Response of *Esherichia coli* to the Alcoholic Extract of Green Alga *Chlorococcum humicola*

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Abstract: The response of *Escherichia coli* bacteria witch was isolated from contaminated water to *Chlorococcum humicola* alcoholic algae extract was studied on growth and different parameters. The Extract concentration of *C. humicola* wear 0.07, 0.15, 0.31, 0.2 and 1.25 mg L^{-1}. showed a high efficiency in significant reducing numbers of *E. coli*, the Growth Account (GA) showed a highest value at concentration 0.07 mg L^{-1} which was 303, 200 and 168 CFU mL^{-1} and less value at. 1.25 mg L^{-1} which was 99, 61 and 49 CFU mL^{-1} in 24, 48 and 72 h, respectively. While removal account (RA) appeared highest value at 1.25 mg L^{-1}, 333, 419 and 431 CFU mL^{-1} and less value at concentration 0.07 mg L^{-1} which was 150 and 27.312 CFU mL^{-1}. Also the mortality percent (MP) appeared highest value at 1.25 mg L^{-1} (68, 87 and 89 CFU mL^{-1}) and less value at 0.07 mg L^{-1} witch was 31, 58 and 65 CFU mL^{-1} as well as the removing percentage of *E. coli* by green algae *C. humicola* which was 60, 80 and 82% in 24, 48 and 72 h, respectively. The result of study improved that the species *E. coli* is high sensitive to *C. humicola* alga extracts toxicity of algal extracts were time and concentration dependent. The aim of the study refers to can be used as biodegradation agent in order to disposal of pollution by *E. coli* witch represent the bacterial water pollutant, with possibility to used the alcoholic extract of green alga to reducing the time and cost the water treatment.

Keywords: Alcoholic algae extract, toxicity, polluted water

INTRODUCTION

Water Sources, especially surface water, is exposed to the dangers of pollution, which leads, directly or indirectly, to the ecosystem. Water pollution means any change in the physical, chemical and biological characteristics of water, as well as the pathogens resulting from the effects of microorganisms such as bacteria, fungi and viruses (Cervenka *et al.*, 2006).

The rivers are the most water bodies susceptible to pollution because of the discharges resulting from the various human activities that make them unusable only after the adoption of additional treatment and liquidation units of high and cost technology.

The presence of *E. coli* and other faucal indicator organisms such as Streptococci in surface waters can indicate a human health hazard because faecal contamination increases the risk of euteric pathogenic microorganisms being endemic transport of FIOs from land to bathing water (SEPA., 2002) and to river water abstracted for irrigation of ready to eat vegetables (Beuchat, 1996) are there for of public concern, the regulation of such contamination is covered in European union by directives such as the bathing waters directive and more recently the water framework directive (EU, 2000).

The *E. coli* was associated with watery or bloody diarrhea hemorrhagic colitis and hemolytic uremic syndrome (Stephan and Untermann, 1999). The organisms typically colonizes the infant gastrointestinal tract within hours of life there after *E. coli* and the host derive mutual benefit. The organisms is distributed in the environment as well as in the bowel of human and animals. It is also present in water supplies as an indicator of resent fecal contamination and potential presence of enteric pathogens (Collee *et al.*, 1996).

Algae are mainly aquatic simple plants found in marine and fresh water as well as terrestrial habitats such as wet rocks or moist soils (NCERT., 2008) microalgae can be a rich source of mane chemically diverse.

Compounds that having used as a bioactive compounds as antimicrobial agents (Stengel and Connan, 2015). making the research use it in human pathology and also in aquaculture as biodegradation which is one of the most effective methods to remediating environmental systems in both situremid and engineered schemes, to reduce costs and environmentally-friendly than traditional detoxifying methods in contaminated environments (Mahmoudi, 2013).

In addition to the use of chemical sterilizers. The algae are microorganisms that are highly efficient in inhibiting the growth and effectiveness of various microorganisms (Katırcıoğlu *et al.*, 2004).

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It also contributes to the process of self-purification in the water bodies through the photosynthesis process, where they releases the dissolved oxygen gas, which causes the gas balance between oxygen and carbon dioxide between the atmosphere and water, that have a biological, medical and economic importance and are necessary to sustain life (Likens, 2010).

As well as algae have recently received a lot of attention as a new biomass source for the production of a new energy (Zhao *et al.*, 2008). Some of the main characteristics which set algae apart from other biomass sources are that algae can have a high oil or starch content do not require agricultural land. Fresh water is not essential and nutrients that can be supplied by waste water and CO_2 by combustion gas (Marina *et al.*, 2013). The first distinction that needs to be made is between macro algae or versus microalgae (Xu *et al.*, 2004).

