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The Characteristics of Microbial Ecosystem Response with the Changes of Hydrolic Retention Time on an Aerobic Fixed-Biofilm Biological Nutrient Removal System

Hai-Uk Nam*, Tae-Ho Lee*, Young-O Kim†, Seung-Han Park and Tae-Joo Park**

Dept. of Environmental Engineering, *Institute for Environmental Technology and Industry,
Pusan National University, Busan 609-735, Korea
**Safety and Environment Team, SK Corp., Ulsan 680-130, Korea
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Abstract−The influence of an aerobic fixed-biofilm activity, microbial ecosystem and mass transfer with respect to HRT variation in a BNR (biological nutrient removal) system has been investigated in this study. The process used in this study was an anoxic (1)/aerobic (1)/anoxic (2)/aerobic (2) system. The study was demonstrated by several kinds of techniques such as INT-dehydrogenase activity (DHA), INT (2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride), DAPI (4',6'-diamidino-2-phenylindole hydrochloride), and microelectrode. The study used by synthetic wastewater and HRT variation demonstrated that the DHA activity, density and heterotrophs/autotrophs ratio increased, as the HRT decreased from 8 hr to 4 hr. In comparing two aerobic reactors in fixed-biofilm process, the first aerobic reactor of the higher C/N ratio showed higher heterotrophs/autotrophs ratio and microbial activity than the second aerobic reactor. It was therefore concluded that the heterotrophs/autotrophs ratio and microbial activity were a greater influence on the first aerobic reactor, as organic loading rate was increased by HRT variation.

Key words: Fixed Bed, Biofilm, pH, NH_4^+-N, NO_3^--N and DO Profile, DAPI, Activity

INTRODUCTION

As industries have been rapidly developing, various kinds of wastewater discharged from the plants include high concentration organics and nutrients, with their contents composed of various complex materials. Some materials in the chemical wastewater inhibit growth of microbes and are very slowly biodegraded by the biomass. To treat these materials effectively, many investigators have studied various types of processes. As parts of the strategies for nutrient removal under these circumstances, many research programs using a biofilm process were studied in Korea, where the goal was to develop, test, and document the effect of nitrogen removal processes [Carrand et al., 1990; Wang et al., 1991]. These processes had to be compact, economical, and superior to the existing municipal wastewater treatment plant with an activated sludge system.

Aerobic biological treatment processes are widely used for removal of organics and nutrient from the wastewater [Su and Ouyang, 1996]. In particular, an aerated submerged biological process has been used for more than 60 years and has received considerable attention recently. Its numerous advantages include operating stability and long retention of microorganisms in the fixed-film process, which proved to be advantageous in the treatment of various wastewaters. However, the biological nutrient removal processes have been focused on process development and promoting its efficiency rather than biofilm formation, activity and microorganisms, which are concerned with wastewater treatment directly.

Recent studies have concentrated on structure, formation, mass transfer and microorganism distribution in biofilm [De Beer et al., 1994; Horn and Hempel, 1995; Liu and Capdeville, 1996; Zhang et al., 1994]. A biofilm is known from the matrix of cell, extra cellular polymers and other byproducts attached to a solid surface. A better understanding of biofilm characteristics for using advanced tools and methods such as INT, DHA, DAPI, fluorescence *in situ* hybridization (FISH) and microelectrode can enhance improvements of the biofilm process and reduce operating cost [Okabe et al., 1999; Park et al., 2003]. In order to acquire information about the role of a biofilm and microorganisms in a practical process, we studied the anoxic/aerobic/anoxic/aerobic (AOAO) process combined with biofilm process as a biological simultaneous nutrient removal process.

MATERIALS AND METHODS

One unit of a laboratory scale reactor capable of performing continuous experiments for nutrient removal was used, including anoxic (1)/aerobic (1)/anoxic (2)/aerobic (2) reactors in series. Fig. 1 shows the fixed biofilm reactor.

Operating conditions are provided in Table 1. The substrate used in this study was synthetic wastewater. The mean concentrations of COD, NH_4^+-N and T-P in the influent were 100 mg/L, 40 mg/L and 8 mg/L, respectively.

