CHAPTER ONE

1.1 Introduction

The world has a progressively increasing demand for energy as more and more countries are becoming developed. The reserves of fossil fuels have been exhausted and limited and only found in certain areas in the world, some of them politically unstable, with increasing prices and uncertain energy supply as a result. Fossil fuels also emit carbon dioxide when burnt thus contributing to global warming. So other more sustainable and secure sources of energy are wanted.

During recent decades, ethanol production has increased significantly all over the world, due to its application as alternative fuel (Pejin, *et al*. 2015). According to Renewables Fuel Association report 2011 approximately 67% of global ethanol production is used for fuel (Rajagopal, *et al*. 2014). In United States and South America the sugar industry has been developed for a long time; the production of sugar shifted its focus towards ethanol production. This more sustainable fuel has played a great role for the economy in many countries in South America (Braunbeck, 2000) .

Ethanol can be produced from many feed stock such as sugar crops, starch crops and/or cellulosic crops. Like in every process there are by-products, in ethanol production the main residue is vinasse. Vinasse has also been known as spent wash distillery, distillery wastewater or stillage (Dowd, *et al*. 1994). It is produced in large quantities; normally a ratio of 9-14 dm^3 of vinasse are produced per dm^{3m} of ethanol, the characteristics of vinasse is variable depending on soil quality, raw materials and distillery processes used for producing ethanol (España-Gamboa, *et al*. 2011). The main characteristics of vinasse is acidity, dark brown color and high biological oxygen demand (BOD) and chemical oxygen demand (COD) with high content of organic and inorganic loads (Satyawali and Balakrishnan. 2008). Disposal the large quantities of vinasse directly without treatments will cause a

serious environmental problems (Mohana, *et al*. 2009). The vinasses had been disposed directly to the flowing water that effected water quality, ecosystem and human health, but only few countries improved upon these issues by making stringent amendments and still many countries have a long way to go about it (EPA, 2012).

Sudan has been producing ethanol from sugar cane since 2009 in White Nile state namely in Kenana Sugar Company; no any effective treatment or alternative utilization methods are applied for vinasses. It is disposed directly to the environment which causes many environmental problems.

The present study was the first attempt worldwide applying sulfate radical based on advanced oxidation process for vinasse treatment. The aim is also to characterize and reduce vinasse pollution.

Ethanol or ethyl alcohol has been made since ancient times by the fermentation processes. Ethanol is colorless and flammable liquid of characteristic odor with density of 0.7939 kg/m^3 at 15°C and boiling point of 78.32°C (at 760 mmHg). Ethyl alcohol is soluble in water and ether. Its net calorific value is 27.723 kJ/kg, with empirical formula C_2H_6O. (Alhassan, 2010).

1.2 Background of Ethanol

Ethyl alcohol has probably been used by man since the dawn of history, it must have been originally produced by the spontaneous fermentation of sugar and utilized by the ancients. Gradually, man learned to control the fermentation to produce alcoholic beverages. With the advance of synthetic organic chemistry in the second half of the 19th century, alcohol become an indispensable fuel, a solvent, an antiseptic and an intermediate for the production of a number of organic compounds (Alhassan, 2010).

In the 20th century the demand for industrial alcohol increased considerably during World War I and II. To meet this need some of the ethyl alcohol was produced

synthetically, mainly in USA, from ethylene derived from petroleum refinery waste gases. The end of the Second World War brought decreasing demand which was progressively accentuated by replacement of ethanol as a chemical base. From the middle eighties of 20th century, the domestic rise in cost of gasoline fuel made many devolved countries more aware of the possible importance of locally produced alcohol as a fuel. Since the raw material from which ethanol is derived is renewable. Brazil and USA are on the head of countries which produced ethanol fuel (70% of total production), Figure 1-1. (Zuurbier and De Vooren. 2008).

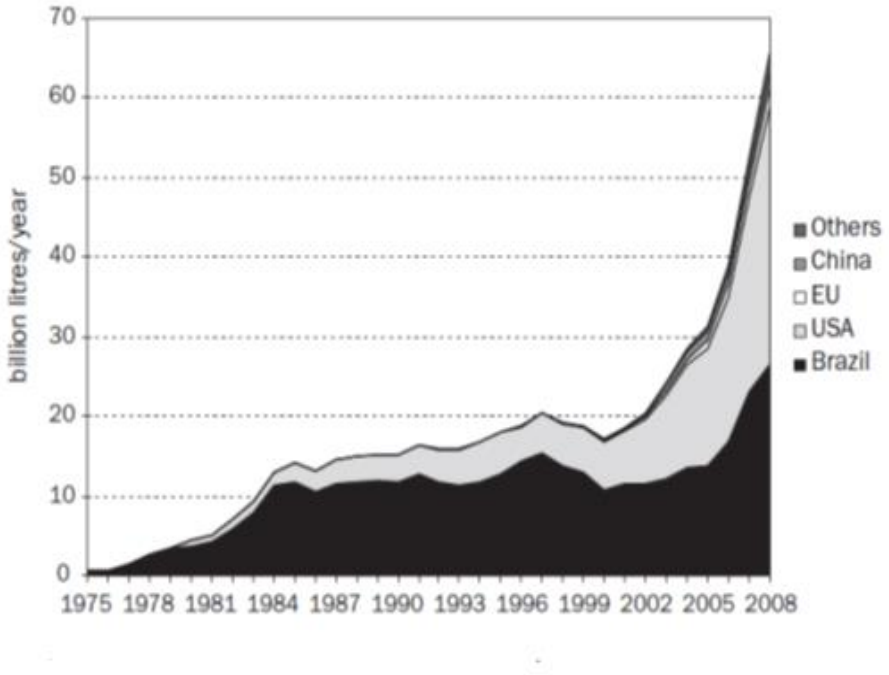


Figure 1.1 World Fuel ethanol production. (Zuurbier, *et al*. 2008)

1.3 Grades of ethanol

There are four grades of ethanol which are classified according to the concentration of ethanol in ethanol/water solution.

i. **Industrial alcohol.** Used for industrial and technical purposes as a solvent, fuel and raw material to produce numerous chemical products.

ii. **Denatured spirit.** Is a term used in certain countries to indicate industrial alcohol which has been denatured and colored, and is generally used for heating and lighting.

iii. **Fine alcohol**. Is a purer type of alcohol used mainly for pharmaceutical, domestic, cosmetics preparation and for human consumptions.

iv. **Absolute or anhydrous or Bio- fuel.** Is a term given to water-free ethyl alcohol and is generally applied to the pure product of pharmaceutical grade, and by extension to anhydrous denatured ethyl alcohol, which find a use as a fuel alone or as a fuel for internal combustions engines when mixed with petrol (gasoline) in ratios 10-15 %.

1.4 Production of ethanol

The process of ethanol production is based on three main steps:

i. The fermentation of carbohydrates raw materials

The substrate which contains carbohydrate is brought into contact with yeast culture under appropriate conditions of pH, temperature, nutrients and time to allow the enzymes to transform carbohydrates into ethanol.

ii. The separation of alcoholic solution

After the fermentation processes ends by reaching the inhibitory alcohol concentration or the temperature limit (around 40°C), the fermented mash is usually separated into alcoholic solution and yeast sludge by concentration.

iii. The separation of alcohol by distillation

The produced alcohol is distilled using a counter-current of steam through three consecutive distillation columns. In the first distillation column, the steam strips the alcohol from the fermented mash and leaves the column as a top product, whereas, the residue leaves as a bottom product representing the residue of the distillery. In the second column the alcohol- steam condensate from the first column is concentrated and purified to a content of 60-80% alcohol top product.

In the third column (rectification column) the ethanol produced from the second column is further concentrated to 95-96% alcohol. If absolute alcohol (Bio-ethanol, 99.5- 99.8%) is required, the rectified alcohol from the third column is passed through a dehydration unit (Alhassan, 2010).

1.4.2 Production of ethanol from renewable materials

Ethanol is produced through different methods (chemical and biochemical). The bulk of ethanol produced nowadays is produced by the fermentation of agriculture materials (Figure 1-2). The main type of agriculture materials are:

- Saccharine products such as molasses and sugar juice from either sugar cane or sugar beet, Saccharine (sugar containing) materials in which the carbohydrate is present in the form of simple, directly, fermentable six and twelve carbon sugar molecules such as glucose, fructose and maltose. Such materials include sugar cane, sugar beet and fruit.
- Starchy products that contain more complex carbohydrates, such as starch and inulin that can be broken down into simpler six and twelve carbon sugar by hydrolysis with acid or by the action of enzymes in processes called malting. Such material include corn, grain and wheat.
- Cellulosic products such as wood, wood waste, paper, straw and cotton, which contain materials that can be hydrolyzed with acid, enzymes or otherwise converted into fermentable sugars.

The above raw materials can be converted biologically to alcohol using yeast fermentation as in equation:

$$C_{12}H_{22}O_{11}+H_2O \rightarrow 2C_6H_{12}O_6 \qquad (1\text{-}1)$$

$$C_6H_{12}O_6 + \text{Yeast} \rightarrow 2CO_2+2C_2H_5OH \qquad (1\text{-}2)$$

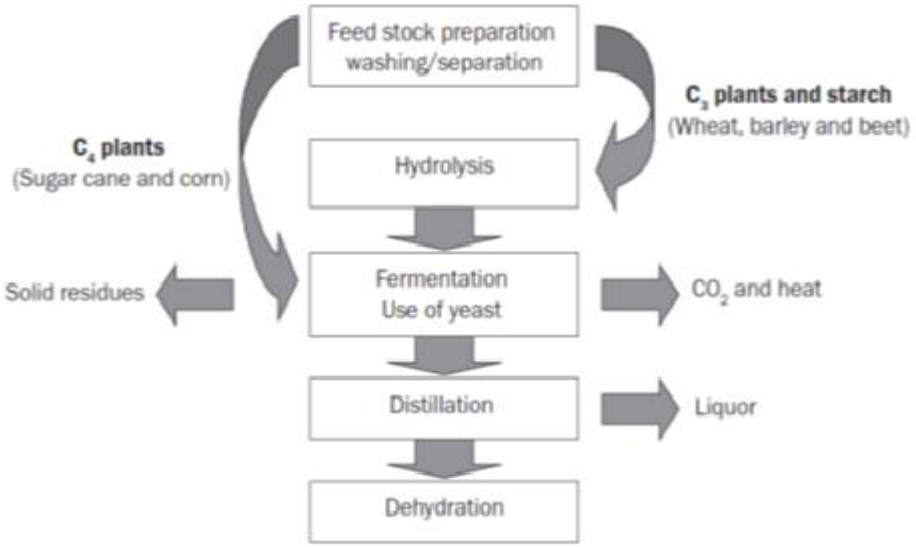


Figure 1.2 Flow chart of ethanol production from different feed stocks.

(Zuurbier *et al*., 2008)

1.5 Vinasse

Vinasse is an aqueous by- product from the ethanol distillation processes. The main charctrasitcs of vinasse are: acidity (pH 3.5 – 5), dark brown slurry (España-Gamboa *et al*., 2011) and with a very high organic content. Its COD and BOD concentration are high, with range up to 90 - 210 g/dm3 and 45 -100 g/dm3 respectively (Satyawali and Balakrishnan. 2008). Chemical composition of vinasse is variable depending on the raw materials and the processes of ethanol production. However most of vinasse types contain melanoidins, humic acid and furfural (Mohana, *et al*. 2009).

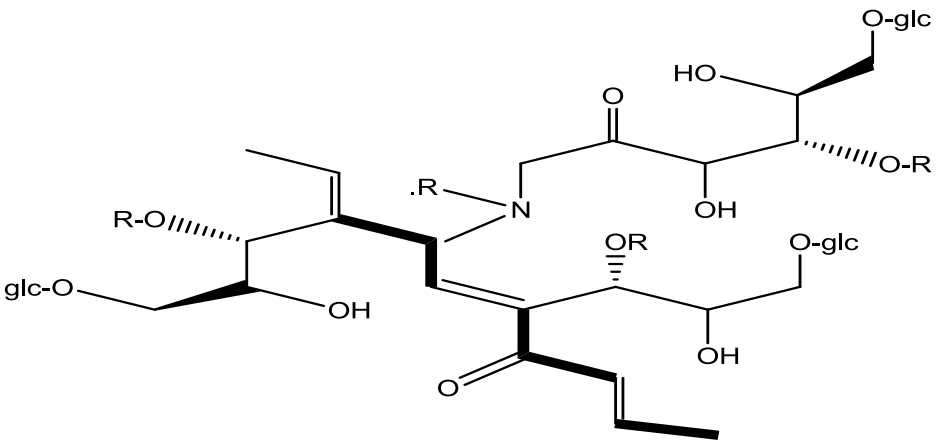


Figure 1.3 Melanoidin structure

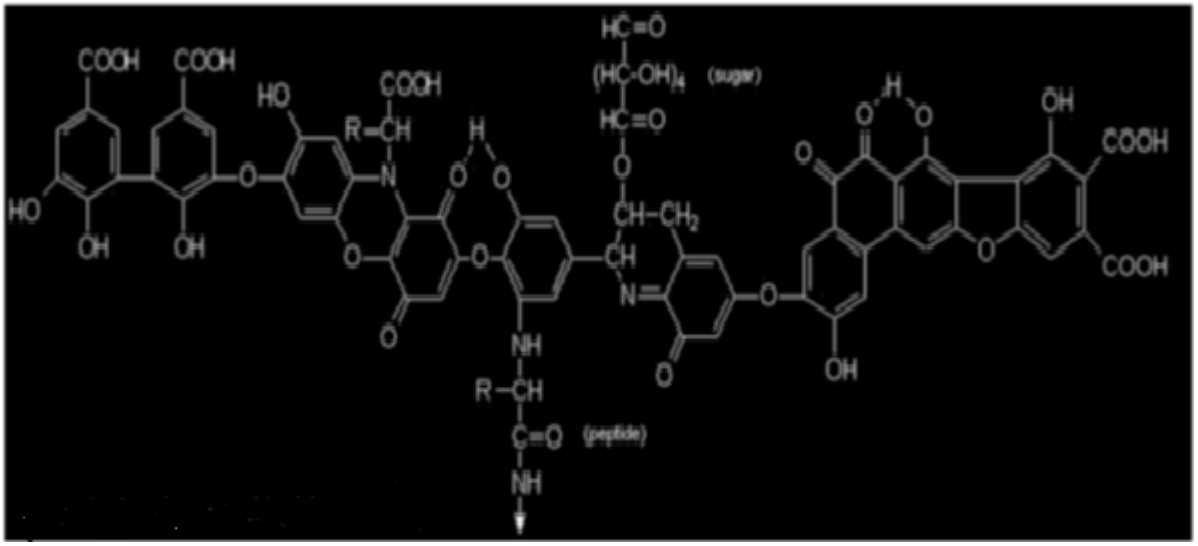


Figure 1.4 Humic acid structure

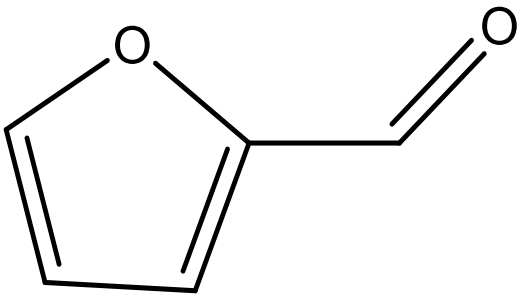


Figure 1.5 Furfural structure

There are many types of vinasses classified according to the feed stock and raw materials used such as:

1.5.1 Sugar cane vinasse

The production of ethanol from sugar cane in the form of either cane juice or molasses, the raw material constitutes about 40–70% of the production cost (Cardona, *et al*. 2010). In many countries ethanol is produced primarily from molasses, a by-product of the sugar industry, while in Brazil ethanol is produced from sugar cane juice. (Da silva, *et al*. 2005). Hence, integrated sugar cane factories which have sugar manufacturing co-located with an ethanol distillery can use molasses as a feedstock for ethanol in addition to raw cane juice directly from the mill. A significant number of sugar cane factories in Brazil and several hundred others around the world are of this type (Gopal and Kammen. 2009). Sugar cane molasses is a product of the concentration of juice and the precipitation of sugar and some non-sugar impurities present in the juice which are separated by the addition of chemical reactants such as sulfur and lime, among others (Ensinas, *et al.* 2009). Due to the crystallization process, molasses has higher concentrations of potassium, phosphates, sulfates, calcium, iron, sodium, chlorides, carbon source and other trace elements than that present in sugar cane juice (Wilkie, *et al*. 2000; Nandy, *et al*. 2002; Cardona and Sánchez. 2007; Parnaudeau, *et al*. 2008; Salomon and Lora. 2009).

Information available in the literature suggests that the major organic components of sugar cane vinasse are glycerol, lactic acid, ethanol, acetic acid, oxalate, malate and other alcoholic compounds, carbohydrates and a high content of phenols (Parnaudeau, *et al.* 2008). The nitrogen in the vinasses comes from microbial biomass and also depends on the origin of the molasses. The latter has been confirmed by the relatively similar proportions of nitrogen found in insoluble

acids in molasses and vinasses, which were derived from the same raw material source (Wilkie, *et al*. 2000).

Table 1.1 The major organic components of sugarcane vinasse.

Compound	Concentrations g/dm^3
Ethanol	3.83
Propylene glycol	0.084
Glycerol	5.86 (0.45)
Formic acid	0.582
Erythritol	0.088
Sucrose	0.222
Acetic acid	1.56
Acetic acid	7.74

1.5.2 Sugar beet vinasses

Ethanol is produced from sugar beet molasses due to the high cost of sugar beet juice. Its vinasses contain a high concentration of a compound rich in nitrogen called betain, which tolerates temperatures as high as 200°C (Parnaudeau, *et al*. 2008) , although there is also a significant glycerol content. Betain has two main metabolic functions, acting as osmoprotector and as a donor of methyl radicals. As in sugar cane vinasses, the main organic acids present in the vinasses are oxalic, lactic, acetate and maleic. These vinasses present higher protein content compared to sugar cane vinasses (Stemme, *et al*. 2005).

Sugar beet vinasses have high nitrogen concentrations and consequently lower carbon–nitrogen ratios; organic nitrogen is approximately 40% of the total nitrogen content (Wilkie *et al*., 2000). Similarly, there is high potassium content, approximately one-third of the mineral content, and sulfates resulting from the sulfatizing process used in sugar production (Stemme, *et al*. 2005).

Table 1.2 Characterizations of sugarcane molasses, sugar juice and sugar beet vinasse (España-Gamboa, *et al*. 2011).

	Cane juice	**Cane molasses**	**Beet molasses**
BOD g/dm^3	16.7	39.5	27.5–44.9
COD g/dm^3	30.4	84.9–95	55.5–91.1
Total N mg/dm^3	102–628	153–1230	1800–4750
Total P mg/dm^3	71–130	1–190	160–163
K mg/dm^3	1733–1952	4893–11000	10000–10030
Total S mg/dm^3	1356	1500–3480	3500–3720
pH	4.04–4.6	4.46–4.8	4.3–5.35
Cu mg/dm^3	4	0.27–1.71	2.1–5
Cd mg/dm^3	NA	0.04–1.36	<1
Pb mg/dm^3	NA	0.02–0.48	<5
Fe mg/dm^3	16	12.8–157.5	203–226
Phenols mg/dm^3	NA	34	450

NA= not available

1.5.3 Starch crops vinasse

Corn is a food source based on starch, which together with wheat, comprise the most popular crops for ethanol production in the U.S. and Europe (Cardona and Sa´nchez. 2007). The solid and liquid fractions remaining after distillation are referred to as ‘whole stillage’. Whole stillage includes the fibre, oil, and protein components of the grain, as well as non-fermented starch (Bothast and Schlicher. 2005).

Corn vinasses are rich of nitrogen which can be found as amino acids and peptides rather than proteins, although other materials containing nitrogen are also present. Traces of amino acids are found in all vinasses examined and corn vinasses contain high levels of alanine and proline (Wilkie, *et al*. 2000), while Wheat

vinasse contain less than 2% starch and protein, and fiber contents are higher than those of corn vinasses. Another significant difference between them is the higher lipid content in corn vinasses (Lee, *et al*. 1991). As with barley vinasses, there is a high nitrogen content (4.09– 8.8 g/dm^3) in these vinasses, due to their high protein content (Hutnan, *et al*. 2003).

1.5.4 Cellulose vinasses

The most important sources of cellulose for ethanol production are herbaceous and wood biomass (soft and hard respectively), along with industrial and municipal solid organic waste (Wilkie, *et al*. 2000; Rabinovich, 2006). These materials mainly consist of a mixture of carbohydrate polymers (cellulose and hemi-cellulose), lignin, extractables and ashes. Lignin is a very complex cross-linked racemic polymer structured by phenylpropanoid units joined in a three-dimensional structure. Soft wood usually contains more lignin than hardwood (Taherzadeh and Karimi. 2007). The extractable compounds are not soluble in water and they are generally found in small proportions in cellulosic sources. These compounds can be classified into four groups: steroids, fats, phenolic constituents and inorganic compounds. Lignin is the principal solid residue found in these vinasses due to its resistance to chemical and enzymatic degradation. Its concentration depends on the type of raw material employed in the fermentation process. Other compounds found are: acetic acid, furfural, hydroxyl methyl furfural, residual sugars, and others (Taherzadeh and Karimi. 2007). In general, the characteristics of cellulose vinasses are comparable to those obtained from sugar and starch vinasses (Wilkie, *et al*. 2000) with two possible exceptions: they may contain a higher level of heavy metals, due to the acid hydrolysis process applied and the presence of unusual inhibitors, such as extractable and phenolic compounds found in the raw material, and these phenolic compounds inhibit the biological digestion of this wastewater. Vinasse from cellulose sources are the

darkest vinasse due to the presence of phenolic compounds released during the degradation of lignin and melanoidins, over-heated sugar caramels. (Taherzadeh and Karimi. 2007).

1.6 Environmental impact of vinasses

While the production and the characteristics of vinasse are highly variable and dependent on the raw material used and various aspects of the ethanol production process, thus the environmental impacts of vinasse are variable. Recalcitrant nature of vinasse is due to presence of the brown polymers, melanoidins, which are formed by Maillard amino carbonyl reaction. These compounds have antioxidant properties, which render them toxic to many microorganisms such as those typically present in wastewater treatment processes (Mohana, *et al*. 2009). The defiance of melanoidins to degradation is apparent from the fact that these compounds escape various stages of wastewater treatment plants and finally enters into the environment. Apart from melanoidins, the other recalcitrant compounds present in the waste are caramel, variety of sugar decomposition products, anthocyanins, tannins and different xenobiotic compounds (Mohana, *et al*. 2009).

Disposal of vinasse into the environment is hazardous and has high pollution potential. High COD, total nitrogen and total phosphate content of the effluent may result in eutrophication of natural water bodies (Kumar, *et al*. 1997). Direct disposal of vinasse into the aquatic environments tend to increase the organic content of water and consequently causes the proliferation of bacteria that depleted the dissolved oxygen and water quality (Mohana, *et al*. 2009). The highly colored components of the vinasse reduce sunlight penetration in rivers, lakes or lagoons which in turn decrease both photosynthetic activity and dissolved oxygen concentration affecting aquatic life (Kumar, *et al*. 1997).

Saxena and Chauhan (2003) investigated the influence of distillery effluent on oxygen consumption in fresh water fish, on the other hand the presence of inorganic and organic salts in the effluent interfered with the respiration in the fish. The coagulation of gill mucous decreased dissolved oxygen consumption causing asphyxiation (Mohana, *et al*. 2009). Matkar and Gangotri (2003) observed concentration dependent toxicity of distillery effluent on the fresh water crab.Disposal of distillery spent wash on land is equally hazardous to the vegetation. It is reported to reduce soil alkalinity and manganese availability, thus inhibiting seed germination (Kumar, *et al.* 1997). Kannan and Upreti (2008) reported highly toxic effects of raw distillery effluent on the growth and germination of Vigna radiata seeds even at low concentration of 5% (v/v). Leaching of protein and carbohydrates from the seeds as well as decrease in activities of important enzymes like alkaline phosphatase.

Application of distillery effluent to soil without proper monitoring, perilously affects the groundwater quality by altering its physicochemical properties such as color, pH, electrical conductivity (EC), etc. due to leaching down of the organic and inorganic ions (Jain, *et al*. 2005). In a study carried out by Dhembare and Amin (2002), indices indicating soil quality like Sodium Absorption Ratio (SAR) and Soluble Sodium Percentage (SSP) were reported to be adversely affected in the soil amended with distillery effluent. Constant disposal/irrigation of the soil with the effluent led to deleterious effect on the soil properties. Soil microorganisms are an essential component of the soil ecosystem and are involved in regulating the various processes of nutrient recycling in soil. Any type of interference with their activity may affect soil productivity as they are the indices of soil fertility. Juwarkar and Dutta (1990) evaluated the impact of application of distillery effluent on soil microflora. Irrigation with raw distillery effluent resulted in low overall bacterial and actinomycetes count. However, population of fungi increased. Nitrogen fixing bacteria Rhizobium and Azotobacter also reduced

considerably. Anaerobically treated effluent also showed similar results but not as much as that of the raw effluent.

The unpleasant odor of the effluent is due to the presence of skatole, indole and other sulphur compounds, which are not effectively decomposed by yeast during distillation (Sharma, 2007).

1.7 Utilizations of vinasse

In order to reduce the disposal of vinasse, different alternative methods for the utilization of vinasses such as fertirrigation and yeast production have been introduced (Figure 1-6).

1.7.1 Fertirrigation

The first studies on the application of vinasse to the soil in Brazil started in the 1950s and were conducted by the Luiz de Queiroz College of Agriculture (ESALQ) (Camargo, *et al*. 2009). The use as fertilizer in fertirrigation became common in sugarcane refineries beginning in the 1980s (Cintya, *et al*. 2013). Fertirrigation consists of the infiltration of raw vinasse in the soil by irrigation of sugarcane crops (Camargo, *et al*. 2009). When applied in nature to the soil, sugarcane vinasse in addition to irrigation, fertilizes the crop, lowering the costs with chemical fertilizers (Laime, *et al*. 2011). The use of vinasse in fertirrigation is an alternative that focuses on the rational use of natural resources, preventing the discharge of vinasse in rivers, while fertilizing agricultural land (Gianchini and Ferraz. 2009). Among the alternatives for the use of vinasse developed around the world, fertirigation is the most commonly used, as it requires a low initial investment (tubes, pumps, trucks, and decantation tanks), low maintenance cost, fast application, does not require complex technologies, and increases crop yield. This practice has totally or partially replaced the use of chemical fertilizers, mainly those containing phosphorus (Camargo, *et al*. 2009; Santana and Machado. 2008). About 75–80% sugarcane crops in Brazil irrigated with vinasse

(Fronzalia, 2007; Junior, et al. 2008). However, according to the literature, the direct application of vinasse in the soil can cause salinization, leaching of metals present in the soil to groundwater, changes in soil quality due to unbalance of nutrients, mainly manganese (Agrawal and Pandey. 1994), alkalinity reduction, crop losses increase of phytotoxicity and unpleasant odor (Navarro *et al*. 2000; Santana and Machado. 2008). According to Santana and Machado (2008), fertirrigation may be a palliative practice that provides a false impression of solving efficiently the problem of vinasse disposal. On the other hand, certain environmental parameters need to be accounted for in fertirrigation, such as soil type, distance from water bodies, soil field capacity (water retention) percentage of salts in the soil (Laime, *et al*. 2011). Some studies indicated that doses of 300 m^3/ha of vinasse with potassium levels between 3 and 4 kg/m^3 of vinasse, regardless of the type of soil, do not alter physical, chemical and biological properties of the soil (Penatti, *et al*. 1999).

1.7.2 Concentration by evaporation

The concentration of sugarcane vinasse by evaporation is an alternative for the use of this residue, since fertirrigation cannot always dispose of total volume of vinasse produced. The product obtained in this process is used in the production of livestock feed and to improve the quality of vinasse as fertilizer. It can also be burned in special boilers generating energy or decreasing the water use in the facility, and the condensate removed by evaporation can be treated and reused by the factory. High energy demand is probably the main constraint of vinasse concentration. Some methods have been described in the literature for the treatment and concentration of vinasse (Gomes, *et al*. 2011). Larsson and Tengberg (2014) reported, it was found possible to evaporate vinasse to a high dry solid content of at least 70 %. However, according to Fitzgibbon *et al.* (1995) and Navarro *et al.* (2000), vinasse concentration and incineration are the only methods

that can satisfactorily solve the pollution problem. In the concentration process, water is removed from vinasse (without loss of solids), reducing its volume. The first vinasse concentration plants were installed in 1942 in Australia by the Australian company. This process can reduce the costs with transportation in tanker trucks, increasing radius of vinasse application, where fertirrigation in ducts is unfeasible (Cintya, *et al*. 2013). However, this process has problems associated with the fast incrustation of evaporators and spontaneous crystallization as the concentration of solids increases (Rodrigues, 2008). In Brazil, only one plant concentrates vinasse. Installed as a demonstration facility more than 20 years ago, it processes 5% of the vinasse produced, concentrating it to 40% (Cintya, *et al*. 2013). When not used as fertilizer, concentrated vinasse can be used in the production of livestock feed, due to its high levels of nutrients. The production of livestock feed from vinasse has also been studied in the 1980s (Laime, *et al*. 2011). The residue needs to have level of potassium reduced, and can be used as feed for cattle, pigs, and poultry. The feed produced does not interfere in the taste or odor of milk or dairy products, is well accepted by animals, and the conversion rate (weight gain in relation to feed consumption) is adequate. However, dosage limits should be observed (Corazza and Salles Filho. 2000). According to Waliszewski *et al*. (1997), in some countries, dry vinasse is used to substitute molasses, mainly to feed ruminants. For these animals, the feed produced from vinasse should not be over 10% of the daily feed; and under 2% to 3% for pigs, (Corazza and Salles Filho. 2000; Laime, *et al*. 2011). However, the high levels of salts and low quantities of carbohydrates limit its use as poultry feed due to the low level of metabolizable energy (Waliszewski, *et al*. 1997).

1.7.3 Energy production

An alternative that has increasingly being used in the ethanol industry is the anaerobic bio-digestion of the organic load of vinasse. This process consists of the

biodegradation of the organic load of vinasse to produce biogas and biodigested vinasse (Cortez, *et al*. 2007). The anaerobic process of biodigestion occurs in two stages, the acidogenic and methanogenic phases. In the acidogenic phase, the organic compounds of complex chains, such as lipids, carbohydrates and proteins are hydrolyzed until the formation of compounds with smaller carbon chains. These smaller-chain compounds are biologically oxidized and converted to organic acids, such as acetic acid (CH_3COOH) and propionic acid (CH_3CH_2COOH) by facultative and obligate anaerobic bacteria. The reduction of the organic load of the effluent occurs in this phase (Cortez, *et al*. 2007). In the methanogenic phase, acids are converted into methane, carbon dioxide and organic acids, or carbon dioxide is reduced until the formation of methane by anaerobic microorganisms. This is the slowest phase of the process and controls the conversion rates (Cortez, *et al*. 2007). The bio digested vinasse is later used as fertilizer. Although it presents a reduced organic load, it maintains its original properties as fertilizer. On the other hand, biogas is mainly used to produce energy, due to its high methane content.

In the sugar-ethanol industry, biogas can be used to: operate gas turbines combined to an electric generator; substitute part of the fuels used in the agro-industry during the harvesting time; or use in boilers to generate vapors and to mill sugarcane (Cortez *et al*. 2007; Szymanski *et al*. 2010). Anaerobic biodigestion has received more attention only after the development of high performance reactors, such as the UASB (Upflow Anaerobic Sludge Blanket), which is the most adapted to vinasse. In this type of system, the sludge at the bottom of the reactor adsorbs most of the organic matter, while gas is produced in the reaction compartment as bubbles during the anaerobic process, and removed to a separate compartment (Von Sperling, 2005).

This treatment in bio-digesters and reactors has the advantage of producing biogas that can be used in the production of energy; in addition to have a low electric

energy consumption, little production of biological sludge to be disposed and low polluting potential – mainly due to the reduction of the vinasse organic load, since most of the BOD is converted into biogas (Freire and Cortez. 2000). However, Cortez *et al*. (2007) describes the longer detention time compared to aerobic systems and production of corrosive gases with unpleasant odor as the main inconvenient of the anaerobic digestion. Therefore, anaerobic bio-digestion is an alternative of great economic as well as environmental interest in the treatment of vinasse, as the biogas produced, once purified, has calorific value similar to that of natural gas, with the advantage of being a renewable and easily available fuel (Szymanski, *et al.* 2010).

1.7.4 Yeast production

The yeast production from the vinasse is also an alternative technology that can reduce the discharge of this residue. However, two factors contribute to the rising costs of this alternative:

i. The fact of being necessary to add ammonium and magnesium salts to the vinasse

ii. The fact that high energy consumption for the evaporation of water from vinasse required in this process (Corazza and Salles Filho. 2000; Laime, et al. 2011).

