# What will it take for algae to overturn Malthus' predictions on fuel and food security?

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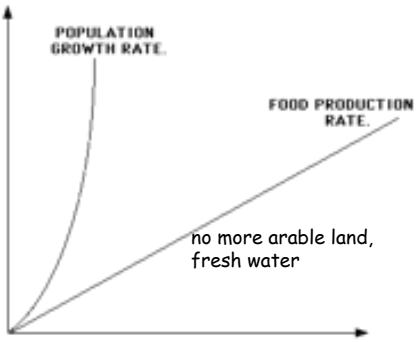
AERI
Dec. 23, 2012

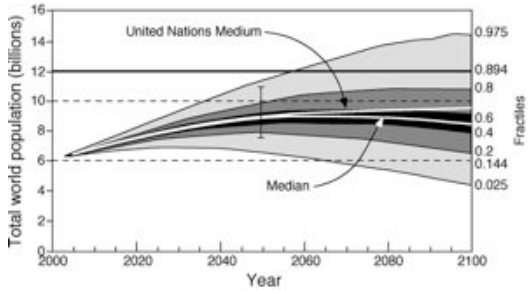
## We have a problem: the world is overloaded

and is being led by…….
Won't be enough food and fuel

## Thomas Malthus – 1766-1834
1798 "An essay on the Principle of Population"




## Must feed a growing world population




The world is resource limited:- in food - in fuel - in fresh water - in arable land – all competing with each other
New solutions must be found

Nails in Malthus's coffin came from quantum leaps – followed by incremental additions
Next quantum leaps from biotechnology

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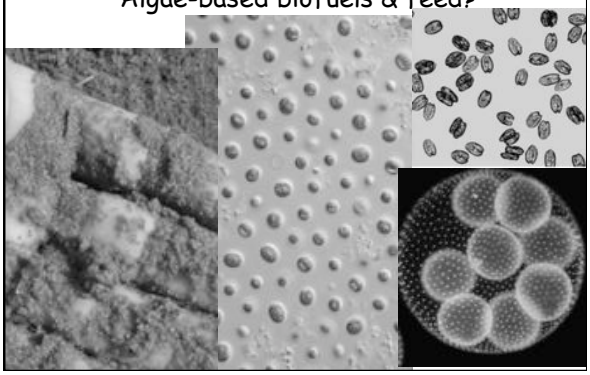
Good news / Bad news

Good news: It won' t be 13 billion,
it will be 8.5 billion

Bad news: The 8.5 billion will be middle class
they will want animal protein and to drive cars.

The food production requirement will like for >13 billion
Fuel???

Is the solution to Malthus' resource crises: Algae-based biofuels & feed?



Algae have >20x potential of crops

not $CO_2$ or $H_2O$ limited; use all photons – all year - 100% harvested/used

<1% freshwater, <5% land, <30% fertilizer

What will it take to go from lab?

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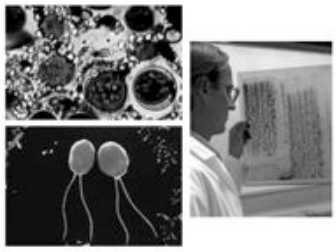
National Renewable Energy Laboratory

NREL/TP-580-24190

A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae



Close-Out Report

Tried in past

1978-1996 DOE Projects

Closed because breakeven only at $70/barrel petroleum

Most current groups using same technologies

DOE Project had "alchemical engineers

"Frankly, I'd be satisfied now if I could even turn gold into lead."

Seeded one algae – another grew

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Cultures often crashed – quorum sensing

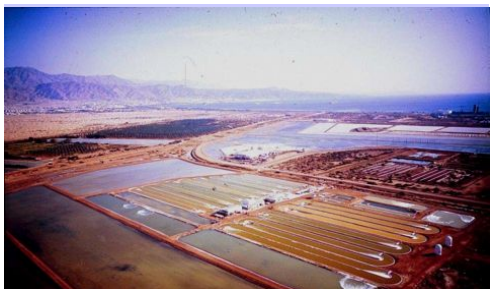
**Open Microalgae Ponds**
**Taiwan & Japan**

