Integrated Processes for Converting CO_2 and Organic Residues to Lipid-based Biofuels

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Bioprocessing and Bioproduct Engineering Laboratory - Program Goal

- Advance Industrial Biosystems Engineering sciences and technologies for producing biofuels and biochemicals
- Contribute to the establishment of a bioenergy industry in Washington State and the nation;
- Support the creation and growth of various companies along the value chains of feedstock production, conversion, and product distribution;
- Contribute to the development of a world bioeconomy.

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Bioprocessing and Bioproduct Engineering Laboratory – Competence and Facts

- Core competences
  - Industrial Biosystems Engineering Approach and multi-scale mathematical modeling;
  - Cell factory design and optimization via pathway modification and environment control;
  - High rate bioprocessing through microbial immobilization and effective mass transfer.
- Numbers and facts
  - 139 refereed publications, 12 book chapters;11 patent applications; 170 conference presentations; Over $10M grants and contracts
  - Currently 33 staff including 20 graduate students
  - A large number of industrial and agency partners
  - A broad range of research collaborations

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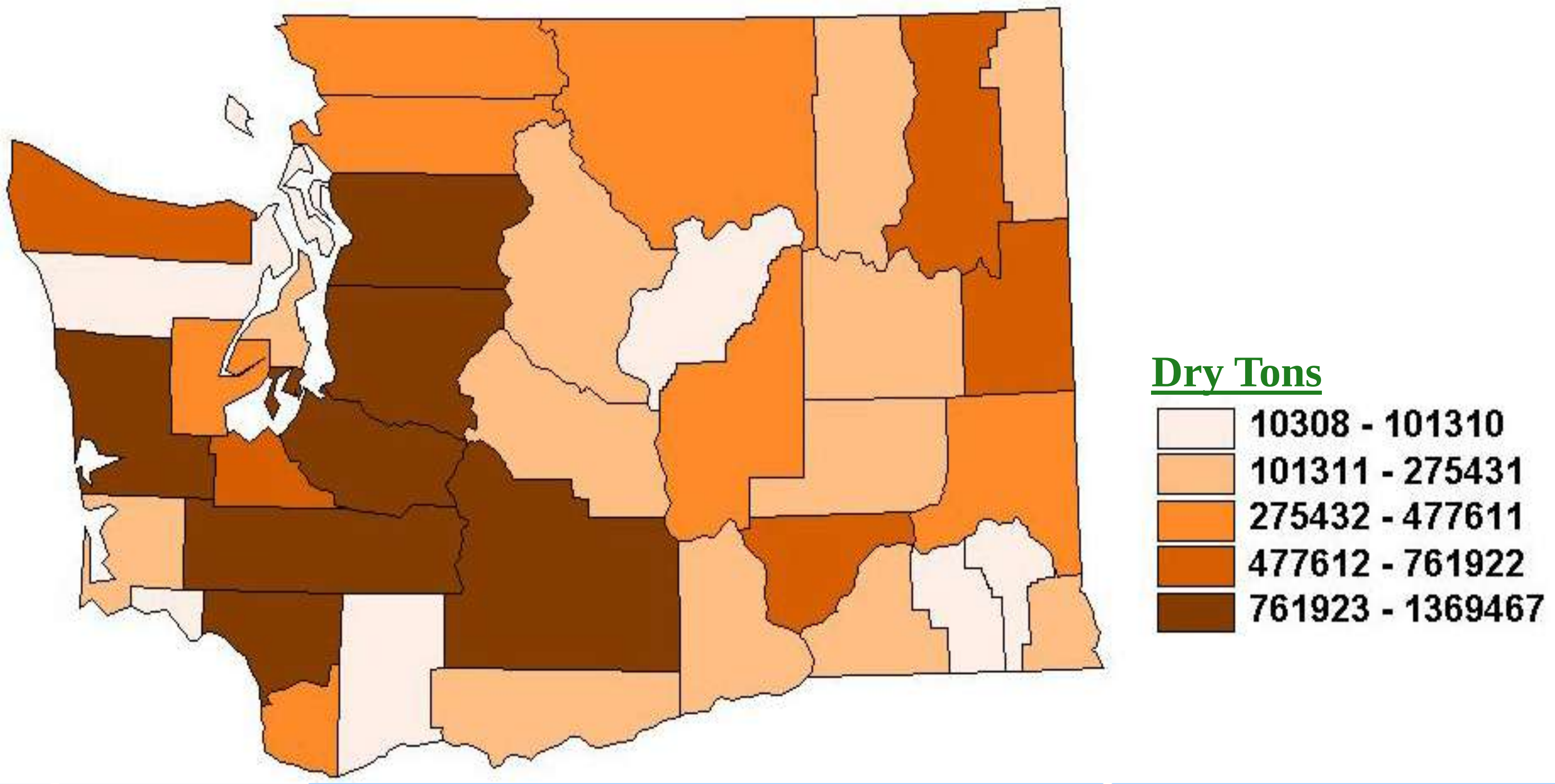
Washington State's great potential as a leader in the upcoming bioeconomy - Biomass by County



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Algae for a Green Washington

With our strong agricultural base and available resources, Washington has the potential to become a major player in algae biofuel

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Vision: Biorefining Biomass to Biofuels

Feedstocks → **Conversion Processes** → **Bio-Products**

Algae
Crop residues
Animal Wastes
Municipal Solid Waste
Food Processing waste
Forest by-products

Biological Pretreatment and enzymatic hydrolysis
Biological conersion
Hydrothermal extraction and conversion
Fermentation
Anaerobic Digestion

Jet fuel
Biodiesel
Biogas for power and transportation fuel
Nutraceuticals
Fine chemicals
Fertilizers

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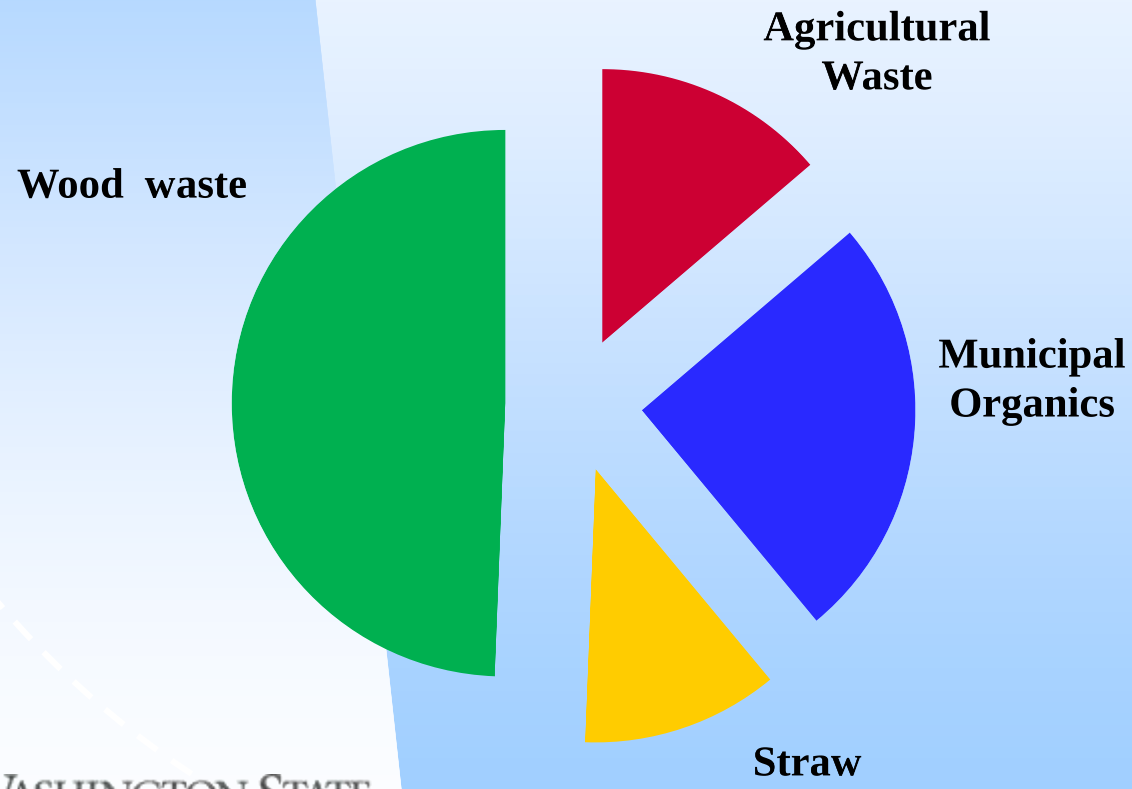
Organic Waste for Heterotrophic Culture
----Feedstock (Washington State as an Example)



