National Pingtung University
Of Science & Technology




Mathematical model of processing parameters for mycelia growth and polysaccharides production from *Ganoderma tsugae* in Submerged Fermentation

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CONTENT

1. INTRODUCTION

Ganoderma (Ganodermataceae) is a folk medicine and also a functional food due to its antitumor and certain physiological benefits. Several biologically active triterpenes and sterols have been isolated from this mushroom and been proved their effectiveness to be a cytotoxic, antiviral (e.g., anti HIV), or anti-inflammatory agent.

Introduction con't

Ganoderma is also found to be active in several other therapeutic effects, including anti-bacteria, anti-parasite, blood pressure regulation, immunomodulation, kidney toning, liver protection, nerve toning, sexual potentiating, etc.

Ganoderma tsugae Murrill, also called Song Shan Ling Chih, has been revered for centuries as a symbol of success and well being, meaning "marvelous herbs" or "mushroom of immortality" (Mau *et al*., 2005; Tseng *et al.*, 2008).

The metabolites of *G. tsugae* consist of mainly polysaccharides and triterpenes, which have various applications in food, pharmaceutical industries (Russell and Paterson, 2006).

- Currently, there have been a lot of efforts on cultivating the fungus in solid media for fruiting body production. However, the method takes long time and has high risk of contamination due to open cultivation and medium ingredients from nature (Lee *et al*., 2004; Shih *et al*., 2008).
- Thus, submerged cultivation to produce fungus' metabolites have been received a lot of attention because of short time cultivation, high productivity, fewer chances of contamination, and easy recovery of producing metabolites (Huang and Liu, 2008; Kim *et al*., 2007).

1. **Culture Condition**
   - Agitation
   - Aeration
   - pH , Temp , DO
   - Time

2. **Nutrients Component**
   - Carbon source
   - Nitrogen source
   - Inorganic salt

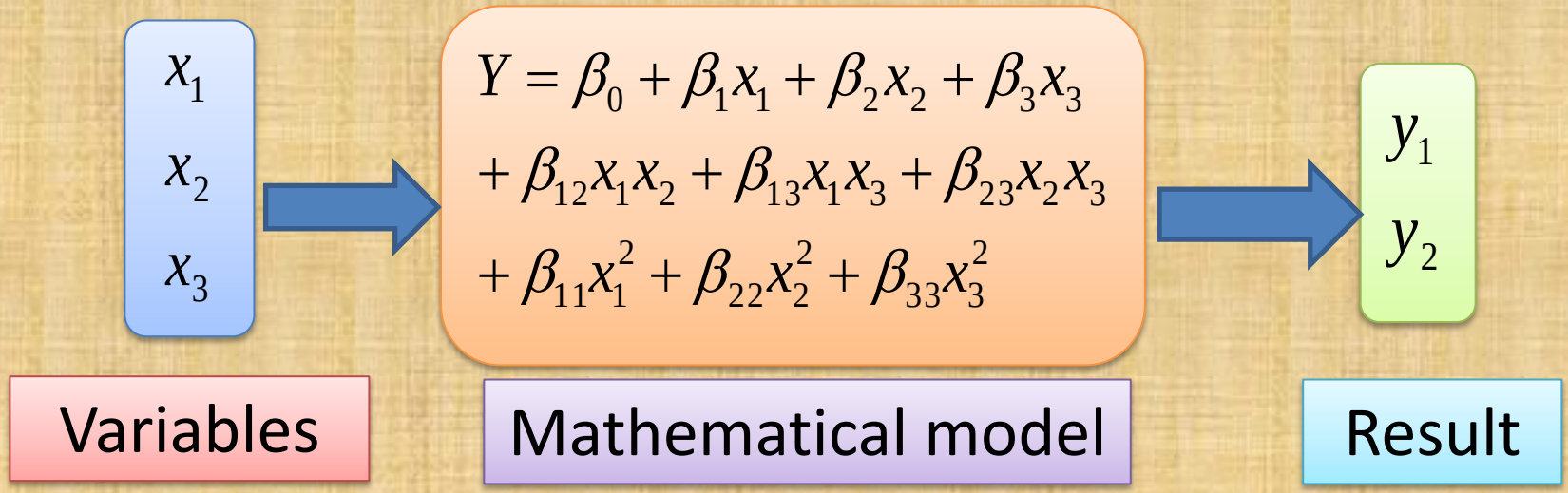
The best product

1. Quantity
2. Quality

Many engineering parameter, such as agitation speed, aeration rate, temperature, pH, dissolved oxygen, nutrients component and fermentation time (Cui *et al.,* 1998; Liu *et al.,* 2008). are critical in submerge fermentation.

Therefore, the ways to study those engineering parameters for optimizing fermentation conditions have attracted rather high interest from industry.

Mathematical model



Many researches reports that the mathematical model of response surface method has been applied in the optimization of biochemical and physical processes (Oscar *et al*, 1999; Muthukumar *et al*, 2003; Wang and Lu, 2005; Duta *et al*, 2006; Gao and Gu, 2007; Xu *et al*, 2008; Zhang *et al*, 2010) because of its reasonable design and excellent outcomes.

Our preliminary results suggested that oxygen supply were one of the important environmental factors significantly influencing the growth and metabolite production of *G. tsugae*. In submerged-state fermentation, oxygen supply can be easily controlled by medium volume and mixing speed aerobically.

Therefore in current research, various mathematical models for biomass and polysaccharide productions during submerged cultivation of *G. tsugae* using Erlenmeyer's flasks were developed. The factors of shaking speed and medium volume were investigated to evaluate the effects of oxygen supply on the cultivation and metabolite productions of *G. tsugae*

2.OBJECTIVE

To establish mathematical models of the oxygen supply parameters during cultured *Ganoderma tsugae* in submerge fermentation for prediction of fermentation progression and for design of automation controls.

3. MATERIALS AND METHODS

- **3.1 Inoculum preparation and submerged culture**
- **3.2 Response Surface Methodology**
- **3.2 Experimental design (The factorial design)**

3.1 Inoculum preparation and submerged culture

The strain was first cultured on solid medium (PDA) at 27°C for 7d in Petri dish.

The culture was transferred to 500 ml Erlenmeyer flasks containing 200 ml of liquid medium (PDB).

The culture was incubated at 150 rpm, at 27°C for 7d.

The oxygen supply factors

1. Shaking speed (0,50,100,150,200 rpm)
2. Medium volume (100,150,200 mL)

1. Mycelia growth
2. Polysaccharides production

3.2 Response Surface Method

3.2.1 Experimental Designs

1. Full factorial design
2. Box-Behken design
3. Central Composite design
4. Plackett-Burman Design

3.2.2 Analysis and Build the Math Model

- SAS®
- Statistica ®
- Minitab ®
- Design-Expert®
- Microsoft Excel ® (Regression)

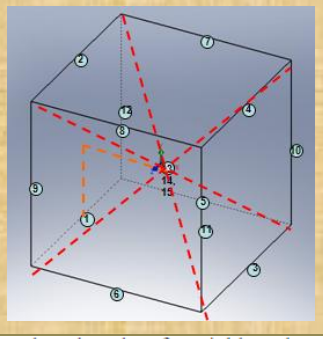
$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2$$

3.2.3 Find the optimal value

- Microsoft Excel® (solve, plot 3D and contour graph)
- Statistica ®
- Minitab ®
- Design-Expert®
- Matlab®
- Sigma Plot® (plot 3D and contour egraph)

Optimization of processing parameters for the mycelia growth and extracellular polysaccharide production by *Boletus* spp. ACCC 50328.

Wang, Y.X. and Lu, Z.X. (2004)



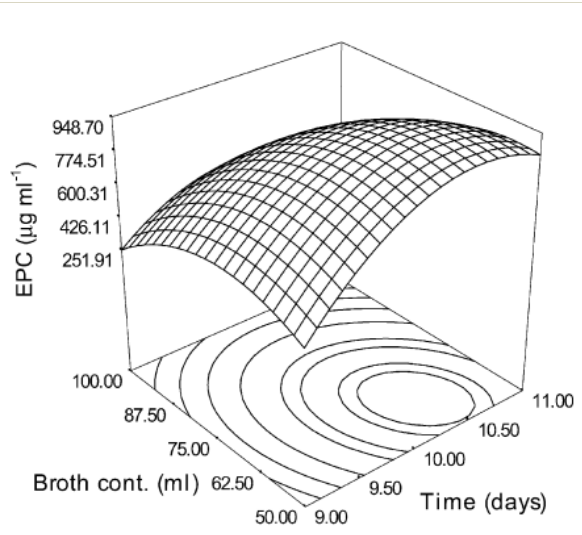
Level and code of variables chose for Box–Behnken design

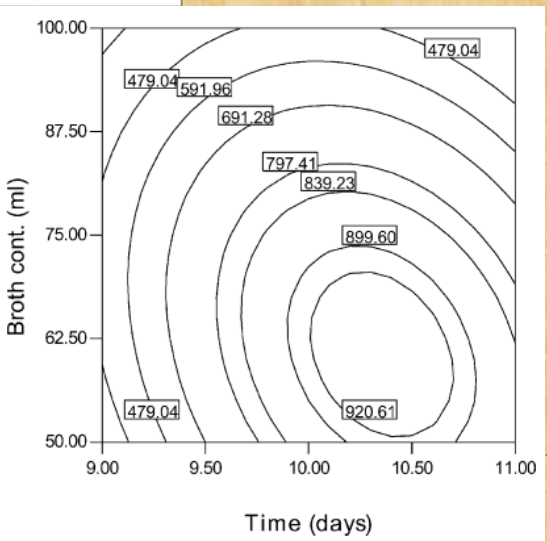
Variables	Symbols		Coded levels		
	Uncoded	Coded	−1	0	1
Temperature (°C)	X_1	x_1	24	27	30
Fermentation time (days)	X_2	x_2	9	10	11
Broth content (ml)	X_3	x_3	50	75	100

x_1 (temperature) $= 27 \pm 3$; x_2 (time) $= 10$; x_3 (broth content) $= 75 \pm 25$.

