Biotecnia
Journal of biological and health sciences
http://biotecnia.unison.mx
Universidad de Sonora
ISSN: 1665-1456

Potential of *Verrucodesmus verrucosus* in the removal of nitrogen and phosphorus structures from pig farms wastewater

Potencial de *Verrucodesmus verrucosus* en la remoción de estructuras de nitrógeno y fósforo de aguas residuales de granjas porcinas

Ana Gabriela Zenteno Carballo[1], Yazmin Sánchez Roque[1*], Sergio Saldaña Trinidad[1], Miguel Angel Canseco Pérez[1], Roberto Berrones Hernández[1] and Yolanda del Carmen Pérez Luna[1*]

[1] Research laboratory, Universidad Politécnica de Chiapas, Carretera Tuxtla-Villaflores KM. 1+500, Las Brisas, C. P. 29150, Suchiapa, Chiapas, México.

ABSTRACT

Verrucodesmus verrucosus in residual water from a pig farm located in Suchiapa, Chiapas, Mexico, was evaluated as a mixotrophic culture medium. The evaluation was carried out for 40 days, under a 12:12 light / dark cycle; two growth stages were evaluated (piglet and fattening) and the 50:50 mixture of these, as well as to the residual water two pre-treatments were applied, this consisted of a filtration process using a 15 µm diameter nylon filter and a sterilization process. The microalgal species *Verrucodesmus verrucosus* was shown to have bioremediation potential by growing in wastewater and producing biomass, demonstrating high efficiency in removing contaminants. The maximum Chemical Oxygen Demand (COD) removal was in the Ps treatment (sterile piglet) where the removal of 96.8 % was reached, while the Biochemical Oxygen Demand (BOD) had a maximum removal of 96.7 % in the Pf treatment (filtered piglet). The removal of total nitrogen, ammonia and nitrate was demonstrated with a percentage of 85.5 %, 74 % and 91 % respectively. As for the maximum removal of phosphorus and phosphate, they reach values of 97.9 % and 82 % respectively. On the other hand, it was possible to demonstrate the antagonistic capacity of this microalgae with respect to *Escherichia coli,* where 100 % elimination was achieved.

Keywords: biomass; maximum removal; mixotrophic; wastewater

RESUMEN

En la presente investigación se evaluó el potencial de biorremediación de la especie de microalga *Verrucodesmus verrucosus* en aguas residuales de una granja porcina ubicada en Suchiapa, Chiapas, México, como medio de cultivo mixotrófico. La evaluación se realizó durante 40 d, bajo el ciclo 12:12 luz/oscuridad, se evaluaron dos etapas de crecimiento (lechón y engorde) y la mezcla 50:50 de estos, así también al agua residual se le realizaron dos pretratamientos, estos consistieron en un proceso de filtración mediante un filtro de nylon de 15 µm de diámetro y un proceso de esterilización. Se demostró que la especie de microalgas *Verrucodesmus verrucosus* tiene potencial de biorremediación al crecer en aguas residuales y producir biomasa, demostrando una alta eficiencia en la eliminación de contaminantes. La máxima remoción de la Demanda Química de Oxígeno (DQO) fue en el tratamiento Ps (lechón estéril) donde se alcanzó la remoción del 96,8 %, mientras que la Demanda Bioquímica de Oxígeno (DBO) tuvo una remoción máxima del 96,7 % en el tratamiento Pf (lechón filtrado). Se demostró la remoción de nitrógeno total, amoniaco y nitrato con un porcentaje de 85.5 %, 74 % y 91 % respectivamente. En cuanto a la remoción máxima de fósforo y fosfato, alcanzan valores de 97.9 % y 82 % respectivamente. Por otro lado, se pudo demostrar la capacidad antagónica de esta microalga con respecto a *Escherichia coli*, donde se logró el 100 % de eliminación.

Palabras clave: biomasa; eliminación máxima; mixotrófico; aguas residuales

INTRODUCTION

At present, there is an exponential increase in swine farms around the world to meet the increasing demand for proteins, resulting in a significant amount of swine/piggery wastewater (Nagarajan *et al*., 2019), which has become a serious environmental concern, due to the high levels of nutrients and toxic contaminants that significantly impact on the ecosystem and public health (Cheng *et al*., 2019).

Swine wastewater is categorized as one of the agricultural wastewaters with high contents of organics and nutrients including nitrogen and phosphorus, which may lead to environmental eutrophication. So too in slaughterhouse process, and wastewaters are considered as a hotspot for antibiotic-resistant bacteria and antimicrobial residues and may thus play an important role for their dissemination into the environment (Banach *et al*., 2018; Li *et al*., 2018; Savin *et al*., 2020). In this sense, insufficient technologies to remove those nutrients could lead to environmental problems after discharge. Several physical and chemical methods have been applied to treat the swine wastewater, the degradation capacity of these conventional treatment technologies is limited, especially regarding heavy metals, nutrients, and xenobiotics, steering the researchers to bioremediation using microalgae (Phycoremediation). In this sense, bioremediation can be defined as the use of microalgae for removal or biotransformation of pollutants and CO_2 from wastewater with concomitant biomass production. Bioremediation with microalgae is particularly effective because of their capabilities of converting solar energy into useful biomasses and assimilate nutrients such as phosphorus and nitrogen which

*Author for correspondence: Yolanda del Carmen Pérez Luna, Yazmin Sánchez Roque
e-mail: yperez@upchiapas.edu.mx; ysanchez@ia.upchiapas.edu.mx

Received: November 13, 2023
Accepted: April 2, 2024
Published: May 2, 2024

cause eutrophication in the photosynthesis process. In tertiary wastewater treatment, microalgae offer an effective low-cost approach to remove contaminants and excess nutrients, and produce potentially valuable biomass, because of its high ability for inorganic nutrient uptake, showing high ability to remove inorganic nutrients from artificial domestic secondary effluents (Wang *et al*., 2016; Feng *et al*., 2020; Lu *et al*., 2020). Thus, biological treatments are considered as the promising methods due to the cost effectiveness and performance efficiency, along with the production of valuable products and bioenergies (Cheng *et al*., 2020; Chen *et al*., 2020 a,b).

The use of a microalgal biomass is a powerful tool to adequately operate wastewater treatment processes, providing valuable information to model wastewater treatment systems (Sánchez-Zurano *et al*., 2020). So too, several types of microalgae such as *Chlorella* and *Dunaliella* have proved their applicability in wastewaters treatment, reporting average removals of NH_4–N (40 – 51 %), PO_4–P (33 – 43 %) and Chemical Oxygen Demand (38 – 63 %) (Wang *et al*., 2014; Cai *et al*., 2017; Li *et al*., 2018; Chen *et al*., 2020). However, it is important to identify other microalgae with potential for bioremediation, which could achieve higher removal values, since each microalgal morphotype responds differently under specific growth conditions, so also with respect to its tolerance and resistance to the growth medium composition (Pei *et al*., 2018).

Due to the foregoing, the present research work aimed to evaluate the microalgal biomass of *Verrucodesmus verrucosus* in bioremediation of wastewater from pig farms, as well as the removal of *Escherichia coli* as a pathogenic microorganism.

MATERIAL AND METHODS

Sampling

The samples were taken from the pig farm called "El Chano" in the municipality of Suchiapa, Chiapas, located at 16°37′05″N 93°05′39″W. One liter of residual water was collected, from the piglet and fattening growth stages, accumulated during the day, considering that cleaning is carried out daily. The residual water was stored in previously sterilized plastic bottles, at 4 °C (NMX-AA-034-SCFI-2015).

