Transgenically domesticating biofuel crops

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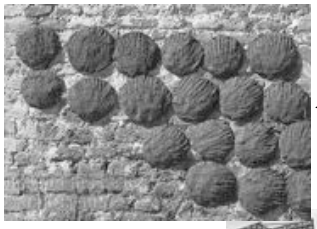
Bioeconomy in Argentina: Present and Future
Sheraton, Buenos Aires, March 21, 2013



Zero generation biofuels

← India

Africa ↓

Inefficient
Polluting
Environmentally negative
Can we do better?

The zero generation biofuel in temperate agriculture was

Grown on ca. 20% of cultivated land

Oats fueled all of farming

Fueled: mules, horses and laborers

What are world implications?

Biofuels: Good News/Bad News to developing world

Bad news: no more cheap/free grain for food security in time of famine

Good news: US not "dumping" subsidized grain, sold below production costs

Argentina farmers get better price for grain

Developing world farmers can now compete easily triple yields

Biofuels: Good News/Bad News to developed world farmers

Good news to grain farmers - prices stable

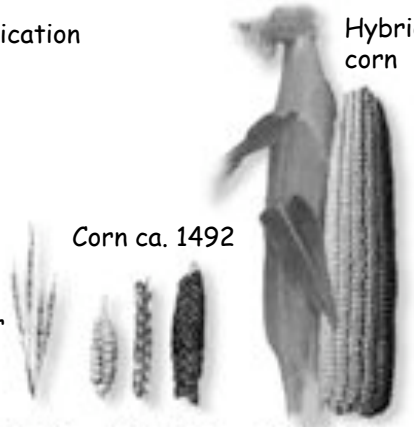
Bad news to dairy/beef/chicken/hog farmers - grain prices high…

Bad news to consumers - do not lower fuel prices, higher food costs

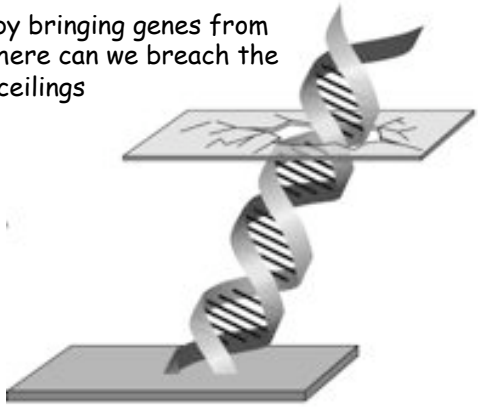
Deutsche Welle
Energy | 23.04.2007
Germany's Cheap Beer Tradition Under Threat From Biofuels
The popularity of biofuels is affecting the price of Germany's most cherished beverage

Germans will have to dig deeper in their pockets to enjoy their beloved beer in the next few months as barley is increasingly displaced in the country's fields by heavily subsidized crops used for biofuels.

Domestication



Only by bringing genes from elsewhere can we breach the glass ceilings



To get rapid domestication of biofuel crops

Genetic Engineering allows:
- Introducing new genes
- Silencing unwanted genes
  - in whole plant
  - in specific tissues
- Upregulating genes
- Modifying pathways

The zero generation biofuels
- not sustainable with present population

The first generation
- not sustainable in near/medium term

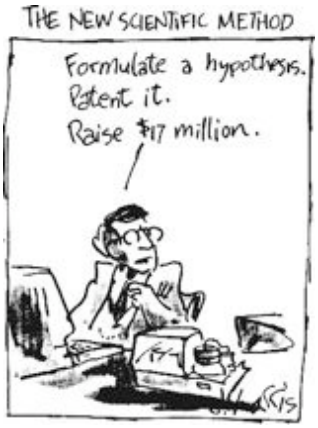
The second generation
– *better late, solutions just coming online*

Cultivating biofuel dedicated crops
- perennial oilseeds
- perennial lignocellulosics

