BU R&D Journal
25 (1): 18-30 | ISSN (Print): 0016-4139
journal.bicol-u.edu.ph | ISSN (Online): 2719-082X
doi: 10.47789/burdj.mbtcbbgs.20222501.02

Qualifying Factor Effects on the Antibiotic Removal Capacity of Titania Impregnated Adsorbents Through An Empirical Model

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Abstract

This work focused on the development of an empirical model which encapsulated factor effects and factor interactions that affect the capacity of titanium dioxide (IV) (titania) impregnated rice husk ash (RHA) and coco coir dust (CCD) to remove amoxicillin dissolve in aqueous solution. Variables that were considered in this work were the following: initial amoxicillin concentration (ppm), time (min), titania impregnated adsorbent dosage (mg), and type of adsorbent (RHA and CCD). A 16 run general multi-level categoric (GMC) experimental design was then implemented to determine a suitable format for the built empirical model. Collation and analysis of the 16-point experimental data derived out of the implemented GMC experimental design identified that the data are in strong agreement with a two-factor interaction (2FI) mathematical model which considered the amoxicillin removal of titania impregnated adsorbents as the response variable. Results of analysis of variance using the collated experimental data validated that the initial amoxicillin concentration, titania impregnated adsorbent dosage, type of adsorbent, and the interaction between the titania impregnated adsorbent dosage and type of adsorbent were statistically significant terms of the formulated 2FI empirical model. Application of a regression analysis validated that the adjusted and predictive coefficients of determination were very near to unity. In addition, confirmatory experiments conducted indicated an average error 1.30% when the predicted amoxicillin removals were compared to the amoxicillin removals calculated by the formulated 2FI empirical model. This substantial evidence strongly implied that the developed 2FI empirical model was accurate and precise in determining a possible maximum amoxicillin removal in the solution space captured by the 16-point experimental data. Moreover, the predicted and experimentally observed amoxicillin removals were consistent in identifying RHA as the most suitable adsorbent for titania particle immobilization which yielded an amoxicillin removal capacity of about 50%.

Keywords: *titania, adsorbent, antibiotics, empirical model*

Introduction

Antibiotics, as emerging pollutants, have rapidly spread to the environment due to their persistent occurrence in the soil and unwarranted discharge of antibiotic containing effluents into various bodies of water (Mückter and Human, 2006; Triebskorn et al., 2007). Presently, the growing problem with the uncontrolled release of antibiotics in the environment could be attributed to at least 4000 pharmaceuticals that are being actively consumed by both humans and animals (Mompelat et al., 2009; Termes and Joss, 2006). It is a widely known fact that after consumption of these pharmaceuticals, only a portion of the discharged effluent are effectively treated in wastewater treatment plants that are specialized in antibiotic removal (Aukidy et al., 2012; Kinney et al., 2006). The presence of these antibiotics in very minute concentrations in the surroundings then significantly changes the behavior of natural flora and fauna in the environment (Stuart et al., 2012; Pounds et al., 2008). As a result of this change, the natural metabolic pathways followed by microorganisms are heavily disrupted and thus cultivate cultures of these microbes with antibiotic resistant genes (Schnell et al., 2009; Schreurs et al., 2005). These observed mutations of these microorganisms is now known as the primary cause of the existing of superbugs in the environment. Moreover, the lack of safety nets such as government support and stringent policies that could hold a generator of such wastes accountable makes low-income communities most vulnerable to drug-resistant pathogens.

Developing countries that lack the technology and corresponding budget to implement state of the art antibiotic contaminated effluent treatments are seen to be critically threatened by this emerging problem. As reviewed literature suggests, photocatalytic treatment processes, particularly advance oxidation methods, have been proven to be the most efficient in the removal of minute antibiotic residues in both soil and aqueous solutions (Lofrano et al., 2018; Tawar et al., 2018). This broadly used effluent treatment, however, is highly technical, very costly, and its continuous operation in the long term is unpractical and unsustainable (Dong et al., 2015). Inevitably, these countries would not have the capacity to mitigate this growing environmental concern. Hence, there is an immediate need to explore other alternative treatment technologies that would be suitable to the conditions and technical capacities of these low-income countries.

Rice husk ash (RHA) and coco coir dust (CCD) are known to be low-cost adsorbents common to agricultural and developing countries. Its capacity to remove various contaminants in solution has already been demonstrated in literature (Ahmaruzzaman and Gupta, 2011). For example, RHA and CCD have been proven to efficiently capture dyes in aqueous media (Sunil and Jayant, 2013; Ahmaruzzaman and Gupta, 2011; Chandrasekhar and Pramada, 2006). In a different study, RHA and CCD were shown to achieve high removal of heavy metals in solution (Vieira et al., 2014; Sunil and Jayant, 2013; Ahmaruzzaman and Gupta, 2011). RHA and CCD has also been used for the removal of selected organic pollutants (e.g. antibiotics) in water (Sunil and Jayant, 2013; Ahmaruzzaman and Gupta, 2011). Moreover, related studies have also explored modification of the surfaces of these adsorbents to enhance their efficacy and be implemented as nanoporous adsorbents in biomedical applications (Patel, 2018; Sumathi and Suriyahprakash 2016; Cheah et al., 2016; Fernandez et al., 2015). These cited evidences, hence, establish the potential of RHA and CCD as viable materials suitable to various environmental applications. Unfortunately, despite this fact, the RHA and CCD were reported to be underutilized (Sunil and Jayant, 2013; Ahmaruzzaman and Gupta, 2011; Chandrasekhar and Pramada, 2006).

