Proteomic Profiling of Microalgae During Long-Term Ensiling Reveals Routes of Nutrient Cycling and Dark Adaptation

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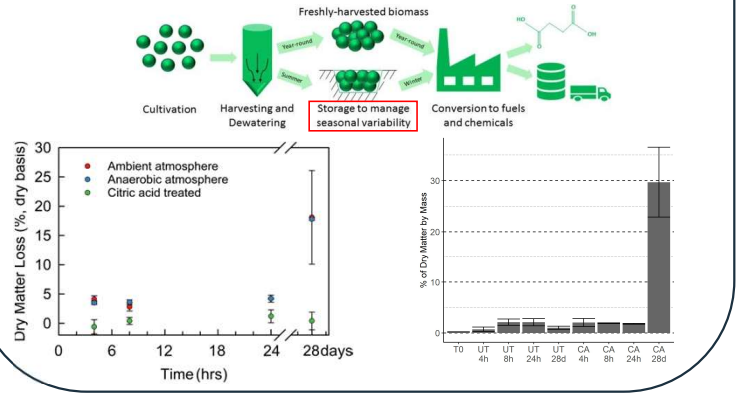
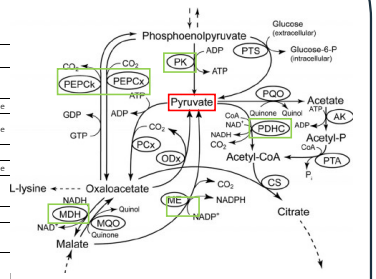
Algae ensiling mitigates problems with seasonal variability

Optimal algae production occurs during in summer months due to optimal temperatures and sunlight. Due to lower growth rates occurring in winter, algae biomass produced during peak months needs to be stored for conversion in the winter. Some value-add co-products created during storage can also help to make algal biofuels more cost-effective.

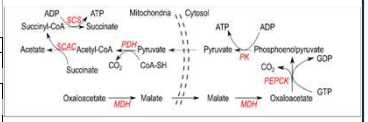


Carbohydrate metabolism

Protein	Isoforms	Relative Protein Abundance: Untreated 28 day	Relative Protein Abundance: Citric acid 28 day	Pathway
Citrate synthase	I	1.98	1.47	TCA cycle
	II	1.54	1.14	
Aconitase	I	1.18	1.02	TCA cycle/Reductive TCA cycle
Isocitrate dehydrogenase	I	1.27	1.96	TCA cycle/Reductive TCA cycle
	II	1.65	1.07	
α-ketoglutarate dehydrogenase	I	0.91	1.19	TCA cycle
Succinyl-CoA synthetase	I	2.23	1.24	TCA cycle/Reductive TCA cycle
Succinate dehydrogenase	I	1.50	1.31	TCA cycle
	II	1.87	1.50	
Fumarase	I	1.04	1.53	TCA Cycle
ATP-citrate lyase	I	1.42	1.42	Reductive TCA cycle
	II	2.38	1.94	
Ferrodoxin	I	0.46	0.50	Reductive TCA cycle
	II	0.53	0.74	
	III	0.41	0.43	
Malate dehydrogenase	I	1.88	2.05	TCA/Reductive TCA cycle
	II	0.69	1.29	
	III	1.96	1.35	
	IV	1.77	1.25	
Isocitrate lyase	I	1.81	1.46	Glyoxylate Cycle
Glutamate synthase	I	2.07	2.12	GABA Shunt
	II	0.77	0.93	
Pyruvate dehydrognease	I	3.61	1.40	
	II	2.53	1.43	
	III	2.39	1.16	



Klaffl, S. (2013)



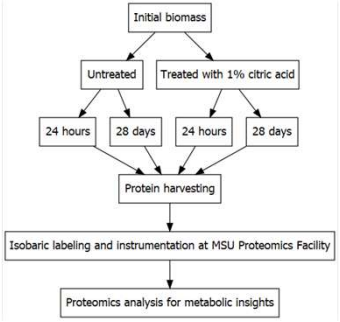
Nitrogen metabolism and cycling

Total nitrogen is constant, but protein is being degraded

Treatment	N (%)	%Protein: Total N*	%Protein: Total AA
Initial Biomass	9.5	45.3	42.4
Untreated 24 hrs	10.0	47.6	30.5
Untreated 28 days	9.6	46.1	29.9
Citric acid 24 hrs	9.4	45.1	40.7
Citric acid 28 days	9.2	44.2	38.8

