



## Application of Response Surface Methodology for Sago Wastewater Treatment by Ozonation

G. Anju<sup>1</sup>, B. Subha<sup>2</sup>, M. Muthukumar<sup>3</sup>, T. Sangeetha<sup>4\*</sup>

<sup>1</sup> School of Ocean Engineering and Underwater Technology, Kerala University of Fisheries and Ocean studies, Panangad, Kerala, In dia

<sup>2</sup> Department of Environmental Engineering, Korea Maritime and Ocean University, Busan, South Korea

<sup>3</sup> Department of Environmental Science, School of Earth Science Systems, Central University of Kerala, Kerala, India

<sup>4</sup> Department of Energy and Refrigerating Air-Conditioning Engineering, Research Centre of Energy Conservation for New Generation of Residential, Commercial, and Industrial Sectors, National Taipei University of Technology, Taipei, Taiwan

### P A P E R I N F O

#### Paper history:

Received 15 March 2019

Accepted in revised form 24 June 2019

#### Keywords:

Central Composite Design

Chemical Oxygen Demand

Ozonation

Sago Wastewater

Response Surface Methodology

### A B S T R A C T

A treatment method based on the degradation of sago processing wastewater using ozonation process was conducted in this research study. The optimization of the process variables was designed with the aid of software called Design Expert and the technique was called response surface methodology (RSM) in Central composite design. The effect of ozonation variables like pH, treatment time and ozone concentration on the reduction of chemical oxygen demand (COD) of sago waste water was investigated. Interestingly, two different types of results like maximum removal of COD and optimum removal of COD were observed. Maximum COD removal of 62.45% was at pH 9.8, ozonation time 95.7 min, ozone dose 42% and optimum COD removal of 56.7% was at pH 9.8, ozonation time 35.7 min, and ozone dose 42%. Bacterial count was found to be nil after ozonation and microscopic observation of biomass proved that the sludge content had effectively reduced after ozonation treatment. It was determined that the ozonation of sago wastewater was a promising effort in wastewater treatment.

doi: 10.5829/ijee.2019.10.02.05

## INTRODUCTION

Tapioca (*Manihot esculenta*) is concentrated in the southern peninsular region of India in the states of Tamil Nadu and Andhra Pradesh. Sago, the edible starch globules processed from the tubers of tapioca, is the staple diet of middle income populations in India. The tubers are the raw material and they are converted into commercial sago through indigenous technology [1]. There are more than 1000 sago industries in Tamil Nadu and in that 80% are in Salem district. They produce about 15 to 30 tonnes of sago per industry/day and discharge about 40,000 to 50,000 litres of sago wastewater per tonne of sago. They generate more than 85% of the total wastewater output and about 400 units discharge directly into rivers. Sago wastewater is complex and acidic in nature with high organic matter, intense COD and BOD, suspended solids, obnoxious odour and irritating colour [2]. Many investigators reported that the wastewater from sago mills are of serious concern and the Central Pollution Control Board (CPCB) has classified sago industries as "Red" industries. Most of the low cost and conventional treatment methods have low treatment efficiency due to high concentration of suspended solids and insoluble fibres present in the wastewater [3]. According to literature various technologies

have been implied to treat sago wastewater. Some widely used methods are high-rate anaerobic treatment such as Microbes [4]; anaerobic reactors [5-8], Three phase fluidized bed bioreactor [9]; activated sludge and cow dung slurry [10], sago-sludge treatment using earthworm [11] and utilizing sago waste as an adsorbent for removing Cu ions [12].

Ozone is the triatomic form of oxygen, which is composed of three oxygen atoms. It is a powerful oxidizing agent with high oxidation potential ( $E_0 = 2.07V$ ) compared to chlorine ( $E_0 = 1.36V$ ) and  $H_2O_2$  ( $E_0 = 1.78V$ ) which on dissolution in an aqueous medium can react with a variety of organic molecules to cause partial oxidation or complete mineralization, i.e.  $CO_2$  and water [13]. The use of ozone was first pioneered in the early 1970's and has been successfully used in a vast number of applications. Various types of organic and inorganic wastewaters like distillery wastewater [14], drinking water [15], domestic wastewater [16], textile wastewater [17] Landfill leachate [18] have been successfully treated by ozonation. The main effects of ozonation are decolorization, elimination of taste and odors, degradation of organics and disinfection. The advantage of ozonation is that its final products are  $CO_2$  and  $H_2O$  and the residual ozone in the system changes in few minutes to oxygen ( $O_2$ ).

