Notes on isolation of *Leptolyngbya* (*plectonema) nostocorum* capable to withstand liquid hands detergent

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Abstract:

The pattern growth of *Leptolyngbya* (*plectonema) nostocorum* on layer of porcelain of washing basin was remarkable, such finding attracts our interest to conduct this research. Despite the unfavorable conditions, the growth of alga was not affected. It was subjected to pressure as a result of flowing of water from tap with ranges from 0.1- 7.6 liters per minute. This process is daily repeated for about 10 times a day for periods of up to 2.5 hours. The light intensity was 100 lux and the temperature ranges between 25- 30 ºC as a result of process of opening and closing the tap, respectively. In addition to the previous stress, organism has been exposing to the amount of liquid soap to wash hands as a result of daily non-stop process of human activities. Description and identification of algae has been made. Basic physical and chemical analysis of tap water has been also conducted, drinking water in Saudi Arabia is stored in underground tanks and replenished as water runs out. The analysis of the water showed that pH was alkaline (8.7) and the total soluble salts was 56.7mg/L. The total alkalinity was 28 mg/L as well as the total hardness was 18 mg/L, Calcium hardness, Magnesium hardness were 14, 4 mg/L, respectively. Calcium, Magnesium and Chloride are represented at concentrations of 5.6, 0.96 and 4 mg/L, respectively. The concentration of nitrate was 0.56 and the concentration of iron was 0.18 mg/L. For the growth curve of alga, the best growth (0.271, 2630.46 mg/L) was achieved for optical density, dry weight, respectively after 16 days of incubation. For different concentrations of the detergent, it was found that the least concentration did not have any inhibition zone to the growth of alga was 0.2%. Above this concentration, there has a steady increase in the diameter of inhibition zones.

Keywords: Analysis of the water**,** hands detergent, inhibition zone, *Leptolyngbya* (*plectonema) nostocorum***,** Saudi Arabia.

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Introduction

The climate of Saudi Arabia is characterized by severe drought with high temperatures causing scarcity of living organisms, especially algae. Makkah region relies on desalinated water from the sea for daily use and as a result of the drought and increasing of water use, there are permanent deficit of the amount of water and this in turn causes changes in the nature of the existing algal flora and makes it always conditioned by the presence of gelatinous sheaths that protect and cover them from high temperatures. Cyanobacteria are characterized by their adaptation to such conditions especially filamentous species. Cyanobacteria flora is still unknown cryptically, especially in tropical and arid regions such as Saudi Arabia (**Mohamed and Al-Shehri, 2008**). The dominant genera of filamentous cyanobacteria in hot desert soils are *Microcoleus*, *Phormidium*, *Plectonema*, *Schizothrix*, *Nostoc*, *Tolypothrix* and *Scytonema* (**Shields and Drouet, 1962**) Several phototrophic microorganisms, principally Cyanobacteria and chlorococcal green algae have been reported to occur as biofilms on the exposed surface of stone substratum of culturally important monuments or any layers of rocks and porcelain and building blocks, especially in humid environments of several countries of the globe (**Darienko and Hoffmann, 2003**; **Gärtner and Stoyneva, 2003**; **Uher *et al.*, 2006**).Such communities accelerate the weathering of the substratum they colonize, hence considered as potential threat to the monuments (**Adhikary and Kovacik, 2010**). *Leptolyngbya* is a cosmopolitan world distribution genus evidently widespread in terrestrial, freshwater and marine habitats. **John *et al.* (2002)** on their account of the British cyanobacteria has shown that the narrow trichomes (about 2.5 µm in diameter),in sheaths that are colorless to distinctly yellow under microscope, do not easily match any of *Lyngbya* or *Phormidium*. It was noted that recent studies have moved many of these taxa into a new genus, *Leptolyngbya* Anagnostidis & Komárek characterized by narrow trichomes and firm, narrow sheaths. The genus *Plectonema* resembles *Lyngbya* in that single trichomes are enclosed in gelatinous sheaths, which tend to be thin, firm and often colored. It differs from *Lyngbya* primarily by the occurrence of false branching. **Issa and Ismail (1994)** were found some algal species were very sensitive to all detergents used while others were tolerant to the

same detergents and sensitive to others. Such results have been reported by **Yamane (1984) and Abdel-Hamid (1986)**. The tested synthetic detergents inhibited completely the growth of *Chlorella pyrenoidosa* and *Thalassiosira pseudonana* at concentrations above 10 mg/l (**Kondo *et al.*, 1984)**.