These approaches showed some major drawbacks such as unable to remove organic substances from vinasse as well as causing surface and ground water pollution (Campos, *et al.* 2014).

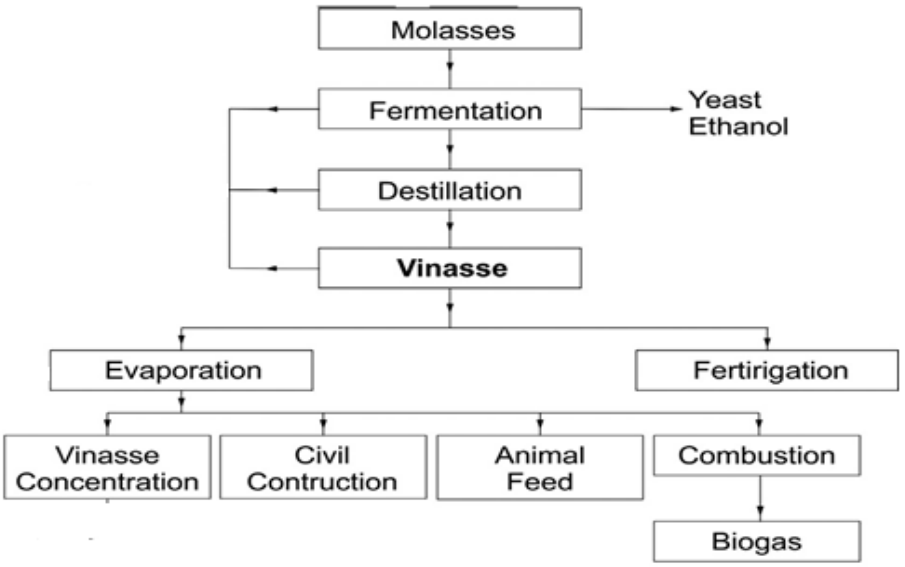


Figure 1. 6: Flow chart of the utilizations of sugarcane vinasse.(Cintya, *et al.* 2013)

1.8 Vinasse treatments

A number of technologies have been explored for reducing the pollution load of vinasse. In general, the control of pollutants in water and wastewater treatment can be divided into two basic techniques.

1.8.1 Treatments based on biological methods

1.8.1.1 Fungal treatment

In recent years, several basidiomycetes and ascomycetes type fungi have been used in the decolorization of natural and synthetic melanoidins in connection with color reduction of wastewaters from distilleries. The aim of fungal treatment is to purify the effluent by consumption of organic substances, thus, reducing its COD and BOD, and at the same time to obtain some valuable products, such as fungal biomass for protein-rich animal feed or some specific fungal metabolite. Several fungi have been investigated for their ability to remove color and COD (Satyawali and Balakrishnan. 2008).

1.8.1.2 Anaerobic systems

The high organic content of molasses spentwash makes anaerobic treatment attractive in comparison to direct aerobic treatment. Therefore, biomethanation is the primary treatment step and is often followed by two-stage aerobic treatment before discharge into a water body or on land for irrigation (Nandy, *et al.* 2002). Aerobic treatment alone is not feasible due to the high energy consumption for aeration, cooling, etc. Moreover, 50% of the COD is converted to sludge after aerobic treatment (Sennitt, 2005). In contrast, anaerobic treatment converts over half of the effluent COD into biogas (Wilkie, *et al.* 2000).

1.8.1.3 Aerobic systems

The post-anaerobic treatment stage effluent still has high organic loading and is dark brown in color, hence it is generally followed by a secondary, aerobic treatment. Solar drying of biomethanated spentwash is one option but the large land area requirement limits this practice.

The presence of some recalcitrant organic compounds in vinasse have been found to be resistant against conventional biological treatments, these compounds have antioxidant properties, which render them toxic to many microorganisms. (De Heredia, et al. 2005; Luty, et al. 2008; Siqueira, et al. 2013). Biological treatments of vinasse also have some disadvantages such as time consuming, required large land area for treatment, producing sludge (Chopra, et al., 2011) and required cooling procedure. (Ribas, et al., 2009).

1.8.2. Treatments based on physiochemical methods

1.8.2.1 Membrane treatment

Pre-treatment of spentwash with ceramic membranes prior to anaerobic digestion is reported to have the COD from 36,000 to 18,000 mg/dm^3 (Chang, *et al*. 1994). The total membrane area was 0.2m^2 and the system was operated at a fluid velocity of 6.08 m/s and 0.5 bar transmembrane pressure. In addition to COD

reduction, the pre-treatment also improved the efficiency of the anaerobic process possibly due to the removal of inhibiting substances. Kumaresan *et al*. (2003) employed emulsion liquid membrane (ELM) technique in a batch process for spentwash treatment. Water–oil–water type of emulsion was used to separate and concentrate the solutes resulting in 86% and 97% decrease in COD and BOD, respectively. Electrodialysis has been explored for desalting spentwash using cation and anion exchange membranes resulting in 50–60% reduction in potassium content (de Wilde, 1987). In another study, Vlyssides *et al*. (1997) reported the treatment of vinasse by electrodialysis using a stainless steel cathode, titanium alloy anode and 4% w/v NaCl as electrolytic agent. Up to 88% COD reduction at pH 9.5 was obtained; however, the COD removal percentage decreased at higher wastewater feeding rates. In addition, reverse osmosis (RO) has also been employed for distillery wastewater treatment. A unit in western India is currently processing effluent obtained after anaerobic digestion, followed by hold-up in a tank maintained under aerobic conditions, in a RO system (Satyawali and Balakrishnan. 2008).

Nataraj *et al*. (2006) reported pilot trials on distillery spentwash using a hybrid nanofiltration (NF) and RO process. Both the NF and RO stages employed thin film composite (TFC) membranes in spiral wound configuration with module dimensions of 2.5 inches diameter and 21 inches length. NF was primarily effective in removing the color and colloidal particles accompanied by 80%, 95% and 45% reduction in total dissolved solids (TDS), conductivity and chloride concentration, respectively, at an optimum feed pressure of 30–50 bar. The subsequent RO operation at a feed pressure of 50 bar resulted in 99% reduction each in COD, potassium and residual TDS (Satyawali and Balakrishnan. 2008).

1.8.2.2 Electrochemical oxidation process

Electrochemical treatment is widely used to eliminate color from industrial effluents. This process uses electrons as a main reagent, but also requires the presence of supporting electrolytes. During the electrochemical process, pollutants are destroyed by any oxidation process, direct or indirect. In a direct anodic oxidation process, the pollutants are first adsorbed onto the surface of the anode and subsequently destroyed by the transfer reaction of anodic electrons. In an indirect oxidation process, strong oxidants such as hypochlorite/chlorine, ozone, and hydrogen peroxide are generated electrochemically. The pollutants are destroyed in the volume solution by the oxidation reaction of the generated oxidant. All of the oxidants are generated in situ and are used immediately (Prasad and Srivastava. 2009). In the use of electro-oxidation, the type of anode employed and the presence of oxidizing agents have a significant influence on the treatment process. Manisankar *et al*. (2003) achieved complete color degradation of vinasses diluted 10 times with an insoluble anode of titanium substrate (Ti/RuO_2). A maximum of 92% in COD reduction, 98.2% in BOD reduction and a 99.5% reduction.

Prasad and Srivastava (2009) studied electrochemical degradation of vinasses using ruthenium oxide covered with titanium mesh functioning as anode and stainless steel as cathode. A maximum color removal of 83.3% and COD degradation of 39.6% was achieved for a current density of $14.3 mAcm^{-2}$. Electrolysis time was 3 hour with a vinasse at 10% dilution and a pH of 5.5.

1.8.2.3 Adsorption

Activated carbon is a widely used adsorbent for the removal of organic pollutants from wastewater but the relatively high cost restricts its usage. Activated carbon, with a significant distribution of micropores and mesopores, has proved to be very efficient in the adsorption of melanoidins and dark composites in vinasses.

Decolorization of synthetic melanoidin using commercially available activated carbon as well as activated carbon produced from sugarcane bagasse was investigated by Bernardo *et al*. (1997). The adsorptive capacity of the different activated carbons was found to be quite comparable. Chemically modified bagasse using 2-diethylaminoethyl chloride hydrochloride and 3-chloro-2-hydroxypropyltrimethylammonium chloride was capable of decolorizing diluted spentwash (Mane, *et al*. 2006).Significant decolorization was observed in packed bed studies on anaerobically treated spentwash using commercial activated charcoal with a surface area of 1400m^2/g (Chandra and Pandey. 2000). Almost complete decolorization (99%) was obtained with 70% of the eluted sample, which also displayed over 90% BOD and COD removal. In contrast, other workers have reported adsorption by activated carbon to be ineffective in the treatment of distillery effluent (Sekar and Murthy. 1998; Mandal, *et al*. 2003).

Mall and Kumar (1997) compared the color removal using commercial activated carbon and bagasse flyash. 58% color removal was reported with 30 g/dm^3 of bagasse flyash and 80.7% with 20 g/dm^3 of commercial activated carbon.

1.8.2.4 Coagulation and flocculation

Coagulation and flocculation constitute the backbone processes in most water and advanced wastewater treatment plants. Their objective is to enhance the separation of particulate species in downstream processes such as sedimentation and filtration. Colloidal particles and other finely divided matter are brought together and agglomerated to form larger size particles that can subsequently be removed in a more efficient technique (Lawrence, *et al*. 2005).

Coagulation flocculation processes have been used in water treatment and industrial waste treatment as well as vinasse treatment. Most researchers prefer using coagulation and flocculation combined with other technique for enhanced vinasse treatment (Espan˜a-Gamboa, *et al*. 2010). Zayas, *et al*. (2007) investigated

the use of coagulation/flocculation and electrochemical oxidation processes to purify vinasse. Also Inanc *et al*. (1999) explored lime and ozone treatment with anaerobically digested effluent. An integrated process of Fenton-coagulation/flocculation was also evaluated by (Beltran, *et al*. 2005).

1.8.2.4.1 Coagulation

Refers to particle neutralization and destabilization by the reduction of the repulsive potential of the double layer surrounding the particles. As applied to water, is the application of certain chemicals in small quantities to produce a precipitate. (Hassan, 2012).

1.8.2.4.2 Flocculation

Implies a chemical bridging mechanism that helps to accumulate the suspended particles in water in a three dimensional network. Coagulation is applied to the overall processes of particle aggregation, including both particle destabilization and particle transport. The term flocculation is used to describe only the transport step. (Tchobanoglous, *et al*. 2003).

1.8.2.4.3 Colloids

Colloids are very small particles that have extremely large surface area. Colloidal particles are larger than atoms and ions but are small enough that they are usually not visible to the naked eye. They range in size from 0.001 to 10 μm resulting in a very small ratio of mass to surface area.

Because of their tremendous surface, colloidal particles have the tendency to adsorb various ions from the surrounding medium that impart to the colloids an electrostatic charge relative to the bulk of surrounding water. The developed electrostatic repulsive forces prevent the colloids from coming together and, consequently, contribute to their dispersion and stability (Reynolds. 1982).

1.8.2.4.4 Colloids structure and stability

The stability of colloidal particulate matter is dependent on their electro-kinetic property. Solid particles in aqueous dispersion move in an electric field which

indicates that they carry electric charge. The sign of this charge can be positive or negative, although most colloids in water carry negative charge. The sign and magnitude of the primarily charges are frequently affected by the pH and the ionic content of the aqueous phase. A colloidal dispersion does not have a net electrical charge, so that the primarily charge on the particles must be counterbalanced in the aqueous phase. As a result, an electric double layer exists at every interface between a solid and water. This double layer consists of the charged particle and an equivalent excess of ions of opposite charge (counter ions) which accumulate in the water near the surface of the particle.

Certain attractive forces in a form of Van der Waals' forces exist between colloids are responsible for aggregation of many colloidal systems. Their magnitude depends upon the kinds of atoms which make up the colloidal particles and their densities. The solid particles in a colloidal dispersion are in constant motion and so they have kinetic energy. At any instant, a distribution of kinetic energy exists, with some particles having a very large kinetic energy, large enough to overcome the activation energy barrier. Irreversible colloidal systems which have high activation energy and/or a low kinetic energy will coagulate very slowly (diuturnal colloids). Colloids with low activation energy and/or a high kinetic energy will coagulate rapidly (caduceus systems) (Hassan, 2012).

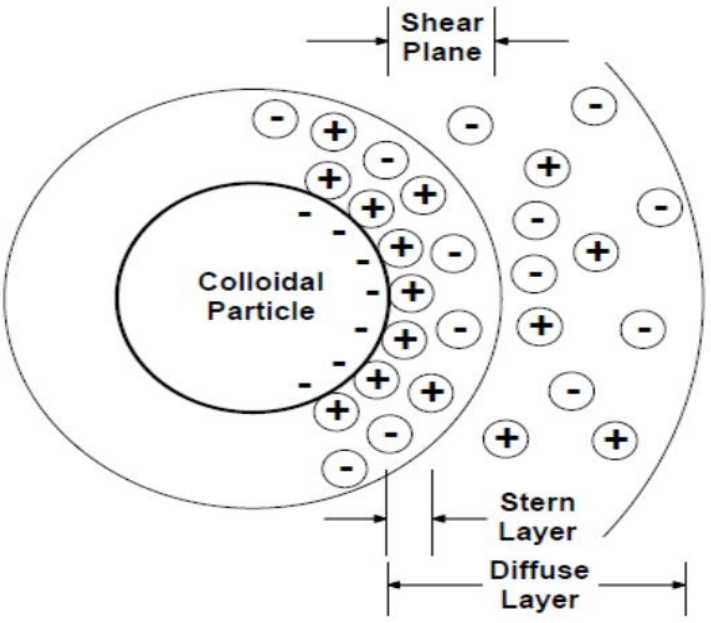


Figure 1.7 Distribution of charge in double layer of colloid particles

1.8.2.4.5 Destabilization of colloidal particles

Destabilization of colloidal particles is accomplished by coagulation through the addition of hydrolyzing electrolytes such as metal salts and/or synthetic organic polymers. Upon being added to the water, the action of the metal salt is complex (Amirtharajah and O'Melia. 1999). It undergoes dissolution, the formation of complex highly charged hydrolyzed metal coagulants (hydroxyoxides of metals), interparticle bridging, and the enmeshment of particles into flocs. Polymers work either on the basis of particle destabilization or bridging between the particles. The destabilization process is achieved by many mechanisms such as double-layer compression, adsorption and charge neutralization, entrapment of particles in precipitate and adsorption and bridging between particles.

1.8.2.4.6 Coagulants

Coagulant is chemicals that are added to the water to achieve coagulation (Mackenzie and Cornwell. 1985), the most commonly used coagulants in water and wastewater treatment include aluminum sulfate (alum), ferric chloride, ferric sulfate, ferrous sulfate (copperas), sodium aluminate, polyaluminum chloride, and

organic polymers. The chemistry of metallic salts is a complex one. It involves dissolution, hydrolysis, and polymerization reactions (olation).

i. Dissolution:

All metal cations in water are present in a hydrated form as aquocomplexes. The simple iron variety does not exist as such in an aqueous solution, rather, the metallic species is present in the aquometal form as $2Fe(H_2O)_6^{3+}$ in the following equation:

$$Fe_2(SO_4)_{3+} 12H_2O \rightarrow 2Fe(H_2O)_6^{3+} + 3SO_4^{2-} \quad (1\text{-}3)$$

ii. Hydrolysis:

The aquometal ions formed in the dissolution of metallic in water are acidic or proton donors. This is demonstrated by the following hydrolytic reactions:

$$Fe(H_2O)_6^{3+} + H_2O \rightarrow Fe(H_2O)_5(OH)^{2+} + H_3O^{+} \quad (1\text{-}4)$$

iii. Polymerization:

The hydroxocomplexes formed as products of hydrolysis may combine to form a variety of hydroxometal polymers such as a ferric dimer $Fe_2(OH)_2^{4+}$

1.8.2.4.7 Factors influencing coagulation

Optimum coagulation treatment of raw water represents the attainment of a complex equilibrium in which many variables are involved. Many conditions affect coagulation such as pH, turbidity, chemical composition of the raw water, type of coagulant, temperature and mixing conditions (Lawrence, *et al*. 2005).

1.8.2.5. Advanced oxidation processes

Advanced oxidation processes (AOPs) are technologies with significant importance in environmental restoration applications (Anipsitakis and Dionysiou. 2003; Bandala *et al*. 2007). The AOPs concept was established by (Glaze, *et al.* 1987; Glaze, et al. 1989; Huang, *et al*. 1993) who defined AOPs as processes

involving the generation of highly reactive oxidizing species able to attack and degrade organic substances (Bolton, 2001). Nowadays AOPs are considered high efficiency physico-chemical processes due to their thermodynamic viability and capablity to produce deep changes in the chemical structure of the contaminants via the participation of free radicals (Domenech, *et al*. 2004). These species, are of particular interest because of their high oxidation capability (Legrini, *et al*. 1993; Goswami and Blake. 1996; Rajeshwar, 1996; Andreozzi, *et al*. 1999; Huston and Pignatello. 1999) Free radicals in AOPs, may be produced by photochemical and non photochemicals procedures (Table 2-3).

Table 1.3 List of some Non- photochemical and photochemical AOPs.

Non-photochemical	**Photochemical**
O_3/H_2O_2	H_2O_2/UV
O_3/US	O_3/UV
O_3/OH^-	$O_3/H_2O_2/UV$
Fe^{2+}/H_2O_2 (Fenton system)	H_2O_2/Fe^{2+} (photo-Fenton)
electro-Fenton	UV/TiO_2
electron beam irradiation	$H_2O_2/TiO_2/UV$
ultrasound (US)	$O_2/TiO_2/UV$
H_2O_2/US	UV/US

In comparison with the other techniques such as adsorption, biological treatment, bio-filtration as well as thermal or catalytic combustion, the employment of new technologies with the generic term advanced oxidation processes leads to the decomposition and mineralisation of many groups of organic materials in both the liquid and gas phases (Munoz *et al* . 2006; Boulamanti *et al* . 2008). AOPs have attracted significant attention in recent years. Depending on the chemical structure of the pollutant molecules, AOPs mineralise numerous pollutants into ultimately

harmless substances like CO_2 and H_2O and therefore avoid the issue of pollution shifting. The particular importance of these technologies appears to be in destroying biologically non-degradable chemical structures as well as ozone-resistant substances such as organic pesticides and herbicides (Meijers, *et al*. 1995; Piera, *et al*. 2000; Sanches, *et al*. 2010), aromatic structures (Zhang, *et al*. 2008) , organo-halogens (Ormad, *et al*. 1997) and petroleum constituents in wastewaters (Saien and Nejati. 2007).

In a general definition, physicochemical procedures which promote in situ generation of free hydroxyl radicals as highly oxidative reagents for the decomposition of pollutants in water or air are described as "advanced oxidation processes". These oxidation processes basically use three different reagents: ozone, hydrogen peroxide and oxygen in many combinations, either combined with each other or applied with UV irradiation and/or various kinds of catalysts (Glaze and Kang. 1989). Due to the generation of increased amounts of $OH^{\bullet}$ radicals, combination of two or more AOPs usually leads to higher oxidation rates. With promising results observed on the laboratory scale, compared with conventional water and wastewater treatment methods, these technologies will likely be more essential for real applications in the near future. Among the different approaches of oxidation and advanced oxidation processes, some of them were used for vinasse treatment.

Oxidation of vinasse with chlorine resulted in 97% color removal but the color reappeared after a few days (Satyawali and Balakrishnan. 2008). Pena, *et al*. (2003) reported that Oxidation by ozone could achieve 80% decolorization for biologically treated vinasse with simultaneous 15–25% COD reduction. It also resulted in improved biodegradability of the effluent. However, ozone only transforms the chromophore groups but does not degrade the dark colored polymeric compounds in the effluent (Alfafara, *et al*. 2000; Pen˜ a, *et al*. 2003). Another option is photocatalytic oxidation that has been studied using solar

radiation and TiO_2 as the photocatalyst (Kulkarni, 1998). Use of TiO_2 was found to be very effective as the destructive oxidation process leads to complete mineralization of effluent to CO_2 and H_2O, while Juliana *et al*. (2012) studied evaluating the efficiency and application of heterogeneous photocatalysis with TiO_2, followed by a biological treatment. De Heredia *et al*. (2005) Also evaluates the Fenton process (H_2O_2/Fe^{2+}) as AOP for the removal of chemical oxygen demand (COD). On the other hand Yavuz (2007) reported that most of oxidation processes can be ineffective individually but effective when used in combinations.

Various vinasse oxidation technologies, that had been widely studied, were limited by unavailability of large-scale devices, or had too high operational costs (e.g. electrochemical oxidation and ozonation). Ozonation was showed no significant result in removal of organic compounds in vinasses. Thus, development of a technically and economically feasible vinasse treatment method is strategically important.

Persulfate ($S_2O_8^{2-}$) and peroxymonosulfate (HSO^{5-}) are the newest oxidant. Moreover, a low cost of persulfate salt (Huling and Pivetz. 2006) facilitates SR-AOP for potential application in wastewater treatment. With a strong oxidative capacity, SR can react selectively with organic compounds via electron transfer reaction, hydrogen abstraction and addition reaction.

1.8.2.5.1 Sulfate radical as AOPs

Persulfates have been used in industry for a considerable length of time as a bleach, as an etchant and as a polymerization agent, their use for environmental applications only began in the late 1990s. In contrast to peroxide and permanganate, persulfates did not have widespread use for industrial waste treatment prior to their use for in situ treatment. As a result, there is no a significant background of information on the reactivity and application of persulfates. The environmental chemistry of persulfate is still under development. Persulfate salts dissociate in water and forms the persulfate anion ($S_2O_8^{2-}$). The persulfate anion

is a strong oxidant (E_o = 2.01 V) as compared to 1.8 V for hydrogen peroxide (H_2O_2) and 1.4 V for the peroxymonosulfate anion (HSO^{5-}) (Table 1.4). But the use of it in treatment processes has kinetic limitations as it reacts much slower than other oxidants (e.g. hydroxyl radicals) (Liang. *et al*. 2007). When the persulfate anion and peroxymonosulfate anion is activated, it produces the sulfate free radical ($SO_4^{\bullet-}$), which is a stronger oxidant (Liang, *et al*. 2007).

Table 1.4 The potential reactive species potentially present in activated persulfate systems

Species		Potential (V)
Persulfate anion	$S_2O_8^{-2}$	+2.01
Monopersulfate anion	HSO_5^-	+1.4
Sulfate radical	$SO_4^{\bullet-}$	+2.6
Hydrogen peroxide	H_2O_2	+1.8
Hydroxyl radical	$OH\bullet$	+2.8

Persulfate-based sulfate radical oxidation has several advantages over other oxidant systems.

i. The sulfate radical is more stable than the hydroxyl radical and thus able to travel greater distances in the subsurface.

ii. Persulfate has less affinity for natural soil organic matter than does the permanganate ion (Brown, *et al*. 2003). And is thus more efficient in high organic content soils.

iii. Kinetically fast

These attributes combine to make persulfate a viable option for the chemical oxidation of a broad range of contaminants. Persulfate is very soluble, at 73 g/100ml of water at 25°C. High solubility makes it easy to apply concentrated solutions of persulfate. Sulfate radicals can be generated by activation of

persulfate or peroxymonosulfate by UV, heat and transition metal. The generation of $SO_4^{\bullet-}$ resembles an AOP based on H_2O_2 in that a similar radical precursor can be used for generation of $SO_4^{\bullet-}$ (i.e., $S_2O_8^{2-}$). This might enable retrofitting existing AOPs based on H_2O_2 by dosing of $S_2O_8^{2-}$.

Sulfate radicals are frequently reported as an alternative oxidant for pollutant treatment (Lutze, *et al*. 2014) and its efficiency in the treatment of various wastewaters such as landfill leachate (Zhang, *et al*. 2014) saline wastewater (Yuan, *et al*. 2014) and dyeing wastewater has been evaluated (Zhao, *et al*. 2014).

1.8.2.5.2 Generation of sulfate radical

Advanced techniques such as photolysis and radiolysis are used for the activation of the oxidants and thus provide a clean way of generating highly oxidizing radical species. Activation of persulfate by heat can produce sulfate radical as thermal persulfate oxidation as follows:

$$S_2O_8^{2-} + \text{heat} \rightarrow 2SO_4^{\bullet-} \quad (E_a = 33.5 \text{ kcal/mol}) \quad (1\text{-}5)$$

Subsequently, $SO_4^{\bullet-}$ may initiate production of other intermediate highly reactive oxygen species (ROS) such as hydroxyl radicals ($OH^{\cdot}$)

$$SO_4^{\bullet-} + H_2O \rightarrow OH^{\bullet} + HSO_4^{-} \quad (1\text{-}6)$$

These ROS can initiate a series of radical propagation and termination chain reactions where organics are partially and even fully decomposed (Yang and Casey. 2011). Philip *et al*. (2004) reported that both metal catalysis and heat activation, at temperatures above 20°C, to oxidize organic contaminants.

Introducing UV illumination into the reaction pathways serves as an alternative route to effectively activate persulfates and peroxymonosulfate , leading to producing $SO_4^{\bullet-}$ without the help of transition metals, thereby creating a powerful oxidant with promising applications to degrade organic contaminants (Yunqing, *et al*. 2013).

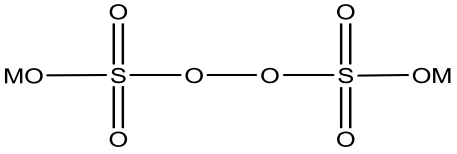
Activation of symmetrical and unsymmetrical peroxides under UV light radiation leads to the generation of $SO_4^{\bullet-}$ as the primary oxidants, through the homolytic

cleavage of the peroxide (–O–O–) bond (Anipsitakis, *et al*. 2004). Since persulfate are symmetrical oxidants (Figure 1.8), activation with UV radiation results in the formation of two $SO_4^{\bullet-}$

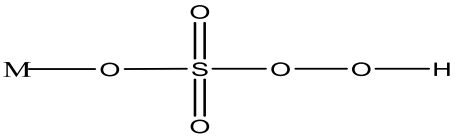
$$S_2O_8^{-2} + h\upsilon \rightarrow 2\ SO_4^{\bullet-} \quad (1\text{ -}7)$$

On the other hand, peroxymonosulfate is unsymmetrical around the peroxide bond (Figure 1.8), resulting in its cleavage to $OH^{\bullet-}$ and $SO_4^{\bullet-}$.

$$HSO_5^{-} + h\upsilon \rightarrow SO_4^{\bullet-} + {\bullet}OH^{-} \quad (1\text{ -}8)$$



Persulfate



Peroxymonosulfate

Figure 1.8 Structure of persulfate and peroxymonosulfate

Anipsitakis *et al*. (2004) employed UV (254 nm)/Co^{+2} activated peroxydisulfate for the oxidation degradation of 2, 4- dichlorophenol. While Yunqing *et al*. (2013), reported UV (365 nm)/peroxydisulfate oxidative process for facile, effective, and environment-friendly degradation of sulfamonomethoxine.

The use of transition metals, in most cases in catalytic amounts, provides another option of radical generation. Perhaps the most well-known of such systems is the Fenton reagent, which leads to the generation of hydroxyl radicals according to the following reaction.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + h \quad (1\text{ -}9)$$

Georgep and Dionysios (2004) demonstrated significant reactivity toward transforming of 2,4-dichlorophenol. Ag(I), Ce(III), Co(II), Fe(II), Fe(III), Mn(II), Ni(II), Ru(III), and V(III) were tested for the activation of three inorganic oxidants: hydrogen peroxide, potassium peroxymonosulfate, and persulfate. Ag(I) is a very efficient activator of the decomposition of persulfate, while Co(II) is best activator of potassium peroxymonosulfate.

$$Ag^{+} + S_2O8^{2-} \rightarrow Ag^{2+} + SO_4^{\bullet-} + SO_4^{2-} \quad (1\text{-}10)$$

$$Co^{2+} + HSO_5^- \rightarrow Co^{3+} + SO_4^{\bullet-} + OH^- \quad (1\text{-}11)$$

However, concern has been raised over the impact of dissolved cobalt on human health, since it has been proven that the Co (II) had an adverse effect on the environment (Yamagami, *et al*. 1994; Pandey and Sharma. 2002).

Among the transition metals, only iron in both the divalent and trivalent forms showed reactivity in mediating the decomposition of two oxidants at a significant extent (Georgep and Dionysios. 2004). Moreover Fe has many advantages as an oxidant activator such as being cheap, less toxic, and naturally abundant (Nfodzo and Choi. 2011).

The rate of reaction between persulfate, peroxymonosulfate and ferrous iron is reliant on the concentration of each reactant. Zou, *et al*. 2013 and Ayoub, *et al*. 2014 reported that SR were generated by the following chemical equations.

$$S_2O_8^{2-} + Fe^{2+} \longrightarrow SO_4^{\bullet-} + Fe^{3+} + SO_4^{2-} \quad k = 2.0\times10\ M^{-1}s^{-1} \quad (1\text{-}12)$$

$$HSO_5^- + Fe^{2+} \longrightarrow SO_4^{\bullet-} + Fe^{3+} + OH^- \quad k = 3.0\times10^4\ M^{-1}s^{-1} \quad (1\text{-}13)$$

Generally with transition metal activation, Balazs *et al*. (2000) pointed out that the mechanism is dependent on catalyst type, organic substrate and oxidant concentration.

1.9 Monitoring parameters for wastewater treatment plants

The biochemical oxygen demand test (BOD_5) has traditionally been used in wastewater analysis but due to problems of repeatability and inhibition by commonly occurring wastewater ions and compounds, is frequently replaced by the chemical oxygen demand test (COD). Chemical oxygen demand is widely used for wastewater monitoring, design, modeling and plant operational analysis (Mittal and Ratra. 2000).

1.9.1 Biochemical oxygen demand (BOD)

The biochemical oxygen demand (BOD) is a standard test for assaying the oxygen demanding concentration of microbes to degrade organic matter over a given time

period, usually 5 days but can be extended to 30 days (Ganjar and Sarwoko. 2010). The test BOD has traditionally been used in wastewater analysis but due to problems of repeatability (Fitzmaurice and Gray. 1987; Fitzmaurice and Gray. 1989), and inhibition by commonly occurring wastewater ions and compounds, is frequently replaced by Chemical Oxygen Demand test (ISO, 1989; ISO, 2002), for wastewater monitoring, design, modeling and plant operational analysis (Mittal and Ratra. 2000; Gray, 2004; Kim, *et al*. 2007).

Moreover the BOD test also requires a 5 day incubation period while COD results are completed within the same day. Also, the COD test is less unaffected by the presence of toxic substances, which is leading to achieve better precision and reliability (Dubber and Gray. 2010).

1.9.2 Chemical oxygen demand (COD)

Clesceri *et al*. (1998) defined chemical oxygen demand (COD) as the amount of dichromate ion ($Cr_2O_7^{-2}$) that reacts with the sample under controlled conditions. The quantity of dichromate ion consumed is expressed in terms of its oxygen equivalence. COD is a defined test, whereby the extent of the sample oxidation can be affected by digestion time, reagent strength, and sample COD concentration. Gomez, *et al*. (2002) and Beltrá, *et al*. (2003) defined COD as the amount of oxygen necessary to oxidize the organic matter present in the water. Fair (2005) reported that COD measures only chemical oxidizable matter. The reaction is illustrated with potassium hydrogen phthalate as an example:

$$2KC_8H_5O_4 + 10K_2Cr_2O_7 + 41H_2SO_4 \rightarrow 16CO_2 + 46H_2O + 10\,Cr_2(SO_4)_3 + 11K_2SO_4 \quad (1\text{-}14)$$

Chemical oxygen demand results are expressed as the amount of oxygen consumed during the oxidation of organic matter. With oxygen as the primary oxidant in the oxidation of potassium hydrogen phthalate, the reaction is illustrated as an example:

$$2KC_8H_5O_4 + 15O_2 \rightarrow 16CO_2 + 4H_2O + 2KOH \quad (1\text{-}15)$$

In the treatment of wastewater, most of the carbon is converted to carbon dioxide and the hydrogen in the sample is converted to water, during the oxidation of organic material by dichromate in sulphuric acid.

Some organic material, such as aromatic hydrocarbons and pyridines, may not be oxidized during the COD test. This may lead to inaccurate results and poor estimates of COD in treatment plant.

Marais and Ekema (1984) further stated that the COD test relies on a time dependent reaction and the specified reflux period of 2 hours must be adhered to, so as to ensure complete or near complete oxidation. Furthermore, different final test volumes with equivalent masses of the same organic compound, will yield different results for COD, because the temperature at which the reflux step takes place also affects the oxidation rate. The reflux temperature, in turn, is dependent on the concentration of sulphuric acid in the test. The temperature at which the refluxing takes place also affects the oxidation rate, and the refluxing temperature is dependent on the concentration of the sulphuric acid in the test. Therefore the COD test will give only consistent results, if the test is done in strict accordance with set procedures.