Therefore, the aim of the study was to eliminate *Escherichia coli*, using the Alcoholic algae extract.

MATERIAL AND METHODS

Isolation and diagnosis of bacterial: The bacteria *Escherichia coli*, were isolated from the drainage water of the Rustmiya Sewage Treatment Plant in Baghdad using Prescott method (Kumar *et al.*, 2010) grown on the Nutrient agar medium, then (Cervenka *et al.*, 2006) 1 mL were taken from pure isolation into glass vials containing broth nutrient medium and incubated at 37°C.

Preparation of dried algae: *Chlorococcum humicola* was isolated from small stream near the University of Baghdad-Jadriya, from different places where several samples were collected, depends on method of Stein (1973).

Then the alga was laboratory diagnosed using a microscope based on the diagnosis method (APHA., 2005).

The alga was cultured under constant laboratory conditions and temperature of 25±2°C, 3000 lux and 16:8 light:dark as photo system in Chu 13 media (Yamaguchi *et al.*, 1987) (incubated in the incubation room for 18 days, harvested by Centrifuge with of 3000 rpm speed for 15 min, the sedimentation dried in oven at 50°C for 48 h, all samples keeps in 25°C antal used (Jawad, 1982; Eppley *et al.*, 1977).

Extraction of active substances from algae: About 15 g of the dried algae was dissolved in 250 mL of Chloroform and then placed in shaking incubator at 25°C with 170 rpm for 15 min and then dried at 40°C (Taskin *et al.*, 2007).

Preparation of concentrations of algae extract: Cervenka *et al.*, (2006) 1 mL of bacterial suspension was taken for each isolation of bacterial isolates and placed in a sterile glass flask containing different concentrations of the extract: 0.07, 0.15, 0.31, 0.62 and 1.25 mg L^{-1} prepared from the primary concentration by the dilutions and then the volume was completed to 100 mL of sterile sewage water with SEPA. (2002) repeated of each bacterial isolation. In addition to the control treatment which was free of the algae extract and incubated at 37°C for 72 h (Kumar *et al.*, 2010).

The number of bacterial cells was calculated in Cervenka *et al.* (2006) mL of bacterial suspension using the method of Hemocytometer (Chamber counting) where the calculation was daily for three days to draw growth carve and account the growth account (GA), removal account (RA), mortality percent (MP) and removing percentage (RP) according to Dahiru and Obidoa (2007).

RESULTS AND DISCUSSION

The result showed exposure of *E. coli* bacteria to different concentrations of *C. humicola* alcoholic extract clearly effect on the growth curve decreed depends on these cells within 72 h. From exposure a decrease was observed within increased emphasis and register a growth curve rapprochement and non-significant differences of the cells number at concentrations 0.13, 0.62 and 1.25 mg L^{-1} in 48 h (Fig. 1). while growth account (GA) showed a clear decline as significant for all concentrations compared with control witch was not exposed to algae extract witch registered (168, 200 and 303) and 133, 177 and 303 CFU mL^{-1} in concentration of 0.07 mg L^{-1} and 100, 100 and 248 CFU mL^{-1} by concentration of 0.15 mg L^{-1} and 100, 100 and 248 CFU mL^{-1} by concentration of 0.31 mg L^{-1} while registered the two concentration 1.25 and 0.62 mg L^{-1} value reached (61, 72 and 180) and (49, 61 and 99) after 24, 48 and 72 h, respectively while the control which was register 480 CFU mL^{-1} after same period (Table 1).

Also register the removal account (RA) a value with significant and increased his concentration and the value converged with non-significant after 24, 48 and 72 h. Between 1.25 and 0.62 mg L^{-1} and the signification between the high concentration compared with the less two concentration which used at the study witch all the values significant compared with control (Table 2).