To address this gap, the authors explored the application of RHA and CCD as materials suitable for the removal of antibiotic residuals in solution. Through an extensive review of literature conducted, the specific research objective, as seen in Figure 1, was thus formulated (Chollom et al., 2020; Sultan et al., 2019; Masse et al., 2018; Patel, 2018; Mäkelä, 2017; Sumathi and Suriyahprakash 2016; Sunil and Jayant, 2013; Ahmaruzzaman and Gupta, 2011; Kowalski et al., 2000). In an attempt then to demonstrate the efficacy of RHA and CCD as potential adsorbents capable of mitigating the effects of residual drugs in aqueous media, this work focused on investigating the extent to which these adsorbents sequestrate amoxicillin dissolved in aqueous solution if these titanium dioxide (IV) particles are immobilized on the surfaces of these adsorbents (see Figure 1). Factors affecting the observed amoxicillin removal of these impregnated adsorbents were then thoroughly investigated through the utilization of a

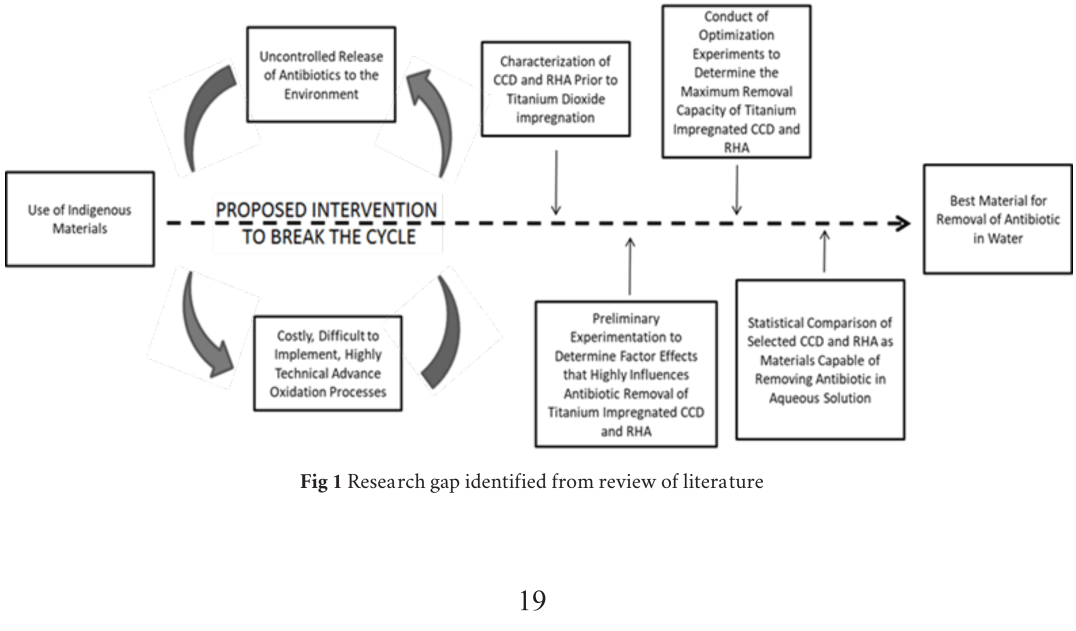

Fig 1 Research gap identified from review of literature

general multi-level categoric experimental design. Out of this approach, a two-factor interaction (2FI) empirical model qualifying the influence of initial amoxicillin concentration, time, titanium dioxide (IV) adsorbent dosage, and type of adsorbent was derived. As the final output of this work, it was consistently identified that RHA impregnated with titanium dioxide (IV) particles was relatively superior in removing amoxicillin particles dissolved in solution than that of CCD. Hence, with the experimental evidences presented in this work, RHA was strongly recommended as a very promising adsorbent for sequestration of residual drugs in aqueous media.

Materials and Methods

Materials

RHA and CCD were investigated in this work as the potential carriers of the titania particles for the photocatalytic degradation of amoxicillin dissolved in aqueous medium. RHA was sourced from a local biomass plant located in Pili, Camarines Sur while CCD was purchased from a local supplier in Legazpi City, Albay. RHA and CCD were ground and washed vigorously to remove excess carbonaceous material according to the procedure described by Etim et al. (2021). The UV lamp that was used in this work had a UV lamp power of 5 W and emitted UV rays at a wavelength of 254 nm. All chemical reagents that were utilized in this work were of analytical grade quality. Deionized (DI) water was used in all of the experiments conducted.

Preparation of Titania Stock Solution

Preparation of the titania stock solution was primarily based on the procedure described by Asiltürk and Şener (2012). Titanium (IV) – iso – propoxide (TTIP; ≥ 97%) was used as the source of titania particles to be immobilized on the surface of RHA and CCD. Briefly, TTIP was diluted in anhydrous n-propanol and this mixture was coded as mixture A. Subsequently, a second solution was prepared by mixing DI water with anhydrous n-propanol and hydrochloric acid, and this second mixture was coded as mixture B. 1:100:0.2:50 for TTIP/n-propanol/HCL/DI water was followed as the molar ratios of mixture A and mixture B. Titania stock solution was freshly prepared for each experiment conducted to minimize potential ageing effects.

Preparation of Titania Impregnated Rice Husk Ash and Coco Coir Dust

Titania particles were immobilized on the surfaces of RHA and CCD according to a modified procedure detailed in the work of Asiltürk and Şener (2012). Briefly, mixture B was individually added onto dried and sieved RHA and CCD particles (10% weight proportion). This solution was continuously stirred until homogeneity of the solution was achieved. These resulting suspensions for RHA and CCD were individually transferred into a previously prepared mixture A and vigorous mixing at room temperature was employed to the separate RHA and CCD suspensions for 1 hour. The final resulting mixtures were then subjected to hydrothermal treatment through the use of a Teflon-lined autoclave reactor at a temperature of 130°C. Hydrothermal treatment for the RHA and CCD final mixtures were conducted for 3 hours. Subsequently, the hydrothermally treated RHA and CCD mixtures were cooled near to room temperature and the titania impregnated RHA and CCD particles were collected by allowing the heavy matter in solution to settle for 1 hour. Excess titania stock solution was removed by thorough rinsing of the collected titania impregnated particles using DI water. Finally, the washed titania impregnated RHA (TI-RHA) and CCD (TI-CCD) were dried over night at 70°C. These dried particles were then stored in a clean and dry place at room temperature prior to experimentation.