**Round & oblong open ponds**
**mostly fresh water mixotrophic cultivation**
**Since 1950**

ex Ami Ben-Amotz


ex Ami Ben-Amotz

**NBT Ltd., Nikken Sohonsha Co. (1975)**
***Dunaliella* 10 Hectares Plant, Eilat, Israel**



ex Ami Ben-Amotz

High energy cost to mixing – 95% cells in dark – most bubbled $CO_2$ wasted

Seambiotic

**Scale-Up Cultivation of**
***Nannochloropsis sp***

ex Ami Ben-Amotz

Source: http://www.algae-biotech.com/production.html (Astaxa GmbH)


also – flat plate bioreactors– if outside – lots of steel – or blow away – high capital costs

***Dunaliella* Harvesting**
**Westphalia Ltd., Continuous Centrifuges**


ex Ami Ben-Amotz

***Dunaliella* β-Carotene Capsules**
**100 yen/capsule**


ex Ami Ben-Amotz

After visiting the


Realized: No one had domesticated algae

The algae have not been domesticated as a crop!

Hybrid corn

Corn ca. 1492

Teosinte- the progenitor

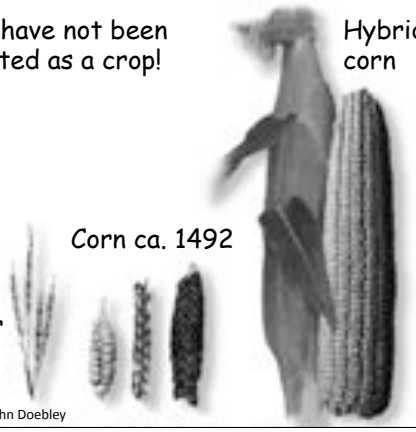
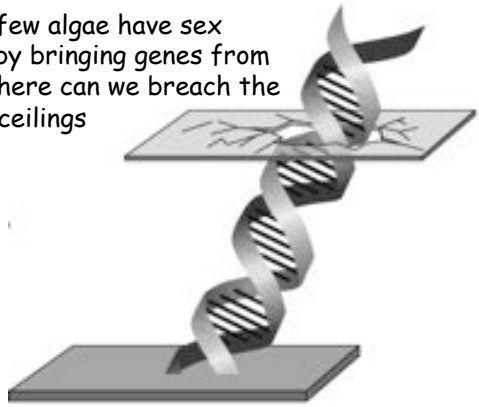


Photo courtesy of John Doebley

Very few algae have sex
Only by bringing genes from elsewhere can we breach the glass ceilings



To quickly domesticate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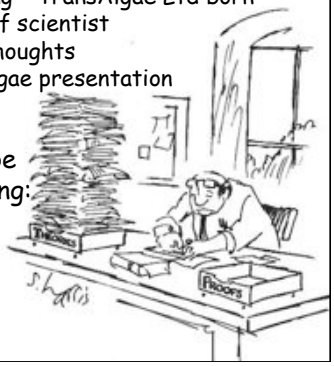
I wrote a report on genes need for domestication
- turned into a business plan
- an investor came along – TransAlgae Ltd born
- spent 3 years as chief scientist
- some results/some thoughts
- This is <u>not</u> a TransAlgae presentation

Problems only can be solved by engineering:
- genetic
-production



Problems to be solved
Domestication – How?
- Choice of organisms – algae or cyanobacteria
  – triglyceride lipids -no quorum sensing
- Contamination by unwanted organisms
- Needed valuable co-products
- Oil content and composition
- If transgenic – spillage into environment

Cooling

Production and harvest costs
Need a reliable robust platform

**Oil Yield**

Cultivating Algae for Liquid Fuel Production
(http://oakhavenpc.org/cultivating_algae.htm)

**Gallons of Oil per Acre per Year**

| | | |
|---|---|---|
| Corn | → | 20 - 30 |
| Soybeans | → | 50 |
| Sunflower | → | 110 |
| Rapeseed | → | 130 |
| Oil Palm | → | 600 |
| **Microalgae** | → | **5.000-25.000 and up to 50.000** |

**50.000-500.000 L oil per ha and year**

Possible? **NO**

ex. Mario Tredici

Target fuel biofeedstocks at $600-800/T?

