The Use of Duckweed for Wastewater Bioremediation and Biofuel Production

Engineering to sustain

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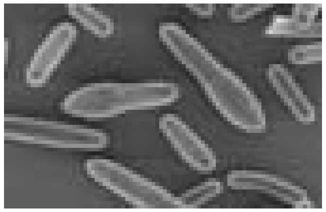
Introduction

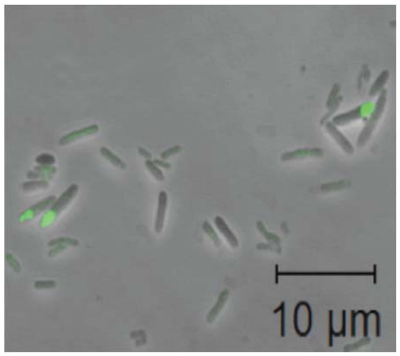


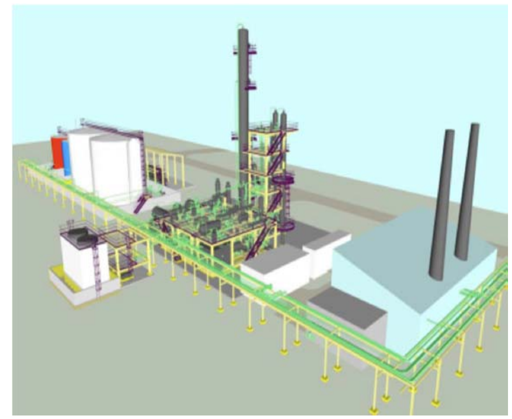
- **Elcriton**
  - Genetic engineering & process development company
  - Biochemical, Platform Technology – Bio-butanol (1st product)
- **Everyday chemicals**
  - Beer & ethanol (gasoline blending agent) – made by yeast & E. coli
  - Butanol – a better biofuel – drop-in fuel
    - Need to price competitively with gasoline and E10 blends
- **Clostridia** *(C. acetobutyliticum)* **technology can potentially decrease butanol production cost by <u>>40%</u>**
  - Can use any renewable biomass
  - A.B.E. fermentation process – acetone, butanol, ethanol

Product & Services

Product - Biochemical technologies (strains & fermentation schemes)

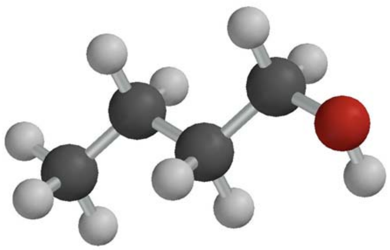
Services – lab demo & commercial-scale simulation





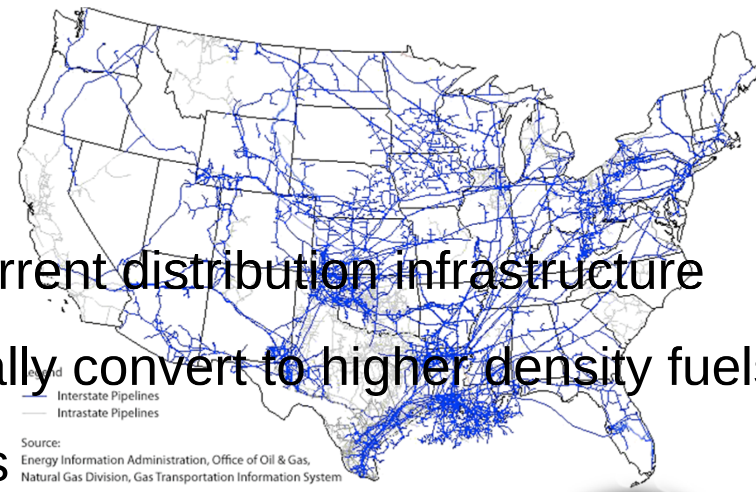
- **State of development:** Moving to pilot-scale demo

Sustainable Biobutanol Production



Why butanol?

- Better than ethanol
- Drop in fuel that utilizes current distribution infrastructure
- Available technologies to catalytically convert to higher density fuels
- Large chemical market currently exists



Why clostridia?

- Best native producers known
- Large and long industrial history of the ABE fermentation
- Anaerobic fermentation and potentially valuable co-products

Biobutanol (n-butanol) advantages

- Ethanol - 1st generation
  - Low energy content - poor MPG
  - High blends require flexfuel - need a new car
  - Absorbs water easily & corrosive – can't pump it
  - Cannibalization of the food chain (corn)
- Biobutanol – next generation
  - Higher energy content, more like gasoline – better MPG
  - 1992 Buick runs on 100% butanol - - Unmodified engine, from Ohio to California
  - Less hygroscopic + less corrosive -- send it through gasoline pipelines
  - Variety of feedstocks (>corn)
  - Can readily retrofit ethanol facility into a butanol facility

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Potential of biobutanol

US Renewable Fuel Standard program (RFS2) – 2022 target: 36 billion gallons of biofuel into domestic auto and truck fuel supply

“Freaking out over USDA estimates that 400 new biorefineries are needed to meet RFS goals, by 2022?

