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Activity of *Chlorella vulgaris* Associated by *Escherichia coli* W3110 on Removal of Total Organic Carbon in Continuous River Water Flow System

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We investigated the association of *Chlorella vulgaris* and *E. coli* W3110 in removal of total organic carbon with the lab-scaled continuous river water flow system (CRWFS). Artificial synthetic wastewater was applied at two levels of organic carbon concentration; 1,335 mg $\cdot l^{-1}$ in the treatment (T)-1 and 267 mg $\cdot l^{-1}$ in T-2. The highest densities of *C. vulgaris* were 8.3 x 10^6 cells $\cdot$ ml^{-1} in T-1 and 6.9 x 10^6 cells $\cdot$ ml^{-1} in T-2. The maximum densities of *E. coli* W3110 were 2.0 x 10^8 colony forming unit (CFU) $\cdot$ ml^{-1} in T-1 and 3.9 x 10^8 CFU $\cdot$ ml^{-1} in T-2. The densities increased during the first 11 days in T-1 and 4 days in T-2, and decreased rapidly till 35th day, then increased slightly afterwards. This trend was prominent in T-2. It was implied that wider range of nutrients was required in the growth of heterotrophic bacteria in T-2 than in T-1. The algal biomass should be increased effectively for the successful removal of organic carbon.

Key Words: algal biomass, association of algae and bacterium, *Chlorella vulgaris*, *Escherichia coli* W3110

INTRODUCTION

The wastewater treatment method using algae has been led traditionally by the use of oxidation ditch/conventional waste stabilization pond (Shelef 1982; Nie 1991). Since the experimental runs had been performed successively with the effectively developed small scale plant, high rate pond (HRP), the noticeable removal efficiencies were being nominated as total nitrogen (inorganic and organic nitrogen; 70-100%); total phosphorus (inorganic and organic phosphorus; about 50%); and COD (up to 70%) within 7-9 days of wastewater detention time (Mara 1990; Picot 1991).

Using solar energy algae uptake CO_2, nitrogen, and phosphorus from the abiotic surroundings of water ecosystem, and produce organic matter and oxygen. Algae show S type of growth pattern and it represents kinetic characteristics as rapid absorption of nutrients when logarithmic proliferation phase. Algae keep high density and it could be maintained under the optimal environmental conditions such as water temperature, light intensity, pH and nutrients like N, P (Goldman and Ryther 1975; Volesky 1989). Algae are the photosynthetic organism like aquatic plant and keep metabolic characteristics of photoautotrophs. Therefore, among the various physicochemical conditions, the environmental factors governing optimum yield of algal biomass are light intensity; water temperature; concentration of nutrients like NO_3^--N, NH_4^+-N, PO_4^{3-}-P; and pH (Goldman and Ryther 1975; Volesky 1989).

Basically, the algal and bacterial interactions can purify water in river water ecosystem (Handricks and Pote 1974; Nakazato 1998). The metabolic activity of aerobic and heterotrophic bacteria produces CO_2, NH_4^+, NO_3^-, PO_4^{3-} etc. Algae uptake these substances and produce organic matter, O_2 and H_2O. Oxygen produced by algae can be used by the aerobic heterotrophic bacteria.

Meanwhile, the water purification system using algae and aquatic plants has been employed in order to furnish the ecological and biological stabilities of aquatic environments (Wang 1991; Stanley and Smith 1992). The concept of water purification by algae and aquatic plants has been established thoroughly on the base of recipro-

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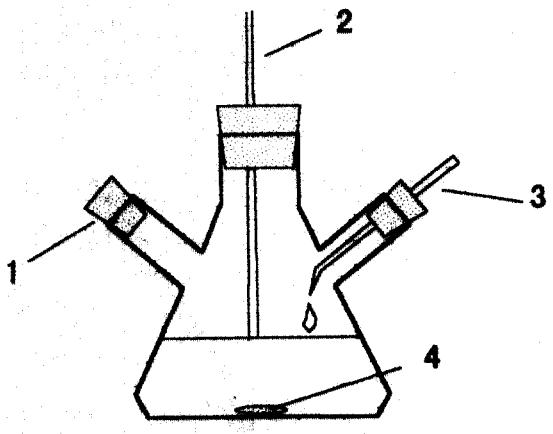
Table 1. Composition of synthetic wastewater used in this study