Table 2
Box–Behnken design matrix along with the experimental and predicted values of EPC and DCW

Std.	Temperature (x_1)	Time (x_2)	Broth content (x_3)	EPC (μg ml^{-1})		DCW (mg ml^{-1})	
				Experimental	Predicted	Experimental	Predicted
1	−1	−1	0	241.412	266.794	2.747	2.795
2	1	−1	0	367.449	399.833	2.893	2.991
3	−1	1	0	787.572	755.187	3.533	3.436
4	1	1	0	395.457	370.074	3.320	3.272
5	−1	0	−1	920.611	928.488	3.300	3.347
6	1	0	−1	451.473	452.348	3.080	3.078
7	−1	0	1	220.405	219.530	2.750	2.752
8	1	0	1	451.473	443.596	3.100	3.053
9	0	−1	−1	423.465	390.205	3.780	3.685
10	0	1	−1	815.580	840.087	4.080	4.130
11	0	−1	1	276.422	251.915	3.410	3.360
12	0	1	1	227.408	260.667	3.740	3.835
13	0	0	0	892.602	877.198	3.987	4.083
14	0	0	0	857.592	877.198	4.067	4.083
15	0	0	0	899.604	877.198	4.147	4.083
16	0	0	0	850.590	877.198	4.093	4.083
17	0	0	0	885.600	877.198	4.120	4.083





$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2$$

$$Y_{EPC} = 877.2 - 63.02x_1 + 114.66x_2 - 179.43x_3 - 176.98x_1^2 - 252.25x_2^2 - 89.23x_3^2 - 129.54x_1x_2 + 175.05x_1x_3 - 110.28x_2x_3$$

3.3 Experimental design (The 5X3 of factorial design)

Independent variable	Symbols		Level				
	Actual	Code	-1	-0.5	0	0.5	1
Shaking speed (rpm)	X_1	x_1	0	50	100	150	200
Medium volume (mL)	X_2	x_2	100	-	150	-	200

Actual Value		Code Value	
Rpm	volume	rpm	volume
X_1	X_2	x_1	x_2
0	100	-1	-1
0	150	-1	0
0	200	-1	1
50	100	-0.5	-1
50	150	-0.5	0
50	200	-0.5	1
100	100	0	-1
100	150	0	0
100	200	0	1
150	100	0.5	-1
150	150	0.5	0
150	200	0.5	1
200	100	1	-1
200	150	1	0
200	200	1	1

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2$$

4. RESULTS AND DISCUSSIONS

- 4.1. Mathematical model of mycelia growth
- 4.2. Mathematical model of polysaccharide Production
- 4.3 Mathematical model of Dry cell mass -Polysaccharides diagram of *Ganoderma tsugae*

4. RESULTS AND DISCUSSIONS

Actual Value		Code Value		Total dry cell mass		Polysaccharides yield	
Rpm	volume	rpm	volume	(mg/flask)		(mg/mL)	
X_1	X_2	x_1	x_2	Experimental	Predict	Experimental	Predict
0	100	-1	-1	140.4		149.853	
0	150	-1	0	136.4		145.896	
0	200	-1	1	196.933		161.796	
50	100	-0.5	-1	282.4		143.409	
50	150	-0.5	0	279.1		145.517	
50	200	-0.5	1	262.733		137.312	
100	100	0	-1	260.1		206.127	
100	150	0	0	342.233		218.134	
100	200	0	1	505.4		187.837	
150	100	0.5	-1	194.687		223.924	
150	150	0.5	0	252.833		270.452	
150	200	0.5	1	369.533		239.872	
200	100	1	-1	133.433		281.442	
200	150	1	0	272.933		308.259	
200	200	1	1	324		281.66	

4.1 Mathematical model of mycelia growth

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.89937442
R Square	0.808874347
Adjusted R Square	0.702693429
Standard Error	54.10063445
Observations	15

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2$$

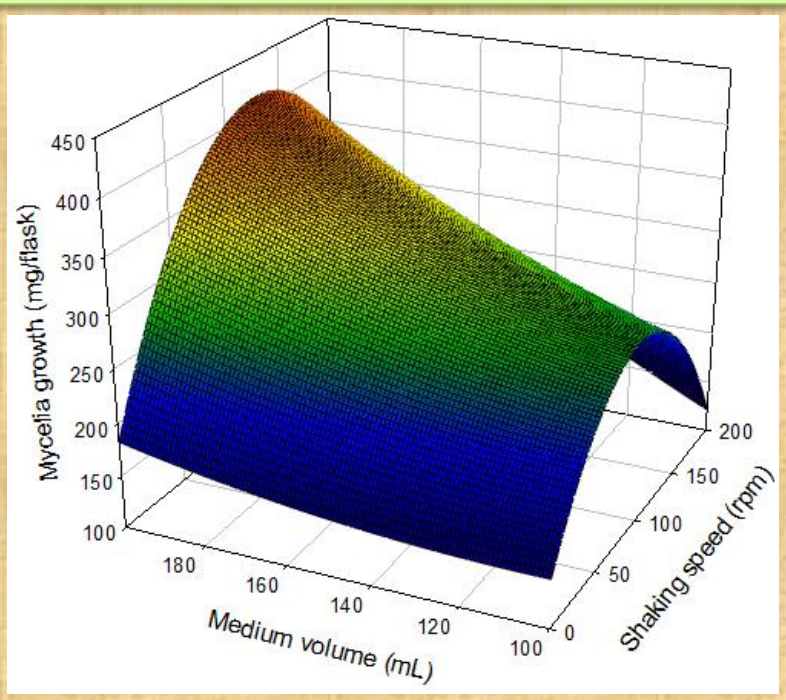
ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	5	[illegible]	[illegible]	[illegible]	[illegible]
Residual	9	26341.90783	2926.88		
Total	14	137825.077			

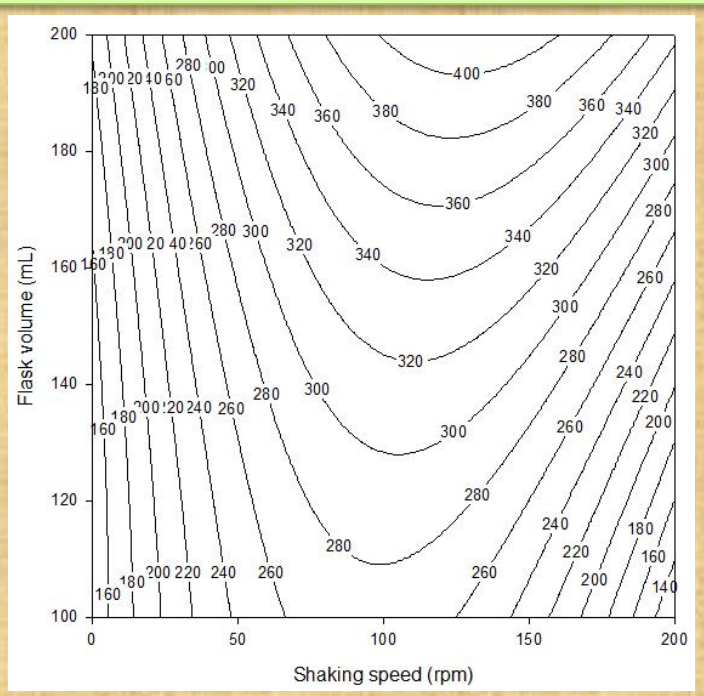
Variable	*Coefficients*	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
β_0	325.679	29.39602145	11.079	1.5E-06	259.18031	392.17715	259.18031	392.1771505
β_1	33.739	19.75475858	1.7079	0.12183	-10.949257	78.42748	-10.9492574	78.42747963
β_2	64.758	17.10812277	3.78522	0.00431	26.0567376	103.45926	26.0567376	103.4592624
β_{12}	46.258	24.19453925	1.91192	0.08819	-8.4738502	100.98985	-8.47385016	100.9898502
β_1^2	-137.957	33.39163652	-4.1315	0.00255	-213.49459	-62.42033	-213.49459	-62.42033073
β_2^2	10.262	29.63213786	0.34631	0.73707	-56.770553	77.294553	-56.7705528	77.29455278

Response surface plots of mycelia growth

$$Y_{DCM} = 325.679 + 33.739x_1 + 64.758x_2 + 46.258x_1x_2 - 137.957x_1^2 + 10.262x_2^2$$