Dry Tons per Year

Agricultural Waste
2,255,385

Municipal Organics
4,141,453

Straws
1,909,534

Wood waste
8,103,686

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Modes of lipid production

Photoautotrophic Growth

CO_2 + Water + Light + nutrients → Algae biomass + O_2

Heterotrophic Growth

Organic substrate (glucose, acetate) + O_2 → Microbial biomass + CO_2

Mixotrophic Growth

Be able to grow with either phototrophic or heterotrophic mode

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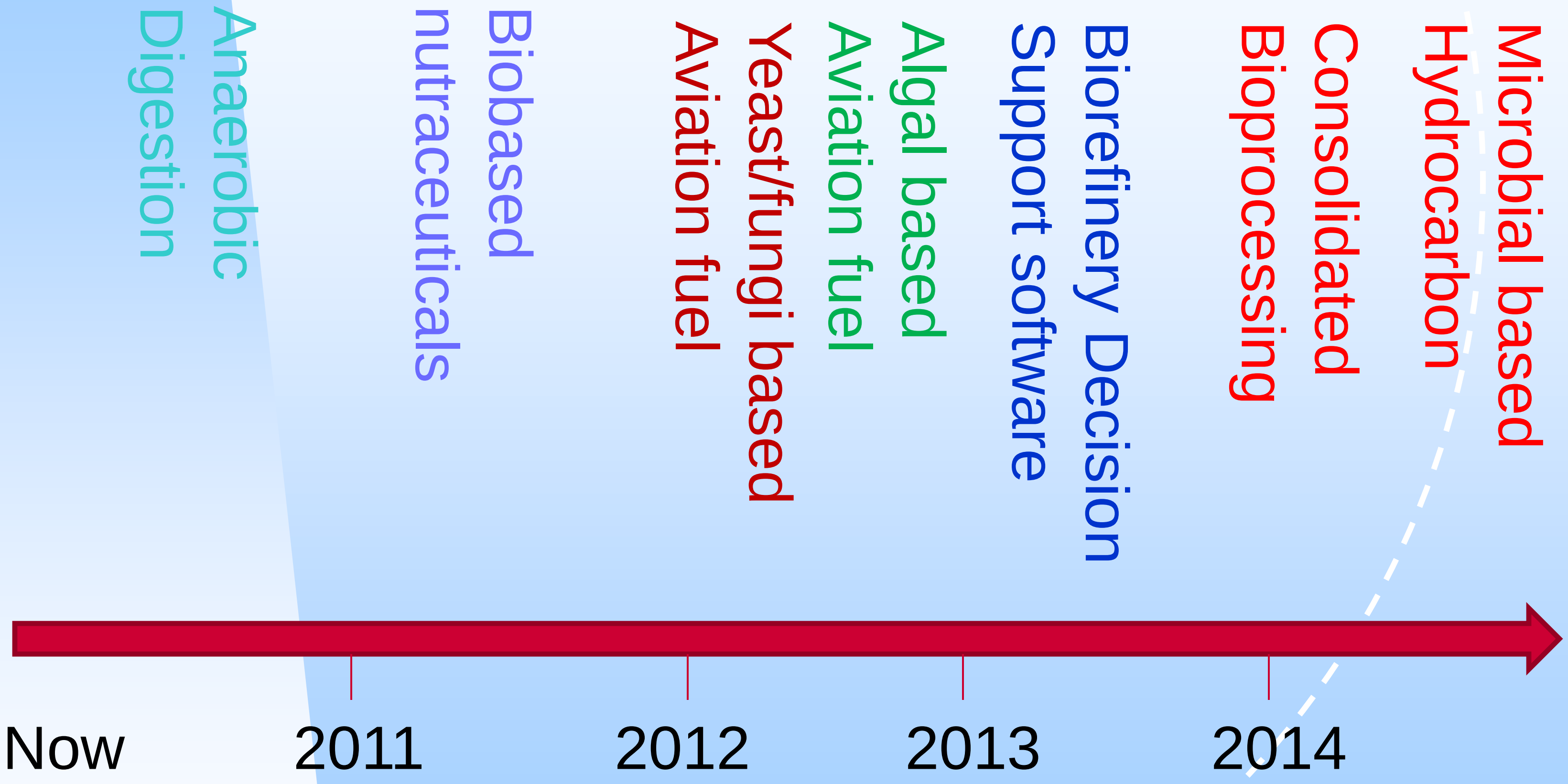
Heterotrophic Micro-Organisms with High Lipid Content

	Lipid Content (% w/w)	Major Fatty Acid Profile				
		14:0	16:0	18:0	18:1	18:2
Yeast						
Cryptococcus curvatus	58		32	15	44	8
Lipomyces starkeyi	63		34	5	51	3
Rhodosporidium toruloidies	66		18	3	66	
Rhodotorula glutinis	72		37	3	47	8
Yarrowia lipolytica	36		11	1	28	51
Fungi						
Entomophthora coronata	43	31	9	2	14	2
Cunninghamella japonica	60		16	14	48	14
Mortirella alpina	50		19	8	28	9
Pythium ultimum	48	7	15	2	20	16
Heterotrophic Algae						
Crpthecodinium cohnii	40	16	16		21	1
Schizochytrium limacinum	50	4	56	1		

Core Technologies under Development

1. Algae culture and conversion systems for aviation fuel
2. Yeast/fungi based aviation fuel from cellulosic biomass
3. Anaerobic digestion systems with nutrient recovery
4. Biobased nutraceuticals and fine chemicals
5. Consolidated bioprocessing and enzyme production for obtaining sugar from lignocellulose
6. Biorefinery decision support software package
7. Microbial hydrocarbon production