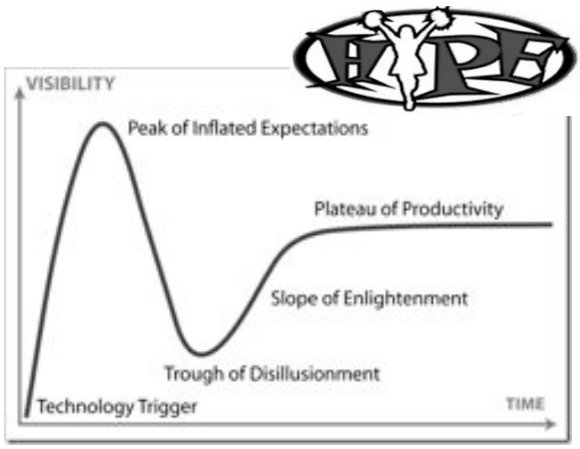
Using agricultural wastes
- lignocellulosics

The third generation
Single-celled algae
– *better late, solutions just coming online*

The old scientific method is not being used in biofuel R&D

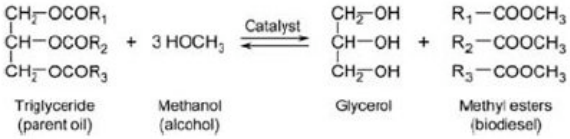


The new method is being used - hard to distinguish between data and hype



Biodiesel from various sources

Transesterification of triglycerides



First generation uses vegetable oils – value in oil and meal – not the case in second generation

30% oil - seeds get US$140/ton (optimistic)
- fruits hand harvested/dried in the shade
- seeds removed by hand

Not domesticated!

Is Jatropha a gimmick to keep the poor poor?

7.5 hrs harvest + dehull per kg oil

<u>Info not in sites promoting *Jatropha curcas*</u>

common plant names: Black vomit nut, Purge nut, etc.
common oil names: hell oil, oleum infernale, etc.

Toxins: Curcin (a toxalbumin) - similar to ricin
Phorbol esters - diterpenoids Alkaloids
skin tumor promoters

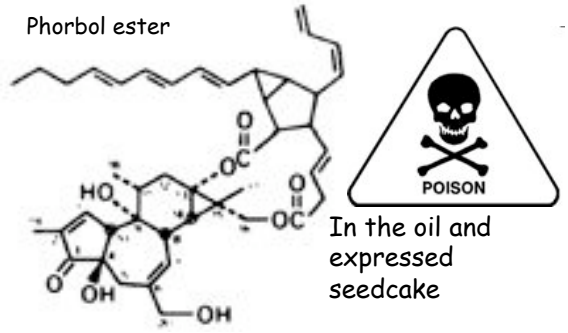
No antidote known

See: http://www.inchem.org/documents/pims/plant/jcurc.htm

Also: A case of Jatropha poisoning resembling organophosphate intoxication Clin. Tox. 44 337,2006

Could one release a transgenic crop with such components? What to do with toxic byproducts?

Phorbol ester



In the oil and expressed seedcake

Hirota et al. A new tumor promoter from the seed oil of *Jatropha curcas* L., Cancer Res 48(20): 5800–5804, 1988

Is "non-toxic"-Mexican *Jatropha* not toxic?

	curcin[a]	phorbol esters[b]	trypsin inhibitor[c]	phytate[d]	saponins[e]
3 *Jatropha* varieties (average)	102	2.39	20.3	8.9	2.2
"non-toxic"-Mexican *Jatropha*	51	0.11	26.5	8.9	3.4
soybeans (control)	<0.5	-	3.9	1.5	4.7

[a]measured as lectin haemagglutination; [b]mg/g kernal; [c]mg/g meal; [d] % in dry matter meal; [e] measured as % diosgenin equivalents in the meal. Source: Modified from Makkar et al. [18]

18. Makkar, H.P.S., Aderibigbe, A.O. and Becker, K. (1998) Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. Food Chemistry 62, 207-215.

Websites claim "curcin is heat degradable"
Quoted citation says "degradable by prolonged autoclaving" New technologies: Heat and base
Better to modify plants

Castor oil for biodiesel

What to do with toxic byproducts?