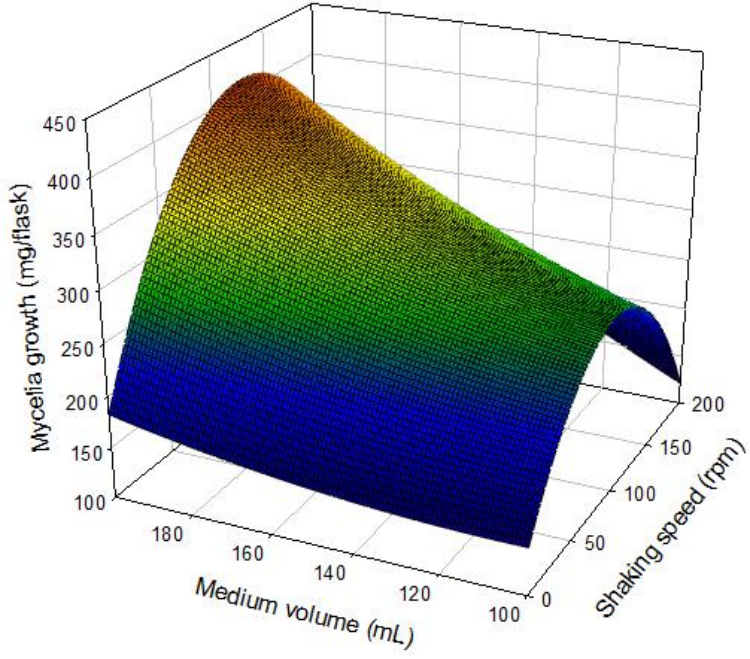


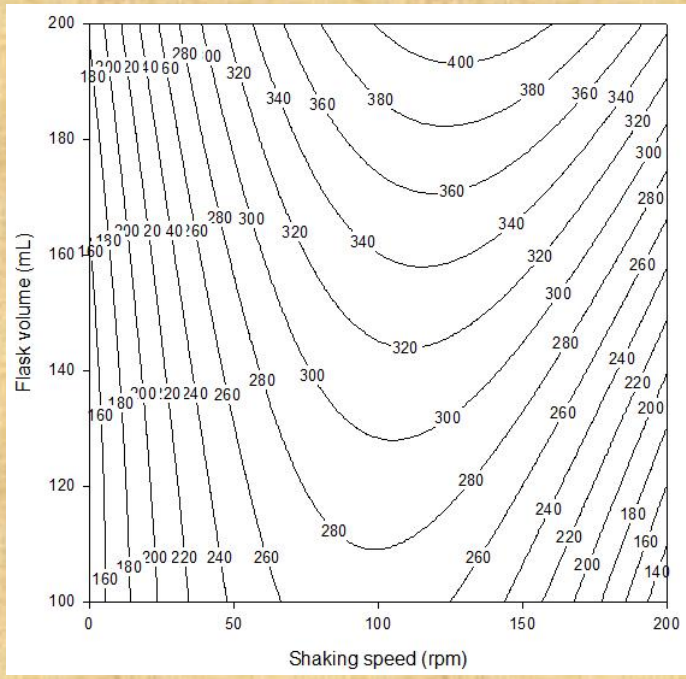
(1a) 3D plots



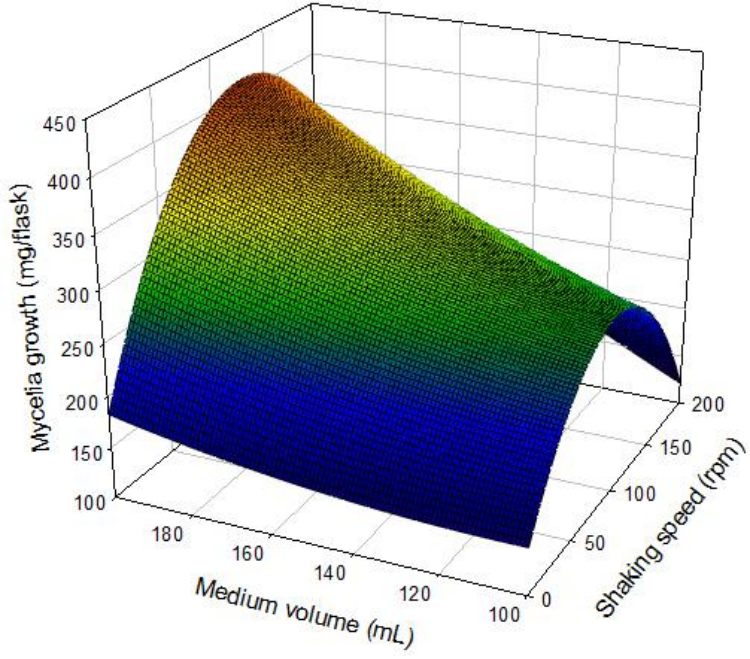
(1b) contour plots

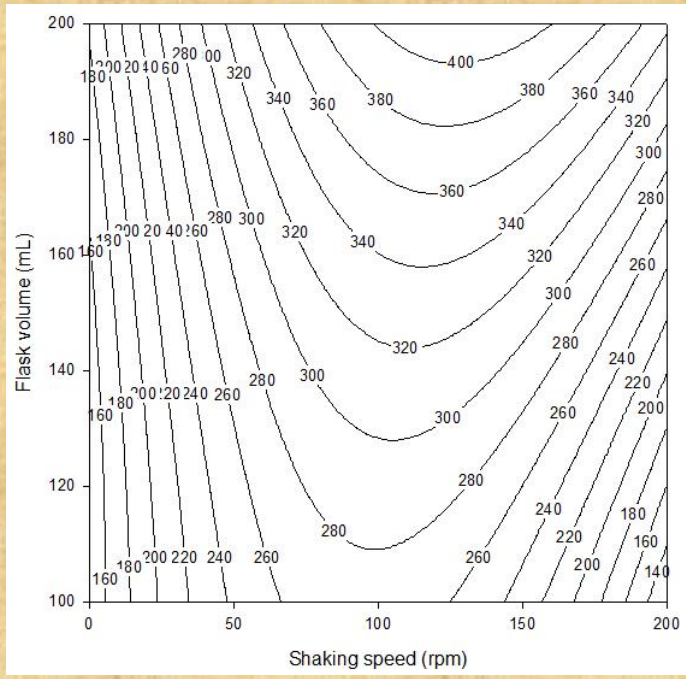
Figure 1. Effect of the shaking speed and medium volume on the total dry cell mass (mg/flask).





- When consider from medium volume, because of at high level of medium volume was abundant nutrient in medium, cause the mycelia growth was high production at highest medium volume.
- However, should consider the shaking speed at the same time, because agitation was important factor of the mixture of oxygen and nutrient. Agitation or shaking speed in submerged cultivation could affect fungi in several ways; damage to cell structure, morphological change, as well as variations in growth rate and product formation (Papagianni, 2004).





- The large mycelia size was produced from low agitation. Because at low agitation speed, cause the fungi pellet cannot segregate out to several small pellet. When the seed inoculums pellet cannot segregate, cause to the seed pellets received the excess oxygen and overabundant nutrient supply. So, the pellets were larger and eventually death.
- Therefore, the appropriate shaking speed at 129 rpm can be break a larger pellet into several smaller pellet which the amount of tiny particle pellet had a great influence on the final number of mycelia production (Yang *et al*, 2009; Kim *et al*, 2007; Enshasy *et al*, 2006).

4.2 Mathematical model of polysaccharide

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.96550279
R Square	0.93219563
Adjusted R Square	0.89452654
Standard Error	19.2088853
Observations	15

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2$$

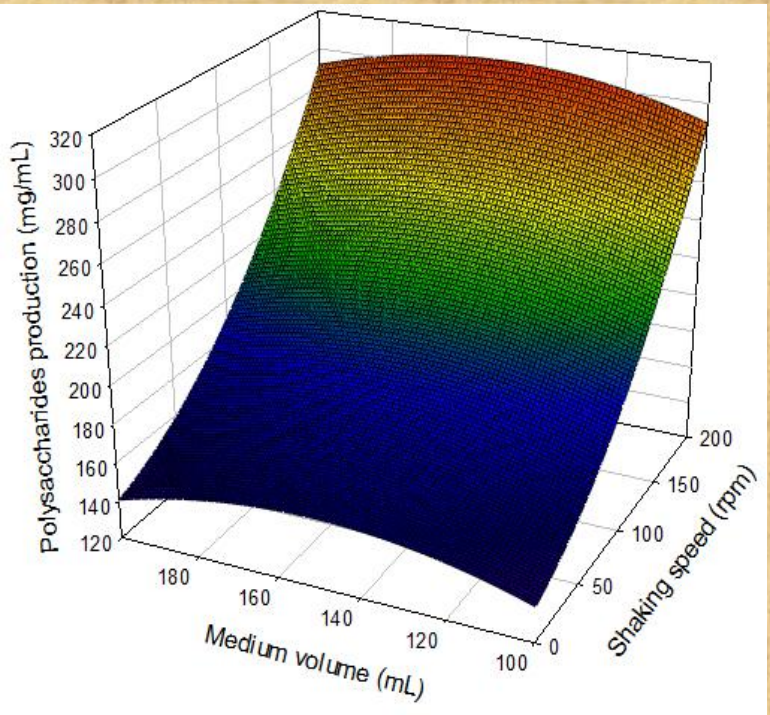
ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	5	45655.82799	9131.17	24.747	5.228E-05
Residual	9	3320.831463	368.981		
Total	14	48976.65945			

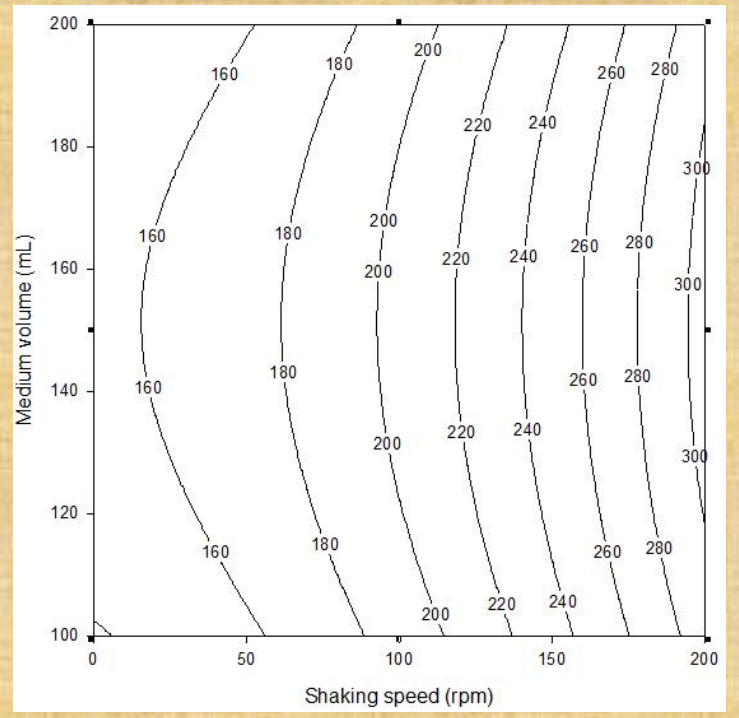
Variable	*Coefficients*	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
β_0	204.645	10.43730465	19.6071	1.1E-08	181.03463	228.25628	181.0346331	228.2562799
β_1	75.710	7.014093182	10.7939	1.9E-06	59.842537	91.576499	59.84253656	91.57649876
β_2	0.372	6.07438288	0.06128	0.95247	-13.36895	14.113463	-13.3689542	14.11346323
β_{12}	-0.141	8.590474652	-0.0164	0.98731	-19.57351	19.2925	-19.5735076	19.29249985
β_1^2	26.012	11.85598139	2.194	0.05588	-0.808035	52.832152	-0.8080346	52.83215174
β_2^2	-16.328	10.52113977	-1.552	0.15509	-40.1288	7.4721448	-40.1287985	7.472144775

Response surface plots of polysaccharides production

$$Y_{PL} = 204.646 + 75.710x_1 + 0.372x_2 - 0.141x_1x_2 + 26.012x_1^2 - 16.328x_2^2$$

