

APPLICATION OF RESPONSE SURFACE METHODOLOGY FOR OPTIMAL COD REDUCTION IN BALLAST WATER TREATED USING A YEAST BIOFILTER

*Amenaghawon, N.A., Salokun, O. and Okhonmina, J.O

¹*Department of Chemical Engineering
Faculty of Engineering
University of Benin, PMB 1154, Benin City, Nigeria
E mail: andrew.amenaghawon@uniben.edu

Abstract

In this study, raw ballast water was treated using a yeast biofilter according to a three variable Box-Behnken design. The effect of three factors (ballast water pH, volume of raw ballast water and treatment time) on the chemical oxygen demand (COD) of the treated ballast water was investigated. Response surface methodology (RSM) was used to optimise the reduction of COD of the ballast water. The experimental design was used to develop a statistical model to predict the value of COD. The model was statistically significant ($p < 0.05$) with a low standard deviation (0.34) and showed a good fit with the experimental data ($R^2 = 0.85$). The treatment process was positively influenced by the treatment time while the reverse was the case for the volume of water treated. Intermediate levels of pH was favourable for ballast water treatment. Results obtained from RSM showed that the minimum residual COD of the treated ballast water was 7.08 mg/L and this was obtained at a pH of 7.8, a ballast water volume of 1 L and a treatment time of 4 hours. Comparison of the COD of the treated and raw ballast water showed that there was a reduction of about 76%. Other properties such as dissolved oxygen (DO) and biochemical oxygen demand showed improvements of 57% and 94% respectively. The properties of the treated ballast water were all within the limits stipulated by the Federal environmental protection agency (FEPA).

Keywords: Ballast water, Yeast, Box-Behnken Design, Optimisation, ANOVA, COD

Introduction

Ballast water (BW) is water carried by ships and oceangoing vessels to ensure stability, maneuverability, trim and structural integrity (Olorunfemi et al.,

2014). The ballast water is discharged into the sea when a ship takes on cargo. International seaborne traffic has been reported to be responsible for the discharge of about 3 to 5 billion tonnes of ballast

water annually (*Tsolaki and Diamadopoulos, 2010*).

Marine pollution resulting from the discharge of dirty ballast water particularly from dirty oil tankers has become a serious problem in recent times. The ballast water contains many microorganisms, phytoplankton, zooplankton and other contaminants (Bax et al., 2003). Some of the constituents of the ballast water may be invasive species and when these nonindigenous species are introduced into the new environments, they may cause serious ecological and economical problems (Champ, 2002). The nonindigenous species can potentially multiply and significantly alter the biodiversity of the new environment if the new environment does not contain natural predators that can feed on the nonindigenous species. Ruiz et al. (2001) reported that the economic impact of invasive species in the United States of America was in the order of over 1 billion dollars per year. Thus ballast water treatment and management is very essential to the protection of marine environments and the international maritime organisation (IMO) has legislated regulations stipulating the quality of ballast water at the time of discharge (*Delacroix et al., 2013*).

Currently, reballasting as recommended by the IMO is the most common method of ballast water management. However, its use is hampered by serious ship safety limitations. Hence filtration has been adopted as the most frequently used method for the treatment of ballast water

(*Tsolaki and Diamadopoulos, 2010*). This process can be carried out during ballasting operations using shipboard filtration systems. Biofiltration is a filtration method that offers the option of bioremediation of ballast water by making use of a biofilter bed containing microorganisms capable of biodegrading the contaminants in the ballast water (Ashoka et al., 2002). Several studies have been carried out to establish the efficiency of using microorganism as biofilters for wastewater remediation (Asamudo et al., 2005; Garcia-Pena et al., 2005; Rabah et al., 2011; Woertz et al., 2001).

