

Summary

This study was deal with compounds that never had been studied in Iraq called taste and odour compounds, The majority of all biologically caused taste and odour outbreaks in drinking water characterized worldwide are caused by microbial production geosmin and 2-Methylisoborneol are compounds that cause musty, earthy odours in public water supply reservoirs and are mainly produced by blue-green algae (cyanophyta), its considered the first of its kind in Iraq.

In this study 27 cyanophyta species were isolated, only two of them were identified as producers for taste and odour compound, the first was *Phormidium retzii* produced geosmin and *Microcoleus vaginatus* produced 2-Methylisoborneol which were recorded for the first time in the world as a producer. The headspace solid phase microextraction method was used to extract these two compounds from aqueous solution, which was given good recoveries and linearity of geosmin and 2-Methylisoborneol standards (1-1000 ng/l) and production by cyanophyta species, this method is considered the most efficient methods in the world used to detect many volatile compounds especially geosmin and 2-Methylisoborneol, which were used for the first time in Iraq.

The results showed that there was no relationship between an increase temperature and geosmin and 2-Methylisoborneol production, but there was between geosmin and 2-Methylisoborneol production and algal biomass when using three temperature 10, 25 and 33 C° and their effect on production of geosmin and 2-Methylisoborneol, maximal production and algal biomass occurred at 25 C° which were reached 96.6, 135.8 ng/l total geosmin and 2-Methylisoborneol respectively, while the concentrations of total geosmin and 2-Methylisoborneol were 67.2, 61.44 ng/l and 48.4, 39.8 ng/l at 10 and 33 C° respectively in the late of exponential phase.

Concerning Light intensity, the second environmental factor used in this study, also no relationship was found between increase light intensity and geosmin/2-Methylisoborneol production when three light intensities (17, 33 and 50 $\mu\text{E m}^{-2} \text{s}^{-1}$) were used to estimate their effect of the production of these two compounds. High concentration of geosmin and 2-Methylisoborneol registered at (17 $\mu\text{E m}^{-2} \text{s}^{-1}$) which was reached 96.6, 135.8 ng/l total geosmin and 2-Methylisoborneol respectively, while the concentrations of total geosmin and 2-Methylisoborneol were 48.4, 50.9 ng/l and 60.5, 59 ng/l at 33 and 50 $\mu\text{E m}^{-2} \text{s}^{-1}$ respectively in the late of exponential phase.

Nitrate was used as a nitrogen source for observing its effect upon the production of geosmin and 2-Methylisoborneol by two cyanophyta species. Four

concentrations of nitrate are used (97, 350, 861 and 1500 μgNO_3^- -N/L). The results illustrated that the geosmin and 2-Methylisoborneol concentrations were affected by the concentration of nitrate positively significant relationship. The maximal production of geosmin was 281.38 ng/l occurred at 861 $\mu\text{g NO}_3^-$ -N/L, whereas the higher production of 2-Methylisoborneol was 296 ng/l occurred at the same concentration in the late of exponential phase. The high concentration of nitrate (1500 μgNO_3^- -N/L) led to suppressed geosmin and 2-Methylisoborneol productions.

The results demonstrated a positive significant relationship between phosphate as phosphorus source and geosmin/2-Methylisoborneol production when used four concentrations of phosphate (12, 40, 400 and 870 μgPO_4^- -P/L), noticed that the production of these two compounds increased with increasing phosphate concentration. The maximal production of geosmin was 132.2 ng/l occurred at 870 $\mu\text{g PO}_4^-$ -P/L, whereas the higher production of 2-Methylisoborneol was 167.2 ng/l occurred at the same concentration in the late exponential phase.

Likewise, the study showed that the concentration of 2-Methylisoborneol higher than of geosmin concentration which were 296 and 281.38 ng/l registered at the late exponential phase respectively in 861 $\mu\text{g NO}_3^-$ -N/L.

The intra- extracellular distribution of both compounds geosmin and 2-Methylisoborneol was studied, the results showed that the environmental factors affect intra-extracellular geosmin and 2-Methylisoborneol. Where it was observed that the geosmin and 2-Methylisoborneol retained intracellularly in lag phase, a small portion will be released in the exponential phase and the highest portion of two compounds will be released to the medium during stationary and decline phase for all environmental factors because the cell became senescent and lyses. The highest portion of intracellular geosmin and 2-Methylisoborneol were occurred in the late exponential phase ranged between 77-88% at 33 $\mu\text{E m}^{-2} \text{ s}^{-1}$, 33 $^\circ\text{C}$ and 870 $\mu\text{g PO}_4^-$ -P/L for geosmin respectively, while 76-94% at 12 $\mu\text{g PO}_4^-$ -P/L and 861 $\mu\text{g NO}_3^-$ -N/L for 2-Methylisoborneol respectively at the same phase.

The results showed that a higher portion of extracellular geosmin and 2-Methylisoborneol were released during the decline phase, which were 63% and 60% respectively at higher temperature 33 $^\circ\text{C}$ and 61% for both extracellular geosmin and 2-Methylisoborneol which registered in decline and stationary phase respectively at light intensity 33 $\mu\text{E m}^{-2} \text{ s}^{-1}$. The maximal extracellular portion was 64% geosmin and 56% 2-Methylisoborneol which occurred at decline phase for 350 and 1500 $\mu\text{g NO}_3^-$ -N/L respectively. About 60% and 56%

of extracellular geosmin and 2-Methylisoborneol observed in the decline phase for phosphate 12 and 40µg PO₄-P/L respectively.

The statistical analysis gave positive significant correlation between geosmin/2-Methylisoborneol production, extra-intracellular geosmin/2-Methylisoborneol, environmental factors and growth phases at the probability 0.05.

The primers designed in this study for detection of genes responsible of geosmin production by *Phormidium retzii* and 2-Methylisoborneol production by *Microcoleus vaginatus* when using genetic analysis by PCR technique which were *geo1*, *geo2*, *geo3* and *2-MIB1*, *2-MIB2*, *2-MIB3* derived from cyanophyta species exist online in National centre of biotechnology information (NCBI) site were discovered before which were producing geosmin and 2-Methylisoborneol. The primers gave good consequences for detection the genes responsible of these two compounds. Thus, genetic analysis was considered one of the important methods and complementary for Instrumental Analysis (Chromatographic) for the detection of such compounds.

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