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Effects of temperature and pH on the growth and vital products of the cyanobacteria species, *Anabaena oryzae*

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ABSTRACT

The effects of temperature and pH on the growth and quantity of vital products extracted from the cyanobacterial species *Anabaena oryzae* in the ASM-1 culture media were studied. Three levels of temperature (18 ± 2 °C, 27 ± 2 °C and 30 ± 2°C) and pH (6.8, 7.6 and 8.4) were selected. It was found that the best daily growth and the largest amount of vital products occur at a temperature of 27 ± 2 ° C and pH 7.6. The vital products were diagnosed by a GC-MS device, and the results of the diagnosis have shown the presence of the compounds dihydroxyphenyl glycol, n -acetyl-Dglucosamine, linalool, niacinamide and gibberellins.

Keywords: Cyanobacteria, *Anabaena oryza*e, Vital products.
Article type: Research Article.

INTRODUCTION

Cyanobacteria are among the oldest living organisms on the Earth's surface (Schopf 1993), dating back to 3.5 billion years ago (Schopf & Walter 1982). They are responsible for the presence of oxygen in the Earth's gaseous atmosphere (Harlin & Darley 1988). Moreover, it has distinctive characteristics among microorganisms such as carrying out the nitrogen fixation process as in *Anabaena*, *Nostoc* and *Oscillatoria*. Some release hydrogen as an accidental product of the nitrogen fixation process (Fogg 1969), as well as excreting toxic substances, including the neurotoxin *Anatoxin-a* and the hepatotoxin *Microcystin* (Carmichael *et al.* 1988). Cyanobacteria is one of the groups of microorganisms capable of producing different types of antibiotics against germs, bacteria and fungi (Issa 1999). Cyanobacteria are characterized by their wide spread in various environments to withstand extreme environmental conditions (Khaliullina 2021), where some of their types are found in the arctic snows such as *Phormidium*, while some other types are found in relatively high temperatures such as *Oscillatoria* (Robarts & Zohary 1987). Cyanobacteria are characterized by their preference for a basal pH, which ranges between 7.5 and 8.5. This study is conducted to investigate the effects of temperature and pH on the daily growth and biological products of *Anabaena oryzae*, to diagnose the biological products and to detect the types of toxins in these bacteria.

MATERIALS AND METHODS

Sample collection

Samples are collected from rocky areas on the banks of the Tigris River in Al-Dour district.

Growth, isolation and purification medium of cyanobacteria

The selective medium ASM-1 was used to grow cyanobacteria. The samples collected from the aforementioned rocky environment were cultured on solid ASM-1 medium in petri dishes, then left to grow for 4-6 weeks in an incubator at a temperature of 25 °C and an intensity of illumination 2500 lux. The developing colonies were

transferred separately to petri dishes containing freshly-prepared ASM-1 medium, and left under the same conditions to grow in a pure form. The pure colonies of *Anabaena oryzae* were transferred to 250-mL glass flasks containing ASM-1 liquid medium and incubated under the same conditions mentioned above. It was then transferred to 5-liter flasks containing 3.5 L ASM-1, supplied with air via a motor and left to grow under the same conditions of temperature, PH and intensity of illumination.

Measuring the daily growth of cyanobacteria

The daily growth of the selected type of cyanobacteria is measured in the freshly-prepared liquid ASM-1 selective medium placed in the shaker incubator, at a temperature of 25 ± 1 ºC, pH 7.6 and light intensity of 2500 lux for a period of 22 days. Its daily growth was measured in terms of the optical density and at a wavelength of 436 nm using a Spectrophotometer (Gibson & Fay 1983).

Measuring the effect of temperature on the daily growth of cyanobacteria and the amount of extracted vital products

The selected cyanobacterial species, *A. oryzae,* was grown in a liquid ASM-1 selective culture medium and placed in the shaker incubator at the separate temperatures of 18 ± 2 °C, 27 ± 2°C and 30 ± 2 °C, under 2500 lux and PH 7.6. The daily growth is measured according to what is mentioned above, and the vital products are extracted according to the method that is to be mentioned later. The extracted quantities are also measured.

