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PROCESS OPTIMIZATION CONDITION TREATMENT OF HIGH SALT SEAFOOD PROCESSING WASTEWATER BY RESPONSE SURFACE METHODOLOGY (RSM).

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Key words: seafood processing wastewater, salt concentration, activated sludge, anaerobic, aerobic.

ABSTRACT

This research aimed to using the Response surface methodology (RSM) was applied to find the optimum parameters for the treatment efficiency of seafood processing wastewater by the use of a laboratory-scale bioreactor, which is operated in anaerobic combining aerobic system. The independent variables considered were retention time, salt concentration, and activated sludge content. Optimization of parameters was performed by analysis of variance (ANOVA). Quadratic regression equation was suggested as a model for prediction of chemical oxygen demand (COD) and removal nutrients (nitrogen and phosphorus). The results indicated that with wastewater from seafood processing with the chemical input parameters of pH = 7 - 8.5, COD = 2000 mg / L, total nitrate nitrogen = 150 mg / L, NH_4^+ = 90 mg / L, total phosphorus = 50 mg / L. In anaerobic tank, the values of optimal conditions for independent variables were obtained as follows: salt concentration (1%), the activated sludge content (8000 mg/l), the retention time (17.59 hours) the efficiencies COD = 44.28 %, NH_4^+=42.20% và PO_4^{3-}= 39.76%, with R^2 = 0.869 and in aerobic tank salt concentration (1%), the activated sludge content (6642.31 mg/l), the retention time (6.95 hours) the efficiencies COD = 98.21%, NH_4^+=94.28% và PO_4^{3-}= 84.64% with R^2 = 0.967 with the acclimatization of 7% bacteria Bacillus velezensis at high salinity. This study suggests that the parameters output wastewater was treated according to standards QCVN 11-MT:2015/BTNMT (column B).

INTRODUCTION

Seafood processing operations generate a high strength wastewater, which contain organic pollutants in soluble, colloidal, particulate form and salt content. Saline wastewater is usually treated through physical-chemical methods, biological methods, or a combination of both (In integrated treatment systems, sedimentation, dissolved air flotation and pH adjustment are usually employed as primary treatment steps [1]. The biological processes (aerobic and anaerobic) are known to be the more appropriate for organics removal [2,3]. However, the elimination of salts is usually expensive, and, on the other hand, the high salinity and the seasonal variation of the effluent characteristics make difficult to remove the organic matter by a biological process. Biological treatment systems can convert approximately one-third of the colloidal and dissolved organic matter into stable end products and convert the remaining two-thirds into microbial cells that can be removed through gravity separation. The organic load present is incorporated in part as biomass by the microbial populations, and almost all the rest is liberated gas. Carbon dioxide (CO_2) is produced in aerobic treatments, whereas anaerobic treatments produce both carbon dioxide and methane (CH_4). In seafood-processing wastewaters, the nonbiodegradable portion is very low.

In classic optimization method, one variable changes at a time, while other parameters are kept constant [4,5,6]. But the classic method is not able to determine the complex interaction between the variables and responses [7]. RSM has been derived from statistical and mathematical techniques which can be used for studying the effect of different factors at various levels and their interactions [8]. This method consists of four main stages including experiment design, model fitting, model verification, and determining the optimal conditions. The central composite design (CCD) is one of the most frequently used technique among RSM due to the need for fewer number of experiments [9,10]. The aim of this study was to optimize the variables which affect parameters output wastewater to standards QCVN 11-MT:2015/BTNMT (column B) by using RSM.

MATERIAL AND METHOD

Material

Seafood procesing wastewater preparation

Fish processing wastewater and fish blood were collected from the processing of edible fish species, which were purchased from a local fish market. The processing of fish involves hand-skinning, filleting, and washing with tap water. The fish processing wash water and fish blood were collected immediately in a beaker and homogenized by agitation on the stirrer plate for 30 min. The wastewater was then kept in a polyethylene bottle and subsequently stored in the freezer below 0^0C for future use. To make the influent for feeding into the bioreactor, the raw wastewater was diluted with distilled water to achieve the required concentration. The wastewater from seafood processing with the chemical input parameters of pH = 7 - 8.5, COD = 2000 mg / L, NH_4^+= 150 mg / L, total phosphorus = 50 mg /L [11,12] and at different salt concentrations (1.0%, 3.0%, 5.0% w/v NaCl) and without salt content (0.0% w/v NaCl) [13,14]. The wastewater used as feed was maintained in a refrigerator at 4^0C. It was maintained in a feed reservoir and mixing was applied manually at regular intervals.

Biological treatment

The biological treatment was applied to the seafood processing wastewater after sedimentation/flotation and coagulation/flocculation steps in order to evaluate the organic matter removal efficiency by activated sludge. The experiments for this study were performed in a biological system that consists of a 7,5 L feed tank containing the wastewater to be treated, an anaerobic and an aeration tank, height (H) 30.5 cm, edge 15.5cm working volume (V) 5 L.

Fig 2.9: Anaerobic tanks and SBR used in the study

Activated Sludge Systems

In an activated sludge treatment system, an acclimatized, mixed, biological growth of microorganisms (sludge) interacts with organic materials in the wastewater in the presence of excess dissolved oxygen and nutrients (nitrogen and phosphorus). The microorganisms convert the soluble organic compounds to carbon dioxide and cellular materials.

