Fe \end{bmatrix} \begin{bmatrix} (HCO3)_2 \\ SO_4 \\ Cl_2 \end{bmatrix} \rightarrow \begin{matrix} CaR \\ MgR \\ FeR \end{matrix} + \begin{matrix} 2NaHCO_3 \\ Na_2SO_4 \\ 2NaCl \end{matrix}$$

  Resin liquid phase Resin liquid phase

  - Hydrogen form, Reaction with bicarbonate

$$H_2R + \begin{bmatrix} Ca \\ Mg \\ Na_2 \end{bmatrix} \begin{bmatrix} (HCO3)_2 \\ (HCO3)_2 \\ (HCO3)_2 \end{bmatrix} \rightarrow \begin{matrix} CaR \\ MgR \\ Na_2R \end{matrix} + 2CO_2 + 2H_2O$$

  Resin liquid phase Resin liquid phase

  - Hydrogen form, Reaction with sulfates and chlorides

$$H_2R + \begin{bmatrix} Ca \\ Mg \\ Na_2 \end{bmatrix} \begin{bmatrix} SO_4 \\ Cl_2 \end{bmatrix} \rightarrow \begin{matrix} CaR \\ MgR \\ Na_2R \end{matrix} + \begin{matrix} H_2SO_4 \\ Cl_2SO_4 \end{matrix}$$

  Resin liquid phase Resin liquid phase

- **Regeneration reaction**

  - Sodium form

$$\begin{matrix} CaR \\ MgR \\ FeR \end{matrix} + 2NaCl \rightarrow Na_2R + \begin{matrix} CaCl_2 \\ MgCl_2 \\ FeCl_2 \end{matrix}$$

  Resin liquid phase Resin liquid phase

- Hydrogen form

$$\begin{matrix} CaR \\ MgR \\ Na_2R \end{matrix} + H_2SO_4 \rightarrow H_2R + \begin{matrix} CaSO_4 \\ MgSO_4 \\ NaSO_4 \end{matrix}$$

Resin liquid phase Resin liquid phase

### 3.1.2 Weak acid cation exchange resin (WAC)

Weak acid ion exchange has carboxylic group (-COOH). This less ability to protonate at low pH conditions, so this type of resin is not possible to exchange ions with strong acid salts. At pH > 4, cations can be exchanged in this condition [294].

- **Adsorption reaction**

$$RCOOH + NaCl \rightarrow \text{No reaction}$$

$$RCOOH + CaCl_2 \rightarrow \text{No reaction}$$

$$RCOOH + NaOH \rightarrow RCOONa + 2H_2O$$

$$RCOOH + NaOH \rightarrow RCOONa + 2H_2O + CO_2$$

$$2RCOOH + \begin{bmatrix} Ca \\ Mg \\ 2Na \end{bmatrix}(HCO_3)_2 \rightarrow 2RCOO\begin{bmatrix} Ca \\ Mg \\ 2Na \end{bmatrix} + 2H_2CO_3$$

Resin liquid phase Resin liquid phase

- **Regeneration reaction**

$$RCOONa + HCl \rightarrow RCOOH + NaCl$$

$$2RCOO\begin{bmatrix} Ca \\ Mg \\ 2Na \end{bmatrix} + 2HCl \rightarrow 2RCOOH + 2CaCl_2$$

Resin liquid phase Resin liquid phase

### 3.1.3 Weak basic anion exchange resin (WBA)

In normally most of the common function group of this type of IER is tertiary amine (R-NR'$_2$). Sometimes maybe primary amine (R-NH$_2$) or secondary amine (R-NHR'). This type of resin can exchange ion with strong acids only such as HCl, $H_2SO_4$, $HNO_3$ [295]. The capacity of this resin increases when pH of the solution decreases.

WBA can be regenerated by using weak bases such as ammonia or sodium carbonate.

- **Adsorption reaction**

$$\begin{aligned} &RNH_2 + \begin{matrix} HCl \\ H_2SO_4 \end{matrix} \rightarrow \begin{matrix} RNH_3Cl \\ (RNH_3)_2SO_4 \end{matrix} \\ &2RNH_3OH + \begin{matrix} 2HCl \\ 2HNO_3 \end{matrix} \rightarrow \begin{matrix} 2RNH_3Cl \\ 2RNH_3NO_3 \end{matrix} + 2H_2O \end{aligned}$$

Resin liquid phase Resin liquid phase

- **Regeneration reaction**

$$\begin{aligned} &RNH_3Cl + NaOH \rightarrow RNH_2 + H_2O + NaCl \\ &(RNH_3)_2SO_4 \\ &\begin{matrix} 2RNH_3Cl \\ 2RNH_3NO_3 \end{matrix} + Na_2CO_3 \rightarrow 2RNH_3OH + \begin{matrix} Na_2SO_4 \\ 2NaCl \\ 2NaCO_3 \end{matrix} + CO_2 + 2H_2 \end{aligned}$$

Resin liquid phase Resin liquid phase

### 3.1.4 Strong basic anion exchange resin (SBA)

SBA resin has quaternary ammonium functional group. This has 2 ionic forms there are hydroxide form (R-NOH) and Chloride form (R-NCl). SBA functional groups can be divided into 2 types there are type 1 (Benzyl trimethyl ammonium, -CH2 N (CH3) 3+) and type 2 (Benzyl dimethyl ethanolamine, -CH2N (CH3) 2 (CH2CH2OH) +). The alkalinity of each type of SBA is different. Type 1 has a higher alkalinity than Type 2 . Type 1 resin has high chemical stability and can be used in higher temperatures conditions [296].

- **Adsorption reaction**

$$2RNOH + \begin{matrix} H_2SO_4 \\ 2HCl \\ 2HNO_3 \\ 2H_2CO_3 \\ 2H_2SiO_3 \end{matrix} \rightarrow \begin{matrix} (RN)_2SO_4 \\ 2RNCl \\ 2RNHCO_3 \\ 2RNNO_3 \\ 2RNHSiO_3 \end{matrix} + 2H_2O$$

Resin liquid phase Resin liquid phase

- **Regeneration reaction**

$$\begin{matrix}(RN)_2SO_4 \\ 2RNCl \\ 2RNHCO_3 \\ 2RNNO_3 \\ 2RNHSiO_3\end{matrix} + 2NaOH \rightarrow 2RNOH + \begin{matrix}Na_2SO_4 \\ 2NaCl \\ 2NaHCO_3 \\ 2NaNO_3 \\ 2NaHSiO_3\end{matrix}$$

Resin liquid phase Resin liquid phase

**3.2 Static adsorption calculation**

$$q = \frac{(C_0 \times V_1 - C \times V_2)}{W} \quad (20)$$

Where q is equilibrium adsorption capacity (mg Acidity/g of dry resin)

$C_0$ is the initial concentration of acidity in solution (mg/L)

C is the equilibrium concentration of acidity (mg/L)

$V_1$ is the initial solution volume (L)

$V_2$ is solution volume at equilibrium (L)

W is dry weight of resin (g)

**3.3 Dynamic adsorption calculation**

**3.3.1 Thomas's model**

This model can be used to determine the adsorbent efficiency in columns. This model is based on Langmuir, which neglects axial dispersion. In order to design the ion exchange resin column Saturation loading capacity of an adsorbent is necessary. Saturation loading capacity can be evaluated by using this model. Linear equation of Thomas model can be expressed as (21).

$$\ln\left[\left(\frac{C_0}{C_t}\right) - 1\right] = \frac{k_{TH} q_e X}{Q} - k_{TH} C_0 t \quad (21)$$

Where $C_0$ is the influent concentration (mg/l)

$C_t$ is the effluent concentration at sampled time t (mg/l)

$K_{TH}$ is the Thomas rate constant (L/mg×min)

$q_e$ is the saturation loading capacity of resin (mg/g)

X is the amount of adsorbent in the column (mg)

Q is volumetric flow rate (mL/min)

t is sampling time (min)

### 3.3.2 Yoon-Nelson Model

This model is less complicated and no requires characteristics of adsorbent details, adsorbent type, physical properties of adsorbent, axial dispersion. This model is based on the rate of reduction in the adsorption probability of each adsorbent molecule is proportional to the probability of adsorption on the adsorbent and the probability of breakthrough in the adsorbents [297] [298].

$$\ln\left(\frac{C_t}{C_0 - C_t}\right) = k_{YN}t - \tau k_{YN} \quad (22)$$

Where $K_{YN}$ is the rate velocity constant ($min^{-1}$)

t is sampling time (min)

$\tau$ is time required for 50 % adsorbate breakthrough (min)

$C_t$ is the effluent concentration at sampled time t (mg/l)

$C_0$ is initial concentration (mg/l)

### 3.3.3 Adam-Bohart Model

This model is based on assumption that the adsorption rate is proportional to the residual capacity of adsorbent and the concentration of the solute. This model does not consider axial dispersion [297] [299].

$$\ln\left[\left(\frac{C_0}{C_t}\right) - 1\right] = \frac{k_{AB}N_0Z}{u} - k_{BA}C_0t \quad (23)$$

Where $C_0$ is Initial acidity concentration of ethanol (mg/L)

$C_t$ is the effluent acidity concentration of ethanol at time t (mg/L)

$K_{AB}$ is Adam-Bohart rate constant (L/mg×min)

$N_0$ is maximum sorption capacity of resin (mg/L)

Z is bed dept (cm)

u is the superficial or linear velocity (cm/min)

t is sampling time (min)

### 3.4 Scale up

Method of scale-up fixed-bed column can be divided into 2 alternatives include scale-up approach and kinetics approach.

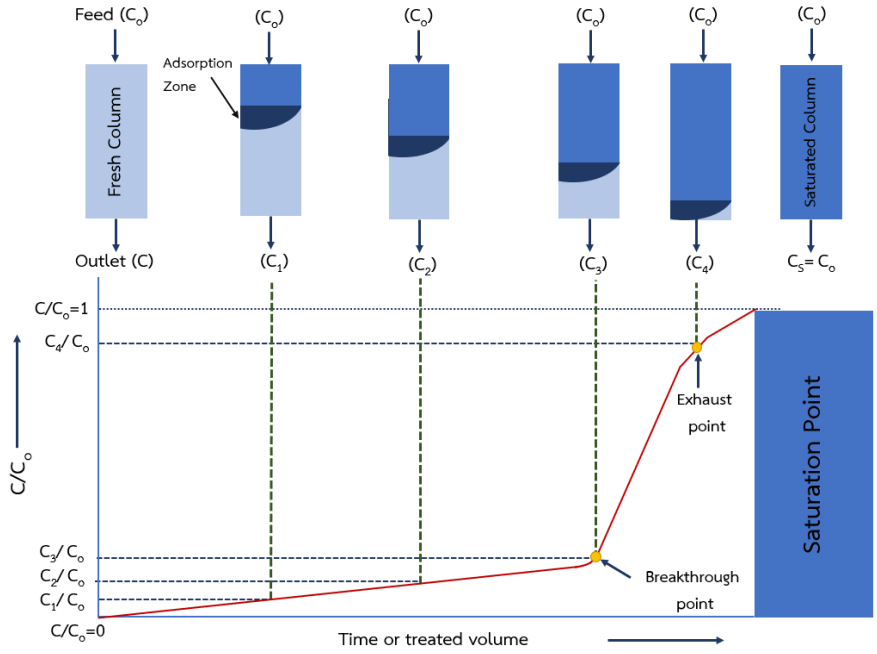



**Figure 16** Breakthrough curve modified from [300]

**1) Scale up approach**

1.1) Calculate design bed volume (BV).

$$\mathrm{BV/hr} = \frac{\mathrm{Q}}{\mathrm{t}} \quad (24)$$

Where BV/hr is bed volume per hours of teste column.