BBEL Technology and Commercialization Timeframe



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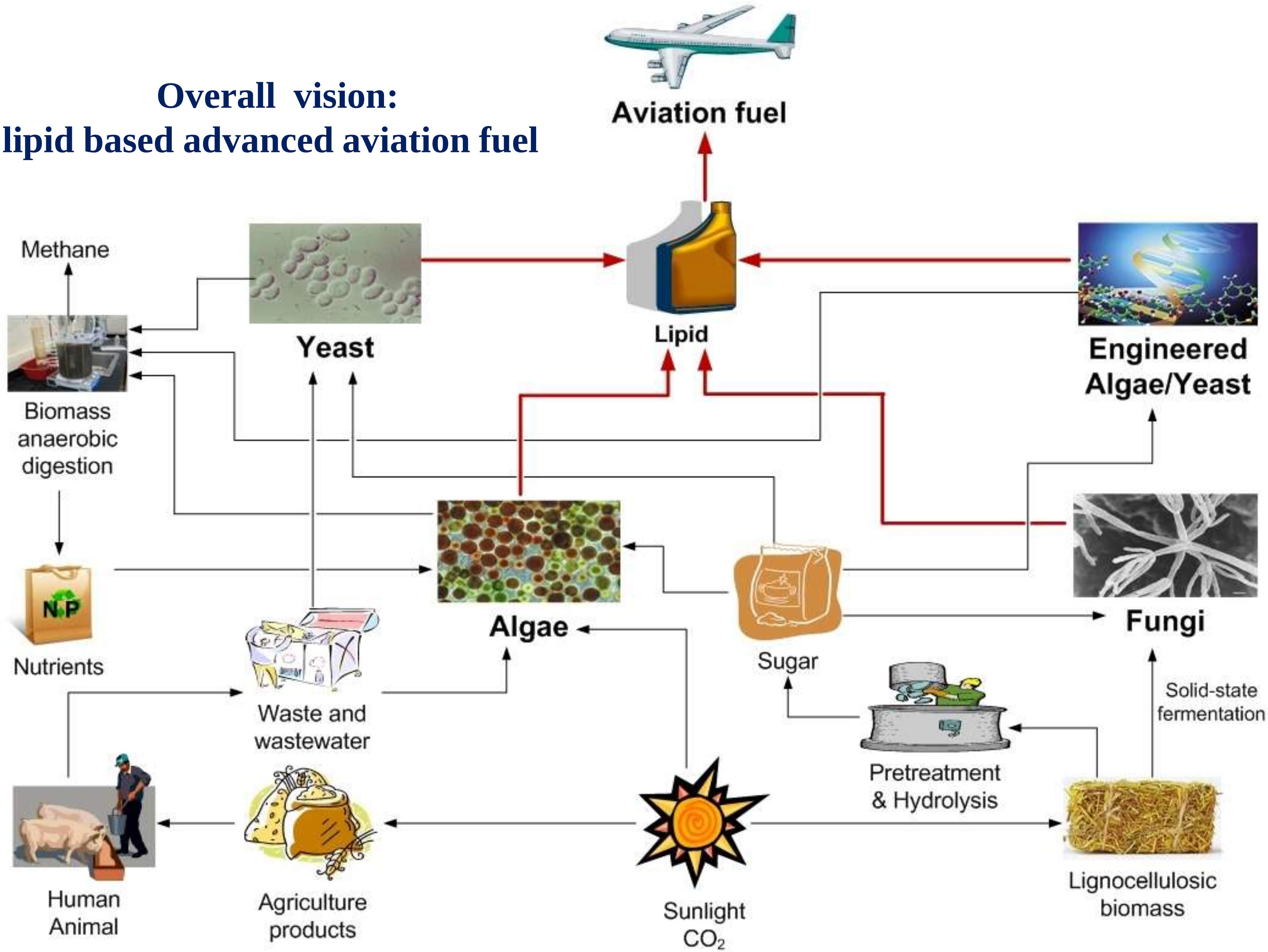
Overall vision:
lipid based advanced aviation fuel



Technologies for Utilizing Existing Biomass such as organic wastes and crop residues

- Producing lipid as biofuel feedstock by growing oleaginous yeast using food wastes;
- Producing lipid as biofuel feedstock by growing oleaginous yeast using pretreatment hydrolysate from straw and woody biomass;
- Producing lipid as biofuel feedstock by growing oleaginous fungi using straw and woody biomass.

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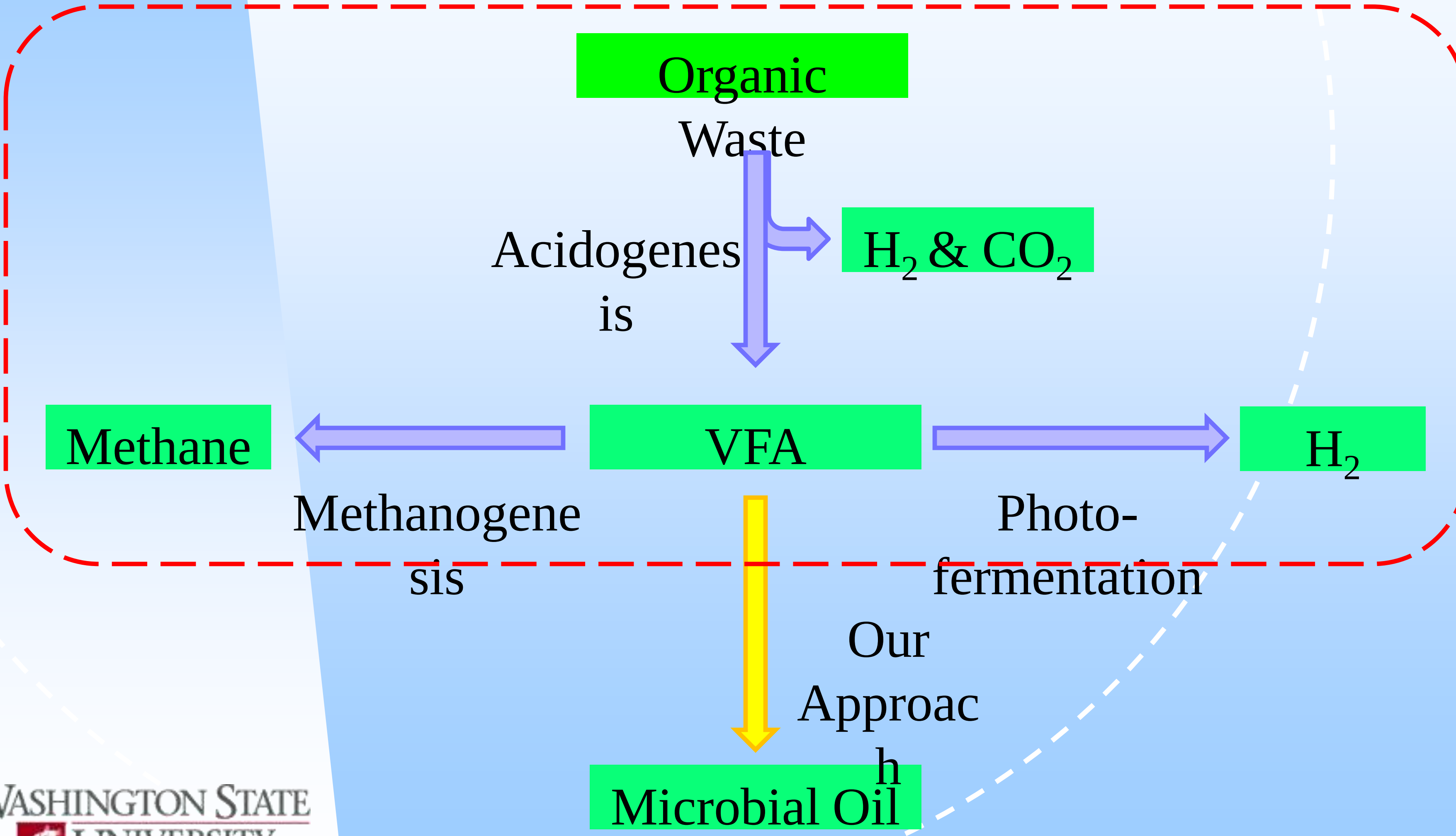
Utilization of Waste Carbon



