



Performance of an UASB Reactor at Lab-Scale Treating Domestic Wastewater with Low Concentrations of Copper

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Authors' contributions

This work was carried out in collaboration between all authors. Author IDBQ designed the study and provided financial support. Author MLSP designed the study, wrote the first draft of the manuscript and managed literature searches. Author JGA collaborated with sequential extractions and atomic absorption spectrophotometry analysis. Author MTGG managed literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The objective was to investigate the effect of copper at low concentrations in an Upflow Anaerobic Sludge Blanket (UASB) reactor performance under continuous conditions and its geochemical distribution in anaerobic sludge through sequential extraction.

Study Design: Continuous operation.

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Place and Duration of Study: Department of Basic Science, Universidad Autónoma Metropolitana -Azcapotzalco, between July 2013 and December 2013.

Methodology: A lab scale UASB reactor was inoculated with sludge from a full-scale UASB reactor treating brewery wastewater and it was allowed to acclimate during two months. After acclimation period, three different copper concentrations were added to UASB influent: 0.25, 0.65 and 1.35 mg l⁻¹, during one month each one. At the end of the experiment, freeze-dried samples of inoculum and UASB reactor sludge were treated with a series of solvents to perform sequential extractions. pH, temperature, oxidation reduction potential (ORP), total chemical oxygen demand (COD), the ratio between partial and total alkalinity, total suspended solids and volatile suspended solids were measured in UASB reactor influent and effluent.

Results: Sequential extractions showed that the fraction with the major affinity with copper was organic/sulfide bound, with a percentage distribution of 96%. Regarding UASB performance, copper caused a total alkalinity diminution in the effluent; however, the system buffer capacity was enough to overcome such instability, as partial alkalinity/total alkalinity ratio results confirmed. COD removal decreased 15% when 1.35 mg l⁻¹ Cu was added in comparison to acclimation period.

Conclusion: Copper addition in UASB reactor influent at low concentrations affected adversely its performance; though, did not cause complete inhibition to anaerobic microorganisms. Metal speciation results in the inoculum and the UASB reactor evidenced that the fraction which had the major affinity with copper was the organically/sulfide bound fraction, however, further studies are needed to establish which is the weight of organic matter and sulfide in copper binding.

Keywords: Anaerobic inoculum; copper geochemical distribution; copper toxic effects; UASB sludge.

1. INTRODUCTION

According to Torres [1], anaerobic treatment is an appropriate and suitable technology to treat municipal wastewater in developing countries located in tropical regions due to its lower investment and operational costs, easy operation and generation of value added products. Therefore, Latin America is the continent that currently has the largest number of wastewater treatment plants of this type. Nevertheless, the presence of inhibitory and toxic substances are often the main cause of anaerobic system failure since they could be present in municipal wastewater. Those substances include ammonia, sulfide, organics and heavy metals [2]. Among the last group, copper has been identified as a major concern for anaerobic treatment systems since it inhibits acetoclastic methanogenesis [3], affects volatile fatty acids (VFA) degradation [4] and has the biggest binding capacity to the anaerobic sludge [5]. Copper is a necessary trace element and is a cofactor for various enzymes [6]; however, copper ions can interact with intracellular iron-sulfur clusters of various proteins and inhibit their function under anoxic conditions [7]. According to the European Commission [8], copper presence in domestic wastewater is associated with corrosion and leaching of plumbing, fungicides (cuprous chloride), pigments, wood preservatives, larvicides (copper acetoarsenite) and antifouling paints.

Although total metal concentrations may indicate the overall level of metals in sludge, the mobility of heavy metals, their bioavailability and related eco-toxicity to plants, depend strongly on their specific chemical forms or ways of binding [9]. Thus, is essential to identify and quantify the different species, forms or phases present in the sludge, in order to assess toxic effects on anaerobic microorganisms [10]. As stated by Tessier et al. [11], this identification and quantification process is known as chemical speciation; and according to Barceló et al. [12], it was divided into five fractions and each fraction possessed distinctive characteristics and migration behavior in the environmental matrix. This means that each fraction might play a different role considering its toxicity to living beings.