Ah, grasshopper – the costs and construction needs are far from that dire. It’s all a matter of utilizing the existing fleet and known technologies.”

BiofuelsDigest
The world's most widely read biofuels daily

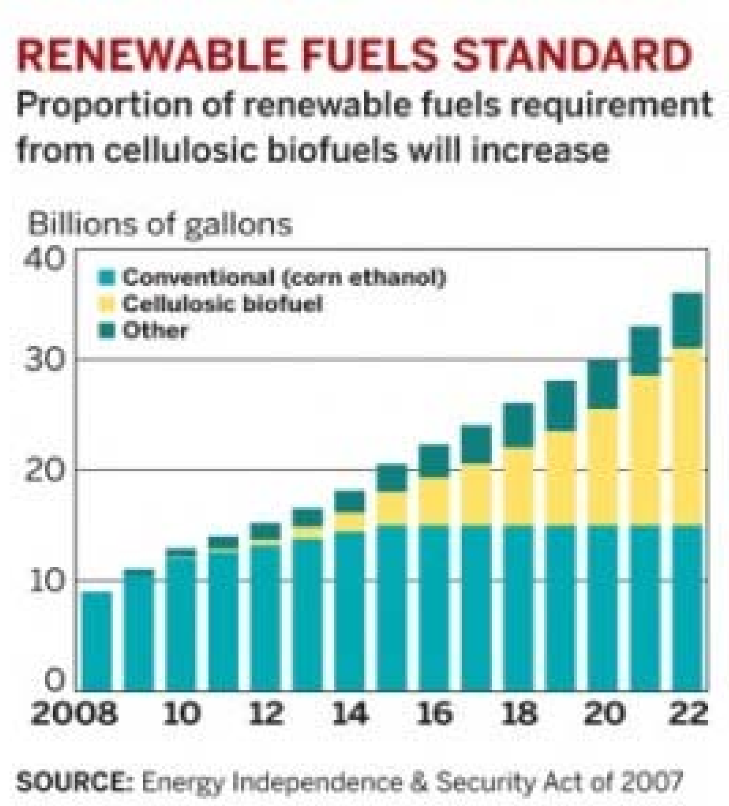
Biofuels Digest, 02/01/12

http://www.biofuelsdigest.com/bdigest/2012/02/01/a-low-cost-low-risk-path-to-meeting-us-biofuels-targets/

Potential of biobutanol

Agricultural residue-based biobutanol :
2.3 billion gallons (existing corn ethanol fleet)
= Equivalent of 3.9 billion gallons ethanol

“Total it up: 36 billion gallons, no E15, no blender pumps, no 400 new refineries, no kidding”



http://www.biofuelsdigest.com/bdigest/2012/02/01/a-low-cost-low-risk-path-to-meeting-us-biofuels-targets/

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Cultivation of Duckweed for Bioremediation of Delaware and Chesapeake Watersheds, and for the Sustainable Production of a Renewable Chemical Feedstock

Planned activities, pending SBIR funding

Planned activities, pending SBIR funding

- Test bioremediation capacity of duckweed on Millsboro Pond water, with and without chicken litter
  - Examine N, P, DO, chlorophyll, coliforms

- Harvest & dry duckweed biomass
  - Examine yield, protein and starch content

- Determine butanol yield in Clostridia fermentations using duckweed as feedstock

Poultry litter

Current annual DE manure production is 300,000 tons

- 200,000 tons used in agricultural applications
- Phosphorous accumulation in soils and groundwater

Mandate for Chesapeake Bay watershed (EPA 2010)
Major concerns for Inland Bays

Bioremediation *in situ* is proposed to decrease eutrophication, as well as provide revenue source for individual growers and biobutanol producers.

Duckweed biomass revenue two-pronged:

