Statistical Evaluation of the Pertinent Parameters in Biosynthesis of Ag/MWf-CNT Composites Using Plackett-Burman Design and Response Surface Methodology

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ABSTRACT: *Green chemistry - also called sustainable chemistry - as the cost-effective and environmentally friendly techniques have been gaining more attention recent years. Here, we introduced a fast, non-toxic and sustainable method to synthesize Ag nanoparticles. Various parameters are involved in the bio-synthesis of Ag nanoparticles/multi-walled carbon nanotubes (Ag/MWf-CNT) composites, including silver nitrate concentration, initial pH, temperature, CNT concentration, agitation time, biomass and stirring rate. The Plackett–Burman Design (PBD) approach indicated that the initial pH, the carbon nanotube concentration and the weight of biomass are the major effects of the Ag/MWf-CNT biosynthesis. A quadratic polynomial model was developed using Central Composite Design (CCD) to statistically evaluate the effect of the initial pH (3.5-7), the carbon nanotubes concentration (0.2-1 g/L) and the weight of wet biomass (6-16 g) on the response- reduction percentage of Ag ions. The significant factors and their interactions in the biosynthesis process were examined by means of analysis of variance (ANOVA). The results showed that the wet biomass weight has the most significant effect on the response compared to the other variables. Additionally, the model predicted that up to 89% of Ag^+ reduction to Ag nanoparticles were obtained at the optimum range conditions– weight of biomass 13 g, the initial pH range 5.5-6.2 and concentration of carbon nanotubes 0.6 g/L.*

KEY WORDS: *Bio-synthesis; Ag/MWf-CNT; Plackett–Burman Design (PBD); Response Surface Methodology (RSM).*

INTRODUCTION

As a matter of fact, nowadays, nanotechnology has a significant impact on various industries and communities. The nanoparticles, as one the most applicable nanostructures, are used as the catalyst in many reactions; CO oxidation, Benzene-to-phenol oxidation, reduction of acetaldehyde gases and oxidation of ethylene to ethylene oxide [1, 2]. Among different nanoparticles, silver (Ag) has distinctive properties such as good conductivity, chemical stability, catalytic activity and antimicrobial activity [3, 4]. Many protocols have been proposed for the synthesis of Ag nanoparticles, including chemical reduction, electrochemical reduction, and photochemical reduction [5]. Generally, these methods use toxic chemical agents for the reduction of Ag ions, and they

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1021-9986/2016/2/51-62 12/$/6.20

produce enormous environmental contaminations and hazardous by-products [1]. Thus, a facile, inexpensive, nontoxic and environmentally friendly method for the synthesis of Ag nanoparticles at ambient temperature, namely “green chemistry”, is favorable [1, 5]. In the “green chemistry” methods the microorganisms are employed for the bio-synthesis of silver nanoparticles [6].

Designing support material is the most important step in the abundant production of catalysts. The stability and recyclability solid-supported catalysts, as well as the large specific surface area and environmental considerations are important concerns in the choice of support materials [7-10]. Multiwall Carbon Nanotubes (MWCNTs) are known as the non-contaminating carriers with high surface areas. Other remarkable characteristics of MWCNTs include unique structure, porous nature, low-density, high strength, good electrical conductivity (facilitate the electron transfers), and thermal properties. Moreover, they are relatively chemically stable which causes the use of them as the catalyst support material (which makes it a better option for using as a catalyst support material) [7, 11-15].

The aim of this work was to optimize the conditions for the bio-synthesis process of Ag nano-particles/multi-walled carbon nanotubes (Ag/MWf-CNT) composite by using *Aspergillus fumigatus*. Regarding the large number of parameters such as initial environmental pH, temperature, concentration of the silver ions, agitation time, stirring rate, concentration of carbon nanotubes and biomass weight which can potentially affect the synthesis process and also, the range of parameters used or reported for the synthesis Ag nanoparticles and Ag/MWf-CNT is relatively broad [7, 16-25], it is essential to screen the parameters. Among various designs that have been frequently used for the parameters screening, Plackett–Burman (PB) has been found to be the well suited to choose the vital parameters and to obtain more information. PB design is quite useful in preliminary studies for selecting variables that can be kept constant or eliminated prior to process optimization with the least number of runs as possible [13, 26].

