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Effect of shutdown on styrene removal in a biofilter inoculated with *Pseudomonas* sp. SR-5

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Abstract

Styrene gas removal was carried out in a biofilter inoculated with a styrene-degrading *Pseudomonas* sp. SR-5 using a mixed packing material of peat and ceramic under the non-sterile condition. More than 86% removal efficiency was obtained at styrene load of 5–93 g m^{-3} h^{-1} for 62 days operation period and 78% carbon of removed styrene was converted to CO_2. Thereafter, three kinds of styrene shutdown experiments were conducted: (i) air and mineral medium were supplied for 4 days, (ii) complete shutdown, namely no styrene, air and moisture supply was conducted for 3 days, and (iii) only air was supplied for 11 days. When styrene gas was re-supplied after (i) and (iii) shutdown experiments, styrene removal efficiency rapidly recovered, but after (ii) shutdown, recovery of styrene removal was significantly delayed. Supply of air during shutdown period was found to be enough to resume microbial activity to degrade styrene.

Keywords: Styrene; Biofilter; *Pseudomonas* sp. SR-5; Shutdown

1. Introduction

Styrene is a widely used chemical, mainly in the manufacturing of plastics and synthetic resins. As styrene represents an important risk of environmental pollution as one of volatile organic compounds (VOCs) and malodorous gases, airborne styrene exhausted from the industry causes a problem to human health because of its toxicity and carcinogenicity [1,2]. Biological processes, such as bioscrubber, trickling filter, and biofilter, are used for the treatment of air stream polluted with VOCs and odorous gases. A biofilter carries a packing material, which is surrounded by biologically active biofilm. Biofiltration offers several advantages compared to chemical and physical treatments of polluted air streams because of its high removal efficiency, low capital and operating costs, safe operating conditions, and low energy consumption if proper operational conditions are maintained. Biofiltration generates least undesirable by-products and converts many organic and inorganic compounds into harmless oxidation products such as water and carbon dioxide [3]. Many studies have been carried out to find ways to improve the effectiveness of biofiltration units in VOCs removal and to obtain the basic data for optimum biofilter construction by changing the operation factors, which affect the biofilter performance. These factors are pH, moisture content, operation temperature, nutrient condition, selection of inoculum and packing materials, flow rate and concentration of the pollutant.

The removal of styrene from industrial waste gases and spills can be achieved by the use of styrene-degrading microorganisms as biocatalysts as an economically feasible method. In the styrene removal by using biofilters [4–9], effect of nutrient [5] and moisture content [7] and packing materials [8] on the removal efficiency have been reported. In practical process, operation is interrupted for a few days in the weekends or holidays, and sometimes for several weeks in the case of plant maintenance or seasonal closures (shutdown), when no pollutant is loaded on the biofilter. Therefore, it is important to evaluate the effect of shutdown periods on biofilter performance when the biofiltration unit is restarted. The times needed for reacclimation after shutdown varied, depending on the kinds of VOCs, microbial populations and inlet loads tested [10–13]. Few reports have been published about the effects of styrene-shutdown on the biofilter performance. In this study, the effect of three kinds of shutdown on biofilter performance inoculated with a styrene-degrading bacterium, *Pseudomonas* sp. SR-5 [14] under open

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system was investigated, and microbial change before and after the shutdown experiments was observed in relation to removal efficiency.

2. Materials and methods

2.1. Inoculum

Pseudomonas sp. SR-5 isolated from soil as a styrene-degrading bacterium was used as inoculum of styrene removal biofilter. Styrene degradation characteristics by SR-5 was analyzed under sterile condition and described in the previous paper [14]. SR-5 was cultured in nutrient broth (NB) containing 5 g meat extract, 10 g peptone, and 5 g NaCl in the 1 l deionized water (pH 7.0) at 30 °C at 120 strokes per minute (spm) for 15 h. The cultured broth was centrifuged at 9200 × *g* for 20 min (RS-20BH, Tomy Seiko Co., Ltd., Tokyo, Japan) and the precipitate was washed twice with sterilized deionized water.

