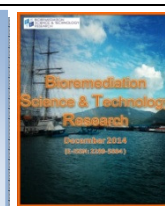




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The Effect of Substrate Concentration on Enzymatic Hydrolysis of Selected Food Waste for Glucose Production

Micky Vincent*, Noor Amalina Mahamad Norrezi, Kopli Bujang, and Dayang Salwani Awang Adeni

Department of Molecular Biology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak

*Corresponding Author: vmicky@frst.unimas.my
Department of Molecular Biology,
Faculty of Resource Science and Technology,
Universiti Malaysia Sarawak,
Sarawak, Malaysia.
Tel: +6082 582985;
Mobile: +6019 8537211

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ABSTRACT

Glucose is an important commodity for various domestic and industrial applications, ranging from the manufacturing of food and pharmaceuticals to biofuel production. It is therefore imperative to explore novel sources of glucose that are easy to process, cheap and environmentally friendly. In this study, food wastes collected from restaurants and cafeteria within Universiti Malaysia Sarawak (UNIMAS) campus were used as feedstock for glucose production. Several feedstock loadings were investigated (7%, 10%, 20% and 30% w/v) in the enzymatic hydrolysis processes that were carried out at pH 5.0, 100 rpm and 58 ± 2 °C for 24 h using glucoamylase and crude protease preparations. Results showed that substrate concentration of 20% (w/v) in batch hydrolysis experiments has the highest glucose production at 140 g/L, with 46.20% glucose recovery. The highest amount of substrate being hydrolyzed (68.35%) within 20 h was also recorded in the same experimental set. We conclude that substrate concentration or feedstock loading during enzymatic hydrolysis is a very crucial parameter for optimal glucose production and recovery from carbohydrate rich food wastes.

INTRODUCTION

Food waste from households and restaurants is a major component of municipal solid waste [1]. In a recent study by Pleisser and Lin (2013) [2], it was reported that approximately 1.3 billion tonnes of untreated food waste is deposited at numerous landfill sites. Based on the current (2014) estimates, approximately 100 million tons of food are wasted annually in the eu alone, and this is expected to rise to about 126 million tonnes by the year 2020 [3].

Food waste contains high concentration of organic compounds, especially carbohydrate rich wastes in the forms of starchy and cellulosic materials [4,5]. Although several treatments such as composting, animal feed supply, incineration and landfilling are conducted in more modern facilities in attempts

to manage excess food waste, these practices fail to exploit the full potential of these organical residues. These organic wastes can serve as cheap feedstocks for the production of simple sugars such as glucose, maltose and cellobiose. Of these sugars, glucose is probably the most flexible starting material. It is an important monosaccharide for multiple domestic and industrial applications, ranging from the manufacturing of food and pharmaceuticals to biofuel production [6,7,8]. Glucose is also an ideal substrate for single cell protein production as most microorganisms would be able to utilize this monosaccharide, and there would be fewer problems with undesirable or toxic residues [8]. In short, cheap glucose produced from food waste that is easy to process and environmentally friendly would find an eager market.

Glucose can be liberated from the polysaccharides in food waste or agricultural residues via enzymatic hydrolysis [9,10,11]. Enzymatic hydrolysis of complex polysaccharides are widely practiced due to its low capital cost, made possible by the reduction of enzyme production [12]. According to Ogier et al. [13], it is possible to obtain cellulose hydrolysis close to 100% by enzymatic hydrolysis. Furthermore, there will not be a severe inhibitory compound in enzymatic hydrolysis [14].

To achieve optimum and favorable glucose production, the enzymatic hydrolysis must be carried out under mild conditions, as the enzymes act at moderate pH and temperature [1]. More importantly, the concentration of enzymes used and the substrate loading have to be optimized. Substrate concentration is one of the main factors that affect the yield and initial rate of enzymatic hydrolysis of complex polysaccharides [10]. Low substrate concentrations normally result in higher yields and reaction rates of the hydrolysis [15]. However, the overall process may not be economical. On the other hand, high substrate loading usually results in inhibition, lowering hydrolysis rate [16]. Therefore, the present study was performed to investigate the effects of substrate concentration on enzymatic hydrolysis of selected food waste that influence glucose production and recoveries, as substrate concentration is a very crucial parameter that affect the yield and initial rate of enzymatic hydrolysis.

