

DOE Bioenergy Technologies Office (BETO) 2017 Project Peer Review

Integration of Nutrient and Water Recycling for Sustainable Algal Biorefineries

03/06/2017

ALGAE TECHNOLOGY AREA

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Goal Statement

Achieve high biomass productivity and recovery at lower cost through:

- Microalgae cultivation in high pH and high alkalinity media.
- Develop methods for harvesting and media recovery (for reuse) AND without use of contaminating chemicals (e.g. flocculants).

Quad Chart Overview

Timeline

- Project start : 2/1/2013
- Project end : 8/31/17
- Percent complete: 90%

Barriers

- Barriers addressed
 - Al-B. Algal Fuel Production
 - Feedstock development and nutrient supply
 - Harvest - Dewatering and water recycle

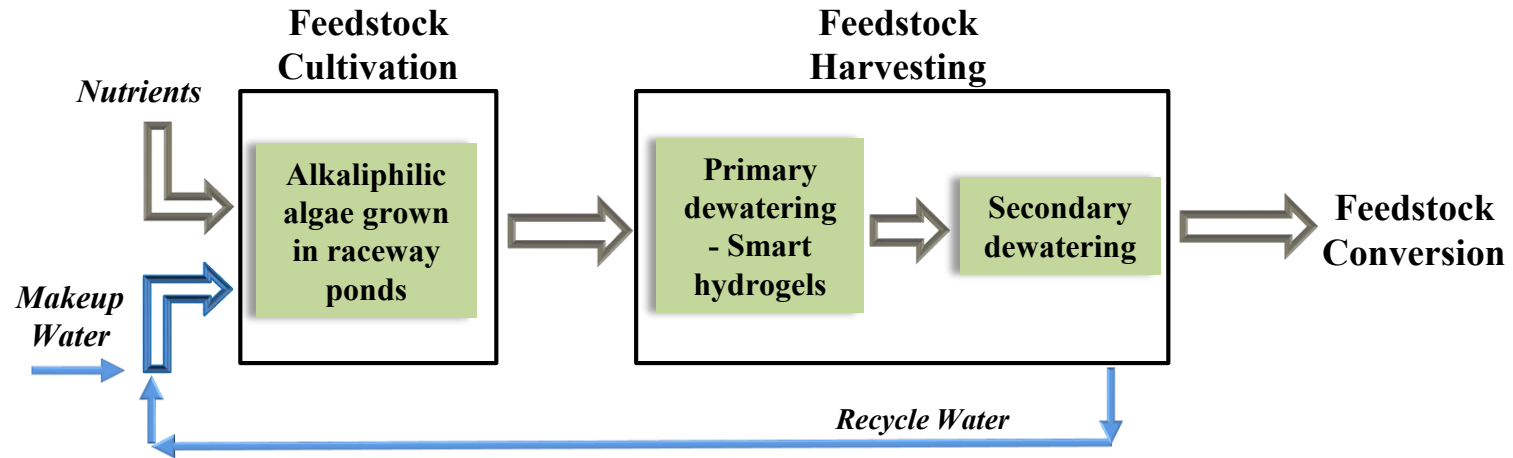
Budget

	Total Costs FY 12 –FY 14	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17- Project End Date)
DOE Funded	868,860	861,717	837,776	431,581
Project Cost Share (Comp.)*	201,377	213,137	221,482	114,097

Partners

- The University of Toledo – Lead (37%)
- Montana State University (37%)
- University of North Carolina (12%)
- University of Minnesota (6%)
- Clearas Water Recovery, Inc. (8%)

Project Overview



PROJECT OBJECTIVES:

- Decrease cost of cultivation through reduction in CO₂ supply cost and improvements in productivity.
- Develop low-cost water-recovery/harvesting methods.
- Characterize the development, structure, and stability of microbial communities in alkaline algal systems[†].
- Perform economic and life cycle assessments (LCA) for sustainable algal biorefineries[‡].

[†]Fields, M.W., et al. (2014) Applied Microbiology and Biotechnology. 98: 4805-4816

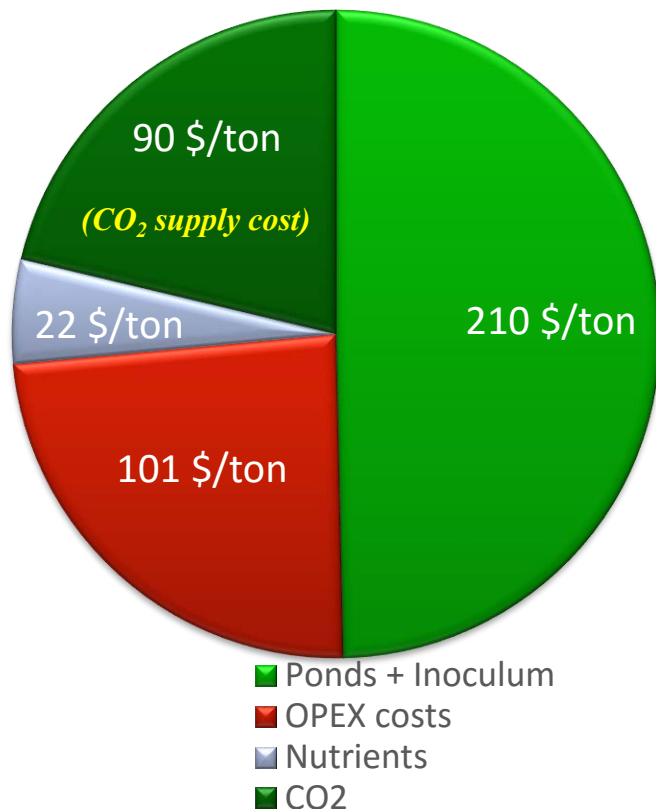
[†]Bell T.A.S., et al. (2016) Frontiers in Microbiology. 6:1480.

[‡]Hise, A.M., et al., (2016) Bioresource Technology. 220: 271-281.

[‡]Kern, J.D., et al., (2017) Bioresource Technology. 225: 418-428.

Technical Approach - Challenges

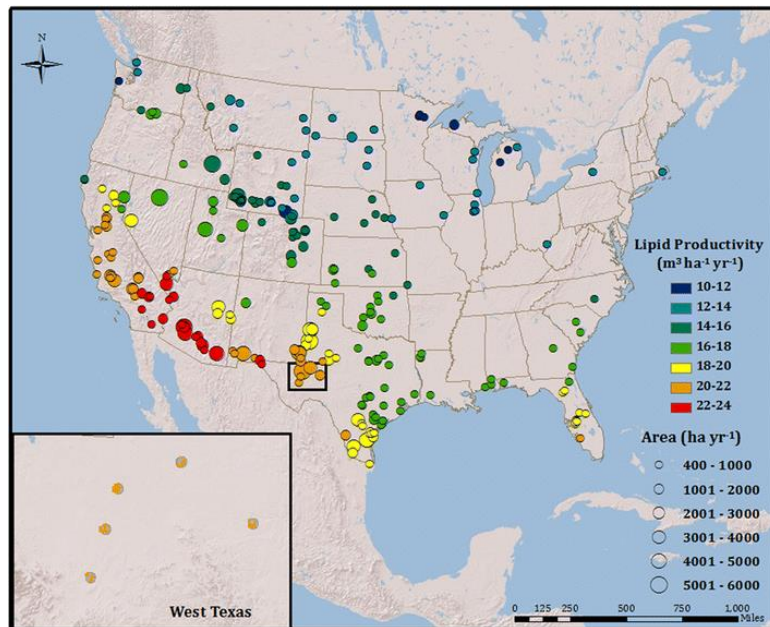
- Challenge 1: High cost of CO₂ supply



- Total algal biomass selling price
≈ **\$420/ton-biomass** (excluding harvesting costs)
- Cost for CO₂ supply
= **\$90/ton-biomass**
>20% of total biomass cost
~50% of variable operating costs

Technical Approach - Challenges

- Challenge 2: Simultaneous availability of land and CO₂ resources

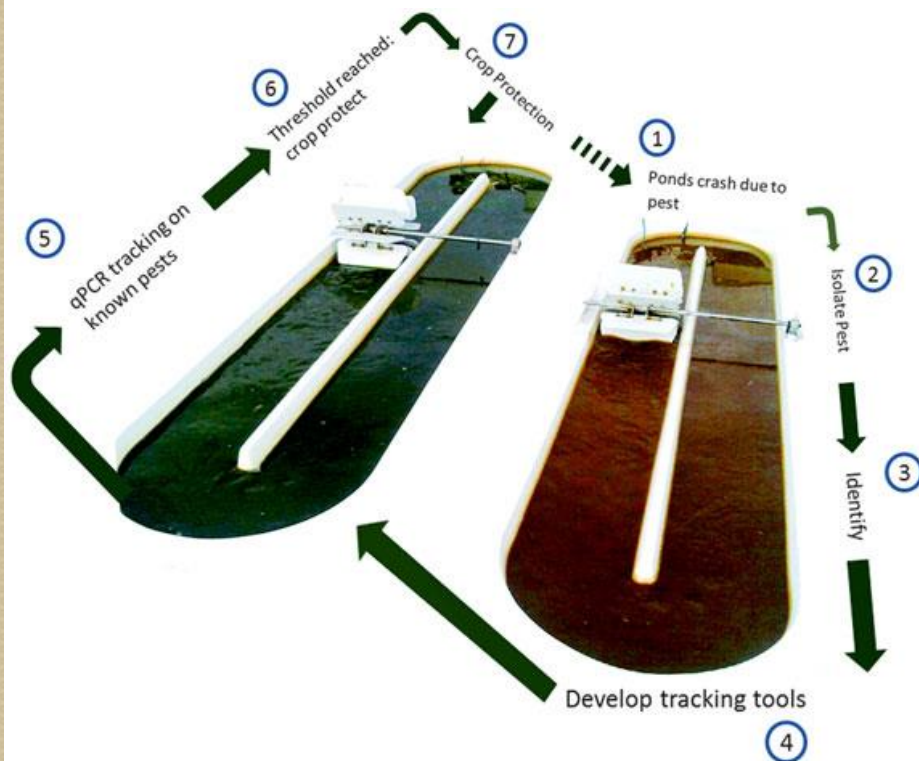


- Constraints
 - Land – *barren, slope < 2%*
- AND
- CO₂ transport distance – *< 4.8 km* (for economically viable CO₂ transport)
- Max. biofuel production
 - = **44 million barrels per year**
- EISA mandate for non-cellulosic advanced biofuel
 - = **100 million barrels per year**

If constrained by CO₂ availability, microalgae biofuels will likely be limited to <50% of the EISA “advanced biofuel” mandate

Technical Approach - Challenges

- Challenge 3: Frequent culture crashes



- Bacteria, viruses, zooplankton, invasive algae
- Productivity loss and/or “predator management costs”

“Possible solution – Cultivation of microalgae at *high alkalinity*”

- Advantage 1: Alkaline solutions scavenge CO₂ from the atmosphere at rapid rates.
 - Costs and geographical constraints associated with CO₂ supply can be mitigated (or eliminated)
- Advantage 2: Harsh pH conditions (pH>10) can mitigate detrimental contamination and predator populations
 - e.g. Daphnia (zooplankton) egg and neonate viability is low
 - Allows sustained maintenance of desired culture
 - e.g. Commercial *Spirulina* production is successfully carried out in high pH media

Approach – Critical success factors

- Biomass productivity of $20\text{g}/\text{m}^2/\text{d}$ or higher
- “Crash free” cultivation for extended periods (>3 months)
- Biomass composition favorable for biofuel production
 - High carbohydrate and/or lipid content
 - Low protein and ash content
- Low residence time ($<3\text{h}$) and high output concentrations ($>20\text{ g/L}$) for the hydrogel harvesting process
- Demonstrated reusability of media after harvesting

CO₂ transfer from the atmosphere into alkaline media

J_{CO_2} = CO₂ transfer flux (mol/m²/h)

$CO_{2(aq)}^*$ = Dissolved CO₂ in equilibrium with the atmosphere; calculated from Henry's constant.