Preparation of Amoxicillin Stock Solution

1000 ppm stock amoxicillin (amoxicillin trihydrate) was prepared according to the procedure described in Li and Yang (2006). Briefly, 0.25 g of amoxicillin was prepared and this was added into a 250 mL of DI water. To ensure that the prepared amoxicillin stock solution was well-preserved, this solution was protected from light and was stored in a chiller at 4°C.

Determination of Amoxicillin Concentration in Solution

Spectrophotometric determination of amoxicillin concentration in aqueous media described by Li and Yang (2006) was adopted in this work. 1.0 mL of the solution containing the amoxicillin antibiotic were initially collected. 2.0 mL of 1,2–naphthoquinone–4–sulfonate and 2.0 mL K2HPO4-K2HPO4 buffer solution (pH 9.00) were then sequentially added. The resulting solution was then diluted using 12.5 mL of DI water with continuous mixing until homogeneity of the solution was achieved. Finally, the well-mixed solution was allowed to stand for 50 min at room temperature and amoxicillin concentrations were then read at a wavelength of 468 nm.

Microscopy Imaging of Titania Impregnated Rice Husk Ash and Coco Coir Dust

The presence of immobilized titania particles on the surfaces of RHA and CCD were confirmed through microscopy imaging. About 30 mg each of TI-RHA and TI-CCD were stored in separate microcentrifuges. Prior to microscopy imaging, both the TI-RHA and TI-CCD were coated with gold-based sputter coating material in order to capture clear microscope images and minimize beam-sensitivity and non-conductivity of the TI-RHA and TI-CCD particles (Fourie, 1982). Finally, images were taken after this sample preparation procedure.

Employed Experimental Design

A general multi-level categoric (GMC) experimental design was implemented to qualify the influence of various factors that could potentially affect the antibiotic removal capacity of the titania impregnated adsorbents investigated in this study. Initial amoxicillin concentration (factor A), time (factor B), titania impregnated adsorbent dosage (factor C), and type of adsorbent (factor D) were the factors identified to be explored in this work. The specifics of these factors (e.g. factor levels) are summarized in Table 1. All other factors not considered as variables in this study were set constant values (temperature = 25°C; UV light wavelength = 254 nm, UV lamp power = 5 W). Level 1 and Level 2 of the chosen factors were based on preliminary exploratory experiments that yielded significant amoxicillin removals (i.e. removal of at least 20%). For example, while amoxicillin removal could be observed for 1-, 2-, 3-, and 4-min intervals, these were not considered since results of preliminary experiments confirmed that 1-4 min intervals yielded amoxicillin removals below 20% (e.g. average removal of 8% ± 0.51%). Using these identified factors, an empirical model was then built using a 16 run GMC experimental design (see Table 2). To minimize human bias in the conduct of all experimental runs, the order where each experimental run is performed where randomized. Moreover, Design Expert 11 Software® (Stat-Ease, USA) was used to facilitate faster calculations for the development of the 16 run GMC experiment mathematical model.

Formulation of the Empirical Model

The 16 run GMC experimental design summarized in Table 2 was primarily used as the basis for the formulation of the empirical model being developed in this work. To quantify the mathematical relationships between the independent and dependent variables, the detailed procedure prescribed by Mäkelä (2017) was implemented in this study. Briefly, the empirical relationship was first built using the general format shown in Equation (1):

$$y = f(\emptyset_1, \emptyset_2, \emptyset_3, \ldots, \emptyset_k) + \varepsilon$$

where y corresponds to the response variable which was expressed in terms of the independent variables ($f(\emptyset_1, \emptyset_2, \emptyset_3, \ldots, \emptyset_k)$) and Ɛ corresponds to the total of all errors incurred during the conduct of experimentation. Consequently, coding of the factor effects caused by the independent variables investigated in this work was derived using Equation (2):

$$x_i = \frac{\emptyset_i - \emptyset_{min}}{\Delta\emptyset/2} - 1$$

where x_i corresponds to the coded value and $\emptyset_i$, $\emptyset_{min}$, and $\Delta\emptyset$ pertain to the respective variable value, minimum level, and variable range in accordance to the original units they were coded unto. As a result

Table 1 Factors Considered in this Study

Factor Name	Unit	Factor Code	Factor Type	Level 1	Level 2
Initial amoxicillin concentration*	ppm	A	Independent Variable	60	100
Time*	min	B	Independent Variable	5	15
Adsorbent Dosage*	mg***	C	Independent Variable	5	15
Type of Adsorbent**	-	D	Independent Variable	TI-RHA	TI-CCD
Amoxicillin Removal	%	R	Dependent Variable	-	-

**Level 1 and Level 2 for these factors represent the low and high factor levels set for the implemented general factorial design.*
***While two levels were investigated, no particular order was given to this factor since it is categoric in nature.*
****Volume of solution was set constant at 50 mL.*

of this formulation, high and low factor levels were coded as experimental design points for establishing the base mathematical model of the targeted empirical relationship containing factors A, B, C, and D. In contrast, factor D (i.e. type of adsorbent) would also be coded as an experimental design point but with the exception of it being identified as a categoric variable. Hence, no particular order was given to factor D since its nature as a variable is nominal. From this formulation, a 2nd order polynomial to predict the amoxicillin removal of TI-RHA and TI-CCD was hence formulated in accordance to Equation (3):

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} x_i x_j + \varepsilon$$

where:

- β_0 corresponds to the average value (intercept) of y
- β_i corresponds to first order terms (without interaction)
- β_{ij} corresponds to interaction coefficients that influence the response
- x_i and x_j corresponds to the coded independent variables
- ε corresponds to the residuals pertinent to the 2nd order polynomial equation.