In addition, Response Surface Methodology (RSM) as a statistical and mathematical technique can be applied to determine the optimal conditions of a multivariable system. Identification and interaction quantification of several physicochemical parameters was carried out by RSM. RSM has been extensively applied to optimize the enzymatic processes [27]. The wide range of values used or reported as optimum for the bios-ynthesis of Ag nanoparticles [26-28]. RSM can quantify the relationships between the response and the input factors in which the objective is to find a desirable location in the design space. The main advantage of RSM is the reduced number of experimental runs needed to provide sufficient information for statistically acceptable results. This method is a faster and less expensive method for gathering results than the classical method, and can be applied for a wide range of chemical reactions involving more than one parameter or response [29]. Central Composite Design (CCD) is the most popular response surface method (RSM) for the experimental design. The CCD allows the construction of statistical models and its graphical representation, and also the response surface. This methodology is useful to predict the optimal conditions to maximize the desired response and gives information about the interaction between variables in relation to the dependent variable. Moreover, this method is less time-consuming and cheaper compared to one-factor-at-a-time methodology [28-30].

EXPERIMENTAL SECTION

Microorganisms and Culture Media

Analytical grade silver nitrate ($AgNO_3$) was used in this study. The functionalized multi-walled carbon nanotubes with hydroxyl and carboxyl reagents (with the length of 10-50 μm and the diameter of less than 10 nm) were provided by the Research Institute of Petroleum Industry of Iran (R.I.P.I). *Aspergillus fumigatus* $U_{B2}60^0$ isolated previously from the Ghazvin Bidestan Factory (Iran) was used in this study. The optimal growth condition is described elsewhere [31].

Experimental Procedures

All experiments were carried out in 250 mL Erlenmeyer flasks in triplicates (the average of the results were reported, with 2% deviation). The experiments were conducted in a rotary shaker. The preparation procedure of fungus cellular metabolite extracts (enzymes and proteins) is described elsewhere [31, 32]. Also, procedure of experiments is described by authors [33]. Given the amounts of carbon nanotubes were sonicated in deionized water to achieve a homogeneous mixture of carbon nanotubes.

Table 1: Levels of variables in the Plackett-Burman experiment of bio-synthesis of Ag/MWf-CNT.

Variable	Symbol	Low (−)	High (+)
Temperature (°C)	T	25	35
stirring rate(rpm)	RPM	160	200
Concentration of Ag ions (mM)	Ag^+	0.5	2
pH of solution	pH	3.5	7
Concentration of MWCNT (g/L)	CNT	0.2	1
Time (hour)	Time	24	72
Weight of wet biomass (g)	W	6	16

Measurements and Analysis

The remaining Ag ion concentration was determined by atomic absorption spectrophotometry (GBC, Australia). The solution pH was measured by Metrohm pH meter model 744 Silver nanoparticles on the carbon nanotube surfaces were observed by using transmission electron microscopy (CEM 902 A, Zeiss, Germany, 200 keV).

The Ag^+ reduction percent was calculated based on the remaining Ag ion concentration in the solutions as Eq. (1):

Reduction of

$$Ag^+\% = \frac{([Ag^+]_{Initial} - [Ag^+]_{Final})}{[Ag^+]_{Initial}} \times 100\% \tag{1}$$

Where $[Ag^+]_{Initial}$ and $[Ag^+]_{Final}$ are the silver ion concentrations at the outset and end of process respectively.

Screening of Parameters by Plackett–Burman (PB) Design

The Plackett–Burman experimental design is a two factorial design, which identifies the critical physico-chemical parameters required for elevated bio-synthesis of Ag/MWf-CNT composites by screening "n" variables in n+1 experiments [34]. The experimental designing for the screening of the variables is described in Table 1.

All the variables were denoted as numerical factors and investigated at two widely spaced intervals designated as -1 (low level) and +1 (high level). In this case, eleven variables comprising of seven independent variables and four dummy variables [H, J, K and L] were organized in 12 runs of triplicates set of experiments (Table 2). Design Expert (7.1.5 Trial) software was used to analyze the experimental design. The experiments were carried out in random order. The dependent variables, i.e. percentage reduction of Ag^+ were selected as the response of the results of experiments (Table 2).