$CO_{2(aq)}^{bulk}$ = Aqueous CO₂ concentration; determined by the equilibrium established with HCO₃⁻, OH⁻ and CO₃²⁻ ions in the medium (Eqs. 1 and 2) $CO_{2(aq)}^{bulk} = \frac{K_2}{K_1} \times \frac{[HCO_3^-]^2}{[CO_3^{2-}]}$

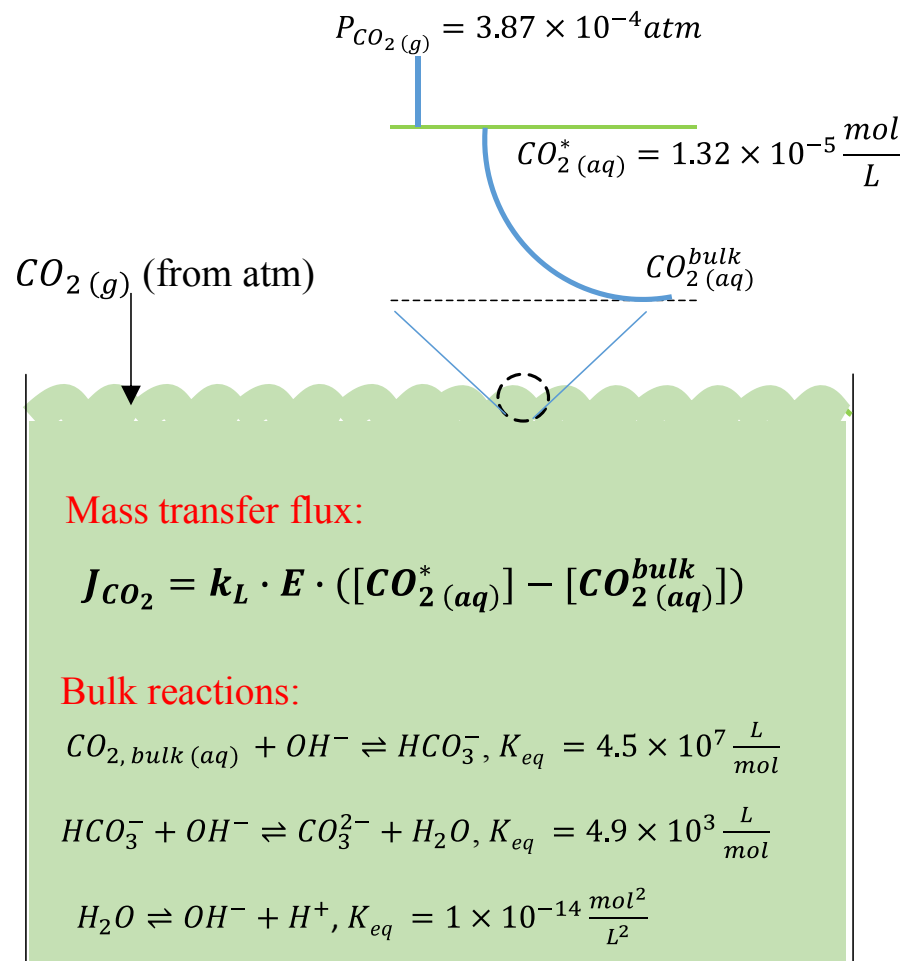
k_L = Mass transfer coefficient; governed by mixing rates and pond depth

= 0.1 m/h for 20 cm ponds mixed at 30 cm/s

E = Enhancement factor for mass transfer due to chemical reaction;

$$= 1 + \frac{D_{OH^-} \cdot D_{HCO_3^-} \cdot K_1 \cdot [OH^-]}{D_{CO_2} (K_1 \cdot [CO_2^* (aq)] \cdot D_{HCO_3^-} + D_{OH^-})}$$

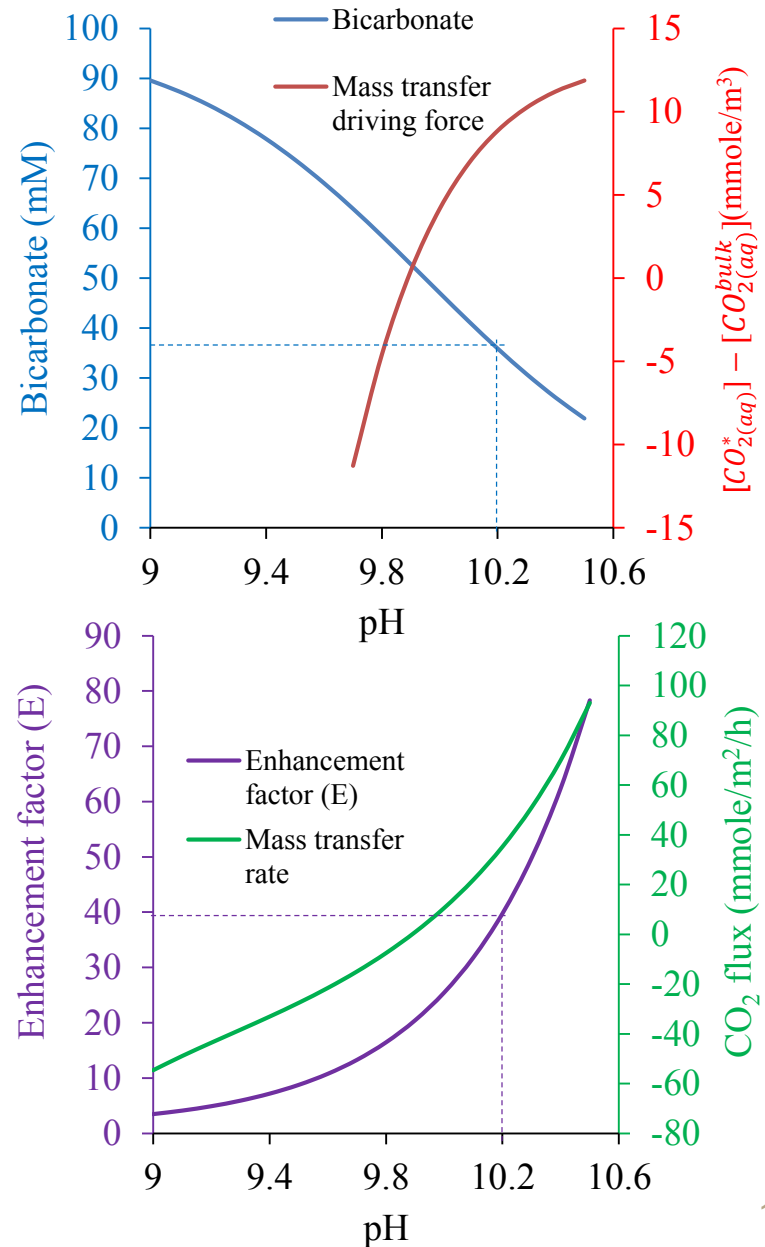
where, the subscripted D 's represent diffusion coefficients of the various dissolved species



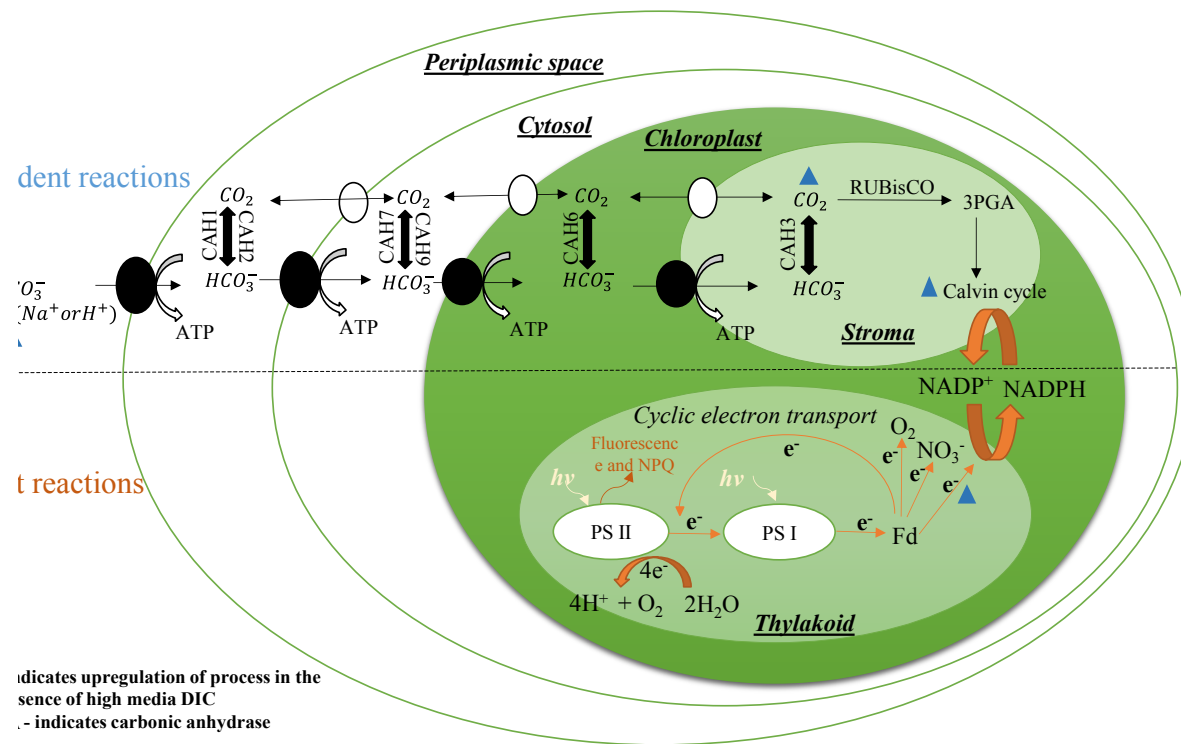
CO₂ transfer from the atmosphere into alkaline media

To maintain high atmospheric CO₂ flux and allow growth without concentrated CO₂ input,

- Maximize mass transfer driving force ($[CO_2^*_{(aq)}] - [CO_2^{bulk}_{(aq)}]$)
- Maximize enhancement factor (E) by maintaining high pH; $E \sim 40$ at pH 10.2
- High media alkalinity to maintain high HCO₃⁻ concentrations in the medium for photosynthesis to occur without inorganic carbon limitations