Q is liquid flowrate in the design column.

t is operating time of design column.w

1.2) Calculate the mass of resin required (M) for scale-up.

$$\mathrm{M} = \mathrm{BV} \times \rho \quad (25)$$

Where M is mass of resin in design column.

$\rho$ is resin density from resin specification data which received from manufacture.

1.3) Calculate treated volume per mass of resin ($\widetilde{V_B}$).

$$\widetilde{V_B} = \frac{V_B}{M} \quad (26)$$

Where $\widetilde{V_B}$ is treated volume per mass of resin.

M is mass of resin in test column.

$V_B$ is breakthrough volume from breakthrough curve.

1.4) Calculate mass of resin exhausted per hour ($M_t$).

$$M_t = \frac{Q}{\widetilde{V_B}} \quad (27)$$

Where $M_t$ is mass of resin exhausted per hour for design column.

1.5) Calculate breakthrough time (T).

$$T = \frac{M}{M_t} \quad (28)$$

Where T is breakthrough time of design column.

M is mass of resin in design column.

1.6) Calculate breakthrough volume for design column.

$$V_B^* = QT \quad (29)$$

Where $V^*_B$ is break through volume for design column.

**2) Kinetics approach**

**2.1) Thomas's model [301]**

2.1.1) Create a breakthrough curve, the relationship between breakthrough fraction ($C_t/C_0$) versus time (t).

2.1.2) Create a linear graph between $\ln\left[\left(\frac{C_0}{C_t}\right)-1\right]$ and sampling time (t).

2.1.3) Calculate Thomas constant rate ($K_{TH}$) by substitution of initial acidity ($C_0$) in slope term of linear equation.

2.1.4) Saturation loading capacity of adsorbent ($q_e$) can be calculated by substitution of Thomas constant rate ($K_{TH}$), flowrate (Q), mass of resin (X) in intercept term of linear equation.

2.1.5) Substitute calculated design parameter ($K_{TH}$, $q_e$) into equation (21) to calculate resin weight (m) at the design flow rate (Q), and allowable effluent concentration (C).

2.1.6) Calculate the required resin volume from the data obtained from resin specification such as dry weight, wet weight, and density by using following equation.

$$\text{Resin volume} = \text{mass of resin} \times \%\ \text{moisture of resin} \times \text{density of resin} \quad (30)$$

$$\%\ \text{moisture of resin} = \frac{\text{mass of resin moist basis}}{\text{mass of resin dry basis}} \quad (31)$$

2.1.7) Set bed depth equal to 2 times of bed diameter (L = 2D).

2.1.8) Calculate column diameter (D) by using the following equation.

$$\text{Resin volume} = \left(\frac{\pi D^2}{4}\right) \times 2D \quad (32)$$

Where D is diameter of design column.

L is bed dept of design column.

2.1.9) Calculate the bed dept (L) from L = 2D.

**2.2) Yoon-Nelson Model**

2.2.1) Create a breakthrough curve, the relationship between breakthrough fraction (Ct/C0) versus time (t).

2.2.2) Create a linear graph between $\ln\left(\frac{C_t}{C_0 - C_t}\right)$ and Time (t).

2.2.3) Calculate design parameters ($K_{YN}$, $\boldsymbol{\tau}$) from the slope and intercept of the linear equation.

2.2.4) Calculate dynamic adsorption equilibrium (q) using the following formula. Optionally, the trapezoidal rule can be applied to calculate the area under a plotted curve.

$$q = \frac{C_0 Q}{1000m} \int_0^t (1 - \frac{C_t}{C_0}) dt \quad (33)$$

Where Q is the volumetric flow rate.

m is the mass of resin in tested column.

$C_0$ is the initial acidity concentration of ethanol flowing through the tested column.

$C_t$ is the outlet ethanol's acidity concentration of the tested column at sampling time (t).

2.2.5) Substitute design parameters ($K_{YN}$, $\boldsymbol{\tau}$) obtained from the previous step to predict the breakthrough curve for scale-up condition.

2.2.6) Calculate mass of resin required for the design column by substituting previously obtained adsorption capacity and other variables in following equation.

$$q = \frac{C_0 Q}{1000m}\int_0^t (1 - \frac{C_t}{C_0})dt \quad 34$$

Where Q is the volumetric flow rate of design column.

m is the mass of resin required for the scale-up condition.

$C_0$ is the initial acidity concentration of ethanol for the scale-up condition.

$C_t$ is the outlet acidity concentration of ethanol predicted by the Yoon-Nelson model.

2.2.7) Calculate resin volume required from the resin specification such as dry weight, wet weight, density by using the following equation.

$$\text{Resin volume} = \text{mass of resin} \times \%\ \text{moisture of resin} \times \text{density of resin} \quad 35$$

$$\%\ \text{moisture of resin} = \frac{\text{mass ofresin moist basis}}{\text{mass ofresin dry basis}} \quad 36$$

*2.2.8) Set bed depth equal to 2 times of bed diameter (L = 2D).*

2.2.9) Calculate column diameter (D) by using the following equation.

$$\text{Resin volume} = \left(\frac{\pi D^2}{4}\right) \times 2D \quad 37$$

Where D is diameter of design column.

L is bed dept of design column.

2.2.10) Calculate the bed dept (L) from L = 2D.

**2.3) Adam-Bohart Model**

2.3.1) Plot Breakthrough curve which is the relationship between breakthrough fraction (Ct/C0) and sampling time (t).

2.3.2) Plot a linear graph between $\ln\left(\frac{C_t}{C_0}\right)$ versus time (t).

2.3.3) Determine slope and Intercept of linearized Adams-Bohart equation.

2.3.4) Calculate the design parameters of Adam Bohart ($N_0$, $K_{AB}$) from slope and intercept.

2.3.5) Calculate dynamic adsorption equilibrium (q) using the following formula. Optionally, the trapezoidal rule can be applied to calculate the area under a plotted curve.

$$q = \frac{C_0 Q}{1000m}\int_0^t (1 - \frac{C_t}{C_0})dt \qquad 38$$

Where Q is the volumetric flow rate.

m is the mass of resin in tested column.

$C_0$ is the initial acidity concentration of ethanol flowing through the tested column.

$C_t$ is the outlet ethanol's acidity concentration of the tested column at sampling time (t).

2.3.6) Substitute design parameters ($N_0$, $K_{AB}$) obtained from the previous step to predict the breakthrough curve for scale-up condition.

2.3.7) Calculate mass of resin required for design column by substituting previously obtained adsorption capacity in following equation.

$$q = \frac{C_0 Q}{1000m}\int_0^t (1 - \frac{C_t}{C_0})dt \qquad 39$$

Where Q is the volumetric flow rate of design column.

m is the mass of resin required for the scale-up condition.

$C_0$ is the initial acidity concentration of ethanol at the scale-up condition.

$C_t$ is the effluent acidity concentration of ethanol predicted by the Adam-bohart model.

2.3.8) Calculate the volume of resin required for scaling up from the maximum sorption capacity of resin ($N_0$).

2.3.9) Calculate resin mass required from the resin specification such as dry weight, wet weight, density by using the following equation.

$$\text{Resin volume} = \text{mass of resin} \times \%\ \text{moisture of resin} \times \text{density of resin} \quad (40)$$

$$\%\ \text{moisture of resin} = \frac{\text{mass ofresin moist basis}}{\text{mass ofresin dry basis}} \quad (41)$$

2.3.1) Set bed depth equal to 2 times of bed diameter (L = 2D).

2.3.2) Calculate column diameter (D) by using the following equation.

$$\text{Resin volume} = \left(\frac{\pi D^2}{4}\right) \times 2D \quad (42)$$

Where D is diameter of design column.

L is bed dept of design column.

2.3.3) Calculate the bed dept (L) from L = 2D.

# Chapter IV

# Experimental

## 4.1 Chemical

**Table 7** Chemical used in the experiment

| Chemical | Formula | Grade | Manufacture |
|---|---|---|---|
| **Sodium hydroxide** | NaOH | AR | ACI Labscan |
| **99.99% Ethanol** | $C_2H_5OH$ | AR | QReC |
| **99.8 % Ethanol** | $C_2H_5OH$ | Industrial grade | L-Pure |
| **99.9 % Ethanol** | $C_2H_5OH$ | Industrial grade | SASOL-South Africa |
| **Phenolphthalein** | $C_{20}H_{14}O_4$ | AR | QReC |
| **Hydrogen peroxide** | $H_2O_2$ | AR | QReC |
| **Acetic acid** | $C_2H_4O_2$ | AR | ANaPURE |

## 4.2 Instruments

**1)** Suppressor-type Ion exchange chromatograph

**2)** Automatic Karl Fischer volumetric titrator

**3)** Conductometer

**4)** pH meter with ethanol electrode

**5)** Magnetic stirrer and magnetic bar

**6)** 12V Peristaltic pump

**7)** Pump control

**8)** AC to DC adaptor

**9)** Stand and clamp

**10)** Strong-based anion exchange resin (Amberlite Anion HPR4800 OH-) with hydroxide form

**11)** Chromatography column with 2 cm inner diameter and 80 cm in height

**4.3 Ethanol characteristics measurement**

**1) Existent and Potential Sulfate and Inorganic Chloride**

**Equipment: Suppressor-type Ion exchange chromatograph**

Ion chromatography was used for measuring existent sulfate, potential sulfate (inorganic sulfate presence after the sample was oxidized), and organic chloride in anhydrous ethanol. This measurement follows the standard method of ASTM D7319-17. In this analysis, suppressor-type ion chromatography is used because suppressor can increase the sensitivity by increasing the conductivity of the sample and reducing the conductivity of eluents [1][2]. This method can measure the existence sulfate or potential inorganic sulfate with a concentration from 1.0 - 20 mg/kg, and inorganic chloride can range from 1 - 50 mg/kg [302] [303].

