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**An ASABE – CSBE/ASABE
Joint Meeting Presentation**

Paper Number: 141898047

Life cycle assessment (LCA) and techno economic analysis (TEA) of algal biomass and biodiesel production from pyrolytic substrate

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**Written for presentation at the
2014 ASABE and CSBE/SCGAB Annual International Meeting
Sponsored by ASABE
Montreal, Quebec Canada
July 13 – 16, 2014**

Abstract

Hybrid processing of cellulosic biomass, composed of thermochemical-based pyrolysis of biomass into fermentative substrates followed by biochemical-based algal fermentation into lipid-rich biomass was developed. The hybrid process has proven an effective way for producing biofuel from lignocellulosic biomass. In this work, life cycle assessment and techno economic analysis were performed for algal fermentation of the acetic-acid rich stage fraction of bio-oil under different scales and fermentation conditions. These results will provide guidance for choosing optimal algal fermentation parameters. Moreover, with more biodiesel produced, increased environmental and economic benefits per gallon of biodiesel can be expected.

Keywords: fermentation, algae, economic analysis, economic evaluation, pyrolysis

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INTRODUCTION

Lignocellulosic biomass is a scalable non-food substrate for biodiesel production [Kim *et al.*, 2013; Brown *et al.*, 2013]. Recently, hybrid processing of cellulosic biomass, composed of thermochemical-based pyrolysis of biomass into fermentative substrates followed by biochemical-based algal fermentation into lipid-rich biomass was developed [Jarboe *et al.*, 2011]. Hybrid processes focus on fast pyrolysis – fermentation to produce alcohols, lipids and other chemicals [Jarboe *et al.*, 2011; Yi *et al.*, 2012; Zhao *et al.*, 2013; Layton *et al.*, 2011; Lian *et al.*, 2012; Lian *et al.*, 2013]. These processes have many advantages, such as flexible feedstock, utilization of both carbohydrate and lignin, yields densified biomass (bio-oil) for easy transportation and storage, and does not require enzymes to produce sugars. However, the main challenge for fermentation of products of thermochemical processing is the inhibition of many contaminants containing pyrolytic substrates or syngas [Xiu *et al.*, 2012; Jarboe *et al.*, 2011]. Consequently, the complexity hinders the complete identification, detoxification and improvement of those substrates. Some work shows that alkaline treatment is effective for the detoxification of pyrolytic substrates while perfusion can alleviate the accumulation of contaminants in microorganism fermentation, which are helpful for the scaling up of hybrid processing of lignocellulosic biomass [Zhao *et al.*, 2013].

Fast pyrolysis, one kind of pyrolysis, is able to produce bio-oil with higher quality and comparatively higher oil yield than other processes through a rapid thermal decomposition of biomass in the absence of oxygen [Goyal *et al.*, 2008]. Bio-oil, the liquid product of fast pyrolysis, can be used to produce drop-in fuel directly via upgrading and refinery [Mortensen *et al.*, 2011]. In addition, bio-oil can provide various substrates for microorganism fermentations to get bioethanol or other value-added products, like succinic acid and lipid [Jarboe *et al.*, 2011; Yi

et al., 2012; Zhao *et al.*, 2013; Layton *et al.*, 2011; Lian *et al.*, 2012; Lian *et al.*, 2013]. However, high inhibition on fermentation, high water content, high viscosity, high ash content, high oxygen content and high corrosiveness, are the main barriers for bio-oil application [Xiu *et al.*, 2012; Jarboe *et al.*, 2011].

To effectively utilize raw bio-oil, a unique pyrolysis-product fractionating system was developed at Iowa State University [Pollard *et al.*, 2012]. Fluidized with nitrogen at 500 °C in this system, raw bio-oil was separated into five stage fractions (SFs) with distinct chemical and physical properties. Stage fraction #1 (SF1) and stage fraction #2 (SF2) contain 3% to 5% levoglucosan, the anhydrosugar of glucose and substrate for bioethanol production. Other stage fractions contain notable acetic acid, which can be utilized by microalgae to produce lipids. The microalgae *Chlamydomonas reinhardtii*s, with the capability of heterotrophic growth on acetate [Chen *et al.*, 1994; Chen *et al.*, 1996] seems promising to utilize acetic acid- rich Stage Fraction #5 (SF5). Another advantage for this strain is that enhanced lipid content of *C. reinhardtii*s has been achieved by genetically modification [Li *et al.*, 2010; Work *et al.*, 2010].

However, due to its complexity, SF5 has a significant inhibition on the growth performance of *C. reinhardtii*s. As a result, when *C. reinhardtii*s is cultured in Tris-Phosphate (TAP) medium, only 0.05 wt% or less of SF5 can be added and higher concentration of SF5 will eliminate any growth of this strain [Yi *et al.*, 2012]. Nevertheless, more than 4.00% of SF5 treated via over liming can be fermented by *C. reinhardtii*s, with no pure acetic acid added [Zhao *et al.*, 2013].

To further improve the fermentability of SF5, perfusion design is needed for fermenters. The advantages of perfusion operation design in substrates detoxification, biomass productivity and bio-product yield have been investigated previously [Wen *et al.*, 2001; Wen *et al.*, 2002a; Wen *et al.*, 2002b; Wen *et al.*, 2003]. In this work, perfusion for contaminants, common continuous and

perfusion – bleeding fermenters were compared via life cycle assessment (LCA) and techno economic analysis to get more knowledge for future commercialized fast pyrolysis –algal fermentation of lignocellulosic biomass.

DESIGN BASIS

Plant size

In the present work, the size of the SF5 fermenter is 2,000 cubic meters, or 528,344 gallons, for small scale design, 20,000 cubic meters for intermediate scale design and 200,000 cubic meters for large scale design.