A search of available literature revealed that no attempt has been made to apply experimental design method coupled with response surface methodology to carry out optimisation of ballast water treatment using biofilters. Hence the focus of this study was to apply response surface methodology for the treatment of ballast water with the primary aim of achieving maximum COD reduction. RSM has been reported to be very useful in optimising multivariate processes and it has a lot of advantages over the traditional one factor at a time method (Montgomery 2005). COD was chosen as the response in this study because it is a useful measure of water quality and it provides an index for assessing the effect discharged wastewater will have on the receiving environment.

2. Materials and Methods

2.1 Ballast water collection

The ballast water used for the study was collected from a seagoing crude oil

tanker berthed at the Lagos Port Complex located in Apapa, Lagos state, Nigeria.

2.2 Isolation and identification of yeast

Isolation and identification of the yeast used as the biofilter microorganism was done according to the method reported by Rabah et al. (2011). Potato peels used as filter bed for the growth of yeast was obtained locally in Benin City, Edo State, Nigeria.

2.3 Design of biofilter and biofiltration of ballast

The biofiltration unit was made of perspex glass with a dimension of 50cm by 50cm by 50cm as shown in Figure 1. The unit was made up of three compartments which were separated by perforated partitions also made of perspex glass. The top two compartments served as filter bed

holding the potato peels while the last compartment served for the collection of the treated water. The bottom compartment also contained a tap for dispensing the treated water. The potato peels serving as filter bed was ground to obtain smaller particles and placed on both perforated partitions. The bed was wetted and then inoculated with the yeast and left for one week at the prevailing laboratory conditions (28 °C and 1 atm) for acclimatisation and growth of cells. The ballast water was then introduced into the biofiltration unit and left to stand for a period as specified by the experimental design. Other conditions of treatment such as volume of water treated and the pH of the water were also specified by the experimental design.



Figure 1: Different views of the biofiltration unit

Analytical methods

The physicochemical properties of the raw ballast water were determined prior to treatment according to the method of Ademoroti (1996). The properties measured included pH, total dissolved solid, electrical conductivity, biochemical oxygen demand, dissolved oxygen, chemical oxygen demand, chloride, metals, carbonate, sulphate, nitrate etc. Microbiological analysis was done according to the method of Adesemoye et al. (2006) to determine the level of *E.coli*, *Aspergillus niger*, *Coliform*, *Lactobacillus brevis*, *Penicillium ech*, *Mucor spp*. After treatment, liquid samples were taken from the treated water stock, filtered using a Whatman's No 4 filter paper and analysed.

2.5 Experimental design

A three variable Box-Behnken design (BBD) for response surface methodology was used to develop a statistical model for the biofiltration process. The ranges of the variables that were optimised (pH, volume of untreated water and treatment time) are as shown in Table 1. The experimental design made up of 17 runs was developed using Design Expert® 7.0.0 (Stat-ease, Inc. Minneapolis, USA). The response (dependent variable) chosen for optimisation was the COD of the treated ballast water. The following generalised second order polynomial equation was used to estimate the response of the dependent variable.

$$Y_i = b_o + \sum b_i X_j + \sum b_{ij} X_i X_j + \sum b_{ii} X_i^R + e_i \tag{1}$$

where Y_i is the dependent variable or predicted response, X_i and X_j are the independent variables, b_o is offset term, b_i and b_{ij} are the single and interaction effect coefficients and e_i is the error term.

Table 1: Experimental range and levels of independent variables

Independent Variable	Symbols	Coded and Actual Levels		
		-1	0	+1
pH	XQ	6	7.5	9
Volume (L)	XR	1	2.0	3
Time (h)	X ₃	2	3.0	4

Results and Discussion

3.1 Statistical Modelling and Analysis

Table 2 shows the result of the 17 experimental runs carried out according to the BBD. Multiple regression analysis was applied to the experimental data to obtain

Equation (2) which was used to estimate the response (COD). The values of COD predicted by Equation (2) are also shown in Table 2 along with the experimental/actual data. The significance of fit of the COD model was assessed by performing analysis of variance (ANOVA).

