Human Health Risk Assessment of Algae Production Systems

May 21, 2013

BETO Platform Review: *Algal Sustainability*

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Goals

- **Explore the potential for environmental or human health risks associated with large scale production of biofuel algae**
  - Potential reservoir for human pathogens or toxin producing microbes (SRNL)
  - Accumulation of metals or metalloids in algal biomass and media (LANL)
  - Evaluation of human liver cells for toxic responses to algae and media (LANL)
  - Emission of noxious, odorous, or reactive volatile organic compound (VOCs) (TAMU-CC)
  - Production of cytotoxic metabolites (NOAA)
- **Broader Relevance and Programmatic Fit**
  - Human health risks: cursory examination
  - Implications for expanding the algal fuel cycle; waste to resource
  - Lends considerations to devise and operate ‘smart’ systems
  - Emerging issues of production but aspects of risk will be system specific & unique

Quad Chart Overview

Timeline

9/01/10 - 9/30/12
NCTE thru April 2013

Project is complete

Barriers

- Feedstock
  - Safety
  - Best Practices
- Sustainability
  - Social Acceptance
  - Land Use
  - Resource Use and reuse

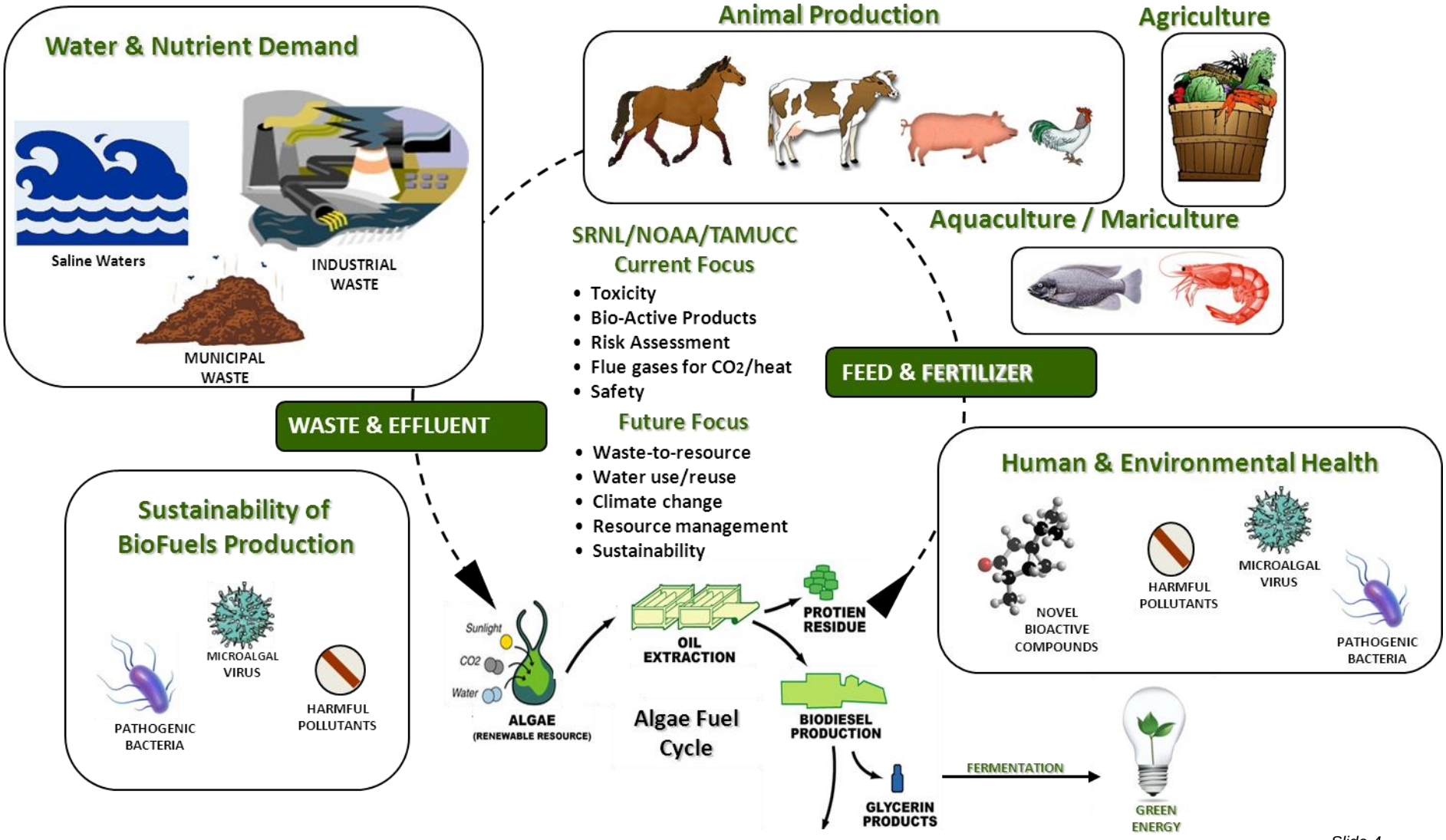
Budget

Award	$1,340,319 (both labs)
FY10	$740,319 ($370K)
	(LANL share 50%)
FY11	$600,000 ($180K)
	(LANL share 33%)

Partners

- Kitt Bagwell (SRNL)
- Paul Zimba (TAMU – CC)
- Peter Moeller (NOAA-NOS)

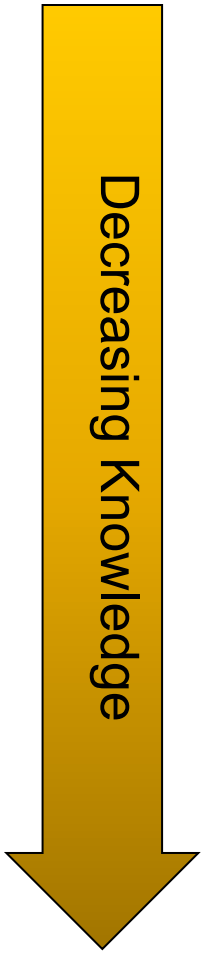
Project Overview-Algae Biofuel Life Cycle



Literature Review

- **Significant literature exists covering metals bioconcentration pathways in algae, including multiple species and both laboratory and field conditions, *as related to environmental remediation*.**
- **Literature exists relating to occurrence and causes of harmful algae blooms (HABs) in the natural environment. *Toxin induction from HABs is complex and not well understood.***
- **Some literature exists relating to hazards or environmental impacts in the *aquaculture* industry. Odorous substances are significant issues.**

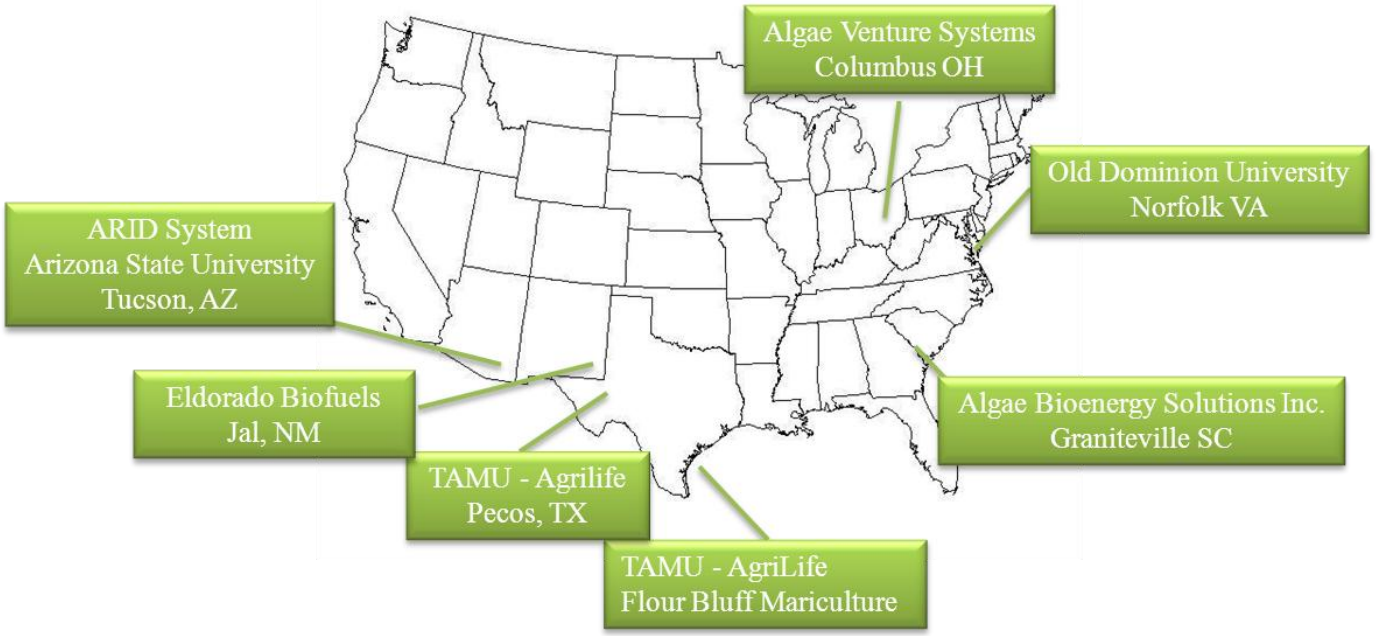
Little literature exists examining risks or hazards of cultivation in the biofuels industry. Conditions specific to the industry that may induce risks to human health or the environment are unknown.