Finally, the GMC-based empirical model was developed using Equation (3) and this derived empirical model included the four factors (i.e. A, B, C, and D) identified in this work.

To qualify the capacity of the formulated empirical model to predict the magnitude of the dependent variable (i.e. amoxicillin removal), regression analysis of the 16 run GMC experimental data was performed according to the procedure detailed in Mäkelä (2017). The variation that is expected from the derived model was expressed through the coefficient of determination (i.e. R^2). This was calculated using Equation (4):

$$R^2 = \frac{SS_{mod}}{SS_{tot}} = 1 - \frac{SS_{res}}{SS_{tot}}$$

where R^2 is the coefficient of determination, *SSmod* is the model sum of squares, *SStot* is the total sum of squares, SSres is the residual sum of squares. Overfitting due to the addition of more model terms is a known problem that invalidates the coefficient of determination returning a value near to unity. Hence, other fit statistic is needed in order to validate the calculated the coefficient of determination (Chollom et al., 2020; Sultan et al., 2019; Masse et al., 2018; Mäkelä, 2017; Kowalski et al., 2000). It is therefore necessary to adjust the calculated coefficient of determination according to the number of terms added to the derived

Table 2 Employed General Multi-Level Categoric Experimental Design in this Study

Experimental Run Number	Factor A: Initial Amoxicillin Concentration (ppm)	Factor B: Time (min)	Factor C: Adsorbent Dosage (mg)	Factor D: Type of Adsorbent
Run 1	60	15	15	TI-RHA
Run 2	60	5	15	TI-RHA
Run 3	60	5	15	TI-CCD
Run 4	100	5	15	TI-RHA
Run 5	100	15	15	TI-CCD
Run 6	100	5	5	TI-RHA
Run 7	100	5	15	TI-CCD
Run 8	60	5	5	TI-CCD
Run 9	100	15	5	TI-RHA
Run 10	60	15	5	TI-CCD
Run 11	60	5	5	TI-RHA
Run 12	60	15	15	TI-CCD
Run 13	100	5	5	TI-CCD
Run 14	100	15	15	TI-RHA
Run 15	100	15	5	TI-CCD
Run 16	60	15	5	TI-RHA

Implemented Regression Analysis

empirical model. This was calculated using Equation (5):

$$R^2_{adj} = 1 - \frac{\frac{SS_{res}}{n-p}}{\frac{SS_{tot}}{n-1}}$$

where R^2adj is the corrected coefficient of determination, $n - p$ is the degrees of freedom of SSres, and $n - 1$ is the degrees of freedom of *SStot*. Additionally, the capacity of the derived empirical relationship to predict the amoxicillin removal exhibited by TI-RHA and TI-CCD was also investigated. In order to quantify this, the sum of squares of the prediction error was also determined in accordance to Equation (6):

$$R^2_{pre} = 1 - \frac{SS_{pre}}{SS_{tot}}$$

where R^2pre is the predictive coefficient of determination and SSpre is the predictive sum of squares. Collectively, these criteria were used in order to evaluate the validity of the developed GMC empirical model.

Results and Discussion

Immobilization of Titania Particles on Rice Husk Ash and Coco Coir Dust Surfaces

The immobilization of titania particles on the surfaces of TI-RHA and TI-CCD were confirmed through the use of microscope imaging. It was observed that the titania particles were largely retained on the surfaces of TI-RHA and TI-CCD as shown on Figure 2B and Figure 2D. Instances of particle crowding, particularly that of TI-RHA as imaged on Figure 2D, signified that TI-RHA was saturated with more titania particles in comparison to TI-CCD. The results of these imaging also implied that a higher antibiotic removal capacity of TI-RHA would be observed since more titania particles were captured on the surface of TI-RHA. Moreover, no desorption of the immobilized titania particles (i.e. negligible absorbance readings for titania particles suspended in rinsing effluent) were observed for both TI-RHA and TI-CCD even after multiple rinsing were employed using DI water. Overall, the confirmed immobilization of titania particles on the surfaces of TI-RHA and TI-CCD strongly implied that antibiotic removal would indeed be observed upon the implementation of the 16 run GMC experimental design.

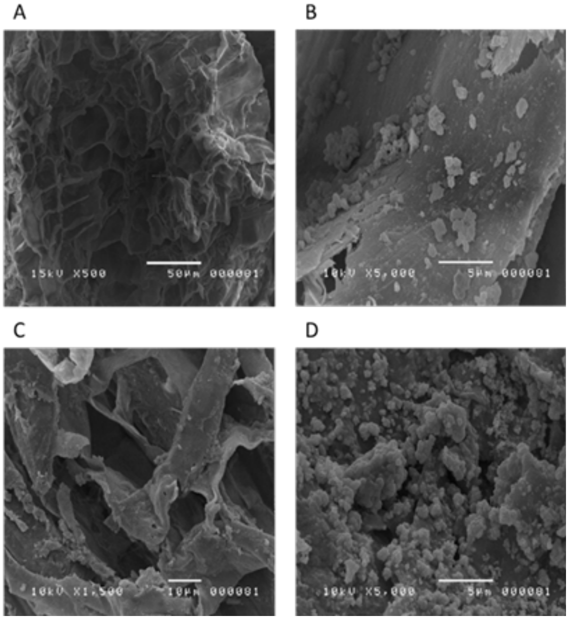

Fig 2 Microscope images of the titania impregnated adsorbents. Comparison of microscope images taken for the unimpregnated CCD (A) and RHA (C) confirmed that titania particles were successfully immobilized on the surfaces of TI-CCD (B) and TI-RHA (D).

