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Anaerobic digestion effluents for corn and sunflower production in Mexico

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Abstract
Anaerobic digestion is an alternative for wastewater treatment and its effluent could be used in agricultural production because it may contain nutrients; however, its harmlessness is a limiting factor. This research evaluated the potential of a self-cleaning biodigester (SCB) effluent to increase corn and sunflower production of a school plot. The SCB was located at the Telebachillerato Comunitario in San Felipe del Progreso, State of Mexico, Mexico. A randomized block design was used with three fertilization levels of N, P and K, irrigated with SCB effluent and potable water and 3 replications. Physical, chemical and biological parameters (total and fecal coliforms, Salmonella and Escherichia coli) were evaluated in the SCB effluent before being used as fertilizer on the corn and sunflower crops. Finally, the grain yield and the harvested seeds' safety were determined. In the SCB effluent, high K and Ca, high salinity, moderate total coliforms, fecal coliforms and E. coli were observed; Salmonella was not detected. In corn and sunflower seed, 98% of total coliforms were reduced, with no presence of fecal coliforms and E. coli compared to the SCB effluent. The grain yield of sunflower irrigated with SCB effluent increased up to 72% compared to that irrigated with potable water and in corn no significant differences were found between SCB effluent and potable water irrigation.

Keywords
Anaerobic digestion, biofertilizer, treated wastewater, innocuousness

Introduction

Accelerated population growth has increased wastewater (WW) production (Wijaya and Soedjono, 2018). The final disposal of untreated WW is a problem that causes pollution in the environment; untreated WW puts biodiversity and human health at risk due to its content of viruses, pathogens, or heavy metals (Gerardi and Zimmerman 2005; Chen et al., 2019; McCall et al., 2020). In some sites, treated and untreated WW is used as a source for crop irrigation and fertilization. One of the alternatives proposed to solve this problem is anaerobic digestion, which is a process that converts organic waste into energy and fertilizer in the absence of oxygen (Manyi-Loh et al., 2013). Self-cleaning biodigesters (SCB) use anaerobic digestion as a small-scale treatment alternative. The SCB are efficient and environmentally friendly, their installation is practical and economical, whereas its effluent (treated WW) can be used as biofertilizer (Fuentes and Viscaino, 2018).

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Studies on the use of SCB effluents in agriculture mention that they increase agricultural production because they still retain valuable nutrients for crop production, such as potassium (K), nitrogen (N) and phosphorus (P) (Wang et al., 2022). Therefore, during the use of SCB effluent in corn cultivation, grain and foliar yields have increased (Montiel et al., 1996; Oron et al., 1999; Tsadilas and Vakalis 2003). During sunflower cultivation, grain yield, plant height, leaf length and number of leaves have increased after using effluent SCB as fertilizer, although their microbial composition is not known (Chatzakis et al., 2011; Safi-Naz and Shaaban, 2015). The use of SCB effluent in crops promotes an economic benefit by reducing fertilization expenditure for producers (Tsadilas and Vakalis 2003; Tavassoli et al., 2010; Belabhir et al., 2021; Khaskhoussy et al., 2022).

Making WW treatment systems available to the population that reduce biological risks and contribute to the agricultural sector is therefore an alternative that should be evaluated in more detail. This is why in the present study the effect of SCB effluent on corn and sunflower crops was evaluated in terms of safety and production.

Materials and methods

Study area

The present study was conducted at the Telebachillerato Comunitario (TBC) number 404 of Ejido de San Juan Jalpa, municipality of San Felipe del Progreso, Mexico, where a school crop was located. The school crop was irrigated with the effluent of a SCB located at the TBC number 164 of San Juan Cote, municipality of San Felipe del Progreso, Mexico. TBC-164 is located at coordinates 99°58'16.943"W y 19°36'28.756"N. TBC-404 is located at coordinates 99°54'37.171"W y 19°41'31.539"N, with an altitude of 2541 m, with a temperate sub-humid climate: C (w1) (w) b (i') g and with a flat soil type.