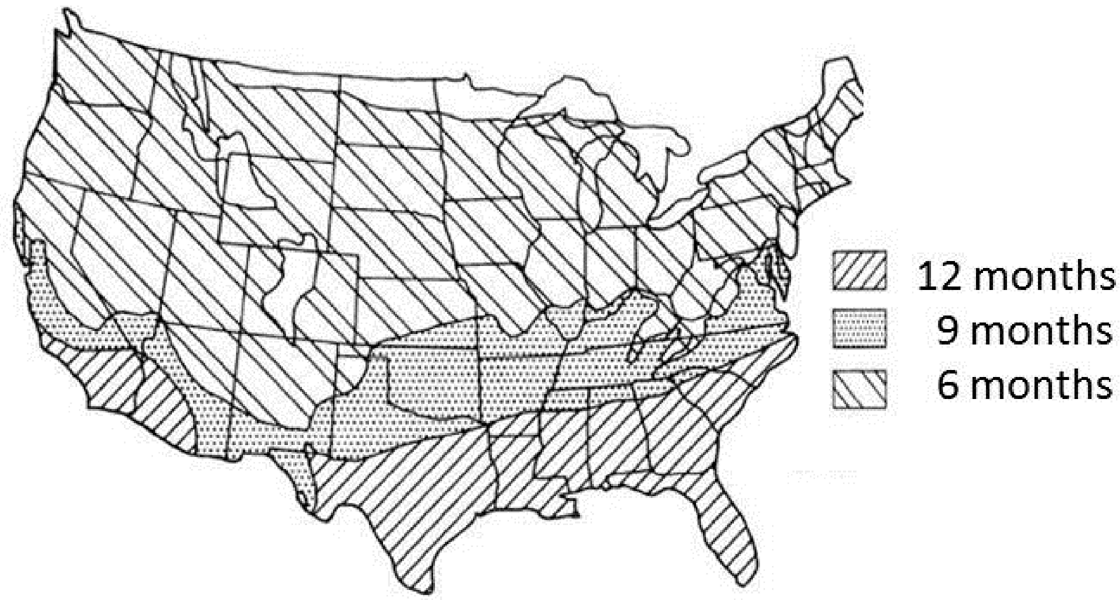
- Renewable feedstock for biobutanol
- Dried distillers grain or directly as poultry feed supplement (layers and finisher diets for broilers)

Duckweed is ubiquitous

Growth range and seasonal temperature tolerance in the United States for duckweed plants

Lemna minor (lesser duckweed)
Wolffia (water meal)

most common on the Delmarva Peninsula



Zirschky, J. and S.C. Reed, *The Use of Duckweed for Wastewater Treatment.* Journal (Water Pollution Control Federation), 1988. **60**(7): p. 1253-1258.

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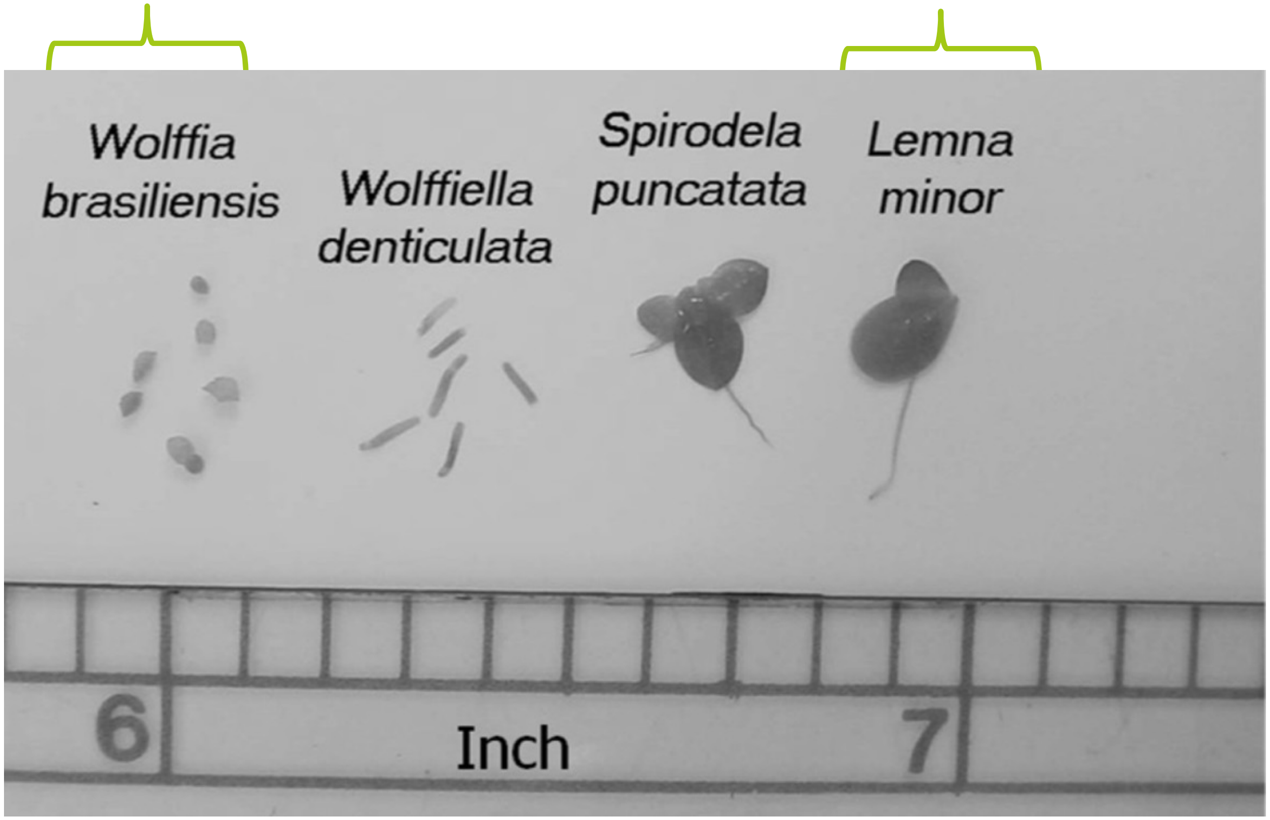
Duckweed – world's smallest flowering plant

Most common on the Delmarva Peninsula, usually a mixture.



