

Bio-immobilization of Cr (VI) and its Impact on the Performance of a Pilot Scale Anaerobic Sludge Reactor Treating Municipal Wastewater

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THIS study aimed to emphasize the effect of hexavalent chromium Cr (VI) on the performance of a pilot scale hybrid up-flow anaerobic sludge blanket (H-UASB). The system was packed with non-woven polyester fabric (NWPF) and continuously fed with real municipal wastewater at a hydraulic retention time (HRT) 7.5 h and average organic loading rate (OLR) 1.24 Kg COD m³/d. The system achieved satisfactory removal rates for total suspended solids (TSS) (86.44 %), chemical oxygen demand (COD) (74.18 %) and biological oxygen demand (BOD₅) (84.8%) without the addition of Cr (VI). Results indicated that feeding the reactor with different concentrations of chromium ions ranged from 0.1 – 1.3 mg/l (i.e: 600 mg / m³. d to 6000 mg / m³. d) decreased the residual concentration of Cr (VI) in the final effluent to 0.019 mg/l, while the accumulation of chromium ion increased in the biobed (160 mg/kg) and in excess sludge (165.44 mg/kg). This was due to the biological reduction of Cr (VI) to Cr (III) by the action of sulphate and chromate reducing bacteria. Increasing the concentration of Cr (VI) up to 6000 mg / m³. d reduce the biochemical reactions of the anaerobic microorganisms and consequently decrease the removal efficiency of TSS from 86.44% to 62.06, COD from 74.2 % to 57.07 % and BOD₅ from 84.8% to 61.92%.

Keywords: Anaerobic digestion, hazardous wastewater, hexavalent chromium, hybrid up-flow anaerobic sludge blanket, non-woven polyester fabric.

Introduction

The available clean water and freshwater supplies are depleting due to the increase of population growth, industrialization and agricultural activities. Heavy metals are one of the emerging contaminants in the environment. They represent a serious problem due to their potential toxicity for human beings and environment as they are non-degradable and can bio-accumulate through food chain. Tchounwou et al. [1], indicated that the main sources of heavy metal pollution are burning of fossil fuels, municipal waste, pesticides, fertilizers, sewage sludge, electroplating industry, mining and smelting of metallic ferrous ores.

Also, heavy metals can enter a water supply by industrial consumer wastes, or even from acidic rain breaking down soils and releasing heavy metals in stream, lakes, river and ground water. The untreated industrial effluents and domestic

wastewater contain variable concentrations of heavy metals such as nickel, lead, chromium, arsenic, cadmium, copper, zinc and mercury [2]. These heavy metals have a noticeable effect on the aquatic flora and fauna which through bio-magnification enter the food chain and consequently affect the human beings as well.

Chromium is considered one of the most hazardous heavy metal ions. It originates from industrial wastewater such as electroplating, leather tanning, wood preservation, mine tailings and others [3]. Chromium can be detected in domestic wastewater from household products (cleaning and personal care) or from commercial sources such as car washes. It can exist in two states either hexavalent (VI) or in trivalent (III), Cr (VI) is more toxic than Cr (III). The Agency for Toxic Substances and Diseases Registry (ATSDR) classifies Cr (VI) as the 16th most hazardous substance while, the US-EPA regulate a threshold

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limit of Cr (VI) to be in domestic water supplies as 0.05 mg/l [4]. Hexavalent chromium is reduced to Cr (III) in the presence of any reducing agent such as: organic matter, hydrogen sulfide, ammonium nitrate, sulfur, iron sulfide and nitrates [5,6].

Under anaerobic conditions especially in the UASB, the pH ranges from 6.5-8.5 and Cr (VI) can exist as CrO_4^{2-} which is reduced directly to Cr (III) by sulphate and chromate reducing bacteria. They utilize the organic matter present in wastewater as an electron donor. Moreover, Cr (VI) can undergo biological degradation via microbial conversion into Cr (III). Recently anaerobic up flow sludge blanket packed with different materials (H-UASB) has been used successfully for the removal of heavy metals in wastewater. Packing materials are considered as one of the factors affecting the removal efficiency of different pollutants. Abou-Elela *et al.* [7-9] used a Non-Woven Polyester Fabric (NWPF) as a novel and effective bio-bed. Due to its high porosity (43.8 μm) and surface area (2000 $\text{m}^2.\text{m}^3$) and non-toxic effect it enhanced the performance of the UASB.