As stated by Ortner et al. [13], some work has been done to investigate the effects of copper on granular sludge in Upflow Anaerobic Sludge Blanket (UASB) reactors, including analysis of metal speciation. Leighton and Forster [14] treated synthetic wastewater containing copper, among other metals, in order to examine the impact of short-term (30 hours) copper exposure on thermophilic methanogenesis. Osuna et al. [15] investigated the effect of the absence of copper on the conversion of a mixture of volatile fatty acids by a distillery anaerobic granular sludge. Van Hullebusch et al. [16] compared the extraction efficiencies of three operationally

defined extraction procedures, when applied to leaching of metals from anaerobic methanogenic granules from full-scale UASB reactors. Zandvoort et al. [17] evaluated trace element content and deficiency effects in granular sludge from full-scale UASB reactors. Aquino and Stuckey [18] investigated the effect of copper complexation on bioavailability and toxicity in a laboratory continuously stirred tank reactor (CSRT).

Up to date, there is no investigation on copper geochemical distribution in granular sludge from UASB reactors through metal speciation when UASB reactor is used to treat real municipal wastewater containing copper at low concentrations at long-term operation. Thus, the objective of this work was to investigate the effect of copper at low concentrations in UASB reactor performance under continuous conditions and its geochemical distribution in anaerobic sludge through sequential extraction by analyzing the results obtained in an experiment at lab-scale in which UASB reactor was operated under hydraulic retention time (HRT) 8 hours, sludge retention time (SRT) of 90 days and fed with copper added municipal wastewater.

2. MATERIALS AND METHODS

2.1 Experimental Set Up

A lab scale UASB reactor (6.1 l volume, PVC) was used in this study. The UASB reactor was inoculated with sludge from a full-scale UASB reactor treating brewery wastewater and it was allowed to acclimate during two months. A sample of inoculum was stored at 4°C in order to carry out metal speciation and total copper analysis. UASB reactor treated municipal wastewater obtained after primary sedimentation in the wetland system constructed at the Metropolitan Autonomous University - Azcapotzalco Campus and it was sampled daily at the same hour to reduce the load variations that normally occur during the day. Characterization of municipal wastewater is provided in Table 1. According to the Mexican law, the maximum permissible values for direct discard in rivers are 75 mg l⁻¹ for biological chemical demand (BOD), 40 mg l⁻¹ for nitrogen, 20 mg l⁻¹ for phosphorous and 75 mg l⁻¹ for suspended solids (SS).

UASB reactor was maintained at environmental temperature (approx. 21°C) and operated under

a hydraulic retention time (HRT) of 8 hours. During the experiment was not carry out any discharge of sludge, given a sludge retention time of approximately 90 days, including start up time. Municipal wastewater was pumped to the UASB reactor using a peristaltic pump (Model Masterflex 7553-30, Cole-Parmer, USA). After an acclimation period of two months, a concentrate solution (1900 mg l⁻¹ as Cu) of cupric nitrate (Cu(NO₃)₂·3H₂O) (Analytical grade, J.T. Baker) was diluted in the municipal wastewater, in order to obtain three different copper concentrations in UASB reactor influent: 0.25±0.02 mg l⁻¹, 0.65±0.15 mg l⁻¹ and 1.35±0.17 mg l⁻¹, which are similar to those found in José Antonio Alzate Dam, located in Lerma River Upper Basin, Mexico. The Lerma River is the second largest river in Mexico and also serves as a source of drinking water and electricity to Mexico City [19]. Each copper concentration was applied to the UASB reactor during a month. At the end of the experiment, a sample of UASB reactor sludge was stored at 4°C with the purpose of carrying out metal speciation and total copper analysis.