2.2. Biofilter setup and operation

A laboratory scale biofilter system used for this experiment was the same as that in the previous paper [14]. However, in this study a CO_2 analyzer was set at the outlet of the biofilter. Glass columns (5 cm inner diameter × 37 cm length) were packed with 74 g dry weight of a mixed packing material of peat (Takahashi Peat Moss Co., Hokkaido, Japan) and ceramic (Kubota Co., Tokyo, Japan) at a ratio of 1:1 on dry weight basis to a height of 17 cm. The physical and chemical properties of peat and ceramic were described in the previous paper [14]. The reason for use of a mixed packing material of peat and ceramic is to take advantage of a high removal efficiency of the peat and a lower pressure drop of ceramic [14]. Peat was neutralized with 0.4 mol $Ca(OH)_2$ (kg dry peat)$^{-1}$ before use. The initial moisture content was adjusted to about 64%. To maintain moisture content, and pH, and supply nutrients in biofilter, 100 ml of mineral medium was supplied daily from the top of the column using a peristaltic pump. The mineral medium consists of 1.55 g of K_2HPO_4, 0.85 g of $NaH_2PO_4{\cdot}2H_2O$, 2.0 g of $(NH_4)_2SO_4$, 0.1 g of $MgCl_2{\cdot}6H_2O$, 10.0 mg of EDTA, 2.0 mg of $ZnSO_4{\cdot}7H_2O$, 1.0 mg of $CaCl_2{\cdot}2H_2O$, 5.0 mg of $FeSO_4{\cdot}7H_2O$, 1.0 mg of $Na_2MoO_4{\cdot}2H_2O$, 0.2 mg of $CuSO_4{\cdot}5H_2O$, 0.4 mg of $CoCl_2{\cdot}6H_2O$, and 1 mg of $MnCl_2{\cdot}4H_2O$ in 1 l of deionized water (pH 7.0).

Styrene gas was supplied to the biofilter by passing air from a compressor through a broad-neck bottle in which a reagent bottle containing 15 ml of styrene was set. Inlet styrene load was changed from 5 to 93 g m^{-3} h^{-1} by adjusting styrene concentration (0.5–2.6 g m^{-3}) and space velocity (10–57 h^{-1}). The experiment was performed at room temperature of 16 ± 4 °C.

Details of the biofilter operation are shown in Table 1. Shutdown experiments were carried out after operation of biofilter in open system was stabilized in 62 days. The first shutdown experiment was carried out by supplying only air and mineral medium to maintain the moisture content for 4 days from 63 to 66 days (A period in Fig. 4). The second shutdown was carried out by completely stopping supply of styrene, air and mineral medium for 3 days from 73 to 75 days (B period in Fig. 4). The third shutdown was carried out by supplying only air for 11 days from 85 to 95 days (C period in Fig. 4).

Table 1
Experimental plans during biofilter operation

Operation periods (days)	Contents
1–62	Supply of styrene gas and 100 ml day^{-1} mineral medium
63–66	Supply of 300 ml min^{-1} air and 100 ml day^{-1} mineral medium (fist shutdown)
67–72	Re-supply of styrene gas and 100 ml day^{-1} mineral medium
73–75	No supply of styrene gas and mineral medium (second shutdown)
76–84	Re-supply of styrene gas and 100 ml day^{-1} mineral medium
85–95	Supply of 300 ml min^{-1} air (third shutdown)
96–103	Re-supply of styrene gas and 100 ml day^{-1} mineral medium

2.3. Analytical method

Inlet and outlet concentrations of styrene sampled with a gas-tight syringe (MS-GANX00, Ito Co., Japan) were determined by gas chromatography (GC) (GC-14A, Shimadzu, Japan) equipped with flame ionization detector (FID) and a capillary column (URBON HR-1, Shimadzu, Japan, 0.53 mm inner diameter × 30 m length). Injection and detection temperatures were 150 °C, oven temperature was 60–120 °C by 2 °C min^{-1}, and a flow rate of He as carrier gas was 15 ml min^{-1}. Removal efficiency (RE) and elimination capacity (EC) of styrene were calculated as follows:

$$\mathrm{RE}\,(\%) = \left(\frac{C_{\mathrm{in}} - C_{\mathrm{out}}}{C_{\mathrm{in}}}\right) \times 100$$

$$\mathrm{EC}\,(\mathrm{g\,m^{-3}\,h^{-1}}) = (C_{\mathrm{in}} - C_{\mathrm{out}}) \times \frac{F}{V}$$

where C_{in}, inlet styrene concentration (g m^{-3}), C_{out}, outlet styrene concentration (g m^{-3}), F, gas flow rate (m^3 h^{-1}), and V, column volume (m^3).