Component	Concentration ($mg \cdot l^{-1}$)	Remarks
$C_6H_{12}O_6$	1,335	Treatment 1
	267	Treatment 2
$(NH4)_2CO_3$	102.9(30 mg NH_3-N $\cdot l^{-1}$)	
Na_2HPO_4	41.4(9 mg PO_4-P $\cdot l^{-1}$)	
$MgSO_4 \cdot 7H_2O$	250	
$CaCl_2 \cdot 2H_2O$	15.47	
$Fe_2(SO_4)_3$	4.06	
$NaHCO_3$	167.97	
Na_2EDTA	4.88	
$MnSO_4 \cdot 5H_2O$	1.41×10^{-3}	
$ZnSO_4 \cdot 7H_2O$	0.2	
$CuSO_4 \cdot 5H_2O$	0.08	
H_3BO_3	3.13×10^{-3}	
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.13	
$CoCl_2 \cdot 6H_2O$	0.04	

cal interactions in river water ecosystem. Algal pond could be positioned as pre-process if both ponds are connected. Because allelopathic chemicals such as gallic acid, ellagic acid, queretin, α-asarone, 6z-, 9z-, 12z-, 15z-octadecatetraenoic acid, stigmast-4-ene3,6-dione, 4-methy-lthio-1,2-dithioiane and other physiologically active compounds from macrophytes like water hyacinth (*Eichhornia crassipes*) can affect algal growth as antialgal bioactive compounds (Saito 1989; Aliota 1990). Equally, the natural antibacterial bioactive conditions could have been obtained through the experimental operation of maturation pond as the concept of wastewater treatment. There are several factors that control the antibacterial bioactive conditions such as temperature, sunlight, pH, lytic action of bacteriophages, protozoan predation, attachment to solids, antibacterial extracellular algal compounds and depletion of nutrients etc. (Fernandez *et al.* 1992; Saqqar and Pescod 1992; Bitton 1994).

The algal and bacterial interaction is basic and significant in the purification of river water ecosystem. However, the clear evidence on same effect has not been offered yet, and particularly it is equivocal in dealing with the open channel, of which water flows continuously.

The experimental apparatus, lab-scale continuous river water flow system (CRWFS), was installed in the green house to evaluate the algal activity associated with bacteria in the removal of TOC, and the algal and bacterial population densities and the changes of TOC were investigated.



1. Sampling port 2. Effluent outlet 3. Influent inlet 4. Magnetic stirrer bar

Fig. 1. Experimental apparatus

MATERIALS AND METHODS

A hexahedron structure of steel angle plate was installed in the green house. Air temperature was maintained at 25°C. Especially, fluorescent lamps were attached to lower part of this structure and Erlenmeyer flasks were installed under fluorescent lamps (Fig. 1).

Chlorella vulgaris was tested as algal component, because it is well known for its characteristics of light utilization and commercially available (Lee and Palsson 1996). Light was provided by the white fluorescent lamp at 3,500 Lux (1.75×10^{-2} $gcal \cdot cm^{-2} \cdot min^{-1}$) on water surface of Erlenmeyer flask (Fallowfield *et al.* 1992). Algae and bacteria were acclimated for 5 days. Synthetic waste-

Table 2. Conditions of culture

Items	Operational Condition
Experimental Microbial Communities	
- Alga	Seeding with *C. vulgaris*
- Bacterium	Seeding with *E. coli* W3110
Culture Volume	200 m*l*
Temperature	25°C
Illumination	3,500 LUX

Table 3. Conditions of experiment

Seeding Microorganisms	Glucose Concentration ($mg \cdot l^{-1}$)	Treatment
E. coli W3110 and *C. vulgaris*	1,335	1
E. coli W3110 and *C. vulgaris*	267	2

water was fed to this culture medium. From 5th day using the peristaltic pump, synthetic wastewater was fed continuously to the flask through port 3 at 18 $ml \cdot hr^{-1}$. The culture volume was maintained at 200 m*l* level by removing the excess water through the port 2 (Fig. 1).

Samples were taken everyday through port 1 and were filtered (Whatmann 0.2 μm pore size). The concentrations of organic carbon were analyzed by TOC analyzer (Shimadzu TOC-5000A). In order to see the change of dissolved organic carbon (DOC) and the non-filtered samples were used to count densities of algae and bacterium.

The numbers of *C. vulgaris* were counted with direct counting method under epifluorescent microscope using a counting chamber. Colonies of *E. coli* also were enumerated with plate count method (viable count method) using agar plate of the same medium applied in the incubation experiments. After inoculation, the plates were incubated at 25°C for a week, and the numbers of colony on the plate were counted. Dilution rate was 0.42 day^{-1}.

RESULTS

Growth rates of *E. coli* on agar plate

The numbers of *E. coli* in the treatment T-1 increased from 2.4 x 10^7 colony forming unit (CFU)$\cdot ml^{-1}$ to 2.0 x 10^8 CFU$\cdot ml^{-1}$ up to 11th day, but sharply decreased afterwards. This decreasing trend continued to 35th day. After 35th day, there seemed to be recovered in the numbers of *E. coli* up to the end of experiment (Fig. 1).