Harvest by skimming with fine mesh nets, or ProSkimmer

Phytomediation: Considerations

+ and - Effects
+Nutrient removal
+Algal suppression
+/- Seasonal affects
+Heavy metal and other contaminants (coliforms)
-Lignin content→
-Need to Monitor:
pH
COD
TSS
Algal growth

Biomass components for ABE fermentation & Poultry feed Supplement

- Cellulose
- Hemicellulose
- Lignin
- Mono- and oligomeric sugars
- Proteins
- Lipids

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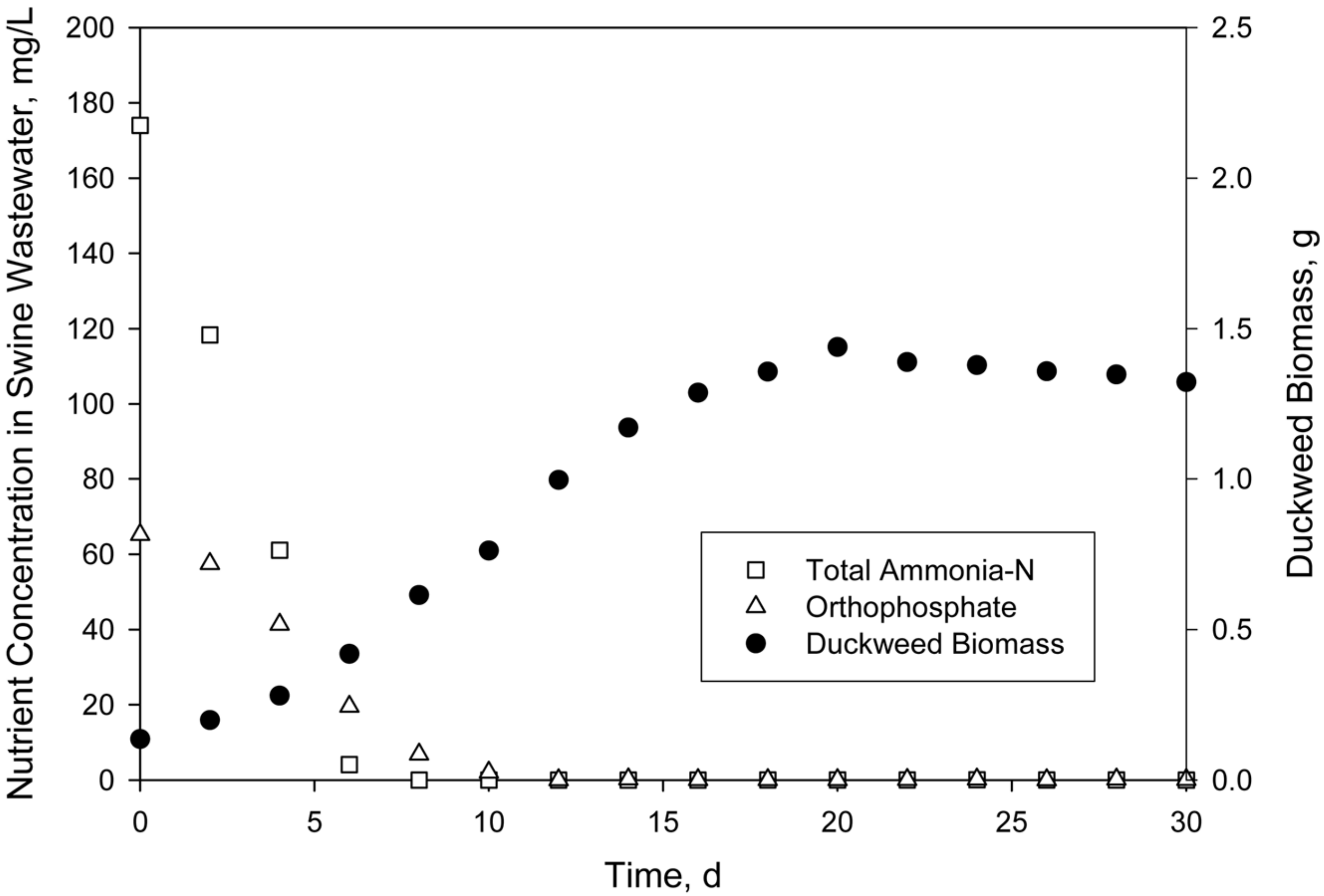
Duckweed biomediation



Serve as feedstock for ABE fermentation

Cheng and Stomp (2009) Clean, 37 (1), 17 – 26.

Duckweed biomediation

Nutrient sink, uptake of N and P well-documented on hog waste.

