Supplementary Information

Enzymatic processing of mussel shells to produce biorenewable calcium carbonate in seawater

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Table S5: Experimental data for 2^4 factorial design with center points using Multifect 6L to clean raw, shell-on mussels. Optimization of temperature, time, amount of enzyme solution, and reaction medium to yield the minimum meat residue.

Run	Temperature (°C) (X1)	Enzyme Loading (µL/g) (X2)	Time (h) (X3)	Medium[a] (X4)	Wt% Meat Remaining (Response)
1	35.0	1.0	4	NS	2.3
2	55.0	2.0	4	NS	0
3	35.0	2.0	2	NS	5.3
4	35.0	2.0	2	TW	12.6
5	55.0	2.0	4	NS	0
6	35.0	1.0	2	TW	8.4
7	35.0	1.0	2	TW	6.4
8	35.0	2.0	4	NS	6.4
9	55.0	1.0	4	TW	0.15
10	35.0	2.0	2	NS	9.1
11	35.0	2.0	4	TW	2.5
12	55.0	1.0	2	TW	0.91
13	35.0	2.0	2	TW	1.3
14	35.0	1.0	4	TW	2.7
15	55.0	2.0	2	NS	0.13
16	35.0	1.0	4	NS	0.93
17	35.0	2.0	2	NS	3.1
18	35.0	2.0	4	TW	1.4
19	55.0	2.0	2	TW	0
20	55.0	2.0	4	TW	0
21	45.0	1.5	3	NS	3.1
22	55.0	2.0	2	TW	0.21
23	35.0	1.0	4	NS	5.1
24	55.0	2.0	2	NS	0.8
25	55.0	1.0	2	TW	0.82
26	35.0	1.0	2	TW	1.2
27	55.0	1.0	4	TW	0.71
28	35.0	2.0	2	TW	0.91
29	35.0	1.0	2	NS	5.1
30	35.0	1.0	2	NS	8.1
31	55.0	1.0	2	TW	1.9
32	55.0	1.0	2	NS	0.52
33	55.0	2.0	4	NS	0.44
34	35.0	1.0	4	TW	6.4
35	55.0	1.0	4	NS	0.32
36	55.0	1.0	4	TW	0

37	55.0	2.0	4	TW	0.08
38	55.0	1.0	4	NS	0.52
39	55.0	1.0	4	NS	0
40	35.0	2.0	4	NS	0.94
41	55.0	1.0	2	NS	1.2
42	45.0	1.5	3	TW	2.1
43	35.0	1.0	2	NS	6.8
44	45.0	1.5	3	TW	3.4
45	35.0	2.0	4	TW	3.8
46	55.0	2.0	2	NS	0.92
47	35.0	2.0	4	NS	3.7
48	55.0	2.0	4	TW	0.11
49	35.0	1.0	4	TW	3.2
50	55.0	1.0	2	NS	1.8
51	45.0	1.5	3	NS	2.5
52	55.0	2.0	2	TW	0.62

[a]NS=natural seawater and TW=tap water

Table S6 and Design Summary: Analysis of variance and partial sum of squares for the 2^4 factorial design using Multifect 6L to clean raw, shell on mussels.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	26.07	2	13.04	46.11	5.47 E-12	significant
X1-Temperature	23.21	1	23.21	82.09	4.80 E-12	
X3-Time	2.86	1	2.86	10.12	0.0026	
Residual	13.86	49	0.28			
Lack of Fit	2.51	15	0.17	0.50	0.9233	not significant
Pure Error	11.35	34	0.33			
Cor Total	39.93	51				

Std. Dev.	0.53	**R-Squared**	0.6530
Mean	1.33	**Adj R-Squared**	0.6388
C.V. %	40.05	**Pred R-Squared**	0.6078
PRESS	15.66	**Adeq Precision**	14.713
-2 Log Likelihood	78.80	**BIC**	90.650
AICc	85.30		

The model has a square root transform with a constant, k=0.0126.

