Identification And Quantification Of Hydrocarbons Produced From The Acid-Pretreated Kitchen Waste By Using Fungal And Bacterial Strains

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Abstract

This study was conducted to identify and quantify hydrocarbons produced during bio-fuel production using kitchen waste (KW). KW is a complex mixture of hardly digestible compounds, mainly lignin, cellulose and hemicellulose, and easily digestible compounds, mostly starchy materials. Therefore, KW has a high potential for the production of biofuel after the chemical hydrolysis of lignocellulose, starch and carbohydrates. In this study, after the physically pretreatment (dried and crushed) of KW, dilute-acid hydrolysis was used for the hydrolysis of lignocellulose and starchy materials, eliminating the enzymes requirement. The dilute acid hydrolysis was conducted with 1, 3 and 5% (w/w) sulfuric acid at 90 and 120°C for 30, 60, 90 and 120 min. The hydrolysis with 5% acid at 120°C for 120 min resulted in the hydrolysate with the highest reducing sugar concentration of 97.917 ± 0.5 g/kg and Energy of 1.567 ± 0.008 MJ/kg. The reducing sugars were used as substrate in fermentation by fungal strain *Aspergillus niger*, bacterial strains *Lacto-bacillus* and *Escherichia coli*, to produce hydrocarbons. The fermented product was quantified after every day till the fermentation time is over i.e. no more products were formed. Biofuel production from *Aspergillus niger, Escherichia coli* and *Lacto-bacillus* was 64%, 45% and 50% after 72 hr. Fermented product contains mainly hydrocarbons as identified by GC-MS analysis. Calorific value of sample and biofuel determined on Differential Scanning Calorimetry were 0.6MJ kg^{-1} for sample before fermentation and 3.56 MJ kg^{-1}, 3.33 MJ kg^{-1} and 2.67 MJ kg^{-1} for KW fermented by *Aspergillus niger, Escherichia coli* and *Lacto-bacillus*, respectively. Hence, maximum of 64% reducing sugars were converted into hydrocarbons (biofuel) after fermentation by *Aspergillus niger.*

Novelty

Traditionally the Biofuel production is associated with open fermentation which is good in producing furfurals compounds but bad for good yields of hydrocarbons. For better quality and quantity of Hydrocarbons, optimized conditions along with fermentation was performed in this study. The results not only indicated better quality but also convert macromolecules to hydrocarbons with good calorific values. Comparison of results of this study with traditional fermentation to produce biofuel showed better of study results (Quality and Quantity of Hydrocarbons) than the previous results available in literature.

Introduction

Abundant usage of fossil fuel for transportation has adverse effects on environment, not only climate changes are visible, but depletion of fossil fuel is also a threat. This leads to use of alternative energy recourses. Environmental friendly and renewable fuel always attracts attention for the protection of the environment and supplies our needs by reducing dependence on non-renewable energy sources and petroleum [1]. Many countries, including Pakistan, are moving towards bio-fuel to reduce the emission of gasses and economic burden (imported petroleum for transportation and industrial plants).

Bio-fuel, which is an attractive alternative fuel, has the potential to meet the increasing demand of energy for different industrial processes, power generation and transportation [2]. Biofuel may be produced by

food crops like sugar beet and rapeseed (1st generation) and nonedible byproduct of food crops e.g. agricultural residue, grass, sawdust, wood chips, waste cooking oil and municipal solid waste (MSW) etc. (2nd generation) [3]. In many countries, biofuel is produced from sugarcane and approximately 60% of global ethanol production based on this raw material while in United States 90% ethanol is produced from corn [4]. Many other sources of biofuel production are also known e.g. starch, palm oil [5], canola oil [6], animal fat [7], waste cooking oil [8], wheat straw [9], rice husk [10] and algae [11]. Moreover, production of biofuel from food source like sugar and corn cause increase in the crop price. Therefore, it is essential to research inexpensive and alternative biomass for ethanol production at a reduced cost. This issue turned the concern of researchers toward Municipal Solid Waste (MSW) as a raw material to produce biofuel.

Kitchen waste (KW) is a major portion of MSW and it is disposed of from restaurants, hotels, industrial and household kitchen at increasing amount all over the world. In Pakistan 90% of the collected waste goes for open dumping and cause environmental pollution. About 18,113 tons per day KW produced in Pakistan that is approximately 30.7% of municipal solid waste if treated or used properly then we can save our mother land from pollution [12, 13]. Current practices for KW treatment involves composting, incineration, anaerobic digestion, land filling, open dumping as well as drying for animal feed [14, 15]. Composting of KW provides a valuable soil conditioner and it reduces the mass and volume of waste as well. As KW has high moisture content so causes unusual level of leachate in composting that affects the performance of whole process by dropping oxygen availability and weak the strength of pile. KW can be utilized as an animal feed but due to its high moisture content and variable composition which favors microbial contamination it can be environmental unfriendly [14]. Incineration of solid waste causes emission of noxious gases and dioxins that are source of air pollution [16]. Landfill that is an engineered practice for waste disposal also causes serious environmental pollution. Its gas emissions are one of the largest anthropogenic sources of methane (cause of air pollution and ultimately global warming) especially because of KW. Leachate production from landfills is another serious environmental issue that causes ground water pollution and soil contamination with dangerous chemicals [17].