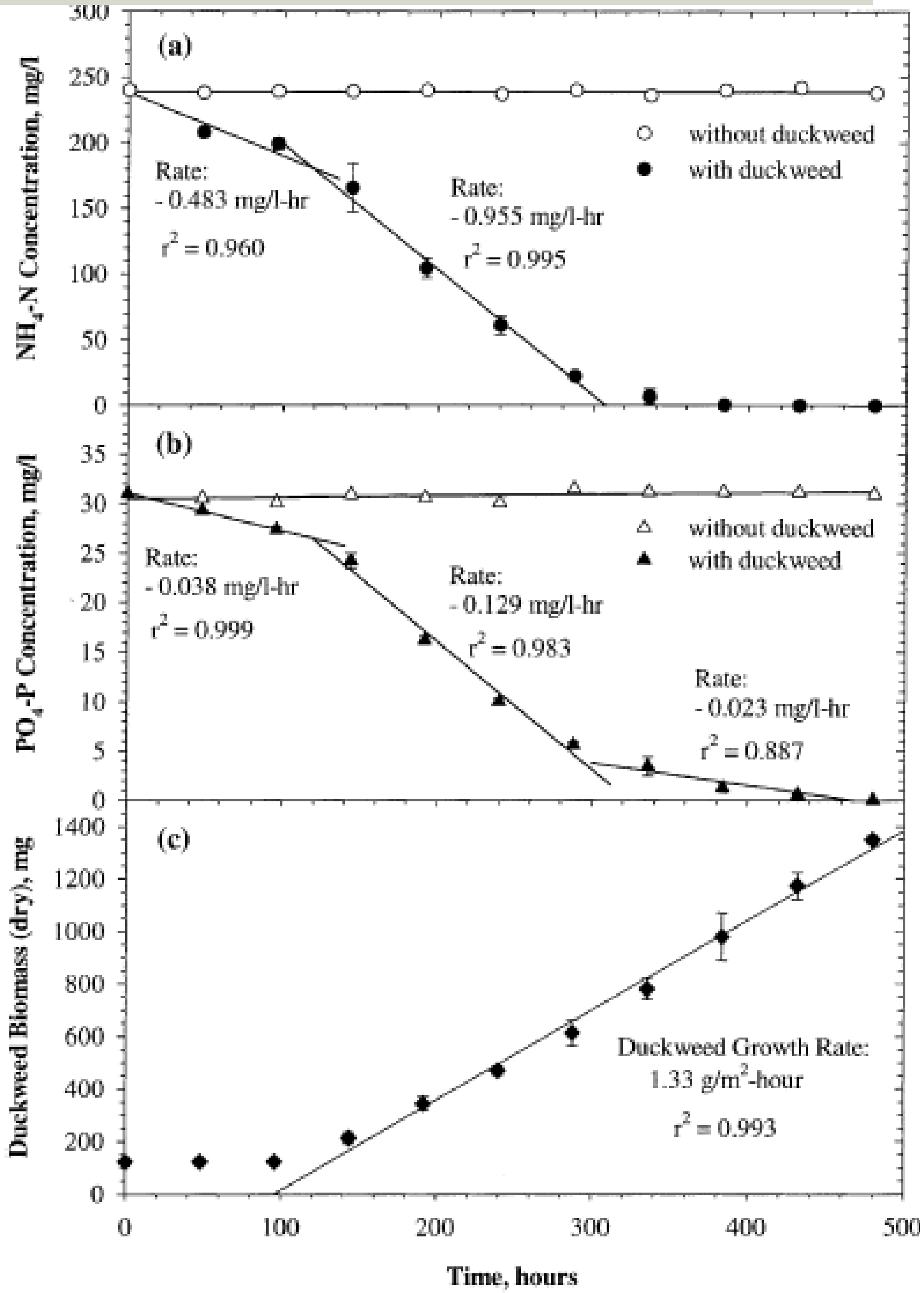
Reduction >70%
Seasonal affects <30%

Also CO_2 sink, fixed into:

Cell walls: cellulose, hemicelluloses, and lignin

Cellular content: mono- and oligomeric sugars, proteins, and lipids.

Important components for ABE fermentation



Cheng and Stomp (2002) Bioresource Technology 81

Duckweed biomass – starch content

Treat with cold (tap) water
- Sudden drop in temperature and nutrients
- Increases starchy turions, starch content of biomass
- Turions = 40-70% starch content

Seasonal affects
- Decreased temperature *in situ*
- Also, light intensity, photoperiod affect turion growth

Duckweed biomass as feed

Soy costs are prohibitive

Duckweed dried distillers grain (DDG).

A lower weight of DDG (compared to total duckweed) can replace conventional feed

Protein, fat and fiber content- concentrated in ABE fermentations

Fresh or DDG can comprise significant % of diet

Layer hens 15-40%

Recent research on egg protein content, Omega3 levels**

Late stage broilers 3-8%

Chantiratikul, A., et al., *Effect of Replacement of Protein from Soybean Meal with Protein from Wolffia Meal [Wolffia globosa (L). Wimm.] on Performance and Egg Production in Laying Hens.* International Journal of Poultry Science, 2010.

