NREL
Transforming ENERGY

Pretreatment of Algae for the Production of Fuels and Chemical Coproducts

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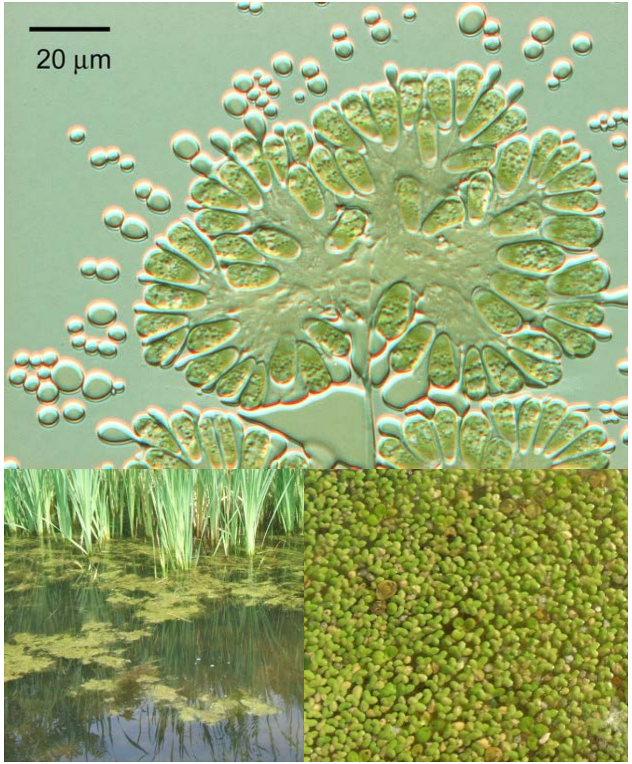
Advantages of Algae Biomass

- **Limitations on terrestrial biomass**
  - Terrestrial biomass can replace ~30% of petroleum consumption[1]
- **Aquatic biomass can help**
  - Can have faster growth rates
  - Can have high content of components desirable for specific applications
    - Oil
    - Carbohydrates
    - Protein
    - Inorganic nutrients



[1]M. Downing et al., *U.S. Billion-Ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry*, **2011**

