APPLICATION NOTE

Astaxanthin Production

Summary

OriginClear's Electro Water Separation™ (EWS) technology helps to industrialize the production of astaxanthin by sanitizing and concentrating the Hematococcus pluvialis (HP) algae for more effective stressing and greater levels of astaxanthin production.

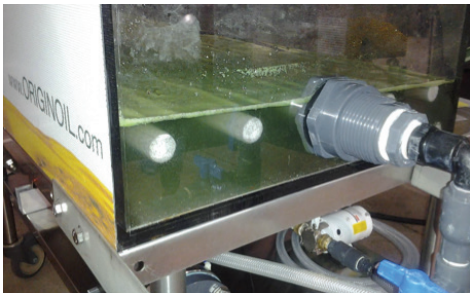
Information

From April 15 to May 5 2013, OriginClear's algae conditioning and harvesting test system, the EWS Algae™ Model A12 was tested for effectiveness in helping to produce astaxanthin in bioreactors at Garden State BioEnterprises Systems (GSBioE) in Woodbine, New Jersey.

Jose Sanchez, general manager of OriginClear's algae division, installed and commissioned the system under the supervision of site director Paul Mulligan and CEO Andrew Greene.

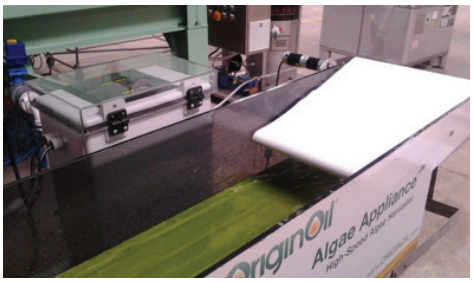
Sanchez Field Report 15 April 2013:

> *"Today I performed tests harvesting Hematococcus Pluvialis, the algae that makes Astaxanthin... The customer is delighted by the results so far, especially because the Hematococcus cells seem unharmed (see photos). The centrifuge next to our A12 just splatters cells that look like 'bugs in a windshield' in a microscope. Tomorrow we will perform some tests to assure that the Hematococcus is alive."*



Sanchez Field Report 16 April 2013:

> *"Today we performed several harvests on the Hematococcus Pluvialis. Our equipment performed as it is supposed to, harvesting the algae out of the water and leaving pretty clear effluent water out.*
>
> *"We analyzed the algae paste in the microscope and the Hematococcus cells seemed intact and unharmed, not like the squished bug-like that the centrifuge produced."*



Conversion to Astaxanthin

Following the successful concentration of the HP culture, it was stressed conventionally and one week later, the algal concentration encysted with successful carotogenesis.

Adoption by Customer

On 9 May 2013, OriginClear and GSBioE jointly announced that GSBioE has adopted OriginClear's harvesting technology as a key component of its proprietary production system for the high-value product Astaxanthin.

"By deploying the OriginClear Algae Appliance, we were able to harvest the algae to a 5% solids concentration while keeping the algae cells viable."

President Andrew Greene stated:

> *"We are very pleased with the successful results of our recent trial of OriginClear's Algae Appliance. By deploying the OriginClear Algae Appliance, we were able to harvest the algae to a 5% solids concentration while keeping the algae cells viable. We were then able to stress them successfully into an Astaxanthin-bearing product.*
>
> *"We are satisfied with the performance of the Algae Appliance. Together with its ability to reduce bacteria, we expect its proven harvesting capability to give us a competitive edge in the production of natural Astaxanthin as we plan to make it a key component of our state-of-the-art Astaxanthin production system."*

The companies are now in discussion regarding GSBioE's 2013 commercialization plans and the potential for expanded use of OriginClear technology.

Conclusion

OriginClear's EWS Algae has been shown to assist the astaxanthin production process by concentrating and sanitizing the HP culture prior to the stressing phase.

Hematococcus pluvialis is notoriously fickle and prone to "crashes" caused by bacteria and other factors. Using EWS, the HP culture can safely be sanitized and concentrated without losing its ability to produce the Astaxanthin-bearing shell.

It is believed that the reduction in bacteria may also help maximize encystment by reducing loss of HP cells during the metamorphosis.

OriginClear's EWS Algae has been shown to assist the astaxanthin production process by concentrating and sanitizing the HP culture prior to the stressing phase.

Additional Findings

The following tentative conclusions are being actively tested for validation with the assistance of the customer:

- The electropulsing regime may accelerate the transformation of HP to the red phase.
- The ability of EWS to create a nitrate deficiency and remove other nutrients may assist in the stressing process.