RSM is a mathematical and statistical technique that is

\* Corresponding Author Email: sangeetha@mail.ntut.edu.tw (Sangeetha Thangavel)

useful for the optimization of chemical reactions and industrial processes and is commonly used for experimental designs [19]. It is an efficient statistical tool to optimize the operating conditions in multivariable systems by considering the interactions between variables. Using RSM, with a minimum number of experiments, an optimal response could be achieved [20, 21]. The objective of this research study was to evaluate the COD removal efficiency in starch processing wastewater employing ozonation process and optimizing it by response surface methodology (RSM) in central composite design. Mathematical correlations between pH, ozonation time and ozone dose on the removal of COD in wastewater were estimated.

## EXPERIMENTAL PROTOCOLS

### Wastewater and its characterisation

Sago wastewater was collected from SPAC Tapioca Products (India) Ltd Erode district, Tamil Nadu, India. Sample collection and characterization was performed according to the standard methods [22] and initial parameters analysed are tabulated in Table 1.

### Experimental setup

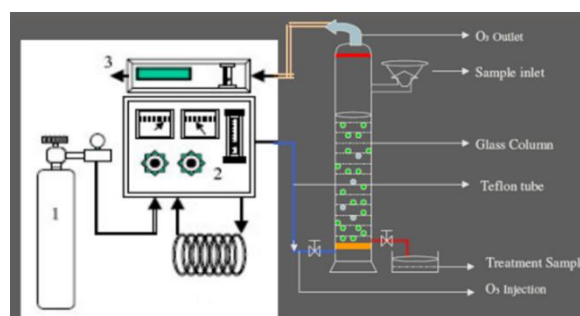
Schematic diagram of the lab-scale experimental set up is shown in Figure 1. It consisted of an oxygen concentrator (SimO<sub>2</sub> plus, Italy), ozone generator (Ozonetek Ltd., India) with built-in oil-free compressor and reaction column. A controlled flow rate of 2L/min of oxygen was used to produce 2g/h of ozone. The reactor consisted of a glass column of 72cm height, outer diameter of 4.5cm, an inner diameter of 3.5cm and holding capacity of 1500mL of effluent. It also had sample ports at various points, an ozone gas inlet at the bottom with an air diffuser over the inlet port to diffuse the oxygen/ozone gas mixture through the column, and a closed top with a collection port to collect the unreacted ozone gas. Teflon tube was used for connecting the ozone outlet port from the ozone generator to the ozone reaction chamber.

### Experimental procedure

**Ozonation process** The sample was transferred to the ozonation chamber and ozone was passed into the sample. The ozonation dose was varied from 10-50% and the ozonation time was varied from 1-121 minutes to investigate

**TABLE 1.** Initial Physicochemical parameters for the Sago effluent

Parameters	Values
pH	4-5
COD	7700mg/L
Total Suspended Solids (TSS)	3000mg/L
Volatile Suspended Solids (VSS)	1600mg/L
Phosphate	45 mg/L
Carbohydrate	10mg/L
Starch	5 mg/L
Sulphate	65 mg/L



1. Oxygen concentrator 2. Ozone generator 3. Excess ozone outlet  
**Figure 1.** Schematic representation of the experimental set up employed in this study

the effects of ozonation parameters on COD removal. After ozonation, the sample was transferred in to a 500 mL conical flask and sealed tightly with a rubber cork. The experiment was carried out at room temperature [23].

**Central composite design procedure** Response surface methodology (RSM) consists of statistical tools and techniques for estimating functional relationship between variables and responses [24]. A three-level factorial design was established with the help of the Design Expert software (Central Composite Design Expert Version 8.0.3, Stat Ease, Minneapolis, USA). In this study, central composite design (CCD) was used for RSM in the experimental design, which is well suited for fitting a quadratic surface and usually works well for process optimization. The variables selected were pH (X<sub>1</sub>) time (X<sub>2</sub>) and ozone dosage (X<sub>3</sub>) while the response observed was COD. The behaviour of the system was explained by the following second-degree quadratic polynomial equation.