1.9.3 Limitations and disadvantages of COD

The samples containing particulates and refractories are not well oxidized as measured by the COD method. Also volatile compounds, such as benzene, are not oxidized to any appreciable extent, because they escape into the vapour phase. Belkin, *et al*. (1983) remarked that analytical difficulties in the determination of COD is due to the presence of free chloride (up to 25 g/dm^3), bromide (up to 5 g/dm^3) and ammonia (up to 2 g/dm^3) concentration levels. Zuev, *et al*. (2004) found that during the evaluation of a rapid determination of COD in water, an under estimation of results were observed when the sample contained volatiles like ethanol.

COD is a time consuming test, with the digestion phase alone taking two hours and involving many chemicals, such as mercury sulphate, to eliminate halide interference, and sulfuric acid to eliminate nitrite interference. Baker, *et al*. (1999) observed that straight chain carboxylic acids are not oxidized without a catalyst and may not even be completely oxidized in the presence of suitable catalysts. They further stated that volatile compounds are only oxidized as long as they stay in contact with the liquid medium. Lastly, they maintained that some hydrophobic organic chemicals have such low solubility that even in saturated solutions they will yield an erroneous COD reading.

Dold, *et al*. (1991) reported that the COD of all domestic wastewater can be divided into biodegradable and un-biodegradable fractions with each fraction sub-divided into soluble and particulate portions. The measurements of all four COD components are required in the optimization of the effluent plant.

A major drawback in the use of COD analysis is the production of hazardous wastes including mercury, hexavalent chromium, sulphuric acid, silver, and other hazardous materials depending on the method used (Clesceri, *et al*. 1998; Bourgeois, *et al*. 2001).This necessitates all the waste material from the COD test being collected for hazardous waste disposal. In the USA the disposal of spent COD waste is regulated by the US environmental Protection Agency (EPA) under the Resource Conservation and Recovery Act with disposal regulations listed in the 40 Code of Federal Regulations Protection of the Environment Parts 260 to 280 (Hach, 2009). Although there is much interest in developing cleaner methods for the COD, for example using near-infrared reflectance (Sousa, *et al*. 2007) or copper electrodes as electro catalytic sensors (Silva, *et al*. 2009), the need to eliminate waste streams within analytical laboratories coupled with the cost of disposal has resulted in greater interest in the use of alternatives to the COD test that neither use nor generate potentially harmful chemicals (Miller, *et al*. 2001; Sousa, *et al*. 2007; Silva, *et al*. 2009.).

Moreover, COD cannot reflect the degree of industrial wastewater precisely owing to the impacts of types and concentration of oxidants, reaction temperature and time, the acidity of reaction solution and catalyst (Sun, *et al*. 1998; Wang and Li, 2001). The procedure of the test is intricate, which always brings secondary effluents (Hua, *et al*. 2011).

1.9.4 Total organic carbon (TOC)

According to Clesceri, *et al*. (1998), TOC is a more convenient and direct expression of total organic content than COD. TOC is independent of the oxidation state of the organic matter and does not measure other organically bound elements, such as nitrogen and hydrogen. Inorganics can also contribute to the oxygen demand measured by biological oxygen demand (BOD) and COD.

Total organic carbon (TOC) is a potential alternative to both the COD and the BOD tests and has the advantage of being both faster and potentially more precise than the COD test (Stevens, *et al*. 2006). While it has previously been considered as a potential replacement for BOD_5 and COD analysis,(Aziz and Tebbutt, 1980; Fadini, *et al*. 2004), more accurate detection methods have been developed making the test even more reliable and precise, especially for more complex materials such as industrial wastewaters (Bourgeois, *et al*. 2001; Rene and Saidutta, 2008). However, little research has been published on the possibility of replacing COD with TOC in wastewater analysis, especially for domestic or municipal wastewater characterization and routine performance analysis.

Also total organic carbon (TOC) can reflect the strength of organic pollution accurately because its method contains many advantages, such as its short time consuming process, fast and accurate results, stability and reliability (Shi, *et al*. 2002).

1.9.5 Relationship between COD and TOC

Many of researchers attempted to establish a relationship between BOD, COD and TOC ratios. They found it difficult to correlate BOD, COD and TOC for industrial water but found relative good correlation ratios for domestic effluents. Researchers concluded that the TOC and COD ratio for industrial wastewater varied between 1.67 and 6.65. According to a study by Louw, *et al*. (2003) a comparison between COD and DOC was found at specific sampling points with cleaner samples. They could not find repeatable ratios between different sampling points on the same plant.

Theoretically, there is a correlation between COD and TOC (Wang, *et al*. 2001). Actually, many scholars have found significant correlation between COD and TOC of industrial effluent, specific classes of organic chemicals and surface water (Brocca, *et al*. 1997; Zhang, *et al*. 2007; Li, *et al*. 2007).The correlations between these indicators are weak in some natural water (Choi, *et al*. 2004).

On the other hand there are significant linear relationships between BOD_5, COD and TOC for settled (influent) domestic and municipal wastewaters. For the treated final effluents a strong correlation was observed between COD and TOC but not between BOD_5 and TOC. The treatment performance monitoring COD can be reliably replaced with the TOC test. The variability between TOC and COD is further reduced when using specific wastewater streams and treatments (Dubber and Gray. 2010).

Recently Environmental Protection Agency (EPA) has used TOC to evaluate the performance of water treatment and as the key indicator for disinfection by-products that produced during water treatment (EPA).

1.10 Problem Statement

The principal objective of waste water treatment is generally to allow human and industrial effluents to be disposed of, without danger to human health or

unacceptable damage to the natural environment. The existing of extensive amount of vinasse in the environment leads to serious problems in ecosystem, therefore the current study will be conducted to achieve the following objectives:-

- Investigation of the environmental impact of vinasse, a by- product of Kenana Sugar Company Factory (Sudan), by studying some of the physicochemical characteristics.
- Application of coagulation-flocculation technology to remove color of organic constituents and the amount of (AOC) of vinasse. Color removal is evaluated by investigation the effects of some parameters such as doses of reagent and pH values.
- Assessment of coagulation-flocculation treatments followed by sulfate radicals based advanced oxidation processes (SR-AOPs) by monitoring TOC in vinasse.
- Application of combined technique of advanced oxidation processes (sulfate radicals and hydroxyl radicals) to enhance reduction of environmental pollution by monitoring TOC in vinasse.

CHAPTER TWO

Materials and methods

2.1 Materials

2.1.1 Collection of samples

The vinasse used in this work was obtained from an ethanol distillery located at White Nile State, Sudan. Two Samples were collected directly from distillery column after the distillation at different time of the day, vinasse (1) was collected at 8 am, while vinasse (2) was collected at 8 pm.

2.1.2 Chemicals

All chemicals and reagents used in the experiments were of analytical grade, and the chemicals are: Ammonium chloride (NH_4Cl), Scharlau, Spain. Ammonium molybdate Merck, Germany. Calcium chloride ($CaCl_2$), CDH, India. Dichloromethane DCM, Suprasolv- Germany. Dipotassium hydrogen phosphate (K_2HPO_4), Carlo erba, Italy. Disodium hydrogen phosphate ($Na_2HPO_4.7H_2O$), Carlo erba, Italy. Ferrous ammonium sulphate Fe $(NH_4)_2(SO_4)_2.6H_2O$. Lab tech, India. Ferroin indicator solution, SDFCL, India. Ferric chloride ($FeCl_3.6H_2O$), Lab tech, India. Glass wool, Aps finechem, Australia. Hexane, Merck, Germany. Hydrochloric acid, Scharlau, Spian. Hydrogen peroxide H_2O_2 (30%), suprasolv Germany. Iron (III) chloride, Merck, Germany. Iron (II) sulfate heptahydrate (99.5%), Acros Organics USA. Magnesium sulfate ($MgSO_4.7H_2O$), CDH, India. Manganese sulphate, Lab tech, India. Mercuric iodide, SDFCL, India. Mercuric sulphate, SDFCL, India. Potassium bromide, Merck, Germany. Potassium dihydrogen phosphate (KH_2PO_4), Carlo erba, Italy. Potassium persulfate (PS) (99%), Acros Organics USA. Potassium peroxomonosulfate (PMS), Acros Organics USA. Potassium iodide (99%) Merck, Germany. Potassium dichromate ($K_2Cr_2O_7$), CDH, India. Potassium iodide, Burgoyne, India. Sulfuric acid,

Scharlau, Spian. Sodium hydroxide, Lab tech, India. Sodium iodide, Lab tech, India. Sodium izide, Loba chemie, India. Sodium thiosulphate, Lab tech, India. Starch, Lab tech, India. Silver sulphate, Avis chemical, India. Sodium hydroxide, Fluka Germany. Sand, Sigma Aldrich, Germany. Acros Organics USA. Sodium thiosulfate (99%), Merck, Germany. Sodium sulfate, Merk- Germany. Sodium sulfate (Na_2SO_4) Merck, Germany. Sulfuric acid (98%), Merck, Germany. Trimethylchlorosilane (TMCS), supelco USA.

2.1.3 Apparatus

Aeration device. Aluminum weighting dishes. BOD Glassware. Drying oven. Desiccators. Evaporation dishes. Filtration apparatus. Glass fiber disk. Magnets. Water bath.

2.1.4 Instrumentations

- **Atomic absorption spectrometer.**

Atomic absorption spectrometer (AAS), model 210 GP-Puck Scientific, USA.

- **Infrared spectroscopy**

Infrared spectroscopy **(**IR) Model, Perkin Elmer, USA.

- **Gas chromatography mass spectrometer.**

Gas chromatography mass spectrometer (GC-MS) Shimadzu, model GC-MS QP2010-Plus, Kyoto, Japan. The column Hewlett- Packard, Model 6890 gas chromatograph (30 m Length, 0.25 mm I.D., 0.25 μm film thickness). The detector a Hewlett- Packard Model, 6890 mass selective. Data acquisition system is ChemStation.

- **Total organic carbon**

Total organic carbon (TOC) Shimadzu TOC analyzer, model TOC-L CPN, Japan. Detector is an infrared gas analyzer (NDIR) (Figure 2.2).

- **UV/Vis spectrophotometer**

UV/Vis spectrophotometer (200-800 nm) single beam model, G10S UV-VIS, USA.

2.2 Methods

2.2.1 Reagents and nutrients

- **Phosphate buffer solution**

8.5g potassium dihydrogen phosphate (KH_2PO_4), 21.75g dipotassium hydrogen phosphate (K_2HPO_4), 33.4g disodium hydrogen phosphate ($Na_2HPO_4.7H_2O$) and 1.7g of ammonium chloride (NH_4Cl) were dissolved in about 500ml distilled water and diluted to 1 dm^3.

- **Magnesium sulfate solution**

22.5g of anhydrous $MgSO_4.7H_2O$ were dissolved in distilled water and diluted to 1 dm^3.

- **Calcium chloride solution**

27.5g of anhydrous $CaCl_2$ were dissolved in distilled water and diluted to 1 dm^3.

- **Ferric chloride solution**

0.25g of $FeCl_3.6H_2O$ was dissolved in distilled water and diluted in 1 dm^3.

- **Manganese sulphate solution**

480g $MnSO_4$ were dissolved in distilled water, then filtered and made up to 1 dm^3.

- **Iodide-azide reagent**

500g of NaOH and 135g of NaI were dissolved in distilled water and diluted to 1 dm^3; then 10g of sodium azide NaN_3 dissolved in 40ml distilled water were added.

- **Starch indicator solution**

5g of starch and 0.01g of mercuric iodide were dissolved in 30ml of cold distilled water and diluted to 1 dm^3.

- **Standard sodium thiosulphate solution**

6.205g of $Na_2S_3O_3.5H_2O$ were dissolved in distilled water and made up to 1 dm^3 and then standardized against potassium dichromate.

- **Sulphuric acid reagent**

543.5ml of H_2SO_4 were added to 5.5g of $AgSO_4$ and left overnight.

- **Standard potassium dichromate**

4.903g of $K_2Cr_2O_7$ were dissolved in 167ml H_2SO_4, then 33.3g of $HgSO_4$ were added and the solution was diluted to 1 dm^3.

- **Standard ferrous ammonium sulphate (FAS)**

39.2g of Fe $(NH_4)_2.6H_2O$ were dissolved in distilled water, and 20ml of conc H_2SO_4 were added and the solution was diluted to 1 dm^3.

- **Sample preparation for TOC analysis**

10ml of raw vinasse were diluted to 1 dm^3, and then filtered for TOC analysis.

- **Preparation of standard stock solution of total carbon**

2.125g of potassium hydrogen phthalate reagent were weighted accurately and transferred to 1000ml volumetric flask. Zero water was added up to the 1000ml mark and mixed well. 1000mg/dm^3 of standard stock solution were prepared.

- **Preparation of standard stock solution of inorganic carbon**

3.497g of sodium hydrogen carbonate and 4.412g of sodium carbonate reagents were weighed accurately and transferred to 1000ml volumetric flask. Zero water were added up to the 1000ml mark and mixed well. 1000mg/dm^3 of standard stock solution were prepared.

- **Preparation of standard stock solution of total nitrogen**

7.219g of potassium nitrate reagent was weighted accurately and transferred to 1000ml volumetric flask. Zero water were added up to the 1000ml mark and mixed well. 1000mg/dm^3 of standard **stock** solution were prepared.

- **Preparation of PS stock solution**

0.05g of PS was dissolved in 1ml of deionized water.

- **Preparation of Fe (II) stock solution**

0.1g of Fe (II) was dissolved in 1ml of deionized water.

- **Preparation of PMS stock solution**

0.05g of PMS was dissolved in 1ml of deionized water.

- **Potassium iodide (1%)**

10g of potassium iodide were dissolve in 100ml of distilled water.

- **Acid mixture**

0.018g of ammonium molybdate [$Mo_7O_{24}(NH_4)_5.4H_2O$] were dissolve in 75ml of distilled water. 30ml of conc H_2SO_4 were added slowly while stirring.

- **Starch solution (10 g/** dm^3**)**

In 150ml beaker 1g of soluble starch was added, and 5ml of distilled water were added gradually while stirring until a paste was formed. 100ml of paste were added to boiling water. After cooling, 5g of potassium iodide were added and stirred until dissolution was complete and then transferred to a plastic bottle.

- **Sodium thiosulfate (0.1M)**

6.2g of sodium thiosulfate [$Na_2S_2O_3.5H_2O$] were weighed and transferred to a 250ml volumetric flask. 50ml of distilled water were added, agitated until dissolution was complete, diluted to the volume mark and well mixed

2.2.2 Vinasse characterization

All glassware were washed with detergent. The glassware were rinsed four times in tap water and once in distilled water. Ultrapure water (Elga, USA) was used for the preparation of all aqueous solutions. All stock solutions were kept in a 4°C refrigerator until use. The physico-chemical parameters, i.e., BOD, COD, TDS, TSS, TS and some metals were determined according to standard methods for examination of water and wastewater (Clesceri, *et al*. 1998).

2.2.2.1 Determination of BOD

Oxygenated water were prepared by using aeration device. 2ml of each of phosphate buffer, magnesium sulfate, calcium chloride and ferric chloride solutions were added in 1 dm3 volumetric flask. 5ml of sample with the

oxygenated water were added into the mixture, and pH were adjusted to 7 by using either 1N of NaOH or H_2SO_4, and then 0.03, 0.07 and 0.1ml of dilution mixture were placed in BOD bottles. Initial dissolved oxygen (DO_1) of each dilution were calculated immediately, others were stoppered tightly and incubated for 5 days at 20°C.

2.2.2.2 Determination of dissolved oxygen

2ml of manganese sulfate and 2ml of iodide-azide reagent were injected into the samples under test, and the sample bottles were tightly stoppered to exclude air bubbles completely, and then the content were mixed well. The mixture was allowed to settle until 100ml of clear solution were observed on the surface, and 2ml of sulphuric acid were added. The bottle was stoppered and shake continually until solution was homogenous. 200ml of sample were titrated with standard sodium thiosulphate solution until pale yellow color was seen. 1-2ml of starch solution were added until the end point. After 5 days the dissolved oxygen have been calculated to determine dissolved oxygen (DO_2).

Calculations:

$$\text{BOD} = \frac{(DO1-DO2)}{Volume} \text{ X } 1000$$

Where DO_1= initial dissolved oxygen

DO_2=dissolved oxygen after 5 days

Volume= volume of sample

2.2.2.3 Determination of COD

In COD reactor tubes 2.5ml of the diluted sample, 1.5ml of $K_2Cr_2O_7$ and then 3.5ml of H_2SO_4 reagent were added. The tubes were closed firmly and arranged in steel rake and put in the oven to digest for 2 hour at 150 °C. The tubes were

removed from the oven and cooled at room temperature, then titrated against 0.1M FAS using 1-2 drop of ferroin indicator.

Calculations: $\text{COD mg/dm}^3 = \frac{(\boldsymbol{Blank\ value - sample\ value} \times 8 \times 0.1 \times 1000)}{\boldsymbol{Volume\ of\ sample}}$

2.2.2.4 Determination of total solids (TS)

Clean dish was heated to 103-105 °C for 1 hour, cooled and weighed. 50ml of well mixed sample were added to pre weighed dish. Which was then evaporated to dryness on water bath.

Calculation:

$$\text{Total solid (TS mg/dm}^3) = \frac{(A-B)\times 1000}{V}$$

Where A= Wight of dried residue + dish

B=Wight of dish

V= volume of sample

2.2.2.5 Determination of total dissolved solids (TDS)

The disk with wrinkled side was inserted thoroughly into filtration apparatus, and washed with distilled water. Vacuum was applied until traces of water were removed, clean dish was heated to 180 °C for 1 hour, cooled, weighed and then stored in desiccator. Then 5ml of well mixed sample were pipetted into glass fiber disk. Filtrate was transferred to preweighed dish and evaporated to dryness on water bath. Evaporated sample was dried for 1 hour at 180 °C, cooled in desiccator and weighed.

Calculations:

$$\text{Total dissolved solid TDS mg/dm}^3 = \frac{(A-B)\times 1000}{V}$$

Where A= Wight of dried residue + dish

B=Wight of dish

V= volume of sample

2.2.2.6 Determination of total suspended solids (TSS)

The disk with wrinkled side was inserted thoroughly into filtration apparatus, then washed with distilled water and vacuum was applied to remove traces of water. The filtration apparatus was transferred to an inert aluminum weighing dish and dried at 103-105°C, cooled, weighed and stored in desiccator. Then 5ml of vinasse were pipetted into the seated glass fiber filter. The filter was washed three time with 10ml successive distilled water and dried, carefully removed from filtration apparatus and transferred to aluminum weighting dish as support, dried for 1 hour at 103-105° C in an oven, cooled in desiccator and weighed.

Calculations: Total suspended solid TSS mg/dm3 = $\frac{(\boldsymbol{A-B})\times\mathbf{1000}}{\boldsymbol{V}}$

Where A= Weight of dried residue + filter

B=Weight of dish

V= volume of sample

2.2.2.7 Determination of K, Na and Ca using AAS

10ml of HCl were added to 20ml of sample in 100ml volumetric flask and the volume made up to the mark with deionized water. 1% Ianthanum solution was added, shaked well and centrifuged.

2.2.2.8 Determination of total organic carbon

The 680°C combustion catalytic oxidation method achieved total combustion of samples by heating them to 680°C in an oxygen-rich (Figure 2-1) environment inside TC combustion tubes filled with a platinum catalyst. The carbon dioxide

generated by oxidation is cooled, dehumidified and detected using an infrared gas analyzer (NDIR).

Calculation: TOC = $TC - IC$

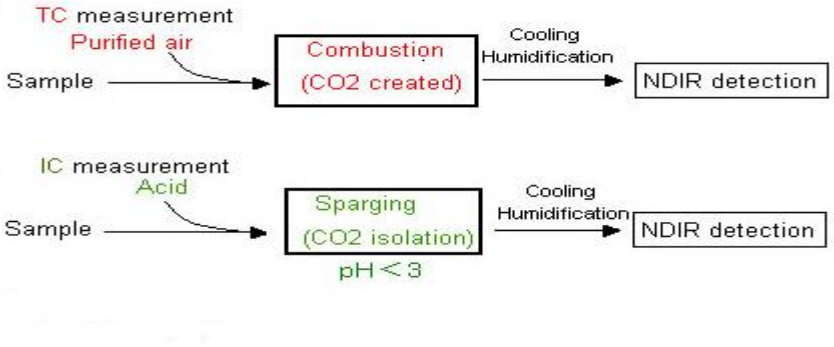


Figure 2-1 TOC measurement diagram

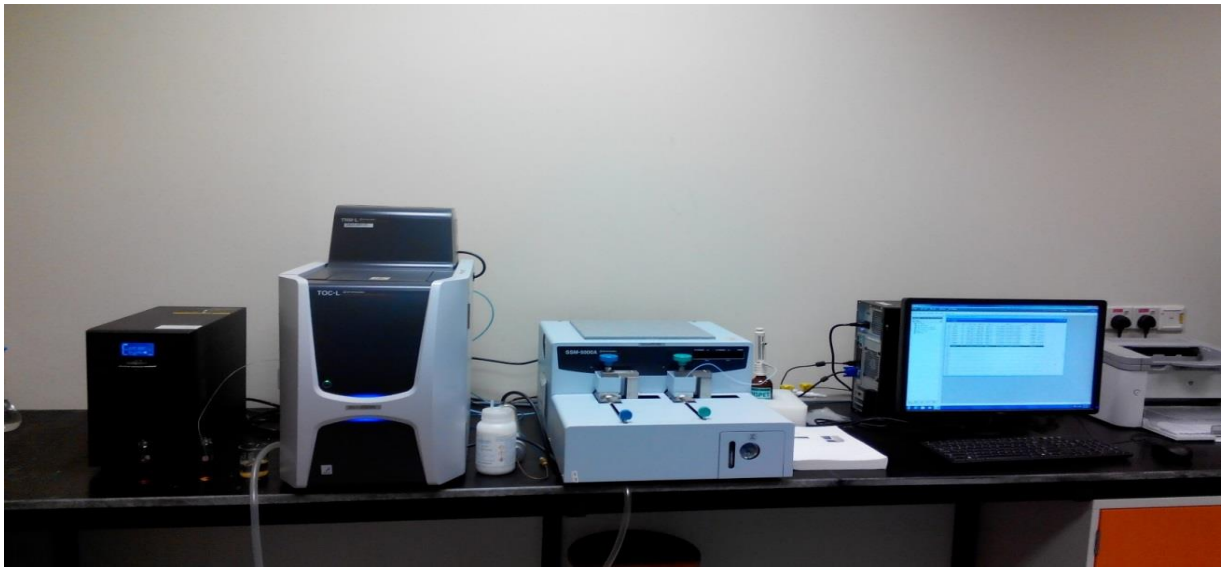


Figure 2.2 TOC instrument

2.2.2.9 Infrared spectroscopy

Vinasse (1) and vinasse (2) were mixed well, and 250ml of vinasse mixture was dried, 2-3mg of dried vinasse were mixed with approximately 0.5- 1g of KBr. and then mixture was grinded to fine powder for IR analysis.

2.2.2.10 Gas chromatography mass spectrometry

Vinasse (1) and vinasse (2) were mixed well, and 500ml of vinasse mixture were used for GC-MS analysis.

- **Samples preparation and derivatization**

The sample of vinasse was prepared for GC-MS analysis by solvent extraction method. Hexane and dichloromethane (DCM) were used as solvents. The sample and the solvent (1:4) were shaken, manually, for 5 min. The solvent was removed from organic layer by a rotary evaporator (37 °C). Extracted organic layer was reduced under a gentle nitrogen stream (approximately 50 min). Trimethylchlorosilane (TMCS) was added to DCM extraction, then heated to 70°C for 4 hour, dried under nitrogen stream and then stored in fridge until GC-MS analysis.

- **GC-MS analysis**

The analyses of the extract were performed by Gas Chromatography mass spectrometry. These analyses were carried out on a Hewlett- Packard Model 6890 gas chromatograph with splitless injector and a VB-5 5% phenylmethylepolysiloxane column (30 m Length, 0.25 mm I.D., 0.25 μm film thickness) equipped with a Hewlett- Packard Model 6890 mass selective detector provided with a HP ChemStation data acquisition system. Helium (purity 99.999%) was used as a carrier gas. The chromatographic conditions are present in Table (2.1). The data for analysis was acquired from electron impact (EI) mode 70 (eV), scanning from 50-550 amu at 1.5 sec/scan.

Table 2.1 The chromatographic conditions of GC-MS

Chromatographic	Conditions
Oven Temperature program	Initial oven temperature 60°C, hold for 2 minutes; then up to 280°C at 6°C/min, then held at 280°C for 20 min
Gas flow rates	1.2ml/min
Injection port temperature	290°C
Injection mode	Splitless (1 min) (1.0-1.4 µl; hot needle technique)
Column inlet pressure	10.4 pis
Average Velocity	40 cm/s
Temperature of transfer line	300°C
Solvent delay	4 min

2.2.3 Vinasse treatment

All glassware were washed with detergent. The glassware were rinsed four times in tap water and once in distilled water. The two samples were mixed well together for treatment methods. All stock solutions were kept in a 4°C refrigerator until use.

2.2.3.1 Coagulation-flocculation process

All experiments were carried out in batch mode at room temperature in duplicate. All reagents were of analytical grade and only deionized water was used. Coagulation-flocculation treatment of vinasse was performed using jar testing which is a pilot scale test, using chemicals for treatment in a particular water plant Satterfield (2005). UV/Vis spectrophotometer was used of monitoring color and AOC removal.

- **Effect of coagulant doses on removal of color, AOC and TOC**

In each 1000ml beaker, 10ml of vinasse were placed and diluted to the mark. Then 5, 7.5, 10, 12.5, and 15g of iron (III) chloride were added in each beaker. The pH was not adjusted. The mixture was stirred at 120 rpm for 1 min and followed by

stirring at 30 rpm for 30 min. After stirring, the floc was allowed to settle for 1.5 hour. (Carvajal-Zarrabal, *et al*. 2012). Then the reaction mixture was filtered through a syringe filter with the pore size of 0.45 μm before TOC, color and AOC analysis.

Calculation:

$$\text{Color and AOC removal \%} = \frac{A(1) - A(2)}{A(1)} \times 100$$

A (1) = sample absorbance prior treatment

A (2) = sample absorbance after treatment

$$\text{TOC removal \%} = \frac{C(1) - C(2)}{C(1)} \times 100$$

C (1) = concentration (mg/dm^3) of sample prior treatment

C (2) = concentration (mg/dm^3) of sample after treatment

- **Effect of pH on removal of color, AOC and TOC**

In each of 1000ml beaker 10ml of vinasse were placed and diluted to the mark with deionized water. Each sample was dosed with 10g of iron (III) chloride, and the pH values were adjusted to 3, 5, 7, 9 and 11 by adding NaOH and/or H_2SO_4. The mixture was stirred at 120 rpm for 1 min and followed by stirring at 30 rpm for 30 min. After stirring, the floc was allowed to settle for 1.5 hour, filtered through a syringe filter with the pore size of 0.45 μm before TOC, color and AOC analysis.

Calculation:

$$\text{Color and AOC removal \%} = \frac{A(1) - A(2)}{A(1)} \times 100$$

A (1) = sample absorbance prior treatment

A (2) = sample absorbance after treatment

$$\text{TOC removal \%} = \frac{C(1) - C(2)}{C(1)} \times 100$$

C (1) = concentration (mg/dm^3) of sample prior treatment

C (2) = concentration (mg/dm^3) of sample after treatment

2.2.3.2 Sand filtration

The treated vinasse was passed through a rapid gravity sand filter according to the method which was described by Tatari *et al*. (2013).

2.2.3.3 Reduction of TOC using SR-AOP process

All experiments were performed in batch mode in triplicated. Experiments were conducted at room temperature in 30ml glass vials. All reagents were of analytical grade and only deionized water was used. Oxidation was initiated by the addition of PS or PMS solution to the vial that containing pretreated vinasse.

2.2.3.3.1 PS-Fe (II) system

- **Effect of PS doses on TOC removal**

In each 30ml glass vial, 20ml of pretreated vinasse were placed. Each sample was dosed with 1ml of iron (II) sulfate as activator. Then 0.97, 1.94, 3.88 and 7.76ml of PS were added (Table 2.2). Vials were shaken at 150 rpm by using an orbital shaker. After reaction, mixture was filtered through a syringe filter with the pore size of 0.45 μm before TOC analysis

Calculation:

$$\text{TOC removal \%} = \frac{C(1) - C(2)}{C(1)} \times 100$$

C (1) = concentration (mg/dm^3) of sample prior treatment

C (2) = concentration (mg/dm^3) of sample after treatment

Table 2.2 Doses of PS added to 20ml vinasse at constant dose of Fe (II)

Ratio of Fe(II): PS	**1:0.5**	**1:1**	**1:2**	**1:4**
Vinasse (ml)	20	20	20	20
PS (ml)	0.97	1.94	3.88	7.76
PS (mM)	0.18	0.36	0.72	1.44
Fe(II) (ml)	1	1	1	1
Fe(II) (mM)	0.36	0.36	0.36	0.36
Water (ml)	7.03	6.06	4.12	0.24
Total	29	29	29	29

- **Effect of iron (II) sulfate doses on TOC removal**

In each 30ml glass vial, 20ml of vinasse were placed. Each sample was dosed with 3.88ml of PS. Then 1, 2, 4 and 5ml of iron (II) sulfate were added respectively (Table 2.3). Vials were shaken at 150 rpm by using an orbital shaker. After the reaction, mixture was filtered through a syringe filter with the pore size of 0.45 μm before TOC analysis.

Calculation: $\text{TOC removal \%} = \frac{C(1) - C(2)}{C(1)} \times 100$

C (1) = concentration (mg/dm^3) of sample prior treatment

C (2) = concentration (mg/dm^3) of sample after treatment

Table 2.3 PS: Fe (II) ratios

Ratio of PS: Fe(II)	**1:0.5**	**1:1**	**1:2**	**1:2.5**
Vinasse (ml)	20	20	20	20
PS (ml)	3.88	3.88	3.88	3.88
PS (mM)	0.72	0.72	0.72	0.72
Fe(II) (ml)	1	2	4	5
Fe(II) (mM)	0.36	0.72	1.44	1.8
Water (ml)	4.12	3.12	1.12	0.12
Total	29	29	29	29

Effect of pH on TOC removal in PS-Fe (II) system

In each 30ml glass vial, 20ml of vinasse were placed. Each sample was dosed with 3.88ml of PS and 1ml of iron (II) sulfate as activator. pH values were adjusted to 3, 5, 7, 8 and 11 by adding NaOH and/or H_2SO_4 solution. Vials were shaken at 150 rpm by using an orbital shaker. After the reaction, mixture was filtered through a syringe filter with the pore size of 0.45 μm before TOC analysis.

Calculation:

$$\text{TOC removal \%} = \frac{C(1) - C(2)}{C(1)} \times 100$$

C (1) = concentration (mg/dm^3) of sample prior treatment

C (2) = concentration (mg/dm^3) of sample after treatment

- **Effect of reaction time on TOC removal in PS-Fe (II) system**

In each 30ml glass vial, 20ml of vinasse were placed. Each sample was dosed with and 3.88 ml, 1 ml of PS and iron (II) sulfate, respectively at pH 7. Reaction time were adjusted to 0.0833, 0.5, 4, 8 and 24 hour. Vials were shaken at 150 rpm by using an orbital shaker in different time. After the reaction, the reaction mixture was filtered through a syringe filter with the pore size of 0.45 μm before TOC analysis.

Calculation:

$$\text{TOC removal \%} = \frac{C(1) - C(2)}{C(1)} \times 100$$

C (1) = concentration (mg/dm^3) of sample prior treatment

C (2) = concentration (mg/dm^3) of sample after treatment

- **Optimization of PS**

Optimum ratio of PS: Fe (II) were placed in vial with 20ml of vinasse. The optimum condition of pH and time reaction were adjusted. Optimum conditions

were increased to obtain the maximum removal of TOC. Vials were shaken at 150 rpm by using an orbital shaker. After the reaction, the reaction mixture was filtered through a syringe filter with the pore size of 0.45 μm before TOC analysis.

2.2.3.3.2 PMS-Fe (II) system

- **Effect of PMS dose on TOC removal**

20ml of vinasse were placed in each 30ml glass vial. Each sample was dosed with 1 ml of iron (II) sulfate as activator. Then 2.21, 4.42, 2.94 and 5.89ml of PMS were added (Table 2.4). Vials were shaken at 150 rpm by using an orbital shaker. After the reaction, mixture was filtered through a syringe filter with the pore size of 0.45 μm before TOC analysis.

