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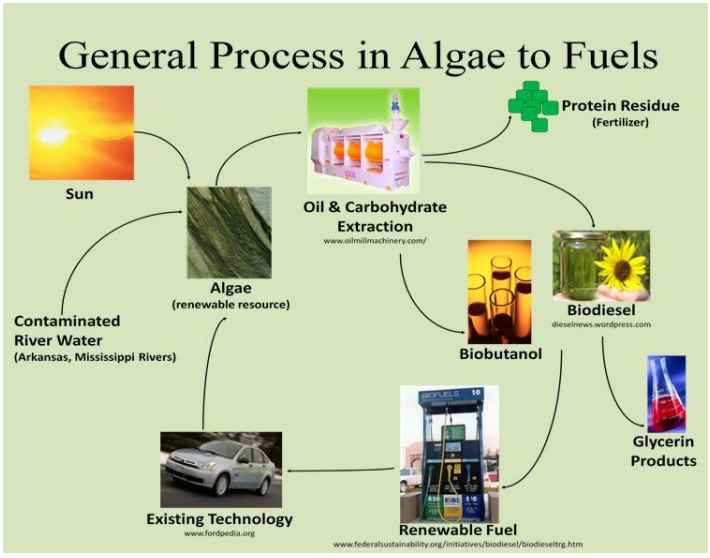
# The production of butanol fuel from renewable systems using a membrane assisted fermentation system, MBTC DOT-3018

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## EXECUTIVE SUMMARY


Fermentation Reactor

The U.S. presently imports over 60% of the crude oil that is used to generate most of the 180 billion gallons of gasoline and diesel fuel that are annually consumed in this country. It is estimated that in the U.S., there are at least 500 million dry tons of biomass available annually. This biomass is in the form of forest residues, mill residues, dedicated energy crops, urban wood waste, and agricultural residues. Our research investigates the use of another feedstock, algae, as a new raw material for transportation fuel. Algae grown in a native algal raceway system removes pollutants from the water as well as making biofuels. Our specific research aim was to transform native algae strains, grown to clean contaminated water, into butanol (1-butyl alcohol).

The algae used were from sources inside of Arkansas and New York City. The first process step was drying the algae. It was found that enough water could be removed in 2-3 days by air drying in a greenhouse to make algae dry enough for subsequent processing.

The next processing step was to hydrolyze the algae and extract carbohydrates for ultimate butanol production. We found that at a temperature of 110 C, a short time (30 minutes or less) combined with a high acid concentration (as high as 30 g/L) was optimal for maximum carbohydrate production.

The next processing step was to ferment carbohydrates into butanol using clostridium spp. We first attempted to use a *C. beijenerckii* but found it was unsuitable for growth with algae. We found, however, that *C. sacchroperbutylacetonicum* was suitable for growth and was able to produce butanol from algae. We also found that the butanol could be separated efficiently using a 2-step distillation with phase separation.

A PhD student, Tom Potts, used this grant to help start his doctoral work. During the project period, he was selected as the winner of the annual Jack Buffington Poster competition. We are in the process of preparing a publication covering this work and will submit the paper by the end of March. Additionally, we are leveraging this work to help support projects from the DOE and the Department of Environmental Protection of the City of New York.

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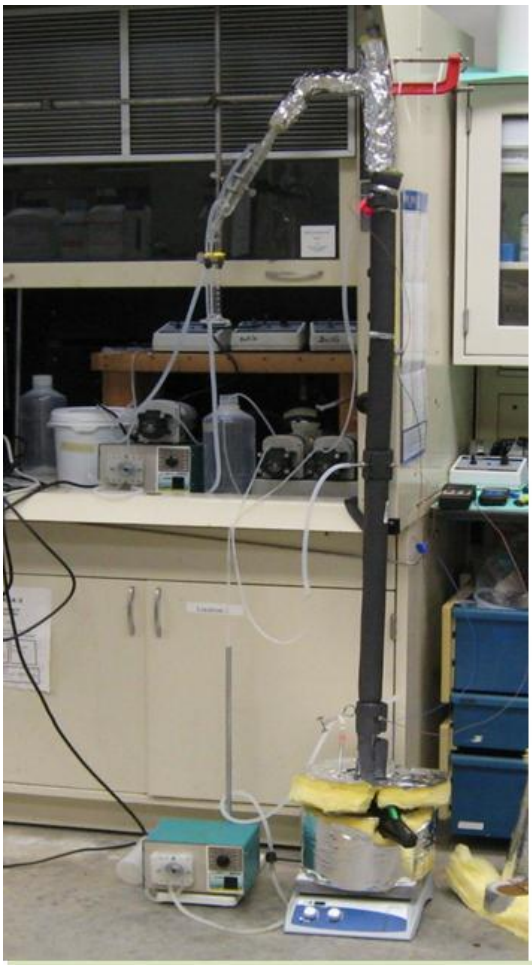