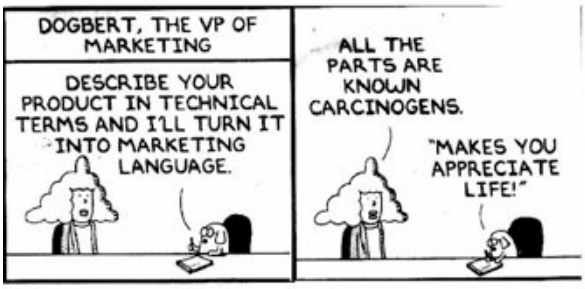
Websites suggest - Use residue as manure
no environmental impact studies

Jatrofraud! ?

Could one release a transgenic crop with such components?

Remember - with soybeans there is much value from meal; not just oil….
Where are the economics of discarding or processing "castropha" meal?

Hype for toxic oilseeds



Castor has similar problems as Jatropha
Seeds contain 0.2 to 3% ricin

1 mg/kg toxic
fill car with 50 liters (13 gallons diesel)
enough ricin byproduct to kill 3 people
at lowest content, 45 at highest

Not transgenic - no environmental impact studies needed - no regulatory scrutiny

Ricin protein "easy" to eliminate transgenically!

Two Oklahoma legislators introduced a bill to outlaw production and transportation of castor
.
Jatropha banned in Western Australia
as "toxic to man and livestock"

Approach should be to:
ban the toxins – not the crop.

stimulate R&D on domestication

If you want "Castropha" as an oil crop - Engineer or breed:
- dwarfing (increase harvest index)
- single stalk (high IAA?)
- Antishattering - fruits dry on stems
  -machine harvesting and threshing
- RNAi curcin /ricin & agglutinin genes
  gene excision + RNAi
- RNAi terpene synthase to rid of phorbols
- RNAi pathways to other toxins/allergens
- better yield, oil content / quality

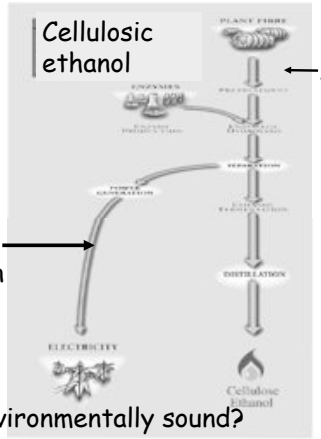
The engineered crop might then be safe to grow and use without hand labor and chemical processing

Can reduce by breeding
- why not continue breeding?

Ricin production dominant
pollen from neighbors

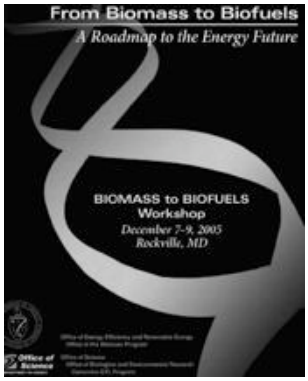
RNAi/antisense dominant for non-production

Override pollen



Is this environmentally sound?

Approach has been:
optimize chem process/ not crops



Executive Summary:
"The key to a new biofuel industry based on cellulose to ethanol is to understand plant cell wall chemical and physical structures.

With this knowledge, innovative biofuel crops can be developed with new biology-based treatment and conversion methods."

Harvesting perennial Miscanthus

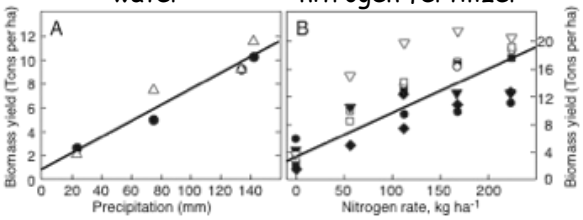
http://www.regensw.co.uk/images/miscanthus_harvesting.jpg

Multi-cut Switchgrass in Italy

www.switchgrass.nl/ photo_gallery.htm

Switchgrass does not defy the law of conservation of matter - grows best with

water nitrogen fertilizer



Data of Lee et al. and Muir et al, collated in Gressel, "Genetic Glass Ceilings, Hopkins, 2007

The non-degraded switchgrass residue is burnt - energy for process

Contains 5-10% ash, >60% of ash=silica
On burning releases 50% more non-precipitable silica than coal*

Same with sugarcane bagasse/other grasses
Major respiratory problems nearby

*Blevins, L.G., and Cauley, T.H. (2005) Fine particulate formation during switchgrass/coal cofiring. *Journal of Engineering for Gas Turbines and Power-Transactions of the ASME* 127, 457-463

Silicon not a required element for plants
small amounts may be useful
but not the high amounts in many grasses, including sugarcane

Silicon transporters being discovered in plants
antisense/RNAi to lower levels?