The research is the first study to isolate this alga in Saudi Arabia with attention to the effect of synthetic detergents on algal growth and to reach the appropriate concentration of detergent that does not interfere with its growth as well as inhibit organism activity.

Materials and Methods

Alga was isolated from a hand washing basin from Makkah Al-Mukarramah Teachers' College Which lies between latitudes and longitudes with coordinates of 21° 25' 21.0360" N and 39° 49' 34.2048" E, respectively (Fig. 1) in 2013, isolation and re-cultivation of alga was carried out by Scratching the algal growth by sterilized sharp blade and then immediately transferred to sterilized Petri dishes containing solidified Z- medium **(Staub, 1961).** The culture was incubated under continuous light intensity at 200 lux and the temperature was at 25 °C. Growth of cyanobacterium began to appear after about 2 weeks in Z medium and incubated at a temperature of 25-30 ° C and light intensity up to 100 lux for a period up to 28 days. Identification of alga was done according to **Alwathnani and Johansen (2011).** The nature of algal growth was photographed in the wash basin as well as the growth pattern in Petri dishes containing Z-solid medium was observed. Analysis of the physical and chemical properties of water was conducted according to **(APHA, 2005).**

Inhibition zone activity test: Standard wells were made on the agar plate (1cm in diameter). One ml of the detergent at different concentrations were added into the wells and incubated for 5 days at room temperature. Then the diameters of the zones of inhibition were measured in millimeters.

Figure (1): The location of Makkah and a map showing the locations of the samples.

Results

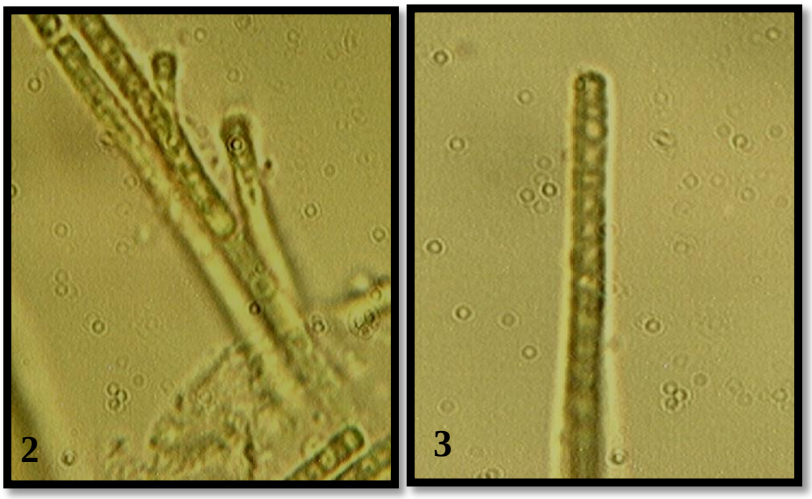
Leptolyngbya nostocorum (Bornet ex. Gomont) Anagnostidis & Komárek (Pl.3, Fig. 23)

Synonym: *Plectonema nostocorum* Borent ex Gomont 1892.

Description of species (figs. 2&3) (1000 X magnification)

Thallus densely intertwined like carpet forming mucilaginous masses, sheath thin , colorless adherent to cells, can be seen clearly at the ends of the filaments; filaments wrapped, overlapping sometimes spiral or tortuous. Cells quadrate or short barrel-shaped, (1-)1.5-2(-2.5)µ width, 1-2(-3) µ length, clearly constricted at the cross walls. Trichomes fragile, fragmented. Branches, few

usually solitary. Empty dead cells are marked between cells. Filaments (1.5-) 2-2.5(-3) µ in diameter. Apical cells rounded isolated from layer of porcelain of washing basin.



Figures (2&3): *Leptolyngbya nostocorum*

Figure 4 shows the growth of alga was like thin flakes of bluish green, especially in the upper region of basin. While thick growth was increased in gelatinous form and the color thallus tended to be blackened with blue in the lower region of basin.

The growth pattern of *Leptolyngbya nostocorum* in Petri dishes was soft, superficial and resembling the carpet and ranged in color from bluish-green to blackish green (Fig. 5). The picture shows also color variation from gray color to brown color.

Figure (4): The growth Pattern of Cyanobacterium in the upper(A) and lower(B) parts of basin.

