Cellulosic Ethanol Stillage as a Cultivation Medium for *Spirulina*

Kimberly D. Hafner[1] and Ann C. Wilkie[2]

[1]Agricultural and Biological Engineering Department, McNair Scholar

[2]Mentor, Soil and Water Science Department, University of Florida-IFAS, Gainesville, FL.

Abstract

Algae are high-yielding plants and a potential alternative to conventional fossil fuels that can alleviate the greenhouse effect while simultaneously treating wastewater. Cultivating algae requires high nitrogen inputs to sustain growth and produce feedstock biomass, providing a possible bioremediation option for high-ammonia wastewaters such as stillage from cellulosic ethanol production. Cultivating algae on stillage can help to offset the energy consumed in the pretreatment and distillation operations of bioethanol production as it combines nutrient removal and algal production for potential use as a biofuel feedstock. Thus, this creates a sustainable, closed-loop process. The objective of this study was to determine the growth and remediation potential of *Spirulina*, a filamentous, blue-green algae with high biomass productivity, on sugarcane bagasse stillage. Cultures were prepared with 10% inoculum in 125 mL Erlenmeyer flasks (50 mL active volume). The treatment mediums consisted of 100% thin stillage (negative control), *Spirulina* Medium (positive control), and 2%, 5% and 10% concentrations of thin stillage with 1% (v/v) sodium bicarbonate. The cultures were cultivated for 96 hours under 120 µmol photons/m^2/s fluorescent lighting on a 24:0 (light:dark) photoperiod. Cells were subjected to moderate mixing (120 rpm) provided by a mechanical shaker. Algal growth was monitored spectrophotometrically using absorbance at 680 nm. The data revealed that thin stillage in dilutions is promising for cultivating *Spirulina*. Compared to algal growth in *Spirulina* Medium (1.96 g/L), the 2%, 5% and 10% thin stillage mediums exhibited max biomass yields of 1.47, 1.89, and 2.14 g/L, respectively.

Introduction

- During bioethanol production, approximately 20L of stillage is generated per L of ethanol [1]
- Successful practices for remediating stillage and other wastewaters on a large scale are necessary to alleviate environmental impacts [2,3]
- Ideally, algae can be used in a dual purpose microalgae based system that treats wastewater and agro-industrial wastes while simultaneously producing biomass for bioenergy production
- *Spirulina* is relatively large and filamentous, which promotes efficient harvesting [4]
- The algae's ease of cultivation is facilitated by a reduction in predation, competition and contamination [5]