Measuring the effect of pH on the daily growth of cyanobacteria and the amount of extracted vital products

The selected cyanobacteria were grown in the liquid ASM-1 selective culture medium and placed in the shaker incubator at a temperature of 25 °C, and under 2500 lux. Three separate degrees of pH (6.8, 7.6 and 8.4) were used to observe its effect on the growth of *A. oryzae* and the daily growth was measured according to what was mentioned above. The vital products are extracted according to the method that to be mentioned later, and the extracted quantities are also measured.

Vital product extraction

After obtaining the growth of cyanobacterial colonies, *A. oryzae* cells were collected using a centrifuge at a speed of 3000 revolutions per min for 5 minutes. The precipitate was taken and dissolved in ethanol (1 g 10 mL; Al-Shahri 1997). The cells were disintegrated using an ultrasound device (Ultrasonic) at 24000 frequency per second for 15 seconds alternating with pauses to cool the device and maintain at a temperature of 4 °C. The process was repeated several times to completely break up and disintegrate the cells. The solution was placed in centrifugal tubes at a speed of 3000 rpm for 10 minutes. The filtrate was taken and the ethanol was evaporated at a temperature of 35 °C (Mustafa 1995). The extract was re-dissolved with distilled water and the proteins (vital products) were precipitated using ammonium sulphate at a concentration of 70%. The precipitate was separated from the filtrate by centrifugation at a speed of 3000 rpm for 30 minutes, and the precipitate containing the proteins was taken. Then, the ammonium sulphate was removed by the method of membrane sorting (Dialysis). The protein was dried by a Desiccator device, and the protein containing the vital products was preserved until examination.

Diagnosing vital products using the GC-MS technology

The compounds of the vital product samples of the cyanobacteria were separated by dissolving them in a methanol solvent to evaporate the solvent and keep the dissolved particles. It was then directed by pure helium gas 99.9% towards the MS device, where this worked to generate charged and ionized particles. In the gas phase, it is easy to treat it electrically or magnetically to facilitate its detection, measurement and determination of its molecular weights by means of a *Shimadzu GCMS-QP2010 Gas Chromatography Mass Spectrometer* (David 2005).

RESULTS AND DISCUSSION

Isolation and diagnosis of cyanobacteria

Cyanobacteria of the type *Anabaena oryzae* were isolated from rocky areas on the banks of the Tigris River in Al-Dour district. This type of filamentous forms contains heterocysts. In addition, this form of cyanobacteria (*Anabaena*) is prevalent in the Iraqi environment and this was proven by previous studies (Al-Douri 2005). It was diagnosed by a light microscope of a photographic camera with two magnifications (400 X and 1000 X), and the diagnosis was conducted based on Desikachary (1959) and Rippka *et al*. (1979).

Effect of temperature on the daily growth of isolated cyanobacteria and on the amount of extracted vital products

Fig. 1 shows the effect of different temperatures on the daily growth of *A. oryzae*. Notably, the growth at 27 ± 2° C was better than those at 18 ± 2 °C and 30 ± 2 °C, although the daily growth at 30 ± 2° C was better in the first five days of the culturing life than at 18 ± 2° C and 27 ± 2 °C. However, it decreased gradually starting from the sixth day, albeit 30 ± 2 °C was better in elevating the daily growth than 18 ± 2 °C until the day 23. in the case of temperature of 27 ± 2 °C, it was the most appropriate to gradually upraise the daily growth until the 23rd day of the culturing life, which began to decline at that time after the depletion of the cyanobacterial nutrients in the ASM-1 culture medium. In the case of the temperature of 18 ± 2 °C, the elevation in daily growth remained low during the study period. This confirms that this type of cyanobacteria prefers moderate temperatures and can adapt and live in relatively high temperatures, and can hardly adapt to live with low temperatures.

