# Effects of algal hydrolyzate as reaction medium on enzymatic hydrolysis of lignocelluloses

Rui Chen [a], Benjamin D. Thomas [a], Yan (Susie) Liu [a], Walter Mulbry [b], Wei Liao [a,*]

[a] Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI 48824, USA
[b] Environmental Management and Byproduct Utilization Laboratory, USDA, Beltsville, MD 20705, USA

## ARTICLE INFO

## ABSTRACT

Effects of an algal hydrolyzate on the enzymatic hydrolysis of lignocelluloses were examined using four bioenergy feedstocks (poplar, corn stover, switchgrass, and anaerobically digested manure fiber). Feedstocks were pretreated using dilute acid or alkali prior to hydrolysis. Hydrolysis reactions were conducted using the neutralized algal hydrolyzate, citrate buffer, or deionized water as reaction media. Results demonstrated that algal hydrolyzate significantly improved the efficiency of enzymatic hydrolysis of lignin-rich or structurally recalcitrant biomass such as poplar and anaerobically digested manure fiber. This study showed that algal biomass can be used as not only a biofuel feedstock for direct diesel and ethanol production, but also a supplemental feedstock to enhance the performance of lignocellulosic biorefining.

## 1. Introduction

As aquatic photosynthesizing organisms, algae are considered as one of the most promising alternative bio-resources that could gradually replace fossil-based transportation fuels [1,2]. Compared to terrestrial energy crops, algae have major advantages such as using marginal land, faster growth, higher photosynthetic efficiency, year-around production, efficient uptake of nutrients in waste streams, and alleviating the global aquatic eutrophication potential [3—6]. Tremendous efforts have been made in the past decades to develop economic algal biofuel production [7]. However, due to the complex composition of algal biomass, conversion processes that focus only on a single component, such as algal lipid, jeopardize the economic viability of algal biorefining. Full utilization of all algal components is critical to realizing the potential of algal biofuel and chemical products at commercial scale.

Lignocellulosic materials such as agricultural and forestry residues, as well as herbaceous and short rotation woody crops, have also been extensively examined as feedstock for biofuel production. At present, the major challenge of biofuel production from lignocell/ulose is to increase the productivity while minimizing the use of expensive chemicals and enzymes [8—10]. Altering the lignin/cellulosic structure [11] and introducing lignin-binding surfactants [12] are two feasible solutions to expose more cellulose and hemicellulose in lignocellulosic materials to enzymatic attack. A recent study

on converting algal biomass to bioethanol showed that a hydrolyzate from algae grown in dairy manure improved the efficiency of enzymatic hydrolysis of a recalcitrant lignocellulosic material (anaerobically digested manure fiber) by 50–80% [13]. This result suggests that, in addition to direct production of biofuels using algae, components such as proteins, carbohydrates, and lipids from algal biomass can also be utilized to enhance lignocellulose conversion of biofuel production.

The objective of this study was to extend our understanding of the effect of algal hydrolyzate on the enzymatic hydrolysis of other lignocellulosic materials. In this study, we determined the hydrolytic efficiencies of four different lignocellulosic feedstocks: poplar, corn stover, switchgrass, and anaerobically digested manure fiber (AD fiber). These results can be applied to develop efficient and integrated biorefining processes on lignocellulosic materials and algal biomass.

## 2. Methods and materials

### 2.1. Lignocellulosic feedstock

Corn stover was harvested and collected in 2009 from a private farm in Muir, MI. Switchgrass was collected from the Michigan State University Crop and Soil Science Teaching and Research Field Facility in 2010. Poplar hybrids were planted in 1998 using a uniform spacing of $8 \times 8$ feet and harvested in fall of 2009. All of above feedstocks were dried and ground using an electric mill (Willey Mill, Standard Model No. 3; Arthur H. Thomas, Philadelphia, PA) with a sieve size of 2 mm. AD fiber was collected from the Scenic View Freeport Dairy Farm (Freeport, MI). The farm's dairy cows were fed alfalfa and corn silage, blended according to standard total mixed rations by the Natural Research Council [14]. The farm's anaerobic digester was a continuously stirred tank reactor (CSTR) operated at temperature of 35 °C with a hydraulic retention time of 30 days. AD fiber was separated from the liquid digestate using a 5.5 kW FAN screw press with 2 mm screen. The AD fiber was dried overnight at 75 °C prior to use.

