Appendix

Appendix-I: Extended phylogenetic trees:

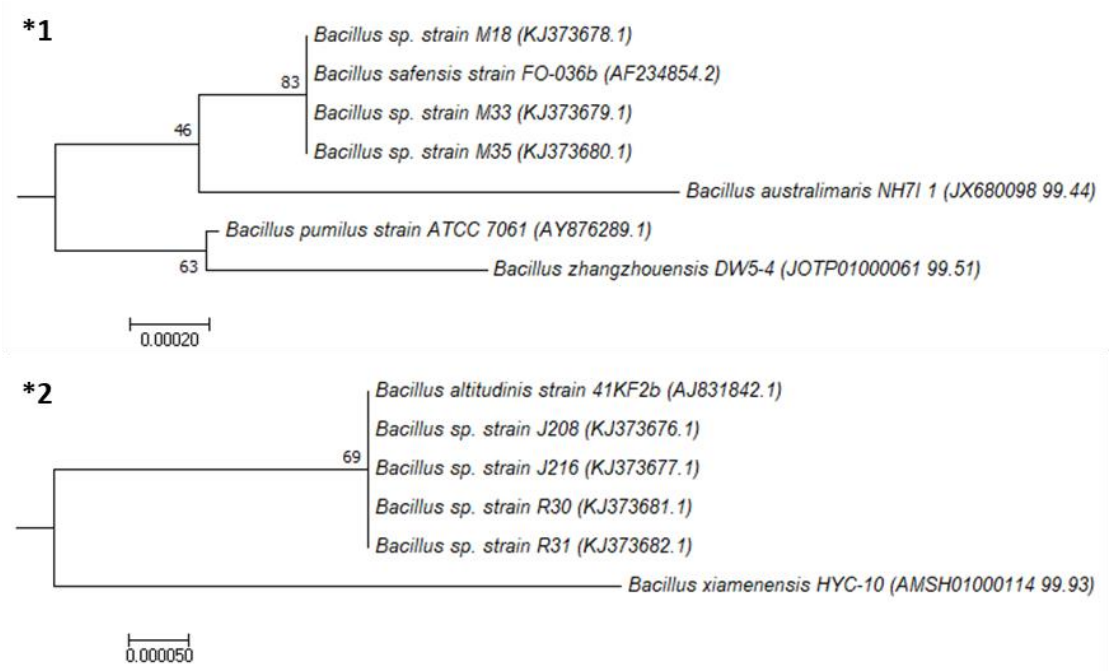

Figure A2.1. Extended phylogenetic tree of *B. safensis* (*1) and *B. altitudinis* (*2) cluster based on 16S rRNA gene sequence analysis from Figure 2.7.

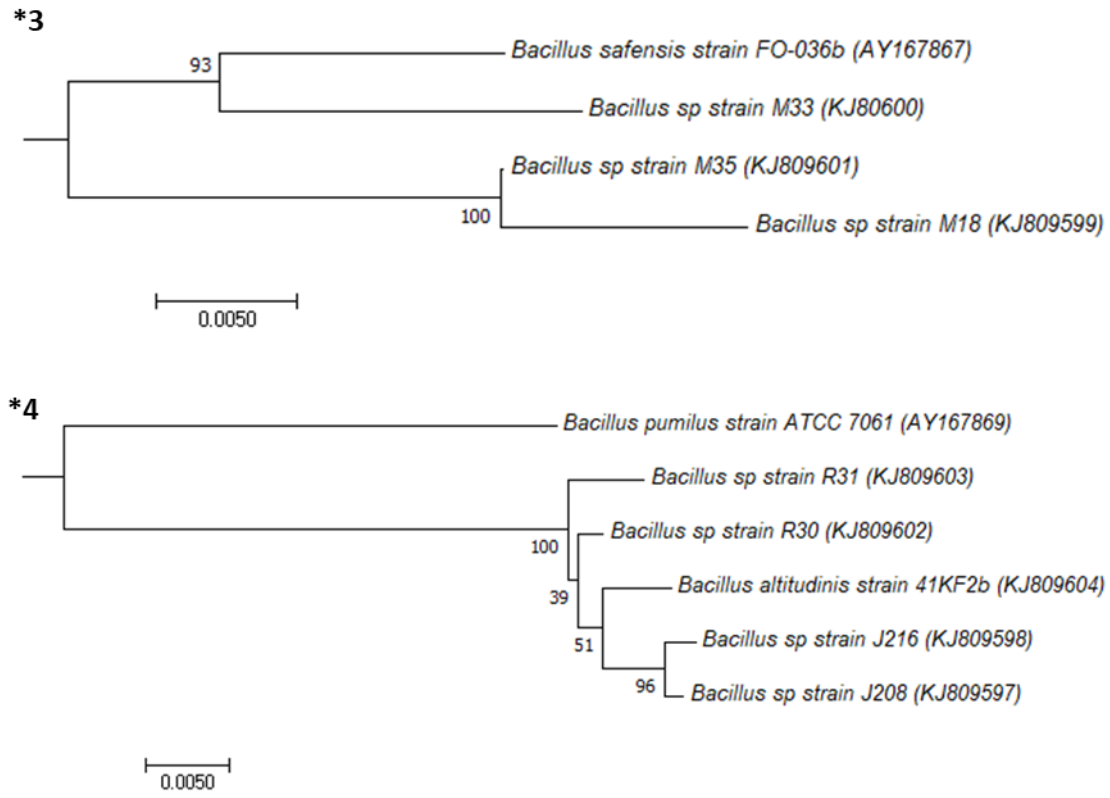

Figure A2.2. Extended phylogenetic tree of *B. safensis* (*3) and *B. altitudinis* (*4) cluster based on *gyrB* gene sequence analysis from Figure 2.10.

Appendix -II: Statistical analysis and model diagnostic plots for inducer substrate optimization using CCD-RSM:

A2.1. Effect of individual factors and their interactions on xylanase production response from different isolates:

A.2.1.1. M35-xylanase production:

During sequential analysis of the response surface for M35 xylanase production, it was observed that the quadratic model fits well with the data. As shown in Table A2.1, the quadratic model was significant above 99.99 % confidence level with p-value < 0.0001. Similarly, the Lack of Fit F-value for quadratic model was 0.2267 suggesting that Lack of Fit was not significant, relative to the pure error and there is a 22.67% chance that a "Lack of Fit F-value" this large could occur due to noise.

The quadratic model values of correlation coefficient (R^2), adjusted R^2 and predicted R^2 were 0.997, 0.993 and 0.979 respectively. Values of R^2 as well as adjusted R^2 were > 0.7. The predicted R^2 was in reasonable agreement with the adjusted R^2. PRESS value of 7.00 was also suitable as it was least in comparison to other models. All these results from Table A2.1 indicated that the quadratic model was the most appropriate one for analysis of xylanase production data.

Therefore, analysis of variance (ANOVA) for M35 xylanase production response was performed using the quadratic model. The values of sum of squares, mean squares, F-value, and *p*-value for model, selected factors and their interactions are given in Table A2.2. P values < 0.05 indicates that the model terms are significant. In this case the Model F-value of 485.54 implies the model was highly significant with p-values < 0.0001 (with confidance level > 99.99%) and there is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. *p*-values less than 0.05 (at confidence level of 95%) indicates the significance of the model terms.