Phase I. Survey of biofuel algae production systems

Potential human pathogen and confirmed toxin producing microbe occurrence

- **Evidence for metal/metalloid bioaccumulation in algal biomass and media**
- **Evidence of human cell toxicity in assays**
- **Emission of noxious or hazardous VOCs and cytotoxic metabolites.**



Lab	**Responsibility**	**Method**	**Samples**
SRNL	Pathogens & Toxic Algae	QPCR	Total Biomass
LANL	Metals/Human cell toxicity	ICP-MS/flow cytometry	Water & Biomass
NOAA	Cytotoxicity Assay	*In vivo*, LC-MS	Biomass
TAMU-CC	VOCs	SPME / GC-MS. NIST	Algae - Water

1-Approach: Metals/metalloid chemistry in media and algae

- **Media**
  - *Phase 1*: 5 sites, grab samples across ponds; *Phase 2*: outdoor, multiple species
  - EPA standard methodology for sampling.
  - Total (centrifuged) and dissolved (<0.45 µm) metals/metalloids by ICP-MS and ICP-OES; major and trace elements. (MDLs
- **Algae Bioaccumulation (1 site, 8 cycles algae and media)**
  - EPA standard methodology for sampling-simultaneously with media
  - Acid extracted for metals/metalloids
  - Total metals/metalloids by ICP-MS and ICP-OES
  - Performed for 1 location (Pecos) for multiple sample harvests over time.
  - Recycled media; active production site with known system controls.
- **Survey goals:**
  - Determine existence/concentrations in media of constituents that may be toxic via contact, ingestion (unlikely) or constitute an environmental risk if discharged.
  - Determine if bioconcentration occurs in algae over time during cultivation.

Site types observed and factors related to risk assessment

Location	Pond Type	Pond Location	Water Type	Environmental Pathways	Human Health Pathways
Ohio	Bubbled-low circulation Bioreactors Zinc or plastic lined	Indoor Greenhouse	Fresh (<500 ppm TDS) Public Supply	Discharge to sewer Pond liner failure Air emissions	-Contact or ingestion Acute/Chronic -Inhalation
Virginia	Circulating paddlewheels Plastic lined	Outdoors	Fresh (<500 ppm TDS) Public Supply	Discharge to sewer or outfall Pond liner failure Air Emissions	-Contact or ingestion Acute/Chronic
Southeast NM	Circulating paddlewheels Plastic lined	Outdoors	Brackish Oil and Gas produced Stock well ~1000-8000 ppm TDS	Pond liner failure Air Emissions	-Contact or ingestion Acute/Chronic
East Texas	Circulating Paddlewheels Bioreactor Zinc or plastic lined	Outdoors Bioreactor indoors	Marine Variable salinity (~20,000-35,000 ppm TDS)	Pond liner failure Air Emissions Discharge to Bay or sewer	-Contact or ingestion Acute/Chronic -Inhalation
West Texas	Circulating Paddlewheels Plastic lined	Outdoors	Brackish Well ~3000 ppm TDS Fresh Public Supply	Pond liner failure Air Emissions Discharge to storage pond	-Contact or ingestion Acute/Chronic

Phase I survey metals/metalloids frequency of detection (media/algae)