The biomass, in case of KW, has a great potential to be used as sustainable energy source if converted to ethanol or another biofuel, while simultaneously treating kitchen waste. KW is primarily composed of cellulose (insoluble carbohydrate), hemicelluloses, lignin, proteins, fat, soluble sugars such as glucose, fructose and sucrose. Cellulose, glycogen and starch components of kitchen waste can be hydrolyzed to monomeric sugars. Therefore, due to abundant source of fermentable carbohydrates this sugar can be used as a substrate in microbial fermentation for the production of useful products such as biofuel [16, 18]. Young et. al [19] used food residue for ethanol production and maximum yield of 25 g/L ethanol per 100 g/L food residues was achieved using *Saccharomyces cerevisiae.* In another study it has been reported that hydrothermal pretreatment enhanced the 13.16% of ethanol production levels and 107.58 g/kg of final ethanol yield was achieved using household food waste [20]. However, in the literature there are very few studies on the utilization of KW for the production of ethanol.

The production of biofuel from biomass includes two main processes: hydrolysis of polysaccharide sugars into reducing sugars and fermentation of the reducing sugars to biofuel [21]. The conventional methods for hydrolysis process are acid hydrolysis and enzymatic hydrolysis. Dilute acid hydrolysis is successful for pretreatment of biomass. High reaction rates can be achieved by the dilute sulfuric acid pretreatment and it significantly improves hydrolysis of cellulose and glycogen [22]. Moreover, dilute acid hydrolysis is favorable at high temperature [23]. Dilute acid hydrolysis of KW converts polysaccharides into reducing sugars like sucrose and fructose [24]. These can be converted into valuable products such as hydrocarbons (HC's), Lactic Acid (LA) and Ethanol (ET) after microbial and fungal fermentation [3]. Yanuar et. al. investigated that 87–90 g/L sugar concentration from hydrolysate was achieved at optimum conditions using lignocellulose biomass [25]. Several microbial species have the ability to ferment the hydrolysate of KW into biofuel. *Aspergillus Niger, Lacto-bacillus* and *Escherichia coli* are commonly used microbial strains in fermentation of biomass as these ferment the hexose sugars in to high ethanol yield [26]. Published studies have reported ethanol production from agro-industrial biomass using *Aspergillus Niger* and *Trichoderma reesei* [27]. Bruce et al. reported ethanol production from lignocellulose biomass using *Escherichia coli* [28]. Moreover, there are only few studies in the literature of microorganisms grown in monoculture on KW hydrolysates with the aim of producing renewable chemicals or biofuels. Usually, conversion of biofuel from KW is performed using open fermentation. Open fermentation has reduced production of furfural compounds which is inhibitor for bacterial growth [29, 30] but produces less hydrocarbons as macromolecules are difficult to ferment. To increase the quantity and quality of hydrocarbon produced as biofuel, this study was performed. The aim of this study was to investigate the effect of hydrolysis temperature, hydrolysis reaction time and acid concentration toward reducing sugar recovery from KW. The acid hydrolysate produced after acid hydrolysis of KW was fermented by *Aspergillus Niger, Lacto-bacillus* and *Escherichia coli* for biofuel production and production of biofuel from these strains was compared. Moreover, the quantity and quality of produced hydrocarbons were analyzed on GC-MS and calorimeter.

Materials And Methods

2.1 Microorganism and Inoculum preparation

Aspergillus Niger FCBP-0198, *Lacto-bacillus* FCBP-0004 and *Escherichia coli* FCBP-0011 purchased from Punjab University fungal bank, Lahore Pakistan, were used in fermentation. Fungus was grown with 20g/L Malt extract (CM0059) and 20g/L Agar (CM0463). The culture media for *Aspergillus Niger* was sterilized at 121°C, 15 psi for 15 min in an autoclave. For inoculum preparation, fungus was inoculated to 20 ml of Maltextract and Agar media and been put to an incubator for 5–7 days at 25°C to 27°C. *Lacto-bacillus* was grown with 20g/L Malt extract (CM0059) and 20g/L Agar (CM0463) and media was sterilized in an autoclave at 121°C, 15 psi for 15 min. For inoculum preparation, bacteria was inoculated to 20 ml of Maltextract and Agar media and been put to an incubator for 24 h at 35°C. While for *Escherichia coli*, bacteria was grown with 5g/L yeast extract (LP0021), 10g/L tryptone (LP0042), 10g/L NaCl and 20g/L Agar (CM0463) and media was sterilized in an autoclave at 121°C, 15 psi for 15 min. For

inoculum preparation, bacteria was inoculated to 20 ml of media and been put to an incubator for 24 h at 35°C. All growth media were purchased from Merck Private Ltd., Pakistan. All cultures were preserved at 4^{o}C to maintain viability.