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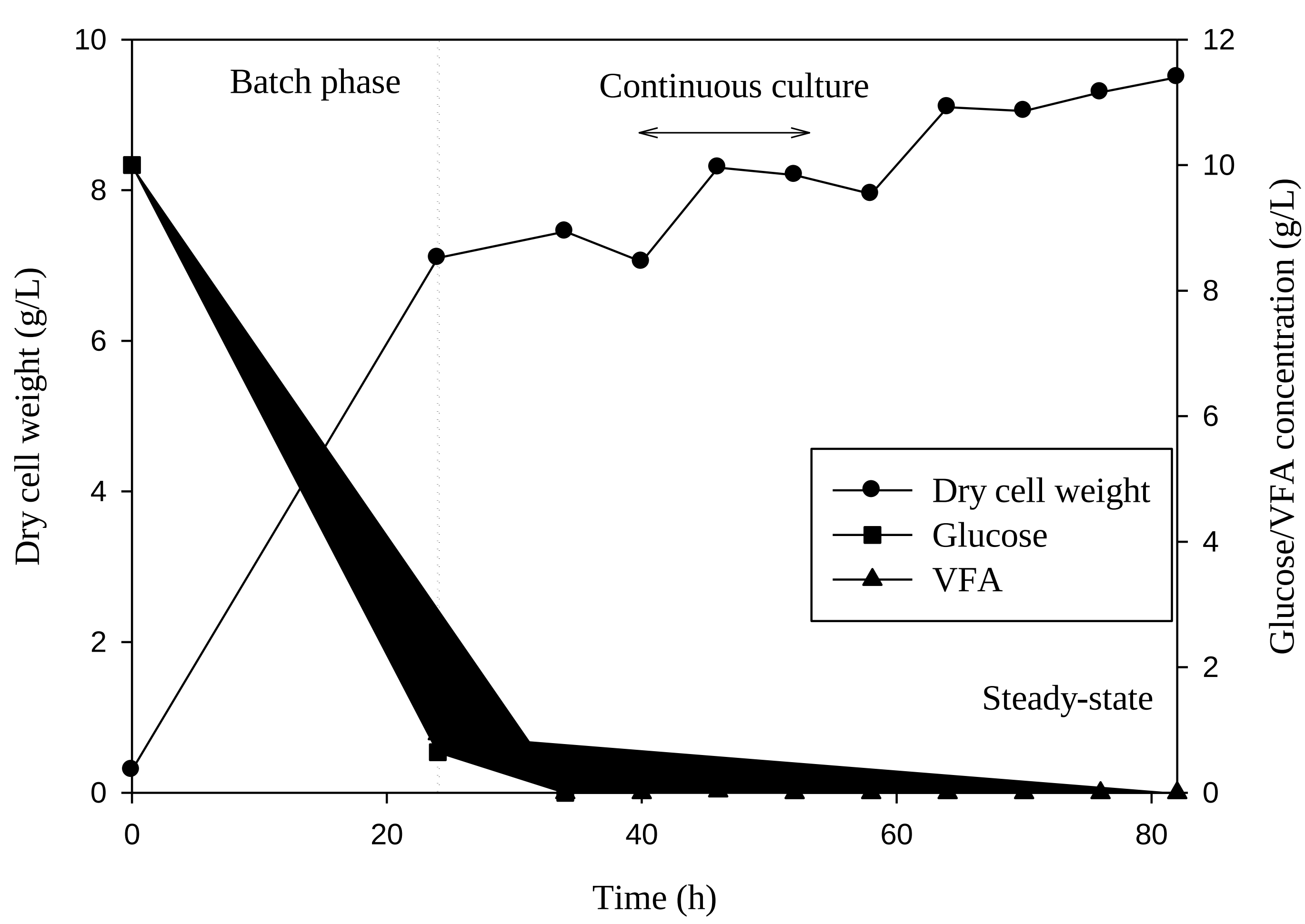
Continuous Culture of Oleaginous Yeast with Food Waste



The Lipid Content and Profile in Produced Yeast Biomass

Fatty Acids	Ratio to Total Fatty Acids (TFA)
Myristic (C 14:0)	0.7 ± 0.0%
Palmitic (C16:0)	24.2 ± 0.9%
Stearic (C18:0)	18.7 ± 0.2%
Oleic (C18:1)	46.9 ± 0.8%
Linoleic (C18:2)	4.1 ± 0.2%
Arachidic (C20:0)	0.7 ± 0.0%
Behenic (C22:0)	< 0.1%
TFA/Biomass	56.6 ± 1.8%

Lipid Production and COD Removal from Food Waste

	Waste stream	Effluent from Yeast Culture
Biomass productivity (g/L/d)		8.0
Lipid content (%)		15.0
Lipid productivity (g/L/d)		1.2
VFA (g/L)	19.5	< 0.1
COD (g/L)	43.7	5.2
TN (g/L)	2.8	1.3

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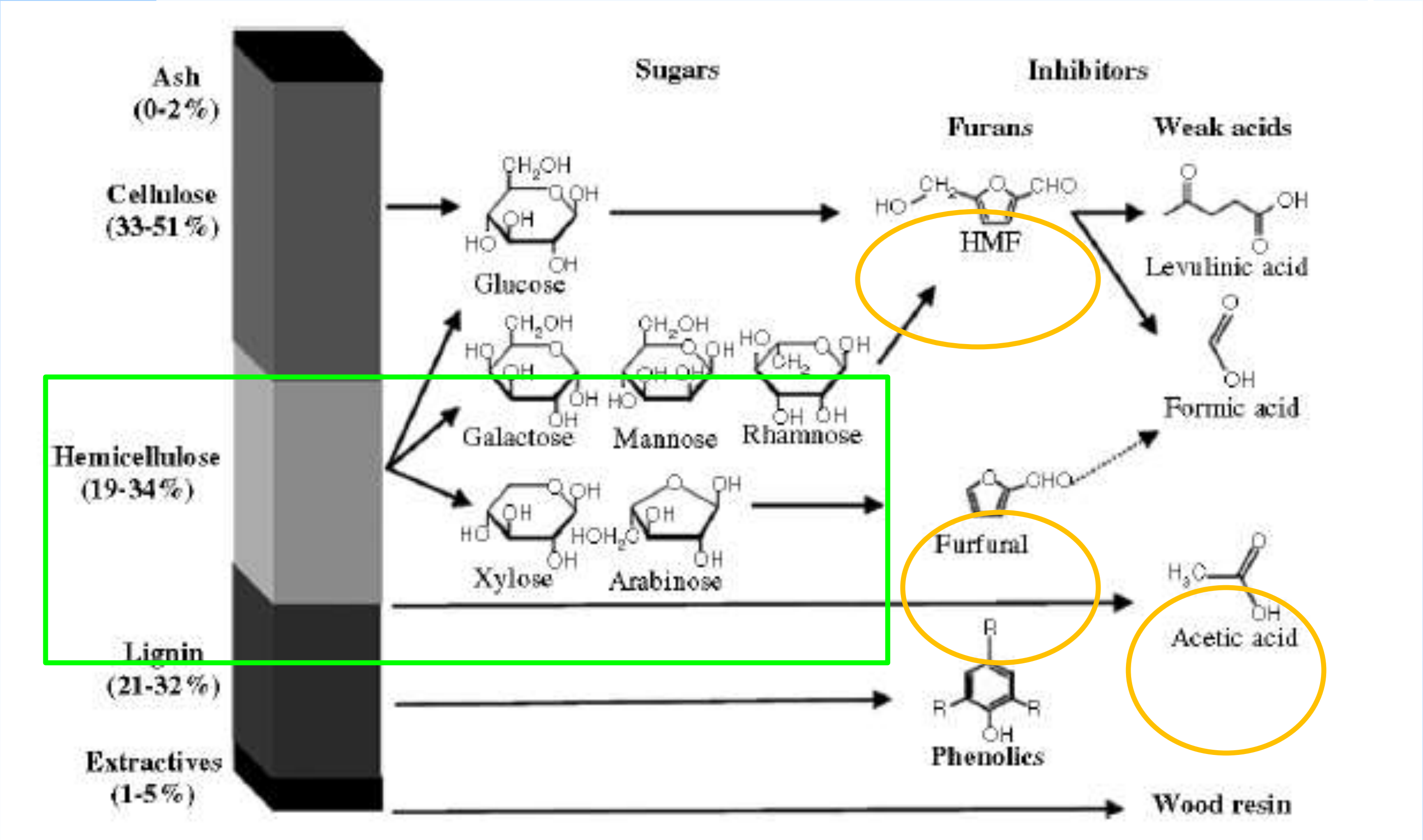
Main Components in Dilute Acid Hydrolysate