Heterogeneous Azeotropic Distillation

## INTRODUCTION

The U.S. presently imports over 60% of the crude oil that is used to generate most of the 180 billion gallons of gasoline and diesel fuel that are consumed annually in this country. It is estimated that in the U.S., there are at least 500 million dry tons of biomass available annually. This biomass is in the form of forest residues, mill residues, dedicated energy crops, urban wood waste, and agricultural residues (Bain *et al.*, NREL/TP-510-33132, 2003). Along with these well-studied feedstocks, there remains a need for other 'crop' based feedstocks to produce liquid biofuels. Switch grass (Pimental and Patzek, 2005) is one potential feedstock that is currently being explored because it could grow on marginal land and thus it should not cause food prices to rise. However, the use of switch grass still requires conversion of cellulose to fermentable sugars, a currently expensive process but one that still shows tremendous potential (Wyman, 2007). Interest in the potential use of algae as a feedstock in biofuel production is gaining momentum in the United States and Europe. The reasons for the interest in algae is because of their ability to grow on marginal land, the high concentrations of carbohydrates and lipids in their cell mass (Xu *et al.*, 2006), and the ability to clean nitrogen and phosphorus contaminants from water (Mulbry *et al.*, 2008). In fact, ongoing research at the University of Arkansas has found that the nitrogen and phosphorous in the Mississippi River could provide a source for as much as 250 million gallons of biofuel per year, while also providing a cleaner water source. However, although biodiesel from algae has been studied extensively (Xu *et al.*, 2006), there have been far fewer studies on the conversion of algae to fuel oxygenates and no studies on the conversion of algae to butanol. Since the DOE has identified biobutanol as a 2$^{nd}$ generation biofuel, research of converting algae into biobutanol is important and could lead to a sustainable fuel alternative. Thus, the overall objective of this project was to show that algae could be used as a feedstock for butanol production. This included drying, extraction of carbohydrates, conversion of carbohydrates into butanol, isolation and purification of the butanol, and leveraging this work into other algae related projects. Detailed results are given below.

Butanol is a viable alternative to ethanol as a fuel oxygenate for gasoline, and has advantageous benefits as a liquid fuel in three key areas:

- Low vapor pressure (loss due to evaporation is decreased).
- High energy density (less volume required for storage).
- Compatible with existing infrastructure (issues of blending and transport eliminated).

However, butanol production by the fermentation of sugars is a complex process and requires significant development to make it commercially feasible. Three major problems limit the commercial application of the carbohydrate to butanol process:

- Typical fermentation products from a carbohydrate to butanol fermentation include a mixture of organic acids and alcohols, as well as hydrogen and $CO_2$
- The fermentation also suffers from product inhibition, which limits the cell concentration, yield and concentration of butanol in the product stream.
- Butanol is partially miscible in water, forming two liquid phases in equilibrium with a single vapor phase.

## BACKGROUND

*ABE Fermentation Process*

The acetone-butanol-ethanol (ABE) fermentation has traditionally employed the bacterium *C. acetobutylicum* to ferment carbohydrates to butanol. Other strains including *C. beijerinckii* and *C. saccharoperbutylacetonicum* have also been used for the fermentation with success. The fermentation passes through two morphologies: the acidogenesis phase, where carbohydrates are fermented to acetic, butyric and lactic acids; and the solventogenesis phase, where the acids from acidogenesis are fermented to acetone, butanol, ethanol and isopropanol. In addition to forming multiple products, the fermentation also suffers from product (and, in particular, butanol) inhibition, which limits the cell concentration, yield and concentration of butanol in the product stream. As was noted above, a number of techniques have been employed to circumvent these problems. A comparison of ABE fermentation results with different organisms, reactor/recovery schemes and product recovery techniques is shown in Table 1. Yields of 0.40-0.47 g ABE per g sugar utilized ($gg^{-1}$) are common. The ABE productivity of *C. beijerinckii* ranged from 0.34 $gl^{-1}h^{-1}$ for batch culture to 15.8 $gl^{-1}h^{-1}$ for culture in a continuous immobilized cell reactor. Other high productivity reactors include a continuous reactor with cell recycle using *C. saccharoperbutylacetonicum* (11.0 $gl^{-1}h^{-1}$) and a membrane cell recycle reactor with *C. beijerinckii* (6.5 $gl^{-1}h^{-1}$). The ratio of butanol/acetone/ethanol in the product streams was not stated in all of these studies, but is generally 6:3:1 on a weight basis.

*Cell Recycle*

In fermentation systems where production is coupled to cell growth, the productivity of a continuous stirred tank fermenter increases with feed rate until it reaches a maximum value. As the feed rate is further increased, the productivity decreases abruptly as cells are washed out of the reactor because cell generation is less than cell loss in the outlet stream from the reactor. There are two generally accepted methods for increasing productivity beyond this maximum, cell immobilization and cell recycle. Cell immobilization is a technique for retaining cells inside the reactor through attachment to a surface (Hu and Dodge, 1985), entrapment within porous matrices (Cheetham *et al.*, 1979), and containment behind a barrier or self-aggregation (Karel *et al.*, 1985). Cell recycle is a technique for separating the cells from the product stream by centrifugation, filtration or settling in a conical tank, followed by returning the cells back to the

reactor (Shuler and Kargi, 2002). Of these two methods, cell immobilization is generally restricted to the laboratory because of significant fouling. In assessing cell recycle technologies, centrifugation to remove cells can be cost prohibitive, and simple settling with or without the addition of flocculating agents requires large tanks because of the similarity in densities between cells and the fermentation broth. Many improvements have been made in axial flow filtration, which have helped to reduce the cost of commercial application of these systems (Clausen, 2007).