Optimization of the bio-synthesis process by Response Surface Methodology (RSM)

Based on the screening based design (PBD) results and significant parameter definition, Response Surface Methodology (RSM) was employed for optimization of the important parameters in the bio-synthesis of Ag/MWf-CNT composites. The levels employed for the different factors (initial environmental pH, wet biomass weight and carbon nanotube concentration), according to CCD, the range employed by previous investigators (see the introduction section) is listed in Table 3.

In Table 4 the CCD was adopted to study three factors at 3 levels. Seventeen experimental runs consisting of one star point (star distance was 0) and 3 center points were generated with 3 factors and 3 levels by the principle of RSM using Design Expert (7.1.5 Trial) software.

The quadratic polynomial regression model (Eq. (2)) was chosen for predicting the response variable in terms of the 3 independent variables:

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3} b_{ij} X_i X_j \tag{2}$$

In Eq. (2) Y is the response variable (i.e. Percentage reduction of Ag^+ to Ag nanoparticles), b_0, b_i, b_{ii}, and b_{ij} are the coefficients of the intercept, linear, quadratic and interaction terms, respectively, and X_i and X_j represent the four independent variables (i.e. CNT (g/L), W (g) and pH). The confirmation of the above polynomial model can be assessed by the coefficient of determination R^2 [35]. The experiments were carried out with three

Table 2: Placket-Burman experimental design for screening significant process variables affecting bio-synthesis of Ag/MWf-CNT.

Std Order	Run Order	T	RPM	Ag^+	pH	CNT	Time	W	H	J	K	L	Reduction of Ag^+ %
2	1	35	200	2	3.5	1	72	16	-1	-1	-1	1	81.9
10	2	35	200	2	7	0.1	24	6	1	-1	1	1	63.9
6	3	25	160	0.5	7	0.1	72	16	-1	1	1	1	72.2
8	4	35	200	0.5	3.5	0.1	72	6	1	1	-1	1	73.4
1	5	35	200	0.5	7	1	72	6	-1	-1	1	-1	69.5
11	6	25	160	2	7	1	24	6	-1	1	-1	1	68.7
7	7	25	160	0.5	3.5	1	24	16	1	-1	1	1	89.3
5	8	25	160	2	3.5	1	72	6	1	1	1	-1	75.6
12	9	25	160	0.5	3.5	0.1	24	6	-1	-1	-1	-1	71.6
4	10	35	200	0.5	7	1	24	16	1	1	-1	-1	82.8
3	11	25	160	2	7	0.1	72	16	1	-1	-1	-1	66.7
9	12	35	200	2	3.5	0.1	24	16	-1	1	1	-1	82.1

Table 3: Independent variables Levels and codes for central composite design.

Independent variables	Levels		
	-1	0	+1
Concentration of carbon nanotube (g/L)	0.2	0.6	1
Weight of wet biomass (g)	6	11	16
Initial pH	3.5	5.25	7

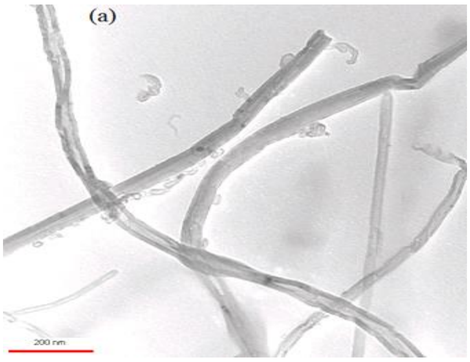
Table 4: The central composite design matrix and the experimental results.

Std.	Run	CNT	W	pH	Reduction of Ag^+ %
17	1	0.6	11	5.25	86.4
10	2	1	11	5.25	87.88
4	3	1	16	3.5	76.88
7	4	0.2	16	7	79.68
9	5	0.2	11	5.25	81.53
13	6	0.6	11	3.5	74.96
2	7	1	6	3.5	71.04
1	8	0.2	6	3.5	61.44
5	9	0.2	6	7	65.28
3	10	0.2	16	3.5	68.28
15	11	0.6	11	5.25	85.92
8	12	1	16	7	88.44
12	13	0.6	16	5.25	87.36
6	14	1	6	7	72.96
11	15	0.6	6	5.25	80.64
16	16	0.6	11	5.25	84.48
14	17	0.6	11	7	82.12

Table 5: Summary of ANOVA of Plackett–Burman screening design batches.