The amount of CO_2 evolved in the biofilter was analyzed by an infrared CO_2 analyzer (EX-1562-1, ABLE, Japan) at the outlet of the biofilter. Viable cell numbers in biofilter were measured as follows. Ten grams wet weight of a packing material was homogenized (EX-3 homogenizer, Nihon Seiki Ltd., Japan) in 90 ml of sterilized distilled water at 9200 × *g* for 20 min. The homogenized solution was diluted with sterilized distilled water. Then, the suspended solutions were spread on nutrient agar (NA) plate. After incubation at 30 °C for 48 h appeared colonies were counted and cell numbers were represented as colony forming units (cfu) per gram dry packing material. NA plate is composed of NB medium and 15 g l^{-1} of agar. The homogenized suspension was also used for pH measurement (*Φ*300 pH meter, Beckman, USA). Moisture content of the packing material was determined by weighing the change of water in the packing material after drying for 12 h at 105 °C.

Organic compounds in drain water in biofilter were extracted with diethyl ether after centrifugation at 9200 × *g* for 20 min,

and then the solvent was evaporated. The residue was dissolved with ethyl acetate and analyzed by GC. Analytical condition of GC was identical with that described above.

2.4. Carbon mass (C-mass) balance of styrene degradation

C-mass balance was determined to evaluate the characteristics of styrene degradation by SR-5 in the biofilter for 2 weeks from 47 to 60 days. During the period, styrene load to biofilter, the amount of styrene detected at the outlet, increased amount of the cell mass in biofilter, the amount of metabolic intermediates accumulated in the drain water and the packing material, and the amount of evolved CO_2 were measured and calculated on a carbon basis.

3. Results and discussion

3.1. Biofilter performance

Styrene removal in the biofilter using a mixed packing material of peat and ceramic inoculated with *Pseudomonas* sp. SR-5 under non-sterilized condition is shown in Fig. 1. The 100% removal efficiency observed at the start-up of the experiment was attributed to the physical and chemical interactions between the wet packing materials and styrene vapor. Then, the 100% removal efficiency lasted even in increased inlet load, indicating that the inoculated SR-5 effectively worked to shorten the acclimation time, which generally last a few weeks to a few months. The non-biological styrene removal capacity of the mixed packing material of peat and ceramic mainly due to adsorption of styrene on the packing material was estimated to be 115 g m^{-3} in the control experiment without SR-5 for 2 days. After acclimation period, a steady state removal of the biofilter was observed for 62 days. More than 86% styrene removal efficiency was obtained in the range of 5 to 93 g m^{-3} h^{-1} styrene load. Plot of styrene load versus elimination capacity (Fig. 2) revealed that the maximum elimination capacity at in the 92% removal efficiency was 86 g m^{-3} h^{-1} and completely elimination capacity which guarantees 100% removal of styrene was 43 g m^{-3} h^{-1}. The maximum elimination capacity obtained in this study was similar to the values of 79 and 62 g m^{-3} h^{-1} by *Exophiala jeanselmei* [4,7], and 63 g m^{-3} h^{-1} by *R. rhodochrous* NCIMB 13259 [15].

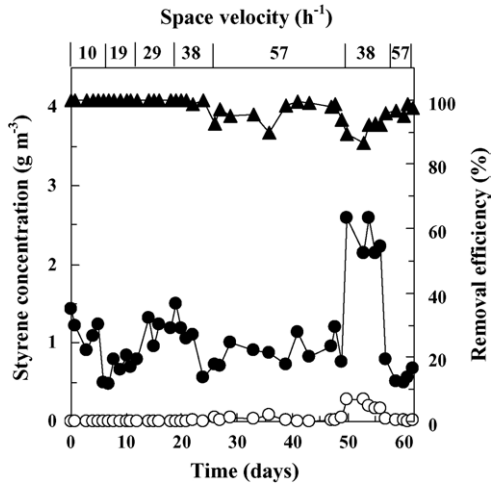

Fig. 1. Biofilter performances inoculated with *Pseudomonas* sp. SR-5 under non-sterile condition. Closed circles, inlet styrene concentration; open circles, outlet styrene concentration; closed triangles, removal efficiency.