Final equation in terms of coded factors for the use of Multifect 6L to clean raw, shell on mussels:

$$Sqrt\ (wt.\ \%\ meat\ remaining + 0.01) = 1.33 - 0.69(X1) - 0.24(X3)$$

Residual Diagnostics for the model resulting from the 2^4 factorial design with center points using Multifect 6L to clean raw, shell-on mussels are shown in Figures S1-S3.

The analysis of variance (ANOVA) gave two significant effects, temperature (X1) and time (X3), for the 2^4 factorial design for the optimization of cleaning raw, shell on mussels with Multifect 6L. As shown in Figure S12, when temperature and time are increased, the less residual meat that remains. Ultimately meaning, at higher temperature and longer reaction times, we get cleaner mussel shells.

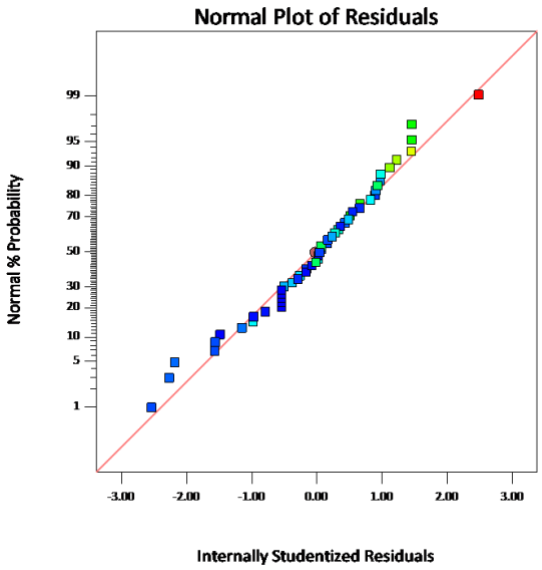

Figure S9: Normal Plot of residuals for the model developed from the 2^4 factorial design used to optimize the use of Multifect 6L to clean raw, shell on mussel.

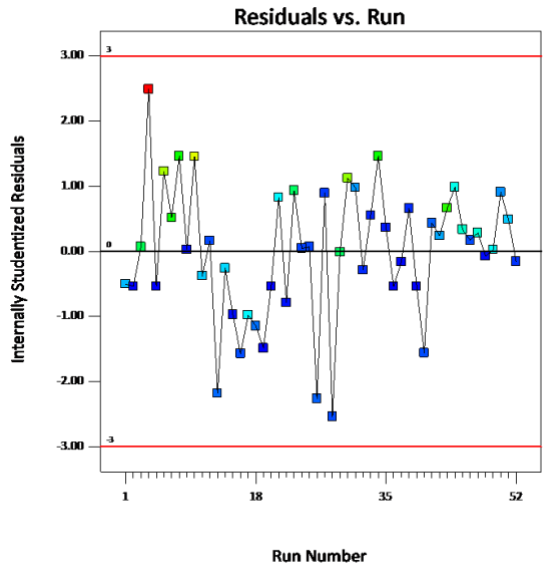

Figure S10: Residuals vs. run for the model developed from the 2^4 factorial design used to optimize the use of Multifect 6L to clean raw, shell on mussel.

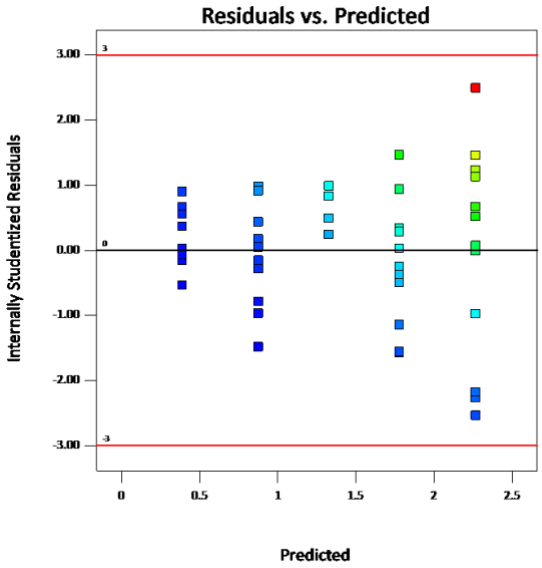

Figure S11: Residuals vs. predicted for the model developed from the 2^4 factorial design used to optimize the use of Multifect 6L to clean raw, shell on mussel.

