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Removal of Microcystins from an Aqueous Cells Extract of some toxic Cyanobacterial species by using activated carbon

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Abstract

In present study, some physical and chemical parameters are measured for the raw water of Al-Harthah drinking water station at Basrah city during July 2010. temperature was at 29ºC, pH value on alkaline side 8.3 , electrical conductivity was 1592 µmos /cm and dissolved oxygen at 6.7 mg /L , generally results showed close similarity to the Iraqi waters characteristics. At the biological side, Four species of cyanobacteria were identified in this study : *Anabaena affinis*, *Microcystis aeruginosa* , *Nostoc linkia,* and *oscillatoria tenuis* are classified as a toxic cyanobacteria in the world, two of them , *M. aeruginosa* and *O. tenuis* , were isolated in laboratory as axenic cultures and detected for producing the toxins by extracted by gel filtration and then detected by TLC technique that showed these species can producing two types of hepatotoxins called Microcystins: MC-LR and MC-YR. In addition, this paper confirm a highly efficiency of activated carbon to remove of microcystins from the aqueous extracted of present isolates by two methods, direct filtration through activated carbon and store with it for one day.

1- Introduction

Cyanobacteria are one of many divisions include different species that lives in water, in last year's there are many reports concentrated on the dangerous toxins (cyanotoxin) that produced by it (Rinehart *et al.* ,1994 ; Carmichael,1997; Falconer,1999), these toxins caused illness and death to animals like cattle and sheep (Jackson *et al.*,1983 ;Frazier *et al.*,1998; Gupta ,1998), dogs (Harding *et al.*,1995), Rhinoceros (Soll and Williams,1985), horses and rabbits (Carmichael,1992 ; Heriksen *et al.*, 1997; Gupta ,1998), fishes

(Jewel *et al*.,2003) and birds (Matsunaga *et al*.,1999), and can also effect on the growth of plants (Abe *et al*., 1996 ; Metcalf and codd , 2004).

Human also undergo illness and death that caused by cyanotoxins (Chorus and Bartram,1999), over fifty patient at renal dialysis center in Caruaru, Brazil, are died because exposure to cyanotoxin through hemodialysis treatment (Pouria *et al*.,1998; Jochimsen *et al*.,1998 ; Azevedo *et al*., 2002). Uneo *et al*.(1996) referred to the relationship between cyanotoxin and primary liver cancer (PLC), and also promotion the cancer in other organs (Falconer and Buckley,1989 ; Falconer, 1991; Falconer and Humpage,1996).

The classic treatments of raw water to produce drinking water cannot decreased or removed the cyanotoxins, but in contrast, they led to release toxins from cells to water (Volterra *et al*.,1990). Mohamed *et al*.(1999) referred to ineffective treatments by flocculation and sedimentation together with sand filtration and chlorination that used in drinking water stations for removable of cyanotoxins, as well as, the soluble cyanotoxins were passed through the filters.

The first research about toxic cyanobactria in Iraq by Al-Shaheen (2002) it is recorded six species of cyanobacteria can produce dangerous toxins to drinking water at three drinking water stations in Basrah city at Iraq; in the same line Al-Reqabi(2002) record the effects of temperature and light density on the toxic cyanobactria *Microcystis aeruginosa*, in 2007, Al-hilfi study on some biological effects of *M. aeruginosa* toxin's on the labrotary mice, also Al-Sultan (2007) and Al-Aaragy and Al-Sultan (2008) isolated and purification the toxin MC-LR in four species of cyanobacteria isolated from sewage water at Basrah city and study it is effects on mice ,artemia and fish . Generally, in Iraq as well as in Basrah city the drinking water plants were used the classic treatment (flocculation, sediment-ation, sand filtration and chlorination) for drinking water supply.