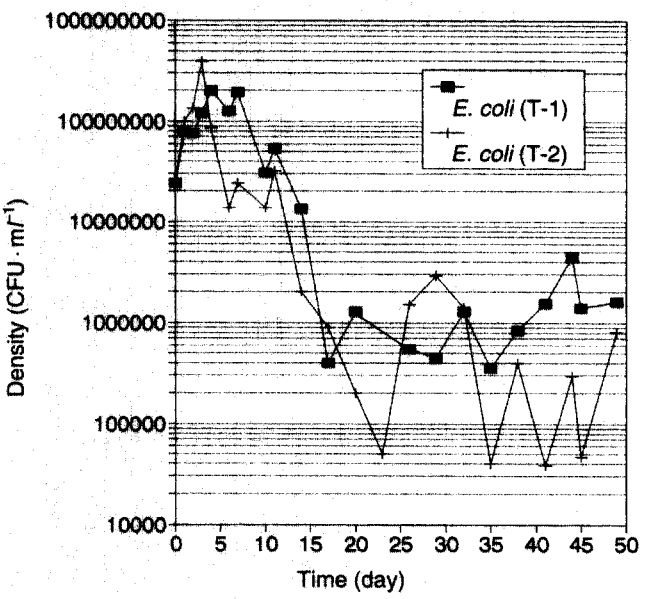


Fig. 2. Changes of the density of *E. coli* W3110 population on the culture using an agar plate

In T-2, the density increased to 3.9 x 10^8 CFU$\cdot ml^{-1}$ during the first 4days. But it decreased afterwards and fluctuated between 5.0 x 10^4-2.9 x 10^6 CFU$\cdot ml^{-1}$ for the rest of incubation period (Fig. 2).

Changes in the density of *C. vulgaris* population in the culture of *C. vulgaris* and *E. coli* W3110

The initial density of *C. vulgaris* population was 1.3 x 10^4 cells$\cdot ml^{-1}$. The densities increased rapidly and reached at their maximal of 8.3 x 10^6 cells$\cdot ml^{-1}$ in T-1 and 6.9 x 10^6 cells$\cdot ml^{-1}$ in T-2 on 10th day, then decreased slowly afterwards.

Changes of DOC

The values of DOC were ranged from 49.0 $mg \cdot l^{-1}$ (11th day) to 249.2 $mg \cdot l^{-1}$ (32nd day) in T-1 and from 11.5 $mg \cdot l^{-1}$ (11th day) to 51.6 $mg \cdot l^{-1}$ (32nd day). During the study period, DOC increased linearly with the slopes of 10.06 and 1.96 (mg DOC$\cdot ml^{-1} \cdot day^{-1}$) with correlation

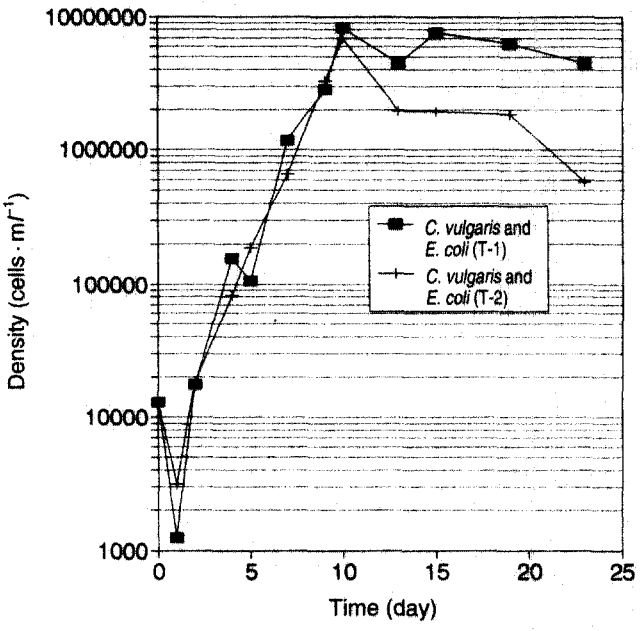


Fig 3. Changes in the density of *C. vulgaris* population in the culture of *C. vulgaris* and *E. coli* W3110

