Biodegradation (2005) 16: 493–499

Bioconversion of lignocellulosic waste from selected dumping sites in Dar es Salaam, Tanzania

Godliving Mtui[1] & Yoshitoshi Nakamura[2,*]
[1]*Applied Microbiology Unit, Department of Botany, University of Dar es Salaam, P. O. Box 35060, Dar es Salaam, Tanzania;* [2]*Graduate School of Natural Science & Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan (*author for correspondence: e-mail: ynakamu@t.kanazawa-u.ac.jp)*

Accepted 4 November 2004

Key words: bioconversion, ethanol, glucose, lignocellulose, solid wastes

Abstract

The poor management of solid wastes in Tanzania urban centers is a chronic problem that has increasingly become a source of environmental pollution. Bioconversion offers a cheap and safe method of not only disposing these wastes, but also it has the potential to convert lignocellulosic wastes into usable forms such as reducing sugars that could be used as food. This paper reports a preliminary study on the physical characteristics, acid pretreatment, saccharification by cellulase from *Trichoderma reesei* and fermentation by *Saccharomyces cerevisiae* of the lignocellulosic component of the solid wastes collected from various dumping sites located in Kinondoni Municipality, Dar es Salaam city. The results showed that overall, the lignocellulosic component constitute about 50% of solid wastes dumped in the study areas. Maximum production of reducing sugars was obtained after 6 h of saccharification while highest concentrations of bioethanol were achieved after 48 h of fermentation. Microbial bioconversion of lignocellulose component yielded up to 21% bioethanol.

Introduction

The dwindling fossil fuel resources and their increasing prices have led to a worldwide search for alternative energy resources. Lignocellulosic materials represent the largest reservoir of potentially fermentable carbohydrates on the earth. It is estimated that approximately 50 billion tons of cellulose and hemicellulose are produced worldwide (Bruce 1985). Due to this abundance and renewability, there has been a great deal of interest in utilizing cellulose as energy resource and as feedstock to material production processes. One of the promising routes to utilizing cellulose for such a purpose is bioconversion processes such as enzymatic hydrolysis to glucose that can subsequently be converted to ethanol. This microbiologically mediated process is a selective, specific and nontoxic natural process which pose less hazards to the environment (Kamley et al. 1990).

However, the enzymatic degradation rate of lignocellulosic materials is low due to the resistant crystalline structure of cellulose and the physical barrier formed by lignin that surrounds the cellulose. Pretreatment, therefore, is an essential prerequisite to disrupt the lignin seal and thereby enhancing the susceptibility of lignocellulosic materials to enzyme action. Efficient pretreatment reduces the lignin content, cellulose crystallinity and increase the surface area for enzymatic reactions (Millett et al. 1975; Mtui 2000). Pretreatments can be classified into mechanical, physical and chemical methods. Mechanical method involves the size reduction of lignocellulosic materials by chopping, grinding and milling while physicochemical methods such as steam explosion, aqueous alkali swelling and acid hydrolysis disrupt the crystalline structure and break down the chemical bonds of the long chain molecules in order to increase susceptibility to enzymatic hydrolysis and microbial conversion (Cowling & Kirk 1976). Processes capable of efficiently converting cellulose and hemicellulose hydrolyzates to reducing sugars and subsequent fermentation to alcohol utilize a wide range of microbial enzymes. Microorganisms capable of converting polysaccharides to monossacharides include cellulase-producing *Trichoderma reesei* and *Trichoderma viride* while S*accharomyces cereviciae* and *Zymomonas mobilis* are favored for ethanol production from reducing sugars (Ramos & Saddler 1994; Stanbury & Whitaker 1989).

Tanzania is faced with an acute problem of poor management of solid wastes in its urban centers resulting to filthy towns and widespread outbreak of infectious diseases. Since lignocellulose constitute a considerable fraction of the solid wastes littering the Dar es Salaam suburbs, microbial bioconversion of these wastes offers a dual solution: it ensures environmental cleanup and production of useful materials. Currently, very little information is known about the status of lignocellulosic waste in Dar es Salaam, and no bioconversion research has been done on this subject. Therefore, it is necessary for the environmental protection in Tanzania to elucidate the composition and biodegradability of lignocellulosic wastes of urban Tanzania because these results give baseline data upon which more thorough research can be conducted.