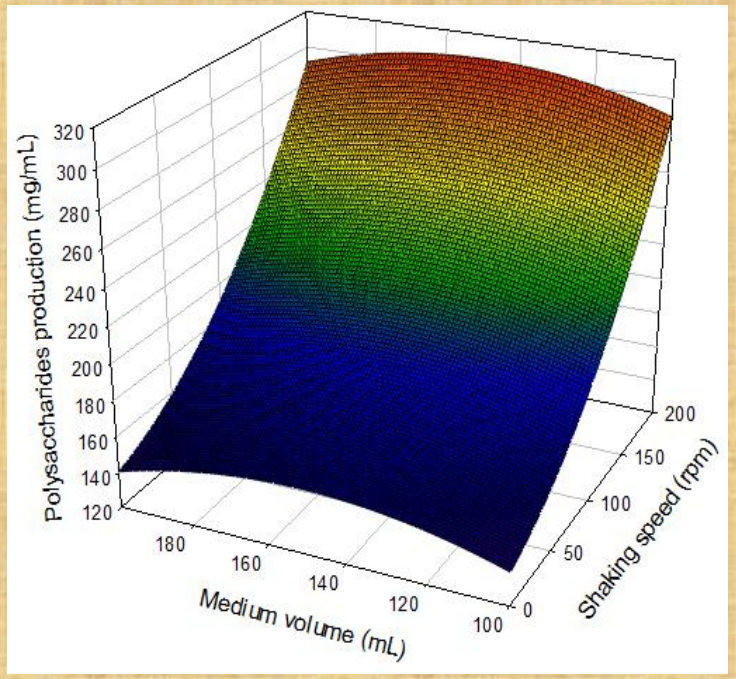


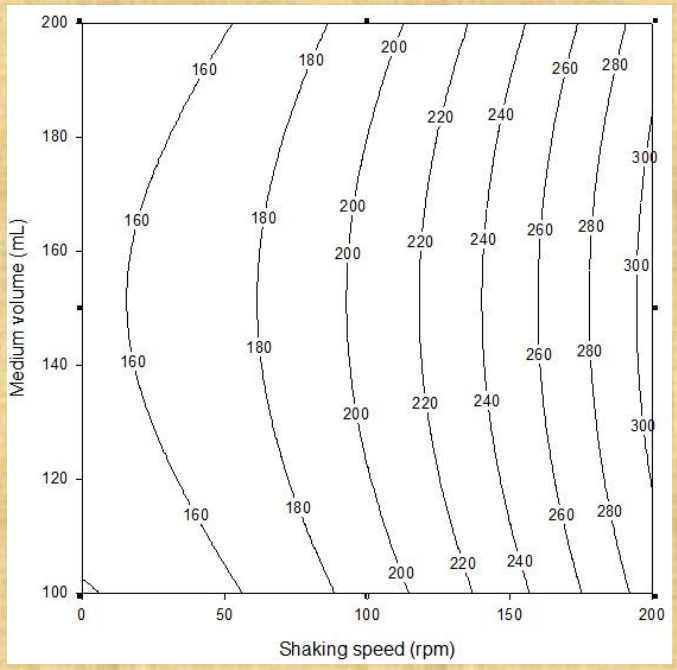
(2a) 3D plots



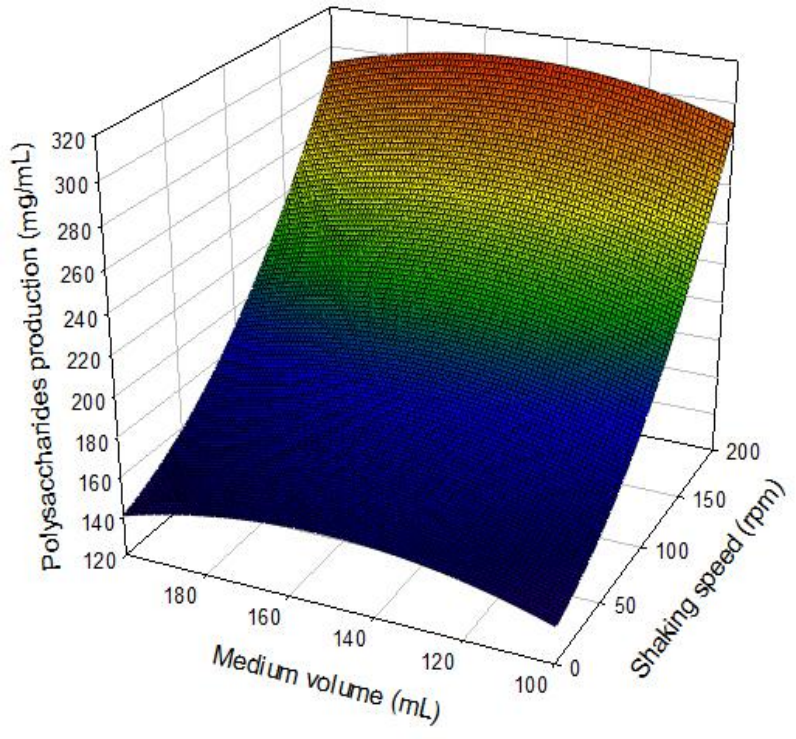
(2b) contour plots

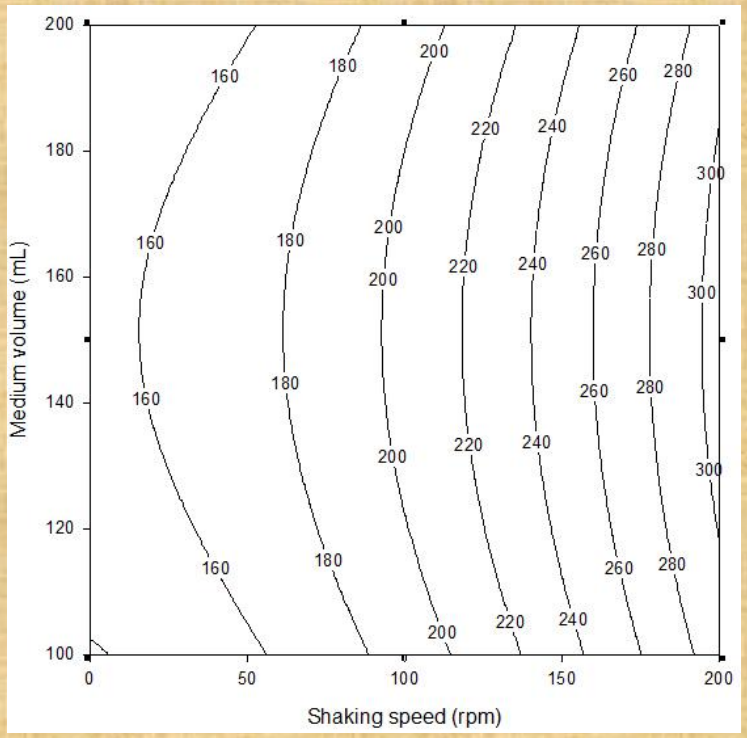
Figure 2. Effect of the shaking speed and medium volume on the total polysaccharides production(mg/mL).





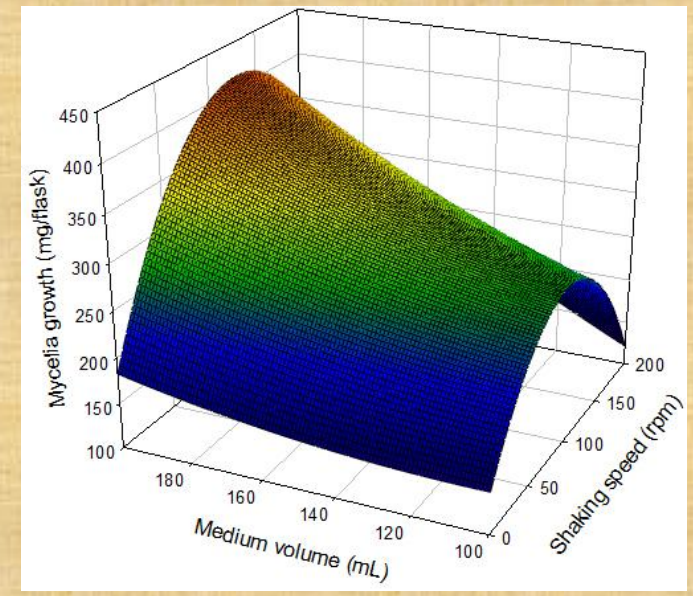
- The effect of polysaccharides production was observed a highest level of polysaccharides production which achieved at the highest shaking speed.
- Because, polysaccharides production processes require high agitation to promote good mass transfer and achieve high product concentrations (Giavasis *et al*, 2006).
- Also, the result agreed with other reports (Yang and Liau, 1998; Lopez *et al*, 2003; Oh *et al*, 2007; Henriques *et al*, 2006; Rau *et al*, 1992) showing that agitation and shaking speed has a positive effect on the polysaccharides production but negative effect on mycelia growth.

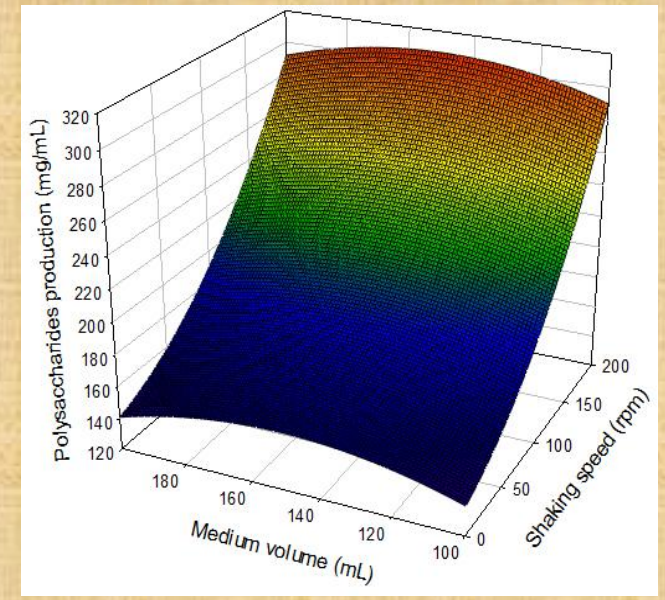




- When considering from a medium volume, the highest polysaccharides production was obtained at 150.35 ml of medium volume.
- Because of aeration or medium volume aids to produce secondary metabolite by promoting the mass transfer of substrates, products and oxygen.
- Therefore, the appropriate aeration intensity is an important factor in polysaccharides production.