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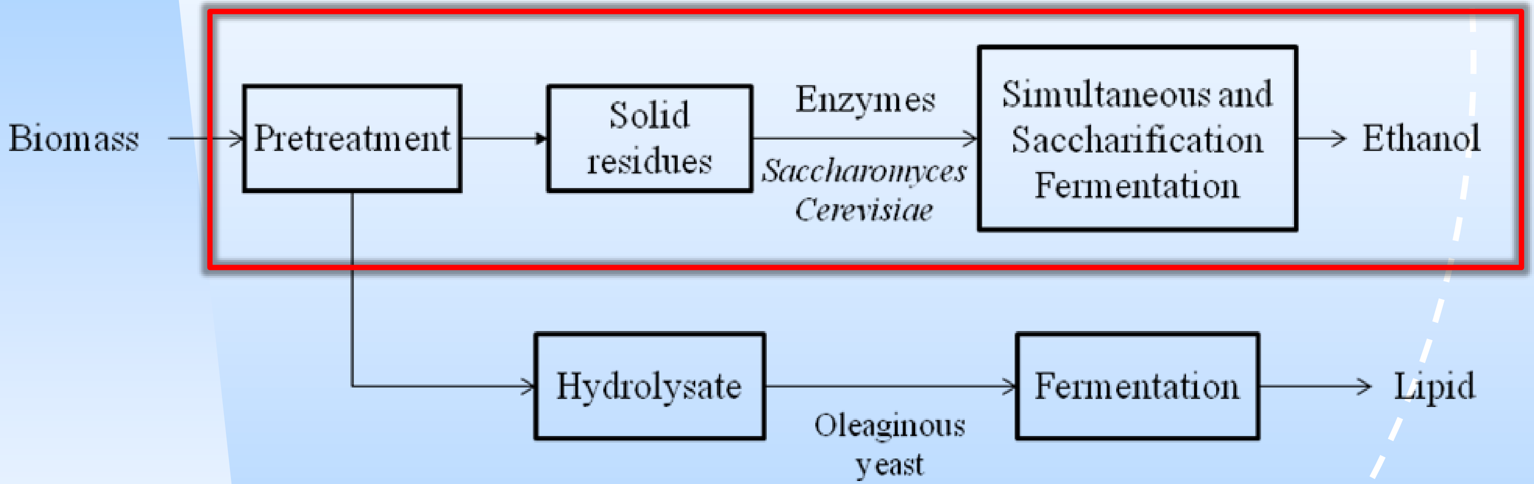
An Alternative Strategy of Converting Biomass to Bioethanol and Lipid



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Effects of Yeast Strains on Biomass and Lipid Production with Hydrolysate

Strain	Biomass (g/L)		Lipid content (%)		Lipid concentration in medium (g/L)	
	NDLH	DLH	NDLH	DLH	NDLH	DLH
Y. lipolytica	7.82 ± 0.76	7.17 ± 0.16	4.60 ± 0.21	4.36 ± 0.30	0.38	0.31
C. curvatus	17.2 ± 0.35	15.6 ± 0.04	33.5 ± 0.14	27.1 ± 0.57	**5.77**	**4.22**
R. glutinis	13.8 ± 0.33	11.8 ± 0.34	25.0 ± 0.57	20.7 ± 0.92	3.46	2.45
R. toruloides	N/D	9.87 ± 0.01	N/D	24.6 ± 0.68	N/A	2.45
L. starkeyi	14.7 ± 0.45	12.7 ± 0.23	31.2 ± 1.27	29.1 ± 0.52	4.57	3.71

- The non-detoxified hydrolysates did not have a significantly negative impact on lipid accumulation of four of the five yeasts.
- Under the concentrations tested, the *Y. lipolytica*, *C. curvatus*, *R. glutinis* and *L. starkeyi* could survive and produce lipids unrestrictedly in the presence of acetic acid, furfural and HMF.
- These results demonstrate that the hydrolysates from different types of feedstocks have different concentrations of inhibitors as well oleaginous yeasts exhibited different tolerances to inhibitors. The strains in our work had a higher tolerance than *T. fermentans* applied.

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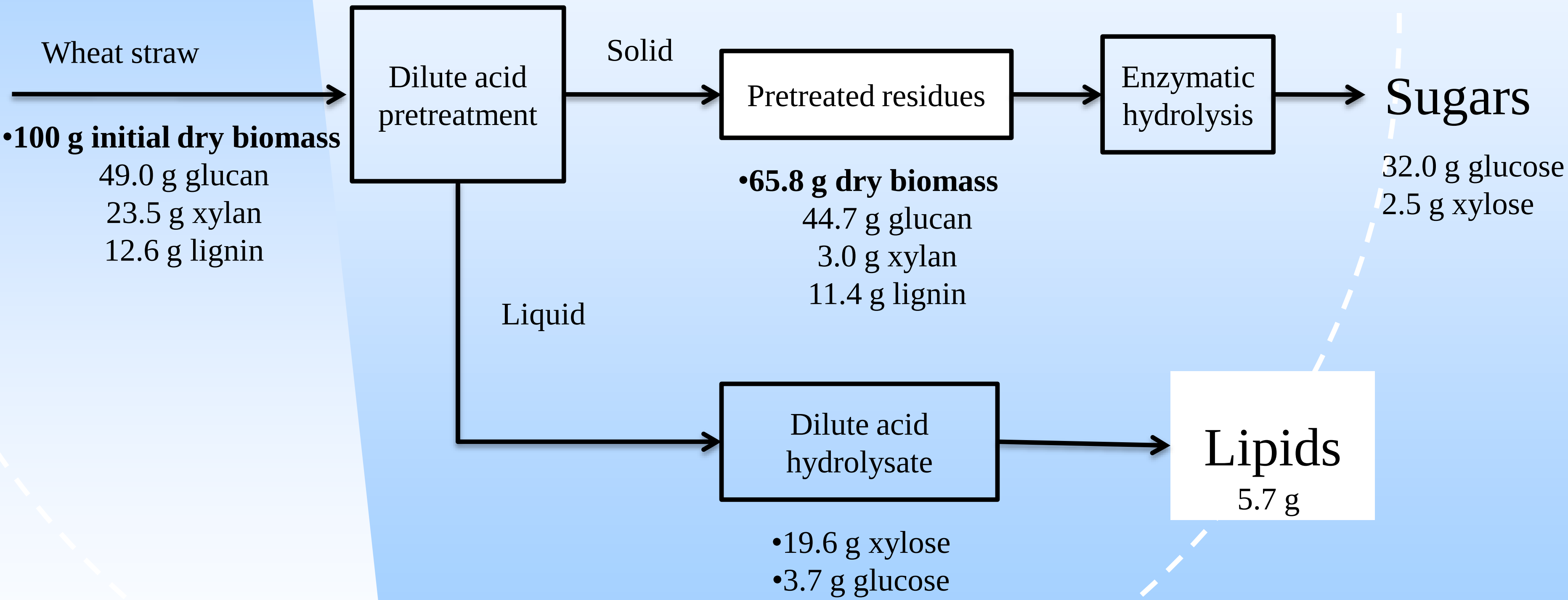
Mass Balance