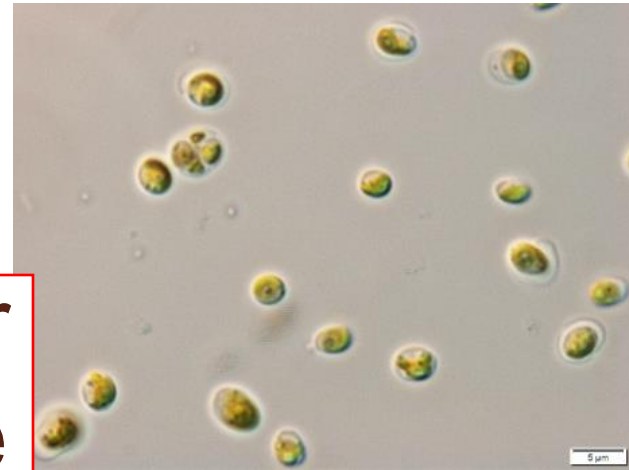
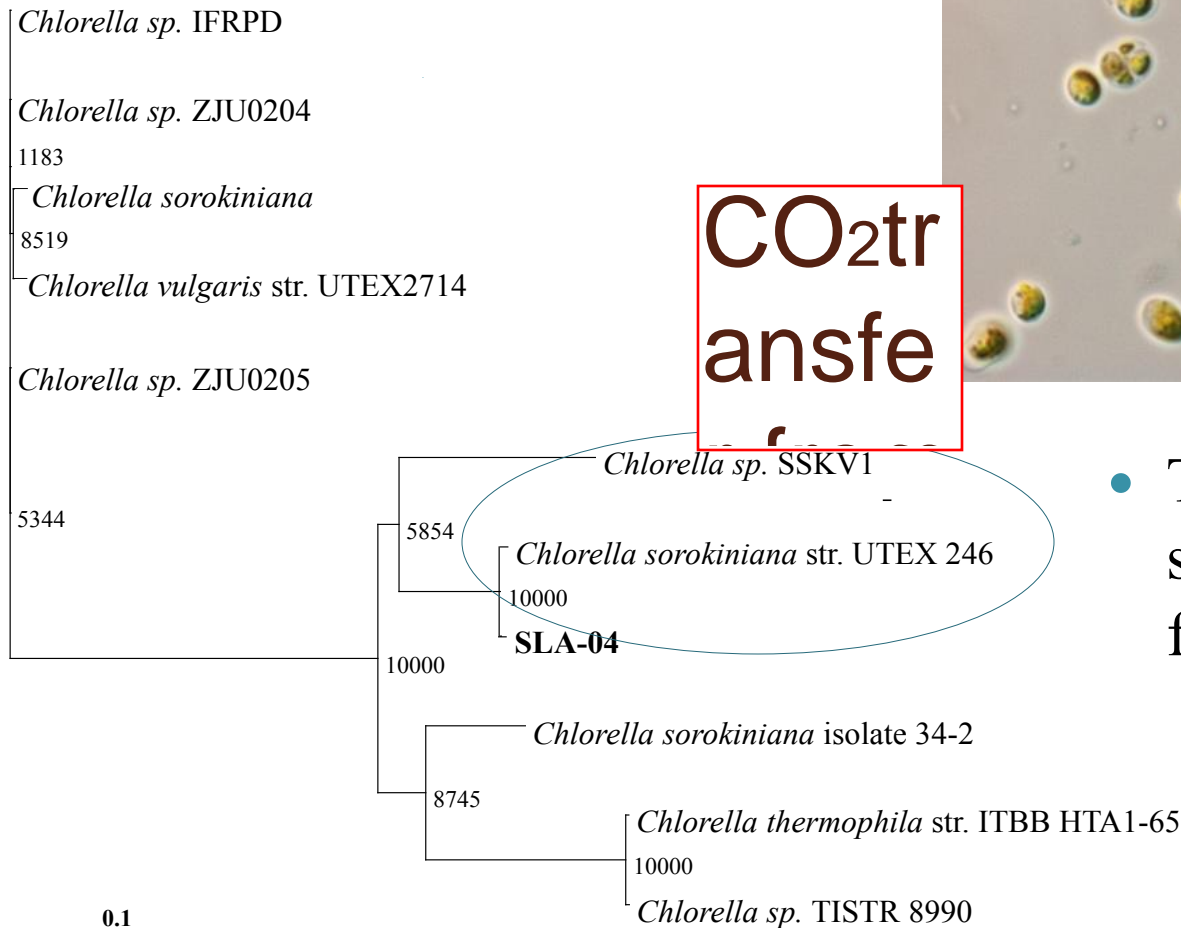


Cellular DIC transport and fixation in alkaline media



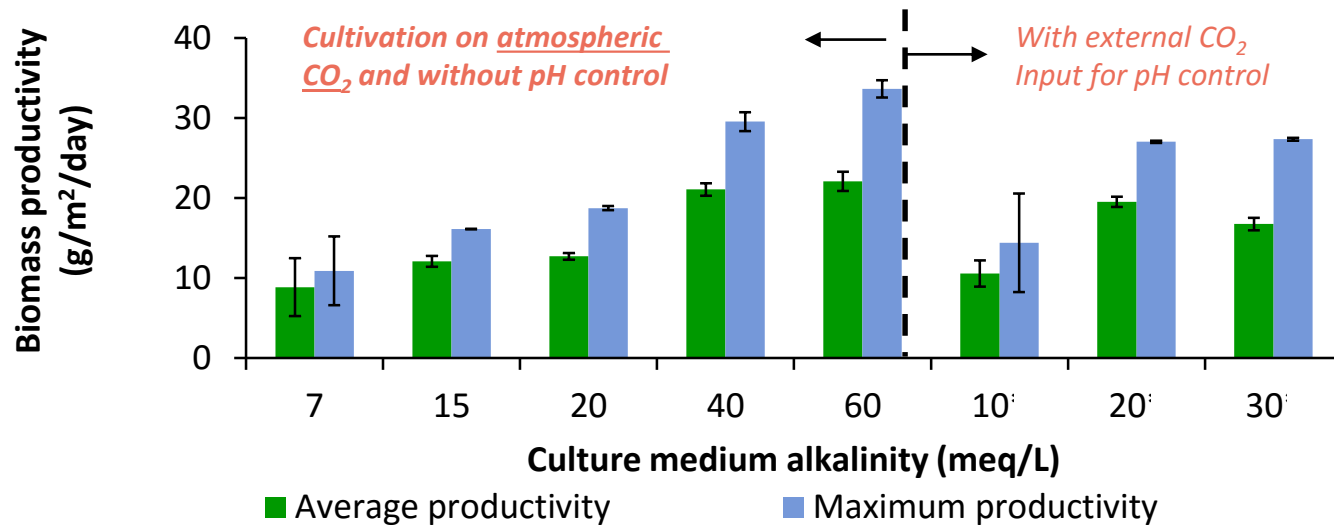
- Under alkaline conditions, DIC uptake occurs via CCMs
- High media DIC increases rate of cellular DIC transport
- Simultaneously, the high cellular DIC flux is also expected to drive the light dependent reactions towards higher production of NADPH for use in carbon fixation.

Results – Isolation and strain identification



- The alkaliphilic strain was isolated from Soap Lake, WA.

Results – Cultivation in high pH and high alkalinity media



- Experiments were performed in 30L outdoor raceway ponds.
- Without concentrated CO₂ inputs in high alkalinity media (40-60 meq/L),
 - Average areal productivities were **22 g/m²/d**
 - Maximum productivity of **32 g/m²/d** was measured.
- Average productivities of cultures grown without concentrated CO₂ inputs were similar to productivities of cultures grown with concentrated CO₂ input (pH maintained at 8.5).

Results – Cultivation in high pH and high alkalinity media

Energy flow	Description	Notation	High Alk. (60meq/L)	Low Alk. (7meq/L)
Towards carbon fixation	Effective PS II quantum yield <i>(photons utilized per incident photons)</i>	Y(II)	0.409	0.252
	Photosynthetic efficiency <i>(electrons per photon)</i>	α	0.217	0.13
	Maximum electron transfer rate <i>($\mu\text{mole}/\text{m}^2/\text{s}$)</i>	ETR _{max}	33.2	13.8
Dissipation	Quantum yield of regulated dissipation <i>(photons dissipated per incident photon)</i>	Y(NPQ)	0.047	0.085
	Quantum yield of unregulated dissipation <i>(photons dissipated per incident photon)</i>	Y(NO)	0.544	0.663

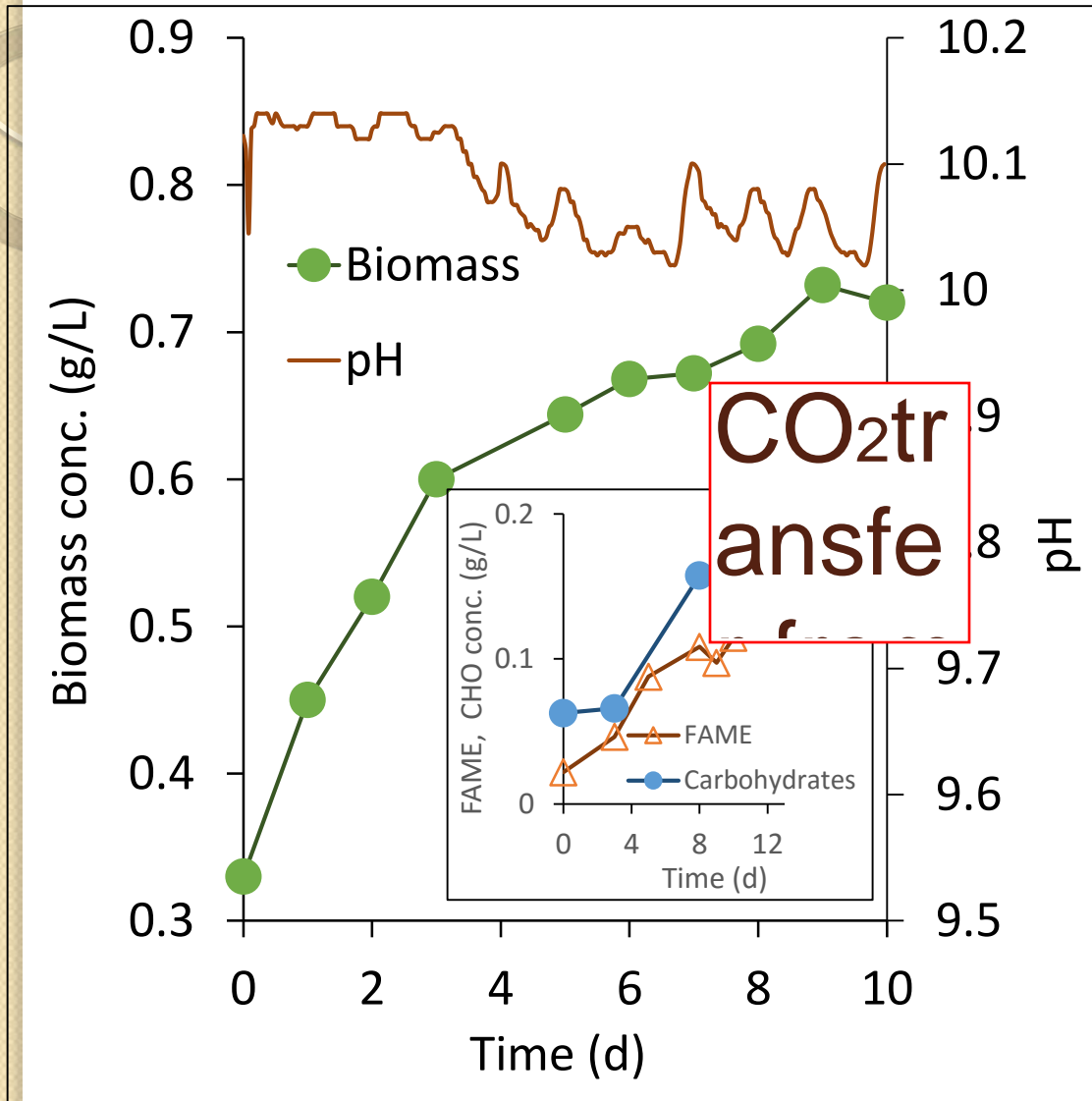
- Cultures growing at pH>10 and in the presence of high media DIC show high ETR_{max}, Y(II), and α values.
 - Better utilization of incident light for photosynthetic carbon fixation
- Cultures growing in low DIC media (pH>10) show high dissipation of electrons (cyclic electron transport)
 - Electron generation is inhibited due to low availability of cellular DIC.

Results – Correlation of theoretical mass transfer prediction with experimental measurements

- CO₂ transfer rates were experimentally determined in alkaline cultures and abiotic controls.
- Measurements of DIC increase were made at night by assessing changes in carbonate alkalinity
- k_L values were estimated from previous experimental measurements corrected using scaling factors (for linear velocity and pond depth) recommended by Weissman et al.
- E was estimated from measured pH.

