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Investigation of potentials Water hyacinth (*Ecchornia crassipes*) grown at Lake Koka and Lake Abaya for bioethanol production

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Abstract

Scarcity, environmental pollution and increasing prices of fossil fuels are issues that forced human to search for alternative energy sources such as fuels from biomass (i.e., biofuels). Bioethanol is one of the biofuels that can be produced from lignocellulosic biomass. Water hyacinth (Ecchornia crassipes) is a lignocellulosic biomass that contains high cellulose and hemicellulose with low lignin that made it good candidate biomass for bioethanol production. However, the lignocellulosic composition of water hyacinth depends on the nutritional conditions habitats where the plant grows. This study was conducted to investigate water hyacinth grown in Lake Koka and Abaya, Ethiopia, for bioethanol production. The biomass collected from these sites (Lakes) were subjected to analysis of their mineral (N, K and P) compositions of water samples and lignocellulosic (cellulose, Hemicellulose and Lignin) compositions of Water hyacinth. The results revealed that the N, P and K levels to be 5.62, 0.81 and 0.64 mg/L, respectively, for Water sample from Lake Koka whereas the corresponding values were 3.68, 0.42 and 0.42 mg/L for water samples from Lake Abaya. The cellulose, Hemicellulose and Lignin values were 30.49, 41.30 and 4.51% for water hyacinth from Lake Koka whereas composition of for the biomass from Lake Abaya values were 25.02%, 39.93% and 8.42%, respectively, for cellulose, Hemicellulose and Lignin. The values for biomass from Lake Koka were slightly higher than that of biomass from Lake Abaya. The differences were attributed to differences in mineral composition of water samples of the lakes. The optimum condition for hydrolysis of lignocellulosic for bioethanol production from water hyacinth collected from Lake Koka were 1% sulphuric acid and 60 minutes whereas for water hyacinth from Lake Abaya the corresponding conditions were 1.5% sulphuric acid and 90 minutes. The ethanol yields were 37.22 and 31.22% for water hyacinth from Lake Koka and Lake Abaya, respectively. The bioethanol product was confirmed by boiling point and FTIR spectroscopic data. From this study it was concluded that higher ethanol product from biomass collected from Lake Koka could be attributed to higher cellulose and hemicellulose compositions and lower lignin as compared to the biomass collected from Lake Abaya. The biomass from Lake Koka is preferred for ethanol production not only because of higher ethanol yield but also it requires less concentrated acid (1% H_2SO_4*) and shorter hydrolysis time (60 minutes). Thus, water hyacinth should be given a due attention as candidate biomass for bioethanol production as it is non-edible lignocellulosic biomass. However, continues efforts are recommended to develop optimum conditions that are cost effective for production of bioethanol from water hyacinth.*

Keywords: Bioethanol, Chemical composition, Lake Abaya, Lake Koka, Total reducing sugar, Water hyacinth.

Introduction

Fossil fuels have been used as energy sources for centuries[1]. The energy demand in different sectors is increasing from time to time. Reports showed that the demand increase by 1.2% per annum from 13.7 billion tonnes oil in 2014 and projected to be to 18.9 tonnes in 2040[2]. This made it difficult to satisfy energy demand of different sectors as the world faced scarcity (due to depletion of existing fuel reserves) and increasing price or price fluctuation. Moreover, environmental pollution, political and military influences by exporting countries on importing countries are also problems associated with utilization of fossil fuels[3,4]. Therefore, human being has been forced to look for alternative and renewable energy sources that are renewable (inexhaustible). These renewable energy sources are environmentally friendly as they cause less pollution to environment[5-8]. Some of the alternative energy sources are biomass energy, wind energy, solar energy, hydropower, fuel cells, nuclear energy and geothermal energy[9-11].

Among these alternatives, biofuel energy sources are given attention as they are abundant, renewable, and relatively clean energy resources that can be used for the generation of in different forms of energy[12]. Thus, there is a pressing need to adapt to the use of biofuel as a substitute for fossil fuels to help address energy cost, energy renewability, energy security, energy diversification, and reducing greenhouse gas emissions associated with utilization of fossil fuels[1]. Bioethanol is one of the biofuels that is considered as a potential liquid fuel. It is biodegradable, causes less environmental pollution and is also a far less toxic and cleaner than fossil-based fuels[13,14]. The global ethanol market is increasing for the reasons mentioned above. For instance, the global market has been reported to increase from US$ 33.703 billion (in 2020) and expected to increase to US$44.261 billion by 2027. The rate is estimated to be 3.97%[15].

The production of bioethanol depends on several renewable biomasses that can be divided into three main categories namely sugar-based materials, starch-based materials, and lignocellulose-based materials[16,17]. Sugar-based raw materials are biomasses that contain high levels of glucose such as sugarcane, sugar beet, sweet sorghum, and various fruits[17]. The other potential bioethanol raw materials are starch-containing crops such as cereal grains, potato, sweet potato, and cassava[18]. However, sugar-containing crops and starch-containing crops are all in human food chain. Therefore, except for some processing residues, they are generally too expensive to be used for fuel ethanol production[19]. Lignocellulose-based feed stocks such as leaves, stems, and stalks from sources like corn cobs, corn stover, sugarcane bagasse, dedicated energy crops such as perennial grasses including switch grass and different aquatic plants are sources of lignocellulose-based feed stocks[16,20,21].