Limited information and research works are available about the bio-sorption and removal of heavy metals in such treating system. Therefore, the aim of this work was to investigate and evaluate the impact of Cr (VI) on the performance of a hybrid up flow anaerobic sludge blanket treating municipal wastewater with a special emphasis on its adsorption/biosorption. The novelty of the work is that the use of the NWPF as a biobed in the reactor enhanced the biochemical reaction in the H-UASB and aided the deposition of $\text{Cr}(\text{OH})_3$ in/on the packing material.

Material and Methods

Experimental set-up

The pilot scale treatment system was installed in a nearby WWTP. It consists of a mixing tank for receiving natural pre-screened raw wastewater as well as different concentrations of Cr (VI) followed by H-UASB and an inclined plate settler. A block diagram of the treatment system used in this study is shown in Fig.1.

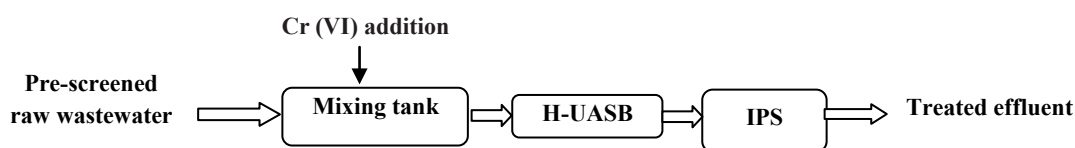


Fig. 1. Block diagram for the pilot scale H-UASB treating municipal wastewater with different addition of Cr (VI).

Description of the H-UASB/IPS

The system was similar to that used by Abou-Elela *et al.*, [8]. It was manufactured from PVC sheets of 10 mm thickness. It was designed based on a flow rate of 5-6 m^3/d with average hydraulic retention time (HRT) 7.5 h. The effective volume of the reactor was 1.81 m^3 with internal dimensions of 102 cm x 116 cm and a height of 153 cm. The reactor was supported by NWPF fixed in a stainless-steel basket (60 x 60 cm) under the gas, liquid and solid separator (GLS) in the upper part of the up-flow anaerobic sludge blanket. The height of the packing material was 0.3 m and placed above the sludge blanket zone. The reactor was provided with piping arrangement for influent and effluent wastewater. Wastewater sampling points, drainage pipe and three sludge sampling points were arranged at different heights along the reactor at 9 cm, 35 cm and 50 cm from the reactor's bottom.

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Description of feeding tank

To study the effect of Cr (VI) on the performance of H-UASB, a feeding tank for chromium addition was manufactured and established prior to H-UASB to receive both the municipal wastewater as well as different concentrations of Cr (VI). The tank was made of polypropylene with an effective volume of 0.8 m^3 , internal dimensions of 100 cm x 100 cm and a height of 80 cm. It was provided by a mixer to obtain a homogeneous wastewater sample. Different known concentrations of potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) were prepared for injection to the screened wastewater prior treatment in H-UASB. Four experimental runs (R1, R2, R3, R4) were carried out for a duration of 245 days. The daily feeding concentrations of Cr (VI) were 600 $\text{mg} / \text{m}^3. \text{d}$ for 110 days (R1), 1800 $\text{mg} / \text{m}^3. \text{d}$ for 55 days (R2), 3000 $\text{mg} / \text{m}^3. \text{d}$ for 50 days (R3) and 6000 $\text{mg} / \text{m}^3. \text{d}$ for 30 days (R4).

Start-up and operating conditions of the system

The system was operated continuously for almost two years from June 2015 to June 2017. In order to reach the steady state conditions (without addition of heavy metals), the system was operated at a flow rate of 1.0 m³/d then gradually increased to 3.0 m³/d, until it reached 5.76 m³/d. This was carried out to minimize the sludge washout from the H-UASB reactor as well as to give enough time for the development of the biofilm upon the supporting material in the UASB reactor. The system reached the steady state after acclimatization period of 125 days from the first inoculation of the sludge into the H-UASB reactor. This was assured by constant measurements of COD for the influent and the effluent.