Recently, there are many new techniques using to removed the cyanotoxins from drinking water by degredation it like UV irradiation, titanium dioxide, hydrogen peroxide and ozonation (Fritz *et al*., 1999 ; Cornish *et al*., 2000; Senogles *et al*., 2001 ; Hoeger *et al*., 2002; Qiao, *et al*., 2005) also activated carbon or active charcoal can be successfully decreased or removed the cyanotoxins from drinking water by absorbance or adsorbance of toxins. (Lambert *et al*.,1996 ; Mohamed *et al*., 1999 ; Pendleton *et*

al., 2001 ; Cook and Newcombe,2002 ; Huang *et al*. ,2007).

The aim of present study is used (activated carbon) technique against the cyanotoxins which produce from some toxic cyanobacterial species isolated from Iraqi waters and study the efficiency of activated carbon to remove toxins.

2-Materiales and Methods

Physical and chemical factors

Temperature, pH, electrical conductivity and dissolved oxygen are measured to raw water by using of water quality instrument (LOVIBOND) model: Sensodirect 150, Germany.

Samples and taxonomy

Water samples were collected from AL-Harthah station of drinking water at Basrah city in July 2010 by phytoplankton net 30cm in diameter and 20 μm mesh. Water Samples were divided into two subsamples, first one fixed by 4% formalin to microscopically identification of cyanobacterial species according to the references: Smith(1950), Diesikachary (1959); and Prescott (1975) ,While the second subsample were taken to isolate species in unialgal culture.

Culture

Strains were isolated by spreading 0.1-0.2 ml of water sample into petri dishes containing Chu-10 medium with 1.5-2% agar according to Stein (1973) and Al-Mousawi (1984), Unialgal culture of two species cyanobacteria *Microcystis aeruginosa* and *Oscillatoria tenuis* were obtained and cultivated in 250ml conical flasks containing Chu-10 medium at 27 ±2 ºc with a photo period of 8:16 hrs. Axenic culture of two species cyanobacteria *M. aeruginosa* and *O. tenuis* were done according to Weidman *et al*.(1984) and then cultivated in 3 liter conical flask containing 2 liters of Chu-10 medium under above conditions then harvested in the late exponential phase and concentrated by centrifugation at 3000rpm/15min and lyophilized by lyophilizer (Labconco) under -100ºc and 0.006 bar for 24hrs.(Brittain *et al*.,2000 ; Al-Shaheen , 2002).

Extraction and detection of toxin

The toxins was extracted according to Namikoshi *et al.* (1993), lyophilized cells (300 mg) mixed with solvent mixture methanol- n-butanol- water with ratio (4:1:15) in conical flask with stirring for one hour and repeated extraction three time, the supernatant was obtain by centrifugation and concentrated to 3ml by air stream .

The method of purified toxin was carried out according to Namikoshi *et al.* (1992 , 1993), the above extract was passed through glass column (2× 15 cm) containing silica gel (100-200 mesh), the column washed with water then methanol

20% and finally toxins eluted by methanol 80% with flow rate 3 ml/ min .

The last fraction checked for their content of toxins by thin layer chromatography (TLC), about 10 µl of fraction were applied to silica gel plate (5 ×20 cm) then put it in a container contain solvent, two solvents are used in this study the first: water – ethyl acetate – 1-propyl alcohol (3:3:4) (Pelander *et al*. 1997), and the second : n- propanol –ethyl acetate-water (32.5: 47.5: 20)(Pelander *et al*. 1998) ,then dried the plate and the spots are detected by Ultraviolet light at 238 (Lawton, *et al.*, 1994 ; Namikoshi, *et al.*,1998 ; Meriluoto *et al*., 2000; Ahmed , *et al*., 2008).

Activated carbon experiment

A modified method of Mohamed *et al*.(1999) was carried out ,dried cells (300 mg) of each *M. aeruginosa* and *O. tenuis* were extracted twice in sterile double deionized water, the aqueous extracts were centrifuged at 10000 rpm/30 min. and the supernatants were filtered through GF/C filters.

