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Algae-based feed ingredients for aquaculture: synergies from microalgal fuel industry

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Outline

- Issues with fish oil and fishmeal
  - Aquaculture versus wild catch and demand/pricing
- Microalgae as a source of nutrition
  - Proximate analysis
  - Protein source
  - Lipid source
  - Micronutrients
- Microalgae environmental advantage
  - Water and nutrients
- Synergies with
  - Biofuels
  - CO_2 capture
- Production of microalgae at scale
  - Issues of cost and scale
- Synthetic Genomics' strategy
  - Facilities
  - Enhancement of desired traits

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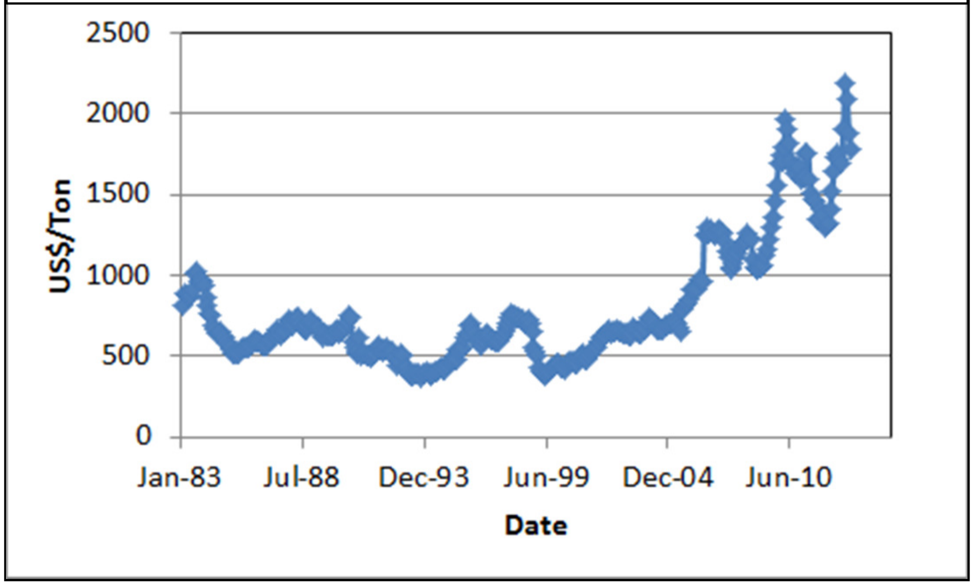
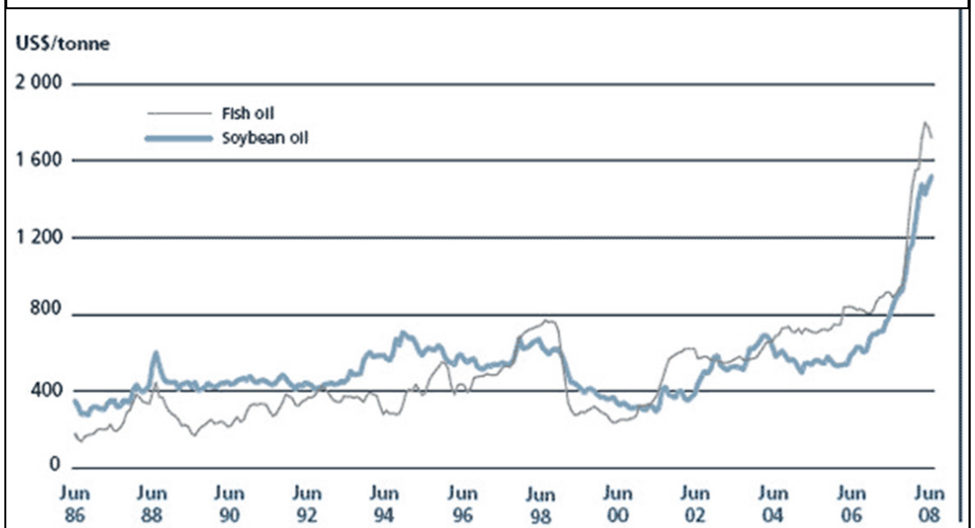
Issues with fish oil and fishmeal

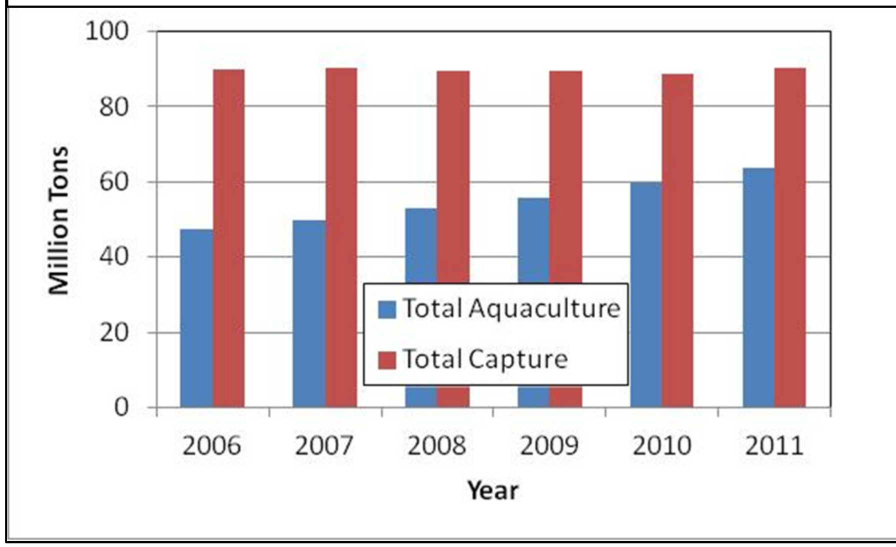
Fishmeal, Peru Fish meal/pellets 65% protein, US$/Metric Ton (http://www.indexmundi.com/)



Fish oil and soybean oil prices in the Netherlands (http://www.greenfacts.org/en/fisheries/)



Fisheries vs. aquaculture production of aquatic food 2006-2011. It is expected that aquaculture production will surpass fisheries within the next 5 years. Data from FAO, 2012.