Table 1. Characterization of municipal wastewater

Parameter	Value
pH	8.09±1.04
Temperature (°C)	21.11±1.06
Dissolved oxygen (mg l ⁻¹)	0.22±0.01
ORP (mV)	-275.74±10.75
Total COD (mg l ⁻¹)	209.81±21.49
Alkalinity (mg l ⁻¹ CaCO ₃)	224.76±11.23
Copper (mg l ⁻¹)	0.14±0.01

2.2 Analytical Methods

pH, temperature and oxidation reduction potential (ORP) were measured daily in UASB reactor influent and effluent using a Ion specific electrode (ISE) for each parameter (Model LabQuest, Vernier, USA) and total chemical oxygen demand (COD) and alkalinity were measured twice per week according to the Standard Methods [20]. The ratio between partial and total alkalinity (PA/TA ratio) was calculated according to Soto et al. [21]. Total suspended solids (TSS) and volatile suspended solids (VSS) were determined in the inoculum and sludge from UASB reactor, according to the Standard Methods [20]. Copper concentration in UASB influent and effluent was measured once per week by atomic absorption spectrophotometry (Model SOLAAR M6, Thermo Elemental, USA).

2.3 Copper Speciation

Prior to metal speciation, samples of inoculum and UASB reactor sludge previously refrigerated were frozen and then freeze-dried using a liophilizer (Model Benchtop SLC, VirTis, USA). After that, inoculum and UASB reactor sludge samples for metal speciation were separated into fractions by a multi-extract technique [12]; which is a modified technique from Tessier et al. [11] and Calmano and Forster [22]. In this technique, metals are removed sequentially based on the strength of their attachment to the sludge. Freeze-dried samples of inoculum and UASB reactor sludge (0.5 g) were treated with a series of solvents (Table 2) in order to perform sequential extractions, which consist of several sequential steps which gave rise to five different fractions.

Blanks were analyzed in the fractions where analytical grade reagents were used. The samples were stirred using an orbital shaker (Model SW23, Julabo, Germany) at 50 RPM. After the addition of each solvent, liquid was then separated using a centrifuge (Model Allegra 21, Beckman Coulter, USA) at 4500 RPM for 5 min, the solids were washed twice with de-ionized water (10 ml) and recovered by further centrifugation and then freeze-dried.

The liquids obtained from each fraction were filtered through a glass microfiber paper (Whatman, GF/B). The filtrate obtained was acidified to pH 2 with nitric acid. Copper concentration in each one of these filtrates was measured in duplicate by atomic absorption spectrophotometry (Model SOLAAR M6, Thermo Elemental, USA).

The residual fraction (F6) contains the residual solids that occlude the metals in their crystalline structures [23]; these crystalline structures are associated with silicates, which, due to the characteristics of the anaerobic sludge, are not present in it [24]; for this reason this fraction was not carried out in the samples.

2.4 Acid Digestions

Total copper concentration in inoculum and UASB reactor sludge were determined in triplicate by acid digestions with 0.5 g of freeze-dried sample, 5 ml of nitric acid (Analytical grade, J.T. Baker), 1 ml of hydrofluoric acid (Analytical grade, J.T. Baker) and 5 ml of de-ionized water. The samples were placed in a Teflon vessel and digested during 30 min using a microwave digestion system (Model Mars 5, CEM, USA). Acid digestions were performed three times until the samples were completely dissolved. Blanks were carried out on both samples.

2.5 Atomic Absorption Spectrophotometry

Copper concentration in each sample was analyzed in triplicate by atomic absorption spectrophotometry (Model SOLAAR M6, Thermo Elemental, USA). A 5 mg l⁻¹ Cu reference standard was also measured in order to guarantee the analytical results. This reference standard was prepared diluting a high purity Cu standard of 1000 mg l⁻¹ (Merck) in de-ionized water and acidified with 1 ml of acid nitric (High purity, Merck). The mean values which resulted from this had standard deviations of less than 5%. Measures were carried out using a wavelength of 324.3 nm, which is the most sensible for Cu, a slit of 0.5 nm and the method detection limit was 0.03 mg l⁻¹ Cu.