Experiment	Predicted			Experimental
	k_L (m·h ⁻¹)	$k_L \cdot E$ (m·h ⁻¹)	Mass transfer flux (g-C·m ⁻² ·d ⁻¹)	Mass transfer flux (g-C·m ⁻² ·d ⁻¹)
Trial 1	0.04	1.51	5.25	6.18 ± 0.61
Trial 2	0.09	2.48	7.80	8.63 ± 0.85
Trial 3	0.13	4.70	7.46	8.25 ± 0.81
Abiotic Control	0.05	2.55	8.4	7.7 ± 0.2

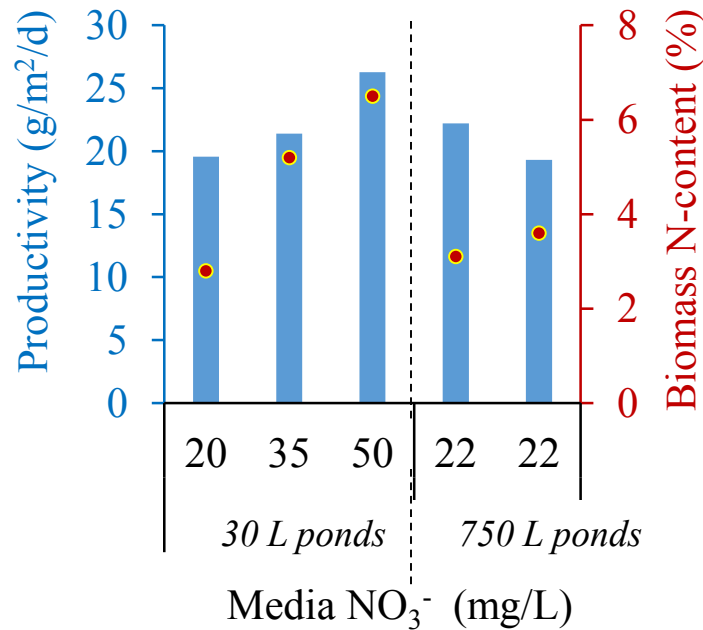
Results – Outdoor cultivation at 750L



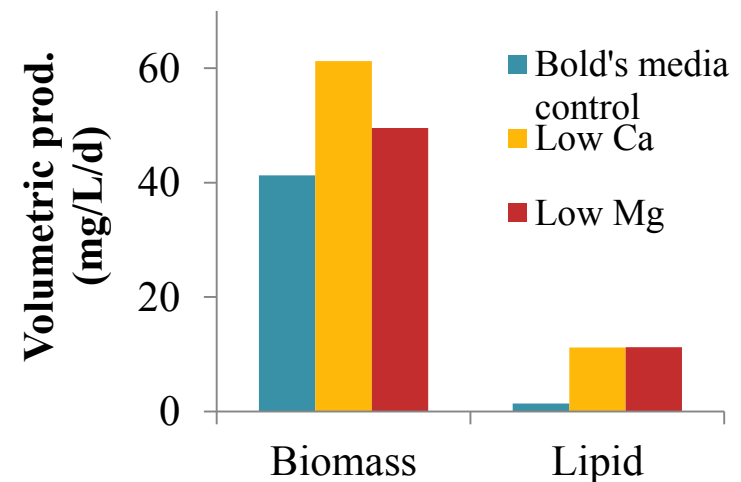
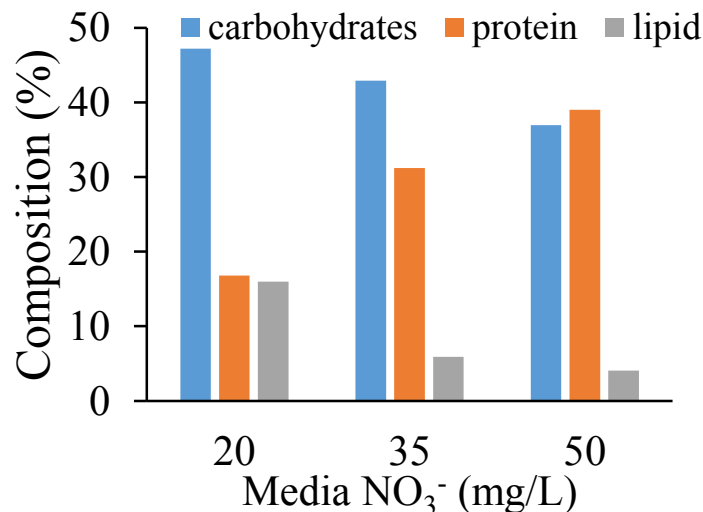
Phototrophic cultivation

- Biomass productivity = 23 g/m²/day
- Lipid productivity = 2 g/m²/day
- Carbohydrate productivity = 1.8 g/m²/day

Results – Macro/micro nutrient utilization



- High biomass productivities were maintained with low N in the media
- The resulting biomass also has a low N-content and higher carbohydrate and lipid content
 - Low-N biomass is desirable for conversion processes such as hydrothermal liquefaction
- Biomass and lipid productivities are improved (up to 33%) in low-Ca (1.5 mg/L and low-Mg (0.5mg/L) media

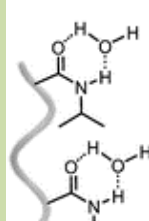
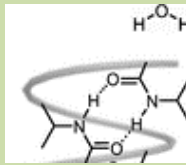
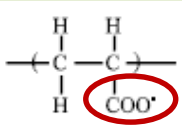
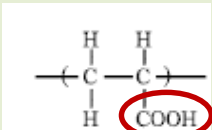


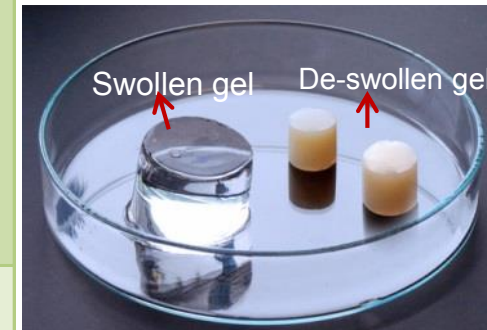
Summary of cultivation studies

- High media pH (>10) drives rapid transfer of CO₂ from the atmosphere to growth media
- High DIC concentrations “buffer” the media allow high media concentration of HCO₃⁻
 - Improves “electron transfer rates” – Likely due to higher rate of delivery of CO₂ to RuBisCO
- Under high-pH AND high-alkalinity conditions, cultures achieve high productivity *even in the absence of* concentrated CO₂ inputs.
- In outdoor cultivation experiments over 2 years, we haven’t observed a “culture crash”
- Biomass composition can be improved by “adjusting” nutrient composition without significantly compromising biomass productivity

Media recovery and harvesting through use of stimuli-sensitive hydrogels

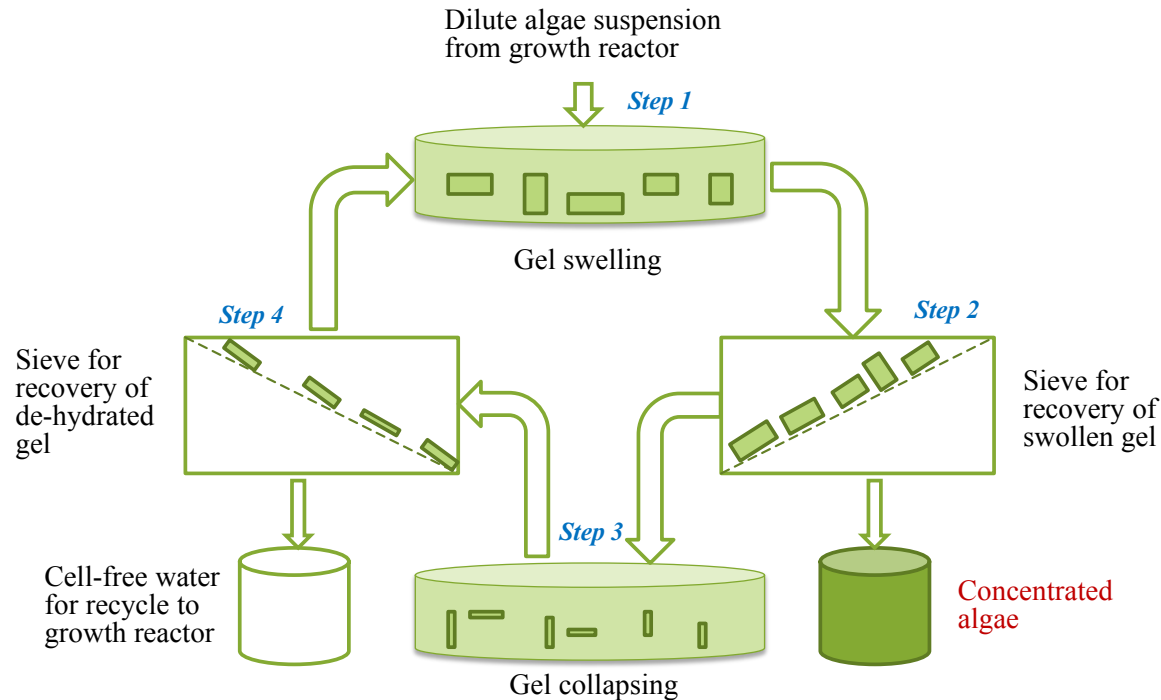
- Hydrogels that absorb and release water in response to an external stimulus

Stimulus type	Water absorption	Water release
Temperature	T < 30°C 	> 33°C 
pH sensitive	high pH (>7) 	low pH (<5) 



- Examples –
 - N-isopropyl acrylamide (pNIPAAm) is a temperature-sensitive hydrogel
 - Poly acrylic acid (PAA) is a pH-sensitive hydrogel