Fishmeal is a major source for missing animal nutrients $1400-2000/T
Grain is missing key nutrients for monogastric animals – fish, poultry swine
add enzymes, synthetic DL-methionine
Carnivorous fish allergic to soybean
Fishoil – same price range – smaller market

Feedstock market – larger potential size

Domesticate algae for both markets
Economy of scale/experience to reduce costs

Initial major technical problems
choice of organisms (discussed)
transformation
beginning: sporadic – colonies in 6 weeks
now: repeatable – colonies 7-10 days

Later realized problems
expression levels

Two stages:
1. Developing a reliable platform
2. Engineering value-added traits

Developing a reliable platform

Overcoming barriers to domestication:
solving system instability
with herbicide resistance
anti-microbial proteins
both have secondary uses
selectable marker
replacement of feed
antibiotics

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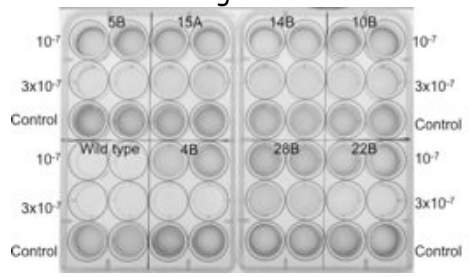
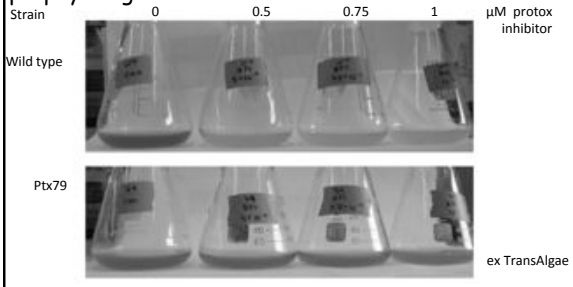
Engineered resistance to a phytoene desaturase-inhibiting herbicide




ex TransAlgae Ltd.

Did the same with a herbicide inhibiting proto-porphyrinogen IX oxidase




Protox useful as a selectable marker and a needed trait

Amused:Sapphire glyphosate R; 1 mM needed to kill WT

Needed platform domestication traits/genes for algae

| Trait | Gene |
|---|---|
| *Herbicide resistance for resistance to algal contamination* | |
| glyphosate | Modified epsp synthase |
| glufosinate | bar |
| fluorochloridone | Mutant phytoene desaturase |
| butafenacil | Mutant protoporphyrinogen oxidase |
| *Resistance to microorganisms* | |
| *Bacteria/fungi* | |
| antimicrobial proteins | e.g. lactoferricin |
| *Viruses* | |
| RNAi or overexpression | Specific pieces of viral DNA or cDNA |
| *Resistance to zooplankton* | |
| protozoans | antimicrobial peptides |
| sea lice | avermectins |
| No quorum sensing | anti apoptosis genes |
| *Maximum growth* | |
| smaller PSII antennae | *tla*1 gene |
| systems/synthetic biology | New light reactions |
| | New dark reactions |
| Heat tolerance | *psbA* double mutant and/or polygenes |
| *Inability to grow in nature* | |

Δ = deleted section of gene resulting in inactivity.

**Value added domestication traits for algae used in aquaculture**

| Trait | Gene |
|---|---|
| Enhanced self digestibility | suppressed cell wall glycosyl transferases |
| Enhanced feed digestibility | vacuolar sequestered carbohydrases |
| Increasing methionine content | cystathionine synthase + zein peptide |
| Increasing lysine content | insensitive dihydrodipicolinate synthase |
| Enriching omega 3 fatty acids | ALA, EPA and elongases |
| Release bound $PO_4$, Fe, Zn | phytase |
| Increase iron content | Inactive ferritin |
| Increase Cu and Zn | Inactive CuZn superoxide dismutase |
| Remove fishy odor | Express trimethylamine oxidase |
| Feed efficiency enhancement | Antimicrobial peptides |
| Controlling sea lice | Avermectins |
| Vaccines | various genes |
| Increase growth rate of fish | Fish growth hormone |

Algae already a good replacement for fish oil

In log phase ca. 25% lipid

**Micro-algae as sources of valuable fatty acids (approx. % of lipid)**

| | | *Nanno-chloris* | *Nanno-chloropsis* | *Phaeo-dactylum* | *Isochrysis* |
|---|---|---|---|---|---|
| **α linolenic** | 18:3 n-3 | **32** | **3** | | |
| **arachidonic** | 20:4 n-6 | **2** | **5** | | **0.1** |
| **eicoisentanoic** | 20:5 n-3 | - | **28** | | **0.6** |
| **DHA** | 22:6 n-3 | - | - | **30** | **13** |

Some variation with growing conditions – values from literature

e.g. *Nannochloris* could be engineered to produce a balance of other needed fatty acids