**Procedures:**

1. The sample for analysis was prepared by using 9 .5 milliliters of the sample and followed by adding 0.5 mL of 30% of hydrogen peroxide.
2. Then the samples were injected into chromatograph. Ions in the sample are separated according to their affinity with the ion exchange resin in chromatograph.
3. From the external calibration curve, ions quantity in the sample can be calculated into concentrations [303].

**2) Water content**

**Equipment: Karl Fischer Volumetric Titrator**

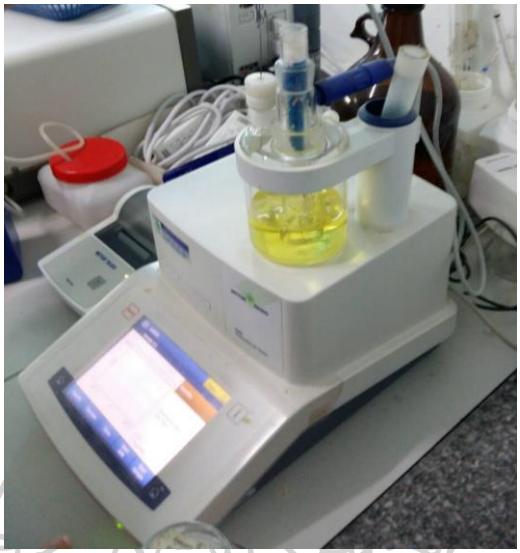


**Figure 17** Mettler Toledo™ C20 Compact Karl Fischer Coulometer

Karl Fischer volumetric titrator is used to determine water content in ethanol or hydrocarbon blends. The measurement follows the standard method of ASTM D7923-19. This method can be used to measure water content in gasoline, denatured fuel ethanol, or other hydrocarbon blend stock.

This method utilizes the Karl Fischer (KF) reaction to measure water content. The alcohol reacts with sulfur dioxide and bases to form an alkyl sulfite salt. Then, the alkyl sulfite salt is oxidized with iodine to an alkyl sulfate salt, as in the equation below. During this step, the mole ratio of water and iodine is consumed in the proportion of 1:1. When the water is consumed completely results in excess of iodine and becomes the endpoint of the titration [304].

**Procedures:**

1. The sample was pulled up into the syringe. (The desired volume depends on the type of equipment and manufacturer.
2. Expel air bubbles.
3. Weigh the syringe.
4. Inject the sample into Karl Fischer Volumetric Titrator.
5. Weight syringe after injection to measure injected sample weight.
6. Enter the weight of the injected sample and press "cal" to get the water content result.

**3) Electrical conductivity**

**Equipment: Conductometer**

The electrical conductivity in anhydrous ethanol was measured by conductometer. This characteristic relates to the amount of corrosive ion presence in ethanol such as sulfate, chloride, etc. [305].

Conductometer can measure the electrical conductivity of an electrolyte by applying alternating current (AC) to 2 electrodes. Anion moves to a positive charge electrode, and cation move to a negative charge electrode. The potential difference between the 2 electrodes was measured. Electrical conductivity can be evaluated by calculation following OHM law by using the distance between 2 electrodes and surface area.

**Procedures:**

1. Adjust the sample temperature to 25 °C (since temperature affects electrical conductivity).
2. Dip the probe into the sample to measure electrical conductivity.

**4) pHe Equipment: pH meter with ethanol electrode**


**Figure 18** Mettler Toledo™ SevenCompact pH/Ion meter

pHe is the measurement of acid strength in high-content ethanol fuel. Since ethanol pH cannot be directly compared to aqueous solution's pH [306]. pHe is used to control the concentration of strong acids, such as sulfuric acid, hydrochloric acid, and phosphoric acid, to meet the standard [97].

**Procedures:**

1. Put ethanol sample into a beaker.
2. Adjust the temperature of the sample to 20 °C.
3. Rinse the probe with DI water
4. Dip the probe into the ethanol sample to measure pHe.

**5) Total acidity**

**Equipment: Burette and magnetic stirrer**

Acidity is the measurement of acid content in ethanol or ethanol blended with gasoline. Acidity is similar to pHe, but acidity suits for measurements very dilute aqueous solutions of low-molecular-weight

organic acids such as acetic acid, which are considered to be the main factors affecting acidity [10].

In this work, the acidity measurement method is based on ASTM D 6423. This method requires 1% of phenolphthalein in ethanol as an indicator. The pH range and color change of 1% of phenolphthalein in ethanol and other indicators can be illustrated in Figure 19 below.

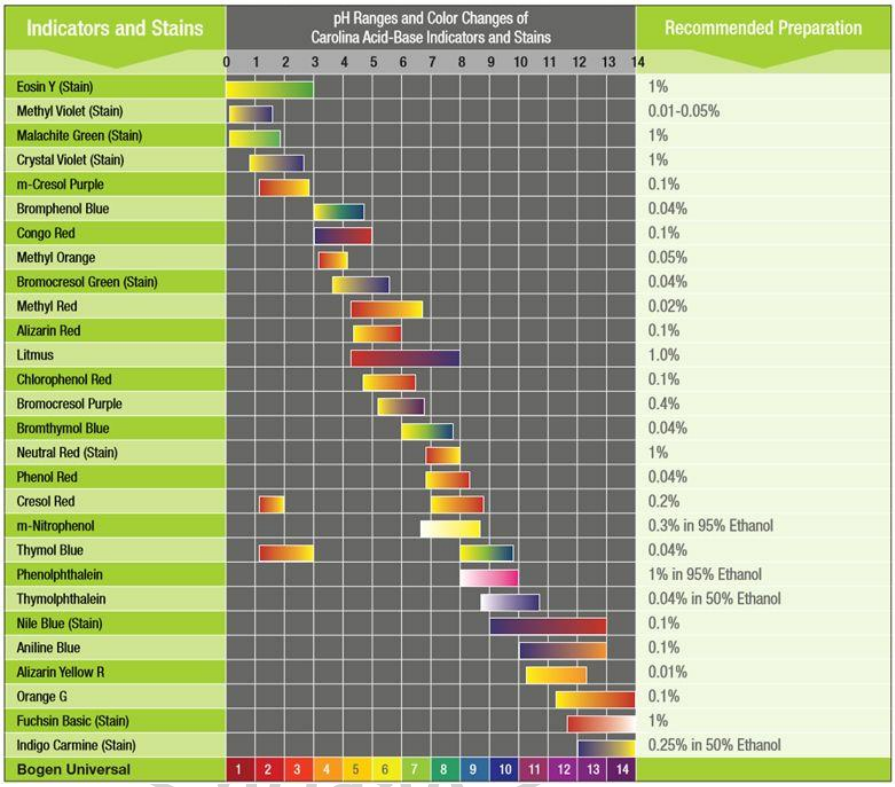



**Figure 19** Color of acid-Base Indicators at different pH [307]

Some concern about titration with phenolphthalein indicator and other indicators is the difficulty in figuring out which shade of color is indicative of being close to the end point or which visually represents overshooting the end point [308]. However, many available applications can use the camera function to analyze the shade of pink to provide titration accuracy, e.g., Titration ColorDart [308] and Titration ColorCam [309]. Figure 20 shows how Titration ColorDart scores titration.

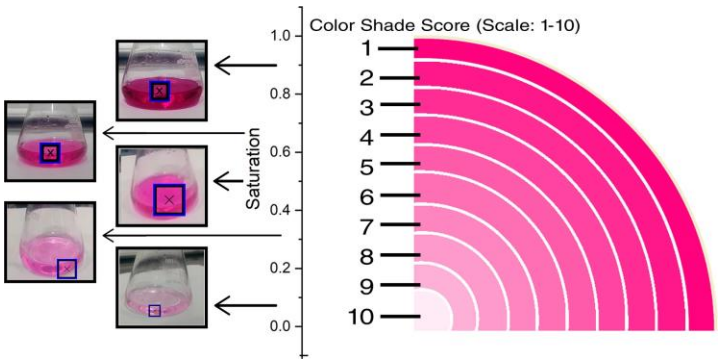



**Figure 20** Titration ColorDart score depending on the saturation of pink color

**Procedures:**

1. Pour 50 ml of DI water into the flask.
2. Pipet 0.5 ml of 1% phenolphthalein into the flask.
3. Pipet 0.05 N NaOH into the flask until the light pink color appear.
4. Pour 50 ml of ethanol into the same flask then the pink color is disappeared.
5. Titrate with 0.05 N NaOH until the light pink color appear.
6. Calculate the total acidity of the ethanol sample by substituting the NaOH volume that is used to reach end-point into the following equation.

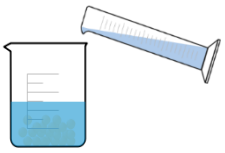
$$\text{Total acidity} = \frac{\text{Volume NaOH} \times 0.0488 \times 600000}{50} \quad (43)$$

**4.4 Static adsorption**

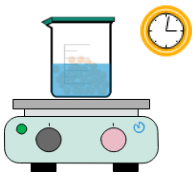
- Put 0.5 g of resin in a 250 ml beaker.

- Pour 200 ml of ethanol 99.9% into each beaker.

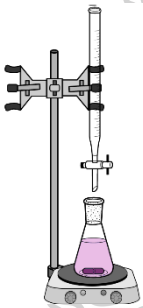
- Adjust acidity with acetic acid to achieve the desired concentration.



- Stir for 10 hours by using a magnetic stirrer.



- After finish 10 hours of stirring, acidity by titration method



- Calculate the equilibrium adsorption capacity (q) from the experimental data using the eq (20).

**4.5 Dynamic adsorption**

1. Ethanol tank
2. Pump control
3. Peristaltic pump
6. Fixed bed column with resin
7. Beaker as sample container

**Figure 21** Experimental Setup for Dynamic adsorption experiment

**1.** Pack resin in the fixed bed column.

2. Prepare a solution between acetic and ethanol by adjusting the acidity concentration in ethanol.
3. Feed acidified ethanol through the resin in a fixed-bed column.
4. Collect treated ethanol samples and measure acidity with the titration method.
5. Develop breakthrough curve
6. Construct the linear plot for each model following Table 8.

**Table 8** Linear plot for each adsorption model

| Model | Plot | Design parameter |
|---|---|---|
| **Thomas** | $\ln\left[\left(\frac{C_0}{C_t}\right)-1\right]$ versus time | $K_{TH}$, $q_0$ |
| **Yoon-Nelson** | $\ln\left(\frac{C_t}{C_0-C_t}\right)$ versus time | $K_{YN}$, **τ** |
| **Adam-Bohart** | $\ln\left(\frac{C_t}{C_0}\right)$ versus time | $K_{AB}$, $N_0$ |

7. Calculate the mass of resin required for scale-up by using linear equation model eq (21 - (23).

# Chapter V Results and Discussion

## Part 1 - Results of the review

The quality of fuel ethanol is regulated by the standard specification for denatured anhydrous ethanol because the impurities in ethanol impact vehicle engine. The 2nd generation ethanol has more impurities than 1st generation ethanol. Furthermore, the increasing ethanol mandate requires a stricter revision of the ethanol standard.

