Characterization of Cellulosic Ethanol Stillage and Use as an Algal Growth Medium

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Abstract

Cellulosic ethanol is a potential alternative to petroleum-based fuels and, because it is created from lignocellulose found in woody plant materials, it does not compete directly with food production, unlike corn-based ethanol. One obstacle in cellulosic ethanol production is dealing with the stillage by-product that is typically high in nutrients and chemical oxygen demand. Growing algae for biofuels and high-value pigments like β –carotene or astaxanthin requires high nitrogen inputs to sustain growth and produce more biomass, providing a possible bioremediation option for stillage. Various treatment options exist for stillage including anaerobic digestion that has been shown to substantially reduce chemical oxygen demand. In addition, algae can serve as a potential feedstock for anaerobic digestion and the subsequent creation of biogas introducing a conceivable link between multiple bioenergy prospects. The objective of this study was to cultivate algae using stillage as a nutrient source. Sugarcane bagasse stillage from the UF-IFAS Stan Mayfield Biorefinery Pilot Plant was characterized, measuring pH, electrical conductivity, light transmission, total nitrogen, total ammoniacal nitrogen, total and soluble phosphorus, and total and soluble chemical oxygen demand. A strain of the microfilamentous cyanobacterial algae *Spirulina* sp. was isolated using a modified *Spirulina* standard culture medium, replacing nitrate ion with ammonium as the nitrogen source. This culture was then inoculated into flasks with 2% dilutions of stillage supplemented with *Spirulina* nutrients using *Spirulina's* preferred sodium bicarbonate as a carbon source. The experimental group using the 2% stillage dilutions produced more biomass than the control under the same conditions, as measured by optical density absorbance readings. Lipid analysis using nuclear magnetic resonance based against a triolein standard showed that the algae biomass had low neutral oil content and was not ideal for algal biodiesel production. However, the biomass growth under experimental conditions points towards a potential use of stillage as a nutrient source in algae production. The algal biomass can be utilized as feedstock for biogas production via anaerobic digestion.

Introduction

- Stillage is an obstacle to the sustainability of Cellulosic Ethanol fuels with high levels of Chemical Oxygen Demand (COD) and Total Nitrogen (TN).
- Algae can be used as a bioremediation tool and can effectively lower TN and COD (Wilkie *et al.* 2011).
- A bottleneck in the production of algae is finding a sustainable Nitrogen source.

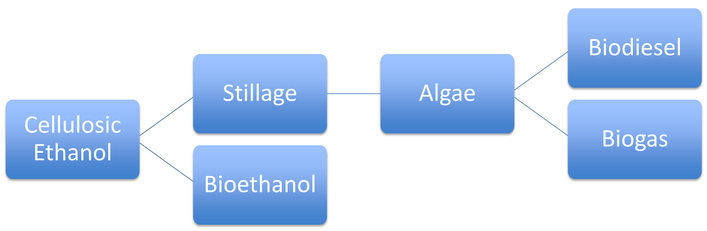

Figure 1: Holistic bioenergy creation from the production of cellulosic ethanol and stillage by-product.

Hypothesis

A dilution of cellulosic ethanol stillage can be used as a nitrogen source for a culture medium for *Spirulina sp.* when supplemented with bicarbonate and micronutrients.

Methodology

- A culture of mixed morphology curly and straight *Spirulina sp.* was obtained (figure 2) and a spiral strain (figure 3) was isolated by serial dilution.
- The algae was cultured using *Spirulina* growth medium (Andersen 2005).
- Cellulosic Ethanol Stillage was obtained from the UF-IFAS Stan Mayfield Biorefinery Pilot Plant and characterized.
- pH, Electrical Conductivity, Optical Density (Absorbance), Total and Soluble COD, TN, Total Ammoniacal Nitrogen (TAN) and Total Phosphorus were measured (APHA 2005).
- 250ml Erlenmeyer flasks were filled with *Spirulina* Medium and 2% dilutions of stillage supernatant were then inoculated with *Spirulin*a from isolated culture.