Differences of three fermenters

Figure 1 shows examples of perfusion, continuous and perfusion – bleeding fermentation procedures. Perfusion fermentation system adds a retention device to common continuous culture system to separate algal cells and cell - free medium [Wen *et al*, 2002a]. The biomass was returned to fermenter and spent contaminants – concentrated medium would be removed from the fermenter and sent to a wastewater treatment system. The flow rate was termed the perfusion rate. Perfusion fermentation can help the cells to adapt to medium with high concentrations of inhibitors; however, it will decrease the biomass productivity and continuous algae harvest cannot be carried out in this system. Perfusion – bleeding adds another retention device to continuously harvest algal cells to perfusion system [Wen *et al*, 2001]. The flow rate during harvest procedure was named the bleeding rate. Perfusion – bleeding system shows higher productivities than other culture systems. Both perfusion and perfusion – bleeding systems need more labors for operation than continuous culture system. The data from literature about the best operation conditions for microalgal fermentation via each of these three systems are listed in table 1.

SF5 concentration and lime usage

Based on the test of fermentability of treated SF5 in our lab, 5.00% of treated SF5 can be fermented in batch culture, which will supply 4 g/L of acetic acid for the microalgae. Perfusion culture needs higher initial substrate concentration than continuous and perfusion – bleeding culture. As a result, acetic acid and SF5 concentration for three different fermenters were designed as 4, 2, 2 g/L, and 50, 25, 25 g/L, respectively. The quantity of $\text{Ca}(\text{OH})_2$ needed in over-liming treatment was obtained in our lab, which was 1 g per g SF5.

Biodiesel productivity and properties

The productivity of biomass was based on reference data, 2.09, 2.82 and 6.75 $\text{g}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ for perfusion, continuous and perfusion – bleeding culture. In previous research on batch culture systems, lipid concentration in this strain was ~10% when cultured with acetic acid provided from treated SF5. The weight loss of biodiesel during cell harvest and oil extraction was made up via oil transesterification. Hence the productivity of biodiesel for each system would be calculated. In this work, the biodiesel productivities for the three systems were calculated as 498, 672 and 1609 kg/day, respectively. The combustion property of biodiesel was investigated with reasonable assumptions in previous work, and was assumed the same as the property of diesel derived from fossil fuels. In this work, the price of biodiesel was set as the same as the commercialized diesel, which was 3.88 \$/gallon.

Equipment, energy, CO₂ emissions and water recycle rate

This work was not aiming to do a complete TEA and LCA analysis for the whole procedure of hybrid processing, but instead to compare the three different fermentation systems via economic and environmental impacts. Therefore, the analysis focused on the equipment for the three systems. Equipment related to this work is listed in Appendix A. The energy used in this plant

was assumed to be obtained from solar thermal energy. Also, the CO₂ produced in the fermentation was assumed to be absorbed by cells. Therefore there was no CO₂ emissions in this analysis. The water recycle rate was set as 90% for all of the systems.

Flow chart, boundaries and functional units

Figure 2 shows the flow chart for the fermentation systems. For the perfusion system, total flow rate F equals perfusion flow rate F_1 . For the common continuous culture system, total flow rate equals bleeding flow rate F_2 . For the perfusion – bleeding system, total flow rate equals the sum of F_1 and F_2 . The dash line shows the boundary of the analysis in this work. The functional unit chosen in this work is one gallon of biodiesel.

RESULT AND DISCUSSION

TEA Results

Total costs comparisons

Figures 3-7 show the TEA and LCA results. Detailed calculation procedures and assumptions are listed in the Appendixes. Figure 3a) shows the annual total costs for the three systems with different scales. For each of the three systems, annual total costs increased with the increase of scale. For each scale, the total costs show same order: continuous < perfusion < perfusion – bleeding system. Figure 3b) shows the total costs per gallon of biodiesel for the three systems with different scales. For each of the three systems, total costs per gallon of biodiesel decreased with the increase of scale. For each size, the total costs per gallon biodiesel shows same order, perfusion – bleeding system < continuous < perfusion. With the increase of scale, the differences of total costs per function unit among the three systems become smaller. Scale efficiency can be found with these three systems and perfusion – bleeding system has the least total costs per function units.

Profit comparisons

Figure 4a) and Figure 4b) show the total profits and profits per gallon of biodiesel for the three systems with different scales. For all of these three systems, with larger scale, profits and profits per function unit increase. For all of the three scales, the profits and profits per functional unit show the same order, perfusion < continuous < perfusion – bleeding system. With an increase of scale, the differences of profits per functional unit among the three systems become smaller. Perfusion – bleeding is most profitable among these three systems.

LCA Results

Energy consumption comparisons

Figure 5a) shows the annual energy consumption for the three systems with different scales. For each of the three systems, annual energy consumption increased with the increase of scale. For each scale, the energy consumption shows the same order, perfusion < continuous < perfusion – bleeding system. Figure 5b) shows the energy consumption per gallon of biodiesel for the three systems with different scales. For each of the three systems, energy consumption per gallon of biodiesel decreased with an increase of scale. For each size, the energy consumption per gallon of biodiesel shows the same order, perfusion – bleeding system < continuous < perfusion. With an increase of scale, the differences of energy consumption per functional unit among the three systems become smaller. Scale efficiency can be seen and the perfusion - bleeding system has the least energy consumption per functional unit.

Net energy production comparisons

Figure 6a) and Figure 6b) show the net energy production and net energy production per gallon of biodiesel for the three systems with different scales. For all of these three systems, with larger scale, net energy production and net energy production per gallon of biodiesel increase. For all of

the three scales, the net energy production and net energy production per functional unit show the same order, perfusion < continuous < perfusion – bleeding system. With the increase of scale, the differences in net energy per functional unit among the three systems become smaller. Perfusion – bleeding produces the most net energy among these three systems.