Table 2. Sequential extractions for copper speciation

Fraction	Solvent	Stirring time
Exchangeable, F1	10 ml of ammonia acetate (CH ₃ COONH ₄) [1M] Suprapur (Merck)	2 h
Acid-soluble, F2	10 ml of sodium acetate (CH ₃ COONa) [1M] Suprapur (Merck)	5 h
Fe and Mnoxihydroxides, F3 and F4	10 ml of hydroxylamine hydrochloride (NH ₂ OH.HCl) [0.1M] (Analytical grade, J.T. Baker)	12 h
Organically bound and sulfides, F5	10 ml of hydrogen peroxide (H ₂ O ₂) (30%) (Analytical grade, J.T. Baker) and 4 ml of acid nitric 65% Suprapur (Merck)	24 h at 60°C

3. RESULTS AND DISCUSSION

3.1 Copper Speciation in the Inoculum and UASB Reactor Sludge

The results obtained with the multi-extract technique in the inoculum and UASB reactor sludge are summarized in Tables 3 and 4.

Table 3. Chemical fraction of copper (mg Cu/kg sludge) in inoculum and UASB sludge

Fraction	Inoculum	UASB sludge
Exchangeable, F1	0.17	128.44
Acid-soluble, F2	ND ^a	131.42
Fe and Mn oxihydroxides, F3 and F4	ND ^a	56.41
Organically bound and sulfides, F5	98.61	7553.86
Sum ^b	98.78	7870.13
Copper obtained through acid digestion	104.71	7161.81
Recovery (%) ^c	94.3%	91%

^a Below method detection limit (0.03 mg l⁻¹), ^b Sum of all fractions analyzed, ^c Ratio Sum/Total

Table 4. Chemical fraction of copper (%) in inoculum and UASB sludge

Fraction	Inoculum	UASB sludge
Exchangeable, F1	0.17	1.63
Acid-soluble, F2	0	1.67
Fe and Mn oxihydroxides, F3 and F4	0	0.71
Organically bound and sulfides, F5	99.83	95.99

Table 3 shows the mass of copper recovered after application of the sequential extraction scheme. As can be seen, the sum of all fractions is reasonably similar to the total contents obtained after acid digestion with nitric acid of the original samples. Recoveries of 90-95% were reached in both samples, values that are very similar to those reported in the literature [10,25], which confirms the validity and reliability of the obtained results. Table 3 also shows that, in comparison with UASB reactor sludge, the inoculum has practically no copper; therefore, UASB sludge effectively retained an important part of the copper added to the influent reactor; besides, copper concentrations in UASB effluent were 0.09±0.01 mg l⁻¹, 0.22±0.04 mg l⁻¹ and 0.17±0.05 mg l⁻¹ when the doses of 0.25±0.02 mg

l⁻¹, 0.65±0.15 mg l⁻¹ and 1.35±0.17 mg l⁻¹, respectively. These results indicate that copper removal in UASB reactor ranged from 55% to 90%, confirming the previous affirmation. With the copper values obtained in UASB influent and effluent and knowing the mass of sludge present in the reactor (0.93 kg) it was possible to calculate approximately the mass of copper retained in the UASB reactor, which was 7255.56 mg Cu/kg sludge, result that is in good agreement with values presented in Table 3.

Copper is a necessary trace element and is a cofactor for various enzymes [26]; however, is not biodegradable and can bioaccumulate to potentially toxic concentrations [7]. Although heavy metals are bound to the various types of sludge, the way in which they are bound will affect the impact they have on the sludge itself and, subsequently, on the surfaces with proteins and acid groups that serve as binding sites [27]. Copper presence in organic fraction has been also ascribed to the reaction with carboxyl group to form chelates [7,25]. Besides, copper binding and sequestration by extracellular polymeric substances (EPS) has also been demonstrated [28]. Extracellular polymeric substances (EPS) are major components of granular matrix and are essential for microbial metabolism as well as for the formation and stability of biofilm [29]. EPS consist of different insoluble macromolecules such as polysaccharides, proteins, nucleic acids and other polymeric materials found on cell surfaces or in the intercellular space of microbial aggregates [30]. They have multiple functions that include cell aggregation, formation of a protective barrier, retention of water and adhesion to surfaces. EPS also can bind metals [31]. Furthermore, the bacterial interface can also function as an important metal binding surface [32]. The strong sorption of copper by organic material (EPS and bacterial cells) in granules of UASB reactor possibly occurs to precipitation, co-precipitation, adsorption and binding [33].