Sampling points

Samples were collected from different points as follows:

- A. Raw wastewater without heavy metal addition,
- B. Influent wastewater to H-UASB after the addition of different concentrations of Cr (VI),
- C. H-UASB effluent after sedimentation,
- D. Mixed sludge samples at different heights in the H-UASB,
- E. Sludge accumulated in the packing material from the top, middle and bottom of the NWPF container.
- F. Excess sludge.

Analyses

Physico-chemical analyses

Performances of the H-UASB as well as the removals of Cr (VI) were monitored by complete physico-chemical analyses. All the analyses, unless otherwise specified, were carried out according to the American Public Health Association for the Examination of Water and Wastewater [10]. The analyses include pH, COD, BOD₅, TSS, Total Nitrogen (TN), Ammonia (NH₃), Total Phosphate (TP) and Hydrogen Sulfide (H₂S). Chromium ion concentration was analyzed in the influent and effluent using Plasma Emission Spectroscopy, ICP-OES 7300DV (Perkin Elmer, U.K), Method 3120. Sludge analyses in the H-UASB effluent and accumulated on the packing material were carried out. It includes total sludge weight (TS) at 105°C, volatile sludge (VSS) at 550°C and total fixed solids (TFS). In order to determine the sludge retained on the surface of the NWPF, different pieces from the top, middle and the bottom in the stainless-steel basket of NWPF

were collected and washed using distilled water for complete removal of any attached biomass. The eluted biomass was analyzed for TS and VSS.

Toxicity characteristic leaching procedure (TCLP)

The Egyptian regulation for Toxicity characteristic leaching procedure (TCLP) (law 4/1994) follows the same regulation stated by United States Environmental Protection Agency (US-EPA). Triplicate samples of five grams sludge were transferred to a shaking bottle. Leaching with an amount of extraction fluid equal to 20 times the sludge weight (leaching fluid: 5.7 ml glacial acetic acid (99.5% CH₃COOH, BDH) diluted with deionized water to a volume of one liter, when correctly prepared, the pH of this fluid will be 2.88 ± 0.05). Slowly add the amount of appropriate leaching fluid to the extractor vessel. Close the extractor bottle tightly (Teflon tape was used to ensure a tight seal), secure in shaking device, mechanical reciprocating shaker "Julapo-SW- 20C", and rotate at 30 ± 2 rpm for 18 ± 2 h at ambient temperature (i.e. room temperature in which leaching takes place). The pH was monitored during the experiments and additional acid was added as necessary to maintain the pH of the sludge within (0.2 pH units of the desired value and then filtered through a 0.6 – 0.8 μm glass fiber filter. Leached chromium was analyzed in the filtrate using ICP-OES 7300DV (Perkin Elmer, U.K) [11].

Results and Discussion

Performance of the treatment system without Cr (VI) addition (control)

The average concentration of Cr (VI) was detected as a trace value (0.065 mg/l) in the raw municipal wastewater fed to the H-UASB. Table 1 shows the efficiency of the H-UASB without the addition of Cr (VI) ion. The average percentage removal of TSS, COD, BOD₅ were 86.44%, 74.2%, 84.8%, respectively. The use of NWPF as a bio-bed in the UASB enhanced the biomass entrapment, increased the surface area and reduced the sludge washout [9]. These results are better than that obtained by De la Varga et al [12], where the percentage removal of COD was 51.7 %, BOD₅ 43.04 % and TSS 77.5 %. Also, the results revealed that residual ammonia concentration increased in the effluent of H-UASB. It ranged between 25.0 and 38.0 mg N/l with a mean value of 29.4 mg N/l compared with the average concentration in the influent raw wastewater

(18.95 mg N/l). Ammonia is produced due to degradation of proteinases organic material and conversion of organic nitrogen under anaerobic conditions [13]. The concentration of hydrogen sulfide in the effluent increased with a mean value of 8 mg/l compared with the concentration in influent raw wastewater (2.82 mg/l). These results agree with Chen *et al.* [14]. They stated that in anaerobic reactors sulphate is reduced to sulphide by sulphate reducing bacteria (SRB).