Calculation: $\text{TOC removal \%} = \frac{C(1)-C(2)}{C(1)} \times 100$

C (1) = concentration (mg/dm^3) of sample prior treatment

C (2) = concentration (mg/dm^3) of sample after treatment

Table 2.4 Doses of PMS added to 20ml vinasse at constant dose of Fe (II)

Ratio of Fe(II): PMS	**1:0.5**	**1:1**	**1:2**	**1:4**
Vinasse (ml)	20	20	20	20
PMS (ml)	2.21	4.42	2.94	5.89
PMS (mM)	0.18	0.36	0.72	1.44
Fe(II) (ml)	1	1	1	1
Fe(II) (mM)	0.3596	0.3596	0.3596	0.3596
Water (ml)	5.79	3.58	5.06	2.11
Total	29	29	29	29

- **Effect of iron (II) sulfate doses on TOC removal**

In each 30ml glass vial, 20ml of vinasse were placed. Each sample was dosed with 4.42ml of PMS. 0.5, 1, 2 and 2.5ml of iron (II) sulfate were added (Table 2.5). Vials were shaken at 150 rpm by using an orbital shaker. After the reaction, the

reaction mixture was filtered through a syringe filter with the pore size of 0.45 μm before TOC analysis.

Calculation:

$$\text{TOC removal \%} = \frac{C(1) - C(2)}{C(1)} \times 100$$

C (1) = concentration (mg/dm^3) of sample prior treatment

C (2) = concentration (mg/dm^3) of sample after treatment

Table 2.5 PMS: Fe (II) ratios

Ratio of PMS: Fe(II)	**1:0.5**	**1:1**	**1:2**	**1:2.5**
Vinasse (ml)	20	20	20	20
PMS (ml)	4.42	4.42	4.42	4.42
PMS (mM)	0.36	0.36	0.36	0.36
Fe(II) (ml)	0.5	1	2	2.5
Fe(II) (mM)	0.18	0.36	0.72	0.9
Water (ml)	4.08	3.58	2.58	2.08
Total	29	29	29	29

- **Effect of pH on TOC removal in PMS-Fe (II) system**

In each 30ml glass vial, 20ml of vinasse were placed. Each sample was dosed with 4.42ml of PMS and 2.5ml of iron (II) sulfate as activator. pH values were adjusted to 3, 5, 7, 8 and 11 by adding NaOH and/or H_2SO_4 solution. Vials were shaken at 150 rpm by using an orbital shaker. After the reaction, the reaction mixture was filtered through a syringe filter with the pore size of 0.45 μm before TOC analysis.

Calculation:

$$\text{TOC removal \%} = \frac{C(1) - C(2)}{C(1)} \times 100$$

C (1) = concentration (mg/dm^3) of sample prior treatment

C (2) = concentration (mg/dm^3) of sample after treatment

- **Effect of reaction time on TOC removal PMS-Fe(II) system**

In each 30ml glass vial, 20ml of vinasse were placed. Each sample was dosed with 4.42 and 2.5ml of PMS and iron (II) sulfate respectively at pH 7. Reaction time were adjusted to 0.0833, 0.5, 4, 8 and 24 hour. Vials were shaken at 150 rpm by using an orbital shaker in different time. After the reaction, the reaction mixture was filtered through a syringe filter with the pore size of 0.45 μm before TOC analysis.

Calculation: TOC removal % = $\frac{C(1)- C(2)}{C(1)} \times 100$

C (1) = concentration (mg/dm^3) of sample prior treatment

C (2) = concentration (mg/dm^3) of sample after treatment

- **Optimization of PMS**

Optimum ratio of PS: Fe (II) were placed in vial with 20ml of vinasse. The optimum condition of pH and time reaction were adjusted. Optimum conditions were increased to obtain the maximum removal of TOC. Vials were shaken at 150 rpm by using an orbital shaker. After the reaction, the reaction mixture was filtered through a syringe filter with the pore size of 0.45 μm before TOC analysis.

2.2.3.4 Enhancement of TOC removal by using hydrogen peroxide

i. 2.643ml of sample were added into 500ml Erlenmeyer flask.
ii. 200ml of water, 20ml of potassium iodide solution and 25ml of the acid mixture were added.
iii. The solution was mixed well, stoppered and left to stand five minutes.
iv. The solution was titrated with sodium thiosulfate in 50ml burette until the brown triiodide color had been reduced to a light straw color.
v. A few drops of starch solution were added and the titration was continued until the color of the solution changed sharply from blue to colorless. Volume was recorded as (A).

vi. The above steps were repeat without sample as blank (B).

Calculations: Hydrogen Peroxide % w/w = $\frac{(A-B)X\ M\ X\ 1.7007}{Sample\ weight}$

Where A= equivalent volume of sodium thiosulfate for sample

B= equivalent volume of sodium thiosulfate for blank

M= molarity of sodium thiosulfate

2.2.3.4.1 Reduction of TOC by coupling of sulfate and hydroxide radicals

The optimum conditions of PS-Fe (II) and PMS –Fe (II) system were used. All experiments were performed in batch mode in triplicated. Experiments were conducted at room temperature in 30mL glass vials. All reagents were of analytical grade and only deionized water was used. Oxidation was initiated by the addition of PS or PMS solution plus H_2O_2 to the vial that contained pretreated vinasse.

Effect of H_2O_2 doses in TOC removal in PS system

In each 30ml glass vial, 20ml of vinasse were placed. Each sample was dosed with 0.388g of PS and 0.198g of Fe (II). Then 0.2, 0.4, 0.6 and 0.8ml of H_2O_2 were added. Deionized water were added to complete volume to 29ml for each vial. Vials were shaken at 150 rpm by using an orbital shaker. After the reaction, the reaction mixture was filtered through a syringe filter with the pore size of 0.45 μm before TOC analysis.

Calculations:

$$\text{TOC removal \%} = \frac{C(1)-C(2)}{C(1)} \times 100$$

C (1) = concentration (mg/dm^3) of sample prior treatment

C (2) = concentration (mg/dm^3) of sample after treatment

- **Effect of H_2O_2 doses in TOC removal in PMS system**

In each 30ml glass vial, 20ml of vinasse were placed. Each sample was dosed with 0.442g of PMS and 0.5g of Fe (II). Then 0.08, 0.162, 0.243 and 0.324ml of H_2O_2 were added. Deionized water were added to complete volume to 29ml for each vial. Vials were shaken at 150 rpm by using an orbital shaker. After the reaction, the reaction mixture was filtered through a syringe filter with the pore size of 0.45 μm before TOC analysis.

Calculations:

$$\text{TOC removal \%} = \frac{C(1) - C(2)}{C(1)} \times 10$$

C (1) = concentration (mg/dm^3) of sample prior treatment

C (2) = concentration (mg/dm^3) of sample after treatm.

CHAPTER THREE

Results and Discussions

3.1 Vinasse characterization

3.1.1 Physicochemical characteristics of vinasse

The physicochemical characteristics are important to select the suitable method for waste water treatment. High levels of pollutants mainly organic matter in water causes an increase in BOD, COD, TDS and TSS. They make the water unsuitable for drinking, irrigation or any other use (Shrivastava, *et al*. 2012).

Table 3.1Some of the physicochemical characteristics of vinasse

Parameters	Units	Vinasse 1	Vinasse 2
pH	-	3.5	4.85
Color	-	Dark brown	Dark brown
BOD	mg/dm^3	68.978	65.000
COD	mg/dm^3	125.777	200.000
TOC	mg/dm^3	489.60	480.4
TC	mg/dm^3	490.00	484.5
IC	mg/dm^3	0.40	4.077
TN	mg/dm^3	6.669	8.309
TS	mg/dm^3	111.46	119.57
TDS	mg/dm^3	63.8	97.47
TSS	mg/dm^3	13.0	17.1
K	mg/dm^3	121.3	92.3
Na	mg/dm^3	120.55	87.9
Ca	mg/dm^3	73.75	22.65

As shows in Table 3.1 the pH values were 3.5 and 4.85 which indicate the acidity nature of the samples. Therefore, disposed vinasse to the land without treatment will cause soil salinization (Mohana, *et al*. 2009). The results shows high BOD of 68.978 and 65.000 mg/dm^3, COD of 125.777 and 200.000 mg/dm^3 for vinasse (1) and (2) respectively, indicating that vinasse has high pollution effects, and in agreement with those of Beltran, *et al*. (2005), Jime´nez, *et al*. (2006), and Pant and Adholeya, (2007a). On the other hand, the large amount of vinasse produced

made vinasse a serious environmental problem. Thus proper treatments are required to reduce the high organic content as well as acidity and color. The physical characteristics TS, TDS and TSS are important to select the suitable method for wastewater treatment (Punmia and Ashok. 1998). Results of TS, TDS and TSS contents of wastewater are useful in the design and process control of wastewater treatment facilities. Moreover, TSS result is commonly used to evaluate the degree of pollution in natural water and serves as a key process control parameter for wastewater treatment operation. As results show the concentration of K was 121.3 and 92.3 mg/dm^3 for vinasse (1) and (2), respectively, whereas those of Na and Ca were 120.55, and 73.75 mg/dm^3 for vinasse (1), 87.9 and 22.65 mg/dm^3 for vinasse (2). According to the obtained results vinasse could be used in irrigation as fertirrigation, and they were in agreement with those described by Ahmed, *et al*. (2013). The cost of use of vinasse as fertirrigation is lower than that of chemical fertilizers (Laime, *et al*. 2011).

3.1.2 IR analysis

Figure 3.1 depicts the IR spectrum of vinasse. A strong and broad band at about 3400 cm^{-1} indicates the presence of OH group, and a relatively weak band at 2930 cm^{-1} is attributed to CH- stretching. The bands at 1600 cm^{-1} is attributed to the C=C in aromatic compounds, also a relatively weak and broad band at 1420 cm^{-1} is attributed to aliphatic C-H bending, and a sharp and weak band at 1130 cm^{-1} is attributed to stretching of the C-O bond as in ether.

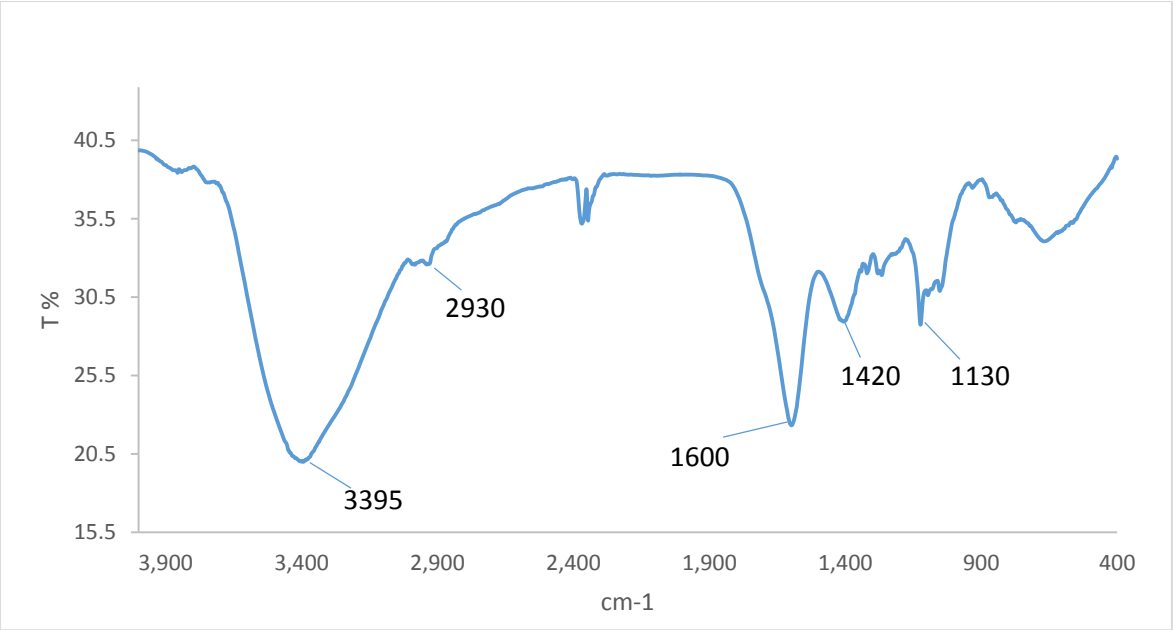


Figure 3.1. IR spectrum of vinasse

3.1.3GC-MS analysis

The identification of unknown compounds was initially accomplished by comparison with the MS library (NIST) and comforted by using Chemo bio draw program version ultra 11.0, and/or by interpreting the fragmentation pattern of the mass spectra. The comparison of the mass spectrums with the data base on MS library gave about 72% - 96% match as well as confirmatory compound structure match.

3.1.3.1 Identification of solvent extracted compounds from vinasse

Various compounds extracted by hexane from vinasse were analyzed by GC- MS. The typical total ion chromatograms (TIC) of compounds extracted by hexane from vinasse are shown in Figure 3.2 and appendix B (1-12). Table (3.2) represent the compounds extracted by hexane.

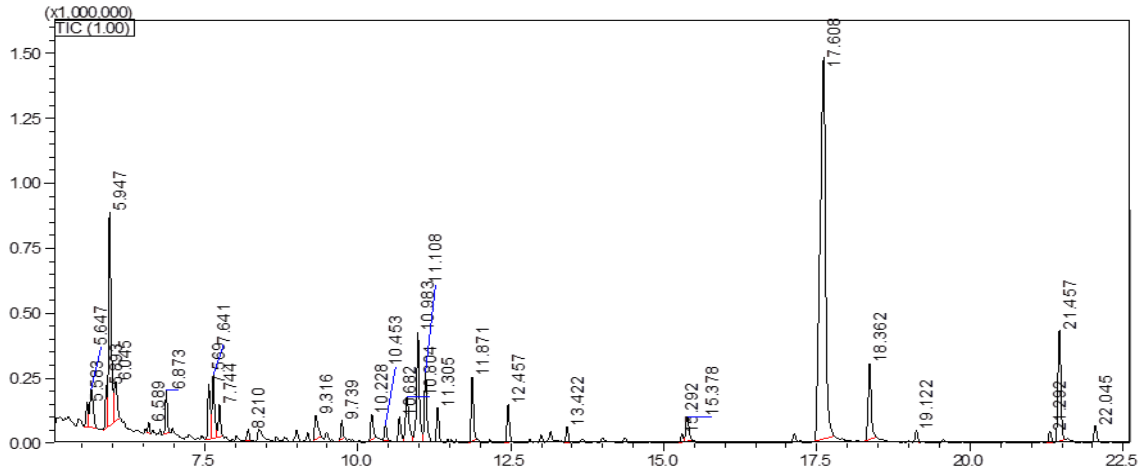


Figure 3.2. Total ion chromatogram of compounds extracted from vinasse by hexane. Peak numbers as R.T

3.1.3.1.1 Identification of hexane extracted compounds

- **Identification of 2-phenyl ethanol**

The EI mass spectrum of 2-phenyl ethanol. MW 122.16 (Figure 3.3). The base peak is found at m/z 91 corresponding to $M[C_7H_7]^{+\cdot}$ as a result from the loss of $[CH_3O]^{\cdot}$. The 2-phenyl ethanol appears at R.T of 5.647 in total ion chromatogram.

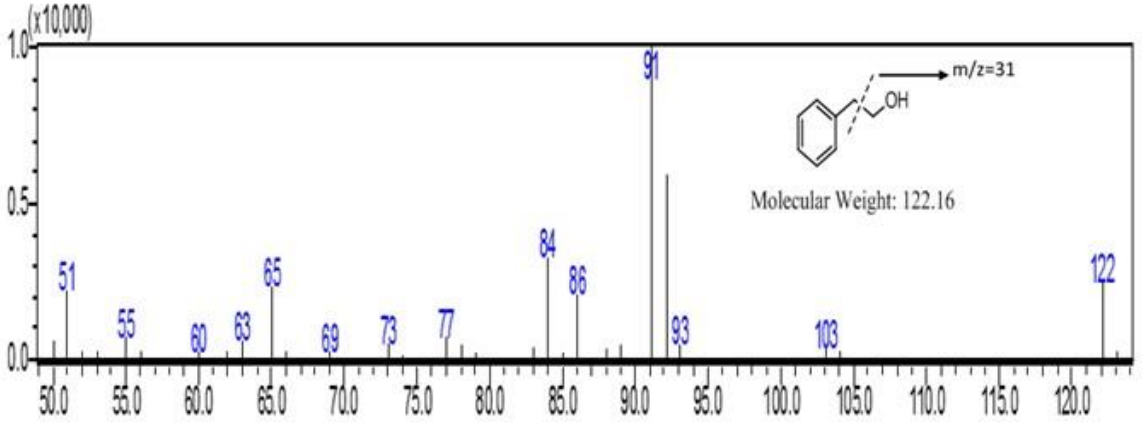


Figure 3.3 The mass spectrum analysis of 2-phenyl ethanol

Identification of 4-ethyl-2-methoxy phenol

The EI mass spectrum of 4-ethyl-2-methoxy phenol MW 152.19 (Figure 3.4). The base peak is found at m/z 137 corresponding to $M[C_8H_9O_2]^{+\cdot}$ as the result of

los s of $[CH_3]^{\cdot}$. The 4-ethyl-2-methoxy phenol appears at R.T 6.873 in total ion chromatogram.

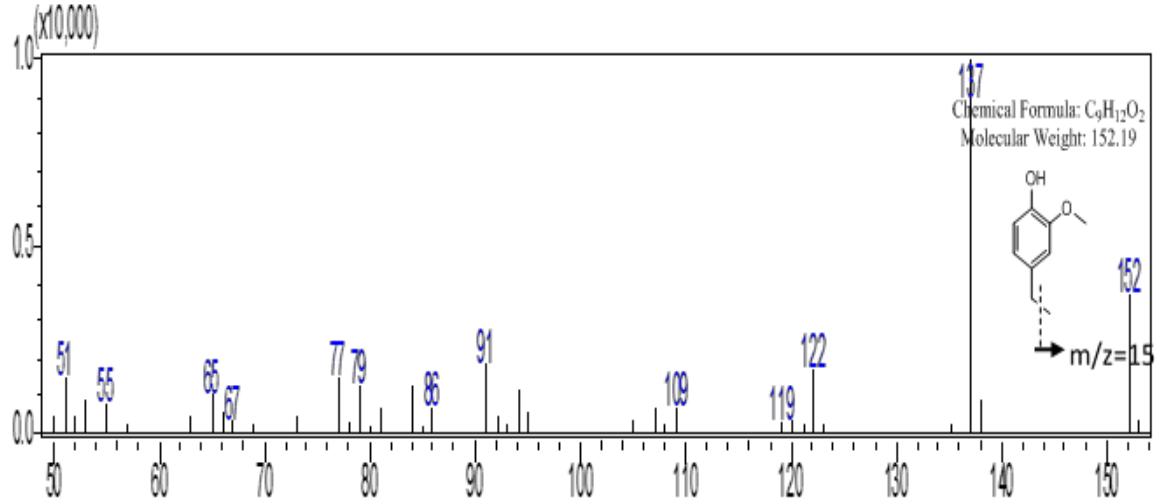


Figure 3.4 The mass spectrum analysis of 4-ethyl-2-methoxy phenol

- **Identification of 2,6- dimethoxy phenol**

The EI mass spectrum of 2,6- dimethoxy phenol MW 154.16 (Figure 3.5). The base peak is found at m/z 139 corresponding to $M[C_7H_7O_3]^{+\cdot}$ as a result from the loss of $[CH_3]^{\cdot}$. 2,6- dimethoxy phenol appears at R.T 7.575 in total ion chromatogram.

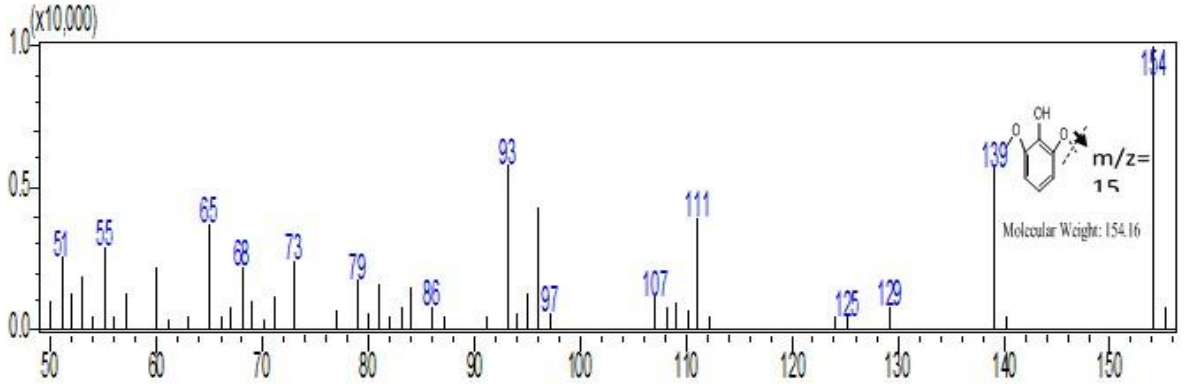


Figure 3.5 The mass spectrum analysis of 2,6-dimethoxy phenol

- **Identification of 1,2,3- triethoxy-5-methyl benzene**

The EI mass spectrum of 1,2,3- triethoxy-5-methyl benzene MW 182.22 (Figure 3.6). The MW of 183 is probably due to the isotope of ^{13}C. The base peak is found at m/z 167 corresponding to $M[C_9H_{11}O_3]^{+\cdot}$ as the result of the loss of $[CH_3]\cdot$. 1,2,3- triethoxy-5-methyl benzene appears at R.T 9.742 in total ion chromatogram.

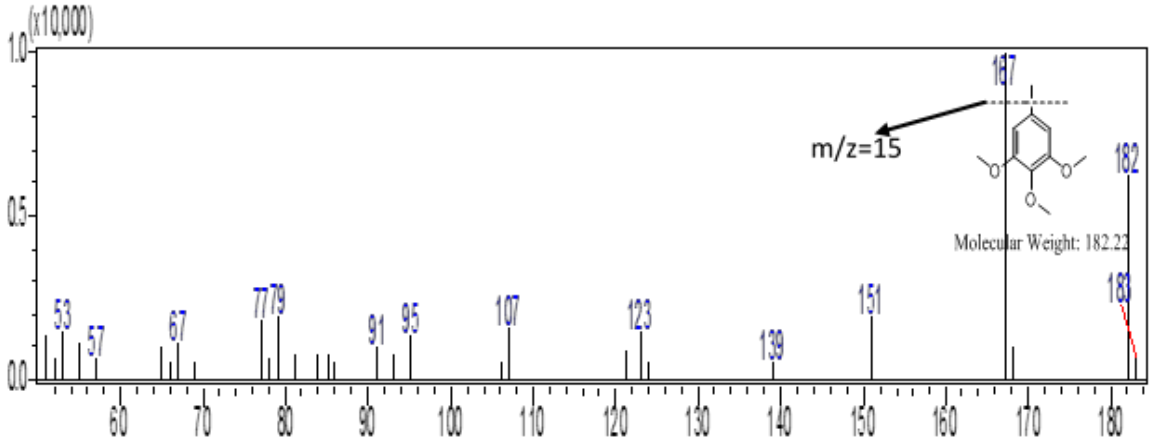


Figure 3.6. The mass spectrum analysis of 1,2,3- triethoxy-5-methyl benzene

- **Identification of dodecanoic acid**

The EI mass spectrum of Dodecanoic acid MW 200.32 (Figure 3.7). The loss of $[C_2H_5]\cdot$ results in $M[C_{10}H_{19}O_2]^{+\cdot}$ at m/z 171 .Meanwhile, the base peak is found at m/z 73, Dodecanoic acid appears at R.T 10.225 in total ion chromatogram.

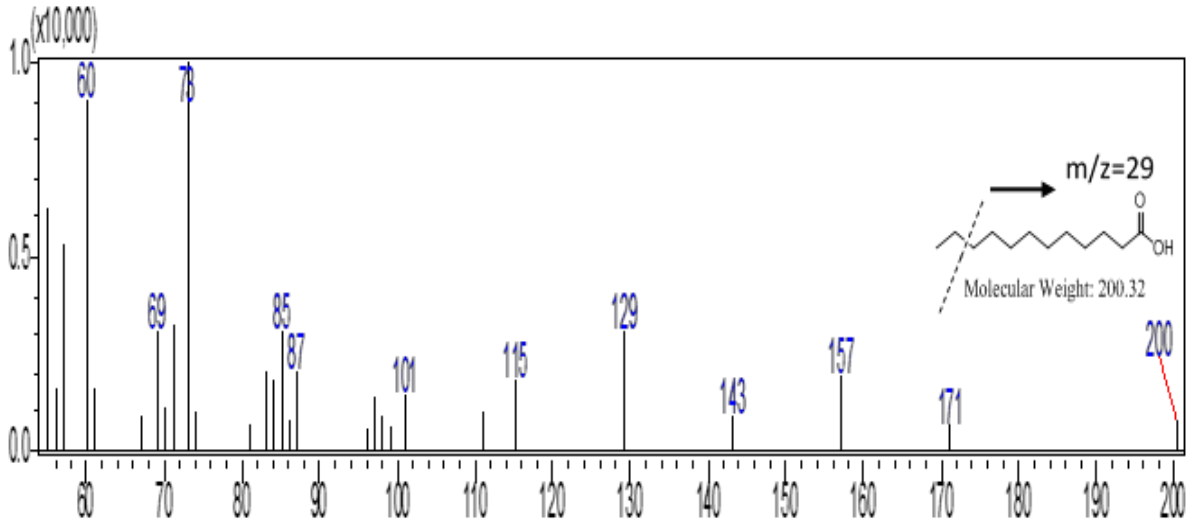


Figure 3.7 The mass spectrum analysis of Dodecanoic acid

- **Identification of 3,4,5- trimethoxy phenol**

The EI mass spectrum of 3,4,5- trimethoxy phenol MW 184.19 (Figure 3.8). The base peak is found at m/z 169 corresponding to M[$C_8H_9O_4$] $^{+\cdot}$ as the result of the loss of [CH_3]$\cdot$. The 3,4,5- trimethoxy phenol appears at R.T 10.675 in total ion chromatogram.

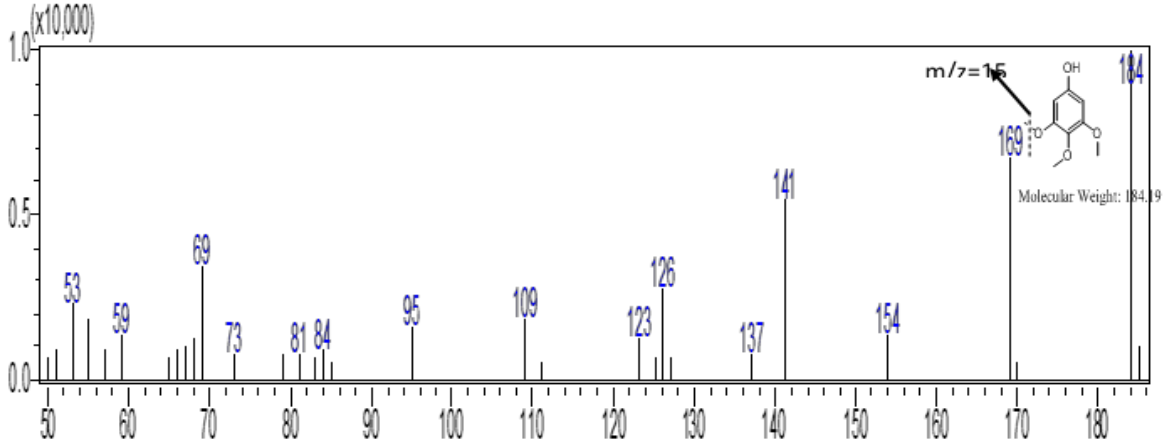


Figure 3.8 The mass spectrum analysis of 3,4,5- trimethoxy phenol

- **Identification of 4-allyl-2, 6-dimethoxy phenol**

The EI mass spectrum of 4-allyl-2, 6-dimethoxy phenol MW 194.23 (Figure

3.9). The loss of [CH_3]· result in the appearance of M [$C_{10}H_{11}O_3$] $^{+\cdot}$ at m/z 179 . Meanwhile, the base peak is found at m/z 91, 4-allyl-2,6-dimethoxy phenol appears at R.T 10.983 in total ion chromatogram.

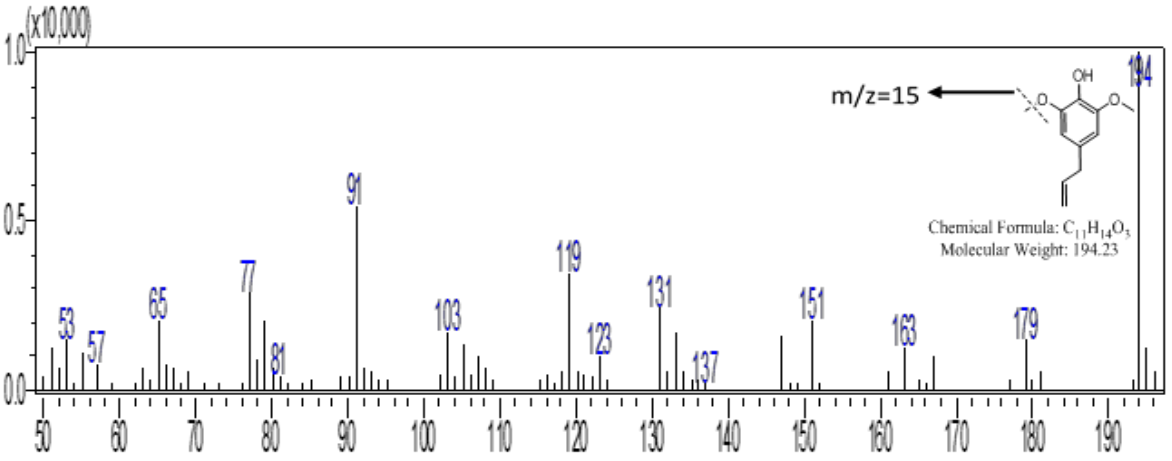


Figure 3.9 The mass spectrum analysis of 4-allyl-2,6-dimethoxy phenol

- **Identification of (E) -1-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)but- 2-en-1-one**

The EI mass spectrum of (E) -1-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-one MW 208.30 (Figure 3.10). The loss of [CH_3]· result in the appearance of M[$C_{12}H_{17}O_2$] $^{+\cdot}$ at m/z 193 . Meanwhile, the base peak is found at m/z 69, (E) -1-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-one appears at R.T 11.300 in total ion chromatogram.

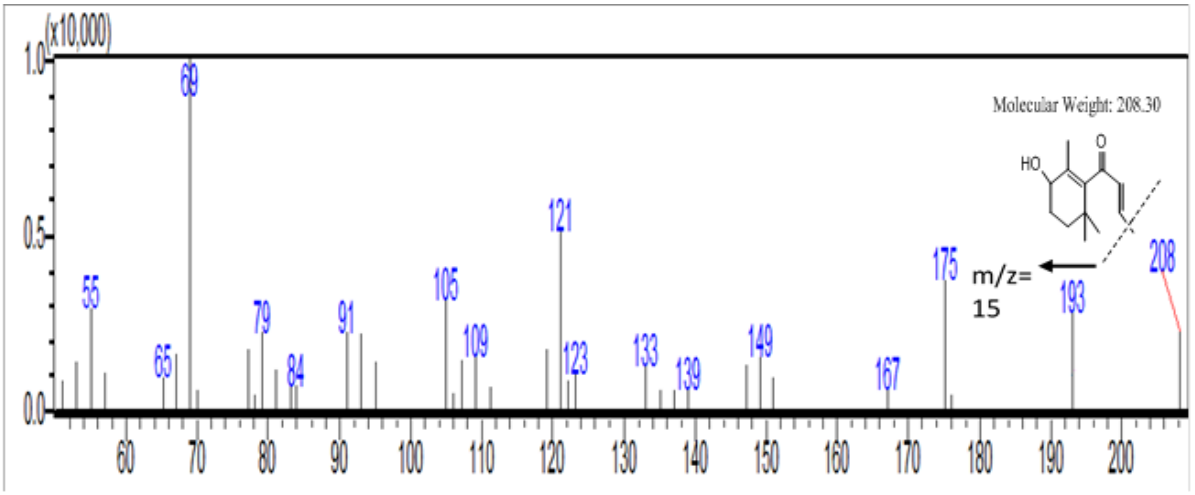


Figure 3.10 The mass spectrum analysis of (E) -1-(3-hydroxy-2,6,6-trimethylcyclohex-1- enyl)but-2-en-1-one

- **Identification of 1,1,4,4-tetramethyl-2,5-dimethylenecyclohexane**

The EI mass spectrum of of 1,1,4,4-tetramethyl-2,5-dimethylenecyclohexane MW 164.29 (Figure 3.11). The base peak is found at m/z 149 corresponding to M $[C_{11}H_{17}]^{+\cdot}$ as the result of the loss of $[CH_3]^{\cdot}$. The 1,1,4,4-tetramethyl-2,5-dimethylenecyclohexane appears at R.T 13.150 in total ion chromatogram.