**Anderson, K.E., et al., *Duckweed as a Feed Ingredient in Laying Hen Diets and its Effect on Egg Production and Composition. International Journal of Poultry Science, 2011.*

Haustein, A.T., et al., *DUCKWEED, A USEFUL STRATEGY FOR FEEDING CHICKENS – PERFORMANCE OF LAYERS FED WITH SEWAGE-GROWN LEMNACEA SPECIES. Poultry Science, 1990.*

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Duckweed biomass as feed

TABLE 6. *Protein content of eggs from HyLine Leghorn hens fed diets containing 15 or 25%* Lemna *species or an isonitrogenous, isocaloric control diet*

Protein content	Treatments		
	Control	15% *Lemna*	25% *Lemna*
	(%)		
Albumin	$84.302 \pm .332^{C}$	$84.746 \pm .168^{B}$	$86.095 \pm .576^{A}$
Yolk	$15.642 \pm .232^{C}$	$16.283 \pm .125^{B}$	$17.238 \pm .141^{A}$

$^{A-C}$Means within a row for the same trait with no common superscripts differ significantly (P<.001).

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Duckweed biomass as feed

Table 2. Amino Acid Composition of Bulk Protein of *Lemnaceae* Species, Grains, Legumes, and Casein.

Amino Acids	*L. gibba*[a]	*S. punctata*[a]	*S. polyrrhiza*[a]	Green Grass[b]	Soybean Meal[b]	Peanut[b]	Rice[b]	Corn Gluten Meal[c]	Casein[b]	Recommended Levels of Essential Amino Acids for Chicken Feed[c]
	g/100 g protein									
Leu	7.15	6.88	6.85	10	8.0	6.7	8.2	15.3	10.0	7.5
Ile	3.87	3.76	3.75	5	6.0	4.6	5.2	4.9	7.5	5.0
Val	4.96	4.71	4.40	5	5.3	4.4	6.2	5.1	7.7	5.0
Met	0.83	1.07	0.83	2.5	1.7	1.0	3	2.35	3.5	2.0
Cys	NA[d]	NA	NA	2.0	1.9	1.6	1.3	1.65	0.4	3.6[e]
Phe	4.45	4.38	4.20	5−6	5.3	5.1	5.0	5.6	6.3	4.4
Tyr	2.91	3.14	3.05	5.0	4.0	4.4	5.7	2.3	6.4	6.4[f]
Lys	4.13	4.26	4.30	5.5	6.8	3.0	3.2	1.85	8.5	4.0
Thr	3.20	3.31	3.45	5.4	3.9	1.6	3.8	3.0	4.5	3.5
Trp	NA	NA	NA	2.2	1.4	1.0	1.3	0.5	1.3	1.0
His	1.89	1.90	2.15	2.0	2.9	2.1	1.7	2.1	3.2	1.9
Arg	4.29	4.86	5.25	7.0	7.3	11.3	7.2	3.25	4.2	5.0
Ser	2.61	2.83	2.80	5	4.2	NA	NA	NA	3.3	NA
Pro	2.93	2.95	3.28	NA	5.0	NA	NA	NA	13.1	NA
Gly	3.79	3.93	3.95	NA	NA	5.0	NA	NA	2.1	NA
Glu	7.60	7.69	8.00	11.5	18.4	17.7	NA	NA	23.0	NA
Asp	7.12	7.38	7.55	5.3	NA	NA	NA	NA	7.0	NA

[a] Rusoff et al. [38];
[b] Block and Bollings [49];
[c] Scott et al. [50]; laying hen requirement;
[d] Not available;
[e] Sum of phenylalanine and tyrosine;
[f] Sum of phenylalanine and tyrosine.

Cheng and Stomp (2009) Clean, 37 (1), 17 – 26.

Duckweed as biobutanol feedstock

Initial analysis of *Cac* growth on duckweed biomass. Duckweed was pretreated by autoclaving only, and medias were not supplemented with salts or yeast extract.

Conditions	Concentration (g/L)		
	Organic acids (acetate & butyrate)	Solvent (Acetone, Butanol & Ethanol)	Glucose Equivalent *
50g/L autoclaved	3.0	0.3	12.4
100g/L autoclaved	4.2	1.6	20.2
50g/L, autoclaved + 5g/L xylose	7.3	1.4	32.9 **
50g/L autoclaved + 5g/L xylan	3.6	0.3	15.0

*Calculated glucose equivalents from 2 acetate/ethanol and 1 butyrate/butanol per mole of glucose.

**Xylose stimulates greater xylanase activity, and subsequently more hemicellulose from the duckweed was utilized in these cultures.

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Requests from CIB/associated DNREC

- Feedback today
  - Suggestions on designs, locations

- Future collaborations in specific locations
  - Bioremediation *in situ* (without litter)
  - Monitoring, harvesting?