Cyanobactrial toxins (Microcystins) detection in each extract was carried out by using Ultraviolet Spectrophotometer model (Pye - Unicam SP8-100) at 238 nm. The pH value of extracts was adjusted to 8 , then each extract was divided into three parts ,the first (part 1)placed in 25ml screw bottles which had been dosed with activated carbon at concentration 500mg /L , and the control (part 3) remain without activated carbon, each extracts and control were prepared in duplicate. The bottles were filled to the top with extract to exclude head space, and capped with caps then all the bottles covered with foil .The bottles were placed in rotary tumbler at room temperature 27-30ºc and rotated at 10 rpm for one day. After that, the extracts will filtrated through GF/C glass microfiber filters and detected absorbance by spectrophotometer at 238 nm. Also present study carried out another modified method of Falconer *et al.*(1989) ,the second part (part 2) were filtrated through 15 × 2 cm glass column containing three grams of activated carbon, then the supernatant detected absorbance by using spectrophotometer at 238 nm.

3-Results

Some ecological factors

Some ecological factors in raw water of AL-Harthah station of drinking water were measured represented by temperature was at 29ºC, pH value on alkaline side 8.3 , electrical conductivity was 1592 µmos /cm and dissolved oxygen at 6.7 mg /L as showed in table 1.

Cyanobacterial species isolates

In this study, four Cyanobactrial species was identified : *Anabaena affinis, Microcystis aeruginosa* , *Nostoc linckia* ,and *Oscillatoria tenuis,* these species known as toxic cyanobacteria in the world. Two of them , *M. aeruginosa* and *O. tenuis* are successful obtained in axenic culture and showed ability to produce toxins normaly microcystin.

M. aeruginosa shown as gelatinous colonies consist of aggregation condensed cells, the cells (3.5-6.8) µm in diameter, spherical, granulated with gas vacuoles. While *O. tenuis* shown as trichomes solitary, straight, constricted, some of it bent at the ends and apical cell are truncately rounded but not capitates; the cells (5.2-6.9) µm in diameter and (2.5-4.7) µm long ; cells blue green , granulated contents .

The taxonomy of *M. aeruginosa* and *O. tenuis* are:

Division: Cyanophyta
Division: Cyanophyta
Class: Cyanophyceae
Class: Cyanophyceae
Order: Chroococales
Order: Nostocales
Family: Chroococcaceae
Family: Oscillatoriaceae
Genus: *Microcystis*
Genus: *Oscillatoria*
Species: *aeruginosa*
Species: *tenuis*

Extraction and identification of toxin

The TLC of extract showed two value to R_f (Rate of Flow), these R_f are 0.72 to *M. aeruginosa* by use the first solvent and R_f of *O.tenuis* are 0.53 by use the second solvent.

Activated carbon experiment

The results showed that the absorbance value of *M. aeruginosa* aqueous extract are reduced from 0.159 to 0.015 by first method (part 1) and 0.035 by second method (part 2) and the percentage of removal toxins are proximally 90% by first method and 78% by second method ,while the absorbance values of *O.tenuis* aqueous extract are reduced from 0.263 to 0.030 by first method (part 1) and 0.040 by second method (part 2) and the percentage of removal toxins are proximally 88% by first method and 85% by second method .

Table (1): Physical and chemical factors of raw water of study station at July 2010.

Physical and Chemical Factors	Results
Temperature	29ºc
pH	8.3
Electrical Conductivity	1592 µmos /cm
Dissolved Oxygen	6.7 mg / L

Table (2): Absorbance of aqueous extracts at (238 nm) of two cyanobactrial species *M. aeruginosa* and *O. tenuis* before and after treatment by activated carbon.

Absorbance at 238nm / Species	Extract before treatment by activated carbon (Control)	After one day (part 1)	After filtration (part 2)
M. aeroginosa	0.159* 100%^{TE}	0.015* 9.433%^{T} (90.567%^{RT})	0.035* 22.012%^{T} (77.988%^{RT})
O. tenuis	0.263* 100%^{TE}	0.030* 11.406%^{T} (88.594%^{RT})	0.040* 15.209%^{T} (84.791%^{RT})

* Absorbance value.