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Microalgae nutritional advantage: proximate analysis

microalgae can provide complete protein with lipids

Source	Protein crude%	Pepsin digestibility%	Oil %	Fiber crude%
Menhaden	64.2	92.8	8.9	
Target	*65.0*	*>85*	*12.0*	
Tetraselmis	*49.1*	*93.2*	*13.7*	*0.4*
Rhodomonas	*45.7*	*90.1*	*25.1*	*2.1*
Pavlova	57.5	92.1	82.7	0.0
Nannochloropsis salina	*51.0*	*82.7*	*20.4*	*1.5*
Nannochloropsis gaditana	*45.7*	*63.0*	*18.7*	*1.0*
Navicula	*48.9*	*85.0*	*18.8*	*0.4*
SGI573 (Haptophyte)	*46.6*	*92.1*	*25.1*	*0.1*
Isochrysis	*41.2*	*92.1*	*17.0*	*0.1*
Pophyridium-a	*35.9*	*79.3*	*7.7*	*0.7*
Pophyridium-b	*40.0*	*83.9*	*10.4*	*0.0*
SGI286 (Prymnesiophyte)	*39.0*	*89.1*	*26.5*	*0.4*
Other non-SGI strains				
Maximum	*86.0*	*65.5*	*48.5*	
Minimium	*2.0*	*4.0*	*11.0*	
Average	*20.1*	*45.5*	*22.7*	

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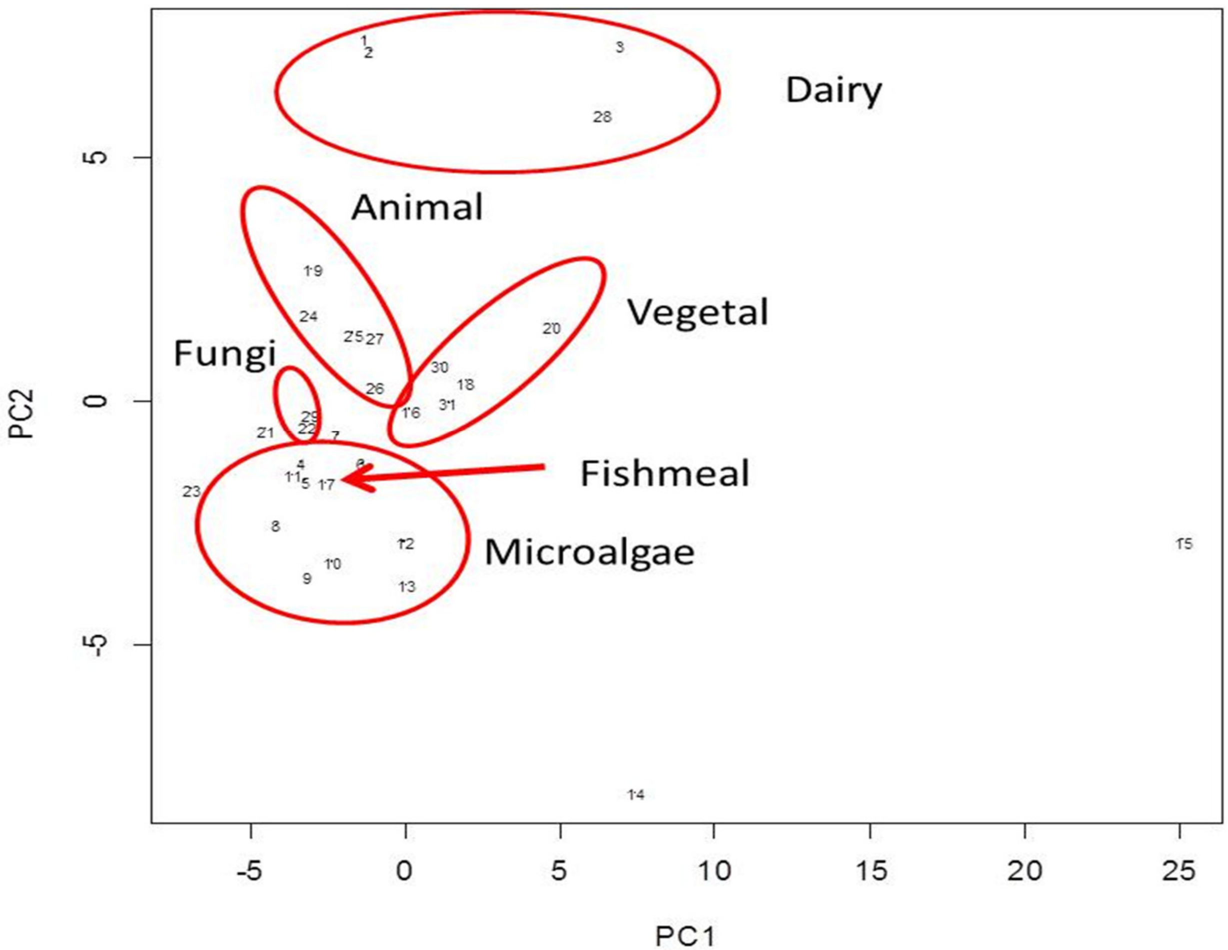
Microalgae nutritional advantage: proteins

similarity with fishmeal

Labels	Source	Data source
1	Whey isolate	SGI
2	Whey concentrate	SGI
3	Rennet casein	SGI
4	SGIC0739	SGI
5	SGIC0609	SGI
6	SGIC0537	SGI
7	SGIC0463	SGI
8	SGIC0675	SGI
9	SGIC1328	SGI
10	SGIC0907	SGI
11	SGIC0908	SGI
12	SGIS0250	SGI
13	SGIS0573	SGI
14	SGIS0285	SGI
15	SGIS0886	SGI
16	Schizochytrium	Pyle et al., 2008
17	Fishmeal	IAFMM, 1970
18	Soymeal	Miller, 1970
19	Egg	Becker, 2007
20	Soy bean	Becker, 2007
21	Chlorella	Becker, 2007
22	Arthrospira	Becker, 2007
23	Spirulina	Becker, 2007
24	Eggwhite	Miller, 1970
25	Tuna	Miller, 1970
26	Beef	Miller, 1970
27	Chicken	Miller, 1970
28	Casein	Miller, 1970
29	Yeast	Miller, 1970
30	Greenpea	Iqbal et al., 2006
31	Chickpea	Iqbal et al., 2006



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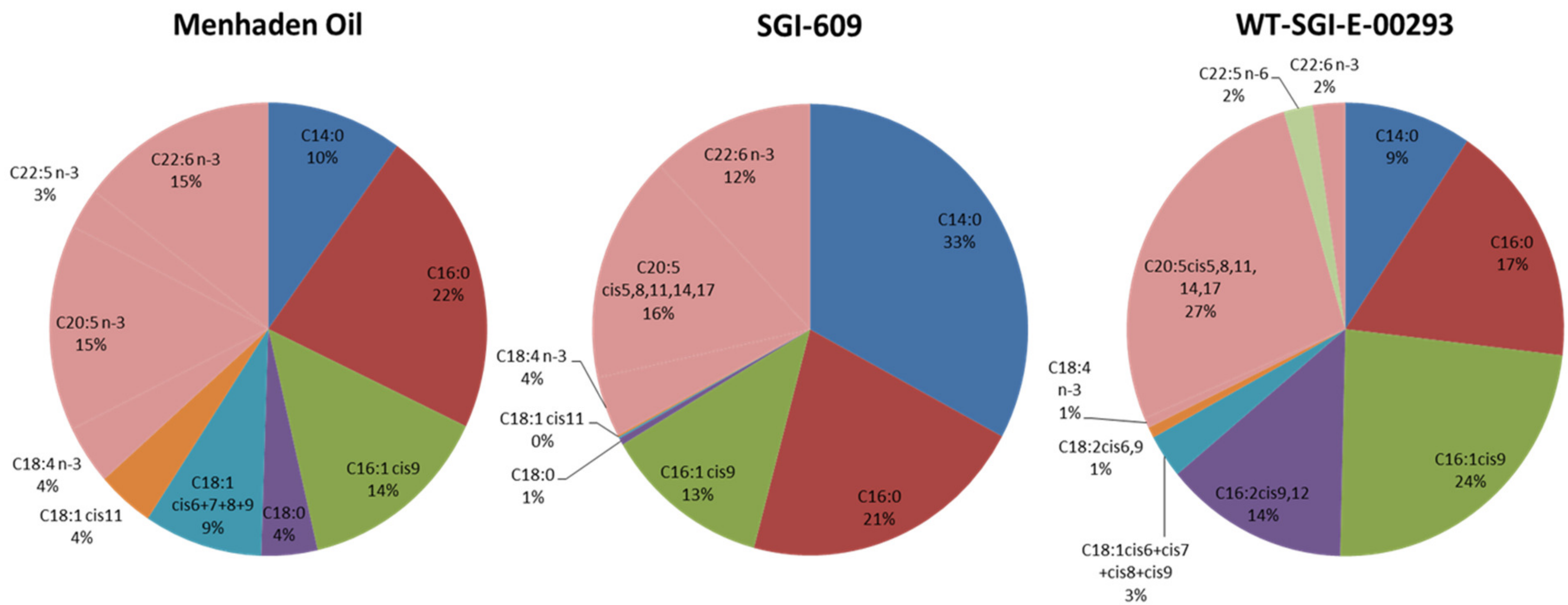
Microalgae nutritional advantage: lipids