Most of the activated sludge systems utilized in the seafood-processing industry are of the extended aeration types: that is, they combine long aeration times with low applied organic loadings. The detention times are 1 to 2 days. The suspended solids concentrations are maintained at moderate levels to facilitate treatment of the low-strength wastes, in experiment have used aerobic activated sludge available at the laboratory of Material Technology Center, Institute of Applied Technology, activated sludge is fed by domestic wastewater, has yellow brown color, activated sludge concentration about 6000 mg/L, with the ratio of MLVSS/MLSS 0.7 - 0.8. Beside, we have used Anaerobic sludge Obtaining from anaerobic BHT tank at the Institute of Environmental Science and Technology, Hanoi University of Science and Technology. Anaerobic sludge is black, BHT concentration is about 8000 - 10000 mg/L, with MLVSS/MLSS ratio 0.7 - 0.75.

METHOD

2.2.1 Effect of salt concentration, the retention time (HRT) and the activated sludge content to efficiency COD, NH^{4+} and $PO4^{3-}$by the treatment of *wastewater with anaerobic activated sludge system combining aerobic in two tank different*

In experiment with anaerobic treatment tanks are supplemented with different ratio: salt concentration (1%, 3%, 5%), the activated sludge content (8000 mg/l, 10000 mg/l, 12000 mg/l) and the retention time (16, 20, 24 hours)

In experiment with aerobic treatment tanks are supplemented with different ratio: salt concentration (1%, 3%, 5%), the activated sludge content (4000 mg/l, 6000 mg/l, 8000 mg/l) and the retention time (6, 7, 8 hours)

Experimental design

The experimental design and statistical analysis were performed with Stat-Ease software (Design Expert version 7.0.0). RSM was used to optimize variables influencing the treatment of COD, NH_4^+ and PO_4^{-3} in high salt seafood processing wastewater.

In the research, a 3-level variables second order Central Composite Design with quadratic model was employed (Table 1,3) [17,18]. The factors (independent variables) were salt concentration (X1), the activated sludge content - BHT (X2) and the retention time - HRT (X3) at three coded levels (−1, 0, +1) . The experimental design in the coded (x) and actual (X) levels of variables is shown in table 1, table 3. The range of factors was chosen based on literature and preliminary results [19]. In each experiment, processing efficiency of COD (Y1), NH_4^+ (Y2) and PO_4^{-3} (Y3) were determined as the responses (dependent variables). The complete design consisted of 14 combinations independent variables. All the experiments were carried out in random order (table 1,3). The variance for each factor assessed was partitioned into linear, quadratic and interactive components and were represented using a second order polynomial for 3 factors, as follows:

$$\mathbf{Y= b_0+b_1x_1+b_2x_2+b_3x_3+b_{11}x_2^2+b_{22}x_2^2+b_{33}x_3^2+b_{12}x_1x_2+b_{13}x_1x_3+b_{23}x_2x_3}$$

The coefficients of the polynomial were represented by b0 (constant term) b1, b2 and b3 (linear effects), b_{11}, b_{22} and b_{33} (quadratic effects) and b_{12}, b_{13} and b_{23} (interaction effects). Analysis of variance (ANOVA) tables were generated and the effects of individual linear quadratic and interactive terms were been determined [17]. The significance of all the terms in the polynomial was judged statistically by computing the F-value at a probability (p) of 0.05. Terms that were not significant were deleted one at a time (stepwise deletion) and the polynomial was recalculated.

Analytical method

Standard Methods for the Examination of Water and Wastewater were adopted for the measurement in table [15,16].

Parameters	Analytical methods	Equipment and machinery used
pH	TCVN 6492:2011 (ISO 10523:2008)	PH measurement electrode (E01581 Thermo, USA)
COD	Standard method (5220 D)	Heating block (DRB200, USA); Photometric machine (Thermo Scienfic, USA)
Total Nitrogen	Persulfate Digestion	HACH DR 6000
NH_4^+	Standard method (4500-NH_3, F)	Ammonium measuring electrode (E01581 Thermo, USA)
Total Phosphorus	Molybdovanadate uses TNT pipes	HACH DR6000
Turbidity	USEPA Method 180.1	Turbidity meter HI 98703 (Hanna, Italy)
SS, MLSS	TCVN 6625:2000 (ISO 11923:1997)	Drying oven (Daihan / Korea), analytical (HR 200, Japan)
Salinity		Salinity and EXTECH temperature meter EC170

The reported values represent the average of at least two measurements; in most cases each sample was injected three times, validation being performed by the apparatus only if the coefficient of variation (CV)was smaller than 5%.

RESULTS AND DISCUSSION

3.1 Optimization condition effect of salt concentration, the retention time (HRT) and the activated sludge content (BHT) to efficiency COD, NH_4^+ and $PO_4^{3-}$by the treatment of wastewater in anaerobic tank.

Experimental study of the effect of salt content (salinity) on processing efficiency, to survey how much salt content will affect the processing efficiency of the system. Characteristics of seafood processing wastewater used in the experiment with input parameters: pH = 7 - 8.5, COD = 2000 mg / L, NH_4^+ = 150 mg /L, PO_4^{3-} = 50 mg / L
The results of the complete three-factor three level factorial experiment designs in **Table 1**.