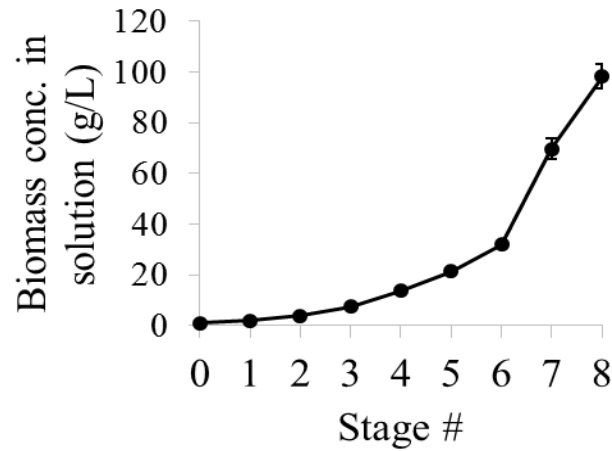
Hydrogel dewatering overview



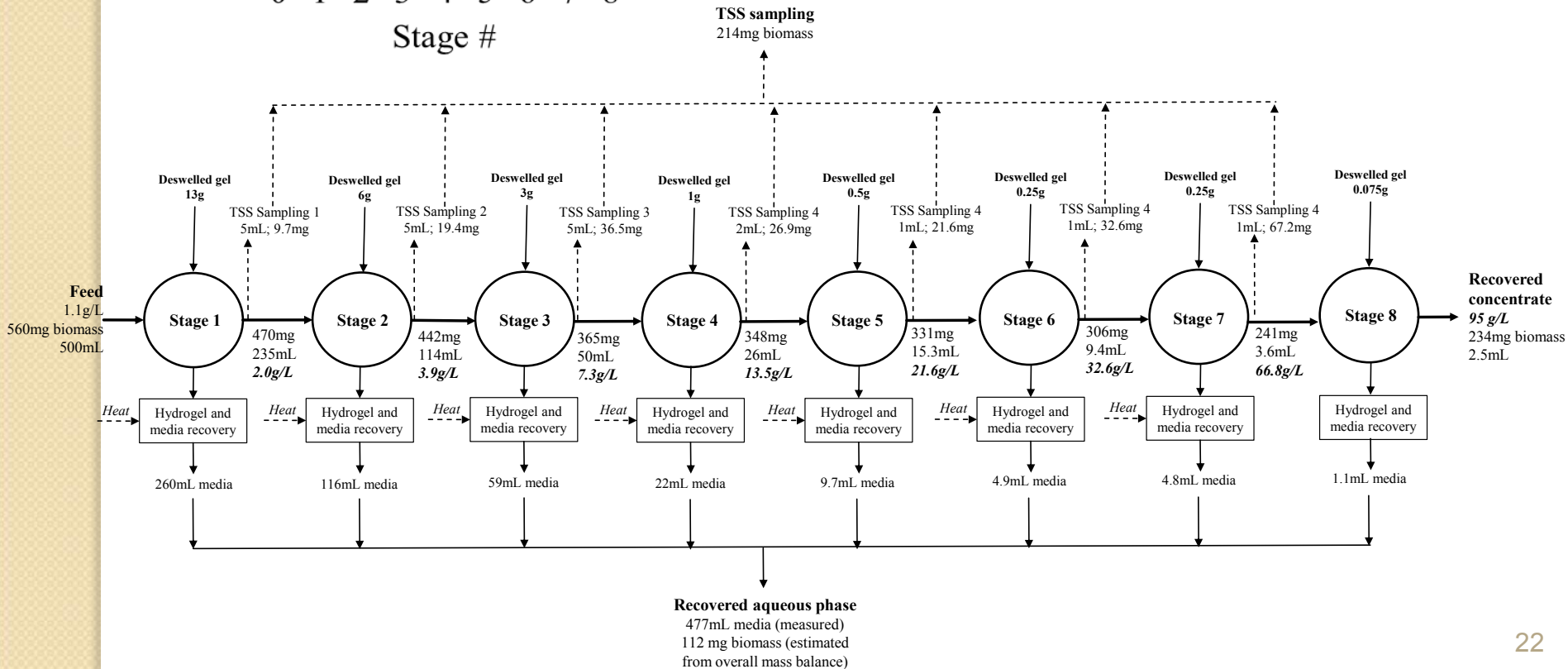
Key process parameters:

- Swelling and de-swelling rates in culture medium
- Water uptake per gram of de-hydrated gel (Swelling ratio)
- Operating conditions
 - Swelling and de-swelling period
 - Culture-swollen gel volume ratio

Stage-wise concentration of microalgae cultures using PNIPAAm hydrogels

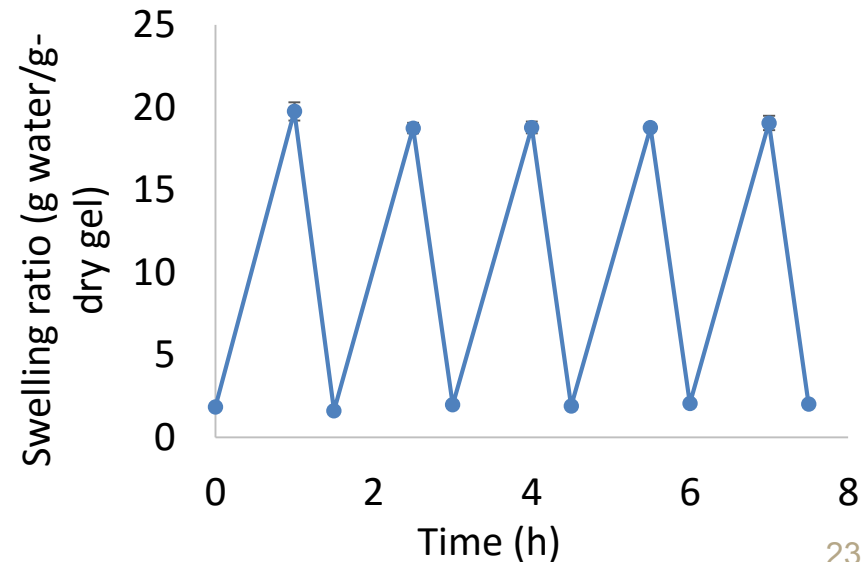
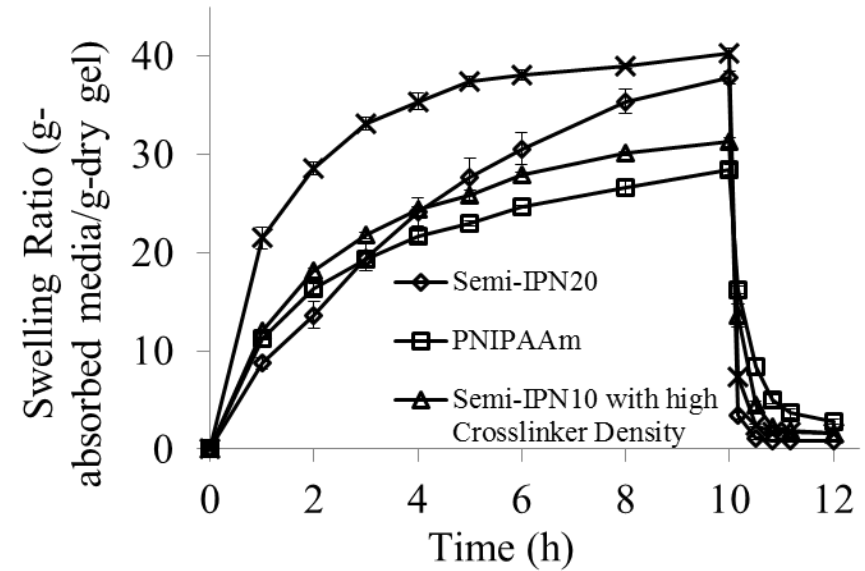


- Concentration was increased from 1g/L to 100 g/L BUT 2h duration per stage (lengthy process time)
 - Slow swelling of PNIPAAm
 - Several stages due to the low mass of absorbent gels used in each stage

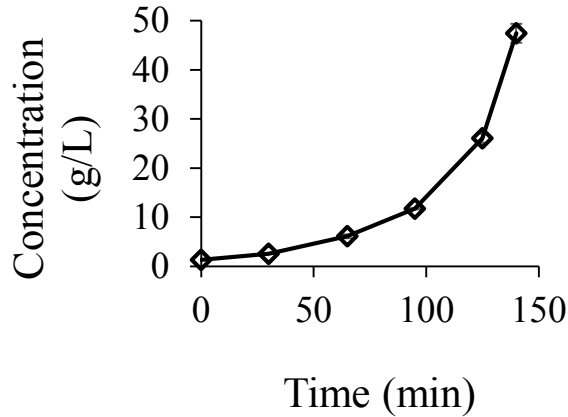


Improvements in performance of temperature-sensitive gels

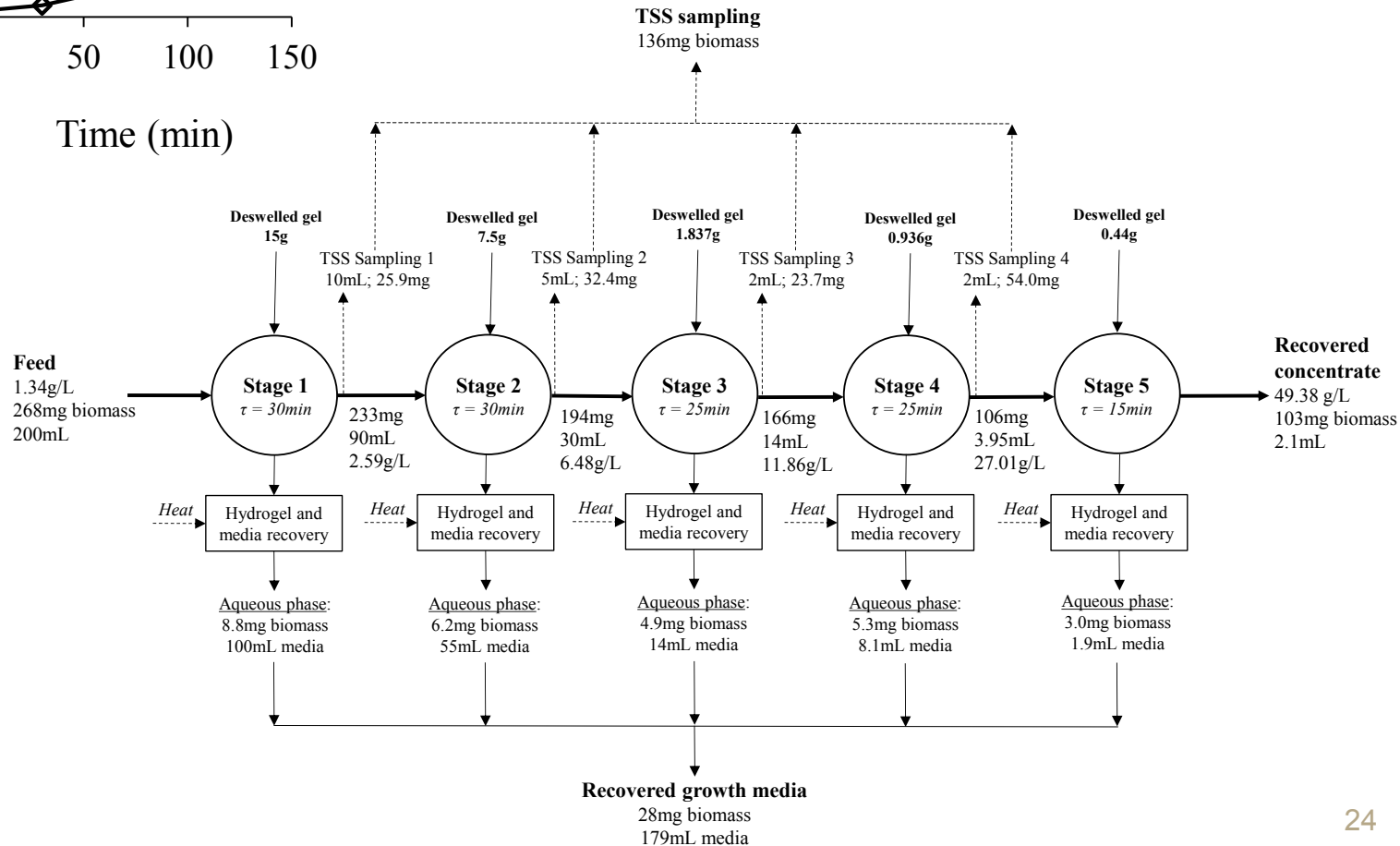
- Semi-IPN 10 gels (10% PVA + 90% p-NIPAAm) showed more rapid swelling and deswelling
- Gels retain performance over multiple cycles
- Semi-IPN gels have greater mechanical strength



