Article **Journal of Environmental Sciences and Natural Resources**
December 2022, Vol.8 No.22 25-33

Analysis of correlations in the growth of biogranules from synthetic wastewater of industrial origin

Análisis de correlaciones en el crecimiento de biogránulos a partir de aguas residuales sintéticas de origen industrial

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DOI: 10.35429/JESN.2022.22.8.25.33 Received: July 25, 2022; Accepted December 30, 2022

Abstract

Pearson correlation is a statistical method used to identify dependence of variables in a system. This paper proposed this correlation method to analyze the relations between variables in a biogranulation process using a synthetic wastewater. These later was prepared assuming an industrial textile origin. The analysis was divided in aerobic, anaerobic and combinate cycles. Results obtained indicated Pearson correlation identified a relation in pH variable between dissolved oxygen and conductivity in aerobic cycle. Conversely, relations with conductivity between density are found in anaerobic cycles. Evaluating total data (in both cycles) relation pH variable between conductivity and mixing were found as a strong correlation in the system. Finally, the importance of identify relationship between variables it is important to monitoring those variables that might affect the system in each formation cycle of biogranulation. For example, in the aerobic cycle the desnitrification is crucial to decomposed until nitrates (N-NO2) and nitrites (N-NO3), or in the anaerobic cycle the reduction to nitrogen gas (N2).

Correlation, Statistical, Dependence, Synthetic, Wastewater

Resumen

La correlación de Pearson es un método estadístico empleado para identificar dependencias entre variables. Este artículo propone emplear esta correlación para analizar las relaciones entre variables en un proceso de biogranulación usando agua sintética residual. Esta última fue preparada asumiendo un origen industrial textil. El análisis fue dividido en los ciclos aerobio, anaerobio y combinados. Los resultados obtenidos indican una relación del pH con respecto a las variables de oxígeno disuelto y conductividad en el ciclo aerobio. Mientras que, en el ciclo anaerobio se encontraron relaciones entre la conductividad y la densidad. En la evaluación de los datos totales (para ambos ciclos) la relación entre el pH y las variables de conductividad y mezclado mostraron una alta correlación en el sistema. Finalmente, la importancia de identificar relaciones entre las variables es importante para monitorear aquellas variables que pudieran afectar el sistema en cada uno de los ciclos de formación de los biogranulos. Por ejemplo, en los ciclos aerobios el proceso de desnitrificación es crucial para la descomposición hasta nitratos (N-NO2) y nitritos (N-NO3), o en el ciclo anaerobio la reducción a nitrógeno gas (N2).

Correlación, Estadística, Dependencia, Sintética, Agua residual

Citation: MIRANDA-FLORES, German, SANCHEZ-SANCHEZ, Celina and MORENO-RODRIGUEZ, Ernestina. Analysis of correlations in the growth of biogranules from synthetic wastewater of industrial origin. Journal of Environmental Sciences and Natural Resources. 2022. 8-22:25-33.

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Introduction

An important concern worldwide is the contamination of rivers, water aquifers and underground rivers. In this topic, Wastewater treatment plants (WWTPs) are the main resource to treat domestic wastewater, agricultural wastewater, or industrial wastewater. Domestic wastewater is divided into two categories, i.e. black water (discharge from toilets that contains high organic, nitrogen & phosphorus content) and grey water (all the other wastewater except the toilet, & contains low organic persistent compounds) (Wulan et al., 2022). The most common pollutants coming from agricultural activities are high loads of oxygen-demanding organic compounds, associated nutrients (particularly nitrogen & phosphorus) and a variety of organic xenobiotic substances applied to cultures, soils or used in livestock, such as pesticides and pharmaceuticals (Dordio & Carvalho, 2013). In the case of industrial wastewater usually contains organic pollutants, heavy metals, and non-disintegrating materials (Abdelbasir & Shalan, 2019). The sectors that produces pollutants are iron-steel; textiles and paper; petrochemicals-refineries; non-ferrous metals; microelectronics; and mining (Hanchang, 2009).

