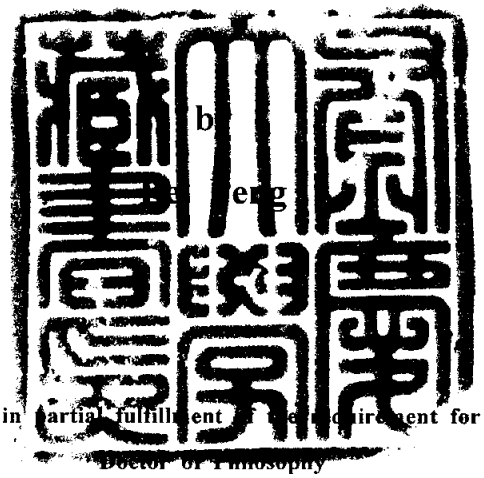
Design, management and performance of a laboratory scale seawater recirculating system for Korean rockfish *Sebastes schlegeli* culture

Advisor: Jae-Yoon Jo



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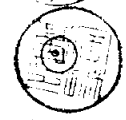
부 심 이학박사 전 임 기 

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Design, management and performance of a laboratory scale seawater recirculating system for Korean rockfish *Sebastes schlegeli* culture

A Dissertation

by

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Contents

조피볼락 양식을 위한 실험실 규모에서의 순환여과 시스템의 설계와 성능

본 실험은 각각 3가지 조건에서 실험이 수행되었으며, 첫 번째 포말분리장치를 이용하여 각각에 작용 요소에 따른 총 부유 고형물(TSS)과 단백질 제거를 연구하였으며, 두 번째 실험은 Hydraulic loading rates, 총암모니아 부하율, 유기물 부하율에 따른 3개의 다른 생물학적 여과재료의 질산화 효율을 확인하였다. 마지막으로 세 번째는 앞에의 실험결과를 토대로 해수 순환여과 시스템을 설계하여 성능을 확인하였으며, 특히 포말 분리 장치와 생물여과의 기능 중요시 하였다.

첫 번째 실험은 모의 해수 양식 시스템을 이용하여 포말분리장치의 각각 공탑유속(SAV), 원료 물리학적 체류시간(HRT), 단백질 농도, 포말층 높이에 따른 부유물과 단백질 제거를 조사하였다. 5개의 consecutive trials에서 공탑유속을 빠르게하고 짧은 물리학적 체류시간의 조건하에 사육수의 TSS와 단백질 농도는 급격히 감소하였다. Batch trials에서는 SAV의 증가에 따라 TSS와 단백질 제거 속도도 함께 증가하였다. 그러나, HRT에서의 TSS와 단백질 제거 속도가 감소하였다. 원료내(Bulk solution)에 단백질 농도가 증가함에 따라 TSS와 단백질 제거 속도는 증가하였다. 포말층 높이가 높아짐에 따라 수집된 거품 condensate 내에 TSS와 단백질 농도는 증가하였며, 포말유출속도는 감소하였다. 첫 번째 실험에서 포말분리장치는 부유물과 단백질 제거에 효과적이다.

두 번째 실험은 3가지의 여과재료(입자큰 모래, 황토 Bead, 스티로폼 Bead)를 이용하여 질산화 효율을 조사하였다. 실험 1에서의 총암모니아 제거 속도는 모든 여과재를 이용하였을 경우 3개월 후부터 안정화 되었다. 그러나 아질산의 농도는 높았다. 실험 2에서의 TAN와 아질산의 제서 속도는 HRT의 증가에 따라 증가하였으나, 유기물의 첨가에 따라서는 감소하였다. 스티로폼 Bead에서의 총암모니아 재거 속도는 유기물을 이용하지 않을 경우보다 9, 18kg O_2/m^3·day에서 낮은 결과를 나타내었다. 9,

18kg O_2/m^3·day에서의 경우 TAN 제거 속도는 유의적인 차이가 없었다. 18kg O_2/m^3·day의 경우 284g TAN/m^3·day, 0 kg O_2/m^3·day의 경우 540g TAN/m^3·day의 수치를 확인할수 있었다. COD/TAN율의 증가함에 따라 질산화에 유기물 영향은 0kg O_2/m^3·day와 9kg O_2/m^3·day에서 보다 9kg O_2/m^3·day와 18kg O_2/m^3·day의 사이에서의 수치가 낮게 나타났다. 결과적으로 유기물은 질산화 효율의 가장 중요한 요소중의 하나이다. 스티로폼 Bead는 3가지 여과재료 중에 가장 좋은 결과를 나타내었다. 18kg O_2/m^3·day에서의 얻은 결과는 순환여과 시스템 내에 생물 여과조로 설계하는데 이용된다. 실험 3에서는 유입수의 TAN 농도에 따른 Areal TAN 제거 속도를 조사하였다.

세 번째 실험은 원형 수조, 두개의 스티로폼 Bead 여과조, 탈질화 모래 여과조, 침전조, 포말 분리장치, 수집조, 펌프를 이용하여 순환여과 시스템을 설계하였다. 총사육수는 1.1m^3이며, 시스템내에 0.4kg/day 사료를 급이하였을 경우 수질에 영향을 끼치지 않았다. 스티로폼 비드 약 42L의 양이면 이스템 내의 생성된 암모니아를 제거 할 수 있다. 상대적으로 큰 탈질화 모래 여과조를 이용하였다. 초기무게 20kg의 조피볼락 130마리를 2002년 11월 13일에 사육하여 2003년 2월 28일 종료하였으며 그때의 무게는 33kg이었다. 상업용 배합사료를 일일 2회 어체중의 1-1.5% 급이하였다. 실험기간에 사육수의 TAN의 농도는 1mg/L, 아질산의 농도는 1-3mg/L의 수치를 나타내었다. 사육수조내에 높은 아질산 농도는 스티로폼 비드 여과조, 침전조 내에 아질산이 생성되었기 때문이다. 스티로폼 비드 여과조의 TAN 제거 속도는 두 번째 실험에서보다 낮은 결과를 나타내었다. 순환여과 시스템에서 포말분리장치는 좋은 결과를 확인시켜 주었다. 포말분리장치에서는 TSS 10.9g, 단백질 제거 1.4g을 기대할수 있으나, 용존 무기 질소 형태에서는 기대할수 없다. 107일동안 사육하였으며, 초기 어류 밀도는 33.3kg/m^3이었으며, 최종 사육시 밀도는 51.7kg/m^3이었다. 본실험은 실험실 내에서 이루어졌으며, 차후 상업적으로 실현 가능성을 재현해봐야 할것이며, 재현 실험을 위한 많은 정보를 제공할 것 이다.

Design, management, and performance of a laboratory scale seawater recirculating system for Korean rockfish *Sebastes schlegeli* culture

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Abstract

Present experiments were conducted to investigate the effect of different operating factors on total suspended solid (TSS) and protein removal by foam fractionator; nitrification efficiencies of three different biofilter media at different hydraulic loading rates (HLR), total ammonia nitrogen (TAN) loading rates, and organic matter loading rates; and the performance of a seawater recirculating system designed based on the previous experimental results and the existing information, with the emphasis on function of foam fractionation and biofiltrition.

1. TSS and protein removal by a foam fractionator

Effects of different operating factors including superficial air velocity (SAV), hydraulic residence time (HRT), protein concentration, and foam overflow height on solids and protein removal by foam fractionator in simulated seawater aquaculture system were investigated. This experiment was conducted on batch and consecutive modes for different combinations of the affecting factors. The foam fractionator used had a diameter of 20 cm and

a height of 120 cm and the experiment was conducted in a simulated recirculating system with synthetic wastewater. In 5 consecutive trials, TSS and protein concentrations in culture tank water decreased more quickly when the foam fractionator was operated at high SAV and low HRT. In batch trials, TSS and protein removal rates increased with increase of SAV but decreased with the increase of HRTs. Increase in protein concentrations in the bulk solution increased TSS and protein removal rates. TSS and protein concentrations in the collected foam condensates increased but the foam overflow rates decreased with the increase of foam overflow heights. The results of this experiment indicate that foam fractionation would be an effective way for solids and protein removal in seawater aquaculture systems and the performance of the foam fractionator depends largely on the operating parameters, especially superficial air velocity.

2. Nitrification efficiencies of three different biofilter media

Nitrification efficiencies of fixed film biofilters were studied. Three biofilter media, coarse sand, loess bead and styrofoam bead, were tested in biofilter columns 1 meter high and 30 cm in diameter. Synthetic wastewater was supplied to the culture tank in order to maintain TAN concentrations in inlet water to biofilters at around 8 mg TAN/L. In trial 1, HLR was set at 200 $m^3/m^2 \cdot day$. TAN conversion rate stabilized after about 3 months conditioning for all filter media. However, net productions of nitrite nitrogen (NO_2-N) were encountered for all the media tested. On volumetric basis, TAN and NO_2-N conversion rates were highest in styrofoam bead filter, followed by decreased conversion rates in sand filter and loess bead filters.

In trial 2, TAN and NO_2-N conversion rates increased with increase of HLR. However, the improvement in biofilter performance was not linearly correlated to HLR in styrofoam bed filter. This is mainly due to the

characteristics of the styrofoam bead used. TAN conversion rates of sand filter increased with the increase of HLR up to 200 m^3/m^2 •day. No increase in TAN conversion rate was observed at highest HLR since flooding on the media surface took place. HLR had significant impact on the TAN conversion rates in loess bead filter up to the highest HLR tested.

TAN conversion rates were much less at organic matter loading rates of 9 and 18 kg O_2/m^3 • day than those without organic carbon addition in styrofoam bead filter. The addition of glucose resulted in reduction of TAN conversion rate from 540 to 284 g TAN/m^3 • day. No significant difference of TAN conversion rates between the two organic matter loading rate was found. This indicates that the impact of organic matter on nitrification becomes less and less sensitive with increase in the COD/TAN ratio. At organic matter loading rate of 9 kg O_2/m^3 •day, great reduction of TAN conversion rates were found in sand filter and loess bead filter. Clearly, organic matter can be one of the most important impacting factors on nitrification efficiency. NO_2-N conversion rates showed similar trend as for TAN. Based on the TAN and nitrite conversion rates, styrofoam bead showed the best performance among the three filter media tested. Also, the low gravity and price of styrofoam bead make the handling easier and commercial application more cost-effective. The results obtained at the highest organic matter loading rates can be used in the biofilter design in recirculating system. Areal TAN conversion rates (ATR) at the highest organic matter loading rate of 18 kg O_2/m^3 • day and different inlet TAN concentrations were investigated in trial 3.

3. Design, management, and performance of a laboratory scale seawater recirculating system for Korean rockfish *Sebastes schlegeli* culture

The designed system consisted of a circular culture tank (volume, 0.75 m^3), two nitrification styrofoam bead filters, a denitrification sand filter, a

sedimentation basin, a foam fractionator, a main water sump, and pumps. The total water volume of the system is 1.1 m^3. Dual drain system was used for in-tank solids separation. The high solids outlet was connected to the sedimentation basin. The system was designed to support feed loading rate of 0.4 kg feed/day and the volume of styrofoam beads needed for nitrification was calculated to be about 42 L. A relatively large dimension of denitrification sand filter was used. This whole system was conditioned for 2 months before stocking. Twenty-kg Korean rockfish (130 fish) with an average body weight of 153.8 g was stocked to the culture tank on November 13, 2002 and harvested on February 28, 2003. Fish were fed twice daily with commercial formulated feed.

TAN concentrations were below 1 mg/L and NO_2-N concentrations were within the range of 1-3 mg/L on most sampling days. TAN was removed in bead and sand filters and it was removed or produced in the sedimentation basin. Basically, nitrite was removed in the bead and sand filters while it was either removed or produced in the sedimentation basin. Nitrate was produced in the bead filters and removed in the sand filter and sedimentation basin. Production of TAN in the sedimentation basin attributed to the elevated TAN concentrations in the last experimental period. High nitrite concentrations in the culture tank water was partially due to the production of nitrite in the bead filters at high inlet TAN concentrations and partially due to the production of nitrite in the sedimentation basin, especially in the last experimental period. TAN conversion rates were lower than those obtained in previous experiment when compared on an equal basis of inlet TAN concentrations. The estimated threshold point where TAN removal equals nitrite removal was around TAN removal of 0.33 mg/L.

Foam fractionator performed well in the recirculating system. TSS and protein enrichment factors were within the range of 6.4-39.4 and 1.6-7.3, respectively. Low values of PO_4-P, TAN, NO_2-N, and NO_3-N enrichment

factors were obtained and this indicates that foam fractionation is not an effective way for removal of dissolved inorganic nitrogen forms. The calculated maximal daily removal values for TSS and protein were 10.9 g and 1.4 g, respectively.

Over 107-day culture period, fish reached final density of 51.7 kg/m^3 (initial density, 33.3 kg/m^3) when calculated on the culture tank volume basis. On a daily basis, water addition was 3.4% of the total water volume in the system.

Nevertheless, whole water quality parameters were within the levels commonly recommended for fish culture on most of the sampling days. However, further studies are needed to evaluate the commercial feasibility of this system because of the small-scale system used in present experiment. At least, present study still provides some basic information for further studies of this kind of system.

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Ⅰ. General Introduction

It is widely acknowledged that fish supplies from the world fisheries are unlikely to increase substantially and the expansion of the aquaculture sector will probably provide the solution to the problem of projected shortfalls (Chamberlain and Rosanthal, 1995). Not only has this led to the quick development of aquaculture systems, but also there is a shift towards the intensification of fish culture system because of the shortage of water resources and high land costs. In intensive aquaculture system, water quality tends to deteriorate rapidly due to the accumulation of uneaten feed, feces, and inorganic metabolic wastes, especially ammonia excreted by fish or decomposition of organic matter. Recirculating aquaculture systems (RAS) are specially developed to address these problems. Prohibitive release of nutrient water into the environment by environmental regulations, high expense associated with pumping large amounts of water, and the danger of introducing pathogens also initiated the development of RAS. Besides, recirculating aquaculture systems provide many advantages compared with traditional aquaculture systems including conserving water and heat, maintaining better control over environmental factors, and providing a quality controlled product. The advantages have been well documented for many years (Liao and Mayo, 1974; Losordo, 1991).