This study focused on collection of solid wastes from selected Dar es Salaam dumping sites to determine their lignocellulose content; pretreatment of the lignocellulose fraction by using mechanical, physical and chemical methods; and enzymatic saccharification and fermentation to obtain sugar and bioethanol.

Materials and methods

The collection of solid wastes was done in Kinondoni municipality, Dar es Salaam region at different sites including University of Dar es Salaam (UDSM), Sinza, Mwenge, Manzense, and Ubungo as shown in Figure 1. Sampling was done at dumping sites of Kinondoni district because it is a focal point of a Government proposal to launch Project on 'Bioconversion of Lignocellulose Waste to produce Renewable Energy' (bioethanol and biogas). In each sampling location, five samples were taken. The samples were brought to the laboratory, accurately weighed into 100 g portions each and then lignocellulosic waste was sorted out from non-lignocellulosic ones. 'Lignocellulosic waste' was defined as being all wastes of plant origin including sawdust, waste paper, hay, straws, bagasse, vegetables fruit remains and alike. The lignocellulosic wastes were shredded to small-size particles and then crushed by a grinding miller to powder form in order to increase its susceptibility to chemical pretreatment. Each sample was analyzed separately and the average results were presented for each location.

In physicochemical pretreatment, 1 g of the sample from each sampling location was dissolved in 980 ml distilled water and then 20 ml of 0.5 M sulfuric acid was added. After mixing thoroughly, 50 ml of this mixture was pipetted into a 100 ml conical flask and then autoclaved at 120 °C for 15 min at 1 atm. The pH was maintained at 5.0. After cooling, saccharification was done by adding 10 mg cellulase enzyme extracted from *Trichoderma reesei* and the mixture was incubated at 55 °C for 8 h. Sampling was carried out at 2 h intervals. Reducing sugar concentration was determined by dinitrosalicylic acid (DNSA) reagent method (Miller 1959). Glucose was determined spectrophotometrically at 570 nm. Glucose standard curve was drawn upon which the reducing sugar content of the samples was read.

Fermentation of the reaction mixture containing reducing sugar was carried out by using *Saccharomyces cerevisiae* at an incubation temperature of 35 °C. Sampling was done after 24, 48 and 72 h of incubation. Ethanol concentration was determined by gas chromatography (GC) using 120/80 6.6% carbowax column. The GC was set at 90 °C oven temperature, 170 °C injection temperature and 175 °C detection temperature. Standards were prepared by using 1 g methanol, 5 g ethanol, 1 g propanol, 1 g butanol, and 2 g iso-butyl alcohol. Distilled water was added into 120 ml serum bottle to make up a total of 100 g. Ethanol concentration was calculated based on the peak areas of the samples and standard solutions (Ramos & Saddler 1994).

Results and discussion

The overall composition of Kinondoni municipal solid waste is given in Table 1. The lignocellulosic