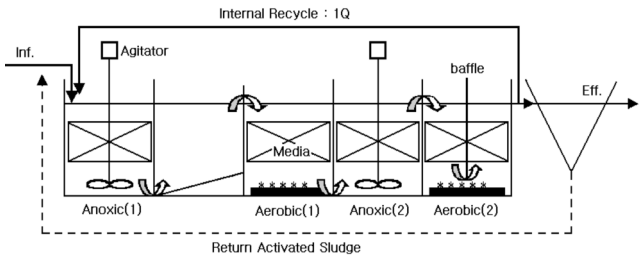


Fig. 1. Schematic diagram of a fixed-biofilm reactor.

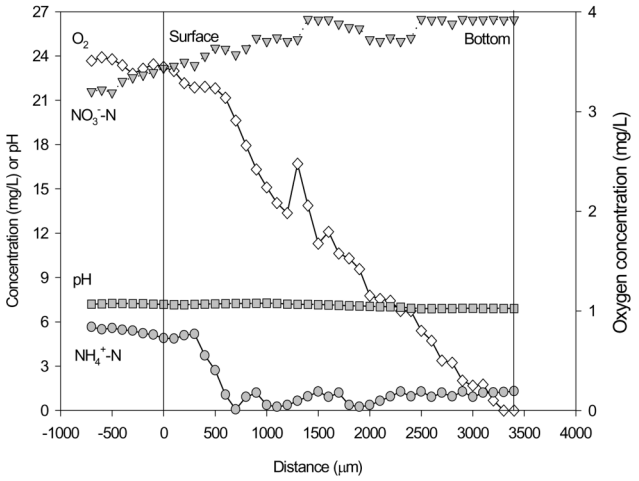
†To whom correspondence should be addressed.
E-mail: yokim@pusan.ac.kr

Table 1. Operating conditions and characteristics

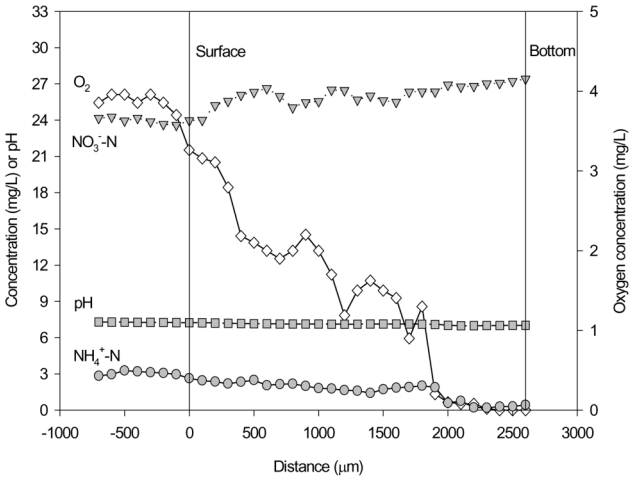
Parameters	AOAO process
Reactor arrangement	Anoxic (1)/Aerobic (1)/Anoxic (2)/ Aerobic(2)
Internal recycle ratio (%)	100
Volume (L)	6.7/6.7/6.7/6.7
HRT (hr)	8, 6, 4
Media	Porous media covered with PVA (Poly Vinyl Alcohol)
Packing type	Submerged bed
Packing ratio (%)	20

The influent and effluent samples were collected from the end of each reactor. The biofilm samples attached on the substratum were collected after minimizing detachment of the biofilm and then each biofilm samples of the media were detached by centrifugation (Hanil Science Industrial Co., Model No. MF80-3,000 rpm, 10 min). System performance parameters routinely analyzed for this study included COD, NH_4^+-N, NO_3^--N, NO_2^--N, pH, alkalinity, DO and temperature. Samples for the determination of soluble components were immediately filtered by using 0.45 μm filter paper and cooled to prevent further reaction after sampling.

The INT method could detect heterotroph and autotroph bacteria participated in oxidation of carbon source and conversion of nitrogen source and also detect *Nitrosomonas* spp. and *Nitrobacter* spp. by Allythiourea (ATU) and Sodium chlorate ($NaClO_3$) reagents [Suthersan et al., 1986]. The cell count samples were determined by combination of staining with DAPI dye and epifluorescence microscopy. The thickness of the samples was detected by microelectrode and microtome method. The analytical methods and equipment used in this paper are summarized in Table 2.