Water usage comparisons

Figure 7a) shows the annual water usage for the three systems with different scales. For each of the three systems, annual water usage increases with the increase of the plant scale. For each scale, the water usage shows same order, perfusion < continuous < perfusion – bleeding system. Figure 7b) shows the water usage per gallon of biodiesel for the three systems with different scales. For each of the three systems, water usage per gallon of biodiesel does not increase with the increase of the plant scale. For each size, the water usage per gallon biodiesel shows same order, perfusion < perfusion – bleeding system < continuous. Scale efficiency could not be found and perfusion system had the least energy consumption per function unit.

CONCLUSIONS

Scale efficiency can be found both for TEA and LCA results, except for water usage. Perfusion system had the least water usage per functional unit, while perfusion – bleeding system had the lowest total costs per functional unit, highest profits per functional unit, lowest energy consumption per functional unit, and highest net energy production per functional unit. In conclusion, the perfusion - bleeding system had the advantage over other two systems but needs a higher water recycle rate.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge NSF Iowa ESPCoR, NSF Energy for Sustainability (CBET-1133319), Iowa Energy Center (#12-06), and Iowa State University Bailey Award for financial support of this project.

Table 1 Comparison of the parameters obtained by different culture methods [Wen *et al*, 2003]

Category	Unit	perfusion	Continuous	Perfusion-bleeding
Max. biomass productivity	$\text{g}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$	2.09	2.82	6.75
Biodiesel productivity	$\text{g}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$	0.25	0.34	0.80
Dilution rate	day^{-1}	0.27	0.6	1.27*
Substrate assimilation efficiency	%	78.5	50	82.5
Feed substrate concentration	$\text{g}\cdot\text{L}^{-1}$	4	2	2
Advantage		Alleviating metabolites inhibition	Continuous cell-harvest	Continuous cell-harvest and alleviating inhibition
Disadvantage		Continuous cell-harvest not available	Metabolites inhibition	Complex in operation

*perfusion rate is 0.6 day^{-1} and bleeding rate is 0.67 day^{-1}

Perfusion

$$F = F_1$$

Continuous

$$F = F_2$$

Perfusion – Bleeding

$$F = F_1 + F_2$$

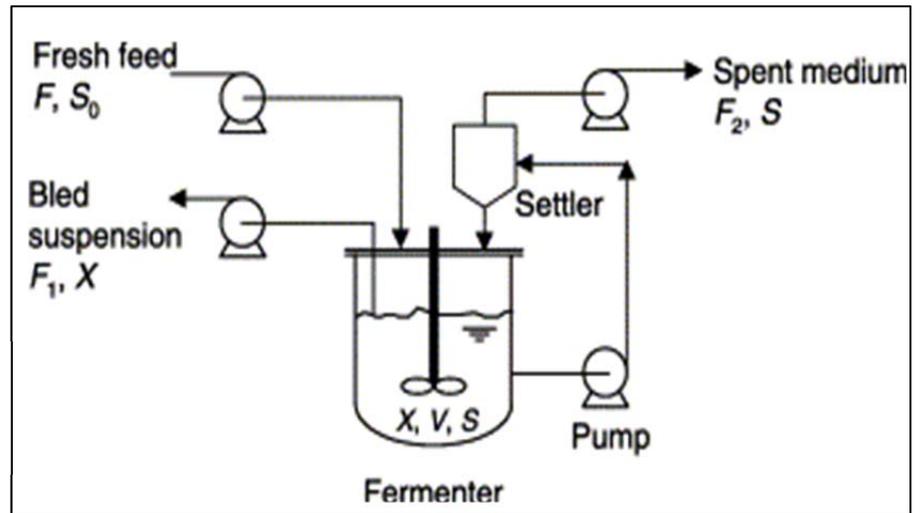
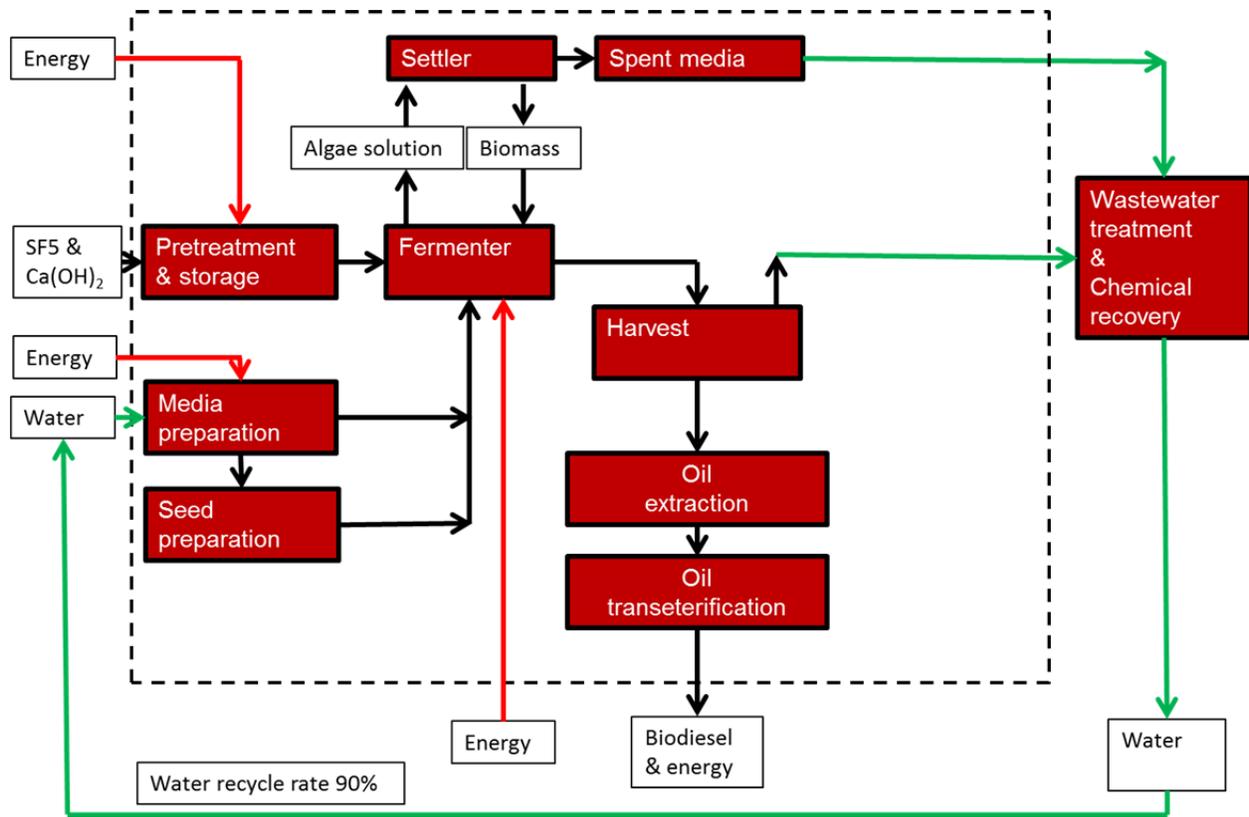