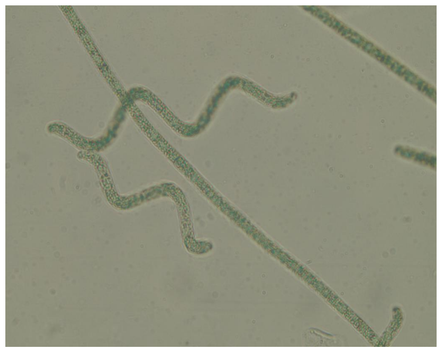

Figure 2: *Spirulina* algae with curly and straight morphologies. Photograph taken at 40x magnification.

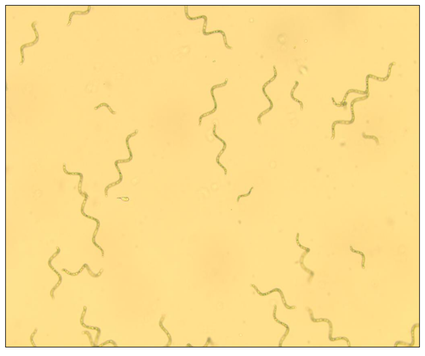

Figure 3: Curly morphology Spirulina isolated from the mixed culture by serial dilution.

Results

Table 1: Characterization of Cellulosic Ethanol Stillage.

	TN (mg/L)	TP (mg/L)	TAN (mg/L)	SRP (mg/L)	TCOD (mg/L)	SCOD (mg/L)	pH	EC (mS/cm)	%TS	% VS
1	1100	577	603	52	58100	34600	6.52	8.8	4.45	97.34
2	1300	583	595	53	57800	37000	6.52	8.8	4.86	97.36
3	1100	572	609	52	59700	38500	6.52	8.7	4.26	96.77
Avg.	1167	577	602	52.3	58533	36700	6.52	8.77	4.52	97.15
STD	115.47	5.51	7.02	0.40	1021.44	1967.23	0	0.06	0.30	0.33

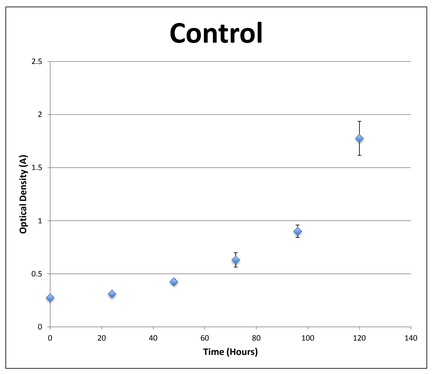

Figure 4: Increase in biomass in the control as measured by increase of optical density.

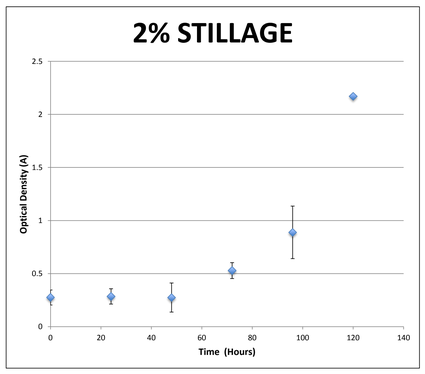

Figure 5: Increase in biomass in the experimental group using 2% dilutions of stillage as nitrogen source.

Conclusions

- Preliminary tests show that algae can grow on the stillage and that it may even be a preferred medium.
- Results from TN and TAN tests indicate that a substantial fraction of nitrogen in the stillage is the result of residual lignin protein.
- While nuclear magnetic resonance testing did not show substantial lipid content, there may still be ample opportunity to produce biogas from algae via anaerobic digestion.

Future Research

- Future experiments should concentrate on the effectiveness of bioremediation.
- Trials should be run to test the viability of the stillage as a nutrient source for other strains of algae potentially higher in oil content.
- Experiments should look at the viability of *Spirulina* as a feedstock for biogas through anaerobic digestion.

Acknowledgements

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