Bioethanol production in Ethiopia is linked mainly with sugar factories[22]. The aims are import substitution of petroleum-based products, enhance agricultural development and agro-processing, job creation, and export earnings. However, only a small fraction (5% and 10% ethanol blend) of the potentials have been utilized[23-25].

As mentioned above, it is a well-known fact that the biomasses for bioethanol production are plant-based that include sugar-based materials, starch-based materials, and lignocellulose-based materials[16]. It is also recommended that the plant-based biomass should be non-edible biomass such as weeds, crop plant residues in order to avoid food competition[26]. The weed water hyacinth (***Eichhornia crassipes)*** could be a good candidate in this regard.

Water hyacinth is one of the invasive weeds that cause severe environmental pollution by invading water bodies and agricultural lands leading to reduction of productivity[27-31]. There are three known control mechanisms to curb the environmental pollution of this invasive weed. These are physical method, biological method and chemical methods[32-35]. However, none of these methods have been found to be effective for complete control of the weed. Therefore, alternative options of control mechanisms are being sought. Attempts from our research group suggested that use of alkali solutions of ashes of invasive weeds for soap making can be used as an alternative option to control environmental pollution caused by such weeds[36,37].

The use of Water hyacinth as a biomass for ethanol production can also be used for two main purposes: i. for substitution of imported fossil fuels and to control its environmental pollution. ii. there are reports stated that high cellulose and hemicellulose contents and low lignin content made it suitable biomass to produce bioethanol[30,38-41]. However, it is necessary to assess lignocellulosic composition before its use for bioethanol production. This is because variations in lignocellulosic compositions and ethanol yields may happen due to factors such as methods of pretreatment, mineral composition, habitats (soil/water), geography where water hyacinth grows, growth state, etc[40,42,43]. Similar to elsewhere in the world, water bodies and farm lands of Ethiopia are highly infested by this weed[33,34]. The present study was aimed at investigating the potential of production of bioethanol, determination of lignocellulosic composition and determination of mineral composition (using UV-Vis) of water hyacinth biomass growing in Lake Abaya and Lake Koka. There are no similar study reports of water hyacinth biomass growing in aforementioned lakes of Ethiopia.

Material and Methods

Chemicals and Equipments: Sulfuric acid, sodium hydroxide, Benedict solution (reagent), yeast extracts (agar), urea, dextrose sugar, standard ethanol (98%), distilled water, chromic acid reagent (5 g of potassium chromate, H_2O, 50ml concentrated sulfuric acid), magnesium sulphate hydrated and peptone were chemicals and reagents used in in the study. All the chemicals and reagents were analytical grade.

Equipments/Instruments: Oven (Gallenkamp), grinder, sieves (mesh size of 1.18 mm, Sortmks-3332, pfeuffr, Germany), electronics weighing balance (Adam, pw-124), Soxhlet extraction set up, vacuum pump filter (model-bn 3 Staatliche, Berlin), UV-Vis spectrophotometer (Spector uv-vis double beam pc 8 scanning auto cell uvd-3200), pH meter (pH meter-3310, Jenway), photometer, autoclave (Electrical Heated Vertical Steam Sterilizer Model LX-B50 LC Digital), water-bath, shaker incubator (Model. DH-500A), distillation set up, digital density meter (DMA 4100 M).

Water sample collection and Analyses: The water samples were collected from the Lakes where the Water hyacinth biomass collected following the APHA standard protocols. The parameters analyzed from the collected water samples were nitrogen (N), phosphorus (P) and potassium (K). The selected parameters and the method used to analyze them were adopted from literature reported procedures[44].

Collection of Water hyacinth biomass and preparation: The biomass was collected before flowering stage from Lake Koka ($8^0$2' to $8^0$26'N latitude, 39^0 to $39^0$10'E longitude, and an altitude elevation of 1660m asl) [45] and Lake Abaya ($5^0$36'39''N; $37^0$33' 20''E and an altitude of 1350m asl)[46]. The collected biomasses were transported to Hawassa University Environmental Engineering laboratory.

They were washed with distilled water to remove adhering dirt, and were cut to small pieces. It was put in an oven and dried at 80^0C for 48 hr. The dried plant materials were ground to powder using electric grinder. It was then sieved using a sieve to get powder with high surface area in order to increase contact between the plant materials and the H_2SO_4 solution during pretreatment[47,48].

The chemical composition of the water hyacinth biomass: Determining extractives in the biomass: Three grams of the biomass was loaded into the cellulose thimble of a Soxhlet extractor set up. Acetone (150mL) was used as a solvent for to carry out the extraction and it was carried out for 6hrs. After removing the extractives, the residues were air-dried at room temperature. Then the % (w/w) of the extractives was evaluated as the difference in weight between the raw extractive (sample with extractive) and extractive free sample and was calculated using the formula reported in literature[49].