In designing recirculating aquaculture systems, the main factors that should be considered for maintaining a good water quality are solids removal, ammonia removal, and aeration. Solids usually consist of uneaten feed, feces, and bacteria consortia. Different solids removal technologies have been studied including sedimentation, hydrocyclones, and mechanical filtrations. Among them, sedimentation is the simplest one. Sedimentation basins require little energy input, are cheap for construction and easy for management. So,

they have been the most common solids removal methods used not only in the municipal wastewater treatment system but also in many aquaculture systems (Shnel et al., 2002; Jones et al., 2001). However, sedimentations are usually believed to be not effective for fine suspended solids removal (Chen et al., 1994a). Another disadvantage is the low HLR required for effective solids removal. Nevertheless, sedimentation is now commonly used in aquaculture systems.

Recently, foam fractionation process is employed in aquaculture system for solids removal and has already been considered a necessary treatment process in recirculating aquaculture systems (Wheaton, 1977; Spotte, 1992; Huguenin and Colt, 1989; Dwivedy, 1973). Foam fractionation is commonly believed to be effective for fine solids removal (Chen, 1991). Also, foam fractionation is preferred due to the low construction cost, low energy consumption, easy management, and easy adaptation to almost all kinds of recirculating aquaculture systems. Lomax (1976) found that, in terms of cost and effectiveness, biofilter with foam fractionation was the best design combination after examination of several fish culture systems. Others also reported that foam fractionation can be used to remove fine solids and excessive nutrients (Chen, 1991; Chen et al., 1993a, b, c; Chen et al., 1994b, c; Dwivedy, 1973). However, Huguenin and Colt (1989) pointed out that there is still a lack of the actual performance data and it is necessary to identify and quantify the organic components involved in foam fractionation process. Weeks et al. (1992) and Suh et al. (2002) evaluated the performances of foam fractionators in freshwater aquaculture systems.

The second critical water quality parameter in recirculating aquaculture system is ammonia nitrogen, usually referred to as TAN. Ammonia should be removed as soon as possible since ammonia is toxic to fish and produces bad flavors. The most commonly used method for TAN removal is biological filtration for nitrification and fixed film biofilters are mostly employed in RAS

(Greiner and Timmons, 1998; Malone and Beecher, 2000; Reyes and Lawson, 1996; Sastry et al., 1999; Singh et al., 1999; van Rijn and Rivera, 1990). Among them, trickling filter and submerged filter have many advantages including low construction cost, easy management and maintenance, and well adaptation to different water and waste loading rates. At present, down flow trickling or submerged biofilters are commonly used in aquaculture systems in Korea. However, not much information about the performance of biofilters in seawater aquaculture systems is available.

The key elements for biofilter design are media to be used and operational parameters. For optimal nitrification and less clogging of biofilter, biofilter media should have high surface area and low specific gravity (Lekang and Kleppe, 2000; Wheaton et al., 1994a). Recently, styrofoam beads have been tested in freshwater system and showed reasonable nitrification capacity in fish farm at Pukyong National University. This kind of beads has very low specific gravity and high specific surface area. No information about the nitrification efficiency of this bead in seawater system is available till now.

Many recirculating aquaculture systems have been developed (Chen et al., 1989; Davis and Arnold, 1998; Greiner and Timmons, 1998; Heinem et al., 1996; Lorsodo et al., 2000; Menasveta et al., 1989, 1991; Millamena et al., 1991; Ridha and Cruz, 2001; Singh et al., 1999; Twarowska et al., 1997) and these systems maintained good water quality for fish or shrimp culture. Still, these systems can exert considerable environmental impact since concentrations of organic matter and nutrients in their effluents are high. Though nitrate is generally considered nontoxic to fishes (Bromage et al., 1988), high nitrate levels are reported to impact growth rates (Muir, 1982). Hrubec et al. (1996) found that prolonged exposure to elevated levels of nitrate may decrease immune response, induce hematological and biochemical changes which are indicative of pathologic response, and may increase mortality. In terms of total nitrogen management another biological process, denitrification, must be

included (Spotte, 1979). The development of an integrated nitrogen removal system that includes nitrification and denitrification processes is essential to the advent of commercial biosecure aquaculture systems (Lee et al., 1995, 2000). Success of a truly closed, recirculating design will allow the fish culture system to be located in any area where seawater could be transported or artificial seawater could be made.

Closed, seawater recirculating systems for shrimp culture have been developed (Millamena et al., 1991). In this system, external carbon sources were used to mediate denitrification. Recently, Shnel et al. (2002) evaluated the performance of a zero-discharge tilapia recirculating system featured an anoxic treatment stage where sludge was biologically digested and nitrate was reduced to nitrogen gas, eliminating the requirement of external carbon sources. The technical feasibility of zero-discharge recirculating systems incorporating denitrifiers fed with endogenously produced carbon has been studied by many researchers (Arbiv and van Rijn, 1994; Kaiser and Schmitz, 1988; Schuster and Steltz, 1988; van Rijn and Rivera, 1990).

In present experiments, a closed recirculating system was developed for Korean rockfish *Sebastes schlegeli* culture since Korean rockfish is one of the main cultured marine species. The performance of foam fractionator was first evaluated in simulated seawater aquaculture system under various operational conditions and then the performance was further evaluated in the recirculating system. Second experiment was conducted for selecting media exhibiting the better nitrification capacity and optimal operational parameters. Biofilter media including coarse sand, styrofoam bead, and loess bead were tested under various operational conditions. First trial was conducted to compare start-up period and nitrification efficiencies at constant HRT without addition of organic matters. Second trial was conducted to evaluate the effects of different HRT and organic matter loading rates on nitrification efficiencies and the best operational factors were determined. The last trial was conducted to

determine TAN removal rates at various inlet TAN concentrations. The results obtained from these trials were used in the design of nitrification biofilter in the closed recirculating system later.

The principal compartments of the recirculating system include: a sedimentation basin and a foam fractionator for solids removal; two styrofoam bead filters for TAN and NO_2-N removal; a sand filter for nitrate removal; a circular tank for fish culture; air blower for aeration; and a heating system for maintaining temperature not to drop to too low levels. The dimension and operating parameters of different compartments were determined according to the results obtained in previous experiments or existing information and common practices. It should be noted that a variety of commercially viable designs and technologies exist, present design is based on the concept that the system should be constructed at low cost and operated with easy management.

II. Solids and protein removal by a foam fractionator in simulated seawater aquaculture system

1. Introduction

Recirculating aquaculture systems have many advantages compared with traditional aquaculture systems, for example, reduced water quantities used and wastewater volume discharged, reduced environmental effects, enhanced siting flexibility of culture facilities, and most important, improved water quality for better fish growth. At present, the obstacles for faster development and commercial application of recirculating system are high fixed and production costs. This makes the production of fish in recirculating aquaculture system with low or even no profit. So, the components of a recirculating system should be designed technologically and economically feasible. Nowadays, seawater recirculating aquaculture systems attract more attention since more expensive fish species and shrimp can be cultured to makeup the profit margin. Also, increased concern on marine environmental protection and consumption of live seafood promoted the development of seawater recirculating aquaculture systems.

The success of a recirculating system depends largely on the treatment efficiency of wastes generated in the system. Wastes that are of critical concern are ammonia and solids. Ammonia can be oxidized to less toxic nitrogen forms or removed through biofiltrition. The generated solids can be divided into two categories: settleable solids and non-settleable solids. Typically, closed aquaculture system units are subject to accumulation of fine suspended solids and dissolved organics (Timmons et al., 1995). Chen et al. (1993a) found that 95% of the suspended particles in three recirculating

aquaculture systems had a diameter less than 20 microns. Fine, non-settleable suspended solids, which are more difficult to control, cause the most problems in recirculating system. Fine solids were suspected to be responsible for fish kill in a recirculating system (Timmons et al., 1987). Major (1988) found that damage to fish gills can occur at moderate TSS levels of 44 mg/L. Chapman et al. (1987) also found that the accumulation of fine particles was associated with lethal effects. Others have reported the adverse affects of solids on fish health and gill damage (Stickney, 1979; Wickins, 1980). Biofilter could be easily clogged in recirculating systems with high solids concentration. Also, solids could generate more ammonia nitrogen and oxygen demand if not removed out of recirculating systems as soon as possible. The recommended limit of suspended solid concentration in a recirculating system is 15 mg/L (FIFAC, 1980; Reinemann, 1987; Timmons et al., 1987).

Different methods are developed for solids removal in aquaculture systems. These processes can be classified as gravity separation (sedimentation), filtration (screen, granular media, and porous media separator), and flotation (foam fractionation). Sedimentation usually is effective for removing particles greater than 100 micron (Rudolfs and Balmat, 1952). Low settling velocity of fine particles makes this method impractical. Chen and Malone (1991) also found that filtration methods require fine media or screen filters and thus making the removal more expensive due to pressure losses or frequent backwashing. The majority of fine particles remain even after passing through biofilters (Muir, 1978).

Lomax (1976) found that, in terms of cost and effectiveness, biofilter with foam fractionation was the best design combination after examination of several fish culture systems. Dwivedy (1973) found that foam fractionator removed solids and helped to maintain pH in an oyster culture system. Besides, foam fractionator can serve as a gas-stripping unit, which is usually a necessary treatment process in recirculating aquaculture systems (Chen et al., 1993a).

Others also reported that foam fractionation can be used to remove fine solids and excessive nutrients (Chen, 1991; Chen et al., 1993b,c; Chen et al., 1994b,c; Weeks et al., 1992).

Foam fractionation has also been used successfully to separate surface-active materials such as enzymes and other proteins (Charm, 1972) and has already been considered a treatment process in recirculating aquaculture systems (Wheaton, 1977; Spotte, 1992; Huguenin and Colt, 1989; Dwivedy, 1973). Surfactants, which are key elements in foam fractionation process, occur naturally in aquaculture systems (Chen et al., 1993b). Although fatty acids could be possible candidate for surfactants, Chen et al. (1993b) measured relatively low fatty acid concentrations in foam condensates when compared with protein concentrations and they concluded that fatty acids are negligible. Usually, protein is considered the main surfactant in aquaculture systems since protein is a major component of fish feed, usually constituting from 30 to 50 percent of the formulated feed (Downey, 1981), and protein leached from uneaten feed and fish feces or directly excreted by fish should be substantial.

Concentration of protein in aquaculture systems is affected not only by the feed supplied and the fish species cultured, but also, to a large extent, by the culture system. Chen et al. (1993b) analyzed protein and TSS concentrations in culture waters from three different culture systems and found great differences between systems. Up to 127 mg/L protein was found in the system with low water exchange rate. Decomposition of these proteins would contribute to high ammonia concentrations in aquaculture systems. So, proteins contained in volatile solids along with dissolved proteins should be removed out of aquaculture system. Foam fractionation is preferred for this purpose due to the low construction cost, low energy consumption, easy management, and easy adaptation to almost all kinds of recirculating aquaculture systems.

Many researches have been done on protein removal by foam fractionation.

Chen et al. (1993b) determined that only 11% of the proteins could act as surfactant and be removed from the aquaculture water and they modeled protein removal in freshwater systems (Chen et al., 1994b,c). Suh et al. (2002), Suh and Lee (1995) also found a partial protein removal by foam fractionator in tilapia culture system. Lomax (1976) confirmed solids removal by foam fractionator and recommended that the substances responsible for foam fractionation should be identified. However, all these experiments were done in freshwater systems. Recently, Suh et al. (2000a) investigated protein removal characteristics in seawater systems using synthetic wastewater, which was made by mixing collected foam condensate with seawater, but without giving the detailed operating parameters. Suh et al. (1999) modeled the protein removal by foam fractionation in seawater system using egg white as protein sources. However, protein concentrations used in their experiment were higher than commonly reported in aquaculture system (Chen et al., 1993b). Also, the use of egg white instead of natural proteins produced in aquaculture system makes direct application of their findings questionable. Huguenin and Colt (1989) already pointed out the lack of the actual performance data and the need to identify and quantify the organic components involved in foam fractionation process.

Spotte (1979) has stated that the main factors affecting the efficiency of foam fractionation include HRT, bubble size, air flow rate, diffuser submergence depth, foam overflow height, and the configuration of foam fractionator itself. For an existed foam fractionator, the factors affecting foam fractionation become air flow rate, water flow rate, and foam overflow height (Weeks et al., 1992).

Here in this experiment, TSS and protein removal efficiencies of an air drift foam fractionator were evaluated at different foam overflow heights, superficial air velocities, and hydraulic residence times in a simulated seawater aquaculture system. Synthetic wastewater was obtained by mixing waste

collected from a freshwater recirculating aquaculture system with artificial seawater. Protein and solids contents of the synthetic wastewater were within the ranges that usually reported in recirculating aquaculture systems. The obtained data would be helpful for selecting operational parameters in applying foam fractionation in seawater aquaculture systems.

2. Materials and methods

2.1. System configuration and procedure

The experiment system consisted of a round, 300-L plastic culture tank, a recirculating pump, a foam fractionator, an air distribution system, and foam collection facilities (Fig. 1). Synthetic wastewater was pumped from the culture tank into the foam fractionator and then back to the culture tank or wasted according to the different set of trials. A bypass was connected to the main outflow from pump for adjusting the water flow rate to the foam fractionator.

In order to obtain equal solid and surfactant concentrations in culture tank water for each set of trials, sediments from first sedimentation basin of a recirculating system in Pukyong National University were collected and mixed by electric stirrer, and then equal aliquots were frozen stored in refrigerator. The sedimentation basin was cleaned once a day, so the collected sediments are relatively fresh. They should be mainly consisted of feces and uneaten feed, which are the main solid wastes in fish culture system. Foam condensates produced in the same recirculating aquaculture system were also collected and stored as did for sediments. Foam condensate and sediments were mixed together to form the synthetic wastewater. All the tests were conducted at water temperature of 20 °C and pH values were within the range of 7.8-7.9.