By 2050, global water demand will increase by 20-30% due to increased industrial and domestic water use (WWAP (UNESCO World Water Assessment Programme), 2019). Through scientific including a reasonable treatment and reclamation, wastewater can be transformed into safe reclaimed water, which can not only reduce environmental pollution, also effectively increase water resources, realize the safe and efficient use of alternative water resources (Liao et al., 2021).

Movement and wastewater treatment systems are important energy consumers around 3%-4% of total U.S. electricity consumption employed (Us, 2008; Galbraith, 2011; Goldstein & Smith, 2002). The operational modifications could lead in some instabilities and hence an increase in energy consumption. As a result, the need for research to focus on improve the operational conditions during the operational processes is so important.

Some biological wastewater treatment processes (BWWTPs) are employed in conventional WWTPs depending on microorganisms available in the system (Meng et al., 2020). Aerobic Granular Sludge (AGS) technology becomes a very competitive method to activated sludge system. Its main advantages include: high energy efficiency, low investment costs, improved sludge settling (Arrojo et al., 2004; Czarnota et al., 2018; Lee et al., 2010; Ni et al., 2009; Othman et al., 2013; Su & Yu, 2005). The use of biogranule in BWWTPs imply their formation, growth and stability of dense and irregular clusters.

Aerobic granular sludge have been applied for treatment for high strength organic wastewater (Moy et al., 2002), degrade phenol (Adav et al., 2007a; Chou et al., 2004; Jiang et al., 2002), remove toxic organic compounds (Xie, 2003), also can degrade pyridine (by products of coal gasification) (Adav et al. id., 2007b). Aerobic granules played a promising role in adsorption of toxic chemicals (Adav et al., 2008), just for mention the highly toxic heavy metals had been removed with sludge granules by biosorption (Wei et. al., 2018; Pagliaccia, et. al., 2022).

There are many factors that can affect the capacity of WWTPs to remove pollutants or micropollutants. The main drawbacks of aerobic granules implementation are the loss of stability, granule break-up, and filament overgrowth (Adav et al., loc. cit.; Liu & Liu , 2006; Su & Yu, loc. cit.). Overcoming the obstacles, during the granule formation is important to identify the most favorable operation conditions (physical & chemical parameters) for granule stability, and increased removal of nitrogen, phosphorus, heavy metals, or particle matter, just for mention some conditions.

Some investigations demonstrate the Pearson correlation is used to identify linear and nonlinear relationship between random variables such as wastewater treatment rate, reclaimed water use rate, phosphate accumulation, eco-efficiency factors, community structure , abiotic parameters, sewage temperature, power consumption, volumetric flow rate and other pollutant parameters (Günther et al., 2012; Hu et al., 2019; Liao et al., 2021; Rashid & Liu, 2020).

In this regard, the aim from this study is demonstrate that some variables are correlated. Therefore, physic-chemical parameters such as pH, conductivity, temperature, dissolved oxygen, and density were correlated during biogranulation in aerobic and anaerobic cycle times.

Methodology

A. Sludge source

A sample of sludge were obtained from the wastewater treatment plant at Universidad de las Americas Puebla, Mexico. The sample was obtained before the conventional aeration process. The collected sample was settled for 5 min to remove excess water to obtain the residual sludge that will be inoculated in the bioreactor.

B. Synthetic wastewater

The concentrations of the effluent from the textile industry were simulated considering only the spinning and weaving process. The synthetic wastewater was prepared using component listed in Table 1. It´s important to mention that colorants and dyes were omitted in the synthetic wastewater.

Component	Formula	Dosed sewage	Unit
Sodium acetate	CH_3COONa	1.5	g/L
Phenol	C_6H_6O	0.20	
Magnesium sulphate	$MgSO_4$	5	
Calcium chloride	$CaCl_2$	2	
Iron(III) chloride	$FeCl_3$	110	mg/L
Copper sulphate	$CuSO_4$	50	
Potassium chloride	KCl	100	
Aluminum chloride	$AlCl_3$	90	
Zinc sulfate hepta-hydrate	$ZnSO_4.7H_2O$	120	

Table 1 Composition of synthetic substrate
Source: (Muda et al., 2010)

C. Bioreactor conditions

A Photo-Sequential Bach Reactor (SBR) model 2F-5000 (Figure 1) was used to analyze the formation, and growing in biogranulation process. The samples were composed in a 2:1 ratio, that is, 2 L of synthetic substrate per 1 L of suspended solids (SS). The SBR was kept at 25°C±1°C using temperature control SEV FC10, and was shaken at 50 rpm with an F2-5000 magnetic stirrer. The air system was controlled with a pump Model BD-15 at 1 L/min.