Currently there are many research topics related to ethanol impurities in fuel ethanol [22] [14] [20] [98] [21]. Many reports and scientific research point out the effect of contaminants in fuel ethanol on vehicle engine, e.g. sulfate [11] [12] [19] [305], acetic acid [14] [287], chloride salt [287], and so on. The impurity profile is different depending on raw materials, production process, and storage procedures. With regard to fuel quality specifications in the U.S. today, the ASTM (American Society for Testing and Materials) International standard specifications for fuel ethanol have been based on traditional corn feedstock production [3]. With so many new feedstocks entering the marketplace, there will be a need to review and, if necessary, update the required quality control testing to ensure that the final blended fuel will not adversely impact vehicle system components and driving performance. There are many challenging aspects to control ethanol quality, as mentioned before. However, currently the industry guidelines specification and procedures for blended gasoline provided by RFA are available [23] but there is no specific guideline related to anhydrous ethanol impurities and quality control for the entire pro-duction step till storage periods, so we have reviewed and purposed specific guideline to coverage ethanol quality control both 1st and 2nd generation fuel ethanol. Table 9, possible contaminations in each production step's entire storage period are summarized along with control strategies that can mitigate the effect of contamination.

Table 9 Summary overall contamination in ethanol production

| Stage | Source of contaminants | | Contaminants | Concern | Control strategies |
|---|---|---|---|---|---|
| Conversion of feedstock  | Sugarcane  | Juice clarification | Sulfur dioxide | • Sulfur dioxide in a dilute aqueous solution can occur in many forms ($SO_2 \cdot H_2O$, $HSO_3^-$ and $SO_3^{2-}$) depending on pH [11].<br>• Increase sulfur dioxide content in ethanol [11] [235].<br>• The addition of sulfur dioxide can leave the residual sulfite in clarified juice [310] [311]. | • Sulfite can be removed by evaporation [311]. |
| | Cassava  | Cassava composition | Cysteine<br>Methionine | • This sulfur-containing compound ends up in the DDGs fraction, not in the ethanol product stream [83] [19]. | - |
| | Lignocellulosic biomass | Lignocellulosic feedstock components | Lignin | • Lignin can degrade into phenolic compounds and benzoquinone. These compounds can inhibit fermentative yeast [115] [168] [169] [166]. | • Select less recalcitrant feedstock that can be pretreated under mild |

Table 9 Summary overall contamination in ethanol production

| Stage | Source of contaminants | Contaminants | Concern | Control strategies |
|---|---|---|---|---|
| | | Hemicellulose | • Hemicellulose can degrade to undesired products such as furfurals and hydroxymethyl furfurals [110].<br>• Degradation products of hemicellulose can inhibit fermentative yeast [166]. | conditions producing fewer inhibitors during pretreatment [115]. |
| |  Pretreatment | HMF<br>Furfural<br>Acetic acid<br>Formic acid<br>Levulinic acid<br>Phenolic compounds<br>2-furoic acid | • These inhibitors can negatively influence both the enzymatic hydrolysis and fermentation yeast [166]. | • Remove inhibitors in lignocellulosic hydrolysate by suitable detoxification methods.<br>• Change fermentation strategies.<br>• Metabolic engineering |
| | | Furanic compounds | • Furanic compounds, specifically 2,5-dimethyfuran and 2-methylfuran have poor oxidative stability in blended gasoline [16].<br>• These compounds show potential for forming dangerous organic peroxides [16]. | |

**Table 9 Summary overall contamination in ethanol production**

| Stage | Source of contaminants | Contaminants | Concern | Control strategies |
|---|---|---|---|---|
| | Water | Calcium | • Increase osmotic stress to yeast [211].<br>• Calcium damage the distillation equipment [221]. | • The quality of water used in fermentation affects enzymatic activity. Thus, water testing and water treatment should be carried out. |
| | | Magnesium | • Increase osmotic stress to yeast [211] [312]. | |
| | | Sodium | • Increase osmotic stress to yeast [313] [314].<br>• Increase the conductivity of ethanol [97] [315].<br>• $Na^+$ content increases looser floc formation (smaller agglomerates) during the juice clarification process [221].<br>• Sodium accumulates in the vehicle combustion chamber and causes corrosion [97] [316].<br>• Ion present in ethanol would impact the corrosion inhibitor's storage stability and effectiveness [3]. | |
| | | Chloride | • Increase osmotic stress to yeast [211].<br>• Chloride increases ethanol corrosivity [97] [5].<br>• It can increase ethanol conductivity [97] [315] [305].<br>• In the presence of water, hydrochloric acid (HCl) can form [97].<br>• Chloride in ethanol can cause injector plugging, fuel pump failure, and intake valve deposits [3]. | |

Table 9 Summary overall contamination in ethanol production

| Stage | Source of contaminants | Contaminants | Concern | Control strategies |
|---|---|---|---|---|
| | | | • It also causes failure on the fuel sender card [3].<br>• Ion present in ethanol would impact the corrosion inhibitor's storage stability and effectiveness [3]. | |
| | | Sulfate | • Increase osmotic stress to yeast [211].<br>• Sulfate content increases ethanol conductivity [97] [315].<br>• It can increase ethanol corrosivity even in a small concentration and accelerates the corrosion of vehicle fuel system parts [316].<br>• Sulfates (present as $SO_3$ and $SO_4$) form a gum with petrol and cause scale formation in engine pipes [97].<br>• Sulfate depositing cause injector clogging in vehicle engine [97] [3]. | |
| **Fermentation**  | Nitrogen source | Ammonium sulfate | • Ammonium sulfate is possible to increase sulfate residual in ethanol.<br>• Sulfate content in ethanol correlates with ethanol pHe and conductivity [18] [97].<br>• Sulfate content increases ethanol corrosivity [316]. | • Select suitable nitrogen source due to addition of nitrogen source in the form of sulfate possibly causes an increase in sulfate residual. |
| | Control of Aldehyde Emissions | Sodium bisulfite | • It can react with acetaldehyde converting to 1-hydroxy-ethane sulfonic acid salt [19]. | • Minimize sodium bisulfite used [317]. |

Table 9 Summary overall contamination in ethanol production

| Stage | Source of contaminants | Contaminants | Concern | Control strategies |
|---|---|---|---|---|
| | | | • It reacts with acrolein resulting in sulfonic acid salt [19].<br>• Sodium bisulfite can decompose to sulfur dioxide and cause increasing in acidity [258].<br>• Overuse sodium bisulfite will contribute to sulfur levels and stress the yeast to produce more glycerol, thus reducing ethanol yield [317]. | • In most ethanol plants, the emission of VOCs is controlled by scrubber, which requires sodium bisulfite. Thus alternative VOCs removal methods might be used instead of scrubbers such as bio-trickling filter [250]. |
| | Fermentation byproducts | Carbon dioxide | • Presence of water, carbon dioxide can be converted into carbonic acid [318].<br>• High carbon dioxide concentration can reduce growth and general metabolic activity. The recommended carbon dioxide concentration in most industries, $Pco_2$ value should be below 0.15-0.2 atm [319]. | |
| | | Acetaldehyde | • The reaction with sodium bisulfite produces 1-hydroxy-ethane sulfonic acid salt [19].<br>• It shows the inhibitory effect on fermentative yeast [233] [320]. | • Minimize $SO_2$ addition because $SO_2$ addition during fermentation |

Table 9 Summary overall contamination in ethanol production

| Stage | Source of contaminants | Contaminants | Concern | Control strategies |
|---|---|---|---|---|
| | | | | induces acetaldehyde production [321] [322].<br>● Sodium bisulfite addition should be optimized when it is used to remove acetaldehydes in the scrubber [317].<br>● Alternative VOCs removal methods might be used instead of scrubbers such as bio-trickling filter [250]. |
| | | Acrolein | ● It reacts with sodium bisulfite and converts to sulfonic acid salt [19]. | ● Alternative VOCs removal methods might be used instead of scrubbers such as bio-trickling filter [250]. |
| | | Acetic acid | ● Increase the acidity of fuel ethanol [15].<br>● It shows the inhibitory effect on yeast [211] [323].<br>● Acetic acid can increase ethanol corrosivity [5]. | ● The most common bacterial contaminants found in ethanol |

Table 9 Summary overall contamination in ethanol production

| Stage | Source of contaminants | Contaminants | Concern | Control strategies |
|---|---|---|---|---|
| | | | | production are lactic acid bacteria (LAB) which can produce lactic and acetic acids. Therefore, bacterial contamination must be carefully monitored [229].<br>● Control fermentation condition (oxygen, medium composition, temperature) [324]. |
| | | Sulfite | ● Sulfite can occur naturally as a product of yeast metabolism.<br>● It shows the inhibitory effect on fermentative yeast [314]. | ● Optimize sulfur dioxide addition.<br>● Screen fewer sulfite-producing yeast [235]. |
| | | 1-Propanol | ● It has chemical interference (change cell morphology) [233] [320]. | ● Change fermentation condition (Temperature, oxygen content, medium composition) [324]. |

Table 9 Summary overall contamination in ethanol production

| Stage | Source of contaminants | Contaminants | Concern | Control strategies |
|---|---|---|---|---|
| | | Formic acid | • It show inhibitory effect on yeast [211] [323] [233] [325] [313] [326].<br>• Formic acid enhances ethanol corrosivity [5]. | • Control fermentation condition (pH, Nitrogen level, thiamin content, $SO_2$ content) [327]. |
| | | Glycerol | • Glycerol affects osmotic pressure on yeast cells [233] [320]. | • Metabolic engineering [328] [329] |
| | | Lactic acid | • It has chemical interference with cell maintenance functions [233] [314]. | • Control bacteria contamination [229] |
| | | Methanol | • Methanol enhances pump and fuel sender card failure [3]. | • Control microbial contamination because the methanol contamination can be linked to microbes producing pectin methylesterase (PME) that can produce methanol from pectin-rich feedstocks [330]. |

Table 9 Summary overall contamination in ethanol production

| Stage | Source of contaminants | Contaminants | Concern | Control strategies |
|---|---|---|---|---|
| | | | | • Use low pectin content feedstock because pectin content affects methanol production in alcohol fermentation [330] [331]. |
| | pH regulator, antimicrobial agent | Sulfuric acid | • Sulfuric acid can react with ethanol and convert to ethyl sulfate and diethyl sulfate [18] [242] [243].<br>• Sulfate introduced from sulfuric utilization remains in the byproduct stream, not in the ethanol product stream [19].<br>• Sulfuric utilization increases sulfur residual in DDGs [83]. | • Use acetic acid to control pH instead of sulfuric [332]. |
| **Ethanol Recovery**  | Distillation | Sulfite | • During ethanol distillation sulfite can vaporize with ethanol resulting in ethanol contamination [11] [19] [18] [266]. | • Treat first distillated with calcium oxide, powdered activated charcoal, or hydrogen peroxide [266]. |
| | | | • Volatile compounds (other alcohols, aldehydes, ketones, fatty acids, esters, sulfite, cyclic, and heterocyclic compounds) can contaminate in distillate [98]. | • Treat distillated with ozonation and physical adsorption [98]. |
| | Dehydration | - | - | - |