(a) aerobic 1



(b) aerobic 2

Fig. 2. The component profiles on the biofilm in aerobic 1 and 2.

RESULTS AND DISCUSSION

1. Component Profiles

Fig. 2 shows component profiles within a fixed-biofilm in aerobic 1 obtained under HRT 8 hr to 4 hr. The oxygen profile showed that those are "voids", "channels", "cavities", "pores", and "filaments" within a biofilm (variation in 1,000-1,500 μm), as the results of de Beer [1994]. Microprofiles of O_2, NH_4^+-N, and NO_3^--N demonstrated the occurrence of complete nitrification in the inner 700 μm of the biofilm.

Component profiles within a fixed-biofilm in aerobic 2 obtained

Table 2. Sample analytical methods

Parameters	Methods
DO	DO meter, model 58 (YSI Inc., USA)
pH	pH meter, HM-14P (TOA Electronics, Japan)
COD	Open reflux methods (Standard Method 19_{th} edition)
NH_4^+-N	Nesslerization method (Standard Method 19_{th} edition)
NO_x-N	AA3 (Bram$^+$luebbe, German)
T-P	Stannous chloride method (Standard Method 19_{th} edition)
Alkalinity	Titration method (Standard Method 19_{th} edition)
Biofilm thickness	Chemical Microsensor system (Diamond General corp.) and Microtome (Leica corp.)
Activity	DHA and INT method
Component profile	NH_4^+-N, NO_x-N, pH and DO microelectrode (Diamond General corp.)
Total cell number	DAPI method

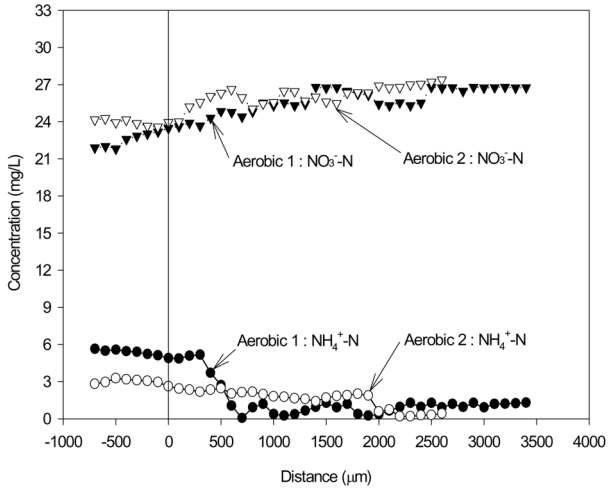


Fig. 3. The NH_4^+-N and NO_3^--N profiles in aerobic 1 and 2.

under HRT 8 to 4 hr are similar to the profiles in aerobic 1. But the depth of mass transfer is the shorter than that of aerobic 1. The profiles of NH_4^+-N and NO_3^--N show that nitrification did occur within this piece of biofilm. The pH profile shows there was a pH drop inside the biofilm, a result of nitrification. The oxygen penetration depth in this case was about 2,700 μm.

Fig. 3 shows that the sequential transformation of NH_4^+-N via NO_3^--N is typically catalyzed by two phylogenetically distinct groups of bacteria, i.e., ammonia-oxidizing bacteria and nitrate-oxidizing bacteria. Experiments showed that competition in biofilm resulted in non-uniform spatial distributions.

Fig. 4 shows oxygen profiles measured in aerobic 1 according to HRT variation 8 hr to 4 hr. The depth of oxygen transfer through biofilm was decreased as to HRT 8 hr to 4 hr, otherwise the results of Zhang and Bishop [1994] who measured the oxygen penetration depth in a biofilm according to the different velocity. Shear stress increased on the surface of biofilm decreased the thickness of biofilm and the depth of oxygen penetration.