**Table 1. Production of ABE by Clostridia in Fermentation/Recovery Systems**

| Clostridium Strain | Laboratory System | ABE Yield ($gg^{-1}$) | Productivity ($gl^{-1}h^{-1}$) | Reference |
|---|---|---|---|---|
| *beijerinckii* | Batch | 0.42 | 0.34 | Evans and Wang, 1988 |
| *beijerinckii* | Batch with gas stripping | 0.47 | 0.60 | Maddox *et al.*, 1995 |
| *beijerinckii* | Fed-batch with gas stripping | 0.47 | 1.16 | Quereshi and Maddox, 1991 |
| *beijerinckii* | Continuous with gas stripping | 0.40 | 0.91 | Ezeji *et al.*, 2004 |
| *beijerinckii* | Batch with pervaporation | 0.42 | 0.50 | Evans and Wang, 1988 |
| *beijerinckii* | Fed-batch with pervaporation | 0.43 | 0.98 | Groot *et al.*, 1984 |
| *beijerinckii* | Immobilized cell continuous reactor | N.A. | 15.8 | Quereshi *et al*, 2000 |
| *beijerinckii* | Membrane cell recycle reactor | N.A. | 6.5 | Afschar *et al.*, 1985; Pierrot *et al.*, 1986 |
| *Saccharoperbutyl-acetonicum* | Continuous | N.A. | 1.85 | Tashiro *et al.*, 2005 |
| *Saccharoperbutyl-acetonicum* | Continuous with cell recycle | N.A. | 11.0 | Tashiro *et al.*, 2005 |
| *acetobutylicum* | Immobilized cell continuous reactor | 0.42 | 4.6 | Huang *et al.*, 2004 |

*In-Situ Product Recovery*

*In-situ* product removal is designed to increase the yield and productivity of a fermentation process by (Freeman *et al.*, 1993):

1. Minimizing the effects of product inhibition on the producing cell, thus allowing for continuous expression at the maximum production level;
2. Minimizing product losses resulting from cross-interaction with the producing cell, environmental conditions or uncontrolled removal from the system (e.g. by evaporation); or
3. Reducing the number of subsequent downstream processing steps.

The product yield is set by overall stoichiometry, the production of cells and cell maintenance. However, in fermentation systems that produce multiple liquid phase products, selective *in-situ* removal of one of the products *may* cause the fermentation system to overproduce that product, and thereby increase the yield of that product relative to the other products in the product matrix. This phenomenon is illustrated in the following examples. Wu and Yang (2003), in fermenting glucose to butyric and acetic acids using *C. tyrobutyricum* with and without *in-situ* removal of products by solvent extraction, utilized an amine-based solvent system that preferentially (but not totally) extracted butyric acid over acetic acid. Without product removal, their fed-batch system gave a butyric acid yield of 0.34 $gg^{-1}$ and an acetic acid yield of 0.12 $gg^{-1}$, for a product selectivity of 0.74. With product extraction, the overall butyric acid yield was 0.45 $gg^{-1}$ and the acetic acid yield was 0.11$gg^{-1}$, for a product selectivity of 0.80.

Similarly, Grobben *et al.* (2003), in fermenting potato wastes to acetone, butanol and ethanol using *C. acetobutylicum* with and without *in-situ* removal of products by perstraction, utilized a solvent system that preferentially removed butanol (K=3.5) over acetone (K=0.65) and ethanol (K=0.2). Without product removal, their fed-batch system steadied at 12 $gl^{-1}$ of butanol, 4 $gl^{-1}$ of acetone and just under 1 $gl^{-1}$ of ethanol. With product removal, the butanol concentration (both extracted and in the fermenter) reached 39 $gl^{-1}$ and the acetone concentration reached 11.5 $gl^{-1}$. In both of these fermentation systems, the preferentially extracted product (butyric acid in the *C. tyrobutyricum* system and butanol in the *C. acetobutylicum* system) was preferentially produced over the lesser extracted product.

## OBJECTIVES

As shown above, much work has been done on converting simple carbohydrates into butanol. However, little or no work has been done to investigate the use of algae as a source of fermentable sugars once they are recovered from the cellular material. Thus, our overall research objectives were to:

- Investigate the processing of algae into an appropriate feedstock for fermentation
- Demonstrate the production of fuel grade butanol via fermentation
- Build a pilot scale system capable of butanol refining

## MATERIALS AND METHODS

A custom made fermentation system was used. This consisted of a 2 L glass continuous tank stirred reactor. The media varied from a mixture of simple carbohydrates to algal lysate. The fermenter itself had the ability to be operated in continuous or batch mode, with control (pH, agitation, temperature, feed rate, purge rate, cell recycle). We obtained and installed a programmable logic control (PLC) system as part of this fermenter which makes it easy to use and control. The fermenter system is shown in Figure 1.