In this case A, B, AB, A^2, B^2 are significant model terms. *p*-values obtained for both individual factors, viz., A (wheat bran, WB) and B (citrus peel, CP) were 0.0012 and <0.0001 respectively suggesting that CP had more significant effect on xylanase production than WB. The *p*-value of AB factor (0.0355) suggested that the interaction between WB and CP was significant for xylanase production at 95% confidence level.

The adequate precision value (39.928) of signal to noise ratio for M35 xylanase production indicated an adequate signal and the model can be used to navigate the design space. The following equation shows fitted quadratic model in terms of actual factors:

Xylanase = -7.00033 + 17.99698*WB + 11.67553*CP – 0.61709*WB*CP – 5.24935*WB^2 – 4.43148*CP^2.

Table A2.1 Fit summary plot for M35-xylanase production obtained using CCD:

1. Sequential Model Sum of Squares [Type I]						
Source	Sum of Squares	DOF	Mean Square	F-value	*p*-value, Prob>F	Prediction
Mean vs Total	485.32	1	485.32			
Linear vs Mean	76.84	2	38.42	1.24	0.3398	
2FI vs Linear	1.80	1	1.80	0.051	0.8274	
Quadratic vs 2FI	**245.13**	**2**	**122.57**	**555.55**	**< 0.0001**	**Suggested**
Cubic vs Quadratic	0.89	2	0.45	6.37	0.0832	Aliased
Residual	0.21	3	0.070			
Total	810.20	11	73.65			
2. Lack of Fit Tests						
Source	Sum of Squares	DOF	Mean Square	F-value	*p*-value, Prob>F	
Linear	247.86	6	41.31	475.52	0.0021	
2FI	246.06	5	49.21	566.48	0.0018	
Quadratic	**0.93**	**3**	**0.31**	**3.57**	**0.2267**	**Suggested**
Cubic	0.037	1	0.037	0.42	0.5832	Aliased
Pure Error	0.17	2	0.087			
3. Model Summary Statistics						
Source	Std. Deviation	R^2	Adjusted R^2	PredictedR^2	PRESS	
Linear	5.57	0.2365	0.0457	-0.1654	378.63	
2FI	5.93	0.2421	-0.0828	-0.5134	491.68	
Quadratic	**0.47**	**0.9966**	**0.9932**	**0.9785**	**7.00**	**Suggested**
Cubic	0.26	0.9994	0.9978	0.9916	2.73	Aliased

Table A2.2 ANOVA for M35-xylanase production obtained using CCD:

ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	DOF	Mean Square	F-value	p-value, Prob>F	
Model	323.78	5	64.76	293.51	< 0.0001	Significant
A-WB	9.51	1	9.51	43.11	0.0012	
B-CP	67.33	1	67.33	305.19	< 0.0001	
AB	1.80	1	1.80	8.17	0.0355	
A^2	184.14	1	184.14	834.64	< 0.0001	
B^2	131.23	1	131.23	594.82	< 0.0001	
Residual	1.10	5	0.22			
Lack of Fit	0.93	3	0.31	3.57	0.2267	not significant
Pure Error	0.17	2	0.087			
Cor Total	324.88	10				

A2.1.2. R31-xylanase production:

During sequential analysis of the response surface for R31 xylanase production, it was observed that the quadratic model fits well with the data. As shown in Table A2.3, the quadratic model was significant above 99.99 % confidence level with p-value < 0.0001. Similarly, the Lack of Fit F-value for quadratic model was 0.6019 suggesting that Lack of Fit was not significant, relative to the pure error and there is a 60.19% chance that a "Lack of Fit F-value" this large could occur due to noise.

The quadratic model values of correlation coefficient (R^2), adjusted R^2 and predicted R^2 were 0.998, 0.996 and 0.990 respectively. Values of R^2 and adjusted R^2 were > 0.7. The predicted R^2 was in reasonable agreement with the adjusted R^2. PRESS value of 2.74 was also suitable as it was least in comparison to other models. All these results from Table A2.3 indicated that the quadratic model was the most appropriate one for analysis of xylanase production data.

Therefore, analysis of variance (ANOVA) for R31 xylanase production response was performed using the quadratic model. The values of sum of squares, mean squares, F-value, and *p*-value for model, selected factors and their interactions are given in Table A2.4. *p*-values < 0.05 indicates that the model terms are significant. In this case the Model F-value of 252.10 implies the model was highly significant with *p*-values <0.0001 (with confidance level > 99.99%) and there is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. *p*-values less than 0.05 (at confidence level of 95%) indicates the significance of the model terms.

In this case A, B, AB, A^2, B^2 are significant model terms. *p*-values obtained for both individual factors, viz., A (wheat bran, WB) and B (citrus peel, CP) were < 0.0001 suggesting that CP and WB both had significant effect on xylanase production. The *p*-value of AB factor (< 0.0001) suggested that the interaction between WB and CP also was significant for xylanase production at 99.99% confidence level.

The adequate precision value (55.712) of signal to noise ratio for R31 xylanase production indicated an adequate signal and the model can be used to navigate the design space. The following equation shows fitted quadratic model in terms of actual factors:

$$\text{Xylanase} = -0.77577 + 12.42360*WB + 2.37047*CP - 2.08562*WB*CP - 2.31111*WB^2 - 1.10729*CP^2.$$

Table A2.3. Fit summary plot for R31-xylanase production obtained using CCD:

1. Sequential Model Sum of Squares [Type I]						
Source	Sum of Squares	DOF	Mean Square	F-value	*p*-value, Prob>F	Prediction
Mean vs Total	433.89	1	433.89			
Linear vs Mean	194.48	2	97.24	13.37	0.0028	
2FI vs Linear	20.59	1	20.59	3.83	0.0911	
Quadratic vs 2FI	**37.03**	**2**	**18.51**	**164.80**	**< 0.0001**	**Suggested**
Cubic vs Quadratic	0.26	2	0.13	1.30	0.3914	Aliased
Residual	0.30	3	0.10			
Total	686.55	11	62.41			
2. Lack of Fit Tests						
Source	Sum of Squares	DOF	Mean Square	F-value	*p*-value, Prob>F	
Linear	57.92	6	9.65	74.92	0.0132	
2FI	37.33	5	7.47	57.94	0.0171	
Quadratic	**0.30**	**3**	**0.10**	**0.79**	**0.6019**	**Suggested**
Cubic	0.043	1	0.043	0.33	0.6225	Aliased
Pure Error	0.26	2	0.13			
3. Model Summary Statistics						
Source	Std. Deviation	R^2	Adjusted R^2	PredictedR^2	PRESS	
Linear	2.70	0.7697	0.7122	0.5659	109.69	
2FI	2.32	0.8512	0.7875	0.7001	75.78	
Quadratic	**0.34**	**0.9978**	**0.9956**	**0.9891**	**2.74**	**Suggested**
Cubic	0.32	0.9988	0.9960	0.9869	3.32	Aliased

Table A2.4. ANOVA for R31-xylanase production obtained using CCD:

ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	DOF	Mean Square	F-value	p-value, Prob>F	
Model	252.10	5	50.42	448.80	< 0.0001	significant
A-WB	41.90	1	41.90	372.92	< 0.0001	
B-CP	152.59	1	152.59	1358.21	< 0.0001	
AB	20.59	1	20.59	183.27	< 0.0001	
A^2	35.69	1	35.69	317.70	< 0.0001	
B^2	8.19	1	8.19	72.93	0.0004	
Residual	0.56	5	0.11			
Lack of Fit	0.30	3	0.10	0.79	0.6019	not significant
Pure Error	0.26	2	0.13			
Cor Total	252.66	10				55.712

A2.1.3. J208-xylanase production:

Through the sequential analysis of the response surface for J208 xylanase production, it was observed that the quadratic model fits well with data. As shown in Table A2.5, the quadratic model was significant above 99.99 % confidence level with p-value 0.0001. Similarly, the Lack of Fit F-value for quadratic model was 0.3268 suggesting that Lack of Fit was not significant, relative to the pure error and there is a 32.68% chance that a "Lack of Fit F-value" this large could occur due to noise.