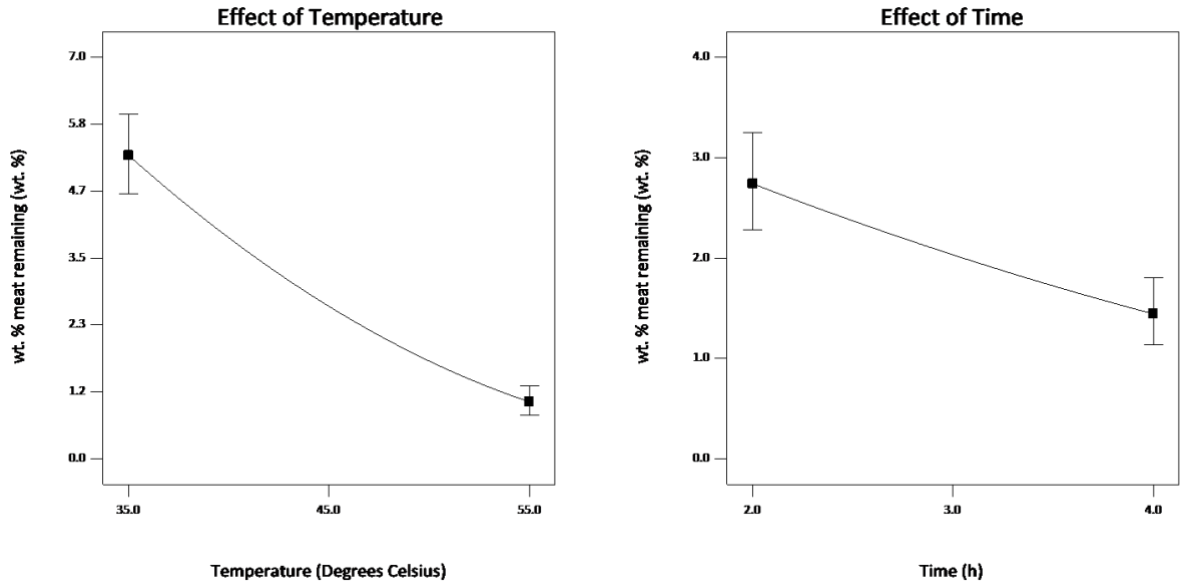

Figure S12: One factor plots for the 2^4 factorial design for the optimization of reaction conditions with respect to temperature, amount of enzyme solution, time and medium for the cleaning of raw, shell on mussels using Multifect 6L. The effect of temperature (X1), left and the effect of time (X3), right.

Table S7: Experimental data for the I-optimal custom design (a response surface method) with 4 replicate points and 8 center points using Multifect 7L to clean cooked, shell-on mussels. Optimization of time, amount of enzyme solution, and reaction medium to yield the minimum meat residue.[a]

Run	Enzyme Loading (µL/g) (Z1)	Time (h) (Z2)	Medium[b] (Z3)	Wt% Meat Remaining (Response)
1	3.0	2	NS	1.5
2	4.5	6	NS	0.28
3	4.5	6	SS	0.25
4	6.0	2	NS	1.1
5	4.5	6	NS	0.2
6	6.0	10	NS	0
7	6.0	10	SS	0
8	4.5	6	SS	0.23
9	4.5	6	NS	0.2
10	3.0	2	SS	1.7
11	3.0	10	NS	0.12
12	3.0	10	NS	0.86
13	4.5	6	SS	0.24
14	3.0	2	NS	1.7
15	3.0	10	SS	0.13
16	4.5	6	NS	0.23
17	6.0	10	NS	0.09
18	6.0	2	NS	0.62
19	4.5	6	SS	0.17
20	3.0	2	SS	0.48
21	6.0	2	SS	0.58
22	6.0	2	SS	2.28
23	3.0	10	SS	0.95
24	6.0	10	SS	0.22

a. All reactions performed at room temperature; b. NS = natural seawater (salinity, 25 ppt) and SS = synthetic seawater (salinity, 35 ppt)