**Figure 1: Fermentation system used in this research was custom built for flexibility.**

High pressure liquid chromatography (HPLC) was used to analyze carbohydrate content. The HPLC system was fitted with a Shodex SPO810 column for quantification. The solvent was a very dilute (0.5 millimolar) sulfuric acid operating isocratically at a flow rate of 1.0 ml/minute. This column was selected to measure the solvents from the fermentation broth but it was discovered early in the research that interferences with the organic acids limited its utility to measure acetone and ethanol. The column and solvent flow rates did give very good resolution of the butanol, and this instrument was used for that purpose. Additionally, the column gave a fairly well isolated glucose peak when the fermentation broth was grown in PYG or TYG media. Standard solutions of glucose in water were used to generate a calibration curve of glucose concentration as a function of integrator area. Insult testing with other solutions of known glucose concentration confirmed the usability of the method for determining glucose concentration in water solutions. At one point, a sample of the hydrolyzed algal media was analyzed by an outside testing facility and it was discovered that in addition to glucose, the algal media contained large amounts of arabinose and lesser but still significant amounts of xylose. Standard solutions of arabinose and xylose were injected into the HPLC and it was discovered that each of the pentoses could be detected with the existing setup. However, quantification was problematic because of the overlap of the individual peaks. When fermentation broth was injected, the peaks became so overlapped that de-convolution was not possible. The response factors for the individual sugars are nearly the same, so the merged peaks were treated as a single peak and a number for the combined glucose, arabinose, and xylose concentration was obtained.

To complement the HPLC method, gas chromatography (GC) analysis of the hydrolysate and fermentation broth was used. The column chosen (Supelco Inc., Bellefonte, PA) was glass (2 m x 2 mm) packed with 80/120 Carbopack BAW/6.6% Carbowax 20M. The oven temperature was programmed from 125 °C to 195 °C at a rate of 10 °C/min after an initial holding time of 7 minutes. A final holding time of 11 minutes allowed sufficient time for the butyric acid to elute.

The injector and detector temperatures were set at 250 °C and250 °C, respectively. Helium was the carrier gas set at a flow rate of 30 ml/min.

Invert sugars in the hydrolysate and fermentation broth were also measured with the 3,5 DNS (dinitrosalicylic acid acid) method. Samples were filtered with a 0.45 micron syringe filter prior to analysis. One ml of the filtrate and one ml of the DNS solution was added to a test tube immersed in a boiling water bath for 6 minutes. At the end of the 6 minute reaction time, the samples were quenched for 10 minutes in an ice water bath. The sample was then diluted with 8 mls of water. One ml of this analyzate was transferred to a disposable cuvette. A spectrophotometer (Spectronic 21) was used to read the absorbance of the sample at 580 nm. Standard glucose solutions were used to generate a calibration curve from which the concentration of the sample was determined.

**RESULTS AND DISCUSSION**

*Drying*

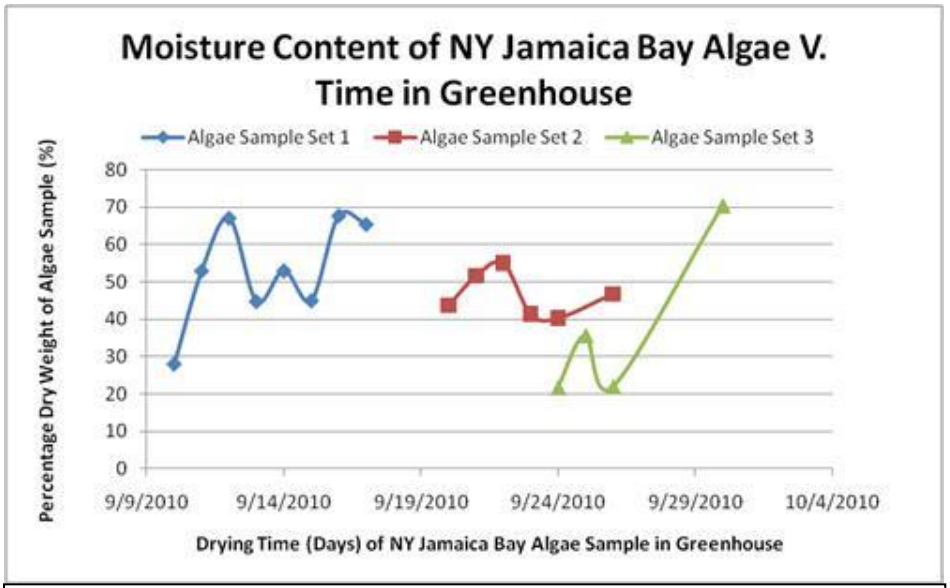


**Figure 2: Algae showed very different drying patterns not necessarily based on initial conditions**

Algae, as received, are often quite wet and difficult to process. Traditional methods of breaking up wet cells either are not successful in lysing the cells or consume too much power. In this research, we air dried the algae in a greenhouse to remove much of the water before trying to hydrolyze and extract the sugars. Air drying in a greenhouse is similar to "field drying" that is commonly used for other biomass processing. The procedure was to receive the wet algae from New York, for example, and measure its moisture content, and then lay the algae on metal benches in a greenhouse where it was left to dry over a period of several days.