Physical, chemical and biological character-rization of the effluent

In the Fertilab laboratory, in April 2023, a 2-L sample of SBC effluent from TBC-164 was evaluated. pH was determined according to the potentiometer method (Bila et al., 2021); electrical conductivity (EC) was determined by the conductimetry method (Bersinger et al., 2013); calcium (Ca), sodium (Na), magnesium (Mg), potassium (K), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), boron (B) and sulfur (S) were determined by the acidification method with HNO_3 and inductively coupled plasma optical emission spectrometry (ICP-OES) (Rocha et al., 2022); sulfates ($S\text{-}SO_4^{2-}$) were determined by the turbidimeter method with UV-Visible spectrophotometry (Thomas et al., 2017): phosphates (PO_4^{3-}) and nitrates ($N\text{-}NO_3^-$) were determined by the UV-Visible spectrophotometry method (Thomas, O. and Burgess, 2017); carbonates (CO_3^{2-}) and bicarbonates (HCO_3^-) were determined by the acid-base titration method (Sun et al., 2016) and chlorides (Cl) with the ion-specific electrode method (Bratovcic and Odobasic, 2011) with the EPA Method 6010.

Chemical Oxygen Demand (COD) was measured in October 2023 in a 25 mL effluent sample, using the method established in NMX-AA-030/1-SCFI-2012. Total suspended solids (TSS) and volatile suspended solids (VSS) were evaluated in 3 samples of 10 mL of SCB effluent in aluminum trays, with the method established in NMX-AA-034-SCFI-2015. These evaluations were performed in the laboratory of the Inter-American Institute of Technology and Water Sciences (IITCA) of the Autonomous University of the State of Mexico (UAEMex).

Quantification of total coliforms, fecal coliforms, E. coli and Salmonella was performed. For this purpose, 1-L samples were collected in April 2023, by NOM-210-SSA1-2014. The samples were analyzed at the Fertilab laboratory.

Experiment with the use of effluents in corn and sunflowers

To evaluate the effect of SCB effluent on crops, a randomized split-plot experimental design with three repetitions was used in the field. The large plot was irrigated with potable water and SCB effluent (Fig. 1). In the small plot, three mineral fertilization treatments were tested: N, P and K, with high (120-80-40), medium (60-40-20) and low (30-20-10) doses (Fig. 1). The experimental units had seven, six and two rows. Each experimental unit row was 2.6 m long and 2 m of the two center rows in the TCB were considered as useful plots (Fig. 1).

Experiment management

The management of the experimental plot consisted of harrowing with a tractor and planting with a horse yoke in June 2023 at a planting density of 60,000 plants per hectare.

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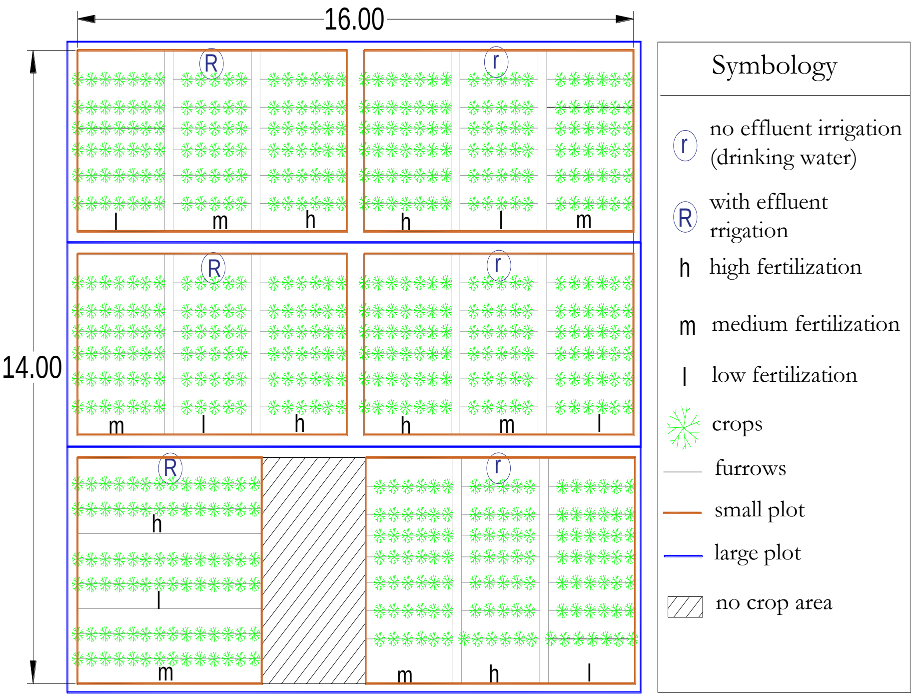

Figure 1.
Sketch of the experiment in the plot

The application of mineral fertilizer was made at two moments of the crop: at 45 days after planting (DAP) and at 82 DAP during weeding. In the first application, half of the N, all the P and K were applied; the second fertilization the rest of the N was applied. The irrigation with the SCB effluent was done every third day in the plot from the first day after sowing. Two liters of effluent were placed in each small plot, equivalent to an irrigation sheet of 30 cm. The seed was harvested manually.