The quadratic model values of correlation coefficient (R^2), adjusted R^2 and predicted R^2 were 0.996, 0.992 and 0.975 respectively. Values of R^2 and adjusted R^2 were > 0.7 and predicted R^2 was in reasonable agreement with the adjusted R^2. PRESS value of 7.39 was also suitable as it was least in comparison to other models. All these results from Table A2.5 indicated that the quadratic model was the most appropriate one for analysis of xylanase production data.

Therefore, analysis of variance (ANOVA) for J208 xylanase production response was performed using the quadratic model. The values of sum of squares, mean squares, F-value, and *p*-value for model, selected factors and their interactions are given in Table A2.6. *p*-values < 0.05 indicates that the model terms are significant. In this case the Model F-value of 294.84 implies the model was highly significant with p-values <0.0001 (with confidance level > 99.99%) and there is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. *p*-values less than 0.05 (at confidence level of 95%) indicates the significance of the model terms.

In this case A, B, AB, A^2, B^2 are significant model terms. *p*-values obtained for both individual factors, viz., A (wheat bran, WB) and B (citrus peel, CP) were <0.0001 suggesting that WB and CP both had significant effect on xylanase production. The *p*-value of AB factor (0.0002) suggested that the interaction between WB and CP was significant for xylanase production at 99.9% confidence level.

The adequate precision value (40.005) of signal to noise ratio for J208 xylanase production indicated an adequate signal and the model can be used to navigate the design space. The following equation shows fitted quadratic model in terms of actual factors:

Xylanase = -0.41634 + 13.69187*WB +1.41756*CP - 2.19675*WB*CP - $2.66641*WB^2$ - $0.86562*CP^2$.

Table A2.5. Fit summary plot for J208-xylanase production obtained using CCD:

1. Sequential Model Sum of Squares [Type I]						
Source	Sum of Squares	DOF	Mean Square	F-value	*p*-value, Prob>F	Prediction
Mean vs Total	465.93	1	465.93			
Linear vs Mean	224.44	2	112.22	12.53	0.0034	
2FI vs Linear	22.84	1	22.84	3.28	0.1132	
Quadratic vs 2FI	**47.56**	**2**	**23.78**	**96.31**	**0.0001**	**Suggested**
Cubic vs Quadratic	0.80	2	0.40	2.77	0.2084	Aliased
Residual	0.43	3	0.14			
Total	762.00	11	69.27			
2. Lack of Fit Tests						
Source	Sum of Squares	DOF	Mean Square	F-value	*p*-value, Prob>F	
Linear	71.35	6	11.89	83.08	0.0119	
2FI	48.51	5	9.70	67.78	0.0146	
Quadratic	**0.95**	**3**	**0.32**	**2.21**	**0.3268**	**Suggested**
Cubic	0.15	1	0.15	1.03	0.4166	Aliased
Pure Error	0.29	2	0.14			
3. Model Summary Statistics						
Source	Std. Deviation	R^2	Adjusted R^2	PredictedR^2	PRESS	
Linear	2.99	0.7580	0.6976	0.5305	139.01	
2FI	2.64	0.8352	0.7646	0.6336	108.49	
Quadratic	**0.50**	**0.9958**	**0.9917**	**0.9751**	**7.39**	**Suggested**
Cubic	0.38	0.9985	0.9951	0.9659	10.10	Aliased

Table A2.6. ANOVA for J208-xylanase production obtained using CCD:

ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	DOF	Mean Square	F-value	p-value, Prob>F	Significant
Model	294.84	5	58.97	238.84	< 0.0001	significant
A-WB	42.48	1	42.48	172.04	< 0.0001	
B-CP	181.96	1	181.96	737.00	< 0.0001	
AB	22.84	1	22.84	92.52	0.0002	
A^2	47.51	1	47.51	192.43	< 0.0001	
B^2	5.01	1	5.01	20.28	0.0064	
Residual	1.23	5	0.25			
Lack of Fit	0.95	3	0.32	2.21	0.3268	not significant
Pure Error	0.29	2	0.14			
Cor Total	296.07	10				

A2.2. Effect of individual factors and their interactions on pectinase production response from different isolates:

A2.2.1. M35-pectinase production:

During sequential analysis of the response surface for M35 pectinase production, it was observed that the quadratic model fits well with data. As shown in Table A2.7, the quadratic model was significant above 99.99 % confidence level with *p*-value < 0.0001. Similarly, the Lack of Fit F-value for quadratic model was 0.1544 suggesting that Lack of Fit was not significant, relative to the pure error and there is a 15.44% chance that a "Lack of Fit F-value" this large could occur due to noise.

The quadratic model values of correlation coefficient (R^2), adjusted R^2 and predicted R^2 were 0.998, 0.989 and 0.963 respectively. Values of R^2 as well as adjusted R^2 were > 0.7. The predicted R^2 was in reasonable agreement with the adjusted R^2. PRESS value of 8384.31 was also suitable as it was least in comparison to other models. All these results from Table A2.7 indicated that the quadratic model was the most appropriate one for analysis of pectinase production data.

Therefore, analysis of variance (ANOVA) for M35 pectinase production response was performed using the quadratic model. The values of sum of squares, mean squares, F-value, and *p*-value for model, selected factors and their interactions are given in Table A2.8. *p*-values < 0.05 indicates that the model terms are significant. In this case the Model F-value of $2.2x10^5$ implies the model was highly significant with p-values <0.0001 (with confidance level > 99.99%) and there is only a 0.1% chance that a "Model F-Value" this large could occur due to noise. *p*-values less than 0.05 (at confidence level of 95%) indicates the significance of the model terms. In this case A, B, A^2, B^2 are significant model terms. *p*-values obtained for both individual factors, viz., A (wheat bran, WB) and B (citrus peel, CP) were 0.0388 and 0.0001 respectively suggesting that CP had more significant effect on pectinase production than WB. The *p*-value of AB factor (0.4860) suggested that the interaction between WB and CP was not significant for pectinase production at 95% confidence level also.

The adequate precision value 31.832 of signal to noise ratio for M35 pectinase production indicated an adequate signal and the model can be used to navigate the design space. The following equation shows fitted quadratic model in terms of actual factors:

Pectinase = -175.34669 + 468.44025 * WB + 283.31450*CP + 5.51010*WB*CP - 151.41107*WB^2 - 115.41191*CP^2.