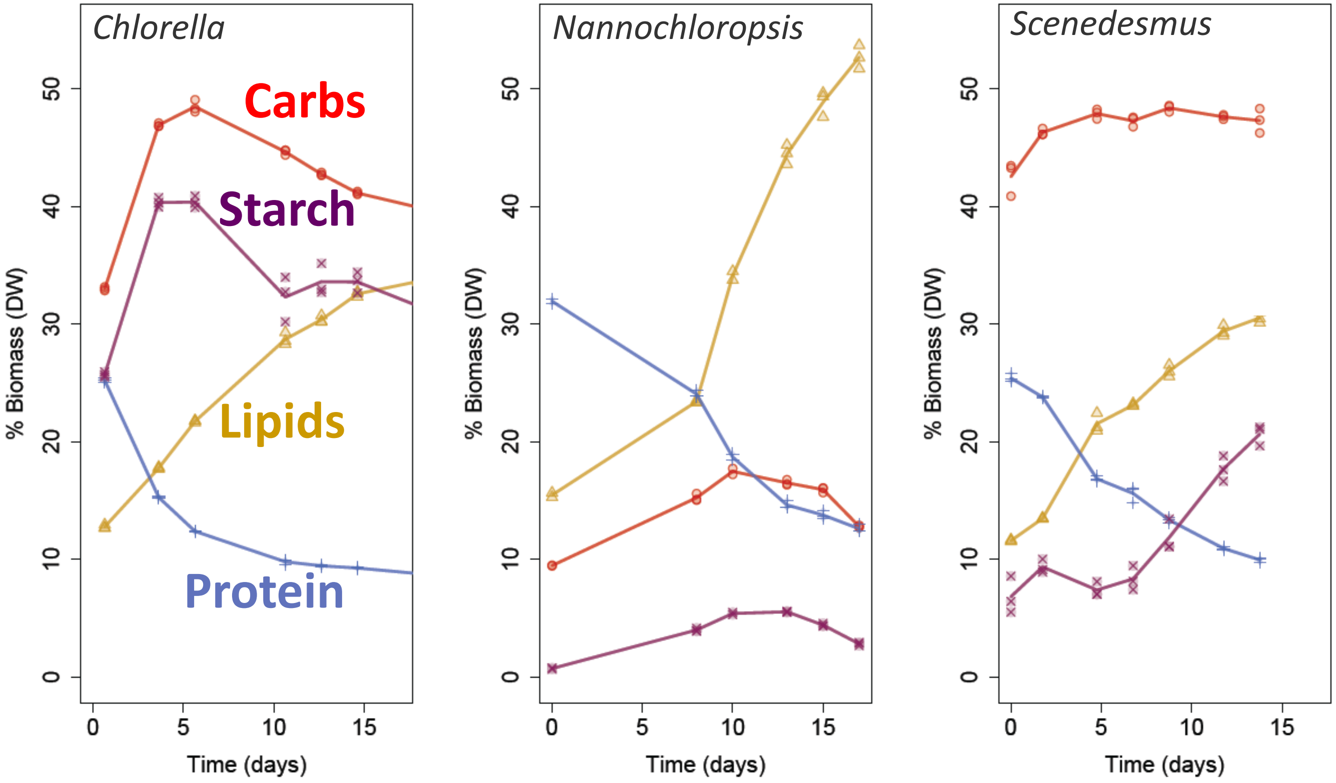
Algae Primary Composition

Composition depends on species and growth phase

Early: high protein

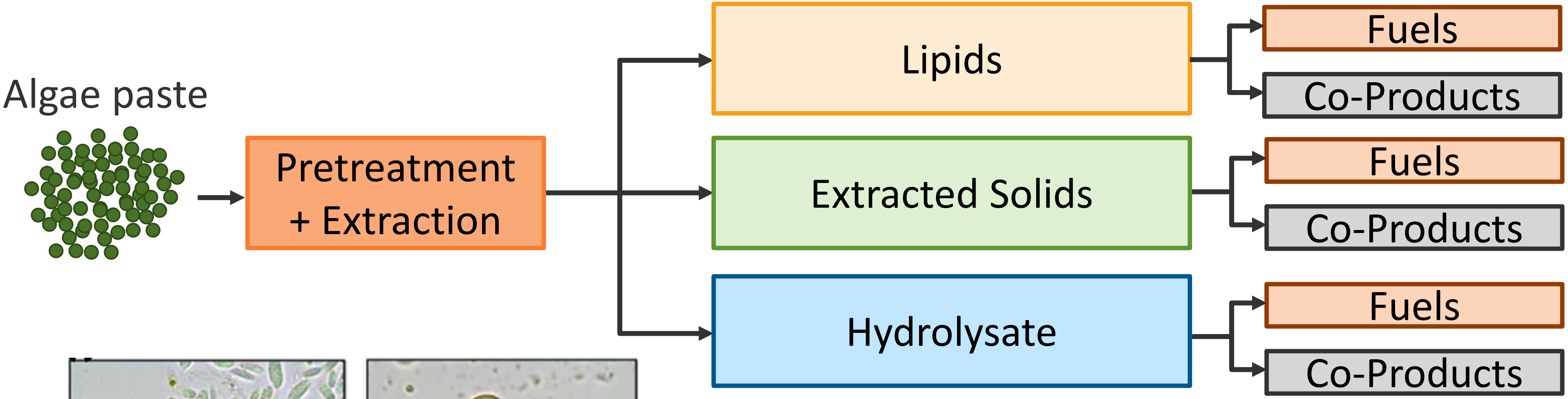
Mid: high carb

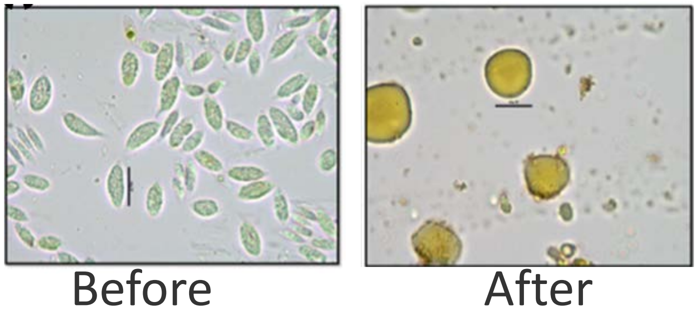
Late: high lipid



Combined Algal Processing (CAP)

- Staged processing: pretreatment, fractionation, tailored upgrading





How does pretreatment effectiveness vary across algae composition?

Algae Sample Selection

Type	Code	Harvest	Form
Nannochloropsis	*Nanno*	Early	Flake
Wastewater Treatment #1	WT1	Early	Treated Flake
Wastewater Treatment #2	WT2	Early	Paste
Scenedesmus IITRIND2	IIT	Early	Paste
Tetraselmis	*Tetra*	Early	Paste
Scenedesmus obliquus UTEX393	393	Early	Paste
Monoraphidium minutum 26BAM	Mono	Early	Paste
Picochlorum celerei	*Pico*	Early	Paste
Scenedesmus acutus 0401	0401	Mid	Paste

Algae Composition

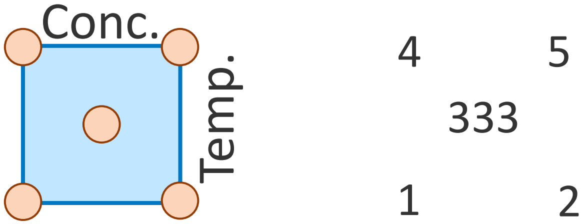
Type	Ash	Protein	Carbs	FAME
Nannochloropsis	25.6	32.2	6.9	8.5
Wastewater Treatment #1	**38.4**	**23.8**	**5.0**	**2.1**
Wastewater Treatment #2	12.3	43.8	10.0	7.0
Scenedesmus IITRIND2	8.3	43.9	6.0	8.8
Tetraselmis	19.5	35.8	6.8	7.3
Scenedesmus obliquus UTEX393	7.3	**47.5**	10.1	7.1
Monoraphidium minutum 26BAM	**6.7**	41.1	**11.1**	9.3
Picochlorum celerei	17.5	42.7	5.3	**9.6**
Scenedesmus acutus 0401	2.2	11.3	46.8	24.6

Pretreatment Selection

Pretreatment	Agent	Motivation
"Normal" Dilute Acid	H_2SO_4	Literature baseline
"Twitchell" Dilute Acid	H_2SO_4	Reduce severity, integration
Lewis Acid	$FeCl_3$	Integration
Flash Hydrolysis	N/A	Literature baseline
Enzymatic	Enzymes	Reduce severity, integration
Dilute alkali	NaOH	Integration