Source	Sum of Squars	Degree of Freedom	Mean Square	F-Value	P-Value
Model	627.97	7	89.71	18.60	0.0067
RPM	0.24	1	0.24	0.050	0.8341
Temperature	7.52	1	7.52	1.56	0.2799
Ag^+	33.00	1	33.00	6.48	0.0591
pH	209.17	1	184.87	43.36	0.0028
CNT	19.70	1	209.17	24.81	0.0076
time	30.40	1	119.70	6.30	0.0660
W	227.94	1	30.40	47.25	0.0023
Residual	19.30	4	4.82		
Corrected total	747.27	11			

R^2=0.970; Adj. R^2=0.918.



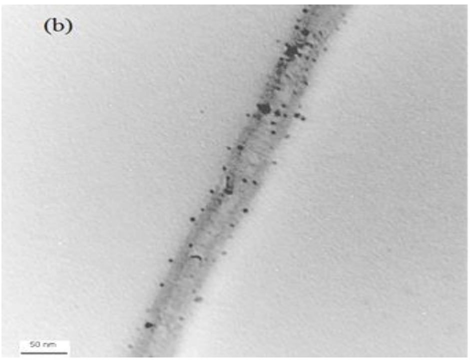


Fig. 1: TEM images of carbon nanotubes before (a) and after (b) of bio-synthesized nano silver particles.

replicates and conducted in a randomized orderly to avoid systematic bias. The statistical significance of the full quadratic models predicted was evaluated by the analysis of variance (ANOVA). The signs and the magnitude of the effects estimates for each variable and quadratic interaction were also determined. Unless otherwise stated, the significance level employed in the analysis was 5%. Finally, the model was used to predict both the optimum value and optimum region of the factor level, which results in maximum or fairly high Ag^+ percentage reduction. All the analysis was carried out using Design Expert (7.1.5 Trial) software.

RESULTS AND DISCUSSION

Transmission electron microscopy micrographs

The UV–Visible spectra, Fourier Transform Infrared Spectroscopy (FT-IR) and X-Ray Diffraction (XRD) were used to verify the Ag nanoparticles formation on the carbon nanotubs [31].

We further studied the carbon nanotubs before and after the bio-synthesis process using TEM micrographs (Fig. 1). Fig. 1b reveals the homogeneous distribution of the roughly spherical nanoparticles with an average size of 10 nm. In overall, the particles were well separated with inisignificant agglomerations.

Screening of Factors Using Plackett–Burman Design

The statistical evaluation of the results (Table 5), leads to develop the standardized main effect Pareto chart (Fig. 2).

The Pareto chart indicates a minimum t-value limit of 2.78 at a confidence level of 95.0% and Bonferroni limit of 5.75 (Fig. 2). In Fig. 2 the length of each factor is proportional to the absolute values of estimated effects and t-value and Bonferroni are included as horizontal reference lines. Therefore, the terms are supporting the hierarchy. In Pareto Chart, the effects above the Bonferroni limit are significant and effects above

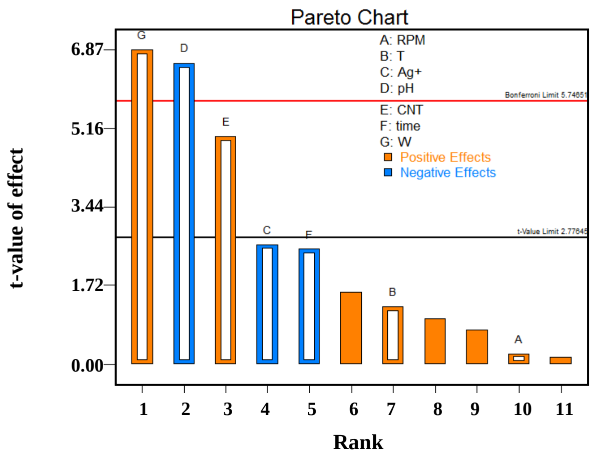


Fig. 2: Pareto chart for the Plackett–Burman design showing the effect and the direction of factors affecting the bio-synthesis of Ag/MWf-CNT.

