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Bacterial Die-Off in Continuous River Water Flow System

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It was examined carefully that the bacterial die-off between *Chlorella vulgaris* and *E. coli.* W3110 was tested through adding TOC (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/l in treatment type 1 and 267 mg/l in type 2. In both types, the population densities of *Chlorella vulgaris* were similar in a maximum 8.25×10^6 cells/ml (type 1) and 6.925×10^6 cells/ml (type 2). The maximum densities of *E. coli.* W3110 were 2.0 x 10^8 colony forming unit (CFU)/ml in type 1 and 3.9×10^8 CFU/ml in type 2. The densities increased for 11 days in type 1 and 4 days in type 2, then decreased rapidly till the 35th day, then slightly increased again. This trend was prominent in type 2. It implied that a wider range of nutrients was required in the growth of heterotrophic bacteria in type 2 than in type 1. We could not expect successful bacterial die-off if the wastewater retention time was not furnished sufficiently.

Key words : *Chlorella vulgaris, E. coli.* W3110, survival pattern, wastewater retention time

1. Introduction

Each unit basin of the Waste Stabilization Pond (WSP) can be arranged in a single line or in rows and in a mixed type by the same ones. And such a line arrangement can be operated in a single floor or in multiple floors, which are designed with a Richmond System[1] parallel meandering channel with a racetrack type. Especially, it is known to society that the single line arrangement of basins is advantageous on removal of high concentration of BOD or of *E. coli.* as indirect indicator of pathogenic bacteria[2]. However effluent water always creates public health problems. This is because the effluent water drained usually from these typical systems has been treated finally into the soil treatment or into the cultivation of aquatic plants as the concept of washing effluents. It is real fact that nobody can deny that pathogenic bacteria may survive on crops, particularly leafly vegetables, irrigated by wastewater.

The algal and bacterial symbiotic reaction reacts most basically on water quality purification of WSP. And these symbiotic reaction have been investigated with the concept of algal and bacterial mutual action in river water ecosystems.[3] The aerobic heterotrophic bacterium's metabolic action is originated from its instinctive survival. Such an instinctual action

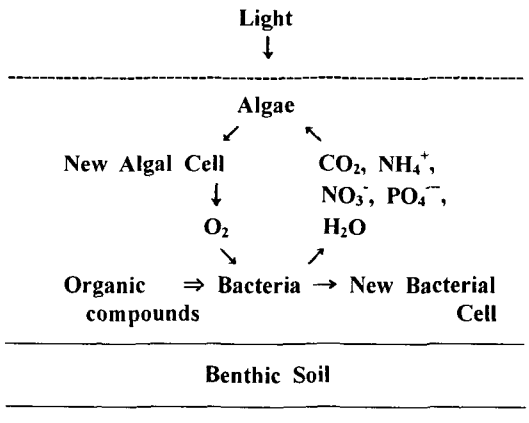

Fig. 1. Algal and bacterial symbiotic reaction.

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produces CO_2. NH_4^++NO_3^-, PO_4^{3-} etc.. Alga intakes such substances into its cell body and produces O_2 and H_2O. The produced O_2 is utilized rotationally by heterotrophic bacterium for its catabolism.

The real antibacterial bioactive conditions have been obtained mainly through experimental runs of a maturation pond as the concept of wastewater treatment. And it has been officially agreed that these conditions are : temperature, sunlight, pH, lytic action of bacteriophages, predation by macroorganisms, attachment to settleable solids, antibacterial extracellular algal compounds, depletion of nutrients etc.[4~6] In addition, especially, Saqqar and Pescod[7] assert that Coliform die-off in ponds increases with an increase in temperature, retention time, and pH, but decreases with an increase of BOD_5/TOC (total organic carbon) and pond depth.

Meanwhile, clear evidence on bacterial die-off has not been offered as much, and particularly it is equivocal in dealing with open channels, of where water flows continuously. In this study, the experimental apparatus, a lab scale continuous river water flow system (CRWFS), was installed in a green house to evaluate bacterial die-off on the same system, The algal and bacterial population densities and the changes of TOC were investigated.

2. Materials and Methods

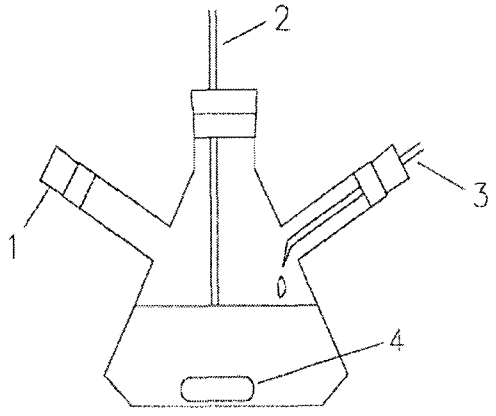
2.1. Green House and Experimental Runs

A hexahedron structure of steel angled plates were installed in the green house. Air temperature was maintained at 25℃. Especially, fluorescent lamps were attached to the lower part of this structure and Erlenmeyer flasks were installed under fluorescent lamps (Fig. 2).