Preliminary Experimentations

Initial experimentations were implemented in order to identify the high and low factor levels for factor A, B, and factor C. For the case of factor A, the high and low factor levels were set at 100 and 60 ppm, respectively. The low factor level was set at 60 ppm since no more amoxicillin removals (< 2.0%) could be observed at initial amoxicillin concentrations below this set value. In contrast, the high factor level for factor A was set at 100 ppm since amoxicillin removals observed for TI-RHA and TI-CCD start to plateau at 50% and 40%, respectively. These set values then represented the range where photocatalytic activity of the titania impregnated adsorbents was significant. Similarly, the slowest and fastest rates of the photocatalytic activity observed for TI-RHA and TI-CCD were investigated by varying the time where amoxicillin molecules are exposed to the titania impregnated adsorbents subjected under UV light. Varying the time at 5 min intervals identified the 5 min mark as the instance where photocatalytic activity was first observed while the 15 min mark as the instance where photocatalytic activity of TI-RHA and TI-CCD neither increased nor decreased (i.e. remained constant). Therefore, the 15 min and 5 min mark were then set as the high and low factor levels for factor B. Correspondingly, the same approach was also implemented for the identification of the high and low factor levels for factor C. Amoxicillin removals when factor C was varied were most significant at adsorbent dosages ranging between 5 mg and 15 mg for treating amoxicillin solutions having a volume of 50 mL. Hence, 15 mg and 5 mg were set as the high and low factor levels, respectively, for factor C. Moreover, no high or low factor levels were set for factor D since this factor is nominal in nature. Finally, the amoxicillin removals of the titania impregnated adsorbents were found to be statistically different (observed antibiotic removal capacity were at least 5.0% higher than that of the unimpregnated adsorbents) with that of the performance of their bare counterparts. Collectively, the results of these preliminary experimentations affirmed that the planned 16 run GMC experimental design could be implemented at the set high and low factor levels for the identified variables.

Specifying the Format of the Formulated Empirical Model

To build the mathematical model which would define the relationship between the identified independent and dependent variables studied in this work, the statistically sound format of the empirical model was first investigated. Using the 16 run GMC experimental design, linear (e.g. main effects alone), two-factor interaction (2FI), three-factor interaction (3FI), and four-factor interaction (4FI) formats of the empirical model were tested and these were found to be statistically significant at a confidence level of 95% (except for the 4FI empirical model). Using Equation (1) to Equation (6), the soundness of these empirical models was also compared by maximizing the R^2_{adj} and R^2_{pre} for the statistically significant (P-values lesser than 0.05) empirical model formats. The results of this comparison are summarized in Table 3. Based on the calculated maximized coefficients of determination and P-values, the 2FI model format (e.g. Equation (3)) was strongly recommended as the most appropriate format to the experimentally observed results of the 16 run GMC experimental design implemented. In addition, this strong fit of the 16 run GMC experimental data is a very strong evidence that point to the use of the 2FI empirical model format (Chollom et al., 2020; Sultan et al., 2019; Masse et al., 2018; Mäkelä, 2017; Kowalski et al., 2000). Hence, the 2FI model was chosen as the primary basis of the built empirical model.

Selection and Identification of Factor Combinations

Out of the four factors considered in this work (A, B, C, and D), 15 factor combinations (excluding the intercept) were identified to be potential terms of the 2FI empirical model. These identified factor combinations are summarized in Table 4. It could be observed that 3FI and 4FI terms were also part of the identified factor combinations but since a 2FI empirical model was found to be the most appropriate empirical model format describing the relationship among factor combinations of A, B, C, and D, these terms were not anymore considered for the built model. To further validate this observation, additional experiments were done to calculate for the statistical significance of the 3FI and 4FI identified factor combinations. As seen from Table 4, 3FI factor combinations were statistically proven to not have significant contribution to the fitted data (i.e. P-values were calculated to be greater than 0.05). Moreover, while the 4FI factor combination indicated a perfect fit to the experimental data, its P-value cannot be calculated due to overfitting (e.g. R2 = 1.0000). Hence, the 2FI empirical model was pursued for final statistical evaluation of its validity.

Determination of Final Empirical Model Terms

From the 15 factor combinations, an iterative calculation process was implemented in order to eliminate factor combinations identified to have the least effect on the dependent variable (i.e. amoxicillin removal) being considered. The results of this iterative calculations are summarized in Table 5. As seen from Table 5, it could be observed that the empirical model was built on the 4th iteration where the final included factor combination D was considered to

Table 3 Fit Statistics of Linear, 2FI, 3FI, and 4FI Empirical Model Formats

Empirical Model Format	P-value	R^2	R^2_{adj}	R^2_{pre}
Linear	< 0.0001	0.8740	0.8282	0.7335
2FI*	< 0.0001	0.9996	0.9988	0.9960
3FI	0.0171	0.9999	0.9995	0.9913
4FI	NA**	1.0000	NA**	NA**

**Applicable empirical model format for the 16 run GMC experimental design due to most significant P-value and fit statistics*

***Values cannot be calculated due to overfitting of the empirical model (e.g. 0 values for the sum of squares of the residuals)*

Table 4 List of Factor Combinations Considered

Factor Combination	Factor Combination Type	P-value*	Remark
A	Main Effect	0.0104	Significant Effect
B	Main Effect	0.3202	No Significant Effect
C	Main Effect	0.0044	Significant Effect
D	Main Effect	0.0245	Significant Effect
AB	2FI	0.6740	No Significant Effect
AC	2FI	0.3559	No Significant Effect
AD	2FI	0.3246	No Significant Effect
BC	2FI	0.3809	No Significant Effect
BD	2FI	0.8149	No Significant Effect
CD	2FI	0.0105	Significant Effect
ABC	3FI	0.4649	No Significant Effect
ABD	3FI	0.7030	No Significant Effect
ACD	3FI	0.2048	No Significant Effect
BCD	3FI	0.8495	No Significant Effect
ABCD	4FI	NA**	No Significant Effect

**P-values were calculated by fitting the experimental data to 2FI, 3FI and 4FI empirical models.*

***P-value for the 4FI term cannot be calculated since the R^2 was already at 1.0000. This is a strong indication of overfitting.*

be statistically significant together with main effect factor combinations (A, C, and D) and the observed factor interaction term (CD). The remaining 2FI, 3FI, and 4FI terms, however, were considered as error terms for the built 2FI empirical model since these factor combinations were statistically validated to not have significant influence on the dependent variable (Mäkelä, 2017). Hence, the factor combinations A, C, D, and CD were selected as the final terms of the 2FI empirical model investigated in this work.