microalgae provide required HUFA



Fatty acid composition of menhaden oil and microalgae strains from SGI culture collection.
(*Pavlova* SGI-609 and *Cyclotella* WT-SGI-E-00293)

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Microalgae nutritional advantage: micronutrients

microalgae also represent good or excellent sources of important micronutrients

Micronutrients	Salmonid requirements (mg/kg dry feed)	Algae average (mg in 100g algae or per kg of feed at 10% inclusion)	Algae provides (at 10% in feed it provides % needed)
HUFAs			
DHA Omega-3	*10000*	*640*	*6%*
EPA Omega-3	*10000*	*1200*	*12%*
DHA + EPA (Australia-NZ)	*10000*		
Vitamins			
Vitamin A and β-carotene	*1.35*	*10.5*	*777.78%*
Vitamin B1 (thiamine)	*12.5*	*2.2*	*18%*
Vitamin B2 (riboflavin)	*25*	*2.5*	*10%*
Vitamin B6 (pyridoxine)	*15*	*0.22*	*1%*
Vitamin B12 (cobalamin)	*0.0175*	*0.06*	*342.86%*
Vitamin C	*125*	*14.3*	*11.4%*
Vitamin E	*75*	*31.6*	*42%*
Folate	*8*	*0.35*	*0%*

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Microalgae environmental advantage

Water footprint: kg water/kg biofuel

Feedstock	Water footpring kg water/kg biofuel
Maize	4015
Sugarcane	*3931*
Potatoes	*3748*
Soybean	*13676*
Switchgrass	2189
Microalgae	*591-3650*†
†Range due to variations in recycle rate	

Nutrient footprint: kg nutrient/10 gal fuel

Nutrient	Corn grain ethanol[1]	Soybean diesel[1]	Algae biodiesel[2]	Algae w/ recycled biomass[2,3]
Nitrogen	7	0.1	5	1.5
Phosphorus	2.6	0.2	0.2	0.2
1 Hill et al 2006				
2 Pate et al 2011				
†Assuming a 70% recycle efficiency				

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Possible synergies (Biofuels)

- Biofuels require very large facilities
- Microalgal biofuels may produce large quantities of by-products
  - High in protein
  - Likely defatted: but distillation possible
- Microalgal biofuels will not be here for many years
  - Issues with cost
  - Issues with scale
  - Differences in scale
- Of course, if we solve for biofuels, we will have solved for feeds.

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Possible synergies (CO_2 capture)

Examples of microalgal cultures grown on flue gases and waste heat.

But regulatory hurdles???
Will there be a strong enough incentives???

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Microalgae production at commercial scale

Problem of cost

Estimated ranges of costs for microalgal biomass and microalgal products.

	Green water	*Arthospira*	*Haematococcus*
US$/kg dry biomass	$0.10	$5.00	$100.00
Production and Processing	None	Minimal	Significant
Cost of 1 kg of			
70% component	$0.14	$7.14	$142.86
30% component	$0.33	$16.67	$333.33
3% component	$3.33	$167	$3,333
1% component	$10	$500	$10,000

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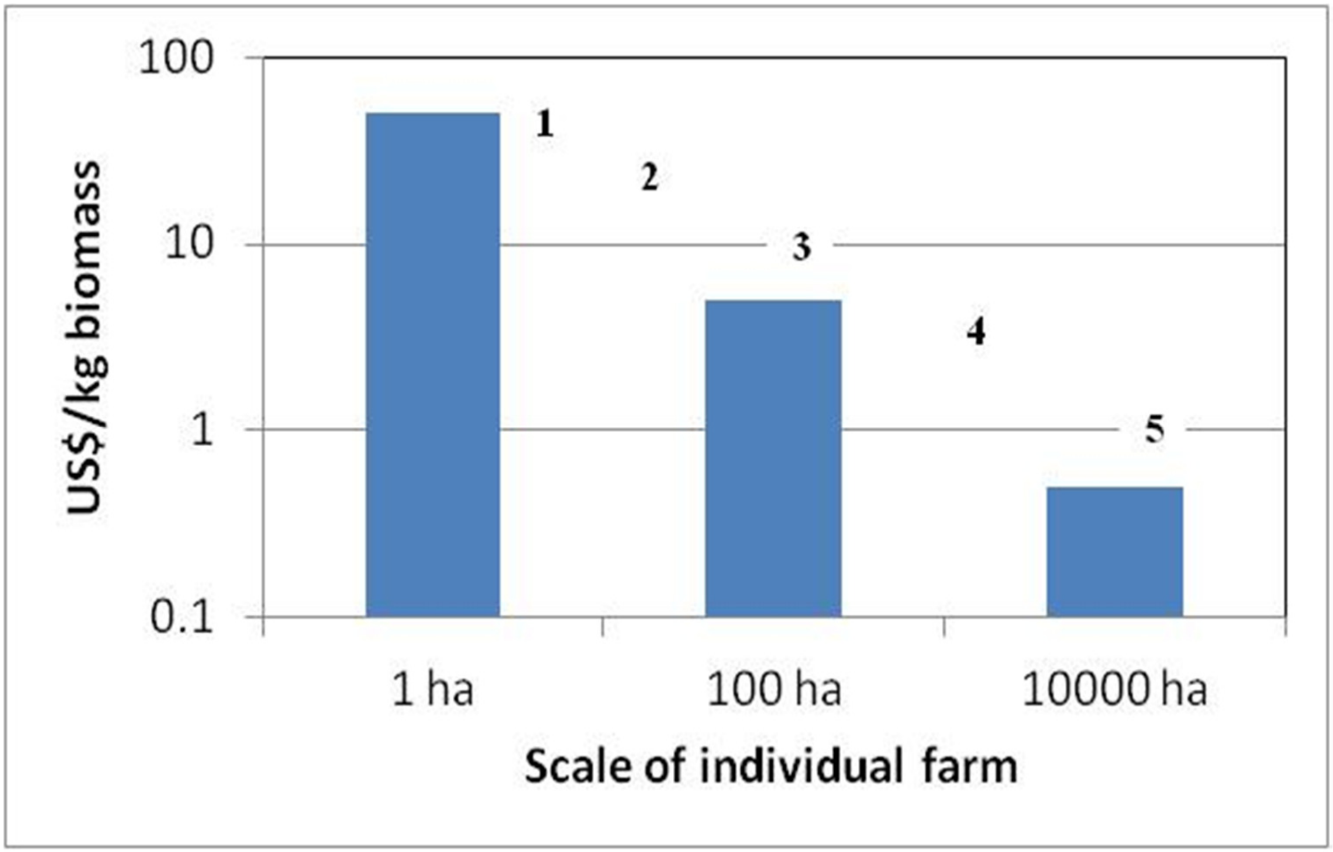
Microalgae production at commercial scale