The freshwater algae, *Chlorella* is simply an example of taking the best available, as seen in common and familiar surroundings in Korea/ Japan, from what currently is commercially available.[8] Therefore *Chlorella vulgaris* was tested as the algal component. It is well known for its characteristics of light utilization.[9] Light was provided by a white fluorescent lamp at 3,500 Lux (1.75×10^{-2} · g · cal · cm^{-2} · min^{-1}) on the water surface of an Erlenmeyer flask.[10] Algae and bacteria were cultured for 5 days. Synthetic wastewater was fed into this culture medium. From the 5th day, using the peristaltic pump, synthetic wastewater was fed continuously to the flask through port 3 at 18 ml · hr^{-1}. The culture volume was maintained at 200 ml by removing the excess water through port 2 (Fig. 2). 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 investigate the change of dissolved organic carbon (DOC), non-filtered samples were used to count densities of algae and bacterium. The numbers of *C. vulgaris* were counted with a direct counting method under epifluorescent microscope. Colonies of *E. coli* also were enumerated with a plate count method (viable count method) using an agar plate of the same medium applied in the incubation experiments. Then, the plates were incubated at 25℃ for a week, and the numbers of colonies on the plate were counted. Dilution rate was 0.42 day^{-1}.

3. Results and Discussion

For the purpose of evaluating the bacterial die-off between *Chlorella vulgaris* and *E. coli.* W3110 in the lab scaled CRWFS (continuous river water flow system), the experimental apparatus was installed in a green house and the algal and bacterial population densities were investigated.



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

Fig. 2. Experimental apparatus.

Table 1. Composition of synthetic wastewater used in this study

Component	Concentration (mg/l)	Remarks
$C_6H_{12}O_6$	1,335	type 1 Experiment
	267	type 2 Experiment
$(NH_4)_2CO_3$	102.9(30 mg NH_3-N/l)	
Na_2HPO_4	41.4(9 mg PO_4-P/l)	
$MgSO_4 \cdot 7H_2O$	250	
$CaCl_2 \cdot 2H_2O$	15.47	
$Fe_2(SO_4)_3$	4.069	
$NaHCO_3$	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}	
$(NH_4)_6Mo_7O_4 \cdot 4H_2O$	0.13	
$CoCl_2 \cdot 6H_2O$	0.04	

3.1. Changes of the *E. coli.* W3110 population on agar plate

As shown in fig. 3, the numbers of *E. coli.* in the experiment (type 1) increased from 2.4 x 10^7 colony forming unit (CFU)/ml (initial seeding density) to 5.45×10^7 CFU/ml up to the 11th day, but sharply decreased afterwards. This decreasing trend continued to 35th day. After then 35th day, there seemed to be a recovery in the numbers of *E. coli.* up to the end of experiment. In the experiment (type 2), the density increased to 3.9×10^8 CFU/ml during the first 4 days. But it decreased afterwards and fluctuated between $5.0 \times 10^4 \sim 2.9 \times 10^6$ CFU/ml for the rest of the incubation period.

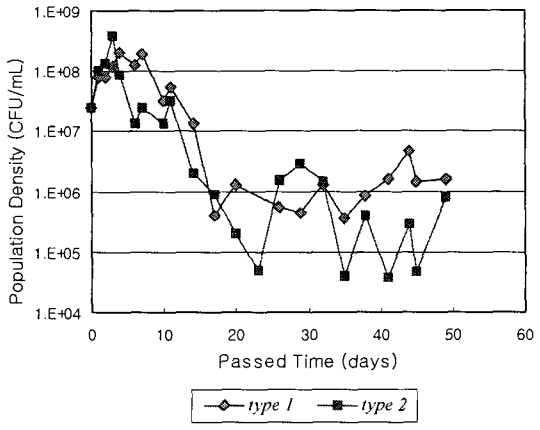


Fig. 3. Changes of the *E. coli* W3110 population on agar plate.