MATERIALS AND METHODOLOGY

Food waste collection and pretreatment

Fresh food waste was collected from several cafeterias within the Universiti Malaysia Sarawak (UNIMAS) campus. The food waste was sorted out manually by separating the hard non-carbohydrate solids (bones and shells), mixed together as a composite sample and stored at -20 °C until further use.

Food waste pH and moisture content analysis

Prior to enzymatic hydrolysis of the food waste, initial analysis was carried out to determine the pH and moisture content of the food waste. The food waste pH was determined using a pH meter (MW100, Milwaukee Instruments Inc., USA) while the moisture content was determined via hot oven drying at 105 °C for 3 days. Then, it was cooled to room temperature in a desiccator and weighed again. The process was repeated until constant weight was achieved, and, thus making the food waste solids free of moisture [16]. Moisture content was calculated as follows:

% moisture content =

$$\frac{W1 - W2}{W} \times 100\%$$

Where:

W= Weight of the initial sample, g

W1= Weight of the sample + container before drying, g

W2= Weight of the sample + container after drying, g

Batch enzymatic hydrolysis of food waste

After the drying process, the food waste was ground into fine powder to be used as substrate for batch enzymatic hydrolysis. In batch enzymatic hydrolysis, four different substrate concentrations 7%, 10%, 20% and 30% (w/v) in a final volume of 100 ml working volume were prepared. Then, the enzymes were added

simultaneously at 10% (v/w) enzyme per substrate loading to the 7, 10, 20, and 30 g of food waste solids, respectively. The enzymes used in this study were amyloglucosidase (AMG, Novozyme, USA) and in-house crude protease preparations. The enzymatic hydrolyses were performed in an orbital incubator shaker (Gyromax™ 708, Amerex Instruments Inc., USA) at constant pH 5.0, 100 rpm and 58 ± 2 °C for 24 hours. The pH was adjusted using 2 M sodium hydroxide (NaOH).

Collection of samples

Samples were taken at intervals of 4 h in the first 16 h at 0, 4, 8, 12 and 16 h, and every 2 hours for the next 8 hours. For each process, 2.0 ml of sample was pipetted aseptically from the second layer of the settled hydrolysate and transfer into sterile 2.0 ml tubes. All the tubes were labeled accordingly and centrifuged at 14,000 rpm for 10 minutes. Then, the aliquot from the centrifuged samples were filtered using 0.2 µm syringe filters and was kept at -20 °C prior to further analyses.

Glucose analysis

Glucose concentrations were determined by using a glucose analyzer (Biosensor BF-5, Oji Scientific Instrument, Japan). This was done by initially diluting the samples 100 times before injecting them into the analyzer with the running buffer (pH 5 to pH 7). The results from analyzer were shown in percentages and were then converted to glucose concentrations.

RESULTS AND DISCUSSION

The initial part of this study was to determine the pH and moisture content of the food waste. The pH of the selected food waste mixtures were acidic, at approximately pH 4.56. This was due to the presence of considerable amount of acidified food residues. Moisture content of the selected food waste was 71.03%, indicating that only 28.97% were actually solid components. These characteristics were very similar to other works reported elsewhere [4, 17]. The dried food solids were then subjected to enzymatic hydrolysis at four different substrate concentrations of 7%, 10%, 20% and 30% (w/v) for glucose production. At 7% (w/v) food waste loading, there was a drastic increase in the glucose concentration from 0 to 25 g/L between 0 to 12 h, as shown in Fig. 1. At 22 h, glucose production was the highest (31 g/l) and plateauing thereafter. Undigested food solid was recorded at 4.12 g (Table 1), indicating that less than half (41.14%) of the food waste was hydrolyzed (Fig. 2).