Problem of cost

Predicted relationship between crop value and farm size based on present knowledge.



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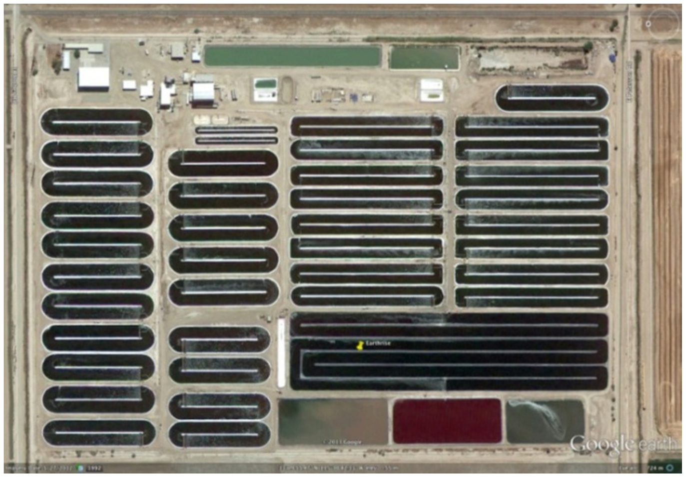
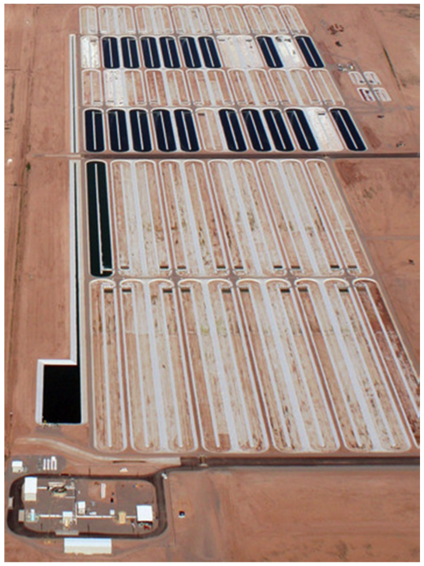
Microalgae production at commercial scale

Problem of scale

Scale of large microalgal farms. From left to right and top to bottom: Earthrise, Cyanotech, Sapphire and Parry Nutraceuticals/Valensa.





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Synthetic Genomics' Strategy

- Facilities
  - Laboratory and Greenhouse
  - Field station in Imperial County
- Process optimization
  - Field productivity
    - Dilution rate
    - Batch vs continuous
  - Harvest strategy
    - Lamellar settling
    - Hydrocyclones
    - Flocculation
    - Centrifugation
- Biology optimization
  - Robustness
  - Photosynthetic efficiency
  - Carbon partitioning
- Products and markets

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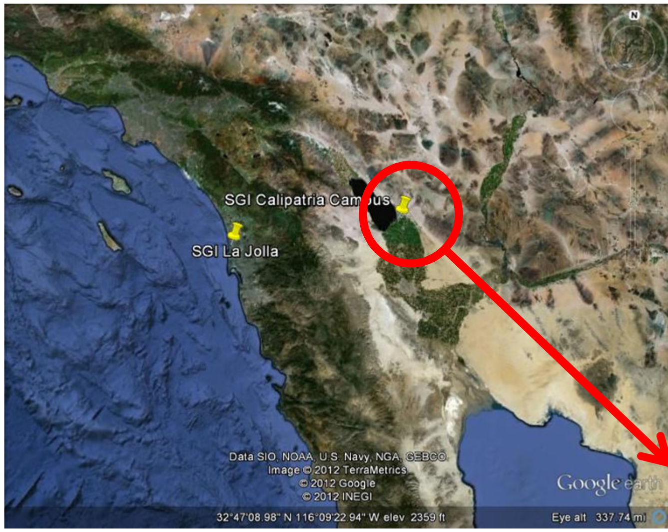
SGI San Diego and Imperial facilities



- Growth units
  - 20 x 1.9 m^2 ponds
  - 3 x 15 m^2 ponds
  - 3 x 70 m^2 ponds
  - 3 x 192 m^2 ponds
  - 6 x 400 m^2 ponds
  - 4 x 3200 m^2 ponds
  - 7 x 4000 m^2 ponds
  - Several racks with 100 L enclosed PBRs
- Other structures
  - 2 buildings for control room, offices and general day use (110 m^2 and 160 m^2)
  - 2 buildings for laboratory, small scale cultivation, processing and shop space (400 m^2 and 700 m^2)