Stage-wise concentration of microalgae cultures using semi-IPN10 hydrogels



- Total process time reduced to ~2.5h.
- Final culture concentration = 50g/L



Conclusions for hydrogel-based harvesting

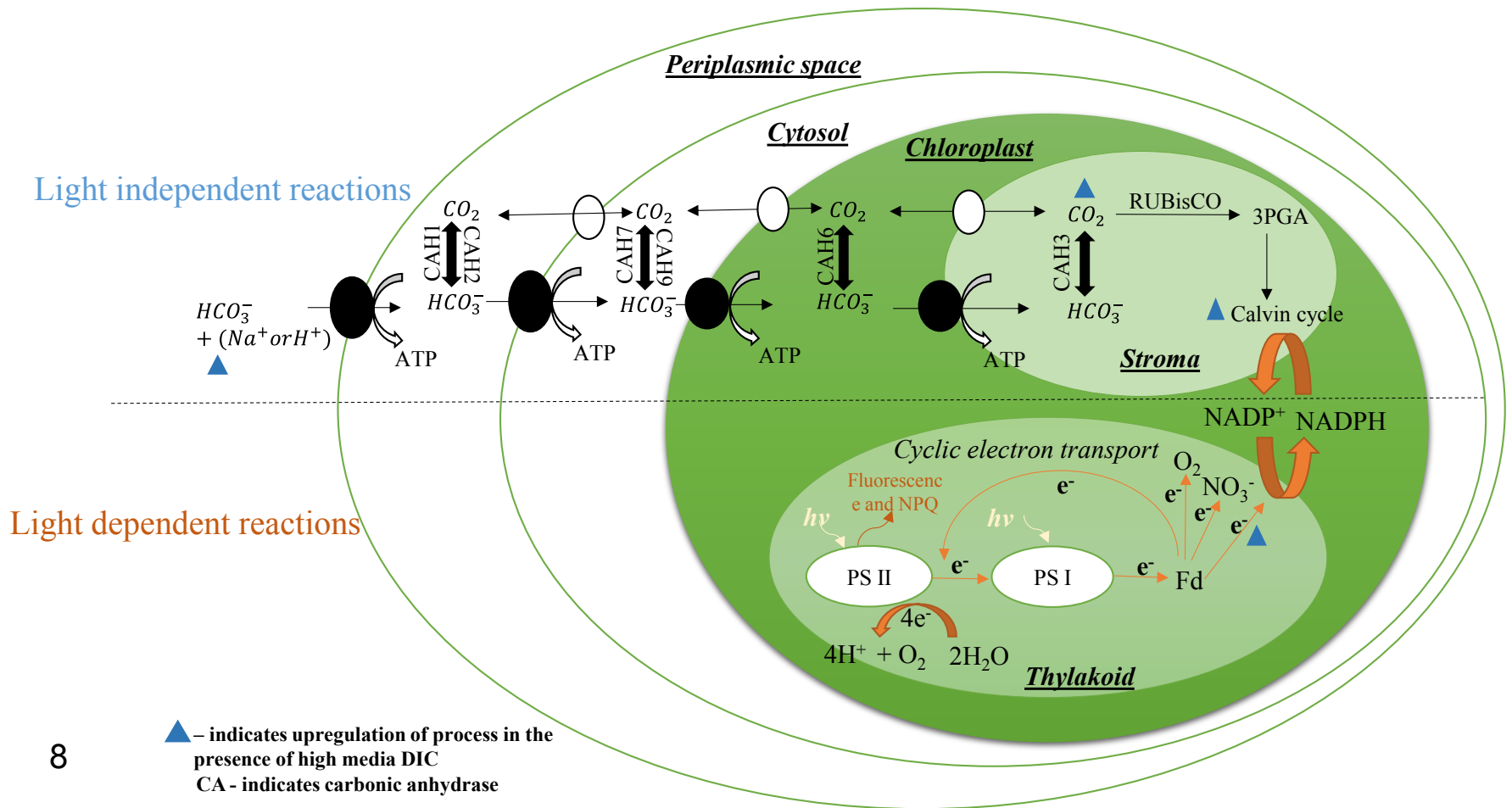
- With the hydrogel dewatering method, concentrations of up to ~100 g/L can be achieved.
- Gels can be re-used over multiple cycles without loss of gel functionality
 - High mechanical strength and elasticity
- Overall processing time could be <3h – comparable with residence times of other conventional processes
- The energy costs associated with the hydrogel-dewatering could be minimized by integration with low-grade waste heat

Additional Slides

(Not a template slide – for information purposes only)

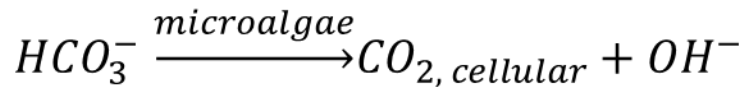
- *The following slides are to be included in your submission for Peer Evaluation purposes, but will **not** be part of your oral presentation –*
- *You may refer to them during the Q&A period if they are helpful to you in explaining certain points.*

Cellular DIC transport and fixation mechanisms in alkaline media

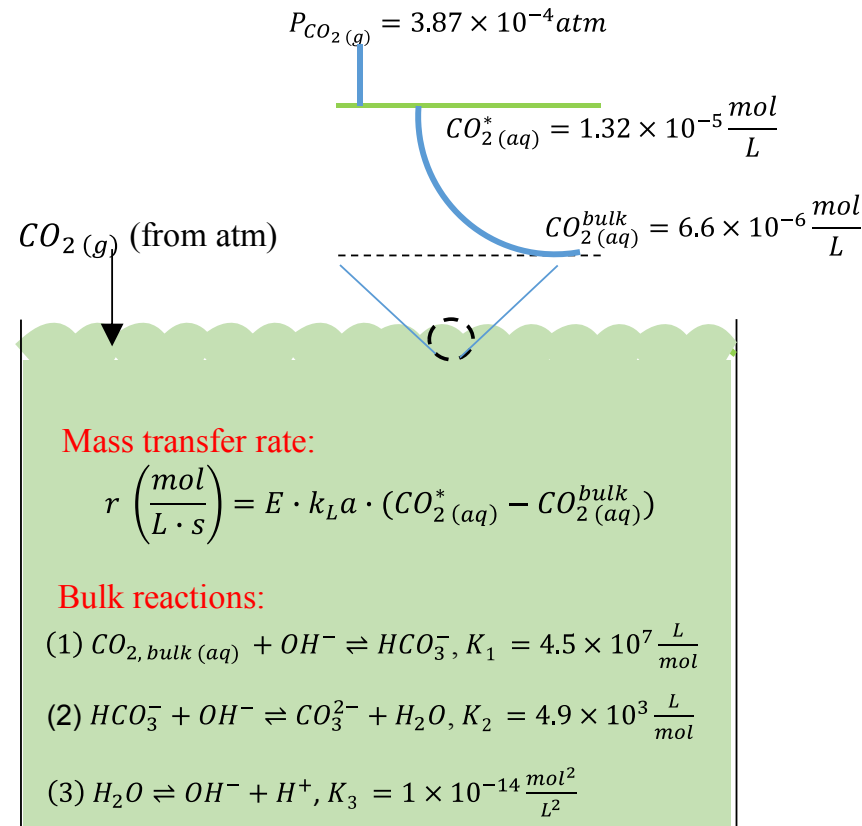


CO₂ transfer from the atmosphere into alkaline media

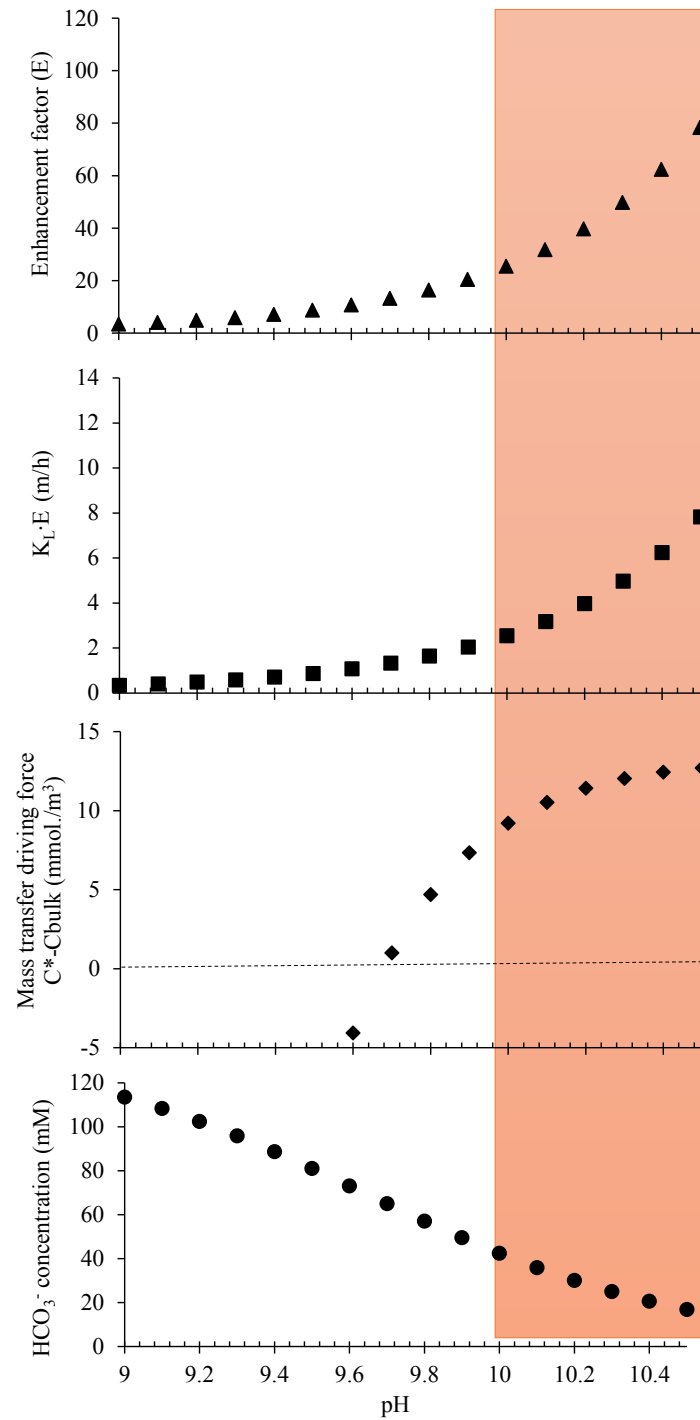
- During microalgae growth, HCO₃⁻ is taken up and CO₂ is fixed resulting in a net release of OH⁻

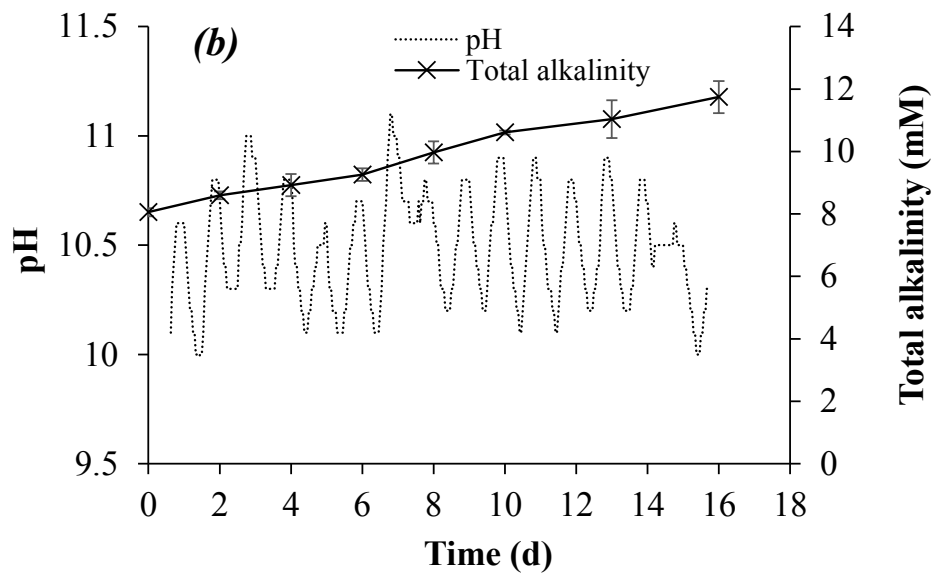


- Shifts the inorganic carbon equilibrium towards CO₃²⁻ (Eq. 2); microalgae are unable to uptake or utilize CO₃²⁻
- Depletion of HCO₃⁻ can be mitigated by
 - Maintaining high alkalinity in media (i.e. high buffer concentration) and
 - Replenishment from the atmosphere,



$$E = 1 + \frac{D_{OH^-} \cdot D_{HCO_3^-} \cdot K_1 \cdot [OH^-]}{D_{CO_2} \cdot (K_1 \cdot CO_{2(aq)}^* \cdot D_{HCO_3^-} + D_{OH^-})}$$





Detailed calculations for mass transfer rates of DIC (shown as Δ DIC).