Treatment establishment

Nine wastewater treatments were established from the collection of two growth stages: piglet (P) and fattening (F), including a combination of these (50/50); the wastewater was evaluated as it was collected (crude), so too, wastewater was filtered (f) using a 15 µm diameter sieve and sterilized (s) in autoclave at 121°C for 15 min, as established in Table 1.

After having identified the treatments, they were characterized by the quantification of ammonium, nitrate, phosphorus, phosphate and nitrogen, to subsequently inoculate *Verrucodesmus verrucosus* and evaluate for 40 days its behavior, through growth kinetics and its subsequent removal capacity as well as its antagonistic capacity against *Escherichia coli*, as described below and shown in Figure 1.

Table 1. Establishment of treatments from porcine wastewater under the bioremediation of *Verrucodesmus verrucosus*.

Tabla 1. Establecimiento de los tratamientos a partir de aguas residuales porcinas bajo biorremediación con *Verrucodesmus verrucosus*.

Treatments	Growth stage	Levels		
		Filtered waste water	Sterile waste water	Crude waste water
Pf	Piglet	X		
Ps	Piglet		X	
Pc	Piglet			X
Ff	Fattening	X		
Fs	Fattening		X	
Fc	Fattening			X
50/50f	50 Piglet /50 Fattening	X		
50/50s	50 Piglet /50 Fattening		X	
50/50c	50 Piglet /50 Fattening			X

Growth stage [P = Piglet; F= Fattening] Treatments [s = Sterile; c = Crude; f = Filtered] Mix [50/50 = 50 % of piglet and 50 % of fattening]. Each treatment was analyzed the mean of three repetitions.

Physical and chemical analyses of water samples

For the characterization of residual water, the total nitrogen (N) was analyzed by Kjeldahl method (Stafilov *et al*., 2020), potential of hydrogen (pH) with the use of a potentiometer (HANNA HI 2211-01) (NMX-AA-008-SCFI-2011), according to Jin *et al.* (2019). Also, ammonium, nitrate, total phosphorous and phosphate were analyzed by quantitative colorimetric analysis according to Pei *et al*. (2018), Zhu *et al.* (2008), Feng *et al.* (2020) and Wilfert *et al.* (2018), respectively. Finally, the determinations of Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) were performed according to NMX-AA-030/1-SCFI-2012 and NMX-AA-028-SCFI-2000, respectively.

Microorganism and culture conditions

Verrucodesmus verrucosus was provided by the UAMI Applied Fiction Laboratory (Universidad Autónoma Metropolitana Iztapalapa). The cells are elliptical, without appendages and curved edges. *V. verrucosus* can form cenobium obes containing 4, 8, 16, or more cells between 4 and 6 µm in size. The specie was recently described by Hegewald *et al*. (2013) and López-Mendoza *et al*. (2015), with GenBank accession number JQ240289. *Verrucodesmus verrucosus* was evaluated in the nine wastewater treatments in a period of 40 d with an initial inoculation of 25 CFU mL^{-1} at a temperature of 25 °C ± 2.

Escherichia coli quantification

The quantity of the coliform group microorganisms was determined according to the Mexican Standard NMX-AA-42-1987, and according to the total coliform quantification protocols established by Banach *et al*. (2018).

Productivity and growth kinetics

Growth kinetics were performed by cell count in a Neubauer chamber, and plate count by colony forming units (CFU). The cultures were shaken in each of the flasks to allow a uniform

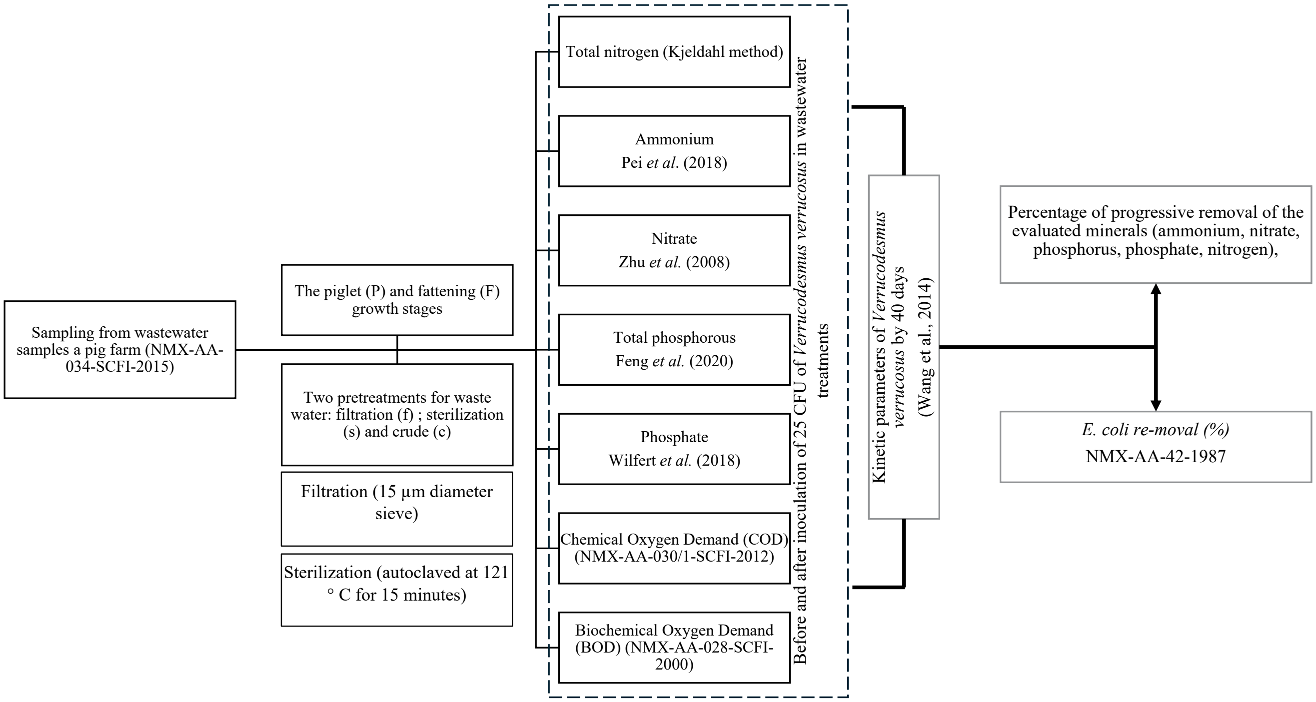


Figure 1. Methodological development for the evaluation of *Verrucodesmus verrucosus* potential in the removal of nitrogen and phosphorus structures from wastewater from pig farms.

Figura 1. Desarrollo metodológico para la evaluación del potencial de *Verrucodesmus verrucosus* en la remoción de estructuras de nitrógeno y fósforo de aguas residuales de granjas porcinas.

distribution of cells, and 1 mL samples were taken and placed in previously washed tubes. The camera was observed in the microscope with 100X objective. The cell concentration (CFU mL^{-1}) was calculated using the following formula, Eq. (1):

$$C = N * 10^4 * \mathrm{dil} \quad (1)$$

Where C is the cell concentration (CFU mL^{-1}), N is the average of cells present in 1mm^2 (0.1µL), dil the dilution factor and 10^4 the conversion factor from 0.1µL to 1mL. From these data the specific growth rate was determined, which was calculated with the following exponential equation (Wang *et al*., 2014), Eq. (2):

$$\mu_e = (\ln N_t - \ln N_0)/(t_2 - t_1) \quad (2)$$

Where, µ is the specific growth rate in days, ln is the natural logarithm, N_0 corresponds to the initial population concentration (CFU mL^{-1}), N_t concentration after the final growth time (CFU mL^{-1}), t_1 initial time for the growth interval of interest in days, and t_2 final time of growth of the interval in days.