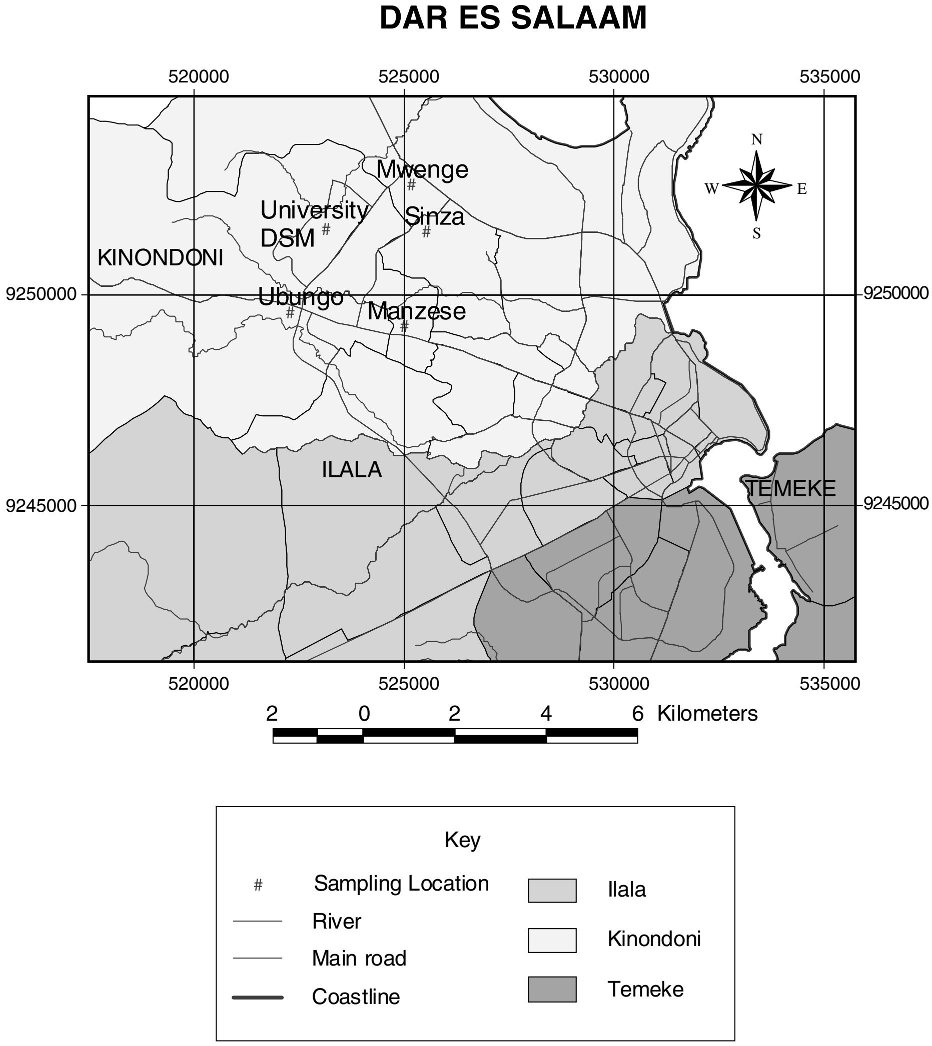

Figure 1. Site map showing the location of solid waste sampling points at selected dumping sites in Kinondoni Municipality, Dar es Salaam, Tanzania.

waste was conspicuously present in all the studied locations. Non-lignocellulosic residential trash consisted of metallic materials, plastics, rubber, organics (foodstuff remains, decomposed matter), and other unidentifiable matter. The data obtained in Table 1 is comparable to Western Europe and North American big cities where the residential municipal solid waste consists of an averaged 37% lignocellulosics (Wayman & Parekh 1990).

Percentage weight composition of non-lignolcelluosics versus lignocellulosics from different sampling locations are given in Figure 2. Lignocellulosic materials constituted the bulk fraction of the solid waste at the University of in Dar es Salaam dumping sites due to the nature of the wastes being mainly waste paper, leaf litter and other plant residues. In Sinza, the percent weight composition of lignocellulosic and non-lignocellulosic materials were equal while in Mwenge it was found that only 16% of the solid waste was composed of lignocellulosic materials with high content of sawdust. Manzese had 18% lignocellulosic materials, mainly fruit residues and vegetable waste. Lowest values of lignocellulose (14%) were recorded at Ubungo sampling site. With exception to UDSM and Sinza, the percent

Table 1. Percent weight composition of overall solid wastes in Kinondoni Municipality, Dar es Salaam, Tanzania

	UDSM	Sinza	Mwenge	Manzese	Ubungo
Lignocellulose	93	50	16	18	14
Metals	0.2	12	21	28	24
Plastics	2	11	12	9	16
Organics	2	12	28	26	21
Glass	1	5	8	8	6
Rubber	1	8	11	7	9
Others	0.8	2	4	4	10

composition of lignocellulosics dumped in Kinondoni Municipality is rather uniform, suggesting that the data can represent the overall status of the waste of lignocellulose nature in the whole of Dar es Salaam city.