According to feast famine approach, in the biogranules formation were considered cycles of initial feeding followed by 96 h aerobic cycle (AC) and 72 h anaerobic cycle (ANC) without food for twelve cycles over a period of 12±1 day (Sun, et al., 2019),. Samples were taken at the end of each cycle and observed under a light microscope with a 4× objective lens (Nikon Eclipse E200) to verify biogranule formation, growth, and stability. A multiparametric equipment Thermo Scientific Orion was used to monitor the pH, temperature, conductivity. The density was estimated using a weight mass pycnometer method. Also, the dissolved oxygen (DO) was measured with a dissolved oxygen equipment model YSI Pro2030.

Figure 1 Photo-Sequential Bach Reactor (SBR) used in experimental biogranulation process

D. Pearson correlation analysis

The Pearson correlation coefficient (PCC) that refers to a measure of the linear and nonlinear relationship between two random variables defines as (Zou et. al., 2003)

$$r_{xy} = \frac{E[xy]}{\sigma_x \sigma_y} = \frac{\sum(x_i-\bar{x})\,\sum(y_i-\bar{y})}{\sqrt{\sum(x_i-\bar{x})^2}\sqrt{\sum(y_i-\bar{y})^2}} \quad (1)$$

Where:

$\bar{x} = \frac{1}{n}\sum_{i=1}^{N} x_i$, is the mean of x

$\bar{y} = \frac{1}{n}\sum_{i=1}^{N} y_i$, is the mean of y

r_{xy}, is the correlation coefficient

$E[xy]$, is the cross correlation between x and y

The correlation coefficient varies between -1 and +1. The value "-1" corresponds to a negative correlation between variables. Meanwhile the value "+1" corresponds to a positive correlation. See Table 2 for details and interpretation.

Correlation coefficient value	Direction and Strength of Correlation
-1.0	Perfectly negative (negatively correlated)
-0.8	Strongly negative
-0.5	Moderately negative
-0.2	Weakly negative
0.0	Uncorrelated (No association)
+0.2	Weakly positive
+0.5	Moderately positive
+0.8	Strongly positive
+1	Perfectly positive correlated (positively correlated)

Table 2 Interpretation of correlation coefficient
Source: (Zou et. al., 2003)

During operational conditions of WWTPS are common nonnormal data, due to the complex behavior of the system involve. Due to non-normal distributions, some variables were normalized before implement Pearson correlation analysis. In this study, the analysis was carried out to identify the relation between variables during the cycles of formation (AC & ANC), growth and stability of biogranules using Minitab® 21.2. Two main cycles were proposed, in the AC occurs the Nitrogen (*N*) ammoniacal oxidation ($N\text{-}NH_4^+$) until nitrates ($N\text{-}NO_2$) and nitrites ($N\text{-}NO_3$). In the ANC, denitrifying bacteria reduce nitrites and nitrates to *N* gas (N_2). Pearson correlation coefficients were computed using the total transformed data and for each AC and ANC. Figure 2 shows the schematic diagram to estimate the Pearson correlations in the SBR at different cycles. The data analyzed were divided in three group:

- Aerobic cycle (AC), cycle of 4 days
- Anaerobic cycles (ANC), cycle of 3 days
- Both cycles (AC & ANC), total data

Some models were obtained to predict main variables that dominate the system behaviors in each group of data.