Table 1: Experimental design matrix and physical attributes scores of the treatment seafood wastewater processing in anaerobic tank

STT	Salt concentration (%)		BHT (mg/l		HRT(hours)		Processing efficiency (%)		
	X_1	**x_1**	**X_2**	**x_2**	**X_3**	**x_3**	**COD (Y1)**	**NH_4^+ (Y2)**	**PO_4^{-3} (Y3)**
1	-1	1	-1	8000	-1	16	44.47	42.66	39.84
2	1	5	-1	8000	-1	16	24.2	10.1	2.08

3	-1	1	1	12000	-1	16	46.1	49.5	42.45
4	1	5	1	12000	-1	16	25	11.2	2.98
5	-1	1	-1	8000	1	24	46	43.1	40.2
6	1	5	-1	8000	1	24	22	9.87	2
7	-1	1	1	12000	1	24	47.2	49.9	43.1
8	1	5	1	12000	1	24	24.8	10.5	2.88
9	-1	1	0	10000	0	20	45	43	39.2
10	1	5	0	10000	0	20	24.4	11	2.24
11	0	3	-1	8000	0	20	36.46	23.85	14.4
12	0	3	1	12000	0	20	40	25.1	15
13	0	3	0	10000	-1	16	39.9	25.05	14.8
14	0	3	0	10000	1	24	38.7	24	13.2

Model analysis, which included checking the validity of the model with the help of various relevant statistical aids, such as F-value, coefficient of determination (R^2) and coefficient of variation (c.v.) revealed that all the models were statistically adequate [9,10]. The result of the regression analysis and analysis of variance (ANOVA) for all the models is reported in Table 2 (Appendix).

From table 2, all the quadratic parameters possess positive effect ($p < 0.001$) and R^2=0.9864 (R^2 values above 90% are considered very well). The quadratic model obtained for regression analysis for yield in terms of coded levels of variables is given below:

$$\mathbf{Y_1 = 38.63-10.84X_1+1.00X_2-0.091X_3+0.096X_1X_2-0.63X_1X_3+0.20X_2X_3-3.93X_1^2-0.4X_2^2+0.67X_3^2}$$ **(Eqs.1)**

$$\mathbf{Y_2 = 23.82-17.55X_1+1.66X_2-0.11X_3-1.49X_1X_2-0.22X_1X_3-0.64X_2X_3+3.18X_1^2+0.65X_2^2+0.7X_3^2}$$ **(Eqs.2)**

$$\mathbf{Y_3 =13.74-19.26X_1+0.79X_2-0.077X_3-0.47X_1X_2-0.15X_1X_3+0.034X_2X_3+6.98X_1^2+0.96X_2^2+0.26X_3^2}$$ **(Eqs.3)**

Furthermore, the model's adequacy can be evaluated using diagnostic diagrams including normal probability distribution diagram of residuals, the diagram of predicted values versus real values. Fig. 1 shows the distribution of normal probability percentage versus studentized residuals for COD (Fig. 1a), NH_4^+ (Fig. 1b) and PO_4^{3-} (Fig 1.c) removal levels. Further, the distribution residuals versus fitted indicates values for COD (Fig. 1d) NH_4^+ (Fig. 1e) and PO_4^{3-} (Fig 1.f) removal levels. As can be seen in these diagrams, the points lie on a relatively straight line, suggesting the constancy of the variance and normal distribution. In the normal probability distribution diagram of residuals, the points are aligned along an almost straight line. Some of the scattered points are even expected in normal distribution of the data. According to Fig. 1, good correlations between predicted values and real values regarding COD, NH_4^+ and PO_4^{3-} removal confirm the adequacy of the models in predicting the removal of them pollutants.