What if there is an inadvertent leak?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What happens when?
- Hurricane
- Earthquake
- Tsunami
- human failure

www.nanovoltaix.com/markets/algae.php

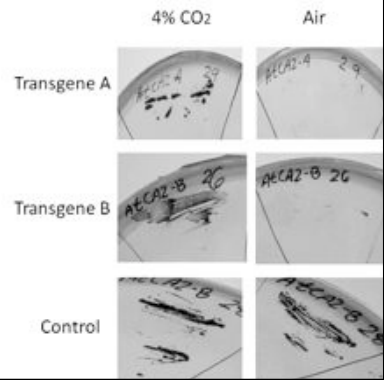
www.energypowershift.com/developments.htm

Spill to natural ecosystems
Swamping native organisms

**Mitigation – suppressed carbon capture**

Engineer high $CO_2$ requiring transgenic algae

- antisense or RNAi carbonic anhydrase




Ex TransAlgae

Other possible mitigators:
- (anti) nitrate/nitrite reductases
- (anti) cilia/flagella
- reduced PS2 antennae
- reduced metabolite storage

Good for transgenic algae
Double whammy concept (coup de grâce)
David Sourdive +
1. Site directed delete unneeded gene (meganuclease, Zn finger, etc.) – 2. insert mitigator
- -dominant mitigator protects from sex
- - recessive deletion protects from mitigator silencing

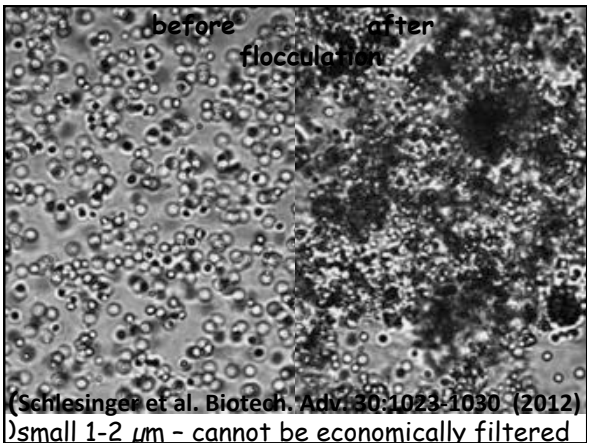
What about non-transgenic algae?
- no environmental risks from spills?

Hans Bergmans:
Can be more dangerous than transgenic if:
- non-native
- "The paradox of the plankton"
  - one thing in lab
  - another in the field
- native predictable

Many jurisdictions: "you may not introduce non- native strains!!!" Hampers development
Solution: deletion mutation of unneeded genes – e.g. chlorate resistant mutations

Cannot achieve no risk

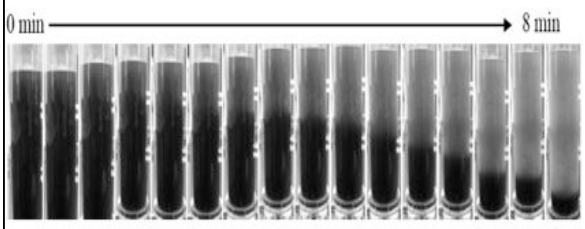

HERE LIES A PSEUDO-SCIENTIST




(Schlesinger et al. Biotech. Adv. 30:1023-1030 (2012)

)small 1-2 μm – cannot be economically filtered

Rapid and inexpensive sedimentation of floc

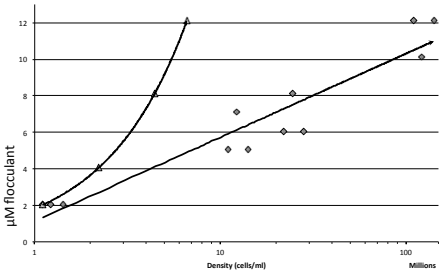



After sedimentation – further, easy dewatering by:
- low speed centrifugation
- filter press, belt drier, etc.