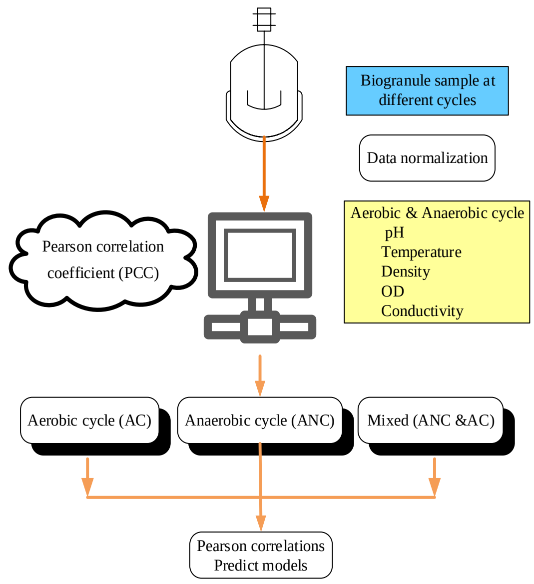


Figure 2 Schematic procedure to estimate the Pearson correlations in the SBR at different cycles

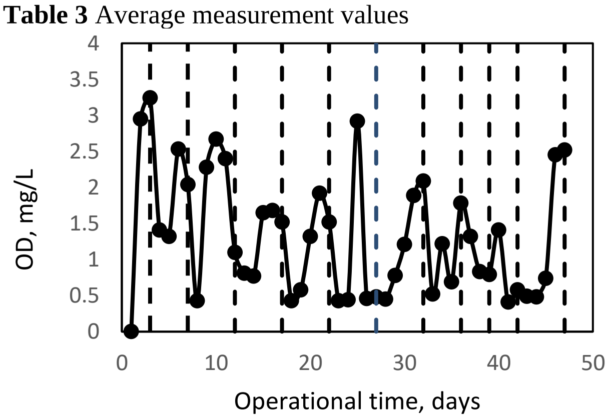
Results and discussion

A. Both cycles (AC & ANC)

Considering total data monitored, Graphic 1 shows variable´s fluctuations where discontinues line represents a new cycle (aerobic or anaerobic). In average the OD was 1.35 mg/L, with a 0.41 mg/L as minimum value and a 3.24 mg/L as maximum value.

Condition	Average value
pH	8.25
T, °C	24.63
OD, mg/L	1.35
Cond, mS/cm	5.76
Rpm	54.48
ρ, g/cm^3	0.98

Table 3 Average measurement values

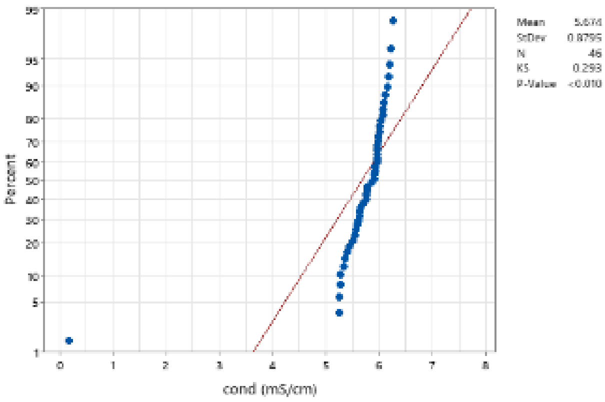


Graphic 1 Variations of the OD in the SBR

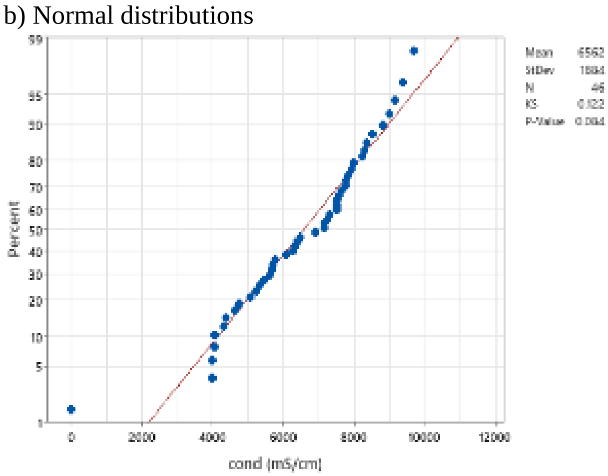
ISSN: 2444-4936

Due to non-normal distributions of data, a normalization analysis was done using Box Cox transformation approach (λ=0.5). Variables like temperature, mixing, and conductivity were normalized before implement Pearson correlation analysis. For example, conductivity of the system using normalized Box Cox method is shown in Graphic 2 (a) original data distributions (p value of 0.004) and (b) after normalized data (p value < 0.070).