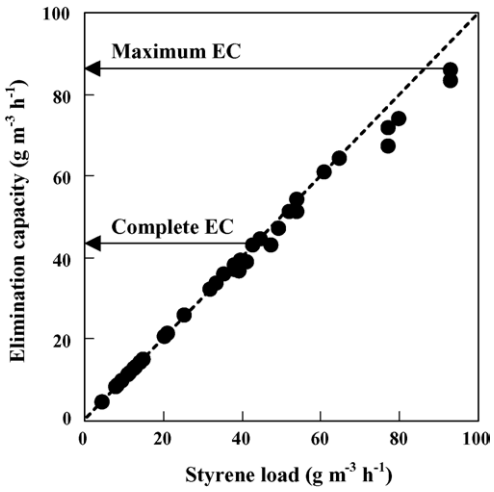

Fig. 2. Relationship between styrene load and elimination capacity. Dotted line shows 100% styrene removal.

3.2. Kinetic analysis

In the steady state operation periods after the end of acclimation, kinetic analysis of the biological removal of styrene was conducted using the following equation by assuming a plug air flow and homogeneous distribution of styrene-degrading bacterium in the filter [16,17].

$$\frac{C_{\ln}}{R} = \frac{C_{\ln}}{V_m} + \frac{K_s}{V_m}$$

where V_m, maximum removal rate (g-styrene/kg-dry packing material/day); K_s, saturation constant (ppm); $C_{\ln} = (C_0 - C_e)/\ln(C_0/C_e)$; C_0, inlet concentration of styrene (ppm); C_e, outlet concentration of styrene (ppm); $R = \mathrm{SV}(C_0 - C_e)/\beta$; SV, space velocity ($\mathrm{day}^{-1}$); β, conversion coefficient ((kg-dry material) (g-C)$^{-1}$). Fig. 3 shows the result of kinetic analysis of styrene removal by using the equation. The maximum removal rate (V_m) obtained was 121 g m^{-3} h^{-1} and saturation constant (K_s) was 111 ppm. V_m corresponds to the extrapolated value of elimination capacity (EC), namely maximum elimination capacity, in Fig. 2. In the previous paper where styrene removal was conducted under sterile condition using the same packing materials [14], the maximum elimination capacity of styrene by SR-5 was 170 g m^{-3} h^{-1}. Thus, in this non-sterile operation, the value was only 30% reduced compared with that in sterile operation.

3.3. C-mass balance

The result of C-mass balance in the biofilter for 10 days from 47 to 56 days of biofilter operation is shown in Table 2. Inlet

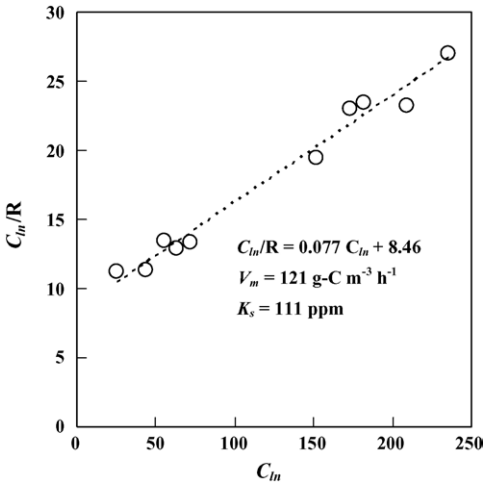

Fig. 3. Kinetic analysis of styrene removal in the biofilter inoculated with *Pseudomonas* sp. SR-5 using a mixed packing material of peat and ceramic.