Statistical analysis

The parameters of the evaluations were estimated using a sigmoidal model, and Gompertz (Sigma plot ® v.11.0) was used for the correlation evaluation between points. An analysis of variance (ANOVA) was performed in order to evaluate the differences between groups. The means of each treatment were compared using the Tukey method ($p \leq 0.05$). The analyzes were carried out using the statistical software Minitab18®.

RESULTS

Wastewater characterization

The characterization of the residual water from a pig farm was carried out at two stages of growth (piglet and fattening) and the mixture between these (50/50), subjected to two treatments (sterilization and filtering), which is why it is possible to identify the higher concentrations in the fattening crude stage for ammonium, nitrate, phosphorous, phosphate, nitrogen, chemical oxygen demand and biochemical oxygen demand in the values of 1992.38 mg mL^{-1}, 9546.76 mg mL^{-1}, 1410.14 mg mL^{-1}, 2999.58 mg mL^{-1}, 49%, 1629.56 mg mL^{-1} and 896.25 mg mL^{-1}, respectively (Table 2).

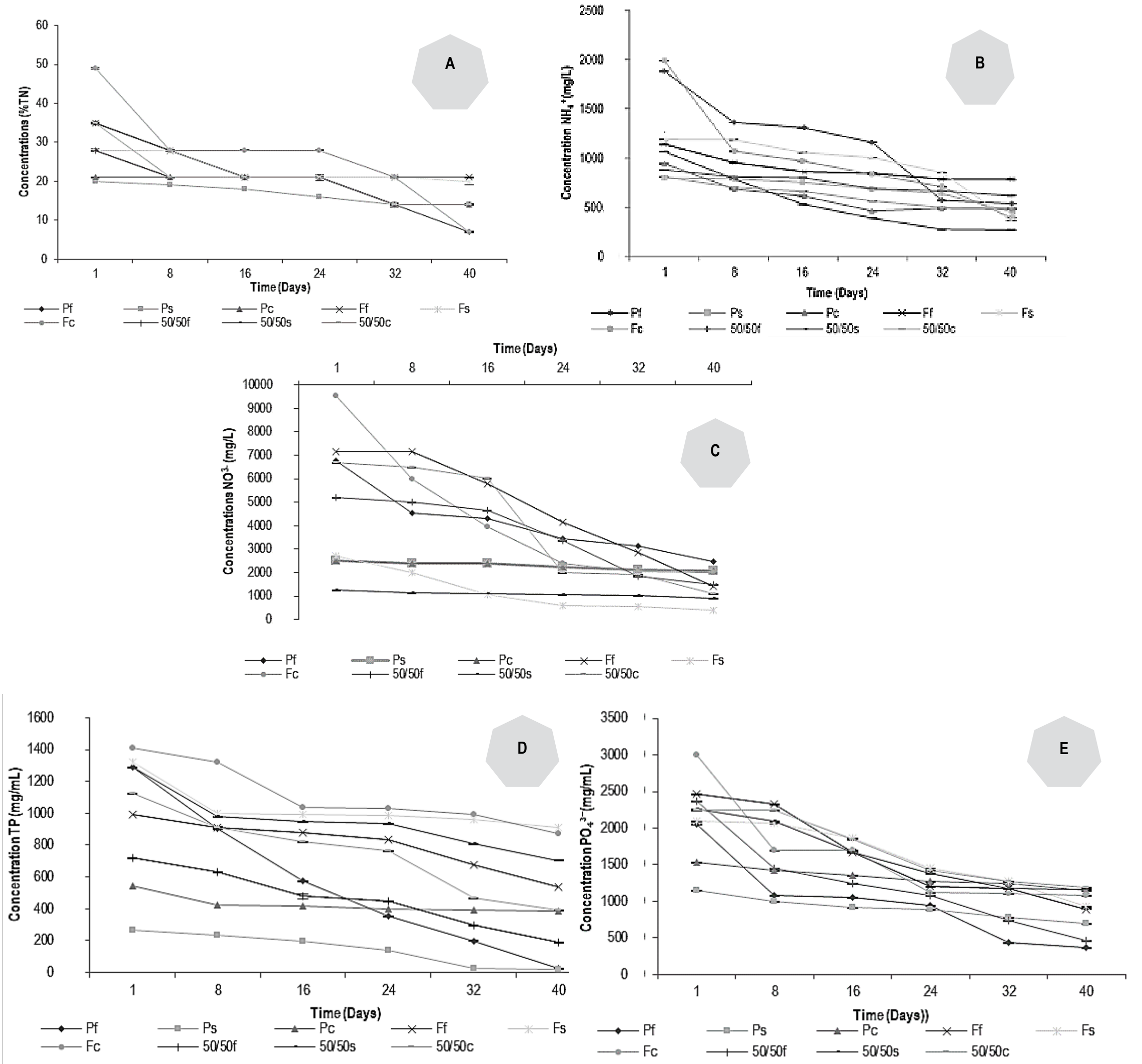
Mineral kinetics

In the kinetics of minerals, the progressive removal of the evaluated minerals is observed (ammonium, nitrate, phosphorous, phosphate, nitrogen), however it is important to mention that *Verrucodesmus verrucosus* demonstrated to start the decrease of minerals in the medium at day eight, reaching levels of highest removal at day 32 of evaluation, so it was determined in this research that the average removal time for these microalgae is 32 to 40 days (Figure 2 a to e).

Table 2. Characterization of wastewater from pig farms taken from two growth stages.
Tabla 2. Caracterización de aguas residuales de granjas porcinas tomadas de dos etapas de crecimiento.

Treatments	Ammonium NH_4^+ (mg L^{-1})	Nitrate NO_3^- (mg L^{-1})	Phosphorus P_4 (mg L^{-1})	Phosphate PO_4^{3-} (mg L^{-1})	Nitrogen N (%)	COD (mg L^{-1})	BOD (mg L^{-1})
Pf	$1884.73 \pm 0.07^{b*}$	6752.24 ± 0.06^{b}	1288.54 ± 0.04^{b}	2057.57 ± 0.00^{d}	35 ± 0.01^{b}	262.68 ± 0.04^{d}	144.47 ± 0.12^{d}
Ps	800.01 ± 0.04^{d}	2504.23 ± 0.01^{d}	263.17 ± 0.00^{e}	1145.95 ± 0.00^{f}	20 ± 0.12^{d}	1335.81 ± 0.14^{b}	734.69 ± 0.00^{b}
Pc	944.61 ± 0.01^{cd}	5509.04 ± 0.04^{c}	545.41 ± 0.00^{d}	1530.99 ± 0.08^{e}	21 ± 0.04^{d}	474.56 ± 0.11^{d}	261.00 ± 0.03^{d}
Ff	1141.09 ± 0.00^{c}	7165.07 ± 0.01^{b}	992.79 ± 0.01^{c}	2471.83 ± 0.11^{b}	35 ± 0.11^{b}	1207.68 ± 0.09^{b}	664.22 ± 0.03^{b}
Fs	1191.62 ± 0.00^{c}	2693.26 ± 0.00^{d}	1323.07 ± 0.05^{a}	2091.52 ± 0.12^{d}	28 ± 0.13^{c}	1670.81 ± 0.11^{a}	918.94 ± 0.00^{a}
Fc	1992.38 ± 0.01^{a}	9546.76 ± 0.07^{a}	1410.14 ± 0.10^{a}	2999.58 ± 0.01^{a}	49 ± 0.03^{a}	1629.56 ± 0.09^{a}	896.25 ± 0.14^{a}
50/50f	881.64 ± 0.06^{d}	5202.56 ± 0.03^{c}	$719.56 + 0.12^{c}$	2369.82 ± 0.04^{bc}	28 ± 0.00^{c}	830.18 ± 0.05^{c}	456.60 ± 0.07^{bc}
50/50s	1068.31 ± 0.05^{c}	1242.38 ± 0.00^{e}	1291.54 ± 0.08^{b}	2253.47 ± 0.06^{c}	28 ± 0.06^{c}	841.43 ± 0.11^{c}	462.79 ± 0.11^{bc}
50/50c	809.66 ± 0.05^{d}	6677.36 ± 0.00^{b}	1126.40 ± 0.00^{b}	2249.25 ± 0.08^{c}	35 ± 0.00^{b}	1645.18 ± 0.00^{a}	904.85 ± 0.05^{a}