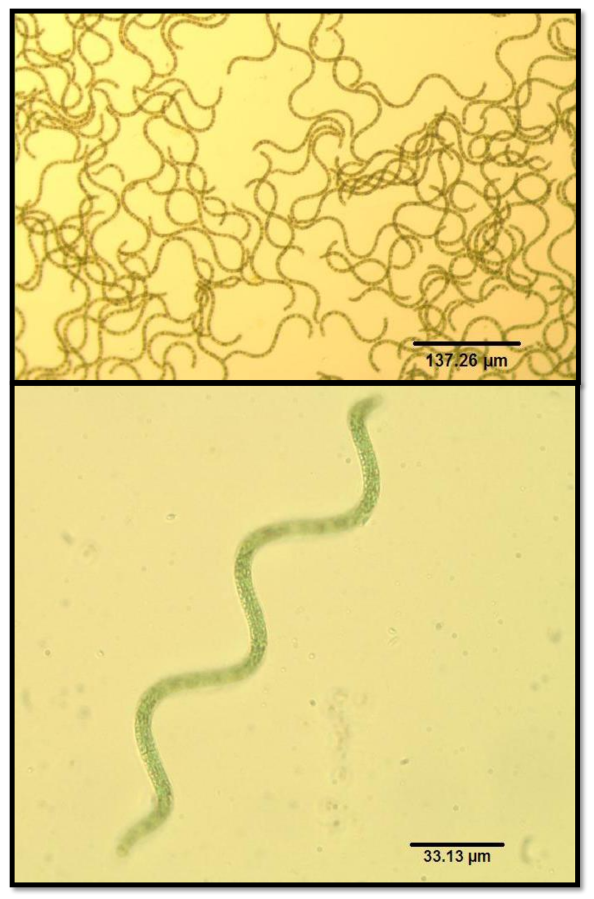


Figure 1: Spirulina under 10x (top) and 40x (bottom) magnifications

Objectives

1. Characterize sugarcane bagasse stillage
2. Determine *Spirulina* growth on sugarcane bagasse stillage

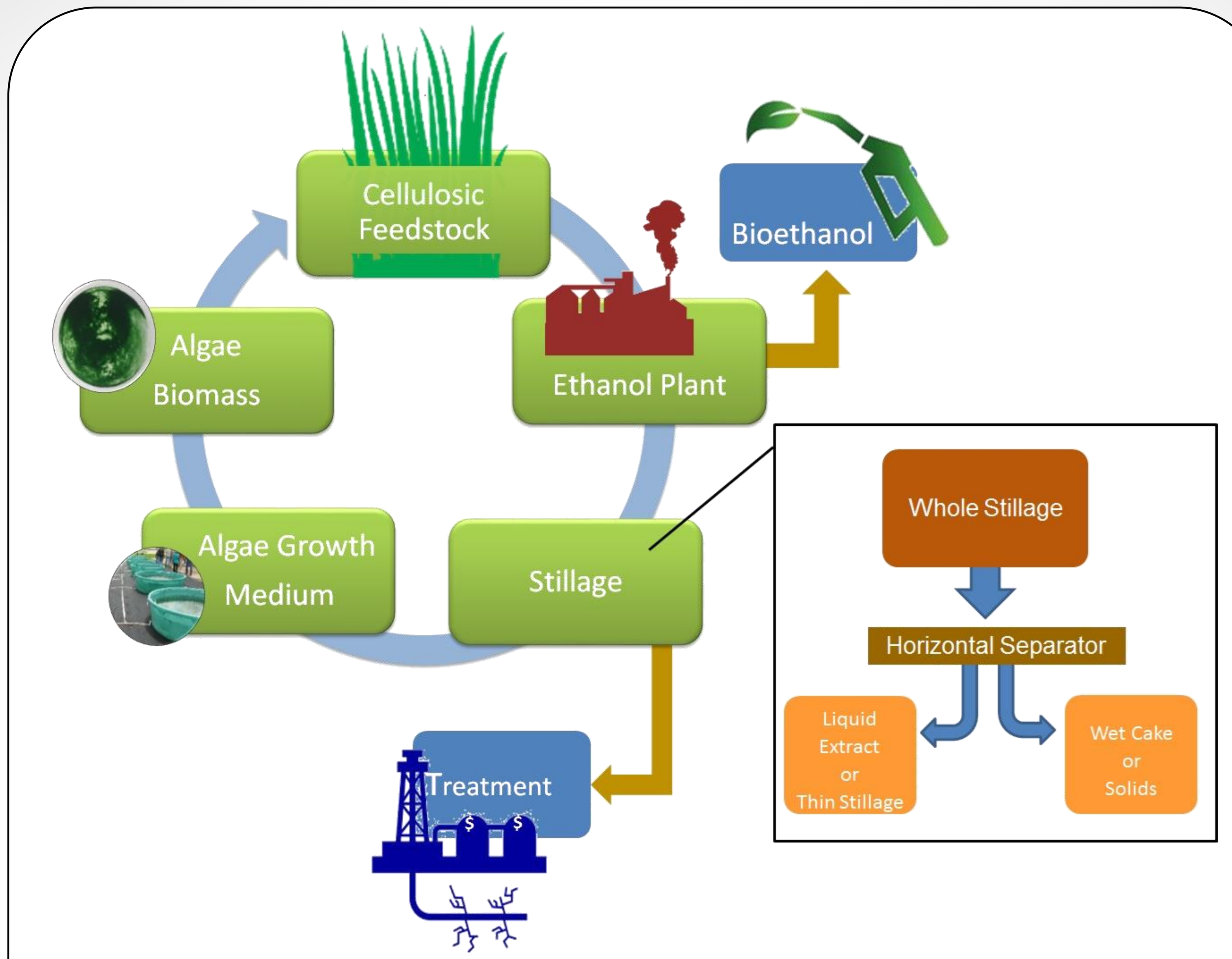
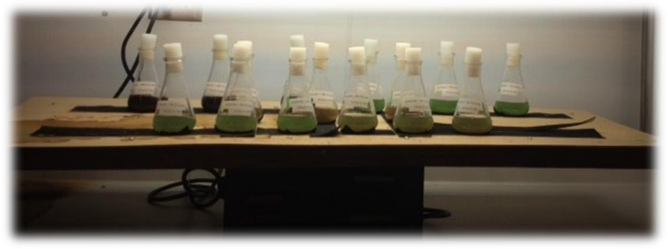


Figure 2: A sustainable, closed loop process with the utilization of algae as an alternative treatment (left) for remediating whole stillage (right)