a) Non-normal distribution

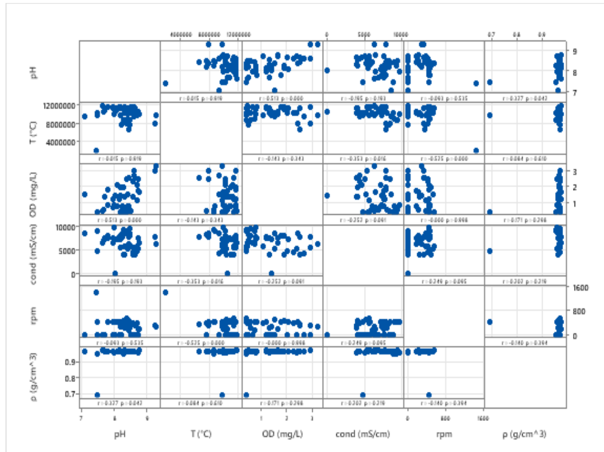


b) Normal distributions



Graphic 2 Normalization of conductivity data in the SBR

Correlations analysis of all data indicate some clusters identifed in the matrix plot (see Graphic 3) that are related with their Pearson coefficient and represented the relations between variables.



Graphic 3 Matrix plot of variable system AC & ANC.

Correlation models were estimated using the highest positive or negatives *Pearson* value in the matrix plot. Conversely no higher correlation was obtained, only two correlations were found as moderate positive and moderate negative correlations. It was found a positive correlation between pH and OD. Yin et al., (2016) demonstrated that the combined effects of DO and pH affects the nitrogen removal rate, where oxygen-consuming bacteria (nitrifiers) were implicitly inhibited by the low pH and less oxygen consumption.

Meanwhile, the temperature as a dependent variable were related with conductivity and mixing as independent variables. These variables are related with water distribution in the wet mass, can give a measure of the uniformity of liquid distribution and also a measure of the change of packing density of the damp mass during the massing stage of the granulation process (Spring, 1983). The correlations models are shown in Table 4 where it can be seen the correlation coefficient (R^2) value.

Correlation	Model	R^2(%)
Moderate positive	$pH = 7.904 + 0.269\ OD$	26.33
Moderate negative	$T = 11.9x10^6 - 224\ Cond - 37952\ Mix$	13.44

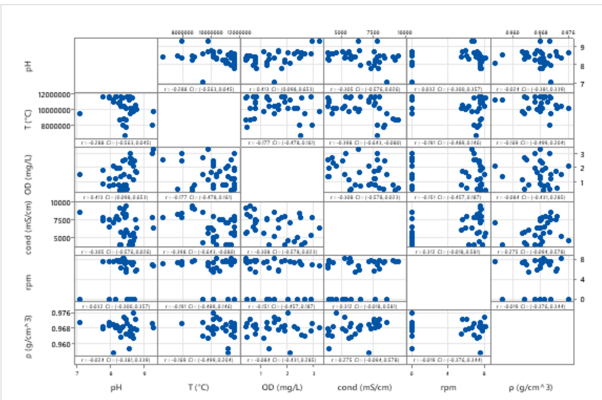
Table 4 Model correlation for both cycles (AC &ANC)

Where: pH, hydrogen potential; OD, Oxygen dissolved [mg/L]; T, Temperature [°C]; Cond, Conductivity [mS/cm]; Mix, Mixing [rpm].

ISSN: 2444-4936

B. Aerobic cycle (AC)

During AC cycle a degradation phase in the SBR was done followed by an aerobic starvation phase with no substrate feeding. Many factors can be related with the performance in the aerobic cycle where is desired the characteristics like good granulation, excellent settling time, regular and smooth compactness, or strong microbial structure, and high biomass retention. Pearson correlation analysis were done to identify relationship between variables of the system.