Table 9 Summary overall contamination in ethanol production

| Stage | Source of contaminants | Contaminants | Concern | Control strategies |
|---|---|---|---|---|
| Ethanol storage  | Sulfite oxidation | Sulfate | • In storage periods, sulfite in ethanol can be converted into sulfate resulting in ethanol pHe, conductivity change over time [18] [3].<br>• In ethanol with a high sulfate ion concentration, high conductivity ethanol can be observed [3].<br>• The reaction of sulfite oxidation to sulfate is the function of ethanol pHe. Ethanol pHe decreased during the reaction [18] [333] [279].<br>• Sulfate content increases the electrical conductivity of ethanol [18] [334] [3].<br>• It can increase ethanol corrosivity even in a small concentration and accelerate corrosion of vehicle fuel system parts [315] [316] [305].<br>• Sulfates (present as $SO_3$ and $SO_4$) form a gum with petrol and result in scale in engine pipes [97].<br>• Sulfate depositing cause injector clogging in vehicle engine [97] [3].<br>• Ion present in ethanol would impact the corrosion inhibitor's storage stability and effectiveness [3]. | • Nitrogen blanketing prevents air and other contaminants which cause oxidative degradation.<br>• Using corrosion inhibitor that contains antioxidants, the oxidation reaction can be minimized.<br>• Use anion exchange resin to adsorb sulfate ions in ethanol.<br>• Determine potential sulfate since potential sulfate can be oxidized into sulfate during the storage period [18]. |

**Table 9 Summary overall contamination in ethanol production**

| Stage | Source of contaminants | Contaminants | Concern | Control strategies |
|---|---|---|---|---|
| | Water pickup | Water | • It increases water content over the storage period [97] [279] [335].<br>• Water can hydrolyze ester to produce carboxylic acid [287].<br>• It effect to ethanol corrosivity [336] [279] [337] [338].<br>• Water can increase ethanol conductivity [97] [339] [3] [305].<br>• Water reacts with carbon dioxide produces carbonic acid [340] [285] [23].<br>• Increasing water content reduces ethanol pHe [341] [342].<br>• Increasing water content would markedly reduce oxygen solubility in ethanol [274]. | • Purging with nitrogen can prevent air and moisture in the storage tank. |
| | The reaction of carbon dioxide and water | Carbonic acid | • When ethanol contacts atmospheric air, it can absorb $CO_2$ [343].<br>• Carbonic formation leads to acidity overestimation [340] [23] [338] [343] [344].<br>• Increasing carbon dioxide in ethanol is not responsible for the corrosiveness of anhydrous ethanol on carbon steel [343].<br>• pHe is a function of carbonic acid content in ethanol [340]. | • Purging with nitrogen can control the carbon dioxide level in the storage tank. |
| | Ester hydrolysis | Carboxylic acid and alcohol | • Ester hydrolysis leads to increasing acidity over storage periods [287]. | • Purging with nitrogen prevents air and moisture |

Table 9 Summary overall contamination in ethanol production

| Stage | Source of contaminants | Contaminants | Concern | Control strategies |
|---|---|---|---|---|
| | | | • Organic acid impurity can cause fuel pump and fuel sender card failure [3]. | involved in the ester hydrolysis. |
| | Ethanol oxidation | Acetaldehyde and acetic acid | • Ethanol has high oxygen solubility [274].<br>• Ethanol oxidation causes the forming of acetaldehyde, acetic acid, and ethyl acetate [273] [276] [98] [345] [277].<br>• This reaction increases ethanol acidity [15] [277].<br>• It increases ethanol corrosivity [23].<br>• It can increase the water content [346].<br>• Organic acid impurity can cause fuel pump and fuel sender card failure [3].<br>• Acetic acid reduces ethanol pHe [305]. | • Use nitrogen blanketing to prevent air and other contaminants which cause oxidative degradation.<br>• Using corrosion inhibitor that contains antioxidants, the oxidation reaction can be minimized.<br>• Anion exchange resin can remove the acetic acid in ethanol [15]. |
| | Esterification between acetic and ethanol | Ethyl acetate | • The corrosive action of alcoholic solutions is considerably affected by acetate, which can result from the manufacturing process, improper handling and storage, and illegal adulteration [288]. | • Nitrogen blanketing can be applied to remove both oxygen and water |

Table 9 Summary overall contamination in ethanol production

| Stage | Source of contaminants | Contaminants | Concern | Control strategies |
|---|---|---|---|---|
| | | | • This reaction can change electrical conductivity because hydrogen ion ($H^+$) and acetate anion ($CH_3COO^-$) are formed by acetic acid dissociation [347]. | vapor from the storage vessel and prevent oxidation which causes the formation of acetic acid.<br>• The corrosion inhibitor can maintain ethanol pHe by neutralizing strong acids. |

## Part 2 - Results of the experiments

### Acidity reduction by addition of bases

The first part of the experiment is corporately conducted with Fakwantip ethanol plant to find ways to reduce off-spec ethanol's acidity by adding chemicals. Sodium hydroxide, 30% ammonium hydroxide, and 99% Triethanolamine were chosen as acidity-neutral agent.

#### 1) Sodium hydroxide

Table 10 below showed pH, acidity, and conductivity of ethanol when 500 ml of anhydrous ethanol was adjusted pH and acidity with NaOH. The addition of Sodium hydroxide can reduce the acidity and increase pH value in anhydrous ethanol. However, it could increase the conductivity of ethanol. Since the addition of NaOH increases metallic ion (sodium ion) presence in ethanol, high electrical conductivity can be expected. The electrical conductivity indicates the risk of corrosion and thus clogging of the fuel systems and injector deposits [97] [316].

**Table 10** The result of acidity reduction in anhydrous ethanol by NaOH addition

| | NaOH addition (%w/v) | pH (6.5 - 9.0) | Acidity (< 30 ppm) | Conductivity (< 500 µS/m) |
|---|---|---|---|---|
| Before NaOH addition | - | 6.16 | 55.40 | 64.9 |
| After NaOH addition | $4.44 \times 10^{-4}$ | 7.18 | 52.49 | - |
| | $2.43 \times 10^{-3}$ | 8.11 | 29.16 | 2250 |

In Brazil, sometimes NaOH is used to correct the pHe of ethanol when it is dropped due to long-term storage. Although it is predicted that conductivity will rise, this does not always occur. The addition of NaOH to increase pHe could cause sediment in the tank. It is recommended that Brazilian fuel stations install filters. However, many do not have them.

**2) 30% ammonium hydroxide**

When fuel ethanol has low pH, it could corrode the engine. Naegeli, Lacey [18] investigated the effect of ammonium hydroxide addition in low pH ethanol. According to NACE tests, Ammonium hydroxide addition results in the reduction of ethanol corrosivity. Table 11 below showed the pH, acidity, and conductivity of ethanol when 500 ml of anhydrous ethanol was adjusted the acidity and pH with 30% ammonium hydroxide. In our finding, the addition of 30% sodium hydroxide can lift the pH value and decrease the acidity of ethanol. Nevertheless, it could increase the electrical conductivity.

**Table 11** The result of acidity reduction in anhydrous ethanol by ammonium hydroxide addition

| | Ammonium hydroxide addition (%w/v) | pH (6.5-9.0) | Acidity (< 30 ppm) | Conductivity (< 500 µs/m) |
|---|---|---|---|---|
| Before ammonium hydroxide addition | - | 6.53 | 90.27 | 113.1 |
| After ammonium hydroxide addition | $3.0 \times 10^{-3}$ | 8.75 | 60.18 | 1400 |

**3) 99% Triethanolamine**

Triethanolamine is used as a corrosion inhibitor in ethanol [348]. Besides, it is commonly used as pH adjuster [349]. Table 12 showed ethanol's pH, acidity, and conductivity when 500 ml of anhydrous ethanol was adjusted pH and acidity with Triethanolamine. The addition can increase the pH value and decrease the acidity of ethanol. However, it increases the electrical conductivity.

**Table 12** The result of acidity reduction in anhydrous ethanol by triethanolamine addition

| | Triethanolamine addition (%w/v) | pH (6.5-9.0) | Acidity (< 30 ppm) | Conductivity (< 500 µs/m) |
|---|---|---|---|---|
| Before Triethanolamine addition | - | 6.53 | 90.27 | 113.1 |
| After Triethanolamine addition | $3.96 \times 10^{-2}$ | 7.92 | 60.18 | 630 |
| | 0.12 | 8.31 | 60.18 | 804 |

**Identification of the cause of the problem in Fakwantip ethanol plant**

**1) Determination of the amount of total sulfate**

From the literature review, sulfate concentration correlates with the pH of ethanol. The ethanol with low pH, the acidity is usually high. To clarify whether the amount of sulfate in the ethanol sample is high or not, the amount of total sulfate needs to be determined. Since total sulfate = existence sulfate + potential sulfate, total sulfate can be determined by oxidizing a 9.5 ml anhydrous ethanol sample with 0.5 ml of 30% hydrogen peroxide. 20 µL of obtained sample is injected into ion chromatography, as shown in Figure 22.

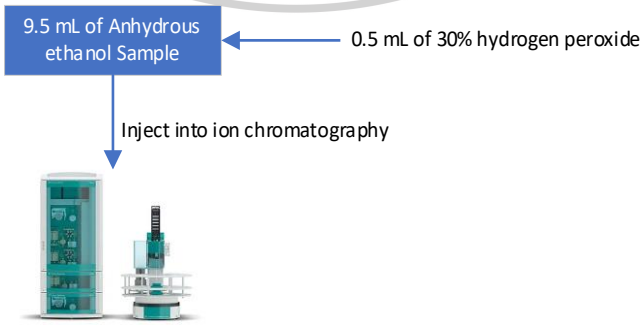



**Figure 22** Determination of total sulfate by ion chromatography

The amount of sulfate in the oxidized sample determined by ion chromatography was lower than 0.2 ppm. However, the actual total concentration of sulfate can be calculated. The total sulfate in anhydrous ethanol was equal to 0.21 ppm, which is lower than the limitation in ASTM 4806. It can be concluded that the amount of sulfate was not a cause of this problem.

**2) Comparision the change in the functional group between fresh and aged ethanol**

IR spectrums of two different ethanol samples collected from Fakwantip Co. Ltd ethanol storage tank were compared in this section. The difference between these two samples is storage time. Figure 23 shows the IR spectrum of ethanol taken from the storage tank and immediately analyzed. Figure 24 shows the IR spectrum of the ethanol sample stored for seven months in the bottle with a cap.