Materials & Methods

- *Spirulina* inoculum cells obtained from Bioenergy and Sustainable Technology Lab and cultivated in standard *Spirulina* Medium
- Characterized sugarcane bagasse thin stillage obtained from the Stan Mayfield Biorefinery Pilot Plant in Perry, Florida (July 23, 2015)
  - pH, conductivity, total solids (TS), volatile solids (VS), and chemical oxygen demand (COD)
- Subcultures with 10% inoculum for 5 different treatment media:
  - 100% thin stillage (negative control)
  - *Spirulina* Medium (positive control)
  - 2%, 5% and 10% concentrations of thin stillage with 1% (v/v) $NaHCO_3$
- Mixing with mechanical shaker at 120 rpm
- Light intensity of 120 µmol photons/m^2/s on a 24:0 photoperiod
- Growth period of 96 hours
- Biomass growth determined by optical density (OD) measured with spectrophotometer (680 nm) [6]
- pH monitored using Orion pH meter
- Dry weights calculated from OD after standardization of algal biomass dried to constant weight at 105°C



Experimental Setup

Results

Table 1: Thin Stillage Characterization

Component	Thin Stillage
pH	4.49 ± 0.00
Conductivity (mS/cm)	12.24 ± 0.01
TS (%)	2.71 ± 0.03
VS (%TS)	90.41 ± 0.08
COD (mg COD/L)	56,107 ± 4541