Proposed experimental design

Millsboro Pond water as model eutrophic system

- Average P 3.19 mg/L
- Designated by EPA as impaired body of water
- Outflow routinely hand-monitored AND outfitted with real-time monitoring (Aqualab®) temperature, salinity, turbidity, pH, DO, nitrate NO_3 and orthophosphate PO_4

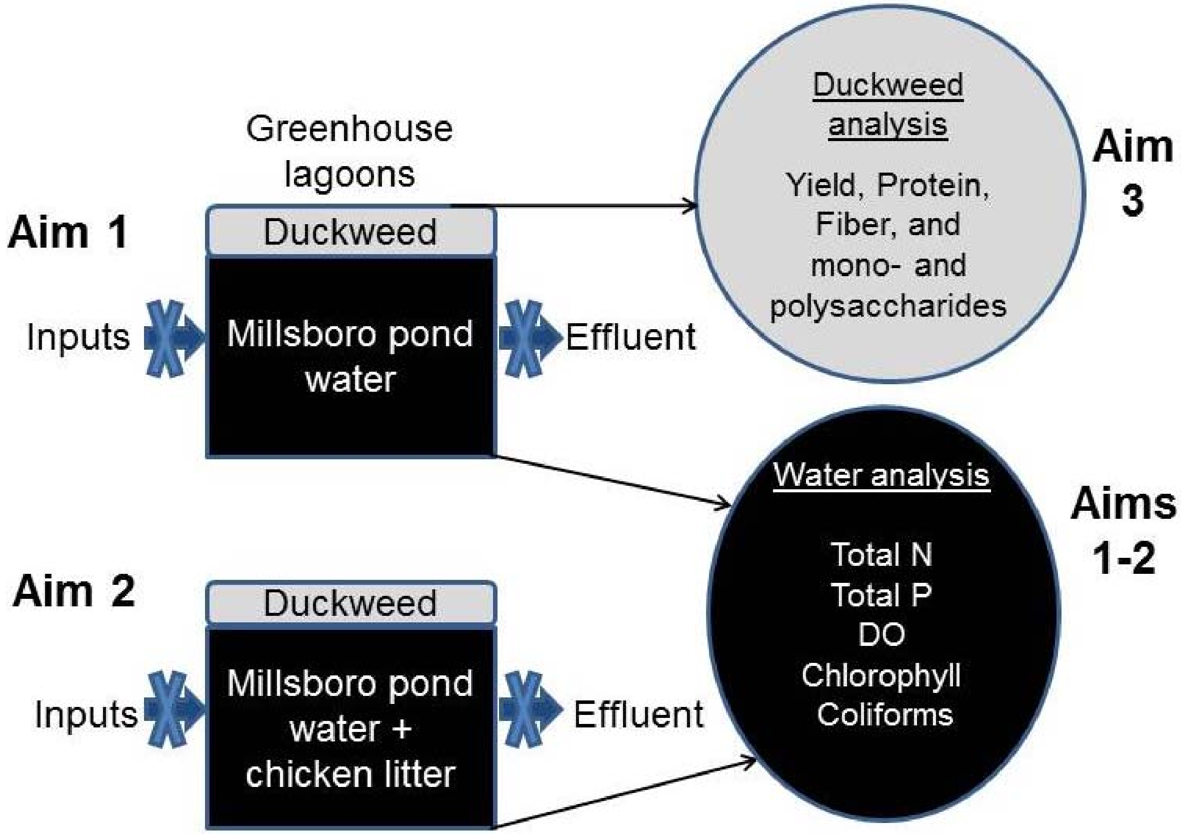
Four purposes of bioremediation testing

(1&2) Known impaired body of water to be bioremediated with duckweed, +/-poultry litter

(3&4) Examination of biomass for biobutanol and feed supplementation.

Proposed experimental design

Millsboro Pond water in artificial lagoons, with and without poultry litter.
Lagoons will not be subjected to environmental inputs; and no effluent will be released



(3&4) Biomass for biobutanol and poultry feed supplement.

(1&2) Assess bioremediation capacity.