Compositional analyses of the glucan, xylan and lignin content of feedstocks were conducted according to the analytical procedures for determination of structural carbohydrates and lignin in biomass provided by National Renewable Energy Laboratory (NREL) [15].

### 2.2. Algae

Algal biomass was grown using dilute AD dairy manure liquid effluent (USDA Dairy Research Unit, Beltsville, MD) recirculated in pilot-scale algal turf scrubber (ATS) raceways [16]. Dominant species of the filamentous green algae assembly included *Rhizoclonium hieroglyphicum* (C.A. Agardh), *Microspora willeana* Lagerh., *Ulothrix ozonata* (Weber and Mohr) Kütz, *R. hieroglyphicum* (C.A. Agardh) Kütz and *Oedogonium* sp [13]. Wet algal biomass was first dewatered using 2 mm mesh nylon netting and then air dried to approximately 90% of total solids. Dry algae was milled to pass a 3 mm sieve and stored at room temperature [17]. The carbohydrate profile of algal biomass was analyzed using a concentrated acid hydrolysis

method described by Chen et al. [13]. Briefly, dry algal biomass was mixed with 75% (wt) sulfuric acid to a 3:5 sample-to-acid ratio (wt). The mixture reacted at room temperature for 30 min and was then heated at 130 °C for 10 min in an autoclave. Total protein and total fatty acid contents were determined by the Experiment Station Chemical Laboratories at the University of Missouri.

### 2.3. Pretreatment of feedstock and algal biomass

The four feedstocks (AD fiber, switchgrass, poplar, and corn stover) were pretreated using either dilute sulfuric acid ($H_2SO_4$) or sodium hydroxide (NaOH) solutions under the optimal conditions determined in previous studies [18–21] (Table 1). Pretreated sample was neutralized to pH 5 using 30% NaOH or 20% $H_2SO_4$ solutions and washed five times each with 500 mL deionized water until the supernatant was transparent. Pretreated sample was dewatered using eight-layer cheese cloth and then oven-dried overnight at 75 °C. Glucan, xylan and lignin contents of the feedstocks were determined before and after pre-treatment.

Algal biomass was hydrolyzed using a 5% TS loading with 4% (wt) $H_2SO_4$ at 116 °C for 30 min [13]. The hydrolyzed mixture was neutralized to pH 5 using calcium carbonate ($CaCO_3$). Solid residues were completely removed by centrifugation ($2846 \times g$, 10 min). The liquid hydrolyzate was saved and its carbohydrate profile was determined using high performance liquid chromatography (HPLC) and its protein content was measured using a bicinchoninic acid protein assay kit (BCA1 and B9643, Sigma–Aldrich, St. Louis, MO).

### 2.4. Enzymatic hydrolysis

In order to determine the effect of the reaction medium on the enzymatic hydrolysis of the four feedstocks, separate hydrolysis reactions were conducted using pretreated feedstocks and de-ionized water, sodium citrate buffer (50 mM, pH 4.8) and neutralized algal hydrolyzate. Aliquots (2 g DW) of each pretreated feedstock were combined with 20 g of each reaction medium and 17 g of de-ionized water. The resulting mixtures were sterilized using an autoclave (15 min, 120 °C) and cooled to room temperature prior to the addition of cellulase (Accelerase 1500®, Genencor, Rochester, NY) to a final concentration of 25 FPU $g^{-1}$ dry feedstock. Samples were mixed using a shaker table orbiting at 150 rpm at 50 °C for 72 h. Samples (1 mL) of hydrolyzates were taken at hour 0, 24, 48 and 72. Hydrolyzate samples were boiled for 5 min and filtered using Millex-GS 0.22 μm syringe filters prior to analysis of monosaccharide content. The net sugar concentrations

| Feedstock | Acid pretreatment condition | Alkali pretreatment condition |
|---|---|---|
| AD fiber | 3% $H_2SO_4$, 130 °C, 2 h [19] | 2% NaOH, 130 °C, 3 h [19] |
| Poplar | 5% $H_2SO_4$, 130 °C, 2 h (unpublished data) | 2.5% NaOH, 100 °C, 2 h [20] |
| Corn stover | 2% $H_2SO_4$, 130 °C, 1 h [21] | 1% NaOH, 130 °C, 2 h [18] |
| Switchgrass | 2% $H_2SO_4$, 130 °C, 2 h [21] | 1% NaOH, 130 °C, 2 h [18] |

Table 1 – Pretreatment conditions for each feedstock (all concentrations are in wt%).