(photo: TransAlgae (Israel) Ltd)

HARVESTING – DISCOVERY THAT ACCEPTED KNOWLEDGE WRONG

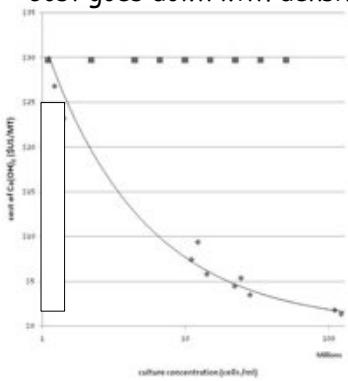
ALL CHARGES ON A CELL MUST BE NEUTRALIZED TO FLOCCULATE




**The amount of flocculant needed to induce flocculation is not a direct linear function, but is a logarithmic function.**

Schlesinger et al. Biotechnology Advances 30:1023-1030 (2012)

## Cost goes down with density

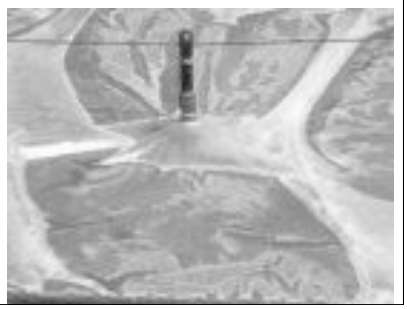


Schlesinger et al. Biotechnology Advances 30:1023-1030 (2012)

## How will algae be cultured?

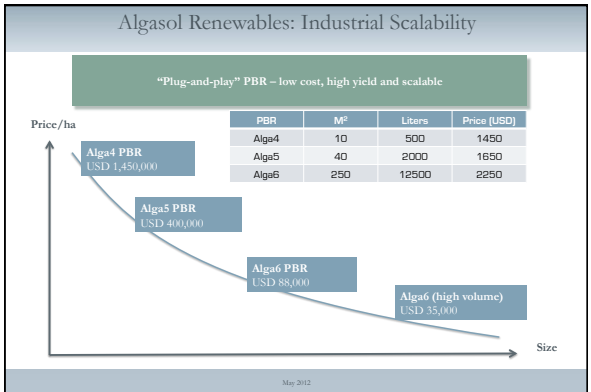

## My guess on ground/water

Algasol Renewables – floats on sea -freshwater algae



>5 cm depth
Sparging (bubbling)
$CO_2$+ air

## Algasol Renewables: Industrial Scalability




Claim: Ave. yield: 115 T dry biomass/ha/yr

Algasol Renewables

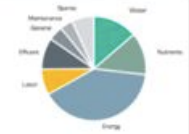
1,000 ha production facility with 20-year lifetime

- Biomass production: 115,500 tons/year
  - Yield of 35 g/m²*day (330 days)
- CAPEX: 134.2 USDm
  - 3-year lifetime of PBR (7x replacement)
- OPEX: 17.5 USDm
- Depreciation: 6.7 USDm

Biomass production cost: 210 USD/ton

CAPEX

OPEX



May 2012 Algasol Renewables - Proprietary photobioreactor technology

The ideal structure will be:
Hundreds meters long
5-10 meters wide
Floating on coolant
Inflated with $CO_2$
- No superstructure

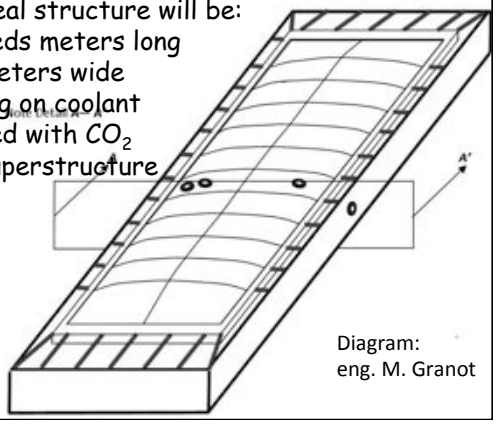
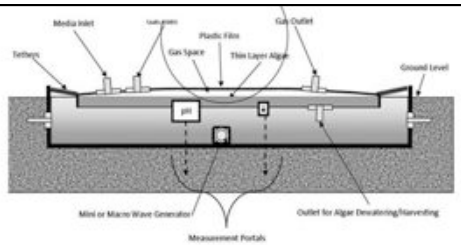


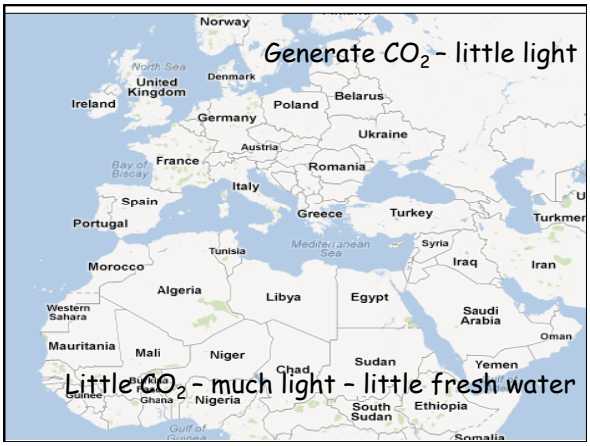
Diagram:
eng. M. Granot



Ultra low volume algae (3-5 mm thick)
less medium to sterilize – easier dewatering
less pumping – cooling simpler – NIR passes
How do we get $CO_2$ in without sparging?
How do we mix cells? Get rid of $O_2$?
-generate waves!

