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Metabolic and kinetic changes of activated sludge because of failures in the aeration system in a WWTP

Cambios metabólicos y cinéticos de los lodos activados como consecuencia de las fallas en el sistema de aireación en una PTAR

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Abstract

The classic question from wastewater treatment plant (WWTP) operators is, how long can a secondary reactor be kept unaerated after an aeration system failure? Therefore, in the present research, samples of activated sludge (AS) were taken from a WWTP and left without aeration for 12, 18, 24, and 36 h; simulating an aeration failure. Subsequently, the activity of AS lacking aeration was evaluated in batch cultures. The results showed that the lack of AS oxygenation affected its metabolism. For example, the heterotrophic activity stopped completely when AS was not aerated for 24 h. The nitrifying activity, in the experiments from 12 to 24 h without aeration, the ammonium removal did not change, but there was an increase in nitrite concentration. In the 36-h experiment without aeration, the ammonium consumption rate and removal efficiency decreased by 56% and 43%, respectively, regarding the control. N_2O was produced in the period when the AS was not aerated, which could be responsible for these metabolic and kinetic changes. Finally, this study suggested don't leave for more than 24 h the activated sludge without aeration for a quick recovery or to diminish the damage.

Keywords: activated-sludge, aeration, inhibition, nitrification, heterotrophic-activity.

Resumen

Una pregunta permanente de los operadores de las Plantas de Tratamiento de Aguas Residuales (PTARs) es ¿por cuánto tiempo se puede mantener un reactor secundario sin airear después de una falla o mantenimiento en el sistema de aireación? Por lo que, en la presente investigación, se tomaron muestras de lodos activados (LA) de una PTAR y se dejaron sin airear por 12, 18, 24 y 36 h; con esto se simuló alguna falla en la aireación. Posteriormente, se evaluó la actividad del LA, en cultivos lote. Los resultados mostraron que la falta de aireación del LA sí afectó el metabolismo de este. Por ejemplo, la actividad heterotrófica se detuvo completamente cuando el LA estuvo 24 h sin airearse. La actividad nitrificante, en los experimentos de 36 h sin aireación, la tasa de consumo de amonio y la eficiencia disminuyeron en un 56% y 43%, respectivamente, respecto al control. El gas N_2O se produjo cuando el LA no estaba siendo aireado y podría ser el responsable de estos cambios metabólicos y cinéticos. Finalmente, el estudio sugiere no dejar por más de 24 h los lodos activados sin aireación para una rápida recuperación o para disminuir el daño.

Palabras clave: lodo activado, aireación, inhibición, nitrificación, actividad heterotrófica.

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1 Introduction

The activated sludge system is widely used worldwide for treating industrial or municipal wastewater. The main goal of the activated sludge system is organic matter removal, where the primary microorganisms involved are heterotrophs. If solid retention time (SRT) is above eight days, it allows the development and permanence of the nitrifying bacteria inside the biological reactor involved in the nitrogen biotransformation. Nitrification is the ammonium oxidation up to nitrate (NO_3^-) via hydroxylamine (NH_2O) and nitrite (NO_2^-) as transitory intermediaries. Both biological processes depend on the dissolved oxygen concentration as the oxidizing source (Espinosa-Rodríguez *et al*., 2019).

Dissolved oxygen is an important parameter that affect the biomass activity (García-Cabrera *et al*,, 2021; López-Taborda *et al*., 2022). Oxygen is essential to complete nitrification and oxidize all organic matter, but high aeration costs have forced operators to limit oxygen or use intermittent aeration. Nonetheless, oxygen availability significantly impacts the nitrogen cycle pathway. At low oxygen concentration, nitrous oxide (N_2O) is produced via nitrification, whereas the denitrification process is affected at a high oxygen concentration (Kampschreur *et al*., 2009). In denitrification, nitrous oxide reductase is more sensitive to oxygen than the others, being the product N_2O instead of N_2 (Kampschreur *et al*., 2009). Ammonium oxidation is commonly associated with the nitrification process, but at low dissolved oxygen concentration, the heterotrophic bacteria also have the metabolic capability to remove it (Van Niel *et al*., 1993). Anderson *et al*. (1993) observed that heterotrophic nitrification could emit more nitrous oxide than autotrophic nitrification in pure cultures. It is well-known that N_2O is not an intermediary in the metabolic pathway of the nitrification process, but ammonium oxidizing bacteria can produce this greenhouse gas, or inclusive N_2O can be produced chemically between nitrite and hydroxylamine (Van Cleemput, 1998; Colliver and Stephenson, 2000). There are few studies evidencing the adverse effects of this greenhouse gas on the metabolic activities of bacteria; for example, Baden and Monk (1981) observed that N_2O decreased the viability of the bacteria *Salmonella Typhimurium*. Yin *et al*. (2018) observed that N_2O concentrations in the low micromolar range decreased dechlorination rates in *Geobacter lovleyi*. Also, the methanogenesis process has been inhibited by N_2O (Yin *et al*., 2018).