With "switchcanthus", land must be bought, dedicated to cultivation, watered, fertilized and harvested.
Straw is available "free" - a byproduct of grain production

World grain production (≈straw production)

wheat	rice	maize	sorghum	millet
million metric tons				
568	579	602	55	26

Total grain (total straw) ≈2,000 million tons
Source: FAO statistics – 2004

Why not use 2 billion T of free waste biomass?

How much cellulosic wastes available?

Production of Agricultural Wastes in Argentina (FAOStat 2011)

Crop	Approx. Million Tons
Maize	23.8
Soybeans	48.9
Wheat	16.3
Total	89.0

-20% for soil organic material = 71.2 M tons as well as 89 M tons of valuable grain

Straw has negative economic/environmental value

- harbors pathogens if not burnt
  - -requires fungicides on next crop
- releases CO_2 if burnt
- binds nutrients while biodegrading
  - requires more fertilizer - pollution

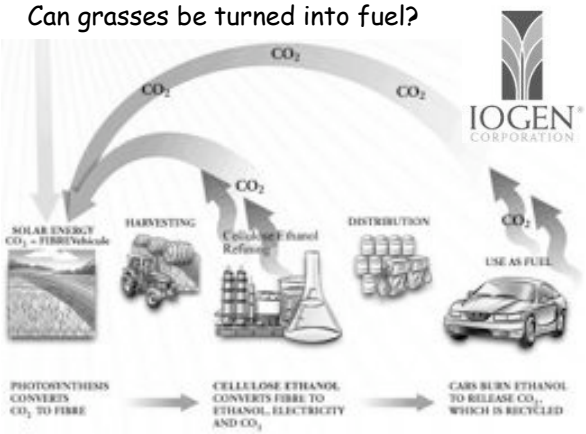
Straw has little value as animal feed or as a feedstock for bioethanol production.

- despite ca. 70% carbohydrate
- less than half digested

Can we turn straw into something valuable?

Maybe not into gold, but into bioethanol

Can grasses be turned into fuel?



The higher the lignin content the lower the digestibility

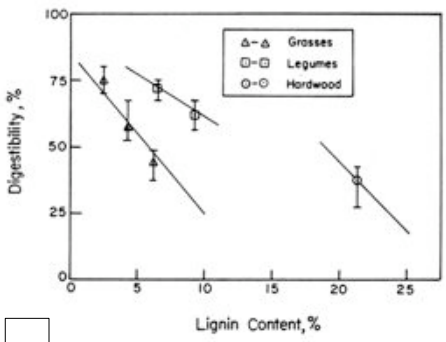


Fig. 6. Relationship between the dry matter digestibility and lignin content

The more lignocellulose is delignified
the greater the digestibility by cellulases

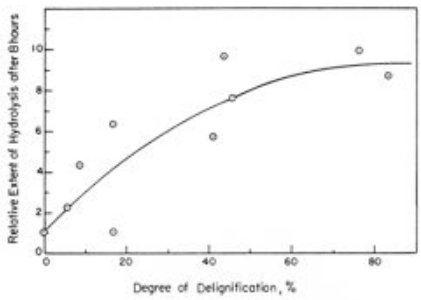
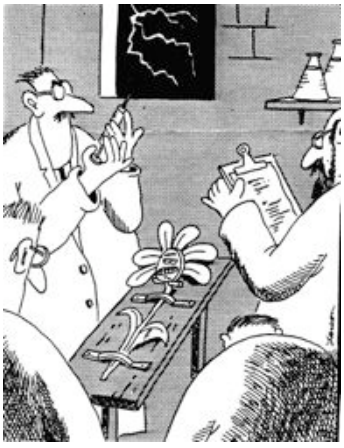


