Okara Waste as a Substrate for the Microalgae *Phaeodactylum tricornutum* Enhances the Production of Algal Biomass, Fucoxanthin, and Polyunsaturated Fatty Acids

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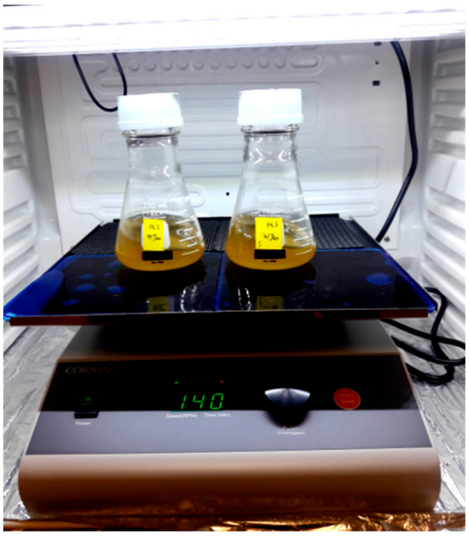
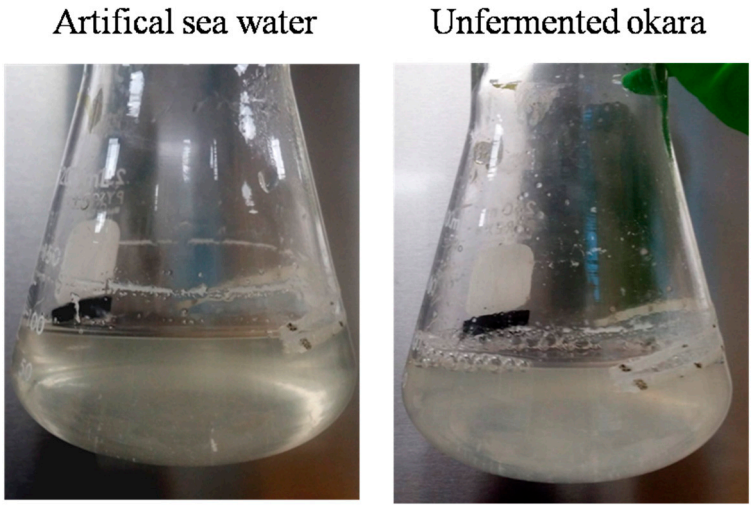
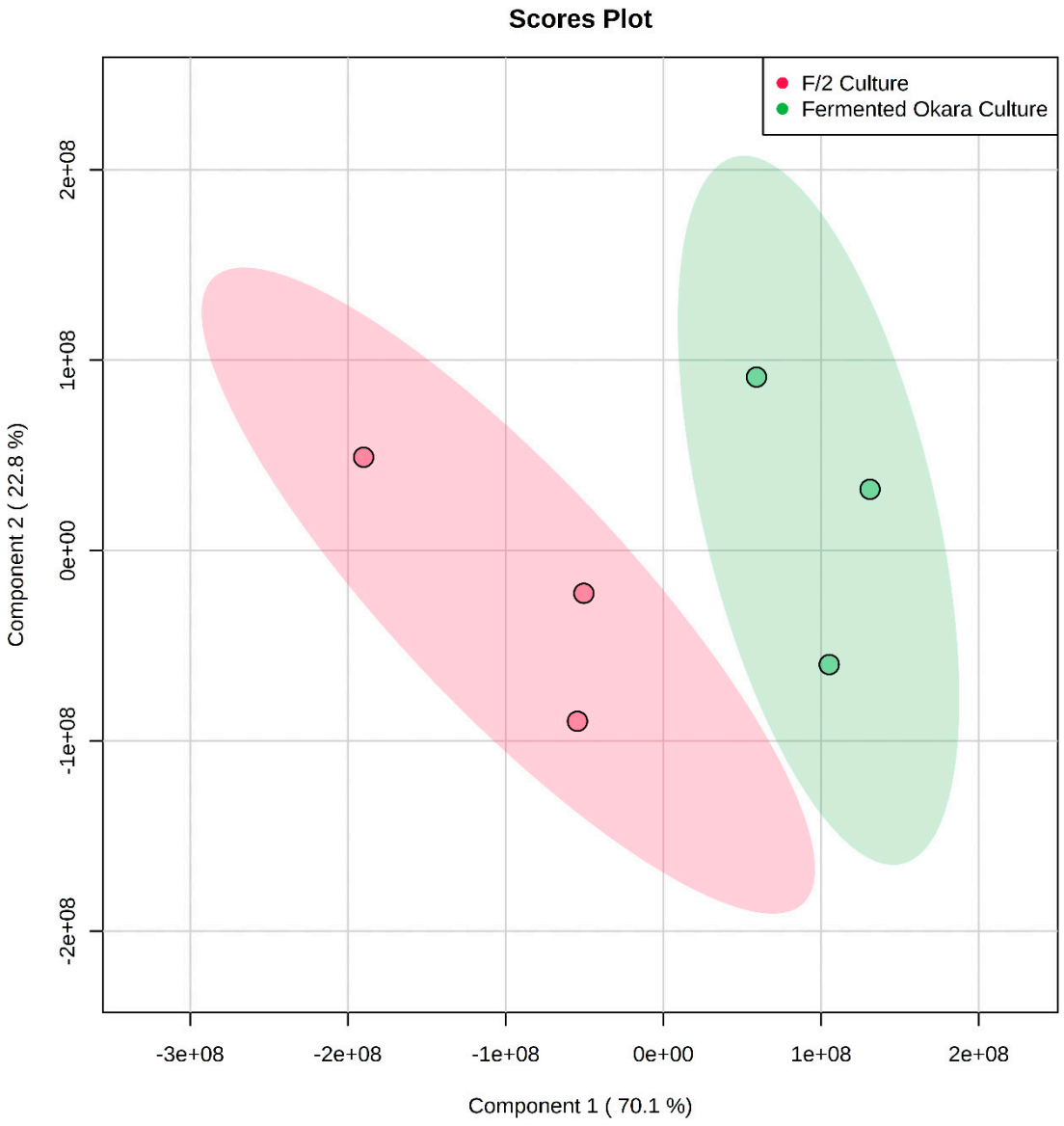
Supplementary Figures