2. Biofilm Activity

The COD concentration in the effluents of HRT 8 hr, 6 hr, and

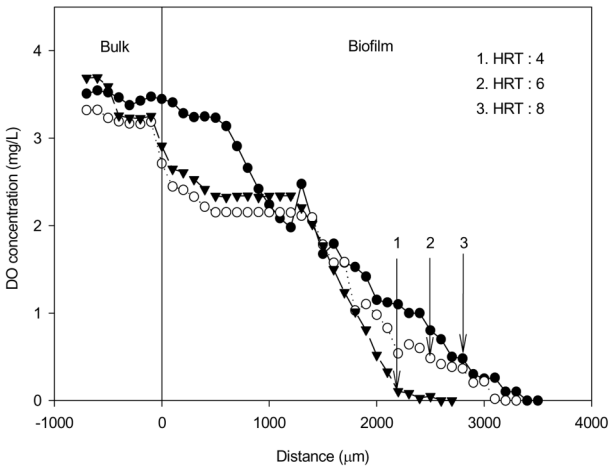


Fig. 4. The oxygen profile with HRT variation.

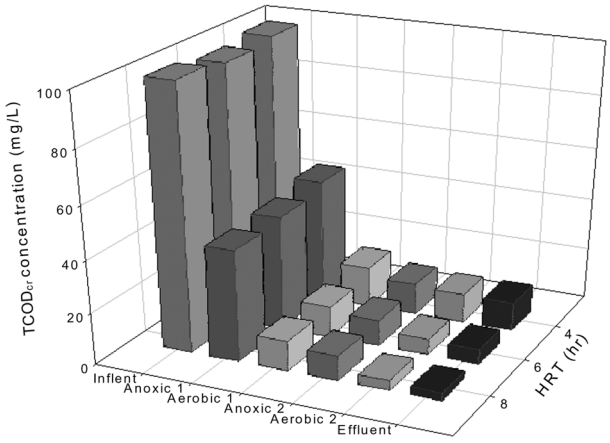


Fig. 5. The effluent COD concentration in each reactor.

4 hr is shown in Fig. 5. Fig. 5 presents the results obtained from the three different operation conditions, in which the different HRT from 8 hr to 4 hr were applied by using only the one waste strength of 100 mg COD/L. All three cases indicate that the effluent COD concentrations were mostly constant although the HRT was decreased. The amounts of reduced COD in anoxic 1 and aerobic 1 were higher than that of anoxic 2 and aerobic 2. The differences of COD removal efficiency in each anoxic and aerobic reactor resulted from the external recycle flow and microbial functions of each reactor. COD concentrations removed in the anoxic reactor were caused by dilution with the 100% external recycle flow and consumption as the denitrification source in anoxic 1. It was found that the COD removal efficiencies were 97.0%, 94.3% and 89.0% in HRT 8 hr, 6 hr, and 4 hr, respectively.

Fig. 6 shows the relationship between the biofilm density and thickness. According to the results of Peyton [1996], biofilm density has been related with following equation:

$$X_f = k_{p\,1/2} (L_f)^{1/2} \quad (1)$$

where X_f is dry density, $k_{p\,1/2}$ is density coefficient, and L_f is the thickness of a biofilm. The $k_{p\,1/2}$ was 0.029, 0.032, and 0.039 in aerobic

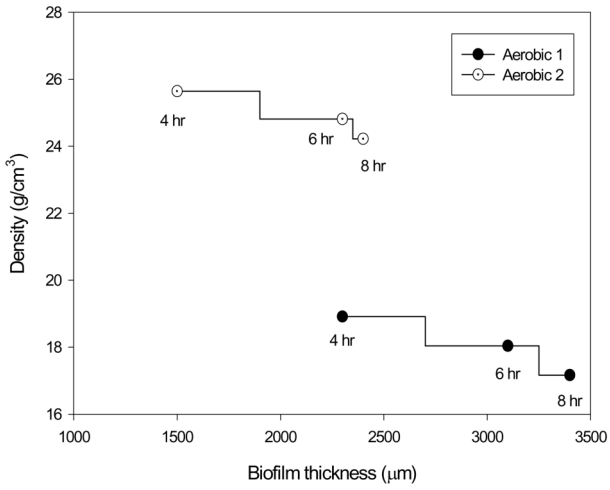


Fig. 6. The relationship between biofilm thickness and biofilm density.