Table A2.7. Fit summary plot for M35-pectinase production obtained using CCD:

1. Sequential Model Sum of Squares [Type I]						
Source	Sum of Squares	DOF	Mean Square	F-value	*p*-value, Prob>F	Prediction
Mean vs Total	255000.00	1	255000.00			
Linear vs Mean	33599.79	2	16799.89	0.70	0.5236	
2FI vs Linear	143.71	1	143.71	0.01	0.9442	
Quadratic vs 2FI	**189900.00**	**2**	**94972.90**	**373.64**	**< 0.0001**	**Suggested**
Cubic vs Quadratic	1.12	2	0.56	0.00	0.9987	Aliased
Residual	1269.81	3	423.27			
Total	480000.00	11	43637.14			
2. Lack of Fit Tests						
Source	Sum of Squares	DOF	Mean Square	F-value	*p*-value, Prob>F	
Linear	191200.00	6	31871.00	474.21	0.0021	
2FI	191100.00	5	38216.46	568.63	0.0018	
Quadratic	**1136.51**	**3**	**378.84**	**5.64**	**0.1544**	**Suggested**
Cubic	1135.39	1	1135.39	16.89	0.0544	Aliased
Pure Error	134.42	2	67.21			
3. Model Summary Statistics						
Source	Std. Deviation	R^2	Adjusted R^2	PredictedR^2	PRESS	
Linear	154.66	0.1494	-0.0633	-0.3086	294400.00	
2FI	165.28	0.1500	-0.2143	-0.6025	360500.00	
Quadratic	**15.94**	**0.9944**	**0.9887**	**0.9627**	**8384.31**	**Suggested**
Cubic	20.57	0.9944	0.9812	0.6756	72967.57	

A2.8. ANOVA for M35-pectinase production obtained using CCD:

ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	DOF	Mean Square	F-value	p-value, Prob>F	
Model	223700.00	5	44737.86	176	< 0.0001	significant
A-WB	1968.36	1	1968.36	7.74	0.0388	
B-CP	31631.43	1	31631.43	124.44	0.0001	
AB	143.71	1	143.71	0.57	0.4860	
A^2	153200.00	1	1.53E+05	602.69	< 0.0001	
B^2	89008.53	1	89008.53	350.17	< 0.0001	
Residual	1270.93	5	254.19			
Lack of Fit	1136.51	3	378.84	5.64	0.1544	not significant
Pure Error	134.42	2	67.21			
Cor Total	225000.00	10				

A2.2.2. R31-pectinase production:

During sequential analysis of the response surface for R31 pectinase, it was observed that the quadratic model fits well with pectinase production data. As shown in Table A2.9, the quadratic model was significant above 99.9 % confidence level with *p*-value < 0.001. Similarly, the Lack of Fit F-value for quadratic model was 0.1146 suggesting that Lack of Fit was not significant, relative to the pure error and there is a 11.46% chance that a "Lack of Fit F-value" this large could occur due to noise.

The quadratic model values of correlation coefficient (R^2), adjusted R^2 and predicted R^2 were 0.973, 0.946 and 0.818 respectively. Values of R^2 and adjusted R^2 were > 0.7. The predicted R^2 was also in a reasonable agreement with the adjusted R^2. PRESS value of 122600 was also suitable as it was least in comparison to other models. All these results from TableA2.9 indicated that the quadratic model was the most appropriate one for analysis of xylanase production data.

Therefore, analysis of variance (ANOVA) for R31 pectinase production response was performed using the quadratic model. The values of sum of squares, mean squares, F-value, and *p*-value for model, selected factors and their interactions are given in Table A2.10 *p*-values < 0.05 indicates that the model terms are significant. In this case the Model F-value of 6.5 x 10^5 implies the model was highly significant with *p*-values < 0.001 (with confidance level > 99.9 %) and there is only a 0.1% chance that a "Model F-Value" this large could occur due to noise. *p*-values less than 0.05 (at confidence level of 95%) indicates the significance of the model terms.

In this case A, B, AB, A^2, B^2 are significant model terms. *p*-values obtained for both individual factors, viz., A (wheat bran, WB) and B (citrus peel, CP) were 0.0258 and 0.0011 suggesting that CP had more significant effect on pectinase production than WB. The *p*-value of AB factor (0.7168) suggested that the interaction between WB and CP was not significant for pectinase production at 95.00% confidence level also.

The adequate precision value (15.203) of signal to noise ratio for R31 pectinase production indicated an adequate signal and this model can be used to navigate the design space. The following equation shows fitted quadratic model in terms of actual factors:

Pectinase = -199.06642 + 696.91749*WB + 477.78140*CP + 10.65257*WB*CP - 212.78253*WB^2 - 206.83482*CP^2.

Table A2.9. Fit summary plot for R31-pectinase production obtained using CCD:

1. Sequential Model Sum of Squares [Type I]						
Source	Sum of Squares	DOF	Mean Square	F-value	*p*-value, Prob>F	Prediction
Mean vs Total	1052000.00	1	1052000.00			
Linear vs Mean	198700.00	2	99374.55	1.68	0.2461	
2FI vs Linear	537.13	1	537.13	0.01	0.9315	
Quadratic vs 2FI	**454800.00**	**2**	**227400.00**	**62.42**	**0.0003**	**Suggested**
Cubic vs Quadratic	16591.69	2	8295.84	15.34	0.0266	Aliased
Residual	1622.13	3	540.71			
Total	1724000.00	11	156800.00			
2. Lack of Fit Tests						
Source	Sum of Squares	DOF	Mean Square	F-value	*p*-value, Prob>F	
Linear	472100.00	6	78683.84	110.85	0.0090	
2FI	471600.00	5	94313.18	132.87	0.0075	
Quadratic	**16794.17**	**3**	**5598.06**	**7.89**	**0.1146**	**Suggested**
Cubic	202.49	1	202.49	0.29	0.6467	Aliased
Pure Error	1419.65	2	709.82			
3. Model Summary Statistics						
Source	Std. Deviation	R^2	Adjusted R^2	PredictedR^2	PRESS	
Linear	243.29	0.2956	0.1195	-0.0821	727500.00	
2FI	259.94	0.2964	-0.0051	-0.4922	1003000.00	
Quadratic	**60.36**	**0.9729**	**0.9458**	**0.8176**	**122600.00**	**Suggested**
Cubic	23.25	0.9976	0.9920	0.9760	16153.42	Aliased

Table A2.10 ANOVA for R31-pectinase production obtained using CCD:

ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	DOF	Mean Square	F-value	p-value, Prob>F	
Model	654100.00	5	130800.00	35.91	0.0006	significant
A-WB	35841.69	1	35841.69	9.84	0.0258	
B-CP	162900.00	1	162900.00	44.72	0.0011	
AB	537.13	1	537.13	0.15	0.7168	
A^2	302600.00	1	302600.00	83.06	0.0003	
B^2	285900.00	1	285900.00	78.48	0.0003	
Residual	18213.82	5	3642.76			
Lack of Fit	16794.17	3	5598.06	7.89	0.1146	not significant
Pure Error	1419.65	2	709.82			
Cor Total	672300.00	10				

A2.2.3. J208-pectinase production:

Through the sequential analysis of the response surface for J208 pectinase production, it was observed that the quadratic model fits well with data. As shown in Table A2.11, the quadratic model was significant above 99.9 % confidence level with *p*-value 0.001. Similarly, the Lack of Fit F-value for quadratic model was 0.2793 suggesting that Lack of Fit was not significant, relative to the pure error and there is a 27.93% chance that a "Lack of Fit F-value" this large could occur due to noise.