- **Key motivations:** no pressure vessel, integrate with upstream/downstream operations

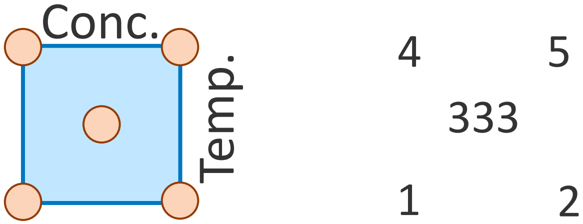
Pretreatment Conditions



Pretreatment	Agent	Time	Temp	Loading
“Normal” Dilute Acid	H_2SO_4	15 min	135 - 175 °C	0.25-0.75 wt%
“Twitchell” Dilute Acid	H_2SO_4	8-16 h	80 - 120 °C	0.75 wt%
Lewis Acid	$FeCl_3$	15 min	135 - 175 °C	0.25-0.75 wt%
Flash Hydrolysis	N/A	9 sec	180-240 °C	1000-1500 psi
Enzymatic	Enzymes	120 h	30-50 °C	2, 5, 15 mg
Dilute alkali	NaOH	15 min	135 - 175 °C	0.25-0.75 wt%

- Algae loading: 7.5% solids, 4 mL scale
- Two-factor, 3 level design with center point triplicates

Pretreatment Approach and Metrics



H_2SO_4	Temp	*Nanno*	WT1	WT2	*IIT*	*Tetra*	*393*	*Mono*	*Pico*	*0401*
0. 25%	135°C	12.4	13.7	20.4	40.2	34.1	26.6	25.5	19.6	41.7
0. 25%	175°C	30.1	27.5	38.1	46.5	48.2	43.8	37.9	36.0	43.8
0. 50%	155°C	28.1	18.2	36.2	45.9	46.0	44.4	40.9	34.4	42.4
0. 50%	155°C	28.4	18.8	34.6	47.0	41.3	42.0	40.4	34.1	41.5
0. 50%	155°C	26.7	19.6	37.9	47.0	41.8	41.7	39.4	36.7	41.9
0. 75%	135°C	23.2	14.9	34.4	40.3	38.7	36.9	39.2	31.1	42.2
0. 75%	175°C	36.7	27.7	45.0	51.1	51.1	49.1	43.8	45.7	45.9

- Three metrics: TOC yield, TN yield into hydrolysate, FAME yield
- Color-coded: Green = 100%. Yellow = 50%, Red = 0% Yield

TOC Yield

- Similar performance across many pretreatments and species
- Some outliers
  - Solid samples lower
  - EH lower
  - FH competitive, but has operational issues
  - *Monoraphidium* responds uniquely well to alkaline

	Nanno	WT1	WT2	*IIT*	*Tetra*	393	*Mono*	*Pico*	0401
H_2SO_4	12.4	13.7	20.4	40.2	34.1	26.6	25.5	19.6	41.7
	30.1	27.5	38.1	46.5	48.2	43.8	37.9	36	43.8
	28.1	18.2	36.2	45.9	46	44.4	40.9	34.4	42.4
	28.4	18.8	34.6	47	41.3	42	40.4	34.1	41.5
	26.7	19.6	37.9	47	41.8	41.7	39.4	36.7	41.9
	23.2	14.9	34.4	40.3	38.7	36.9	39.2	31.1	42.2
	36.7	27.7	45	51.1	51.1	49.1	43.8	45.7	45.9
$FeCl_3$	15.3	12.6	20.7	41.4	29.7	31.2	38.2	23	43.2
	28.5	26.4	39	49.9	42.1	45.5	43.6	37.9	42.7
	29.9	22	38	46.4	34.7	52.1	51.4	38.6	44.3
	32.7	21.7	39.3	46.8	36.5	52.4	49.9	37.9	45.2
	35.2	22.1	39.5	46.3	35.1	52.8	52.8	40.4	45.4
	26.7	18.2	34.2	43.2	34.6	40.3	44.6	34.7	42.9
	41.5	30.6	44.4	51.7	42.9	50.7	50.4	47.5	44.3
NaOH	17.1	18	19.3	46.3	33.6	20.2	29.8	16.5	11.7
	32.4	29.1	34.4	45.6	40.9	39.3	55.6	27	15.3
	45.1	28.4	30.5	44.7	44.2	32	57.9	26.8	15.6
	47	29.6	29.4	44.7	43.7	31.6	63.5	27.1	15.8
	36.6	28.1	23.6	47.5	36.9	30.5	60.6	21.8	15.2
	37.5	28.7	34	42.6	32.7	35.4	63.2	30.5	21.4
	45.1	28.2	47.6	53.8	40.7	51.4	70.5	44.1	29.3
Tw	6.1	5.5	15.4	41.3	32.1	24.9	25.4	16.7	27.3
	16.7	7.3	32.4	49.6	40.9	40.2	40.2	36.8	42.9
	11.6	6.7	24	45	34.5	35.4	31.8	26.9	45.3
	11.2	5.9	23.8	44.4	36	33.7	31.2	24.8	44.1
	12.1	6.2	24.9	44.3	34.3	34.8	32.9	24	45.4
	6.5	5.7	22.4	40.7	34.2	25.4	27	17.8	31.8
	19.4	9.3	39.3	48.6	41.3	41.9	44.2	40.9	48.3
EH	25	18.4	11.9	36.6	28.8	26.6	29.8	11.2	12.4
	38.3	11.3	23.5	47.4	30.5	45.5	49.6	17.3	19.2
	27	12.2	15.2	37	27.3	28.9	35.3	14.1	16.8
	33	11.4	16.3	41	26.2	36.6	36.2	22.6	17.3
	24.9	12.2	14.3	36.9	30.6	38.8	48.3	12.2	18.4
	21.8	7.2	8.6	37.5	27.6	28.5	32.8	5.9	11.6
	32.6	11	23.9	49.1	28.9	51.6	48.2	12.1	23.8
	13.2	10.1	11.4	36.1	27.2	19.2	22.9	15.6	11.4
FH			24.0	50.2	47.9	18.6	?	29.0	28.6
			36.6	52.5	44.4	45.1	?		39.0
			22.7	49.3	43.4	30.7	?	34.0	26.8
			36.0	58.8	49.5	49.2	50.7		44.3

