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Sustainable removal of nutrients (n and p) in a wastewater treatment plant, with eggshell (biocalcium)

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ABSTRACT

Biological treatments have become insufficient to treat municipal wastewater with greater toxicity and excess nitrogen and phosphate species, thus affecting the organisms that consume the water. In this work, a process was implemented for the removal of nutrients through three stages: stage A, complete aeration (24 h, 43 months); stage B, decreased aeration (12 h, 17 months); and stage C, decreased aeration with biocalcium (12 h, 19 months). The addition of biocalcium from eggshell promoted the formation of flocks, which resulted in the removal of nitrites (61 %), nitrates (84 %), total nitrogen (57 %), total phosphorus (8.3 %), sedimentable solids (50 %), total suspended solids (69 %), BOD_5 (76 %), helminth eggs (50 %) and fecal coliforms (54 %). The statistical analyses in the three stages indicated that there is a strong correlation between the concentration of fats and oils and the removal of sedimentable solids and total suspended solids, since these parameters were correlated by 97 and 89 %, respectively. Sedimentable solids were correlated with total suspended solids by 94 %, while nitrates and total nitrogen were correlated 92 %, which favors the removal of nutrients in wastewater. The increase in the concentration of nitrogen in the sludge in stage C generated a C:N ratio of 7.98. This ratio shows that the sludge is feasible for use as a mediator of soils and a biofertilizer because of the high contents of calcium, phosphorus and nitrogen. In addition, biocalcium promoted the precipitation of hydroxyapatite, struvite, calcite and quartz. In general, the three stages of the treatment contributed to the stabilization of the wastewater treatment plant (WWTP) in an efficient, economical, and safe way.

1. Introduction

Water quality changes because of the increase in population and anthropogenic activities, which causes changes in its chemical composition due to the release of industrial, municipal and agricultural wastewater. This puts the integrity of ecosystems, including

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plants, animals and humans, at risk because various toxic compounds are produced that cause various diseases and affect human reproduction [1,2] Biological treatments have become insufficient to treat wastewater with high toxicity [1,3], and large amounts of nitrogen and phosphate species are discharged to natural bodies of water [4,5].

Nitrogen and phosphorus concentrations in the environment have increased because of anthropogenic emissions, such as through the production of fertilizers; these nutrients promote eutrophication and a decrease in dissolved oxygen, which affects the ecosystem [6,7]. For these reasons, treatment systems have focused on nitrogen and phosphorus recovery options as well as biogas and electricity production as part of their integral management strategies to guarantee the sustainability of the ecosystem [8]. In recent years, various techniques have been developed to treat wastewater, including biological treatment, ion exchange, membrane separation, adsorption, and chemical precipitation [6]. Among them, chemical precipitation is considered one of the most promising methods for wastewater treatment due to its removal efficiency and feasibility [7,9,10]. Biological nutrient removal (BNR) processes have been widely employed in large-scale municipal wastewater treatment plants (WWTPs). The anaerobic/anoxic/oxic (A/A/O) process is one of the most popular BNR techniques [11,12]. Gao et al. (2023) studied the denitrifying phosphorus removal (DPR) process for simultaneous nitrogen and phosphorus removal from municipal wastewater. The DPR process was successfully started and operated by an alternating anoxic-anaerobic/aerobic biofilter (A-A/O BF) system, achieving effective synchronous nitrogen and phosphorus removal. In the proposed system, after 60 days of operation, under optimized conditions, the average removal efficiencies of COD, NH_4^+-N, TN and PO_4^{3-}-P were 83.14 %, 99.02 %, 69.75 % and 85.19 %, respectively [13].

In this way, recent works have reported that it is possible to remove nitrogen and phosphorus from wastewater using chemical precipitation. This process allows nutrients to be recovered and value-added products such as struvite and hydroxyapatite to be obtained [7,10]. This type of treatment is dependent on changes in the pH value as well as on the interactions between ionic species present in the wastewater; therefore, to control the recovery of nutrients, it is necessary to measure and control the pH value and the concentrations of ionic species in wastewater, such as Mg^{2+}, NH_4^+, Ca^{2+}, PO_4^{3-}, HCO_3^- and CO_3^{2-}, because the increase in pH decreases the solubility of these species, promoting their chemical precipitation [10]. Madeira et al. (2020) reported that chemical precipitation using lime and hydrated lime has shown various benefits because this process generates suspended particles that can be removed by simultaneous precipitation [14].

Other studies indicate that it is possible to carry out chemical precipitation using CaO, $Ca(OH)_2$ and ($Ba(OH)_2$) to remove Cr^{3+}, SO_4^{2-}, F^-, PO_4^{3-} and NH_3–N from tannery wastewater and landfill leachate. The authors concluded that chemical precipitation using these alkalis removed ionic species quickly, efficiently and safely at a low cost and allowed valuable solids to be recovered [9,15,16].

The current treatments, in addition to seeking greater efficiency, seek to achieve sustainability, which is possible by carrying out waste recycling actions that generate novel, economical treatments to produce added value and positively contribute to the local economy. Some studies have reported the use of eggshell (biocalcium); since this residue is easy to obtain and abundant worldwide, it is reported that approximately 250,000 tons are produced annually [17]. This residue has been used to remove contaminants in wastewater due to its low cost, physicochemical properties and high removal [18]. Biocalcium behaves as a natural adsorbent since it has a mesoporous structure [19,20], and it contains 95 % $CaCO_3$, 1.4 % Mg and other elements in trace percentages [21,22]. In addition, biocalcium was used to precipitate phosphorus and ladimirite in wastewater [21,23].