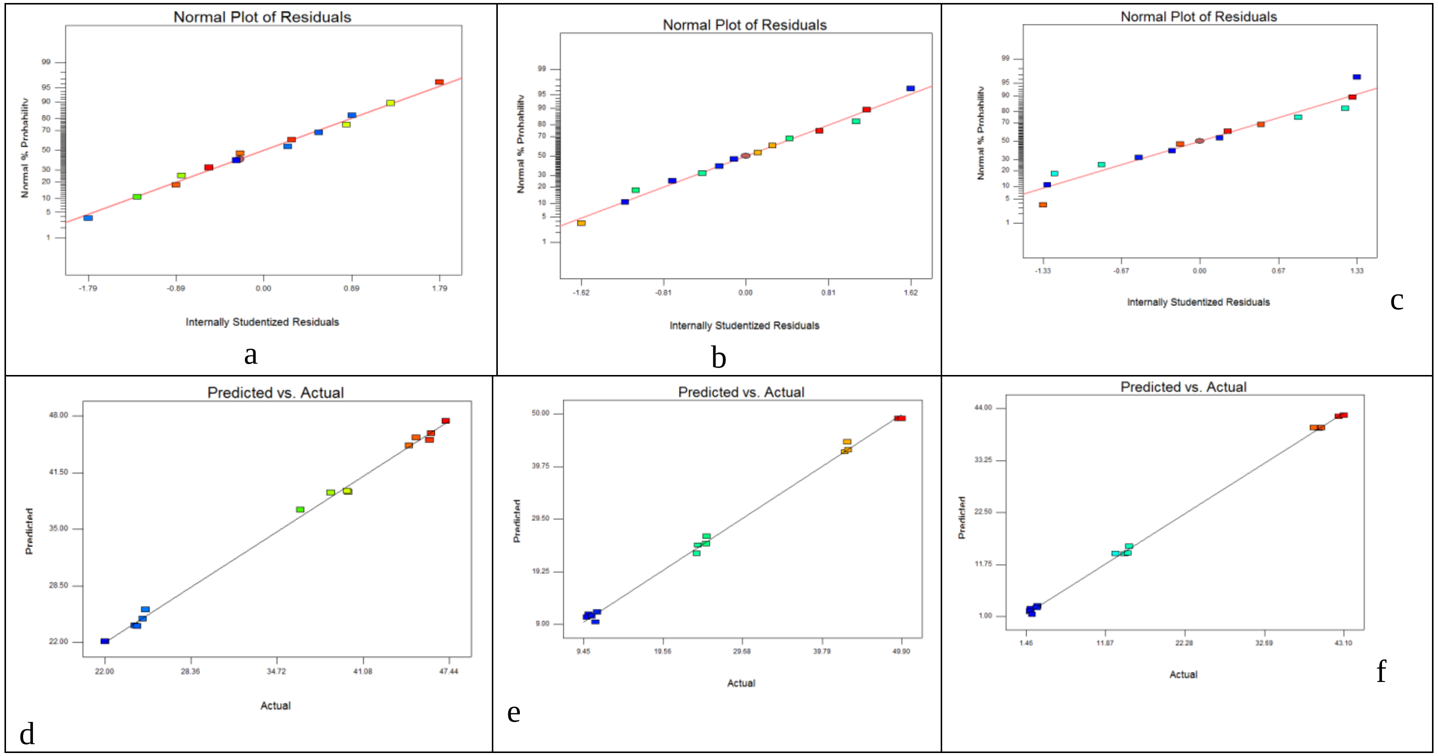

Fig. 1. Normal probability plots of the studentized residuals for COD, NH_4^+ and PO_4^{3-} removal efficiency in anaerobic tank: (a, b, c), and residuals versus run plots for COD, NH_4^+ and PO_4^{3-} removal efficiency (d, e, f).

The interactive reaction between three independent variables and dependent variables (responses) can be plotted based on regression models (Eqs. (1), (2) and (3)) and aligned diagrams of the interactive relationships between them and the response variable.

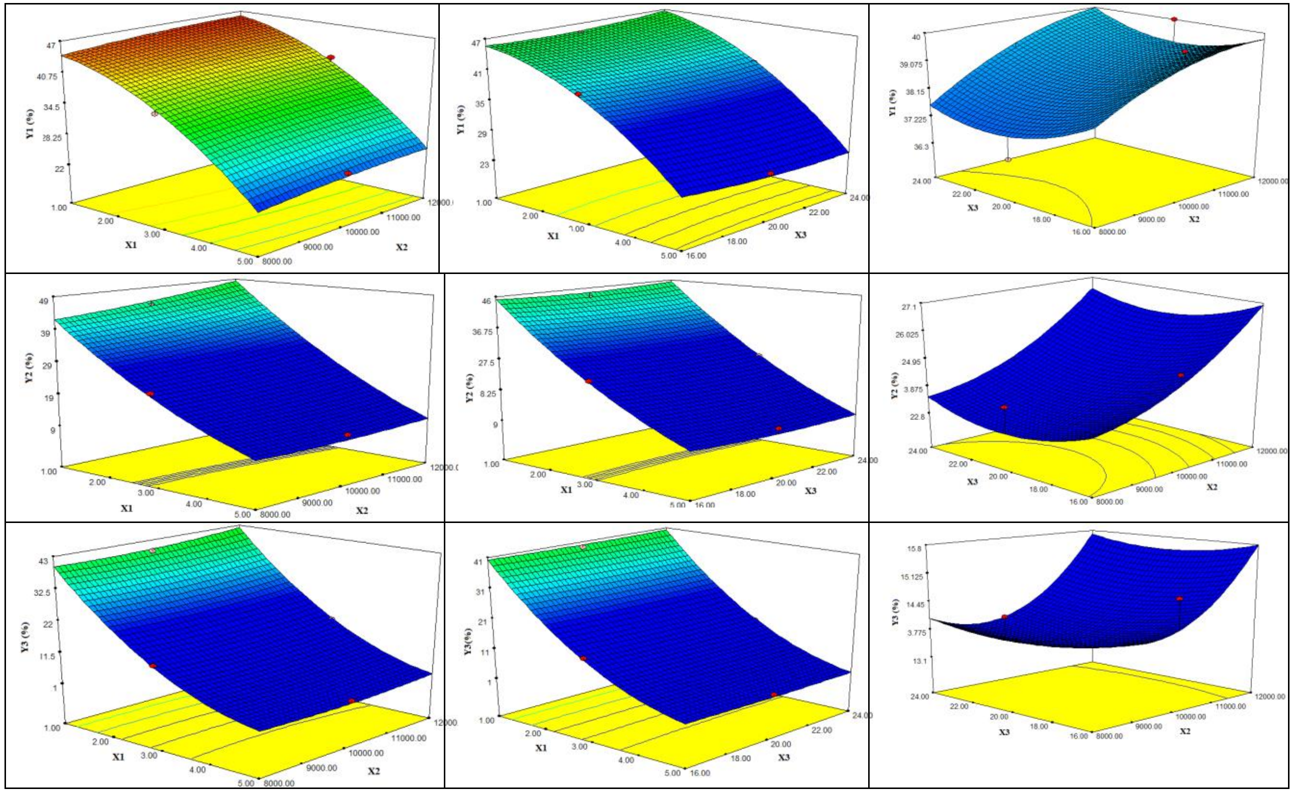

Fig. 2. *Relation between COD (Y1), NH_4^+ (Y2) and PO_4^{3-} (Y3) removal efficiency in anaerobic tank with the interaction terms by 3D plot: interaction between salt concentration (X1) - the actived sludge content (X2), interaction between salt concentration (X1)-the retention time (X3), interaction between the actived sludge content (X2) - the retention time (X3)*

3.2 Optimization condition effect of salt concentration, the retention time (HRT) and the activated sludge content(BHT) to efficiency COD, $NH_4^+$and $PO4^{3-}$by the treatment of wastewater in aerobic tank.