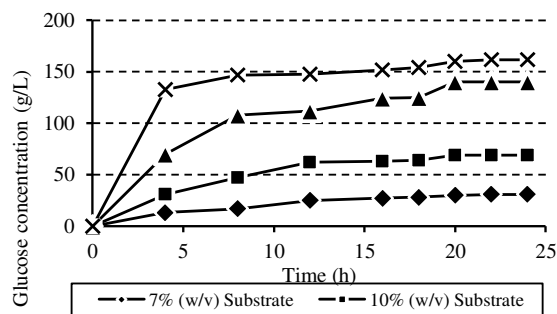


Fig. 1. Batch enzymatic hydrolysis profile of glucose concentration on different substrate concentration.

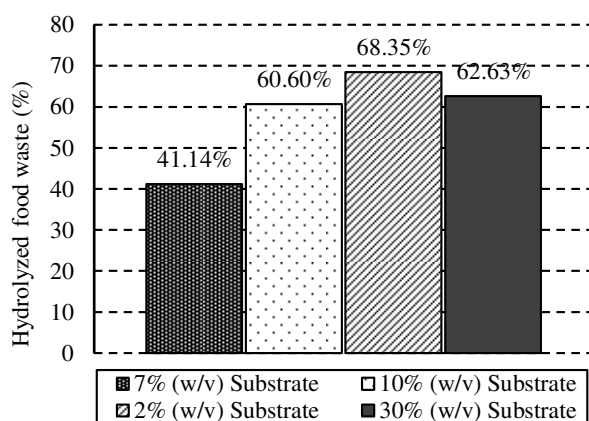


Fig. 2. Percentages of hydrolyzed food waste on different substrate concentration in batch enzymatic hydrolysis.

Table 1. Tabulated data of solid and liquid separation on different substrate concentration of enzymatic hydrolysis of food waste.

Substrate Concentration (w/v)%	Solid and Liquid Separation	
	Solid Residue (g)	Liquid (ml)
7	4.12	75
10	3.94	73
20	6.33	66
30	11.21	57

The drastic increase in the glucose concentration in the first 12 h (0 to 62 g/L) was also observed in the enzymatic hydrolysis of 10% (w/v) loading. From **Fig. 1**, peak glucose production (69 g/L) was seen at 20 h. Hydrolyzed food waste was calculated to be at 60.60%, with 39.40% (3.94 g) residual solids.

The glucose production profiles were different in the experiments with 20% and 30% (w/v) food substrate. For the experiment with 20% and 30% (w/v) substrate loading, the significant increase in glucose production was seen as early as at 8 h, at 108 and 147 g/L, respectively.

Fig. 1 shows that glucose production continued at a steady rate and plateaued after 20 h for the enzymatic hydrolysis with 20% (w/v) substrate, with undigested solid remaining at 6.33 g (31.65%). As for the enzymatic hydrolysis of 30% (w/v) substrate concentration, glucose production peaked at 22 h (162 g/L). At the end of the experiment, approximately 62.63% of the food waste solid was recorded to be hydrolyzed.

Next, glucose recoveries from the fermentation broths were tabulated. Based on **Fig. 3** the glucose recoveries for all substrate concentrations show steady increment. From 0 to 12 h of hydrolysis, the graph shows exponential pattern of recoveries, with slight increment thereafter. After 20 h, the recovery process started to become constant. In comparison with the four substrate load, glucose recovery of 10% and 20% (w/v) substrate concentration showed the highest glucose recoveries at 50.37% and 46.20%, respectively.