This work provides an alternative for nutrient removal in the biological treatment of municipal wastewater. This is the main novelty of the research because several works have reported the use of eggshell to remove nutrients, heavy metals, and organic compounds at the laboratory or pilot scale only and in aqueous solution matrices. The study was carried out in three stages: stage A,

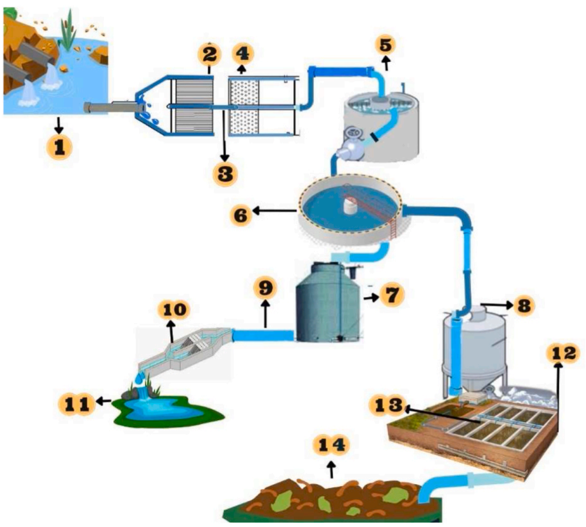


Fig. 1. Wastewater treatment plant process diagram: 1) influent, 2) screen, 3) Parshall Channel, 4) pretreatment tank (eggshell addition), 5) activated sludge reactor, 6) clarifier tank, 7) chlorination tank, 8) sludge thickener, 9) serpentine, 10) output channel, 11) receiving body, 12) sludge stabilization tank, 13) sludge drying, 14) compost area (biofertilizer).

complete aeration (24 h, 48 months); stage B, decrease in aeration (12 h, 21 months); and stage C, decrease in aeration with biocalcium (12 h, 19 months), with the aim of recovering nutrients to be used as biofertilizers or in the bioremediation of soils to increase the C/N ratio. In addition, the wastewater quality was improved and could be used for agricultural irrigation or discharged into a surface water body, so a statistical analysis was carried out with the results of the effluent characterization in the wastewater treatment plant (WWTP) from 2015 to 2022 (20 samplings). The correlation between wastewater quality parameters and their compliance with national legislation was determined to predict the future behavior of WWTPs (NOM-001-SEMARNAT-1996 and 2021) [24,25].

2. Methodology

The WWTP (Fig. 1) is located at coordinates 19° 41′ 57.9999″ north latitude and 99° 47′ 34.9999″ west longitude. The plant, which began operations in 1999 and was remodeled in 2016, treats wastewater at a rate of 30 L/s, and currently receives the wastewater generated by 7965 inhabitants [26], The WWTP works with an activated sludge system. As the first step in the process, there is a manual screening that removes stones, bags, leaves, and sticks. After the water passes through a Parshall channel where the influent is measured, the wastewater enters the biological reactor of activated sludge, and the aeration and digestion of the organic matter is carried out. Later, the water passes to the secondary sedimentation, the gravity separation process and the densest particles are deposited at the bottom of the sedimentation tank. Then, clarified wastewater is disinfected with chlorine to inactivate pathogenic microorganisms. The sedimented sludge is pumped to the sludge thickener, the excess water is removed, and finally, the sludge is directed to the drying beds for the removal of moisture.

2.1. Materials and methods

2.1.1. Biocalcium and sludge

The biocalcium was collected from restaurants, budget kitchens and bakeries. The biocalcium and sludge from the WWTP were characterized before and after adding biocalcium by infrared spectroscopy (IR) with the spectrophotometer Two Lita FT-IR brand PerkinElmer with serial number 100743. In addition, its morphology and composition were determined using a scanning electron microscope (SEM) JSM-IT100LV, and elemental analysis of energy dispersive X-ray spectroscopy (EDS) was performed with a spectrophotometer (Bruker brand dispersion). Additionally, the crystallinity of the sludge was quantitatively and qualitatively analyzed using X-ray diffraction (XRD) with a Bruker D8 Discover diffractometer.

2.1.2. Characterization of treated wastewater

The wastewater characterization was compared with NOM-001-SEMARNAT-1996 and 2021 Mexican standards, and Pearson correlation statistical analysis was carried out to analyze the behavior of nutrient removal in the WWTP.

The wastewater quality parameters were analyzed: pH, temperature, fats and oils, suspended solids, total suspended solids, Kjeldahl nitrogen, nitrites, nitrates, total nitrogen, BOD_5, total phosphorus, cyanides, arsenic, and metals (cadmium, copper, chromium, mercury, nickel, lead and zinc). Additionally, fecal coliforms and helminth eggs were determined by the standard methods of analysis [27].

Subsequently, the results were compared with the maximum permissible limits established in NOM 001-SEMARNAT-1996 and 2021 to evaluate whether the effluent discharged from 2015 to 2022 met the requirements established to be reused in agricultural irrigation and discharged to a water body.

2.1.3. Elemental composition

Finally, the wastewater before and after treatment with biocalcium was characterized by inductively coupled plasma optical emission spectroscopy (ICP−OES) analysis to completely characterize the effluent that is used for agricultural irrigation, which was performed using an iCAP 6500 Duo from Thermo Brand.

2.1.4. Nutrient removal stages (N and P)

Stage A (complete aeration, 24 h)

This first stage corresponds to the evaluation of the treatment of municipal wastewater by the activated sludge process from 2015 to 2019 to determine the concentrations of nitrogen and phosphorus species as total nitrogen, Kjeldahl nitrogen, nitrites, nitrates, and total phosphorus of the effluent that is discharged for agricultural irrigation. Step An includes sampling 1 to sampling 8.

Stage B (Decreased aeration, 12 h)

In the second stage, we sought to decrease the concentration of total nitrogen through the control of the nitrification process and reduce the concentration of nitrates, which was carried out with the decrease in oxygen in the aeration system that was operated intermittently for 12 continuous hours during the day from 6:00 a.m. to 18:00 p.m., maintaining the recirculation process in the activated sludge reactor continuously and its passage to the secondary sediment, from August 28, 2019, to May 27, 2021. Step B includes sampling 9 to sampling 14.

Table 1
Effluent wastewater characterization from WWTPs.