* Mean of three repetitions. ** The averages (± standard error) within each column without common superscript differ significantly at $P < 0.05$. Growth stage [P=Piglet; F= Fattening] Treatments [s = Sterile; c = Crude; f = Filtered] Mix [50/50 = 50% of piglet and 50% of fattening]. COD: Chemical Oxygen Demand; BOD: Biochemical Oxygen Demand



Mean of three repetitions. The averages (± standard error). Growth stage [P=Piglet; F= Fattening] Treatments [s = Sterile; = Crude; f = Filtered] Mix [50/50 = 50% of piglet and 50% of fattening]. A: total nitrogen concentration; B: Ammonium concentration; C: Nitrate concentration; D: Total phosphorus concentration; E: Phosphate concentration

Figure 2. Kinetics of minerals removal by *Verrucodesmus verrucosus* present in the wastewater from pig farms evaluated for 40 days.
Figura 2. Cinética de remoción de minerales por *Verrucodesmus verrucosus* presente en aguas residuales de granjas porcinas evaluadas durante 40 días.

On the other hand, the growth stage with the highest removal was the piglet stage, for the minerals phosphorus and phosphate with 97.98 %, 82.18 % respectively, in the filtered and sterilized treatments. However, the fattening stage has the highest removals in nitrate and nitrogen with 85.65 % and 85.71% respectively, in the sterilized treatments for nitrate. So, it is also important to mention that the decrease in nitrogen is significantly higher compared to the other treatments in its crude form (Table 3 and Figure 2).

At the conclusion of the mineral kinetics, it was determined that *V. verrucosus* has, on average, the ability to remove ammonia, nitrate, phosphorus, phosphates and nitrogen at percentages of 54.13 %, 77.51 %, 57.73 %, 54.22 % and 53.06 % respectively, so that this microalgae has an average mineral removal capacity in pork wastewater of 66.24 %, benefiting the removal processes by applying sterilization and filtration treatments (Table 3).

COD and BOD removal kinetics

During the kinetics evaluation of the chemical and biochemical oxygen demand, a significant removal is observed at 32 days after *V. verrucosus* inoculation, thus also identifying that residual water from the lechon growth stage reaches higher removal rates of 92 and 96 % for BOD and COD, respectively (Figure 3 and Table 3).

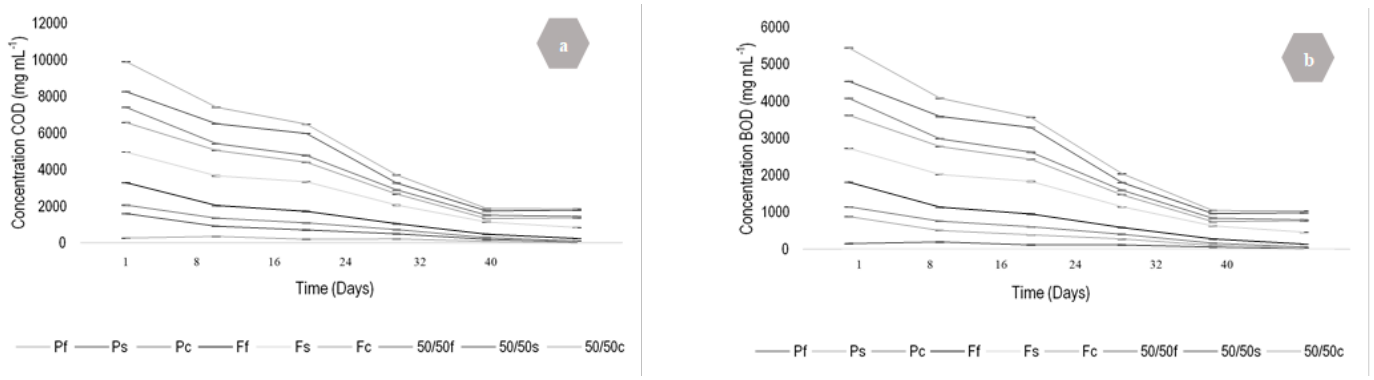
Escherichia coli removal

During the growth kinetics of *V. verrucosus* the start of the exponential stage is observed at 8 days, demonstrating the maximum growth rate of 0.14 CFU / Day in the sterile fattening treatment and the shortest doubling time of 4.31 d, therefore the removal values begin to be important, however the exponential stage ends between 24 and 32 d, data that correlates the higher removal values for the minerals as well as the chemical and biochemical oxygen demands. On the

Table 3. Removal percentage of *Verrucodesmus verrucosus* evaluated in pig wastewater for 40 day.
Table 3. Porcentaje de eliminación de *Verrucodesmus verrucosus* evaluado en aguas residuales porcinas durante 40 días.

	Removal percentage (%)						
Treatments	Ammonium (NH_4^+)	Nitrate (NO_3^-)	Phosphorus (P_4)	Phosphate (PO_4^{3-})	Nitrogen (N)	COD	BOD
Pf	$71.47 \pm 0.02^{a*}$	63.46 ± 0.08^{e}	97.98 ± 0.10^{a}	82.18 ± 0.00^{a}	80 ± 0.01^{b}	92.55 ± 0.14^{a}	96.75 ± 0.12^{a}
Ps	58.68 ± 0.09^{b}	61.28 ± 0.04^{e}	93.55 ± 0.02^{a}	39.66 ± 0.10^{e}	50 ± 0.16^{d}	96.75 ± 0.11^{a}	93.63 ± 0.18^{a}
Pc	48.89 ± 0.07^{c}	69.61 ± 0.01^{d}	28.90 ± 0.00^{f}	24.79 ± 0.16^{f}	33.33 ± 0.08^{f}	93.63 ± 0.19^{a}	92.55 ± 0.05^{a}
Ff	31.15 ± 0.00^{d}	80.07 ± 0.11^{c}	45.97 ± 0.13^{d}	63.80 ± 0.02^{b}	40 ± 0.16^{e}	86.99 ± 0.03^{b}	86.99 ± 0.12^{b}
Fs	69.24 ± 0.00^{a}	85.65 ± 0.11^{b}	31.20 ± 0.07^{d}	55.46 ± 0.00^{c}	28.57 ± 0.01^{g}	65.08 ± 0.17^{c}	65.08 ± 0.10^{c}
Fc	63.91 ± 0.10^{ab}	82.57 ± 0.12^{c}	38.00 ± 0.00^{e}	46.28 ± 0.12^{d}	85.71 ± 0.02^{a}	66.27 ± 0.10^{c}	66.27 ± 0.17^{c}
50/50f	29.84 ± 0.16^{e}	71.65 ± 0.00^{d}	73.44 ± 0.01^{b}	80.51 ± 0.15^{a}	50 ± 0.12^{d}	93.87 ± 0.02^{a}	93.87 ± 0.10^{a}
50/50s	74.60 ± 0.00^{a}	91.92 ± 0.13^{a}	45.44 ± 0.13^{d}	48.26 ± 0.02^{d}	50 ± 0.17^{d}	58.67 ± 0.16^{d}	58.67 ± 0.19^{d}
50/50c	39.45 ± 0.05^{d}	91.44 ± 0.05^{a}	65.17 ± 0.13^{c}	47.09 ± 0.16^{d}	60 ± 0.11^{c}	95.16 ± 0.01^{a}	95.16 ± 0.02^{a}
Mean**	$54.13 \pm 0.05^{c**}$	$77.51 \pm 0.07^{b**}$	$57.73 \pm 0.06^{c**}$	$54.22 \pm 0.08^{c**}$	$53.06 \pm 0.10^{c**}$	$83.24 \pm 0.13^{a**}$	$83.21 \pm 0.11^{a**}$
Total mean	66.24 ± 0.08						