Table S8 and Design Summary: Analysis of variance and partial sum of squares for the I-optimal custom design using Multifect 7L to clean cooked, shell on mussels.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	24.09	2	12.05	14.64	0.0001	significant
Z1-Enzyme Loading	5.25	1	5.25	6.38	0.0196	
Z2-Time	18.84	1	18.84	22.90	< 0.0001	
Residual	17.28	21	0.82			
Lack of Fit	5.22	7	0.75	0.87	0.5548	not significant
Pure Error	12.06	14	0.86			
Cor Total	41.38	23				

Std. Dev.	0.91	**R-Squared**	0.5823
Mean	-1.14	**Adj R-Squared**	0.5426
C.V. %	79.28	**Pred R-Squared**	0.4061
PRESS	24.57	**Adeq Precision**	10.340
-2 Log Likelihood	60.23	**BIC**	69.76
AICc	67.43		

The model has a natural log transform with a constant, k=0.0126.

Final equation in terms of coded factors for the use of Multifect 7L to clean cooked, shell on mussels:

$$ln\ (wt.\ \%\ meat\ remaining + 0.01) = -1.14 - 0.57(Z1) - 1.09(Z2)$$

Residual Diagnostics for the model resulting from the I-optimal design with center points using Multifect 7L to clean cooked, shell-on mussels are shown in Figures S5-S6.

The analysis of variance (ANOVA) gave two significant effects, time (Z1) and amount of enzyme solution (Z2), for the I-optimal design for the optimization of cleaning cooked, shell on mussels with Multifect 7L. As shown in Figure S16, when time and amount of enzyme solution are increased, the less residual meat that remains. Ultimately meaning, the longer reaction times and the higher the enzyme solution loading, the cleaner mussel shells.

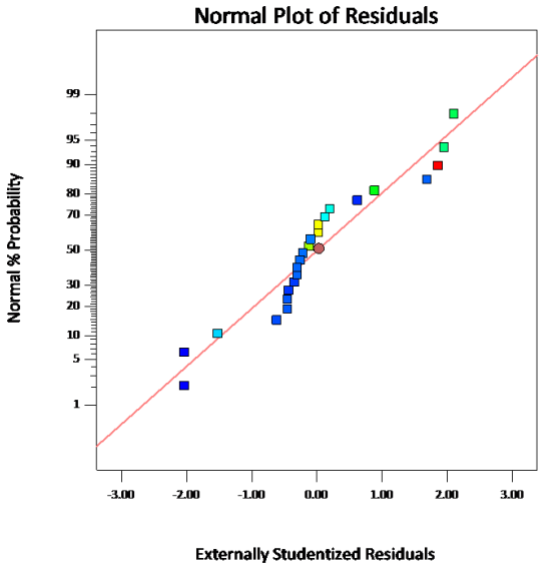

Figure S13: Normal Plot of residuals for the model developed from the I-optimal design used to optimize the use of Multifect 7L to clean cooked, shell on mussel.

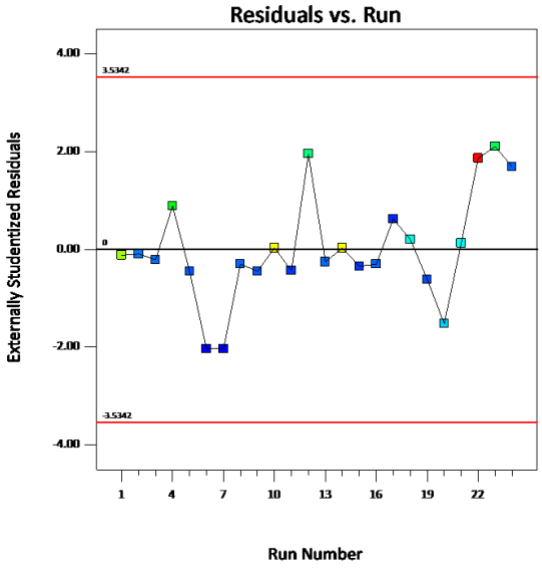

Figure S14: Residuals vs. run for the model developed from the I-optimal design used to optimize the use of Multifect 7L to clean cooked, shell on mussel.