Sample	pH	Fecal coliforms NMP/100	Helminth eggs	Greases and oils	Sedimentable solids	Total suspended solids	BOD_5	Kjeldahl Nitrogen	Nitrites	Nitrates	Total Nitrogen	Total phosphorous
1	7.3	759.66	1	7.6	0.1	12	48.53	7.92	0.02	17.66	25.6	3.12
2	7.4	321.41	1	11.6	0.9	40	51.7	11.68	0.51	14.64	26.84	3.7
3	7.4	293.93	0.2	5	0.1	25.5	62.63	4.06	0.39	3.69	8.15	2.89
4	7.3	172.77	0.2	5.4	0.1	23	63.67	7.41	0.04	17.18	24.63	2.75
5	7.1	107.81	0.2	5	0.1	78	18.37	8.79	1.15	11.34	21.28	2.16
6	7.1	880.81	0.2	5.1	0.1	37.8	26.13	8.03	0.13	2.27	10.43	2.2
7	7.8	29.05	0.2	5	0.1	12	15.32	9.02	0.21	10	19.23	1.33
8	6.6	9.64	0.2	5	0.1	12	31.39	4.28	0.01	18.16	22.46	2.87
9	7.1	3.7	0.2	5	0.5	12	9.39	6.37	0.011	0.3	6.68	3.36
10	6.8	718.23	0.2	5	0.1	12	7.49	4.55	0.08	0.34	4.96	2.3
11	7.8	58.64	0.2	5	0.1	12	17.29	11.22	0.15	0.32	11.69	4.36
12	7.2	250.68	0.2	5	0.1	12	5.32	8.28	0.16	0.32	8.77	4.34
13	7.3	41.61	0.2	36.7	10.9	212	106.6	9.03	0.03	0.1	9.16	4.39
14	7.2	317.61	0.2	5	0.1	12	8	9.71	0.38	0.1	10.186	4.41
15	7.8	378.1	0.2	5	0.1	12	9.14	9.98	0.099	0.108	10.19	3.29
16	7.67	136	0.2	5	0.1	12	11.84	7.26	0.01	0.1	7.37	1.06
17	7.1	365.2	0.2	5	0.1	12	13.12	9.25	0.13	2.05	11.43	3.32
18	7.62	7	0.2	5	0.1	12	12.32	8.09	0.49	0.91	9.49	1.83
19	7.1	7	0.2	10.00	0.1	3	5	3.5	0.02	4.05	7.27	2.5
20	7.2	3	0.2	10.00	0.1	5	5	0.8	0.01	4.12	4.93	2.5

Stage C (Decreased aeration, 12 h with the addition of Biocalcium)

In the third stage, biocalcium from eggshell residues was added, with the aim of increasing sludge production in the WWTP, and 100 kg of biocalcium was added daily in the Parshall channel from 9:00 a.m. to 10:00 a.m., from May 28, 2021, to December 11, 2022. The process of continuous aeration was intermittent as established in stage B. Step C included sampling 15 to sampling 20.

The number of samples in each stage was defined by the municipality's administration according to the budget granted.

2.1.5. Statistical analysis

With the results of the analyses carried out from August 14, 2015, to December 11, 2022, a statistical analysis was performed using IBM SPSS Statistics software. In addition, Tukey's test was performed with Minitab software to monitor the WWTP in the three stages during the removal of nutrients.

3. Results

3.1. Pearson correlation statistical analysis

Tables 1 and 2 show the results from 20 characterizations of the WWTP during the three stages studied. Tables 3 and 4 show the Pearson correlations for each parameter. The statistical analysis indicated that there is a strong correlation between the amount of fats and oils and the removal of sedimentable solids and total suspended solids, since these parameters were correlated by 97 and 89 %, respectively; sedimentable solids with total suspended solids were correlated by 94 % because fats and oils have low miscibility with water, and consequently, the biological treatment is affected [28–30]. The correlation between total suspended solids and BOD_5 was 74 %, which can be explained because a fraction of total suspended solids is biodegradable matter from fats and oils, urine, and fecal feces. Moreover, nitrates and total nitrogen were positively correlated by 92 %, and this strong correlation is because of the oxidation of organic nitrogen to ammonia and then to nitrates [31,32].

The statistical analysis shows that the interactions between metals in solution, cadmium with copper, mercury, nickel, and zinc are 98, 96, 88 and 90 %, respectively. This correlation can be explained because the concentrations of these metals were the highest in the wastewater and pH = 7.2 improved the precipitation mechanisms for the metals, while cadmium with lead was negatively correlated at 82 % because Cd precipitates at pH above 8.5 and lead precipitates in a wide range of pH values (1.3–13). Copper was positively correlated with mercury, nickel and zinc by 98, 95 and 89 %, respectively, due to Hg, Ni and Zn forming hydroxides that adsorb copper oxides at the sample pH (7.2). Copper and chromium were negatively correlated by 94 % because copper forms oxides and chromium remains in solution as bichromate and chromate due to the lack of charge attraction in aqueous media. Chromium was negatively correlated with mercury, nickel and zinc by 85, 98 and 86 %, respectively, for the same reason explained in the last paragraph. Finally, zinc was correlated with mercury and nickel by 83 and 84 %, respectively, due to hydroxide formation at the sample pH, and then the precipitation of these metals was carried out efficiently. The other metals were weakly correlated due to their low concentrations, so they remained in solution.

Charazińska et al. (2022) studied the use of biosorbents for the purification of aqueous solutions from nickel ions. The highest sorption capacity value for the composite/modified material-calcinated eggshells was 769 mg Ni g^{-1}, and the removal mechanisms identified, chemisorption and ion exchange, are considered to be the most common. However, for this material, the sorption phenomenon may be accompanied by precipitation in the presence of hydroxides, which significantly affects the sorption capacity

Table 2
Heavy metals, arsenic, and cyanide characterization from effluent wastewater.

Sample	Arsenic	Cadmium	Cyanide	Copper	Chromium	Mercury	Nickel	Lead	Zinc
1	0.016	0.0427	0.02	0.204	0.215	0.0021	0.18	0.1273	0.211
2	0.0248	0.0427	0.02	0.204	0.215	0.0021	0.18	0.1273	0.211
3	0.0142	0.0423	0.02	0.216	0.201	0.0023	0.2	0.2048	0.202
4	0.0114	0.0395	0.02	0.199	0.198	0.002	0.19	0.193	0.199
5	0.0079	0.0395	0.02	0.199	0.198	0.002	0.19	0.1938	0.199
6	0.0119	0.0395	0.02	0.199	0.198	0.002	0.19	0.1938	0.199
7	0.0129	0.0395	0.02	0.199	0.198	0.002	0.19	0.1938	0.199
8	0.0061	0.0395	0.02	0.199	0.198	0.002	0.19	0.1938	0.199
9	0.0188	0.0395	0.02	0.199	0.198	0.002	0.19	0.1938	0.199
10	0.0174	0.0395	0.02	0.199	0.198	0.002	0.19	0.1938	0.199
11	0.0136	0.0395	0.02	0.199	0.198	0.002	0.19	0.1938	0.199
12	0.0105	0.0395	0.02	0.199	0.198	0.002	0.19	0.1938	0.199
13	0.0164	0.0395	0.02	0.199	0.198	0.002	0.19	0.1938	0.228
14	0.0086	0.0395	0.02	0.199	0.198	0.002	0.19	0.1938	0.199
15	0.0445	0.0395	0.021	0.199	0.198	0.002	0.19	0.1938	0.199
16	0.018	0.039	0.021	0.199	0.198	0.002	0.19	0.193	0.199
17	0.0049	0.04	0.021	0.2008	0.2	0.002	0.2	0.2	0.199
18	0.008	0.04	0.021	0.2008	0.2	0.002	0.2	0.2	0.199
19	0.001	0.05	0.02	0.25	0.1	0.0025	0.25	0.125	0.25
20	0.001	0.05	0.02	0.25	0.1	0.0025	0.25	0.125	0.25