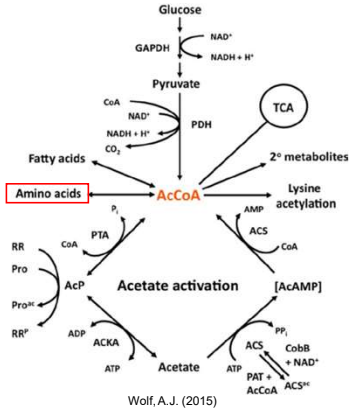
	Initial Biomass	Citric		Untreated	
		24 hr	28 days	24 hr	28 days
Amino acid	gAA/kg algae	gAA/kg initial algae*	gAA/kg initial algae*	gAA/kg initial algae*	gAA/kg initial algae*
L-HydroxyProline	0.00	0.00	0.00	0.00	0.00
ASX[1]	40.4	39.0	37.9	28.3	24.2
L-Threonine	21.9	20.8	20.1	15.1	13.3
L-Serine	18.3	16.8	16.2	12.3	10.3
GLX[1]	49.5	45.6	44.1	33.6	33.3
L-Proline	21.9	21.0	20.3	15.3	12.9
L-Glycine	23.5	23.0	22.4	16.7	18.8
L-Alanine	36.8	34.7	33.9	25.8	32.1
L-Cysteine	5.5	4.7	4.0	5.1	3.1
L-Valine	25.4	25.3	24.6	18.3	22.9
L-Methionine	11.0	9.6	8.6	10.5	8.5
L-Isoleucine	18.3	18.1	17.6	13.1	15.9
L-Leucine	39.3	37.9	36.4	27.6	32.9
L-Tyrosine	17.5	16.8	16.2	12.4	11.5
L-Phenylalanine	25.0	24.2	23.2	17.7	17.8
L-Tryptophan	9.4	9.7	9.4	9.8	8.2
L-Lysine	26.6	26.6	24.8	18.9	13.8
L-Histidine	8.1	8.0	7.4	5.8	5.8
L-Arginine	26.3	25.5	20.7	18.6	14.3
Total	424.4	407.5	387.8	304.8	299.5

Workflow

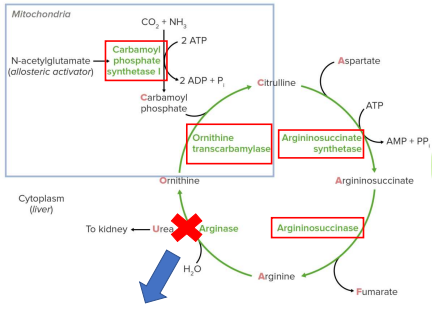


Storage condition		Total number of proteins changed	Number of proteins up-regulated	Number of proteins down-regulated
Citric acid	0hr and 24 hr	20	11	9
	0 hr and 28 days	218	101	117
	24 hr and 28 days	295	131	164
Untreated	0hr and 24 hr	137	69	68
	0 hr and 28 days	924	589	335
	24 hr and 28 days	961	364	597

Relationships between carbohydrate and protein metabolism



Wolf, A.J. (2015)



Other biological amines?

Protein	Isoforms	Relative Abundance: Untreated 28 day	Relative Abundance: Citric Acid 28 day
Carbamoyl phosphate synthetase	I	3.86	2.22
	II	2.3	1.62
Ornithine transcarbamylase	I	3.03	1.74
Argininosuccinate synthetase	I	3.25	2
Arginosuccinase	I	2.83	1.74
	II	2.83	1.74

Research Highlights

- Metabolic analysis reveals novel insights into microalgae during ensiling
- Citric acid treatment completely negates any biomass loss during long-term storage
- Route of production of high value-add co-products identified
- Previously undetected protein degradation results from route of nitrogen cycling in algae

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