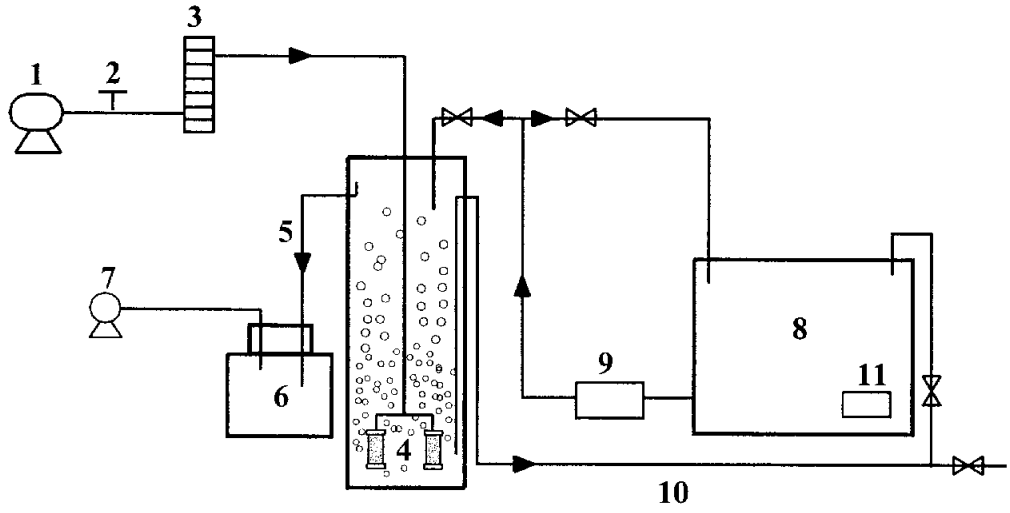

Fig. 1. Schematic diagram of foam fractionation system. 1, Air blower; 2, pressure regulator; 3, air flow meter; 4, air diffuser; 5, foam collection pipe; 6, foam collection bottle; 7, vacuum pump; 8, culture tank; 9, recirculating pump; 10, outflow line; 11, mixing pump.

Protein and solids removal rates were evaluated at 4 different air flow rates of 7, 14, 21, and 28 l/min, 5 HRTs of 1, 2, 3, 4, and 6 min, and 4 foam overflow heights of 1, 3, 5, and 7 cm. Superficial air velocity was used instead of air flow rate since it is a convenient way of expressing air flow velocity through foam fractionator column and corresponding SAV values were 0.371, 0.743, 1.114, and 1.486 cm/sec, respectively.

In first set of trials, selected combinations of operating parameters were tested and were conducted on batch mode. Protein removal rates were tested at different initial protein concentrations. Also, TSS removal rates were tested at different initial protein concentrations. Removal rates were calculated according to Suh et al. (2000).

$$-r_a = \frac{C_{i,a} \times Q_i - C_{o,a} \times Q_i}{V}$$

Where, $-r_a$, removal rate (g/L • day); $C_{i,a}$, TSS or protein concentration in inflow; $C_{o,a}$, TSS or protein concentration in outflow; V, volume of fractionator.

In the second set of trials, changes of TSS and protein concentrations in culture tank water were monitored till no foam could be collected for 5 sets of combinations of HRTs and SAVs and each was conducted on consecutive mode. Foam overflow height was 3 cm for all the 5 consecutive trials. Gas holdup, which is the fractional increase in column liquid height due to supply of aeration, was measured since it is essential for determination of foam overflow height. Gas holdup was determined by measuring the height differences before and after supply of aeration.

2.2. Foam fractionator

Schematic diagram of the foam fractionator used in present experiment is shown in Fig. 2. This foam fractionator is made of acrylic pipe with a diameter of 20 cm and a height of 120 cm. Water outlet was located near the

bottom and inlet water was introduced on top of the column. This formed a counter-current flow pattern in the foam fractionator column. A 40-mm PVC elbow was installed at 90-cm height for foam collection. Foam overflow height was controlled by changing the length of nipple pipe connected to the elbow. Foam outlet was connected to collection bottle and vacuum pump was used for quick collection of foam produced on top of the collection pipe. Air distribution system included an air blower, a pressure regulator, and an air flow meter (Dwyer instruments, model RMA). Two coarse air stones with a diameter of 3.2 cm and length of 9 cm were used to disperse air bubbles.

2.3. Sample and analysis

Samples were taken at 10, 20, 30 minutes and then at half hour intervals in culture tank after start of air supply for 5 consecutive trials to monitor changes of solids and protein concentrations in culture tank water. For trials conducted on batch mode, 4 samples were taken at the inlet and outlet of foam fractionator at intervals of 1-6 minutes. Protein analysis was conducted according to Lowry et al. (1951). TSS was measured according to standard methods (APHA, 1995). Filter paper was rinsed successively 6 times with 20 ml distilled water for removing the salts left on the filter paper.

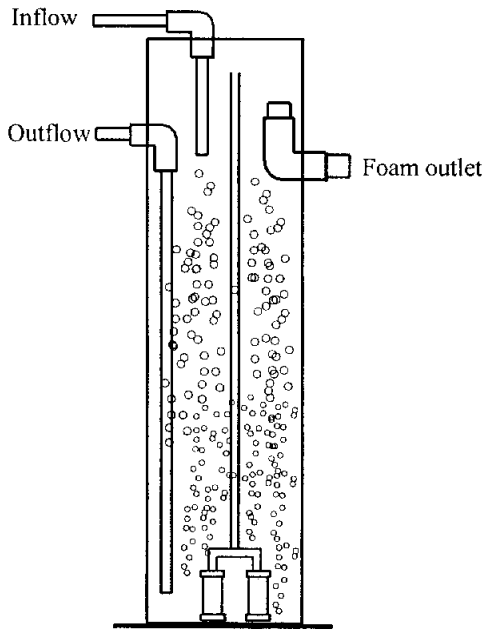

Fig. 2. Schematic diagram of the foam fractionator.

3. Results and discussion

Fig. 3 shows the changes of TSS concentrations in culture tank water at three HRTs of 2, 3, and 6 min and constant SAV of 1.486 cm/sec in the consecutive trials. Initial protein concentrations were 34.7±0.1 mg/L. TSS concentrations in culture tank water decreased faster at lower HRT. In other words, increase in water flow rate through the foam fractionator column increased TSS removal rate. Also, TSS removal rates increased with decrease of HRT at fixed protein concentrations of 32.5±0.1 mg/L when the trials were conducted on batch mode (Fig. 4). Increase in HRT means increase in water and air contact time, which provides longer contact time of solids with air bubbles at water-air interface and thus increasing solids removal efficiency. On the contrary, decrease in HRT increased the contact opportunities of solids with air at the water-air interface and thus increasing the removal rates. This caused the faster drop of TSS concentration in culture tank water at lower HRT.

Suh et al. (2000a) reported relatively higher values of TSS removal rates at different HRTs. However, they did not provide protein concentrations in the bulk solution, and thus making the comparison difficult. A possible explanation may be derived from the fact that the small foam fractionator column used in their experiments since a foam fractionator column with smaller cross-sectional area is considered to be more efficient (Know, 1971).

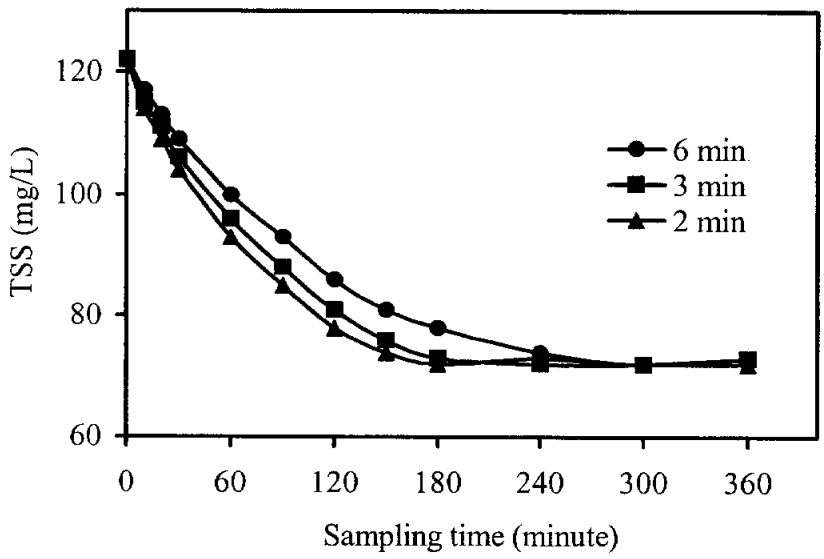

Fig. 3. Change of TSS concentrations in culture tank water at three different hydraulic residence times of 2. 3 and 6 minutes in the simulated seawater aquaculture system.

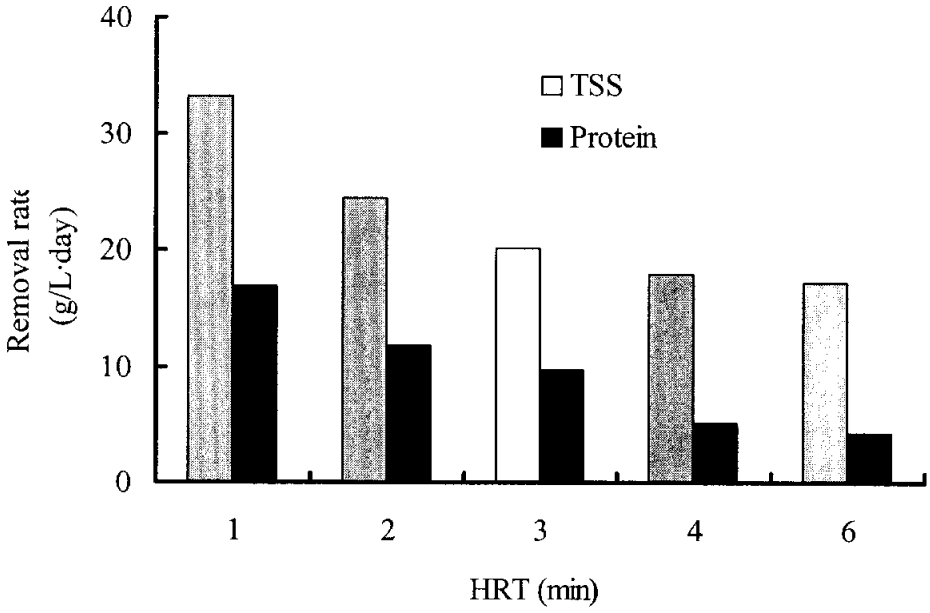

Fig. 4. TSS and protein removal rates at different hydraulic residence time of 1, 2, 3, 4, 6 minutes and fixed protein concentration of 32.5 mg/L.

Fig. 5 shows changes of TSS concentrations in culture tank water at SAVs of 0.734, 1.114, and 1.486 cm/sec and constant HRT of 3 min. Initial TSS concentrations were 122 mg/L. TSS concentrations in culture tank water decreased faster at higher SAVs for the 5 consecutive trials. Higher SAV also greatly increased TSS removal rates when the foam fractionator was operated at HRT of 3 min on batch mode (Fig. 6). The increases of TSS removal rates were nearly proportional to the SAVs tested. Usually, higher SAV increased the areas of air-water interface in a given time period. This, in consequences, increased the opportunities for solids to be adsorbed on the air-water interface and then increased the TSS removal rate. Similar results were reported for foam fractionator operated in seawater system by Suh et al. (2000b). In fresh water aquaculture systems, Suh et al. (1997) also reported the similar trends for TSS removal at different SAVs.

Changes of protein concentrations in culture tank water when the foam fractionator was operated at different SAVs and fixed HRT of 3 minutes is shown in Fig. 7. Initial protein concentrations were 34.8 ± 0.1 mg/L. Protein concentrations in culture tank water decreased faster at higher SAV, with the lowest SAV corresponding to the lowest reduction rate of protein concentrations. Chen et al. (1994b) reported the similar treads in freshwater systems.

Changes of protein concentrations in culture tank water at different HRTs are shown in Fig. 8. Superficial air velocity was set at 1.486 cm/sec. Lower HRT caused rapid removal of protein from culture tank water and thus the quick reduction of protein concentrations. Also, the reduction rates declined in treatment time, which were similar to the results obtained at different SAV. Protein reduction rates in culture tank water declined along with the progressing of experiment. In experiments done with direct fish culture water or synthetic wastewater, Chen et al. (1993b) also found that protein removal rates declined in treatment time. This decline of protein reduction rates must have been induced by the declined protein concentration in culture tank water.

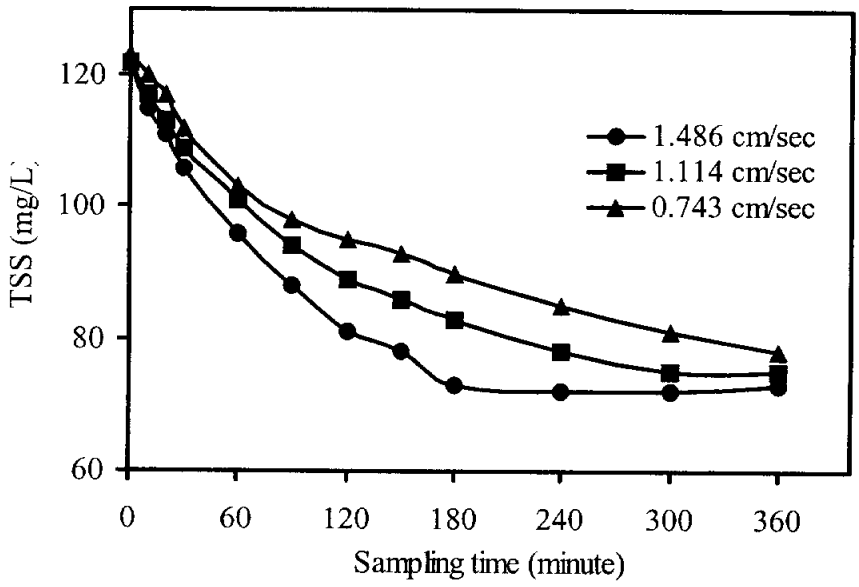

Fig. 5. Changes of TSS concentrations in culture tank water at three different superficial air velocities of 0.743, 1.114, and 1.486 cm/sec in the simulated seawater aquaculture system.

