PHILIPPINE ENGINEERING JOURNAL
PEJ 2011; Vol. 32, No. 2: 13-18

# ENZYMATIC HYDROLYSIS OF STARCH FROM DUCKWEEDS (*LEMNA SPP.*)

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## ABSTRACT

*Duckweeds are another alternative starch feedstock in ethanol production. Enzymatic hydrolysis is one of the key steps to convert the feedstock into fermentable sugar. In this study, the effect of varying glucoamylase concentration on the hydrolysis of duckweed starch was studied. The maximum value of the reducing sugar concentration was found to be 548mg/ml at around 15 hours for the enzyme concentration of 5 ml α-amylase and 3 ml glucoamylase. The results of the experiment show that there is an opportunity in the exploration of the use of duckweed as substrate for starch hydrolysis. However, the results of the study on the effect of the variation of glucoamylase concentrations on the production of reducing sugar is inconclusive.*

**Keywords:** Enzymatic hydrolysis; Bioethanol; Duckweeds (Lemna spp.); Biomass; α-amylase; Glucoamylase; Aquatic plant

## 1. INTRODUCTION

Bioethanol has been produced from agricultural biomass (Martin and Lopez, 2006), kitchen wastes (Ma and Wang, 2008), and aquatic plants (Mishima and Kuniki, 2007). Among these feedstocks, aquatic plants have the advantage of growing on and in bodies of water without competing for other food crops and grains for arable land and can be produced on a large scale because of their rapid growth rate; they have the ability to uptake nutrients in bodies of water and use it for their growth (Mishima and Tateda, 2006).

Like most crops, aquatic plants accumulate sugar resources in the form of carbohydrate polymers including cellulose, hemicelluloses, and starch. These carbohydrates can be hydrolyzed to produce fermentable sugars that can be converted to ethanol. Some of the aquatic plants that have been studied as potential ethanol feedstocks are water hyacinth and water lettuce (Mishima and Kumiki, 2007). Another emerging ethanol feedstock is the duckweed which has the ability to accumulate nutrients and produce starch ranging from 3 to 75% (Cheng and Stomp, 2009).

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*Received: August 10, 2011*
*Accepted: May 25, 2012*

*Lemnaceae* or duckweeds are the smallest floating flowering plants that can grow either in fresh or polluted water. Duckweeds have very tiny roots and no stems, and do not grow beyond 1cm. They contain more nutrition by weight (protein, fat, nitrogen, phosphorus, amino acids), a large concentration of trace minerals and low lignin content compared to other vascular plants which make them an attractive feedstock as they produce a more superior distillers grain (in terms of nutritional content) compared to other feedstocks. Duckweeds can also be used for wastewater treatment and bioremediation of heavy metals or halogenated organics. (Cross, J.W., 1994)

To produce ethanol, the complex carbohydrates from the feedstock need to be hydrolyzed first into reducing sugars. Enzymatic hydrolysis procedure presents certain advantages over pre-treatments based on acid and alkaline digestions. Advantages include the moderate conditions of temperature and pH in which the reaction is carried out, higher yield compared to other processes, and selectivity of the process because enzymes act only on certain chemical bonds. The enzymatic hydrolysis of starch using amylases has been in use in the industry for many years already and is replacing acid hydrolysis. (Aiyer, 2005).

In this study, duckweeds were used as substrate for enzymatic hydrolysis using varying treatments of combinations of α-amylase and glucoamylase. The effect of varying enzyme concentration on the rate of reducing sugar production was determined.

## 2. MATERIALS AND METHODS

**Raw Material:** Duckweeds (*Lemna* spp.) were obtained from a commercial grower in Rizal. The collected samples were washed manually using tap water, dried at 60°C, milled, and then screened (20-40 mesh). The screened samples were stored under dry conditions.

**Enzymes:** The glucoamylase (1500 U/mL) and α-amylase (900 U/mL) used for enzymatic saccharification were obtained from the Enzyme Laboratory division of the National Institute of Molecular Biology and Biotechnology (BIOTECH) at the University of the Philippines–Los Baños (UPLB) in Laguna. The enzyme activity units were provided by the enzyme manufacturers.

**Figure 1.** Fresh Duckweed Samples

**Component Determination of Biomass:** The starch content of duckweeds was analyzed by the Bureau of Plant Industry division of the Department of Agriculture using the standard method of Total Starch Analysis. The dry matter (DM) fraction was determined by drying 3.0 g of sample at 105°C until constant weight was obtained. The starch content was found to be 15.31 mass%. This fraction does not include other polysaccharides that may be found in the sample.

**Enzymatic Hydrolysis Experiment:** To determine the effect of the amount of glucoamylase on enzymatic hydrolysis of duckweed starch to reducing sugars, three sets of mixtures were prepared: A, B and C. Each of them contained 25 g duckweed starch, 450 ml distilled water, and 5.00 ml α-amylase solution. The amount of glucoamylase added to each set of mixture is as follows: 1.00, 3.00 and 5.00 ml for sets A, B, and C, respectively. The sets of treatment for hydrolysis are summarized in Table 1.