Figure 1. Sketches for perfusion, continuous and perfusion – bleeding systems



- Mass flow
- Energy flow
- Water flow
- Boundary of analysis

Figure 2. Flow chart for the fermentation systems

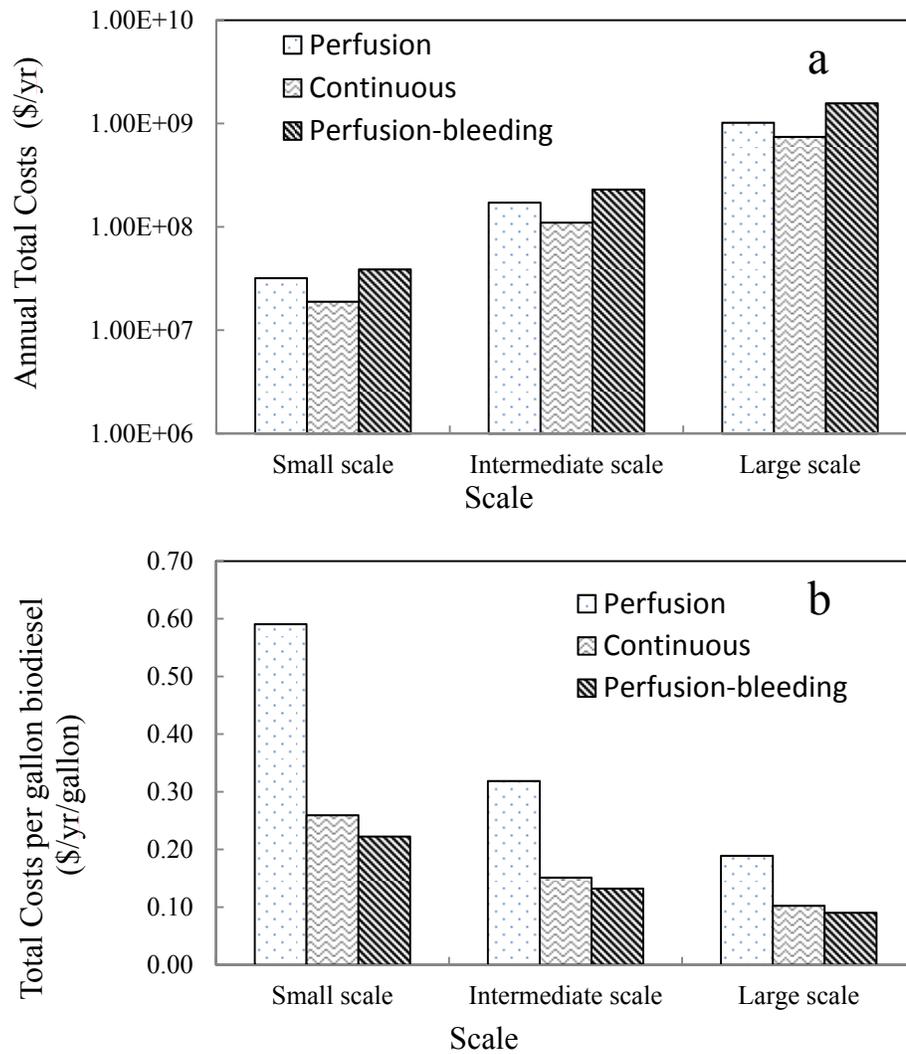


Figure 3. Total costs analysis for three systems with different scales. a) annual total costs comparison b) the total costs per gallon of biodiesel comparison