	Time (d)	pH	Temperature (° C)	Ionic strength (I)	pK ₁	pK ₂	TA (mM)	HCO ₃ ⁻ (mM)	CO ₃ ²⁻ (mM)	DIC (mM)	Δ DIC (mM)	Δ DIC (mmol·m ⁻² ·d ⁻¹) ^c	Δ DIC (g·C·m ⁻² ·d ⁻¹)	
Set 1 ^a	Day 1	1.21	10.2	17.8	0.024	6.33	10.15	2.4	2.7	5.2	0.23	688.1	8.3	
		1.25	10.1	17.3		6.33	10.15	2.9	2.5	5.4				
	Day 4	4.33	10.2	24.0		6.29	10.09	2.2	2.9	5.0	0.22	635.3	7.6	
		4.38	10.1	23.8		6.29	10.09	2.6	2.7	5.3				
	Day 5	5.25	10.2	21.8		6.30	10.11	2.3	2.8	5.1	0.22	641.1	7.7	
		5.29	10.1	21.7		6.30	10.11	2.7	2.6	5.3				
	Day 6	6.29	10.2	20.9		6.31	10.12	8.1	2.3	2.8	5.1	0.22	657.6	7.9
		6.33	10.1	20.6		6.31	10.12		2.7	2.6	5.3			
	Day 10	10.38	10.2	16.6		6.34	10.16	2.5	2.7	5.2	0.23	681.7	8.2	
		10.42	10.1	16.3		6.34	10.16	2.9	2.5	5.4				
	Day 11	11.38	10.2	15.1		6.35	10.18	2.5	2.7	5.2	0.24	690.1	8.3	
		11.42	10.1	14.8		6.35	10.18	3.0	2.5	5.5				
	Day 15	15.25	10.2	23.2		6.29	10.09	2.2	2.8	5.1	0.22	645.0	7.7	
		15.29	10.1	22.9		6.30	10.10	2.6	2.6	5.3				
	Set 2 ^b	Day 1	0.99	10.2		17.8	6.33	10.16	1.4	1.5	2.9	0.14	548.3	6.6
1.03			10.1	17.5	6.34	10.17	1.6	1.4	3.0					
Day 2		1.97	10.2	18.9	6.33	10.15	1.3	1.5	2.8	0.14	617.0	7.4		
		2.00	10.1	18.5	6.33	10.16	1.6	1.4	3.0					
Day 3		2.95	10.2	19.7	6.32	10.14	1.3	1.5	2.8	0.14	618.3	7.4		
		2.98	10.1	19.2	6.32	10.15	1.6	1.4	3.0					
Day 4		3.97	10.2	19.2	6.32	10.15	4.5	1.3	1.5	2.8	0.14	615.5	7.4	
		3.99	10.1	18.8	6.33	10.15		1.6	1.4	3.0				
Day 5		4.77	10.2	20.2	6.32	10.14	1.3	1.5	2.8	0.14	547.4	6.6		
		4.80	10.1	19.7	6.32	10.14	1.6	1.4	3.0					
Day 6		5.77	10.2	20.2	6.32	10.14	1.3	1.5	2.8	0.14	492.7	5.9		
		5.80	10.1	19.7	6.32	10.14	1.6	1.4	3.0					
Day 7		6.72	10.2	20.8	6.31	10.13	1.3	1.5	2.8	0.14	558.4	6.7		
		6.75	10.1	20.0	6.32	10.14	1.5	1.4	3.0					

^a Data obtained from the experiments carried out during December 2013

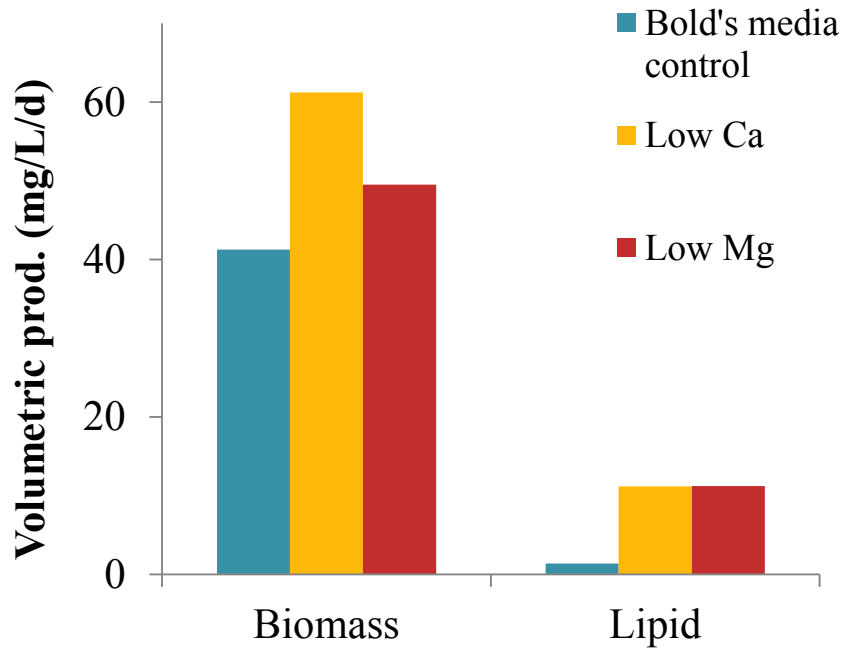
^b Data obtained from the experiments carried out during September 2013

^c Cultivations were carried out in small raceway ponds (surface area = 0.18 m²) and the rate of increase in DIC was calculated using this surface area.

Mass transfer coefficients calculated using the mass transfer rates

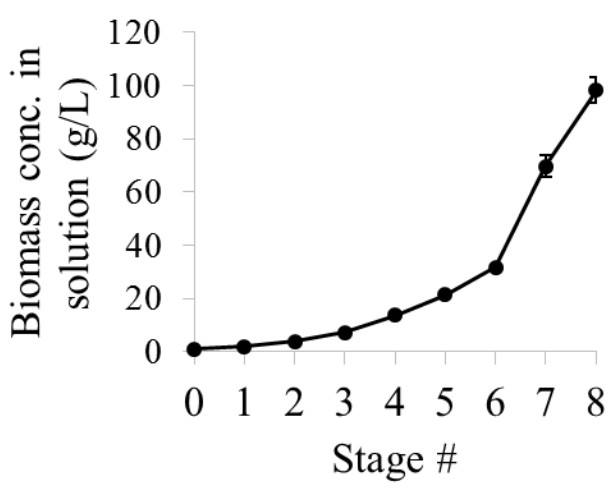
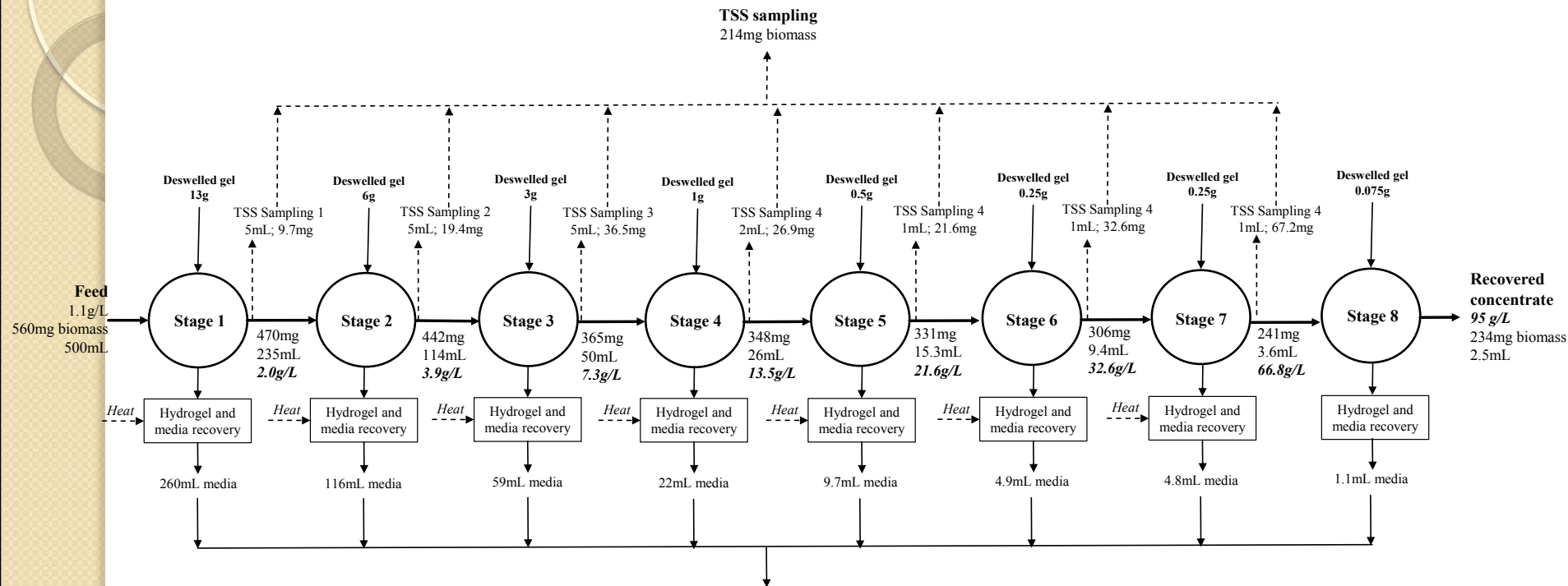
Δ DIC (mmol·m ⁻² ·d ⁻¹)	Mass transfer driving force			$\frac{KL \cdot E}{(m \cdot h^{-1})}$	$\frac{K_L}{(m \cdot h^{-1})}$
	$\frac{CO_2^*_{(aq)}}{(mmol \cdot m^{-3})}$	$\frac{CO_2^{bulk}_{(aq)}}{(mmol \cdot m^{-3})}$	$\frac{CO_2^*_{(aq)} - CO_2^{bulk}_{(aq)}}{(mmol \cdot m^{-3})}$		
688.1	13.2	0.23	12.97	2.21	0.06
635.3		0.18	13.02	2.03	0.05
641.1		0.20	13.00	2.05	0.05
657.6		0.21	12.99	2.11	0.05
681.7		0.24	12.96	2.19	0.06
690.1		0.26	12.94	2.22	0.06
645.0		0.19	13.01	2.07	0.05
548.3		0.20	13.00	1.76	0.04
617.0		0.19	13.01	1.98	0.05
618.3		0.19	13.01	1.98	0.05
615.5		0.19	13.01	1.97	0.05
547.4		0.19	13.01	1.75	0.04
492.7		0.19	13.01	1.58	0.04
558.4		0.18	13.02	1.79	0.04

Results – Micro nutrient utilization



- Biomass and lipid productivities are improved (up to 33%) in low-Ca (1.5 mg/L and low-Mg (0.5mg/L) media
- Standard media have 10× higher Ca and Mg concentrations