$$Y = 33.29 - 6.58X_1 + 1.10X_2 - 1.27X_3 - 0.10X_1X_2 + 0.18X_1X_3 - 0.16X_2X_3 + 0.41X_1^R + 0.078X_2^2 \tag{2}$$

Table 2: Experimental design matrix for the production of particle boards

Run	Factors						Response	
	Coded values			Actual values			COD (mg/L)	
	X ₁	X ₂	X ₃	X ₁	X ₂	X ₃	Actual	Predicted
1	0	0	0	7.5	2	3	6.6	7.2
2	-1	0	-1	6.0	2	2	8.9	8.8
3	1	0	1	9.0	2	4	7.8	8.0
4	1	0	-1	9.0	2	2	7.8	7.9
5	0	0	0	7.5	2	3	7.6	7.2
6	0	0	0	7.5	2	3	7.6	7.2
7	0	1	-1	7.5	3	2	7.9	7.8
8	-1	-1	0	6.0	1	3	8.1	8.0
9	1	-1	0	9.0	1	3	8.2	8.0
10	1	1	0	9.0	3	3	8.0	8.0
11	0	0	0	7.5	2	3	7.0	7.2
12	0	-1	-1	7.5	1	2	7.0	7.2
13	-1	1	0	6.0	3	3	8.5	8.7
14	-1	0	1	6.0	2	4	7.8	7.8
15	0	0	0	7.5	2	3	7.3	7.2
16	0	1	1	7.5	3	4	7.2	7.0
17	0	-1	1	7.5	1	4	6.9	7.0

The quadratic model representing the COD of the treated ballast water was significant with a p value of 0.0125 as shown in Table 3. The "Lack of Fit" p value of 0.6447 implies that there was insignificant lack of fit. The coefficient of determination (R^2) obtained as 0.85 (Table 4) shows that 85% of the variability in the response was explained by the statistical model showing a good fit between the model and the experimental data (Amenaghawon et al., 2014). The standard deviation was observed to be relatively small compared to the mean further validating the good fit

between the model and the experimental observations. A low value of the coefficient of variation (C.V=4.39%) as shown in Table 4 shows that the experimental runs were carried out with a relatively high degree of precision (Montgomery 2005). The Adequate precision value measures the signal to- noise ratio and a value greater than four as obtained in this study shows that the model shows adequate signal and can be used to navigate the design space (Cao et al., 2009).

Table 3: Analysis of variance (ANOVA) for quadratic model

Sources	Sum of Squares	df	Mean Squares	F value	p value
Model	5.06	8	0.63	5.60	0.0125
X ₁	0.25	1	0.25	2.20	0.1760
X ₂	0.23	1	0.23	2.02	0.1930
X ₃	0.46	1	0.46	4.09	0.0779
X ₁ X ₂	0.09	1	0.09	0.83	0.3903
X ₁ X ₃	0.29	1	0.29	2.59	0.1465
X ₂ X ₃	0.10	1	0.10	0.91	0.3685
X ₁ ²	3.56	1	3.56	31.58	0.0005
X ₂ ²	0.03	1	0.03	0.23	0.6447
Residual	0.90	8	0.11		
Lack of Fit	0.19	4	0.05	0.27	0.8855
Pure Error	0.71	4	0.18		
Cor Total	5.96	16			

Table 4: Statistical information for ANOVA

Parameter	Value
R ²	0.85
Mean	7.66
Standard dev.	0.34
CV %	4.39
Adeq. Precision	7.27
Adeq. Precision	19.941

Response Surface Plots

Figures 2 and 3 show the response surface plots generated from the COD model. The plots show the effect of the independent variables on the COD. Figure 2 shows the effect of treatment time and volume of untreated ballast water on the COD of the treated water. COD reduction was observed to be positively influenced by the treatment time. This trend could be attributed to the fact that sufficient time was provided for the microorganisms to

biodegrade the organic contaminants present in the ballast water. The COD gives a measure of the amount of oxidisable soluble and particulate organic matter present in wastewater and it gives an indication of the amount of oxygen consumed in the oxidation of the contaminants during biodegradation. A high COD level in a water body corresponds to a greater amount of oxidisable organics hence more oxygen