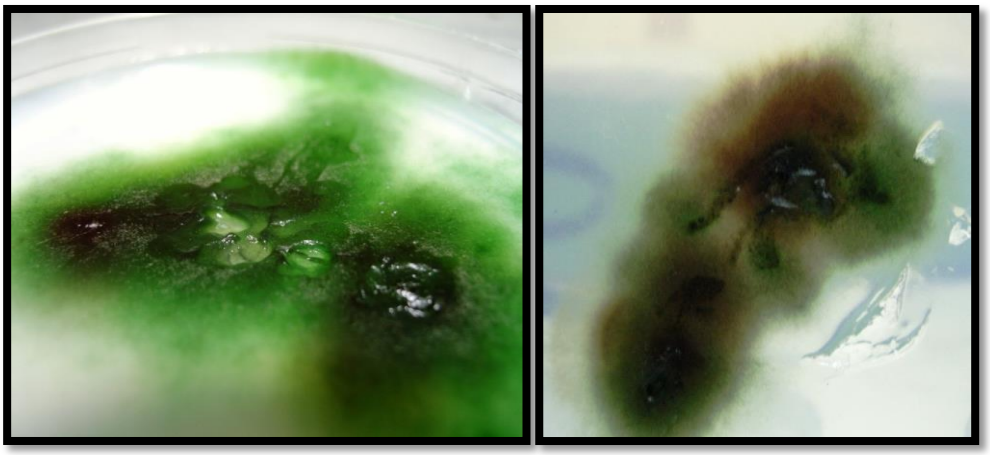


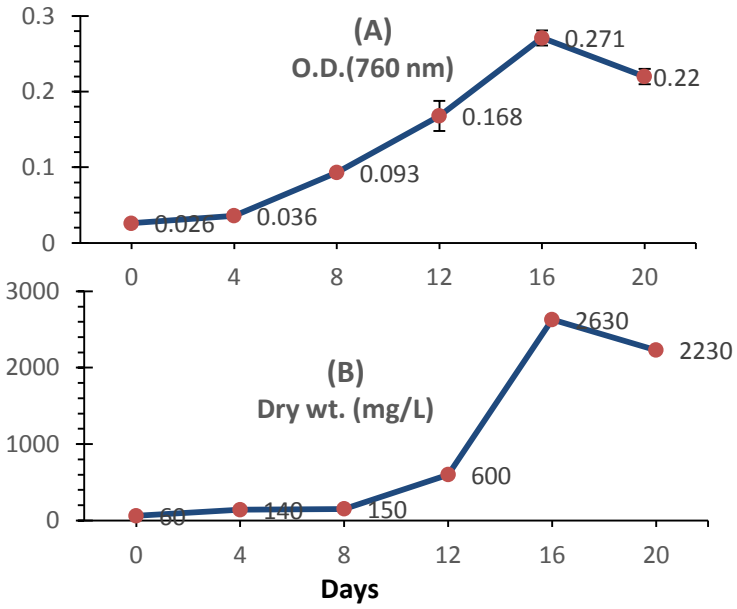
Figure (5): Growth pattern of *Lyptolyngbya nostocorum* in Petri dishes.

As for the physical and chemical properties of water, Table (1) shows that the water was alkaline in nature, since pH was 8.7. For the odor and color of the water the results were negative**.** Total soluble salts recorded 56.7 mg / L. As for the total alkalinity, the result was 28 mg/L. Regarding the total hardness, calcium and Magnesium hardness, the results were as follows 18, 14, and 4 mg/L, respectively. Calcium and magnesium values varied between 5.6 and 0.96 mg / L in water samples. For chlorides and nitrates, the results were as shown in Table (1) recorded 4 and 0.56 mg/L, respectively. Nitrite and manganese were not detect, but iron record average of 0.18 mg/L. Studying the growth curve of algae. It was found that the alga reached the maximum biomass in 16 days of incubation (Figs.6 A&B), achieving optical density of 0.271 and the dry weight of 2630 mg/L under continuous light intensity of 100 lux and a temperature of 30°C. After 16 days, growth of *Lyptolyngbya nostocorum* declined.