^{TE} Percentage of toxins in aqueous extracts.

^{T} Percentage of remaining toxins.

^{RT} Percentage of removal toxins .

4- **Discussion**

The results of the physical and chemical factors of raw water that uses in drinking water station in present study are close similarity to the Iraqi surface waters characteristics at this season of year (AL-Zubaidy, 1985; AL-Aaragy, 1988; AL-Mousawy, 1992; AL-Shaheen, 2002).

Present study successful to identified four dangerous species of cyanobacteria *Microcystis aeruginosa* , *Oscillatoria tenuis, Anabaena affinis* and *Nostoc linckia* that known a toxic cyanobacteria in the world (Carmichael,1997; Falconer,1998 , 1999 ; Brittain *et al*.,2000).

Two axenic culture of cyanobactrial species *M. aeruginosa* and *O. tenuis* was obtained and successful to growth in laboratory. Local studies was isolated these species from drinking water stations and iraqi water and tested to producing microcystins and they found the ability to produce microcystin-LR (MC-LR) by *M. aeruginosa* and microcystin-YR (MC-YR) by *O. tenuis* (AL-Shaheen , 2002 ; AL-hilfi , 2007; AL-Sultan , 2007).

In the world many studies about these species referred to producing a very potent cyanotoxin called Microcystin and identified as dangerous material when found in drinking water of animal and human (Carmichael,1992,1997; Carmich-ael and Falconer,1993 ; Uneu *et al.*,1996 ; Falconer,1999; Chorus and Bartram , 1999; Brittain et al.,2000), Nagata *et al.* (1997) and Park *et al*.(1998) referred to relationship between the occurrence of toxic cyanophyta (specially *Microcystis* and *Oscillatoria*) and the microcystins concentration in drinking water .

TLC technique consider a successful chemical test for detect microcystins and identified their types, also this test is very easy, rapid and low coast (Pelander *et al*. 1996; Pelander, 2000). TLC test in present study showed the ability of isolate *M. aeruginosa* and O. *tenuis* to produce cyanotoxins (microcystins) with a very closely R_f values to the references, isolated toxin of *M. aeruginosa* by first solvent was have $R_f = 0.72$ they close to R_f of microcystin MC-LR in references equal to 0.70 that also isolate from the same species (Carmichael, 1997; Pelander *et al*. 1997; Park *et al*.,2001). Hyesstrand *et al.*(2001) submit the toxin MC-LR are consider as abundant and very toxic cyanotoxins in the world.

The R_f value by second solvent of *O. tenuis* toxin is 0.53 and these result near of the R_f value microcystins MC-LR or MC-YR that equal to 0.54 as in Pelander *et al*. (1998),this is refer that *O. tenuis* may be have one of microcystins(MC-LR or MC-YR) or both them because of a few different between structure and molecular weight of both toxins MC-LR and MC-

YR(Carmichael, 1992, 1997; Rinehart *et al.*, 1994).

Detection of microcystins by spectrophotometer at wavelength 238nm is specific for these toxins in the cyanobactria due to the occurrence of side group in the structures of microcystins called (Adda) (Harada, 1996 ; Akin-oriala and Lawton 2005). These wavelength is also used to detected the microcystins by high performance liquid chromatography technique (HPLC) (Jakobi *et al*., 1996 ; Vezie *et al*., 1997 ; Namikoshi *et al*., 1998 ; Park *et al*., 1998 ; Brittain *et al*., 2000).

The pH value of extracts adjust to 8 to become at the pH range of Iraqi fresh water that will be used for produced drinking water , Huang *et al*, (2007) referred to capability of adsorption of activated carbon are increase with the raised of pH value.