The quadratic model values of correlation coefficient (R^2), adjusted R^2 and predicted R^2 were 0.963, 0.927 and 0.774 respectively. Values of R^2 and adjusted R^2 were > 0.7. Predicted R^2 was in reasonable agreement with the adjusted R^2. PRESS value of 183000 was also suitable as it was least in comparison to other models. All these results from Table A2.11 indicated that the quadratic model was the most appropriate one for analysis of pectinase production data.

Therefore, analysis of variance (ANOVA) for J208 pectinase production response was performed using the quadratic model. The values of sum of squares, mean squares, F-value, and *p*-value for model, selected factors and their interactions are given in table A2.12. *p*-values < 0.05 indicates that the model terms are significant. In this case the Model F-value of $7.7x10^5$ implies the model was highly significant with *p*-values < 0.002 (with confidance level > 99.8%) and there is only a 0.2% chance that a "Model F-Value" this large could occur due to noise. *p*-values less than 0.05 (at confidence level of 95%) indicates the significance of the model terms.

In this case A, B, AB, A^2, B^2 are significant model terms. *p*-values obtained for both individual factors, viz., A (wheat bran, WB) and B (citrus peel, CP) were 0.0166 and 0.0047 suggesting that CP had more significant effect on pectinase production than WB. The *p*-value of AB factor (0.9232) suggested that the interaction between WB and CP was significant for pectinase production at 95.00% confidence level also.

The adequate precision value (12.494) of signal to noise ratio for J208 pectinase production indicated an adequate signal and the model can be used to navigate the design space. The following equation shows fitted quadratic model in terms of actual factors:

Pectinase = -304.18615 + 846.60422*WB + 541.58813*CP + 3.57315*WB*CP - 265.56470*WB^2 - 220.63661*CP^2.

Table A2.11. Fit summary plot for J208-pectinase obtained using CCD:

1. Sequential Model Sum of Squares [Type I]						
Source	Sum of Squares	DOF	Mean Square	F-value	*p*-value, Prob>F	Prediction
Mean vs Total	1028000.00	1	1028000.00			
Linear vs Mean	153400.00	2	76680.96	0.94	0.4282	
2FI vs Linear	60.43	1	60.43	0.00	0.9803	
Quadratic vs 2FI	**619900.00**	**2**	**309900.00**	**52.65**	**0.0004**	**Suggested**
Cubic vs Quadratic	6655.87	2	3327.94	0.44	0.6808	Aliased
Residual	22780.38	3	7593.46			
Total	1831000.00	11	166500.00			
2. Lack of Fit Tests						
Source	Sum of Squares	DOF	Mean Square	F-value	*p*-value, Prob>F	
Linear	643600.00	6	107300.00	37.15	0.0264	
2FI	643500.00	5	128700.00	44.58	0.0221	
Quadratic	**23661.88**	**3**	**7887.29**	**2.73**	**0.2793**	**Suggested**
Cubic	17006.01	1	17006.01	5.89	0.1360	Aliased
Pure Error	5774.36	2	2887.18			
3. Model Summary Statistics						
Source	Std. Deviation	R^2	Adjusted R^2	PredictedR^2	PRESS	
Linear	284.91	0.1910	-0.0112	-0.2505	1004000.00	
2FI	304.56	0.1911	-0.1555	-0.4419	1157000.00	
Quadratic	**76.73**	**0.9633**	**0.9267**	**0.7742**	**181300.00**	**Suggested**
Cubic	87.14	0.9716	0.9054	-0.3720	1101000.00	Aliased

Table A2.12. ANOVA for pectinase production by *B. altitudinis* J208 obtained using CCD:

ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	DOF	Mean Square	F-value	p-value, Prob>F	
Model	773300.00	5	154700.00	26.27	0.0013	Significant
A-WB	15410.44	1	15410.44	2.62	0.0166	
B-CP	138000.00	1	138000.00	23.43	0.0047	
AB	60.43	1	60.43	0.01	0.9232	
A^2	471300.00	1	471300.00	80.05	0.0003	
B^2	325300.00	1	325300.00	55.26	0.0007	
Residual	29436.25	5	5887.25			
Lack of Fit	23661.88	3	7887.29	2.73	0.2793	not significant
Pure Error	5774.36	2	2887.18			
Cor Total	802700.00	10				

A2.3. Model diagnostics:

During analysis of the designed experiment and received responses, the Design expert software provided different diagnostic plots which helped to study the effect of selected experimental variables on responses. Few of such plots like (I) Normal probability plot of residuals, (II) Plot of studentized residuals versus predicted residuals and (III) Plot of actual versus predicted values were used to diagnose the statistical properties of the model and its adequacy, which is an important part of data analysis.

A2.3.1. Normal probability plot of residuals:

Normal probability plot of residuals is the most important part of the diagnostics. These plots indicate whether the residuals follow a normal distribution, i.e., the residual plots will follow a straight line. The normality assumptions for residuals of individual run for xylanase and pectinase production responses from each of three isolates when plotted, were found to be distributed around a straight line suggesting that the quadratic model fits well for both the responses from each isolate as shown in Figure A2.1 (A-F).

A2.3.2. Plots of studentized residuals versus predicted values:

This plot tests the assumption of constant variance. As shown in Figure A2.2 (A-F), the patterns of the plots for xylanase and pectinase production responses showed random distribution of studentized residuals in all the runs, indicated that the assumption of constant variance obtained using the quadratic model was true.

A2.3.3. Plot of actual versus predicted values:

This plot shows point of the actual response values versus the predicted response values. A pattern of the plot of the actual values versus the predicted values for xylanase and pectinase production response were distributed on or around the line and indicated that the predicted data points and actual data points matched and the quadratic model fits well for each response Figure 6.10 (A-F).