Regression Analysis of the Built Empirical Model

Using the statistically determined factor combinations, the 2FI empirical model with its statistically significant terms is then summarized by the coded equation shown in Equation (7):

$$\text{Amoxicillin Removal (\%)} = 29.92 + 5.23\,A - 12.47\,C + 2.22\,D + 5.19\,CD$$

where the *base model* (where no values is given to factor D) of this formulated model corresponds to the influence of RHA on the dependent variable. To further validate the integrity of this developed 2FI model, a regression analysis in accordance to Equation (4) to Equation (6) was implemented. The results of this regression analysis are summarized under Table 6 and Table 7. Parallel to the results summarized in Table 5, Table 6 strongly supported the claim of this work that initial amoxicillin concentration (factor A), titania impregnated adsorbent dosage (factor C), type of adsorbent (factor D) and the interaction (factor CD) that of the titania impregnated adsorbent dosage (factor C) and type of adsorbent (factor D) primarily influenced the observed antibiotic removal capacity of the titania impregnated adsorbents. Furthermore, an R^2 value of 0.9992 (very close to unity) imply that the 16 run GMC experimental design strongly fits the developed 2FI empirical model (Chollom et al., 2020). To dismiss the scenario of a potential overfitting, the R^2_{adj} and R^2_{pre} were also calculated. The difference between R^2 and R^2_{adj}, and the difference between R^2_{adj} and R^2_{pre} were well within the criterion which requires

Table 5 Results of Iteration for Determining the 2FI Empirical Model Specifications

Iteration Number	Empirical Model Specifications	Calculated P-value of the Model Term
Iteration 1	Inclusion of factor C	2.97×10^{-5}
Iteration 2	Inclusion of factor A	0.0054
Iteration 3	Inclusion of factor CD	4.00×10^{-6}
Iteration 4	Inclusion of factor D	1.76×10^{-9}
Iteration 5*	Inclusion of factor B, AB, AC, AD, BC, BD, ABC, ABD, ACD, BCD, and ABCD	> 0.0500

**The insignificant terms (e.g. remaining 2FI, 3FI, and 4FI terms) were considered as error terms.*

a maximum difference of 0.2 between these statistics. As an additional test for empirical model validity, a precision value that is greater than 4.0 (i.e. 164.75) strongly implied that the results being read and calculated are not caused by noise induced by possible errors incurred in the conduct of the 16 run GMC experimental design. Hence, it could be confidently concluded that the developed 2FI empirical model adequately describes the existing relationship between the investigated independent and dependent variables (Chollom et al., 2020; Mäkelä, 2017).

Table 6 Analysis of Variance for the Factor Combinations Included in the Empirical Model

Empirical Model Terms	Calculated P-value
A	< 0.0001
C	< 0.0001
D	< 0.0001
CD	< 0.0001

Table 7 Results of Regression Analysis for the Built 2FI Empirical Model

Regression Statistic	Calculated P-value
R^2	0.9992
R^2_{adj}	0.9989
R^2_{pre}	0.9983
Precision	164.75

Interpretation of the Built Two-Factor Interaction Empirical Model

Using the statistically established 2FI empirical model describing the effects of various factors on the dependent variable, the coefficients of each factor combination calculated as shown in Equation (7) could be utilized to qualify the extent of factor influence on the observed amoxicillin removal of TI-RHA and TI-CCD. The implications of the main effects derived out of factors A, C, and D are visualized in Figure 3. Firstly, the calculated intercept while setting all of the other factor combinations to zero (i.e. base model) predicts that the titania impregnated RHA would garner a predicted amoxicillin removal of 29.92%. Secondly, three main effects that affect the predicted amoxicillin removal of TI-RHA and TI-CCD could be generally observed. Surprisingly, an increase in the initial amoxicillin concentration gave a positive uptake on the expected amoxicillin removal of TI-RHA and TI-CCD. This observation was found to be in contrast with what is usually observed in literature (Belfort and Lee, 1991). It is hypothesized that the relatively high concentrations of particles suspended in bulk solution fueled a faster diffusion rate of amoxicillin molecules to the surface of TI-RHA and TI-CCD which in turn resulted to a significant increase in the photocatalytic activity of the two titania impregnated adsorbents (Das and Mondal, 2011). A positive coefficient on factor D further implied that it is highly possible that the antibiotic removal capacities of TI-RHA and TI-CCD were indeed significantly increased by this hypothesized phenomenon. In the absence of a comprehensive characterization data, it is, however, advised that caution be exercised in accepting this derived hypothesis as the conclusive explanation for this observed unique behavior. Nevertheless, it is then highly suggested that additional characterization experiments (i.e. mapping the movement and potential distribution of amoxicillin molecules in bulk solution when treated with TI-RHA or TI-CCD) be done in order to fully explain this observed phenomenon.