4.3 Mathematical model of Dry cell mass-Polysaccharides diagram of *G. tsugae*





$$Y_{DCM} = 325.679 + 33.739x_1 + 64.758x_2 + 46.258x_1x_2 - 137.957x_1^2 + 10.262x_2^2$$

$$Y_{PL} = 204.646 + 75.710x_1 + 0.372x_2 - 0.141x_1x_2 + 26.012x_1^2 - 16.328x_2^2$$

$$y_{PL(x_1,x_2)} = f(y_{DCM(x_1,x_2)})$$

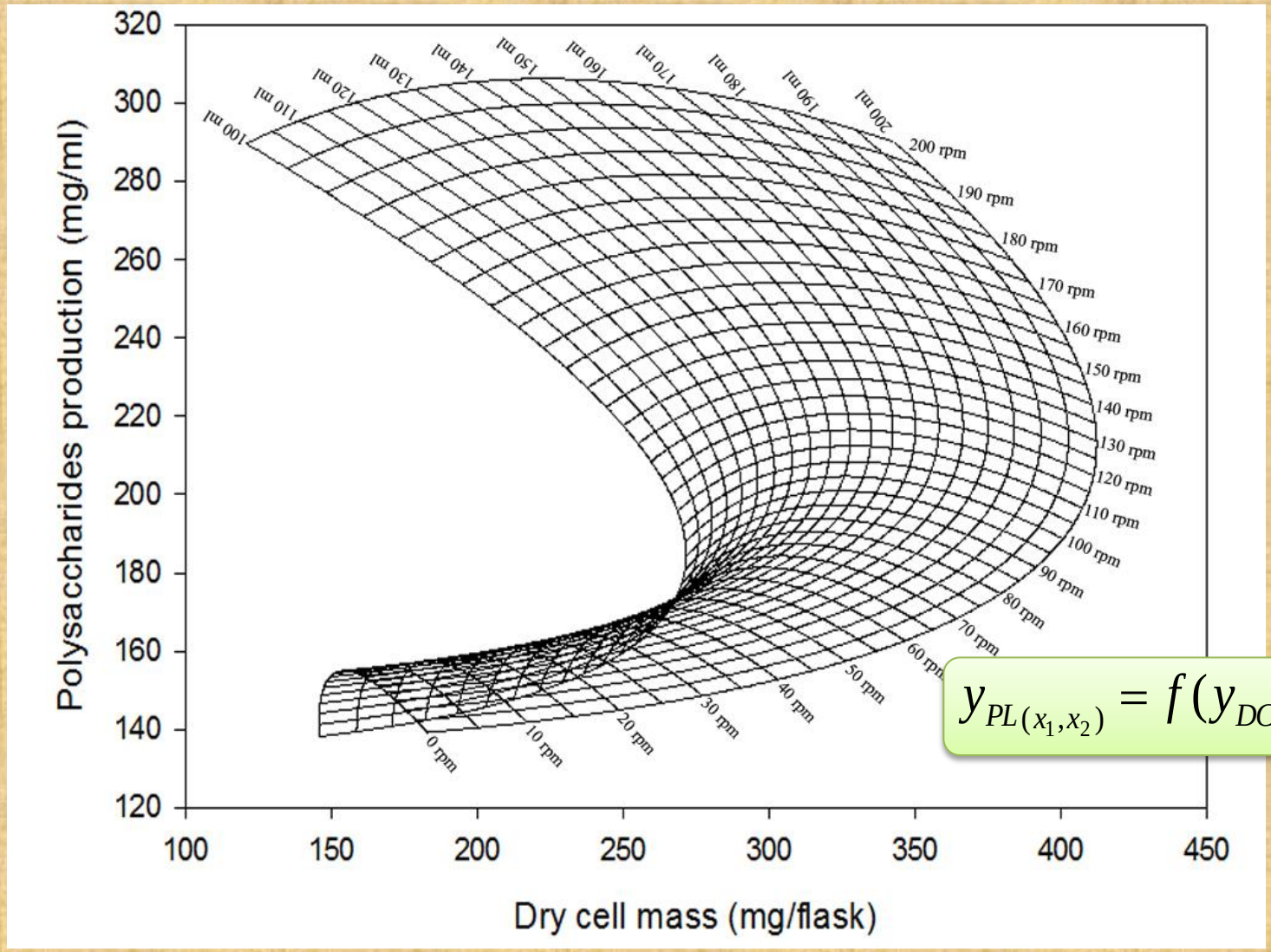
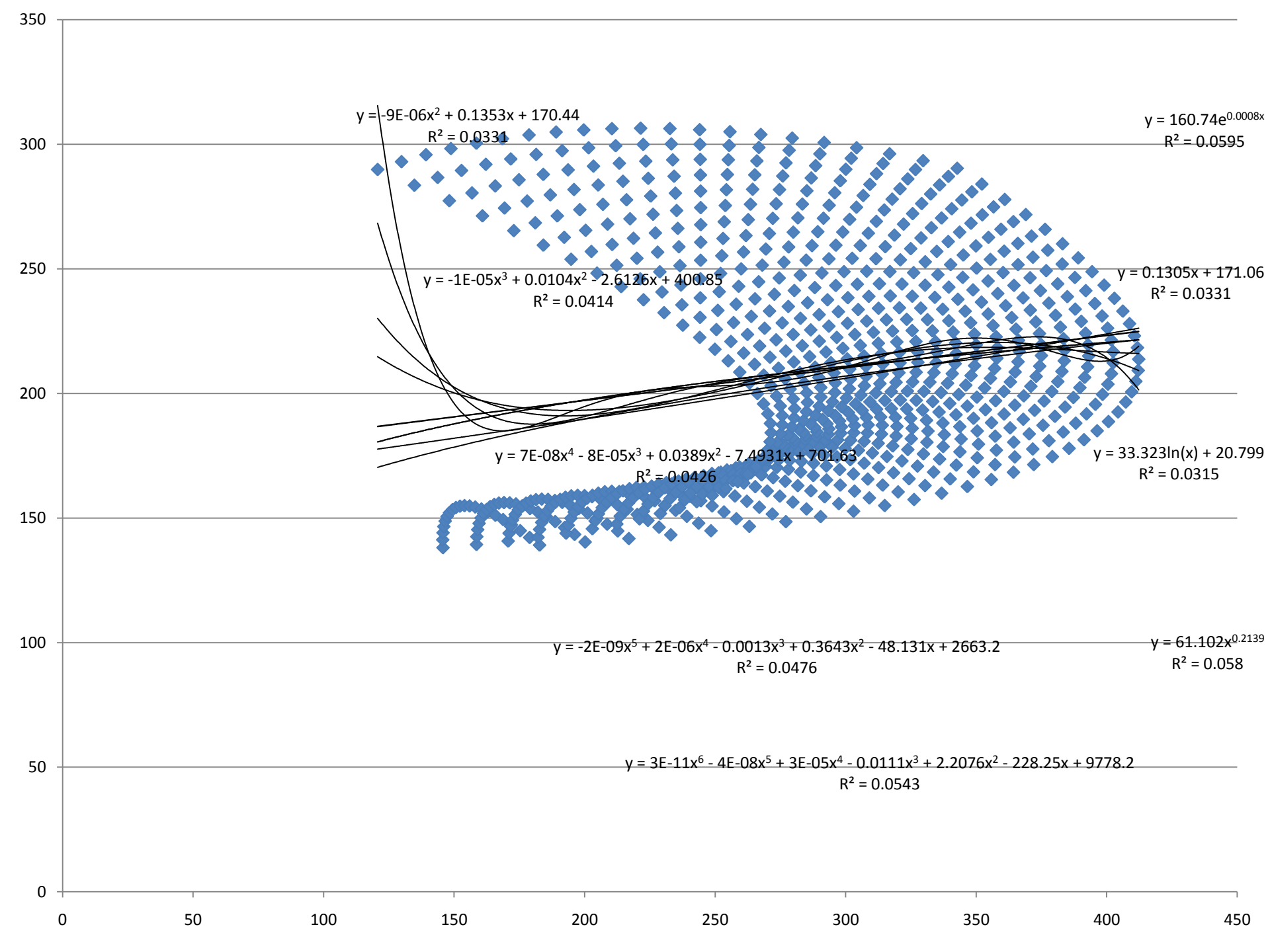


Figure 3. Dry cell mass-Polysaccharides diagram of *Ganoderma tsugae*


y = -9E-06x² + 0.1353x + 170.44
R² = 0.0331
y = 160.74e^0.0008x
R² = 0.0595
y = -1E-05x³ + 0.0104x² - 2.6126x + 400.85
R² = 0.0414
y = 0.1305x + 171.06
R² = 0.0331
y = 7E-08x⁴ - 8E-05x³ + 0.0389x² - 7.4931x + 701.63
R² = 0.0426
y = 33.323ln(x) + 20.799
R² = 0.0315
y = -2E-09x⁵ + 2E-06x⁴ - 0.0013x³ + 0.3643x² - 48.131x + 2663.2
R² = 0.0476
y = 61.102x^0.2139
R² = 0.058
y = 3E-11x⁶ - 4E-08x⁵ + 3E-05x⁴ - 0.0111x³ + 2.2076x² - 228.25x + 9778.2
R² = 0.0543
350
300
250
200
150
100
50
0
0
50
100
150
200
250
300
350
400
450