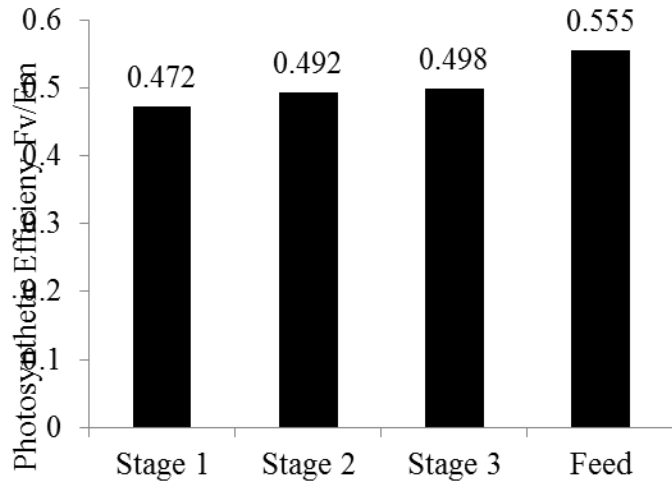
Stage-wise concentration of microalgae cultures using PNIPAAm hydrogels



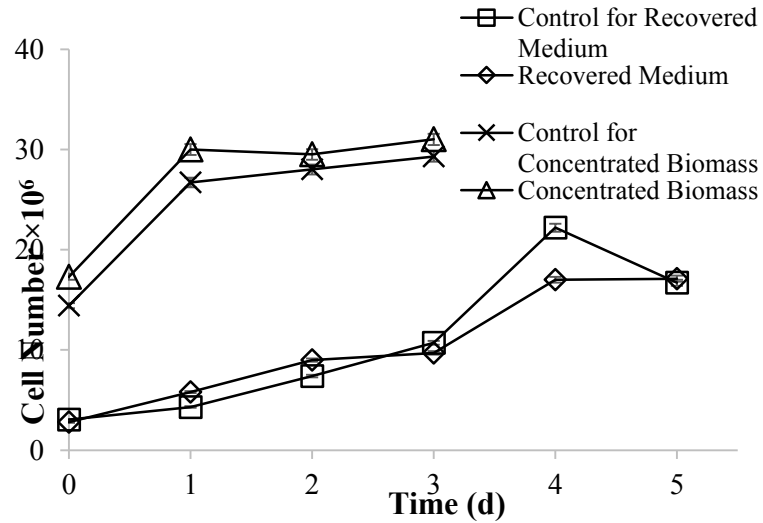
Recovered aqueous phase
 477mL media (measured)
 112 mg biomass (estimated from overall mass balance)

- Concentration was increased from 1g/L to 100 g/L BUT 2h duration per stage (lengthy process time)
 - Slow swelling of PNIPAAm
 - Several stages due to the low mass of absorbent gels used in each stage

Viability of harvested biomass and unrecovered cells

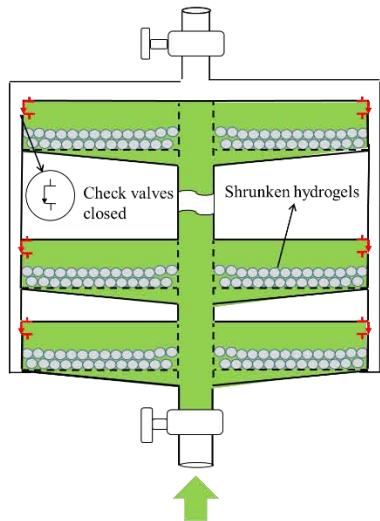


Photosynthetic efficiencies of feed and concentrated cultures

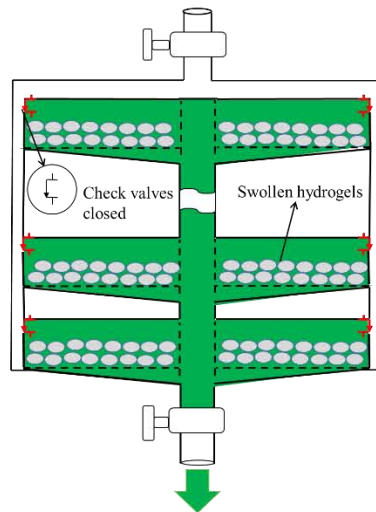


Growth of harvested biomass and unrecovered cells

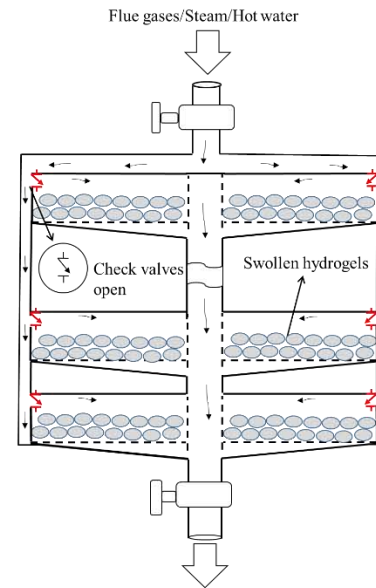
Design of a harvesting device



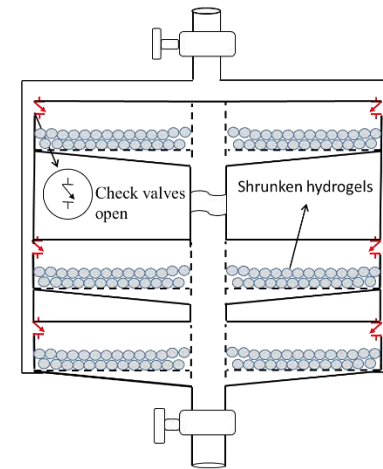
Step 1: Dilute algae solution introduced into inner chamber through the bottom valve. Check valves connected to jacket are kept closed to prevent leakage of algae slurry into jacket.



Step 2: Concentrated algae solution removed from inner chamber leaving behind swollen hydrogels containing absorbed growth media. Check valves connected to jacket remain closed.

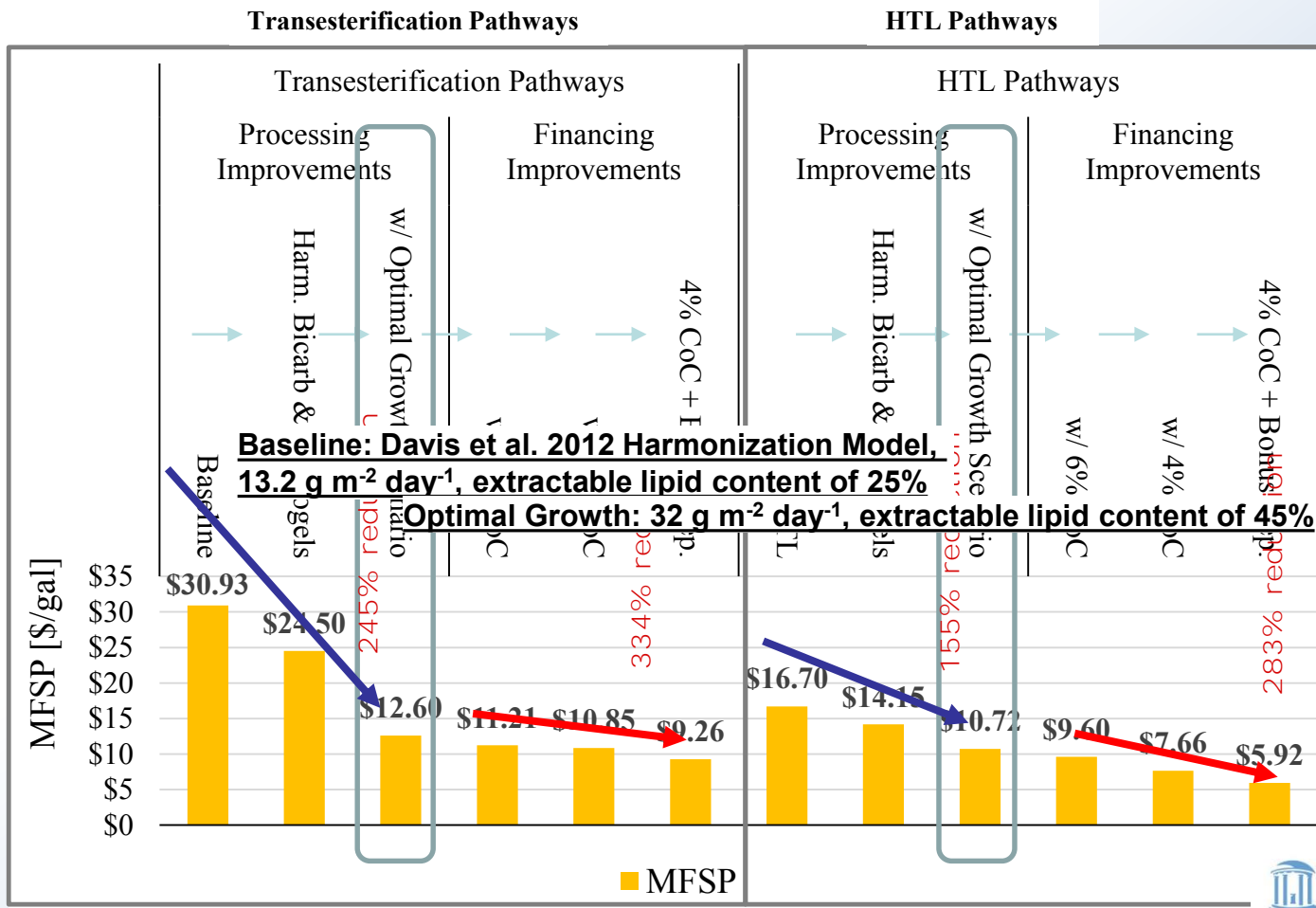


Step 3: Heat or CO_2 is introduced into the jacket through the top valve. Check valves on the jacket are forced open allowing the heat/ CO_2 to enter into the inner chamber. These stimuli cause deswelling of the swollen hydrogels. The desorbed growth media is drained through the bottom valve.



Step 4: Hydrogels are fully shrunken and top valve is closed. Inner chamber has been fully drained and is ready for re-introduction of dilute algae feed.

Impact of Technical Improvements and Financing Incentives



Hise et al. (2016) Evaluating the relative impacts of operational and financial factors on the competitiveness of an algal biofuel production facility. Bioresource Technology. 220:271-281.



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Responses to Previous Reviewers' Comments

- Reviewers suggested that we focus on hydrogel harvesting task and we have improved the method significantly since the previous review
- Reviewers suggested we pivot towards scale-up of cultivation. Since the last peer review we have focused on outdoor cultivation and scale-up.

Note: This slide is for the use of the Peer Reviewers only – it is not to be presented as part of your oral presentation. These Additional Slides will be included in the copy of your presentation that will be made available to the Reviewers.

Publications and Presentations

Publications:

1. Lohman, E. J.; Gardner, R.D.; Halverson, L.; Macur, R.; Peyton, B.M.; Gerlach, R. An Efficient and Scalable Extraction and Quantification Method for Algal Derived Biofuel. *Journal of Microbiological Methods*. Accepted. June 04, 2013. MIMET-D-13-00177. DOI: 10.1016/j.mimet.2013.06.007
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- Maddi B, Viamajala S, Varanasi S. "Pyrolytic fractionation of oleaginous algae." Platform presentation at the 2012 AIChE Annual Meeting, Pittsburg, PA (October 28 - November 2, 2012).

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- Maddi B, Vadlamani A, Viamajala S, Varanasi S. “Triglyceride quantification in oleaginous biomass using thermogravimetry (TG).” Poster presented at the 2012 AIChE Annual Meeting, Pittsburg, PA (October 28 - November 2, 2012).
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Publications and Presentations

Presentations (cont.):

- Jackson, M.C.; Pedersen, T.; Berninghaus, A.; Gardner, R.; Gerlach, R. (2015). *Promoting Lipid Accumulation in Chlorella vulgaris UTEX395 Using Nitrogen Limitation and Bicarbonate Amendment*. 2015 Montana Biofilm Meeting. Bozeman, MT.
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- Maddi B, Viamajala S, Varanasi S (2015). *Pyrolytic fractionation: A thermo-chemical technique for processing oleaginous algae*. Annual AIChE conference. Salt Lake City UT.
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