Results

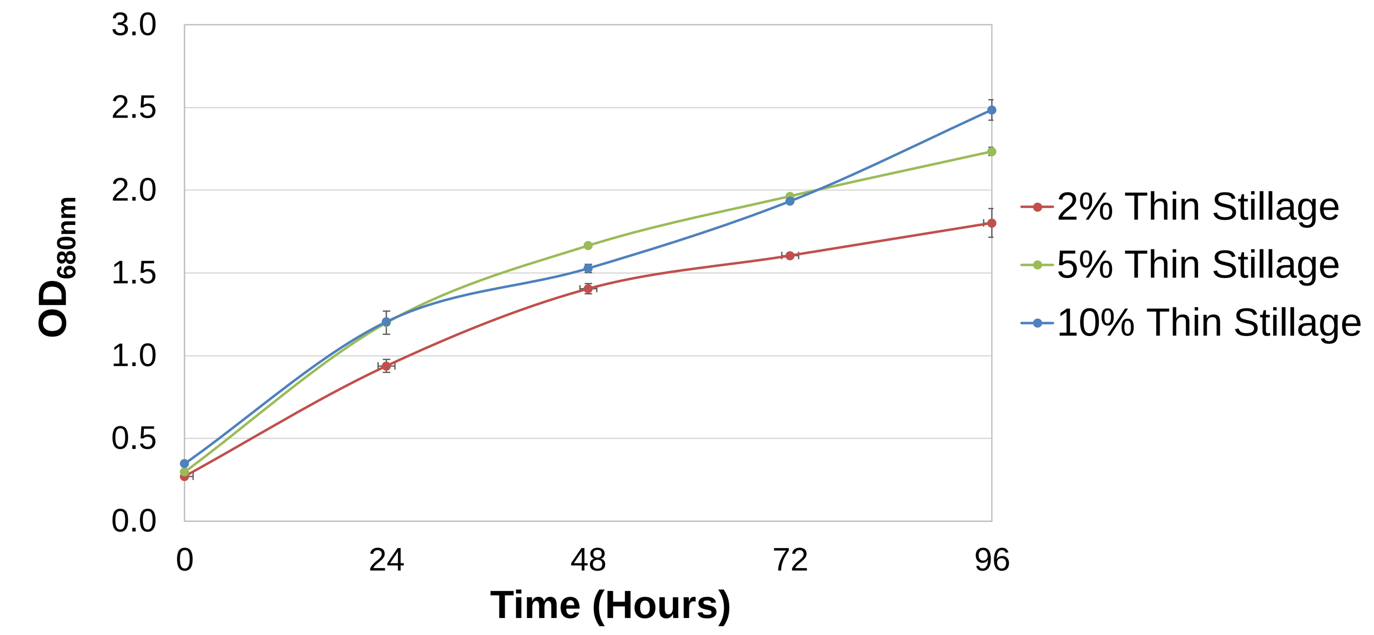


Figure 3: Spirulina Growth Curve

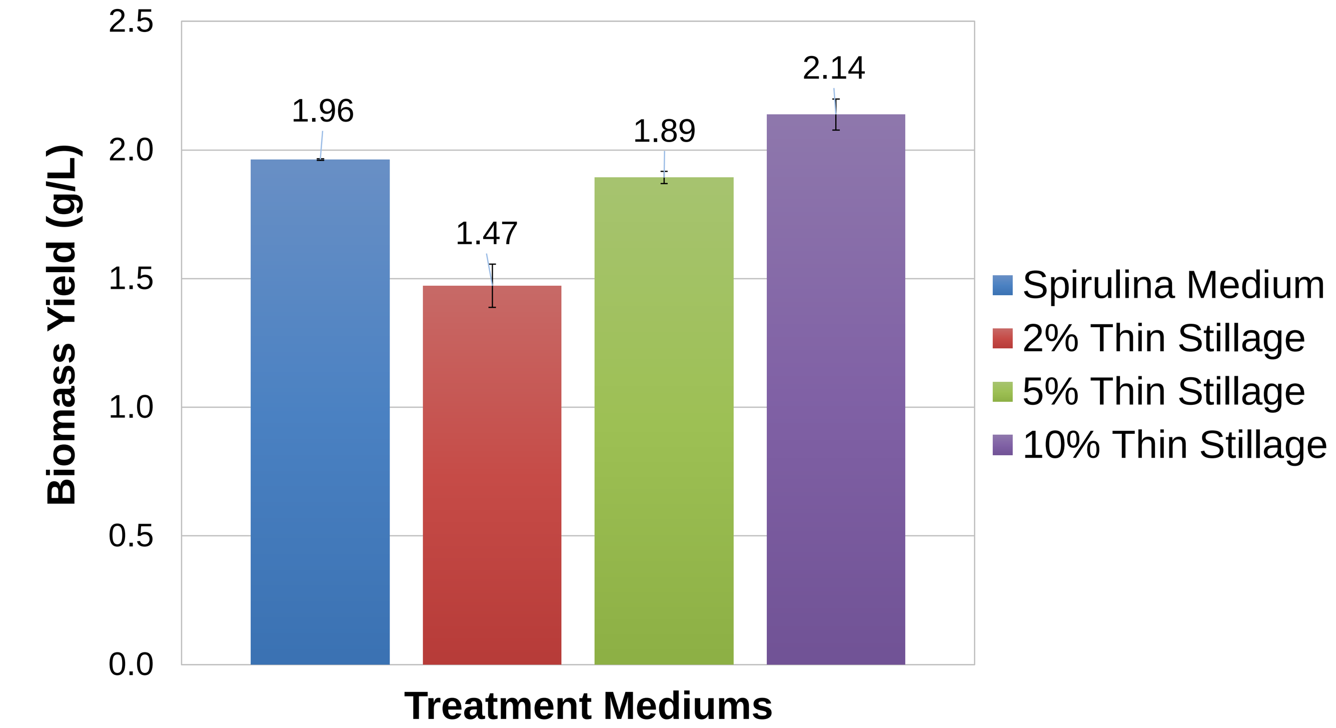


Figure 4: Maximum Algal Biomass Yield

All treatment mediums, except 100% thin stillage (not shown), exhibited growth. Increasing stillage concentrations resulted in higher biomass yields likely because of increasing nutrient availability. The 10% stillage medium exhibited higher growth yields than the basic *Spirulina* medium.

Conclusion

- *Spirulina* acclimated well to 2%, 5% and 10% thin stillage media, showing promise of stillage as an algal cultivation medium
- Utilization of thin stillage as a cultivation medium is a more sustainable and economically favorable option for growing *Spirulina* than the generic medium
- Future research should focus on the remediation potential of *Spirulina* on particular contaminants in stillage

References

1. Wilkie, A. C., Riedesel, K. J., & Owens, J. M. (2000). Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. *Biomass & Bioenergy, 19*(2), 63-102.
2. Edmundson, S.J. and Wilkie, A.C. (2013). Landfill leachate – a water and nutrient resource for algae-based biofuels. *Environ. Technol.* 34(13–14):1849-1857.
3. Markou, G., & Georgakakis, D. (2011). Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review. *Applied Energy, 88*(10), 3389-3401.
4. Vonshak A .(ed) *Spirulina platensis (Arthrospira*): Physiology, Cell-biology and Biotechnology. Taylor & Francis Ltd, London.
5. Aiba, S. and Ogawa, T. (1977). Assessment of growth yield of a blue- green algae *Spirulina platensis*, in axenic and continuous culture. *J. Gen. Microbiol.* 102:179-182.
6. Andersen, R.A. (2005). *Algae Culturing Techniques*. Elsevier Academic Press, Boston, MA.

Acknowledgements

This work was supported by funding from the U.S. Department of Energy's Office of Energy Efficiency and Renewable Energy, Bioenergy Technologies Office and sponsored by the U.S. Department of Energy's International Affairs under award number DE-PI0000031, and by the 2015-2016 McNair Scholars Program at the University of Florida.