Performance of the H- UASB inoculated with different concentrations of Cr (VI)

Four different concentrations Cr (VI) namely; 600, 1800, 3000, 6000 mg/m³. d was added incrementally to the treatment system through four different experimental runs (R1, R2, R3, R4). The percentage removal of Cr (VI) increased

by increasing its concentration in the feeding wastewater. The order of removal efficiency was R4>R3>R2>R1 as shown in Fig. 2. Their corresponding percentages removal were 87.87% at 600 mg/m³.d, 94.22 % at 1800 mg/m³.d, 92.25 % at 3000 mg/m³.d and 98.47 % at 6000 mg/m³.d. Results can be explained that reduction process induced by hexavalent chromium in the presence of biological sulfidogenesis was carried out by SBR (Desulfobacter, Desulfobulbus and Desulfovibrio) [15]. Figure 3 shows that the maximum Cr (VI) reduction rate was 3.95 mg Cr (VI)/L/h and this is in presence of mixed sludge culture formed from anaerobic sludge and the hydrogen sulfide produced in the H-UASB which stimulate the SBR to reduce Cr (VI) into Cr (III).
Mechanism of Cr ions removal in the sludge

TABLE 1. Physico-chemical characteristics of the H-UASB effluent without the addition of Cr (VI)

Parameters	Unit	Raw Wastewater	Treated effluent				Mean %R
		Mean	Min.	Max.	Mean	SD (±)	
pH	--	7.45	7	7.5	7.0-7.5	0.14	---
TSS	mg/l	144.82	16	25	19.64	3.32	86.44
COD	mgO ₂ /l	377.73	79	118	97.00	10.94	74.18
BOD ₅	mgO ₂ /l	227.90	29	45	34.64	4.54	84.8
TN	mg/l	62.40	41.3	55.2	47.20	1.7	24.05
NH ₃ -N	mgN/l	23.80	36.9	42.9	39.60	3.3	---
TP	mgP/l	5.46	1.9	4.1	3.18	0.60	41.76
H ₂ S	mg/l	2.82	4.9	10	8.01	1.64	---