Variables evaluated

In the sunflower plot, the variables evaluated were yield and yield components (weight of the disc, seeds, leaves, stem and root, number of empty and full seeds) in g and pieces, as appropriate. In the case of the corn plot, the weight of the stalk, leaves and cob were measured in g. These data were measured when the crop cycle had ended at 126 DAP in the sunflower plot and 147 DAP in the corn plot. Weight data was taken with a JWS OBI model 207136 high-precision digital scale. The seeds were left to dry at room temperature until the producers considered the product dry to use, approximately 14% moisture. To verify the safety of SCB effluent after irrigation, a 100 g sample of corn and sunflower seeds was analyzed at Fertilab, according to NOM-210-SSA1-2014.

Data analysis

The collected data were tested for assumptions (homogeneity of variances or Bartlett test and normality of errors or Shapiro-Wilks test). When the assumptions were met, an analysis of variance was performed for the split-plot experimental design and Tukey's multiple comparisons test ($P < 0.05$). Data that did not meet any of the assumptions were subjected to Friedman's test. Analyses were performed using the statistical software InfoStat free version.

Results

Physical, chemical and biological effluent characteristics

The physical and chemical analysis (Table 1) indicated that the SC effluent contained an alkaline pH (8.37) due to a high level of HCO3- (19.36 me L^{-1}) and a moderate level of CO32- (0.62 me L^{-1}); the EC and sodium adsorption ratio (SAR) were high (3.30 ds m^{-1} and 5.91, respectively); the hardness was 856.80 mg-CaCO3 L^{-1}, indicating a high content of Ca (12.60 me L^{-1}) and Mg (4.53 me L^{-1}); while the Cl level is high (15.00 me L^{-1}). The results of the nutrient analysis of the SCB effluent (Table 2) indicated that the predominant macronutrient is K with 195 kg ha^{-1}, while the level of N is low with 1 kg ha^{-1}.. The micronutrient that shows a high level in the effluent is Cu with 339 kg ha^{-1} and a low level is Mn with 75 kg ha^{-1}. Although in this research P was not found in the effluent.

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Table 1
Physical and chemical analysis of the SCB effluent from the self-cleaning biodigester.

Parameter evaluated	Remarks
pH, electrical conductivity and sodium adsorption ratio	Alkaline pH was caused by a very saline water as the concentration of sodium, magnesium and calcium were high. Therefore, it should only be used in crops tolerable to this condition.
Bicarbonates	Its level is high, causing the precipitation of soluble calcium in the water as calcium carbonate, preventing some plants from taking advantage of some nutrients and causing clogging in pressurized water systems.
Chlorides	It has a high level and some sensitive crops do not tolerate it.

Table 2
Nutrient supply (kg ha^{-1}) of the effluent from the self-cleaning biodigester in a 30 cm irrigation sheet.

Macronutrient	Content (kg ha^{-1})	Micronutrient	Content (kg ha^{-1})
Potassium	195	Iron	159
Calcium	758	Zinc	75
Magnesium	165	Manganese	51
Sulfur	112	Copper	339
Nitrogen	1	Boron	162

The biological parameters of the SCB effluent indicate that it still contains pathogens: more than 1600 NMP 100 mL^{-1} of total and fecal coliforms, 7.8 NMP 100 mL^{-1} in E. coli, exceeding the maximum permissible limits (< 1.1 NMP 100 mL^{-1}) established in NOM-210-SSA1-2014; and absence of Salmonella. However, according to NOM-001-SEMARNAT-2021, it complies with the permi-ssible limits for agri-cultural use of *E. coli*, where it is established that the content in the effluent must not exceed 600 NMP 100 mL^{-1}; although the maximum limit of fecal and total coliforms are not mentioned in this standard. Results should be < 1.1 NMP 100 mL^{-1} according to NOM-210-SSA1-2014. Measurement of COD in the efflu-ent determined that the SC removes 380 mg L^{-1} of to-tal COD and 370 mg L^{-1} of soluble COD; in other words, the anaerobic digestion process carried out by this system removes 65 % of COD. When evaluating TSS and SSV, the results show that the effluent con-tains 0.93 mg L^{-1} and 0.26 mg L^{-1} respectively. There-fore, the anaerobic digestion of the SC complies with NOM-001-SEMARNAT-2021, which states that, for agricultural use, the TSS content in the effluent must be less than 42 mg L^{-1}

Table 3
Biological parameters of the effluent from the self-cleaning biodigester.