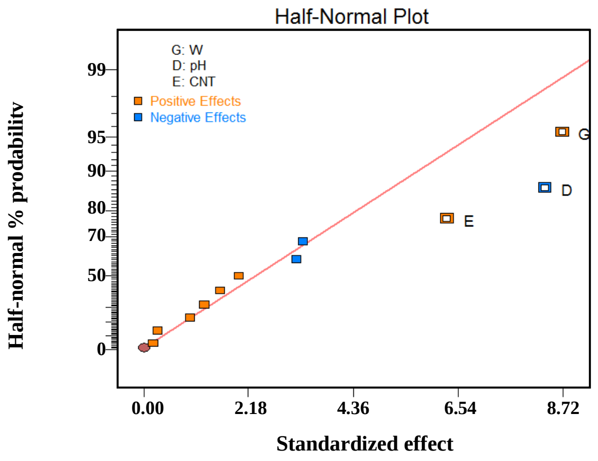


Fig. 3: Half-normal probability plot of effects for choosing the statistically significant effects in Plackett–Burman screening design.

the t-value limit are possibly significant and effects below the t-value limit are not likely to be significant.

The final decision on the most effective parameters requires the assessment of half-normal probability plot (Fig. 3). Furthermore, the positive and negative signs – corresponding to a tangerine and blue bar, respectively – showed that whether the response is improved from the low to high level or not. The Pareto chart shows that biomass weight (W) with a t-value of 6.86 has positive effect, pH with a t-vlue of 6.0 has a negative effect and CNT with a t-value of 5.0 has a positive effect. Therefore from Fig. 2 it can be concluded that parameters namely weight of wet biomass, pH and CNT concentration have the largest influence on the bio-synthesis of Ag/MWf-CNT.

The half-normal probability plot was also used to identify the significant parameters. In this plot, the factors having effects near the straight line through zero are not significant, while those deviating from the straight line are considered to be significant [28]. As shown in Fig. 3, the weight of wet biomass (W), pH and concentration of Carbon NanoTubes (CNT) are statistically significant. Moreover W and CNT demonstrate the positive, and pH shows the negative on the response (Pareto chart). Other points in the Fig. 3 are either insignificant or are dummy variables.

Based on the main effect plots (not shown), an increase in the level of W and CNT – within the range studied – had a statistically significant effect on the rate of Ag^+ reduction. Yet the increase of pH range led to decrease of Ag^+ reduction. Less accumulation of silver at lower pH could be simply due to the inhibition of silver ion accumulation by protons at lower pH [4, 19]. On the other hand, in acidic solutions low negative values of ξ-potential clearly indicates instability of the aggregates and reduction of Ag^+ ions [36].

The protein molecules act as reducing agent for silver nanoparticles. The overall peaks from FT-IR observation confirm the presence of protein in the samples of silver nanoparticles [33]. A protein molecule is made up of different functional group in amino acid sequences such as amino, carboxyl, sulfate groups. The Proteins facilitate the formation of extremely small-sized silver nanoparticles with the narrow particle size distribution. The hydroxyl and sulfonic groups are beneficial for the synthesis of silver nanoparticles with a slightly larger particle size in weak reducing environment. In addition, the proteins present over the silver nanoparticles surface act as capping agent. This indicates secretion of some protein components into the medium by the fungal biomass, which might play important role in the reduction of the metal ions in the form of nanoparticles. Consequently, the proteins also may bind to the nanoparticles and enhance the stability. Currently, the concentrated and separated proteins released by the fungus are employed to determine the exact role in the reduction processes [16, 20, 37]. Hence, the biomass weight is a pivotal parameter in bio-synthesis process to control the formation of nanoparticles.

The challenge in the fabrication of supported nanoparticles catalyst is to avoid of the agglomeration particles.

Table 6: Summary of the analysis of variance (ANOVA) result of the response quadratic model.