Table 3
Pearson correlation statistical analysis for physicochemical parameters from effluent wastewater.

	pH	Fecal coliforms	Helminth eggs	Greases and oils	Sedimentable solids	Total suspended solids	BOD_5	Kjeldahl nitrogen	Nitrites	Nitrates	Total nitrogen	Total phosphorous
pH	1	−0.195	0.059	−0.013	0.004	−0.035	0.010	0.496[a]	0.066	−0.228	−0.017	−0.089
Fecal coliforms		1	0.377	−0.187	−0.179	−0.113	0.036	0.141	−0.082	0.026	0.076	0.017
Helminth eggs			1	0.097	−0.028	−0.018	0.301	0.288	0.079	0.548[a]	0.618[b]	0.164
Greases and oils				1	0.967[b]	0.889[b]	0.701[b]	0.068	−0.154	−0.091	−0.065	0.350
Sedimentable solids					1	0.936[b]	0.713[b]	0.157	−0.133	−0.168	−0.100	0.364
Total suspended solids						1	0.735[b]	0.233	0.159	−0.061	0.040	0.283
BOD_5							1	0.147	−0.034	0.346	0.375	0.278
Kjeldahl nitrogen								1	0.324	−0.022	0.375	0.366
Nitrites									1	0.138	0.290	−0.085
Nitrates										1	0.918[b]	−0.152
Total nitrogen											1	−0.003
Total phosphorous												1

bIt cannot be calculated because, a value is constant.
[a] The correlation is significant at the level 0.05.
[b] The correlation is significant at level 0.01 (two tails).

Table 4
Pearson correlation analysis for heavy metals, arsenic, and cyanide.

	Arsenic	Cadmium	Cyanide	Copper	Chromium	Mercury	Nickel	Lead	Zinc
pH	0.451[a]	−0.125	0.402	−0.126	0.160	−0.103	−0.138	0.081	−0.129
Fecal coliforms	0.277	−0.177	−0.041	−0.257	0.354	−0.197	−0.361	−0.047	−0.272
Helminth eggs	0.252	0.174	−0.167	−0.036	0.271	0.053	−0.297	−0.649[b]	0.086
Greases and oils	0.041	0.125	−0.184	0.101	−0.100	0.094	0.067	−0.171	0.509*
Sedimentable solids	0.101	−0.106	−0.128	−0.105	0.074	−0.111	−0.099	0.074	0.304
Total suspended solids	0.085	−0.189	−0.182	−0.190	0.168	−0.181	−0.194	0.138	0.192
BOD_5	0.134	−0.121	−0.282	−0.172	0.316	−0.070	−0.304	0.000	0.098
Kjeldahl nitrogen	0.452*	−0.609[b]	0.219	−0.692[b]	0.676[b]	−0.688[b]	−0.693[b]	0.265	−0.522[a]
Nitrites	−0.068	−0.162	−0.036	−0.180	0.242	−0.138	−0.196	0.140	−0.255
Nitrates	−0.115	0.085	−0.351	−0.034	0.150	0.016	−0.193	−0.364	−0.016
Total nitrogen	0.066	−0.165	−0.240	−0.307	0.411	−0.257	−0.454[a]	−0.225	−0.228
Total phosphorous	0.142	−0.091	−0.289	−0.141	0.169	−0.116	−0.194	−0.013	0.030
Arsenic	1	−0.378	0.295	−0.427	0.468[a]	−0.369	−0.514[a]	0.135	−0.341
Cadmium		1	−0.222	0.976[b]	−0.876[b]	0.964[b]	0.878[b]	−0.821[b]	0.900[b]
Cyanide			1	−0.188	0.143	−0.238	−0.041	0.276	−0.248
Copper				1	−0.938[b]	0.978[b]	0.948[b]	−0.687[b]	0.887[b]
Chromium					1	−0.853[b]	−0.976[b]	0.552[a]	−0.858[b]
Mercury						1	0.878[b]	−0.687[b]	0.843[b]
Nickel							1	−0.494[a]	0.827[b]
Lead								1	−0.747[b]
Zinc									1

bIt cannot be calculated because a value is constant.
[a] The correlation is significant at the level 0.05.
[b] The correlation is significant at level 0.01 (two tails).

achieved [33].

Kothari et al., 2021 reported that a high correlation coexists between different water quality parameters; for example, there is a significant relationship between the total concentration of iron with total coliforms and fecal coliforms of 20 and 40 %, respectively. In addition, total dissolved solids have a significant correlation with electrical conductivity, sulfates, chloride ions, total alkalinity and total hardness at 90, 90, 70, 80 and 80 %, respectively; moreover, an important correlation is observed between turbidity and nitrates at 80 % [8].

3.2. Comparative analysis for nutrient removal

Fig. 2 shows the comparative analysis of the three stages: A (complete aeration), B (decrease in aeration) and C (decrease in aeration and addition of biocalcium). In step A, from sampling 1 to sampling 8, there was greater variation in the concentrations of total nitrogen (8.15–26.84 mg/L), nitrates (2.27–18.16 mg/L) and Kjeldahl nitrogen (4.06–11.68 mg/L), which reflects greater instability of the WWTP. The concentrations of nitrites (0.01–1.15 mg/L) and total phosphorus (1.01–4.41 mg/L) remained stable throughout the three stages. In stage B, from sampling 9 to 14, a decrease in the concentration of total nitrogen (4.96–11.69 mg/L) was observed, which was found mainly in the chemical form as Kjeldahl nitrogen, because of the decrease in aeration that was established at this stage to reduce the concentration of nitrates (0.01–0.34 mg/L). In step C, from sampling 15 to sampling 20, the addition of biocalcium was carried out to reduce the concentration of Kjeldahl nitrogen (0.8–9.98 mg/L), total nitrogen (4.93–11.43 mg/L) and total phosphorus (1.01–3.32 mg/L) through the precipitation process of Kjeldahl nitrogen and total phosphorus and thus enrich the ratio of C to N in the sludge. It was also observed that there was a recovery of the nitrification process because nitrates increased from sampling 16 (2.05–4.12 mg/L).