TN Yield

- Generally similar features as TOC, but more variability
  - Solid samples lower
  - EH lower
  - Twitchell best at high temp

	Nanno	WT1	WT2	*IIT*	*Tetra*	393	*Mono*	*Pico*	0401
H_2SO_4	12.3	21.6	28.0	39.5	28.2	24.4	37.1	25.7	45.0
	38.4	44.8	47.6	45.0	52.1	43.3	58.7	50.7	46.6
	33.8	28.9	46.2	47.2	47.0	41.7	63.0	48.0	47.7
	36.0	28.8	47.1	50.7	40.2	39.6	63.0	51.6	47.1
	33.7	32.9	45.8	52.2	37.5	37.2	62.0	50.9	56.0
	25.8	21.0	44.8	42.2	42.2	36.0	59.8	44.2	56.0
	45.5	44.0	59.3	56.4	62.6	54.3	76.2	65.8	57.6
$FeCl_3$	18.5	19.8	24.5	37.6	33.5	32.3	59.5	35.3	41.9
	36.9	43.0	51.6	49.9	40.0	48.5	69.1	46.7	52.9
	37.4	34.2	50.2	52.1	34.9	53.9	78.5	56.9	53.9
	33.7	35.1	52.4	53.1	30.9	53.6	76.6	59.8	55.0
	39.7	36.5	50.4	51.0	31.2	51.5	74.7	56.4	55.5
	28.7	20.9	40.5	39.8	32.2	38.0	64.5	36.1	48.2
	50.1	44.7	56.4	58.1	39.7	80.7	77.9	70.3	57.6
NaOH	12.6	23.4	19.6	41.0	34.0	20.7	33.2	21.5	10.0
	28.7	37.1	37.4	44.2	34.4	42.6	70.7	37.9	23.6
	36.2	35.5	19.6	41.1	31.5	28.3	69.4	35.4	23.0
	36.2	35.6	28.4	38.9	31.5	20.1	69.7	37.7	22.0
	34.2	34.9	19.8	42.7	34.2	22.4	69.4	32.9	20.4
	34.6	33.0	24.9	46.8	30.0	24.9	67.2	38.6	34.0
	45.5	46.4	47.8	45.4	40.7	51.1	77.7	55.6	42.4
Tw	7.3	7.6	15.6	50.2	36.0	31.2	36.0	23.1	19.4
	25.4	11.4	48.2	62.7	49.1	56.1	64.3	51.6	46.6
	13.2	9.4	23.1	54.4	38.2	48.6	53.8	30.7	40.3
	10.6	8.6	22.9	54.9	39.5	45.8	52.3	29.5	38.8
	12.9	9.0	25.0	55.7	39.7	47.7	52.2	28.1	38.8
	8.4	7.3	21.5	50.8	37.7	33.7	42.1	25.2	24.1
	29.5	14.7	52.8	64.8	50.1	61.2	68.1	59.4	56.0
EH	26.6	19.9	8.5	37.2	30.7	33.2	35.1	18.9	4.2
	41.2	13.2	14.2	48.1	39.3	59.7	56.8	23.5	17.8
	32.7	20.2	13.4	42.9	34.7	33.7	40.5	13.3	5.8
	30.4	23.4	14.3	-3.7	34.5	46.5	40.6	16.0	7.9
	35.4	20.0	13.8	40.6	37.5	40.1	43.5	15.6	10.0
	23.9	8.5	5.6	34.5	37.5	37.3	33.7	3.8	-16.8
	40.3	24.2	24.9	48.3	44.0	71.2	59.1	18.6	8.4
	10.8	16.0	10.7	38.5	33.9	25.4	28.5	18.1	7.9
FH			22.0	55.4	47.1	31.7	?	32.3	15.0
			38.4	62.5	40.7	46.8	?		38.1
			20.4	57.7	38.8	29.5	?	39.0	28.2
			36.5	73.4	47.7	54.0	68.8		43.8

FAME Yield

- TOC, TN, and FAME yield do not always trend together
  - Lewis acid less favorable for FAME than TOC or TN
- Certain combinations of species and pretreatment uniquely good/bad