After the wastewater is treated in anaerobic tank, it will enter the aerobic tank for treatment. During treatment, the activated sludge content and retention time are kept constant. The results of the complete three-factor three level factorial experiment designs in **Table 3.**

Table 3: Experimental design matrix and physical attributes scores of the treatment seafood wastewater processing in aerobic tank

STT	Salt concentration (%)		BHT (mg/l		HRT(hours)		Processing efficiency (%)		
	X_1	x_1	X_2	x_2	X_3	x_3	COD (Y1)	NH_4^+ (Y2)	PO_4^{-3} (Y3)
1	-1	1	-1	4000	-1	6	91.25	75.83	45.63
2	1	5	-1	4000	-1	6	40.36	26.05	21.16
3	-1	1	1	8000	-1	6	97.42	95.03	84.85
4	1	5	1	8000	-1	6	41.56	27.04	22.47
5	-1	1	-1	4000	1	8	90.45	74.6	44.23
6	1	5	-1	4000	1	8	39.9	24	19.8
7	-1	1	1	8000	1	8	98.14	96.52	85.35
8	1	5	1	8000	1	8	40.24	26	20.78
9	-1	1	0	6000	0	7	98.84	94.82	88.54
10	1	5	0	6000	0	7	41.25	27	22.03
11	0	3	-1	4000	0	7	79.6	50	30
12	0	3	1	8000	0	7	81	52.6	31
13	0	3	0	6000	-1	6	81.04	53	32
14	0	3	0	6000	1	8	80.32	51.6	31.5

The result of the regression analysis and analysis of variance (ANOVA) for all the models is reported in Table 4 (appendix). The probability values of all regression models were less than 0.05, indicating that models terms are significant at a confidence level of 95 %. R^2 values above 90% are considered very well. According to Mirhosseini et al. [20], for a good fitness of model, R^2 should be at least 0.8. Bashir et al. [21] reported that high R^2 values suggest a great accordance between the experimental data and data estimated by the model. The quadratic model obtained for regression analysis processing efficacy in terms of coded levels of variables is given below:

$$Y_1 = 81.81-27.28X_1+1.68X_2-0.26X_3-1.54X_1X_2-0.21X_1X_3+0.083X_2X_3-11.76X_1^2-1.51X_2^2-1.12X_3^2 \text{ (Eqs.4)}$$

$$Y_2 = 54.44-30.67X_1+4.67X_2-0.42X_3-4.77X_1X_2-0.42X_1X_3+0.47X_2X_3+6.47X_1^2-3.14X_2^2-2.14X_3^2 \text{ (Eqs.5)}$$

$$Y_3 = 37.25 - 24.24X_1 + 8.36X_2 - 0.45X_3 - 0.96X_1X_2 - 0.27X_1X_3 + 0.20X_2X_3 + 18.03X_1^2 - 6.75X_2^2 - 5.50X_3^2 \quad \text{(Eqs.6)}$$

From the **Table 4** (appendix), the salt concentration and the activated sludge content affect significantly with the efficiency treatment of wastewater in aerobic tank. The interaction effect between the activated sludge and the retention time (HRT) was not significant ($p > 0.05$). The salt concentration high effects the reduction of chemical oxygen demand (COD) of the activated sludge [22, 23]. However, retention time (HRT) help to increase the adaptation of biomass to saline wastewater improved COD reduction [24].