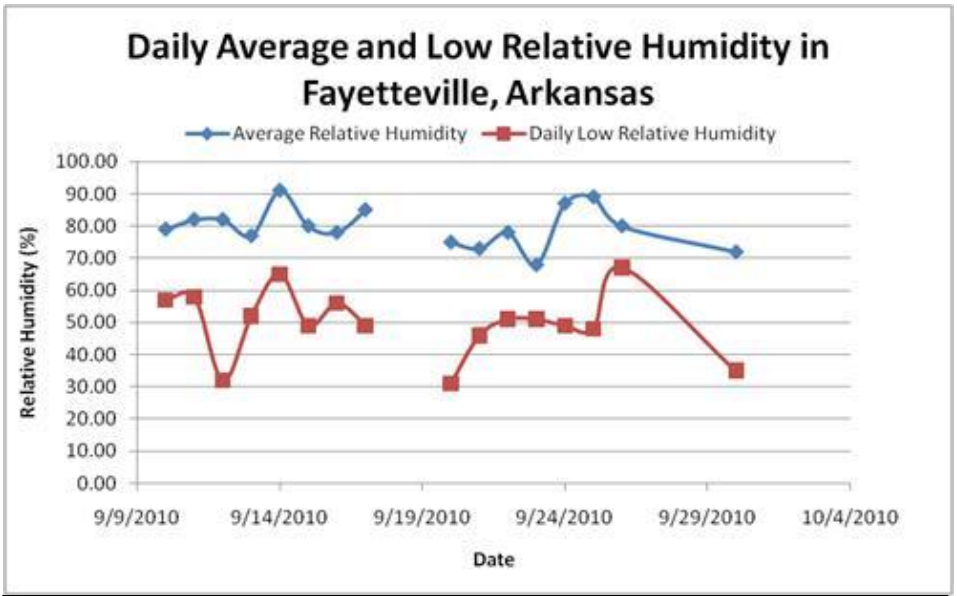


**Figure 3: Relative humidity didn't have a true correlation with drying, but temperature seemed quite important**

Upon receipt at the University of Arkansas – Fayetteville, the algae was removed from its shipping container. It was determined that when layered on the expanded metal screen tables to a depth of about 3 inches, all of the algae dried at about the same rate. If the layer were thicker than 3 inches, the inner algae dried much slower than the top or bottom of the layer. It was assumed that the algae laid on the ground around the tables would have to be thinner to dry evenly, because the ground dried algae had no air circulating below it. However, it was determined that a 3 inch layer of algae on the ground dried evenly throughout its thickness but these samples required 4-6 days to reach the target 70% dry weight. Ulva Algae were subjected to the greenhouse drying. Samples 1 and 2 were dried on the expanded metal tables whereas sample 3 was dried on the ground. Figure 2 shows the percentage dry weight of the samples as a function of time. Figure 3 shows the high and low relative humidity as reported for the Fayetteville weather station for the same time periods. Samples 1 and 3 reached the target 70% dry weight within 3-5 days, but sample 2 remained wetter for the 4 day duration of its drying study. A review of the daily temperatures showed that the high temperature in Fayetteville during the period sample 1 and 3 were consistently close to 90 F, but the high temperature during sample 2 was about 15 degrees colder. It appears that the air drying of the algae is more dependent on air temperature that upon relative humidity.

In conclusion, we found algae easy to "field dry" and thus a simple, cost effective air drying method could be used to make algae processing more economical.

*Extraction and Hydrolysis*

We studied the release of the sugars from several algae samples. It was desired to get maximum carbohydrate yield with expenditure of minimum energy. A DNS invert sugar analysis was used to ascertain if the content of invert sugars in the hydrolysate was sufficient to support bacterial fermentation into butanol. The hydrolysis was performed by grinding the algae in a blender, adding sulfuric acid, and "cooking" in a sealed container for a period of time in an autoclave

(125 C). The results of these experiments can be seen in Figures 4 and 5. Figure 4 shows that a period of time of 30 minutes is as effective as much longer hydrolysis times. Thus, one should use a shorter time as it is more cost effective. Figure 5 shows that as the sulfuric acid concentration is increased at a constant hydrolysis time, more sugars are extracted. Thus, short time with high acid concentration was found to be the best conditions from these experiments. One sample of hydrolysate was subjected to HPLC analysis for measurement of the sugars and sugar by-products. The results of this analysis indicated that the hydrolysate sugars and byproducts consisted of 25% glucose, 15% xylose, 52% arabinose, 8% formic acid, and an unquantifiable trace of furfural. We did not vary temperature as we attempted to keep this as low as possible for process economic considerations. Future studies should include a temperature study.