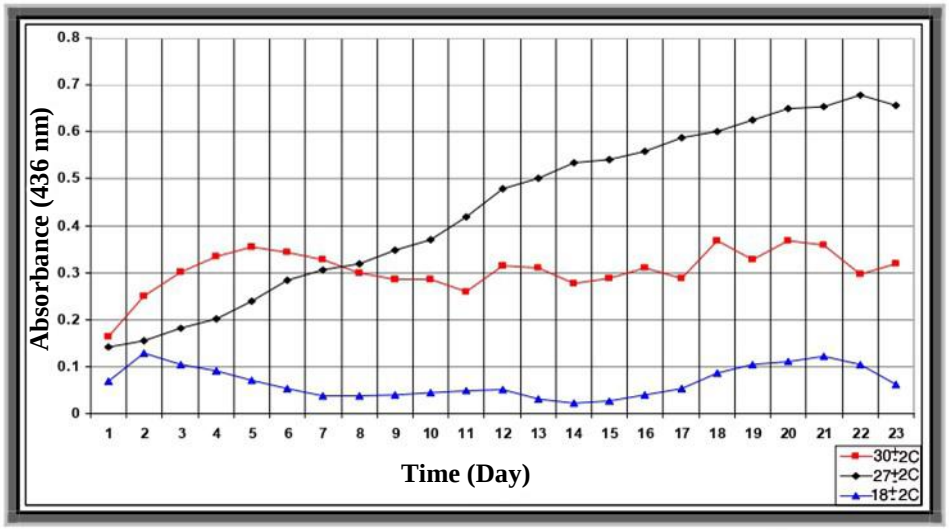

Fig. 1. Effect of different temperatures on the daily growth of the cyanobacteria *Anabaena oryzae.*

The effect of temperature on the amount of biological products of *Anabaena oryzae* is shown in Fig. 2. Notably, the amount of vital products at 18 ± 2 °C was 95 mg L^{-1}, while at 27 ± 2° C was 364 mg L^{-1} and at 30 ± 2 °C was 269 mg L^{-1}. Thus, 18 ± 2 ° C exhibited the lowest amount of vital products, while at 27 ± 2 °C the highest. This confirms what is mentioned above about its temperature preference (moderate). Therefore, the above-mentioned results is in agreement with the assertion by Robarts & Zohary (1987), who emphasized that cyanobacteria differ in their response to temperatures. It is possible that we find some of their species growing on the rocks of Antarctica and in volcanic springs of high temperatures, however there are exceptions.

Effect of pH on the daily growth of isolated cyanobacteria and the amount of extracted vital products

Fig. 3 shows *A. oryzae* at pH 6.8. The amount of daily growth increment according to the culturing age of the cyanobacteria was low compared to the other pH in the first fifteen days of the culturing life. The growth began to increase on the sixteenth day compared to PH 8.4, which at the beginning of the culturing age exhibited more increment in growth than at pH 6.8. The highest daily growth at pH 6.8 was recorded on the 23rd day of the culturing life with 0.34 as optical density, while the highest daily growth at pH 8.4 on the 14th day with 0.186 as an optical density. The best increment in its daily growth was observed at pH 7.6 where the increase in daily growth was noticeable, as the increment continued to grow gradually until the 22nd day reaching 0.69 as an optical density. This indicates that *A. oryzae* tolerates simple acidity and does not tolerate high alkalinity. In the case of its optimum daily growth increment, it is according to the simple alkaline. It is clear from Fig. 4 that the amount of vital product extracted from *A. oryzae* was the highest in the culture medium at pH 7.6, amounting to 370 mg L^{-1}, while at pH 8.4 was the lowest (12.2 mg L^{-1}). It was 255.1 mg L^{-1} at pH 6.8. The reason for the difference in the amounts of vital product for this cyanobacteria may be due to its association with the daily growth under these different pH conditions.