Virginia Site-Fresh Water Example

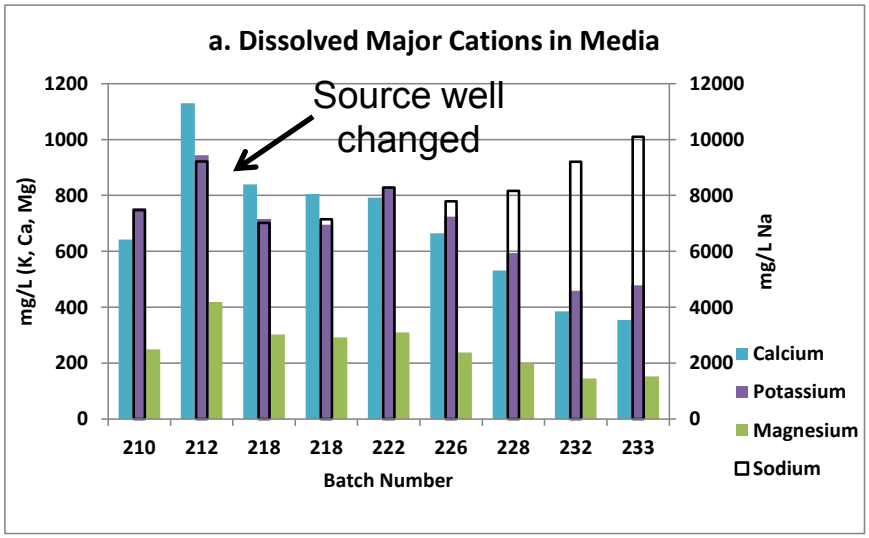
TOTAL METALS				DISSOLVED METALS			
Never detected	Detected 50% or less	Detected over 50%	Always detected	Never detected	Detected 50% or less	Detected over 50%	Always detected
Beryllium	Lithium	Aluminium	Boron	Beryllium	Lithium	Aluminium	Boron
Sulfur	Cesium	Phosphorus	Sodium	Sulfur	Tin	Phosphorus	Sodium
Germanium		Vanadium	Magnesium	Germanium	Cesium	Vanadium	Magnesium
Arsenic		Yttrium	Silicon	Arsenic	Mercury	Yttrium	Silicon
Selenium		Silver	Potassium	Selenium		Silver	Potassium
Niobium		Cerium	Calcium	Niobium		Cerium	Calcium
Ruthenium		Neodymium	Scandium	Ruthenium		Tungsten	Scandium
Rhodium		Mercury	Titanium	Rhodium		Uranium	Titanium
Indium		Uranium	Chromium	Indium			Chromium
Tellurium			Manganese	Tellurium			Manganese
Praeseodymium			Iron	Praeseodymium			Iron
Samarium			Cobalt	Samarium			Cobalt
Europium			Nickel	Europium			Nickel
Terbium			Copper	Terbium			Copper
Dysprosium			Zinc	Dysprosium			Zinc
Holmium			Gallium	Holmium			Gallium
Erbium			Rubidium	Erbium			Rubidium
Thulium			Strontium	Thulium			Strontium
Ytterbium			Zirconium	Ytterbium			Zirconium
Lutetium			Molybdenum	Lutetium			Molybdenum
Hafnium			Palladium	Hafnium			Palladium
Tantalum			Cadmium	Tantalum			Cadmium
Rhenium			Tin	Rhenium			Antimony
Osmium			Antimony	Osmium			Barium
Iridium			Barium	Iridium			Lanthanum
Platinum			Lanthanum	Platinum			Neodymium
Gold			Gadolinium	Gold			Gadolinium
Thallium			Tungsten	Thallium			Lead
Thorium			Lead	Thorium			Bismuth
			Bismuth				