Surprisingly, an increase in the TI-RHA and TI-CCD dosage was predicted to negatively affect the antibiotic removal capacities of these titania impregnated adsorbents. This observed behavior could be attributed to the fact that particle overcrowding of the titania impregnated adsorbents was already evident in bulk solution (Garg et al., 2003). This observation then strongly implied that lower TI-RHA and TI-CCD could potentially achieve higher amoxicillin removals than those which was observed in this work. Moreover, a combinatory effect of the titania impregnated adsorbent dosage (factor C) and type of adsorbent (factor D) was also detected (see Figure 4). While a negative effect on the amoxicillin removals of the titania impregnated adsorbents was previously observed, a positive uptake to the observed amoxicillin removals was also prevalent. In its entirety, an increase in the titania adsorbent dosage might not ultimately lead to a decrease in the observed amoxicillin removals. However, caution should still be exercised when exploring titania impregnated dosages below 5 mg. It is also recommended that extent of titania particle immobilization of the surfaces of the investigated adsorbents be also explored in future work investigating amoxicillin removals of TI-RHA and TI-CCD at dosages below 5 mg. Moreover, it is also suggested that optimization experiments be conducted to further explore experimental conditions at dosages below 5 mg. Nevertheless, these crucial findings point to the fact that amoxicillin removals exhibited by TI-RHA and TI-CCD could possibly be maximized through the understanding of the factor effects that largely influence the antibiotic removal capacities of these titania impregnated adsorbents.

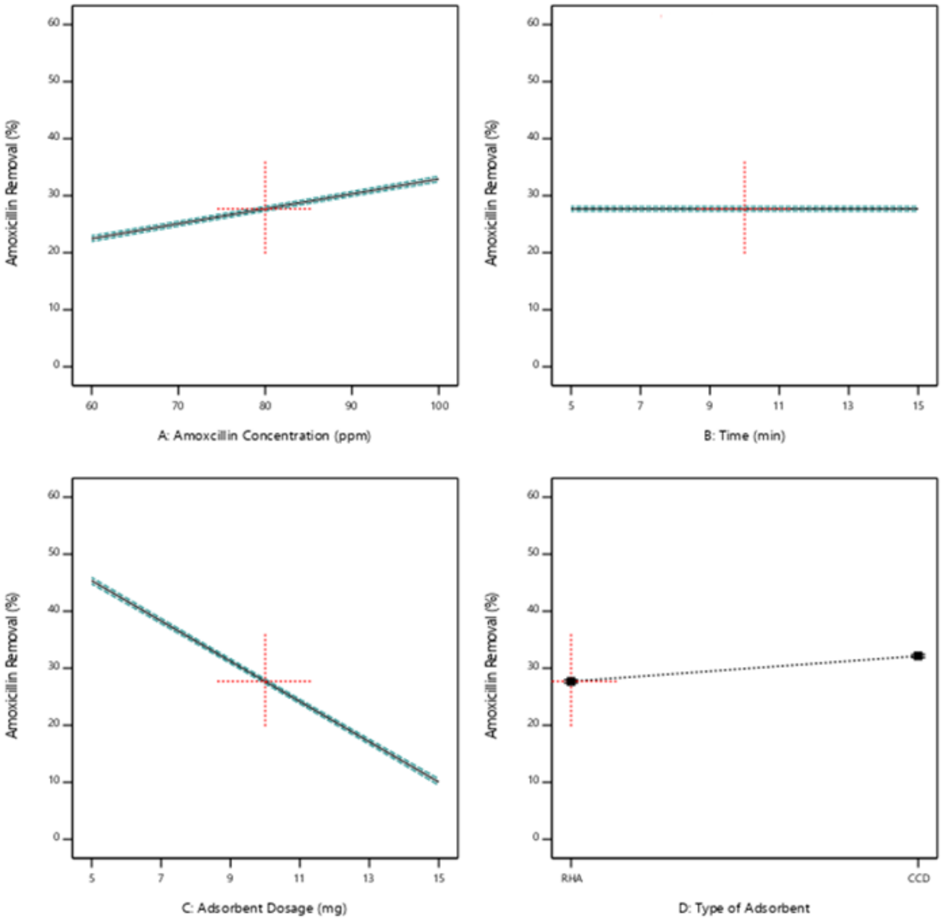

Fig 3 Visualization of the main factor effects affecting the observed amoxicillin removals of the titania impregnated adsorbents. This figure visualizes the magnitude of the observed main effects attributed to the initial amoxicillin concentration (A), titania impregnated adsorbent dosage (C), and type of adsorbent (D) as described in Equation (7). No significant factor effect was observed for time (B).

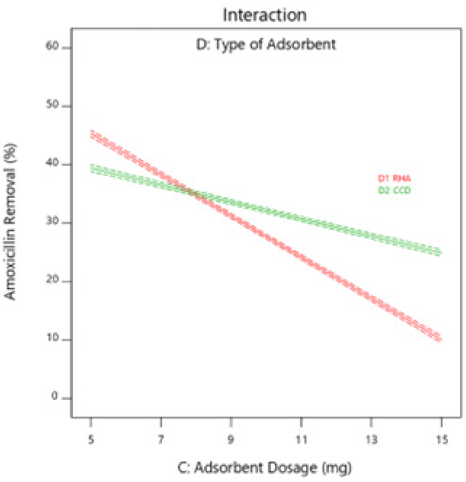

Fig 4 Visualization of the CD factor interaction affecting the observed antibiotic removal capacity of TI-RHA and TI-CCD. The observed intersection between TI-RHA and TI-CCD confirmed the presence of a factor interaction.