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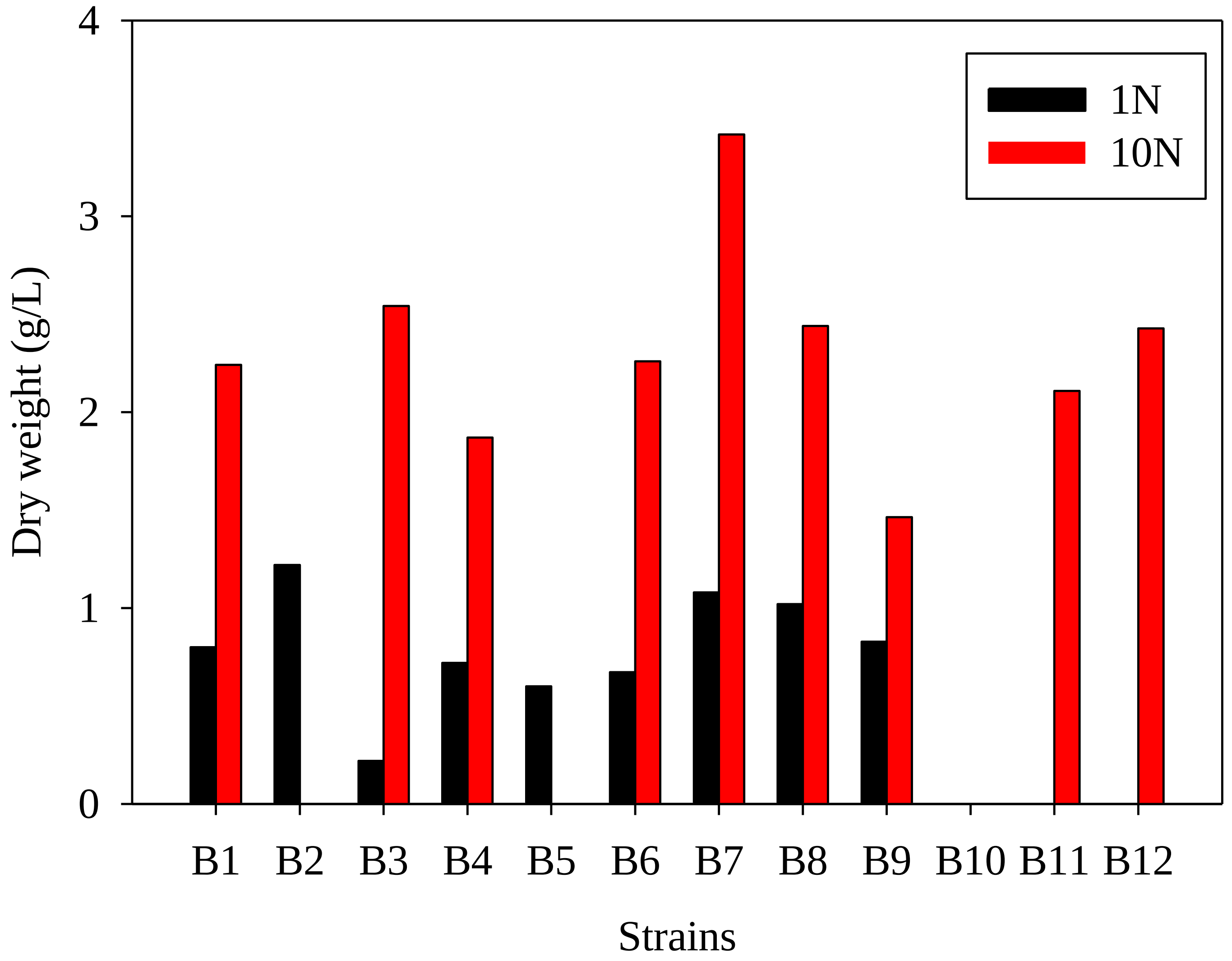
Potential oleaginous fungi

Strain	Number	Abbreviate
Rhizopus oryzae	BBEL01	B1
Neosartorya fischeri	BBEL02	B2
Chaetomium globosum	BBEL03	B3
Aspergillus niger	BBEL04	B4
Mortierella isabellina	BBEL05	B5
Cunninghamella elegans	BBEL06	B6
Mucor circinelloides	BBEL07	B7
Aspergillus terreus	BBEL08	B8
Umbelopsis vinacea	BBEL09	B9
Pythium ultimum	BBEL10	B10
Mortierella alpina	BBEL11	B11
Mucor plumbeus	BBEL12	B12

DW vs temperature on cellulose



Fatty acid profile

		B7	B8	B9	B10	B11	B12
Fatty acid	Structure	% FA	% FA	% FA	% FA	% FA	% FA
Myristic	C14:0	2.07	0.19	0.42	8.79	0.98	4.66
Palmitic	C16:0	21.95	15.97	20.29	22.92	12.83	19.90
Stearic	C18:0	3.23	16.89	3.38	3.91	15.48	9.89
Oleic	C18:1n9	45.63	41.39	49.92	17.08	14.75	27.29
Linoleic	C18:2n6	8.70	22.57	15.76	16.96	7.91	9.22
ϒ- Linolenic	C18:3n6	9.00	0.02	5.27	0.95	5.97	12.39
Linolenic	C18:3n3	0.00	0.35	0.02	0.29	0.00	0.04
EPA	C20:5n3	0.00	0.00	0.00	8.22	0.06	0.00
Saturated		28.07	34.80	25.56	44.11	62.95	44.25
Total Fat (w/w)		39.39	38.60	52.23	6.59	37.66	19.61

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Growing algae biomass using wastewater and CO_2 to increase biofuel production potential in Washington State

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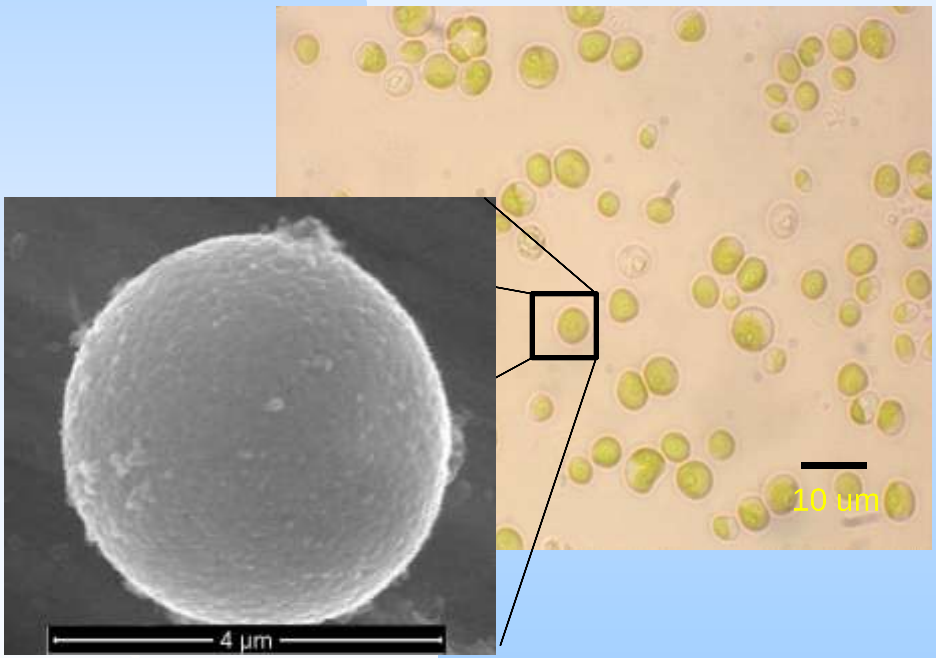
BBEL Algal Fuel Platforms