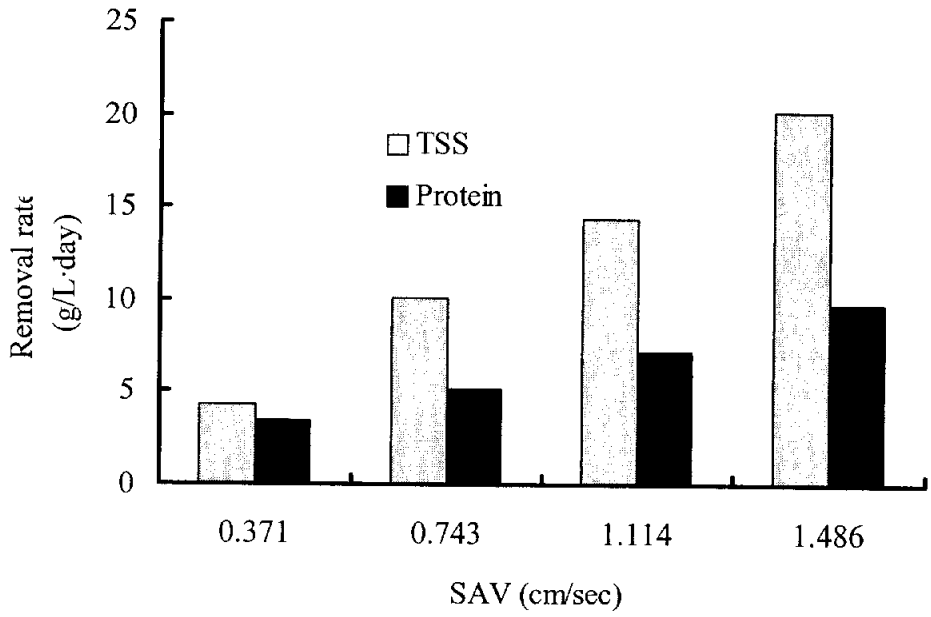

Fig. 6. TSS and protein removal rates at different superficial air velocity of 0.371, 0.743, 1.114, and 1.486 cm/sec and fixed protein concentration of 32.5 mg/L.

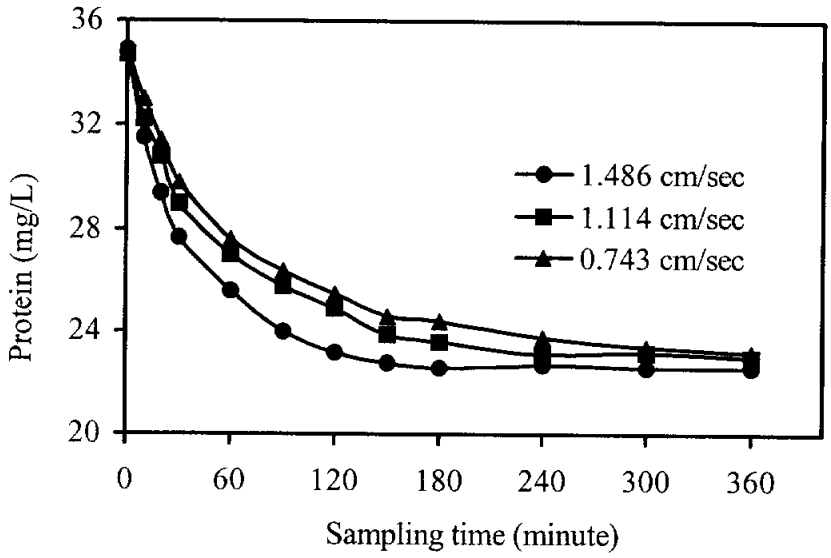

Fig. 7. Changes of protein concentrations in culture tank water at three different superficial air velocities of 0.743, 1.114, and 1.486 cm/sec in the simulated seawater aquaculture system.

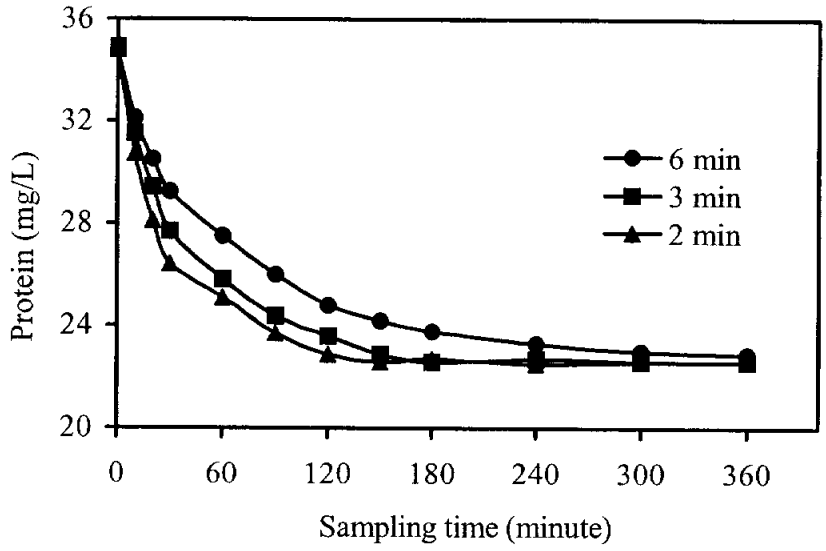

Fig. 8. Changes of protein concentrations in culture tank water at three different hydraulic residence times of 2, 3, and 6 minutes in the simulated seawater aquaculture system.

Percentage protein removal averaged 34.3%, which means incomplete removal of protein from culture tank water. The ratios of removed protein to initial protein concentrations were reported to be 11% (8-15%) by Chen et al. (1993b). These values were lower than the results obtained in present experiment. Synthetic wastewater, which contained foam condensate, might have resulted in the discrepancies. These results also suggested that protein removal was limited and that not all the proteins detected by Lowry's method were surface-active since some proteins might not possess significant surface-active properties under certain conditions when considering their wide range of molecular structures (Chen et al., 1993b). This can be further confirmed by the results obtained in present experiment that at high SAV and low HRT, no further reductions of protein concentrations were detected in the last 3-hour operations though protein concentrations were still relatively high in culture tank water in trials conducted on consecutive mode (Fig. 7, 8).

In batch trials conducted at fixed initial protein concentrations of 32.5 mg/L and HRT of 3 minutes, protein removal rates increased with increase of SAV (Fig. 6). Chen et al. (1994c) also found that protein removal rate in foam fractionation process is closely related to SAV and similar trends were reported. However, protein removal rates decreased with increase of HRTs when foam fractionator was operated at fixed SAV of 1.486 cm/sec (Fig. 4). These results were coincident with those reported by Suh et al. (2000b). Usually, higher superficial air velocity increased the areas of air-water interface in a given time period. This, in consequences, increased the opportunities for proteins to be adsorbed on the air-water interface and then increased the protein removal rate. Lower HRT increased the contact opportunities of protein with air at water-air interface and then increased the removal rates.

Fig. 9 shows TSS and protein removal rates on different initial protein concentrations. TSS removal rates increased with increase of initial protein concentrations. Protein is usually considered the main source of surfactants in

aquaculture water (Chen et al., 1993b) when considering the high protein content of feed. Essentially all proteins are volatile solids (Timmons et al., 1995), which are considered to be the main substances that could be removed by foam fractionation (Weeks et al., 1992). The great impact of protein concentration on TSS removal is easy to be understood.

A linear increase of protein removal rates versus the increase of initial protein concentrations in bulk solutions suggested that the protein removal rates followed a first-order process. Chen et al. (1994c) also reported that protein removal rate is related to its concentration in the bulk solution as first-order reaction in five-trial experiments which were conducted in freshwater system. Suh et al. (2000a) also reported an increase of protein removal rates at higher initial protein concentrations in bulk solution. However, Suh et al. (1999) reported an exponential expression of protein removal rates versus initial protein concentrations and they attributed this discrepancy to the different protein concentrations used and the different operating parameters.

Foam condensates produced in the 5 consecutive trials were collected till the separation process ceased. TSS and protein concentrations in the foam condensates, foam overflow rates, time consumptions, and gas holdup data are summarized in Table 1. Higher SAV resulted in greater foam flow rates but lower TSS concentrations in the foam condensates. Weeks et al. (1992) found the same trend in a freshwater aquaculture system. TSS concentrations of the collected foam condensates in present experiment were about 17.8-32.7 times of the initial TSS concentrations in culture tank water. Weeks et al. (1992) found that TSS concentration in the foam condensate was 25 times higher than that in the untreated fish culture water in a freshwater system. These results show that TSS enrichment in foam condensate can be substantial. Hydraulic residence times have significant effects on foam overflow rate and the time consumption for TSS removal in culture tank water. Time consumptions for removal of TSS from culture tank water were about 2.5, 3, and 5.4 hours at

HRT of 2, 3, and 6 min, respectively. TSS concentration in foam condensate was higher at lower HRT. However, the effects of HRT on TSS concentrations in the foam condensates were not as great as the effects of SAV.

In 5 consecutive trials, incomplete removal of solids was found for the treatments tested. Approximately 40% of TSS was removed from culture tank water. This would be partially due to the low protein concentration in the synthetic culture tank water. On the other hand, the synthetic wastewater was made of collected foam condensate and sediments after frozen-stored, though the collected wastes were well mixed before supply to culture tank water, coagulation of solids would attribute to the low solids removal rates.

Protein concentrations decreased with increase of SAV. At fixed foam overflow height, higher SAV resulted in greater foam overflow rates and lower protein concentrations in the foam condensates. Weeks et al. (1992) also found that as air flow rate increased, condensate production increased but concentration decreased for foam fractionators operated in a freshwater aquaculture system. At fixed SAV of 1.486 cm/sec, longer HRT resulted in higher protein concentrations in the collected foam condensates but lower foam overflow rates. However, the differences of protein concentrations in the foam condensates collected from the trials conducted at different HRT were not as great as those obtained from trials conducted at different SAV. Weeks et al. (1992) already reported that water flow rate did not affect the removal of volatile solids over the range of 11.4-34.1 L/min tested in freshwater systems.

Though the foam condensates were collected in trials conducted on consecutive mode, which means that a continuous reduction of TSS and protein concentrations in culture tank water occurred in treatment time, these results still confirmed that high SAV would induce great protein removal and HRT has less effect on TSS and protein concentrations and volume of foam condensates.

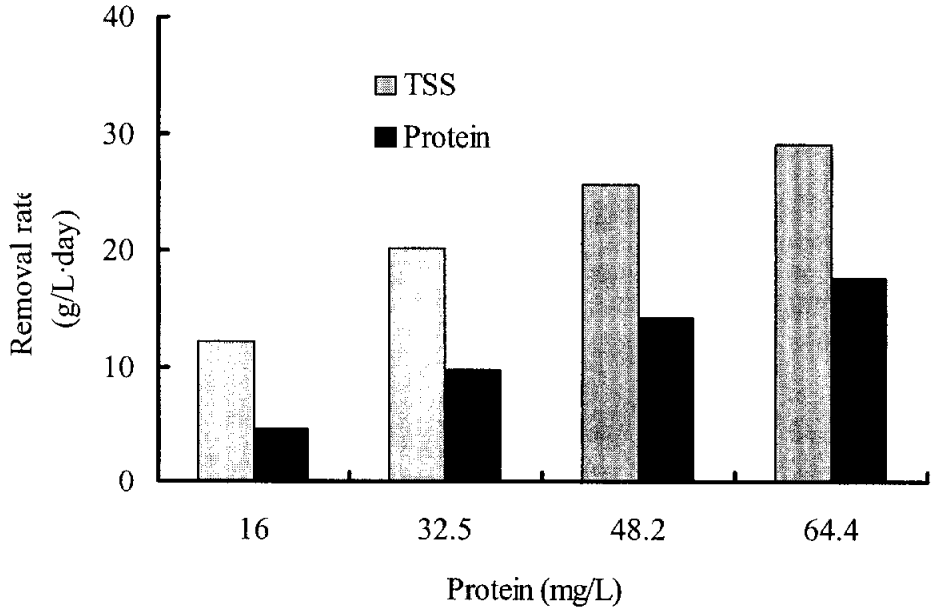

Fig. 9. TSS and protein removal rates at different initial protein concentrations of 16, 32.5, 48.2, 64.4 mg/L and fixed HRT of 3 min.

Table 1. Summary of analysis results of foam condensates collected in 5 consecutive trials.

HRT (min)	SAV (cm/sec)	TSS (mg/L)	Protein (mg/L)	Time (hours)	Flow rate (ml/min)	Holdup (cm)
6	1.486	2790	610	5.4	12	5.6
2	1.486	2135	474	2.5	32.6	5.6
3	1.486	2305	524	3	25.4	5.6
3	1.114	2625	607	3.9	16	4.2
3	0.743	3920	915	5.6	6.8	2.8

The effects of foam overflow heights on performance of foam fractionator are shown in Table 2. Enrichment factor is defined as ratio of the TSS or protein concentrations in foam condensates to those corresponding values in the untreated bulk solutions. TSS and protein concentrations and enrichment factors in the foam condensates increased with increase of foam overflow heights. However, foam overflow rates decreased with increase of foam overflow heights. This is because that foam is swept out at a faster rate at lower foam overflow height, which does not allow excess water to drain from the foam. Higher foam overflow heights would increase TSS and protein concentrations and lower foam volume. Weeks et al. (1992), in a fresh water system, found the same trends but the differences were not as great as found in present experiment. Foam condensate used in the synthetic wastewater should have attributed to this. Suh et al. (1997) also found that an increase of TSS concentration in the foam condensate with the increase of foam overflow height.

These results suggested that high overflow heights may produce extremely concentrated foam condensate, but the production rates maybe extremely low. So, for practical application of foam fractionation in aquaculture systems, the foam overflow height should be selected so that the desired results, e.g. minimizing the effluent volume or maximizing substrate removal, could be obtained.

Gas holdup values were higher for higher SAVs. However, gas holdup values were same at fixed SAV with different HRTs. This indicates that gas holdup is not related to water flow rate through a foam fractionator column. This coincides with the results reported by Chen (1991).

Table 2. Performance data at different foam overflow height (FOH) and fixed TSS concentrations of 120 mg/L and protein concentrations of 34 mg/L.

FOH (cm)	Concentration (mg/L)		Enrichment factor		Foam flow rate (ml/min)
	TSS	Protein	TSS	Protein	
1	936	178	7.8	4.7	76.4
3	2762	524	23	13.8	25.2
5	3994	816	33.3	21.5	15.6
7	4890	920	40.8	24.2	12.3

III. Nitrification efficiencies of biofilters containing different filter media in simulated seawater aquaculture system

1. Introduction

Decreased capture of marine fish and crustaceans, combined with the increased demand for seafood, especially live seafood, have led to the quick development of intensive seawater aquaculture systems. Cage culture is widely used in Korea now. However, in the past few years, the red tide brought disastrous loss to the fish farmers. Recirculating aquaculture system, which can provide a protected environment for cultured species, can be a good substitute. In a closed aquaculture system, water quality tends to deteriorate rapidly due to the accumulation of uneaten feed, feces, and inorganic metabolic wastes, especially ammonia, excreted by fish. Ammonia may lead to suppression of fish growth, sublethal histopathological changes, and even death (Redner and Stickney, 1979), thus ammonia is considered to be toxic to fish and attracted lots of researches to study how to remove it from aquaculture systems. By far, biofilters are commonly used for ammonia removal in aquaculture systems and are considered as the main and crucial component of recirculating systems.