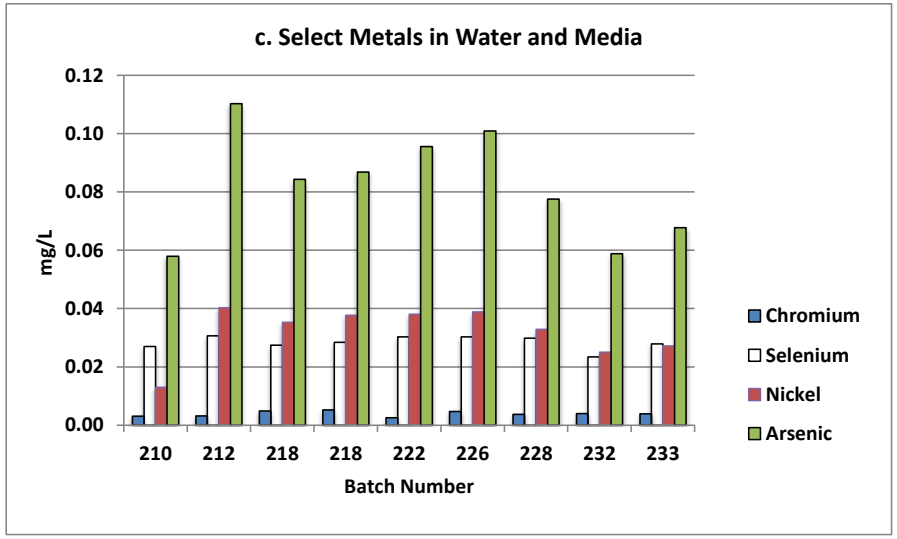
Typical ranges of RCRA 8 and other metals in media Phase 1 site survey (n=55)

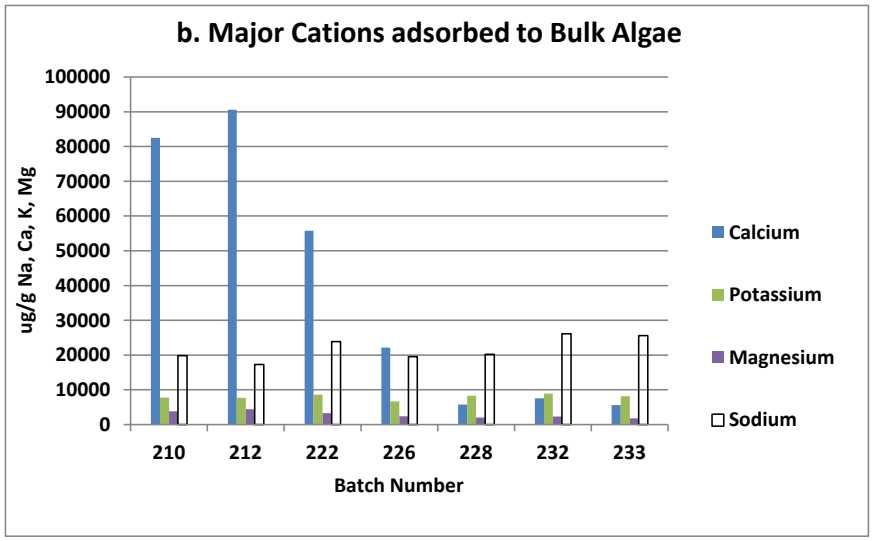
- **RCRA 8 metals are usually low concentrations, occasionally exceed Maximum Contaminant Limits (MCLs) (As, Cd, Se). MCLs also exceeded for U, Fe (not shown).**
- **Some other limits (secondary drinking water limits, World Health Organization (WHO), US EPA Target Limits) may apply (As, Pb, Cu)**
- **MCLs are strict health-based ingestion limits: an indicator of significance**
- **Regulators *may* consider these levels as discharge limitations**