Practical Implications of the Developed 2FI Empirical Model

Aside from the benefit of qualifying factor effects affecting the amoxicillin removal of titania impregnated adsorbents, the derived empirical model was ultimately used to determine and replicate experimental conditions where amoxicillin removals of TI-RHA and TI-CCD are at their highest. With the goal of determining the highest amoxicillin removal that could be possibly achieved given the experimental conditions explored in this study, 11 solution points replicating a maximum amoxicillin removal for the investigated titania impregnated adsorbents were generated. Out of these 11 solutions, maximums for each of the investigated TI-RHA and TI-CCD with acceptable desirabilities were chosen. The specific experimental conditions describing these predicted maximum amoxicillin removals are summarized under Table 8. It could be seen that the TI-RHA was predicted

Table 8 Experimental Conditions Describing a Maximum Amoxicillin Removal

Solution Number	Initial Amoxicillin Concentration (ppm)	Time* (min)	Adsorbent Dosage (g/L)	Type of Adsorbent	Predicted Amoxicillin Removal (%)	Desirability	% Error
Solution 1	100	10	5	RHA	50.59	0.995	0.90
Solution 2	100	10	5	CCD	44.65	0.866	1.70

**Time was identified to be an insignificant factor so this was set constant at 10 min.*

to have a higher antibiotic removal capacity (50.59%) in comparison to that of the TI-CCD (44.65%). This result implied that RHA performed better than that of CCD. To further validate this finding, additional experimental runs were performed at the suggested experimental conditions predicting the highest possible amoxicillin removals to be achieved by TI-RHA and TI-CCD. At the suggested experimental conditions, it was verified that the amoxicillin removal of TI-RHA and TI-CCD were 51.05% and 45.42%, respectively. These experimentally observed results consequently corresponded to an average incurred % error of 1.30%. This strong agreement with the fitted 16 run GMC experimental data is further validated from the visualization of the deviation between the predicted and experimentally observed amoxicillin removals of TI-RHA and TI-CCD. As shown in Figure 5, a very strong correlation between the predicted and experimentally observed values was evident. Hence, these evidences firmly validated the usefulness and predictive capability of the developed 2FI empirical model.

Limitations of the Developed 2FI Empirical Model

Visualization of the solution space as summarized in Table 8 indicated that the antibiotic removal capacities of the titania impregnated adsorbents were calculated without the inclusion of quadratic terms (e.g. B2, C2). This derived experimental conditions only considered solution spaces that solely portrayed 3D planes (see Figure 6). As a consequence, a "true maximum" amoxicillin removal could not be possibly derived since this limitation resulted to the exclusion of curved 3D solution spaces that could also depict the factor effects on the amoxicillin removals of TI-RHA and TI-CCD (Chollom et al., 2020; Sultan et al., 2019; Masse et al., 2018; Mäkelä, 2017; Kowalski et al., 2000). While this perceived limitation strongly suggested that caution be exercised in the use of 2FI empirical model for optimization studies, the extent of factor interactions described in this work are still adequate basis for the further exploration of potential solution spaces that may ultimately identify the optimal experimental conditions where the investigated dependent variable is either minimized or maximized (Mäkelä, 2017).

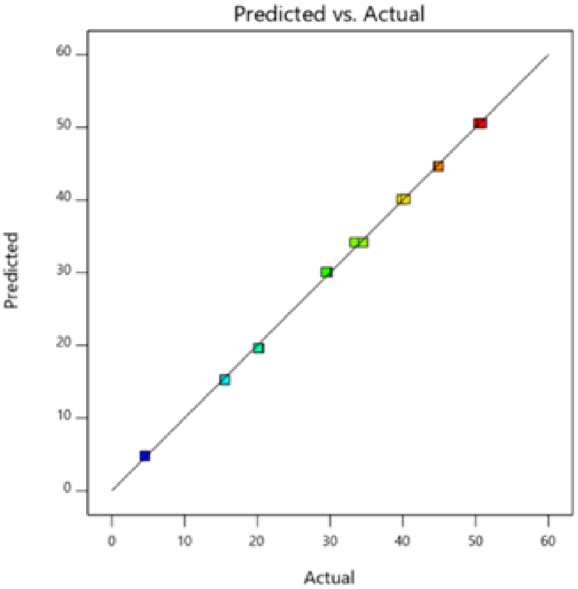

Fig 6 Scatterplot of the experimentally observed amoxicillin removals plotted against the predicted amoxicillin removals of TI-RHA and TI-CCD. Low amoxicillin removals are depicted by the blue hue while high amoxicillin removals are depicted by the red hue. The 45° line represents experimental points that have zero residuals. Experimental points (depicted by squares) that are close to the 45° line are experimental runs that are in good agreement with the predicted values.

Conclusion

This work was able to demonstrate that various factors affecting the antibiotic removal capacities of the titania impregnated adsorbents could be effectively analyzed through a GMC experimental design. Out of the 16 run GMC experimental design implemented in this work, it was experimentally established that a 2FI empirical model successfully quantified the extent of factor effects of factors A, C, D, and CD. Moreover, an average % error of 1.30% strongly implied that the predicted amoxicillin removals for TI-RHA and TI-

CCD were in strong agreement with the experimentally observed values and consistently identified RHA as the potential matrix for immobilizing titania particles. In conclusion, the evidences presented in this work strongly support the claim of this study that RHA is a promising matrix for the immobilization of titania particles for the removal of amoxicillin in aqueous media.

Acknowledgement

Funding for this work was kindly provided by Bicol University channeled through the Bicol University College of Engineering (BUCENG) and the Office of the Vice President for Research, Development and Extension (OVPRDE). This work would also like to thank the support given by Dr. Jocelyn Serrano and Prof. Phil Morano, Dr. Amelia Gonzales, Engr. Junel Borbo, and Engr. Michelle Canaria. Moreover, this work would also like to express its utmost gratitude to the strong administrative support given by Dr. Arnulfo Mascariñas, Dr. Luis Amano, Prof. Ronnel Dioneda, Sr., Dr. Amelia Dorosan, and Dr. Benedicto Balilo, Jr.

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Recommended citation:

Barajas, J.R.B. & Lucero, A.T. (2022). Qualifying Factor Effects on the Antibiotic Removal Capacity of Titania Impregnated Adsorbents Through An Empirical Model . Bicol University Research & Development (BUR&D) Journal. 25 (1), 18-30. doi: 10.47789/burdj.mbtcbbgs.20222501.02