Many types of biofilters with different configurations including trickling filter, submerged filter, sand filter, fluidized bed filter are employed in aquaculture systems. Among them, tricking filter and submerged filter have many advantages including low construction cost, easy management and maintenance, and well adaptation to different water and waste loading rates (Lekang and Kleppe, 2000). At present, down flow, trickling or submerged biofilters are commonly used in aquaculture systems in Korea. However, not

much information about the performance of biofilters in seawater system is available. Hill and Gellman (1977) found that the oxidation of ammonia was almost completely inhibited and a cease of conversion of nitrite to nitrate as a result of the chlorinity of seawater. However, complete nitrifications in seawater systems have been reported (Bower and Turner, 1981; Forster, 1974; Weatherly, 1984). Recent studies in shrimp aquaculture systems with different biofilter media showed promising prospects of the seawater recirculating systems (Davis and Arnold, 1998; Menasveta et al., 2001; Tseng et al., 1998).

Various types of filter media are used to provide surfaces for nitrifying bacteria to grow on in aquaculture and wastewater treatment systems (Greiner, and Timmons, 1998; Lekang and Kleppe, 2000; Nijhof and Bovendeur, 1990; Ridha and Cruz, 2001). For optimal nitrification and less clogging of the biofilter, biofilter media should have a high surface area and low specific gravity (Lekang and Kleppe, 2000; Wheaton et al., 1994a). Coarse sands (2-5 mm) mixed with shell debris, which can provide a high specific surface area, are mainly used in live fish aquarium biofilters in Korea by now. It is believed that this kind of biofilter not only can remove ammonia but also can help maintain the pH of the culture water. Another biofilter media, styrofoam beads, have been tested in freshwater system and showed a reasonable nitrification capacity. This kind of beads has high specific area and low gravity thus make them very suitable as biofilter media. Loess bead, which is a newly developed biofilter media, together with the above-mentioned sand and styrofoam beads, was used in present experiment since, to the best knowledge of the author, no detailed information about the nitrification efficiencies of these media in seawater recirculating systems has been provided.

Many factors influence the performance of biofilters. Ammonia loading rate is commonly considered the crucial factor since the allowable concentration in aquaculture system is so low that it may become the rate-limiting factor in filter nitrification (Hochheimer, 1990). Hydraulic

loading rates also affect the performance of biofilters since the substance loading rates usually are determined by the substance concentration and flow rate through the biofilter. Kaiser and Wheaton (1983) observed that for low ammonia concentration, higher flow rates produce higher ammonia mass removal rates. Organic matter is another factor that affects the performance of nitrification filters including clogging of biofilters and providing substance for heterotrophic bacteria, which compete with nitrifiers for growing space. Pano and Middlebrooks (1983) studied RBC's in wastewater treatment and found that ammonia removal was influenced by organic loading rates with ammonia removal rates decreasing as organic loading rates increased.

Nitrification efficiencies of the three media mentioned above were tested in a simulated aquaculture system at various water, TAN, and organic matter loading rates. The obtained data would be useful for selection of the suitable biofilter media for aquaculture systems. Also, the obtained TAN removal rates in present experiment can serve as database for designing biofilters filled with the media tested.

2. Materials and methods

2.1. System description

The configuration of the whole system is shown in Fig. 10. It consisted of culture tank, recirculating pump, three biofilters (sand filter, styrofoam bead filter and loess bead filter), synthetic wastewater feed tank, metering pump, and thermostatic heating system. Aeration was supplied in the culture tank to increase DO levels and ensure well mixing of the added synthetic wastewater. The volume of the rectangular culture tank is 900 L and the whole water

volume in the system is around 1000 L. The three biofilters are placed on a higher level, so the water can flow back to the culture tank by gravity.

Biofilter columns were made of PVC pipes (Fig. 11) and the dimension of each biofilter was 1 m high and 30 cm in diameter. Two gratings are placed inside each biofilter, one near the bottom to prevent the loss of biofilter media and the other one at the top to ensure even distribution of inlet water. The high outlet position of styrofoam bead filter reduced the headloss of this kind of biofilter. Outlets of the other two biofilters are just near the bottom of the filter columns. Approximately 20 L of biofilter media was used in each biofilter.

2.2. Experiment procedure

The three biofilters were conditioned by feeding synthetic wastewater. Composition of the synthetic wastewater was shown in Table 3. In trial 1, TAN conversion rates were evaluated at high TAN loading rates, around 100 g TAN/m^2 • day which is calculated based on the biofilter surface area, in order to minimize the inhibitive effect of low TAN levels on nitrification efficiencies. TAN concentrations of the inlet water were maintained around the set point by changing feeding rate of the synthetic wastewater. Nitrite and nitrate concentrations were maintained at low levels by water exchange. Water flow through the biofilters was set at 10 L/min, which equals to a HLR of 200 m^3/m^2 • day. This is over the recommended minimum HLR for trickling filter (Roberts, 1985). Water temperature was maintained at 20℃ with thermostatic heating system. This trial was mainly for comparison of the start-up period and the nitrification rates of the different media.

Trial 2 was conducted at different HLRs of 100, 200, and 300 m^3/m^2 • day for evaluating the effects of HLRs on nitrification rates without addition of organic matters and then the biofiters were reconditioned with the addition of

organic matters to test the nitrification rates at fixed HLR of 100 m^3/m^2 • day and different inlet organic matter (COD) concentrations of 0, 25, and 50 mg/L, corresponding to COD loading rates of 0, 9, and 18 kg O_2/m^3 •day, respectively. This trial was only conducted at the lowest HLR tested in present experiment because at higher HLR, sand filter is easily clogged and makes the comparison not practical. Later, the nitrification rates of styrofoam bead filter at various TAN loading rates were conducted in trial 3.

2.3. Characteristics of media

Characteristics of the three filter media are shown in Table 4. Styrofoam bead is round-shaped with an average diameter of 1.4 mm and the calculated specific surface area is 2820 m^2/m^3. The specific gravity is only one thirties of water, so most of the beads in the biofilter tends to float on water surface. Loess bead is cylindrical (1cm long and 1 cm in diameter) with some small holes on the surface. These media have the lowest specific surface area of 225 m^2/m^3. Size distribution of sands is within 2 to 4.5 mm and the estimated specific surface area is about 950 m^2/m^3. Gravels were put on the bottom layer and then coarse sand mixed with shell debris, the middle layer in sand filter. A thin layer of fine sands was put on the surface for evenly distribution of inlet water.

2.4. Sampling and analysis

Water samples were taken once a week in the conditioning period and once every three days after a relatively stable nitrification was observed in trial 1. In trial 2 and 3, water samples were taken randomly after reconditioning. All the water samples were taken at the main inlet and the outlet of each biofilter. TAN, NO_2-N, NO_3-N, and COD were measured using the methods described by

Strickland and Parsons (1972). Temperature and DO were measured with DO meter (KDO 5151, KRK Co.). Total alkalinity was measured by the titration method (Grasshoff et al., 1999) and pH was measured with pH meter (Oregon, model 720 A).

Table 3. Composition of the synthetic wastewater (modified after Liu and Capdeville, 1994).

Ingredients	Concentration (g/L)
NH_4Cl	139
$NaHCO_3$	350
Na_2HPO_4	15.9
KH_2PO_4	15.3
$MnSO_4 \cdot 7H_2O$	3.6

2.5. Performance evaluation

The volumetric TAN conversion rate (VTR), areal TAN conversion rate (ATR), volumetric oxygen consumption rate (OCR), areal nitrite conversion rate (ANR), volumetric nitrite conversion rate (VNR), and volumetric alkalinity consumption rate (VAR) on a daily basis were calculated using the following equations:

$VTR\ (g/m^3 \cdot day) = 1.44\ (TAN_i - TAN_e)\ Q / V$

$ATR\ (g/m^2 \cdot day) = 1.44\ (TAN_i - TAN_e)\ Q / A$

$VNR\ (g/m^3 \cdot day) = 1.44\ [(TAN_i - TAN_e) + (NO_{2i} - NO_{2e})]\ Q / V$

$ANR\ (g/m^2 \cdot day) = 1.44\ [(TAN_i - TAN_e) + (NO_{2i} - NO_{2e})]\ Q / A$

OCR (g/m^3 • day) = 1.44 (DOi-DOe) Q / V

VAR (g/m^3 • day) = 1.44 (TA_i-TA_e) Q / V

Where, TAN_i and TAN_e, inflow and effluent TAN concentrations (mg/L); DO_i and DO_e, inflow and effluent DO concentration (mg/L); NO_{2i} and NO_{2e}, inflow and effluent NO_2-N concentrations (mg/L); TA_i and TA_e, inflow and effluent total alkalinity concentrations (mg $CaCO_3$/L); Q, flow rate through the biofilters (L/min); V, filter media volume (m^3); A, total surface area of the biofilter (m^2); 1.44, conversion factor.

2.6. Statistical analysis

Data were analyzed by one-way ANOVA test (Statistix 3.1, Analytical Software, St. Paul, MN, USA). Least Significant Different (LSD) test was used to compare means. Treatment effects were considered at $P < 0.05$.

Table 4. Characteristics of the media used in present experiment.

Media	Styrofoam bead	Sand	Losses bead
Specific weight	0.033	2.6	2.2
Size (mm)	1.4	3.5	10
Porosity	32%	38%	46%
SSA (m^2/m^3)	2820	950	225
Total volume (L)	20	20	20
PSA (m^2)	0.75	0.75	0.75
TSA (m^2)	57.2	19.8	5.3

PSA, Passive surface area, surface area of filter column and pipes between inlet and outet of biofilter; SSA, specific surface area; TSA, total surface area.

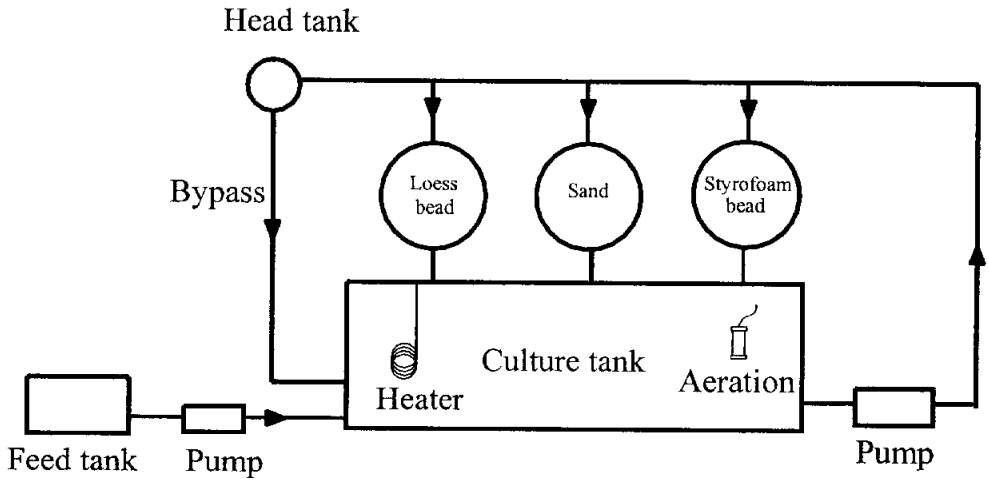

Fig. 10. Schematic diagram of the simulated seawater aquaculture system.

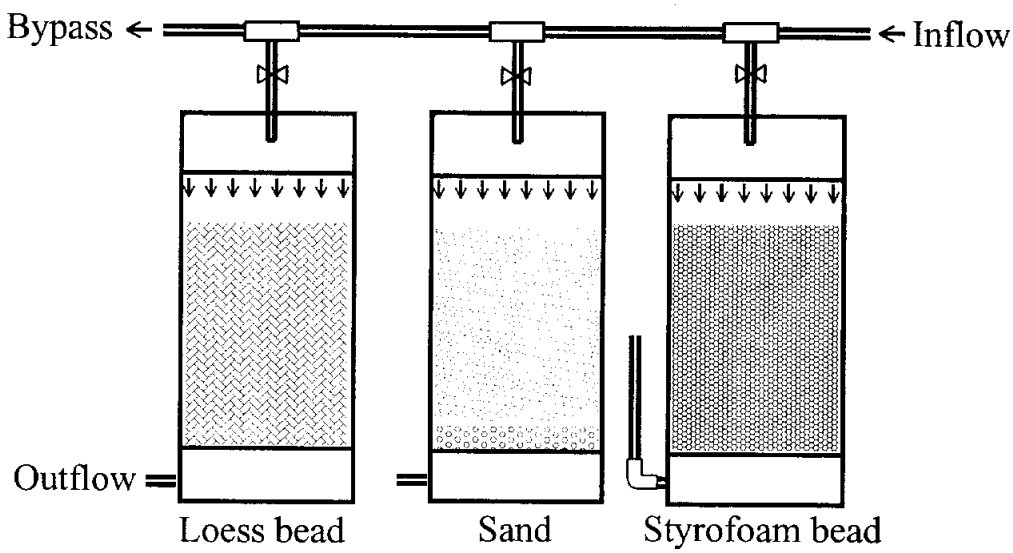

Fig. 11. Configuration and flow patterns of the three biofilters.

3. Results

3.1.1. Changes of water quality parameters

In trial 1, TAN concentration decreased in the outlet water in styrofoam bead filter and sand filter after about 4 weeks of conditioning. Loess bead filter exhibited nitrification after about 6 weeks conditioning. After three-months operating, TAN in the outlet water from all the three biofilters were relatively stable. Greatest decrease of TAN occurred in styrofoam bead filter and less decreases were found in sand filter and loess bead filter (Fig. 12). Average inlet TAN concentration was 7.8 mg/L.

Productions of NO_2-N were found in all three biofilters following the decrease of TAN concentrations. Corresponding to the greatest decrease of TAN, NO_2-N concentrations in the outlet water of the styrofoam bead filter were also high (Fig. 13). Incomplete nitrification occurred in all these three biofilters and this resulted in nitrite accumulation in culture tank water. This phenomenon lasted throughout the experimental period. Water was periodically changed to assure relatively low nitrite levels in culture tank water.

Increases of NO_3-N concentrations were found in the outlet water of these three biofilters after around 30-days conditioning (Fig. 14). NO_3-N concentrations were highest in the outlet water from styrofoam bead filter and lowest in outlet water from the loess bead filter.