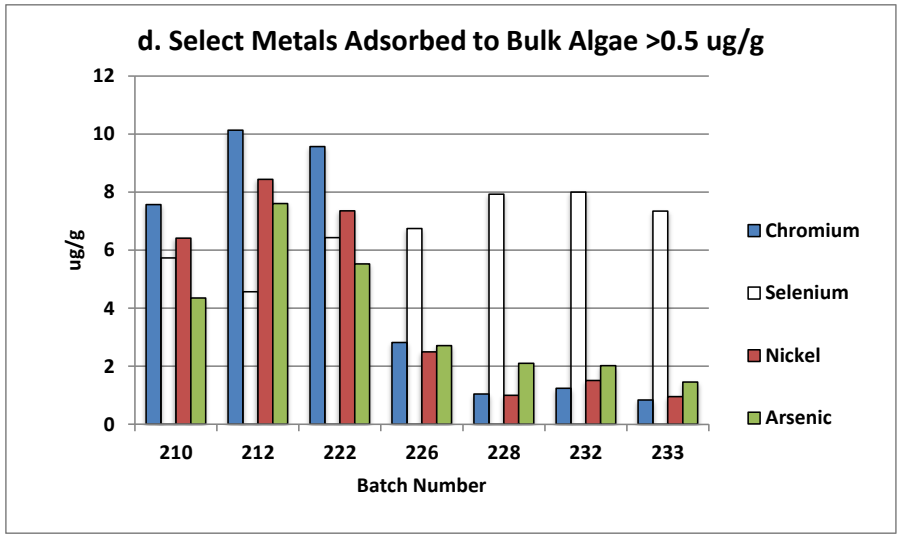
Constituent	Ag	As	Ba	Cd	Cr*	Hg	Pb	Se
Limit(s)	0.05 MCL	0.006 MCL 0.001 WHO	2 MCL	0.005 MCL	0.1 MCL	0.002 MCL	0.015 Action Level	0.05 MCL
Public Health Goal #	0.05	0	2	0.005	0.1	0.002	0	0.05
Range Detected for all locations (# > limit)	0.0001-0.0057 (0)	0.0003-0.11 (16)	0.005-1.44 (0)	0.0001-0.13 (6)	0.0004-0.07 (0)	0.0003-0.002 (0)	0.0001-0.026 (2> action level)	0.0001-0.088 (2)

Bioconcentration in algae over time is media-related
r^2 (divalent) > r^2 (monovalent); can increase or decrease









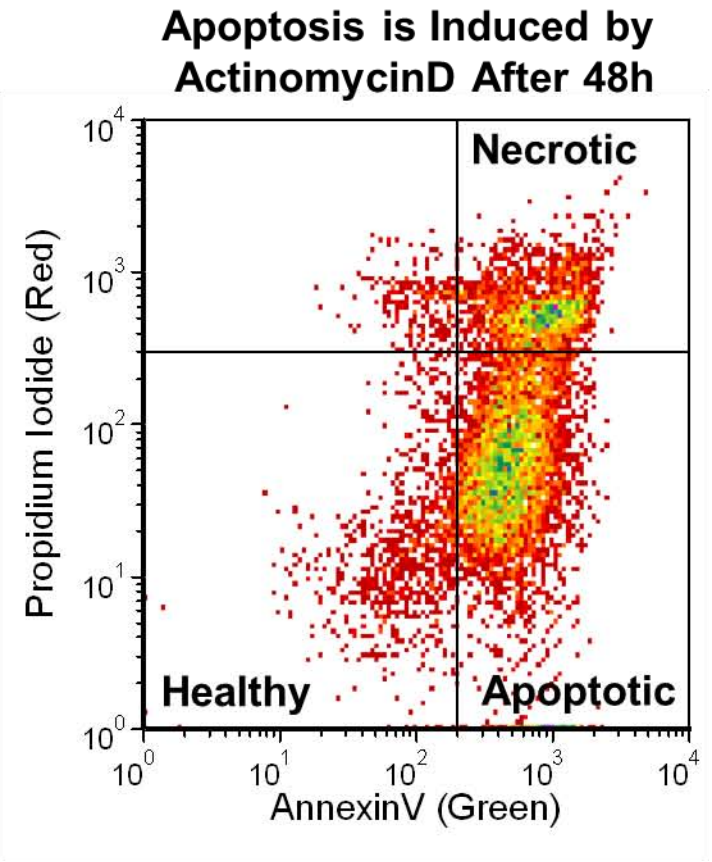
Sullivan et al., Submitted to Algal Research 2013, in review.

Results

- **Algae: media metals composition related to algal composition (equilibria). Both increasing and decreasing metal concentrations in algae can be found. Source water should be monitored, particularly if changes made.**
- **Media:**
  - Total metals or dissolved metals analysis appears sufficient for cultivators to assess site risks (easy, straightforward).
  - Speciation of specific toxics not relevant at the low concentrations measured.
  - As, Cd, Cr most frequently detected constituents of concern; As, Cd exceeded MCLs in some cases (29% and 10%, respectively).
- **Overall potential human health risk of contact for metals, in "typical" media and algae appears low, however, discharge standards may apply for blowdown discharge even for commonly supplemented constituents.**
- **Liner failure/no liner may be a potential route for environmental (ground water) contamination. State regulations could apply.**