Need plastic engineering:

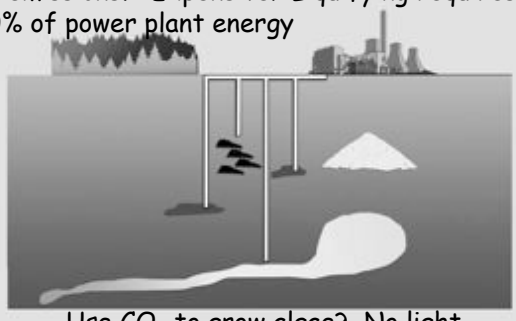
Specifications:
The plastic must be non-toxic to algae.
Anti-ultraviolet coating.
The top sheet should have a low beta value;
i.e. pass excess $O_2$ and retain $CO_2$
Non-reflective coating
not to waste early a.m./late p.m sun
Inner side top sheet: anti-(reflective) droplet coating
Dust repellent
Infrared reflective (with no loss of visible)




Generate $CO_2$ – little light

Little $CO_2$ – much light – little fresh water

In north:
Is sequestering $CO_2$ underground the answer to emissions? Expensive! Liquifying requires 10% of power plant energy

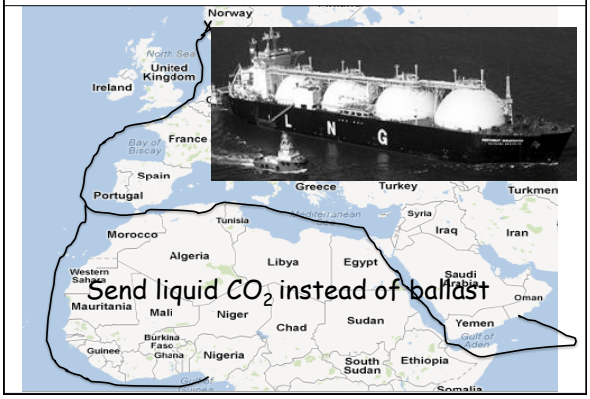


Use $CO_2$ to grow algae? No light

Natural gas comes mixed with $CO_2$
– separated –
Methane ⇨ LNG
$CO_2$ vented

$CO_2$ should go to algae

Ship LNG to Europe – back with ballast




Send liquid $CO_2$ instead of ballast

Science fiction?

In South:
Use energy from $CO_2$ liq ⇨ $CO_2$ gas

to compress methane ⇨ LNG

In North:
Use energy from LNG ⇨ methane

to compress $CO_2$ gas ⇨ $CO_2$ liq

Not perpetuum mobilum – but will save energy

Many sunny deserts near the sea

Appropriate cultivate algae

Use algae as feedstock for labor intensive aquaculture

For employment and food sufficiency

Summary – with proper engineering
Algae can replace biofuel and feed crops
There are the beginnings of:
Genetic engineering solutions to:
reliability
products
Industrial engineering solutions to:
cultivation
harvesting
Need more to extraction and utilization
Crops are multi-purpose – must use all

Balance between fuel, feed, other products depends on markets

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Summary: Marine microalgae

- do not compete for land and water
- sequester industrial carbon dioxide
- fertilizer efficient
- high productivity – multiple products
- need domestication – transgenically for:

  reliability – productivity- composition

Eventually – for feed and fuel

Can be the next two nails in Malthus's coffin; one for fuel limitation, one for feed

Thanks to:
past and present TransAlgae Scientists
Drs. Ofra Chen, Shai Ufaz, Daniella Schatz, Doron Eisenstadt, Ami Schlesinger, Shai Einbinder with whom I have learned a lot

To: Dutch regulators: Cécile J.B. van der Vlugt, and J.E.N. (Hans) Bergmans
To: David Sourdive (Cellectis)
To: Eng. Mike Granot
who is designing new photobioreactors with me

(and to Google images)