Supporting Information: Co-cultivation of Microalgae and Bacteria for Optimal Bioenergy Feedstock Production in Wastewater by Using Response Surface Methodology

Kishore Gopalakrishnan [1, 2], Yongli Z. Wager [1, *], Javad Roostaei [1, 3]

[1] Department of Civil and Environmental Engineering, Wayne state university, 5050 Anthony Wayne Dr, Detroit, MI, U.S. 48201

[*] Corresponding author: zhangyl@wayne.edu. Department of Civil and Environmental Engineering, Wayne state university, 5050 Anthony Wayne Dr., Detroit, MI, U.S. 48201
[2] Current: Department of Biological Sciences, Wayne state university, 5047 Gullen Mall, Detroit, MI, U.S. 48202, kishore.gopalakrishnan@wayne.edu
[3] Current: Senior Principal Engineer, Hazen and Sawyer, JRoostaei@hazenandsawyer.com.

S1. Symbiotic Algae-Bacteria Seed Culture

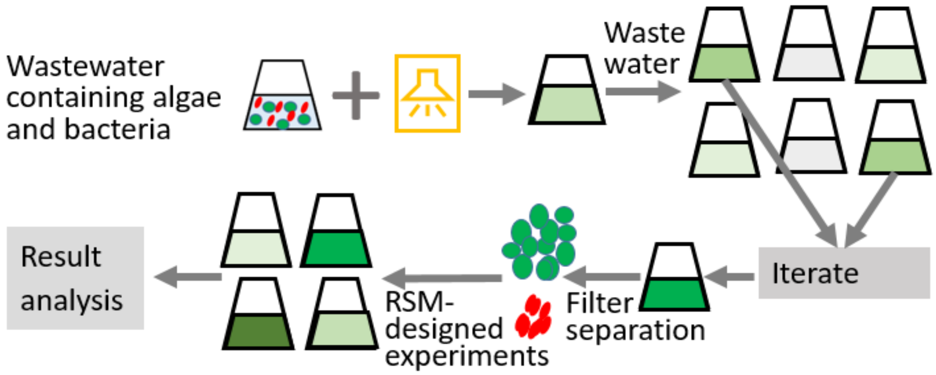

Figure S1: Screening pipeline for the selection of symbiotic algal-bacterial cultures.

S2. Design of experiment

According to the methodology described by Gopalakrishnan et al. (2018) [1], equation S1 shows the correlation between the coded and the actual value in the regression study.

$$v_i = \frac{(V_i - V_i^*)}{\Delta V_i} \tag{S1}$$

- where v_i and V_i are the coded and un-coded value of the i^{th} independent variables respectively; V_i^* is the un-coded value of the i^{th} independent variable at the midpoint; and ΔV_i is the difference in the values.

Table S1 summarizes the experimental design for thirty runs and two responses (algal biomass and lipid productivity). Equation S2 is the fitted quadratic equation [1] to estimate the correlation between the independent variables and responses:

$$y = \alpha_0 + \sum_{b=1}^{c} \alpha_j x_j + \sum_{b=1}^{c} \alpha_{jj} {x^2}_j + \sum_{a=1}^{b-1} \sum_{c=2}^{c} \alpha_{ij}\, x_i x_j + \varepsilon \tag{S2}$$

y = predicted response, α_0= a constant, α_j= linear coefficient, α_{jj}= squared coefficient, and α_{ij}= interaction coefficient, x_i and x_j are the independent variables and ε is noise or error.

S3. Algal biomass and lipid productivity analyses

A comparison between Flow Cytometer Analyses and conventional measurements were performed to calibrate the results from different results. The gravimetric analysis [2] and oven drying method [3] were used for measure total lipid content and algal dry biomass respectively. Significant linear correlations were observed for both algal biomass and lipid productivity (Figures S2-3), which is consistent with other studies [4].