On the other hand, ORP inside the UASB reactor (Fig. 1) is adequate to sulfate reduction [34], therefore copper also can interact with sulfur and produce precipitates [35]. According to Morse and Luther [36], chalcocite (Cu₂S) can be formed in the presence of sulfide and even different Cu sulfide minerals can coexist in the anaerobic sludge matrixes [37]. These metals bound to the sulfides are mainly leached in the organic/sulfides fraction in natural sediment, soil or sludge [38]. Copper may be also adsorbed in large quantity at the surface of pyrite minerals

[39]; however, little evidence has been found in the literature for Cu accumulation in the crystalline lattice of the iron sulfides. Thus, further studies are needed in order to prove which is the weight of organic matter and sulfide in copper binding.

3.2 UASB Reactor Performance

Oxidation reduction potential (ORP) mean values obtained during the experiment were -312.2 ± 28.0 mV in acclimation period, -324.4 ± 28.0 mV when

the dose of 0.25 mg l^{-1} was added, -375.5 ± 21.9 mV for the dose of 0.65 mg l^{-1} and -392.6 ± 10.4 mV for the dose of 1.35 mg l^{-1} . Galli and McCarty [40] reported that methanogenesis occurs with ORP values below than -170 mV, therefore the atmosphere inside the UASB reactor was suitable for methane production.

Figs. 1, 2 and 3 show pH, total alkalinity and the ratio between partial and total alkalinity (PA/TA ratio), respectively, obtained during the entire experiment.