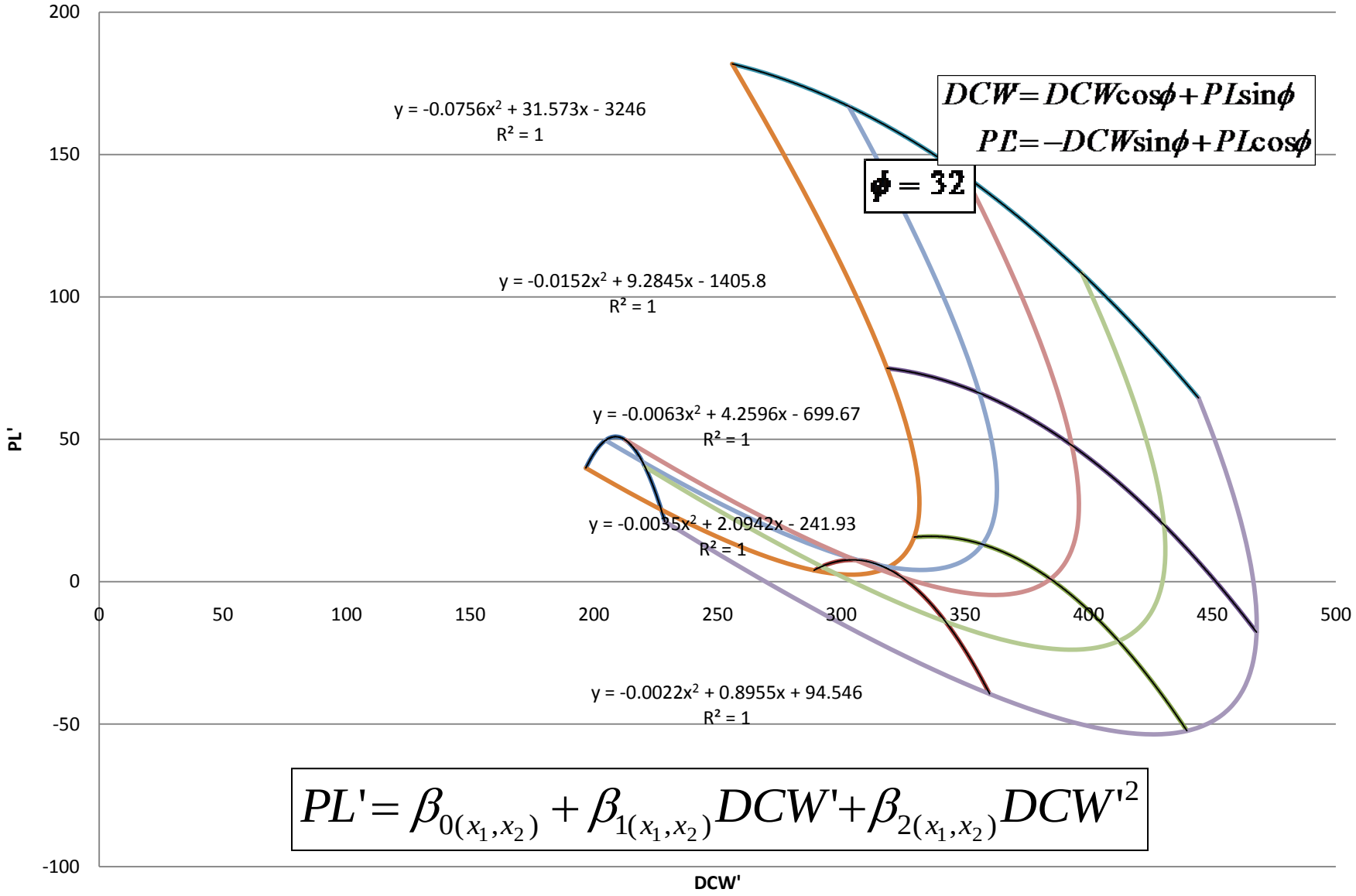


Figure 4. Polynomial equations of each level of shaking speed after rotated the axes

TABLE 1. Analysis polynomial regression of each level of shaking speed by STATISTICA 7®.

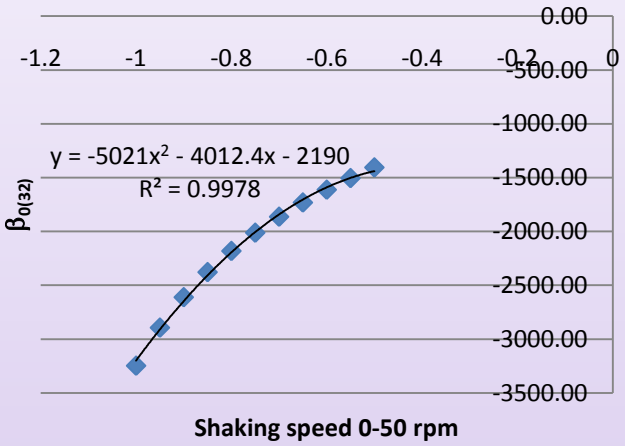
θ = 32	0 rpm	5 rpm	10 rpm	15 rpm	20 rpm	25 rpm	30 rpm	35 rpm	40 rpm	45 rpm	50 rpm
β_0	-3245.99	-2892.71	-2610.28	-2377.56	-2181.04	-2011.68	-1863.27	-1731.34	-1612.64	-1504.76	-1405.83
β_1	31.57	26.54	22.72	19.73	17.34	15.37	13.74	12.36	11.19	10.17	9.28
β_2	-0.08	-0.06	-0.05	-0.04	-0.03	-0.03	-0.03	-0.02	-0.02	-0.02	-0.02

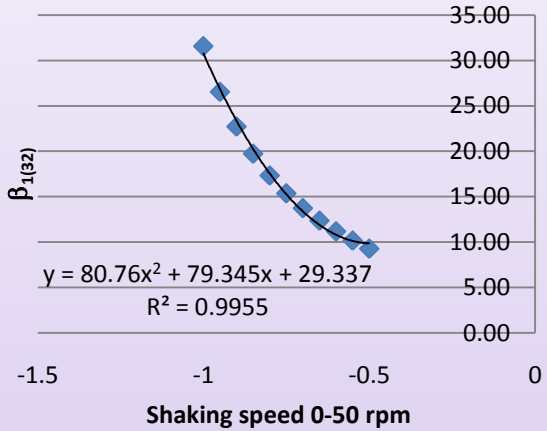
θ = 32	55 rpm	60 rpm	65 rpm	70 rpm	75 rpm	80 rpm	85 rpm	90 rpm	95 rpm	100 rpm
β_0	-1314.42	-1229.40	-1149.87	-1075.11	-1004.51	-937.608	-873.984	-813.302	-755.277	-699.665
β_1	8.51	7.82	7.20	6.65	6.15	5.703	5.293	4.919	4.575	4.260
β_2	-0.01	-0.01	-0.01	-0.01	-0.01	-0.009	-0.008	-0.007	-0.007	-0.006

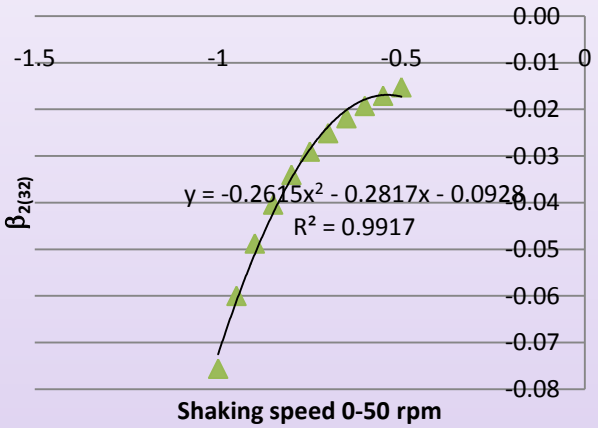
θ = 32	105 rpm	110 rpm	115 rpm	120 rpm	125 rpm	130 rpm	135 rpm	140 rpm	145 rpm	150 rpm
β_0	-646.260	-594.883	-545.380	-497.617	-451.477	-406.857	-363.667	-321.828	-281.269	-241.927
β_1	3.968	3.698	3.447	3.214	2.996	2.792	2.601	2.422	2.253	2.094
β_2	-0.006	-0.006	-0.005	-0.005	-0.005	-0.004	-0.004	-0.004	-0.004	-0.003

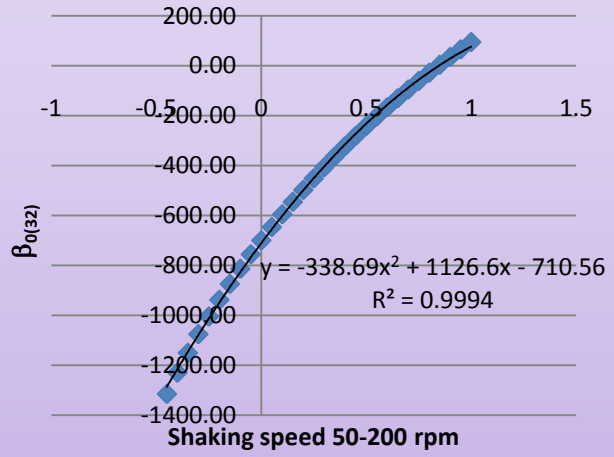
θ = 32	155 rpm	160 rpm	165 rpm	170 rpm	175 rpm	180 rpm	185 rpm	190 rpm	195 rpm	200 rpm
β_0	-203.745	-166.673	-130.666	-95.6830	-61.6876	-28.6461	3.471677	34.69328	65.04381	94.54620
β_1	1.944	1.802	1.668	1.5403	1.4193	1.3042	1.194641	1.09024	0.99063	0.89548
β_2	-0.003	-0.003	-0.003	-0.0028	-0.0027	-0.0026	-0.002464	-0.00236	-0.00226	-0.00217

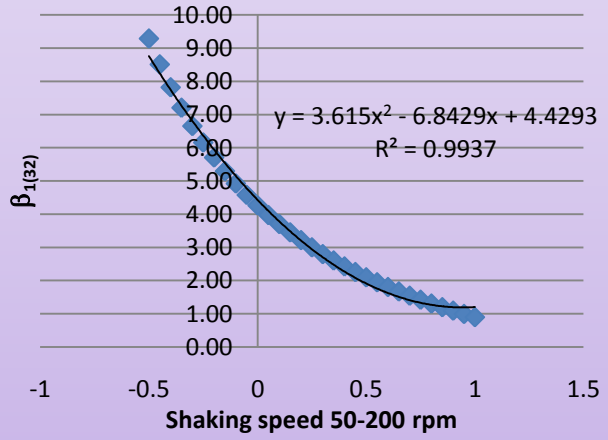
The relationship between the $\beta_{(32)}$ constant ($\beta_{0(32)}$,$\beta_{1(32)}$, $\beta_{2(32)}$)and shaking speed