2.2 Raw Material and Pretreatment

The KW utilized in this present research work was collected from houses, restaurants and cafeterias (Lahore, Pakistan) in summertime. The composition of KW was uncooked vegetables and fruits (51%), cooked meat (16%), uncooked meat (15%), bread (2%), tea leaves (5%), egg shell (6%), miscellaneous (5%). Mixed KW was dried using Laboratory hot air oven (Wise cube WON-105) at 55 ± 2°C for 48 h or more, until constant weight and then was ground in a laboratory grinder to achieve fine particles size of 0.45 mm to 1 mm by using a sieve of 200–400 micron and to increase the surface area of particles. To maintain its physicochemical characteristics during the whole period it was stored at -20 ^{o} C. The characteristics of the KW used in this present study are presented in Table 1. The dry mass of KW was mainly composed of cellulose, hemicellulose, starch sugars, protein and fat, which could be considered as a suitable biomass for ethanol production. These characteristics of KW were very similar to other studies that have been reported [31] [32] [33].

Table 1
Composition of kitchen waste

Parameters	Content (w/w, %)
pH	4.61
Total solid (TS)	20.05 ± 1.29
Volatile total solid (VTS)	18.78 ± 1.32
Ash	1.97 ± 0.58
Moisture	80.54 ± 3.12
Total sugars (based on wet weight)	12.31 ± 1.45
Total sugars (based on dry weight)	61.87 ± 1.98
Cellulose (based on dry weight)	2.01 ± 0.28
Protein (based on dry weight)	21.55 ± 1.74
Lipid (based on dry weight)	11.98 ± 1.17

2.3 Acid Hydrolysis

Acid hydrolysis was performed to produce soluble reducing sugars from KW. The dilute acid hydrolysis of KW with 1%, 3% and 5% (w/w) sulfuric acid was conducted at two different temperatures (90 and 120°C) and four reaction times (30, 60, 90 and 100 min) at a solid to liquid ratio of 1:10 (w/w) (based on total solid) in a 500 mL Erlenmeyer flasks with a working volume of 100 ml. After dilute acid hydrolysis, the

flask was cooled in an ice bath, and the hydrolysate was separated from the solid by filtration. The hydrolysate was then analyzed for its reducing sugar content by using by 3,5-dinitrosalicylic acid method using glucose as the standard following Miller's method [34].

2.4 Fermentation

Reducing sugars produced after acid hydrolysis of KW were subjected to fermentation using fungal and bacterial strains i.e. *Aspergillus Niger FCBP-0198, Lacto-bacillus FCBP-0004 and Escherichia coli FCBP-0011*without adding of any nutrient components. After the adjustment of pH to 6.5 using 5 M NaOH, a final volume 100 mL of hydrolysates in 250 ml Erlenmeyer flasks was fermented with 2% (v/v) of *Aspergillus Niger FCBP-0198, Lacto-bacillus FCBP-0004 and Escherichia coli FCBP-00.* The flasks were sealed with a rubber stopper. These three flasks were incubated in laboratory incubator (Wise Cube WIG-105) at 25^{o}C (*Aspergillus Niger)*, 37^{o}C (*Lacto-bacillus)* and 37^{o}C (*Escherichia coli*) for 7 days by using separate hydrolysis and fermentation. Reducing sugar concentration was measured after every day by collecting 20ml liquid of samples in the flasks, for continuously monitoring of the fermentation substrate. All fermentation experiments were performed with three replicates.

2.5 Analytical methods

The preparation of samples for GCMS and analysis procedure is explained elsewhere [35]. In summary, the fermented product was dried under nitrogen flow and passed through magnesium sulphate column to remove any moisture contents. The dried contents then dissolved in cyclohexane and send for GCMS analysis. A Hewlett-Packard (HP) 5973 Mass Selective Detector (MSD) interfaced to a HP 6890N gas chromatograph (GC) with 30 m × 0.25 mm ID capillary column (DB-5 MS, J & W scientific) was used for the analysis. GC-MS analysis was performed on sample before and after fermentation to identify and quantify the compound present in sample. Calorific value of before and after fermentation was also measured using Differential Scanning Calorimeter (DSC-6000, Perkin Elmer) [36].

2.6 Statistical analysis

Statistical analysis of the results was carried out by two-way ANOVA analysis and Tukey's test using Minitab software Version 17.0. Tukey's test was used to compare the significance between the means of results at the 95% confidence level ($p < 0.05$).

Results And Discussion

3.1 Acid hydrolysis

The use of low cost and abundantly available KW is presently being recognized as raw material for the production of bio-fuel because it contains significant amount of carbohydrates and lipid [37]. Moreover, it also has abundant nutrition, high moisture and organic component. Their key advantages are their abundance, diversity and low cost [38]. KW used in this study was composed of cellulose (insoluble carbohydrate), hemicelluloses, lignin, proteins, fat etc. Hydrolysis of KW was performed to convert

polysaccharides into monosaccharide i.e. reducing sugars, lipids to fatty acids and proteins to amino acids. Optimization of various parameters for hydrolysis was done for maximum reducing sugar production.