Most WWTPs use atmospheric air to supply the oxygen required for bacterial metabolism. Nonetheless, failures in the aeration system cause diverse troubles like foul odors, drops in removal efficiencies, low water quality, and septic zones, among others. But the most critical question of WWTP operators is, how long can I stop aerating my system? Is the metabolic activity of the activated sludge modified? Those questions are addressed in the present experimental work. It is worth mentioning that there is no information in the literature on this subject.

2 Material and methods

Plastic bottles with an operating volume of 200 mL were performed in batch cultures. The activated sludge was taken from WWTP treating municipal wastewater. The WWTP was operating at SRT for above 15 days. The activated sludge samples were withdrawn from the WWTP (i.e., from the secondary tank) and left without aeration for different periods (0 (control test), 12, 18, 24, and 36 h); this experimental step was called pretreatment. The sludge of the control test was not deprived of aeration. After the pretreatment was concluded, the supernatant was removed, then the activated sludge was washed with a physiological solution (9 g NaCl/L) three times to remove organic and inorganic residuals to carry out the batch cultures to evaluate the heterotrophic and nitrifying activities. Batch cultures were spiked with 2 g VSS/L and the following chemical culture media (in g/L): NH_4^+-N/L (0.050), glucose-COD/L (0.400), K_2HPO_4 (3.0), KH_2PO_4 (4.5), $NaHCO_3$ (2.0), $FeCl_3$ (0.015), $CuSO_4{\cdot}5H_2O$ (0.015), $CaCl_2{\cdot}2H_2O$ (0.07), and 0.1 ml/L of trace elements. The chemical composition of trace elements is as follows (in mg/L): $FeSO_4$ (3000), EDTA (5000), $ZnSO_4{\cdot}7H_2O$ (430), $CoCl_2{\cdot}6H_2O$ (240), $MnCl_2$ (600), $CuSO_4{\cdot}5H_2O$ (250), $Na_2MoO_4{\cdot}2H_2O$ (220), $NiCl_2{\cdot}6H_20$ (190) and $MgCl_2$ (500). The batch cultures, including the control, were aerated using a fish tank pump, keeping oxygen dissolved concentration between 3.5-4.5 mg/L. The initial pH of the batch cultures was 7.0. Batch cultures were performed by duplicate. Degradation rates were computed by the Gompertz model using the software Origin 8.0 (OriginLab, Inc®). Tukey's statistical test and data analysis were performed by the Minitab® 19 software, with a

significance level of 0.05.

3 Analytical methods

Ammonium was measured with a selective electrode (pHoenix Electrode Co. Mod. NH331501). Soluble Chemical Oxygen Demand (COD) was measured with the technique of closed reflux (APHA- AWWA-WEF-2018). Volatile suspended solids (VSS) were measured according to the standard methods (APHA-AWWA-WEF-2018). Dissolved oxygen concentration and pH were measured in a multiparameter (HACH sension378). N_2O was detected by gas chromatography, with helium as the carrier gas at a 20 mL/min flow rate, and thermal conductivity detector; the injection port, oven, and detector temperatures were at 80, 30, and 120 °C, respectively, using a stainless-steel column packed with Porapak T (60/80 mesh) (GOW-MAC Model 580 isothermal 120 V, 60 Hz). HPLC Agilent was used to measure nitrate and nitrite with an ionic interchange column (IC-Pak anion HC, 4.6 x 150 mm), using a mobile phase composed of 20 mL de n-butanol, 120 mL of acetonitrile, and 20 mL of borate-gluconate, at 214 nm.