Statistical Terms	Response
	% Reduction of Ag^+
R^2	0.987
R^2 adjusted	0.977
R^2Predicted	0.952
P-Value (Prob.> F)	< 0.0001
F-Value	98.99
Lack of fit (LOF)	0.4083
PRESS	53.21
Adequate precision	29.736
Coefficient of variation	1.61

The functionalized multi-walled carbon nanotubes with hydroxyl and carboxyl reagents provide active sites for the adsorption and precipitation of Ag nanoparticles. (various oxygen groups onto the surface of the MWCNT including C-O and C=O) Thus, the concentration of the CNT in solution is a critical parameter to reduce the particles agglomeration.

The results of analysis of variance are listed in Table 5. The F-value and its corresponding p-value – equal to 18.60 and 0.0067, respectively – imply that the model is significant (at Prob<0.05). The value of R^2(>0.9), indicates that over 90% of the variation in the response can be explained by the model. Among the screened variables W, pH and CNT were identified as the most significant variables influencing the bio-synthesis process having F-values of 47.25, 43.3 and 24.81respectively with Prob<0.05 (Table 5). Moreover results of the regression analysis are in agreement with Pareto chart and half-normal probability plot which were previously discussed (Fig 2 and Fig. 3).

Response Surface Methodology (RSM)

Table 4 lists the reduction percentage of Ag^+ to Ag nanoparticles varied from 61.44 to 88.44% in each of the 17 factor level combinations. The data analysis is carried out for each response variable described in the following sections.

To find the most suitable fitting of the experimental data, a response surface model was developed using the regression analysis by considering different combinations of the linear, quadratic and interaction terms in polynomial equations. The adequacy of each model was checked using the F-values, lack of fit, and R^2-values, and finally a quadratic model was adopted.

The ANOVA results of the quadratic model are summarized in Table 6. The F-value (98.99), p-value (< 0.0001) and lack-of-fit (0.4083) indicate that the relation between the response and the selected parameters is statistically significant [38].

The adequacy of the model developed was evaluated based on the correlation coefficient R^2 and standard deviation value. The closer the R^2 value of unity and the smaller the standard deviation implying more accurate response and repeatability that could be predicted by the model [31, 39 and 40]. According to Table 6 R^2 and R^2-(adj.) for the model obtained were 0.987% and 0.977%, respectively. The compatibility of adjusted R^2 to R^2 means a good adaptation of the theoretical values for the experimental data of the model [41, 42]. This confirms that 98.72% of variation can be explained by the fitted model. The model can be considered a practical model for the prediction of the factors used and within the ranges tested.

From Table 6, the low value of coefficient of variation (1.61) indicates good precision and reliability of the experiments. The adequate precision (29.736) measures the signal to noise ratio and a ratio greater than 4 is generally desirable. Also, the predicted sum of squares (PRESS) is a measure of how a particular model fitted each point in the design [31]. The Model F-value of 98.99 implies the model is significant. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise.

Table 7 presented p-value of the variables included in the model. The values of “Prob. > F” less than 0.05 indicate the model terms are significant. The values greater than 0.10 indicate the model terms are not signed [42].

It can be concluded from Table 7 that all three main variables, i.e. weight of biomass, pH and concentration of carbon nanotube, are clearly significant; while the interactions between CNT with the other two factors (CNT×pH and CNT×W) are statistically insignificant; in other words, the optimum level of concentration of Carbon NanoTube (CNT) in the bio-synthesis process

Table 7: Values of regression coefficients calculated, F-value and P-value for each variable in the response quadratic model.

Independent factor	Regression coefficient	Standard error	F-value	P-value
Constant	-19.898	0.54		
CNT	25.966	0.40	80.79	< 0.0001
W	2.160	0.40	152.41	< 0.0001
pH	27.670	0.40	105.44	< 0.0001
CNT × CNT	13.099	0.77	7.39	0.0237
W × W	-0.112	0.77	13.19	0.0055
pH × pH	-02.697	0.77	114.47	< 0.0001
CNT × W	-	-	-	-
CNT × pH	-	-	-	-
W × pH	0.246	0.45	23.21	0.0010

does not depend on the level of weight of biomass (W) and pH. Also, according to the greater F-value (152.41), the weight of wet biomass is the most important variable of the response, which confirms Plakett-Burman design results. The model terms in the equation (2) were calculated after the elimination of insignificant interactions which have the lowest F-value and P-value greater (≥0.05) to improve the model adequacy.