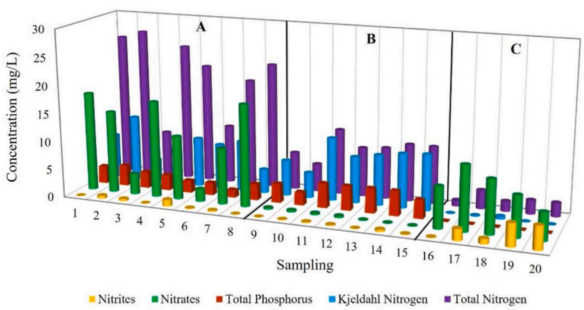


Fig. 2. Comparative analysis for nutrient removal: complete aeration (A), decrease in aeration (B), decrease in aeration and addition of biocalcium (C).

Aeration accounts for 45–75 % of the total energy consumption in a wastewater treatment plant [34]. According to Izadi et al. (2021), intermittent aeration was applied to develop a robust nutrient removal system aimed at achieving high energy savings and removal efficiency. The results showed a higher correspondence of P uptake, polymeric substance synthesis and glycogen degradation in intermittent aeration with longer interval periods compared to continuous aeration. Increasing the intermittent aeration duration from 25 to 50 min resulted in higher process performance, where the system exhibited approximately 30 % higher nutrient removal. The 50 min intermittent aeration favored the growth of *P*-accumulating organisms and nitrogen removal microbial groups, indicating complications related to nutrient removal systems. This process is a promising approach to potentially remove nutrients in high competence, in contrast to optimizing the cost-efficacy of the system [35]. Pryce et al. (2022) showed that integrated fixed-film activated sludge (IFAS) promotes sufficient total nitrogen (TN) removal at a lower DO concentration of 2 mg/L, providing that the carbon:nitrogen (C/N) ratio of the influent was favorable [36]. More recently, Li et al. (2016) recommended a reduction from 8 mg/L to 4 mg/L to be sufficient in an IFAS reactor [37]. Singh et al. (2016) found that a similar level of TN removal could be achieved in a package IFAS system when the aeration was reduced from 4.5 mg/L to 2.5 mg/L. This could be attributed to the occurrence of simultaneous nitrification and denitrification (SND), which is a more efficient form of TN removal than the conventional oxic-anoxic process due to its reduced aeration and footprint requirements [38].

Fig. 3 shows the statistical analysis and the main effects on the concentrations of nitrates, nitrites, Kjeldahl nitrogen, total nitrogen and total phosphorus for stages A, B and C. By Tukey's method, with a level confidence of 95 %, the data show that the concentrations are significantly different for nitrates, total nitrogen, nitrites, Kjeldahl nitrogen, and total phosphorus. The means are not significantly different between the stages, and with the general linear model and the variance analysis, it was determined for p values < 0.05 that treatment is statistically significant for the decrease in the concentration of nitrates and total nitrogen.

3.3. Physicochemical and microbiological characterization before and after adding biocalcium

Table 5 shows the physicochemical and microbiological parameters for the treated wastewater in the three stages. It was observed that the addition of biocalcium and the decrease in aeration promoted the removal of sedimentable solids and total suspended solids by 50 and 69 %, respectively, as well as the removal of BOD_5 by 76 % and total nitrogen by 57 %, nitrites and nitrates by 61 and 84 %, respectively, helminth eggs and total coliforms by 50 and 54 %, and total phosphorus by 8.3 %. In 2020, Madeira and collaborators obtained similar results using lime as a precipitant. In this work, the use of biocalcium is proposed as a novel treatment with low cost and easy application.

The results of treated wastewater characterization after biocalcium addition were compared with the NOM-001-SEMARNAT-2021 Mexican standards. All parameters are accomplished with permissible limits, and effluent can be reused for agricultural irrigation and/or discharged into a water body.

The worldwide consumption of eggs is very high, leading to approximately 250,000 tons of eggshell membrane (ESM) waste annually. Ganesh Saratale et al. (2021) investigated the potential use of ESM as an inexpensive and abundant adsorbent for Reactive Red 120 (RR120) in aqueous solutions, a widespread hydrophilic azo dye used in the textile industry. The maximum monolayer adsorption ability of ESM for RR120 was found to be 191.5 mg/g at 318 K, and the sorption process [39].

Andrade Cruz et al. (2022) studied the influence of calcium particles from eggshell residues on the anaerobic digestion of cassava wastewater. Calcium particles from milled-calcined chicken eggshells were added to the bioreactor, and biogas production was investigated for 21 days. Adding 1 g/L and 3 g/L of calcium particles increased biogas (Bio H_2 + Bio CH_4) production by 195 % and 338 %, respectively, and the COD removal was improved from 76 to 90 % [40].

3.4. Biocalcium characterization

Fig. 4 shows the infrared spectrum of biocalcium. The analysis shows characteristic peaks at 3468 cm^{-1}, which come from the stretching vibration of the OH^- bond. The peak at 1793 cm^{-1} corresponds to the double bond of carbonate C=O, while the peaks at

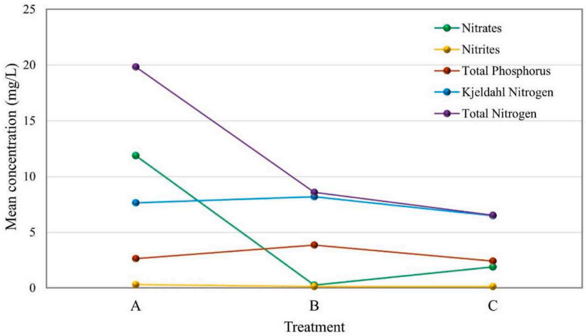


Fig. 3. Main effects of nitrates, nitrites, Kjeldahl nitrogen, total nitrogen, and total phosphorus for the different stages with complete aeration (A), decrease in aeration (B) and decrease in aeration and addition of biocalcium (C).

Table 5
Wastewater characterization after biocalcium addition.