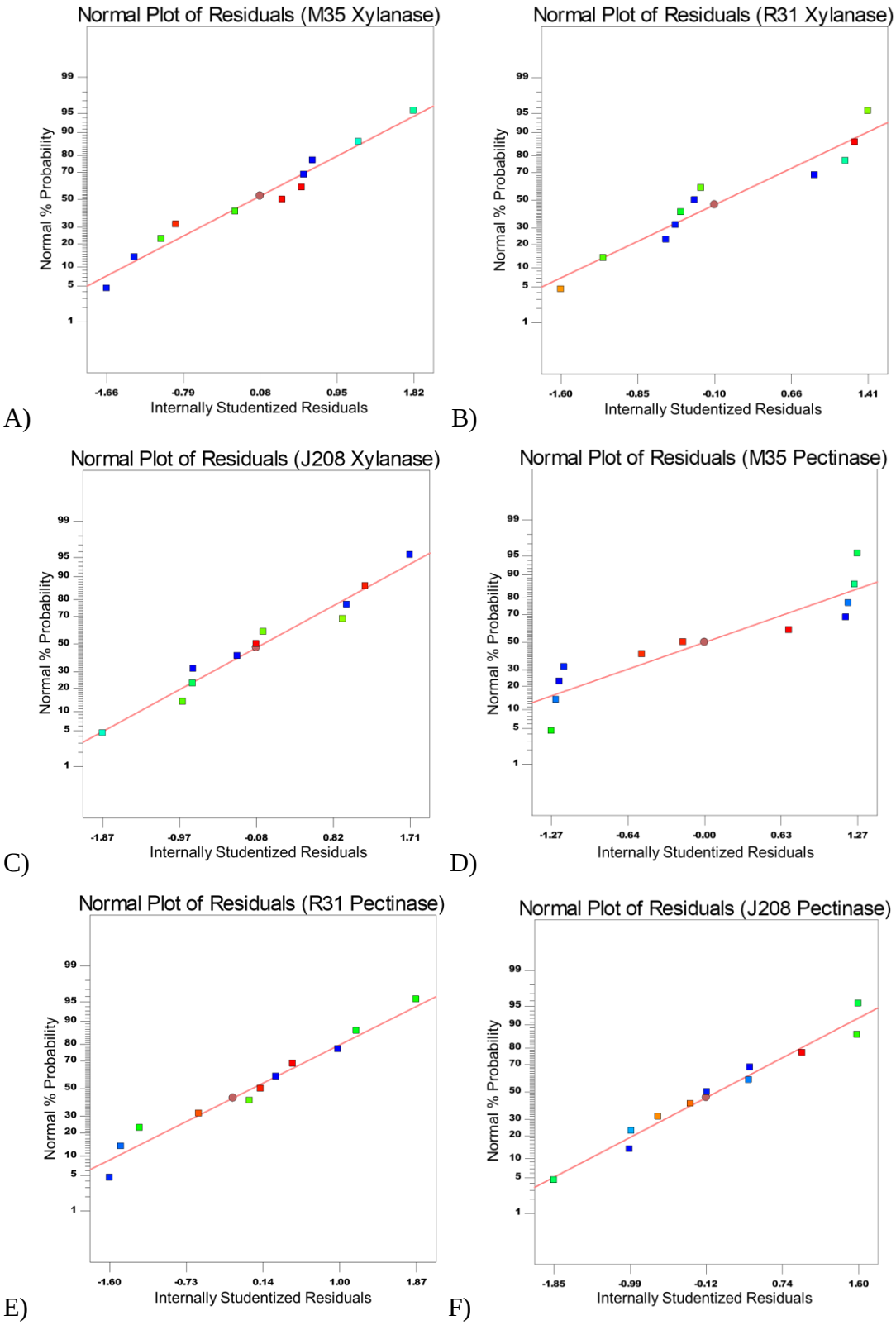

Figure A2.1. Normal probability plot of residuals for xylanase and pectinase production responses obtained using CCD from *Bacillus* spp. isolates:
(A-C): Normal probability plot of residuals for xylanase production response obtained from (A) *B. safensis* M35, (B) *B. altitudinis* R31, (C) *B. altitudinis* J208; (D-E): Normal probability plot of residuals for pectinase production response obtained from (D) *B. safensis* M35, (E) *B. altitudinis* R31, (F) *B. altitudinis* J208.

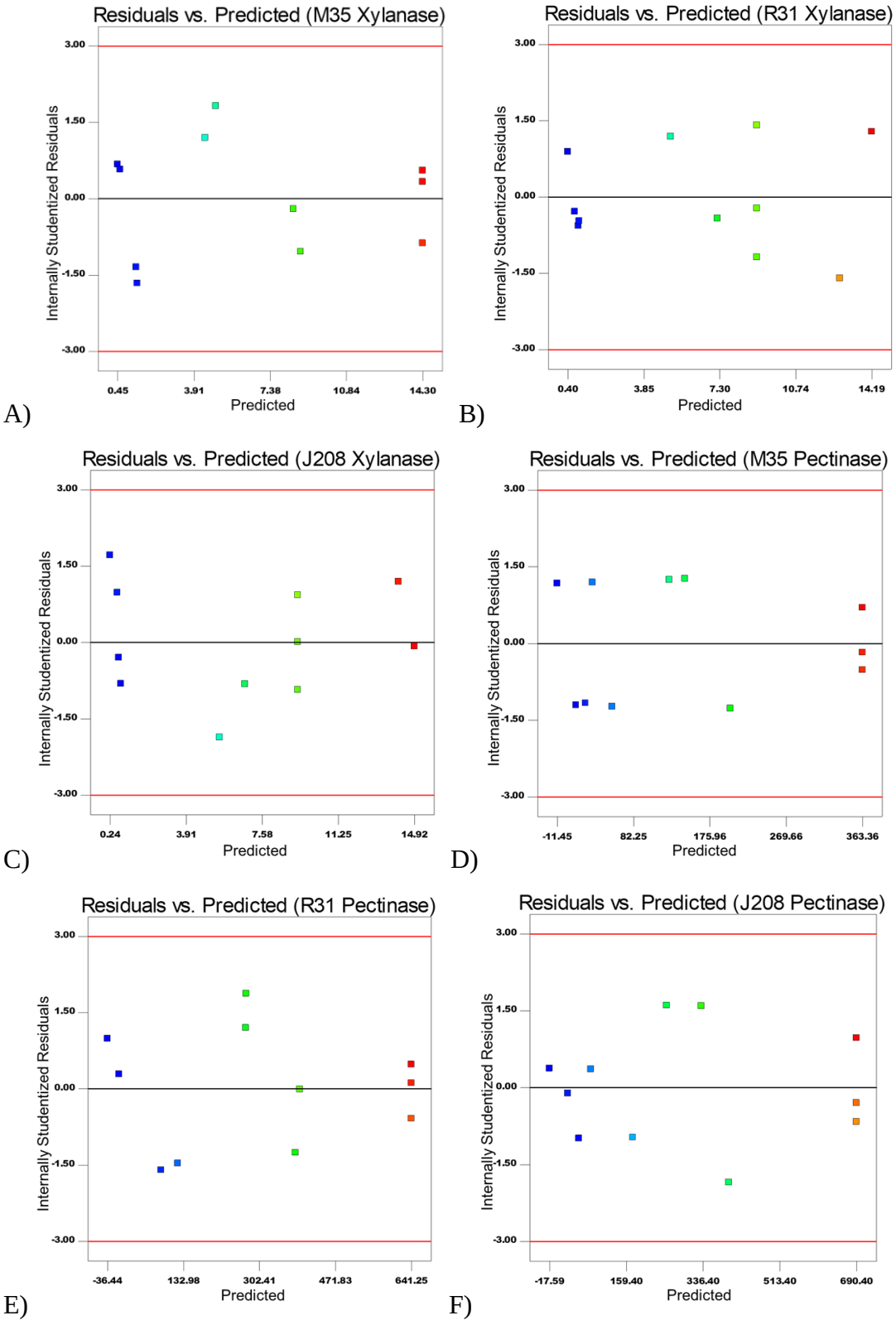

Figure A2.2. Plot of studentized residuals versus predicted values for xylanase and pectinase production responses obtained using CCD from *Bacillus* spp. isolates: (A-C): Plot of studentized residuals versus predicted values for xylanase production response obtained from (A) *B. safensis* M35, (B) *B. altitudinis* R31, (C) *B. altitudinis* J208; (D-E): Plot of studentized residuals versus predicted values for pectinase production response obtained from (D) *B. safensis* M35, (E) *B. altitudinis* R31, (F) *B. altitudinis* J208.