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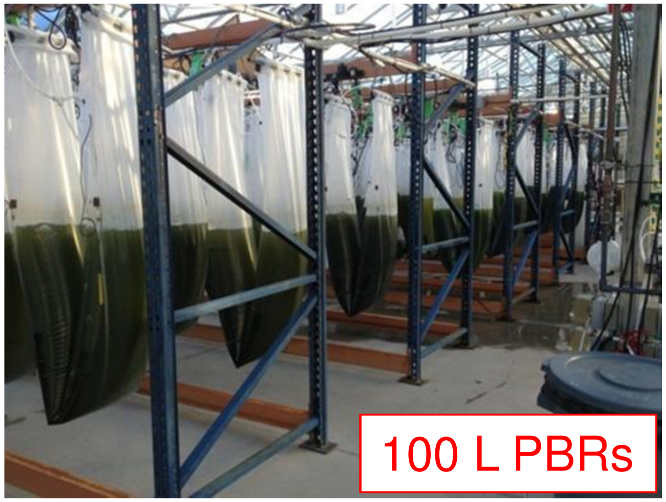
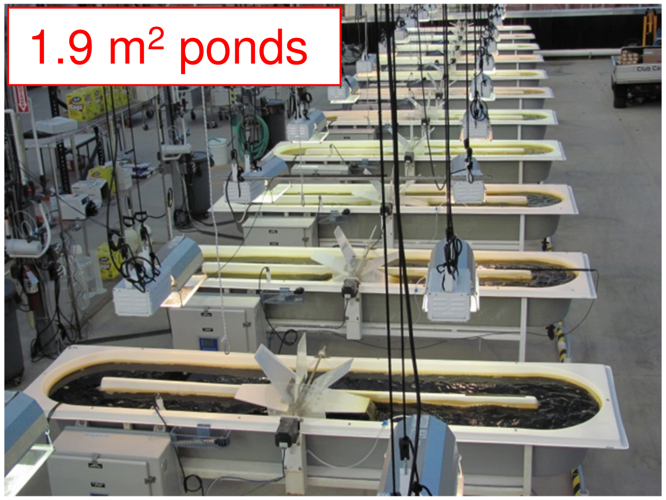
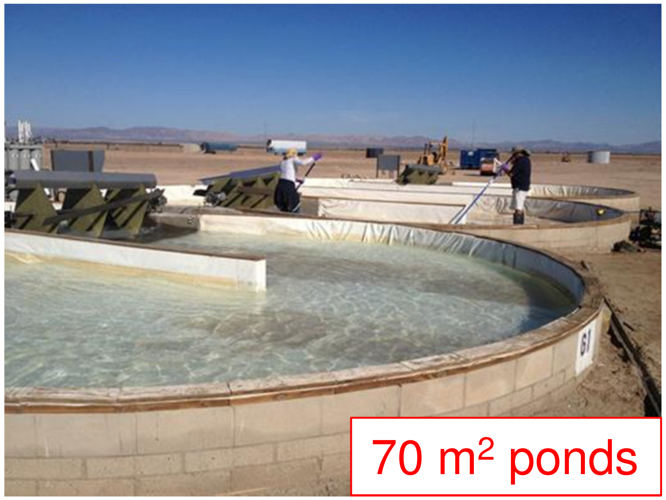
Microalgae production: R&D scale

Pilot scale facilities for microalgal technology research at Synthetic Genomics.

Greenhouse 100 L PBRs and 1.9 m^2 ponds







Outdoor 1.9 and 70 m^2 ponds used for strain robustness

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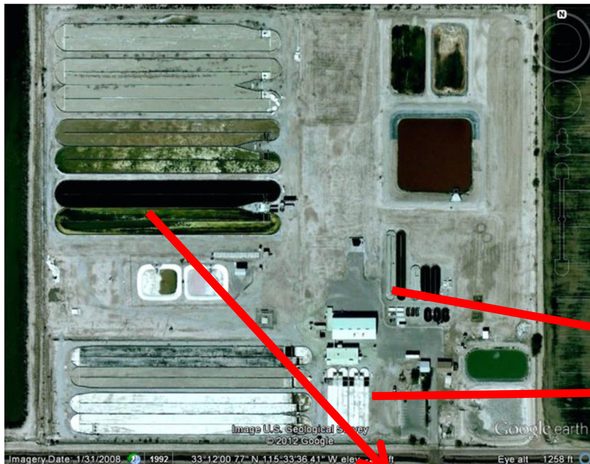
Microalgae production: Production scale

Production scale facilities for microalgal technology research at Synthetic Genomics.



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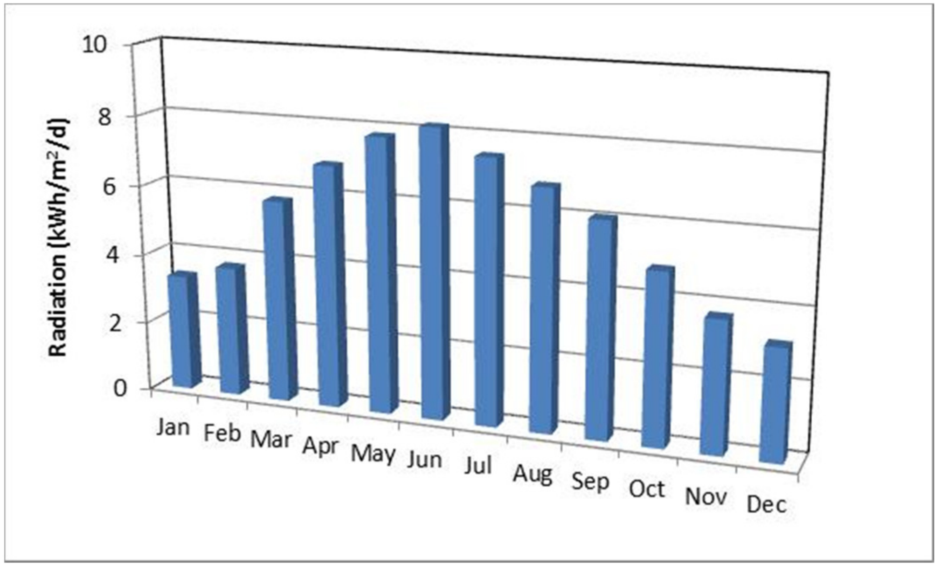
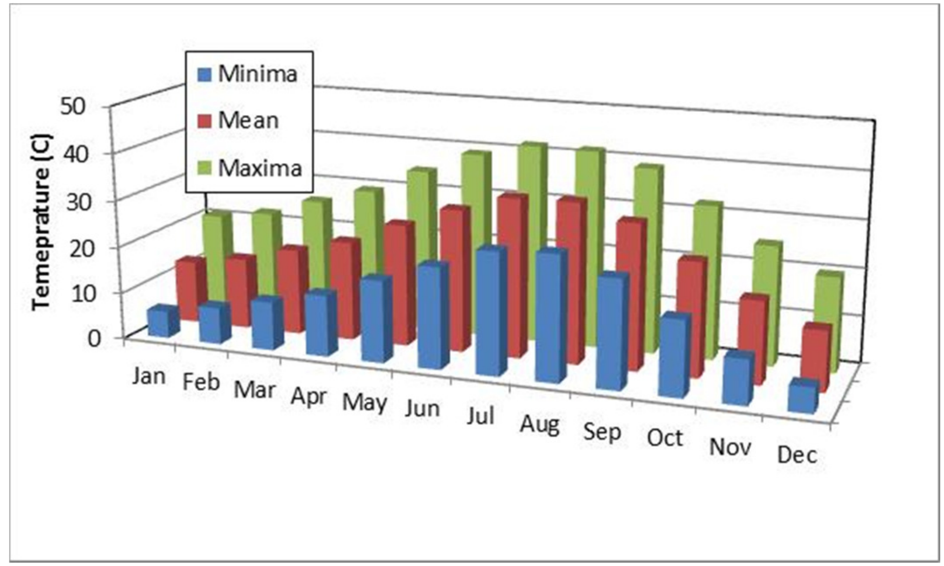
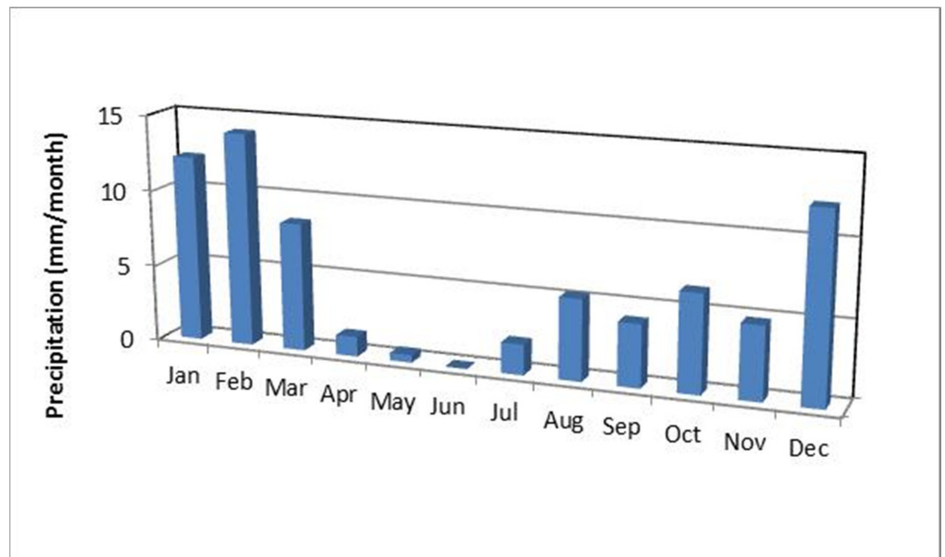
SGI Imperial Valley facility: growth conditions