***Table 1.*** *Mixture Preparation for Enzymatic Hydrolysis Treatment*

| Set | Duckweed starch substrate : water : α-amylase : glucoamylase |
|---|---|
| A | 25 g : 450mL : 5 mL : 1 mL |
| B | 25 g : 450mL : 5 mL : 3 mL |
| C | 25 g : 450mL : 5 mL : 5 mL |

To prepare each mixture, duckweed starch and α-amylase were added to water contained in a 1-L Erlenmeyer flask. The pH of the mixture was adjusted to 4.0-4.5 using 1 M sulfuric acid solution. The mixture was heated at 90°C for 2.5 h, after which time, the mixture has liquefied, i.e., has become homogenous. The glucoamylase was added to the liquefied mixture to start the saccharification still at pH 4.0-4.5 but this time at 60°C. A small aliquot was withdrawn from each batch of starch slurry at various time intervals. The aliquot samples were then quickly separated into solid and liquid fractions by centrifugation for 15 minutes.

**Determination of Reducing Sugar Content:** The liquid phase of the hydrolysate was analyzed for its reducing sugar content using the dinitrosalicylic (DNS) acid method (Miller, 1959 and Ghose, 1987). Absorbance was measured at 550 nm using a UV-Visible Spectrophotometer. Glucose was used as the standard. The absorbance readings for each data set were related to glucose concentration based on the calibration curve obtained using varying concentrations of standard glucose solution. The calibration curve follows a polynomial equation of the second order and has the equation: $Abs = 0.58929c^2 + 0.09257c - 0.00186$, where Abs is the absorbance of the solution and c is the concentration of the reducing sugar, in mg/ml. Each analysis was performed in duplicate.

The concentration of glucose liberated in each set was plotted against time for all the data sets. The experiment was conducted in replicates for all of the sets. The true concentration of the sample was obtained by multiplying it by the aliquot factor of 2000.

## 3. DISCUSSION OF RESULTS

The concentration of reducing sugars in the hydrolysate of the mixtures A, B and C are presented in figure 2.

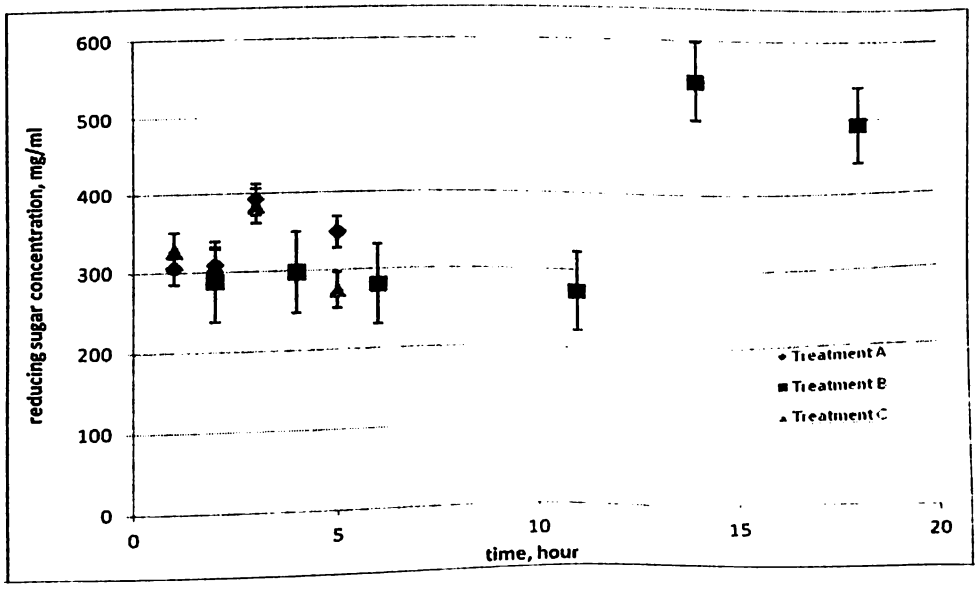


**Figure 2.** Concentration of reducing sugar as a function of time for treatments A, B and C.

As shown in figure 2, during the first 5 to 6 hours of hydrolysis, the amounts of reducing sugar concentration for the three sets of treatment are almost the same. Taking this into consideration, it can be deduced that the variation of the enzyme concentration within the range used in the study in terms of the glucoamylase added into the system does not have a significant effect on the system during the start of the hydrolysis process. The effect of varying glucoamylase concentration on duckweed starch hydrolysis after 5 to 6 hours cannot be deduced from the plot. Considering the results of treatment B, the maximum value of the reducing sugar concentration was found to be 548mg/ml at around 15 hours.

## 4. CONCLUSIONS AND RECOMMENDATIONS

The results of the experiment show that there is an opportunity in the exploration of the use of duckweed as substrate for starch hydrolysis. The results of the study on the effect of the variation of amylase concentrations on the production of reducing sugar is somewhat inconclusive. Future studies on the effect of varying enzyme concentration and other conditions of hydrolysis, such as temperature, and pH, are recommended to optimize the process.

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