Parameter (mg/L)	NOM-001-SEMARNAT-1996	NOM-001-SEMARNAT-2021	Stage A	Stage B	Stage C
Temperature (°C)	40	35	18.25	19.04	20.38
Greases and oils	25	18	6.21	10.28	6.66
Sedimentables solids	2	N/A	0.20	1.97	0.1
Total Suspended Solids	125	24	30.04	45.33	9.33
BOD_5	150	N/A	39.72	25.68	9.40
Total Nitrogen	60	25	19.83	8.57	8.44
Kjeldahl Nitrogen		N/A	7.65	8.19	6.48
Nitrites	N/A	N/A	0.31	0.14	0.12
Nitrates	N/A	N/A	11.87	0.25	1.89
Total phosphorous	30	10	2.63	3.86	2.41
Helminths eggs (eggs/L)	1	N/A	0.4	0.2	0.2
Fecal coliforms MPN/100 ml)	1000	N/A	321.89	231.75	149.38
Escherichia coli (MPN/100 ml)	N/A	500	N/D	N/D	N/D
Fecal enterococci	N/A	400	N/D	N/D	N/D
pH	10	9	7.25	7.23	7.41
TOC	N/A	30	N/D	N/D	N/D
Arsenic	0.4	0.15	0.013	0.014	0.012
Cadmium	0.4	0.15	0.041	0.039	0.043
Cyanide	3	1.5	0.020	0.020	0.020
Copper	6	5	0.202	0.199	0.216
Chromium	1.5	0.75	0.202	0.198	0.166
Mercury	0.02	0.008	0.002	0.002	0.002
Nickel	4	3	0.189	0.190	0.213
Lead	1	0.3	0.178	0.194	0.173
Zinc	20	15	0.202	0.204	0.216
Acute Toxicity (TU)	N/A	2 at 15 min of exposition	N/D	N/D	N/D

N/A: No apply, MPN: More probable number, TU: Toxicity units, N/D: no determined.

868 and 709 cm^{-1} are representative of the in-plane deformation and out-of-plane bending vibration of the (CO_3^{2-}) bond and –OCO–. Similar results were found by Ganesh Saratale et al., 2021 and other authors [41,42].

To investigate the structure and semiquantitative elemental composition, SEM micrographs and EDS

Were taken. Fig. 5 shows the biocalcium morphology, and a rough texture and small pores characteristic of biocalcium were observed. It has been reported that this material behaves as a bioadsorbent since it has pores of approximately 0.894 μm [43].

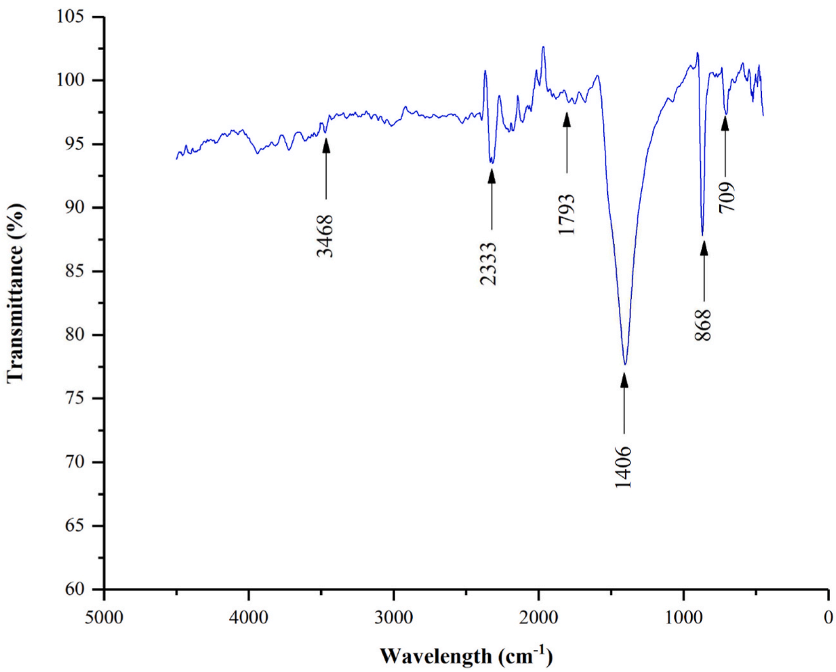


Fig. 4. Biocalcium infrared spectra.

Table 6 shows the EDS analysis of biocalcium, which indicates that biocalcium consists mainly of calcium, magnesium, carbon, and oxygen. Deoli et al. (2021) determined the relative elemental concentrations in eggshells by micro-PIXE analysis, and the eggshell and eggshell membrane revealed the following elements: Al, Si, P, S, Cl, K, Ca, Fe, Cu, and Zn [44].

3.5. Sludge characterization from the WWTP before and after biocalcium treatment

Fig. 6a shows the infrared spectrum of the sludge from the WWTP before biocalcium treatment. A wide peak is observed at 3716-3037 cm^{-1} from the N–H stretch bond of the amino group due to ammonia compounds [45], the bands at 2926, 2856 and 1646 cm^{-1} can be attributed to asymmetric or symmetric bonds from cyclic alkanes ν (C–H) [46,47], and the peak at 939 cm^{-1} is due to the presence of the C–O–H bond that is generated by bending out of the plane [45].

Fig. 6b shows the infrared spectrum of sludge from the WWTP. After the biocalcium treatment. The analysis shows a peak at 1649 cm^{-1}, which is attributed to the presence of organic matter from bacterial biomass [47]. Additionally, the sample shows the presence of biocalcium at 1419, 875 and 713 cm^{-1} [41,48]. The peak located at 1042 cm^{-1} is from the stretching vibrations and Fe–O bond of biogenic Fe(III) oxides [47], and the peak at 1970 cm^{-1} shows the stretching of the OH- bond, as reported in other studies [49].

Fig. 7a shows the scanning electron microscopy of the sludge from the WWTP before the addition of biocalcium. Fig. 7b shows the MEB of the sludge after the addition of biocalcium, where porous structures are visualized.

Table 7 shows the EDS analysis of the sludge from the WWTP before and after the addition of biocalcium. The analysis determined the absence of heavy metals such as mercury, arsenic and lead. This sludge can be widely recommended for agriculture and soil remediation because of its high calcium, phosphorus and nitrogen contents, which can satisfactorily promote plant growth. The sludge after the addition of biocalcium has a C:N ratio of 7.98. It should be noted that the C:N ratio in biofertilizers is of utmost importance since its efficiency in biofertilization depends on it, and both carbon and nitrogen are vital for the survival and flourishing of plants because they are a source of direct energy [50].

The characterization of sludge after the addition of biocalcium shows higher nitrogen values. It has been reported that the amount of nitrogen in sludge from wastewater treatment plants is generally low compared to the initial concentration of the influent because nitrogen is removed as molecular nitrogen during the nitrification and denitrification process [51]. The addition of biocalcium increased the concentration of nitrogen in the sludge of the WWTP due to the addition of biocalcium, which promoted the sorption of organic nitrogen and the increase in the C:N ratio in the sludge, making it a feasible biofertilizer. Some authors reported that the C:N ratio for composting should be within the range of 16–21. However, C:N ratios <12 for municipal waste compost show a good degree of maturity for agricultural applications [52,53].

