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Evaluation of a high-moisture stabilization strategy for harvested microalgae blended with herbaceous biomass: Part I—Storage performance

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ABSTRACT

Algal biomass is becoming increasingly attractive as a feedstock for biofuel production. However, the swing in algal biomass production between summer and winter months poses a challenge for delivering predictable, constant feedstock supply to a conversion facility. Drying is one approach for stabilizing algal biomass produced in excess during high productivity summer months for utilization during low productivity months, yet drying is energy intensive and thus costly. Wet, anaerobic storage, or ensiling, is a low-cost approach that is commonly used to preserve high moisture herbaceous feedstock. The potential for microalgae stabilization without the need for drying was investigated in this study by simulating ensiling, in which oxygen limitation drives anaerobic fermentation of soluble sugars to organic acids, dropping the pH and thereby stabilizing the material. Algal biomass, *Scenedesmus obliquus*, was blended with corn stover and stored in acidic, anaerobic conditions at 60% moisture (wet basis) to simulate wet storage by means of ensiling. Results demonstrate that algae and corn stover blends were successfully preserved in anaerobic, acidic conditions for 30 days with < 2% dry matter loss occurring during storage compared to 21% loss in aerobic, non-acidified conditions. Likewise, *Scenedesmus obliquus* stored alone at 80% moisture (wet basis) in acidified, anaerobic conditions for 30 days, resulted in dry matter losses of 6–14%, compared to 44% loss in neutral pH, anaerobic storage and 37% loss in a neutral pH, aerobically stored condition. Additional experiments were performed at a larger scale in which an algae and corn stover blend was subject to mechanical oxygen exclusion and a *Lactobacillus acidophilus* inoculum, resulting in 8% loss over 35 days and further indicating that acidic, anaerobic conditions can stabilize microalgae biomass. In summary, the stabilization of harvested algae can be achieved through anaerobic storage, securing a feedstock that is labile yet of high value.

1. Introduction

The conversion of algal biomass to biofuels has the potential to offset a significant amount of fossil fuels and fossil fuel based products in the United States [1–3]. The inclusion of algal biomass in the U.S. Department of Energy's 2016 Billion-Ton Report signifies the growing interest in large-scale production of algae biomass [4]. A challenge for algal biomass, like most biomass sources, is that there is a seasonality aspect to production. Herbaceous biomass is generally harvested once a year, and storage is required in order to provide year-round feedstock supply to a conversion facility regardless of whether the biomass is for biofuel production or agricultural uses. For algal biomass, production can occur year-round in many geographical areas, yet the growth rate and biomass yield fluctuates due to seasonal changes in temperature and solar irradiation [5–7]. This poses a challenge for maintaining constant conversion throughput. In the algal biomass conversion designs supported by the U.S. Department of Energy Bioenergy Technologies Office, seasonal fluctuations in productivity are managed by sizing the conversion reactor at approximately 65% of the maximum expected algal biomass levels and drying algae produced in excess of conversion capacity in order to stabilize until winter months [8,9]. Drying of algal biomass is typically assumed to be performed using natural gas fed dryers and can cost over $150 per tonne biomass [8,9].

Wet anaerobic storage (i.e. ensiling) is an alternative to drying and dry storage and has been used for hundreds of years to preserve herbaceous biomass for livestock use. In ensiling, oxygen is limited

Abbreviations: wb, wet basis; db, dry basis; cfu, Colony forming unit; ID, Internal diameter; OD, Outer diameter; HPLC, high-performance liquid chromatography; ANOVA, analysis of variance

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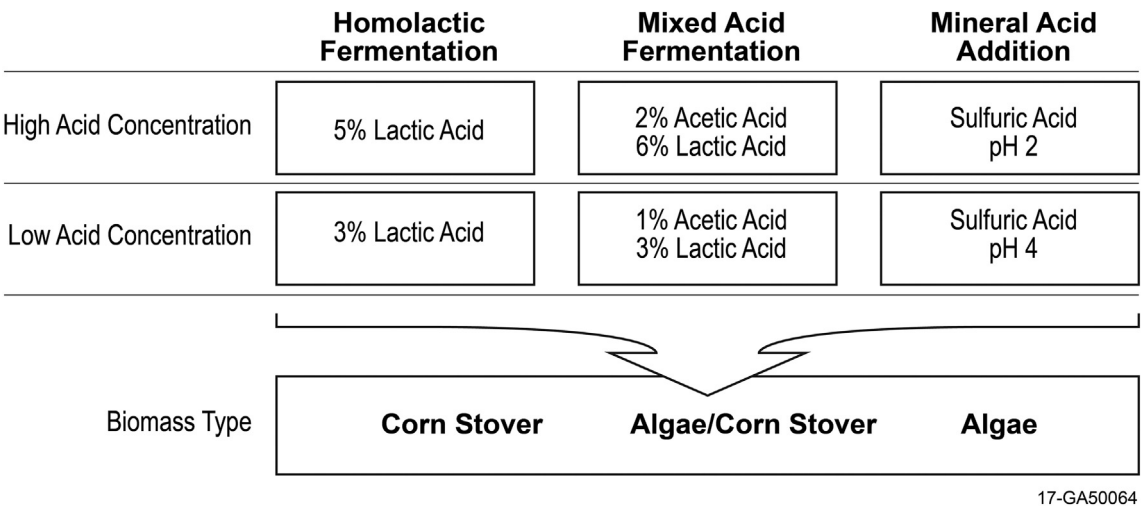