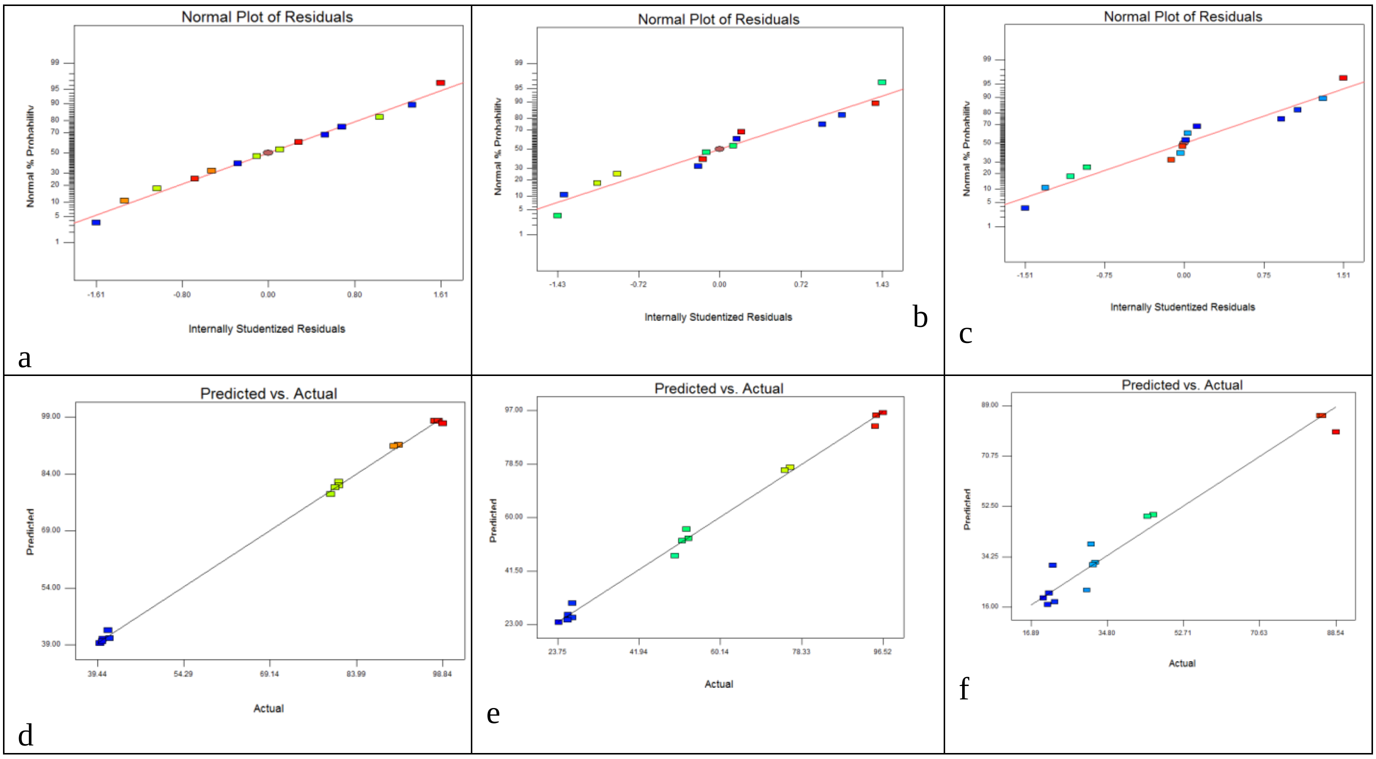

Fig. 3. Normal probability plots of the studentized residuals for COD, $NH_4^+$and PO_4^{3-} removal efficiency in aerobic tank: (a, b, c), and residuals versus run plots for COD, $NH_4^+$and PO_4^{3-} removal efficiency (d, e, f).

As can be seen in these diagrams fig 3, the points lie on a relatively straight line, suggesting the constancy of the variance and normal distribution. In the normal probability distribution diagram of residuals, the points are aligned along an almost straight line. Some of the scattered points are even expected in normal distribution of the data. According to Fig. 3, good correlations between predicted values and real values regarding COD, NH_4^+ and PO_4^{3-} removal confirm the adequacy of the models in predicting the removal of them pollutants.

The interactive reaction between four independent variables and dependent variables (responses) can be plotted based on regression models (Eqs. (4), (5) and (6)) and aligned diagrams of the interactive relationships between them and the response variable.