Fig. 8a shows the X-ray diffraction (XRD) of the sludge before and after biocalcium, and the spectra show low crystallinity because few peaks characteristic of hydroxyapatite are observed ($(Ca_5(PO_4)_3 \cdot XH_2O)$), struvite ($MgNH_4PO_4$ $X(H_2O)$), calcite ($CaCO_3$) and quartz (SiO_2), indicating that there is a noncrystalline or amorphous sample, which may be due to the content of biogenic material, as reported in other studies [47,54]. Fig. 8b shows the XRD analysis of the sludge after biocalcium treatment. In this figure, there is a greater number of peaks that show the presence of hydroxyapatite ($(Ca_5(PO_4)_3 \cdot XH_2O)$), struvite ($MgNH_4PO_4 \cdot X(H_2O)$), calcite ($CaCO_3$) and quartz ($SiO_2$). Some studies have discussed that these peaks show the presence of materials that can be recovered from sludge and obtain added value [7,54].

Yang et al. (2021) studied a novel Ca-modified biochar prepared via copyrolysis of eggshell and sewage sludge (mass ratio 2:1) to recover phosphorus from wastewater. The maximum adsorption capacity reached 106.99 mg P/g, and it exhibited a good ability for phosphate adsorption from solution in a wide range of pH values (2−11) with a removal efficiency of more than 96 % for 50 mg P/L with an adsorbent dosage of 2 g/L. The coexisting NO_3^-, Cl^- and SO_4^{2-} did not significantly influence adsorption performance because of the reduction of adsorption capacity less than 5 %, except for HCO_3^-. Electrostatic attraction and precipitation with hydroxyapatite ($Ca_5(PO_4)_3(OH)$) were the main removal mechanisms [55].

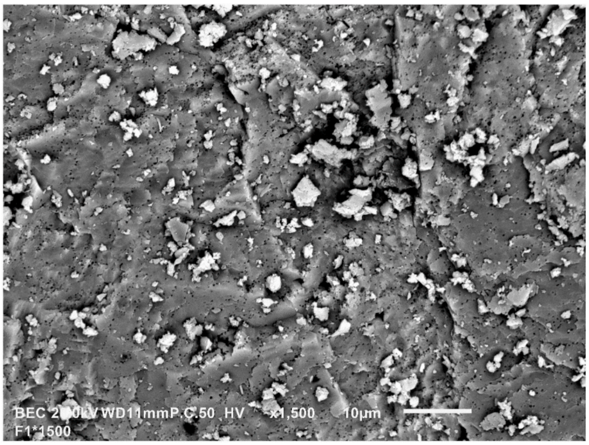
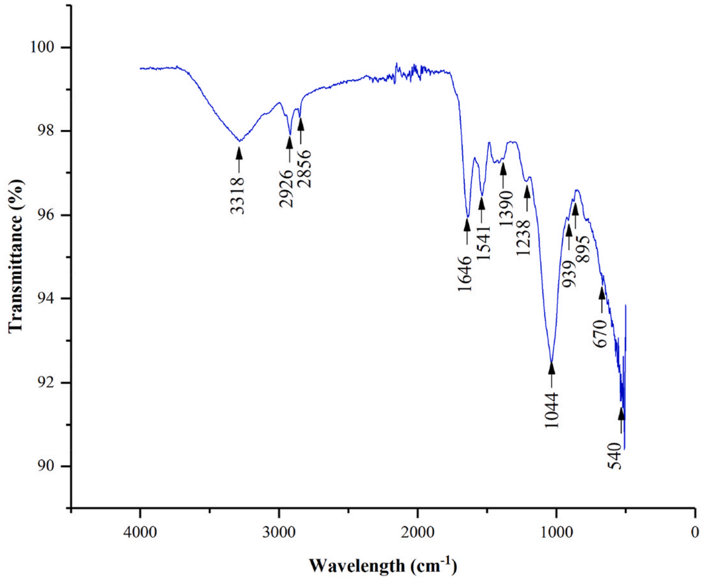


Fig. 5. Scanning electron microscopy of biocalcium.

Table 6
Elemental analysis of biocalcium.

ELEMENT	%
Carbon	10.32
Oxygen	50.84
Calcium	38.32
Magnesium	0.50

a)



b)

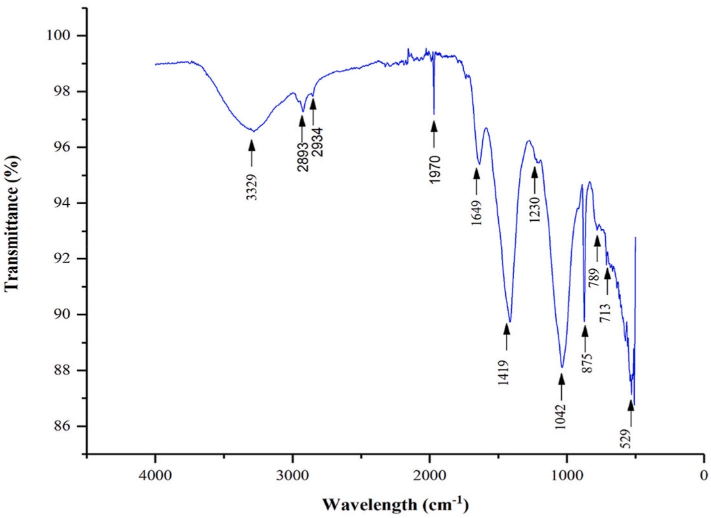


Fig. 6. a)IR spectra for sludge from the WWTP before the addition of biocalcium. 6b) IR spectra after the addition of biocalcium.

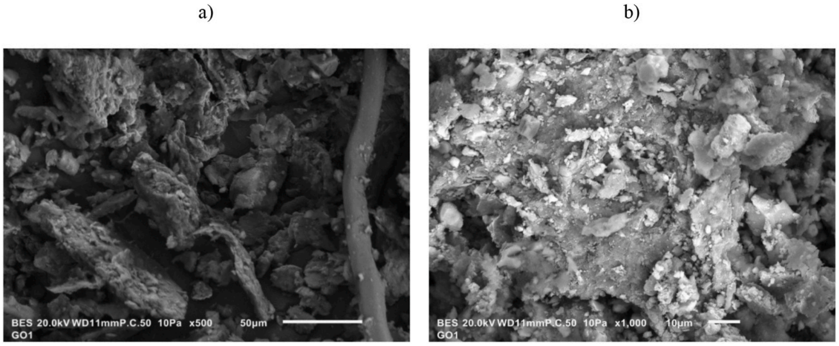


Fig. 7. a)MEB of the raw sludge from the WWTP and 7b) MEB of the sludge after biocalcium treatment.