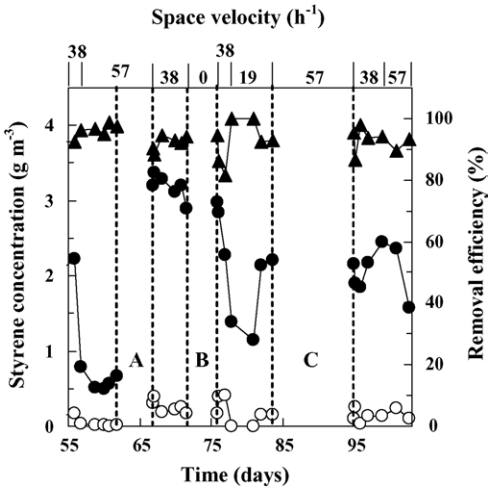

Fig. 4. Effect of shutdown in the biofilter inoculated with *Pseudomonas* sp. SR-5 using a mixed packing material of peat and ceramic. (A) Shutdown of styrene; (B) shutdown of styrene, air, and moisture; (C) shutdown of styrene and moisture. Closed circles, inlet styrene concentration; open circles, outlet styrene concentration; closed triangles, removal efficiency.

styrene load to the biofilter was 18,176 g-C m^{-3} and 1594 g-C m^{-3} was detected at the outlet. Phenylacetic acid and 2-phenylethanol which are the intermediates of styrene degradation by SR-5 [14] detected from the drain water and packing material were 3.4 g-C m^{-3} and 1.4 g-C m^{-3}, respectively. 561 g-C m^{-3} of styrene was converted to the cell mass of SR-5. Produced CO_2 from styrene degradation was 14,260 g-C m^{-3}. Theses results indicate that 78.5% of inlet styrene was converted to CO_2, less than 3.12% of styrene was used for cell growth and degradation products, and 9.66% of styrene was transformed into unknown carbon compounds. This result was similar to the report, showing that 75% of inlet styrene was converted to CO_2 in the perlite biofilter inoculated with fungus [7]. The high conversion of styrene to CO_2 is effective to delay the increase in pressure drop caused by growth of the cell mass during a long-term operation of biofilter.

3.4. Effects of shutdown on styrene removal

The effect of shutdown was investigated after about 2 months steady removal operation under non-sterile condition described above. Removal pattern and removal efficiency during the three kinds of shutdown experiments are shown in Fig. 4. For 4 days from 63 to 66 days, the first shutdown experiment was carried out by flowing only the air at 300 ml min^{-1} without styrene and supplying 100 ml mineral medium per day (Fig. 4, A period). After A period, 115 g m^{-3} h^{-1} of styrene was re-supplied to the biofilter. This level of styrene load was about three times higher than 36 g m^{-3} h^{-1} which was supplied before the shutdown. Styrene removal efficiency was restored quickly and 90% removal efficiency was observed in 1 h after re-supply of styrene and 95% removal was attained within 1 day. One hundred and twelve grams per cubic meter per hour maximum elimination capacity was obtained in the 95% removal efficiency after the first shutdown experiment.

For 3 days from 73 to 75 days, the second shutdown experiment was carried out by stopping supply of styrene, mineral medium and air (Fig. 4, B period). When re-flow of air containing styrene and re-supply of mineral medium were started, 82% removal at 82 g m^{-3} h^{-1} styrene load was observed after 1-day operation. However, 100% of removal efficiency was observed only by the decrease of inlet styrene load to 21 g m^{-3} h^{-1}. Eighty-eight grams per cubic meter per hour maximum elimination capacity was obtained at the 86% removal efficiency after 9 days of the second shutdown experiment.

Finally, the shutdown was carried out by flowing only air without styrene and no supply of mineral medium for 11days from 85 to 95 days (Fig. 4, C period). After restart-up of styrene supply, more than 90% removal efficiency was rapidly recovered. Thereafter even in increase in styrene load to 132 g m^{-3} h^{-1}, stable removal efficiency was observed.

In the first and third shutdown experiments in biofilter inoculated with SR-5, styrene removal efficiency was completely recovered within 1 day, when the load was carefully controlled. However, in the second shutdown experiment, the deterioration of the styrene-removal activity was significant. In the compost biofilter to treat benzene, toluene, and *p*-xylene [12], the shutdown with air supply was lasted for 90 h and 2 weeks, and the reacclimation periods needed were 9 and 43 h, respectively. When no air supply lasted for 40, 90, 11 and 24 h were required

Table 2
Carbon balance of styrene degradation biofilter using a mixed packing material of peat and ceramic inoculated with *Pseudomonas* sp. SR-5 for 7 days

Input styrene	18176 (g-C m^{-3})
Output styrene	1594 (8.77%)[a]
Degradation products[b]	2-PE; 1.4 (0.01%)
	PAA; 3.4 (0.02%)
Biomass	561 (3.09%)
CO_2 emission	14260 (78. 5%)
Unidentified carbon[c]	1756.2 (9.66%)

[a] Percentage against input styrene.
[b] PAA: phenylacetic acid, 2-PE: 2-phenylethanol.
[c] Unidentified carbon presumed extracellular product and polymer.