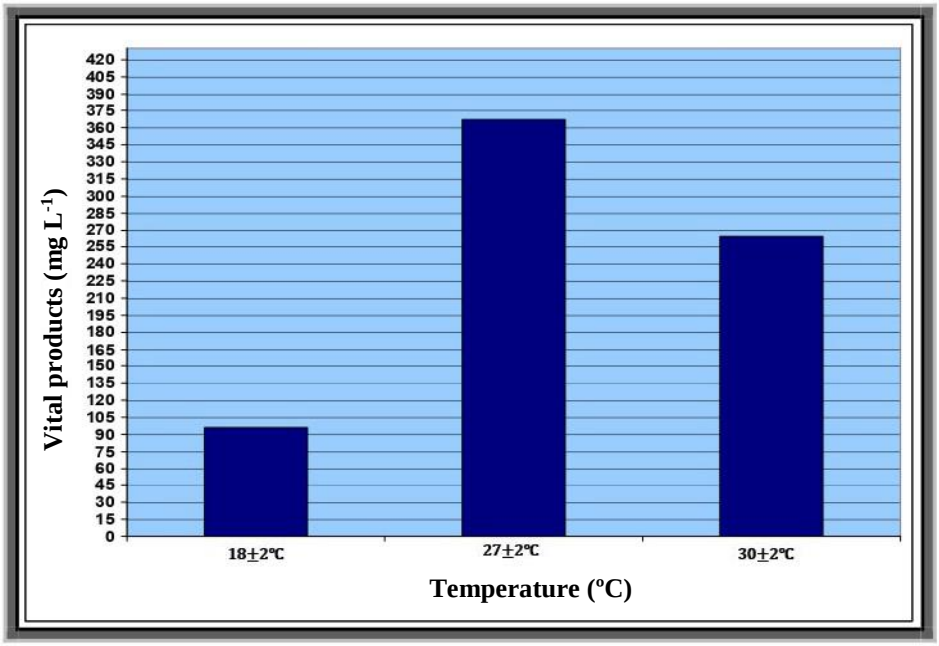

Fig. 2. Effect of different temperatures on the amount of vital products extracted from the cyanobacterial *Anabaena oryzae.*