Determination	Results (NMP 100 mL^{-1})
Fecal coliforms	1600
Total coliforms	1600
Salmonella	Absence
E. coli	7.8

Effect of effluent on sunflower and corn production

The use of effluent on sunflower plants showed statistically significant differences (Table 4) in the different variables measured. The effect of the application of effluent increased the values of the va-riables evaluated in the growth of the plant, disc, leaves, stem, root and quantity of seeds. Although no difference was observed between using or not using mineral fertilization in combination with effluent or potable water (no effluent). In corn plants, the results of the effect of SCB effluent on the different parame-ters measured show that there are no statistically significant differences. In the growth of stem, root, leaves and ear weight, the results were similar when using SCB effluent and potable water in combination with mineral fertilization.

Biological analysis of corn and sunflower seeds

Biological analysis of seeds (sunflower and corn) harvested from crops irrigated with the effluent showed 23 NMP 100 mL^{-1} of total coliforms, decreasing the amount by up to 98 % of what was detected in the SCB effluent; the harvested seed did not contain fecal coliforms and E. coli. according to NOM-210-SSA1-2014.

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Table 4
Effect of self-cleaning biodigester effluent on different variables of sunflower plants

Parameter	Treatment	
	Potable water (no effluent)	With effluent
Disc with seeds (g)	86.50 A ± 51.13	429.67 B ± 293.53
Seeds (g)	24.17 A ± 12.61	95.00 B ± 59.14
Stem (g)	120.00 A ± 64.50	373.33 B ± 213.32
Root (g)	44.17 A ± 21.08	83.33 B ± 43.20
Leaves (g)	27.67 A ± 10.54	251.67 B ± 197.12
Seeds (piece)	217.00 A ± 117.14	573.00 B ± 328.32
Full seeds (piece)	116.00 A ± 44.72	482.00 B ± 260.33
Empty seeds (piece)	101.00 A ± 75.29	91.00 B ± 86.96

*Means with a common letter in rows are not statistically significantly different according to Tukey's test ($p > 0.05$). Mean values n=x ± SD

Discussion

The SCB effluent evaluated in this study proved to be safe for use in crop irrigation and was confirmed by the safety of the seeds harvested in the experiment, so it can be used for irrigation and fertilization due to the nutrients it contains. According to da Silva et al. (2012), the decrease of pathogens in the effluent of anaerobic digestion is remarkable; therefore, it can be used for crop fertigation. Furthermore, Ibekwe (2018) mentions that the soil characteristics show no difference in the presence of pathogens when the effluent is placed and days later. This shows that the effluent does not transmit pathogens in crops as had already shown Valdez et al. (2024), who found in a field experiment of wheat crops irrigated with effluent from a treatment plant a significant reduction of pathogens (< 3 NMP g^{-1}), therefore, they stated that wheat (grain) irrigated with effluent is safe for human and animal use. Likewise, Urbano et al. (2017) mentioned that there are no significant differences in the presence of pathogens in crops irrigated with treated wastewater and potable water.

The characteristics of SC effluent showed an alkaline pH and high EC, characteristic in waters with high HCO_3^- and moderate CO_3^{2-} levels, respectively, a situation that favors Ca precipitation in the water as $CaCO_3$, prevents the plant from taking advantage of the nutrients (Alhendawi et al., 1997). However, although there are some restrictions on HCO_3^- in crops, its content in the effluent is suitable for agricultural irrigation, as already studied by Kaboosi (2017), who mentions that the increase of HCO_3^- in the soil by irrigation with treated wastewater can be suitable in crops according to national and international standards. High levels of effluent hardness are as a result of high levels of Ca and Mg concentration (Bamniya et al., 2010; Rahimi et al., 2018; Gupta et al., 2021). Although this fact does not affect crops, because Ca and Mg are nutrients that plants take advantage of, the damage can be economic, since hardness can form incrustations of small crystals in pipes or equipment intended to fertigate crops. In addition, irrigation of water with high levels of hardness can diminish the effect of agrochemicals on agricultural production. The nutrient supply of SCB effluent could substitute K and Ca fertilization, due to its high content. This result differs from other studies (de da Fonseca et al., 2007; Poustie et al., 2020; Zidan et al., 2024) where they have found that the effluent increases the availability of nutrients such as N and P and not Ca and K. However, the low levels of N and P detected could be supplemented with biofertilization or mineral fertilization (Perulli et al., 2019). In the biological part (Table 3), the effluent parameter evaluated for *E. coli* did not exceed 600 NMP 100 mL^{-1} according to NOM-001-SEMARNAT-2021. Although the maximum permissible limit of fecal and total coliforms in irrigation of crop areas are not mentioned in NOM-001-SEMARNAT-2021. Similar to this research, Cirelli et al. (2012) found a higher level of E. coli and fecal coliforms in crops irrigated with effluent than that stipulated by Italian regulations and did not