4 Results and discussion

4.1 Pretreatments

The activated sludge used in this study presented an initial sludge volume index (SVI) of 140 mL sludge/g TSS. In the activated sludge systems, SVI values of less than 150 are commonly sought since low SVI reflects a good sludge settling and clarifies the treated water (Mesquita *et al*., 2011). The initial oxygen concentration in the activated sludge liquid samples was 1.6 mg/L. In the pretreatments, the initial oxygen concentration declined with the time course due to the biological activity (Figure 1). For instance, in 36 h without aeration, the residual oxygen at the end of the pretreatment dropped to 0.25 mg O_2/L. In addition, during the pretreatment time, in all studies, after 2 hours, the activated sludge began to float, affecting the SVI measurement. This flotation was due to the gas coming from heterotrophic nitrification or aerobic denitrifying activity (Anderson *et al*., 1993; Dotro *et al*., 2011). In this sense, one pretreatment was carried out in a closed serological bottle to retain

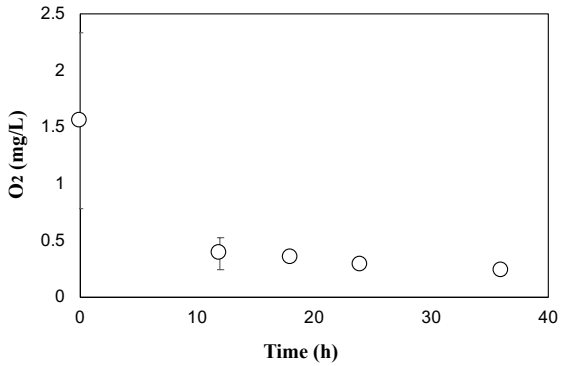

Figure 1 Dissolved oxygen concentration at the end of each pretreatment.

the gas formed inside the system during the aeration deprivation to identify the kind of gas formed by chromatography. At 12 h of the pretreatment, N_2O was detected, and it stayed until 36 h, but unfortunately, its quantification was not possible. The initial N_2 at the begging of the pretreatment in terms of the area did not change regarding the end of the pretreatment, for this reason, this observation suggested that the main gas produced was N_2O. Nitrous oxide is highly soluble in water, at 5 °C, 0.0425 mol/L water, so the gas in the headspace will be in equilibrium with the water depending on temperature (Roper *et al*., 2013). N_2O also has been identified in activated sludge systems fed with intermittent aeration, during the anoxic phase, from 0.07% to 27% N_2O-N/NH_4^+-N oxidized (Dotro *et al*., 2011).

Nitrous oxide has been reported as an inhibitor for bacteria and a greenhouse gas, having a 300-fold more substantial effect than CO_2 (IPCC, 2006). On the other hand, the enzyme N_2O reductase is more sensitive to oxygen than the other enzymes, leading to N_2O emission during denitrification when oxygen is present in low amounts (Otte *et al*., 1996). It is well-known that 0.25 mg O_2/L inhibits the enzyme nitrous oxide reductase; therefore, the final product will be N_2O instead of N_2 (Beristain-Cardoso *et al*. 2009).

It is worth mentioning that N_2O can cross the cell membrane of bacteria and cause some damage (Otte *et al*., 1996). Still, there is scarce information about this effect on the metabolic activity of bacteria, much less in an activated sludge system. In the pretreatments, the remaining dissolved oxygen concentration was equal to or above 2.5 mg/L, evidencing the denitrification process was not complete.

Table 1. Consumption rates, removal efficiencies and yields during batch cultures.