Determination of hemicellulose content: 150mL of 0.5M NaOH was added into a 250-mL flask that contain 1 gram of extractive free water hyacinth biomass sample. The mixture was then boiled for 3 and ½ hrs and then allowed to cool to room temperature. The cold mixture was filtered through vacuum filtration and then washed with water until it reaches neutral pH. It was subjected to drying at 105^0C in an oven. The difference between the sample weight before and after the treatment was considered as the hemicellulose content (%w/w) of dry biomass and it was calculated the formula reported in literature[50].

Determination of Lignin content: There are two types of lignin: acid-insoluble and acid-soluble lignin. The contents of both acid-soluble and acid-insoluble lignins were determined according to NREL protocol reported in literature[49].

Cellulose determination: The cellulose content (% w/w) was calculated according to the methods reported in literature[49] by assuming that extractives, hemicellulose, lignin, ash, and cellulose are the only components of the entire biomass.

Acid pretreatment of biomass: The biomass (water hyacinth plant material) was pretreated at optimum pretreatment conditions for reported water hyacinth biomass in literature[51,52].

Dilute acid hydrolysis of water hyacinth biomass: The cellulose and hemicellulose molecules can be broken down to monomer sugars by hydrolysis before they were fermented to bioethanol. So, the hydrolysis step was carried out in an autoclave using dil. H_2SO_4 of different concentrations (0.5, 1,1.5%) and different reaction times (30,60,90min.) at a maximum temp of autoclave (121^0C) by using 1:10 w/v ratio (sample to acid solution ratio). The variables (H_2SO_4 concentration and hydrolysis time) were varied based on the report of Masami *et al*.[53] who reported that the efficient water hyacinth hydrolysis occurred when the water hyacinth biomass was incubated in 1% H_2SO_4 for 60 min in an autoclave at 121^0C.

Determination of total reducing sugar in the hydrolyzed sample: The total reducing sugar concentration of the hydrolyzed sample was determined using a Spectrophotometer by measuring absorbance at 540 nm wavelength according to the report by Silva *et al.*[54].

Calibration plot for glucose standard: The standard glucose solution was used to plot the calibration curve. The glucose stock solution was prepared to draw a calibration plot. Three (3) grams of glucose was added into a 500mL volumetric flask enough containing distilled water to dissolve the added glucose. Then volume of the solution was adjusted to 300mL mark by adding more distilled water. The final concentration of the stock solution was then adjusted to 10 g/L and it was diluted to make 6 standard solutions (0, 1, 3, 5, 7, 9%). Literature reported procedures were also used to determine total reducing sugar in the hydrolyzed samples[54].

Fermentation and distillation of the samples: Microorganism used for fermentation: Yeast (*S. cerevisiae*) was used for fermentation purposes and it was obtained from the Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia.

Preparation of growth media: The yeast was cultured in the media that was prepared following standard procedures reported in literature[54].

Fermentation and distillation: The pH values of the hydrolyzed samples were adjusted to 5.0 which is optimum for *S. cerevisiae*. The sample and media with the yeast were mixed in 500mL flasks containing a 10% ratio (1% media and 10% sample). Then the fermentation process was carried out by placing the flasks in a shaker incubator at 30^0C with stirring at 180 rpm for 72 hrs[55]. The separation of bioethanol was carried out using simple distillation. The distillate was collected at 78.3^0C.

Determining yield of ethanol: The volume percentage of ethanol was determined using a digital density meter. A sample was injected into the density meter and the volume percentage of each sample was recorded. The yield of ethanol (mL of ethanol per gram of sugar) was then determined using the following using formula reported in literature[55].

Experimental design and Statistical analysis: Experimental design: The experimental design of water sample analysis and chemical composition determination of water hyacinth biomass collected from two different lakes (Lake Koka and Lake Abaya) was a complete randomized design (CRD) with three replications. The hydrolysis step of the experiment was also laid out in factorial arrangement using CRD with three replications.
The treatments studied include water hyacinth biomass and water sample collected from two different sources (from Lake

Koka and Lake Abaya), three levels of concentration of H_2SO_4 acid (0.5%, 1%, and 1.5%) used for hydrolysis, and three levels of hydrolysis time (30 minutes, 60 minutes and 90 minutes).

Statistical analysis: The data were subjected to analysis of variance (Proc Mixed model procedure) using SAS software version 9.0 to test treatments effects on dependent variable. Analysis of variance (ANOVA) was conducted and means comparison was done using least significant difference (LSD) at a 5% level of significance (SAS, 2009)[56].

Results and Discussion

Determination of nutrient contents of water samples: In this study macronutrient (nitrogen (N), phosphorus (P) and potassium (K)) content of water samples collected from the biomass collecting sites were investigated. The nutrients on lignocellulosic composition of water hyacinth biomass were also measured[42,43]. The finding of the present study revealed that macronutrients (N, P and K) of the water samples collected from the two sites (Lake Koka and Lake Abaya) found to be significantly different (Table-1).