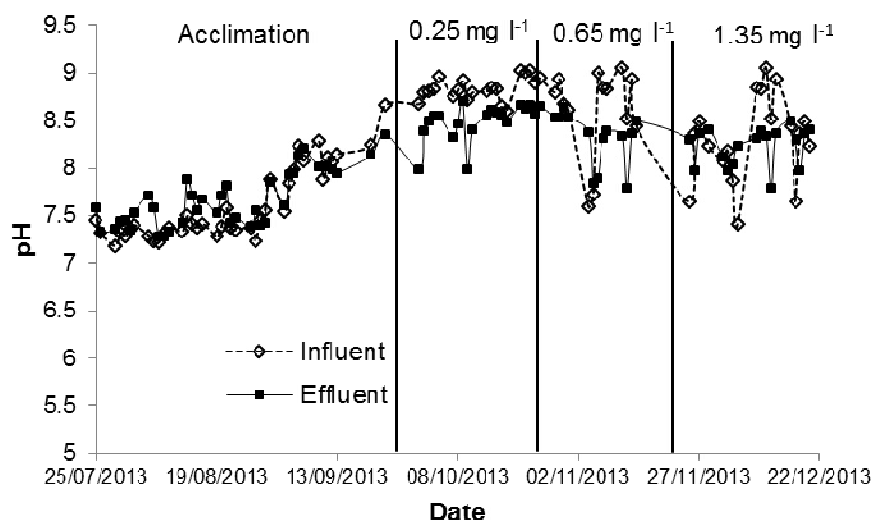


Fig. 1. pH values obtained in UASB reactor influent and effluent

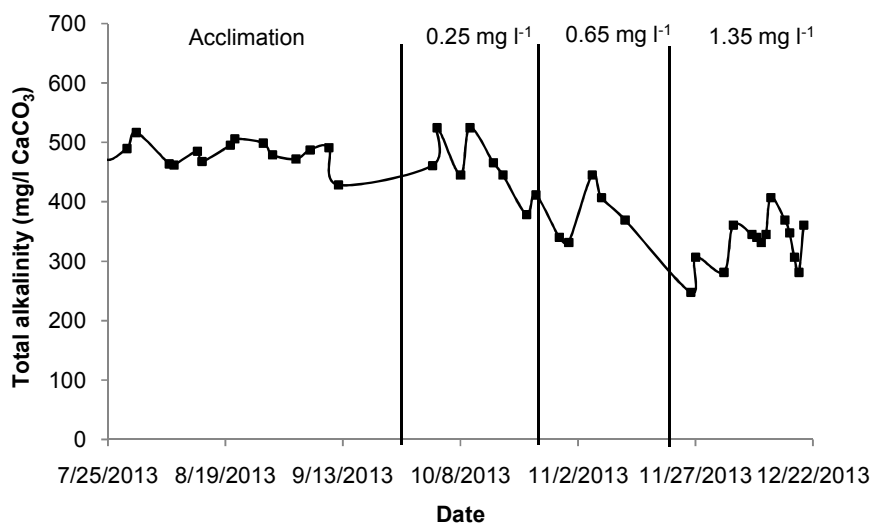


Fig. 2. Total alkalinity values obtained in UASB reactor effluent

Fig. 1 shows that pH values in UASB effluent followed the influent tendency. pH values in municipal wastewater were between 7.5 and 9, which are higher than those that usually occurs in domestic wastewater; this could be associated with the fact that sewage in the university campus also collects wastewater generated in laboratories. On the other hand, pH in UASB reactor was slightly basic (between 7.3 and 8.7) throughout the entire experiment, which is adequate for UASB reactor operation [41]. Total alkalinity values (Fig. 2) were higher during acclimation than in the period where copper was added to UASB influent; probably due to the copper addition caused volatile fatty acids (VFA) accumulation inside the UASB reactor [4] which produced alkalinity consumption inside the UASB reactor and consequently its drop in effluent. Alkalinity values obtained during the experiment show that copper addition in influent caused a slight instability in UASB reactor; however, the system buffer capacity was enough to overcome such instability and pH was not affected.

This affirmation was corroborated by partial and total alkalinity ratio results (Fig. 3); according to Soto et al. [21], the ratio between partial and total alkalinity is a parameter that allows the monitoring VFA accumulation inside anaerobic reactors and it must be maintained near from 1 in order to prevent acidification. During the experiment, PA/TA ratio mean value was 0.95 ± 0.02 , indicating that if VFA accumulation occurred, it was controlled by the buffer capacity inside the UASB reactor.

Chemical oxygen demand (COD) concentrations obtained in the influent and effluent of UASB

reactor depicted in Fig. 4. When the acclimation period started, COD at UASB influent were below 200 mg l^{-1} and, after approximately a month, it increased to stabilize at a value around 300 mg l^{-1} . This rise in the organic load was a consequence of an increase in university campus population due to the end of the holiday period. During the acclimation period COD concentration in UASB effluent remained below 100 mg l^{-1} , but when copper addition started, this parameter increased until 168 mg l^{-1} for 0.25 mg l^{-1} and 0.65 mg l^{-1} Cu doses. When the dose of 1.35 mg l^{-1} was added, the range of COD concentration in UASB effluent was around 145 and 169 mg l^{-1} . Regarding COD percentage removal, it had an average value of $49.6 \pm 2.6\%$ during the acclimation period. This percentage removal is low and could be related with the temperature of the experiment; according to Torres [1], at temperatures below 30°C the maximum rate of anaerobic digestion decreases at a rate of 11% per Celsius degree, thus COD removal efficiency could be affected by temperature in this experiment.

The percentage removal was $49.4 \pm 3.4\%$ when the dose of 0.25 mg l^{-1} was added; after that COD removal decreased approximately 10% when the higher doses were applied ($39.1 \pm 2.0\%$ for 0.65 mg l^{-1} and $38.7 \pm 2.7\%$ for 1.35 mg l^{-1}). The toxicity of Cu ions (Cu^{2+} and Cu^+) to microorganisms is well documented; Cu ions can interact with intracellular iron-sulfur clusters of various proteins and inhibit their function under anoxic conditions [7].

Methane production in UASB reactor is depicted in Fig. 5.