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 \quad (1)$$

where, Y was predicted response, B<sub>0</sub> the constant coefficient, B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub> the linear coefficient, B<sub>11</sub>, B<sub>22</sub>, B<sub>33</sub> the quadratic coefficient, B<sub>11</sub>, B<sub>12</sub>, B<sub>13</sub> the cross-products coefficient, and X<sub>1</sub>, X<sub>2</sub>, and X<sub>3</sub> were input variables (pH, ozonation time and retention time). The variables and their levels were designated as -1.682, -1, 0, +1, and +1.682 as in Table 2. A total of 20 runs were essential for response optimization and the adequacy of the proposed model was then revealed by the diagnostic checking tests provided by analysis of variance (ANOVA). The quality of the fit polynomial model was expressed by the coefficient of determination R<sup>2</sup>, adjusted R<sup>2</sup>, and "adequate precision." The fitted polynomial equation was expressed as three dimensional (3D) surface plots to visualize individual and interactive effect of factors on the responses within the designed range [23].

**Analytical procedure** Chemical Oxygen Demand (COD) was measured in accordance with the standard methods [22]. Bacterial count analysis in the sago wastewater was performed using serial dilution and plating techniques [25]. The colony forming units (CFU) were estimated before and after ozonation treatment of the wastewater. Olympus microscope (Leica Meteor II, Germany) with digital camera at 100X magnification was used in this study to observe wastewater biomass before and after the experiments.

**TABLE 2.** Process variables and their factorial levels

Variables, Unit	Factors		Levels			
	X	-1.682	-1	0	+1	+1.682
pH	X1	3	4.2	7	9.8	11
Time (min)	X2	1	35.7	61	95.7	121
Ozone dose (%)	X3	10	18	30	42	50

## RESULTS AND DISCUSSIONS

### Fitting the second order polynomial equation and statistical analysis

Several factors influence the organic removal in a wastewater during ozonation and pH, ozonation time and dose may play vital roles. Experiments were performed to study the effect of pH, ozone dosage and the treatment time on the substrate removal efficiency from sago wastewater using ozonation process. Table 3 shows the full factorial design of the conducted experiments and the relationship between the actual and predicted values of Y. The regression Equation (2) given below was obtained by the analysis of variance by giving the percentage level of COD reduction.

$$Y = +52.74 + 11.70X_1 + 4.49X_2 + 5.79X_3 - 9.49X_1^2 - 3.93X_2^2 - 6.42X_3^2 + 0.25X_1X_2 + 8.36X_1X_3 - 3.03X_2X_3 \quad (2)$$

**TABLE 3.** Experimental and predicted COD removal values: Run 11- Maximum results; Run 18- Optimized results

Run	pH	Ozonation time (min)	Ozone dose (%)	COD removal (%)	
				Experiment	Predicted
1	7	61	50	46.6	45.9
2	4.2	35.7	18	17	16.6
3	7	61	10	19	19.4
4	4.2	95.7	18	33.7	33.2
5	3	61	30	16.2	16.6
6	11	61	30	45.4	44.5
7	7	61	30	52	51.9
8	7	61	30	51.5	51.9
9	4.2	95.7	42	19	15.2
10	4.2	35.7	42	15.8	19.8
11	9.8	95.7	42	62	62.45
12	7	61	30	51.8	51.9
13	7	61	30	52	51.9
14	7	1	30	25.2	21.8
15	7	121	30	45	49.4
16	9.8	95.7	18	41	37.2
17	7	61	30	52.5	51.9
18	9.8	35.7	42	56	56.7
19	9.8	35.7	18	26	29.9
20	7	61	30	53	51.9

Y is the predicted response in real value, X<sub>1</sub> is the coded value of the variable of pH, X<sub>2</sub> is the coded value of the variable of ozonation time, and X<sub>3</sub> is the coded value of the variable of ozone dosage. For the statistical testing of the significant model equation, Fisher's statistical test and P values for ANOVA were used. They indicated that the model parameters and their interactions were significant [26]. The equation in terms of coded factors can be used to predict the response for the given levels of each factor. The high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Two different tests namely sequential model sum of squares and model summary statistics were employed to decide about the adequacy models to represent the COD removal and the results of these tests are tabulated in Table 4. As per sequential model sum of squares test quadratic model had a probability value of <0.0001, implying that the model was significant. Any factor or interaction of factors with p < 0.05 is considered to be significant [23]. It had maximum adjusted and predicted R<sup>2</sup> values of 0.9673 and 0.9828, respectively. However, Cubic model was aliased so quadratic model was chosen for further analysis. The results showed an F value of 63.36, and the large value of F indicated that most of the variation in response can be explained by the regression equation. The associated P value was used to estimate whether F was large enough to indicate statistical significance. The Predicted R<sup>2</sup> of 0.8216 was in reasonable agreement with the Adjusted R<sup>2</sup> of 0.9673; i.e. the difference was less than 0.2. Adeq Precision measured the signal to noise ratio and a ratio greater than 4 was desirable. The ratio of this study 23.813 indicated an adequate signal. Therefore, this quadratic model can be used to navigate the design space for COD. The ANOVA thus proved that the form of the model chosen to explain the relationship between the factors and the response was correct.