The dilute acid hydrolysis was performed using 1%, 3% and 5% (w/w) sulfuric acid at different temperatures (90°C and 120°C) and reaction times (30, 60, 90 and 120 min). The dilute acid hydrolysis conditions, reducing sugars and energy released from KW are summarized in Table 2. The dilute acid hydrolysis conditions were selected after a series of preliminary experiments. As shown in Table 2 at constant acid concentration and temperature, reducing sugars were increased by increasing the reaction time. Through the dilute acid hydrolysis with 1% acid at 120°C for 120 minutes, 61.287 ± 0.2 g of reducing sugars were released from each kg of KW and 0.981 ± 0.004 MJ energy was produced from each kg of KW. By increasing the temperature to 120°C from 90°C and reaction time to 120 min at 1% acid concentration, release of reducing sugars and energy are increasing. At the temperature of 90°C, when the acid concentration was increased to 3%, maximum release of reducing sugars was 62.483 ± 1.5 from each kg of KW and production of energy was 1.000 ± 0.025 from each kg of KW for 120 minutes. Reducing sugars and energy values at 3% acid concentration at 120°C for 120 minutes are higher than the previous ones it shows that increase in concentration of acid cause increase in production of reducing sugars and energy from each kg of KW. Whereas, increasing the reaction time to 120 min increased 20.747 g reducing sugars and 0.332 MJ energy from each kg of KW. The highest amount of reducing sugars and energy was detected after treatment with 5% acid concentration at 120°C for 120 min. At 5 % acid concentration by increasing temperature to 120°C and reaction time to 120 minutes release of reducing sugars and energy are increasing and maximum 97.917 ± 0.5 of reducing sugars from each kg of KW and 1.567 ± 0.008 MJ of energy from each kg of KW was achieved. Similarly, V. Gupta et al. [39] observed 19.1% reducing sugars in dilute acid hydrolysis by sulfuric acid (0.5 % v/v) at 120 ^{o}C for 60 min of rice straw. Jain et al. [40] carried out dilute sulfuric acid pretreatment and resulted in releasing of 12.52 % reducing sugars using rice straw as raw material. Kshirsagar et al. [41] resulted release of 0.359 g/g reducing sugars after 72 h saccharification of 0.5 % dilute sulfuric acid pretreated rice straw at 120 ^{o}C, for 60 min.

Table 2
Reducing sugars and Energy at different parameters of acid hydrolysis of KW

Acid Concentration (%w/w)	Temperature (°C)	Time (min)	Reducing sugars (g/kg)	Energy (MJ/kg)
1	90	30	37.673 ± 0.3	0.603 ± 0.005
		60	43.613 ± 0.4	0.698 ± 0.006
		90	48.047 ± 0.9	0.769 ± 0.016
		120	53.850 ± 0.7	0.862 ± 0.013
	120	30	41.300 ± 0.4	0.661 ± 0.008
		60	46.197 ± 1.3	0.739 ± 0.021
		90	55.830 ± 0.4	0.893 ± 0.007
		120	61.287 ± 0.2	0.981 ± 0.004
3	90	30	48.980 ± 0.3	0.784 ± 0.006
		60	56.933 ± 0.8	0.911 ± 0.014
		90	59.697 ± 0.8	0.955 ± 0.013
		120	62.483 ± 1.5	1.000 ± 0.025
	120	30	51.407 ± 0.5	0.823 ± 0.008
		60	58.023 ± 0.5	0.928 ± 0.009
		90	64.430 ± 0.6	1.031 ± 0.010
		120	69.727 ± 0.6	1.116 ± 0.011
5	90	30	55.107 ± 1.0	0.882 ± 0.017
		60	61.673 ± 0.8	0.987 ± 0.013
		90	66.497 ± 0.4	1.064 ± 0.007
		120	77.857 ± 0.5	1.246 ± 0.009
	120	30	74.537 ± 0.7	1.193 ± 0.011
		60	81.087 ± 0.6	1.297 ± 0.010
		90	93.203 ± 0.6	1.491 ± 0.011
		120	97.917 ± 0.5	1.567 ± 0.008

3.2 Fermentation

In the present study, dilute sulfuric acid hydrolysate was fermented by Fungus and bacterial species i.e. *Aspergillus Niger* FCBP-0198, *Lacto-bacillus* FCBP-0004 *and Escherichia coli* FCBP-001 to convert reducing sugars in bio-fuel. During fermentation of 72 h change in concentration of reducing sugars was calculated after every 24 hours as shown in Fig. 1. The amount of reducing sugars of KW fermented by *Aspergillus Niger, Lacto-bacillus* and *Escherichia coli* for 72 h showed that with the passage of time the amount of reducing sugars decreased. About 64% reducing sugar was converted into hydrocarbons after fermentation of KW by *Aspergillus Niger* while conversion of reducing sugar into hydrocarbons after fermentation of KW by *Lacto-bacillus* and *Escherichia coli* was 45% and 50% respectively as shown in Fig. 2. It indicates that *Aspergillus Niger* has more potential for conversion of reducing sugars in hydrocarbons as compare to *Lacto-bacillus* and *Escherichia coli. Overall*, maximum percentage of hydrocarbons from KW was produced after fermentation by *Aspergillus Niger.*