Present study modified method of Mohamed *et al*.(1999) by reducing storage period of aqueous extracts from seven days to one day only ,and detected the losses in cyanotoxins by the easy test using the absorbance by spectrophotometer instead of complex test the ELISA , the second method also modified Falconer *et al.*(1989) by using the absorbance by spectrophotometer instead of uses bioassay for testing toxicity of extracts after filtration .

By table 2 can be show the big different between the absorbance value of the control on side and the two parts (part 1 and 2) on other side, that mean the highly absorbance (or adsorbance) of cyanotoxins by activated carbon referred to efficient activated carbon technique to decreasing or removing cyanotoxins (microcystins) from water (Lambert *et al*., 1996 ; Mohamed *et al*.,1999; Pendleton *et al*, 2001 ; Cook and Newcombe, 2002 ; Yan *et al*, 2006). Also the store of extract for one day with activated carbon in our study give good reduction in toxins as well as the store for long time that found in the results of Mohamed *et al.* (1999). Another result showing a few different between the absorbance values of two methods in present study, the storage extract for one day (part 1) removed proximally 89-91% of toxins from cyanobacterial aqueous extracts and also the direct filtration (part 2) removed proximally 78-85% of toxins for the both isolates and that consider a very good results of both methods successful to decreasing the concentration of microcystins in extracts and this results agree with many researches (Falconer *et al*. ,1989 ; Lambert *et al.*,1996 ; Mohamed *et al.*,1999 ; Pendleton *et al.*, 2001), but this technique must be done before the addition of the chlore to water because the chlorine react with activated carbon and caused a

decrease in sorption capacity of cyanotoxin ,and the chlorine at normal treatment plant dosages is not effective for degrading of microcystins (Mohamed *et al*.,1999 ; Huang *et al*, 2006).

So, the both above methods can be used especially direct filtration and repeated it to remove the dangerous cyanotoxins from drinking water at our stations to became safety in uses by populations in Iraq.

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إزالة سموم المايكروسستينات من المستخلص المائي لخلايا بعض انواع السيانوبكتريا السامة بواسطة الكاربون المنشط

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الخلاصة

تضمنت الدراسة الحالية قياس بعض العوامل الفيزيائية والكيميائية للمياة الخام لمحطة الهارثة لتصفية مياة الشرب في مدينة البصرة خلال شهر تموز عام2010 إذ بلغت قيمة درجة الحرارة 29 °م وكانت المياة في الجانب القاعدي للاس الهيدروجيني بقيمة 3,8 بينما بلغت قيمة التوصيلية الكهربائية 1592 مايكروموس/سم وأخيرا فأن نسبة الاوكسجين المذاب بلغ 7,6 ملغم/لتر وعموما" فأن هذة النتائج قريبة جدا" من مواصفات المياة العراقية. اما في الجانب الاحيائي من الدراسة فقد امكن تشخيص اربعة انواع من السيانوبكتريا وهي:

Anabaena affinis , *Microcystis aeruginosa* , *Nostoc linkia* , and *oscillatoria tenuis* وهذه الانواع مصنفه عالميا" ضمن السيانوبكتريا السامة والخطرة على صحة الانسان والحيوان على السواء و تمكنت الدراسة الحالية من الحصول على عزلتين نقيتين للنوعين السامين واختبرت قابليتهما على انتاج السموم استخدمت تقنية *O. tenuis* و *M. aeruginosa* لمعرفة نوعية السموم المستخلصة اذ أظهرت النتائج إن العزلتين بإمكانهما إنتاج السمومMC-YR TLC و MC-LR الكبدية المسماة (المايكروسستينات) وبنوعين .فضلا" عن ذلك فقد اثبتت الدراسة الحالية الكفاءة العالية لمادة الكاربون المنشط على امتصاص سموم المايكروسستينات من المستخلص المائي للعزلتين قيد الدراسة وبطريقتين احداهما بواسطة الترشيح المباشر للمستخلص المائي عبر الكاربون المنشط و الأخرى بواسطة خزن المستخلص المائي مع الكاربون المنشط لمدة يوم واحد.