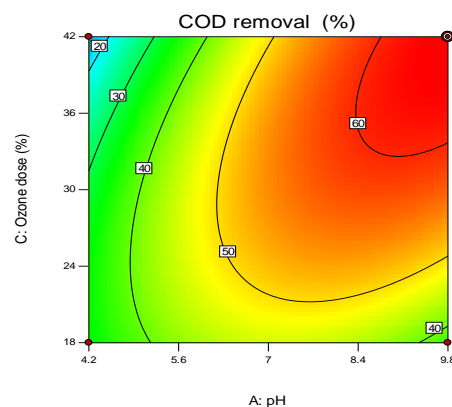
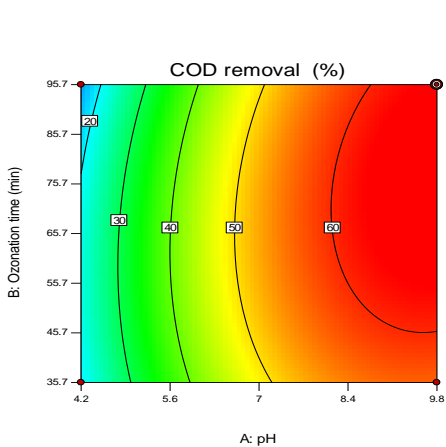
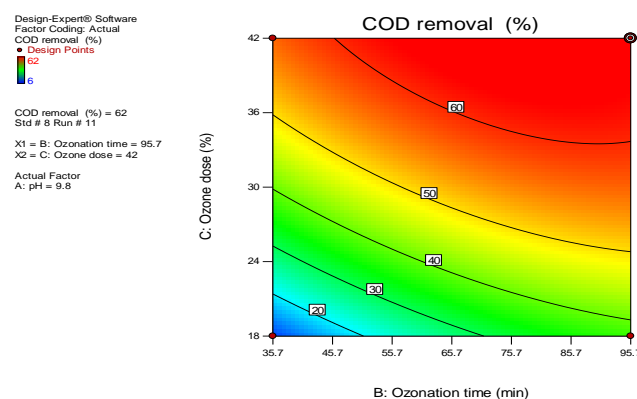
### Effect of variables on COD removal

The initial COD value of the sample was 7700 mg/L. We obtained two types of result such as maximum and optimum. Maximum was the highest COD removal in the study, which was obtained at almost the maximum parameters constraint (Run 11). Optimum was the ideal COD removal which can be obtained by an optimal requirement of parameters (Run 18). The maximum results depicted in Figure 2 illustrate an increase in the COD removal efficiency with increasing pH, ozone dose and ozonation time and maximum removal of 62.45% at pH of 9.8, ozone dose of 42% and ozonation time of 95.7 min. Figure 2A (1,2,3) show the contour graphs of the effects of the three variables on COD removal; Figure 2B (1,2,3) show the three dimensional surface plots for the same and overlay plot for the condition is shown in Figure 2C. The optimum results for COD removal efficiency are portrayed in Figure 3. As mentioned earlier Figure 3A (1,2,3) are contours, Figure 3B (1,2,3) are the 3D graphs and Figure 3C is the overlay plot for the optimized conditions. Generally, the COD removal was better in alkaline pH (9.8) than at acidic pH (see Table 3), because at acidic pH the protons in the solution get reduced to H<sub>2</sub>, and thus the proportion of OH<sup>-</sup> ions produced is less and consequently there is less substrate removal [27]. The maximum removal of COD (61%) observed in this study