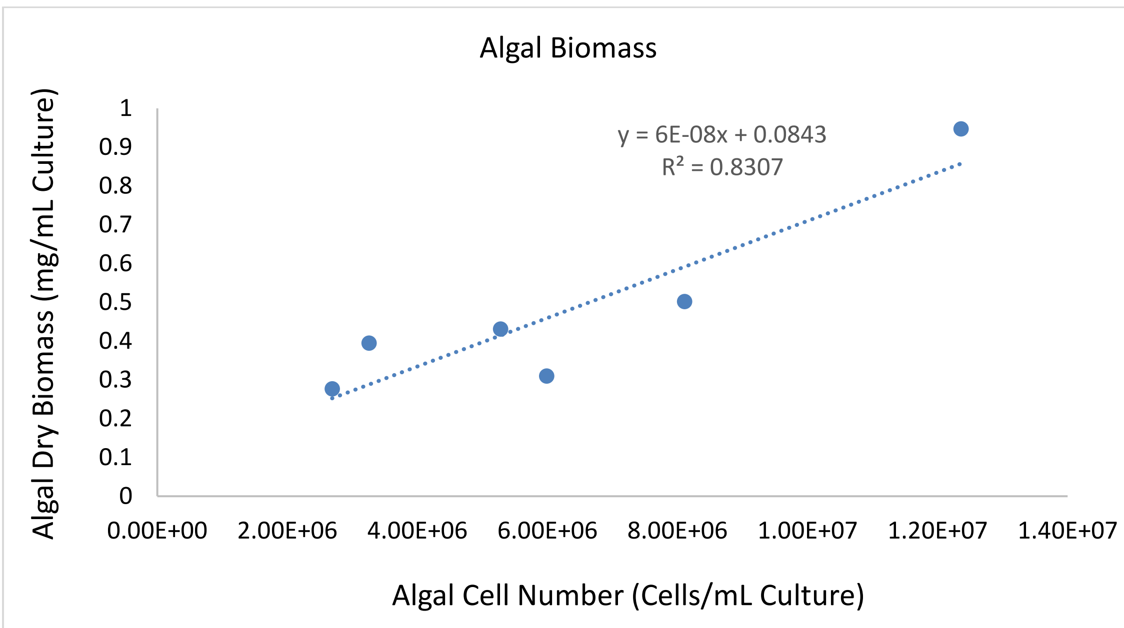

Figure S2: The significant correlation between algal cells measurement obtained from flow cytometer analysis and algal dry biomass.

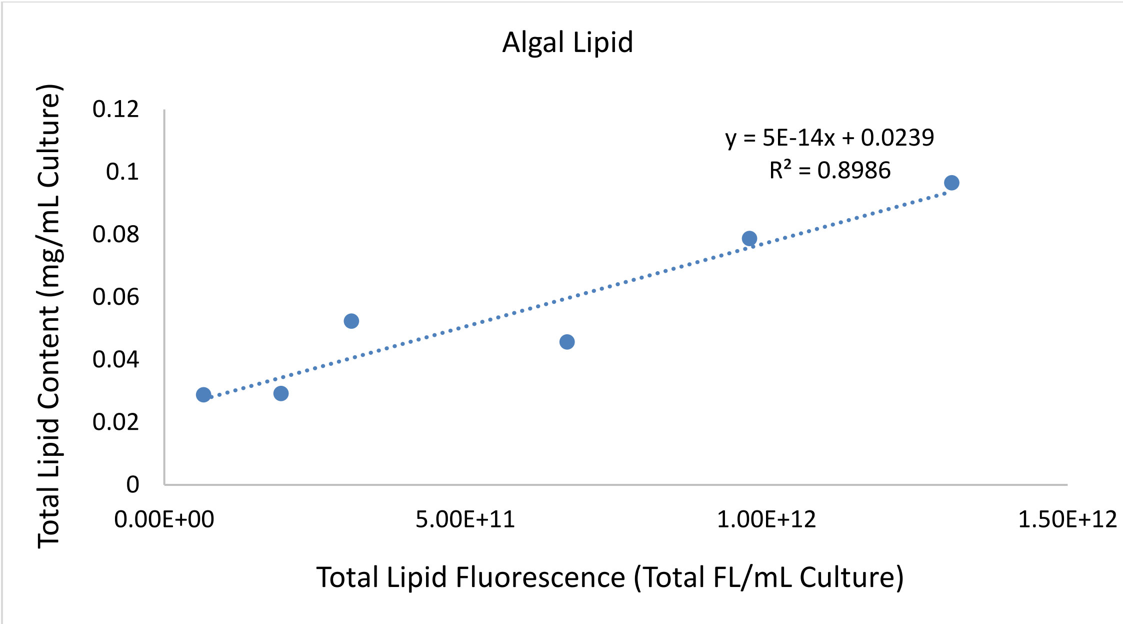

Figure S3: The significant correlation between total lipid fluorescence and gravimetrical determination of total lipids.