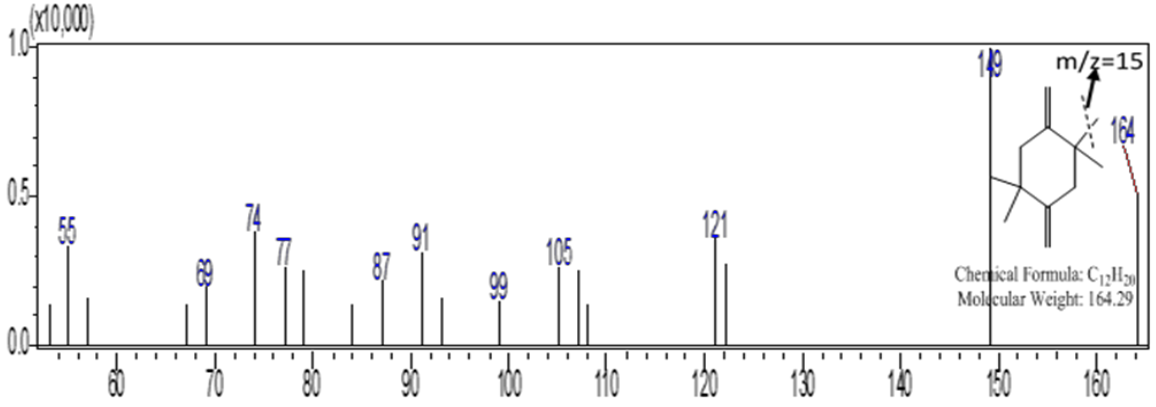


Figure 3.11 The mass spectrum of 1,1,4,4-tetramethyl 2,5-dimethylenecyclohexane

- **Identification of methyl palmitate**

The EI mass spectrum of methyl palmitate. MW 270.45 (Figure. 3.12). The loss of $[C_3H_7]\cdot$ results in the appearance of $[C_{14}H_{27}0_2]^{+\cdot}$ at m/z 227. The base peak is found at m/z 74, the Methyl palmitate appears at R.T 17.608 in total ion chromatogram.

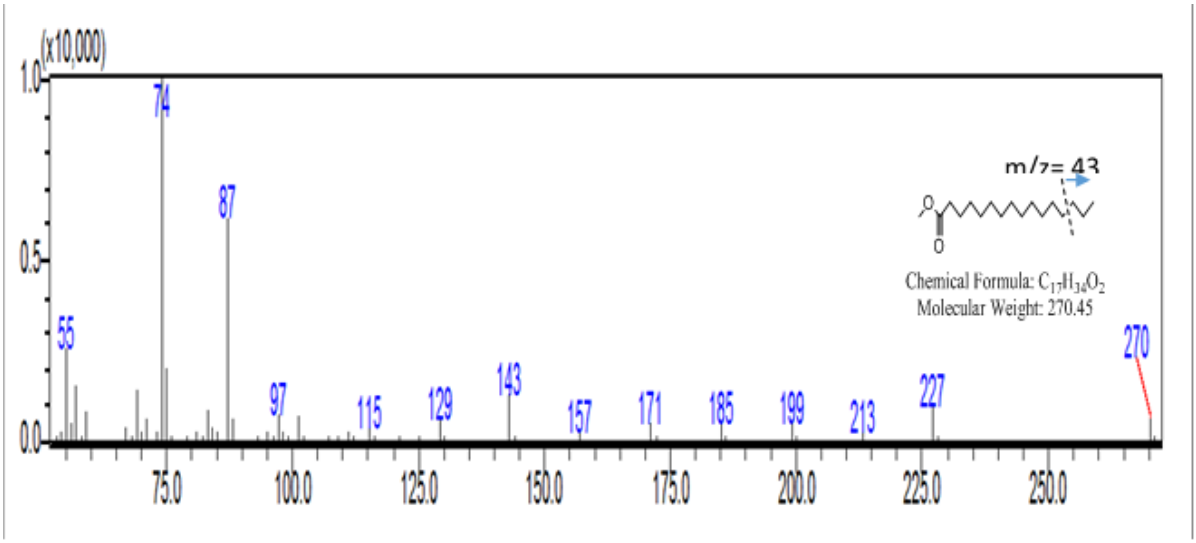


Figure 3.12. The mass spectrum analysis of Methyl palmitate

- **Identification of of palmitic acid**

The EI mass spectrum of Palmitic acid .MW 256.42 (Figure 3.13). The loss of $[C_2H_5]\cdot$ result in the appearance of $M[C_{14}H_{27}0_2]^{+\cdot}$ at m/z 227. Meanwhile, the base peak is found at m/z 73, Palmitic acid appears at R.T 18.367 in total ion chromatogram.

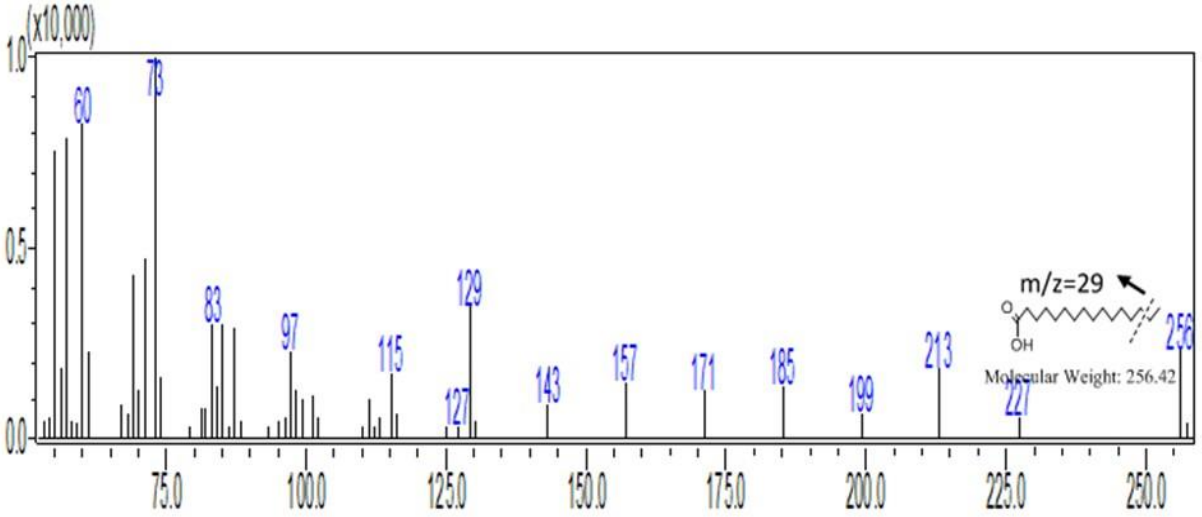


Figure 3.13. The mass spectrum analysis of Palmitic acid

- **Identification of methyl stearate**

The EI mass spectrum of methyl stearate MW 298.5 (Figure3.14). The loss of $[C_3H_7]\cdot$ Result in M $[C_{16}H_{31}0_2]^{+\cdot}$ At m/z 255.Meanwhile, the base peak is found at m/z 74, methyl stearate appears at R.T 22.050 in total ion chromatogram.

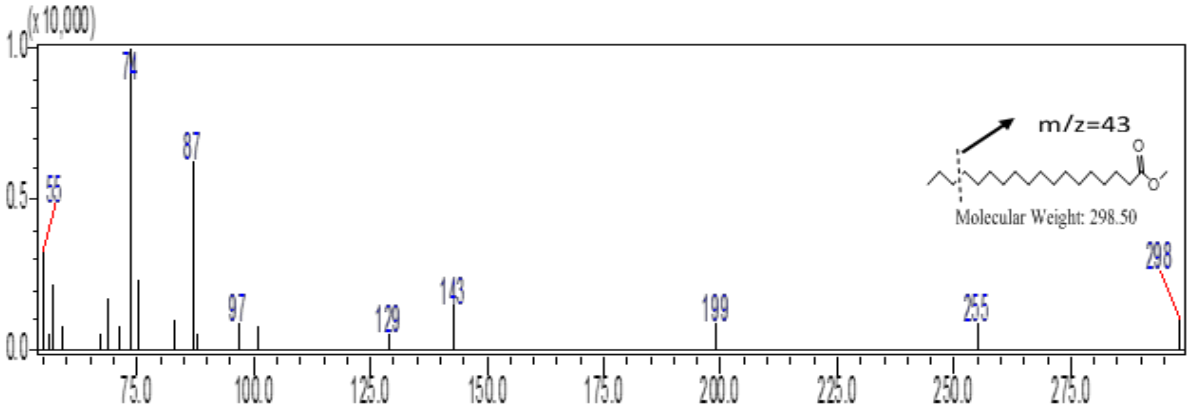
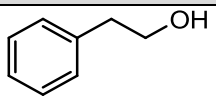
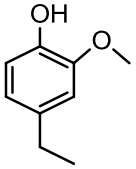
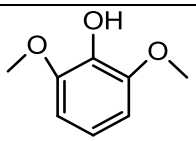
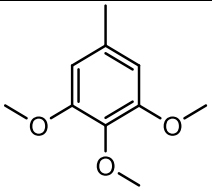
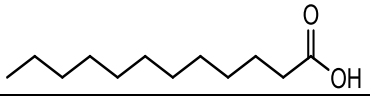
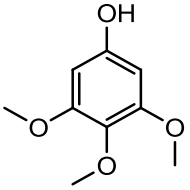
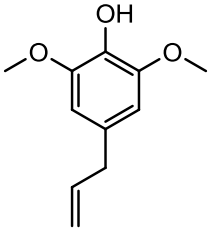
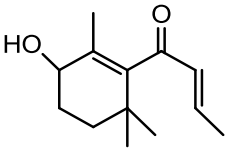
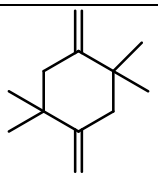
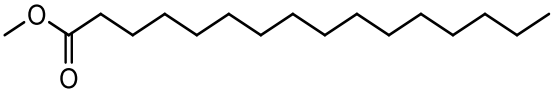
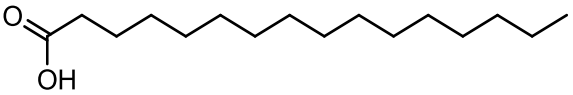
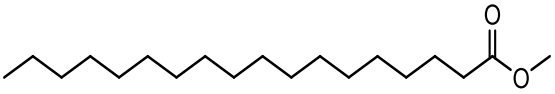


Figure 3.14. The mass spectrum analysis of methyl stearate

Table 3.2 Identification of compounds extracted from vinasse by hexane.

No	R.T	Structure	Name and MW	Match %	Class
1	5.647		2-phenyl ethanol. MW .122.16	86	alcohol
2	6.873		4-ethyl-2-methoxy phenol. MW 152.19	91	Phenol
3	7.575		2,6- dimethoxy phenol. MW 154.16	86	phenol
4	9.742		1,2,3- triethoxy-5-methyl benzene. MW 182.22	75	Aromatic (benzene ring)
5	10.225		Dodecanoic acid. MW 200.32	94	Carboxylic acid
6	10.675		3,4,5-trimethoxy phenol. MW 184.19	80	phenol
7	10.983		4-allyl-2,6-dimethoxy phenol. MW 194.23	87	phenol
8	11.300		(E) -1-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-one. 208.30	86	Ketone

9	13.150		1,1,4,4-tetramethyl-2,5-dimethylenecyclohexane. MW 164.29	72	cycloalkane
10	17.608	 Methyl palmitate. MW 270.45		96	ester
11	18.367	 Palmitic acid. MW 256.42		95	Carboxylic acid
12	22.050	 methyl stearate MW 298.5		92	ester

3.1.3.1.2 Identification of DCM extracted compounds

Various compounds extracted by DCM from vinasse were analyzed by GC-MS. The typical total ion chromatogram (TIC) of compounds extracted by DCM are given in figure 3.15 and appendix C (1-3). Table 3.3 represent the compounds extracted by DCM. Appendix (C).

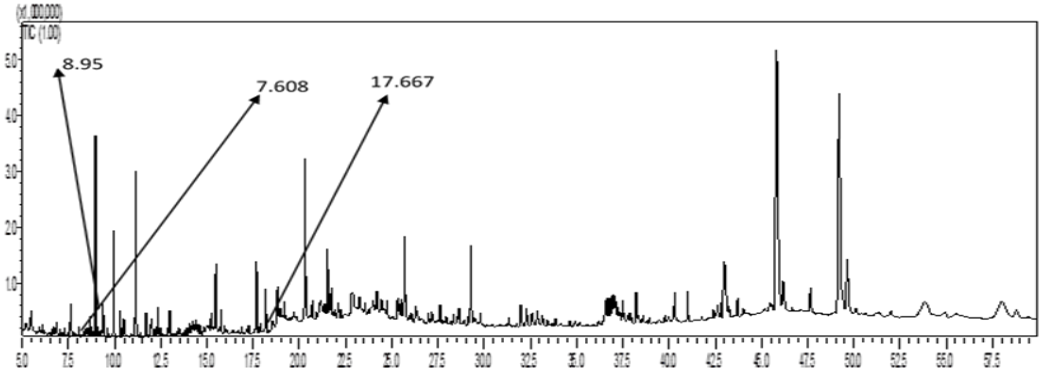


Figure 3.15 Total ion chromatogram of compounds extracted from vinasse by

DCM. Peak numbers as R.T

- **Identification of 2,6 dimethoxy phenol**

The EI mass spectrum of 2,6 dimethoxy phenol MW 154.16 (Figure 3.16). The base peak is found at m/z 154. The loss of $[CH_3]^{\cdot}$ results in $M[C_7H_7O_3]^{+\cdot}$ at m/z 139. The 2,6 dimethoxy phenol appears at R.T 7.608 in total ion chromatogram.

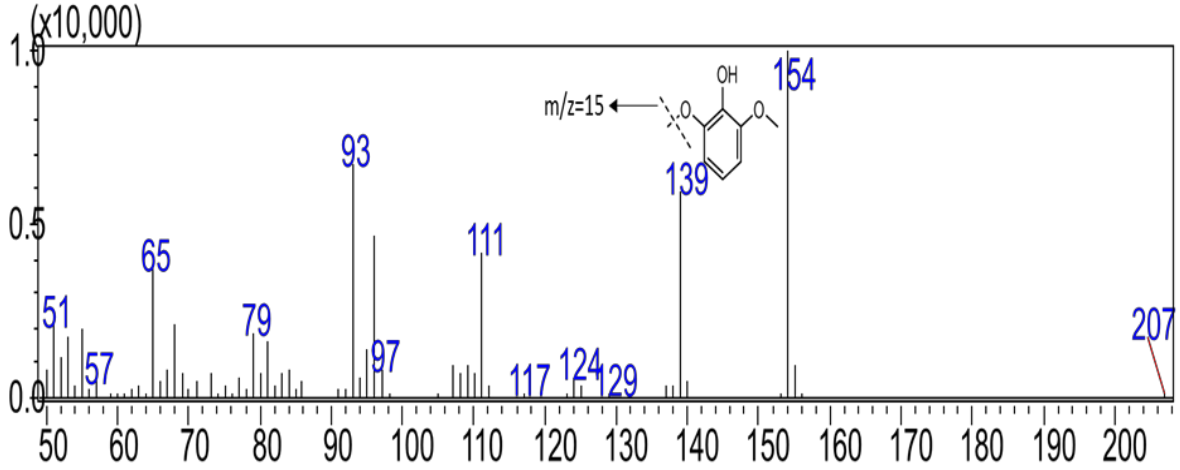


Figure 3.16. The mass spectrum analysis of 2,6 dimethoxyphenol

- **Identification of methyl palmitate**

The EI mass spectrum of Methyl palmitate MW 270.45 (Figure 3.17) the loss of $[CH_3O]^{\cdot}$ results in M $[C_{16}H_{31}O]^{+\cdot}$ at m/z 239 .Meanwhile, the base peak is found at m/z 74, Methyl palmitate appears at R.T 17.66 in total ion chromatogram.

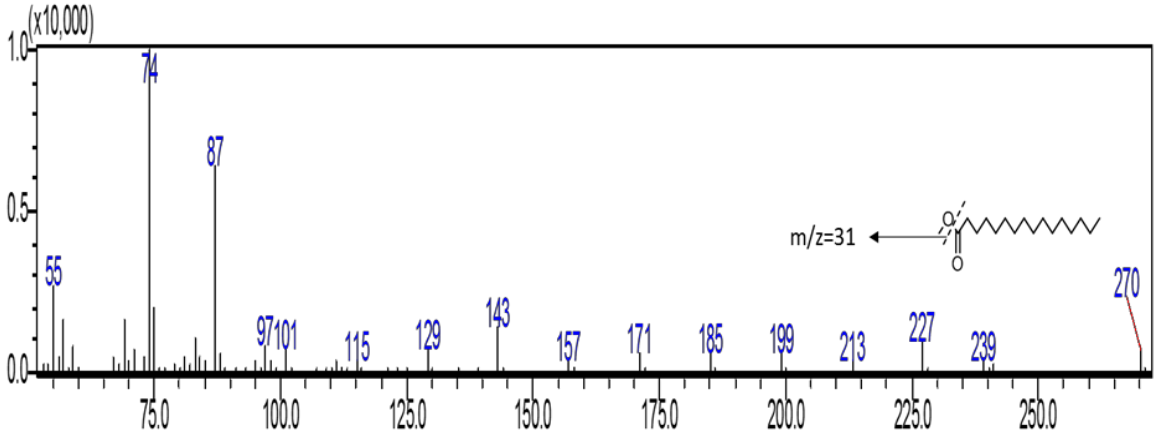
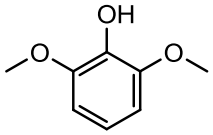
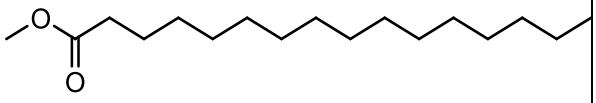
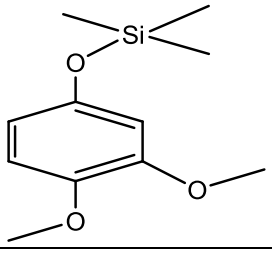


Figure 3.17. The mass spectrum analysis of methyl palmitate

- **Existing of 3,4-dimethoxy phenol trimethylsilane**

3,4-dimethoxy phenol trimethylsilane MW 226.34 appears at R.T 8.95 in total ion chromatogram. Presence of Si is due to the silylation Table (3.3)

Table 3.3 Identification of compounds extracted from vinasse by DCM.

No	R.T	Structure	Name and MW	Match %	Class
1	7.608		2,6 dimethoxyphenol 154.16	92	Phenol
2	17.667		Methyl palmitate 270.45	95	ester
3	8.95		3,4-dimethoxy phenol MW 226.34	94	Phenol

As observed twelve compounds were identified by hexane extraction, whereas three compounds only were identified by DCM; this is due to the exclusion of many compounds that were less than 80% match percentage, and/or to the presence of Si produced come from the silylation such as (3,4-dimethoxy phenoxy) trimethylsilane. Moreover, the derivatives method which is used might result in overlapping of peaks, and consequently in poor chromatographic peaks.

The GC-MS analysis shows that the major compounds detected in vinasse were phenolic compounds. These results were confirmed by the IR spectra, displaying strong broad peaks in (OH) region. Phenols compounds are considered as the most hazardous compounds that may have serious harmful effects on humans

and natural environment (Urszula, *et al*. 2012), thus effective treatment techniques should be used for vinasse treatment.

3.2 Vinasse treatment

The treatment of vinasse was carried out in two stages, coagulation- flocculation as the first stage and followed by SR-AOP as the second stage.

3.2.1 Coagulation-flocculation process

In this part, TOC, UV_{254} and color were used to monitor the efficiency of coagulation-flocculation pretreatment method. UV spectrophotometry (at λ max 254nm) was often used to estimate the amount of AOC, especially for the humic substances in wastewater (Tang, et al. 2014). In visible region, absorbance at 475 nm was often measured to determine the intensity of color. (Mrityunjay and Anil. 2012). The efficiency of this pretreatment method was found to be pH and coagulant doses dependent.

3.2.1.1 Removal of TOC, color and AOC using different doses of $FeCl_3$

Table (3.4) shows that the optimum dose of $FeCl_3$ at which an effective coagulation removing 65.37% of TOC occurred when 10 g/dm^3 of coagulant was applied. By increasing the coagulant dose from 5 to 15 g/dm^3 the percentage of TOC removal was found to increase from 55.8 to 69.7% (Figure 3.18). Therefore, higher TOC removal was achieved by using higher dose of coagulant. This result might be due to the precipitation of ferric hydroxide, formed favorably at adequately high coagulant dose, and it could physically swept the colloidal particles from the suspension (Ayguna and Yilmazb. 2010). The obtained results was in agreement with that of Zayas *et al*. (2007). As shown in Table 3.5 and 4.6, color and AOC removal percentages, respectively, were approaching 100% with coagulant dosage of 5 g/dm^3. This results indicate that the AOC, such as humic substances, that contributed both to color (475 nm) and UV absorption (254 nm) would be effectively removed Figure (3.19). Lower efficiency of TOC removal as

compared with color and AOC removals has also been reported in the pretreatment of olive oil mill wastewater (Rizzo, *et al*. 2008). In general, the results indicate that TOC, color and AOC of vinasse were significantly reduced after coagulation-flocculation (Figure3.20).

Table 3.4 Removal percent of TOC using different doses of $FeCl_3$ coagulant

Doses g/dm^3	TOC 1	TOC 2	TOC mean	S.E	Blank	TOC R%
5	214.0	210.7	212.3500	1.6500	480.9	55.84
7.5	229.8	167.3	198.5500	31.250	480.9	58.71
10	178.1	155	166.5500	11.550	480.9	65.37
12.5	185.2	132.1	158.6500	26.550	480.9	67.01
15	147.6	144.1	145.8500	1.7500	480.9	69.67

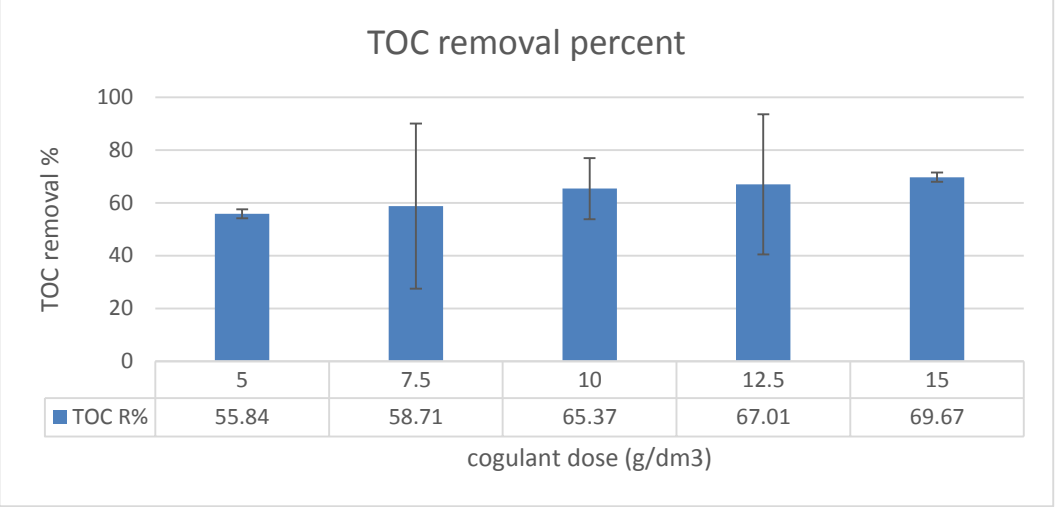


Figure 3.18 Effect of $FeCl_3$ doses g/dm^3 on TOC removal

Table 3.5 Removal percent of color using different doses of $FeCl_3$ coagulant,

Dose g	Blank	5	7.5	10	12.5	15
Sample A 475 nm	0.25	0.001	0.002	0.000	0.001	0.001
Color Removal%		99.6	99.2	100	99.6	99.6

Table 3.6 Removal percent of AOC using different doses of $FeCl_3$ coagulant.

Dose g	Blank	5	7.5	10	12.5	15
Sample A 254 nm	4.8	0.111	0.099	0.073	0.068	0.048
Aromatic Removal%		97.6	97.9	98.4	98.5	99

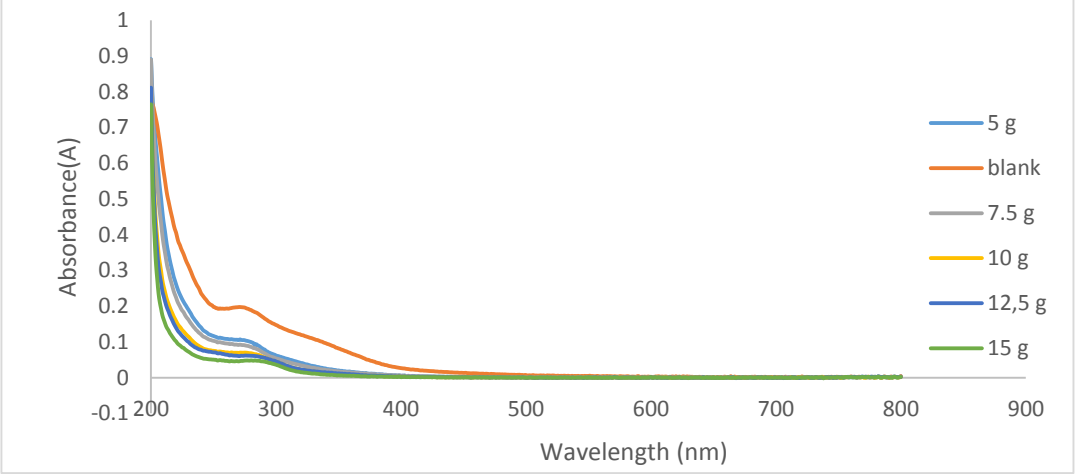


Figure 3.19 UV-Vis spectrum for vinasse in different doses of $FeCl_3$.

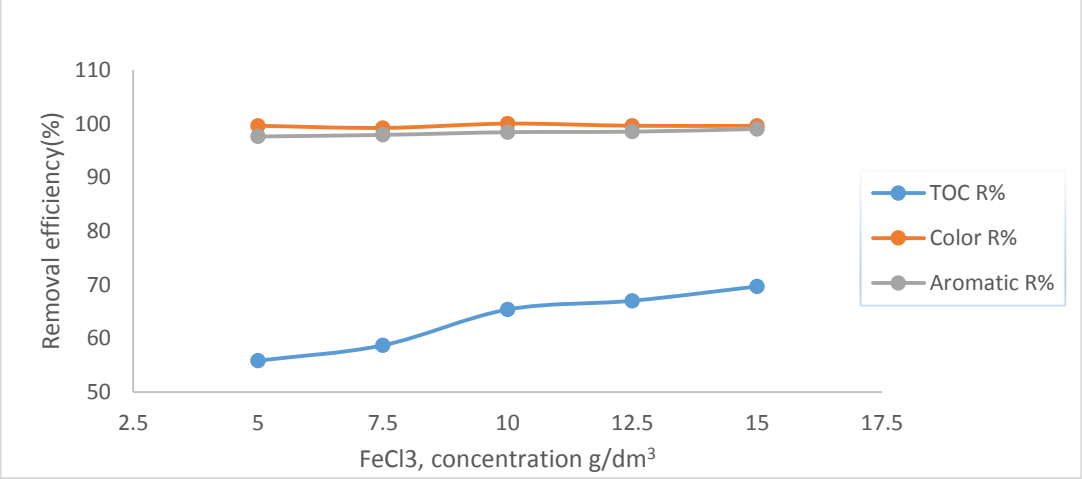


Figure 3.20 Effect of $FeCl_3$ coagulant doses on TOC, color and AOC removal percent

3.2.1.2 Removal of TOC, color and AOC at different pH values

The effect of pH on TOC, color and AOC removal were examined at constant dosage of $FeCl_3$ coagulant at 10 g/dm^3. The removal of TOC, color and AOC was relatively higher when the pretreatment was performed under acidic conditions. The efficiency of coagulation-flocculation pretreatment decreased significantly after pH 7 (Figure 3.23). Most of the vinasses are acidic, therefore, this pretreatment method could be used to remove a large portion of organic content of vinasses without pH adjustment. Table 3.7 and Figure 3.21 shows that the TOC decreased from 84% to 51% when the pH was increased from 3 to11. The effect of pH on coagulation-flocculation for the treatment of vinasse was also examined by spectrophotometric measurement (Figure 3.22). This measurement can provide a simple and qualitative way to monitor the elimination of organic compounds from the aqueous solution. At pH 9 and 11, the adsorption spectra there are no significant reduction in the absorbance, but when the pH was decreased to 7 and below; the absorbance decreased significantly. Therefore, acidic conditions are more favorable operating conditions for the removal of organic compounds from the vinasse through coagulation-flocculation. Results also indicate that further acidification from pH 5 to 3 did not show any significant reduction in absorbance suggesting the need of other treatment method to enhance the removal of organic content of vinasse Tables 3.8 and 3.9 respectively

Table 3.7 Removal percent of TOC at different pH values using 10 g/dm^3 $FeCl_3$

pH	TOC 1	TOC 2	TOC mean	S.E	Blank	TOC R%
3	79.91	82.2	81.0550	1.14500	501.9	83.85
5	87.91	77.5	82.7050	5.20500	471.5	82.46
7	119.1	116.9	118.0000	1.10000	518.4	77.24
9	224.3	242	233.1500	8.85000	556.7	58.16
11	216.8	303	259.9000	43.1000	531.7	51.12

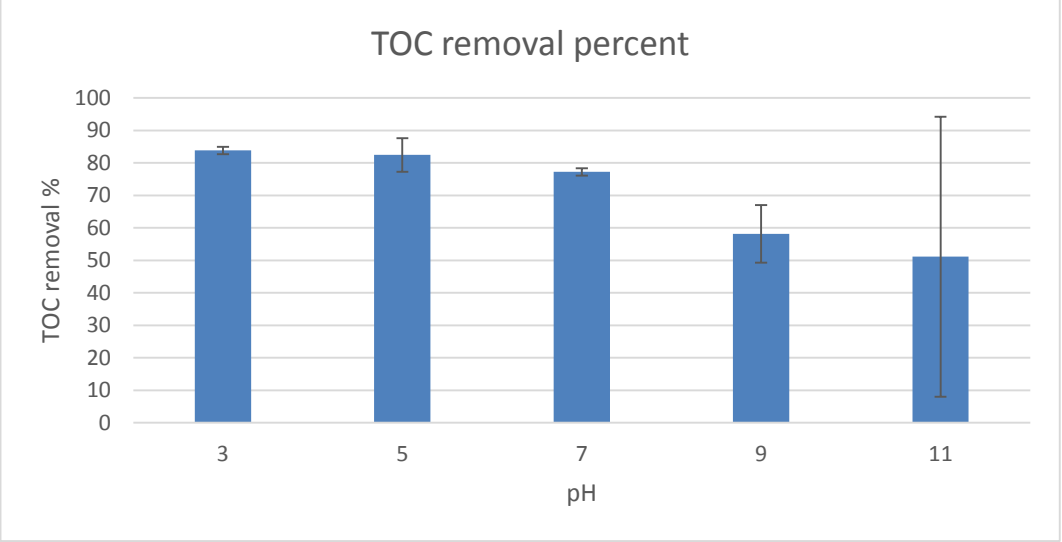


Figure 3.21 Effect of pH on TOC removal by using 10 g/dm^3 of $FeCl_3$

Table 3.8 Removal percent of color at different pH values using 10 g/dm^3 $FeCl_3$

Color Removal	Sample A 475 nm	pH 3	pH 5	pH 7	pH 9	pH 11
Sample	A	0.007	0.004	0.005	0.033	0.049
Blank	A	0.085	0.084	0.104	0.188	0.148
Removal	%	91.76	95.24	95.19	82.45	66.89

Table 3.9 Removal percent of AOC at different pH values using 10 g/dm^3 $FeCl_3$

Aromatic Removal	Sample A 254 nm	pH 3	pH 5	pH 7	pH 9	pH 11
Sample	A	0.320	0.301	0.393	1.048	1.329
Blank	A	0.901	0.868	0.955	1.565	1.658
Removal	%	64.48	65.32	58.85	33.04	19.84

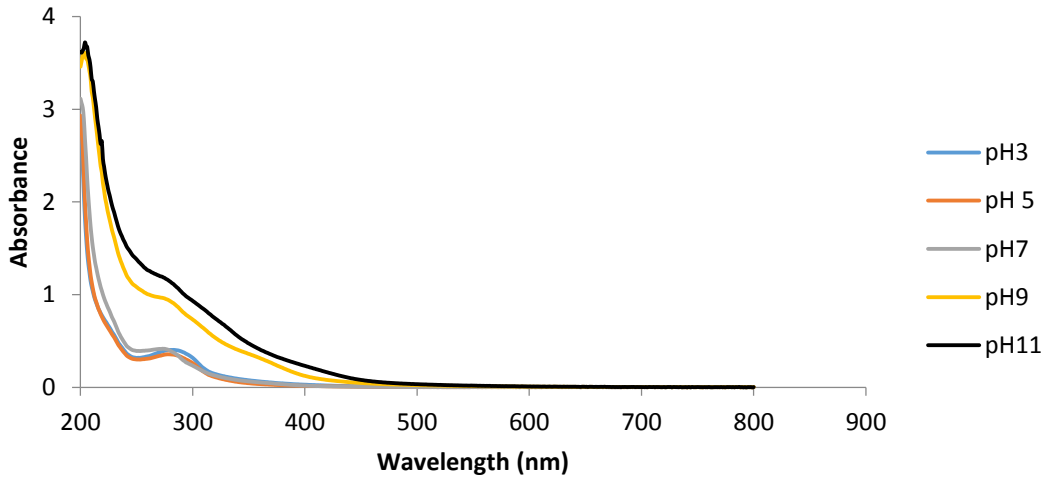


Figure 3.22 UV-Vis spectrum for vinasse at different pH values

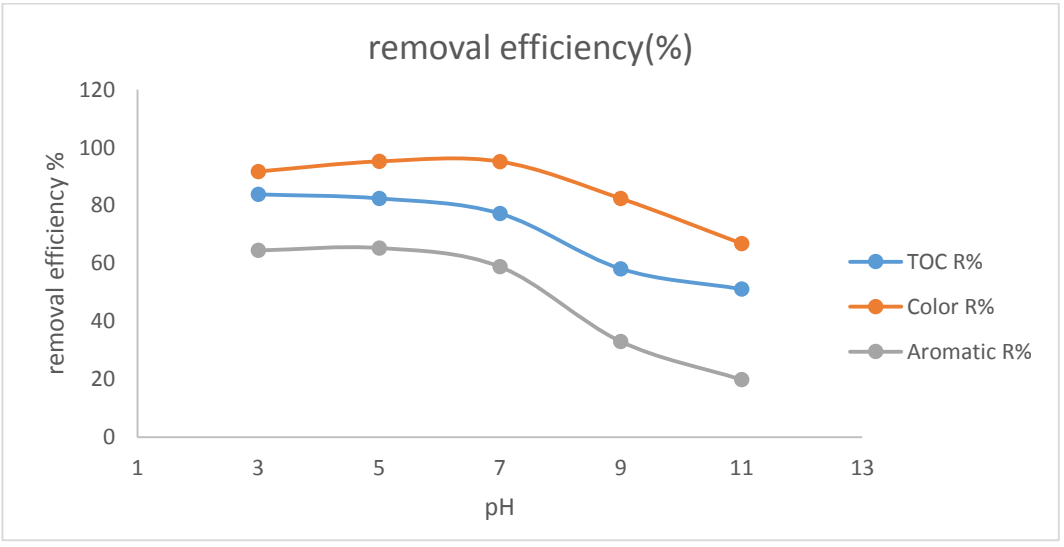


Figure 3.23 Effect of pH on TOC, color and aromatic removal percent

3.2.2 Reduction of TOC using SR-AOP processes

The pretreated vinasse was further treated with sulfate radical-based advanced oxidation process (SR-AOP) after sand filtration. Sand filtration was used to remove the floc that remained in the pretreated vinasse, and no significant of TOC removal was observed.