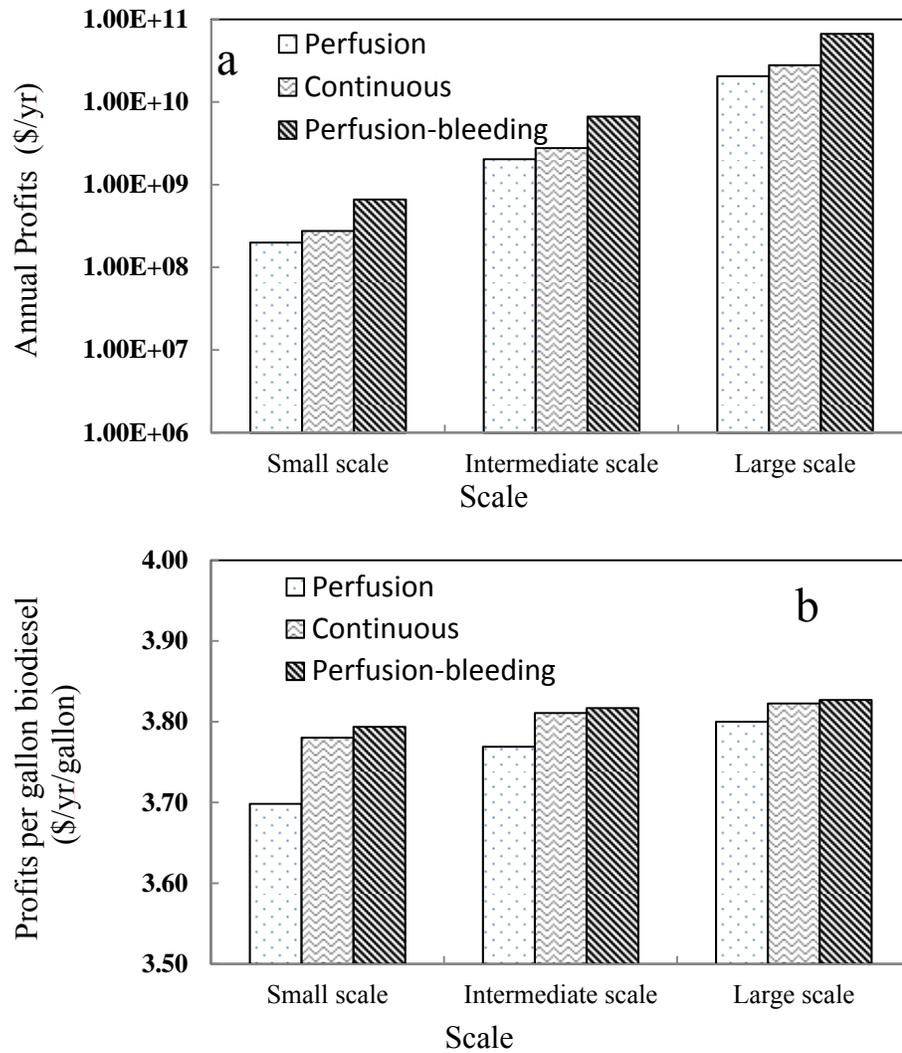


Figure 4. Profits analysis for three systems with different scales. a) annual profits comparison
 b) profits per gallon of biodiesel comparison

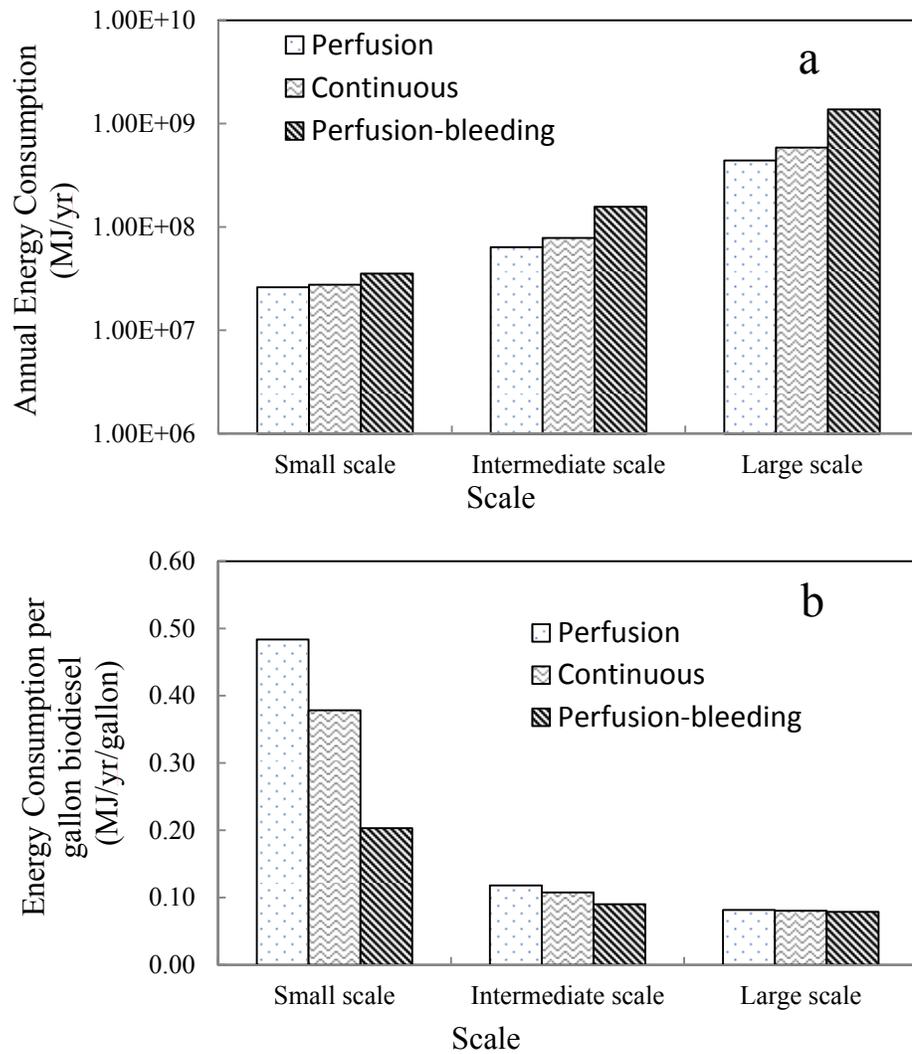


Figure 5. Energy consumption for three systems with different scales. a) annual total energy consumption comparison b) energy consumption per gallon of biodiesel comparison