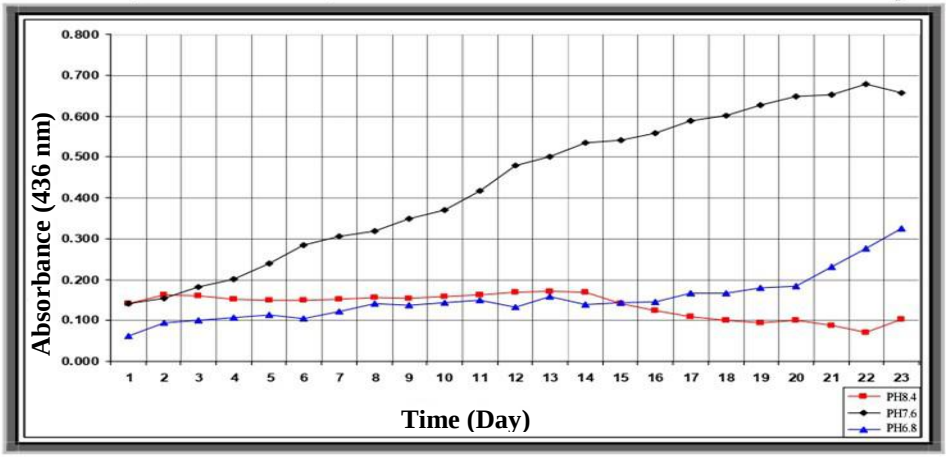

Fig. 3. Effect of pH on the daily growth of the cyanobacteria *Anabaena oryzae.*

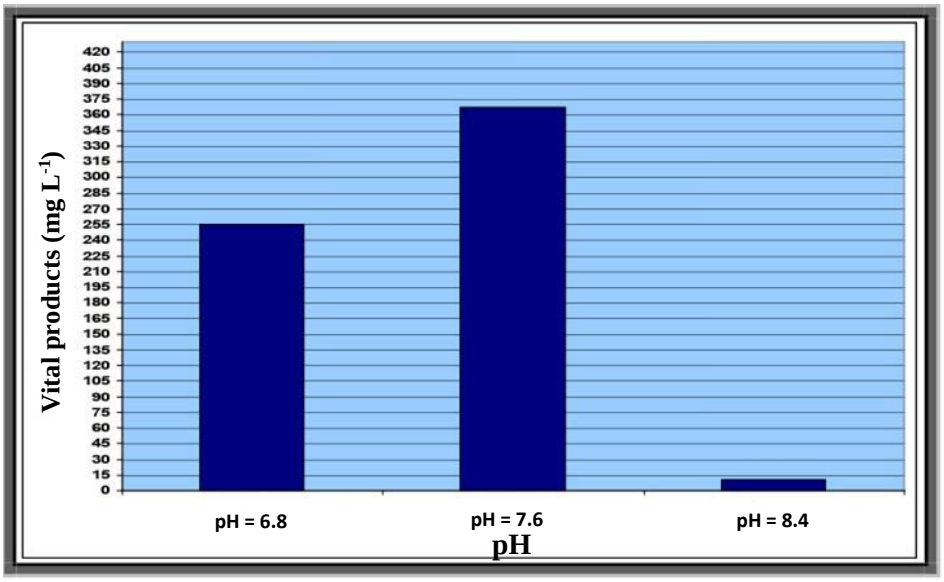

Fig. 4. Effect of pH on the amount of vital products extracted from the cyanobacterial *Anabaena oryzae.*

The pH affects the growth of cyanobacteria and their nitrogen fixation (Pongtep & orenzen 1982; Yahya 1989). Moreover, Stewart (1970) has indicated that most types of cyanobacteria spread in soils with pH 7.5-8.5, and some have been found in waters with pH 9.0-9.5 such as *Anabaena flos-aqua* (Talling 1976). It is also reported by Al-Baldawi (1997) that the optimum pH for nitrogenase enzyme activity and efficiency was around 7.8. It seems that high pH exhibits the most influential factor on the growth of cyanobacteria. This may be due to the nature of this species, which prefers to live and grow in a neutral or slightly alkaline medium, with the possibility of living in a slightly acidic medium. In addition, most types of cyanobacteria are affected in one way or another by the environment in which it lives.

Diagnosing the vital products of *Anabaena oryzae* using the GC-MS device

The vital products of *Anabaena oryzae* are diagnosed by The Gas Chromatography-Mass Spectrometry device (Fig. 5), where it has taken a holding time of 12 minutes.

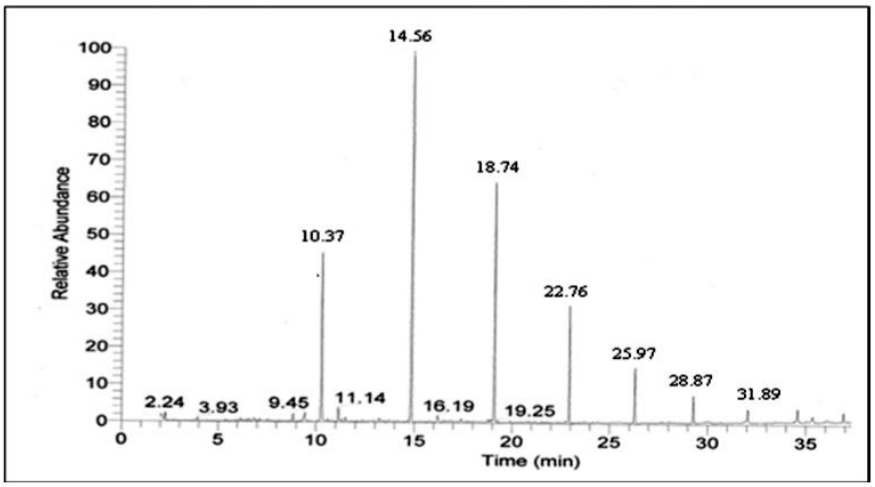

Fig. 5. Diagnosing the vital products of the extracted products from the cyanobacterial *Anabaena oryzae* by the GC-MS device.

The results of the matching compounds consisting of one of the vital products using the computer database in the GC-Mass technique have indicated that it is composed of the following compounds: Dihydroxyphenyl glycol with a molecular weight of 170.16, a chemical composition of ($C_8H_{10}O_4$), the compound n-acetyl-D-glucosamine with a molecular weight of 221.21, the chemical structure ($C_8H_{15}NO_6$), the linalool compound with a molecular weight of 154.25, the chemical structure $(CH_3)_2C=CHCH_2CH_2C(CH_3)(OH)CH=CH_2$, the compounds niacinamide and gibberellins which are components of hepatotoxic microcystins (Natalia & Elzbieta 2015).

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