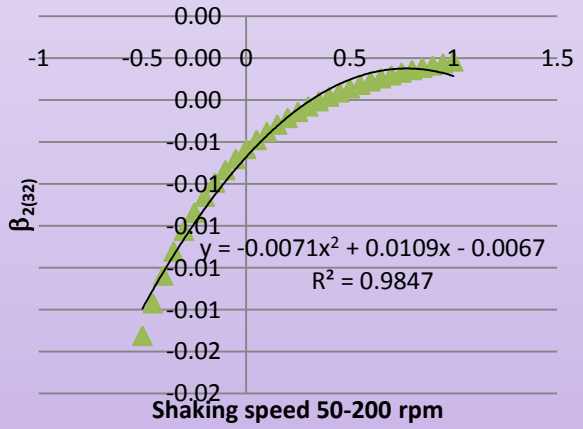












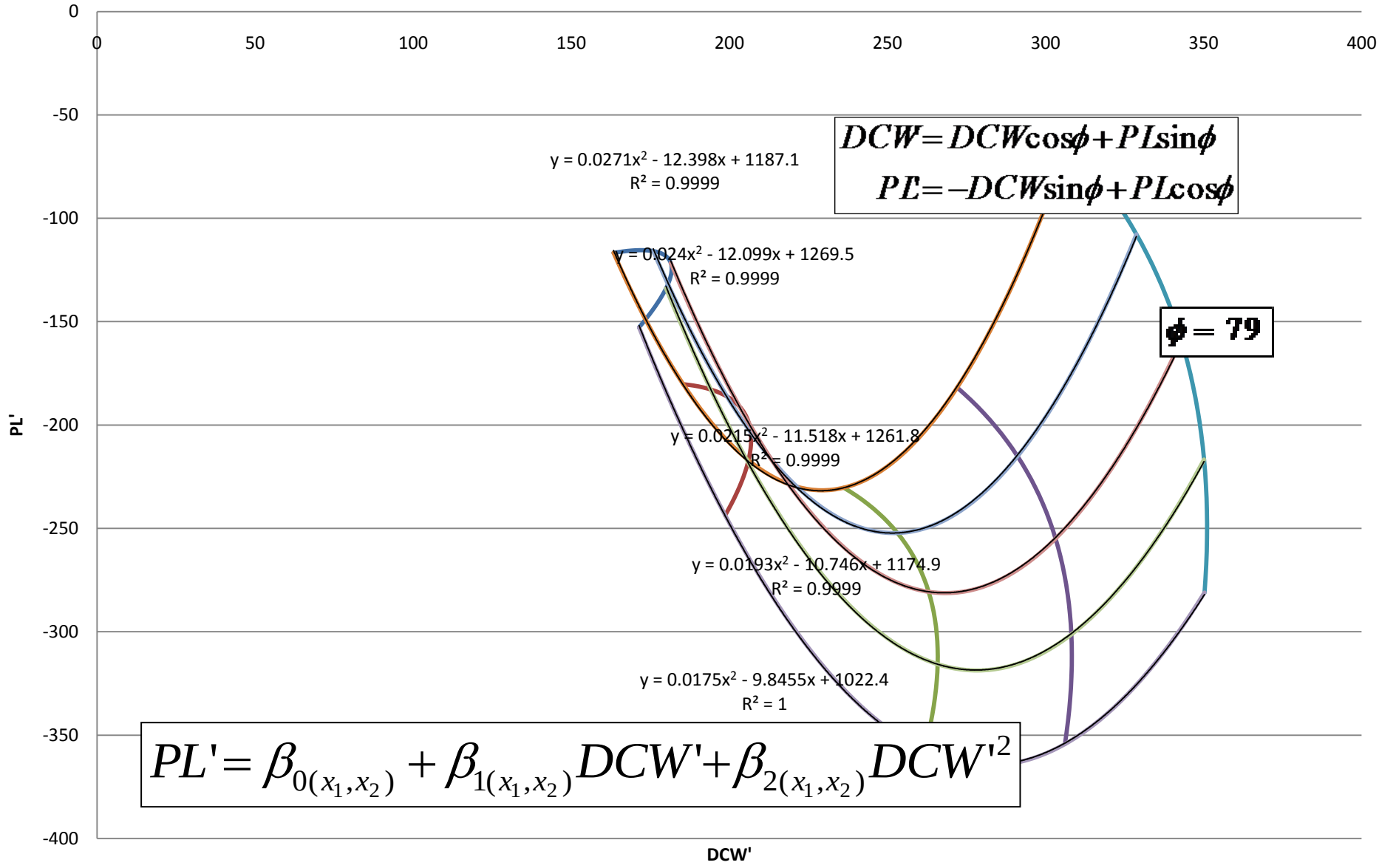


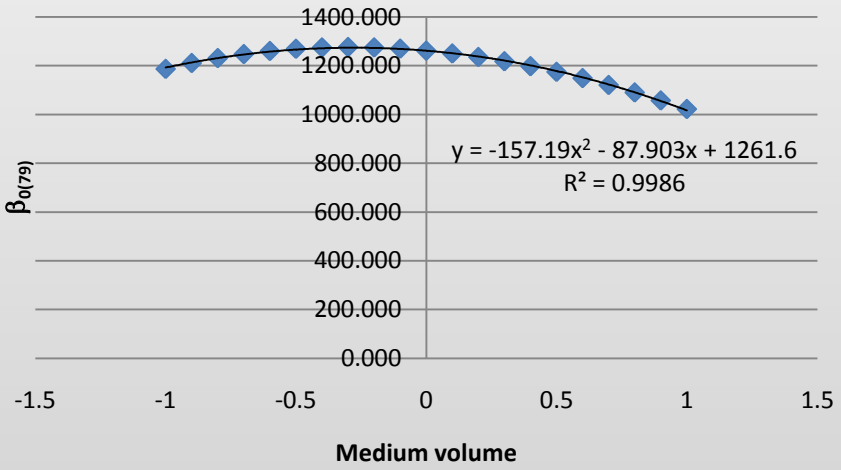
Figure 5. Polynomial equations of each level of medium volume after rotated the axes

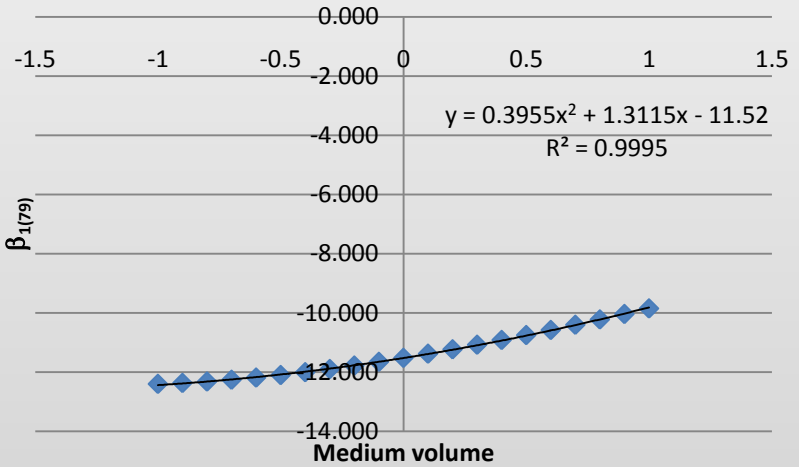
TABLE 2. Analysis polynomial regression of each level of medium volume by STATISTICA 7®.

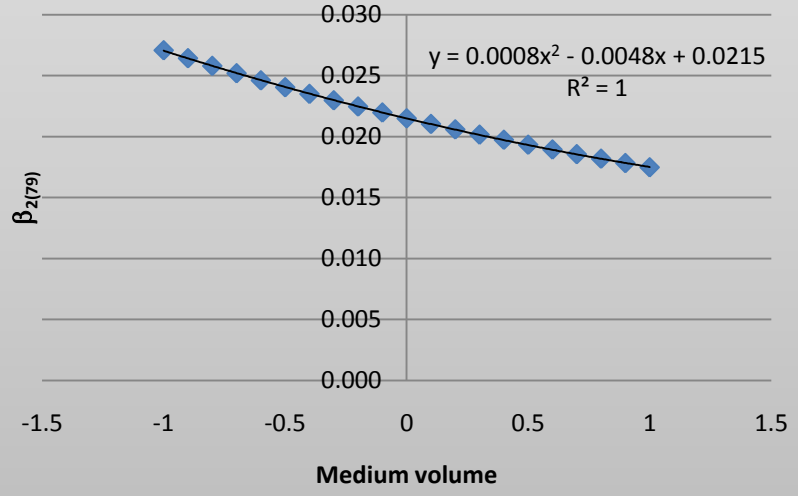
θ = 79	100 mL	105 mL	110 mL	115 mL	120 mL	125 mL	130 mL	135 mL	140 mL	145 mL	150 mL
β_0	1187.069	1211.173	1231.425	1247.865	1260.548	1269.536	1274.901	1276.721	1275.083	1270.077	1261.800
β_1	-12.398	-12.366	-12.320	-12.259	-12.185	-12.099	-12.002	-11.895	-11.778	-11.652	-11.518
β_2	0.027	0.026	0.026	0.025	0.025	0.024	0.024	0.023	0.022	0.022	0.021

θ = 79	155 mL	160 mL	165 mL	170 mL	175 mL	180 mL	185 mL	190 mL	195 mL	200 mL
β_0	1250.351	1235.834	1218.355	1198.022	1174.946	1149.239	1121.012	1090.380	1057.457	1022.356
β_1	-11.377	-11.228	-11.073	-10.913	-10.746	-10.574	-10.398	-10.218	-10.033	-9.845
β_2	0.021	0.021	0.020	0.020	0.019	0.019	0.019	0.018	0.018	0.017

The relationship between the $\beta_{(79)}$ constant ($\beta_{0(79)}$,$\beta_{1(79)}$, $\beta_{2(79)}$)and medium volume







4.3 Mathematical model of Dry cell mass-Polysaccharides diagram of *Ganoderma tsugae*

$$PL' = \beta_{0(\theta)} + \beta_{1(\theta)} DCM' + \beta_{2(\theta)} DCM'^2$$

$$DCM' = DCM\cos\theta + PL\sin\theta$$

$$PL' = -DCM\sin\theta + PL\cos\theta$$

$$(-DCM\sin\theta + PL\cos\theta) = \beta_{0(\theta)} + \beta_{1(\theta)}(DCM\cos\theta + PL\sin\theta) + \beta_{2(\theta)}(DCM\cos\theta + PL\sin\theta)^2$$

when given a constant value at medium volume (x_2) and a variable value at shaking speed (x_1), the angle $\theta = 32$

At the shaking speed 0-50 rpm

$$\beta_{0(32)} = -5021.05x_1^2 - 4012.35x_1 - 2190.04, R^2 = 0.997817$$
$$\beta_{1(32)} = 80.76038x_1^2 + 79.34491x_1 + 29.33729, R^2 = 0.995537$$
$$\beta_{2(32)} = -0.261486x_1^2 - 0.281734x_1 - 0.092795, R^2 = 0.991723$$