Treatment	q COD (mg /L-d)	q NH_4^+ (mg /L-d)	COD removal (%)	NH_4^+ removal (%)	NO_3^--N *Yield	NO_2^--N * Yield
Control (0h)	286.9 ± 51.2	7.6 ± 0.75	46 ± 14	48.4 ± 6.0	0.60 ± 0.04	0.26 ± 0.06
12h	268.5 ± 11.7	14.3 ± 5.4	28.4 ± 2.3	55.4 ± 8.7	0.23 ± 0.02	0.75 ± 0.09
18h	240.4 ± 15.8	7.3 ± 2.6	36.2 ± 2.5	45.4 ± 8.9	0.22 ± 0.05	0.73 ± 0.03
24h	00 ± 00	4.6 ± 0.8	00.0 ± 0.0	45.4 ± 6.3	0.41 ± 0.23	0.71 ± 0.26
36h	00 ± 00	3.3 ± 0.03	00.0 ± 0.0	27.3 ± 5.3	0.26 ± 0.02	0.84 ± 0.11

*Yield: mg NO_3^--N produced/mg NH_4^+-N consumed, and mg NO_2^- -N produced/mg NH_4^+-N consumed

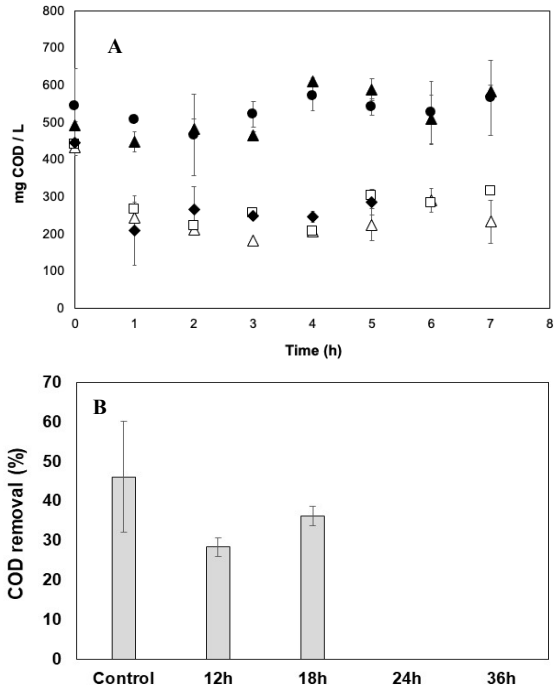

Figure 2 A) COD consumption profiles. (Δ) control, (□) 12 h, (♦)18 h, (▲) 24 h, (●) 36 h. B) COD removal efficiencies in all batch cultures.

4.2 *Influence of the lack of aeration in the heterotrophic and nitrifying metabolism in batch cultures*

Once the activated sludge liquid samples were left without aeration, the sludge activity was evaluated in batch cultures to prove if the lack of aeration might affect the metabolic activity of the heterotrophs and nitrifiers.

Figure 2 shows the COD consumption profiles for an incubation period of 7 h. Control batch culture displayed a COD removal rate of 268.98 ± 51.20 mg COD/L-h, with a removal efficiency of 46.13 ± 13.96 % (Table 1, Figure 2B). In batch cultures with activated sludge deprived of 12 and 18 h aeration, COD removal rates and removal efficiencies did not significantly change in batch cultures (Table 1). Nonetheless, the heterotrophic biological process stopped utterly in the pretreatments deprived of 24 h aeration, with no COD removal. The nitrogen gas produced during the pretreatments might be involved in this biological behavior. In the literature, there is no information about the negative effect of N_2O in activated sludge systems, but there is litter information on other bacteria. For instance, Baden and Monk (1981) observed that N_2O decreased the viability of Salmonella lyphimurium, and Yin *et al*. (2018) observed that N_2O decreased dichlorination rates in *Geobacter lovleyi*. At the same time, Yin *et al*. (2018) observed a methanogenesis inhibition by this greenhouse gas.

Figure 3 shows the nitrogen consumption profiles. The control culture showed an ammonium removal rate of 7.60 ± 0.75 mg NH_4^+-N/L-h, with a removal efficiency of 48.35 ± 6.05 % (Table 1, Figure 3B). The low removal efficiency was because the batch cultures were performed in a short time, 7 h. In the batch cultures where the activated sludge was deprived for 12, 18, and 24 h without aeration, the Turkey statistical test indicated no significant difference in ammonium removal rates or efficiencies. Nonetheless, in the last batch culture evaluated, where the activated sludge was deprived by 36 h without aeration, both ammonium removal rates and ammonium removal efficiencies were significantly affected (Table 1). Comparing this last study with the control test, the ammonium removal rate and efficiency diminished by 56% and 43%, respectively.