Table 1.Physico-Chemical Analysis of Water

Parameters	**Mean≅**
pH	8.7
Odor	Odorless
Color	Colorless
Total Dissolved Solids	56.7
Total Alkalinity (as $CaCO_3$)	28
Total Hardness (as $CaCo_3$)	18
Calcium Hardness (as $CaCO_3$)	14
Magnesium Hardness (as $CaCO_3$)	4
Calcium	5.6
Magnesium	0.96
Chloride	4
Nitrite	N.D
Nitrate	0.56
Iron	0.18
Manganese	N.D

≅Results are expressed in mg/L unless other units are mentioned)
N.D. Not detected.



Figures (6 A &B): Growth curve of *Leptolyngbya nostocorum*

In the study of the effect of different concentrations of liquid hand detergents on algal growth, the results (Table 2, Figs. 7&8) showed that concentrations of 100%, 50%, 8.5% and 1.4 % had an inhibitory effect proportional to the concentrations of the detergents, whereas the lowest concentrations had no effect on the growth.

Table 2. Inhibition zones of different concentrations of detergent.

Zone symbol	Zones of Inhibition for Various Concentrations (mm) after 5 days.
A	17 ±2
B	12 ±1
C	2.5 ±0.2
D	2 ±0.02
E	0
F	0
G	0

A: 100% of concentration of detergent, B: 50 % of the concentration, C: 8.5 % of concentration, D: 1.4 % of concentration, E: 0.2 % of concentration, F: 0.03 % of concentration, G: 0.01 % of concentration.

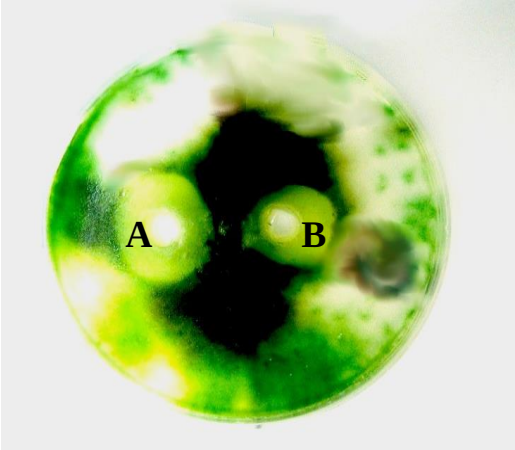


Figure (7): Inhibition zone for A (Original concentration of the detergent) and B (Half original concentration of the detergent

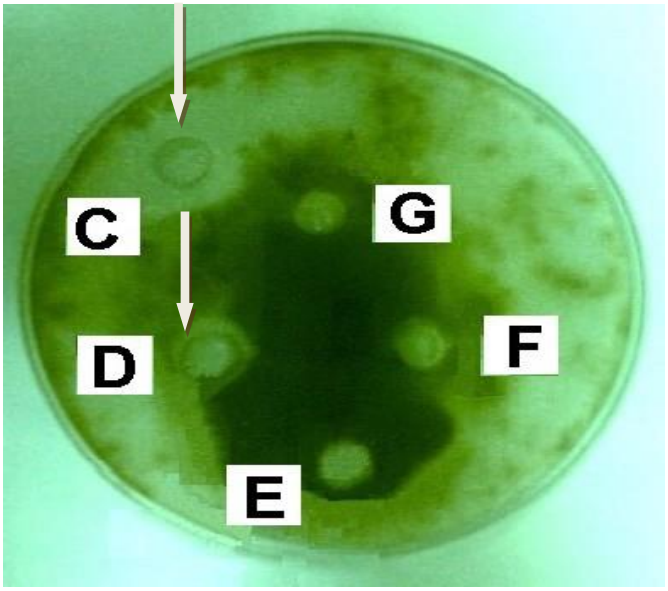


Figure (8): Inhibition zone for C and D, No inhibition zones for E, F and G concentration of detergent, C (8.5 %), D (1.4 %), E (0.2 %), F (0.03 %), G (0.01 %)

Discussion

The study of algae will remain the concern of phycologists as long as explorations and research is underway. Therefore, the algae of the Arab region will remain with more scientific secrets, which will not stop until the completion of the hidden ones. This alga has attracted our attention because of its predominance in a basin that is used to wash hands with industrial detergents. The growth of alga was followed for more than a month to determine its ability to resist or was affected by the detergents used. The expected result was its ability to grow in such a poor environment in the light intensity, which did not exceed 100 lux and variable temperature due to the use or non-use of water. *Leptolyngbya nostocorum* was isolated and identified from hot environments, especially