In this study, SR was generated using Fe(II)-mediated activation of persulfate ($S_2O_8^{2-}$, PS) and peroxymonosulfate (HSO_5^-, PMS) as indicated by the following chemical equations (Ayoub and Ghauch, 2014; Zou *et al*., 2013):

$$S_2O_8^{2-} + Fe^{2+} \longrightarrow SO_4^{\bullet-} + Fe^{3+} + SO_4^{2-} \quad k = 2.0\times10\ M^{-1}s^{-1} \qquad (3\text{- }1)$$

$$HSO_5^{-} + Fe^{2+} \longrightarrow SO_4^{\bullet-} + Fe^{3+} + OH^{-} \quad k = 3.0\times10^{4} M^{-1}s^{-1} \qquad (3\text{- }2)$$

In SR-AOP treatment, the influence of various operating parameters such as amount of activator, type of oxidant (PS and PMS), pH and reaction time, was studied in detail. PS and PMS are oxidants that have been frequently used to generate SR for the treatment of non-biodegradable pollutants (Yang, *et al*. 2010). Both PS and PMS are relatively stable with the changes of the pH (Park, *et al*. 2010), therefore, it can be used to treat the vinasse.

3.2.2.1 Reduction of TOC using PS-Fe (II) system

3.2.2.1.1 Removal of TOC using different doses of PS

Table (3.10) shows the removal percentage of TOC during oxidation at different concentrations of PS. Fe (II) concentration was fixed at 0.36 mM, and the reaction time was fixed at 4 hour. TOC removal increased from 9.4 to 15.5% as PS concentration increased from 0.18 to 1.44 mM. Further increase of PS concentration does not show any significant enhancement in TOC removal (Figure 3.24). Although increasing the PS concentration could lead to the generation of a higher quantity of SR, however, the generation of SR might be limited by the available Fe (II)

Table 3.10 Removal percent of TOC at different doses of PS

PS dose(mM)	TOC 1	TOC 2	TOC 3	TOC mean	S.E	Blank	TOC R%
0.18	131.3	121	122.7	121.85	0.85	134.5	9.4
0.36	129.8	114	113.2	113.6	0.4	134.5	15.54
0.72	123	112.42	115.3	113.86	1.44	134.5	15.3
1.44	122.6	113.4	114	113.7	0.3	134.5	15.5

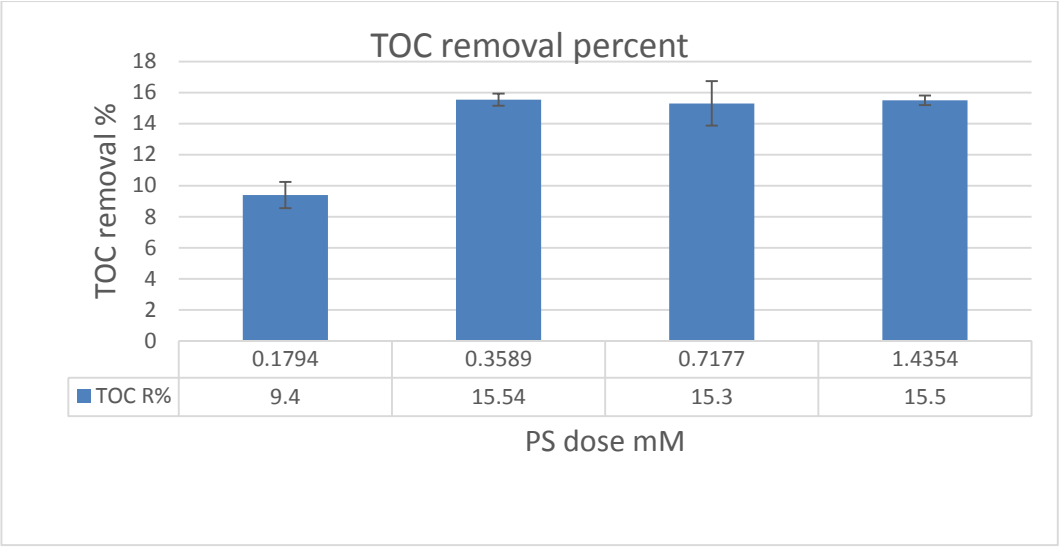


Figure 3.24 Effect of mM PS doses on TOC removal

3.2.2.1.2 Removal of TOC at different PS: Fe (II) ratios

To assess the effect of Fe (II) concentration on TOC removal, the concentration of PS was fixed at 0.72 mM. The concentration of Fe (II) was varied from 0.36 to 1.8 mM. The results from these experiments were presented as TOC removal versus oxidant PS to Fe (II) ratio (Figure 3.25). As shown in Table 3.11 TOC removal was slightly decreased when ratio increased from 1:0.5 to 1:2.5. This might be attributed to the consumption of SR by the excess amount of Fe (II) (Nfodzo and Choi. 2011; Zou, *et al*. 2014). As indicated by Eq 3- 3, the rate of deactivation of SR through the reaction with Fe (II) is much higher than the rate of SR production. Therefore, the presence of higher amount of Fe (II) would limit the amount of SR for organic compound oxidation.

$$SO_4^{\bullet-} + Fe^{2+} \longrightarrow SO_4^{2-} + Fe^{3+} \quad k = 4.9\times10^{9}\ M^{-1}s^{-1} \qquad (3\text{-}3)$$

Table 3.11 Removal percent of TOC at different PS: Fe (II) ratios

PS: Fe(II) ratio	TOC 1	TOC 2	TOC 3	TOC mean	S.E	Blank	TOC R%
1:0.5	108.4	105.4	104.9	106.233	1.09291	134.5	21.02
1:1	109.4	108.5	111.1	109.667	0.76231	134.5	18.46
1:2	113.6	111.1	112.3	112.333	0.72188	134.5	16.48
1:2.5	111.5	113.5	_____	112.5	0.68394	134.5	16.35

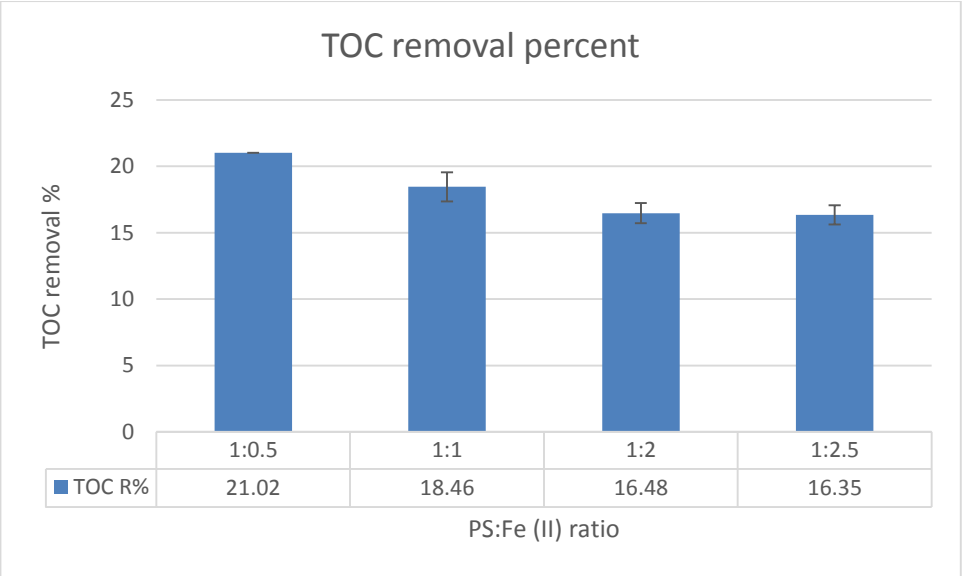


Figure 3.25 Effect of PS: Fe (II) ratios on TOC removal

3.2.2.1.3 Removal of TOC using different pH values

For the effect of pH, the TOC removal was evaluated at pH 3, 5, 7, 8 and 11. The results indicate that the effect of pH significantly influenced the efficiency of TOC removal. As shown in Table (3.12) and (Figure 3.26), TOC removal achieved the highest efficiency at pH 7. At pH 7, TOC removal was found to be 21 %. So far, most of the SR-AOP studies have showed that the degradation of organic pollutants achieved its highest efficiency at acidic conditions due to the formation of SR through acid-catalysis reaction (Zhang, *et al*. 2014). In the present study Fe (II) was thought to complex with soluble organic substances in the vinasse at neutral condition. Complexation could stabilize the Fe (II) from being oxidized to Fe (III) and consequently enhanced the TOC removal. Wu *et al*. (2014) reported that the complexation between Fe (II) with organic ligand could also enhance the removal of organic pollutants by using SR-AOP. At pH 8 and 11, however, TOC removal was retarded, most probably, due to the formation of insoluble Fe (II) hydroxide species reducing the amount of available Fe (II) for the activation of PS.

Table 3.12 Removal percent of TOC at different pH values

pH	TOC 1	TOC 2	TOC 3	TOC mean	S.E	Blank	TOC R%
pH 3	92.64	90.8	95.53	92.99	1.3766	100.1	7.1
pH 5	90.34	92.4	90.79	91.1767	0.62531	100	8.82
pH 7	108.4	105.4	104.9	106.2333	1.09291	134.5	21.02
pH 8	111.2	111	109.9	111.1	0.40415	120.5	7.8
pH 11	101.2	98.1	91.05	96.78	3.003	108.9	11.12

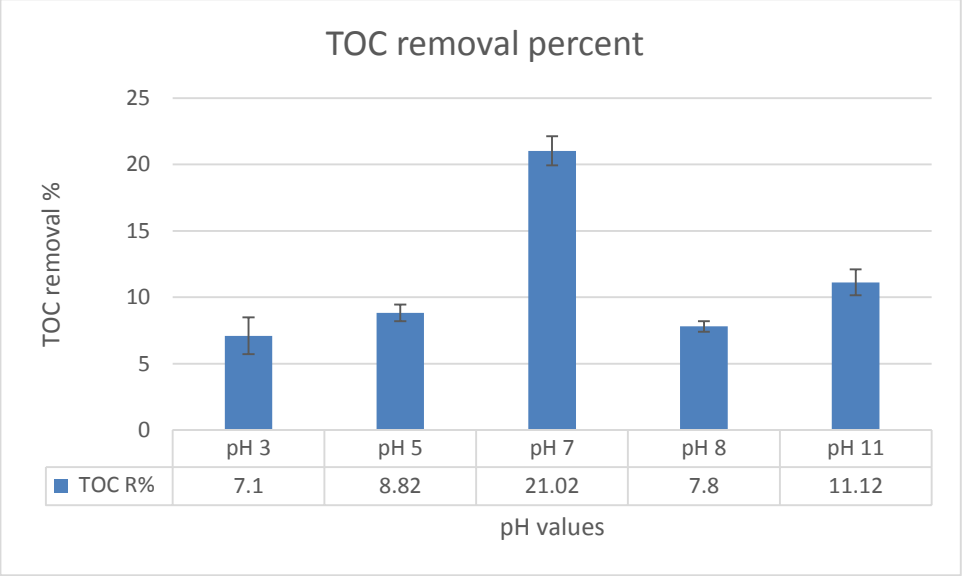


Figure 3.26 Effect of pH values on TOC removal percent in PS-Fe (II) system

3.2.2.1.4 Removal of TOC using different reaction times

To evaluate the effect of reaction time on the TOC removal, the PS-Fe (II) was carried out for 0.083, 0.5, 4, 8 and 24 hour on pretreated vinasses (Table 3.13). The results indicate that the TOC removal was increased significantly for the first 5 min. When the reaction times increased from 5 min to 24 hour, TOC removal increased slowly from 13.0 to 23.7% (Figure 3.27).

Table 3.13 Removal percent of TOC at different reaction times

Time (hour)	TOC 1	TOC 2	TOC 3	TOC mean	S.E	Blank	TOC R%
0.0833	116.1	116.7	118.2	117	0.6245	134.5	13.01
0.5	114.7	114.1	112.1	113.6333	0.78599	134.5	15.51
4	108.4	105.4	104.9	106.2333	1.09291	134.5	21.02
8	103.4	106.6	105.3	105.1	0.92916	134.5	21.86
24	99.52	108.5	99.81	102.61	2.94619	134.5	23.71

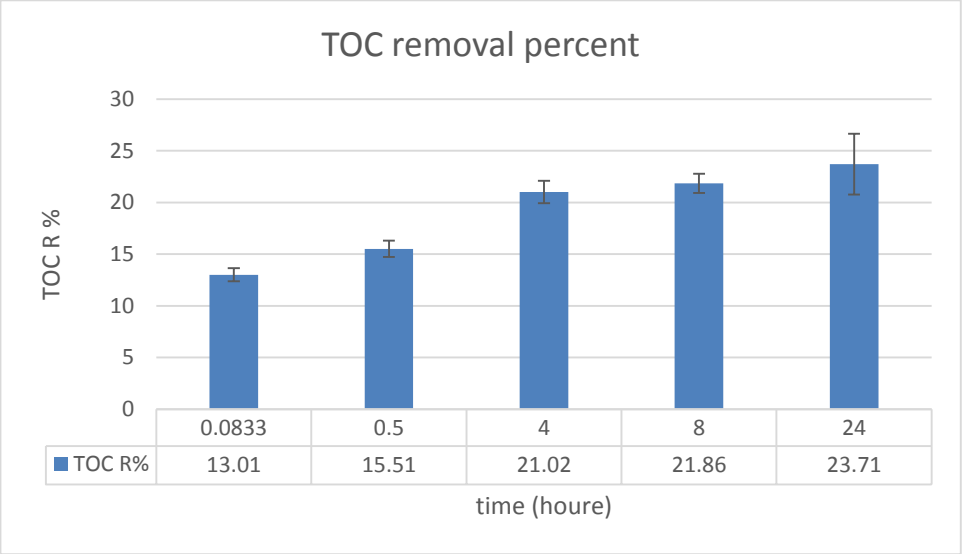


Figure 3.27 Effect of reaction time (hour) on TOC removal percent in PS-Fe(II)system

3.2.2.1..5 PS optimization

The TOC were enhanced by increasing the doses of PS and Fe (II) under the optimum operating conditions, the ratio of PS to Fe (II) was kept at 1:0.5, pH 7 and reaction time was 4 hour. As illustrated in Figure 3.28, TOC removal increased to 48.6% when PS concentration rose to 2.88 mM which was almost 4 times of the initial PS concentration.

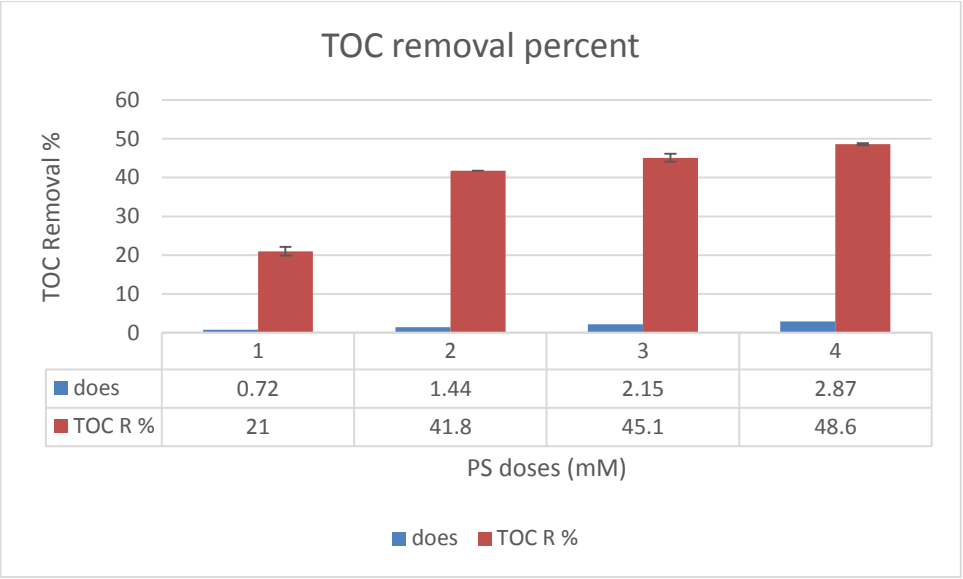


Figure 3.28 Maximum TOC removal by PS-Fe (II) system under optimum conditions

3.2.2.2 Reduction of TOC using PMS-Fe (II) system

3.2.2.2.1 Removal of TOC using different doses of PMS

Table 3.14 shows the effect of PMS concentration on TOC removal. Different PMS concentrations were examined. Fe (II) concentration was fixed at 0.36 mM and the reaction time was fixed at 4 hour. The results illustrate that 22.5% of TOC removal was achieved by using 0.18 mM of PMS. The TOC removal was found to decrease from 22.5 to 17.9% as the PMS concentration increased from 0.18 to 1.44 mM (Figure 3.29). This might be due to the unfavorable consumption of SR by the excessive PMS which leads to the formation of less reactive $SO_5^{\bullet-}$ and scavenged the SR as shown in the following equation (Lin, *et al*. 2014).

$$HSO_5^- + SO_4^{\bullet-} \longrightarrow SO_5^{\bullet-} + SO_4^{2-} + H^+ \qquad (3\text{-}4)$$

Table 3.14 Removal percent of TOC at different doses of PMS

PMS doses	TOC 1	TOC 2	TOC 3	TOC mean	S.E	Blank	TOC R%
0.18	109.4	104.8	103.8	104.3	0.5	134.5	22.46
0.36	104.2	102.1	105.8	105	0.8	134.5	21.94
0.72	107.1	109.3	110.7	109.03	1.04775	134.5	18.94
1.44	105.08	111.2	115.1	110.46	1.91609	134.5	17.87

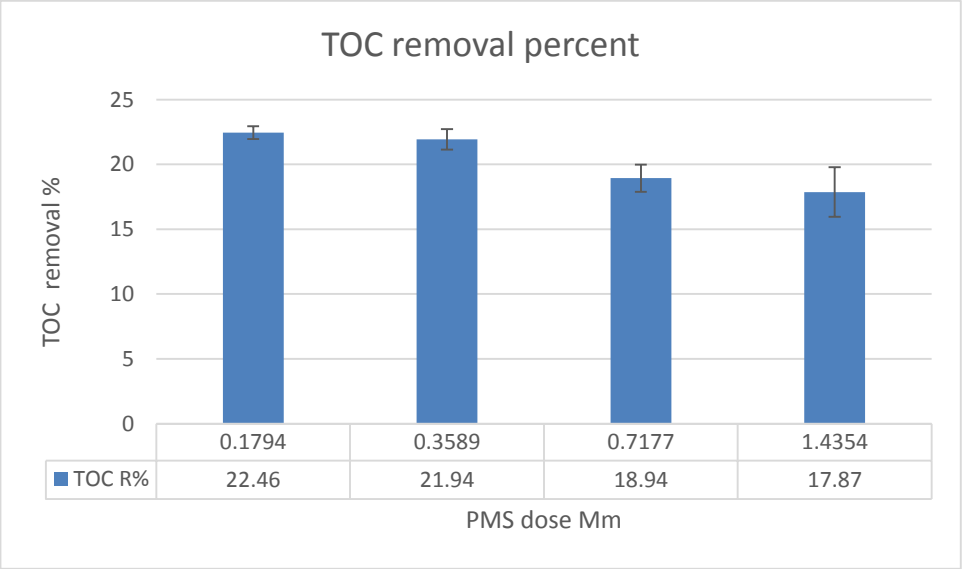


Figure 3.29 Effect of mM PMS doses on TOC removal

3.2.2.2.2 Removal of TOC at different PMS: Fe (II) ratios

To evaluate the effect of Fe (II) concentration on TOC removal, the concentration of PMS were fixed at 0.36 mM. The concentration of Fe (II) was varied from 0.18 to 0.9 mM. The results from these experiments were presented as TOC removal versus oxidant PMS to Fe (II) ratio (Table 3.15).The increasing PMS: Fe (II) ratio show the enhancement of TOC removal efficiency (Figure 3.30). The removal percentage of TOC was 33.3% corresponding to PMS: Fe (II) ratio of 1:2.5. In this case, the efficiency of TOC removal was not influenced by the scavenging effect of SR by Fe (II). As indicated by Eq 3- 5, the rate of reaction between PMS and Fe (II) was about 1500 time higher than the reaction between PS and Fe (II).

Therefore, higher amounts of SR were available in PMS-Fe (II) treatment of vinasse and consequently they increased the efficiency of TOC removal.

$$HSO_5^- + Fe^{2+} \longrightarrow SO_4^{\bullet -} + Fe^{3+} + OH^- \quad k = 3.0\times10^4 M^{-1}s^{-1} \qquad (3\text{- }5)$$

Table 3.15 Removal percent of TOC at different PMS: Fe (II) ratios

PMS: Fe	TOC 1	TOC 2	TOC 3	TOC mean	S.E	Blank	TOC R%
01:0.5	101	98.16	104.9	102.95	1.95	134.5	23.46
1:1	104.2	102.1	_____	103.15	1.05	134.5	23.31
1:2	94.28	94.97	94.23	94.4933	0.23877	134.5	29.74
01:2.5	91.1	90.41	87.63	89.7133	1.06054	134.5	33.3

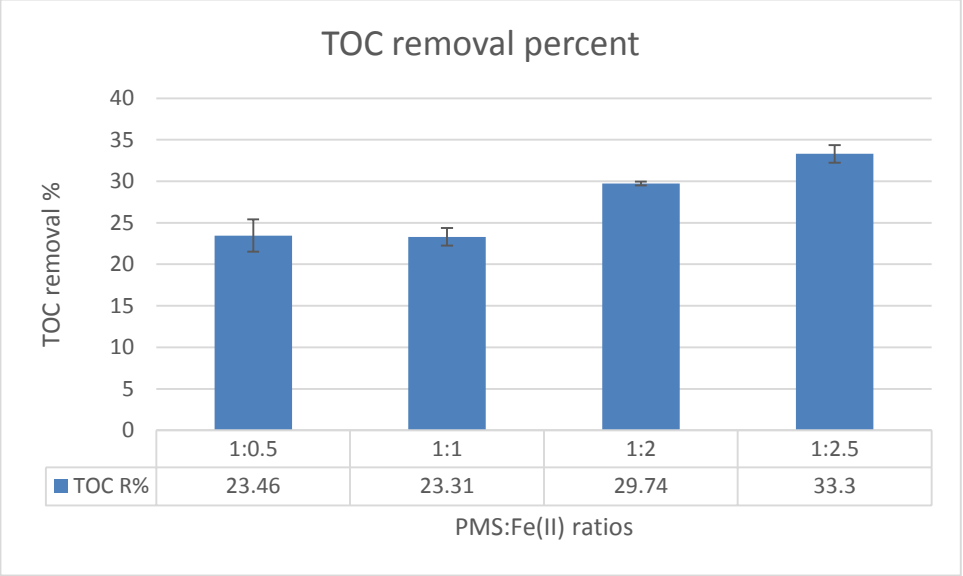


Figure 3.30 Effect of PMS: Fe (II) ratios on TOC removal

3.2.2.2.3 Removal of TOC at different pH values

Values of pH 3, 5, 7, 8 and 11 were measured to assess the effect of pH on TOC removal. Like PS-Fe (II) the PMS-Fe (II) shows significant removal of TOC at pH 7 Table (3.16). As shown in Figure 3.31, TOC removal was 33.3% at pH

7due to the complexation of Fe (II) with the soluble substance at pH 7, the complexation could stabilize the Fe (II) from being oxidized to Fe (III) and consequently enhanced the TOC removal.

Table 3.16 Removal percent of TOC at different pH values

pH	TOC 1	TOC 2	TOC 3	TOC mean	S.E	Blank	TOC R%
pH 3	83	77.5	79.71	80.07	1.59788	88.84	9.87
pH 5	82.14	79.48	81.05	80.89	0.77203	84.92	4.75
pH 7	91.1	90.41	87.63	89.7133	1.06054	134.5	33.3
pH 8	87.99	85.23	82.59	85.27	1.55897	88.79	3.96
pH 11	96.45	89.89	87.86	91.39	2.59211	98.5	7.2

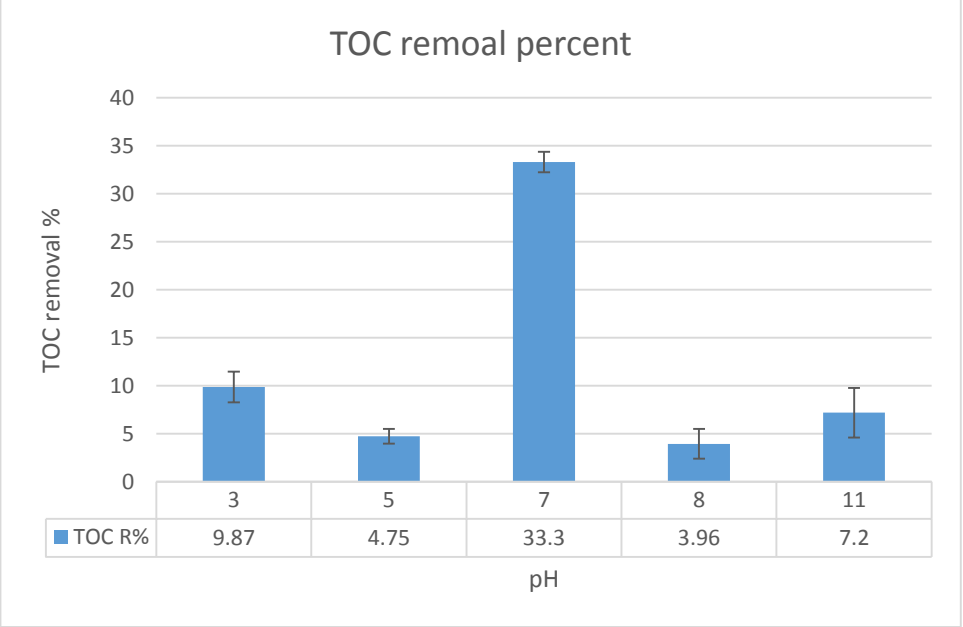


Figure 3.31 Effect of pH values on TOC removal in PMS-Fe (II) system

3.2.2.2.4 Removal of TOC at different reaction times

Times of 0.083, 0.5, 4, 8 and 24 hour were subjected to evaluate the effect of reaction time (Table 3.17). The results indicate that the TOC removal was increased significantly for the first 5 min. When the reaction times increased from 5 min to 24hour, TOC removal increased slowly from 29% to36.4% Figure 3.32.

Table 3.17 Removal percent of TOC at different reaction times

Time (hour)	TOC 1	TOC 2	TOC 3	TOC mean	S.E	Blank	TOC R%
0.0833	93.56	97.3	95.49	95.45	1.07983	134.5	29.03
0.30	97.91	93.64	90.21	93.92	2.2272	134.5	30.17
4	91.1	90.41	87.63	89.7133	1.06054	134.5	33.3
8	87.31	81.99	84.15	84.4833	1.54477	134.5	36,26
24	79.56	90.98	86.04	85.5267	3.30665	134.5	36.41

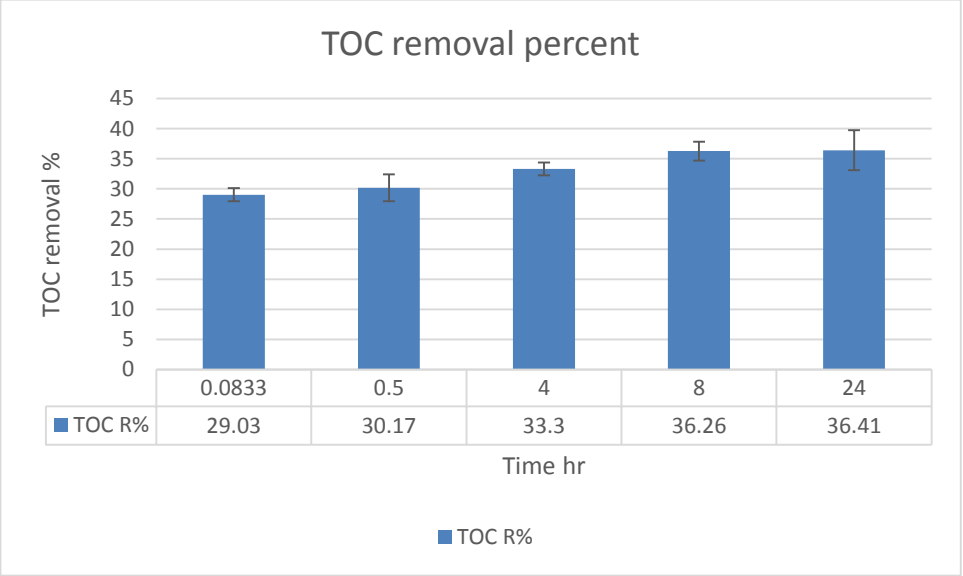


Figure 3.32 Effect of reaction time (hour) on TOC removal in PMS-Fe (II) system

3.2.2.2.5 PMS optimization

The TOC were enhanced by increasing the dose of PMS and Fe (II) under the optimum operating conditions. The ratio of PMS to Fe (II) was kept at 1:2.5, the pH was adjusted to 7 and the TOC for the treated vinasse was measured at reaction time of 4 hour. As can be seen in Figure 3.33 TOC removal could be enhanced by

increasing the dosage of oxidant. Using PMS-Fe (II) system, TOC removal increased from 33.3 to 70 % when PMS concentration increased by 4 times

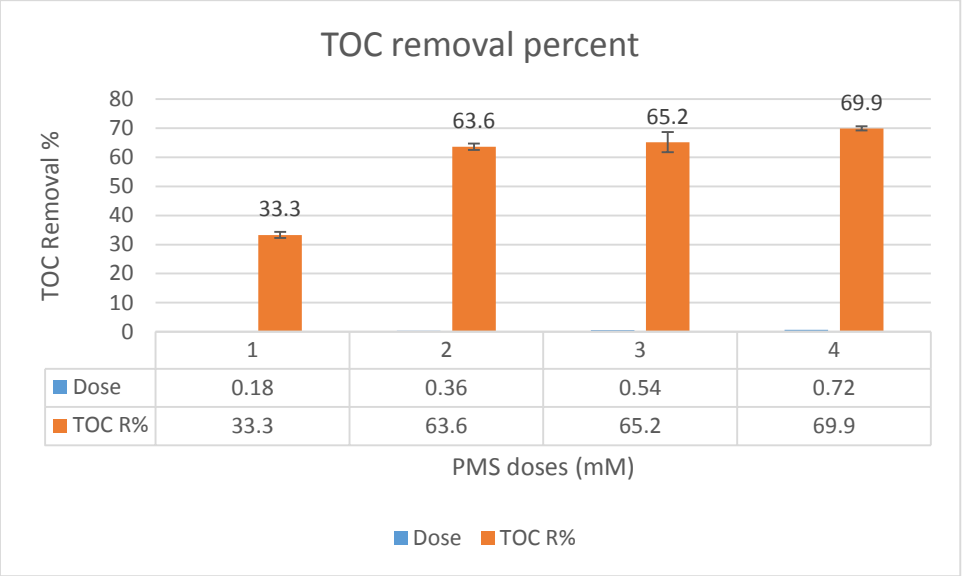


Figure 3.33 Maximum TOC removal by PMS-Fe (II) system under optimum conditions

3.2.3 Enhancement of TOC removal by combined sulfate and hydroxide radicals

3.2.3.1 Enhancement of TOC removal in PS: Fe (II) system by using different doses of H_2O_2

As shown in Table 3.18, TOC removal was increased slightly when H_2O_2 increased from 0.2 to 0.6 ml. Addition of 0.2 ml of H_2O_2 did not show any significant enhancement in TOC removal. Further addition lead to a slight increase of TOC removal (Figure 3.34). This might be due to generation of a lower quantity of OH· Radicals; however, the generation of ·OH might be limited by the available Fe (II).