Nitrate and nitrite were measured at the end of the batch cultures (Table 1). In the control batch culture, the ammonium nitrogen consumption was recovered as NO_3^- and NO_2^-, with yields of 0.60 ± 0.04 mg NO_3^--N/ mg NH_4^+-N consumed and 0.26 ± 0.06 mg NO_2^--N/mg NH_4^+-N consumed, respectively. In the case of the nitrate, the nitrate yield of 0.60 indicates that 60% of ammonium nitrogen consumed

Table 2. Mater balance and nitrogen recovery in batch cultures.

Treatment	NH_4^+-N consumed (mg/L)	NO_3^--N produced (mg/L)	NO_2^--N produced (mg/L)	Recovery percentage (%)
Control (0h)	22.4 ± 5.4	13.5 ± 0.8	5.8 ± 1.4	86
12h	39.4 ± 15.8	9.2 ± 0.8	29.7 ± 3.4	98.8
18h	28.9 ± 19.4	6.4 ± 1.5	21.2 ± 1.0	95.4
24h	27.5 ± 3.8	7.2 ± 0.8	19.7 ± 7.1	97.5
36h	13.6 ± 3.7	3.6 ± 0.3	11.4 ± 1.6	109.9

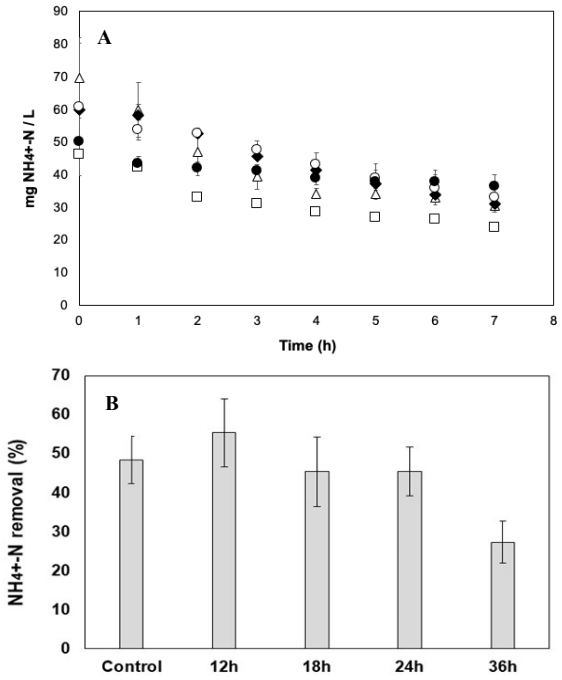

Figure 3 A) Ammonium consumption profiles. (□) control, (Δ) 12 h, (♦)18 h, (*o*) 24 h, (●) 36 h. B) Ammonium removal efficiencies in all batch cultures.

was recovered as nitrate nitrogen. These yields are essential to following the outcome of nitrification. In Table 1, conforming to the AS was left without aeration for more time, the nitrate yield decreased with an increment of nitrite accumulation, showing a nitrite yield of 0.84 ± 0.11 for the last batch culture. For example, there are several enzymes participating in the nitrification process (Suárez-García *et al*., 2019). The general biochemistry pathway is as follows:

$$NH_4^+ \xrightarrow{AMO} NH_2OH \xrightarrow{HAO} NO_2^- \xrightarrow{Nor} NO_3^-$$

Where, AMO: Ammonium monooxygenase, HAO: Hydroxylamine oxidoreductase, and Nor: Nitrite oxidoreductase. Now, how in the present work the nitrite accumulation increased means that the N_2O formation in the pretreatments might have affected the enzyme nitrite oxidoreductase (Nor).

Table 2 shows the nitrogen balance for all batch cultures. In batch cultures of sludge deprived by 12 up to 36 h aeration, nitrogen balances were close to 100%; this means that the nitrifying process was merely catabolic. In the control batch culture, 86% of nitrogen consumed was recovered as nitrate and nitrite. The lack of nitrogen (about 14%) might be in the form of an intermediary of nitrification, for instance, the hydroxylamine (compound not measured).