3.3 GC-MS analysis

After fermentation process samples were analyzed on GC-MS to identify and quantify the compound present in samples before and after fermentation by *Aspergillus Niger, Escherichia coli* and *Lacto bacillus.* Figure 2 shows the peaks of compounds in KW after and before fermentation. Nine different compounds were identified in KW before fermentation and 12 hydrocarbons were identified after fermentation. Figure 2 shows the compounds that were present in KW. These compounds are mostly hydrocarbons and can be used as biofuel. 5-8g of HC were produced per Kg of KW.

3.4 Calorific value of samples

Calorific values of sample before and after fermentation by different strains were determined. Table 3 shows the calorific value of KW before fermentation i.e. 0.6 MJ/kg and calorific value of KW fermented by *Aspergillus Niger, Escherichia coli* and *Lacto-bacillus* are 3.56, 3.34 and 2.67 MJ/kg. Maximum calorific value i.e. 3.56 MJ/kg was achieved by KW fermented by *Aspergillus Niger.* This calorific value of biofuel is before distillation process. So, this value will be improved after distillation.

Table-3: Quantification of Hydrocarbons produced in Biofuel from KW.

Peak I.D.	Compound	Absolute Concentration (g of HC/Kg of KW)
1	Methyl alcohol	6.76
2	Oxalic acid	5.07
3	2-Methyl Pentane	8.45
4	3-Methyl Pentane	6.76
5	2,2-Dimethyl Pentane	5.07
6	4-Methyl 1-Pentol	8.45
7	3,3-Dimethyl Pentane	6.76
8	2-Methyl Hexane	5.07
9	3-Methyl Hexane	8.45
10	2,2,3,3-Tetramethyl Butane	6.76
11	2,3-Dimethyl Hexane	5.07

3.5 Literature comparison

A literature comparison of biofuel calorific values of present study with reported literature values was also done [38] (Table-4). It shows the calorific values of biofuel fermented from different waste streams. Due to pre-treatment and acid hydrolysis, the production of biofuel in enhanced quite much which in indicated by the high calorific values of KW fermentation it is because the other waste was direct fermented to produce biofuel while for KW methodology was modified by doing pretreatment and acid hydrolysis that will increasing the chance for conversion of reducing sugars into biofuel.

Table-4: Calorific value of various samples imported in literature (Biofuels & Biomass 2007)

Raw material	Calorific value (MJ/kg)
Kitchen waste (Present Study)	3.56 (*A. Niger*)
	3.34 *(E. Coli)*
	2.67 *(L. Curvatus)*
Forest wood chip fresh	0.718
Miscanthus	1.580
Rape seed	2.453
Stover rapeseed	1.496
Sunflower	1.998
Wheat	1.496

Conclusions

The KW was used as source to produce biofuel. The acid hydrolysis produced maximum of 37.673g reducing sugars per each kg of KW under optimum conditions i.e. 5 % acid concentration for 2 hours at 120^{o}C. A maximum of 64% reducing sugars were converted into hydrocarbons (biofuel) after fermentation by *Aspergillus Niger*. GCMS analysis showed that the reducing sugars were converted into hydrocarbons having good calorific values i.e. 3.65MJ/Kg.

Declarations

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References

1. Goldemberg, J., *Ethanol for a sustainable energy future.* science, 2007. **315**(5813): p. 808-810.
2. Borines, M.G., R.L. de Leon, and J.L. Cuello, *Bioethanol production from the macroalgae< i> Sargassum</i> spp.* Bioresource technology, 2013. **138**: p. 22-29.
3. Vavouraki, A.I., E.M. Angelis, and M. Kornaros, *Optimization of thermo-chemical hydrolysis of kitchen wastes.* Waste Management, 2013. **33**(3): p. 740-745.
4. Balat, M., H. Balat, and C. Öz, *Progress in bioethanol processing.* Progress in energy and combustion science, 2008. **34**(5): p. 551-573.