- Very sunny
  - Average (year) radiation: 5.6 kWhr/m^2/d
  - Winter low: 3.1 kWhr/m^2/d
  - Summer high: 8.0 kWhr/m^2/d
- Very warm
  - Winter minima: 5.3ºC
  - Summer maxima: 41.8ºC
- Very dry
  - Winter: "wet" season



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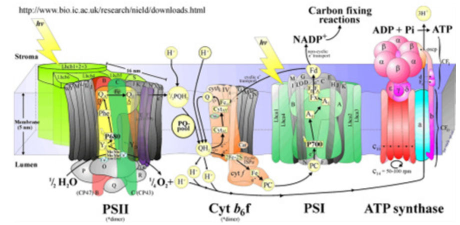
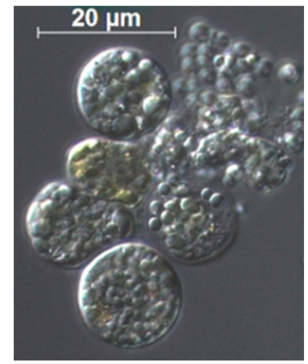
Natural Microalgae Exhibit Individually Desired Traits

Synthetic Biology technologies required to combine traits, and coordinately channel energy into desired commodity product

- Photosynthetic efficiency
  - photosystem antenna size
  - energy-wasting, non-photochemical processes
  - energy coupling reactions
  - futile reaction cycles (e.g. RuBisCO)
- Carbon partitioning to target molecule
  - down-regulate competing pathways
  - <u>constitutively</u> up-regulate biosynthetic pathways
  - precursor and co-factor supply
- Tolerance to production environment
  - temperature
  - halotolerance
  - microbial contaminants & predators





proprietary lipid-accumulating eukaryotic microalgae

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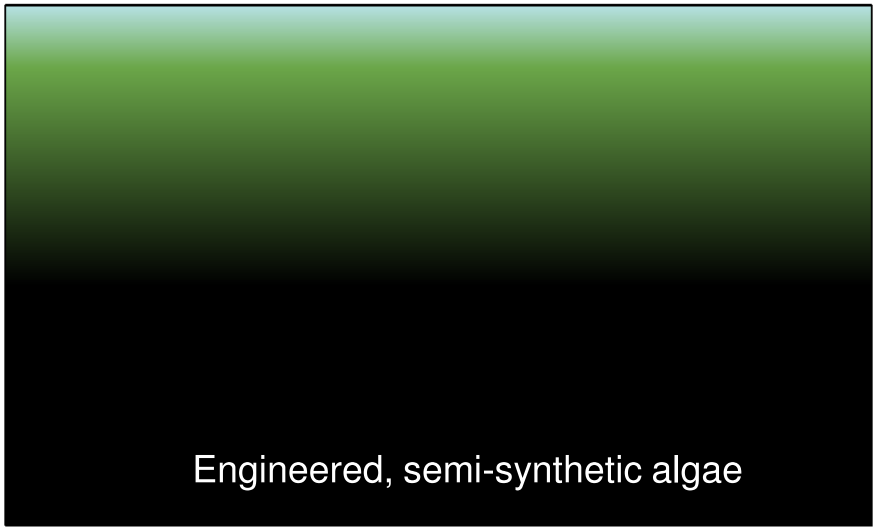
Photosynthetic Efficiency Challenge in Mass Culture

natural algae response to changing light environment limits productivity

low productivity

- Algae respond to self shading by “selfishly” building a large light harvesting antenna
- The larger antenna further exacerbates the self shading leaving much of the pond in darkness
- The larger antenna drives saturation of photosynthesis at low light intensities with the excess absorbed light actively dissipated as heat



high productivity

- Algae engineered to attenuate response to changes in light field
- Less light is absorbed and therefore the penetration of light into the pond is deeper
- The smaller antenna saturates photosynthesis at higher irradiance with less absorbed energy wastefully lost as heat

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Engineered Algae with Desired Phenotype

SGI has engineered algae for increased light penetration and improved photosynthetic efficiency

same cell density

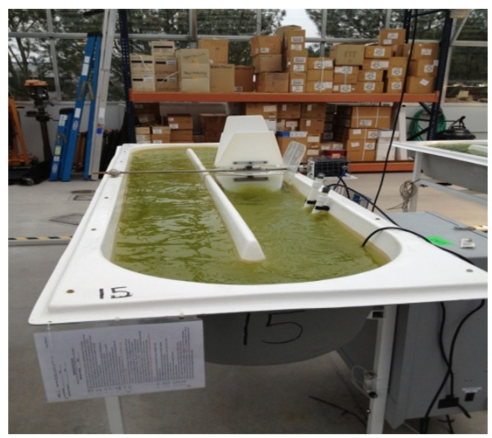
- One third Wild Type levels of chlorophyll
- Rate of photosynthesis per cell unchanged therefore the photosynthesis per unit chlorophyll is almost three times higher
- Much greater light penetration into culture
- Data confirm modified physiological response to changes in light field

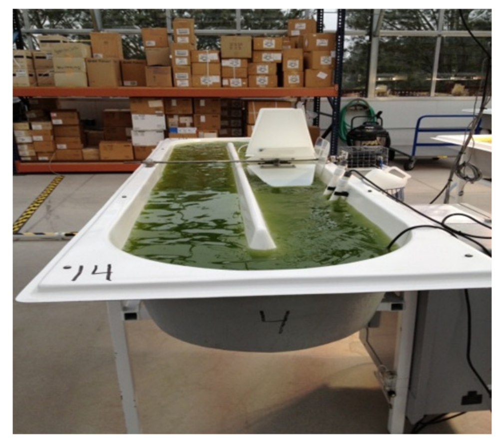
Chlorophyll content per cell (pg/cell)

Wild Type	**0.12**
Engineered species	**0.03**

Max. photosynthetic rate (umol O_2/hour/mg chl)