Table 7
X-ray spectroscopy (EDS) of sludge from the WWTP before and after biocalcium treatment.

Element	Raw Sludge	Biocalcium + sludge
Carbon	66.26	37.19
Oxygen	26.33	44.53
Aluminum	1.01	0.83
Calcium	1.16	7.73
Potassium	0.14	0.09
Sodium	0	0.16
Magnesium	0	0.41
Silicon	4.49	2.12
Iron	0.47	0.25
Phosphorus	0	0.38
Sulfur	0	0.03
Nickel	0.03	0.02
Copper	0.005	0.013
Zinc	0.055	0.01
Nitrogen	0	4.66
Mercury	0	0
Arsenic	0	0
Lead	0	0
C:N ratio	0	7.98

4. Conclusions

Statistical analysis showed that nutrient removal (N and P) increased the efficiency of the WWTP through three stages: stage A, complete aeration (24 h, 43 months); stage B, decreased aeration (12 h, 17 months); and stage C, decreased aeration with biocalcium (12 h, 19 months). First, the stabilization of the biological process was sought; subsequently, the formation of nitrates was controlled through the decrease in aeration, and finally, the addition of biocalcium, a source of calcium carbonate, promoted the improvement of the effluent quality by increasing the pH value (7.41). The effluent characterization showed removal efficiencies of 50 and 69 % for sedimentable solids and total suspended solids, respectively, 76 % for BOD_5, 8.3 % for total phosphorus and 57 % for total nitrogen, and an improvement in the formation of flocks allowed better sedimentation. The sludge can be used safely in agriculture because EDS analysis showed only trace concentrations of arsenic and heavy metals (lead, mercury, cadmium, chromium, nickel, and zinc). The XRD characterization showed that the biocalcium sludge is more crystalline because a greater number of peaks indicated the presence of hydroxyapatite ($(Ca_5(PO_4)_3{\cdot}XH_2O)$), struvite ($MgNH_4PO_4{\cdot}X(H_2O)$), calcite ($CaCO_3$) and quartz ($SiO_2$). In addition, the increase in the nitrogen concentration in the sludge after biocalcium treatment improved the C:N ratio to 7.98; hence, the sludge can be used in agriculture for soil remediation due to its contents of calcium, phosphorus and nitrogen.

Data availability statement

No, Data will be made available on request.

CRediT authorship contribution statement

Laura Garduño Pineda: Formal analysis, Methodology. **Ivonne Linares Hernández:** Writing – original draft, Writing – review & editing. **Verónica Martínez Miranda:** Conceptualization, Project administration, Writing – original draft, Writing – review & editing.

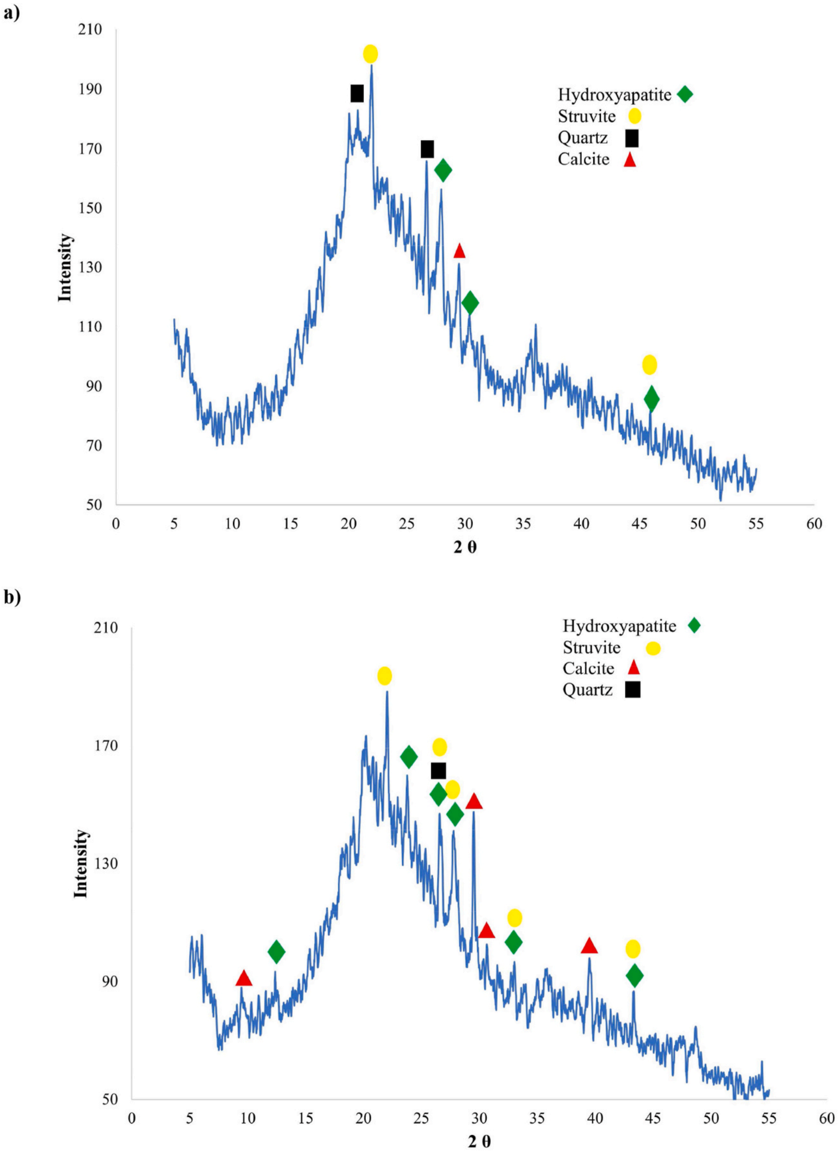


Fig. 8. a)X-ray diffraction of sludge from the WWTP before biocalcium treatment. 8b) X-ray diffraction of WWTP sludge after biocalcium treatment. Hydroxyapatite (◆) ($Ca_5(PO_4)_3{\cdot}XH_2O$), struvite (●)($MgNH_4PO_4$ X(H_2O)), calcite (▲) ($CaCO_3$) and quartz (■) (SiO_2).

Elía Alejandra Teutli Sequeira: Software, Validation. **Jesús Martínez Santa Cruz:** Data curation. **José Juan García Sánchez:** Data curation, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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