Chlorella sorokiniana



Cyanobacteria mix

http://www-cyanosite.bio.purdue.edu/images/lgimages/mystery9.jpg

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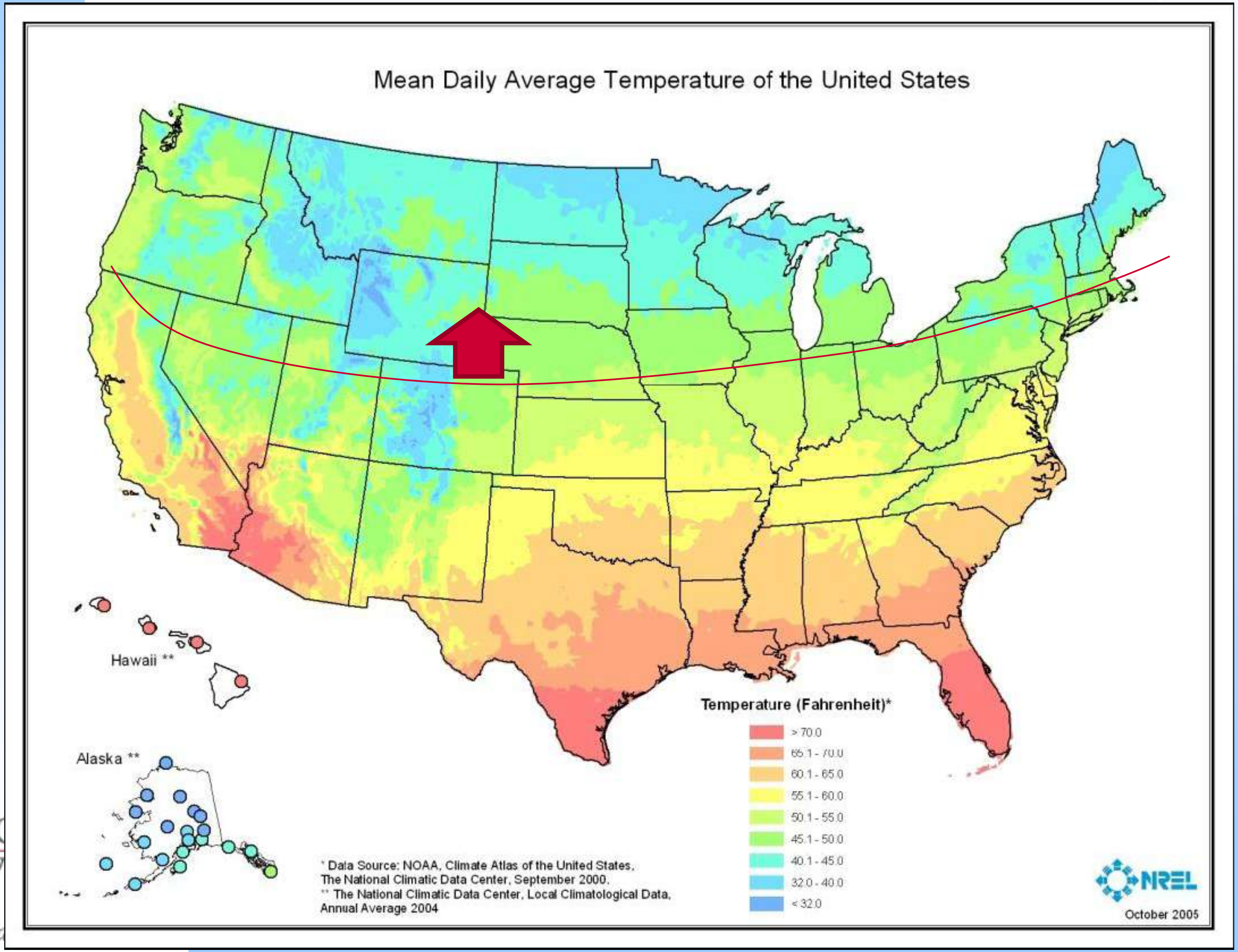
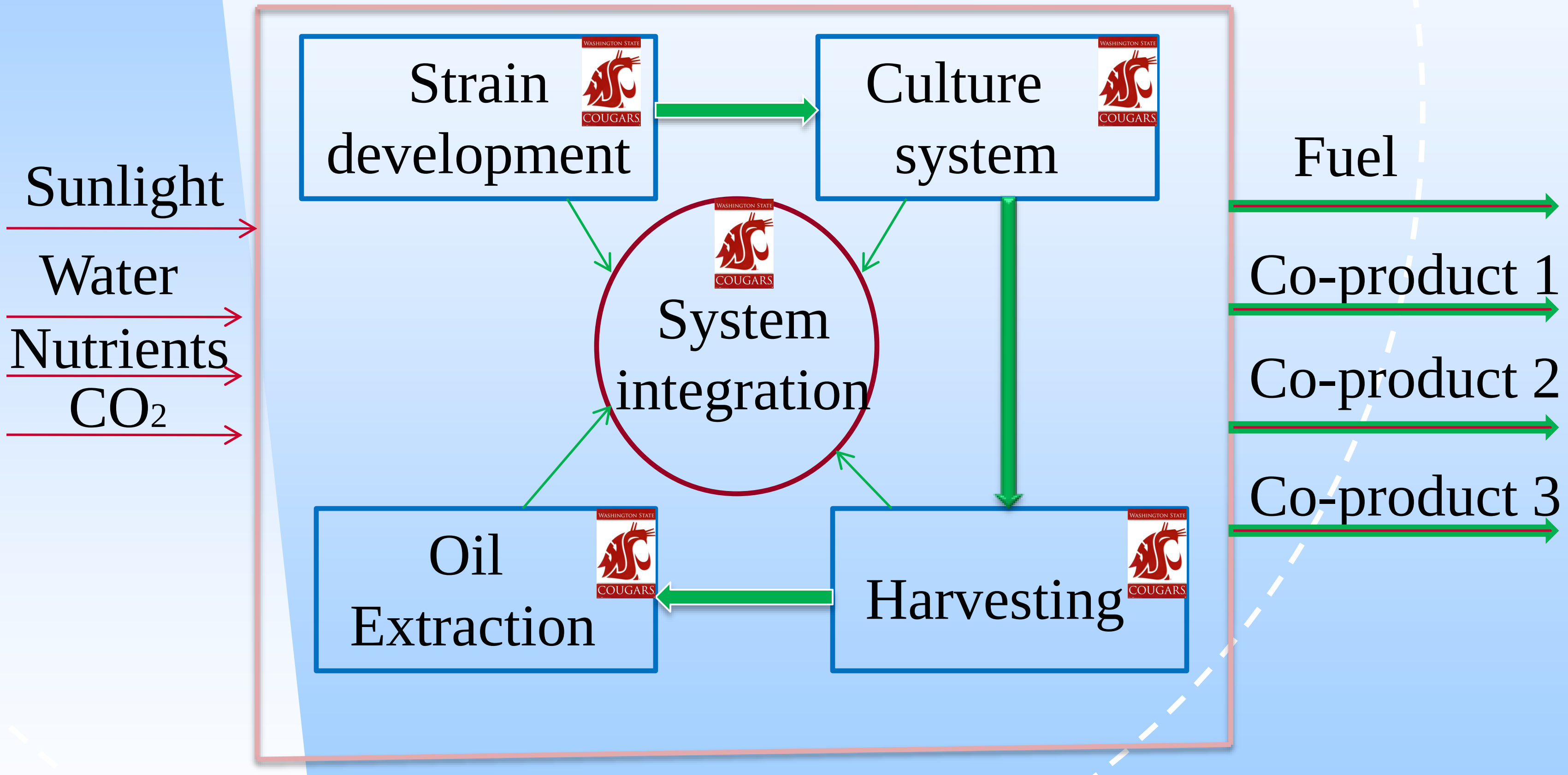
WSU: Improves feasibility of algal fuel production in the Northern states