The study of 36 h without aeration was repeated to evaluate the biological processes, but in an incubation period of 96 h instead of 7 h to assess if the metabolic activity recovery might be a function of time (Figure 4). At the end of the batch cultures, the COD and ammonium removal efficiencies were 53% ± 12.3 and 95.20 ± 6.4 %, respectively. The COD profile showed a lag phase of 50 h, while the nitrification process did not present lag phase. Comparing the COD consumption profile in the recovery test regarding the control test where the ammonium removal was 46.13% in 7 h, in the recovery test the same COD removal percentage was reached about 90 hours. In the case of the nitrifying profile, the ammonium removal of 48.35 % observed in the control test in 7 h, in the recovery test this percentage was reached about 48 h. The extension of the cultivation time improved the removal efficiencies regarding the study of 7 h of incubation, where nitrification displayed a better response. These experimental results might suggest that the enzymes for glucose consumption by aerobic heterotrophic bacteria via glycolysis were more sensitive to the N_2O presence than the enzymes of nitrification.

Finally, these experimental results suggested that the damage caused during the pretreatment due to the lack of aeration might be reversible. However, it will take a long time to get the initial behavior. Finally, this experimental work showed clearly that lack of aeration significantly affects the biological processes involved in an activated sludge process, being the heterotrophic activity the metabolic process most affected.

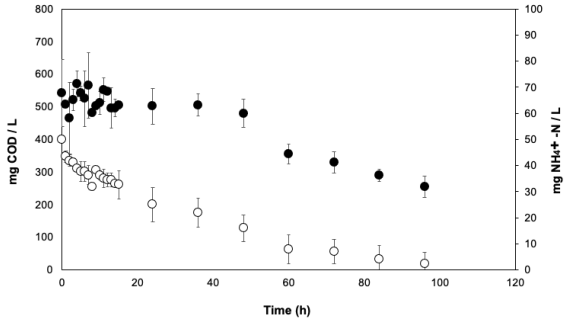

Figure 4 Recovery test in the pretreatment of 36 h. (*o*) Ammonium consumption profile, (•) COD consumption profile.

Conclusions

The lack of aeration of the activated sludge significantly affected the metabolic activities. The biological process most affected was the heterotrophic process since 24 h of aeration deprivation was enough to stop the respiratory process completely. Nitrification took more time to be affected, up to 36 h of aeration deprivation. The metabolic and kinetic changes of the activated sludge were associated with N_2O production in the not aerated systems. Finally, this study suggested don't leave for more than 24 h the activated sludge without aeration for a quick recovery or to diminish the damage.

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References

Anderson, I.C., Poth, M., Homstead, J. and Burdige, D. (1993). A comparison of nitric oxide and nitrous oxide production by the autotrophic nitrifier Nitrosomonas europaea and the heterotrophic nitrifier *Alcaligenes faecalis*. *Applied and Environmental Microbiology 59*, 3525-3533. DOI: 10.1128/aem.59.11.3525-3533.1993

APHA AWWA and WEF (2018). *Standard methods for the examination of water and wastewater* (23rd ed.)

Baden, J.M. and Monk, S.J. (1981). Mutagenicity and toxicity studies with high-pressure nitrous oxide. *Toxicology Letters 7*, 259-262. DOI: 10.1016/0378-4274(81)90078-3

Beristain-Cardoso, R., Texier, A.C., Razo-Flores, E., Méndez-Pampín, R. and Gómez, J. (2009). Biotransformation of aromatic compounds from wastewaters containing N and/or S, by nitrification/denitrification: a review. *Reviews in Environmental Science and Biotechnology 8*, 325-342. https://doi.org/10.1007/s11157-009-9172-0

Colliver, B.B. and Stephenson, T. (2000). Production of nitrogen oxide and dinitrogen oxide by autotrophic nitrifiers. *Biotechnology Advances 18*, 219-232. DOI: 10.1016/s0734-9750(00)00035-5