Fig. 1. Experimental approach to validate wet anaerobic storage as an effective means of preserving algae biomass. Each material, corn stover, algae/corn stover blend (20% algae, db), and algae (20% solids, wb), was subjected to three acid treatments each at two levels. Treatment with lactic acid replicates conditions typical of homolactic acid fermentation, where lactic acid is the predominant fermentation product. Heterolactic acid fermentation was simulated by treating biomass with both lactic and acetic acids. The effect of acid was examined by treating each biomass with sulfuric acid. The concentrations of acids for each treatment are listed. Storage experiments were conducted for 30 days in sealed vials purged with nitrogen gas.

through the combination of biomass compaction and physical barriers [10,11]. As such, anaerobic conditions are quickly created, and naturally-present or inoculated lactic acid bacteria flourish, fermenting soluble sugars into organic acids and thus reducing the pH to around 3.5–4.5 [10]. Dry matter losses can be limited to 5% over 1 year of ensiled storage, and losses primarily occur during the initial step of organic acid fermentation. Typically organic acid levels can range from 2 to 6% [12] and are dependent on the fermentation type: homolactic fermentation occurs when the primary fermentation product is lactic acid, whereas heterolactic fermentation involves the production of acetic acid and carbon dioxide in addition to lactic acid [12]. Ensiling has been utilized to preserve many types of herbaceous biomass destined for bioenergy including corn stover, wheat straw, sweet sorghum, switchgrass, and other grasses [13–17]. Ensiling has been successfully used to preserve macroalgae as well [18–21]. Small quantities of microalgae (< 2.5%) have also been added to silage in order to increase the protein content for feed or for increased yield in anaerobic digestion [22,23]. Microalgae have also been used as a substrate for lactic acid fermentation for human consumption and animal feed production [24]. However, wet storage of significant levels of algae biomass has not been explored previously and deserves attention as a possible approach to storing microalgae produced for bioenergy use.

This paper describes the ability of wet storage to preserve algae stored alone or blended with corn stover. Corn stover represents one type of biomass that is commonly used for bioenergy production and consists of all parts of a corn plant remaining on the field after grain harvest. However, multiple other herbaceous feedstocks exist that would be compatible with this blended storage approach including grass clippings, grain sorghum residue, or biomass sorghum to name a few. By blending algae biomass with herbaceous biomass, the rheological properties of the herbaceous biomass can be maintained such that existing technologies for herbaceous biomass can be utilized. For example, storage structures can range from small scale silage bags to bunkers and drive over piles. An economic analysis of a blended algae/corn stover scenario is presented in Part II of this study. To demonstrate the biological applicability of the blending scenario, multiple storage treatments, simulating various fermentation reactions and extents common to the ensiling process, were performed to understand their impact on the stability of algae and algae blends.

2. Material and methods

2.1. Materials

Scenedesmus obliquus DOE 0152 [25] was cultivated in 1000 L outdoor raceway ponds at the Regional Algal Feedstock Testbed located at the University of Arizona in Tucson, AZ. The media composition was as follows: 0.134 g/L $NaNO_3$; 0.075 g/L $MgSO_4$ ($7H_2O$); 0.013 g/L KH_2PO_4; 0.175 g/L potash; 0.0054 g/L Fecitraplex; 0.0029 g/L H_3BO_3; 0.0018 g/L $MnCl_2$ ($4H_2O$); 0.0014 g/L $ZnSO_4$ ($7H_2O$): 0.0004 g/L Na_2MoO_4 ($2H_2O$);0.00008 g/L $CuSO_4$ (5 H_2O); 0.00006 g/L $Co(NO_3)_2$ (6 H_2O); 0.0001 g/L $NiCl_2$ ($6H_2O$). The pH was maintained at 8.0 with CO_2 injection. Algal biomass was concentrated by centrifugation (Evodos 10, Raamsdonksveer, The Netherlands) to a paste with a moisture content of 80% wet basis (wb), as determined by drying to a constant weight at 105 °C. The algae biomass was transported overnight to Idaho National Laboratory in a cooler on ice. Algal biomass was immediately used for experiments upon arrival.

Single pass corn stover used in this study was collected in Boone County, IA and was ground to pass through a 1-in. sieve using a Vermeer BG480 grinder (Pella, IA) and a Bliss Hammermill (Ponca City, OK) with no screen. This corn stover is a reference material offered to the public through the Bioenergy Feedstock Library at Idaho National Laboratory [26], with full chemical characterization available [27]. In preparation for storage experiments, the corn stover was further size reduced to pass through a 6 mm screen with a Wiley Mill (model 4, Thomas, Swedesboro, NJ). Prior to blending, dried corn stover was rehydrated for 24 h with sufficient water to result in a 20:80 (dry basis, db) algae to corn stover blend with 60% moisture (wb). Likewise, for corn stover-only conditions, dried stover was rehydrated for 24 h to result in a final moisture content of 60% (wb).

2.2. Storage experiments

Storage experiments were conducted with algae/corn stover blends and algae or corn stover unblended as a control. Initial proof-of-principle experiments were conducted in 50 mL serum vials, where algae was blended with corn stover at a ratio of 20:80 (dry basis, db) algae to corn stover. All biomass was acidified with one of three acids or combination of acids (sulfuric acid, pH 4 and pH 2; lactic acid, 3% and 5%; or a mixture of acetic and lactic acids, 1% acetic and 3% lactic; 2% acetic and 6% lactic) to reach a moisture content of 60% (wb) and