Supplementary Figure S1. Microalgae culture setup in a chilling incubator with white fluorescence light and an orbital shaker.



Supplementary Figure S2. Images of *P. tricornutum* grown in artificial seawater (left) and unfermented okara media (right).



Supplementary Figure S3. Partial least square discriminate analysis of intracellular metabolites from fermented okara culture and F/2

Supplementary Table

Supplementary Table S1. Components of F/2 media (G0154, Sigma)

Component (mg/L)	G0154
Biotin	0.005
Cobalt chloride • $6H_2O$	0.01
Cupric sulfate • $5H_2O$	0.01
EDTA disodium • $2H_2O$	4.36
Ferric chloride • $6H_2O$	3.15
Manganese chloride • $4H_2O$	0.18
Sodium molybdate • $2H_2O$	0.006
Sodium nitrate	75.0
Sodium phosphate monobasic	4.411
Thiamine • HCl	0.1
Vitamin B_{12}	0.005
Zinc sulfate • $7H_2O$	0.022

Supplementary Table S2. Metabolite quantitation of cells from fermented okara culture and F/2

Metabolites	Fermented Okara Culture	F/2	Fold Change
L-Alanine	151117955 ± 40248104	55359717 ± 17274417	2.73
L-Valine	20249132 ± 4739954	11174734 ± 1040699	1.81
L-Leucine	13985044 ± 4426860	N.D.	-
L-Proline	366493174 ± 78788977	270164158 ± 107803147	1.36
Succinic acid	12995910 ± 1736148	N.D.	-
Methyltyrosine	5268397 ± 930580	14552927 ± 5665727	0.36
L-Serine	13187098 ± 306213	4498369 ± 1571415	2.93
L-Threonine	21065286 ± 2717681	6169260 ± 1918788	3.41
L-Aspartic Acid	21988273 ± 15688058	N.D.	-
L-Lysine	64648813 ± 40470104	14058399 ± 2779101	4.60
L-Threnoic Acid	3515504 ± 1704135	12633603 ± 1972932	1.48
Phosphoric acid	11340906 ± 2055252	N.D.	-
Ketoglutaric acid	9749749 ± 2509415	N.D.	-
D-Glucose	27849931 ± 7020191	57319747 ± 17733245	0.49
D-Mannose	4573025 ± 1247440	10509429 ± 4330966	0.44
Galactofuranose	11127298 ± 2356787	52582778 ± 4904629	0.21
Inositol	3544007 ± 1276600	6452898 ± 717349	0.55
Myo-Inositol	8220090 ± 2980639	19703952 ± 2499325	0.42
Sedoheptulose	3777560 ± 2112451	107350452 ± 49800832	0.04
D-Galactose	102550085 ± 27630901	58873733 ± 8781061	1.74
Glucopyranose	3200496 ± 1889693	18556617 ± 7687305	0.17
Propane	4579043 ± 1050385	N.D.	-
Glycine	21308777 ± 4261830	14367172 ± 4607780	1.27
Malic acid	6054507 ± 1065589	4763921 ± 4060474	0.28

N.D. = Not Detected

Supplementary Table S3. Maximal growth rate and method of aeration from *P. tricornutum* in this study compared to previous literatures

Species	Maximal growth rate (day^{-1})	Method of aeration	Reference
P. tricornutum (f/2)	0.38	Orbital shaking	This study
P. tricornutum (fermented okara medium)	0.48		
P. tricornutum	<0.3	1% CO_2	[1]
P. tricornutum	<0.2	1% CO_2	[2]
P. tricornutum	0.14	Orbital shaking	[3]
P. tricornutum (six strains)	<0.35	Unknown	[4]

Reference

1. Wang, H.; Zhang, Y.; Chen, L.; Cheng, W.; Liu, T. Combined Production of Fucoxanthin and EPA from Two Diatom Strains Phaeodactylum Tricornutum and Cylindrotheca Fusiformis Cultures. *Bioprocess Biosyst Eng* **2018**, *41* (7), 1061–1071. https://doi.org/10.1007/s00449-018-1935-y.
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4. Wu, H.; Li, T.; Wang, G.; Dai, S.; He, H.; Xiang, W. A Comparative Analysis of Fatty Acid Composition and Fucoxanthin Content in Six Phaeodactylum Tricornutum Strains from Diff Erent Origins. *Chinese Journal of Oceanology and Limnology* **2016**, *34* (2), 391–398. https://doi.org/10.1007/s00343-015-4325-1.