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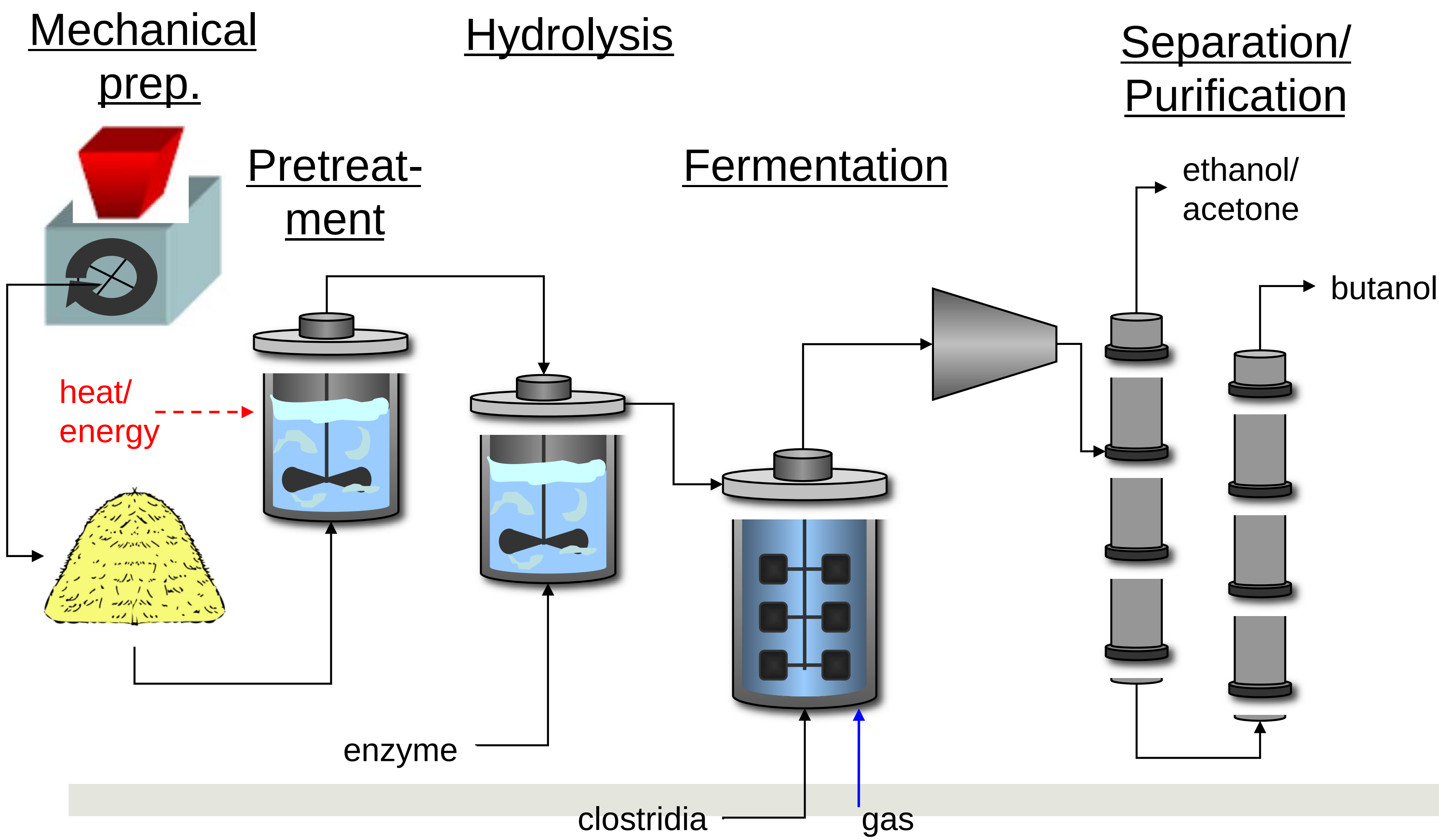
End goal 1:biobutanol from duckweed



End goal 2: duckweed as feed supplement

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$^{A-C}$Means within a row for the same trait with no common superscripts differ significantly (P<.001).

Largest local market for this is Lancaster, PA area. Again, discussions with local growers and hatcheries.

Team and Collaborators

- **Elcriton team:**
  - **Prof. Terry Papoutsakis** - President and founder
    - Eugene DuPont Chair Professor of Chemical Engineering, UD
  - **Dr. Bryan P. Tracy** – CEO & Lead Scientist
    - Biotech research, IP development & commercialization
    - *Clostridia* genetic engineering.
  - **Dr. Lisa A. Waidner** – Principal Research Associate
    - Biotech research, *Clostridia* genetic engineering
    - Graduate work -- environmental parameters of estuarine and freshwaters, including Delaware, Chesapeake, and offshore waters, including bacterial loads, DO, chlorophyll, nutrients, etc.
    - Postdoctoral work with poultry viral diseases(Dr. Robin Morgan, UD).
- **Collaborative and contract help**

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  - **Prof. Terry Papoutsakis** - President and founder
  - **Dr. Bryan P. Tracy** – CEO & Lead Scientist
  - **Dr. Lisa A. Waidner** – Principal Research Associate

- **Collaborative and contract help from:**
  - David O. Rickards, Birdsong Gardens, Environmental Research, Delaware.
    - 21 years as a poultry grower, nutrient management and poultry waste management.
    - Experience with duckweed growth and harvesting.
  - In discussions with DNREC with respect to permits for duckweed harvesting, locations of experimental sites, etc.
  - In discussions with poultry producers with respect to litter and litter by-products
  - **YOU! CIB -- suggestions, design, harvesting, monitoring?**

Requests from CIB/associated DNREC

- Feedback today
  - Suggestions on designs, locations

- Future collaborations in specific locations
  - Bioremediation *in situ* (without litter)
  - Monitoring, harvesting?

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Thank you

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Questions -- Discussion

Potential of biobutanol

US Coast Guard and Oak Ridge National Labs

3-year project to study maximum biobutanol for blends in gasoline engines

Also examining infrastructure for salt water operations

Biofuels Digest, 01/20/12

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