**TABLE 4.** ANOVA results of the quadratic model for COD removal

Source	Sum of squares	df	Mean square	F value	Prob> F	Remark
Model	5527.12	9	614.12	63.36	< 0.0001	significant
A-pH	1244.52	1	1244.52	128.40	< 0.0001	
B-Time	504.96	1	504.96	52.10	< 0.0001	
C-ozone dosage	681.48	1	681.48	70.31	< 0.0001	
AB	54.57	1	54.57	5.63	0.0391	
AC	943.95	1	943.95	97.39	< 0.0001	
BC	231.12	1	231.12	23.85	0.0006	
A <sup>2</sup>	975.64	1	975.64	100.66	< 0.0001	
B <sup>2</sup>	445.24	1	445.24	45.94	< 0.0001	
C <sup>2</sup>	672.43	1	672.43	69.38	< 0.0001	
Residual	96.92	10	9.69			
Lack of Fit	95.49	5	19.10	66.62	0.0001	significant
Pure Error	1.43	5	0.29			
Cor Total	5624.05	19				

at alkaline pH is a result of fast reaction of organic matter with molecular ozone. This was supported by the study of Singh et al. [28] where they suggested that increasing the amount of OH radicals will destroy the organic compounds more effectively. They reported 85% of COD removal in corn-processing wastewater at alkaline pH. The decomposition of aqueous ozone increases with an increase in pH and this will in turn result in an increase in OH radicals and simultaneous increase in removal of the pollutant. Garcia et al. [29] had observed a maximum COD removal of 80% at alkaline pH of 9 than at acidic pH of 3, from dye wastewater. They suggested that hydroxyl radicals had a better ability of organic oxidation. It was also found that as the ozone concentration increased the percentage of COD removal rate simultaneously increased.



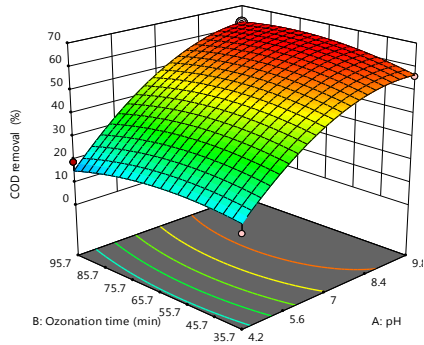
(A)

Design-Expert® Software  
Factor Coding: Actual

**COD removal (%)**  
● Design points above predicted value  
○ Design points below predicted value  
6 62

COD removal (%) = 62  
Std # 8 Run # 11  
X1 = A: pH = 9.8  
X2 = B: Ozonation time = 95.7

**Actual Factor**  
C: Ozone dose = 42

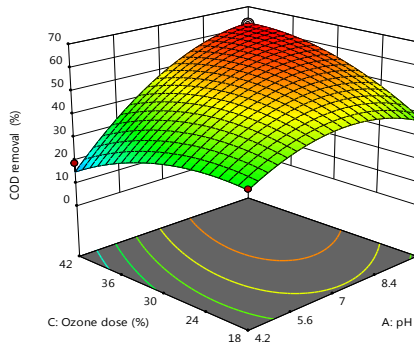


Design-Expert® Software  
Factor Coding: Actual

**COD removal (%)**  
● Design points above predicted value  
○ Design points below predicted value  
6 62

COD removal (%) = 62  
Std # 8 Run # 11  
X1 = A: pH = 9.8  
X2 = C: Ozone dose = 42

**Actual Factor**  
B: Ozonation time = 95.7

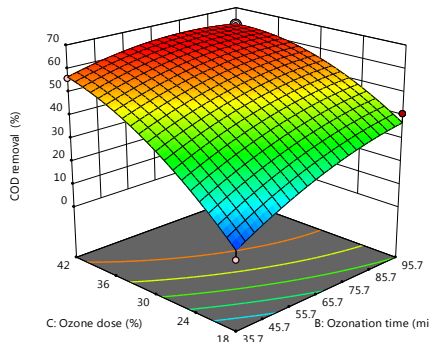


Design-Expert® Software  
Factor Coding: Actual

**COD removal (%)**  
● Design points above predicted value  
○ Design points below predicted value  
6 62

COD removal (%) = 62  
Std # 8 Run # 11  
X1 = B: Ozonation time = 95.7  
X2 = C: Ozone dose = 42

**Actual Factor**  
A: pH = 9.8



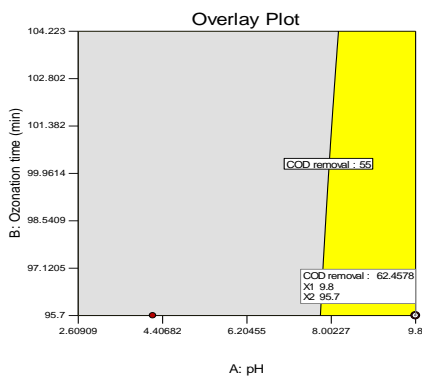
(B)