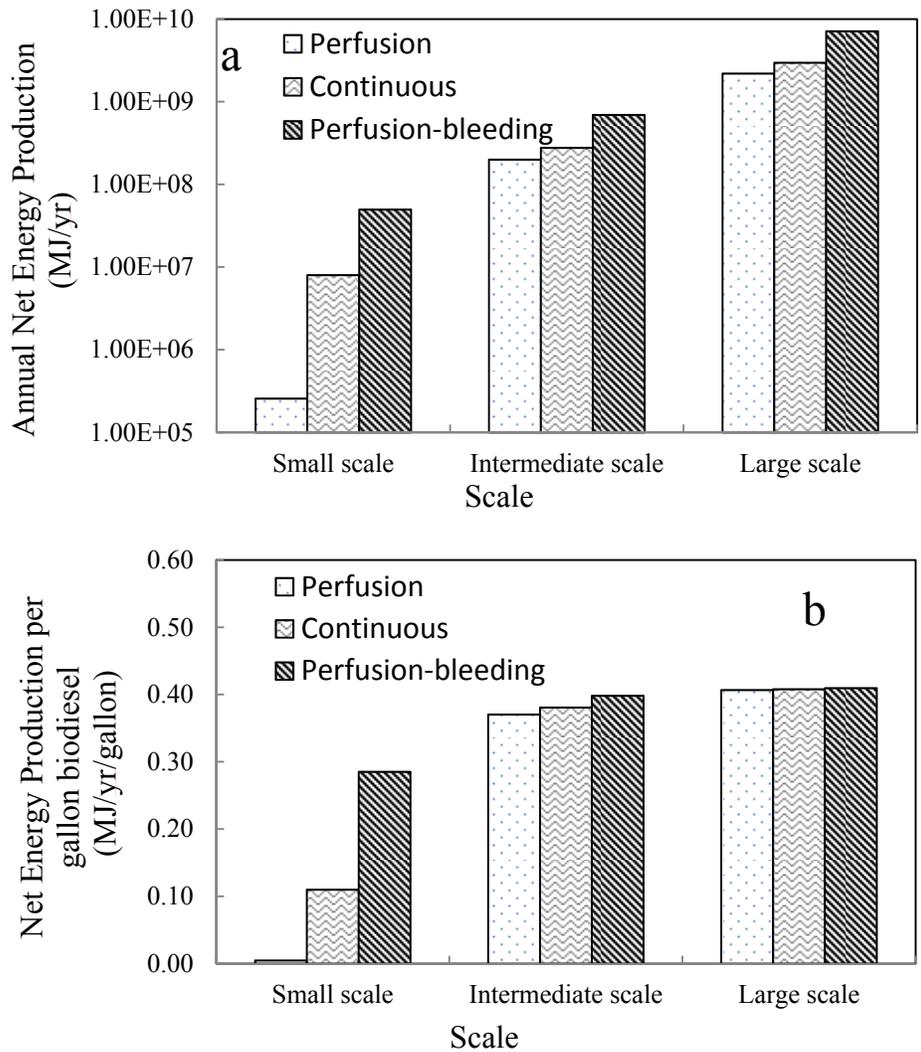


Figure 6. Net energy production analysis for three systems with different scales. a) annual net energy production comparison b) net energy production per gallon of biodiesel comparison

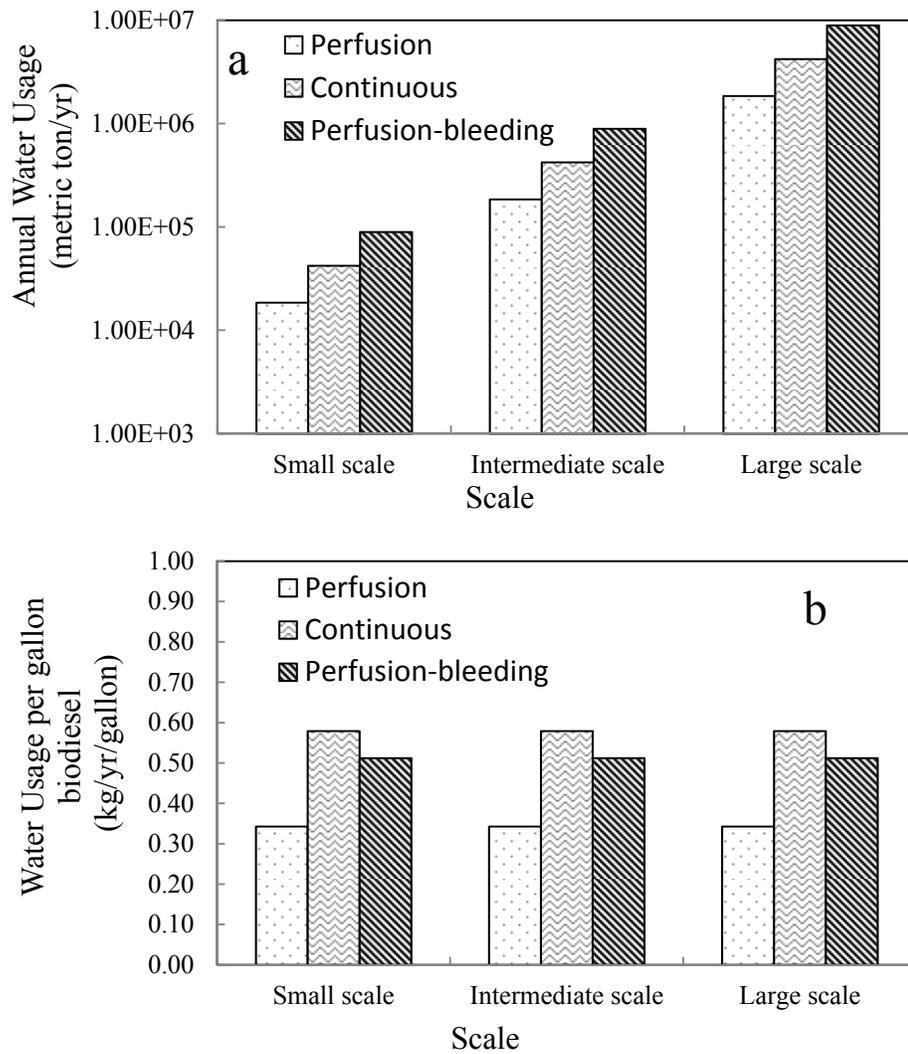


Figure 7. Water usage analysis for three systems with different scales. a) annual water usage comparison b) water usage per gallon of biodiesel comparison