will be required for its oxidation, a process which will reduce the dissolved oxygen (DO) levels of the water body. A reduction in DO to critical levels can lead to anaerobic conditions, which is deleterious to higher aquatic life forms (Amenaghawon et al., 2013). Hence the requirement of good water quality is a low level of COD such that the activities of DO utilising microorganisms will be kept minimum. Figure 2 also shows that treatment of large volumes of ballast water at a time was not favourable to COD reduction. This could be because the optimum rate of biodegradation by the yeast cells could not be sustained when large volumes of ballast water was treated. However, when low volumes of ballast water was treated, significant reduction in COD was observed.

Intermediate level of pH was favourable for COD removal as shown in Figure 3. Significant reductions in COD was observed between pH values of 6.5 to 8.0. This could be attributed to the fact that the yeast cells possess optimum metabolic activity within the range of pH values. Rabah et al. (2011) reported that for optimum growth of microorganisms during biofiltration, the permissible range of pH values should be 6.0 to 9.5. The pH of the biofiltration medium is a very important factor that must be given serious consideration. Operating outside permissible levels of pH could result in adverse growth of the microorganism cells and this can significantly affect the performance of the biofiltration process adversely.

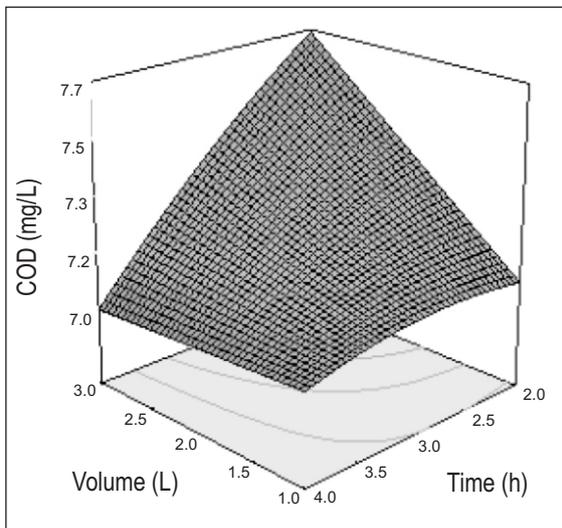


Figure 2: Effect of ballast water volume and treatment time on COD

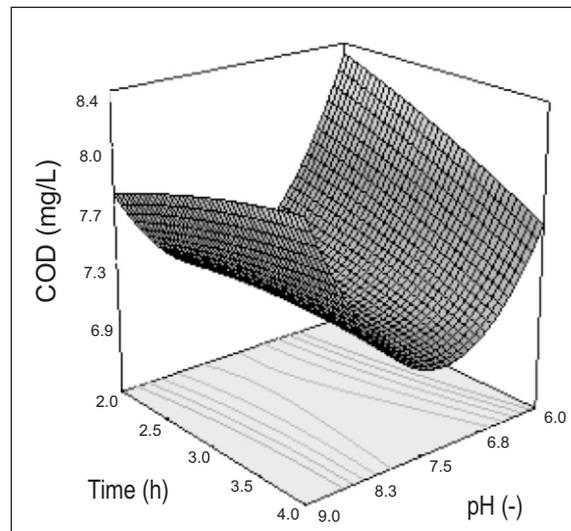


Figure 3: Effect of pH and treatment time on COD

Numerical Optimisation

Results obtained from numerical optimisation carried out using the Design Expert software revealed that the minimum COD of the treated ballast water was 7.08 mg/L. This value was obtained at the optimum treatment conditions of pH of 7.8, ballast water volume of 1 L and a treatment time of 4 hours. These results were validated by carrying out three

replicated experiments at the identified optimum conditions. The results obtained (COD=7.00 mg/L) showed that there was no significant difference between predicted and observed values. The ballast water treated at the optimum conditions was further analysed and its properties were compared with those of the untreated ballast water to determine the extent of treatment and the results are shown in Table 5.