The data on P and K were significantly different at $P<0.01$ whereas that of N were different significantly at $P<0.05$. That means water samples collected from Lake Koka contain higher macronutrients than the water samples collected from Lake Abaya. The results of the study showed that the N, P and K content of water sample collected from Lake Koka were 5.61, 0.8 and 0.63 mg/L, respectively, whereas the corresponding values for water samples collected from Lake Abaya were found to be 3.67, 0.42 and 0.41 mg/L for N, P, and K, respectively. Generally, the mineral contents of water samples from Lake Koka are relatively higher than the corresponding values for water samples for Lake Abaya (Table-1). This could be the reason for the observed relatively higher cellulose and hemicellulose composition of water hyacinth from Lake Koka. However, the mineral contents were found to be lower than the mineral requirement (20 mg/L N, 3 mg/L P, and 53 mg/L K) for optimum growth of water hyacinth[57].

Lignocellulosic composition of water hyacinth biomass: The major chemical compositions of water hyacinth biomass are cellulose, hemicellulose, and lignin[58,59]. Literature reports also indicated that estimation of cellulose, hemicellulose, and lignin is important to predict the conversion efficiency of biomass to bioenergy[60] as there are differences in carbohydrate and lignin composition of water hyacinth biomass growing in different habitats[61]. Thus, in the present study, the lignocellulosic composition of the biomasses that were collected from Lake Koka and Lake Abaya were determined and the results revealed that the significant different from each other (Table-2). The cellulose and lignin content of biomass significantly different at $P<0.01$ whereas the hemicellulose content was significantly different at $P<0.05$.

Table-1: The macronutrients contents of water samples collected from water hyacinth feed stocks collection sites.

Treatments	N	P	K
WSK	5.6167^a	0.8033^a	0.6333^a
WSA	3.6767^b	0.4200^b	0.4133^b
LSD	1.1062	0.1225	0.0162
Significance	*	**	***
CV	6.77	5.7	0.88

Where; WSK = water sample from Lake Koka, WSA = water sample from Lake Abaya, LSD = Least Significant Difference, CV= Coefficient of Variation, Values are expressed as mean and means in different letters are statically significant according to LSD test, (*,**,***) indicates significance (significant at $P<0.05$), highly significance (significant at $P<0.01$) and very highly significance (significant at $P<0.001$) respectively and all parameters were measured in mg/L.

Table-2: Chemical compositions of water hyacinth biomasses collected from Lake Koka and Lake Abaya.

Treatments	Cellulose %	Hemicellulos %	Lignin %
WHK	30.4967^a	41.3033^a	4.5100^b
WHA	25.0267^b	34.9333^b	8.4233^a
LSD	1.5129	3.0183	1.5872
Significance	**	*	**
CV	1.55	2.25	6.98

Where; WHK = Water hyacinth biomass from Lake Koka, WHA = Water hyacinth biomass from Lake Abaya, LSD = Least Significant Difference, CV= Coefficient of Variation, Values are expressed as mean and means in different letters are statically significant according to LSD test and (*, **) indicates significance (significant at $P<0.05$), highly significance (significant at $P<0.01$) respectively.

The biomass collected from Lake Koka was found to contain higher hemicellulose and cellulose but lower lignin than that of the biomass collected from Lake Abaya site. The levels of hemicellulose, cellulose, and lignin of the water hyacinth biomass collected from Lake Koka were 41.30, 30.49, and 4.51%, respectively, whereas the corresponding values of hemicellulose, cellulose, and lignin in water hyacinth biomass collected from Lake Abaya were 34.93, 25.02 and 8.42% (Table 2). The variation of chemical compositions of the biomasses from the two sites could be attributed to the variation of nutrient contents of water bodies (Table-1).

These observed results were in good agreement with the data reported by of Betelihem (2016) who reported 30% cellulose, 48% hemicellulose and 5% lignin for water hyacinth biomass collected from Awash river, Ethiopia[62]. These findings are also consistent with reports of Gunja *et al*.[38] and Madian *et al.*[40] that stated the nutritional conditions of habitats affect the metabolic processes of the plant and this result in different chemical compositions. Thus, the growth of Water hyacinth is directly dependent on the amount of nutrients present in water as it requires a large amount of N, P, and K for its growth and development[62]. The opposite holds true for lignin levels of the above biomasses collected from the two different sites (Lakes). This observation is also in line with the previous studies that revealed high nitrogen results in low level of lignin[38,63].

Analysis of effects of biomass source, acid concentration, and hydrolysis time on total reducing sugar yield: Total reducing sugar concentration (TRSC) yields of the biomasses collected from the two sites were found to be significantly affected by the source of biomass, acid concentration and hydrolysis time (at P<0.001). The results of the two-way interaction of source of biomass with acid concentration, source of biomass with hydrolysis time and acid concentration with hydrolysis time were significantly were found to affect TRS yield at P<0.001.