	Nanno	WT1	WT2	*IIT*	*Tetra*	393	*Mono*	*Pico*	0401
H_2SO_4	32.2	8.4	16.4	63.7	29.3	33.1	46.7	44.2	89.9
	57.9	37.9	70.6	46.2	58.3	61.0	49.9	65.2	71.9
	32.7	38.1	63.7	54.2	64.7	70.4	47.4	46.7	85.1
	52.0	21.4	64.8	50.7	64.7	64.8	59.6	36.9	80.5
	42.8	21.4	69.2	47.6	71.3	70.1	63.3	44.6	84.0
	39.7	24.2	62.3	45.0	79.2	87.8	33.4	49.5	85.9
	57.4	56.8	72.3	62.5	35.6	81.4	59.7	67.2	81.4
$FeCl_3$	29.3	7.3	12.1	26.3	17.5	29.4	39.8	13.1	58.6
	41.4	18.7	30.3	53.3	42.8	32.0	38.5	26.1	52.8
	42.6	17.7	17.0	38.2	33.5	30.5	39.6	42.1	62.9
	48.4	24.9	20.0	43.5	45.1	37.7	35.4	44.0	71.8
	45.3	23.6	20.2	47.8	41.2	37.7	34.9	46.7	68.8
	38.3	66.4	16.5	28.5	40.8	33.5	53.8	34.5	60.1
	60.8	27.5	24.2	27.7	67.4	23.0	38.2	37.8	76.9
NaOH	53.9	9.4	32.3	31.4	73.1	49.3	23.8	27.6	36.9
	70.0	34.6	28.1	74.2	100.2	65.9	22.8	85.6	60.5
	62.1	9.7	41.4	68.9	97.7	52.2	20.3	68.9	66.1
	63.0	14.6	55.2	69.0	98.9	61.8	19.8	67.6	55.1
	57.9	14.8	52.8	65.6	86.8	62.5	20.8	81.9	66.7
	72.4	4.7	48.8	75.2	99.9	56.5	22.1	79.0	68.3
	55.7	23.0	64.8	76.5	91.3	21.8	20.5	76.2	71.1
Tw	40.2	36.3	40.5	68.3	70.2	64.5	56.4	58.3	54.0
	69.4	73.1	47.9	76.9	88.5	82.8	73.5	79.8	80.1
	71.9	47.5	67.1	69.9	78.2	86.3	73.1	78.2	95.7
	72.6	60.1	72.3	74.3	78.4	79.3	75.3	60.1	71.1
	71.9	60.2	76.0	76.7	79.4	94.5	78.2	80.2	76.8
	58.8	77.1	39.2	59.9	85.6	58.9	70.6	37.6	85.4
	87.2	63.5	61.7	71.4	82.6	83.3	74.8	86.7	88.5
EH	96.9	53.3	27.2	50.7	76.4	40.8	51.2	16.8	65.8
	97.1	59.1	28.1	42.2	62.6	60.4	92.3	10.0	81.6
	99.8	61.3	29.6	53.1	59.5	53.6	56.4	16.4	76.9
	90.1	56.4	27.0	50.8	62.2	55.2	48.0	9.8	72.8
	93.0	59.9	26.1	49.0	60.7	55.6	78.0	19.7	76.6
	102.0	63.6	30.3	57.5	53.7	20.9	84.8	24.0	73.1
	92.8	69.2	30.5	40.6	60.3	56.2	102.0	9.9	80.5
	52.8	44.1	25.1	33.1	63.6	51.9	49.7	18.6	77.0
FH			38.6	86.2	114.6	55.0	?	60.2	77.9
			105.7	95.4	125.2	84.6	?		88.9
			41.0	90.0	121.2	53.7	?	64.3	58.4
			89.1	98.1	115.3	114.6	50.5		99.7

Combined Yield

- Combining TOC, TN, and FAME yield gives estimate of overall pretreatment effectiveness
  - TOC Yield x TN Yield x FAME Yield = Combined Yield
- Data more scattered, but still some interesting trends
  - High-carb Scenedesmus most amenable, especially with acid
  - FH promising, when it works