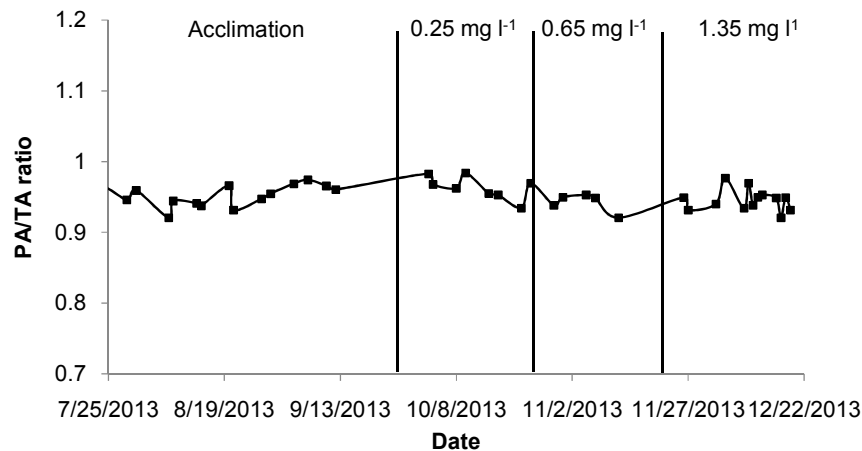


Fig. 3. Partial and total alkalinity (PA/TA) ratios obtained in UASB reactor effluent

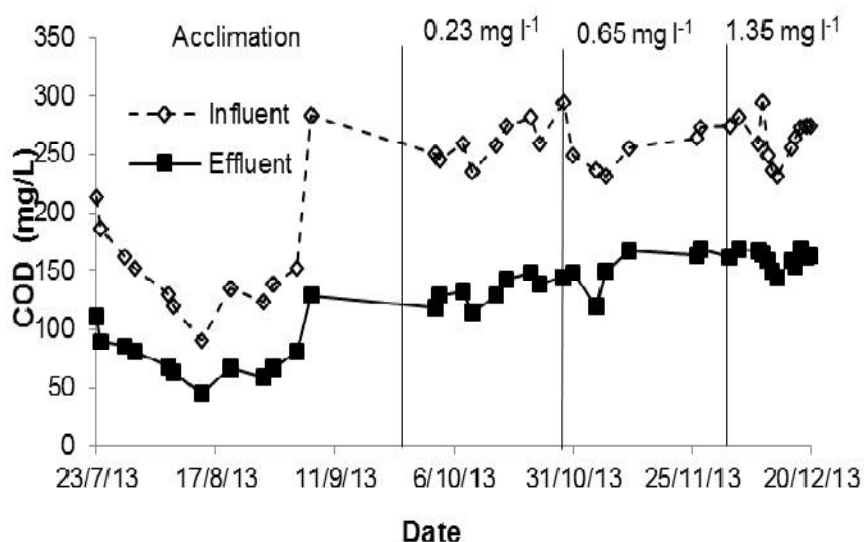


Fig. 4. COD concentration in the influent and effluent of UASB reactor during the experiment

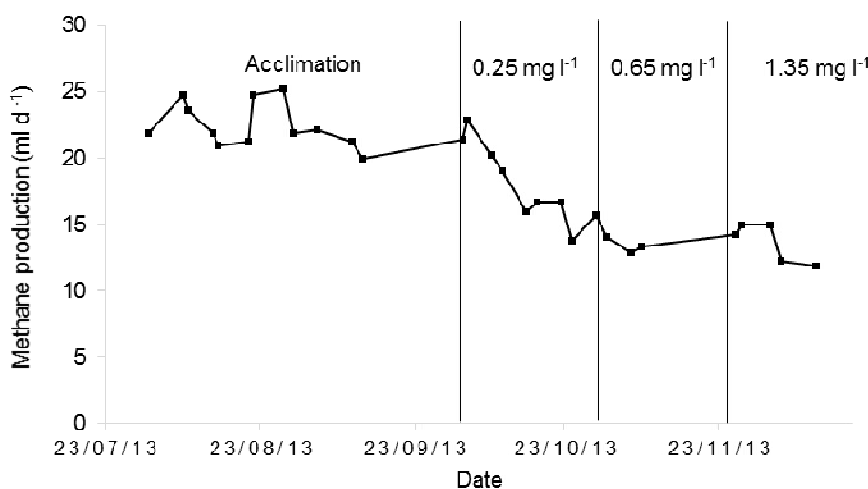


Fig. 5. Methane production in UASB reactor during the experiment

Methane production ranged from 20 to 25 ml d⁻¹ during acclimation period and it sharply decreased when the dose of 0.25 mg l⁻¹ of copper was added until reached approximately 15 ml d⁻¹. After the addition of 0.65 and 1.35 in UASB influent, methane production stabilized, although the lowest methane production (11 ml d⁻¹) was obtained with the highest concentration of copper.