Design-Expert® Software  
Factor Coding: Actual  
Overlay Plot

**COD removal**  
● Design Points

X1 = A: pH  
X2 = B: Ozonation time

**Actual Factor**  
C: Ozone dose = 42



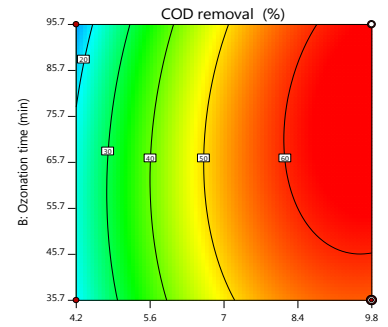
(C)

Design-Expert® Software  
Factor Coding: Actual

**COD removal (%)**  
● Design Points  
6 62

COD removal (%) = 56  
Std # 6 Run # 18  
X1 = A: pH = 9.8  
X2 = B: Ozonation time = 35.7

**Actual Factor**  
C: Ozone dose = 42

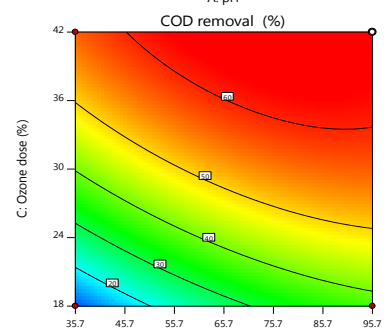


Design-Expert® Software  
Factor Coding: Actual

**COD removal (%)**  
● Design Points  
6 62

X1 = B: Ozonation time  
X2 = C: Ozone dose

**Actual Factor**  
A: pH = 9.8

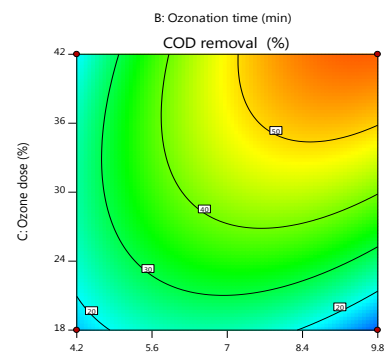


Design-Expert® Software  
Factor Coding: Actual

**COD removal (%)**  
● Design Points  
6 62

X1 = A: pH  
X2 = C: Ozone dose

**Actual Factor**  
B: Ozonation time = 35.7



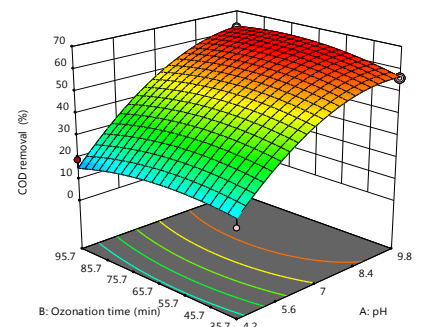
(A)

Design-Expert® Software  
Factor Coding: Actual

**COD removal (%)**  
● Design points above predicted value  
○ Design points below predicted value  
6 62

COD removal (%) = 56  
Std # 6 Run # 18  
X1 = A: pH = 9.8  
X2 = B: Ozonation time = 35.7