Fig. 5. Relationship between the extent of delignification and the hydrolysis rate

Present process:
Heat + acid pre-treatment (delignification)
Enzymatically digest cellulose to sugars
Ferment sugars to ethanol

But half of cellulose is unavailable
208 kg ethanol/ton straw
Claim: with present technology - Canadian wheat straw could provide ethanol for almost all Canadian automobiles
Less heat / less acid will be used if straw "domesticated for biofuel as well as grain production



The solution:
Modify straw for:
-less lignin
or
-modified lignin
or
- more cellulose

Should reduce the acid/heat requirement, add to yield

Use RNAi or antisense technologies
generate many transformants
will suppress to varying levels
screen optimal suppression/modification

Less lignin should = higher grain yield
Despite common suggestions / myth:
no correlation between lignin and strength

No reason to expect increased lodging if lignin slightly modified and / or reduced by a few percent

More cellulose

Engineer over-production of the cellulose binding domain causes over-production of cellulose

Probably best - stack
lignin reduction/modification
cellulose over-production

Proposal: Until Malthus arrives in developed world & until CO_2-free fuel sources available, use modified straw in:
Developed world:
Use technology for bioethanol

Developing world:
Use technology for ruminant feed

Later – everywhere:
Use technology for ruminant feed

All users should get carbon credits

The world is resource limited:- in food - in fuel - in fresh water - in arable land
New solutions must be found

Nails in Malthus's coffin came from quantum leaps – followed by incremental additions
Next quantum leap from algae – feed and fuel

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>20x potential

marine algae yield compared to soy & corn

never CO_2 or water limited – must be fed CO_2
use every photon – all year
100% harvested/used

<1% freshwater, <5% land

<30% fertilizer, <10% pesticides

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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

Cultures often crashed – quorum sensing

Open Microalgae Ponds
Taiwan & Japan

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

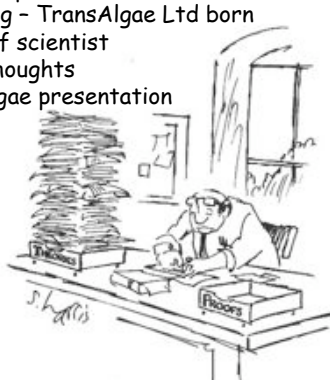
ex Ami Ben-Amotz

ex Ami Ben-Amotz

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

ex Ami Ben-Amotz

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 not a TransAlgae presentation



Problems to be solved

Domestication – Genetic Engineer

-Choice of organisms – algae or cyanobacteri triglyceride lipids

-no quorum sensing –grow dense/less water

-Contamination by unwanted organisms

- Needed co-products
- Oil content and composition
- If transgenic – spillage into environment
- Cooling – engineer heat tolerance

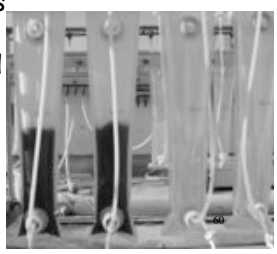
Production and harvest –Production Engineer

Developing a reliable platform

Overcoming Barriers to Algae Domestication:

solving system instability
- with herbicide resistance
- anti-microbial proteins

both have secondary uses
- selectable marker
- replacement of feed antibiotics

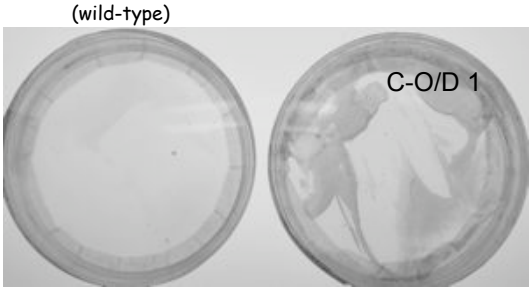


Needed platform domestication traits/genes for algae

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.