	Nanno	**WT1**	**WT2**	***IIT***	***Tetra***	**393**	***Mono***	***Pico***	**0401**
H_2SO_4	0.6	0.3	1.1	11.9	3.2	2.3	5.4	2.7	26.3
	7.9	4.9	14.5	11.3	16.9	12.6	13.5	14.3	22.9
	3.7	2.1	12.0	13.8	16.1	14.1	14.9	9.3	26.8
	6.3	1.2	11.9	14.2	12.4	11.7	18.4	7.8	24.6
	4.6	1.4	13.6	13.7	12.9	11.8	18.8	10.0	30.7
	2.8	0.8	10.8	9.0	14.9	12.6	9.5	8.2	31.7
	11.4	7.3	21.8	21.1	13.1	23.5	24.2	24.3	33.6
$FeCl_3$	1.0	0.2	0.7	4.8	2.0	3.2	11.0	1.3	16.5
	5.2	2.2	6.9	15.6	8.3	7.7	14.1	5.6	18.6
	5.7	1.4	3.7	10.8	4.7	9.3	19.4	11.1	23.5
	6.3	2.0	4.7	12.7	5.8	11.5	16.4	12.0	27.8
	7.5	2.0	4.5	13.2	5.2	11.1	16.7	12.8	27.1
	3.5	2.7	2.6	5.7	5.2	5.6	18.8	5.2	19.4
	15.0	3.9	6.8	9.8	13.2	10.2	18.2	15.2	30.6
NaOH	1.4	0.4	1.4	7.0	9.6	2.2	2.9	1.2	0.7
	7.7	3.9	4.1	17.5	16.2	12.0	10.9	10.5	3.4
	12.0	1.0	2.8	14.8	15.7	5.1	9.9	7.9	3.7
	12.7	1.6	5.2	14.1	15.7	4.3	10.6	8.3	3.0
	8.6	1.5	2.8	15.6	12.6	4.6	10.6	7.1	3.2
	11.2	0.5	4.7	17.6	11.3	5.4	11.4	11.2	7.8
	13.6	3.2	16.7	21.9	17.4	6.2	13.7	22.5	13.8
Tw	0.2	0.2	1.1	16.6	9.4	5.4	6.3	2.7	4.5
	3.5	0.6	8.5	28.0	20.5	20.2	23.1	18.2	25.0
	1.3	0.3	4.2	20.0	11.9	16.1	15.2	7.8	27.3
	1.0	0.3	4.4	21.2	12.8	13.3	14.9	5.3	18.9
	1.3	0.4	5.4	22.2	12.4	17.0	16.3	6.5	21.1
	0.4	0.3	2.1	14.5	12.7	5.5	9.7	2.0	10.2
	5.9	0.9	14.5	26.4	19.7	23.1	27.4	25.3	37.4
EH	7.6	2.1	0.3	8.1	7.8	3.9	6.5	0.4	0.5
	18.2	0.9	1.1	11.3	8.6	17.8	31.6	0.5	4.3
	10.5	1.6	0.7	9.9	6.5	5.7	9.8	0.4	1.2
	10.7	1.6	0.7	-0.9	6.5	10.2	8.6	0.4	1.5
	9.8	1.5	0.6	8.6	8.0	9.4	19.9	0.4	2.2
	6.3	0.4	0.2	8.7	6.4	2.4	11.4	0.1	-2.2
	14.5	1.9	2.1	11.3	8.8	22.4	35.3	0.3	2.5
	0.9	0.7	0.3	5.4	6.7	2.7	3.9	0.6	1.1
FH			2.3	28.1	29.8	3.5	?	6.8	5.2
			16.8	36.7	26.1	19.4	?		20.6
			2.1	30.0	23.5	5.3	?	10.2	6.9
			13.2	49.7	31.4	33.0	21.4		30.2

Best Combined Performance

	Nanno	WT1	WT2	IIT	*Tetra*	393	Mono	*Pico*	0401	Avg	Range
Contr.	1.6	1.6	3.4	10.8	2.7	5.4	8.1	2.6	4.3	4.5	9.2
H_2SO_4	11.4	7.3	21.8	21.1	16.9	23.5	24.2	24.3	33.6	**20.4**	26.3
$FeCl_3$	15.0	3.9	6.9	15.6	13.2	11.5	19.4	15.2	30.6	14.6	26.6
Tw.	5.9	0.9	14.5	28.0	20.5	23.1	27.4	25.3	37.4	**20.3**	36.4
NaOH	13.6	3.9	16.7	21.9	17.4	12.0	13.7	22.5	13.8	15.0	18.5
EH	18.2	2.1	2.1	11.3	8.8	22.4	35.3	0.6	4.3	11.7	34.7
FH	0.0	0.0	16.8	49.7	31.4	33.0	21.4	10.2	30.2	**21.4**	49.7

- Based on top performance, dilute H_2SO_4 and FH are most promising
- Average and range both important
- Move forward with “baseline” dilute H_2SO_4

Best Combined Performance

	Nanno	WT1	WT2	IIT	*Tetra*	393	*Mono*	*Pico*	0401	Avg	Range
Contr.			3.4	10.8	2.7	5.4	8.1	2.6	4.3	5.3	8.1
H_2SO_4			21.8	21.1	16.9	23.5	24.2	24.3	33.6	**23.6**	**16.7**
$FeCl_3$			6.9	15.6	13.2	11.5	19.4	15.2	30.6	16.0	23.7
Tw.			14.5	28.0	20.5	23.1	27.4	25.3	37.4	**25.2**	22.9
NaOH			16.7	21.9	17.4	12.0	13.7	22.5	13.8	16.8	10.5
EH			2.1	11.3	8.8	22.4	35.3	0.6	4.3	12.1	34.7
FH			16.8	49.7	31.4	33.0	21.4	10.2	30.2	**27.5**	39.5

- Based on top performance, dilute H_2SO_4 and FH are most promising
- Average and range both important
- Move forward with “baseline” dilute H_2SO_4

Best Combined Performance

	Nanno	WT1	WT2	IIT	*Tetra*	393	*Mono*	*Pico*	0401	Avg	Range
Contr.			3.4	10.8	2.7	5.4	8.1	2.6		5.5	8.1
H_2SO_4			21.8	21.1	16.9	23.5	24.2	24.3		**22.0**	**7.4**
$FeCl_3$			6.9	15.6	13.2	11.5	19.4	15.2		13.6	12.5
Tw.			14.5	28.0	20.5	23.1	27.4	25.3		23.1	13.6
NaOH			16.7	21.9	17.4	12.0	13.7	22.5		17.3	10.5
EH			2.1	11.3	8.8	22.4	35.3	0.6		13.4	34.7
FH			16.8	49.7	31.4	33.0	21.4	10.2		27.1	39.5