**Actual Factor**  
C: Ozone dose = 42

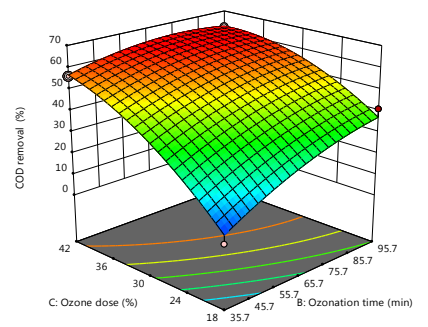


Design-Expert® Software  
Factor Coding: Actual

**COD removal (%)**  
● Design points above predicted value  
○ Design points below predicted value  
6 62

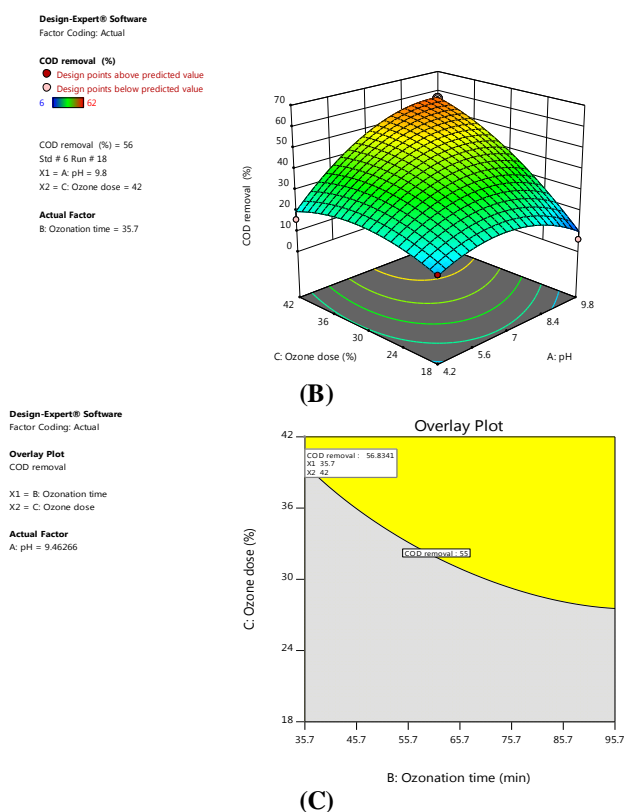
COD removal (%) = 56  
Std # 6 Run # 18  
X1 = B: Ozonation time = 35.7  
X2 = C: Ozone dose = 42

**Actual Factor**  
A: pH = 9.8



**Figure 2.** Maximum COD removal at pH 9.8, ozonation time 95.7 min, ozone dose 42% (A) Contour graphs (B) three dimensional surface plots (C) Overlay plot of the condition





**Figure 3.** Optimum COD removal at pH 9.8, ozonation time 35.7 min, ozone dose 42% (A) Contour graphs (B) three dimensional surface plots (C) Overlay plot of the optimized condition

Sreethawong and Chavadej [30] supported this result, and they reported that the COD removal rate increased from 25% to 50% when the ozone concentration was increased from 0.3 g/h to 1.1 g/h. The COD removal rate increased from 60% to 90% when the ozone dose increased from 7 mg/min to 33 mg/min. This is because, an increase in the influent ozone gas concentration results in an increase in aqueous ozone concentration which either directly reacts with organics or decomposes to produce  $\bullet\text{OH}$  which in turn reacts with the same. Another study by Terry [31], have indicated that the COD removal efficiency increased to 15% when the ozone concentration was increased from 0.053 g/min.L to 0.53 g/min.L. Thus increasing ozone concentration effectively resulted in enhanced removal of COD. It was found that as ozonation time was increased, the percentage COD removal rate also increased simultaneously. Wang et al. [32] also suggested that the COD reduced from 40mg/L to 29.6mg/L as the ozone contact time was increased from 1 min to 4 min. Another study by Ulson et al. [33], have also suggested that the COD value of the dye wastewater was 500 mg/L when the ozonation duration was 60 min, but the values decreased to 250mg/L as the contact time was increased to 100 min. Thus these above mentioned discussions have justified the results of this study.

#### Effect of ozone on the bacterial count

Microbial count studies were conducted to find out the efficiency of ozonation for the removal of bacteria present in sago wastewater. Table 5 shows the bacterial count in sago

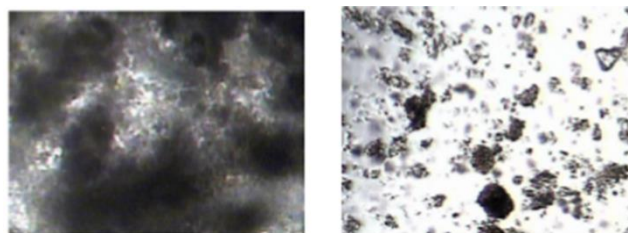
wastewater before and after ozonation. Prior to ozonation the bacterial count was  $10 \times 10^2 \text{CFU/mL}$  and after ozonation, it was found that there were no bacteria present. This is due to the above mentioned fact of bacterial cell lysis due to ozone gas exposure. Subha and Muthukumar [23] reported that most of the activated sludge microorganisms in the ozonation reactor would be killed and oxidized to organic substances. Those organic substances produced from the sludge ozonation can then be degraded in the subsequent biological treatment. Muhlisin et al. [34] have passed ozone gas for decontamination of *Salmonella typhimurium* in chicken meat. They observed a reduction in 80-90% of the CFU of the pathogen due to ozone exposure than control. The effects of gaseous ozone exposure on the bacterial counts and oxidative properties were evaluated in duck and chicken breast meat. Ozone effectively reduced the growth of coliform, aerobic and anaerobic bacteria in both chicken and duck breast [35]. Zhang et al. [36] had examined the mechanism of ozone-induced damage to *Pseudomonas aeruginosa* bacterium. They reported that ozone treatment effectively ruptured the bacteria's cell membrane leading to the leakages of cell contents. In this present study, after 20 minutes of ozonation, there were no microorganisms present in the sample. Digital microscopic appearance of wastewater is shown in Figure 4. It was observed that the biomass was dense before ozonation and after ozonation biomass concentration was reduced. These results and substantiations have clearly indicated that bacterial population reduction was steady due to ozone dosages. It can be concluded that ozonation will be an effective method for the inhibition and elimination of microorganisms.