Table 3.18 Removal percent of TOC at different doses of H_20_2 in PS-Fe (II)

H_2O_2 dose ml	TOC 1	TOC 2	TOC 3	TOC mean	S.E	Blank	TOC R%
0.2	69.66	70.6	64.51	68.26	1.89288	134.5	49.25
0.4	61.48	55.97	55.41	57.62	1.93676	134.5	57.16
0.6	55.23	52.34	52.74	53.44	0.90407	134.5	60.27
0.8	57.78	54.14	54.13	55.35	1.215	134.5	58.85

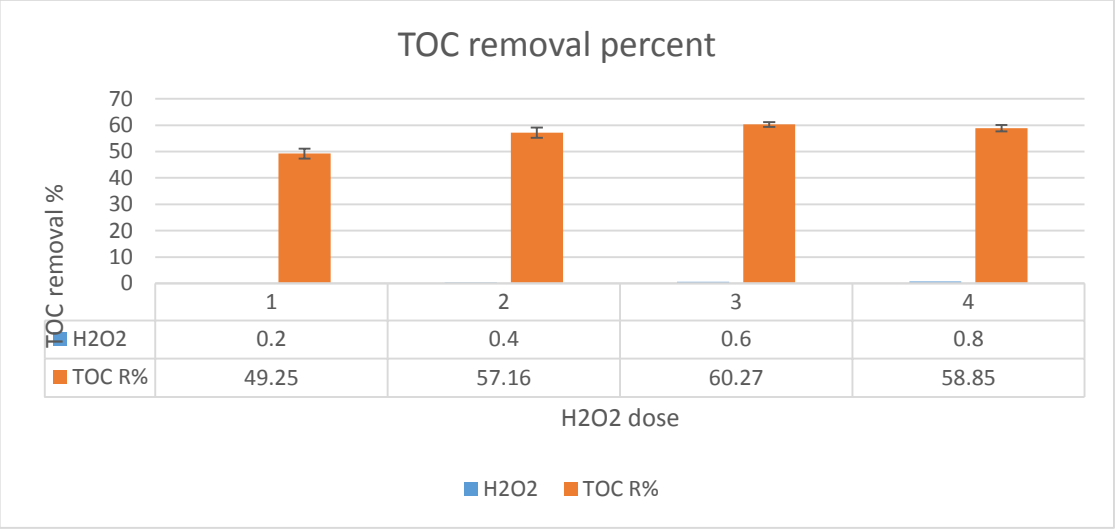


Figure 3.34 Effect of H_2O_2 doses on TOC removal in PS-Fe (II) system

3.2.3.2 Enhancement of TOC removal in PMS: Fe (II) system by using different doses of H_2O_2

As shown in Figure 3.35 and Table 3.19, TOC removal was increased when H_2O_2 increased from 0.081 to 0.324 ml. This might be due to generation of a higher quantity of $OH^{\bullet}$, which is known as Fenton reaction

$$H_2O_2+Fe^{2+} \rightarrow Fe^{3} + OH^{-} +{\cdot}OH \quad (3\text{-}6)$$

Fenton reaction is a mixture of hydrogen peroxide and iron salts (Fe^{2+} or Fe^{3+}) which produces hydroxyl radicals. The Fenton oxidation process has been

employed successfully to treat different industrial wastewaters (De Heredia, *et al*. 2005).

Table 3.19 Removal percent of TOC at different doses of H_2O_2 in PMS-Fe (II)

H_2O_2 dose ml	TOC 1	TOC 2	TOC 3	TOC mean	S.E	Blank	TOC R%
0.081	29.69	25.37	29.67	29.68	1.43668	134.5	77.93
0.162	25.41	25.7	29.9	25.56	0.145	134.5	81
0.243	24.74	23.56		24.15	0.59	134.5	82.04
0.324	22.42	22.32	22.44	22.39	0.3712	134.5	83.35

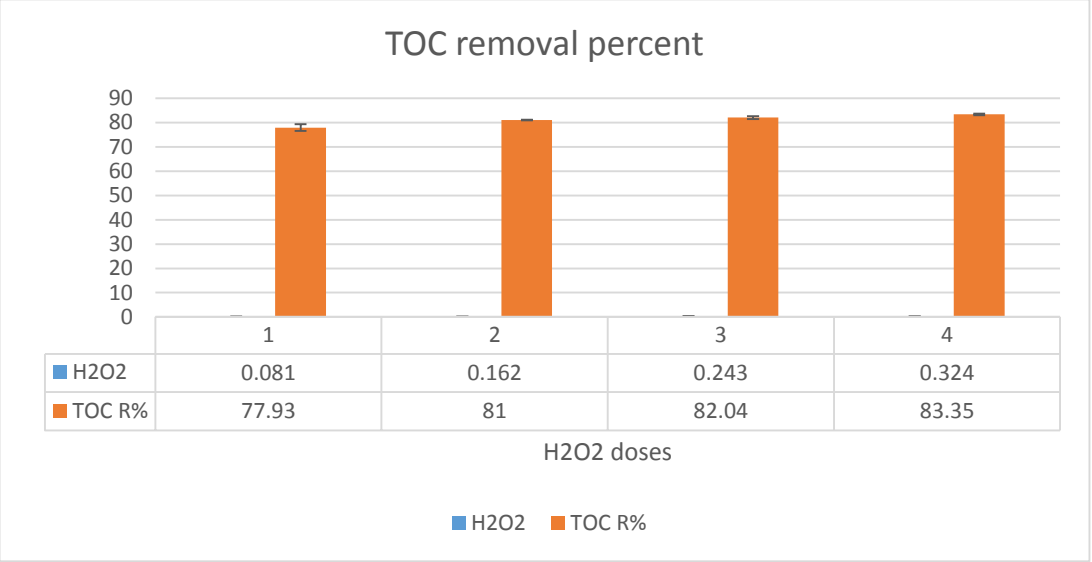


Figure 3.35 Effect of H_2O_2 doses on TOC removal in PMS-Fe (II) system

Conclusion and Recommendations

Vinasse characterization was examined in this study. The results show that the vinasse have high BOD, COD and TOC which is considered as hazardous to the environment. The acidic nature and color of vinasse also causes environmental problems. Most of organic compounds detected by GC.MS classified as phenolic compounds and carboxylic acids. Treatment methods were monitored by TOC rather than COD.

The application of coagulation-flocculation followed by SR-AOP for the treatment of sugarcane vinasse was examined in this study. The result presented in this study demonstrated that the coagulation-flocculation pretreatment followed by SR-AOP is effective method to remove TOC from vinasse. For coagulation-flocculation, 69.7% of TOC was removed from vinasse by using 15 g/dm^3 of coagulant ($FeCl_3$), whereas, nearly 100% of color and AOC removal was achieved by using lower dose of coagulant (5 g/dm^3). In general, the result indicated that the TOC removal was more favorable when the coagulation-flocculation was performed on acidic vinasse. On the other hand, the TOC removal can be enhanced by increasing the amount of $FeCl_3$.

After coagulation-flocculation pretreatment, vinasse was subjected for SR-AOP. This work reports the first study in the application of SR-AOP for vinasse treatment. The results indicate that SR-AOP is an effective oxidation process in the reduction of TOC from pretreated vinasse. Type of oxidant (PMS and PS), initial pH, Fe (II) and oxidant dose all influence the TOC removal efficiency. Based on the results, PMS-Fe (II) was found to be more effective than PS-Fe (II). Nearly 70% and 49 % of TOC removal was achieved when the reaction was carried out at pH 7 by using PMS-Fe (II) system and PS-Fe (II) respectively. Coupling SR-AOP and $HO^{\cdot}$-AOP enhanced the TOC removal. 83.35% and 60.27 % achieved of TOC removal by enhanced PMS-Fe (II)

system and PS-Fe (II) system with H_2O_2.Overall TOC removal of raw vinasse was 91.6% and 85.6% by using coagulation-flocculation flowed by PMS-Fe (II) system and PS-Fe (II) system. This results indicate that the coagulation-flocculation flowed by SR-AOP is conceder to be a good alternative method for vinasse treatment.

Recommendations for future research

- SR-AOPs can be applied after biological treatment to degrade the recalcitrant organic compounds that declined to be degraded by biological treatments.
- Hence SR-AOPs economically feasible, further studies may be carried out on the use of SR-AOPs for industrial wastewater treatment.
- More investigations can conducted on AOPs for vinasse treatment and industrial wastewater in general, by using other AOPs techniques.
- More investigation can be conducted on vinasse utilizing such as yeast production, Energy production, polyhydroxybutyrate production and Fertirrigation.
- Further investigation can be conducted to characterize and utilize the sludge that produced after SR-AOP treatment.
- Further investigation can be conduct to characterize and utilize the sludge that produced after coagulation- flocculation treatment.

CHAPTER FOUR

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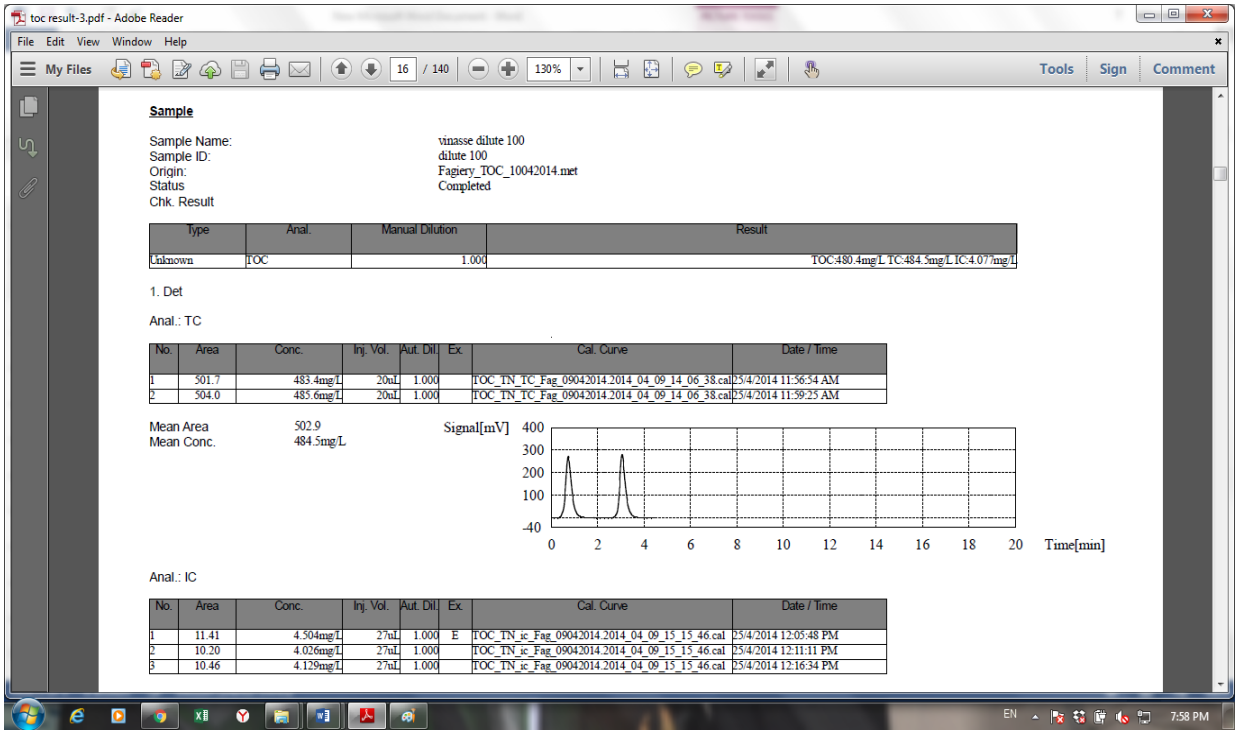
Appendix A.



toc result-3.pdf - Adobe Reader

File Edit View Window Help

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Sample

Sample Name: vinasse dilute 100
Sample ID: dilute 100
Origin: Fagiery_TOC_10042014.met
Status Completed
Chk. Result

Type	Anal.	Manual Dilution	Result
Unknown	TOC	1.000	TOC:480.4mg/L TC:484.5mg/L IC:4.077mg/L

1. Det

Anal.: TC

No.	Area	Conc.	Inj. Vol.	Aut. Dil.	Ex.	Cal. Curve	Date / Time
1	501.7	483.4mg/L	20uL	1.000		TOC_TN_TC_Fag_09042014.2014_04_09_14_06_38.cal	25/4/2014 11:56:54 AM
2	504.0	485.6mg/L	20uL	1.000		TOC_TN_TC_Fag_09042014.2014_04_09_14_06_38.cal	25/4/2014 11:59:25 AM

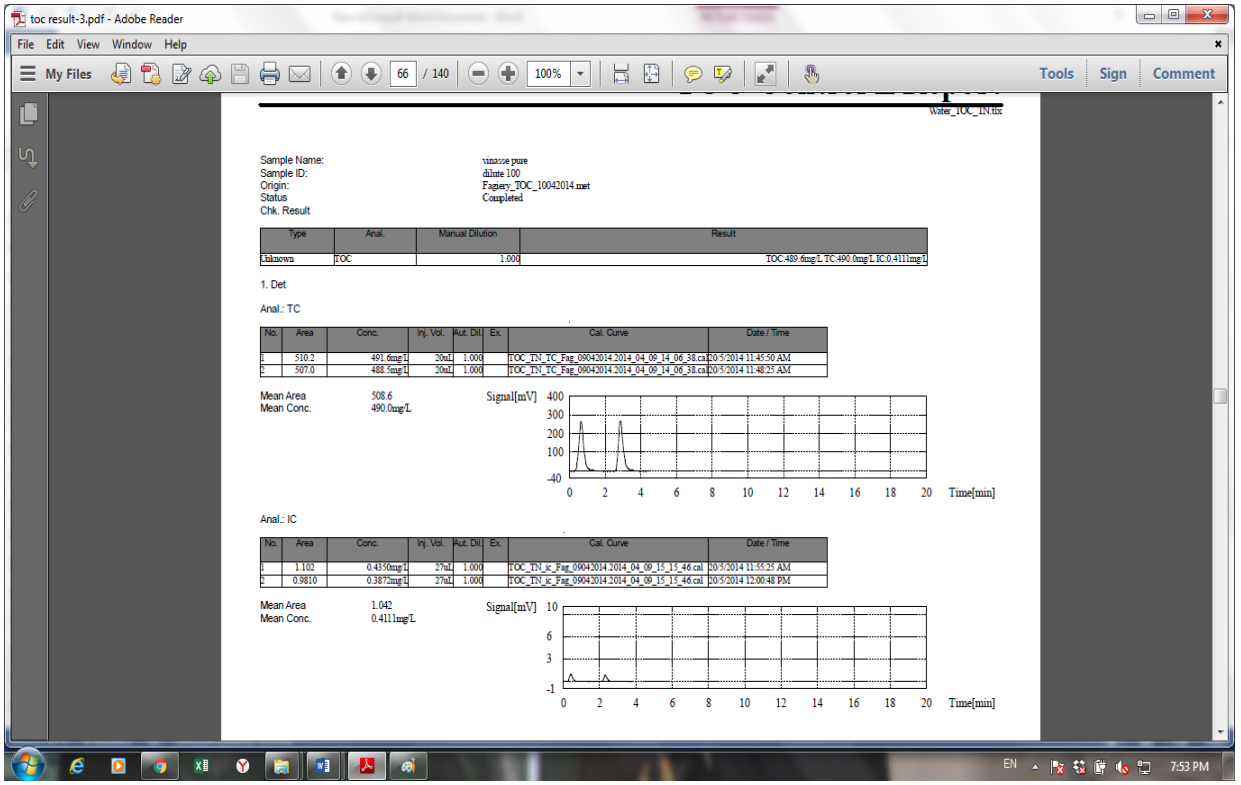
Mean Area 502.9
Mean Conc. 484.5mg/L

Anal.: IC

No.	Area	Conc.	Inj. Vol.	Aut. Dil.	Ex.	Cal. Curve	Date / Time
1	11.41	4.504mg/L	27uL	1.000	E	TOC_TN_ic_Fag_09042014.2014_04_09_15_15_46.cal	25/4/2014 12:05:48 PM
2	10.20	4.026mg/L	27uL	1.000		TOC_TN_ic_Fag_09042014.2014_04_09_15_15_46.cal	25/4/2014 12:11:11 PM
3	10.46	4.129mg/L	27uL	1.000		TOC_TN_ic_Fag_09042014.2014_04_09_15_15_46.cal	25/4/2014 12:16:34 PM

7:58 PM

TOC measurement of vinasse (1)



toc result-3.pdf - Adobe Reader

File Edit View Window Help

My Files 66 / 140 100% Tools Sign Comment

Water_TOC_IN.tix

Sample Name: vinasse pure
Sample ID: dilute 100
Origin: Fagiery_TOC_10042014.met
Status Completed
Chk. Result

Type	Anal.	Manual Dilution	Result
Unknown	TOC	1.000	TOC:489.6mg/L TC:490.0mg/L IC:0.4111mg/L

1. Det

Anal.: TC

No.	Area	Conc.	Inj. Vol.	Aut. Dil.	Ex.	Cal. Curve	Date / Time
1	510.2	491.6mg/L	20uL	1.000		TOC_TN_TC_Fag_09042014.2014_04_09_14_06_38.cal	20/5/2014 11:45:50 AM
2	507.0	488.5mg/L	20uL	1.000		TOC_TN_TC_Fag_09042014.2014_04_09_14_06_38.cal	20/5/2014 11:48:25 AM

Mean Area 508.6
Mean Conc. 490.0mg/L

Anal.: IC

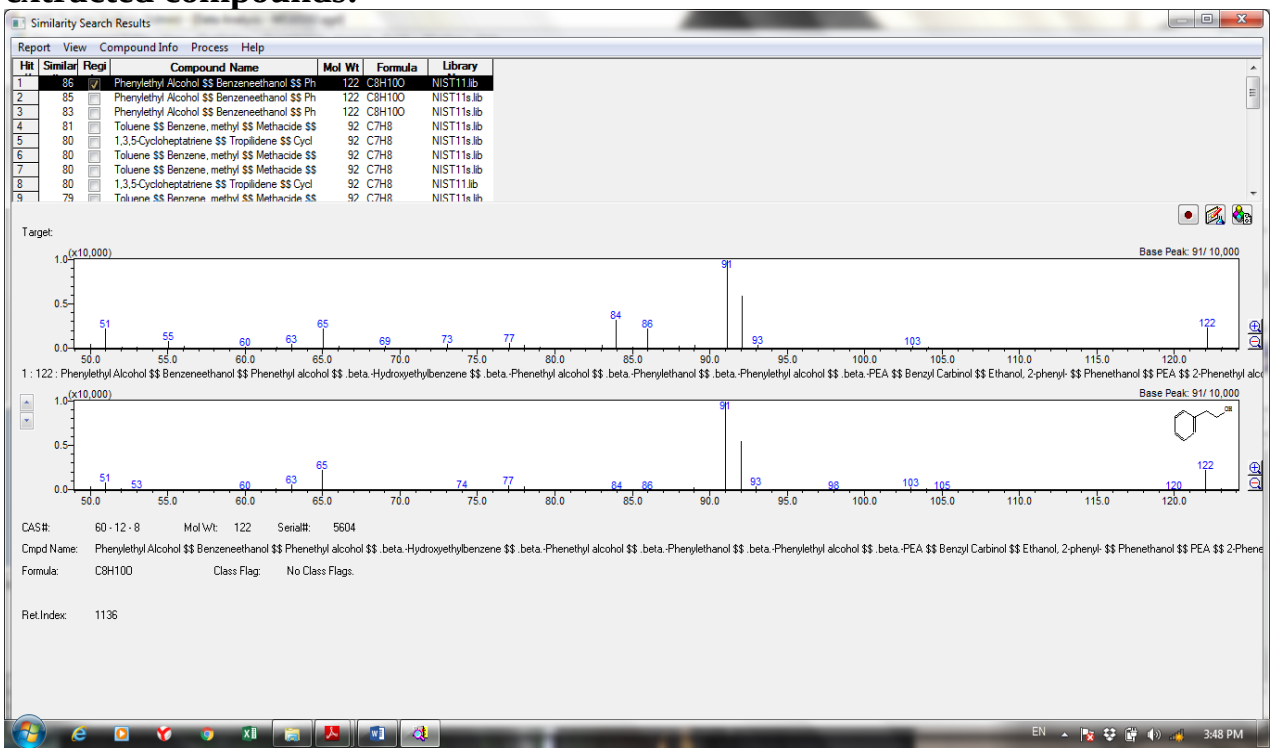
No.	Area	Conc.	Inj. Vol.	Aut. Dil.	Ex.	Cal. Curve	Date / Time
1	1.102	0.4350mg/L	27uL	1.000		TOC_TN_ic_Fag_09042014.2014_04_09_15_15_46.cal	20/5/2014 11:55:25 AM
2	0.9810	0.3872mg/L	27uL	1.000		TOC_TN_ic_Fag_09042014.2014_04_09_15_15_46.cal	20/5/2014 12:00:48 PM

Mean Area 1.042
Mean Conc. 0.4111mg/L

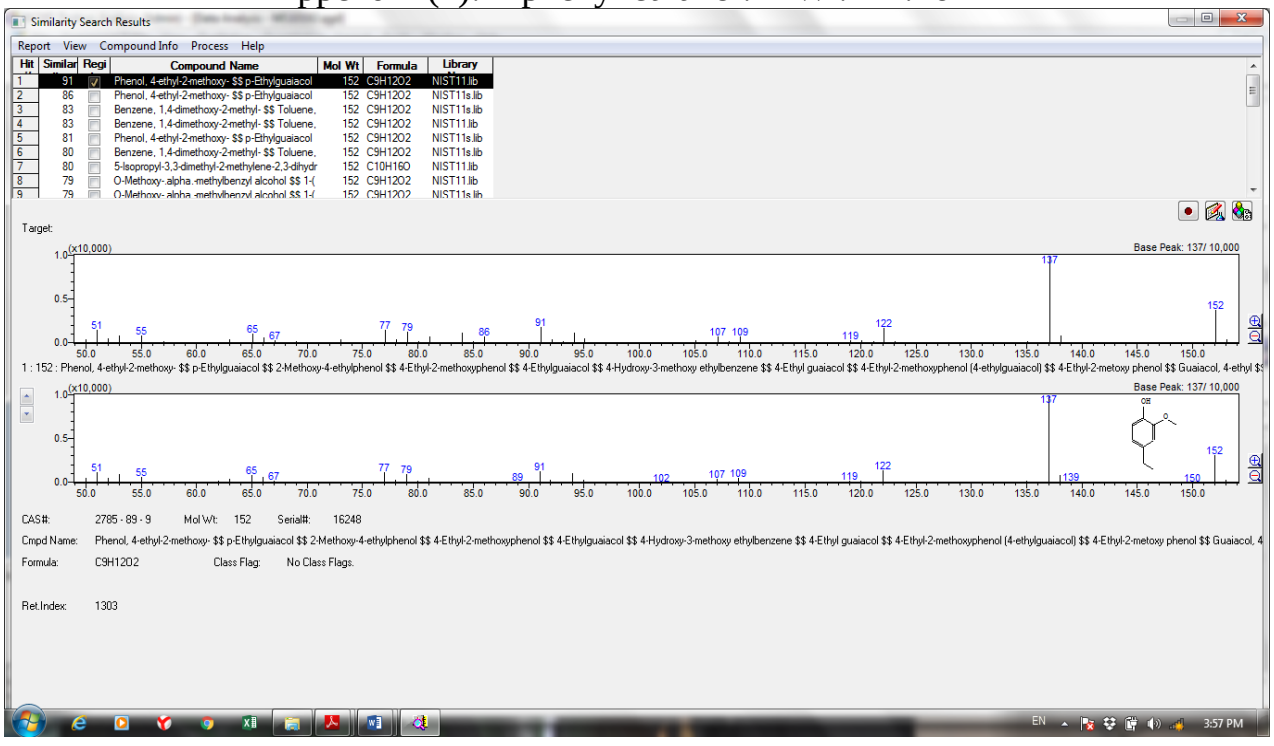
7:53 PM

TOC measurement of vinasse (2)

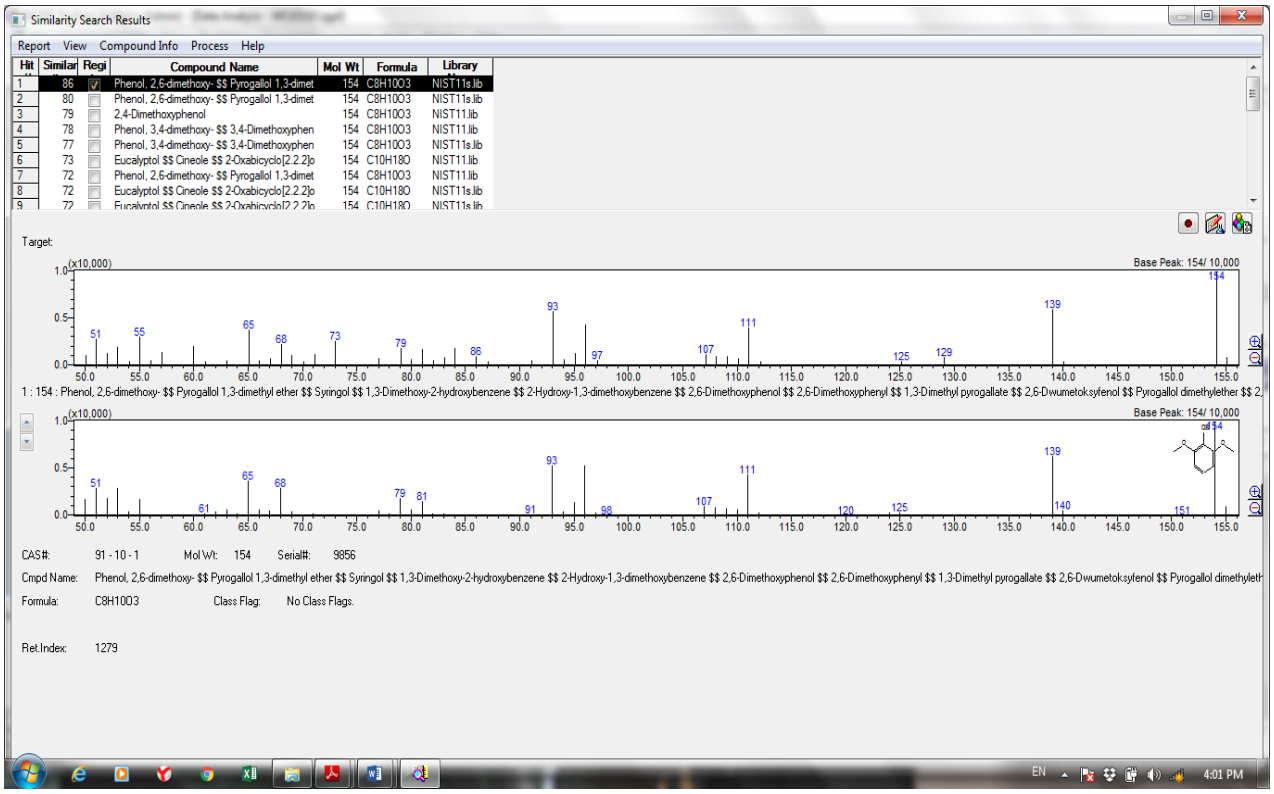
Appendix (B) Similarity search results and mass spectrum of hexane extracted compounds:



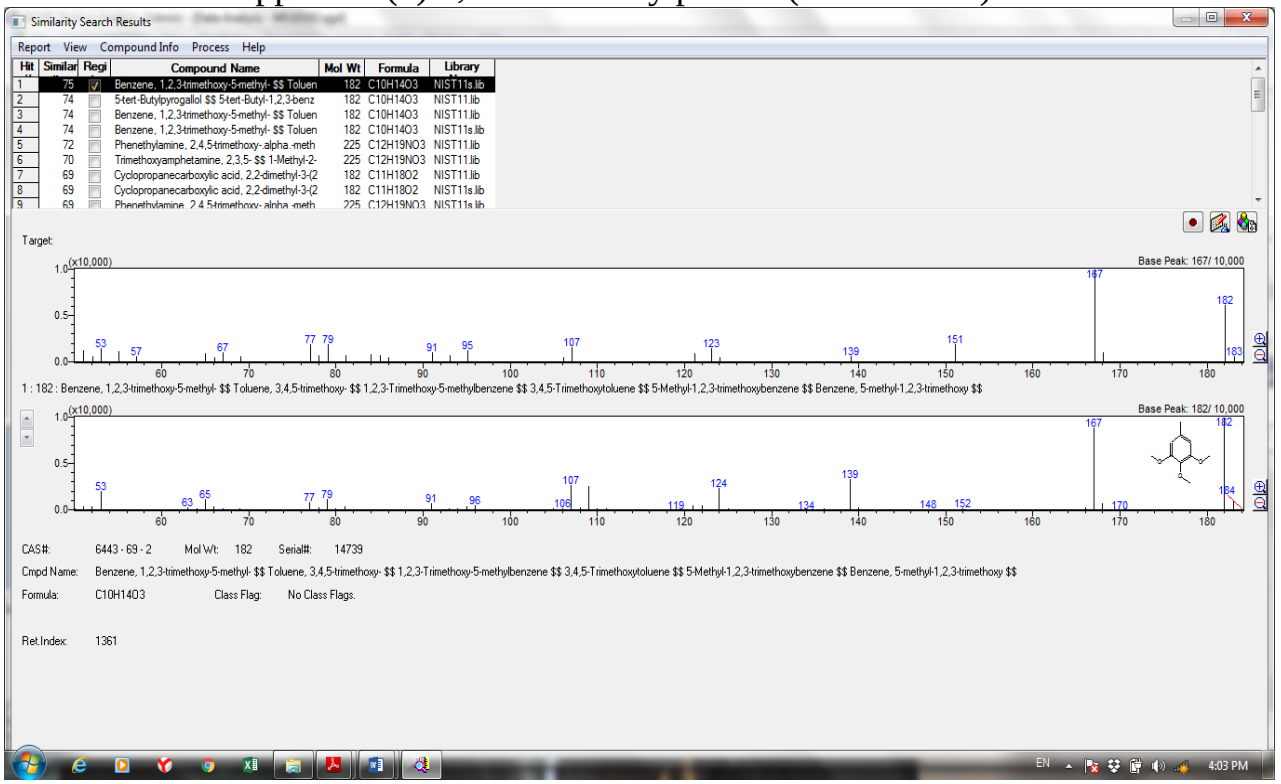
Appendix (1): 2-phenyl ethanol. MW .122.16



Appendix (2) 4-ethyl-2-methoxy phenol. (MW 152.19)