As well as mortality percent (MP) register values reached 31, 58 and 65% at concentration 0.07 mg L^{-1}, 37, 63 and 71% at 0.15 mg L^{-1} and 47, 74 and 79% at 0.31 mg L^{-1} 62, 85 and 87% at concentration 0.62 mg L^{-1} after 24, 48 and 72 h, respectively (Table 3).
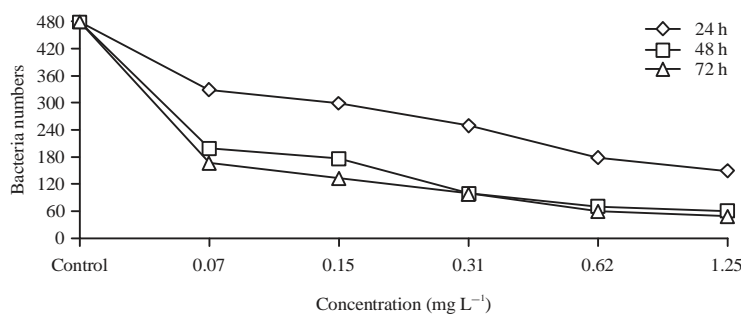
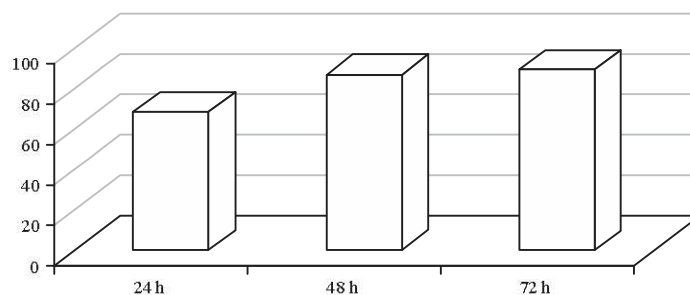Fig. 1: Decreasing of *E. coli* numbers using algae extract during 72 h

Fig. 2: Removing percentage of *E. coli* under deferent concentration of green alga *C. humicola*

Table 1: Growth account for *E. coli* under deferent concentration of green alga *C. humicol*

Concentration (mg L^{-1})	Growth account		
	24 h	48 h	72 h
Control	480.0±11.36[A*]	480.0±10.41[A*]	479.3±9.9[A*]
0.07	303.0±3.33[A*]	200.0±11.55[c*]	168.0±17.50
0.15	303.0±40.44[A*]	177.0±16.80[c*]	133.0±11.00
0.31	248.0±21.28[b*]	100.0±1.0	100.0±5.77
0.62	180.0±14.43[b*]	72.0±2.3[d*]	61.0±10.51
1.25	99.0±72	61.0±2.1[d*]	49.0±6.51

Small letter*: No significant in the same column, Capital letter: No significant in same row at 0.05 level

Table 2: Removal account for *E. coli* under deferent concentration of green alga *C. humicola*

Concentration (mg L^{-1})	Removal account		
	24 h	48 h	72 h
Control	0.0±0.0	0.0±0.0	0.0±0.0
0.07	150.0±13.20[a*]	276.0±19.43[c*]	312.0±13.0[f*]
0.15	180.0±4.62[a*]	303.0±3.51[c*]	347.0±29.6[f*]
0.31	230.0±7.51	380.0±10.44	380.0±40.7[f*]
0.62	300.0±7.53[b*]	408.0±5.29[c*]	419.0±19.0
1.25	333.0±9.53[b*]	419.0±14.53[d*]	431.0±2.00

Table 3: Mortality percent (MP) for *E. coli* under deferent concentration of green alga *C. humicola*

Concentration (mg L^{-1})	Mortality percent		
	24 h	48 h	72 h
Control	0.0±0.0	0.0±0.0	0.00±0.0
0.07	31.3±0.43	58.3±0.33	65.00±2.52
0.15	37.5±5.01	63.1±1.13	71.96±1.28
0.31	47.9±0.53	79.1±0.04	79.17±0.17
0.62	62.5±2.02	85.0±1.73[a*]	87.30±0.84[b*]
1.25	68.8±4.79	87.3±0.29[a*]	89.76±1.76[b*]

While the removing percentage registered 60, 80 and 82% after 24, 48 and 72 h. Which refers to increased the removal rate (Fig. 2).

Also the study agrees with Taskin *et al.* (2010), which indicated the ability of *Cladophora* extract to reduce the number of *E. coli* bacteria, the reason is due to the secretion of many substances such as Ferpenoids, fatty acids. acids, amino, which have inhibitory effect on bacteria (Xu *et al.*, 2004). And they produce a wide variety of chemically active metabolites in their surroundings, potentially as an aid to protect themselves against the other selling organisms (Rao *et al.*, 2007). These active metabolites also known as biogenic compounds, such as halogenated compounds, alcohols, aldehydes, terpenoids, are produced by several species of marine macro and microalgae and have antibacterial, anti-algal and anti-fungal properties which are effective in the prevention of bio fouling and have other uses in therapeutics (Dahiru and Obidoa, 2007).

The algae extract has an inhibitory effect against the positive and negative bacteria (Gram stain) by inhibiting the Enzyme of phosphatase protein, which plays an important role in the process of inserting substances into the body of the organism Dahiru and Obidoa (2007).

The active substances found in the algae extract have an effect on the positive bacteria of (Gram stain) rather than on the negative bacteria.

As indicated by Ordog *et al.* (2004). The reason is that the negative bacteria are less sensitive than the active compounds found in positive bacteria of (Gram stain) due to their cell wall structure, which consists of many layers (Abd-Elnaby, 2010).

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