Table 2. Operational conditions of culture

Contents	Condition
Experimental Microbial Communities	
- Alga	Seeding with *C. vulgaris*
- Bacterium	Seeding with *E. coli.* W3110
Culture Volume	200 ml
Temperature	25 ℃
Illumination	3500 LUX

Table 3. Conditions of experiment

Seeding Microorganisms	Glucose Concentration (mg/l)	type
E. coli. W3110	1335	1
E. coli. W3110	267	2
E. coli. 3110 and *Chlorella vulgaris*	1335	1
E. coli. W3110 and *Chlorella vulgaris*	267	2

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

The inoculation density of *C. vulgaris* was 1.285×10^4 cells/ml, which was the same density of seeding for both types. This density increased rapidly and resulted in a maximum of 8.25×10^6 cells/ml (type 1) and 6.925×10^6 cells/ml (type 2), respectively, before and after the 10th day. After that time, the densities decreased slowly. (Fig. 4)

3.3. Changes of DOC

DOC of type 1 varied from 49 mg/ml at 11 days to 249.2 mg/ml at 32 days. DOC of type 2 also changed from 11.46 mg/ml at 11 days to 51.56 mg/ml at 32 days. In this experiment, DOC increased with the duration of incubation. Furthermore, it indicates that even the glucose concentration in type 1 could not help the decrease of DOC, although the ratio of BOD_5 : T-N : T-P was 84 : 3 : 1, was similar to

the optimum ratio of BOD_5 : T-N : T-P of 100 : 5 : 1. It suggests that the culture system maintains its problems due to the continuous feed of the wastewater. The continuous fluid flow by the peristaltic pump could not satisfactorily be given a chance to form the algal biomass and could not give wastewater detention time for such algal biomass to bio-adsorb sufficiently the organic carbon substrate.

3.4. Changes in the density of *E. coli.* W3110 population between *E. coli.* W3110 and *Chlorella vulgaris* (type 1)

The initial density of the *E. coli.* W3110 population was 2.4×10^7 CFU/ml, which was the same density of seeding for both types. This density increased rapidly and reached a maximum of 6.675×10^8 CFU/ml on the 3rd day and slightly decreased afterwards. This decreasing trend continued to the 23rd day and resulted in minimum 2.5×10^5 CFU/ml. But it again increased rapidly after that time. (Fig. 6)

3.5. Changes in the density of *E. coli.* W3110 population between *E. coli.* W3110 and *Chlorella vulgaris* (type 2)

The initial density 2.4×10^7 CFU/ml increased rapidly for 3 days and reached a maximum 1.205×10^8 CFU/ml. After that time, the densities decreased slowly and reached a minimum

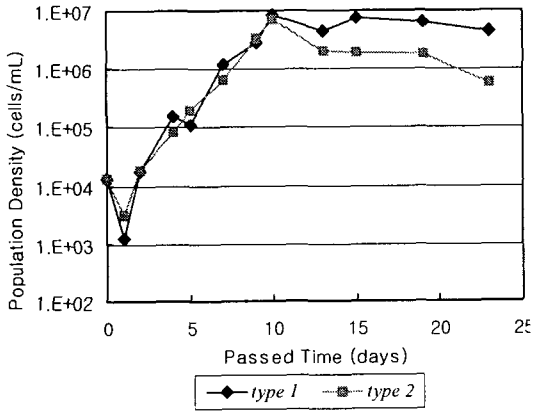


Fig. 4. Changes of the *C. vulgaris* population in the culture of *C. vulgaris* and *E. coli.* W3110.

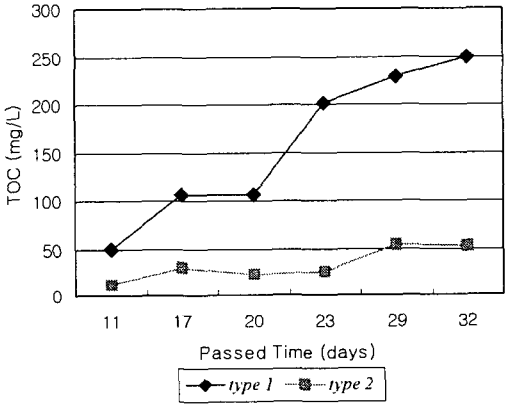


Fig. 5. Changes in concentration of DOC in symbiotic reaction between *E. coli.* W3110 and *Chlorella vulgaris* in synthetic wastewater.

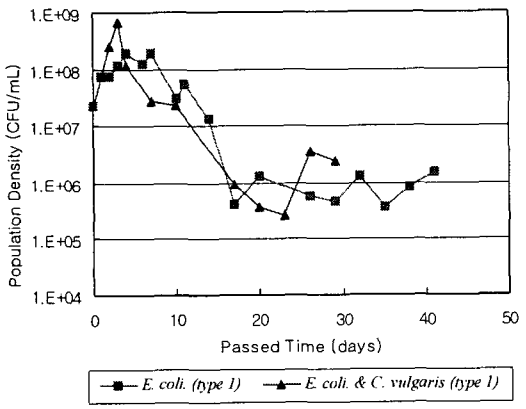


Fig. 6. Changes of the density of *E. coli.* W3110 population between *E. coli.* W3110 and *Chlorella vulgaris*(type 1).