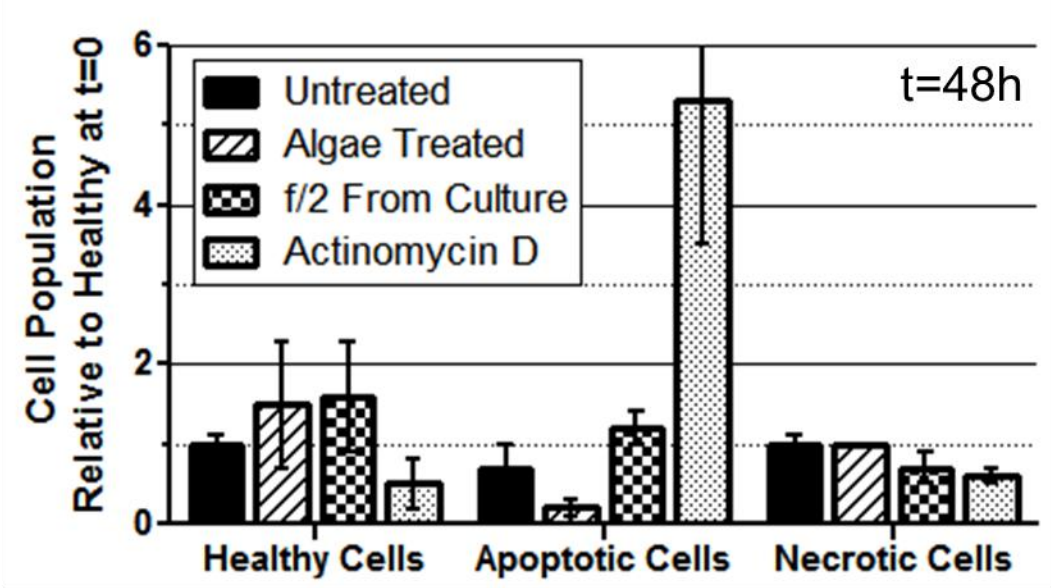
1-Approach: Response of Human Liver Cells to Algae

- A flow cytometry assay monitors cell populations:
  - ✓ **Healthy**, living cells
  - ✓ Early **apoptotic** cells (apoptosis cell death pathway)
  - ✓ **Necrotic**/Late apoptotic cells (dead cells)
- HEPG2 cells (human liver cell line) were used.
- Algae used = *Nannochloropsis salina*
- Time = 0, 24, 48h
- Positive control = ActinomycinD induces apoptosis
- RT-PCR experiments were conducted in tandem to track specific gene expression levels.



Cell counts in each quadrant provide a quantitative measure of cell death

Treatment of Liver Cells with *N. salina* Cultures Does Not Induce Apoptosis



- Cell counts were normalized to the healthy counts at t=0.
- Similar levels of healthy, apoptotic, and necrotic cells are observed for all samples except ActinomycinD-treated (+ control).
- Neither *N. salina* nor culture media pose a large risk to liver cells under this limited study.

Gene Expression Experiments:

- Expression levels of 14 genes were measured & normalized to a housekeeping gene.
- A general trend of gene down-regulation was observed for the Algae-treated sample at 48h, but only ATP-synthase down-regulation was statistically significant (P=0.02).
- Few significant changes were observed for algae-treated cells in this time frame.
- Long-term and chronic exposures were not addressed.

2-Benchmarks from Survey (P1) and Cultivation (P2)

Technical Targets

1. Potential reservoir for human pathogens or toxin producing microbes (SRNL)
2. Accumulation of metals or metalloids in algal biomass and media (LANL)
3. Evaluation of human liver cells for toxic responses to algae and media (LANL)
4. Emission of noxious, odorous, reactive volatile organic compounds (SRNL/TAMUCC)
5. Production of cytotoxic metabolites (SRNL/NOAA)

Assessment Results:

- ✓ Some specific pathogens detected; further in-depth analysis needed.
- ✓ Metals accumulations unlikely to be an ingestion/contact risk, may be regulated on discharge. Regular monitoring is recommended for specific metals.
- ✓ Human liver cell cytotoxic responses are low, but evaluated only for a single system (1 cell type, acute exposure only)
- ✓ Volatile emissions are unlikely to be a risk in open air systems. Ventilation is recommended for closed systems (e.g., greenhouses, closed buildings). Odorous compounds may be an issue for neighboring public.
- ✓ Cytotoxic metabolites are found in many ponds, recommend monitoring, personal protective equipment (PPE) such as gloves, coveralls, controls such as hand washing.