The ester peak ranged between 1750-1735 $cm^{-1}$ was found in the sample stored for seven months. From the literature, ethyl acetate is the main component of ester found in ethanol. During storage periods, acetic acid can be produced from the oxidation reaction of acetaldehyde. Since ethanol contains acetaldehyde as impurities from pyruvate decarboxylation in the fermentation stage [275]. Another source of acetaldehyde is the product of ethanol oxidation. Acetaldehyde can be oxidized to acetic acid during storage periods [273] [98] [276]. Additionally, ethyl acetate can form by the esterification reaction between acetic acid and ethanol [277] [98].

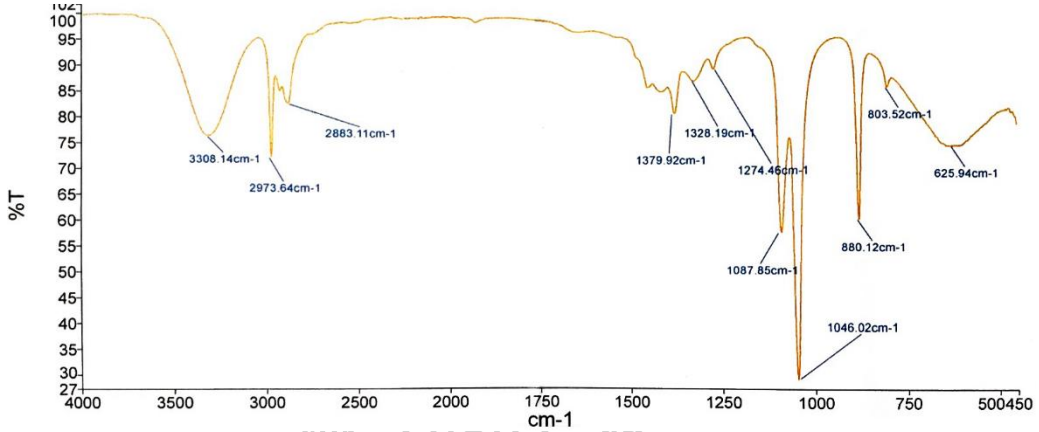



**Figure 23** IR spectrum of fresh ethanol

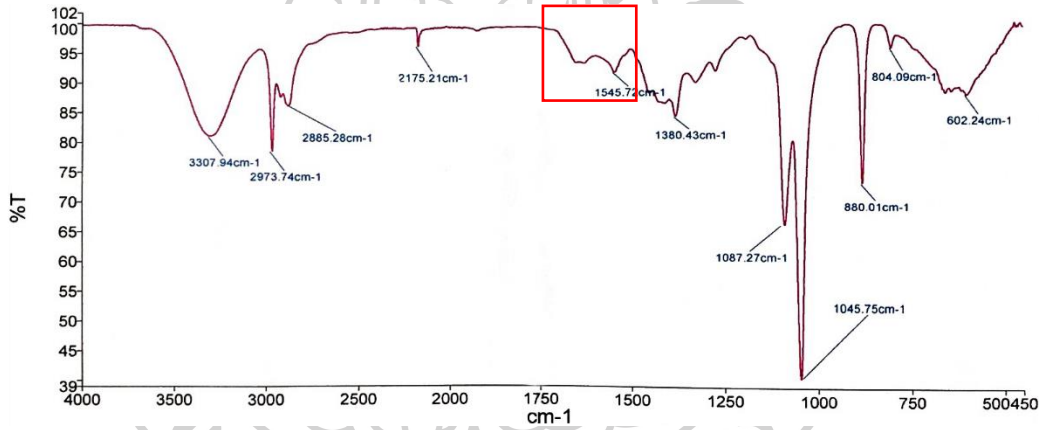



**Figure 24** IR spectrum of aged ethanol

### 3) Determine acid concentration in ethanol by Ion Chromatography

After FTIR analysis of the ethanol sample, acetic acid was suspected to be an acid that forms during the storage period. In this section, fresh and aged ethanol samples were analyzed with Ion Chromatography to measure the concentration of major acids in ethanol which are acetic acid, propionic acid, and formic acid. The results show that acetic acid and acidity concentration increased during the storage period.

**Table 13** Concentration of major acid in ethanol measured by Ion Chromatography

| Acids | Fresh ethanol (ppm) | Aged ethanol (ppm) |
|---|---|---|
| Acidity | 16.20 | 52.48 |
| Acetic acid | 3.77 | 32.07 |
| Propionic acid | 3.42 | < 0.20 |
| Formic | < 0.10 | N.D. |

**N.D.: Not determined.**

## Batch adsorption

Our results agree with previous reports that after ethanol is stored for 3 to 4 months, the acidity of ethanol usually drops due to oxidative degradation. The main component affecting acidity is acetic acid. In this part, static adsorption experiment was performed with Amberlite HPR4800 $OH^-$ anion exchange resin to remove acetic acid and determine some optimum treatment parameters. The results show the adsorption capacity increase when the initial acidity of ethanol is increased. On the contrary, the removal efficiency decreases.

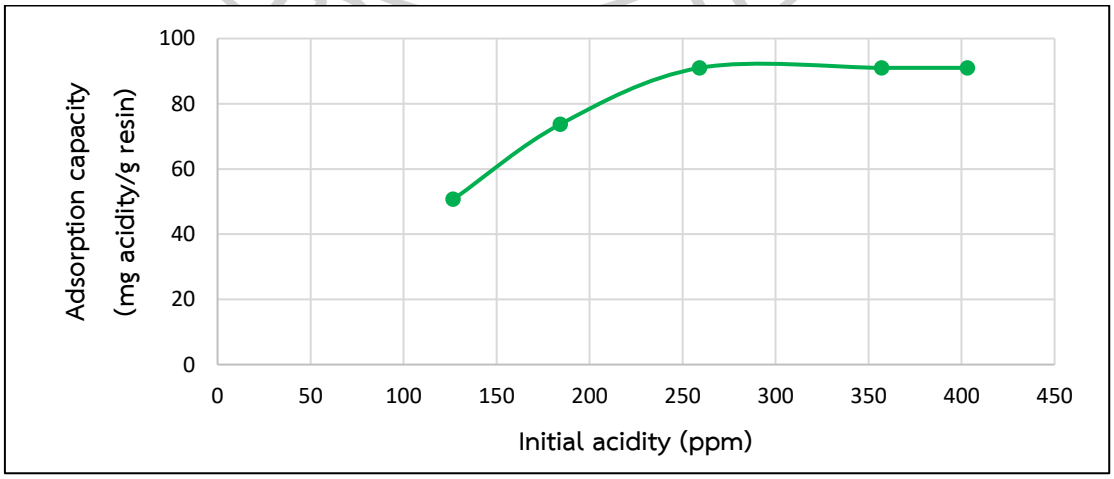



**Figure 25** Static adsorption capacity of HPR4800 $OH^-$

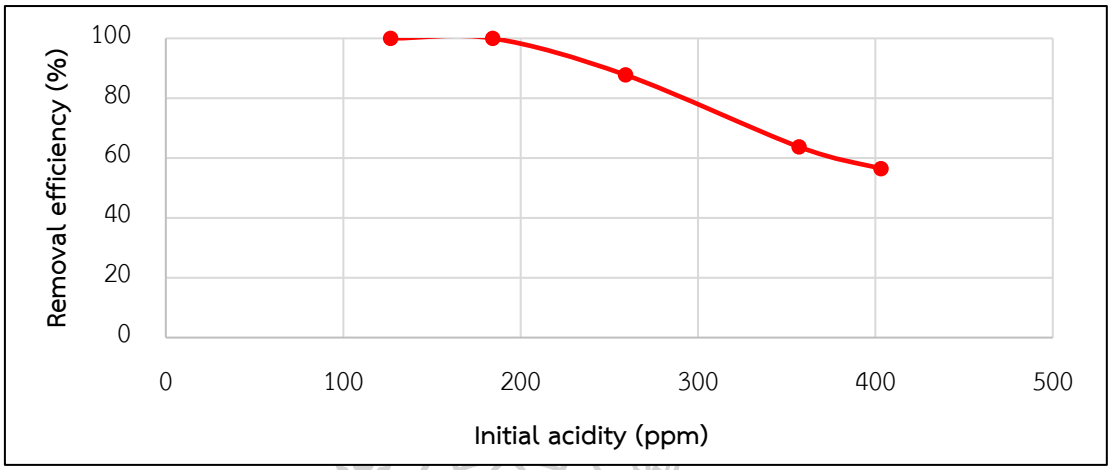



**Figure 26** Removal efficiency of HPR4800 $OH^-$

## Dynamic adsorption

The dynamic adsorption was conducted to investigate the effect of the initial concentration of acidity on adsorption behavior. The results show in breakthrough curve. In the higher initial acidity, the adsorption equilibrium can be achieved faster than the lower initial acidity concentration. After calculating dynamic adsorption equilibrium, higher initial acidity shows higher adsorption equilibrium. From breakthrough curve, dynamic adsorption capacity can be calculated as shown in Table 14

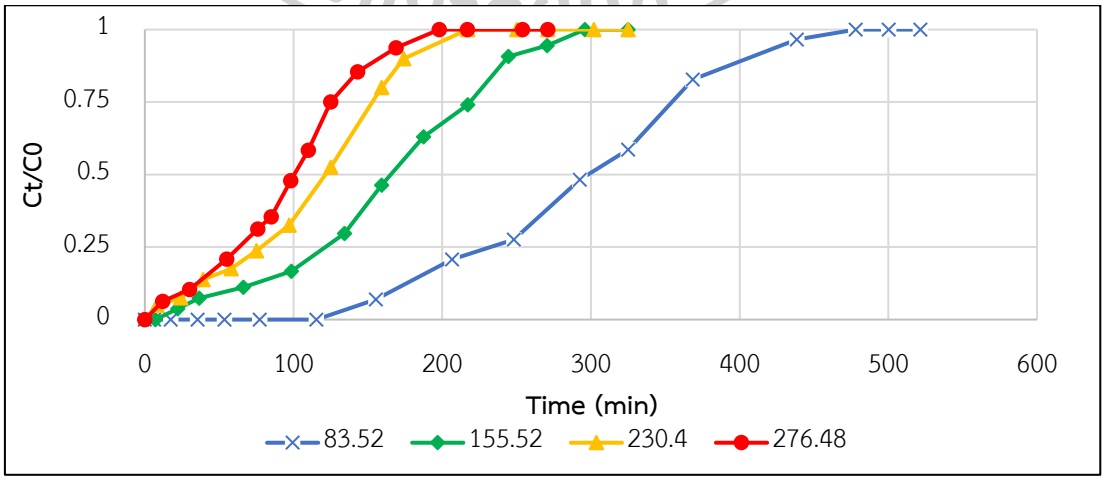



**Figure 27** Dynamic adsorption curve of HPR4800 $OH^-$ on acidity

**Table 14** Dynamic adsorption capacity calculated from area under breakthrough curve

| Initial acidity (ppm) | Dynamic adsorption capacity (mg acidity/g resin) |
|---|---|
| 83.52 | 81.56 |
| 155.52 | 84.38 |
| 230.4 | 37.39 |
| 276.48 | 88.78 |