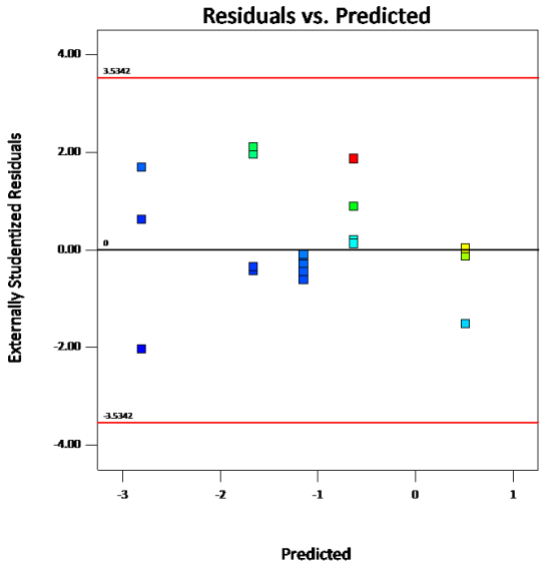

Figure S15: Residuals vs. predicted for the model developed from the I-optimal design used to optimize the use of Multifect 7L to clean cooked, shell on mussel.

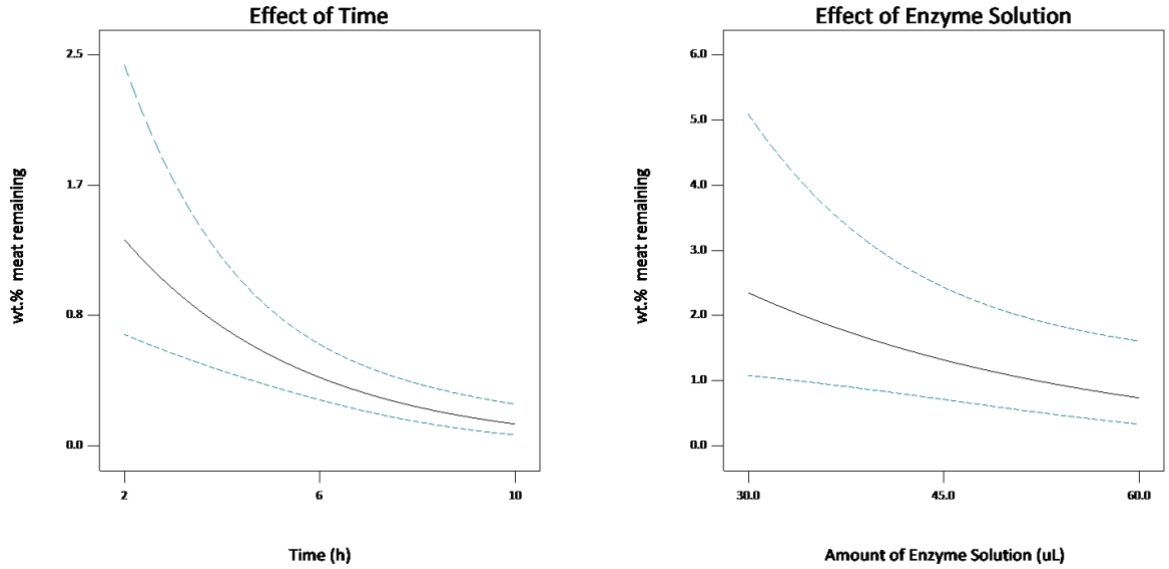

Figure S16: One factor plots for the I-optimal design for the optimization of reaction conditions with respect to time, amount of enzyme solution, and medium for the cleaning of cooked, shell on mussels using Multifect 7L. The effect of time (Z1), left, and the effect of the amount of enzyme solution (Z2), right.