Table 4 shows that the highest reduction of Ag ions to Ag nanoparticlesis 88.44%, which was achieved in Run 12, with the initial pH, CNT and weight of wet biomass of 7, 1 g/L, and 16 g, respectively. The lowest reduction of Ag ions to Ag nanoparticles was 61.44%, which is obtained in Run 8, with initial pH, sucrose, CNT and weight of wet biomass by 3.5, 0.2 g/L, and 6g, respectively.

Based on the calculated regression coefficient values (Table 7), a polynomial regression model equation was proposed as:

$$\%\text{Reduction of Ag}^{+} = -19.898 + 25.966(\text{CNT}) + 2.160(\text{W}) + 27.670(\text{pH}) + 13.099(\text{CNT})^2 - 0.112(\text{W})^2 - 2.697(\text{pH})^2 + 0.246(\text{W}\times\text{pH}) \quad (2)$$

The data were fitted into this equation with 95.24% of the variation.

The surface plot gives more complete information regarding the influence of a factor in the response. The major factor involved in secretion of enzymes and proteins in *Aspergillusis* is pH [43]. For example, the optimum pH recorded for protease production by *Aspergillus fumigatus* is 5, [44] or the extracellular enzymes of *Aspergillus fumigates* are efficient in the pH range of 4.5 to 5.5 [45].

The three-dimensional and contour plots presented in Fig. 4 shows the initial pH and wet biomass weight effects on reduction of Ag ions while the concentration of carbon nanotube is fixed at its center value (0.6 g/L). Verification of surface plot in Fig. 4a reveals that the effect of pH on the Ag^+ reduction rate depends on the wet biomass weight. At all pH, an increase in wet biomass weight (in the range 6–16 g) leads to an increase in the Ag^+ reduction rate. However, with an increase in pH value, the maximum weight of biomass increasing is observed in optimum wet biomass weights.

The contour plot in Fig. 4b shows the pH variation and wet biomass weight effect on the Ag^+ reduction rate in the bio-synthesis process. The contour plots are graphical representations of the regression equation. The maximum predicted value is indicated by the surface confined in the smallest ellipse in the contour diagram is the optimal region. Elliptical contours are obtained when there is a perfect interaction between the independent variables [46]. According to Fig. 4b, the reduction rate of Ag^+ increases with an increase in wet biomass weight at all pH value. However, in the case of biomass weight, there is a pH range optimum. Fig. 4b shows that the pH of 5.5-6.2 is the optimum range for the highest rate of Ag^+ reduction. This has also been reported by other researchers [18, 19]. It is derived from the contour plot

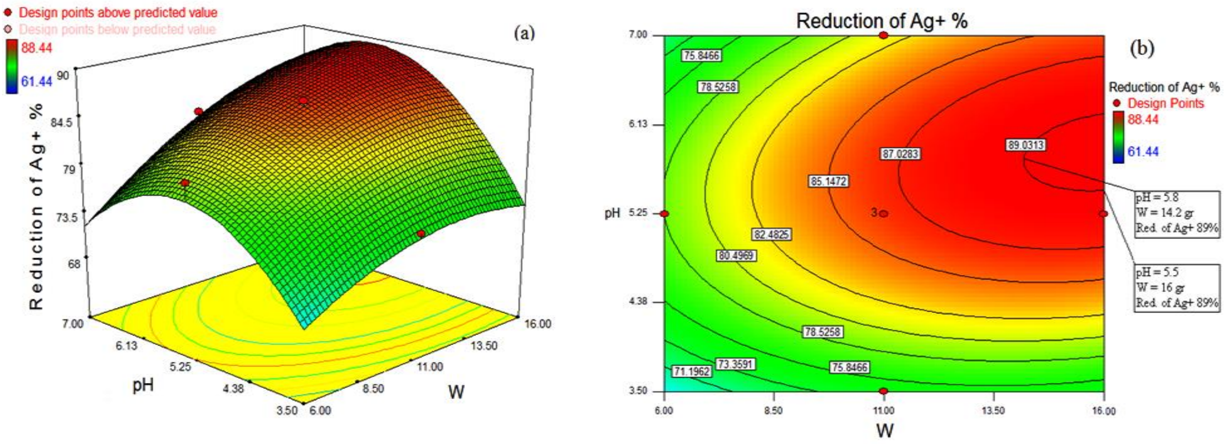


Fig. 4: (a) Surface plot for Ag^+ reduction with respect to weight of biomass and pH (with a concentration of carbon nanotube fixed at middle value). (b) Contour plot for Ag^+ reduction with respect weight of biomass and pH (with a concentration of carbon nanotube fixed at middle value).