Similarly, the three-way interaction was found to significantly affect TRS yield at P<0.001 (Table-3). The results revealed that TRS yield of biomasses collected from Lake Koka and Lake Abaya were 42.97% and 36.42%, respectively (Table-3). Such differences on TRS yields of the biomasses might be attributed to the difference in lignocellulosic composition of the biomass. Reports showed that lignocellulosic biomasses that contain higher cellulose and hemicellulose with lower lignin to give higher TRS yields. Moreover, the amount of lignin in lignocellulosic biomass affects TRS yield as it is very resistant to chemical degradation due to the presence of strong chemical bonds[20].

The difference in concentration of acid gave different TRS yields. The highest TRS yield (41.35%) was obtained at higher acid concentration (1% H_2SO_4). On the other hand, treatment using a lower acid concentration (0.5% H_2SO_4) results in a lower TRS yield (38.21%)(Table 3). In the course of hydrolysis, a biomass is expected to be converted to five- and six-carbon sugars before they get fermented and converted into ethanol. This is because cellulose is a beta-polysaccharide of glucose, and hemicellulose is a complex polymer that consisting of xylose and arabinose and glucose, mannose and galactose. These linkages need to be broken down by acid hydrolysis process in order to facilitate fermentation processes[63].

Table-3: TRSC, TRS in gram and yield in percent with respective biomass source, acid concentration and hydrolysis time.

Treatments	TRSC (%)	TRS (g)	TRSY (%)
Source of biomass			
WHK	22.9466^a	2.2946^a	42.9726^a
WHA	19.5066^b	1.9506^b	36.4285^b
LSD	0.1969	0.0197	0.4208
Significance	***	***	***
H_2SO_4 Acid (%)			
0.5	20.3700^c	2.0370^c	38.2150^c
1	22.1444^a	2.2144^a	41.3522^a
1.5	21.1656^b	2.1165^b	39.5344^b
LSD	0.2411	0.0241	0.5154
Significance level	***	***	***
Time (minute)			
30	19.7822^c	1.9782^c	36.9511^c
60	21.7522^b	2.1752^b	40.6289^b
90	22.1456^a	2.2145^a	41.5217^a
LSD	0.2411	0.5154	0.5154
Significance	***	***	***
CV	1.68	1.68	1.92

Where; WHK = Water hyacinth biomass from Lake Koka, WHA = Water hyacinth biomass from Lake Abaya, TRSC = total reducing sugar concentration, TRSY = total reducing sugar yield, TRS = total reducing sugar in gram, LSD = Least Significant Difference CV= Coefficient of Variation, Values are expressed as mean and means in different letters are statically significant according to LSD test and (***) indicates very highly significance (significant at P<0.001.)

In the present study, 1% H_2SO_4 acid was found to efficiently breakdown the hemicellulose and a beta-polysaccharide of glucose of cellulose found in the biomass used in the study. On the other hand, the lower TRS yield obtained at lower acid concentration (0.5% H_2SO_4) indicated that the hydrolysis process to be inefficient at lower acid concentration (Table-3). The data was in line with report of Masami *et al*. who reported that the efficient water hyacinth biomass hydrolysis occurred when it is hydrolyzed at 1% H_2SO_4 concentration[53].

Hydrolysis time is also a factor that affects the TRS yield[62]. In the present study, the highest TRS yield (41.52%) was obtained at 90 min whereas at hydrolysis of the biomass for 30 min result in the lowest TRS yield (36.95%) (Table-3). The results indicated that the cellulose and the branched structures of hemicellulose of the biomass hydrolyzed efficiently at longer hydrolysis time. The breakdown of the lignin bond, elimination of lignin and increasing the porosity of the material was occurred at the longer hydrolysis time. The data are consistent with literature reports that showed at a longer reaction/hydrolysis time, higher levels of hemicellulose sugars can be hydrolyzed efficiently (converted into simple sugars)[66,67].

Interaction effect of hydrolysis factors on total reducing sugar yield: Interaction effects independent variable impose on one another. All controllable factors are variables that affect the output of the dependent variable. In this study, there are three controllable factors in the hydrolysis process. These are source of biomass, H_2SO_4 concentration and hydrolysis time. The main effects of acid concentration and hydrolysis time depended on the source of the Water hyacinth biomass.

Two-way interaction effect on TRS yield: In the two-way interaction, the combination of source of biomass with H_2SO_4 concentrations, the biomass collected from Lake Koka resulted in TRS of 42.80%, 45.57% and 40.54% when it was hydrolyzed in 0.5%, 1% and 1.5% H_2SO_4 concentration, respectively. The data showed that the highest TRS yield to be 45.57% when that biomass was hydrolyzed with acid 1% H_2SO_4 concentration (Table 3). The findings are in good agreement with a literature reports that stated that efficient biomass hydrolysis occurred when it was hydrolyzed at 1% H_2SO_4 concentration[53]. On the other hand, TRS yield was lower when the biomass was hydrolyzed in 1.5% H_2SO_4 concentration. This could be attributed to one of the following (a) Degradation of monomeric sugars to furfural and HMF[62] (b) Dehydrating or oxidizing of glucose by sulfuric acid and/or (c) The conversion of glucose to levulinic and formic acid which leads to decrease in glucose yield[68].