- Based on top performance, dilute H_2SO_4 and FH are most promising
- Average and range both important
- Move forward with “baseline” dilute H_2SO_4

Scaled up dilute H_2SO_4 pretreatment

Scale	Nanno	WT1	WT2	IIT	*Tetra*	393	*Mono*	*Pico*	0401
Small-scale TOC	36.7	27.7	45.0	51.1	51.1	49.1	43.8	45.7	45.9
Large-scale TOC	28.2	21.6	47.3	47.1	52.0	53.6	64.4	36.6	51.6
Small-scale FAME	57.9	56.8	72.3	62.5	79.2	87.8	63.3	67.2	89.9
Large-scale FAME	51.9	50.2	57.3	57.8	75.6	87.3	62.5	33.4	74.5
Small-scale TN	45.5	44.8	59.3	56.4	62.6	54.3	76.2	65.8	57.6
Large-scale TN	39.4	49.8	54.4	63.1	60.7	52.0	62.4	62.7	83.0

- Generally good agreement for TOC, TN, FAME yields between 4 mL and 200 mL scales

Large Scale Sugar Yields

Type	Mono	Total	Yield
Nannochloropsis	1.40	3.05	69.8%
Wastewater Treatment #1	0.16	1.49	87.4%
Wastewater Treatment #2	1.40	3.87	63.4%
Scenedesmus IITRIND2	0.85	1.40	39.6%
Tetraselmis	1.58	3.02	62.7%
Scenedesmus obliquus UTEX393	1.13	2.53	50.9%
Picochlorum celerei	1.12	1.49	81.7%
Scenedesmus acutus 0401	20.01	20.04	76.9%

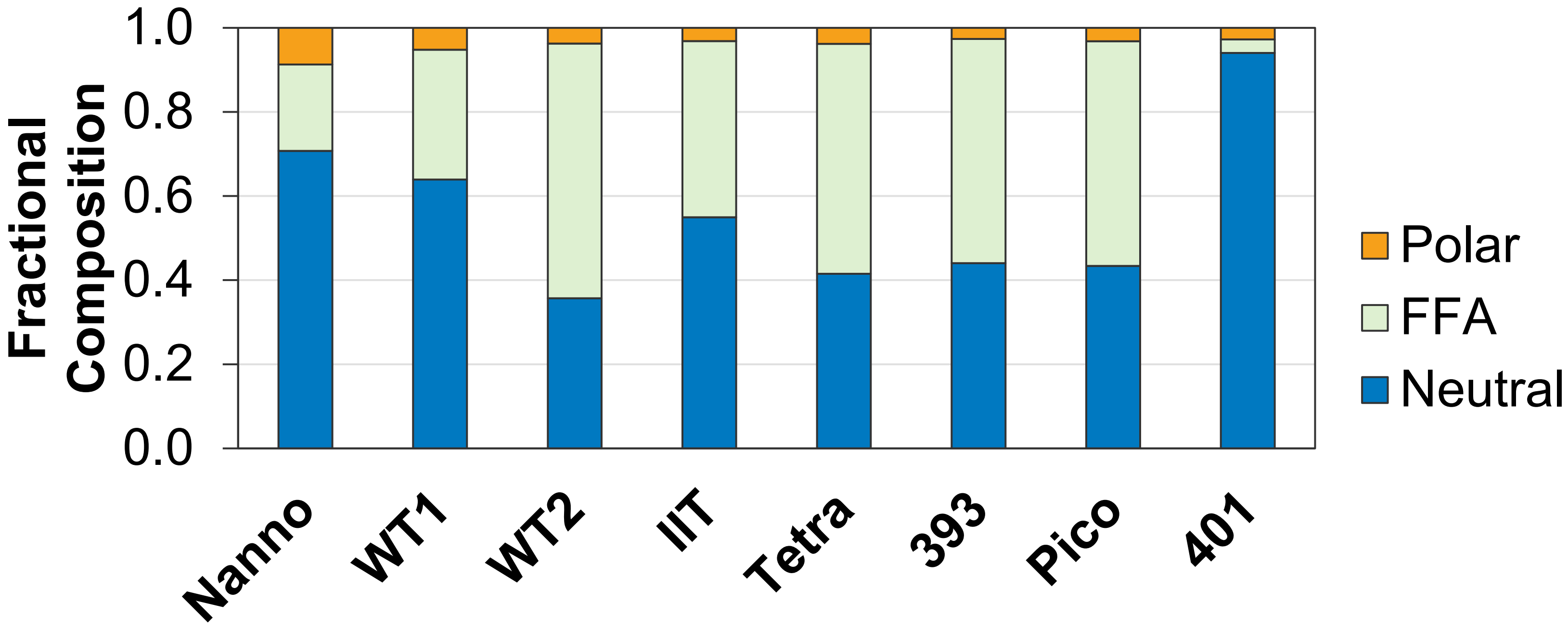
- Sugar yields mostly reasonable, consistent with previous work
- Sugar concentrations low for high-protein strains

Other Fermentable Carbon

Type	Lactic Acid	Glycerol	Acetic Acid
Nannochloropsis	0.0	<LOQ	<LOQ
Wastewater Treatment #1	<LOQ	<LOQ	<LOQ
Wastewater Treatment #2	<LOQ	0.8	<LOQ
Scenedesmus IITRIND2	2.6	0.8	<LOQ
Tetraselmis	2.4	0.5	0.5
Scenedesmus obliquus UTEX393	1.5	0.9	0.5
Picochlorum celerei	<LOQ	<LOQ	<LOQ
Scenedesmus acutus 0401	<LOQ	1.4	<LOQ

- Small amount of other identified carbon present
- Currently quantifying amino acids

Lipid Speciation



- **Most lipid extracts contain significant amount of free fatty acids (FFA)**
- **Some hydrolysis may be from co-extracted lipases**

Conclusions

- Algae pretreatment effectiveness shows significant variation across strains and pretreatment technologies
- Dilute acid pretreatment is among the most robust approaches
- Pretreatment effectiveness metrics (TOC, TN, FAME) do not always correlate
  - To each other or to composition

Thank You!