Algal Fuel Challenges and WSU Technologies



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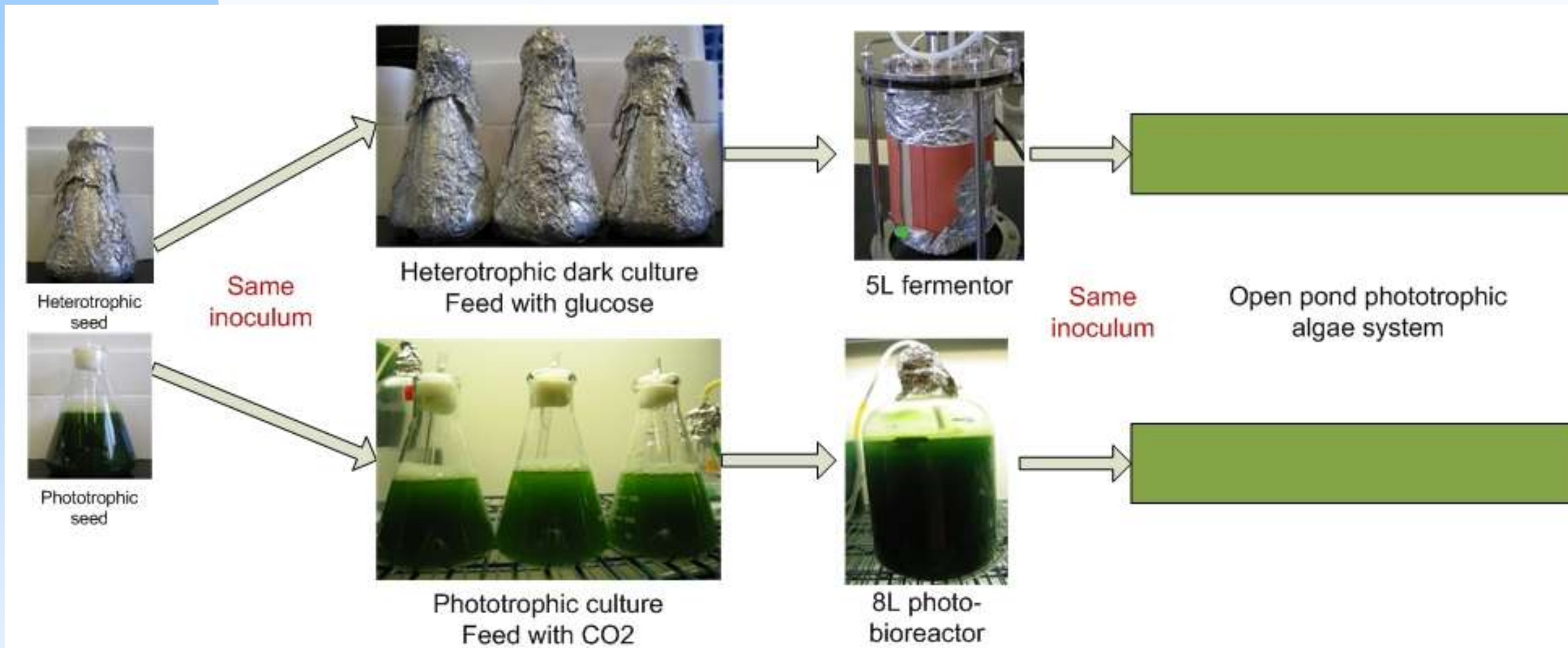
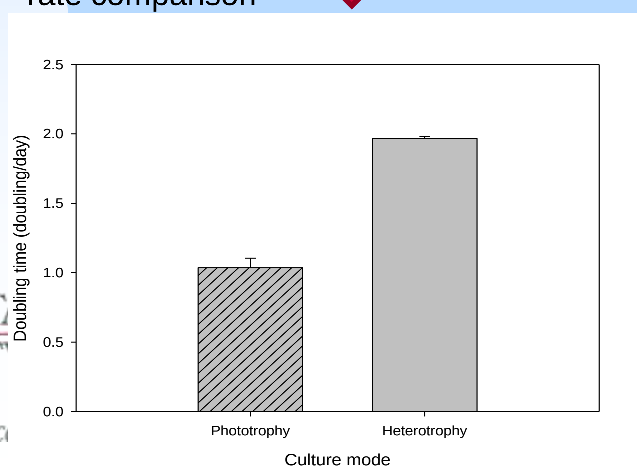
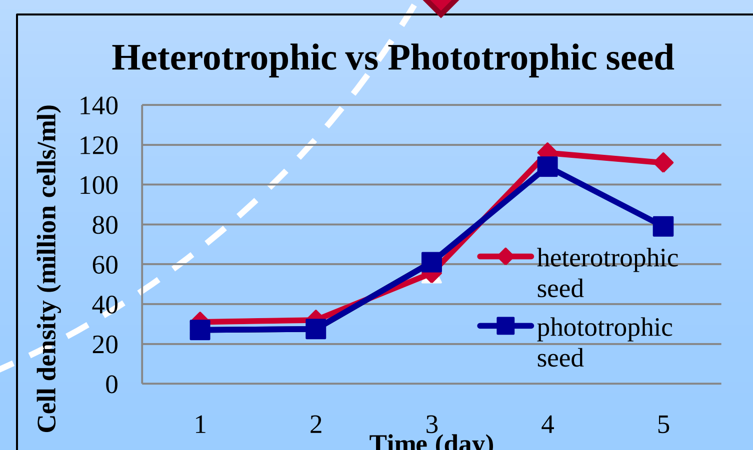
Separated mixotrophic algae seed culture



Algae seed growth rate comparison





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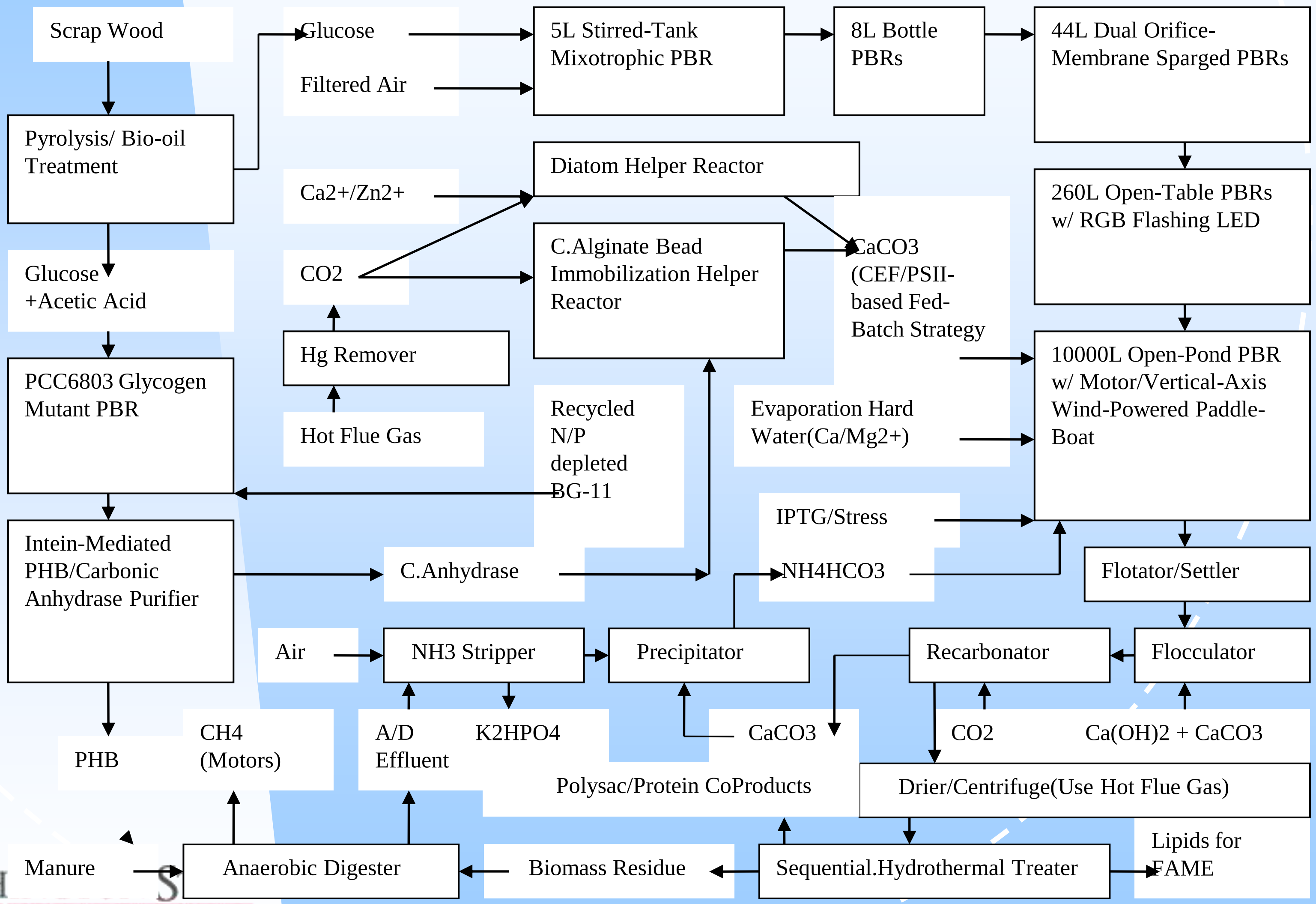
Integrated Cyanobacteria Culture System



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- WSU Agricultural Research Center
- US Department of Agriculture
- Boeing Company
- Bioalgene

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