Wild Type	**161**
Engineered species	**405**





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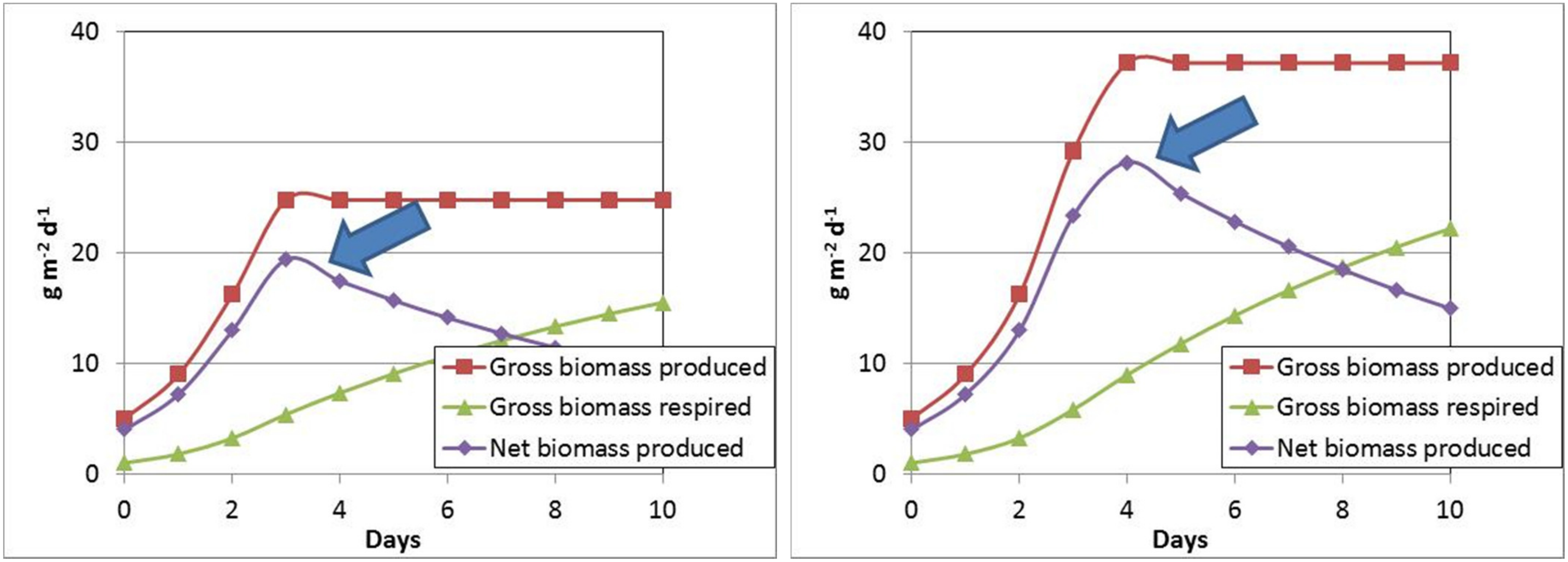
Optimization: photosynthetic efficiency

Effect of a 50% increase in photosynthetic efficiency (from 2%-left to 3%-right) on algal productivity.



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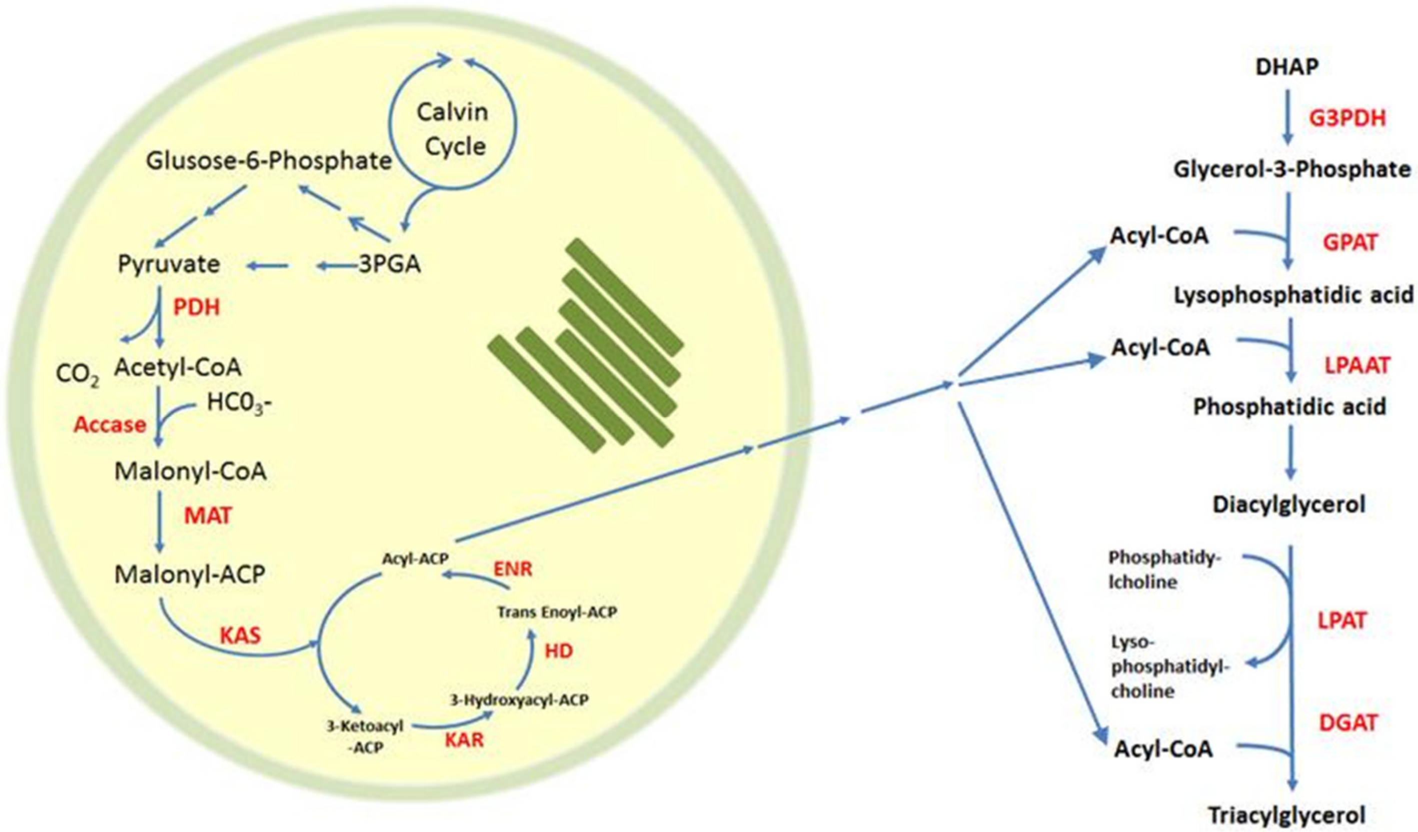
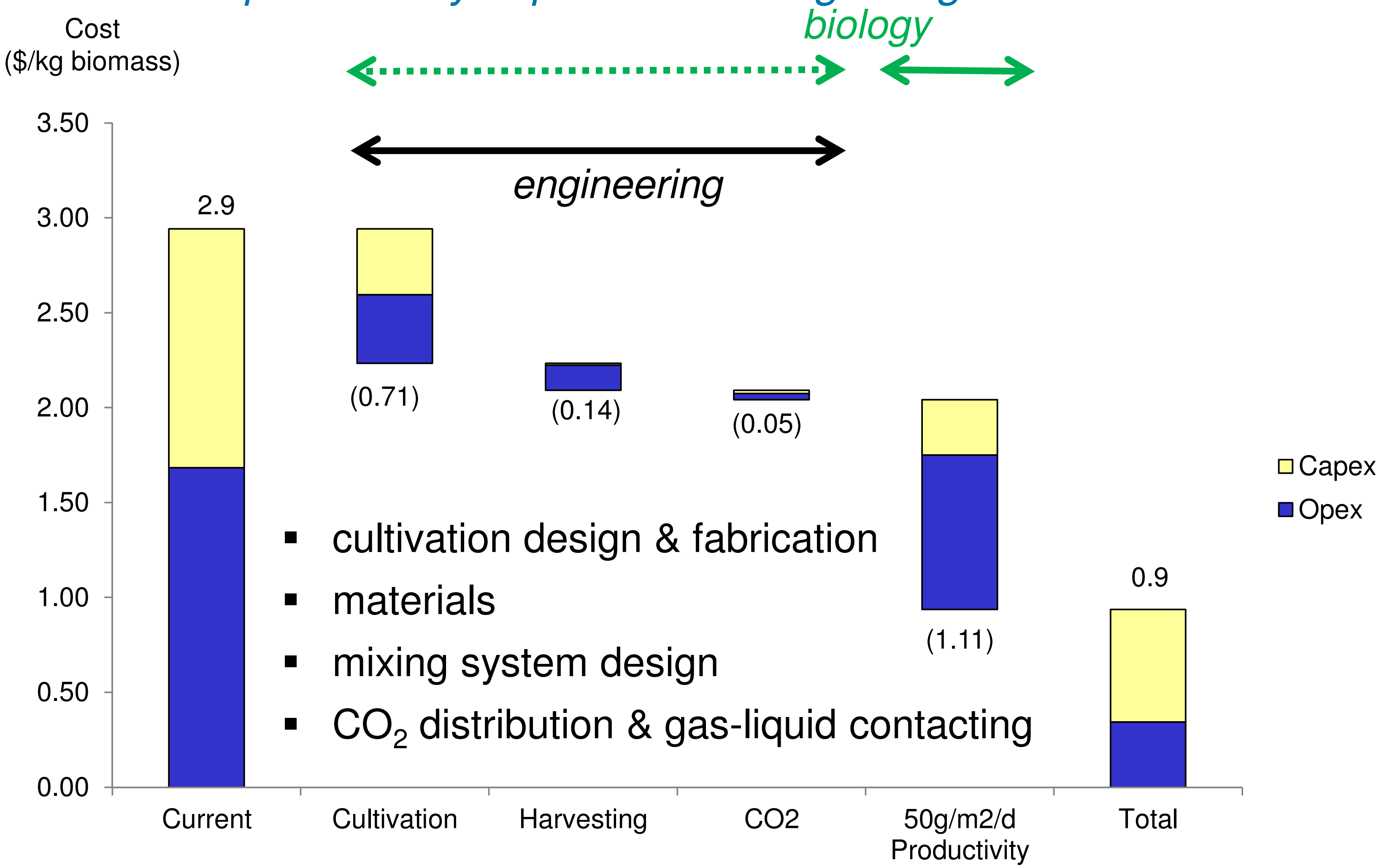
Optimization: carbon partitioning