5. Crabbe, E., et al., *Biodiesel production from crude palm oil and evaluation of butanol extraction and fuel properties.* Process biochemistry, 2001. **37**(1): p. 65-71.
6. Dizge, N. and B. Keskinler, *Enzymatic production of biodiesel from canola oil using immobilized lipase.* Biomass and Bioenergy, 2008. **32**(12): p. 1274-1278.
7. Ma, F., L.D. Clements, and M.A. Hanna, *Biodiesel fuel from animal fat. Ancillary studies on transesterification of beef tallow.* Industrial & engineering chemistry research, 1998. **37**(9): p. 3768-3771.
8. Zhang, Y., et al., *Biodiesel production from waste cooking oil: 1. Process design and technological assessment.* Bioresource technology, 2003. **89**(1): p. 1-16.
9. Talebnia, F., D. Karakashev, and I. Angelidaki, *Production of bioethanol from wheat straw: an overview on pretreatment, hydrolysis and fermentation.* Bioresource technology, 2010. **101**(13): p. 4744-4753.
10. Abbas, A. and S. Ansumali, *Global potential of rice husk as a renewable feedstock for ethanol biofuel production.* Bioenergy Research, 2010. **3**(4): p. 328-334.
11. Khola, G. and B. Ghazala, *Biodiesel production from algae.* Pak J Bot, 2012. **44**(1): p. 379-381.
12. Haydar, S. and J. Masood, *Evaluation of Kitchen Waste Composting and its Comparison with Compost Prepared from Municipal Solid Waste.* Pak. J. Engg. & Appl. Sci. Vol, 2011: p. 26-33.
13. Haydar, S. and J. Masood, *Evaluation of kitchen waste composting and its comparison with compost prepared from municipal solid waste.* Pakistan Journal of Engineering and Applied Sciences, 2016.
14. Uncu, O.N. and D. Cekmecelioglu, *Cost-effective approach to ethanol production and optimization by response surface methodology.* Waste management, 2011. **31**(4): p. 636-643.
15. Karmee, S.K. and C.S.K. Lin, *Valorisation of food waste to biofuel: current trends and technological challenges.* Sustainable Chemical Processes, 2014. **2**(1): p. 1-4.
16. Hong, Y., et al. *Ethanol Production from Food Wastes*. in *The 31st Symposium on Biotechnology for Fuels and Chemicals*. 2009.
17. Adhikari, B.K., S. Barrington, and J. Martinez, *Predicted growth of world urban food waste and methane production.* Waste Management & Research, 2006. **24**(5): p. 421-433.
18. Palmisano, A.C. and M.A. Barlaz, *Microbiology of solid waste*. Vol. 3. 1996: CRC press.
19. Hong, Y.S. and H.H. Yoon, *Ethanol production from food residues.* Biomass and Bioenergy, 2011. **35**(7): p. 3271-3275.
20. Matsakas, L., et al., *Utilization of household food waste for the production of ethanol at high dry material content.* Biotechnology for biofuels, 2014. **7**(1): p. 1-9.
21. Sun, Y. and J. Cheng, *Hydrolysis of lignocellulosic materials for ethanol production: a review.* Bioresource technology, 2002. **83**(1): p. 1-11.
22. Esteghlalian, A., et al., *Modeling and optimization of the dilute-sulfuric-acid pretreatment of corn stover, poplar and switchgrass.* Bioresource Technology, 1997. **59**(2): p. 129-136.

23. McMillan, J.D. *Pretreatment of lignocellulosic biomass*. in *ACS symposium series (USA)*. 1994.
24. Del Campo, I., et al., *Diluted acid hydrolysis pretreatment of agri-food wastes for bioethanol production.* Industrial Crops and Products, 2006. **24**(3): p. 214-221.
25. Wijaya, Y.P., et al., *Comparative study on two-step concentrated acid hydrolysis for the extraction of sugars from lignocellulosic biomass.* Bioresource technology, 2014. **164**: p. 221-231.
26. Limayem, A. and S.C. Ricke, *Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects.* Progress in energy and combustion science, 2012. **38**(4): p. 449-467.
27. Rocha, N.R.d.A.F., et al., *Ethanol production from agroindustrial biomass using a crude enzyme complex produced by Aspergillus niger.* Renewable Energy, 2013. **57**: p. 432-435.
28. Dien, B.S., et al., *Development of new ethanologenic Escherichia coli strains for fermentation of lignocellulosic biomass.* Applied biochemistry and biotechnology, 2000. **84**(1): p. 181-196.
29. Sakai, K. and T. Yamanami, *Thermotolerant Bacillus licheniformis TY7 produces optically active L-lactic acid from kitchen refuse under open condition.* J Biosci Bioeng, 2006. **102**(2): p. 132-134.
30. Akao, S., H. Tsuno, and J. Cheon, *Semi-continuous L-lactate fermentation of garbage without sterile condition and analysis of the microbial structure.* Water Res, 2007. **41**(8): p. 1774-1780.
31. Yan, S., et al., *Enzymatical hydrolysis of food waste and ethanol production from the hydrolysate.* Renewable Energy, 2011. **36**(4): p. 1259-1265.
32. Sakai, K., et al., *Selective proliferation of lactic acid bacteria and accumulation of lactic acid during open fermentation of kitchen refuse with intermittent pH adjustment.* Food science and technology research, 2007. **6**(2): p. 140-145.
33. Shin, H.-S., J.-H. Youn, and S.-H. Kim, *Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis.* International Journal of Hydrogen Energy, 2004. **29**(13): p. 1355-1363.
34. Miller, G.L., *Use of dinitrosalicylic acid reagent for determination of reducing sugar.* Analytical chemistry, 1959. **31**(3): p. 426-428.
35. Jalees, M.I., F. Tahira, and H. Saleem, *Study on the geochemical correlation of crude oils of Palaeocene and Jurassic ages from the Potowar Indus Basin in northern Pakistan.* Chinese Journal of Geochemistry, 2010. **29**(1): p. 82-93.
36. Kumar, J.V. and B.C. Pratt, *Determination of calorific values of some renewable biofuels.* Thermochimica Acta, 1996. **279**: p. 111-120.
37. Ma, H., et al., *The utilization of acid-tolerant bacteria on ethanol production from kitchen garbage.* Renewable Energy, 2009. **34**(6): p. 1466-1470.
38. Biofuels & Biomass. *biofuelsb2b*. 2007 [cited 2016 June 6,]; Available from: http://www.biofuelsb2b.com/.
39. Gupta, V., V.V. Thakur, and R. Jain, *Dilute acid hydrolysis of rice straw for efficient recovery of xylose.* J. Env. Res., 2016. **4**: p. 307-311.