The amounts of reducing sugar (measured as glucose concentration) produced in each respective sites based on 1 g of pretreated lignocellulosic hydrolyzate are presented in the Figure 3. Overall, negligible amounts of reducing sugar were present before saccharification reactions. The values increased with increasing saccharification time and peaked after 6 h of incubation. The peaking of glucose concentration at 6 h could be attributed to depletion of the hydrolysable polysaccharides (Stanbury & Whitaker 1989), while the reason behind the decrease in glucose concentration after 6 h of incubation is unclear. Therefore, this point is a future research subject. Solid waste samples collected at dumping sites of the University of Dar es Salaam recorded the highest glucose concentration (maximum value was 0.13 g/L) followed by samples from Sinza with 0.1 g/L maximum glucose. Mwenge and Manzese lignocellulosic wastes produced 0.06 g/L and 0.07 g/L glucose, respectively. Ubungo sampling sites gave the lowest values (0.05 g/l) glucose.

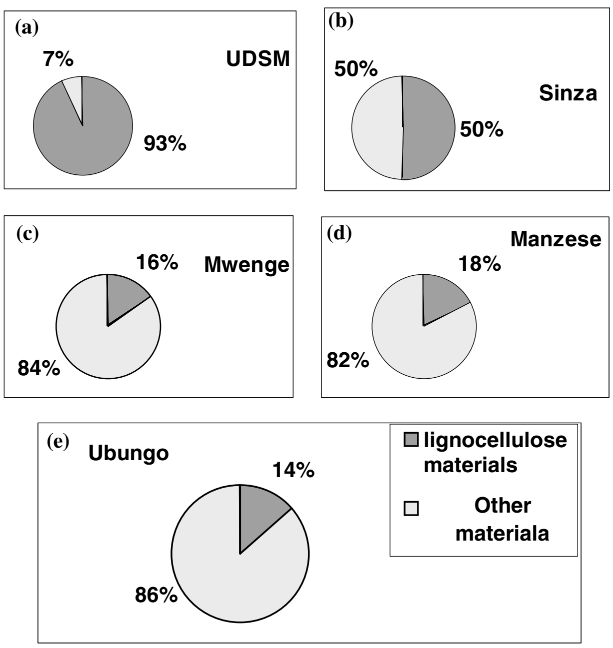

Figure 2. Percent dry weight composition of solid waste collected from different locations in Dar es Salaam, Tanzania (based on 100 g dry weight of solid waste randomly sampled).

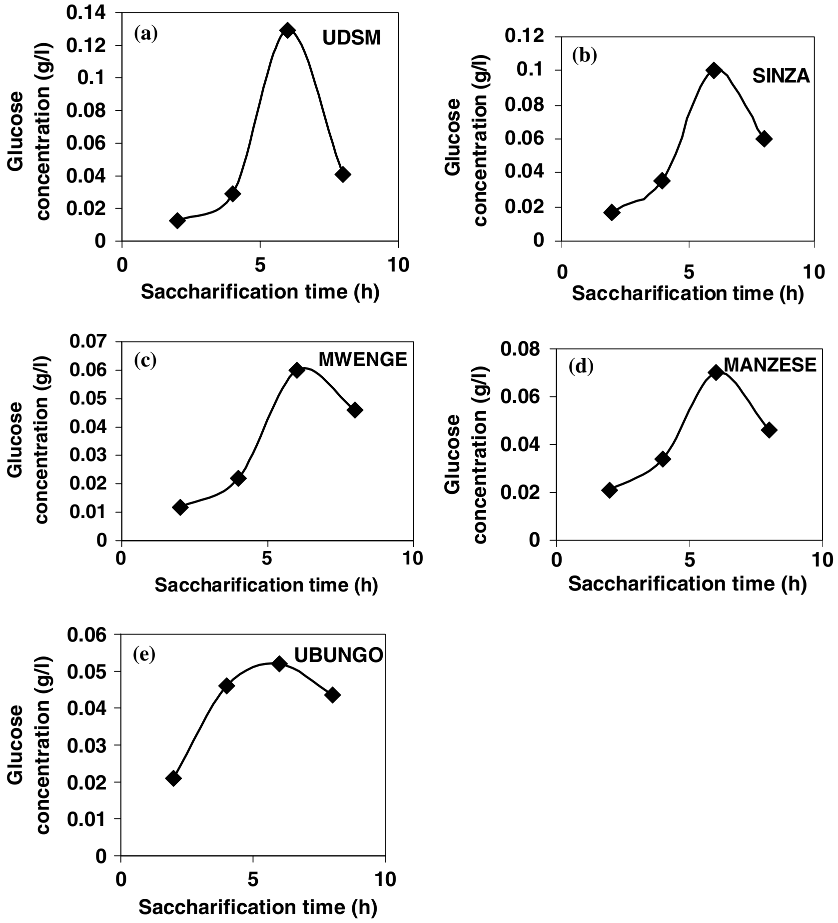

Figure 3. Time courses of glucose production from lignocellulosic waste collected from various locations in Kinondoni Municipality, Dar es Salaam, Tanzania (Enzyme used: Cellulase; Saccharfication temperature: 55 °C).