Proven Effectiveness in Reducing Bacteria

In a 2013 study, an independent university team harvested algae from both open ponds and closed systems, using a well-known centrifugal process on the one hand, and OriginClear's EWS Algae Harvester on the other.

The samples were then sent to an FDA-audited laboratory for analysis. When compared with centrifuging, EWS Algae reduced bacterial colonies by 98% in the case of open ponds, and 99% for bioreactors. The full report follows.

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Pacific Coast Analytical Services

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15751 Roxford Street Unit F Sylmar CA 91342 Tel: 818-364-7470 Fax: 818-364-7472
www.pacificcoastanalytical.com DOHS #2667 LACSD#10255 FDA Audited A2LA Member

Analytical Report

Client:	RL Food Testing Laboratory 3976 Ceanothus Place, Ste. B Calabasas, CA 91302	**Project #:**	[none]
Attention:	Roger Legg	**Work Order #:**	12K0094
Phone:	(949) 309-0105	**Received:**	11/13/2012
FAX:	-		
Client Project Info:	CCMP 1776 Nannochloropsis salina	**Reported:**	11/20/2012

Dear Roger :

The results in this report apply to samples analyzed in accordance with the Chain of Custody document. Pacific Coast Analytical Services certifies that the results meet all requirements for laboratory performance unless noted in the case narrative or in the report with data qualifiers.

This analytical report is confidential and it is only intended for use by Pacific Coast Analytical Services and its client. The Chain of Custody form is an integral part of it. The report can only be reproduced in full with all its attachments and the authorization of Pacific Coast Analytical Services.

Thank you for the opportunity to service your analytical needs. Please feel free to call with any questions.

Reviewed by: _____

Claudio Cardelli, Ph.D. **Lab Director**

/Enclosure: COC

Pacific Coast Analytical Services

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www.pacificcoastanalytical.com DOHS #2667 LACSD#10255 FDA Audited A2LA Member

Analytical Report

Client:	RL Food Testing Laboratory	**Project # :**	[none]
	3976 Ceanothus Place, Ste. B	**Work Order #:**	12K0094
	Calabasas CA, 91302	**Received:**	11/13/2012
Client Project Info:	CCMP 1776 Nannochloropsis salina	**Reported:**	11/20/2012

Client Sample ID #: Grown in 600L raceway & harv. using centrifugal PCAS Sample ID #: 12K0094-01 (Food)

Analyte	Result	Min. Det. Limit	Reporting Limit	Units	Analysis Completed	Method	Qualifers
Aerobic Plate Count, APC	150000000	10	10	CFU/g	11/15/12	FDA-BAM,8th ed,Ch 3	
Coliforms	30	10	10	CFU/g	11/14/12	AOAC 991.14	
Escherichia coli	ND	10	10	CFU/g	11/15/12	AOAC 991.14	
Staph aureus (coag. pos.)	ND	10	10	CFU/g	11/15/12	FDA-BAM,8th ed, Ch 12	
Yeast	1400000	10	10	CFU/g	11/18/12	FDA-BAM,8th ed,Ch18	
Mold	1000	10	10	CFU/g	11/18/12	FDA-BAM,8th ed,Ch18	

Client Sample ID #: Grown in 600L raceway & harv. using origin oil PCAS Sample ID #: 12K0094-02 (Food)

Analyte	Result	Min. Det. Limit	Reporting Limit	Units	Analysis Completed	Method	Qualifers
Aerobic Plate Count, APC	2500000	10	10	CFU/g	11/15/12	FDA-BAM,8th ed,Ch 3	
Coliforms	140	10	10	CFU/g	11/14/12	AOAC 991.14	
Escherichia coli	ND	10	10	CFU/g	11/15/12	AOAC 991.14	
Staph aureus (coag. pos.)	ND	10	10	CFU/g	11/15/12	FDA-BAM,8th ed, Ch 12	
Yeast	3000	10	10	CFU/g	11/18/12	FDA-BAM,8th ed,Ch18	
Mold	3000	10	10	CFU/g	11/18/12	FDA-BAM,8th ed,Ch18	

Client Sample ID #: Grown in PBR & harv. with CentrifugePCAS Sample ID #: 12K0094-03 (Food)