40. Jain, R.K., et al., *Ethanol production from rice straw using thermotolerant Kluyveromyces sp. IIPE453.* Biomass Conversion and Biorefinery, 2015. **5**(4): p. 331-337.
41. Kshirsagar, S.D., et al., *Dilute acid pretreatment of rice straw, structural characterization and optimization of enzymatic hydrolysis conditions by response surface methodology.* RSC Advances, 2015. **5**(58): p. 46525-46533.

Figures

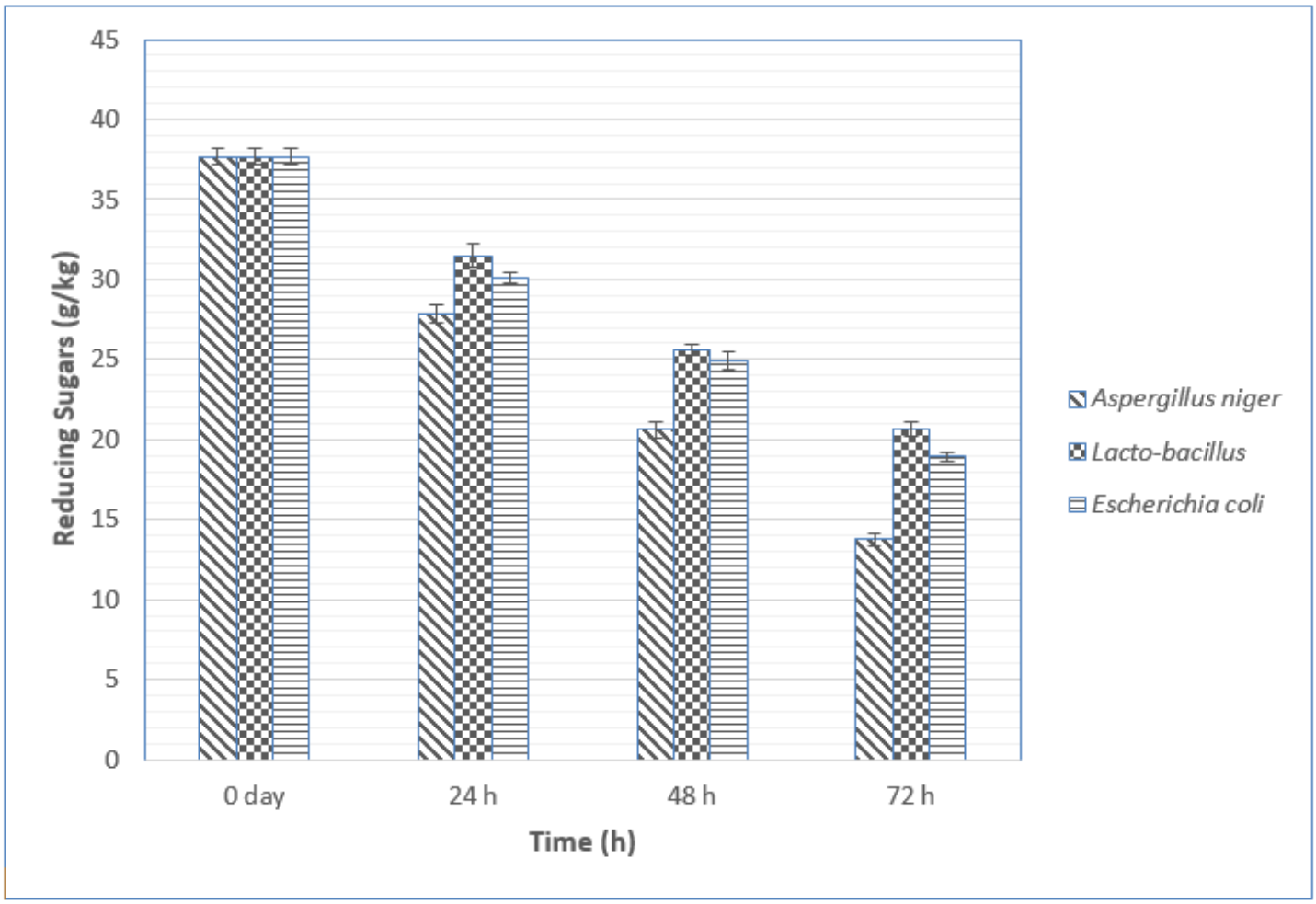

Figure 1

1 Reducing sugars during 72 h of fermentation of KW by Aspergillus niger, Lacto-bacillus and Escherichia coli