APPENDIX A. Equipment list

Equipment List							
Section	EQUIPMENT TITLE	VENDOR	DESCRIPTION	HP	MATERIAL	Number reqd	\$
Pretreatment	In-line Mixer	KOMAX	Kynar Lined - 600 gpm H2O - 5 gpm acid		SS304	1	6,000
	Agitator	Andritz	Side-mounted, 3 x 75 hp. (170 kW)	170 kW	316LSS	1	INCLUDED
Media preparation	In-line Mixer	KOMAX	Kynar Lined - 600 gpm H2O - 5 gpm acid		SS304	1	6,000
	Agitator	Andritz	Side-mounted, 3 x 75 hp. (170 kW)	170 kW	316LSS	1	INCLUDED
Seed preparation	Seed Fermentor	Mueller	80,000 gal, 1 atm, 28 °C, Internal coil		SS316	3	400,500
	Agitator	Lotus		30 hp	SS304	1	52,500
Acetic acid Fermentation	Ethanol Fermentor	Mueller	1,000,000 gallon ea.		304SS	2	844,000
	Agitator	Lotus		30 hp	SS304	1	52,500
	Solid-Liquid Separator for perfusion	Larox					35,000,000
	pump for SF5	ADI	2500 gpm submersible rail mounted	50 hp	CS		231,488
	pump for media	ADI	2500 gpm submersible rail mounted	50 hp	CS		231,488
	pump for seed	ADI	2500 gpm submersible rail mounted	50 hp	CS		231,488
Harvest	Solid-Liquid Separator for Harvest	Larox					35,000,000
Oil extraction	In-line Mixer	KOMAX	Kynar Lined - 600 gpm H2O - 5 gpm acid		SS304	1	6,000
	Agitator	Andritz	Side-mounted, 3 x 75 hp. (170 kW)	170 kW	316LSS	1	INCLUDED
	pump for separator	ADI	2500 gpm submersible rail mounted	50 hp	CS		231,488
	Solid-Liquid Separator for Harvest	Larox					35,000,000

APPENDIX B-1. TEA calculations

		Unit	Perfusion	Continuous	Perfusion bleeding
Small scale	Annualized total costs	\$/year	31841777.70	18861973.96	38709960.47
	Annualized benefit	\$/year	231248238.68	293880931.73	699317029.87
	Annualized total costs per gallon biodiesel	\$/year/gallon	0.59	0.26	0.22
	Annualized total revenues per gallon biodiesel	\$/year/gallon	4.29	4.04	4.02
	Annualized "profits"	\$/year	199406460.99	275018957.77	660607069.40
	Annualized "profits" per gallon biodiesel	\$/year/gallon	3.70	3.78	3.79
Intermediate scale	Annualized total costs	\$/year	171770307.55	109934444.64	230091591.56
	Annualized benefit	\$/year	2203925231.19	2882299724.35	6876675560.60
	Annualized total costs per gallon biodiesel	\$/year/gallon	0.32	0.15	0.13
	Annualized total revenues per gallon biodiesel	\$/year/gallon	4.09	3.96	3.95
	Annualized "profits"	\$/year	2032154923.65	2772365279.70	6646583969.05
	Annualized "profits" per gallon biodiesel	\$/year/gallon	3.77	3.81	3.82
Large scale	Annualized total costs	\$/year	1018288616.41	744036307.94	1569222504.09
	Annualized benefit	\$/year	21507200629.58	28554557013.59	68209973865.74
	Annualized total costs per gallon biodiesel	\$/year/gallon	0.19	0.10	0.09
	Annualized total revenues per gallon biodiesel	\$/year/gallon	3.99	3.92	3.92
	Annualized "profits"	\$/year	20488912013.16	27810520705.65	66640751361.64
	Annualized "profits" per gallon biodiesel	\$/year/gallon	3.80	3.82	3.83

APPENDIX B-2. LCA calculations

		Unit	Perfusion	Continuous	Perfusion-bleeding
Small scale	Energy consumed	MJ/year	26072548.27	27532200.24	35390326.58
	Energy Produced	MJ/year	26329516.09	35525949.94	85035518.47
	Net energy production	MJ/year	256967.82	7993749.71	49645191.90
	CO2-eq	kg/year	0.00	0.00	0.00
	Water usage	metric ton/year	18468.00	42120.00	89154.00
	energy consumption per gallon biodiesel	MJ/year/gallon	0.48	0.38	0.20
	Net energy production per gallon biodiesel	MJ/year/gallon	0.00	0.11	0.29
	Water usage per gallon biodiesel	kg/year/gallon	0.34	0.58	0.51
Intermediate scale	Energy consumed	MJ/year	63683580.45	78280100.12	156861363.52
	Energy Produced	MJ/year	263295160.91	355259499.40	850355184.74
	Net energy production	MJ/year	199611580.46	276979399.29	693493821.22
	CO2-eq	kg/year	0.00	0.00	0.00
	Water usage	metric ton/year	184680.00	421200.00	891540.00
	energy consumption per gallon biodiesel	MJ/year/gallon	0.12	0.11	0.09
	Net energy production per gallon biodiesel	MJ/year/gallon	0.37	0.38	0.40
	Water usage per gallon biodiesel	kg/year/gallon	0.34	0.58	0.51
Large scale	Energy consumed	MJ/year	439793902.26	585759098.92	1371571733.01
	Energy Produced	MJ/year	2632951609.06	3552594994.04	8503551847.44
	Net energy production	MJ/year	2193157706.80	2966835895.12	7131980114.43
	CO2-eq	kg/year	0.00	0.00	0.00
	Water usage	metric ton/year	1846800.00	4212000.00	8915400.00
	energy consumption per gallon biodiesel	MJ/year/gallon	0.08	0.08	0.08
	Net energy production per gallon biodiesel	MJ/year/gallon	0.41	0.41	0.41
	Water usage per gallon biodiesel	kg/year/gallon	0.34	0.58	0.51