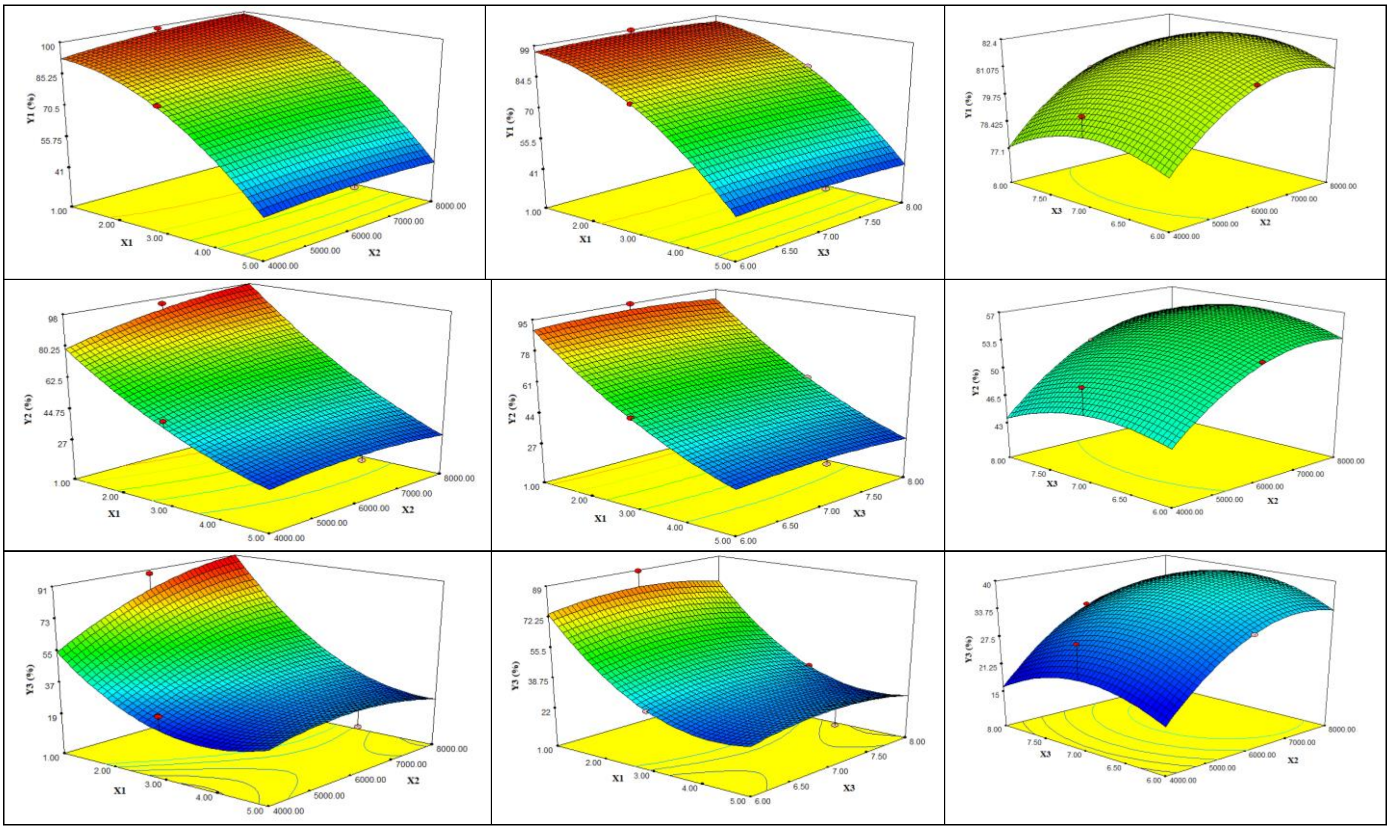

Fig. 4. *Relation between COD (Y1),* NH_4^+ *(Y2) and* PO_4^{3-} *(Y3) removal efficiency in aerobic tank with the interaction terms by 3D plot: interaction between salt concentration (X1) - the actived sludge content (X2), interaction between salt concentration (X1)-the retention time (X3), interaction between the actived sludge content (X2) - the retention time (X3)*

Fig 4 show, salt concentration effect to the treatment efficiency of activate Zd sludge anaerobic combining aerobic system is significantly affected when the salt content of seafood processing wastewater is greater than 3%. High salinity can cause high osmotic stress or the inhibition of the reaction pathways in the organic degradation process. This results in a significant decrease in biological treatment efficiency. In addition, high salt content induces cell lysis, which causes increased effluent solids. The populations of protozoa and filamentous organisms required for proper flocculation are also significantly reduced by the elevated salt content [25,26]. Therefore, high salinity in fish processing wastewater will lead to difficulties in biological treatment processes [25].

3.3 Optimization of the operational conditions for the process anaerobic combining aerobic for treatment of high salt seafood processing wastewater

Optimization of COD, NH_4^+ and PO_4^{3-} removal for determining optimized points for operational conditions and achieving the maximum removal percentage was performed by estimation models above. To achieve the highest removal performance at operational conditions of independent variables, COD, NH_4^+ and PO_4^{3-}removal percentage were selected at maximum value. In anaerobic tank, the values of optimal conditions for independent variables were obtained as follows: salt concentration (1%), the activated sludge content (8000 mg/l), the retention time (17.59 hours). Under these conditions, the degree of desirability of the model was equal to 0.869, while the removal percentage of COD, NH_4^+ and PO_4^{3-} was 44.28%, 42.20% and 39.76% respectively. In aerobic tank, the values of optimal conditions for independent variables were obtained as follows: salt concentration (1%), the activated sludge content (6642.31 mg/l), the retention time (6.95 hours) . Under these conditions, the degree of desirability of the model was equal to 0.967, while the removal percentage of COD, NH_4^+ and PO_4^{3-} was 98.21%, 94.28% and 84.64% respectively.

It can be seen that, with salt content less than 1%, it does not affect the processing efficiency of the system much. When the salt content is 3%, the efficiency of treatment decreases markedly and does not meet the output standard according to QCVN11-MT: 2015 / BTNMT (column B) COD is 150 mg/L, NH_4^+ = 54 mg/L, PO_4^{3-} = 34 mg/L. Study from Joong et al. [28] in the experiment for examination of the salt effect on cellular growth show that there is no effect on cellular growth at concentrations of 1% and 2% NaCl. Burnett et al. reported that operation of activated sludge process at salt contents higher than 20g/L is characterized by poor flocculation, high effluent solids, and a severe decrease in substrate utilization rate [27].

To confirm the adequacy of the models and accuracy of the optimization method, we were performed study on supplementation of saline microorganisms to improve processing efficiency at the obtained optimal conditions. The saline microorganism of *Bacillus velezensis* is isolated from the sea of the Institute of Natural Products Chemistry. Surveying the concentration of microorganisms favoring salinity from 3 - 10% (density of microbial cells equivalent to 10^4 CFU/mL) added to the wastewater treatment process has a salinity level of 3%. The result is as follows:

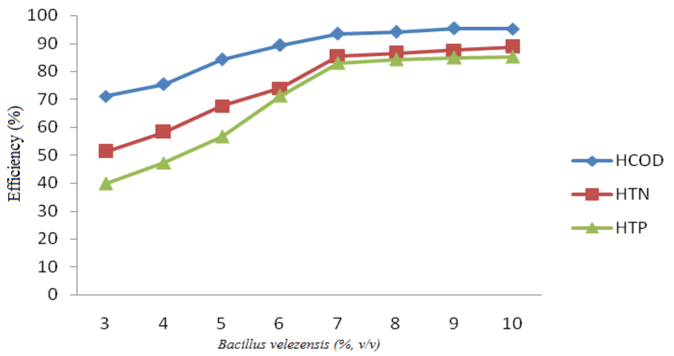

Fig 5: Effect of ration *Bacillus velezensis* additional to processing efficiency

The results showed that treatment efficiency is directly proportional to increasing microbial concentration. However, at a high rate of supplementation (7-10%), treatment efficiency increased but not significantly [31,32,33]. The reason explained is that the nutrients in the environment are exhausted. The appropriate percentage of additional microorganisms for treatment is determined at 7%. The quality of wastewater after treatment with anaerobic activated sludge combining aerobic with additional saline microorganisms the output standard according to QCVN 11-MT:2015/BTNMT (column B). However, as the concentration of salinity exceeds this limit, the tendency of bacteria aggregation or adsorption decreases [29,34].