From the results, it is evident that the values of glucose produced varied from one sampling location to another, mainly due to the nature of the solid wastes dumped. UDSM samples, which had the highest fraction of ligocellulose as shown in Figure 2, produced more than twice the amount of glucose compared to Ubungo sites whose solid waste constituted least lignocellulosic materials. This observation underscores the importance of using wastes rich in lignocellulose in order to achieve high rates of bioconversion products.

Results on bioethanol production from saccharified hydrolyzates are shown in Figure 4. In all glucose hydrolyzate samples, ethanol concentration increased steadily and peaked after 48 h of incubation and then decreased, apparently due to ethanol being oxidized to aldehydes and carboxylic acids (Stanbury & Whitaker 1989). Maximum ethanol production of 0.15 g/L was obtained from lignocellulosic waste collected at UDSM followed with Sinza (0.1 g/L); Manzese (0.07 g/L); Mwenge (0.55 g/L) and Ubungo (0.05 g/L). In some cases, some ethanol concentration values exceeded the amounts of glucose recorded in Figure 3 due to the fact that the DNSA method is unable to detect all the fermentable sugars present in the hydrolyzates (Miller 1959). Considering that the total hydrolysable polysaccharide fraction contained in lignocellulosic waste is about 70% (Fan & Lee 1980; Fan et al. 1985), the maximum conversion of UDSM samples was 21.45% (w/v). This conversion rate is comparable to the literature values obtained from fermentation of polysaccharide substrates although higher conversion rates have been reported by McMillan et al. (1994) when using detoxified and medium-enriched substrates were used. It is