*Average of 11 samples

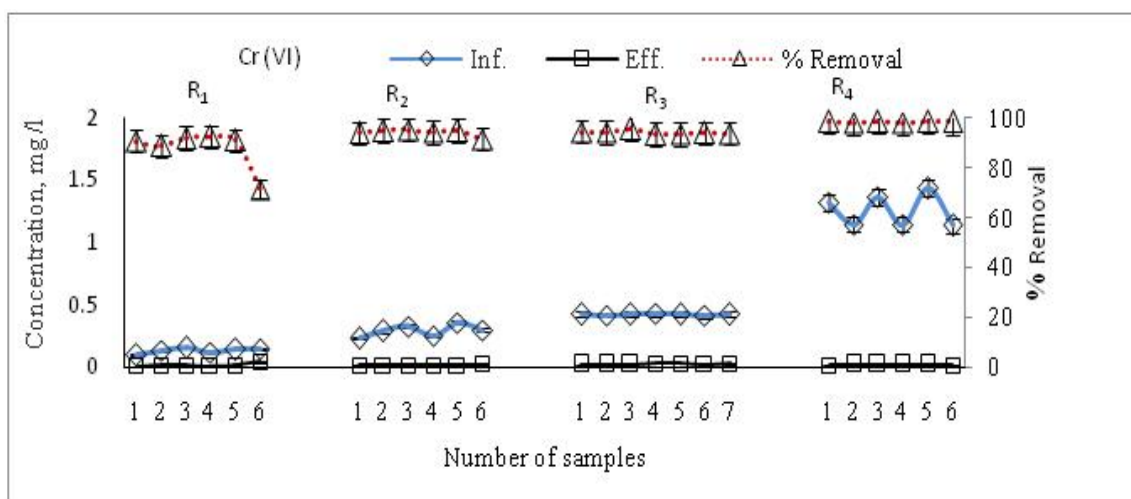


Fig. 2. Removal of Cr (VI) during the different runs

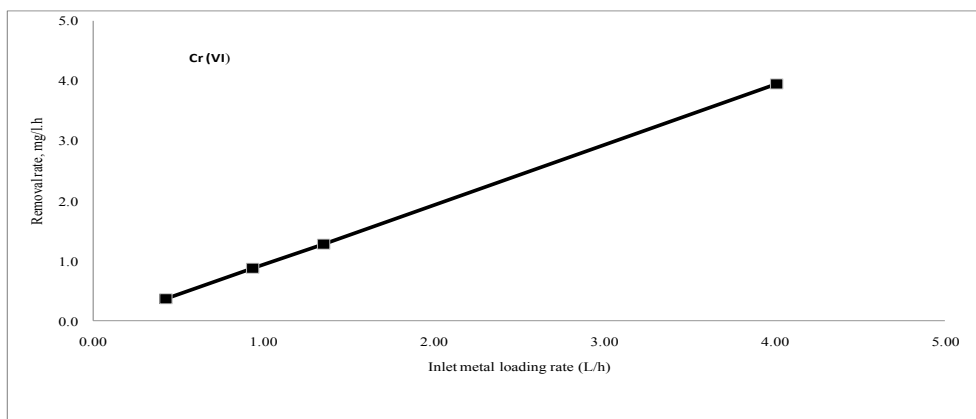
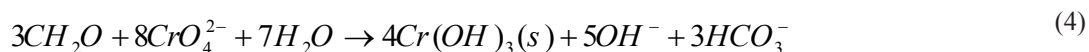
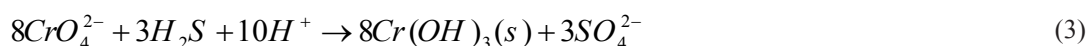
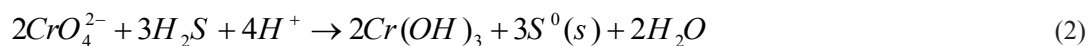


Fig. 3. Removal rate of Cr (VI) at different concentrations

At anaerobic condition, Cr (VI) degraded to Cr (III). This is occurred when high sulphate content is available in wastewater. The phenomena is called sulfidogenesis, where sulfate reduction bacteria (SRB), utilized the organic matter present. This happened only due to the presence of high sulfate content in H-UASB reactor. Sulfate reducing bacteria (SBR) “Desulfobacter, Desulfobulbus and Desulfovibrio” are responsible for the reduction of Cr (VI) to Cr (III) as shown in equations (1-3).

In H-UASB, the pH ranges from 6.5-8.5 and Cr (VI) can exist as CrO_4^{2-} which is reduced directly to Cr (III) by SRB and chromate reducing bacteria using the organic matter as an electron donor and chrome hydroxide sediment is formed on the surface of the microorganisms as indicated in equation (4). Qian *et al* [15] showed that CrO_4^{2-} possesses similar bioactivity like SO_4^{2-} because they have similar structure (four tetrahedrally arranged oxygen atoms and two negative charges).

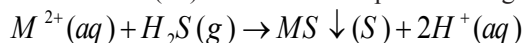


Mechanism of accumulation of Cr ion in the sludge

The removal of Cr (VI) in the H-UASB system is attributed to sulfide precipitation and adsorption

$$\text{Influent} = \text{Effluent (eff. soluble + eff. particulate)} + \text{Sludge} + \text{Adsorption/biosorption} \quad (5)$$

The results revealed that during the control run, the accumulation of chromium on sludge was 75.31 mg/Kg. Simultaneous increase of Cr (VI) concentration in influent wastewater from 600mg / m³.d to 1800 mg / m³.d, to 3000 mg / m³.d and then to 6000 mg / m³.d led to the adsorption/biosorption of Cr (VI) on the surface of sludge granules. The amount of Cr (VI) adsorbed/biosorped on sludge



combined with biosorption on to the NWPF. The adsorption/biosorption of Cr (VI) on sludge can be calculated according to Equation (5).

granules increased from 0.1 mg/l to 0.172 mg/l to 0.27 mg/l and 1.077 mg/l, respectively. Mechanism of removal occurs by both metal sulfide precipitation (Equation 6) and the biomass in NWPF and sludge granules act as either through an ion exchange mechanism, on the surface of the biofilm or surface precipitation of metal hydroxide/sulfide species [16].

(6)

Where; *M*: metal ions *MS*: metal sulfide

The results depicted in Table 2 shows the effect of sludge age on the sludge composition through the different experimental runs in the H-UASB reactor. Total and volatile solids increased with increasing sludge age at the R4 and consequently with increasing the concentration of Cr (VI) in the influent wastewater. TSS and VSS values were 103 g/l and 71.4 g/l. This means that the increase of VSS is due to the presence of active biomass and also confirmed by the increase in the VSS/TSS. It reached up to 1.03 g/l with increasing

the sludge age at the end of the study period compared with 0.68 g/l in all runs. This ratio reflects the quality of sludge characteristics and active growth of the biomass as reported by Rizvi et al [17].