S/No	Parameter	Before treatment (mg/L)	After treatment (mg/L)	FEPA Standard (mg/L)
1	pH Value	8.7	7.8	6-9
2	Total Dissolved Solid	360	255	2000
3	Conductivity(μ s/cm)	540	313	
4	Biochemical oxygen demand	3.9	0.24	30
5	Dissolved Oxygen	7.7	18	2
6	Chemical oxygen demand	29.2	7.00	40
7	Chloride	178	132.16	600
8	Trioxocarbonate (v)	24.2	12.8	
9	Sodium metal	7.84	3.63	
10	Calcium	10.6	3.77	200
11	Magnesium	3.67	2.83	200
12	Nitrate	1.78	1.24	20
13	Iron	0.56	0.25	20
14	Sulphate	5.70	3.64	500

The COD of the untreated ballast water was 29.2 mg/L as shown in Table 5. The minimum COD of the treated ballast water was obtained as 7.00 mg/L. Comparing both values, it can be observed that the COD was reduced by as much as 76%. This shows that ballast water treatment using a yeast biofilter was a fairly efficient process. This observation is mirrored by those of other researchers. Rabah et al. (2011) and Melamene et al. (2007) both reported COD removal efficiencies of 43% and 53% for the treatment of abattoir and wine distillery wastewater respectively. In another study, Jang et al. (2005) reported a removal

efficiency of more than 90% for the treatment of styrene contaminated effluent using a biofilter inoculated with *Pseudomonas spp.* The different level of COD removal reported in these studies could be attributed to the different microorganism, biofilter substrate and treatment conditions used. The values reported in Table 5 shows that the properties of the ballast water significantly improved after treatment. The reduction in COD positively resulted in an increase in the DO by as much as 57% while the BOD was reduced by as much as 94%. The properties of the treated ballast water were

all within the limits stipulated by the Federal environmental protection agency (FEPA) (FEPA, 1997).

Conclusion

In this study, a biofiltration unit consisting a yeast biofilter bed was determined to be effective for the treatment of ballast water. Box-Behnken design was used for designing the treatment process as well as developing a quadratic statistical model suitable for predicting the COD of the treated ballast water. The statistical model was validated and it showed a good fit with the experimental observations ($R^2=0.85$; Standard deviation=0.34). The reduction of COD of the ballast water was favoured by high levels of treatment time while the reverse was the case for the volume of ballast water treated. COD reduction was favoured by intermediate levels of pH (6.5 to 8.0). This suggests the optimum conditions of growth of the yeast cells to enable them carry out the biodegradation process. Optimisation results obtained from RSM showed that the minimum residual COD of the treated ballast water was 7.00 mg/L. Compared with the COD of the raw ballast water, this represents a reduction level of about 76%. Other properties of the treated ballast water also showed significant improvements such as BOD (94%) and DO (57%). The properties of the treated ballast water were all within the limits stipulated by the Federal environmental protection agency (FEPA).

References

- Ademoroti, C.M.A. (1996). Standard methods for water and effluents analysis, 1st edition, Fokudex Press Ltd, Ibadan, pp. 20-79
- Adesemoye, A.O., Opere, B.O. and Makinde, S.C.O. (2006). Microbial content of abattoir wastewater and its contaminated soil in Lagos, Nigeria. *African Journal of Biotechnology*, 5(20), pp. 1963-1968.
- Amenaghawon, N.A., Asegame, P.A. and Obahiagbon, K.O. (2013). Potential Application of Urea and NPK 15: 15: 15 Fertilizers as Biostimulants in the Bioremediation of Domestic Wastewater. *American Journal of Environmental Protection*, 1(4), pp. 91-95.
- Amenaghawon, N.A., Oronsaye, J.E and Ogbeide, S.E. (2014). Statistical Optimisation of Fermentation Conditions for Citric Acid Production from Pineapple Peels. *Nigerian Journal of Technological Research*, 9(2), pp. 20-26.
- Asamudo, N.U., Daba, A.S. and Ezeronye, O.U. (2005). Bioremediation of textile dye effluents using *Phanerochaete chrysosporium*. *African Journal of Biotechnology*, 4(13), pp. 1548-1553.
- Ashoka, C., Geetha, M. S. and Sullia, S.B. (2002). Biobleaching of composite textile-dye effluent using bacterial consortia. *Asian Journal of Microbiology Biotechnology and Environmental Sciences*, 4(1), pp. 65-68.
- Bax, N., Williamson, A., Agüero, M., Gonzalez, E. and Geeves, W. (2003). Marine invasive alien species: a threat to global biodiversity. *Marine Policy*, 27, pp. 313–323.
- Cao, G., Ren, N., Wang, A., Lee, D.J., Guo, W., Liu, B., Feng, Y. and Zhao, Q. (2009). Acid hydrolysis of corn stover for biohydrogen production