In contrast to the data obtained from the biomass collected from Lake Koka, the biomass collected from Lake Abaya resulted in TRS yield of 33.63%, 37.12% and 38.52% when it was hydrolyzed in 0.5%, 1% and 1.5% H_2SO_4 concentration, respectively (Table 3). From this biomass, it was observed that the TRS yield increased when it was hydrolyzed in highest acid concentration (1.5% H_2SO_4). This observation could be attributed to the presence of higher content of lignin in the water hyacinth biomass that was collected from Lake Abaya as compared to that water hyacinth biomass collected from Lake Koka. Higher concentration of acid is used to degrade the chemical structure of lignin that is found in higher level water hyacinth biomass was collected from Lake Abaya (Table-3). The findings are consistent with report by Teirumnieka *et al*. (2010) that states in lignocellulosic biomass, lignin acts as a polymer around the hemicellulose micro fibrils binding the cellulose molecules together and in nature it is very resistant to chemical degradation as it has strong chemical bonds[69]. Therefore, hydrolyzing the biomass that contains higher lignin requires more H_2SO_4 concentration than that of biomass which contains less lignin (Figure-1).

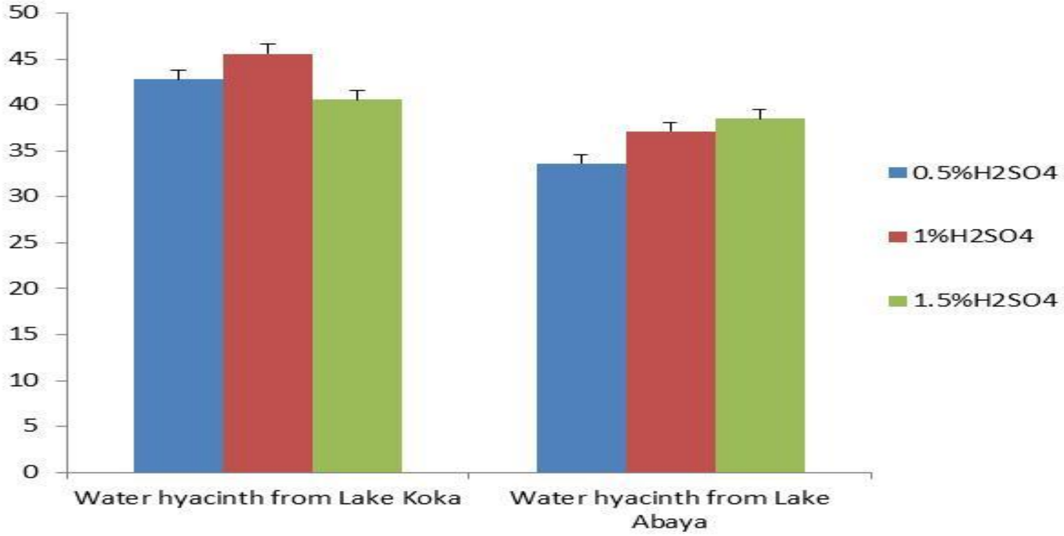

Figure-1: Interaction effect of source of biomass and acid concentration on total reducing sugar yield.

The TRS yield of 40.41%, 45.35% and 43.15% were for plant material obtained from Lake Koka in the corresponding times of extraction of 30, 60 and 90min, respectively. The highest TRS yield (45.35%) was obtained from the water hyacinth biomass that was hydrolyzed for 60 minutes. On the other hand, the TRS obtained from the water hyacinth biomass collected from Lake Abaya yielded were 33.48, 35.90 and 39.89% at 30, 60 and 90 minutes, respectively (Table-3). The observed differences in TRS yield could be attributed to the higher content of lignin in the water hyacinth biomass collected from Lake Abaya as compared to that of Water hyacinth biomass collected from Lake Koka. The interaction effect of source of biomass and hydrolysis time on TRS yield is shown below (Figure-2).

Regardless of the source of the water hyacinth biomass, the highest TRS yield (40.61%) was obtained at the combination of 1%H_2SO_4 acid concentration and 60 min of hydrolysis time. The lowest TRS yield (31.72%) was also obtained at 0.5% H_2SO_4 concentration and 30 min time of hydrolysis (Figure-3).