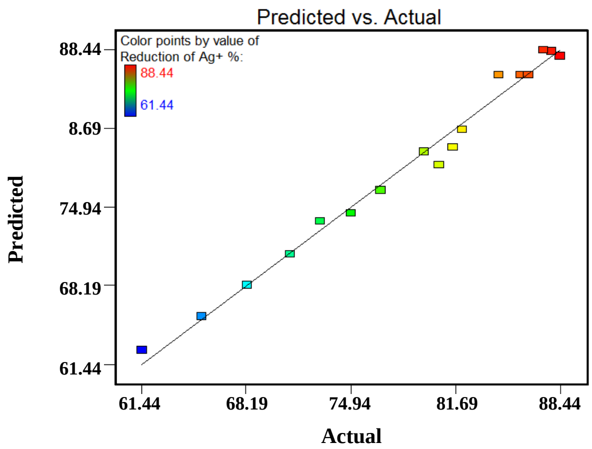


Fig. 5: Plot of experimental values versus predicted values.

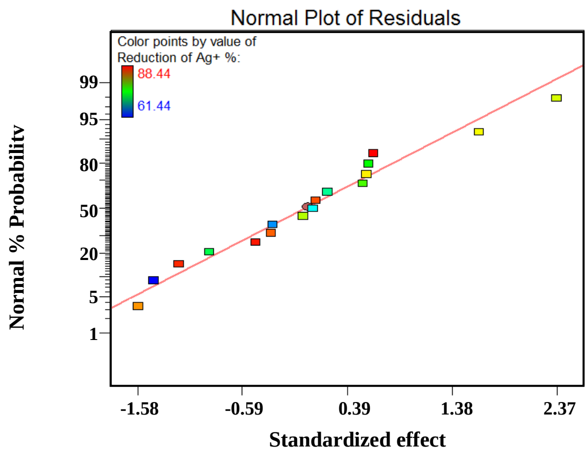


Fig. 6: Normal probability plot of the residuals.

(Fig. 4b) that the reduction of Ag ions increases in the range of 5.5-6.2 pHs, higher than 89%.

The significance of the model was also verified by the plot of predicted values versus the experimental values (Fig. 5). The clustering and concordance of points around the diagonal line together with the slope of the line (very close to 1) confirms the capability of the model to predict the experiments [35, 39 and 47].

Fig. 6 shows the normal probability plot of the residuals. A good correlation is observed between the experiment's distribution data and the linear regression model. Therefore, ANOVA can be validated since Figs. 5 and 6 demonstrate that the model precision represents the influence of the selected factors on the bio-synthesis process [47, 48].

CONCLUSIONS

The impact of various variables in the boi-synthesis of Ag/MWf-CNT process, including the initial pH, temperature, the concentration of the existing silver ions in the solution, the agitation time, stirring rate, the concentration of carbon nanotube and the biomass weight were explored to decipher the main factors in this process.

Using Plackett–Burman design screening, it was found that three variables, including the biomass weight, the initial pH and the concentration of carbon nanotubes, are the most statistically significant factors. It was also indicated whether the factors have direct or inverse effects on the response. Accordingly, increasing the wet biomass weight and carbon nanotube concentration increase rate of bio-synthesis process and conversely decreasing of pH increase the rate of bio-synthesis process.

According to the results of response surface methodology, there is an obvious interaction between the initial pH and the wet biomass weight. An increase in wet biomass weight leads to an increase in the Ag^{+} reduction rate at all pH ranges. The pH range of 5.5-6.2, the weight of biomass 13 g and concentration of carbon nanotubes of 0.6 g/L are the optimum conditions for the bio-synthesis of Ag/MWf-CNT.

Received : Dec. 17, 2014 ; Accepted : Nov. 23, 2015

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