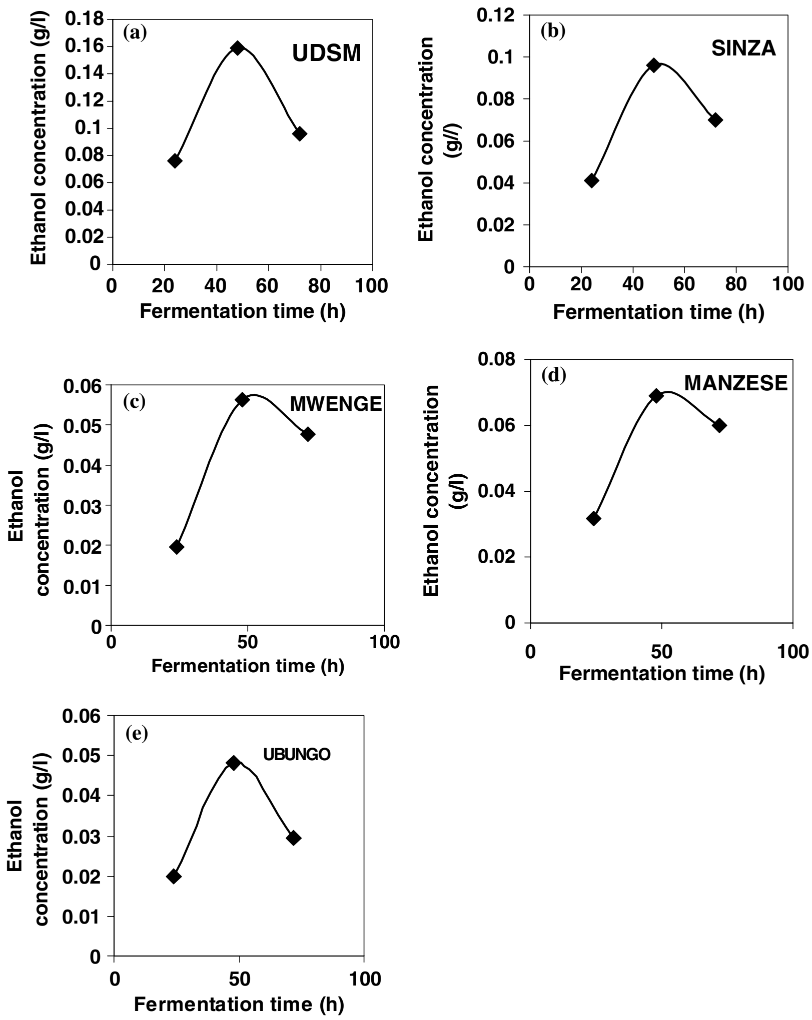

Figure 4. Time courses of ethanol production from lignocellulosic waste collected from various locations in Kinondoni Municipality, Dar es Salaam, Tanzania (Yeast used: *Saccharomyces cerevisiae*; Incubation temperature: 35 °C).

noteworthy that, other alcohols like methanol, propanol, and butanol were produced in a trace amounts. Future research will focus on the optimization of the bioconversion processes and the use of bioengineered microorganisms described by Ramos and Saddler (1994) in the improvement of bioconversion of lignocellulosics.

Conclusion

Environmental management of urban solid wastes demands that sustainable and affordable technologies be applied. This study highlighted the potential of microbial biotechnology to enhance bioconversion of holocellulose waste to sugars and biofuels thus solving the problem of environmental pollution, and at the same time, producing biofuels that are renewable sources of energy. Furthermore, this work provides the basic information for future research on scale-up experimentation for attainment of large-scale applications. In addition, these preliminary findings will assist in the feasibility study of the Government's Project on the bioconversion of solid waste in Dar es Salaam City for bioethanol and biogas production. The proposed Bioethanol/biogas project will not only reduce waste volumes in the city, but it will also alleviate the dependency of charcoal as fuel which has led to serious problem of deforestation. Future studies will also focus on more elaborate research involving intensive sampling and statistical analyses, and it will extend to Ilala and Temeke which other Dar es Salaam administrative districts.

Acknowledgements

The author expresses sincere thanks to Mr. G. Meliyo for his assistance in data collection and analyses. Profound appreciation goes to the technical staff of the Applied Microbiology Unit, University of Dar es Salaam and the Directorate of Traditional Medicine of the Muhimbili University College of Health Sciences for technical support.

References

Bruce E (1985) Annual Reports on Fermentation Process, Vol 8 (pp 299–323). Academic press Inc. Department of Agriculture and Chemical Engineering Colorado State University, USA

Cowling EB & Kirk TK (1976) Properties of cellulose and lignocellulosic materials as substrates for enzymatic conversion processes. Biotechnol. Bioeng. Symp. 6: 95–123

Fan LT & Lee YH (1980) The nature of lignocellulosics and their pretreatment for enzymatics hydrolysis. Biotechnol. Bioeng. 22: 177–186

Fan LT, Young HL & Gharpurai MM (1985) The Nature of Lignocellulosics and their Pretreatments for Enzymatic Hydrolysis (pp 156–157). Johm Wiley & Sons, Inc. New York, USA

Kamely D, Chakrabarty A & Omenn GS (1990) Biotechnology and Biodegradation. Adv. Biotechnol. Series 4. Gulf publishing Co. Tokyo

McMillan JD (1994) Conversion of hemicellulose hydrolyzates to ethanol. In: Enzymatic conversion of biomass for fuel production. ACS Symp. Ser. 566: 410–435

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426–428

Millett MA, Baker AJ & Satter LD (1975) Pretreatment to enhance chemical, enzymatic and microbial attack of cellulose materials. Biotechnol. Bioeng. Symp. 5: 193–219

Mtui GYS (2000) Production of sugars & ethanol from steam exploded rice straw. Proc. scientific conference on research capacity profiles. Faculty of Science, University of Dar es Salaam, Tanzania

Ramos LP & Saddler JN (1994) Bioconversion of wood residue: mechanisms involved in pretreating and hydrolyzing lignocellulosic materials. ACS Symp. Ser. 566: 325–341

Stanbury PF & Whitaker A (1989) Principles of Fermentation Technology (pp 255–256). Pergamon Press, Oxford, UK

Wayman M & Parekh S (1990) Biotechnology of Biomass Conversion: Fuels and Chemicals from Renewable Sources. In: Biotechnology Series, 30 Vol (pp 40–71). Open University Press, UK