Makkah, which is characterized by high temperatures and marked drought. Despite the undisputed importance of molecular data, morphology continues to play an important role in cyanobacterial characterization **(Komárek, 2010; Kauff and Büdel, 2011)**. In addition, the ecological characterization of the cyanobacteria and the relationship with phylogenetic assignments can now be addressed with molecular tools. It has been emphasized that ecological data are an integral part of species characterization (**Komárek and Anagnostidis, 1999; Komárek 2010; Mateo *et al.*, 2011)**. The identification by morphological characteristics is still the corner stone to recognition the organism in addition to some of its environmental needs and this is what has been done in the present study. Criteria such as the nature of the gelatinous sheath, color, cell description, shape and dimensions were the most important criteria used in the identification according to the usual standard methods followed. **Alwathnani and Johansen (2011)** isolated *Leptolyngbya* as the most rich species from soils of Mojave desert ecosystem which characterized by hot and dry desert. They described *Leptolyngbya nostocorum* in which the filaments with single trichome per sheath, with no false branching, 2.0–2.5 (3.0) µ m wide. Sheaths thin, open, colorless, soft. Trichomes slightly flexuous, not tapering, very constricted at the cross-walls, without necridia, 2.0–2.5 µ m wide. Hormogonia few-celled, evidently constricted at the cross-walls, and lacking sheath material. Cells blue green, becoming yellowish to washed-out brown with age, rarely with a small central granule, with thylakoids not visible in light microscopy, generally isodiametric, 2.0–2.5 (4.0) µ m long. End cells slightly conical to bluntly rounded. The local form is similar to the species previously described except my form is slightly less in length. **Samad and Adhikary (2008)** have described such alga as thallus dark green; filament with necridic cells, hormogonia flexuous, with a very thin sheath; cells isodiametric or wider than long, 3-5 µm diameter, slightly constricted at the cross wall; apical cells widely conical, with round apex. This local findings are consistent with the few previous studies, in which green algae were shown to be dominant in temperate regions, whereas cyanobacteria dominant in the tropics, especially the forms with well developed sheaths and /or mucilage in their outer envelope.

Regarding the physical and chemical analysis of water, municipal water is considered desalinated water from the Red sea in Jeddah and transported to Makkah via water transport lines.

The studies focused on physical and chemical analysis of rivers, canals, drains, sewage and agricultural waste-water. There is a lack of studies that deals with desalinated water analysis. Therefore, these analyzes were important where it explain the impact of drinking water treatment on existing parameters in water.

Concerning the physical and chemical properties of water, the results confirmed that the water was alkaline and pH was 8.7. The optimum pH required will vary in different supplies according to the composition of the water and the nature of the construction materials used in the distribution system, but it is usually in the range 6.5–8.5(**WHO, 2011)**

Total soluble salts recorded an average of 56.7 mg/L. This result was lower than recoded by **El-Gamal *et al.* (2019)** with 271 mg/L. This may be due to the different standards of desalinated water treatment from river water.

Total alkalinity recorded 28 mg/L, the current results was much lower representing 14.3 % of the result obtained by **El-Gamal *et al.* (2019)**. Total hardness, Ca hardness and Mg hardness recorded 18, 14 and 4 mg/L, respectively. Hardness is most commonly expresses as milligrams of Calcium carbonate equivalent per liter. Although hardness is caused by cations, it may be also expressed by carbonate (temporary) and non-carbonate(permanent) hardness. Desalination of seawater and brackish water converts water with a high dissolved solids content to water with a very low dissolved solids content **(WHO, 2011)**. Therefore, Water contained very low concentrations of elements compared with drinking water from rivers, where calcium concentration was 5.6 mg/L and magnesium 0.96 mg/L,chlorides was 4 mg/L, nitrates was 0.56 mg/L, iron was 0,18 mg/L, respectively. From an ecological perspective, isolates of *Leptolyngbya* were separated on the basis of their nutritional requirements. *L. boryana* was characteristic of waters with high concentrations of nutrients, where as *L. nostocorum* was associated with low levels of nutrients **(Loza *et al*., 2013)**.