At the shaking speed 50-200 rpm

$$\beta_{0(32)} = -356.020x_1^2 + 1141.012x_1 - 711.136, R^2 = 0.999183$$
$$\beta_{1(32)} = 3.61502x_1^2 - 6.84292x_1 + 4.42930, R^2 = 0.993740$$
$$\beta_{2(32)} = -0.007056x_1^2 - 0.010920x_1 - 0.006725, R^2 = 0.984749$$

$$\cos\theta = \cos\left(\frac{\pi}{180} \times \theta\right)$$
$$\sin\theta = \sin\left(\frac{\pi}{180} \times \theta\right)$$

$$x_1, rpm_{code} = -1 + \frac{rpm_{actual}}{100}$$

$$x_2, MediumVolume_{code} = -3 + \frac{MediumVolume_{actual}}{50}$$

when given a constant value at shaking speed (x_1) and a variable value at medium volume (x_2) ,the angle $\theta = 79^{\circ}$

$$\beta_{0(79)} = -157.193x_2^2 - 87.903x_2 + 1261.645, R^2 = 0.998552$$
$$\beta_{1(79)} = 0.3955x_2^2 + 1.3115x_2 - 11.5203, R^2 = 0.999517$$
$$\beta_{2(79)} = 0.000782x_2^2 - 0.004765x_2 - 0.021495, R^2 = 0.999954$$

$$(-DCMsin\theta + PLcos\theta) = \beta_{0(\theta)} + \beta_{1(\theta)}(DCMcos\theta + PLsin\theta) + \beta_{2(\theta)}(DCMcos\theta + PLsin\theta)^2$$

DCM, y_1	350.000	mg/flask
shaking speed	100	rpm
x_1	0	
θ	32	
sin θ	0.5299	
cos θ	0.848	
$\beta_{0(\theta)}$	-711.136	
$\beta_{1(\theta)}$	4.429	
$\beta_{2(\theta)}$	-0.007	

0-50 rpm	A_θ	A_1	A_2	Multiple R	Multiple R^2	Adjusted R^2
-2190.04	-2190.04	-4012.35	-5021.05	0.998908	0.997817	0.997272
29.33729	29.33729	79.34491	80.76038	0.997766	0.995537	0.994422
-0.09279	-0.092795	-0.281734	-0.261486	0.995853	0.991723	0.989653

50-200 rpm	A_θ	A_1	A_2	Multiple R	Multiple R^2	Adjusted R^2
-711.136	-711.136	1141.012	-356.020	0.999591	0.999183	0.999124
4.4293	4.42930	-6.84292	3.61502	0.996865	0.993740	0.993293
-0.00672	-0.006725	0.010920	-0.007056	0.992345	0.984749	0.983660

$$rpm_{coda} = -1 + \frac{rpm_{actual}}{100}$$

$$a = \beta_2(y_2 \sin\theta)^2 = \beta_2(\sin 32)^2 y_2^2$$

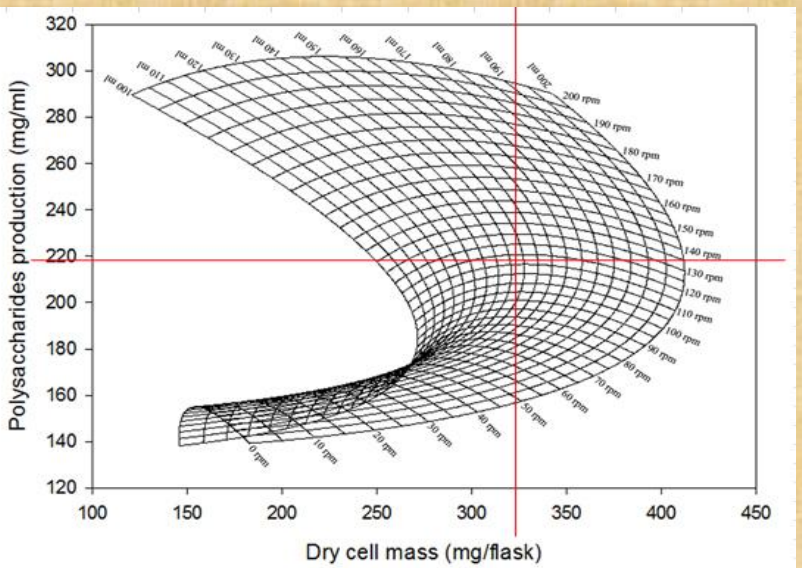
a	-0.002
b	-0.616
c	196.56

$$b = \beta_1 y_2 \sin\theta + 2\beta_2(y_1\cos\theta)(y_2\sin\theta) - y_2\cos\theta$$
$$= (\beta_1 \sin\theta + 2\beta_2(y_1\cos\theta)(\sin\theta) - \cos\theta)y_2$$
$$= (\beta_1 \sin 32 + 2\beta_2(y_1\cos 32)(\sin 32) - \cos 32)y_2$$

PL, y_2	-524.7	and	198.3492	mg/ml

$$PL, y_2 = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

$$c = \beta_0 + \beta_1(y_1\cos\theta) + \beta_2(y_1\cos\theta)^2 + y_1\sin\theta$$
$$= \beta_0 + \beta_1(y_1\cos 32) + \beta_2(y_1\cos 32)^2 + y_1\sin 32$$



$$(-DCMsin\theta + PLcos\theta) = \beta_{0(\theta)} + \beta_{1(\theta)}(DCMcos\theta + PLsin\theta) + \beta_{2(\theta)}(DCMcos\theta + PLsin\theta)^2$$

PL, y_2	260.000	mg/mL
Medium volume	160	ml
x_2	0.2	
θ	79	
sin θ	0.9816	
cos θ	0.1908	
$\beta_{0(\theta)}$	1237.776	
$\beta_{1(\theta)}$	-11.242	
$\beta_{2(\theta)}$	0.021	

$$MediumVolume_{coda} = -3 + \frac{MediumVolume_{actual}}{50}$$

100-200 mL	A_θ	A_1	A_2	Multiple R	Multiple R^2	Adjusted R^2
1237.776	1261.645	-87.903	-157.193	0.999276	0.998552	0.998391
-11.2421	-11.5203	1.3115	0.3955	0.999758	0.999517	0.999463
0.020574	0.021495	-0.004765	0.000782	0.999977	0.999954	0.999949

$$a = \beta_2(y_1\cos\theta)^2 = \beta_2(\cos 79)^2 y_1^2$$

a	0.0007
b	0.8404
c	-340.9

$$b = \beta_1 y_1 \cos\theta + 2\beta_2(y_1\cos\theta)(y_2\sin\theta) + y_1\sin\theta$$
$$= (\beta_1\cos\theta + 2\beta_2(\cos\theta)(y_2\sin\theta) + \sin\theta)y_1$$
$$= (\beta_1\cos 79 + 2\beta_2(\cos 79)(y_2\sin 79) + \sin 79)y_1$$

DCM, y_1	316.45	and	-1438.35	mg/flask

$$DCM, y_1 = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

$$c = \beta_0 + \beta_1(y_2\sin\theta) + \beta_2(y_2\sin\theta)^2 - y_2\cos\theta$$
$$= \beta_0 + \beta_1(y_2\sin 79) + \beta_2(y_2\sin 79)^2 - y_2\cos 79$$

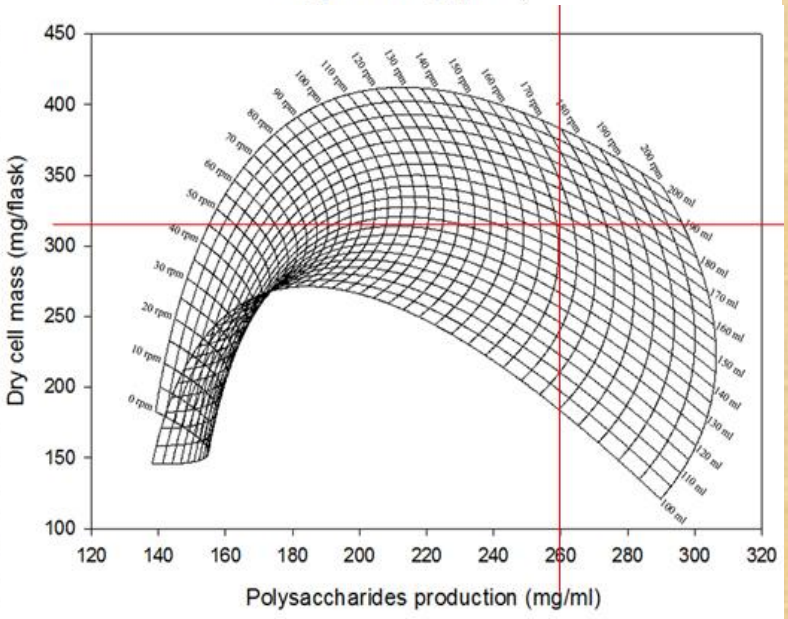


Figure 6. Spreadsheet of *Ganoderma tsugae* production

5. CONCLUSIONS

- Mathematical model of the process parameters for controlling the *Ganoderma tsugae* production by response surface methodology is an appropriate tool for evaluating those variable parameters to look for a suitable outcome from a submerge fermentation process mathematically and statistically.
- Mycelia growth requires the appropriate agitation to break up the large pellet which the broken pellets were used to reseed the formation of new pellet for increasing the mycelia production and highest medium volume to supply the nutrient consumption.
- Polysaccharides production requires the highest agitation and appropriate aeration for encourage the good mass transfer of substrates, products and oxygen.

5. CONCLUSIONS

- DCM-PL diagram was build from calculated data of both production equations while DCM-PL mathematical model was obtained from the combination of polynomial equation and the rotated of axes equation.
- The mathematical model for controlling the productions of mycelia and polysaccharides from *Ganoderma tsugae* in submerged fermentation was obtained as follows:

$$(-DCMsin\theta + PLcos\theta) = \beta_{0(\theta)} + \beta_{1(\theta)}(DCMcos\theta + PLsin\theta) + \beta_{2(\theta)}(DCMcos\theta + PLsin\theta)^2$$

- Moreover, this experiment which culture a *G. tsugae* fungus in an shaking incubator can applies to use in a bioreactor by changing the variables from shaking speed and medium volume are the agitation speed impeller and aeration air flow rate, respectively for building the DCM-PL diagram and mathematical model of the large scale bioreactor which more useful for the submerge fermentation in controlling the fungus production.

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