*Mean of three repetitions. ** The averages (± standard error) within each column without common superscript differ significantly at $P < 0.05$. Growth stage [P=Piglet; F= Fattening] Treatments [s = Sterile; c = Crude; f = Filtered] Mix [50/50 = 50% of piglet and 50% of fattening]. COD: Chemical Oxygen Demand; BOD: Biochemical Oxygen Demand



Mean of three repetitions. The averages (± standard error). Growth stage [P=Piglet; F= Fattening] Treatments [s = Sterile; c = Crude; f = Filtered] Mix [50/50 = 50% of piglet and 50% of fattening]. a: Chemical Oxygen Demand concentration; b: Biochemical Oxygen Demand concentration

Figure 3. Kinetics of removal of Chemical and Biochemical Oxygen Demand (COD, BOD) by ***Verrucodesmus verrucosus*** evaluated in pig waste water for 40 days.
Figura 3. Cinética de remoción de la Demanda Química y Bioquímica de oxígeno (DQO, DBO) por ***Verrucodesmus verrucosus*** evaluado en aguas residuales de granjas porcinas por 40 días.

other hand, it is observed how kinetics of *Escherichia coli* decreases significantly after 8 days of inoculating *V. verrucosus,* decreasing the bacterial population in its entirety at 32 d. Therefore, *V. verrucosus* demonstrated 100 % removal of the *E.coli* bacterial population within 24 d (Figure 4 and Table 4).

DISCUSSION

Wastewater characterization

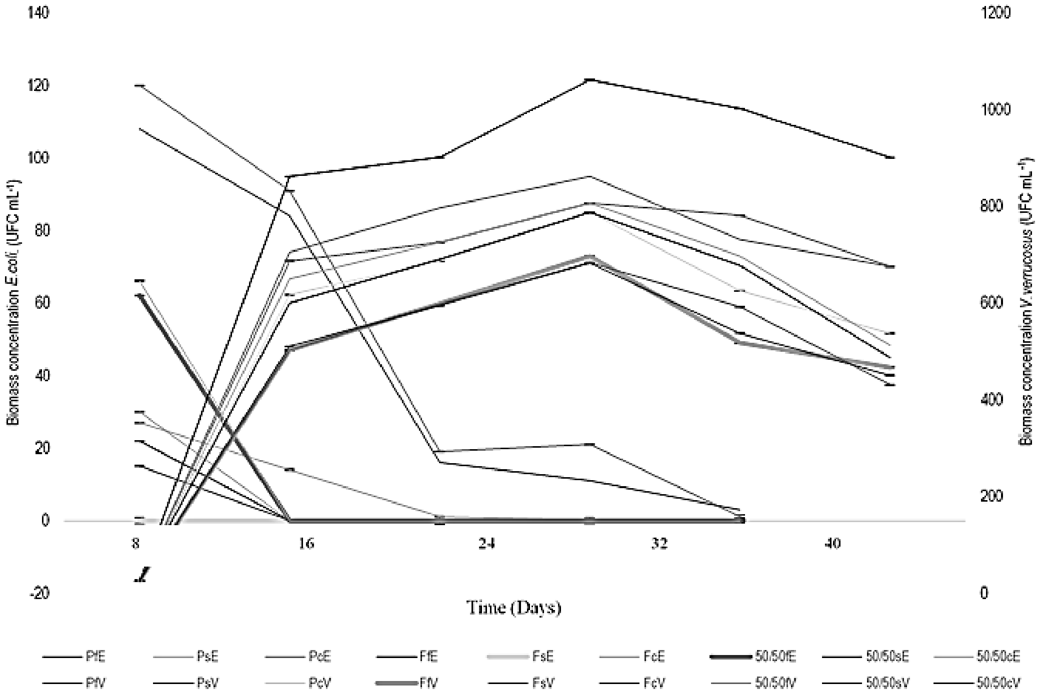
Swine wastewater is rich in nitrogen and organic carbon, however, in the present research it was shown that the wastewater from fattening pigs, corresponding to an adult age, showed the highest concentrations in the different nitrogen

Table 4. Kinetic parameters and antagonistic potential of the *Verrucodesmus verrucosus* microalgae biomass evaluated in pig wastewater for 40 days.

Tabla 4. Parámetros cinéticos y potencial antagónico de la biomasa microalgal de *Verrucodesmus verrucosus* evaluada en aguas residuales porcinas durante 40 días.

Treatment	**Initial biomass (CFU mL^{-1})**		**Final biomass (CFU mL^{-1})**		**µ (CFU Day^{-1})**	**Td (Day)**	***E. coli* removal (%)**
	V. ***v****errucosus*	*E. coli*	*V. verrucosus*	*E.coli*			
Pf	25 ± 0.16^a	108 ± 0.22^b	781 ± 0.21^b	3 ± 0.09^a	0.14 ± 0.06^d	4.63 ± 0.05^d	97.22 ± 0.01^a
Ps	25 ± 0.01^a	27 ± 0.15^d	1002 ± 0.18^a	0 ± 0.01^c	0.16 ± 0.03^a	4.31 ± 0.01^e	100 ± 0.018^a
Pc	25 ± 0.09^a	120 ± 0.18^a	625 ± 0.11^d	1 ± 0.21^b	0.13 ± 0.01^f	4.95 ± 0.09^b	99.16 ± 0.07^a
Ff	25 ± 0.02^a	22 ± 0.27^d	517 ± 0.07^f	0 ± 0.02^c	0.13 ± 0.10^e	5.26 ± 0.12^a	100 ± 0.03^a
Fs	25 ± 0.12^a	0 ± 0.01^f	592 ± 0.0^e	0 ± 0.00^c	0.13 ± 0.11^e	5.03 ± 0.08^b	100 ± 0.09^a
Fc	25 ± 0.2^a	30 ± 0.11^d	537 ± 0.12^e	0 ± 0.00^c	0.13 ± 0.03^f	5.19 ± 0.01^a	100 ± 0.05^a
50/50f	25 ± 0.18^a	62 ± 0.31^c	697 ± 0.09^c	0 ± 0.00^c	0.14 ± 0.11^b	4.79 ± 0.12^c	100 ± 0.12^a
50/50s	25 ± 0.11^a	15 ± 0.17^e	731 ± 0.12^b	0 ± 0.00^c	0.14 ± 0.12^b	4.72 ± 0.01^c	100 ± 0.12^a
50/50c	25 ± 0.12^a	66 ± 0.14^c	677 ± 0.06^c	0 ± 0.10^c	0.14 ± 0.09^c	4.83 ± 0.19^b	100 ± 0.12^a

* Mean of three repetitions. ** The averages (± standard error) within each column without common superscript differ significantly at $P < 0.05$. Growth stage [P=Piglet; F= Fattening] Treatments [s = Sterile; c = Crude; f = Filtered] Mix [50/50 = 50% of piglet and 50% of fattening].



Mean of three repetitions. The averages (± standard error). Growth stage [P=Piglet; F= Fattening] Treatments [s = Sterile; c = Crude; f = Filtered] Mix [50/50 = 50% of piglet and 50% of fattening].