Following the growth curve of *Leptolyngbya nostocorum*, it was found that the highest result of growth at 30°C and100 lux recorded at 16 days of incubation,

either by determination of optical density at 760 nm or by calculating dry weight of algae. The results were 0.271and 2630 mg/L, respectively. **Prihantini *et al*. (2019)** found that *Leptolyngbya* were likely thermo-tolerant cyanobacteria and had optimum cultured temperature 35°C. While, optimal growth rates of *Leptolyngbya* isolate in BG-11 and SOT media were achieved at 30 ° C under 12 h light : 12 h dark cycles at 100 µ mol photons m^2 s^1. The isolate was able to grow under low-light conditions (< 4 µmol photons m^2s^1 Approximately <296 lux) (**Kim *et al*., 2015)**.

From the review of the literature it is concluded that no reports on the effects of the commercial detergents on the algae of Kingdom of Saudi Arabia have been done. It therefore seems important to obtain information on the inhibitory or stimulatory effect of the detergents and to what extent they may affect such algae. In the present study of the effect of different concentrations of liquid hand detergents on algal growth, the results showed that concentrations of 100% ,50%, 8.5% and 1.4 % had an inhibitory effect proportional to the concentrations of the detergents, whereas the lowest concentrations had no effect on the growth. In a similar study, **Issa and Ismail (1994)** were found some algal species were very sensitive to all detergents used while others were tolerant to the same detergents and sensitive to others. Such results have been reported by **Yamane (1984) and Abdel-Hamid (1986)**. The tested synthetic detergents inhibited completely the growth of *Chlorella pyrenoidosa* and *Thalassiosira pseudonana* at concentrations above 10 mg/l (**Kondo *et al*., 1984)**.

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ملاحظات على عزل لبتولينبيا (بلكتونيما) نوستوكورم ذات القدرة على تحمل المنظفات السائلة للأيدي

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كان نمط النمو للبتولينبيا *Leptolyngbya (plectonema) nostocorum* على طبقة من خزف حوض الغسيل ملحوظًا ، جذبت هذه المشاهدة اهتمامنا لإجراء هذا البحث. وعلى الرغم من الظروف الغير مناسبة، لم يتأثر نمو الطحلب. تعرض الطحلب للإجهاد المائي نتيجة لتدفق المياه من الصنبور بمعدلات تتراوح من 0.1 إلى 7.6 لتر في الدقيقة, وتتكرر هذه العملية 10 مرات يوميًا لفترات تصل إلى 2.5 ساعة نتيجة لعملية فتح وإغلاق الصنبور، على التوالي. كان متوسط شدة الاستضاءة المحيطة بالطحلب حوالي 100 لوكس وتراوحت درجة الحرارة بين 25-30 درجة مئوية. بالإضافة إلى الظروف السابقة فقد تعرض الكائن الحي لكميات من الصابون السائل لغسل اليدين نتيجة للأنشطة البشرية. تم إجراء وصف وتعريف الطحلب ومقارنة ذلك مع المراجع المتبعة في هذا الصدد . كما تم إجراء التحليل الفيزيائي والكيميائي الأساسي لمياه الصنبور، أظهرت تحاليل الماء أن الأس الهيدروجيني كان قلوي (8.7) والأملاح الذائبة الكلية 56.7 ملي جرام / لتر. وكانت القلوية الكلية 28 وكذلك الصلادة الكلية 18 ملي جرام / لتر ، صلادة الكالسيوم ، صلادة المغنيسيوم كانت 14 ، 4 ملي جرام / لتر ، على التوالي. تراوحت قيم الكالسيوم والمغنيسيوم والكلوريد بتركيزات 5.6 و 0.96 و 4 ملي جرام / لتر على التوالي. كان تركيز النترات 0.56 وكان تركيز الحديد 0.18 ملي جرام / لتر. بالنسبة لمنحنى نمو الطحالب ، تحقق أفضل نمو (0.271, 2630.46 mg/L) للكثافة الضوئية ، الوزن الجاف ، على التوالي بعد 16 يومًا من فترة التحضين . بالنسبة للتركيزات المختلفة من المنظف ، وجد أن أقل تركيز(0,2%) لم يكن لديه أي تأثير مثبط على نمو الطحلب ,بينما كانت التركيزات الأعلى من هذا التركيز تأثيرات بزيادة مطردة في زيادة قطر مناطق التثبيط على النمو. يعد البحث أول دراسة لعزل هذا الطحلب من المملكة العربية السعودية مع لفت الانتباه إلى تأثير التركيزات الأولية للمنظفات السائلة في ازدهار نمو الطحالب في المسطحات المائية.