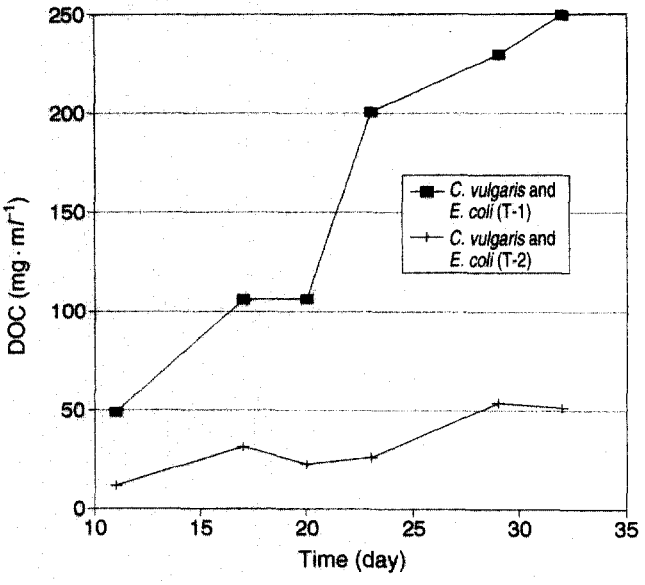


Fig. 4. Changes in concentration of DOC in the culture of *E. coli* W3110 and *C. vulgaris* in synthetic wastewater.

coefficients (R^2) of 0.9340 and 0.8317 in T-1 and T-2, respectively (Fig. 4).

In both experiments, increase of DOC continued during the whole incubation periods. Furthermore, the glucose in T-1 could not help the removal of DOC, although the ratio of BOD_5:T-N:T-P was 84:3:1, which was similar to the general stoichiometric ratio of BOD_5:T-N:T-P of 100:5:1.

DISCUSSION

To evaluate the algal activity associated with bacterium in removal of TOC in the lab scaled continuous river water flow system (CRWFS), the experimental apparatus was installed in the green house. The algal and the bacterial population density and the change of TOC were investigated.

The stoichiometric formula for cellular composition of aerobic bacteria has been reported as $C_5H_7O_2N$ and the synthetic reaction in the aerobic bacterial cell with glucose is given as following equation (Hendricks and Pote 1974);

$$6C_6H_{12}O_6 + 16O_2 + 4NH_3 \xrightarrow{\text{enzyme}} 4C_5H_7O_2N + 16CO_2 + 28H_2O$$

Through the photosynthetic reactions, algae utilize carbon dioxide from the aerobic bacteria and ammonia to produce cell protoplasm and release oxygen molecule (Hendricks and Pote 1974);

$$NH_3 + 8CO_2 + 4.5H_2O \xrightarrow{\text{light}} C_5H_{14}O_3N + 8.75O_2$$

Environmental factors governing algal biomass production are: (1) light intensity, (2) water temperature, (3) pH, (4) macro/micro nutrients, and (5) CO_2 concentration (Volesky 1989; Kong 1996). In the present experiment, the increase of CO_2 due to the heterotrophic bacterial degradation of TOC can be regarded as the 1st primary factor, because other parameters except TOC were maintained constant. However, the high and low concentration of TOC provided with glucose could not affect the growth of *C. vulgaris* significantly. In both treatments,. The densities of *C. vulgaris* population were 8.3×10^6 cells · ml^{-1} in T-1 and 6.9×10^6 cells · ml^{-1} in T-2.

In addition to antibacterial bioactive conditions mentioned in the introduction, especially in continuous river water flow system (CRWFS), long detention time of wastewater can be regarded as the primary factor on antibacterial activities. This factor was regarded as the most important one in the antibacterial reaction because highly alkaline condition generated from algal photosynthesis can hydrolyze the bacterial cell components and affects the dissociation of amino acid of zwitterions on protein molecules (Atlas and Bartha 1994). In addition to long detention time, inactivating effect of light can affect

the desired bacterial growth. The UV-B spectra (280-320 nm) of sunlight can kill bacteria (Moeller and Calkins 1980). The effect of UV increased at high dissolved oxygen concentrations and high pH levels (Curtis *et al.* 1992). However, the growth of *E. coli* W3110 in this experiment represented sigmoid type of growth pattern with the logarithmic growth phase, the decreased growth phase, and the endo-respiration phase. The densities of *E. coli* W3110 increased to the maximal of 2.0 x 10^8 CFU · ml^{-1} in T-1 and 3.9 x 10^8 CFU · ml^{-1} in T-2. The densities decreased rapidly till 35th day, then slightly increased afterwards. This trend in *E. coli* W3110 was more prominent in T-2. This suggested that wider range of nutrients was required for the growth of heterotrophic bacteria in T-2 than in T-1.

The freshwater algae such as *Chlorella* and *Spirulina* are used to make the algal polymer in USA by Bio-Recovery Systems for biosorption of heavy metals (Volesky 1989). The *Chlorella* is not necessarily the best freshwater algae for biosorbing metal ions, but this is commercially available (Volesky 1989).

There was a problem of feeding the wastewater in the continuous culture system. Because the peristaltic pump could not make enough flow nor give satisfactory results in algal biomass bioadsorption. The wastewater detention time should enough to allow the algal and bacterial biosorption. And this must be considered for this culture system in case of the wastewater is supplied continuously at constant rate.

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