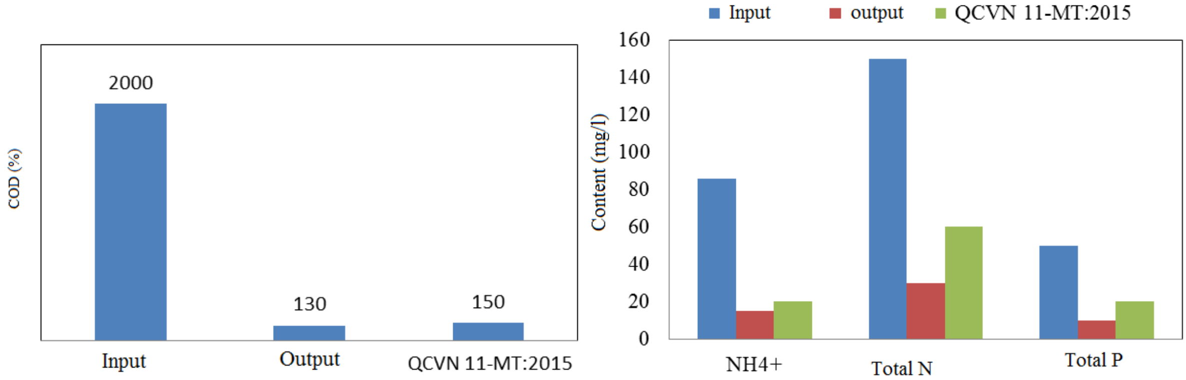

Fig 6: The quality of waste water after treatment with anaerobic activated sludge system combining aerobic with added saline microorganisms

CONCLUSION

The results of this study demonstrated that response surface methodology is a good tool for optimizing of parameters found from the experimental data. A quadratic model was suggested as a good model for the prediction of COD, NH_4^+ and PO_4^{3-} . Furthermore, ANOVA analysis indicated that salt concentration (X1), the activated sludge content - BHT (X2) and the retention time - HRT (X3), square terms and interaction terms of them had significant effects on COD, NH_4^+ and PO_4^{3-} processing efficiency. The findings proved a good agreement between the experimental data and the predicted equation. Therefore, the RSM can be proposed as a useful tool for the optimization of saline wastewater treatment by method biological. The biological system help to reduce environmental contaminant levels and improve the seafood processing effluent water quality so that it can be reused and the protection of environment quality [35,37].

ACKNOWLEDGEMENT

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Appendix

Table 2: Analysis of regression and variance of the second order polynomial models for dependent variables of the treatment seafood wastewater processing in anaerobic tank

Regression coefficient	**Y1**		**Y2**		**Y3**	
	F -value	**p-value**	**F -value**	**p-value**	**F -value**	**p-value**
Model	397.91	< *0.0001*	79.93	< *0.0001*	10.12	0.0198
X_1	3351.74	< *0.0001*	681.75	< *0.0001*	65.51	0.0013
X_2	12.71	0.0235	15.81	0.0165	7.80	0.0042
X_3	0.30	0.6131	0.13	0.7370	0.022	0.8890
X_1X_2	8.55	0.0431	13.17	0.022	8.49	0.0435
X_1X_3	0.16	0.7073	0.10	0.7658	0.006	0.9399
X_2X_3	0.025	0.8831	0.13	0.7405	0.003	0.9561
X_1^2	153.33	0.0002	7.47	0.0523	8.93	0.0040
X_2^2	2.51	0.1882	1.76	0.2557	1.25	0.3260
X_3^2	1.40	0.3018	0.82	0.4176	0.83	0.4136
R-Squared	0.9989		0.9945		0.9579	

Table 4: Analysis of regression and variance of the second order polynomial models for dependent variables of the treatment seafood wastewater processing in aerobic tank

Regression coefficient	**Y1**		**Y2**		**Y3**	
	F -value	**p-value**	**F -value**	**p-value**	**F -value**	**p-value**
Model	152.66	< *0.0001*	154.04	< *0.0001*	500.44	< *0.0001*
X_1	1310.22	< *0.0001*	1348.91	< *0.0001*	4311.86	< *0.0001*
X_2	11.09	0.0291	12.10	0.0254	7.24	0.0547
X_3	0.10	0.7622	0.057	0.82332	0.069	0.8059
X_1X_2	0.083	0.7880	7.77	0.0495	2.02	0.2282
X_1X_3	3.53	0.1335	0.17	0.7000	0.21	0.6736
X_2X_3	0.34	0.5892	0.014	0.9108	0.011	0.9230
X_1^2	42.4	0.0029	10.88	0.0300	139.41	0.0003
X_2^2	0.44	0.5443	0.46	0.5356	2.64	0.1795
X_3^2	1.24	0.3287	0.53	0.5065	0.19	0.6821
R-Squared	0.9971		0.9971		0.9991	

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