S3. Post-run Statistical Analysis

Design Expert® Software Version 10 (Stat-Ease Inc., Minneapolis, USA) was used for the post-run statistical analysis to develop the predicted model based on the method described in our previous study [1]. The results of the statistical analyses were summarized in Table S1.

Table S1: Variance analysis of response surface quadratic model for algal biomass (S2a) and algal lipid productivity (S2b). Sum of squares signifies the deviation of the experimental data from the mean value. Degree of freedom is represented by df that is the number of freedom independent ways the dynamic system can be moved. Mean square is the degree of freedom divided by the df.

Table S1a.

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	1.578×10^{7}	14	1.127×10^{6}	3.29	0.0144	Significant
A-Bacteria ratio	4.963×10^{6}	1	4.963×10^{6}	14.47	0.0017	Significant
B-light intensity	1.426×10^{6}	1	1.426×10^{6}	4.16	0.0595	
C-CO_2	9.364×10^{5}	1	9.364×10^{5}	2.73	0.1193	
D-harvest time	1.950×10^{6}	1	1.950×10^{6}	5.68	0.0308	Significant
AB	2.393×10^{4}	1	2.393×10^{4}	0.070	0.7953	
AC	1.114×10^{5}	1	1.114×10^{5}	0.32	0.5772	
AD	2.244×10^{5}	1	2.244×10^{5}	0.65	0.4313	
BC	3.124×10^{4}	1	3.124×10^{4}	0.091	0.7670	
BD	4.523×10^{4}	1	4.523×10^{4}	0.13	0.7216	
CD	7.962×10^{4}	1	7.962×10^{4}	0.23	0.6370	
A^2	1.935×10^{4}	1	1.935×10^{4}	0.056	0.8155	
B^2	1.609×10^{6}	1	1.609×10^{6}	4.69	0.0469	Significant
C^2	7.454×10^{5}	1	7.454×10^{5}	2.17	0.1612	
D^2	4.740×10^{6}	1	4.740×10^{6}	13.81	0.0021	Significant
Residual	5.147×10^{6}	15	3.431×10^{5}			
Lack of Fit	5.139×10^{6}	10	5.139×10^{5}	2.88	0.1275	
Pure Error	7.722×10^{3}	5	1.544×10^{4}			
Corrected Total	2.093×10^{7}	29				

$R^2 = 0.7541$; Adjusted $R^2 = 0.5246$

Table S1b.

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	8.344×10^{12}	14	5.960×10^{11}	3.11	0.0183	Significant
A-Bacteria	4.488×10^{11}	1	4.488×10^{11}	2.34	0.1466	
B-light intensity	3.293×10^{11}	1	3.293×10^{11}	1.72	0.2094	
C-CO_2	6.581×10^{11}	1	6.581×10^{11}	3.44	0.0835	
D-harvest time	1.421×10^{12}	1	1.421×10^{12}	7.42	0.0157	Significant
AB	2.792×10^{4}	1	2.792×10^{4}	1.459×10^{-7}	0.9997	
AC	2.267×10^{9}	1	2.267×10^{9}	0.012	0.9148	
AD	4.188×10^{11}	1	4.188×10^{11}	2.19	0.1598	
BC	1.142×10^{11}	1	1.142×10^{11}	0.60	0.4519	
BD	1.100×10^{10}	1	1.100×10^{10}	0.057	0.8138	
CD	1.225×10^{12}	1	1.225×10^{12}	6.40	0.0231	Significant
A^2	1.961×10^{11}	1	1.961×10^{11}	1.02	0.3276	
B^2	1.287×10^{12}	1	1.287×10^{12}	6.72	0.0204	Significant
C^2	9.206×10^{11}	1	9.206×10^{11}	4.81	0.0445	Significant
D^2	1.640×10^{12}	1	1.640×10^{12}	8.57	0.0104	Significant
Residual	2.872×10^{12}	15	1.915×10^{11}			
Lack of Fit	2.869×10^{12}	10	2.869×10^{11}	3.65	0.0828	Not significant
Pure Error	2.899×10^{9}	5	5.798×10^{8}			
Corrected Total	1.122×10^{13}	29				

$R^2 = 0.7440$; Adjusted $R^2 = 0.5050$

References

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