REFERENCES

- Bridgwater AV. 2012. Review of fast pyrolysis of biomass and product upgrading. *Biomass and Bioenergy* 38(0):68-94.
- Brown TR, Brown RC. 2013. A review of cellulosic biofuel commercial-scale projects in the United States. *Biofuels, Bioproducts and Biorefining* 7(3):235-245.
- Chen F, Johns MR. 1994. Substrate inhibition of *Chlamydomonas reinhardtii* by acetate in heterotrophic culture. *Process Biochemistry* 29(4):245-252.
- Chen F, Johns MR. 1996. Heterotrophic growth of *Chlamydomonas reinhardtii* on acetate in chemostat culture. *Process Biochemistry* 31(6):601-604.
- Demirbaş A. 2001. Biomass resource facilities and biomass conversion processing for fuels and chemicals. *Energy Conversion and Management* 42(11):1357-1378.
- Gnansounou E. 2010. Production and use of lignocellulosic bioethanol in Europe: Current situation and perspectives. *Bioresource Technology* 101(13):4842-4850.
- Goyal HB, Seal D, Saxena RC. 2008. Bio-fuels from thermochemical conversion of renewable resources: A review. *Renewable and Sustainable Energy Reviews* 12(2):504-517.
- Jarboe L, Wen Z, Choi D, Brown R. 2011. Hybrid thermochemical processing: fermentation of pyrolysis-derived bio-oil. *Applied Microbiology and Biotechnology* 91(6):1519-1523.
- Kim KH, Kim T-S, Lee S-M, Choi D, Yeo H, Choi I-G, Choi JW. 2013. Comparison of physicochemical features of bio oils and biochars produced from various woody biomasses by fast pyrolysis. *Renewable Energy* 50(0):188-195.
- Layton DS, Ajjarapu A, Choi DW, Jarboe LR. 2011. Engineering ethanologenic *Escherichia coli* for levoglucosan utilization. *Bioresource Technology* 102(17):8318-8322.
- Li Y, Han D, Hu G, Sommerfeld M, Hu Q. 2010. Inhibition of starch synthesis results in overproduction of lipids in *Chlamydomonas reinhardtii*. *Biotechnology and Bioengineering* 107(2):258-268.
- Lian J, Garcia-Perez M, Chen S. 2013. Fermentation of levoglucosan with oleaginous yeasts for lipid production. *Bioresource Technology* 133(0):183-189.
- Lian J, Garcia-Perez M, Coates R, Wu H, Chen S. 2012. Yeast fermentation of carboxylic acids obtained from pyrolytic aqueous phases for lipid production. *Bioresource Technology* 118(0):177-186.
- Mortensen PM, Grunwaldt JD, Jensen PA, Knudsen KG, Jensen AD. 2011. A review of catalytic upgrading of bio-oil to engine fuels. *Applied Catalysis A: General* 407(1-2):1-19.
- Naik SN, Goud VV, Rout PK, Dalai AK. 2010. Production of first and second generation biofuels: A comprehensive review. *Renewable and Sustainable Energy Reviews* 14(2):578-597.
- Perez-Garcia O, Escalante FME, de-Bashan LE, Bashan Y. 2011. Heterotrophic cultures of microalgae: Metabolism and potential products. *Water Research* 45(1):11-36.
- Pollard AS, Rover MR, Brown RC. 2012. Characterization of bio-oil recovered as stage fractions with unique chemical and physical properties. *Journal of Analytical and Applied Pyrolysis* 93(0):129-138.

- Sarkar N, Ghosh SK, Bannerjee S, Aikat K. 2012. Bioethanol production from agricultural wastes: An overview. *Renewable Energy* 37(1):19-27.
- Wen Z-Y, Chen F. 2002a. Perfusion culture of the diatom *Nitzschia laevis* for ultra-high yield of eicosapentaenoic acid. *Process Biochemistry* 38(4):523-529.
- Wen Z-Y, Chen F. 2003. Heterotrophic production of eicosapentaenoic acid by microalgae. *Biotechnology Advances* 21(4):273-294.
- Wen ZY, Chen F. 2001. A perfusion–cell bleeding culture strategy for enhancing the productivity of eicosapentaenoic acid by *Nitzschia laevis*. *Applied Microbiology and Biotechnology* 57(3):316-322.
- Wen Z-Y, Chen F. 2002b. Continuous cultivation of the diatom *Nitzschia laevis* for eicosapentaenoic acid production: physiological study and process optimization. (8756-7938 (Print)).
- Work VH, Radakovits R, Jinkerson RE, Meuser JE, Elliott LG, Vinyard DJ, Laurens LML, Dismukes GC, Posewitz MC. 2010. Increased Lipid Accumulation in the *Chlamydomonas reinhardtii* *sta7-10* Starchless Isoamylase Mutant and Increased Carbohydrate Synthesis in Complemented Strains.
- Xiu S, Shahbazi A. 2012. Bio-oil production and upgrading research: A review. *Renewable and Sustainable Energy Reviews* 16(7):4406-4414.
- Yang J, Xu M, Zhang X, Hu Q, Sommerfeld M, Chen Y. 2011. Life-cycle analysis on biodiesel production from microalgae: Water footprint and nutrients balance. *Bioresource Technology* 102(1):159-165.
- Zhao X, Chi Z, Rover M, Brown R, Jarboe L, Wen Z. 2013a. Microalgae fermentation of acetic acid-rich pyrolytic bio-oil: Reducing bio-oil toxicity by alkali treatment. *Environmental Progress & Sustainable Energy* 32(4): DOI 10.1002/ep.