Analyte	Result	Min. Det. Limit	Reporting Limit	Units	Analysis Completed	Method	Qualifers
Aerobic Plate Count, APC	140000000	10	10	CFU/g	11/15/12	FDA-BAM,8th ed,Ch 3	
Coliforms	ND	10	10	CFU/g	11/14/12	AOAC 991.14	
Escherichia coli	ND	10	10	CFU/g	11/15/12	AOAC 991.14	
Staph aureus (coag. pos.)	ND	10	10	CFU/g	11/15/12	FDA-BAM,8th ed, Ch 12	
Yeast	12000	10	10	CFU/g	11/18/12	FDA-BAM,8th ed,Ch18	

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Analytical Report

Client:	RL Food Testing Laboratory	**Project # :**	[none]
	3976 Ceanothus Place, Ste. B	**Work Order #:**	12K0094
	Calabasas CA, 91302	**Received:**	11/13/2012
Client Project Info:	CCMP 1776 Nannochloropsis salina	**Reported:**	11/20/2012

Client Sample ID #: Grown in PBR & harv. with CentrifugePCAS Sample ID #: 12K0094-03 (Food)

Analyte	Result	Min. Det. Limit	Reporting Limit	Units	Analysis Completed	Method	Qualifers
Mold	40000	10	10	CFU/g	11/18/12	FDA-BAM,8th ed,Ch18	

Client Sample ID #: Grown in PBR & harv. with OriginOil AppliancePCAS Sample ID #: 12K0094-04 (Food)

Analyte	Result	Min. Det. Limit	Reporting Limit	Units	Analysis Completed	Method	Qualifers
Aerobic Plate Count, APC	1500000	10	10	CFU/g	11/15/12	FDA-BAM,8th ed,Ch 3	
Coliforms	ND	10	10	CFU/g	11/14/12	AOAC 991.14	
Escherichia coli	ND	10	10	CFU/g	11/15/12	AOAC 991.14	
Staph aureus (coag. pos.)	ND	10	10	CFU/g	11/15/12	FDA-BAM,8th ed, Ch 12	
Yeast	26000	10	10	CFU/g	11/18/12	FDA-BAM,8th ed,Ch18	
Mold	5000	10	10	CFU/g	11/18/12	FDA-BAM,8th ed,Ch18	

NOTE: ND = Non-Detected

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Pacific Coast Analytical Services

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CHAIN OF CUSTODY

Page 1 of 1

Company: RL Food Testing Lab	Bill to:	Turn Around Time: ( ) RUSH (call for upcharge & time) (✓) Normal (10-15 working days)	Work Order: 12K0094
Address: 3976 Ceanothus Place, #B Calabasas, CA 91302	Address:	Method of Transport: ( ) Client ( ) PCAS (✓) Other Fedex / Condition of Sample: (✓) Ambient ( ) Cold _____ °C ( ) Frozen:	Sample Condition (✓) Sealed (✓) Accepted ( ) Chilled ( ) Preserved
Phone: 949-309-0105 Fax:	P.O. #: Prepaid ( ):		
Contact Name: Roger Legg	Project Name: CCMP 1776 *Nannochloropsis salina*	Special Instructions:	
E-mail: roger@rlfoodtestinglaboratory.com	Project #:	*There is a total of 8-50ml. 4 sets with duplicate samples for each set.	
Sampled by: Roger Legg	Signature:		

LAB ID	DATE	TIME	MATRIX	SAMPLE DESCRIPTION	CONT.	ANALYSIS REQUESTED	EXPECTED LEVEL/ METHOD
12K0094 -1	N/A	N/A	Food	Grown in 600L raceway & harvested using centrifuge 11/5/12	2*	APC, Yeast& Mold, Total Coliforms, E.coli & Staph Coagulase	
-2				Grown in 600L raceway & harvested using origin oil appliance 11/5/12	2*	APC, Yeast& Mold, Total Coliforms, E.coli & Staph Coagulase	
-3				Grown in outdoor photobioreactor & harvested using centrifuge 11/6/12	2*	APC, Yeast& Mold, Total Coliforms, E.coli & Staph Coagulase	
-4				Grown in outdoor photobioreactor & harvested using origin oil appliance 11/6/12	2*	APC, Yeast& Mold, Total Coliforms, E.coli & Staph Coagulase	

Relinquished by: Client	Date N/A	Time N/A	Received by: Fedex
Relinquished by: Fedex	Date 11/13/12	Time 15:30	Received by: [illegible]
Relinquished by:	Date	Time	Received by:

Abbreviation:

CONT Container
DW Drinking Water
F Food