#### Selection of optimal levels and estimation of optimum response characteristics

Optimized values are always desirable to justify the feasibility and practical application possibilities of a particular treatment process in large scale. Optimum level of various parameters obtained after examining the response curves and contour plots were pH of 9.8, ozone dose 42% and ozonation time 35.7 min where an experimental maximum removal of 56% and predicted removal of 56.7 were obtained.

**TABLE 5.** Initial and final bacterial count in the effluent

Dilution factor	No. of colonies	
	Before Ozonation	After Ozonation
$10^2$	10CFU/mL	Nil
$10^3$	5 CFU/mL	Nil



**Figure 4.** Microscopic appearance of the biomass in the wastewater (A) before ozonation (B) after ozonation

## SUMMARY

The increase in number of the sago industries has been creating a threat to the environment. The highly organic wastewater has been polluting not only the water bodies but also the land. Ozonation process was successfully employed for treatment of the sago wastewater in this research study. A central composite design was successfully employed for experimental design, analysis of results and optimization of the operating parameters for maximizing the COD removal from sago wastewater by ozonation. It also helps to understand the interaction effect of the variables on responses. The COD removal was found to be affected by pH, ozonation time and ozone dose. A maximum COD removal of 62.45% was predicted and 62% was obtained through experimental studies. But, maximum COD removal was only obtained when all the parameters were provided at almost high ranges like pH of 9.8, ozonation time of 95.7 min and ozone dose of 42%. The optimization of the analysed responses demonstrated that best results for COD removal (experimental 56 and predicted 56.7%) were obtained at a pH of 9.8, ozonation time of 35.7 min and ozone dose of 42%. The results of this study can be of considerable significance for choosing appropriate operating factors at optimum levels with minimum effort and time. The present study and its results demonstrated the applicability of ozonation process for effective organic load removal in sago wastewater. The authors do not have any conflict of interest.

## ACKNOWLEDGEMENTS

The authors would like to acknowledge the funding support from NSC (National Science Council) Taiwan under the MOST (Ministry of Science and Technology) project number MOST 106-2218-E-027-014-MY2 for the Design Expert software used in this research study.

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#### Persian Abstract

DOI: 10.5829/ijee.2018.09.02.05

#### چکیده

در این تحقیق، یک روش تصفیه مبتنی بر تخریب فاضلاب ناشسته ساگو با استفاده از فرایند ازوناسیون انجام شد. بهینه سازی متغیرهای فرایند با کمک نرم افزاری به نام Expert Design طراحی شده و روش شناسی پاسخ سطح (RSM) در طراحی کامپوزیت مرکزی نامیده می شود. تاثیر متغیرهای وزنی، مانند pH، زمان درمان و غلظت ازون در کاهش نیاز اکسیژن شیمیایی (COD) پساب ساگو مورد بررسی قرار گرفت. جالب توجه است، دو نوع مختلف از نتایج مانند حذف حداکثر COD و حذف مطلوب COD مشاهده شد. حداکثر حذف COD از ۶۲/۴۵٪ در pH ۹/۸، زمان بارگذاری ازون ۹۵/۷ دقیقه، دوز ازون ۴۲٪ و حذف COD مطلوب ۵۶/۷٪ در pH ۹/۸، زمان بارگیری در ۳۵/۷ دقیقه و دوز ازون ۴۲٪ بود. شمارش باکتری ها پس از اتمسفری برابر با صفر است و مشاهده میکروسکوپی زیست توده نشان داد که مقدار لجن بعد از اتمسفر به طور معنی داری کاهش می یابد. مشخص شد که ازون دهی فاضلاب ساگو، یک تلاش امیدبخش در تصفیه فاضلاب صنعتی می باشد.