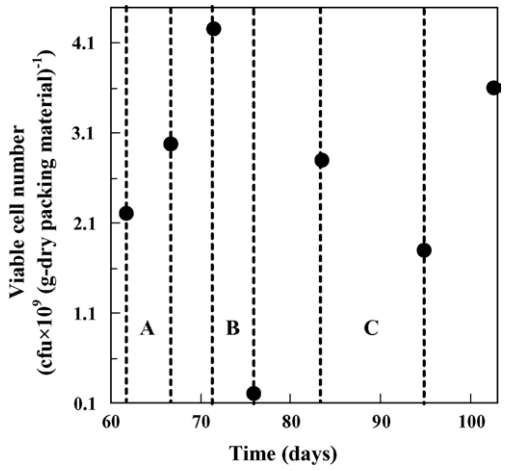

Fig. 5. Change in the total viable cell number in the biofilter before and after shutdown experiments. A, B and C periods correspond to those in Fig. 4.

for reaching a steady removal of styrene again, respectively. In fungal biofilter to treat VOCs, a long-term shutdown required a longer time interval to recover its high removal efficiency, but after 9-day VOCs loading shutdown, only 3.5 h was needed to reach 100% removal efficiency for some VOCs [13]. In styrene removal in this study, at least supply of air and moisture was necessary to minimize the reacclimation period. These indicate that microbial species and the properties of VOCs are closely associated with the recovery of the biofilter after shutdown experiment.

In this biofilter, three shutdowns had no serious adverse effect on the biofilter performance mainly because that (i) strain SR-5 can degrade a high concentration of styrene (0.5–10%, v/v) which is inhibitory to most of microorganisms, (ii) the doubling time of strain SR-5 in styrene medium was 2 h which was significantly shorter, compared with 19 h of *Xanthobacter* sp. 124X [18] and 10 h of *Rhodococcus rhodochrous* NCIMB 13259 [15] and (iii) the mixed packing material of peat and ceramic was appropriate for the stable activity of SR-5. This was reflected in the data that the maximum elimination capacities under sterile and non-sterile conditions did not show remarkable differences.

3.5. Changes in cell number in shutdown experiments

Before the shutdown experiment, the operation was conducted in open system and the stable removal of styrene in Fig. 1 suggests that SR-5 played an important role for degradation of styrene, although the determination of the cell number of SR-5 was not carried out. Change in the total cell number in three shutdown experiments is shown in Fig. 5. At the first shutdown period (A period in Fig. 5), the cell numbers was 2.2×10^9 cfu (g-dry packing material)$^{-1}$. Then, the cell numbers considerably increased to 4.3×10^9 cfu (g-dry packing material)$^{-1}$ after styrene re-supply. This indicates that the supply of air and mineral supply can maintain the microbial activity of styrene removal during shutdown period. The cell number after the second shutdown (B period in Fig. 5) significantly decreased to 2.0×10^8 cfu (g-dry packing material)$^{-1}$ from 4.3×10^9 cfu (g-dry packing material)$^{-1}$ mainly due to no supply from outside. This means that complete shutdown is critical for survival of styrene-degrading microorganisms including SR-5. However, the cell number gradually increased to 2.8×10^9 cfu (g-dry packing material)$^{-1}$ by re-supply of styrene, air, and mineral medium in 10 days. In the third shutdown operation (C period in Fig. 5), the cell number decreased to 1.8×10^8 cfu (g-dry packing material)$^{-1}$ mainly due to dried packing material. However, it increased again to 2.8×10^9 cfu (g-dry packing material)$^{-1}$ by re-flow of air with styrene and re-supply of mineral medium. These results indicate that at least supply of air and moisture is essential to keep the activities of aerobic microbes including *Pseudomonas* sp. SR-5, which are supposed to be responsible for styrene-degradation.

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