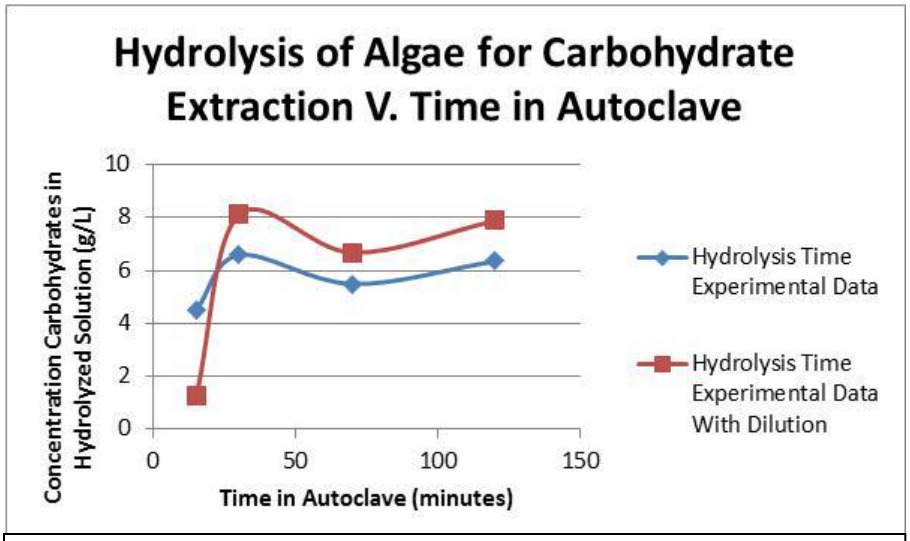


**Figure 4: Hydrolysis time wasn't that important after about 40-50 minutes**

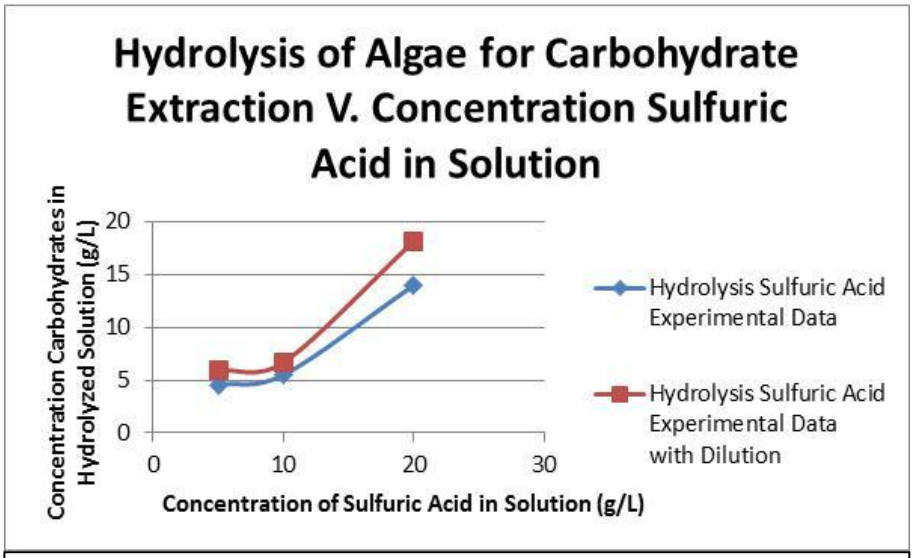


**Figure 5: Sulfuric acid concentration was much more important in hydrolysis**

It is important to note that little or no furfural was made in this process. Furfural is an significant inhibitor to fermentation; its formation is one of the biggest problems in cellulose hydrolysis. Since a high quantity of sugar was made with little furfural, we believe that we have optimized the conditions necessary for the preparation of a quality feedstock for fermentation.

*Fermentation*

Bacterial fermentation was used to produce quantities of fuel grade butanol. Two different organisms were tested. The first, *Clostridium beijenerckii*, was difficult to maintain with algal sugar solutions and its viability was very dependent on pH. Thus, we switched to another organism, *Clostridium sacchroperbutylacetonicum*, which we found to be much more robust (Figure 6). This bacterium grew quickly and produced a significant quantity of butanol with a variety of algae samples. The fermentation reactor was a continuous system with cell recycle through a 50K molecular weight ultrafilter with permeate collection of the butanol rich broth. Butanol in the collected cell-free broth was concentrated with pervaporation.