Moreover, toxicity characteristic leaching procedure (TCLP) was applied in this study to evaluate the efficiency of the stabilization and solidification of heavy metals in sludge and it was <0.05 which is in compliance with the US-EPA TCLP regulatory limits for safe disposal of sludge (5.0 mg/l).

TABLE 2. Sludge compositions as a function of sludge age in H-UASB

Parameters	Unit	Sludge age (days)				
		125	110	55	50	30
		Control	R (1)	R (2)	R (3)	R (4)
VSS	g/l	66.0	66.1	66.1	67.1	71.4
TFS	g/l	29.8	29.9	30.9	30.9	31.6
TS	g/l	95.8	96.0	97.0	98.0	103.0
VSS/TS	--	0.68	0.68	0.68	0.68	1.03

*R1: 600 mg / m³.d, R2:1800 mg / m³.d, R3:3000 mg / m³.d, R4: 6000 mg / m³.d

Organic matter and TSS removal in presence of Cr (VI)

Results in Fig. 4-6 indicated that the order of percentage removals of TSS, COD and BOD₅ were R1>R2>R3 >R4. Their corresponding average removal rates decreased from 86.17, 74.18, 84.84 % to 62.06, 57.07, 61.92 %. The decrease in the removal rate may be explained that increasing chromium concentration in the reactor inhibits the activity of anaerobic microorganisms. These results agree with Lo *et al* [18]. In R4 the removal rate decreased to 62.06, 57.07, 61.92 % for TSS, COD and BOD₅. This is due to the increase in the organic loading rate in the feeding wastewater up to 1.53 Kg COD m³/d. Increasing the concentration of Cr (VI) up to 6000 mg/m³.d in influent wastewater affects greatly the performance of the H-UASB reactor and the average removal rates decreased to 62.06 % for TSS, 57.07 % for COD and 61.92% for BOD₅. These results agreed with Mudhoo and Kumar [19]. They stated that metals like copper,

nickel, zinc, cadmium, chromium have an inhibitory effect and under certain conditions are toxic in biochemical reactions depending on their concentrations. In anaerobic digestion, sulfides are not oxidized, they combine with hydrogen to produce hydrogen sulfide gas. The biological reduction of Cr (VI) in the reactor was successfully achieved through sulphate and chromate reducing bacteria. Moreover, chromium is reduced in presence of hydrogen sulfide to form Cr (OH)₃ as a precipitate. This precipitate is adsorbed combined with biosorption on the surface and inside the NWPF as well as the excess sludge in the H-UASB reactor. The concentration of chromium on sludge adsorbed/biosorbed on NWPF was 160 mg/kg and that accumulated in the H-UASB reactor was 165.44 mg/kg. The residual concentrations of total chromium in the final effluent were 0.0167 mg/l, 0.017 mg/l, 0.024 mg/l, and 0.019 mg/l for initial feeding of 600, 1800, 3000 and 6000 mg Cr (VI)/m³.d.