Figure 4. Growth kinetics and antagonistic potential of *Verrucodesmus verrucosus* microalgal biomass evaluated in pig wastewater for 40 days.

Figura 4. Cinética de crecimiento y potencial antagónico de la biomasa microalgal de *Verrucodesmus verrucosus* evaluada en aguas residuales de granjas porcinas por 40 días.

and phosphorus forms evaluated (Table 2), *data similar to that reported by Wen et al.* (2017), they evaluated the resistance condition of *Chlorella vulgaris* in undiluted swine slurry and artificial wastewater, identifying concentrations higher than 2000 mg / L of the different forms of TN and TP.

This is because fattening pigs consume a more complete diet accompanied by chemical compounds that modulate feed conversion, associated with digestibility coefficient problems, such as bad absorption or bacterial infections that force the use of antibiotics, generating feces loaded with organic and inorganic matter that convert wastewater into a complex mixture. It is also important to mention that the nitrogen and phosphorous retention capacity decreases with the age of the animal (Wang *et al*., 2017; Likiliki *et al*., 2020).

Mineral kinetics

During the mineral removal kinetics, it was determined that *V. verrucosus* has the ability to decrease the concentration of phosphorus and phosphate present in wastewater of piglets, from 82 to 97.98 % of removal respectively. Thus, removal concentrations of 85.71% were demonstrated for nitrogen evaluated in this research work, it is also important to mention that the highest total nitrogen removals are found in crude treatments (Table 3). Similar data has been reported by Garcia *et al*. (2018), who mentioned that results revealed a high diversity and rapid variations in the structure of microalgae populations, *Chlorella sp*., were recorded in the removal efficiencies (REs) of total organic carbon (86 – 87 %), inorganic carbon (62 – 71 %), total nitrogen (82 – 85 %) and total phosphorous (90 – 92 %).

According to the aforementioned, Sudiarto *et al*. (2019) studied the removal of nitrogen and phosphorus from the effluent of treated swine wastewater by *Eichhornia crassipes*, *Pistia stratiotes*, *Limnobium laevigatum*, and *Lemna sp*. *Pistia stratiotes* showed the highest total nitrogen removal (63.15 %) from the treated effluent, *Lemna sp.* showed the highest phosphorus removal of 36.15 % from the treated effluent. Caí *et al.* (2019) also showed that *Spirulina platensis* removal efficiency of ammonium was 99 %, which provides an alternative method for the utilization of Digested Piggery Wastewater (DPW).

However, Chen *et al.* (2020) evaluated *Desmodesmus sp*., a microalgae belonging to the same family of *V. verrucosus*, showing that *Desmodesmus sp.* PW1grew well in diluted and undiluted piggery wastewater, and could effectively remove nitrogen and phosphorus with removal rates up to 90 % and 70 %, respectively. At laboratory scale by 30L photobioreactor, microalgae also performed well in TN (65.3 %) and TP (83.5 %) removal.

It is important to mention that the treatment of fattening without sterilization showed the highest removal values (Table 3), so it is not necessary to implement a treatment to improve the removal capacity of *V. verrucosos*, data that agrees with the work carried out by Wang *et al*. (2016) who evaluated an UV irradiation-based method to treat the piggery wastewater before inoculating the microalgae biomass, in order to reduce microbial competition and benefit the degradation of compounds. However, the microalgae grew well in slurry without treating and achieved outstanding removal efficiencies in total nitrogen (TN) and total phosphorus (TP), with 89.5 % and 85.3 %, respectively (Rasoul-Amini *et al*., 2014).

The assimilation of nutrients is variable and depends on the level of daily consumption or intake, which are affected by factors such as genetics and mainly age or growth phase.

It has been verified that in piglets from fetal life, the gastrointestinal tract is in charge of supplying in its entirety of metabolites and protective substances through nutrients absorption, the endocytosis of immunoglobulins from colostrum and milk, an activity that guarantees the high absorption and retention of nutritional substances. Another reason is associated with the lumen surface of the small intestine, which is made up of numerous villi at the base where are tubular glands (*Lieberkühn crypts*) that descend to the muscularis mucosa benefiting their high absorption, unlike fattening pigs that with advancing age, a certain percentage of intestinal mucosa velocities atrophy, reducing the efficiency of nutrient absorption, associated with competition for the substrate and problems caused by the presence of *Enterobacteriaceae* (Bibbal *et al*., 2018).

With all of the above, it was possible to demonstrate the potential of *V. verrucosus* to remove minerals in mixotrophic conditions, since the swine wastewater is rich in nitrogen and organic carbon which are essential macronutrients for microalgal growth (Chen *et al*., 2020; Sánchez *et al*., 2020).

The ability of microalgae to feed on minerals present in pigs' wastewater has benefited the accumulation of compounds of biotechnological interest, as mentioned by Li *et al.* (2018), who demonstrated that *Coelastrella sp.* could remove nutrients from anaerobically digested swine wastewater (ADSW) effectively, if its responses to the stress of Cu (II) were less. However, they showed that finding high copper concentrations in the medium increases the concentration of the superoxide dismutase enzyme responsible for pigment synthesis and oxidative stability of lipids synthesized by the microalgae.

COD and BOD removal kinetics

In this research an 83 % average removal of chemical and biochemical oxygen demand by *V. verrucosus* was demonstrated (Table 3). These data agree with those obtained by Chen *et al.* (2020), who evaluated the remove capacity from *Chlorella sorokiniana* in swine wastewater, and demonstrated the COD, TN and TP removal efficiency for the swine wastewater was 90.1, 97.0 and 92.8 %, respectively (Zhou *et al*., 2018).

These results tend to be promising when considering the *V. verrucosus* microalgal biomass, as a potential alternative to remove, since removal efficiencies of 50 % have been demonstrated by facultative aerobic bacteria, which are not very effective with respect to microalgae. As demonstrated by Chen *et al.* (2020), who evaluated the COD removal efficiency (55.46 %) in anaerobic digestion treatment of real piggery wastewater: Treatment efficiency and bacterial diversity.

Escherichia coli **removal**

Through the evaluation of the microalgal biomass growth kinetics, it was observed that the exponential stage of *V. verrocosus* is coupled to the stationary and death stage of the growth kinetics of *Escherichia coli* (Figure 4). Said aforementioned behavior was observed in other investigations, as mentioned by Wen *et al.* (2017), they identified the stress tolerance of *Chlorella vulgaris* in wastewater, which was verified in artificial wastewater containing different levels of chemical oxygen demand (COD), corresponding to a high bacterial load.

The microalgae *V. verrucosus* demonstrated antagonistic capacity by removing 100 % of *E. coli* (Figure 4 and Table 4), an enterobacterium present at a high concentration in feces deposited in wastewater, as shown in the present work, since slaughterhouse process and wastewater are considered as a hotspot for antibiotic resistant bacteria and antimicrobial residues, which may thus play an important role for their dissemination into the environment (Savin *et al*., 2020).

This phenomenon has been observed previously, for instance, Li *et al.* (2020) identified the effect of antibiotic residue pollution from swine feedlots to nearby groundwater environment, analyzing the presence of residues from most commonly used antibiotics, including tetracyclines (TCs), fluoroquinolones (FQNs), sulfonamides (SAs), macrolides, and fenicols, therefore, antibiotics discharged from swine feedlots through wastewater could disseminate into surrounding groundwater environments, generating antibiotic resistance genes (AGRs) in different microorganisms.