Decreases in pH values were found in outlet water from all the three biofilters (Fig. 15). The reduction also was greater in styrofoam bead filter than the other two biofilters. A slight decrease in pH values was found in loess bead filter. Decrease in total alkalinity showed the similar trend as for pH. Average DO concentration in the inlet water to each biofilter was 8.2 mg/L (Fig. 16). Greatest decrease of DO was found in styrofoam bead filter.

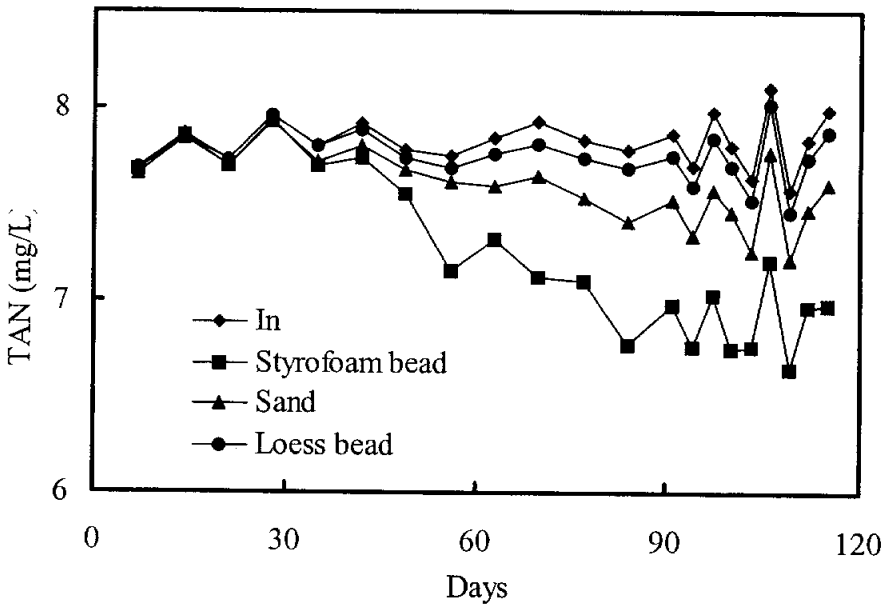

Fig. 12. TAN concentrations in the inlet and outlet water of each biofilter measured over the experimental period.

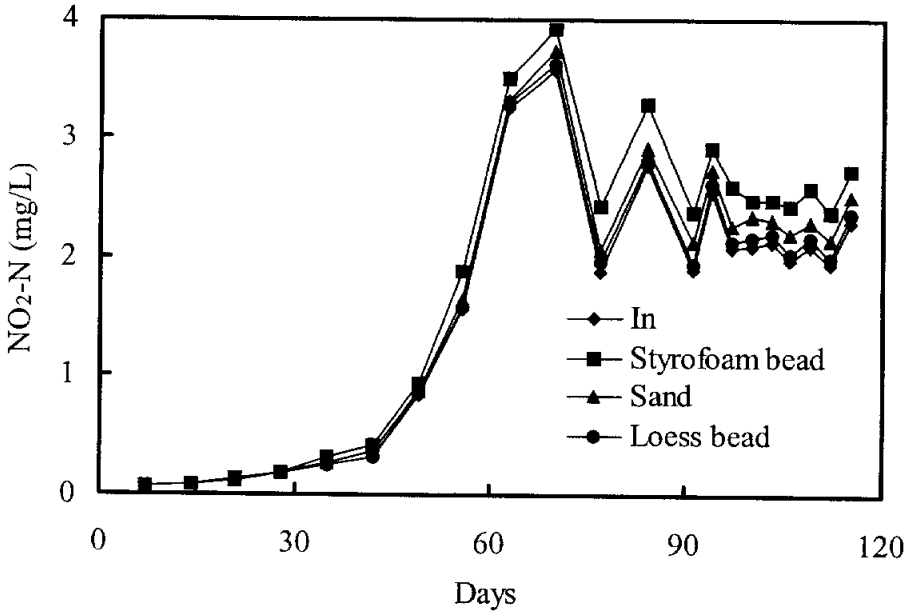

Fig. 13. NO_2-N concentrations in the inlet and outlet water of each biofilter measured over the experimental period.

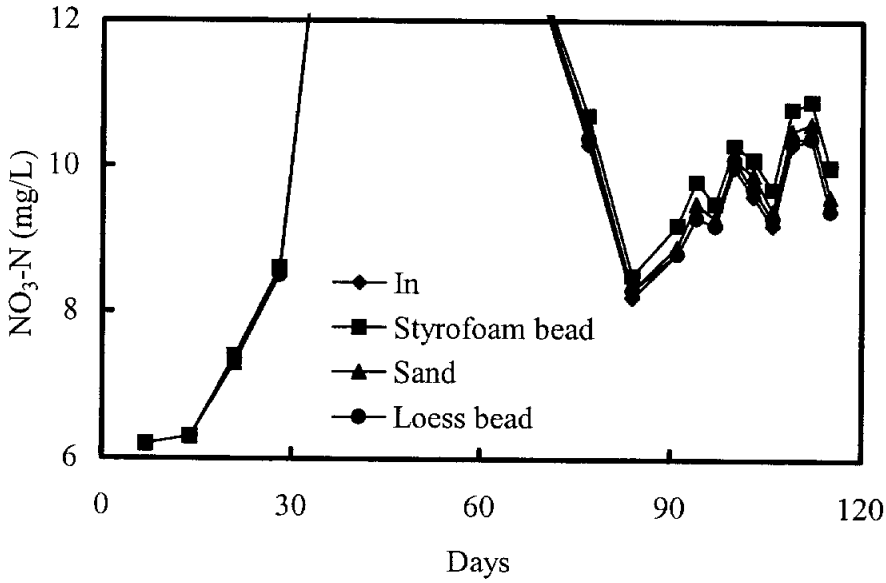

Fig. 14. NO_3-N concentrations in the inlet and outlet water of each biofilter measured over the experimental period.

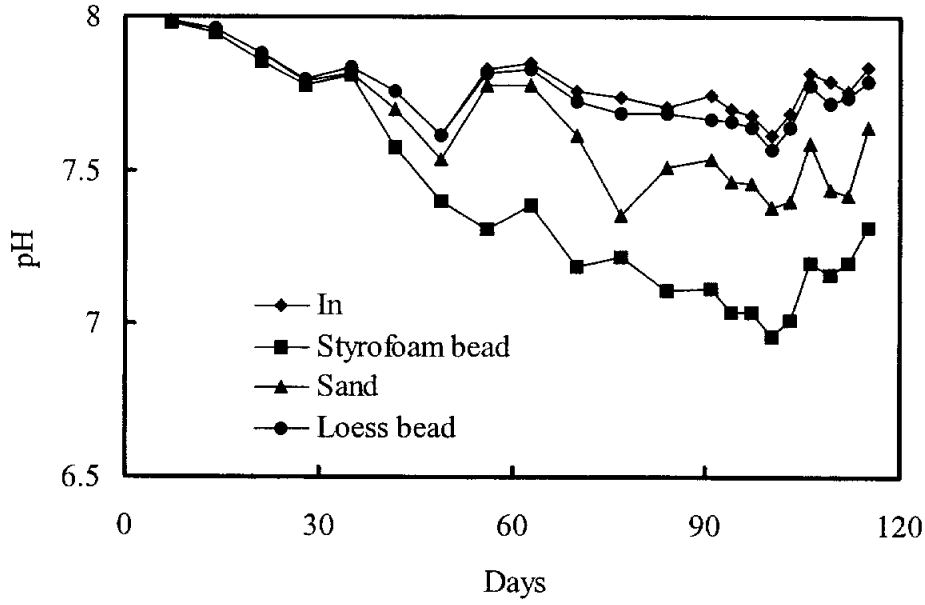

Fig.15. pH values in the inlet and outlet water of each biofilter measured over the experimental period.

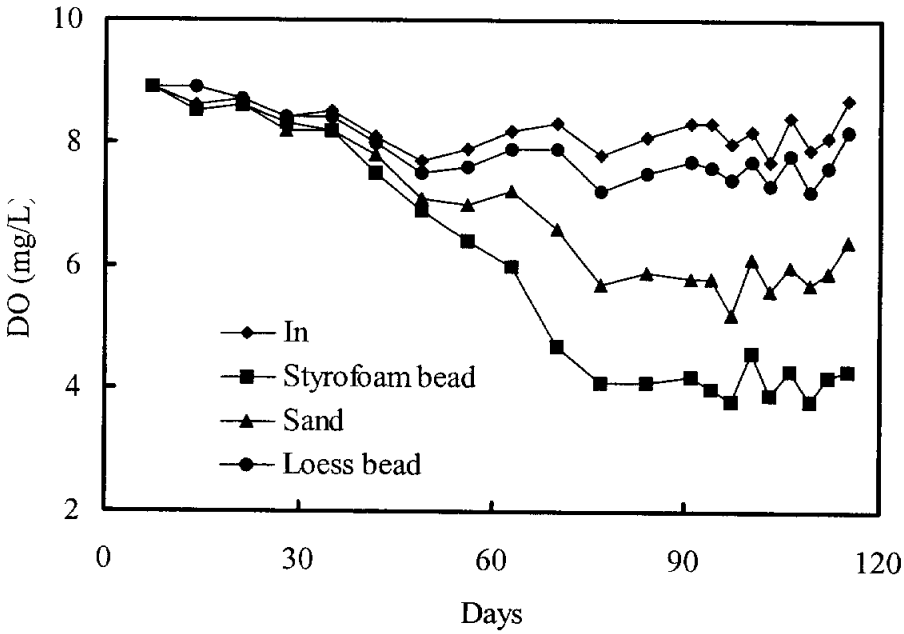

Fig. 16. DO concentrations in the inlet and outlet water of each biofilter measured over the experimental period.

3.1.2. Production or consumption of TAN, NO_2, NO_3, total alkalinity and oxygen

Table 5 shows the calculated values of total nitrification, TAN and NO_2-N conversion rates, and total alkalinity and oxygen consumption rates. Total nitrification refers to difference of TAN concentrations between inlet and outlet water of each biofilter. Total nitrification averaged 0.95 mg/L in styrofoam bead filter. Sand filter showed a lower nitrification and the lowest total nitrification was found in loess bead filter. On volumetric basis, styrofoam bead filter showed significantly higher TAN conversion rate than the other two biofilters and sand filter converted significantly more TAN than did loess bead filter ($P<0.05$). However, on areal basis, no significant difference in TAN conversion rates was found between styrofoam bead filter and sand filter or between sand filter and loess bead filter, while loess bead filter exhibited a significantly higher TAN conversion rates than those of styrofoam bead filter ($P<0.05$).

No significant difference of areal NO_2-N conversion rates was found among these three biofilters. On volumetric basis, the greatest NO_2-N conversion rates were found in styrofaom bead filter and these values were significantly higher than those of the other two biofilters. The lowest values were obtained in loess bead filter.

Oxygen and total alkalinity consumption rates were highest in styrofoam bead filter. On volumetric basis, styrofoam bead filter showed significantly higher oxygen and total alkalinity consumption rates than the other two biofilters ($P < 0.05$).

3.2.1. TAN conversion rates

TAN loading rate in trial 2 was set as used in the former trial. Table 6

shows TAN conversion rates at different water and organic matter loading rates for the three biofilters tested. TAN conversion rates increased with increase of HLR in styrofoam bead filter. TAN conversion rates at HLRs of 200 and 300 m^3/m^2 • day were significantly higher than those at HLR of 100 m^3/m^2 • day. However, no significant difference was found between HLRs of 300 and 200 m^3/m^2 • day. TAN conversion rates were same at high HLR of 200 and 300 m^3/m^2 •day and these values were significantly higher than those at HLR of 100 m^3/m^2 • day in sand filter. TAN conversion rates increased with increase of HLR in loess bead filter and were significantly higher at HLRs of 200 and 300 m^3/m^2 • day than those at HLR of 100 m^3/m^2 • day. However, no significant differences were found between HLRs of 200 and 300 m^3/m^2 • day.

Table 5. Average nitrification, NO_2-N production, total alkalinity and oxygen consumption of three biofilter media tested in trial 1.

Item	Styrofoam bead	Sand	Loess bead
Total nitrification (mg/L)	0.95 ± 0.05^a	0.37 ± 0.03^b	0.11 ± 0.01^c
VTR (g TAN/m^3 • day)	682 ± 35^a	269 ± 8^b	79 ± 5^c
ATR (g TAN/m^2 • day)	0.24 ± 0.01^b	0.27 ± 0.01^{ab}	0.31 ± 0.02^a
Nitrite production (mg/L)	0.44 ± 0.02^a	0.20 ± 0.01^b	0.06 ± 0.01^c
ANR (g NO_2-N/m^2 • day)	0.13 ± 0.01	0.16 ± 0.01	0.15 ± 0.01
VNR (g NO_2-N/m^3 • day)	384 ± 32^a	154 ± 13^b	41 ± 4^c
VAR (g $CaCO_3/m^3$ • day)	4343 ± 361^a	1772 ± 48^b	534 ± 45^c
Oxygen consumption (mg/L)	4.1 ± 0.2^a	2.2 ± 0.0^b	0.6 ± 0.1^c
OCR (g O_2/m^3 • day)	2978 ± 128^a	1608 ± 32^b	320 ± 7^c

[1]Values are means ± SD. Values in each row with a different superscript are significantly different ($p < 0.05$).

rates for all the biofilters tested at HLR of 100 m^3/m^2 • day (Table 6). TAN conversion rates at zero organic matter loading were significantly higher than those obtained with organic matter load in styrofoam bead filter. However, no significant difference of TAN conversion rates was found between organic matter loading rates of 9 and 18 kg O_2/m^3 •day. Also, TAN conversion rates in sand and loess bead filters at zero organic matter loading rate were significantly higher than those obtained at organic matter loading rate of 9 kg O_2/m^3 • day. TAN conversion rates at higher organic matter loading rate of 18 kg O_2/m^3 • day in sand fitter and loess bead filter were not measured because sand filter was easily clogged even with frequent backwashing.

3.2.2. NO_2-N conversion rates

NO_2-N conversion rates decreased with increase of HLR in loess bead filter (Table 7). NO_2-N conversion rates at HLR of 100 m^3/m^2 • day were significantly lower than those at HLRs of 200 and 300 m^3/m^2 • day. However, no significant difference was found between HLR of 200 and 300 m^3/m^2 • day. The increment in NO_2-N conversion rates was not as great as the increment of TAN conversion rates and this caused the increase of NO_2-N concentrations in the outlet water at higher HLRs. For sand filter, NO_2-N conversion rates were same at high HLRs of 200 and 300 m^3/m^2 • day and these values were significantly higher than those at HLR of 100 m^3/m^2 • day. NO_2-N conversion rates increased with increase of HLRs in loess bead filter. No significant differences were found between HLRs of 100 and 200 m^3/m^2 • day or between HLRs of 200 and 300 m^3/m^2 • day.