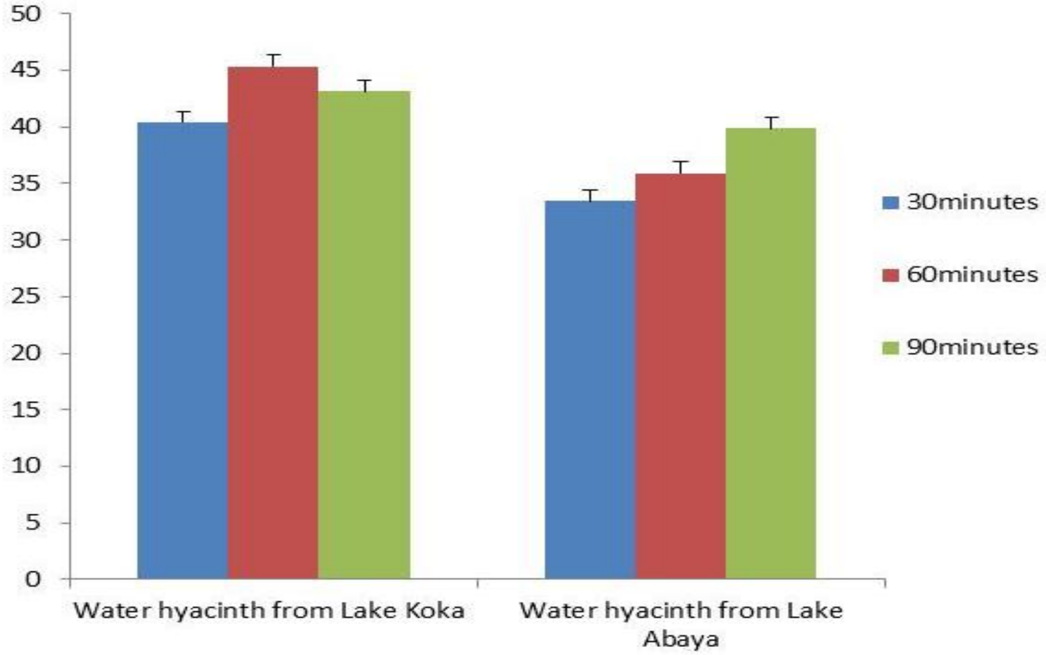

Figure-2: Interaction effect of source of biomass and time of hydrolysis on total reducing sugar yield.

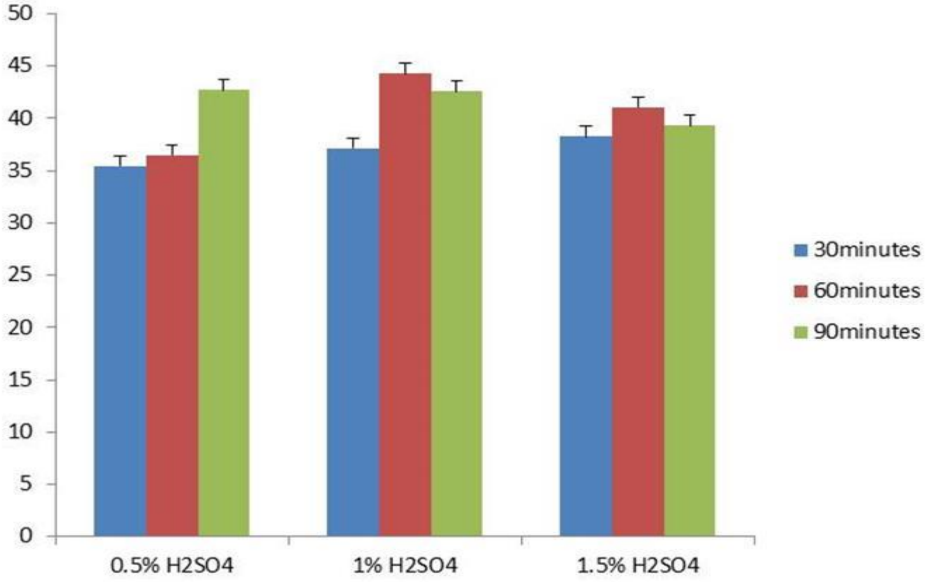

Figure-3: Interaction effect of H_2SO_4 concentration and time of hydrolysis on TRS yield.

Three-way interaction effect on TRS yield: The three-way interaction revealed that the combination of water hyacinth biomass collected from Lake Koka hydrolyzed with 1% H_2SO_4 concentration for 60 min of hydrolysis time resulted in highest (51.22%) TRS yield. The results are found to be in line with literature reports that stated the efficient water hyacinth biomass hydrolysis occurs when it is done in the acid hydrolysis using 1% H_2SO_4 for 60 min in an autoclave at 121^{o}C[53]. However, this was not the case for water hyacinth biomass collected from Lake Abaya as it was found to give the highest TRS (41.46%) when it was hydrolyzed in 1.5% H_2SO_4 concentration for 90 min (Table-3). The differences could be due to variations amounts of cellulose, hemicellulose and lignin in the biomasses collected from two different sites (Lakes). This outcome was also in line with the finding of Masami *et al.* who investigated two feedstock containing different amounts of lignin and they found different results[53]. Timung *et al.* also reported that besides hydrolysis conditions like acid concentration, temperature, and reaction time, the varying yield of total reducing sugar might be attributed to factors such as amount of cellulose, hemicellulose, and lignin content of the biomass[60].

Yield Ethanol product: *The aim of the present study was to prepare bioethanol from* water hyacinth biomass and factors that affect the *yield of bioethanol.* The yield (mL of ethanol/ gram of TRS) of ethanol obtained from water hyacinth biomass grown in Lake Koka and Lake Abaya was significantly different. The results showed that the yields to be 0.47 mL/g (or 37.77%) and 0.39 mL/g (31.22%) of TRS for water hyacinth biomass collected from Lake Koka and Lake Abaya, respectively (Table-4). The higher ethanol yield (EY) obtained from the biomass collected from Lake Koka could be attributed to the relatively lower lignin content and higher carbohydrate (cellulose and hemicellulose) content. The opposite was true for biomass collected from Lake Abaya (Table-4). It known that lignin component of a given biomass cannot be converted into sugars and that would limit the microbiological activities during the fermentation process. Therefore, a biomass that contains a lower lignin, the cellulose and hemicellulose components will easily be converted to fermentable sugar, and gives a higher ethanol yield[39]. The observed boiling point (78.3^{0}C) and FTIR spectral data (not given in the manuscript) confirmed the product to be bioethanol.