Graphic 4 Matrix plot of variable system AC

The results indicated four models that presents the highest *Pearson* value. Graphic 4 shows the matrix correlation for AC where some clusters are identified. Table 5 shows the models obtained where the higher correlation was pH (as dependent variable) versus OD and conductivity (as independent variable). In AC, the negative correlation between temperature and conductivity indicates the relation of packing density during granulation process The later, is have reported as deterioration of the settling properties due to overgrowth of filamentous microbial structures at higher mesophilic temperatures Liu & Liu, loc. cit.

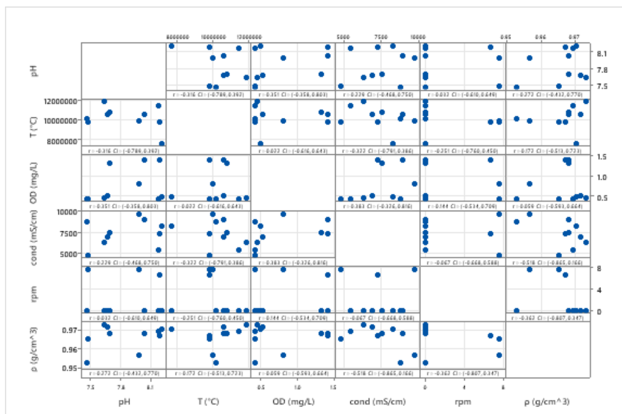
Correlation	Model	R^2(%)
Moderately negative with *OD* Moderately positive with respect to *Cond*	$pH = 8.447 + 0.1652\ OD - 4.4\ x10^{-5}\ Cond$	20.55
Moderately negative	$T = 12.424361x10^6 - 329\ Cond$	15.88
Moderately negative	$OD = 2.553 - 0.000159\ Cond$	9.47
Moderately positive	$Cond = 5727 - 158.5\ rpm$	9.75

Table 5 Model correlation for AC

Where: pH, hydrogen potential; OD, Oxygen dissolved [mg/L]; T, Temperature [°C]; Cond, Conductivity [mS/cm]; Mix, Mixing [rpm]

C. Anaerobic cycle (ANC)

Removal of organic carbon, nitrogen and phosphorus simultaneously in sequential batch reactors combined the aerobic with anaerobic cycle adding anoxic stage (Callado & Foresti, 2001). As can be seen in Table 6, the highest correlation was found between conductivity (dependent variable) versus density (independent variable). The granule formation is related with biomass density, shape, or packing, between others. The biomass density of aerobic granules is related with a higher settling velocity and result from the dense microbial structure (Tay et al., 2001).



Graphic 5 Matrix plot of variable system ANC

Correlation	Model	R^2 (%)
High Moderately positive	$Cond = 128693 - 5022\ Dens$	26.80
Moderately negative	$T = 11.988949x10^6 - 235\ Cond$	10.34
Moderately positive	$OD = -0.012 + 0.000106\ Cond$	14.63
Moderately positive	$pH = 8.516 - 0.243\ OD$	22.80
Moderately negative	$rpm = 191 - 7.80\ Dens$	13.07

Table 6 Model correlation for ANC

Where: pH, hydrogen potential; OD, Oxygen dissolved [mg/L]; T, Temperature [°C]; Cond, Conductivity [mS/cm]; Mix, Mixing [rpm]; Dens, density [g/cm^3]

Conlusions

The results indicated that in AC, ANC or both cycles are some variables correlated significantly. In AC and ANC, were found that OD and pH shows a moderate positive correlation. These relations could also have influence operational factors-values (OD, temperature, aeration) or biogranule characteristic (settling times, microbial community, mass density, high biomass retention). This study provides a basis to find the variables that could enhance aerobic, or anaerobic properties during granulation process.

Acknowledgment

This work has been funded by CONACYT [2018-000068-02NACF-24440]. Also, the authors want to acknowledge the support and facilities provided by Universidad de las Americas Puebla (UDLAP), Mexico.

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