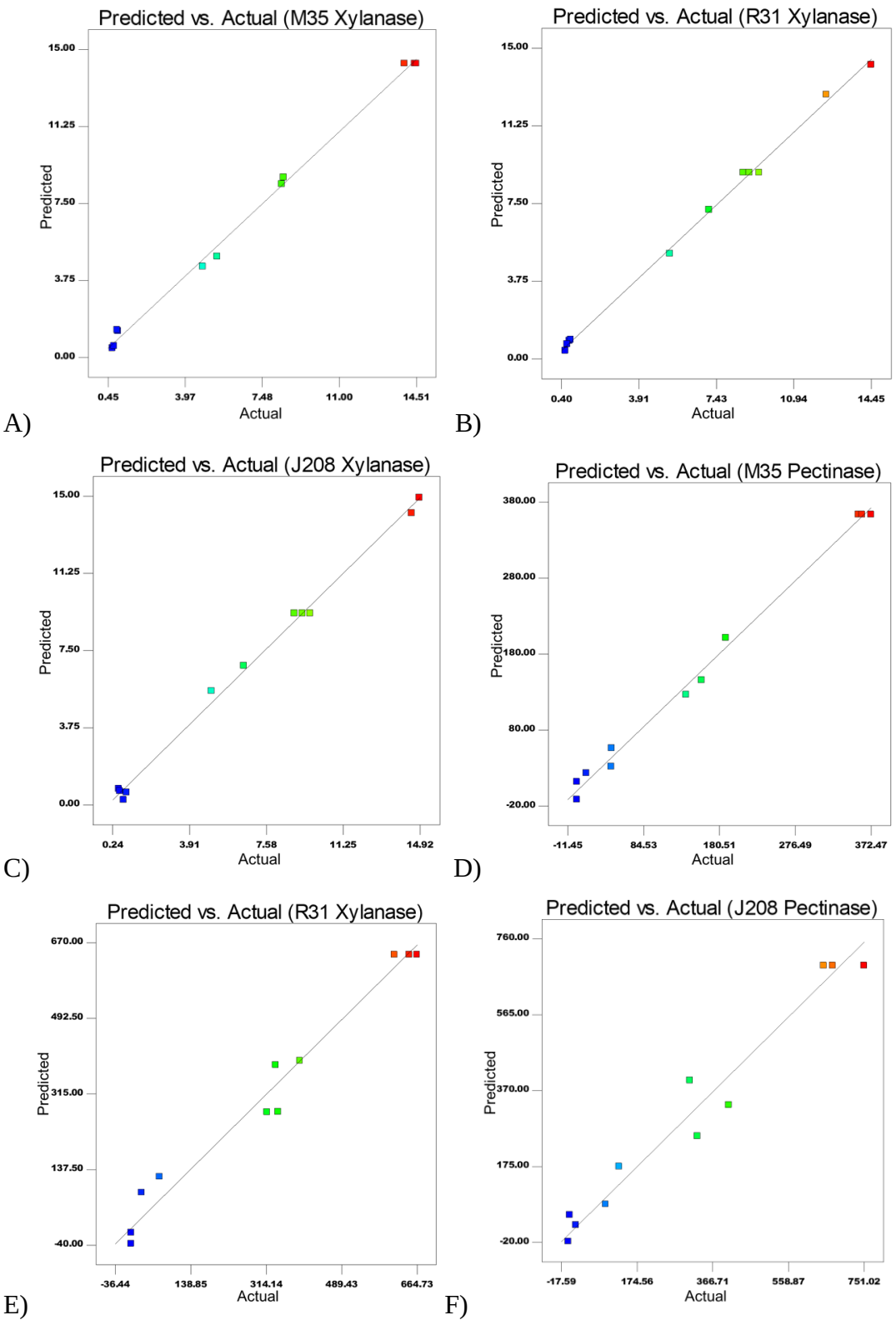

Figure A2.3: Plot of actual versus predicted values for xylanase and pectinase production responses obtained using CCD from *Bacillus* spp. isolates:
(A-C): Plot of actual versus predicted values for xylanase production response obtained from (A) *B. safensis* M35, (B) *B. altitudinis* R31, (C) *B. altitudinis* J208; (D-E): Plot of actual versus predicted values for pectinase production response obtained from (D) *B. safensis* M35, (E) *B. altitudinis* R31, (F) *B. altitudinis* J208.

A2.4 Model graphs:

As diagnosis of residuals revealed no statistical problem and showed that the quadratic models were suitable, further response surface plots of xylanase and pectinase production response for each isolate were generated.

A2.4.1. Xylanase production response:

The quadratic model suited well for xylanase production response. The one factor effect plots representing the xylanase production over changes in independent variable A-WB and B-CP are shown for each of the *Bacillus* isolate, i.e., M35, R31 and J208 in Figure A2.4 (A-F). It was visible that increase of WB and CP concentrations up to certain level, positively affected xylanase production while beyond that concentration, they negatively affect the xylanase production response by *B. safensis* M35 (A, B). While the plots C and E suggested the moderate positive effect of WB on xylanase production response, negative effect of CP on xylanase production response was exhibited by plots D and F for *B. altitudinis* R31 and J208. While. These results are in accordance with the results present in Table, A2.2, A2.4 and A2.6 where it was noted that the CP had more significant effect on xylanase production response than WB.

A2.4.2. Pectinase production response:

The quadratic model suited well for pectinase production response. The one factor effect plots representing the pectinase production over changes in independent variables A-WB and B-CP are shown for each of the *Bacillus* isolate, i.e., M35, R31 and J208 in Figure A2.5 (A-F). It was visible that increase of WB and CP concentrations up to certain level, positively affected pectinase production while beyond that concentration, they negatively affect the xylanase production response by all three isolates *B. safensis* M35, B. altitudinis R31 and J208. These results are in accordance with the results present in Table, A2.8, A2.10 and A2.12 where it was noted that the CP had more significant effect on pectinase production response than WB.