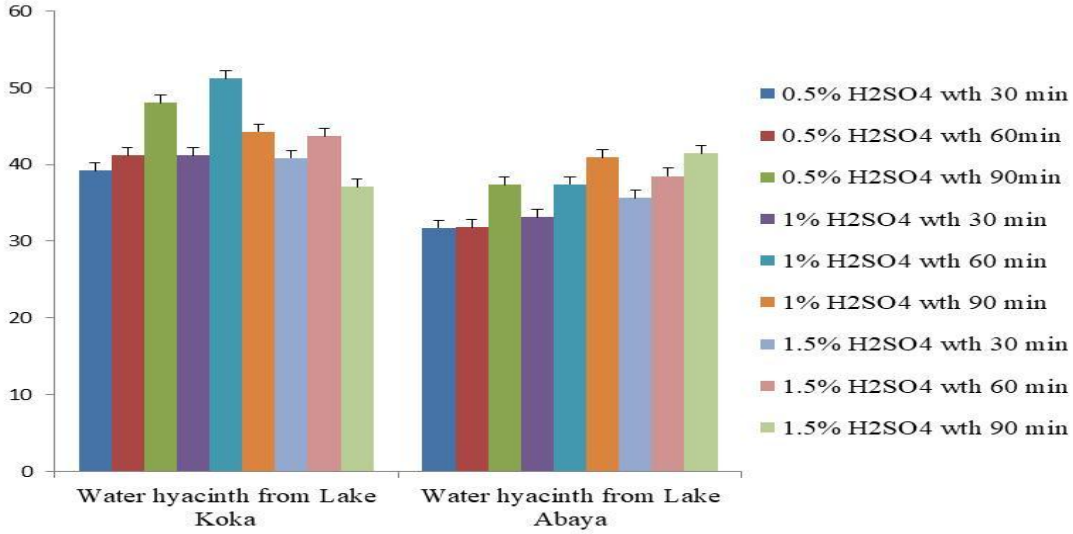

Figure-4: Three-way interaction effects (source of biomass, H_2SO_4 concentration and time of hydrolysis) on TRS yield.

Table-4: Ethanol yield obtained from water hyacinth biomass collected from Lake Koka and Lake Abaya.

Treatments	EV (mL)	EY(mL/g)	EY (%)
Source of biomass			
WHK	1.11092^{a}	0.478763^{a}	37.7781^{a}
WHA	0.77958^{b}	0.395522^{b}	31.2222^{b}
LSD	0.0211	0.0054	0.4314
Significance	***	***	***
CV	4.050653	2.258333	2.265165

Whereas; WHK = Water hyacinth biomass from Lake Koka, WHA = Water hyacinth biomass from Lake Abaya, EV =ethanol in volume, EY = ethanol yield, LSD = Least Significant Difference, CV= Coefficient of Variation, Values are expressed as mean and means in different letters are statically significant according to LSD test and (***) indicates highly significance (significant at P<0.001.).

Conclusion

In this study, it was observed that the water sample collected from Lake Koka contained higher macronutrients (N, P and K) than the water samples collected from Lake Abaya. This was attributed for the observed significant differences in the lignocellulosic compositions of the water hyacinth biomasses collected from the two lakes. The higher hemicellulose and cellulose and the lower lignin content was recorded in water hyacinth biomass that was collected from Lake Koka associated with higher nutrient content. Such biomasses are preferable for bioethanol production.

In line with this fact, the water hyacinth biomass that was collected from Lake Koka resulted in a higher total reducing sugar yield at 1% H_2SO_4 concentration and 60 min of hydrolysis time. However, the biomass collected from Lake Abaya hydrolyzed efficiently at 1.5% H_2SO_4 concentration and 90 min of hydrolysis time. The relatively lower lignin content of water hyacinth biomass collected from Lake Koka is expected to reduce the processing cost by consuming low acid concentration to hydrolyze efficiently. The cellulose and hemicellulose were hydrolyzed easily in a shorter time. This finding confirmed that the biomass with higher lignin needs higher H_2SO_4 concentration and longer reaction time to hydrolyze efficiently. The product was ethanol was confirmed based its FTIR spectrum and boiling point (78.3^{o}C).

Finally, the finding of the present study suggested that bioethanol production from water hyacinth biomass growing in water bodies growing in different parts of Ethiopia needs to be given a due attention. This would help not only to produce ethanol for biofuel but also would serve as means to control environmental pollution caused by this invasive weed.

Data availability statement: All the data that were generated in this study are included in the article and further inquiries can be directed to the corresponding author.

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