Kinetic parameters calculation

1) Thomas adsorption model

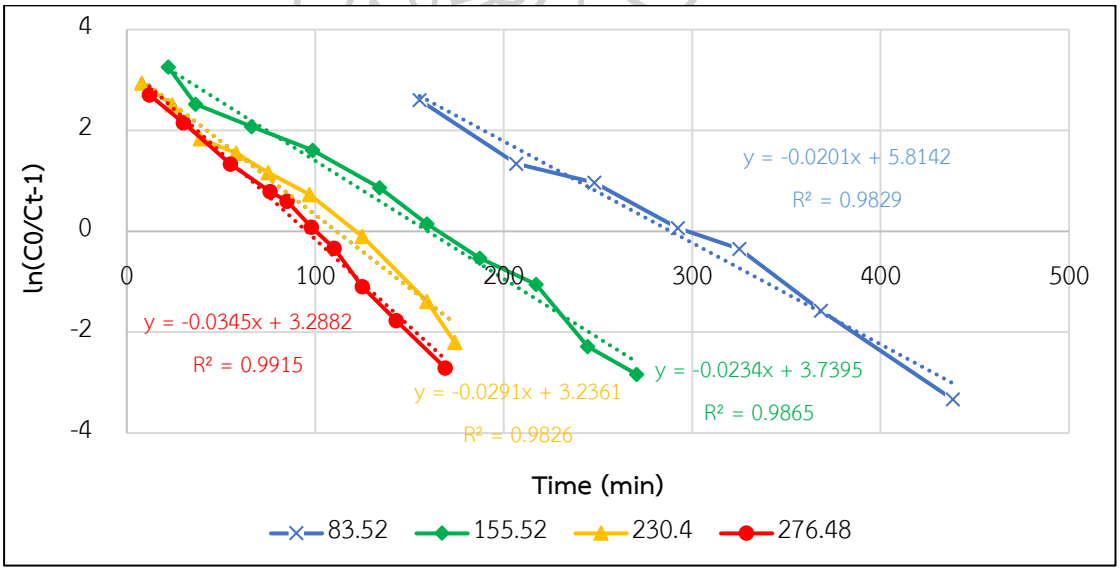



**Figure 28** Linearized Thomas model plot for adsorption of different initial acidity in anhydrous ethanol with 10 mL/min flow rate

Figure 28 shows the linearized Thomas model plot for adsorption of 184.32 ppm acidity at 10 mL/min flow rate. The values of $R^2$ were ranged from 0.9826 to 0.9915.

2) Yoon-nelson adsorption model

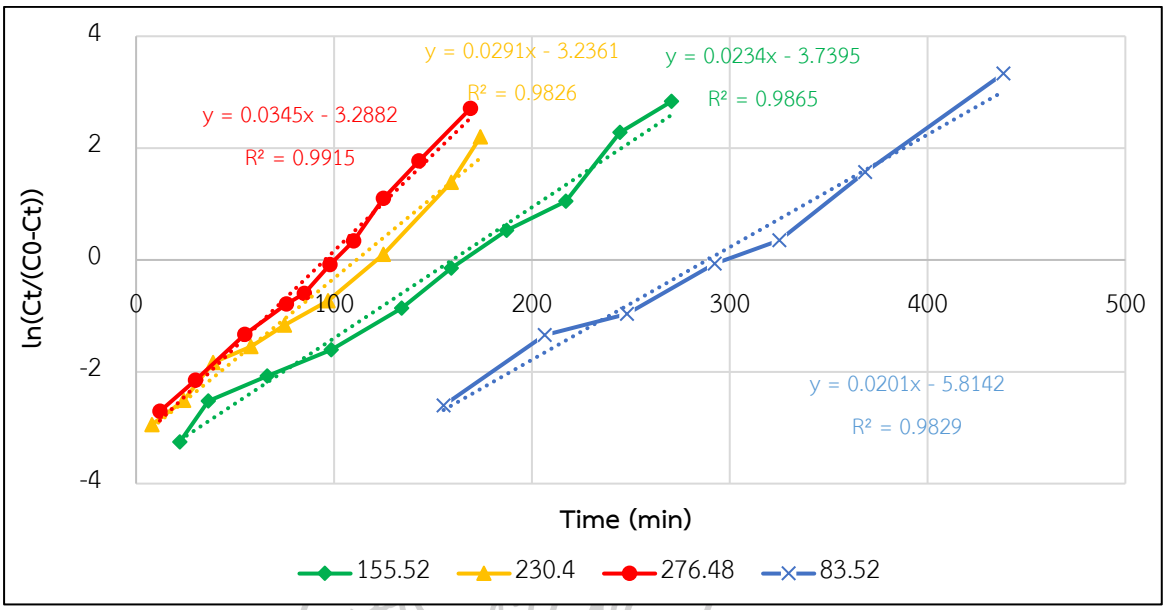



**Figure 29** Linearized Yoon-nelson model plot for adsorption of different initial acidity in anhydrous ethanol with 10 mL/min flow rate

The linearized Yoon-nelson model plot for adsorption of different initial acidity in anhydrous ethanol at 10 mL/min flow rate is shown in Figure 29. The values of $R^2$ were ranged from 0.9826 to 0.9915.

3) Adams-Bohart adsorption model

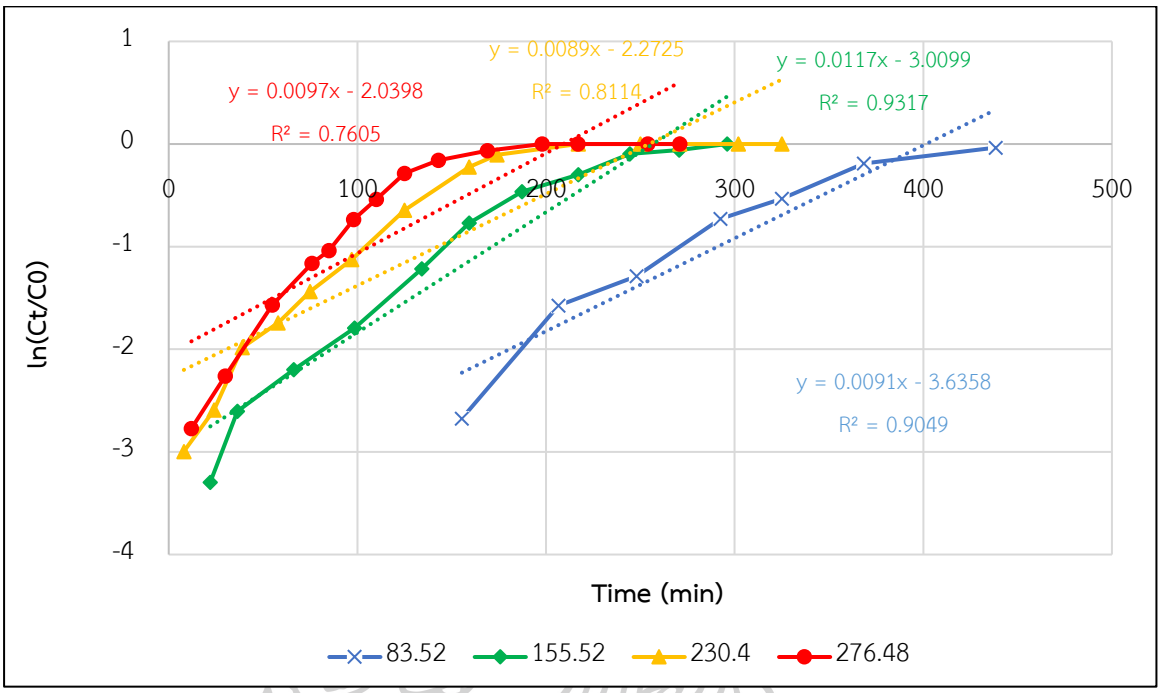



**Figure 30** Linearized Adams-Bohart adsorption model plot for adsorption of different initial acidity in anhydrous ethanol with 10 mL/min flow rate

The linearized Adams-Bohart model plot for adsorption of different initial acidity in anhydrous ethanol at 10 mL/min flow rate is shown in Figure 30. The values of $R^2$ were ranged from 0.7605 to 0.9317.

**4) Comparison of adsorption model**

**Table 15** Comparison of kinetic parameters of various adsorption models for acidity removal at the different initial concentration

| Experimental conditions | | | Thomas model | | | Yoon-nelson | | | Adams-Bohart | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Initial acidity (ppm)** | Flow rate (ml/min) | Bed dept (cm) | Q (mg/g) | $K_{TH}$ (L/(min×mg)) | $R^2$ | $K_{YN}$ ($min^{-1}$) | $\tau$ (min) | $R^2$ | $K_{AB}$ (L/(min×mg)) | $N_0$ (mg/L) | $R^2$ |
| **83.52** | 10 | 0.7 | 80.53 | 0.00024 | 0.9829 | 0.0201 | 289.26 | 0.9829 | $1.09 \times 10^{-4}$ | $1.52 \times 10^{5}$ | 0.9049 |
| **155.52** | 10 | 0.7 | 83.86 | 0.00015 | 0.9865 | 0.0234 | 159.81 | 0.9865 | $6.82 \times 10^{-5}$ | $1.93 \times 10^{5}$ | 0.9317 |
| **230.4** | 10 | 0.7 | 86.02 | 0.00013 | 0.9826 | 0.0291 | 111.21 | 0.9826 | $3.86 \times 10^{-5}$ | $2.68 \times 10^{5}$ | 0.8114 |
| **276.48** | 10 | 0.7 | 87.84 | 0.00012 | 0.9915 | 0.0345 | 95.31 | 0.9915 | $3.51 \times 10^{-5}$ | $2.64\times 10^{5}$ | 0.7605 |

Kinetic adsorption parameters of Thomas, Yoon-nelson, and Adams-Bohart were evaluated. Comparing coefficient of determination ($R^2$) values, Adams-Bohart shows the lowest value as shown in Table 15. Thomas and Yoon-nelson show $R^2$ ranging from 0.9736 to 0.9868 and fit well with the experimental data. Adams-Bohart model shows poor prediction performance of adsorption column. The well-fitting with experimental data of Thomas model indicates that the external and internal diffusion are not the limiting steps [350]. In accordance with the experiment conducted by Lv, Sun [15]. Adsorption of acetic acid from ethanol can be considered as pseudo-second-order model. In this model, the rate-limiting step is chemical sorption [351] [352].