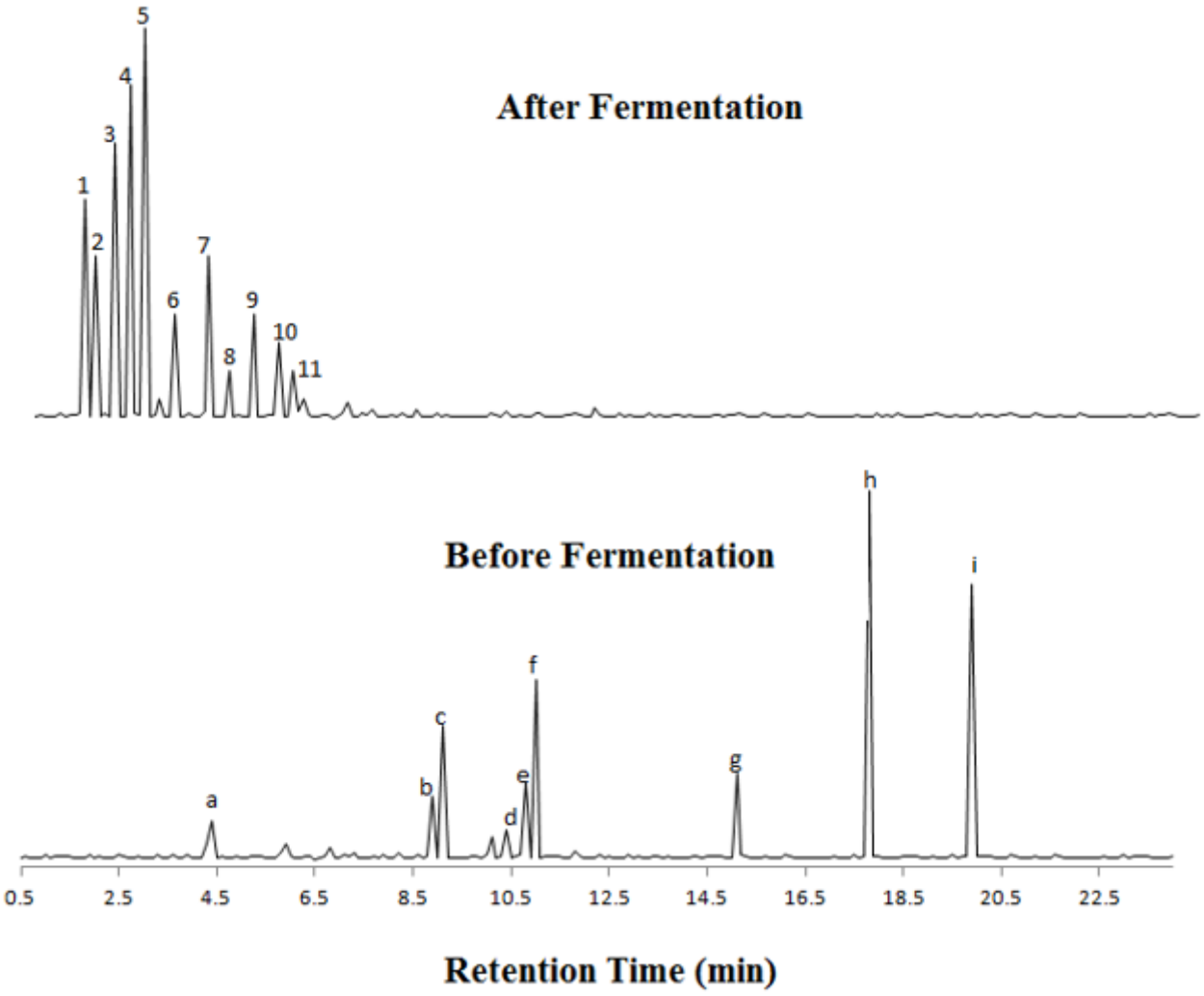

Figure 2

GCMS Analysis of fermentation products of Aspergillus niger used for Sample-1. Peaks are labeled i.e. a: furfural; b: 2,5-Furan dicarboxaldehyde; c: Levoglucosenone; d: 2,4-Decadieral (R); e: 2,4-Decadieral (S); f: 2-Furancarboxaldehyde; 5-(hydroxymethyl); g: D-Allose, h; n-Hexadecanoic acid; i: Octadec-9-enoic acid; 1: Methyl alcohol; 2: Oxalic acid; 3: 2-Methyl Pentane; 4: 3-Methyl Pentane; 5: 2,2-Dimethyl Pentane; 6: 4-Methyl 1-Pentol; 7: 3,3-Dimethyl Pentane; 8: 2-Methyl Hexane; 9: 3-Methyl Hexane; 10: 2,2,3,3-Tetramethyl Butane; 11: 2,3-Dimethyl Hexane