3-Relevance to BETO MYPP-Feedstock R&D and Sustainability Support

- **Social Sustainability**
  - This task addresses key questions that affect social acceptability, social well being, and workforce training (MYPP p. 2-87).
- **Sustainability: "Assess effects across full supply chain"**
  - This task assesses risks in the cultivation phase of the supply chain
- **Sustainability: "Develop Best Practices"**
  - This task recommends that best practices be developed for handling algae and media in ponds; and for discharge of blown down media.
  - This task recommends that a "best practices" informational process be developed, such as a website or online manual to inform workers of potential hazards.
  - This task recommends that a review of relevant environmental and health regulations for cultivation practices be performed
- **This task is cross-cutting and relevant to research, demonstration, and deployment stages of algal biofuel systems.**
- **The task results can be used to inform stakeholders, regulatory and permitting agencies, standards organizations, site operators, and workers of potential risk levels and areas that may need to be assessed on a site-specific basis.**

4-Critical Success Factors and Summary

Factor 1: An initial knowledge of many potential sources of risk in the algae cultivation system was gained.

Factor 2: Findings will advance the development of standard practices and safeguards in the algal biofuel cultivation industry.

Summary:

- Potential for risks and environmental impacts were assessed in specific areas: actual risk was not quantified or assessed
- Most potential acute risks or environmental impacts found are not severe and can be mitigated with simple engineering or process controls
- Chronic human health risks should be more fully examined.
    - "Low hazard" does not mean "no hazard"; an informed workforce is able to make continued assessments of their work environment and changes in processes.
- Results can guide system- and site-specific risk assessments
- Learning from literature basis for aquaculture operations and Harmful Algae Blooms (HABs) is valuable
- Project is completed (2010-2012 timeline).

5-Future Work and Recommendations

- Industry-wide standard operating procedures can identify and mitigate potential risks and would help individual cultivators and researchers in industry and academia.
- Standard practices and a chemical knowledge base to mitigate environmental impacts from emissions or discharges can assist operators
- Development of a central “hazard/risk knowledge base” for cultivators is recommended
- Develop better understanding of chronic exposures to cultivation workers, and the impacts of “scale-up” on water and air emissions.
- Perform a regulatory review of health and environmental regulations that may affect cultivation and other processes.

Response to Peer-Review Comments

- **Need for literature review**
  - We performed a literature review to determine the extent of information available regarding human health or environmental risks associated with algal biomass cultivation for biofuels.
  - Very few publications addressed this specific issue, some related publications were found (some not peer reviewed).
  - A strong literature base exists for aquaculture, harmful algae blooms, and metals sorption to algae. Some of this base is relevant to risk evaluation.
- **Risk Assessment as proposed too complex for cultivators (metals analyses)**
  - Used readily available metals analyses to assess scenarios
  - Speciation not needed if total concentrations remain low for specific toxics (Cr, As, Se), but will be regulation-dependent at specific sites
  - Potential risks, if any, appear to be controllable via simple engineering measures, standard operating procedures, and discharge monitoring awareness.

Presentations and Publications

- **Presentations**
  - Resolving complex microbial interactions for sustainable production of algae. The 34th Symposium on Biotechnology for Fuels and Chemicals (April 30 – May 3, 2012) in New Orleans, LA USA.
  - Cytotoxicity induction in algal strains used in biofuel production. The 2012 Algal Biomass Summit (Sept 24-24, 2012) in Denver, CO USA.
- **Publications**
  - Functional interactions explored between bacteria and chlorophytes. Bagwell et al., In Review.
  - Evaluation of VOC compounds released by *Nannochloropsis* spp. Zimba et al., In Review.
  - Experimental production of bioactive metabolites by *Chlorella* sp. Moeller and Bagwell. In Preparation.
  - Recycling of metals in F/2 brackish water media during microalgae cultivation for algal biofuel production. Sullivan et al. In Review.