NO_2-N conversion rates decreased with increase of organic matter loading rates for all the media tested at HLR of 100 m^3/m^2 • day (Table 7). NO_2-N conversion rates at zero organic matter loading rate were significantly higher than those obtained with organic matter load in styrofoam bead filter.

However, no significant difference of NO_2-N conversion rates was found between organic matter loading rates of 9 and 18 kg O_2/m^3 • day. Also, NO_2-N conversion rates in sand and loess bead filters at zero organic matter loading rate were significantly higher than those corresponding values at organic matter loading rate of 9 kg O_2/m^3 • day.

Table 6. Volumetric TAN conversion rates at different hydraulic loading rate (HLR) and organic matter loading rates (OLR).

Operational parameters		TAN conversion rates (g/m^3 • day)		
HLR (m^3/m^2 • day)	OLR (kg O_2/m^3 •day)	Styrofoam bead	Sand	Loess bead
300	0	737 ± 30^a	264 ± 22^a	91 ± 11^a
200	0	682 ± 35^a	269 ± 8^a	79 ± 5^a
100	0	540 ± 31^b	220 ± 16^b	62 ± 6^b
100	9	301 ± 48^c	129 ± 14^c	40 ± 10^c
100	18	284 ± 23^c	-	-

[1]Values are means ± SD. Values in each column with a different superscript are significantly different ($p < 0.05$).

3.2.3. Oxygen consumption rates

Oxygen consumption rates increased with increase of HLRs for all the biofilters tested in zero organic matter load treatments (Table 8). Oxygen consumption rates were higher for higher HLRs of 200 and 300 m^3/m^2 • day and these values were significantly higher than those at HLR of 100 m^3/m^2 • day in styrofoam bead filter. No significant difference was found between HLRs of 200 and 300 m^3/m^2 • day. Oxygen consumption rates were significantly higher

at HLR of 200 and 300 m^3/m^2 • day than those at low HLR in sand filter and no significant differences existed between HLRs of 200 and 300 m^3/m^2 • day. Significant differences existed among all the hydraulic treatments in loess bead filter.

Oxygen consumption rates increased with increase of organic matter loading rates for all biofilters tested (Table 8). Oxygen consumption rates were significantly higher at organic matter loading rates of 9 and 18 kg O_2/m^3 • day than those at zero organic matter loading rate. No significant difference was found between organic matter loading rates of 9 and 18 kg O_2/m^3 • day. Oxygen consumption rates were significantly higher at organic matter loading rate of 9 kg O_2/m^3 • day than those at zero organic matter loading rates in sand and loess bead filters.

3.2.4. Total alkalinity consumption rates

Total alkalinity (TA) consumption rates increased with increase of HLR in styrofoam bead filter (Table 9). TA consumption rates were significantly higher at HLRs of 200 and 300 m^3/m^2 •day than those at HLR of 100 m^3/m^2 •day. TA consumption rates showed the same trends as found for TAN conversion rates at different HLRs in sand and loess bead filters.

TA consumption rates decreased with increase of organic matter loading rates in styrofoam bead filters (Table 9). This is corresponding to the decreased TAN conversion rates with the increase of organic matter loading rates. TA consumption rates were significantly higher at zero organic matter loading rate than those at higher organic matter loading rates. Consumption rates also decreased with increases of organic matter loading rate in loess bead and sand filters. Average inlet TA concentration was 186 mg $CaCO_3$/L ($\pm$17).

Table 7. Volumetric NO_2-N conversion rates at different hydraulic loading rates (HLR) and organic matter loading rates (OLR).

Operational parameters		NO_2-N conversion rates ($g/m^3 \cdot day$)		
HLR ($m^3/m^2 \cdot day$)	OLR ($kg\ O_2/m^3 \cdot day$)	Styrofoam bead	Sand	Loess bead
300	0	411 ± 20^a	148 ± 10^a	48 ± 7^a
200	0	384 ± 32^a	154 ± 11^a	41 ± 4^{ab}
100	0	348 ± 18^b	132 ± 9^b	32 ± 6^b
100	9	200 ± 28^c	84 ± 5^c	25 ± 10^c
100	18	178 ± 31^c	-	-

[1]Values are means $\pm$ SD. Values in each column with a different superscript are significantly different ($p < 0.05$).

Table 8. Volumetric oxygen consumption rates at different hydraulic loading rates (HLR) and organic matter loading rates (OLR).

Operational parameters		Oxygen consumption rates ($g/m^3 \cdot day$)		
HLR ($m^3/m^2 \cdot day$)	OLR ($kg\ O_2/m^3 \cdot day$)	Styrofoam bead	Sand	Loess bead
300	0	3024 ± 162^a	917 ± 24^b	316 ± 24^a
200	0	2978 ± 128^a	908 ± 32^b	280 ± 7^b
100	0	2097 ± 63^c	821 ± 69^c	239 ± 27^c
100	9	2586 ± 70^b	1523 ± 45^a	505 ± 27^d
100	18	2736 ± 104^b	-	-

[1]Values are means $\pm$ SD. Values in each column with a different superscript are significantly different ($p < 0.05$).

3.3. TAN conversion rates at different initial TAN concentrations.

TAN conversion rates versus the initial inlet TAN concentrations are shown in Fig. 17. HLR was set at 100 m^3/m^2 • day and inlet COD concentration was 50 mg/L. At the higher TAN loading rates, relatively stable TAN conversion rates were observed, indicating that conversion rates are independent of TAN loading rates. As inlet TAN concentration declines to a certain value, TAN conversion rates decreased accordingly, indicating dependence on TAN concentration in the inlet water. TAN conversion rates could be described by the 1-order/zero-order kinetic model. TAN conversion rates versus inlet TAN concentrations can be described by the equation as:

ATR (mg/m^2 • day) = 20.776 Ln (TAN) + 53.38.

Table 9. Volumetric total alkalinity (TA) consumption rates at different hydraulic loading rates (HLR) and organic matter loading rates (OLR).

Operational parameters		TA consumption rates (g/m^3 • day)		
HLR ($m^3/m^2/day$)	OLR (kg $O_2/m^3/day$)	Styrofoam bead	Sand	Loess bead
300	0	5256 ± 540^a	1898 ± 43^a	648 ± 58^a
200	0	4843 ± 361^a	1922 ± 48^a	574 ± 45^b
100	0	3932 ± 198^b	1620 ± 170^b	446 ± 90^c
100	9	2240 ± 140^c	940 ± 84^c	288 ± 32^b
100	18	2010 ± 104^c	-	-

[1]Values are means ± SD. Values in each column with a different superscript are significantly different ($p < 0.05$).

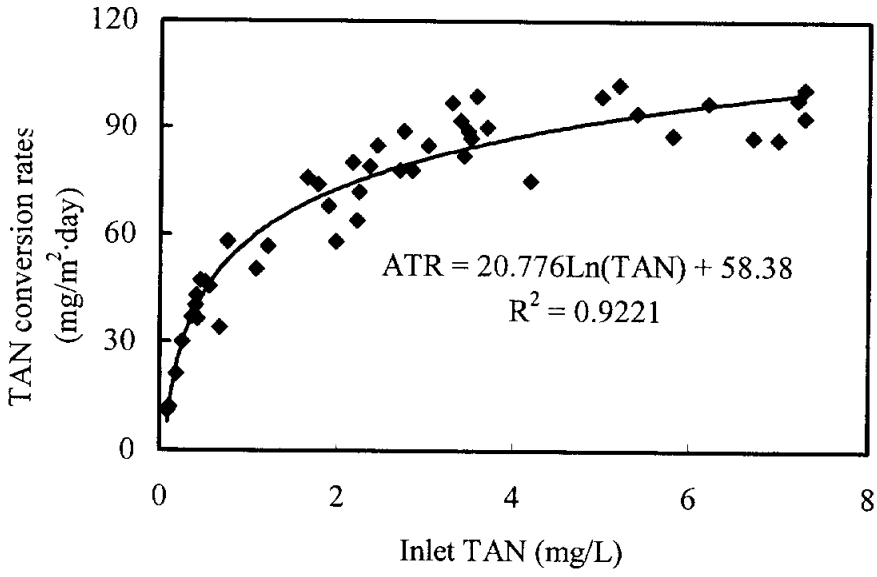

Fig. 17. TAN conversion rates versus various inlet TAN concentrations (HLR, 100 m^3/m^2 • day; COD loading rate, 18 kg O_2/m^3 • day).

4. Discussion

4.1.1. TAN

TAN conversion rates varied significantly between these three biofilters filled with different filter media in trial 1. Under the same HLR, the best results were obtained in the biofilter filled with styrofoam bead. Loess bead filter showed the worst performance among the three filters tested. This was mainly due to the great differences in surface areas provided by the different media. Styrofoam bead had the largest surface area among the three media tested on volumetric basis. Larger specific surface provides more space for nitrifiers to grow on and also results in more contact between the biofilm and water, thus increasing TAN removal per unit volume of filter.

However, on areal basis, loess bead filter converted more TAN than styrofoam bead filter and sand filter, and TAN conversion rates in loess bead filter were significantly higher than those in styrofoam bead filter. Specific gravity and void ratio of the filter media contributed partly to this difference. Usually, high void ratio means less media contact with each other. Loess bead has a higher void ratio than other media, this means that loess beads have relatively more available surface for nitrifiers. Styrofoam beads, in the downflow mode, more beads would contact with each other and this would reduce the surface area available for nitrifiers. In this case, we can say that the calculated surface area for a given volume of media is not the real surface that would be totally available to nitrifiers. Overestimation of the surface area would attribute to the low values of the calculated areal TAN conversion rates. On the other hand, the calculated values were based on the total surface area of the biofilter including the surface area of the media themselves and the passive surface area of filter column and connecting PVC pipes. The passive surface

contributed to about 14% of the total surface area for loess bead filter but only 4% for sand filter and 1.3% for styrofoam bead filter (Table 4). The passive surface would provide effective area for bacteria adhesion and may play an important role in TAN removal. Hargrove et al (1996) reported that passive surfaces were probably supporting between 10-30% of the TAN conversion. Checking the TAN conversion rates without media in the filter column can provide the necessary information for accurate evaluation of the areal TAN removal rates of different media. Since the filters tested in present experiment will continuously be used for other trials, no efforts were made on this.

4.1.2. Nitrite production

Great differences of NO_2-N concentrations in the outlet water were found between the three biofilters. The highest values were found in styrofoam bead filter. Net productions of nitrite were found in all these three bioflters regardless of which kind of filter media used. This caused the accumulation of nitrite in the culture water with the maximum values up to 3.8 mg/L and this value was above those recommended for aquaculture (Liao and Mayor, 1974; Russi et al., 1974).

Many factors including pH (Alleman, 1985), substrate inhibition, DO (Alleman, 1985; Liu and Capdeville, 1994), chlorinity (Hill and Gellman, 1977), and light (Horrigan et al., 1981; Olson, 1981) affect ammonia and nitrite oxidizers and thus cause nitrite accumulation. In present experiment, pH and DO were well within the range for nitrification (Kaiser and Wheaton, 1983; Water Pollution Control Federation, 1983). Light also should not be limiting factor since all the media were in dark conditions.

van Rijn and Rivera (1990), in a fresh water system, found that the high inlet ammonia concentrations caused relatively large amounts of nitrite produced and this nitrite might not be directly available for the nitrite oxidizers.

They also found that nitrite removal by the trickling filter took place when ambient ammonia concentrations were lower than 1 mg/L, while at higher ambient ammonia concentrations nitrite accumulated. Hao and Chen (1994) observed nitrite accumulation in a submerged-bed system and attributed this to an irreversible inhibitory effect of hydroxylamine on the nitrite oxidizers. These findings may partially explain the accumulation of nitrite in present experiment considering the high TAN loading rate.

Alternatively, the accumulation of nitrite might be due to the depth of the media used and HLR. The filter media were only about 30-cm deep and this depth might not be sufficient for the nitrite oxidizers to fully develop and consume all the nitrite produced. Nijhof and Klapwijk (1995) concluded that the occurrence of high nitrite concentrations in trickling filter effluents could be explained by diffusional transport mechanism in combination with the characteristics of the biofilm, and biofilms with a relatively low nitrite oxidation capacity induced high nitrite concentrations. Spotte (1992) also found that nitrite sometimes persists at higher than expected concentrations long after it should have disappeared and been replaced by nitrate, and concluded that this might be caused by dissimilatory activities by other bacteria or incomplete nitrification. The accumulation of nitrite in present experiment also might be induced by an imbalance of ammonia and nitrite oxidation bacteria developed in the biofilters.

Kamstra et al. (1998) found that nitrite oxidation capacity in biofilms seems to be variable and sensitive to environmental disturbances after they assessed the performances of trickling filters on 14 eel farms. Nijhof and Bovendeur (1990), after comparing the nitrification of freshwater and seawater biofilms, concluded that the high accumulation of nitrite in seawater system was due to the slow development of nitrite oxidation capacity during the start-up periods. This phenomenon also cloud have occurred in present experiment since nitrite accumulation could be noted just after the start of TAN

oxidation. This also may indicate a higher growth rate of ammonia oxidation bacteria than nitrite oxidation bacteria as found by Grommen et al. (2001).

In present experiment, the accumulation of nitrite might be due to the incomplete nitrification caused by the short period of conditioning, high ammonia and hudraulic loading rates, and the shallow depth of media.

4.1.3. Oxygen and total alkalinity consumptions

Lekang and Kleppe (2000) found that media having high void ratio usually have a reduced need for taking oxygen from the water in trickling filters and the same results were found for loess bead filter which was resemble to work in a trickling model in present experiment. However, oxygen consumption rate should be mainly related to the nitrification capacity of the biofilter since biofilm with great nitrification capacity consume greater amount of oxygen.

Total alkalinity consumption rates showed the same trend as TAN conversion rates. Total alkalinity consumption was not affected by the filter media when compared with the calculated consumption rate based on the TAN conversion, or the compensation of alkalinity by sand and shell was too small to be detectable.