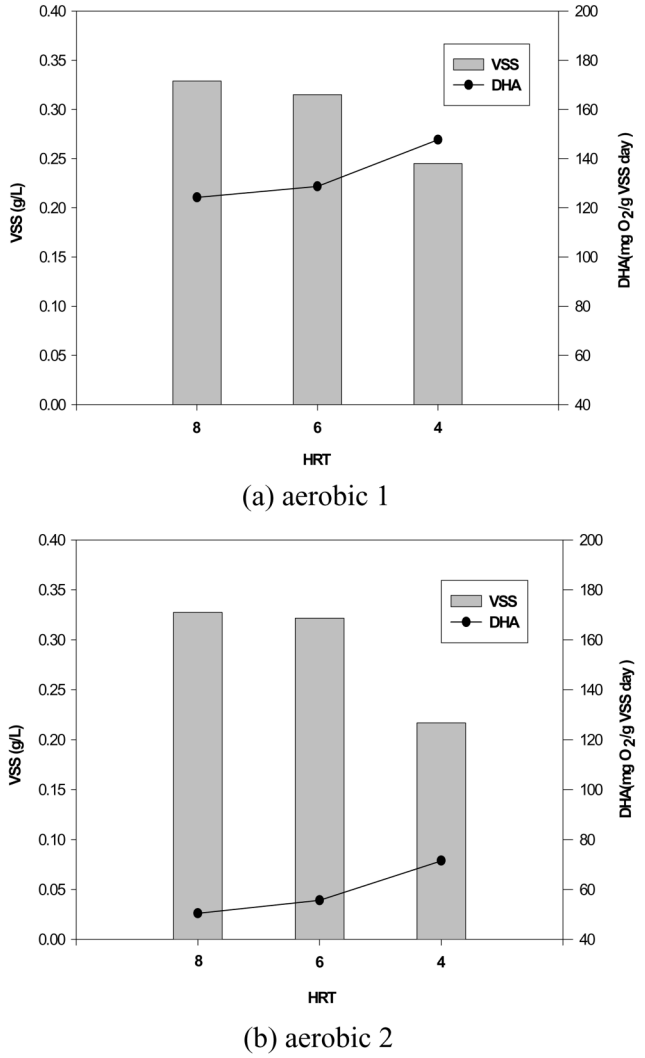


Fig. 7. The relation between VSS concentration and activity with HRT variation in aerobic 1 and 2.

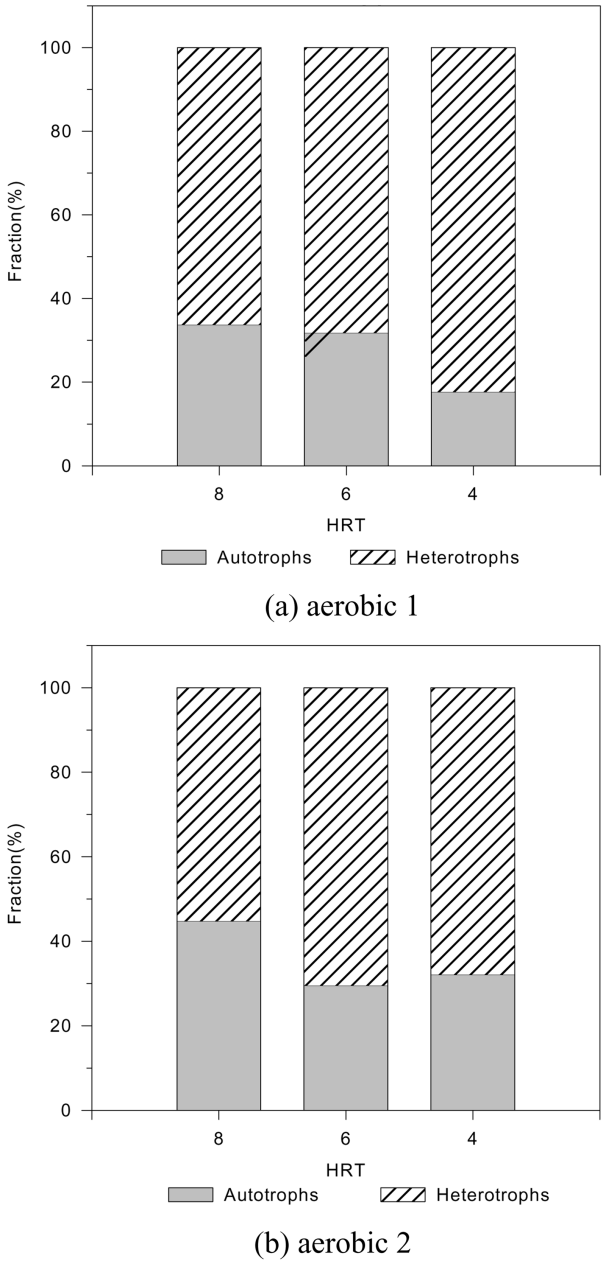


Fig. 8. The changes of the autotrophs/heterotrophs ratio with HRT variation in aerobic 1 and 2.