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detect Salmonella. These results are similar to what was found by da Silva et al. (2012), who found that there is a 99.99 % decrease of *E. coli* in the effluent when the anaerobic digestion process is performed under optimal conditions. However, da Silva et al. (2019), evaluated the influent and effluent of a SC and obtained that the anaerobic digestion system promotes a reduction of more than 90 % in pathogens. The removal of COD by the SC is not sufficient to comply with the provisions of NOM-001-SEMARNAT-2021; the maximum permissible limits of 210 mg L^{-1} are exceeded; this is characteristic of effluents extracted from sanitation systems that do not receive adequate maintenance, as has already been studied by Leitão et al. (2006), who mention that the concentration of COD in anaerobic systems is still under discussion due to the type of adequate operation that they may have. The findings of this research agree with Licata et al. (2022), who mention that a 65 % COD removal rate is obtained in treated wastewater. For Lettinga et al. (1993) and da Silva et al. (2012), anaerobic digestion systems are effective in the reduction of COD, because they manage to stabilize and eliminate organic pollutants in a time of up to 4 hours, with an efficiency of 75 % to 97 %, depending on their management. After the TSS and SSV removal process, 0.10 % remained in the SC effluent, promoting a high removal level of up to 99 % according to this research. According to Rodrigues et al. (2016), the average removal efficiency of TSS and SSV is 92 % when using an anaerobic sanitation system, therefore, high performance in solids and organic load removal is demonstrated in this research. Similarly for Subramani et al. (2024), in an experiment conducted in an anaerobic biodigester of a public toilet, a low level of TSS (8 mg L^{-1} on average) was obtained in the effluent when evaluated; the influent upon entering has on average 150 mg L^{-1} TSS, however, it is chlorinated and decomposes the organic portion of the suspended solid, thus reducing TSS by up to 90 %. It is inferred that the low level of TSS and VSS could be due to the use of chlorinated water. On the other hand, the yield measured in sunflower plants under different variables: disk, leaves, stem, root and number of seeds showed that SCB effluent increases yield by more than 70 % compared to drinking water, as it was also studied by Safi-Naz and Shaaban, (2015). Similarly, for Chatzakis et al. (2011) the use of effluent in sunflower crops has constituted an important source of nutrients and increased plant height, stem and root length, number of leaves and disc growth. In the corn crop of this research no differences were obtained between using effluent or potable water for irrigation, our effluent does not have N and P, however, this differed with what was found by Zidan et al. (2024) and Younas et al. (2020), who mentions that the use of effluent increases corn productivity and increases soil fertility. A study by Bame et al. (2014) found that the use of effluent significantly increased the yield of maize crops in different soil types as opposed to irrigation with potable water, increasing dry matter by 2.67 g per plant. Wang et al. (2022) mention that climate, soil, water or nutrient conditions directly affect crop growth, which is why it could have influenced corn yields in our study because the evaluated cycle was greatly affected by drought conditions, hail and high temperatures.

Conclusion

This study demonstrated that the effluent from an anaerobic biodigester is technically safe and can be used in agricultural production. The characteristics of the SCB effluent are within the permissible limits of pathogens for crop areas according to NOM-001-SEMARNAT-2021; it also has a high content of K and Ca, therefore, it would only be necessary to supplement with the missing nutrients. The use of SCB effluent as a source of fertigation is particularly suitable for the irrigation of sunflower crops. However, more studies are needed in the future to evaluate the effect that the SCB effluent promotes in the long term.

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