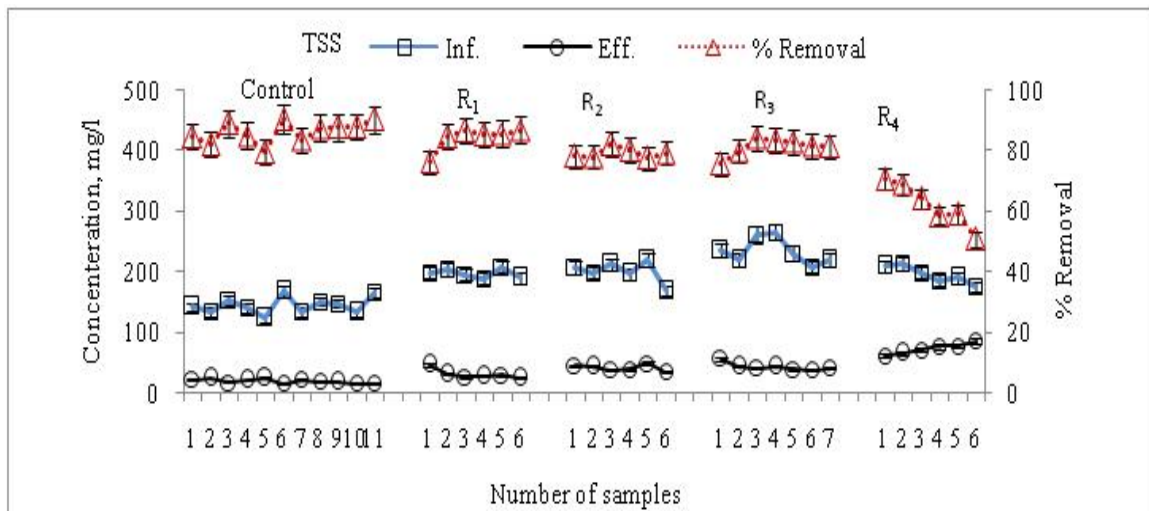


Fig. 4. Removal of TSS during the study period

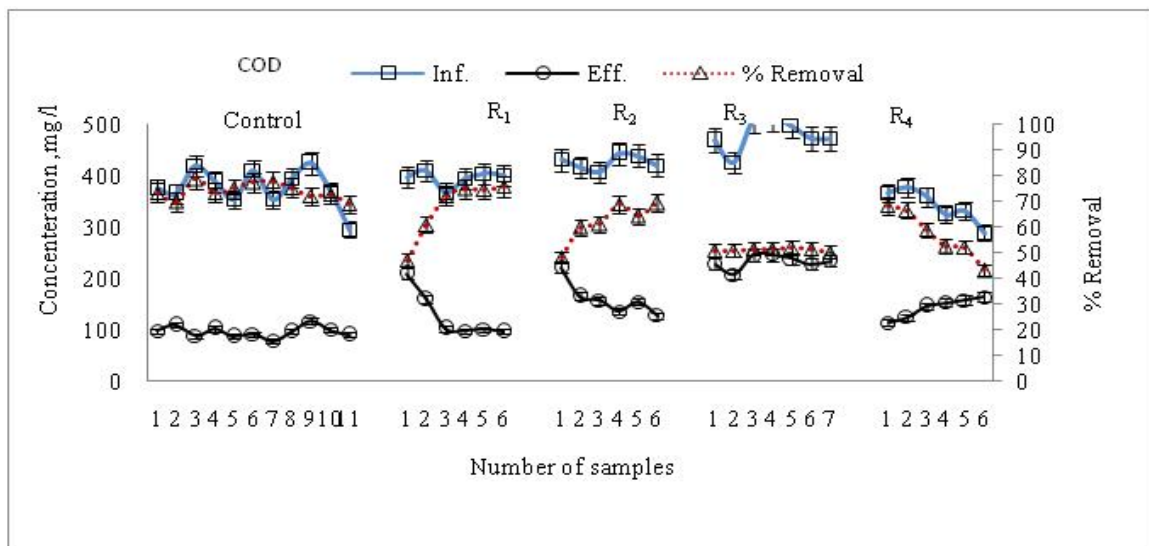


Fig. 5. Removal of COD during the study period

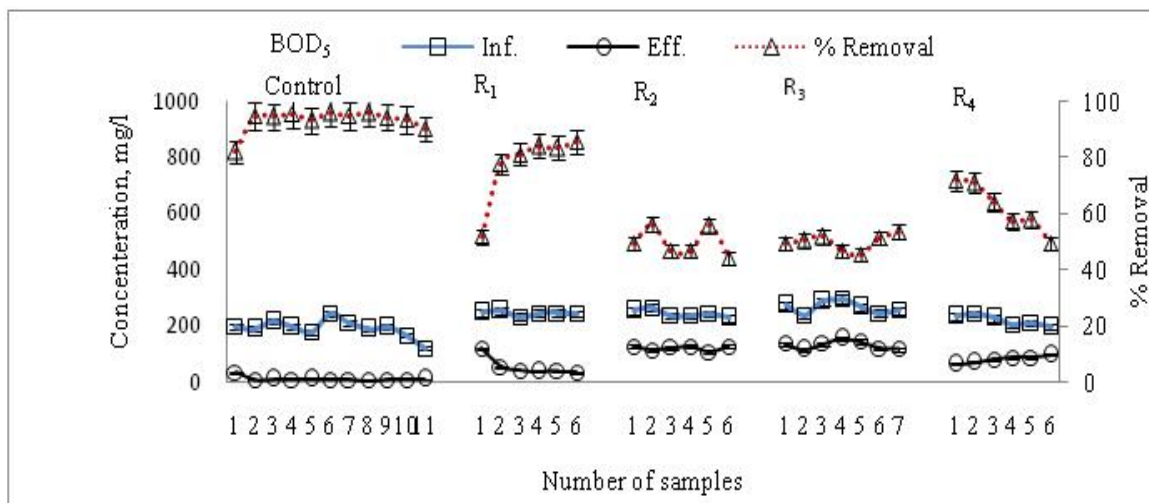


Fig.6. Removal of BOD5 during the study period

Conclusion

In this study, H-USAB was used for treating municipal wastewater. The reactor was packed with NWPF to improve its performance. It was operated at HRT of 7.5 h and OLR ranged from 0.95 to 1.53 Kg COD m³/day. The percentage removal of COD and BOD₅ reached 74.2% and 84.8% in absence of heavy metal ions. To study the impact of Cr (VI) on the performance of the reactor, four different concentrations namely; 600, 1800, 3000, 6000 mg Cr (VI) / m³. d (i.e: 0.1 – 1.3 mg/l) was added intermittently to the reactor. Results indicated that the H-UASB was very efficient for the removal and reduction of Cr (VI) even at high concentration (6000 mg Cr (VI) / m³. d). Its percentage removal in the final effluent was 98.47% with a residual value of 0.019 mg/l. This may be attributed to the biological sulfide reduction of Cr (VI) which has been achieved by sulphate and chromate reducing bacteria. Also, the presence of the NWPF in the reactor enhanced the precipitation of Cr (OH)₃ in/on the packing material. In contrary, the organic matter removal decreased by increasing the concentration of chromium ion because of its inhibitory effect on the anaerobic microorganisms.