Also, Yang *et al.* (2020) demonstrated that in the wastewater of pig farms was a higher abundance of Proteobacteria, including the potential human pathogenic bacteria (HPB) (*Escherichia*, *Shigella*, *Bordetella* and *Morganella*), crop pathogen (Pectobacterium) and denitrifying bacteria (Zobellella); the results showed that the bacteria played an important role in the denitrification, which can provide a new point of penetration for improving the bioremove at pig farms, because eradicating the nitrogen present in the wastewater would eliminate a large population of pathogenic bacteria. For this reason, since *V. verrucosus* is efficient in removing nitrogen forms and is also metabolically more active, it is a disadvantage for pathogenic bacteria that try to compete for the substrate (Wu *et al.*, 2020).

Thus, it is also important to mention that the highest biomass production was reached in wastewater from the fattening stage, identifying a direct relationship between biomass and the concentration of pollutants (Table 4, Figure 4). This effect was identified in an evaluation on *C. pyrenoidosa,* where, the results showed the maximum nutrient removal efficiencies at 50 % in the fattening pig wastewater, since the fattening pig wastewater is rich in nutrients, which can be utilized for algal biomass production (Azam *et al*., 2020).

Finally, it is important to mention that according to the aforementioned results, the residual water from pig farms previously treated with *V. verrucosus* can be used for the irrigation of fruit and vegetable crops.

CONCLUSIONS

The use of *V. verrucosus* is a powerful tool to adequately manage and operate wastewater treatment processes, providing valuable information to model wastewater treatment systems with these microalgae, on the other hand, it was shown that this microalgae species grows well in swine wastewater with large amounts of biomass being produced, despite the impact of various parameters (e.g., nutrients and toxicants levels, cultivation conditions, and bacteria in swine wastewater).

It is important to mention that the harvested microalgae biomass elicits high potential for conversion to bioenergy, because the lipids can be used for biodiesel production, and according to this, the development of renewable and clean energy as well as bio-based fine chemicals technologies are the keys to overcome the problems such as fossil depletion, global warming, and environment pollution.

The strategy is therefore a promising method for microalgae to purify piggery slurry containing high nutrient contents, reducing the environmental impact of contaminated waters that damage soils and consumers of food irrigated with these waters.

ACKNOWLEDGMENTS

This study was supported by the Universidad Politécnica de Chiapas.

CONFLICTS OF INTEREST

The authors have no financial conflicts of interest to declare.

REFERENCES

Azam, R., Kothari, R., Singh, H. M., Ahmad, S., Ashokkumar, V., and Tyagi, V. V. 2020. Production of algal biomass for its biochemical profile using slaughterhouse wastewater for treatment under axenic conditions. *Bioresource Technology*, 306, 123116. doi: https://doi.org/10.1016/j.biortech.2020.123116

Bibbal, D., Um, M. M., Diallo, A. A., Kérourédan, M., Dupouy, V., Toutain, P. L., ... and Brugère, H. 2018. Mixing of Shiga toxin-producing and enteropathogenic *Escherichia coli* in a wastewater treatment plant receiving city and slaughterhouse wastewater. *International Journal of Hygiene and Environmental Health*, 221(2), 355-363. doi: https://doi.org/10.1016/j.ijheh.2017.12.009

Banach, J. L., van Overbeek, L. S., Groot, M. N., Van der Zouwen, P. S., and Van der Fels-Klerx, H. J. 2018. Efficacy of chlorine dioxide on *Escherichia coli* inactivation during pilot-scale fresh-cut lettuce processing. *International Journal of Food Microbiology*, 269, 128-136. doi: https://doi.org/10.1016/j.ijfoodmicro.2018.01.013

Cai, X. B., Yu, Q. Q., Liu, R., Zhao, Y., and Chen, L. J. 2017. Cultivation of *Spirulina platensis* in digested piggery wastewater pretreated by SBR with *Operating Conditions Optimization*. Huan Jing ke Xue= Huanjing Kexue, 38(7), 2910-2916. doi: https://doi.org/10.13227/j.hjkx.201612168

Chen, C. Y., Kuo, E. W., Nagarajan, D., Ho, S. H., Dong, C. D., Lee, D. J., and Chang, J. S. 2020. Cultivating *Chlorella sorokiniana* AK-1 with swine wastewater for simultaneous wastewater treatment and algal biomass production. *Bioresource technology*, 302, 122814. doi: https://doi.org/10.1016/j.biortech.2020.122814

Chen, J., Yang, Y., Liu, Y., Tang, M., Wang, R., Hu, H., ... and Zhang, X. 2020. Effects caused by chlortetracycline and oxytetracycline in anaerobic digestion treatment of real piggery wastewater: treatment efficiency and bacterial diversity. *International Journal of Hydrogen Energy*, 45(15), 9222-9230. doi: https://doi.org/10.1016/j.ijhydene.2020.01.138

Cheng, D. L., Ngo, H. H., Guo, W. S., Chang, S. W., Nguyen, D. D., and Kumar, S. M. 2019. Microalgae biomass from swine wastewater and its conversion to bioenergy. *Bioresource technology*, 275, 109-122. doi: https://doi.org/10.1016/j.biortech.2018.12.019

Cheng, H. H., Narindri, B., Chu, H., and Whang, L. M. 2020. Recent advancement on biological technologies and strategies for resource recovery from swine wastewater. *Bioresource technology*, 303, 122861. doi: https://doi.org/10.1016/j.biortech.2020.122861

Chen, Z., Shao, S., He, Y., Luo, Q., Zheng, M., Zheng, M., ... and Wang, M. 2020. Nutrients removal from piggery wastewater coupled to lipid production by a newly isolated self-flocculating microalga *Desmodesmus* sp. PW1. *Bioresource technology*, 302, 122806. doi: https://doi.org/10.1016/j.biortech.2020.122806

Feng, C., Welles, L., Zhang, X., Pronk, M., de Graaff, D., and van Loosdrecht, M. 2020. Stress-induced assays for polyphosphate quantification by uncoupling acetic acid uptake and anaerobic phosphorus release. *Water research*, 169, 115228. doi: https://doi.org/10.1016/j.watres.2019.115228

García, D., Posadas, E., Blanco, S., Acién, G., García-Encina, P., Bolado, S., and Muñoz, R. 2018. Evaluation of the dynamics of microalgae population structure and process performance during piggery wastewater treatment in algal-bacterial photobioreactors. *Bioresource Technology*, 248, 120-126. doi: https://doi.org/10.1016/j.biortech.2017.06.079

Hegewald, E., Bock, C., and Krienitz, L. 2013. A phylogenetic study on Scenedesmaceae with the description of a new species of *Pectinodesmus* and the new genera *Verrucodesmus* and *Chodatodesmus* (Chlorophyta, Chlorophyceae). Fottea, 13(2), 149-164.

Jin, Y., Lin, Y., Wang, P., Jin, R., Gao, M., Wang, Q., ... and Ma, H. 2019. Volatile fatty acids production from saccharification residue from food waste ethanol fermentation: effect of pH and microbial community. *Bioresource technology*, 292, 121957. doi: https://doi.org/10.1016/j.biortech.2019.121957

Li, X., Yang, W. L., He, H., Wu, S., Zhou, Q., Yang, C., ... and Lou, W. 2018. Responses of microalgae *Coelastrella* sp. to stress of cupric ions in treatment of anaerobically digested swine wastewater. *Bioresource technology*, 251, 274-279. doi: https://doi.org/10.1016/j.biortech.2017.12.058

López-Mendoza, Z., Tavera, R., and Novelo, E. 2015. El fitoplancton de un canal de Xochimilco y la importancia de estudiar ecosistemas acuáticos urbanos. TIP Revista Especializada en Ciencias Químico-Biológicas 18(1): 13-28.