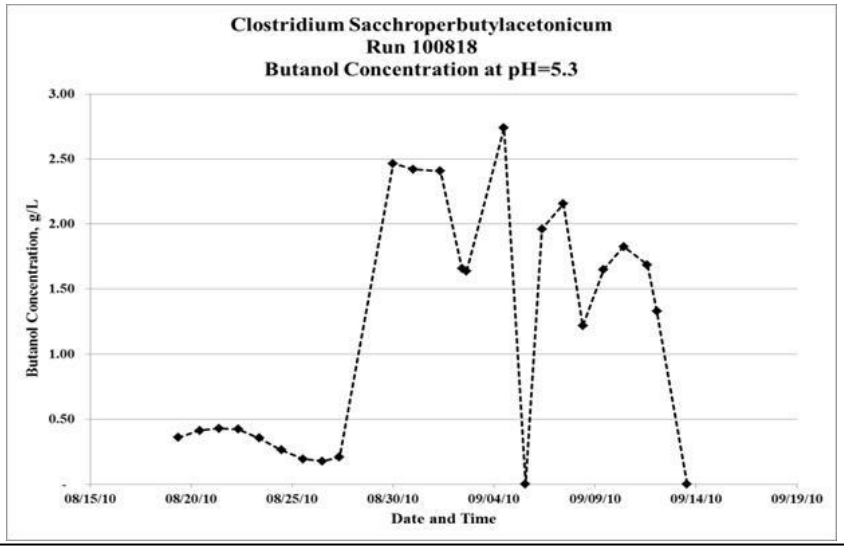


**Figure 6: The organism was quite important in clostridium fermentation**

We also built a heterogeneous azeotropic distillation system to treat the algae. The distillation system consisted of a stainless steel column with a condenser and decanter at the top of the column. This unit was used for both steps of a two-step distillation and produced fuel grade butanol.

*Construction of a pilot scale butanol system and results that this showed*

The pilot-scale distillation unit consisted of an 18/10 stainless steel boiler constructed from a commercial pressure cooker modified to accept and return feed that was continually pumped from a large holding tank, and to deliver vapor and receive liquid from a 1 inch diameter distillation column. The details of the construction follow in the Appendix.

This unit was operated a several times to produce butanol from approximately 30 liters of fermentation broth. Yields obtained from this unit were as high as 0.33 g biobutanol/g sugar indicating that the unit has little losses and works effectively for butanol production. This number compares favorably to the theoretical number of butanol from glucose which is 0.38 g/g. Work is ongoing with this system in continued pilot testing on other projects.

**CONCLUSIONS AND FUTURE WORK**

It was our goal at the beginning of this project to demonstrate that we could turn algae into fuel grade butanol and make a system that could convert significant quantities as such. We feel that we have accomplished this goal and that the project was a success. Other major successes of this project include.

1. Provided support for a PhD student (Tom Potts)
2. Prepared an invited publication to *Environmental Progress and Energy Sustainability*
3. Submitted proposals to the New York Department of Environmental Protection and the United States Department of Energy
4. Created the necessary laboratory infrastructure for the practice of competitive technology now and in the future.

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**APPENDIX**

The pilot-scale distillation unit consisted of a kitchen pressure cooker modified in house with three welded stubs in its top. The pressure cooker was composed of 18/10 stainless steel with a polyurethane gasket. The spring loaded pressure relief valve in the lid of the pressure cooker was found to be prone to leakage and was sealed with silicone. Two of the welded stubs in the lid were ¼" stainless steel tubing and were used for feed and return of the feed solution to the broth holding tank. One tube terminated just inside the lid of the reboiler and was used for the feed to the reboiler. The other tube extended to approximately the mid-point of the reboiler and was the suction line for the peristaltic pump returning processed media to the holding tank. The reboiler was placed on a laboratory hot plate, which served as primary heater. Additionally, the

lower third of the reboiler was wrapped with a 160 watt heating tape driven with a Variac. This supplemental heater was added to provide greater boil-up and thus increase the production rate. A J-type thermocouple was affixed to the side of the reboiler away from the heating tape and read periodically with an Omega digital thermometer. The top and sides of the reboiler were wrapped with a single layer of fiberglass insulation which was faced on one side with an aluminum/cloth composite foil. The third stub on the reboiler lid was 1" stainless steel. It terminated just below the interior of the lid and extended about 4" on the outside. The column was constructed of 1" stainless steel tubing in two modules, each 3 feet long. Each module was packed with 316 stainless steel wool. The two modules were each fitted with a 1" compression union at each end. One compression union connected the two modules, giving a total of three fittings used. The central wrench flats of the compression modules were drilled and tapped with 1/8" NPT female fittings. One of these drilled and tapped fittings was fitted with a 316 S/S 1/8" NPT male by 1/8" compression fitting drilled through with a 1/8" drill bit to allow positioning of a 1/8" thermocouple well into the interior of the 1" fitting. The stainless steel ferrule set was replaced with a single piece PTFE ferrule. This ferrule allowed a tight seal, but also allowed lateral movement of the thermocouple well to make fine adjustments of the position of the thermocouple. The other drilled and tapped fitting was fitted with a 316 S/S 1/8" NPT male by ¼" compression fitting drilled through with a ¼" drill bit to allow positioning of a ¼" tube stub to be used for reflux. The stainless steel ferrule set was replaced with a single piece PTFE ferrule in the same fashion as was done with the thermocouple well fitting. This arrangement provided a thermocouple and feed point at the base of the two-module column, at the midpoint of the column, and at the top of the column. Unused feed points were fitted with a short length of knotted size 15 silicone tubing serving as "caps". These tubing "caps" were not sealed with hose clamps and thus provided pressure relief in the event of column plugging or other event causing excess pressure. The compression fitting on the bottom of the bottom module was fitted with a short (3") stub of 1" 316 S/S tubing. This stub was connected to the stub of the reboiler with a 6" length of 1" I.D. reinforced flexible PVC tubing. The plastic tubing was secured to the stainless steel stubs with hose clamps. The "soft" connection of the plastic tubing was selected because it was felt that this connection would facilitate the process of vertical alignment of the column while still maintaining good thermal contact between the reboiler bottom to the hot plate. The top fitting of the top module was also fitted with a short (3") stub of 1" 316 S/S tubing. This stub was connected to a glass piece of heavy wall 1" O.D. borosilicate glass with a 24/40 standard taper ground glass male fitting on the other end. Connection between the stainless steel stub and the glass connection piece was done with a 6" piece of 1" I.D. reinforced flexible PVC tubing. The plastic tubing was secured to the stainless steel stub and the glass tubing with hose clamps. An inverted glass Y with 24/40 standard taper fittings was connected to the matching fitting on the glass connector piece. The surfaces of the standard taper fittings were coated with stopcock grease prior to assembly. The top fitting of the glass Y pointed vertically and was fitted with a rubber stopper though which protruded a thermocouple well. The rubber stopper was secured in place with a generous wrapping of vinyl tape. The side fitting of the glass Y pointed approximately 45° downward from the vertical. A glass condenser with matching 24/40 standard taper fittings was attached to this side fitting. Stopcock grease and another wrapping with vinyl tape secured the connection between the two glass pieces. A second glass Y was affixed to the lower end of the condenser, also with stopcock grease and vinyl tape. The upper leg was fitted with a PVC cap interiorly greased with stopcock grease and set on top of the glass fitting. The stiction of the grease-to-glass-to-plastic seal provided enough integrity to keep the