Dotro, G., Jefferson, B., Jones, M., Vale, P., Cartmell, E., and Stephenson, T. (2011). A review of the impact and potential of intermittent aeration on continuous flow nitrifying activated sludge. *Environmental Technology 32*, 1685-1697. https://doi.org/10.1080/09593330.2011.597783

Espinosa-Rodríguez, M.A., Ruiz-Sánchez, T.J., Hidalgo-Millán, A., and Delgado-Delgado, R. (2019). Efecto de la carga hidráulica de un filtro percolador en el proceso de nitrificación. *Revista Mexicana de Ingeniería Química 18*, 107-113. https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n1/Espinosa

García-Cabrera, R.I., Valdez-Cruz, N.A., Daniel-Vázquez, A., Blancas-Cabrera, A., and Trujillo-Roldán, M.A. (2021). Roles of culture media and oxygen transfer in the scale-up from shake flasks to pneumatic bioreactor of the plant growth-promoting bacterium *Rhizobium phaseoli*. *Revista Mexicana de Ingeniería Química 20*, 1091-1109. https://doi.org/10.24275/rmiq/Bio2297

IPCC. (2006). IPCC Guidelines for National Greenhouse Gas Inventories. In: Eggleston, H.S., Buendia, L., Miwa, K., Ngara, T., Tanabe, K. (Eds.). IGES, Japan, pp. 6.24-26.26.

Kampschreur, M. J., Temmink, H., Kleerebezem, R., Jetten, M. S. and van Loosdrecht, M.C. (2009). Nitrous oxide emission during wastewater treatment. Water Research 43, 4093-4103. https://doi.org/10.1016/j.watres.2009.03.001

López-Taborda, J.D., Vargas-Zapata, A., Ramírez-Vargas, J.F., Valdez-Cruz, N. A., Trujillo-Roldán, M. A., and Orozco-Sanchez, F. (2022). Development and characterization of an alternative control system to study separately oxygen and momentum transfer effects in stirred tank bioreactors. *Revista Mexicana De Ingeniería Química 21*, Proc2607-Proc2607. https://doi.org/10.24275/rmiq/Proc2607

Mesquita, D.P., Amaral, A.L., and Ferreira, E.C. (2011). Identifying different types of bulking in an activated sludge system through quantitative image analysis. *Chemosphere 85*, 643-652. https://doi.org/10.1016/j.chemosphere.2011.07.012

Otte, S., Grobben, N.G., Robertson, L.A., Jetten, M.S.M. and Kuenen, J.G. (1996). Nitrous oxide production by Alcaligenes faecalis under transient and dynamic aerobic and anaerobic conditions. *Applied and Environmental Microbiology 62*, 2421-2426. DOI: 10.1128/aem.62.7.2421-2426.1996

Roper, J.D., Burton, D.L., Madani, A., and Stratton, G.W. (2013). A simple method for quantifying dissolved nitrous oxide in tile drainage water. *Canadian Journal of Soil Science 93*, 59-64. https://doi.org/10.4141/cjss2012-02

Suárez-García, L.I., Cuervo-López, F.M., and Texier, A.C. (2019). Biological removal of mixtures of ammonium, phenol, cresol isomers, and sulfide in a sequencing batch reactor. *Revista Mexicana de Ingeniería Química 18*, 1189-1202. https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Suarez

Van Cleemput, O. (1998) Subsoils: chemo- and biological denitrification, N_2O, and N_2 emissions. *Nutrient Cycling in Agroecosystems 52*, 187-194. https://doi.org/10.1023/A:1009728125678

Van Niel, E.W.J., Arts, P.A.M., Wesselink, B.J., Robertson, L.A. and Kuenen, J.G. (1993). Competition between heterotrophic and autotrophic nitrifiers for ammonia in chemostat cultures. *FEMS Microbiology Ecology 11*, 109-118. https://doi.org/10.1016/0378-1097(93)90006-N

Yin, Y., Yan, J., Chen, G., Murdoch, F.K., Pfisterer, N. and Löffler, F.E. (2018). Nitrous oxide is a potent inhibitor of bacterial reductive dechlorination. *Environmental Science and Technology 53*, 692-701. https://doi.org/10.1021/acs.est.8b05871