**Adsorption column design**

Thomas and Yoon-nelson can be used to design the adsorption column due to the validation with experimental data. To design the adsorption column for Fakwantip Co., Ltd., the data obtained from Fakwantip Co., Ltd are listed.

- The acidity of off-spec ethanol was 90.27 ppm.
- Volume ethanol to be treated is around 100 $m^3$.
- Assume the adsorption flow rate is 6 $m^3$/hr ($1 \times 10^5$ ml/min). Since this flow rate offers low pressure-drop when estimated from the data provided in resin specification.
- From the ASTM standard anhydrous ethanol for blending with gasoline, the allowable acidity in ethanol was 56 ppm. However, the effluent acidity of treated ethanol should be lower than the maximum allowable value to extend the room for acid formation in the later stage. In this work, allowable acidity was set at 30 ppm of acidity.

**Design adsorption column**

In dynamic adsorption column experiment, all of experiments were conducted by fixing flowrate (10 ml/min), resin weight (3 g). Initial acidity of ethanol is only one parameter that varied in this experiment. In the figure shown the adsorption capacity and Thomas constant at varied initial acidity of ethanol (83.52, 155.52, 230.4, 276.48 ppm).

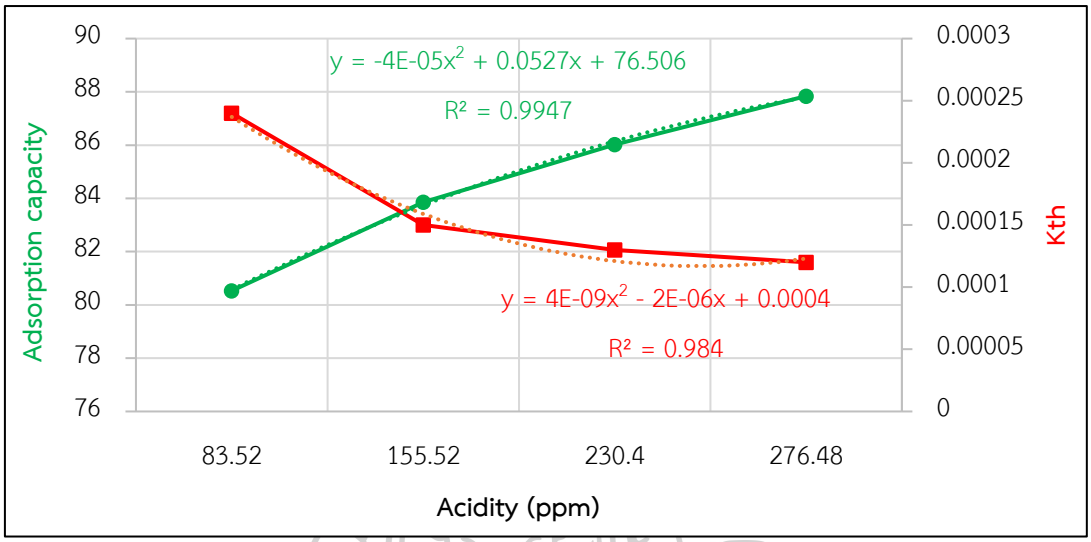



**Figure 31** Adsorption capacity and Thomas constant ($K_{TH}$) in dynamic adsorption of acidity removal from ethanol with HPR4800 $OH^-$ resin

1) From Figure 31, Thomas kinetic parameters (q and $K_{TH}$) can be estimated as 80.94 mg/g and 0.00025 L/min×mg respectively.
2) The mass of resin required to treat off-spec ethanol can be calculated using the Thomas model.

$$\ln\left(\frac{90.27\ \frac{\text{mg}}{\text{L}}}{30\ \frac{\text{mg}}{\text{L}}}-1\right)=\frac{(0.00025\ \frac{\text{L}}{\text{min}\times\text{mg}})(80.94\ \frac{\text{mg}}{\text{g}})\text{X}}{\left(1\times10^{5}\ \frac{\text{ml}}{\text{min}}\right)\left(\frac{1\ \text{L}}{1000\ \text{ml}}\right)}-(0.00025\ \frac{\text{L}}{\text{min}\times\text{mg}})(90.27\ \frac{\text{mg}}{\text{L}})(1000\ \text{min})$$

$$\text{X}=96.19\ \text{kg}$$

3) Bed volume can be determined from particle density or bed density.

$$96.19\ \text{kg}\left(\frac{1000\ \text{g}}{1\ \text{kg}}\right)\left(\frac{\text{ml}}{1.07\ \text{g}}\right)\left(\frac{1\ \text{L}}{1000\ \text{ml}}\right)=89.90\ \text{L}$$

4) Bed dept can be calculated with the assumption that L=2D

$$0.0899\ \text{m}^3=\left(\frac{\pi\text{D}^2}{4}\right)\times 2\text{D}$$

$$\text{D}=\ 0.385\ \text{m}$$

$$\text{L}=2\text{D}=\ 0.771\ \text{m}$$

5) Calculate pressure drop across the bed from operating temperature and linear flow rate

$$\text{Linear flowrate} = \frac{\text{Volumetric flowrate}}{\text{Bed crossection area}} = \frac{6\ \text{m}^3/\text{hr}}{0.117\ \text{m}^2} = 51.48\ \text{m/hr}$$

6) Pressure Drop across the bed can be estimated from the data provided in resin. From Figure 32, at 30°C operating temperature and 51.48 m/h of linear flowrate, pressure drop is approximately 0.5 bar/m.

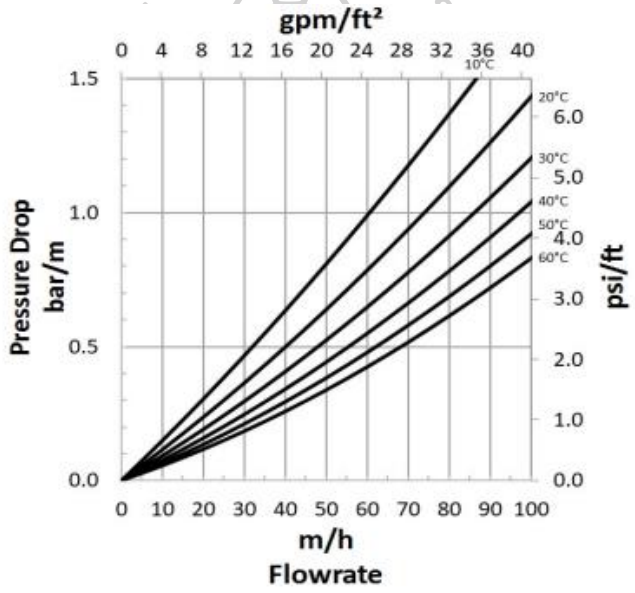



**Figure 32** Estimated pressure drop for AmberLite™ HPR4800 $OH^-$ as a function of service flowrate and temperature

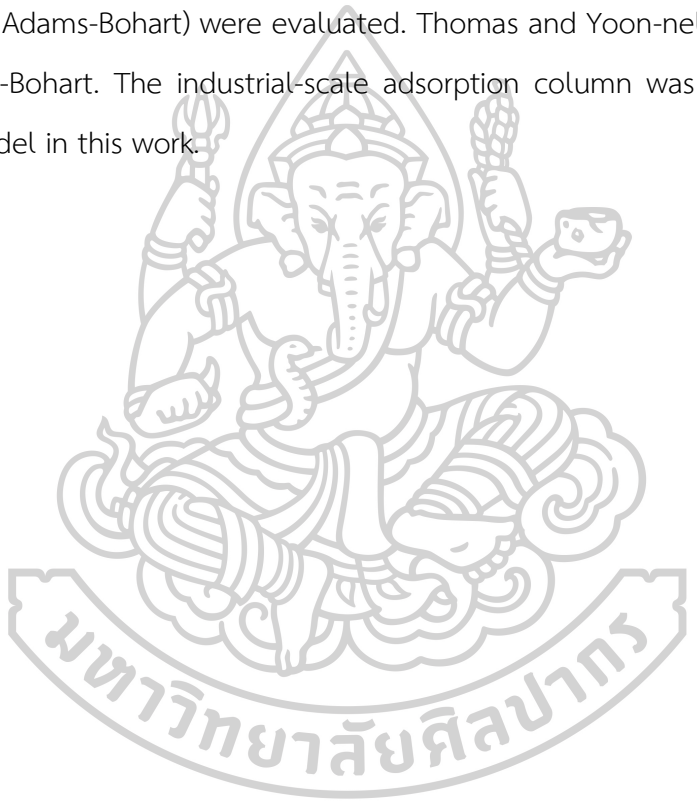
## Chapter VI Conclusion

Anhydrous ethanol was stored for six months in the storage tank of the Fakwantip ethanol plant, the ethanol was considered as off-spec due to high acidity, high water content, and low pH. To correct the acidity and pH of ethanol, the addition of sodium hydroxide, ammonium hydroxide, and triethanolamine were investigated. The addition of these chemicals can reduce acidity and increase the pH of ethanol. Many literatures reported that the addition of NaOH can increase ethanol conductivity. Even though ammonium hydroxide and triethanolamine were recommended to use for reducing ethanol corrosive and pH adjusting, respectively, in our finding, they also increased the conductivity.

In the initial research stage, the problem seems to be related to sulfate formation because sulfite contained in ethanol can be oxidized to sulfate over storage time and reduce ethanol pH. Sulfate is an essential characteristic of ethanol. The existence sulfate and potential sulfate (sulfite that can be oxidized to sulfate) in ethanol should be determined. In this work, the total sulfate concentration of the oxidized ethanol sample was determined by ion chromatography. The result shows that the total sulfate was very low and did not cause this problem. Then the fresh and aged ethanol samples were characterized by FTIR to observe the change in the function group. The FTIR result showed the ester peak appeared in the aged ethanol sample. Since the main ester in ester is acetate, it is possible that oxidation can occur during the storage period. Acetic acid is produced from the oxidation reaction of acetaldehyde which is contained in ethanol as impurities from pyruvate decarboxylation in the fermentation stage. Another source of acetaldehyde is the product of ethanol oxidation. Acetaldehyde can be oxidized to acetic acid during storage periods. Additionally, ethyl acetate can form by the esterification reaction between acetic acid and ethanol. This assumption is supported by the fact that acetic acid is the main component that affects ethanol acidity. Thus, the

concentration of acetic acid in fresh and aged ethanol were measured to confirm this assumption. The result showed that acetic acid concentration was higher in the aged ethanol sample.

To reduce off-spec ethanol acidity, anion resin was employed. Static and dynamic adsorption were conducted. Three adsorption models (Thomas, Yoon-nelson, and Adams-Bohart) were evaluated. Thomas and Yoon-nelson show higher $R^2$ than Adams-Bohart. The industrial-scale adsorption column was designed with the Thomas model in this work.

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