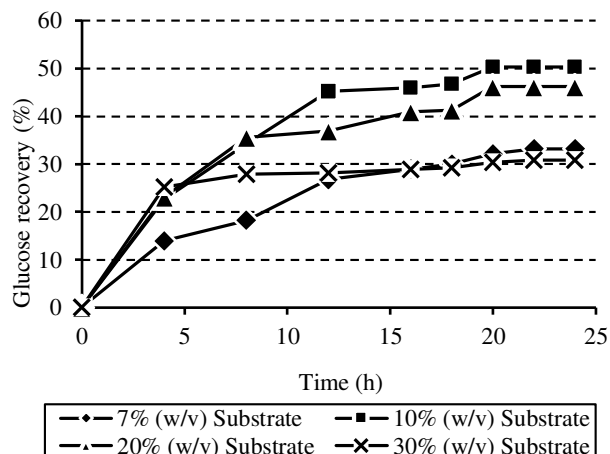


Fig. 3. Glucose recovery profile of different substrate concentration in batch enzymatic hydrolysis.

Efficient conversion of food waste depends mainly on the rate of carbohydrate saccharification and it is well known that higher rates of enzymatic hydrolysis occur at low substrate concentration in batch operation system. However, low substrate concentration would yield low concentrations of sugars [18]. Besides, low substrate concentration would increase both the capital cost of equipment and the operation costs in order to reach a certain production capacity. Therefore, high substrate concentration is more preferable and economically practical than low substrate concentration. However, high substrate concentration can cause substrate inhibition, which substantially lowers the rate of the hydrolysis, and the extent of substrate inhibition depends on the ratio of total substrate to total enzyme [16]. High substrate loading also greatly reduces the amount of free water present for enzymatic hydrolysis to occur. Therefore, the amount of water content is very important in the hydrolysis to enhance the interaction between enzyme and substrate, and, to act as enzyme transport mechanisms throughout the hydrolysis reactions.

High substrate concentration is also detrimental towards glucose recoveries. This is seen in the experiment with 30% (w/v) as shown in **Fig. 3**, whereby enzymatic hydrolysis only resulted in relatively small increase of glucose conversion. There are several possible explanation for this observation. Firstly, high substrate concentration can result in mixing problems, which further hinder effective heat and mass transfers that limit the diffusion of enzyme and end products. Secondly, solid liquefaction will be dampened and sample collection becomes more difficult due to the concentrated media. One way to solve this problem and obtain maximum conversion during enzymatic hydrolysis is to increase enzyme dosages as recommended by Manonmani and Sreekantiah [19]. However, high enzyme dosing is not an economical nor practical solution.

According to **Fig. 2**, the percentages of total substrate being hydrolyzed during enzymatic process are the highest in the substrate concentration of 10% and 20% (w/v). This indicates that substrate loading at these two concentrations could be the optimum substrate loadings. Table 2 shows the comparison

between these two substrate concentrations. From the analysis, even though substrate concentration of 10% (w/v) have better glucose recovery at 50.37%, the value was only slightly higher (4.17%) compared to 20% (w/v) substrate concentration. In addition, 20% (w/v) substrate concentration has a higher production of glucose concentration (140 g/L). Therefore, by considering the relatively higher production of glucose, glucose recovery and amount of final food waste hydrolyzed, 20% (w/v) substrate concentration is the apparent optimum substrate concentration in enzymatic hydrolysis of food waste using glucoamylase and crude protease.

Table 1. Comparison between two substrate concentrations on glucose recovery and food hydrolysis performance.

Analysis	Substrate Concentration (w/v)	
	10% (w/v)	20% (w/v)
Glucose (g)	5.04	9.24
Glucose concentration (g/l)	69.00	140.00
Glucose recovery (%)	50.37	46.20
Hydrolysed food waste (%)	60.60	68.35

CONCLUSION

Glucose production from selected food waste by enzymatic hydrolysis utilizing different amount of substrate concentration was archived in this study. Among four different substrate concentrations of 7%, 10%, 20%, and 30% (w/v) in batch enzymatic hydrolysis, the substrate concentration of 20% (w/v) was found to be the optimum substrate loading by considering the highest glucose concentration produced and recovered at 140 g/L and 46.20%, respectively. Moreover, the highest amount of hydrolyzed substrate (68.35%) was also observed when this loading was tested. Future works should also look further into the mechanisms responsible for the performance of enzymes under conditions of other substrate concentration, and the types of enzymes used and their concentrations coupled with other parameters such as pH, temperature, aeration and agitation.

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