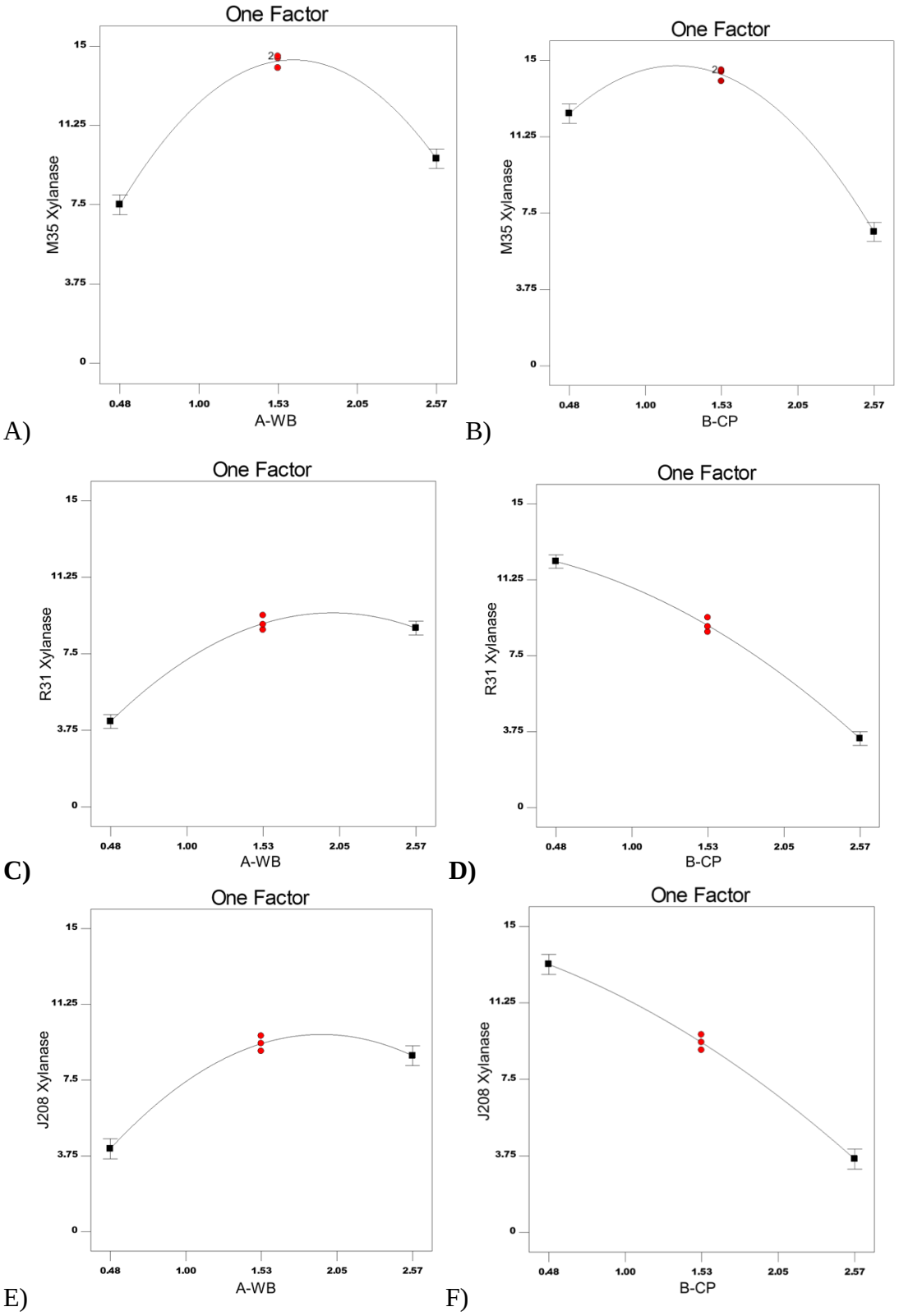

Figure A2.4. One factor plots showing the effect of WB and CP on xylanase production response from *Bacillus* isolates.

(A, B): Effect of (A) WB and (B) CP on xylanase production response by *B. safensis* M35; (C, D): Effect of (C) WB and (D) CP on xylanase production response by *B. altitudinis* R31; (E, F): effect of (E) WB and (F) CP on xylanase production response by *B. altitudinis* J208.

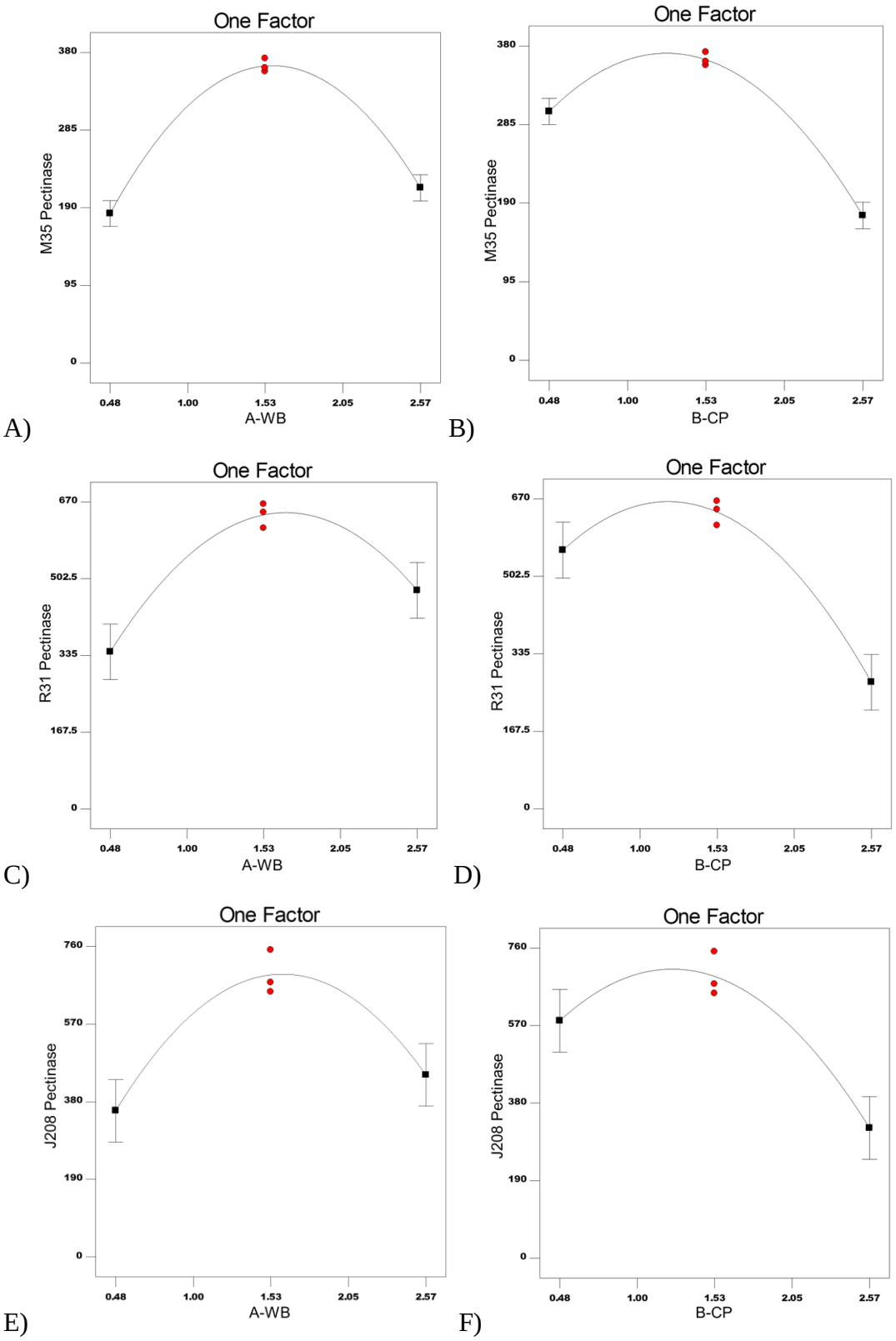

Figure A2.5. One factor plots showing the effect of WB and CP on pectinase production response from *Bacillus* isolates.
(A, B): Effect of **(A)** WB and **(B)** CP on pectinase production response by *B. safensis* M35; **(C, D)**: Effect of **(C)** WB and **(D)** CP on pectinase production response by *B. altitudinis* R31; **(E, F)**: effect of **(E)** WB and **(F)** CP on pectinase production response by *B. altitudinis* J208.