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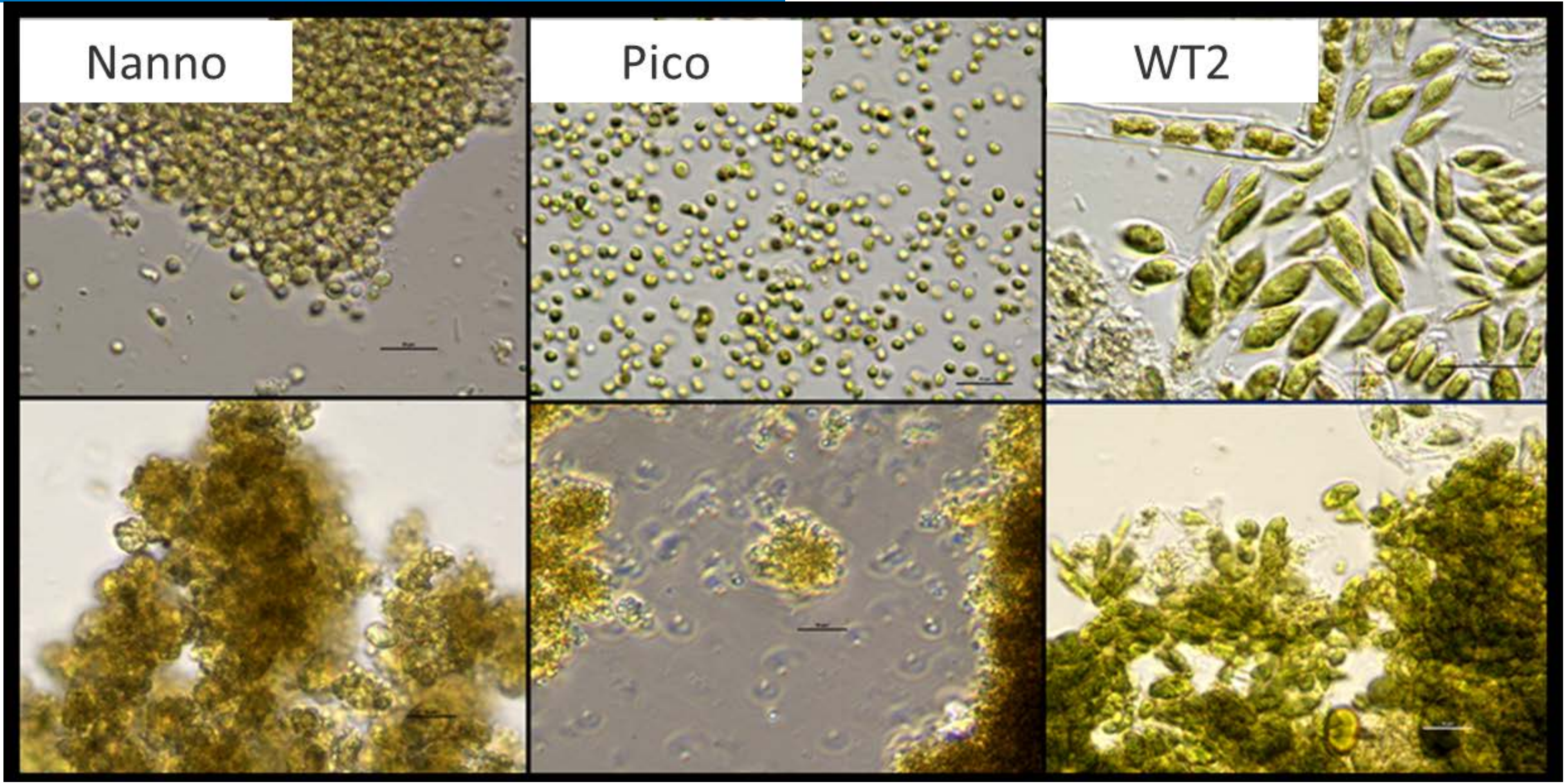
Pretreated Cell Integrity



- Good cell lysis in most cases, some “mostly intact” cell walls in some cases

Lipid Extraction

Type	Biomass FAME	Residuals FAME
Nannochloropsis	8.5	8.6
Wastewater Treatment #1	2.1	0.5
Wastewater Treatment #2	7.0	4.5
Scenedesmus IITRIND2	8.8	8.2
Tetraselmis	7.3	6.5
Scenedesmus obliquus UTEX393	7.1	2.6
Picochlorum celerei	9.6	7.0
Scenedesmus acutus 0401	24.6	14.6

- **Some polar lipids may remain with solids after extraction**