The results found demonstrate the negative impact that low doses of copper have on UASB reactor performance and are in accordance with the findings of several authors [4,14,25,42,43]

which reported that copper caused the highest toxicity to methanogenic microorganisms.

On the other hand, copper was added to UASB influent diluting a solution of cupric nitrate (Cu(NO₃)₂·3H₂O), as described in Material and methods section. Nitrate have been reported to cause low to moderate inhibition of methanogenesis [44]; nevertheless, it is important to point out that nitrate concentrations added to UASB influent (0.49 mg l⁻¹ NO₃⁻ for 0.25 mg l⁻¹ Cu, 1.30 mg l⁻¹ NO₃⁻ for 0.65 mg l⁻¹ Cu and 2.64 mg l⁻¹ NO₃⁻ for 1.35 mg l⁻¹ Cu) were much lower than those reported in literature [45,46], so detrimental effect in COD removal and methane

production in UASB reactor could be associated with copper rather than with nitrate.

Results from copper speciation performed (See copper speciation results section) demonstrated that this metal was mainly associated with the organic fraction and sulfides in anaerobic sludge, thus its detrimental effect on UASB reactor performance derives from this fact. Still, it is important to note that even though system efficiency dropped due to the copper presence in UASB influent, anaerobic digestion was not completely inhibited. The doses studied in this experiment were lower than those described in literature as inhibitory: Karri et al. [47] indicated severe inhibition of anaerobic digestion of sewage sludge with copper concentrations ranging from 70 to 400 mg l⁻¹. The activity of an acetate-degrading methanogenic culture was inhibited by 50% at 12.5 mg l⁻¹ of copper [42]. A mixed methanogenic culture was inhibited by 50% by 10 mg l⁻¹ of copper [43]. For this reason, the copper concentrations dosed to the UASB reactor in this experiment affected its performance without inhibiting it.

The results obtained in this experiment demonstrate that the presence of copper, even at low concentrations, should be taken into consideration if UASB reactors are used to treat wastewater containing this metal, though at low concentrations copper will not cause total inhibition of microbial activity in UASB reactor.

4. CONCLUSION

In this experiment, a lab-scale UASB reactor was operated under a hydraulic retention time (HRT) of 8 hours and fed with municipal wastewater added with three different copper concentrations (0.25±0.02 mg l⁻¹, 0.65±0.15 mg l⁻¹ and 1.35±0.17 mg l⁻¹) in order to analyze the performance of an UASB reactor treating domestic wastewater with low copper concentrations; the following conclusions were drawn:

Copper concentrations studied in this experiment affected adversely UASB reactor performance: addition of 0.25 mg l⁻¹ Cu in UASB reactor influent caused a decrease of approximately 30% in methane production; while the concentration of 0.65 mg l⁻¹ caused a 10% decrease in COD removal regarding the values found in the acclimation period. When the dose of 1.35 mg l⁻¹ was added, COD removal and methane production reached their lowest values (33% and

11 ml d⁻¹, respectively). Similar trends were found in alkalinity results: addition of 0.25 mg l⁻¹ Cu decreased 20% alkalinity values in UASB effluent, whereas 1.35 mg l⁻¹ concentration caused a diminution of around 30%; however, the system buffer capacity was able to overcome such instability, as partial and total alkalinity ratio results evidenced, and pH inside anaerobic reactor was not affected. Concerning copper geochemical distribution in the inoculum and the UASB reactor sludge, metal speciation results evidenced that the fraction which had the major affinity with copper was the organically/sulfide bound fraction (F5), counting for approximately 96%; however, further studies are needed in order to establish which is the weight of organic matter and sulfide in copper binding.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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