1 and 0.049, 0.052, and 0.066 in aerobic 2 as to HRT variation 8 hr to 4 hr. The relationship between the biofilm density and thickness (r^2=0.95, 0.9 in aerobic 1 and 2, respectively) is very close to Peyton's experiment.

Fig. 7 shows the relationship between VSS concentration and DHA activity with HRT variation in aerobic 1 and 2. The trends of the DHA activity and VSS concentration in the aerobic biofilm were contrary to each other with HRT variation. The VSS concentration in two aerobic reactors was decreased from 0.33 g/L to 0.25 g/L and 0.33 g/L to 0.21 g/L, respectively. The DHA activity, however, was increased 124.2 to 147.7 mg O_2/g VSS·day and 73.8 to 76.2 mg O_2/g VSS·day as the HRT was decreased from 8 hr to 4 hr. The result is that the carbon source and nutrient in aerobic 1 was more abundant compared with aerobic 2. Lopes et al. [1986] reported that it is apparent that DHA (expressed on a unit biomass basis) decreases with increasing biomass concentration; this effect was most pronounced at the lower (less than 2 g/L) VSS level.

Fig. 8 presents the changes of the autotrophs/heterotrophs ratio with HRT variation in aerobic 1 and 2.These results of the INT test showed that the relative activity of aerobic 1 was higher as heterotrophs/autotrophs bacteria ratio of 66.3% to 83.3% than that of aerobic 2 of 53.8% to 67.7%, and the heterotrophs/autotrophs bacteria ratio was increased in two reactors as HRT was decreased from 8 hr to 4 hr. This was caused by the differences of the C/N ratio in the two aerobic reactors. The higher C/N ratio was overloaded in aerobic 1. Therefore, the total cells and the biofilm thickness concerned with COD and nutrient removal was superior to that of aerobic 2. The INT test could detect heterotroph bacteria and autotroph bacteria which participated in oxidation of carbon source and conversion of nitrogen source and also detect *Nitrosomonas* spp. and *Nitrobacter* spp. by treating Allythiourea (ATU) and Sodium chlorate ($NaClO_3$) reagents [Antonisen et al., 1976; Suthersan et al., 1986]. But in this study, the media samples collected by the aerobic reactor

were treated for mixture of the two specific inhibition reagents (ATU and $NaClO_3$), and consequently the fraction of *Nitrosomonas* spp. and *Nitrobacter* spp. was not detected.

CONCLUSIONS

The influence of aerobic fixed-biofilm activity, microbial ecosystem and mass transfer on HRT variation in a BNR system has been investigated in this study. The process used in this study was anoxic (1)/aerobic (1)/anoxic (2)/aerobic (2) system. The study demonstrated several kinds of techniques such as DHA, INT, DAPI, and microelectrode. The conclusions are as follows.

1. The study used synthetic wastewater and HRT variation demonstrating that the DHA activity, density and heterotrophs/autotrophs ratio were increased, but biofilm thickness was decreased as the HRT was decreased 8 hr to 4 hr.

2. The study comparing two different reactors in anoxic (1)/aerobic (1)/anoxic (2)/aerobic (2) fixed-biofilm process demonstrated that the first aerobic of the higher COD loading rate was a higher heterotrophs/autotrophs ratio and microbial activity than the second aerobic reactor, and the first aerobic reactor was more reflected in heterotrophs/autotrophs and microbial activity as organic loading rate was increased by HRT variation.

3. The study for microelectrode revealed that the internal diffusivity transfer rate was decreased with the increase of external mass transfer rate.

In the above conclusions, for fixed-biofilm in an aerobic reactor it may be essential that external mass transfer resistance must be decreased and the internal diffusivity must be increased.

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