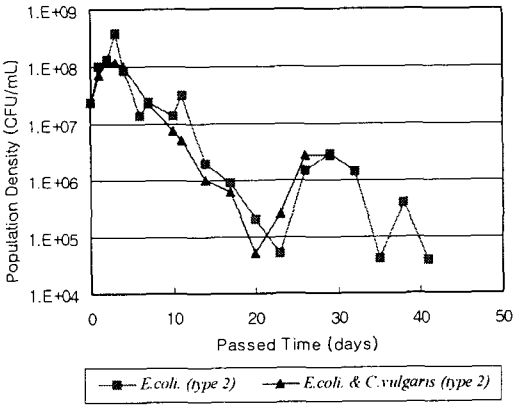


Fig. 7. Changes of *E. coli.* W3110 population in symbiotic reaction between *E. coli.* W3110 and *Chlorella vulgaris* (type 2).

of 5.0×10^4 CFU/ml on the 20th day. But it again increased rapidly after that time.

In addition to the antibacterial bioactive conditions described in the introduction, especially in the CRWFS, long detention times of wastewater can be regarded as primary factors of antibacterial activities. This activity is regarded as the most important one in the antibacterial reaction because highly alkaline conditions generated from algal photosynthesis can hydrolyze the bacterial cell components and affect the dissociation of zwitterions in amino acids of protein molecules[11]. In addition to long detention time, the inactivating effect of light can affect the desired bacterial growth. The UV-B spectrum (wavelength between 280 nm and 320 nm) of light can kill bacterium[12]. Since ultraviolet light does not penetrate into the water column, visible light can affect bacterial die-off. Its impact increases with highly dissolved oxygen concentrations as well as high pH levels[13]. However, the growth of *E. coli* W3110 in this experiment represents an S style of the lag growth phase, of the logarithmic growth phase, of the decreased growth phase, of the endo-respiration phase. The maximum densities of *E. coli* W3110 were 2.025×10^8 colonies · ml^{-1} (type 1) and 3.915×10^8 colonies · ml^{-1} (type 2). The densities decreased rapidly until 35 days of incubation had passed away, but indicated again a slight increase after that time. The trend of such change was more serious in type 2 than that of type 1. Such change of *E. coli* W3110 population implies that the growth of heterotrophic bacteria in type 2 represents the survival pattern, which requires more nutrients than those in type 1.

The stoichimetric composition of aerobic bacteria has been reported as $C_5H_7O_2N$[14,15]. With aerobic bacteria as a product and glucose as a reactant, the cell synthesis reaction is given to us as

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

Through the photosynthesis reactions, alga utilizes carbon dioxide from the aerobic bacteria reaction and ammmonia to produce cell protoplasm and produces oxygen molecules as given in the equation :

$$NH_3 + 8CO_2 + 4.5H_2O \xrightarrow{\text{light}} C_5H_{14}O_3N + 8.75O_2$$[17].

Clearly, it is well known to society that environmental factors governing algal biomass production are : (1) luminosity ; (2) water temperature ; (3) pH conditions ; (4) macro/micro nutrients ; (5) CO_2 concentration[18]. Also in this experiment, the degrees of the heterotrophic bacteria' degradation activity of TOC can be regarded as the primary factor for bacterial die-off since the others, except TOC concentration, remained constant.

In this experiment, the retention time of wastewater can be regarded as the primary factor among several conditions. Really, It indicates that even the glucose concentration in type 1 could not help the decrease of the density of the *E. coli* W3110 population, even though 1335 mg/l glucose (type 1) was higher than 267 mg/l glucose (type 2). It suggests that the culture system maintains its problem due to a continuous feed of the wastewater. Because the continuous circulatory DOC flow system by the peristaltic pump could not satisfactorily give the wastewater retention time for *E. coli* W3110 population to keep its timely respiration.

4. Conclusions

1) The inoculation density 2.4×10^7 CFU/ml of the *E. coli* W3110 population in both types of CRWFS decreased rapidly for 35 days, while these densities increased slowly after that time. The trend of change of type 2 was more severe than for that of type 1.
2) In CRWFS the minimum densities of *E. coli* W3110 population were 2.5×10^5 CFU/ml (type 1) and 5.0×10^4 CFU/ml (type 2).
3) In CRWFS, the growth of heterotrophic bacteria of type 2 shows a survival instinct pattern of a broader requirement of nutrients than bacteria of type 1.
4) The successful bacterial die-off can not be expected in CRWFS if wastewater retention time is not furnished sufficiently.

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