seal intact during normal operation but would lift when the system pressure became large. This provided pressure relief to our glass condenser setup. The bottom leg of the pressure relief glass Y was fitted with an adapter that stepped the glass down to a 3/8" glass tube. A length of size 17 silicone peristaltic tubing was connected to this glass tube. The silicone tubing was routed into the top of a 500 ml seperatory funnel, which served as the decanter. The condenser was chilled by pumping cold water from a reservoir via a peristaltic pump through the condenser and then back into the reservoir. The reservoir was chilled with a -40° C immersion probe. The immersion probe was not equipped with temperature control, so a timer was used on the power supply of the probe and was adjusted to keep some ice in the reservoir but not allow the reservoir to freeze solid. This was accomplished with a little experimentation. The decanter, a 500 ml seperatory funnel, was supported with a ring stand and iron ring which was adjusted so that the condensate gravity fed into the decanter. Experimentation determined that the phase separation occurred faster when the condensate feed line end was below the surface of the liquid in the decanter. Reflux from the bottom layer was taken with a peristaltic pump using size 14 silicone tubing. The suction end of the silicone tubing was fitted to an 8" spinal tap needle and the needle was hand positioned in the decanter so that its tip rested very close to the bottom of the decanter. The delivery end of the reflux tubing as connected with size adapters to the ¼" feed tube on the top of the upper distillation column module. The pump speed was adjusted by the operator so that the over-all liquid level in the decanter remained constant. Product take-off was accomplished with a peristaltic pump setup identical to the reflux setup. The product take-off spinal tap needle was positioned by hand by the operator so that its tip was just above the two-phase interface. Attempts to run the product take-off pump continuously were unsuccessful because of slight variations in the flow of condensate. An operator controlled batch mode for product removal was instead used. Periodically, the product pump would be turned on to remove a "slug" of butanol rich phase. When the top layer had been removed, the product pump would be turned off and the reflux pump would be turned off for a short time to allow the decanter liquid return to its target level. Feed to the reboiler was provided with a peristaltic pump fitted with size 15 silicone tubing. The delivery end of this tube was attached to the stub of the reboiler and the suction end was connected to one of several feed reservoirs. Reservoirs used at various times included 10 liter glass media bottles filled with filtered fermentation broth, 20 liter plastic carboys filled with unfiltered fermentation broth, and towards the end of the pilot scale operation, a 30 gallon stock tank filled from multiple 20 liter carboys of unfiltered fermentation broth. The bulk of the butanol obtained from the distillation campaign was processed from the 30 gallon tank. The feed pump was adjusted to run at a speed that delivered approximately 1.5 liters per minute. Higher feed rates were desired, but it was discovered that when the feed rate exceeded the 1.5 liters per minute, the heat duty available from the reboiler heaters was insufficient to keep distillation boil-up. Return of solution from the reboiler to the feed reservoir was accomplished with another peristaltic pump. This pump was fitted with size 17 silicone tubing and was run at a slightly higher speed than the feed pump. The combination of the larger tubing size and higher pump speed gave a much larger pumping speed on the return than on the feed. This caused the level in the reboiler to remain constant at the level of the tube stub used for the return pump. Butanol content of the feed reservoir was tracked on a time basis by periodically sampling the reservoir content by HPLC. Distillation was terminated when the butanol content of the reservoir was too low to support the formation of two phases in the decanter.