Lu, W., Alam, M. A., Liu, S., Xu, J., and Saldivar, R. P. 2020. Critical processes and variables in microalgae biomass production coupled with bioremediation of nutrients and CO_2 from livestock farms: A review. Science of the Total Environment, 716, 135247. doi: https://doi.org/10.1016/j.scitotenv.2019.135247

Li, X., Liu, C., Chen, Y., Huang, H., and Ren, T. 2018. Antibiotic residues in liquid manure from swine feedlot and their effects on nearby groundwater in regions of North China. *Environmental Science and Pollution Research*, 25, 11565-11575. doi: https://doi.org/10.1007/s11356-018-1339-1

Likiliki, C., Convers, B., and Béline, F. 2020. Dataset on the characteristics of the liquid effluent issued from separation of faeces and urine under slats using V-shaped scraper in swine buildings. *Data in Brief*, 30, 105533. doi: https://doi.org/10.1016/j.dib.2020.105533

Nagarajan, D., Kusmayadi, A., Yen, H. W., Dong, C. D., Lee, D. J., and Chang, J. S. 2019. Current advances in biological swine wastewater treatment using microalgae-based processes. *Bioresource technology*, 289, 121718. doi: https://doi.org/10.1016/j.biortech.2019.121718

NMX-AA-034-SCFI-2015. Water analysis – measurement of salts and solids dissolved in natural water, wastewaters and treated wastewaters - Test method.

NMX-AA-008-SCFI-2011. Water Analysis - pH Determination - Test Method - (Cancels NMX-AA-008- SCFI-2000).

NMX-AA-030/1-SCFI-2012. Water analysis - Determination of the chemical oxygen demand, in natural waters, wastewaters and treated wastewaters - test method - part 1 – Opened reflux method.

NMX-AA-028-SCFI-2000. Water analysis - Determination of the biochemical oxygen demand in natural, wastewaters (bod5) and wastewaters treated - Test method.

NMX-AA-42-1987. Water quality determination of the most likely number (MPN) of total coliforms, fecal coliforms (thermotolerants) and presumptive *Escherichia coli*.

Pei, H., Yang, Z., Nie, C., Hou, Q., Zhang, L., Wang, Y., and Zhang, S. 2018. Using a tubular photosynthetic microbial fuel cell to treat anaerobically digested effluent from kitchen waste: Mechanisms of organics and ammonium removal. *Bioresource technology*, 256, 11-16. doi: https://doi.org/10.1016/j.biortech.2018.01.144

Rasoul-Amini, S., Montazeri-Najafabady, N., Shaker, S., Safari, A., Kazemi, A., Mousavi, P., ... and Ghasemi, Y. 2014. Removal of nitrogen and phosphorus from wastewater using microalgae free cells in bath culture system. *Biocatalysis and Agricultural Biotechnology*, 3(2), 126-131. doi: https://doi.org/10.1016/j.bcab.2013.09.003

Sánchez-Roque, Y., Luna, Y. P., Acosta, J. M., Vázquez, N. F., Sebastian, J. P., and Hernández, R. B. 2020. Optimization for the production of *Verrucodesmus verrucosus* biomass through crops in autotrophic and mixotrophic conditions with potential for the production of biodiesel. *Revista Mexicana de Ingeniería Química*, 19(1), 133-147. doi: https://doi.org/10.24275/rmiq/Bio463

Sánchez-Zurano, A., Gomez-Serrano, C., Acién-Fernández, F. G., Fernández-Sevilla, J. M., and Molina-Grima, E. 2020. A novel photo-respirometry method to characterize consortia in microalgae-related wastewater treatment processes. *Algal Research*, 47, 101858. doi: https://doi.org/10.1016/j.algal.2020.101858

Savin, M., Bierbaum, G., Hammerl, J. A., Heinemann, C., Parcina, M., Sib, E., ... and Kreyenschmidt, J. 2020. Antibiotic-resistant bacteria and antimicrobial residues in wastewater and process water from German pig slaughterhouses and their receiving municipal wastewater treatment plants. *Science of The Total Environment*, 727, 138788. doi: https://doi.org/10.1016/j.scitotenv.2020.138788

Stafilov, T., Špirić, Z., Glad, M., Barandovski, L., Bačeva Andonovska, K., Šajn, R., and Antonić, O. 2020. Study of nitrogen pollution in the Republic of North Macedonia by moss biomonitoring and Kjeldahl method. *Journal of Environmental Science and Health*, Part A, 55(6), 759-764. doi: https://doi.org/10.1080/10934529.2020.1738825

Sudiarto, S. I. A., Renggaman, A., and Choi, H. L. 2019. Floating aquatic plants for total nitrogen and phosphorus removal from treated swine wastewater and their biomass characteristics. *Journal of environmental management*, 231, 763-769. doi: https://doi.org/10.1016/j.jenvman.2018.10.070

Wang, M., Yang, Y., Chen, Z., Chen, Y., Wen, Y., and Chen, B. 2016. Removal of nutrients from undiluted anaerobically treated piggery wastewater by improved microalgae. *Bioresource Technology*, 222, 130-138. doi: https://doi.org/10.1016/j.biortech.2016.09.128

Wang, M., Kuo-Dahab, W. C., Dolan, S., and Park, C. 2014. Kinetics of nutrient removal and expression of extracellular polymeric substances of the microalgae, *Chlorella* sp. and *Micractinium* sp., in wastewater treatment. *Bioresource technology*, 154, 131-137. doi: https://doi.org/10.1016/j.biortech.2013.12.047

Jin, W. A. N. G., Ye, H. A. N., ZHAO, J. Z., ZHOU, Z. J., and Huan, F. A. N. 2017. Consuming fermented distillers' dried grains with solubles (DDGS) feed reveals a shift in the faecal microbiota of growing and fattening pigs using 454 pyrosequencing. *Journal of integrative agriculture*, 16(4), 900-910. doi: https://doi.org/10.1016/S2095-3119(16)61523-X

Wilfert, P., Dugulan, A. I., Goubitz, K., Korving, L., Witkamp, G. J., and Van Loosdrecht, M. C. M. 2018. Vivianite as the main phosphate mineral in digested sewage sludge and its role for phosphate recovery. *Water research*, 144, 312-321. doi: https://doi.org/10.1016/j.watres.2018.07.020

Wen, Y., He, Y., Ji, X., Li, S., Chen, L., Zhou, Y., ... and Chen, B. 2017. Isolation of an indigenous *Chlorella vulgaris* from swine wastewater and characterization of its nutrient removal ability in undiluted sewage. *Bioresource technology*, 243, 247-253. doi: https://doi.org/10.1016/j.biortech.2017.06.094

Wu, W., Cheng, L. C., and Chang, J. S. 2020. Environmental life cycle comparisons of pig farming integrated with anaerobic digestion and algae-based wastewater treatment. *Journal of environmental management*, 264, 110512. doi: https://doi.org/10.1016/j.jenvman.2020.110512

Yang, Y., Xing, S., Li, S., Niu, Y., Li, C., Huang, T., and Liao, X. 2020. Potential regulation of small RNAs on bacterial function activities in pig farm wastewater treatment plants. *Journal of Environmental Sciences*, 91, 292-300. doi: https://doi.org/10.1016/j.jes.2020.02.014

Zhou, W., Wang, Z., Xu, J., and Ma, L. 2018. Cultivation of microalgae *Chlorella zofingiensis* on municipal wastewater and biogas slurry towards bioenergy. *Journal of bioscience and bioengineering*, 126(5), 644-648. doi: https://doi.org/10.1016/j.jbiosc.2018.05.006

Zhu, G., Peng, Y., Li, B., Guo, J., Yang, Q., and Wang, S. 2008. Biological removal of nitrogen from wastewater. Reviews of environmental contamination and toxicology, 159-195. doi:https://doi.org/10.1007/978-0-387-71724-1_5