Mock –Transformed (wild-type) Transformed



Transformed algae are resistant to an inexpensive phytoene desaturase inhibiting herbicide that kills other algae and cyanobacteria

What if there is an inadvertent leak?

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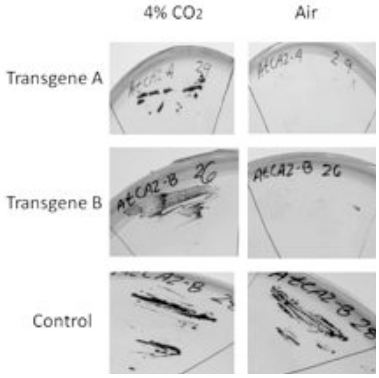
Mitigation – suppressed carbon capture

Engineer high CO_2 requiring transgenic algae

- antisense or RNAi carbonic anhydrase or nitrate reductase- needed in ocean



Fish for fishmeal/oil disappearing
fishmeal/oil $1800/T–biofuel oil $600-800/T

Many studies:
algae can replace fishmeal 1:1 by weight
Fishoil is algae oil
Most peculiar:
- have excellent amino acid composition
- but less protein
- are less digestible
(by definition "probiotic")

Which way is obvious

65

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

Already a good replacement for fish oil
High omega-3 fatty acids

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

Nannochloris could be engineered to produce a balance of other needed fatty acids

How will algae be cultured?

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

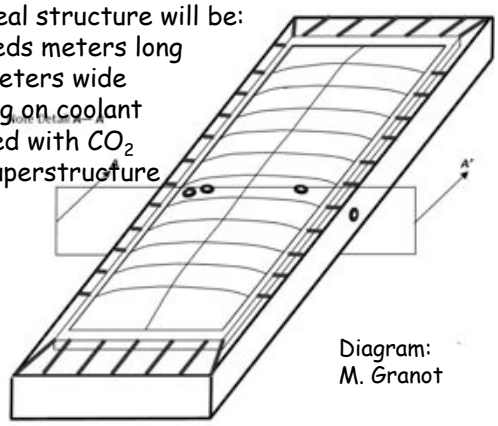
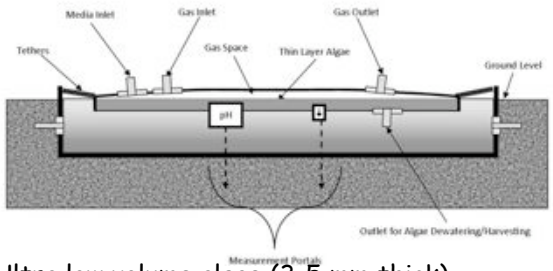


Diagram:
M. Granot



Ultra low volume algae (3-5 mm thick)
less medium to sterilize – easier dewatering
less pumping
How do we get CO_2 in without bubbling?
How do we mix cells? Get rid of O_2?

Where to cultivate algae in Argentina?

Along seacoast
On estuaries
On rivers
On floodplains
Where no agriculture

Intensive algaculture in sunny/warm north

Microalgae

- do not compete for land and fresh 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

Need industrial engineers to lower production costs

Summary predictions:
- In the short term - biofuels will come from food crops
- In the medium term - biofuels will come from cellulosic wastes and algae
- In the long term - only algae;
  cellulosics to ruminant animals

Biofuel feedstocks will be transgenic

For more information – two reviews

Available online at www.sciencedirect.com
ScienceDirect
Plant Science 174 (2008) 246–263
PLANT SCIENCE
www.elsevier.com/locate/plantsci

Review

Transgenics are imperative for biofuel crops

Jonathan Gressel [a,b,*]

Transgenic marine algae for aquaculture: a coupled solution for protein sufficiency

Successful Agricultural Innovation in Emerging Economics: New Genetic Technologies for Global Food Production, eds. David J. Bennett and Richard C. Jennings. Published by Cambridge University Press. © Cambridge University Press 2013. 233

Happy to send you pdfs:
Jonathan.gressel@weizmann.ac.il

Beware of

"A word to the wise is not sufficient - if it makes no sense.
J. Thurber

I hope I have made some sense today-
Thank you