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الإصحاح الحيوي للكروم السداسي و دراسة تأثيره علي أداء المخمر البيولوجي اللاهوائي المعدل لمعالجة مياه الصرف الصحي (وحدة معالجة تجريبية)

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ان الهدف من هذه الدراسة هو الإصحاح الحيوي ودراسة تأثير الكروم السداسي علي أداء المخمر البيولوجي اللاهوائي المعدل. تم تغذية وحدة المعالجة بمياه الصرف الصحي الحقيقي بمتوسط حمل عضوي ١,٢٤ كجم / م^٣ / يوم وعند زمن مكث ٧,٥ ساعة. اوضحت النتائج ان نظام المعالجة حقق نسب إزالة جيدة لكل من المواد العالقة الكلية (٨٦,٤٤%)، الاحتياج الأكسوجيني الكيميائي (٧٤,١٨%) و الاحتياج الأكسوجيني الحيوي (٨٤,٨%) وذلك بدون إضافة الكروم السداسي. وعند إضافة تركيزات من الكروم السداسي تراوحت بين ٦٠٠ إلى ٦٠٠٠ مجم/م^٣. يوم (٠,١ مجم/ لتر – ١,٣ مجم/ لتر) أدت الي انخفاض تركيز الكروم في السبب النهائي الي ٠,٠١٩ مجم/ لتر و زيادة تركيز الكروم في المادة المألثة (١٦٠ مجم/ كجم) و في الحمأة (١٦٥,٤٤ مجم/ كجم). و ذلك نتيجة اختزال الكروم السداسي إلي كروم ثلاثي بواسطة بكتيريا السلفات و الكرومات في المخمر البيولوجي اللاهوائي. و بالوصول إلي تركيز ٦٠٠٠ مجم/ م^٣. يوم ادي الي انخفاض سلسلة التفاعلات الكيميائية للكائنات اللاهوائية و بالتالي انخفضت كفاءة المخمر البيولوجي اللاهوائي لازالة المواد العالقة الكلية من ٨٦,٤٤% إلي ٨٦,٠٦%، الاحتياج الأكسوجيني الكيميائي من ٧٤,١٨% إلي ٥٧,٠٧% و الاحتياج الأكسوجيني الحيوي من ٨٤,٨% إلي ٦١,٩٢%.