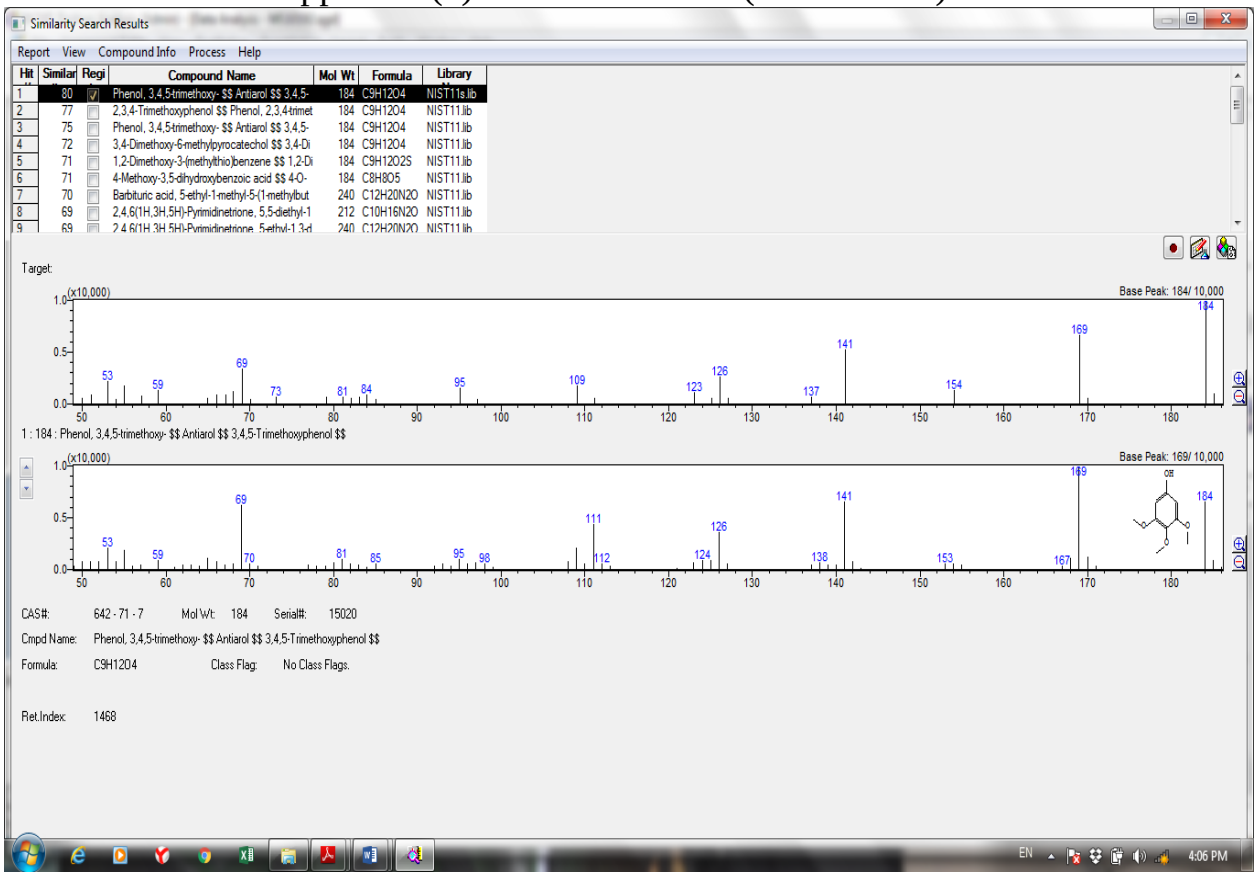


Appendix (3) 2,6- dimethoxy phenol.(MW 154.16)

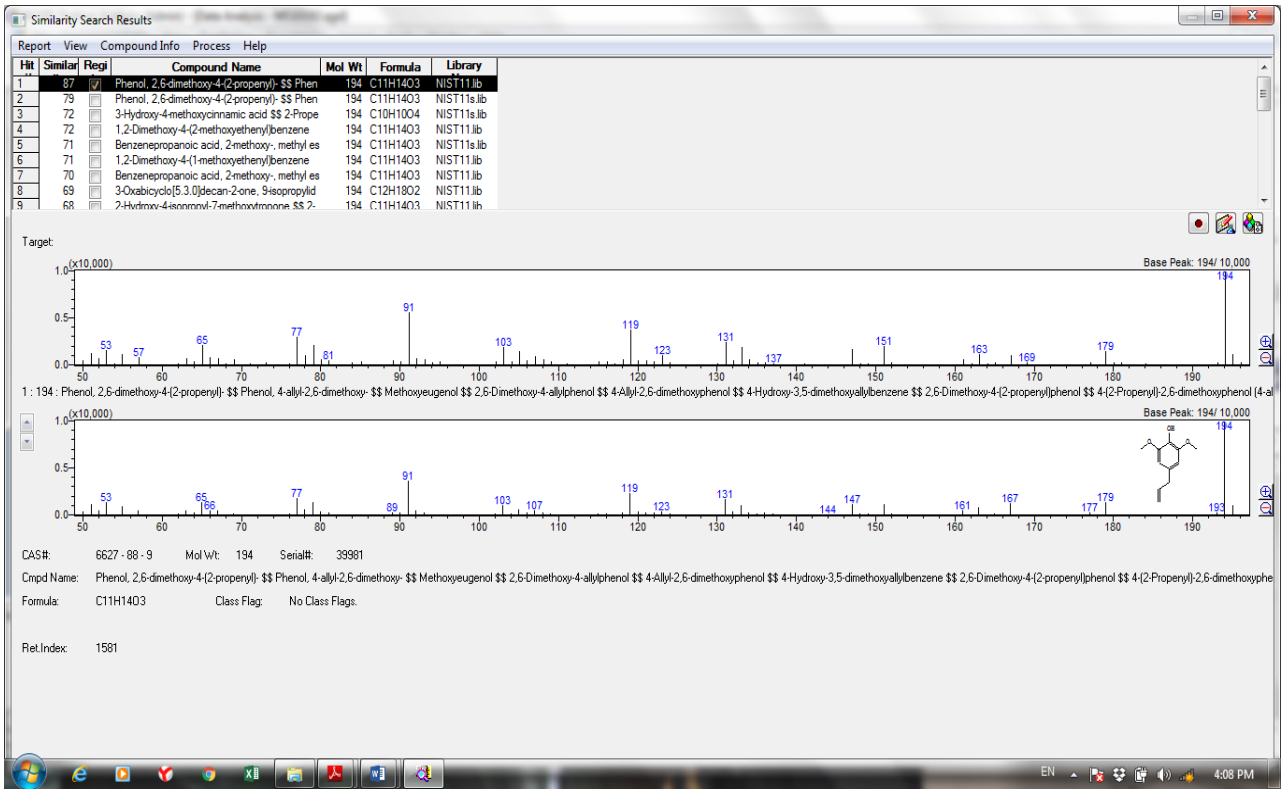


Appendix (4) 1,2,3- triethoxy-5-methyl benzene.(MW 182.22)

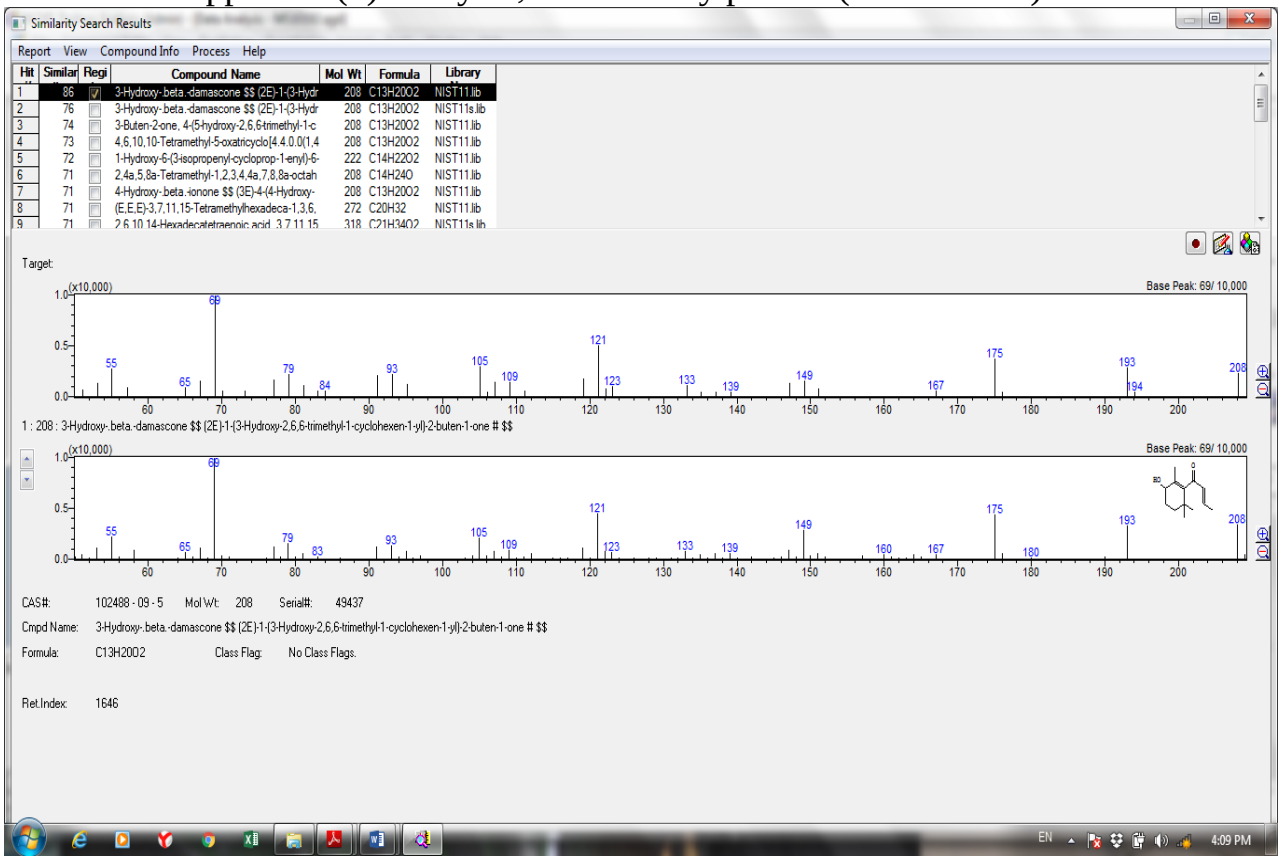
Appendix (5) Dodecanoic acid (MW 200.32)



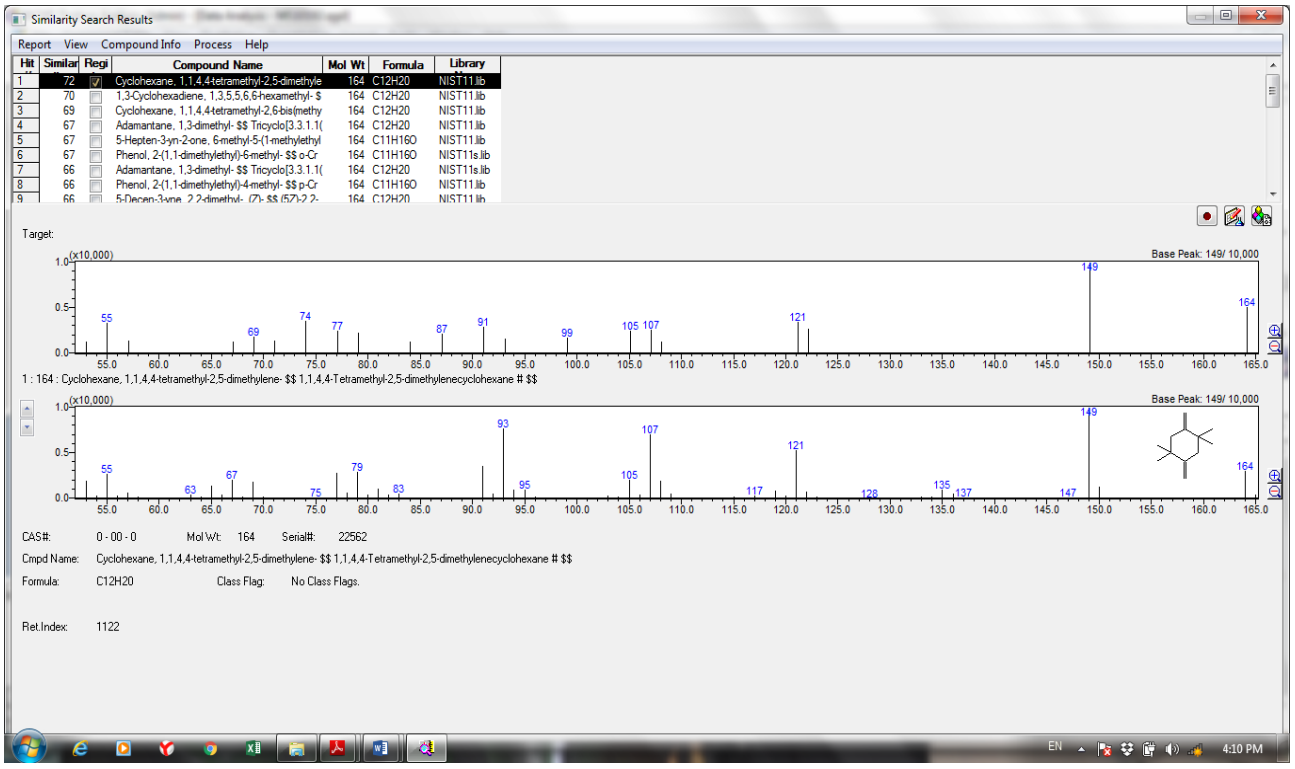
Appendix (6) 3,4,5- trimethoxy phenol. (MW 184.19)



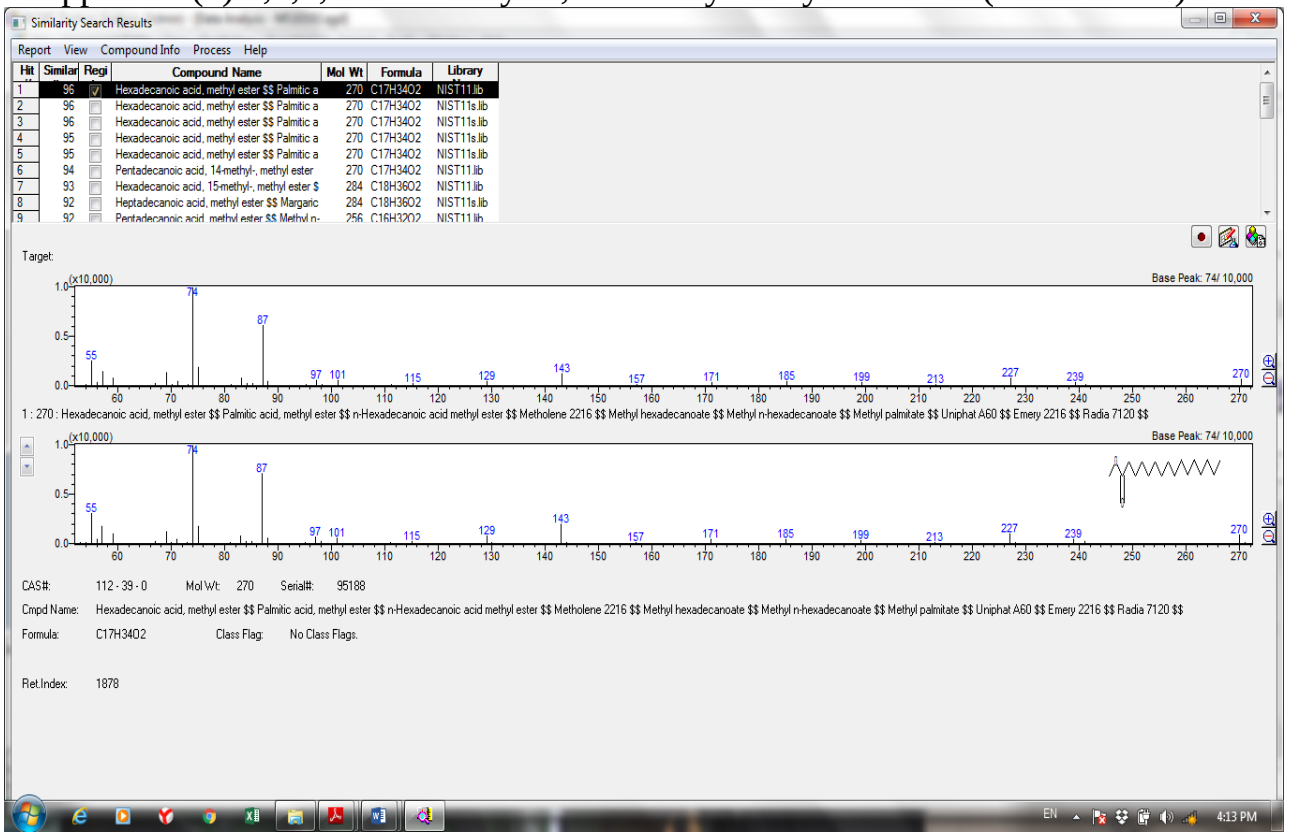
Appendix (7) 4-allyl-2,6-dimethoxy phenol.(MW 194.23)



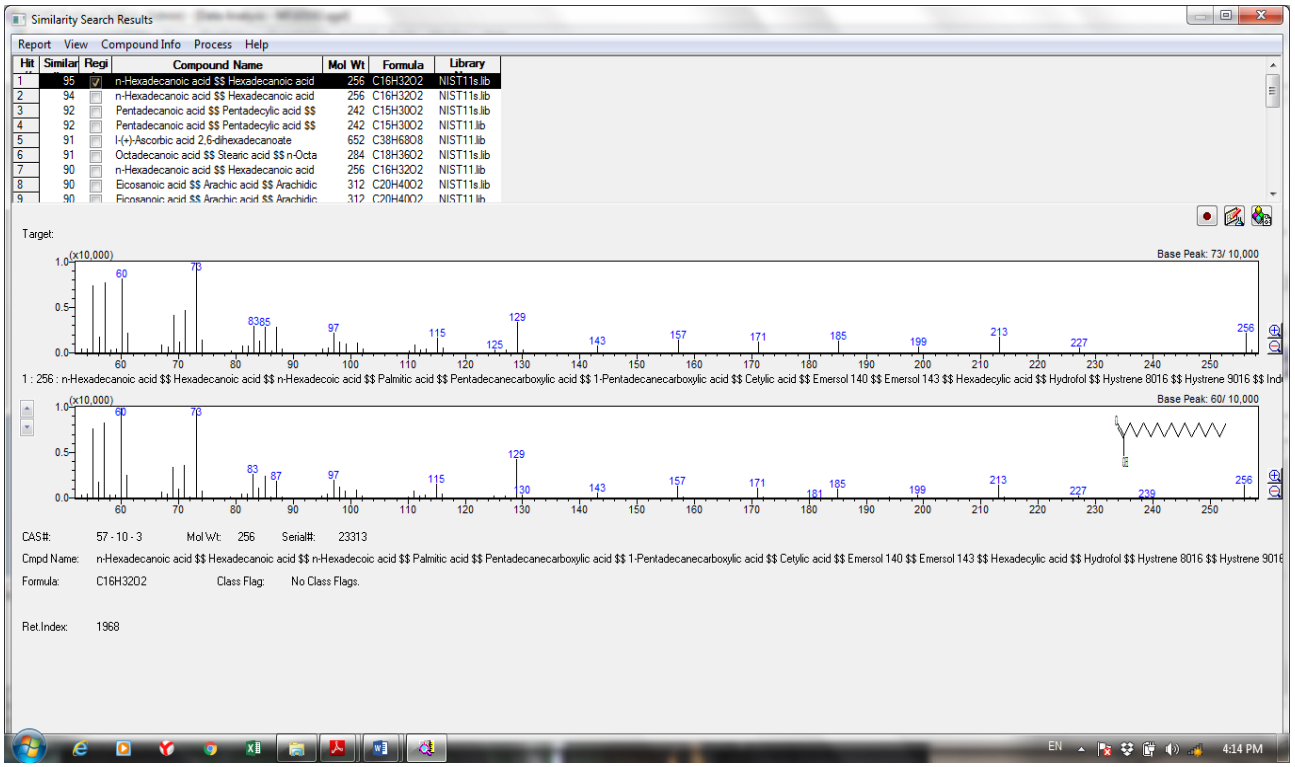
Appendix (8) (E) -1-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-one. (208.30)



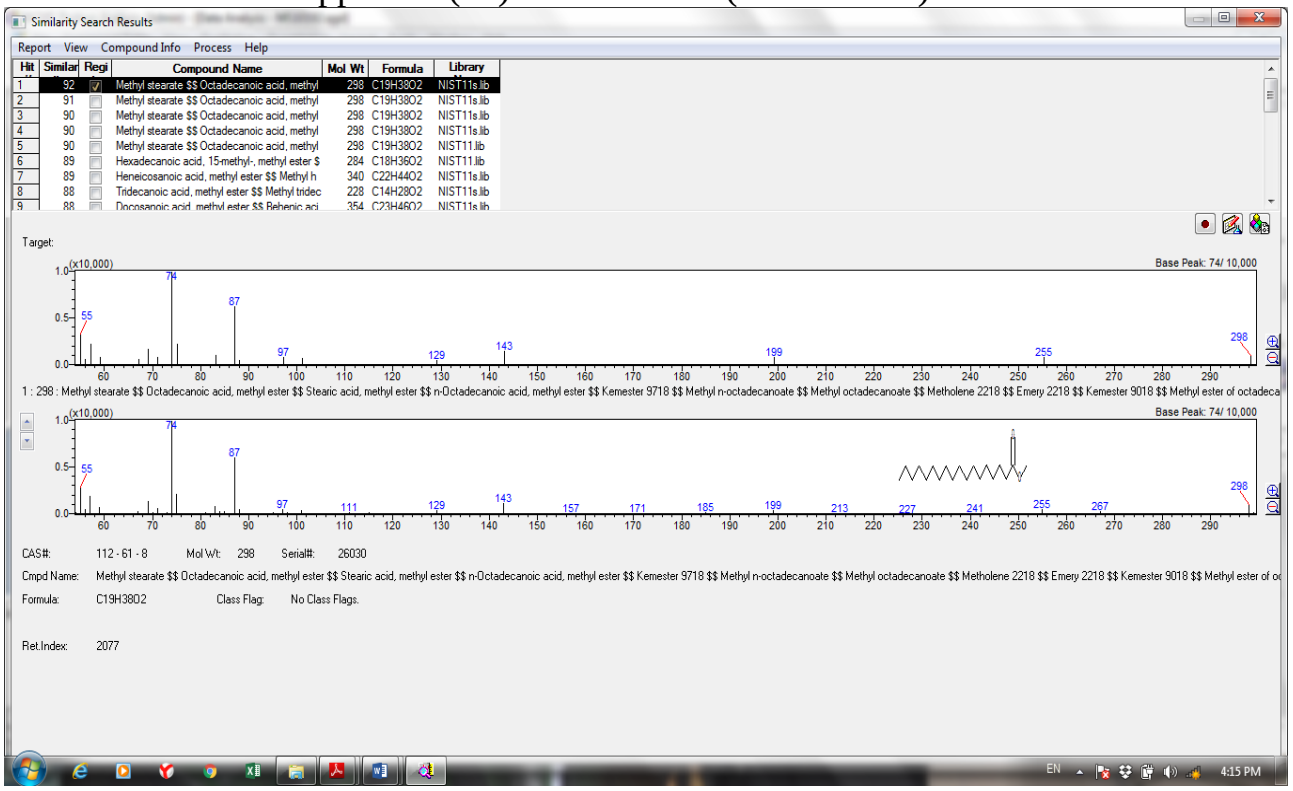
Appendix (9) 1,1,4,4-tetramethyl-2,5-dimethylenecyclohexane.(MW 164.29)



Appendix (10) Methyl palmitate.(MW 270.45)

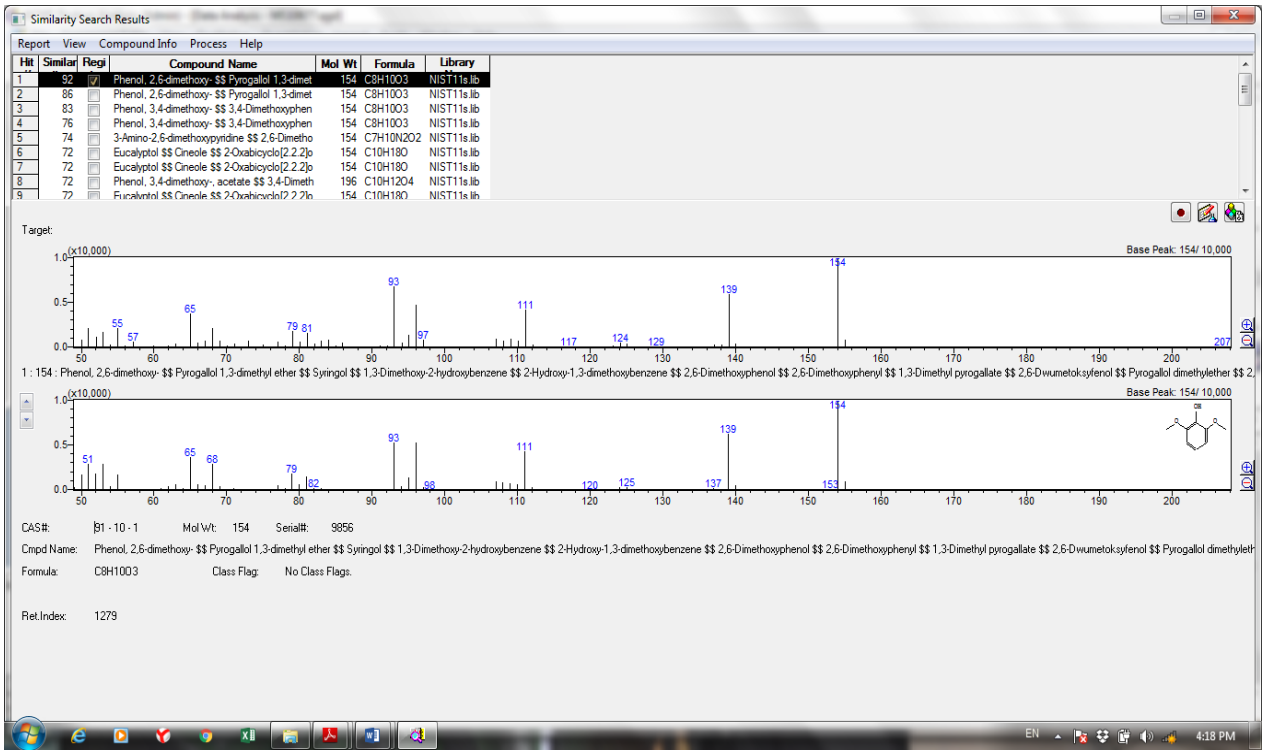


Appendix (11) Palmitic acid.(MW 256.42)

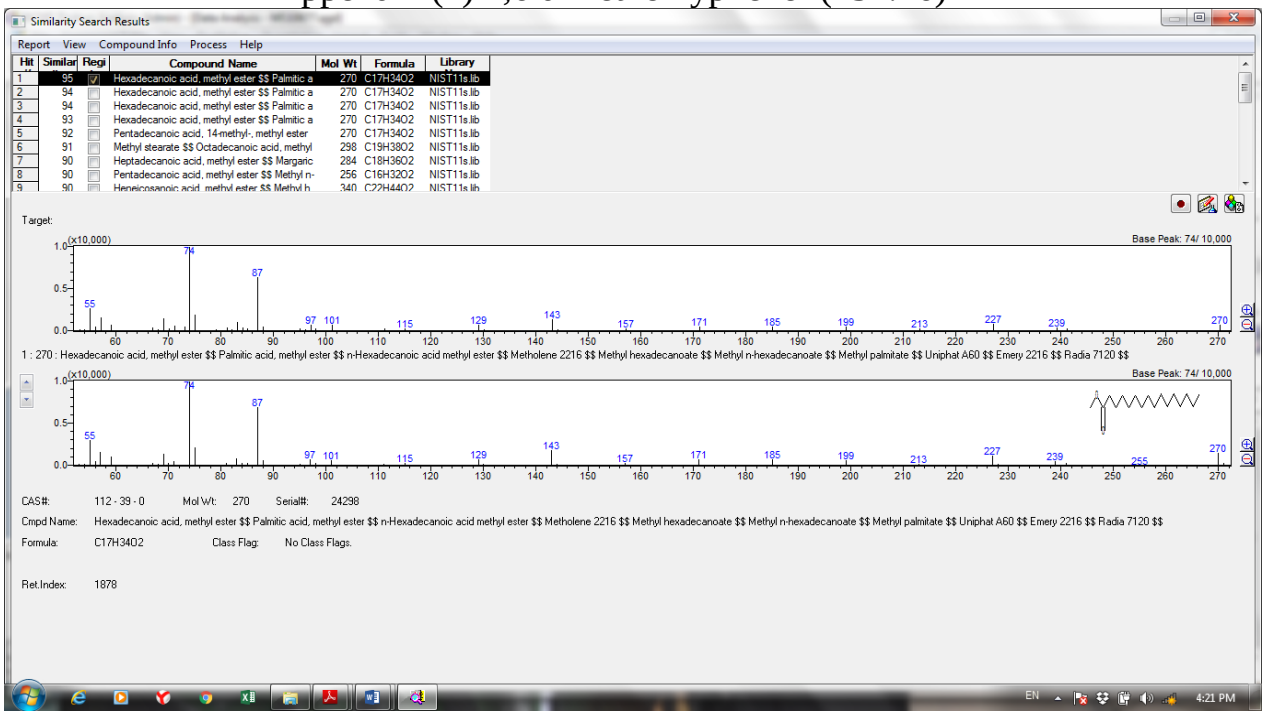


Appendix (12) methyl stearate MW (298.5)

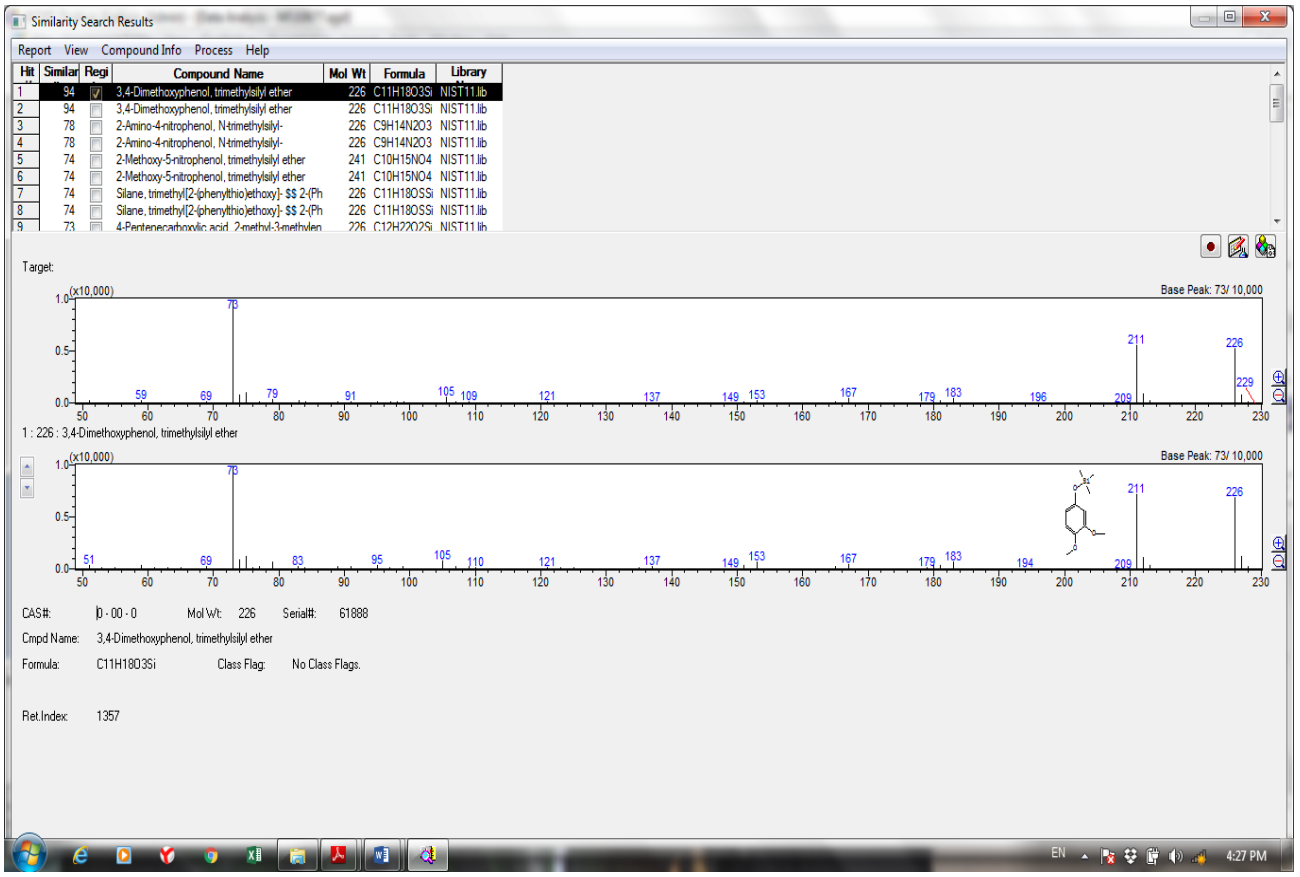
Appendix (C): Similarity search results and mass spectrum of DCM extracted compounds:



Appendix (1) 2,6 dimethox yphenol (154.16)



Appendix (2) Methyl palmitate (270.45)



Appendix (3) 3,4- dimethox y phenol MW (226.34)