Areas of targeted modifications for improved carbon partitioning to lipid.



Minimum Production Cost of Algae Biomass - Future

strain productivity improvement single largest cost driver



- cultivation design & fabrication
- materials
- mixing system design
- CO_2 distribution & gas-liquid contacting

Current assumes 200 ha facility; 17 g/m2/day, inoculum bioreactors, power plant flue gas at 10 km, 70% CO2 utilization, open pond raceways, chemical floc to 1% + centrifuge, evaporation ponds, land cost at $8,750/ha, 10 year depreciation, indirect costs of 83%, contingency of 25%

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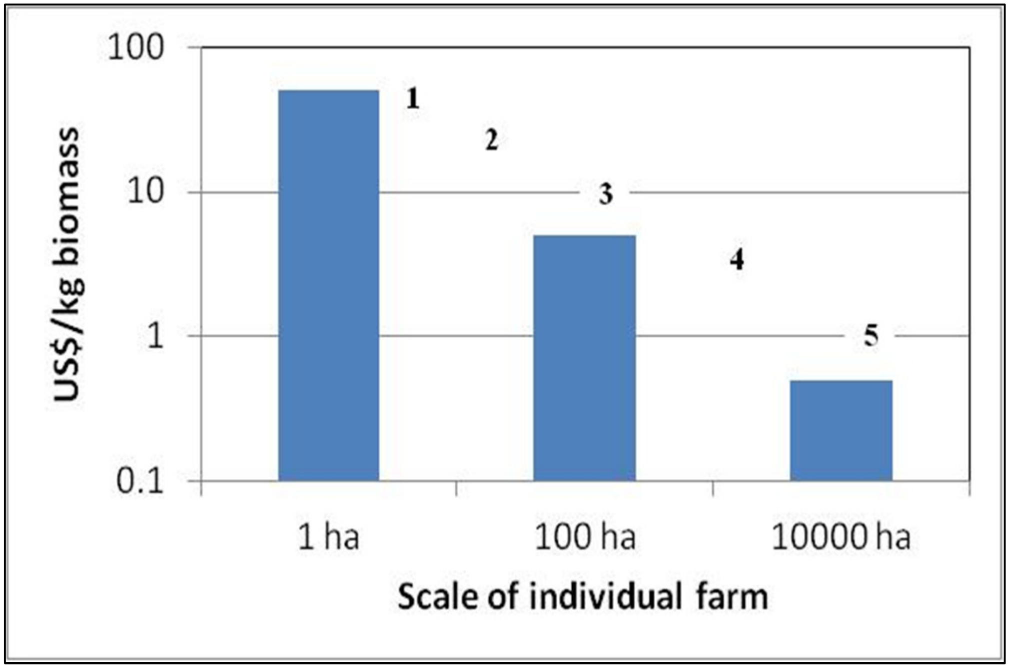
Product and Market

Progression of scale and value

- Need small markets (scale issue) of higher value (1→2)
  - In the feeds area
    - High value ingredients
- Next, larger markets of less value (2→3)
  - Specialty feeds
    - Larval feeds
    - Starting diets
    - Finishing diets
- Finally, commodities (3→4)
  - HUFA

  and
  - Proteins



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Summary

- Demand for fish oil and fishmeal is outstripping supply
- Microalgae are a superior source of fish nutrition
- Transformational innovations are required to establish commercially attractive, sustainable alternatives for commodity feed production
- Cost is high but Synthetic Biology technology is driving the cost down by enhancing
  - Photosynthetic efficiency
  - Carbon partitioning
  - Robustness

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