- using *Thermoanaerobacterium thermosaccharolyticum* W16. *International Journal of Hydrogen Energy*, 34, pp. 7182–7188.
- Champ, M.A. (2002). Marine Testing Board for certification of ballast water treatment technologies. *Marine Pollution Bulletin*, 44, pp. 1327–1335.
- Delacroix, S., Vogelsang, C., Tobiesen, A. and Liltved H, (2013). Disinfection by-products and ecotoxicity of ballast water after oxidative treatment - Results and experiences from seven years of full-scale testing of Ballast Water Management Systems. *Marine Pollution Bulletin*, 73(1), pp. 24-36.
- Federal Environmental Protection Agency (FEPA) (1997). Guidelines and Standards for Environmental Impact Assessment in Nigeria, pp. 87-95.
- García-Peña, I., Hernández, S., Auria, R. and Revah, S. (2005). Correlation of biological activity and reactor performance in biofiltration of toluene with the fungus *Paecilomyces variotii* CBS115145. *Applied and Environmental Microbiology*, 71(8), pp. 4280-4285.
- Jang, J.H., Hirai, M. and Shoda, M. (2005). Performance of a styrene-degrading biofilter inoculated with *Pseudomonas* sp. SR-5. *Journal of Bioscience and Bioengineering*, 100(3), pp. 297-302.
- Melamane, X., Tandlich, R. and Burgess, J. (2007). Anaerobic digestion of fungally pre-treated wine distillery wastewater. *African Journal of Biotechnology*, 6(17), pp. 1990-1993.
- Montgomery, D.C. (2005). *Design and Analysis of Experiments*. 6. ed. New York: John Wiley & Sons, Inc.
- Olorunfemi, D.I., Okieimen, E.A. and Ovwemuvwose, J. (2014). DNA integrity of onion (*Allium cepa* L.) Root cells exposed to ballast water. *Studia Universitatis "Vasile Goldis" Arad. Seria Stiintele Vietii (Life Sciences Series)*, 24(3), pp. 305-309.
- Rabah, A.B., Ibrahim, M.L., Ijah, U.J. and Manga, S.A.B. (2011). Assessment of the efficiency of a yeast biofilter in the treatment of abattoir wastewater. *African Journal of Biotechnology*, 10(46), pp. 9347-9351.
- Ruiz, G.M., Miller, A.W., Lion, K., Steves, B., Arnwine, A. and Collinetti, E., et al. (2001). First biennial report of the National Ballast Information Clearhouse. Status and Trends of Ballast Water Management in the United States, Smithsonian Environmental Research Center, MD, USA
- Tsolaki, E. and Diamadopoulos, E. (2010). Technologies for ballast water treatment: a review. *Journal of Chemical Technology and Biotechnology*, 85(1), pp. 19-32.
- Woertz, J.R., Kinney, K.A., McIntosh, N.D.P. and Szanisab, P.J. (2001). Removal of toluene in a Vapour phase bioreactor containing a strain of the dimorphic black yeast *Exophiala lecanii-cornii*. *Biotechnology and Bioengineering*, 75, pp. 550-558.