4.1.4. Comparison of the nitrification rates

The nitrification rates of the three media tested were between 0.24 and 0.31 $g/m^2 \cdot day$. Similar result, 0.28 $g/m^2 \cdot day$, was reported in a seawater trickling filter by Nijhof and Bovendeur (1990). In freshwater systems, the reported values were between 0.5-2 $g/m^2 \cdot day$ (Anderson et al., 1994; Boller et al., 1994; Parker et al., 1997). The nitrification rates in seawater system were quite lower compared with those in freshwater systems. This probably can be explained by the inhibiting effect of chloride on nitrification, which is reported

to occur at chloride concentrations exceeding 10 mg/L (Richardson, 1985). Salinity is known to affect bacterial metabolic activity, reducing microbial growth and ammonia oxidation rate (Rosa et al., 1998). Sakairi et al. (1996) observed a six-fold reduction in the nitrification rate of synthetic seawater when it was used in an air-lift contactor containing bacteria immobilized in a cellulose carrier. Rosa et al. (1998) also have observed that the presence of NaCl seriously affects biofilm development and induced a two-fold reduction of nitrification in aerated biological filters.

4.1.5. Start-up period

Kawai et al. (1964) and Hirayama (1974) found that it takes about 60 days for a marine biofilter to reach its full nitrifying capacity at temperatures of 20-22 °C. It has also been reported that the start-up period of a biofilter takes from 28 to 60 days in aquaculture systems (Carmignani and Bennett, 1977; Forster, 1974; Perfettini and Bianchi, 1990). However, Nijhof and Bovendeur (1990) found that a relatively longer start-up period of more than 3 months was required for the biofilters to achieve maximum ammonia conversion rate. In present experiment, TAN conversion rate became relatively stable after about three-month conditioning. No obvious differences in the start-up periods were found between these biofilters. However, the incomplete nitrification indicated that a longer conditioning period might be required.

4.2.1. TAN conversion rates in trial 2

TAN conversion rates increased with increase of HLRs. Nijhof and Klapwijk (1995) also found an increased performance of trickling filter with increase of HLR and they concluded that the increase in HLR in combination with the specific filter media used could result in improvement in wettening of

media, which might cause the increased performance. This would be true for the styrofoam bead filter used in present experiment. Higher HLR would induce more even distribution of water through the biofilter media and thus improve the performance. However, the improvement in biofilter performance was not linearly correlated to the HLRs. At the highest HLR of 300 m^3/m^2 • day tested, the improvement was not significantly high. This was mainly due to the characteristics of the styrofoam bead used. Though the specific gravity of this kind of bead is only one thirties of water, part of the beads, around 30% according to the author's observation, would sink in water after supply of water. This would reduce the impact of HLR on the whole performance. TAN conversion rates of sand filter increased with the increase of the HLR up to 200 m^3/m^2 • day. No increase was observed at highest HLR due to the factor that at higher water loading rates, flooding on the media surface took place. HLRs had significant impact on the TAN conversion rates of loess bead filter up to the highest HLR tested. Loess bead filter was operated in real trickling mode. This result was in accordance with the others reported (Forster, 1974; Nijhof and Klapwijk, 1995).

TAN conversion rates were much less at organic matter loading rates of 9 and 18 kg O_2/m^3 •day than those without organic matter input in styrofoam bead filter. The addition of glucose resulted in reduction of TAN conversion rate from 540 to 284 g TAN/m^3 • day. At organic matter loading rate of 9 kg O_2/m^3 • day, great reduction of TAN conversion rates were also found in sand filter and loess bead filter. Clearly, organic matter can be one of the most important impacting factors on nitrification efficiency. This mainly due to the growth of heterotrophic bacteria, which compete for oxygen, nutrients, and space with the autotrophic nitrifiers. TAN conversion rates were much lower than those reported by Bovendeur et al. (1990), Satoh et al. (2000), and Zhu and Chen (2001) in freshwater systems.

No significant difference of TAN conversion rates between the two organic

matter loading rates was found in styrofoam bead filter. This indicates that the impact of organic matter on nitrification becomes less and less sensitive with an increase in COD/TAN ratio. This can be explained by the fact that heterotrophic growth followed the Monod kinetics (Bailey and Ollis, 1986) with substrate concentration. Heterotrophic bacterial growth rates increase with the increase of organic concentration until it reaches a saturation level. Consequently, the potential of heterotrophic inhibitory impact becomes less and less with increase in organic concentrations. Zhu and Chen (2001) also found that the difference of TAN conversion rates between C/N ratio of 1 and 2 in freshwater system was not significant and they calculated the corresponding COD/TAN ratios to be 2.7 for C/N ratio of 1 and 5.4 for C/N ratio of 2. In present experiment, the COD/TAN ratios used were 3.2 and 6.4, which were similar to the case reported in their study. Also, they concluded that the biofilters used in recirculating system usually operated under the condition of C/N ratio equal to 2. So, the results obtained at the highest organic matter loading rates can be used in biofilter design in recirculating aquaculture system.

4.2.2. NO_2-N conversion rates

Increment in NO_2-N conversion rates with increase of HLRs would be due to the fact that the increased conversion of TAN provided more NO_2-N to the nitrafication bacteria. Weatherley (1984) reported that under certain conditions the oxidation of nitrite might be described using first-order kinetics. Sandu et al. (2002) found that TAN and NO_2 removal rates increased with the increase of HLR. Similar results were reported by Malone et al. (1999). Though the removal rates increased with increase of HLRs, NO_2-N concentrations in the outlet water also increased. This would cause the accumulation of nitrite in culture tank water. Sandu et al. (2002) also reported an elevated NO_2 concentration in the effluent water with the increase of HLRs.

Incomplete removal of NO_2 was found for all the filters tested at various HLRs. The reason should be same as discussed in trial 1.

4.2.3. Oxygen and total alkalinity

Volumetric oxygen consumption rates at various HLRs and zero organic matter load rate for all the biofilter media tested showed the same trends as for TAN conversion rates since oxygen was mainly consumed by nitrifiers. Oxygen consumption rates increased with increase of organic matter loading rates since heterotrophic bacteria also consume oxygen. Bovendeur et al. (1990) reported a linear increase of COD removal with increase of COD loading rates. However, they use low organic matter loading rates of only up to 25 $g/m^2 \cdot$ day. The high organic matter loading rates may contribute to the less significant difference of oxygen consumptions at the two organic matter loading rates.

Total alkalinity consumption rates followed the same trends as TAN conversion rates for all the biofilters tested. The ratios of TA consumption to TAN conversion were within the range of 7.10-7.44 with mean value of 7.19.

Ⅳ. Design, management and performance of a laboratory scale seawater recirculating system for Korean rockfish *Sebastes schlegeli* culture

1. Introduction

Aquaculture has been the fastest growing food-producing sector in the world, with an annual growth rate of almost 10% between 1984 and 1995 (FAO, 1997). Decreased capture and increased demands of seafood have led to the quick development of marine aquaculture systems. However, the marine aquaculture system can only be successful if it is cost effective, socially responsive, ecologically sustainable, and scientifically sound (Davis and Arnold, 1998). The outbreak of disease and increased concern about environmental impacts of aquaculture encouraged the development of recirculating aquaculture systems. Compared with the traditional aquaculture systems, RAS has many advantages including a reduced requirement for appropriate water resources, complete environmental control, and the availability of a quality controlled product (Van Gorder, 1994).

The most critical treatment processes for a recirculating aquaculture system are aeration (supplying oxygen), clarification (solids removal), biofiltration (ammonia, nitrite, and BOD removal) and degasification (carbon dioxide removal), which are all linked by means of circulation (Malone and Beecher, 2000). Usually, aeration and degasification can be conducted simultaneously in one operation unit such as low head oxygen contactor, packed column oxygenator, U-tube system or simply, air blower (Dwyer and Peterson, 1993; Vinci et al., 1997; Wagner, et al., 1995; Watten and Todd Beck, 1985). In recirculating aquaculture systems, ammonia and solids removal are of critical

importance.

The generated solids can be divided into two categories: settleable solids and non-settleable solids. The recommended limit of suspended solid concentration in a recirculating system is 15 mg/L (FIFAC, 1980; Reinemann, 1987; Timmons et al., 1987), while Muir (1982) recommended a limit of 20-40 mg/L and Alabaster and Lloyd (1982) found that there is no evidence that concentrations of suspended solids less than 25 mg/L have any harmful effect on fish. Nevertheless, solids should be controlled as low as possible since solids have adverse effects on fish health and gill damage (Stickeny, 1979; Wickins, 1980), clogging of biofilter may be induced by high solids concentration, and solids could generate more ammonia nitrogen and oxygen demand. For control of the solids below the recommend limit, foam fractionation process and sedimentation combined with the separation of solids within the culture tank using dual-drain system were used in present design.

Another crucial compartment of recirculating aquaculture system is biofiltration device since ammonia and nitrite are directly toxic to fish (Meade, 1985; Lewis and Morris, 1986). Fixed film biofilters are now commonly used in fish culture facilities since salt quickly saturates the adsorption sites and thus make ion exchange not applicable (Hochheimer and Wheaton, 1988). Styrofoam bead filter, which was tested previously in simulated seawater system and showed reasonable TAN removal ability, was used for biofiltration in present system considering the low price and easy management of this kind of biofilters. Nitrate levels were controlled mainly by denitrification process in sedimentation basin and sand filter.

The purpose of this study is to present the design criteria based on the existing information and the performance of the laboratory scale seawater recircualting system for Korean rockfish culture. Korean rockfish is commonly cultured and commercially important in Korea. The principal compartments of the recirculating system include: a sedimentation basin and a

foam fractionator for solids removal; styrofoam bead filters for TAN and NO_2-N removal; a sand filter for nitrate removal; a circular tank for fish culture; an air blower for aeration; and heating system for maintaining temperature not to drop to too low levels. It should be noted that though a variety of commercially viable designs and technologies exist, present design is based on the concept that the system should be constructed at low cost and operated with easy management.

2. Materials and methods

2.1. System configuration

Schematic diagram of the designed system is shown in Fig. 18. This system consisted of a circular culture tank (volume, 0.75 m^3), two nitrification bead filters, a denitrification sand filter, a sedimentation basin, a foam fractionator, a main water sump, and pumps. Low solids outlet water was let to two bead filters through standpipe before going to the main water sump. Hydraulic loading rate was set around 100 $m^3/m^2 \cdot$ day for each styrofoam filter. The excess outlet water directly fell to the sump. From the sump, water was pumped back to the culture tank. The flow rate to the culture tank was controlled by a bypass system and excess water flew to the foam fractionator. The high solids outlet water from the culture tank was led to sedimentation basin. A denitrification sand filter was connected to the sedimentation baisn. The excess water from the sedimentation basin overflew to the water sump. Sodium bicarbonate solution was supplied for pH compensation. Characteristics of the system compartments are shown in Table 10.

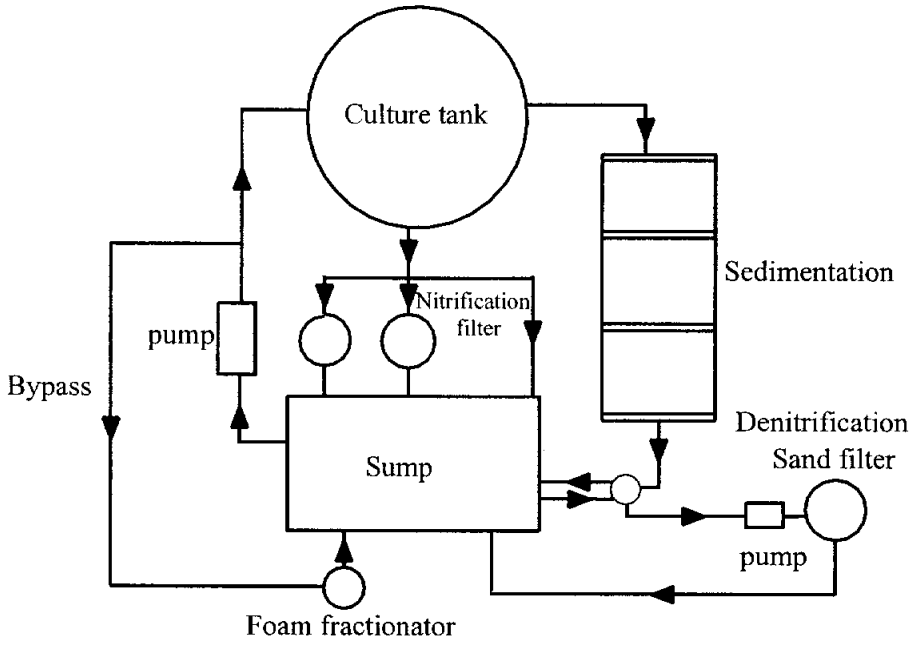

Fig. 18. Schematic diagram of the seawater recirculating aquaculture system. Arrows indicate the direction of water flow (not to scale).

Table 10. Characteristics of the compartments of the seawater recirculating system.

Compartment	Water volume (L)	Flow rate (L/h)	HRT (h)
Culture tank	600	1200	0.5
Bead filter	50	450	-
Sedimentation basin	210	60-120	1.7-3.5
Sand filter	25	3-60	0.5-8
Water sump	120	-	-
Fractionator	30	1200	-

2.2. Design of the seawater recirculating system

2.2.1. Culture tank

Culture tank design has a dominating influence on the use of water resources, feed input, stock management, and waste discharge (Summerfelt et al., 2000). Circular tanks have been widely used because circular tanks have many advantages: they can provide a uniform culture environment and can be operated under a wide range of rotational velocities; they can be used to rapidly concentrate and remove settleable solids; they allow for good feed and fish distribution and visual observation of waste feed to enable satiation feeding (Timmons and Summerfelt, 1997; Timmons et al., 1998). Circular tank with a diameter of 110 cm and a depth of 80 cm (volume, 0.75 m^3) was selected as the culture tank in present design. The bottom of the tank has a slight slope from the base of sidewall to the central drain pool.

Once the shape of the tank is determined, the water distribution device and

the velocity of the water rotating in the circular tank become the crucial factors for determining the tank self-cleaning. Usually, combination of horizontal and vertical branches achieves uniform mixing in the culture tank (Skybakmoen, 1993). The rotational velocity in the tank can be controlled by either the size and number of orifice used, or the inflow rate. The inflow pipe with orifices drilled on it was vertically positioned near the culture tank wall (Fig. 19). The diameter of the orifice is 6 mm and totally 30 orifices were equally spaced along the inflow pipe. A bypass with valve was used to adjust the water flow rate to the culture tank. Losordo and Westers (1994) reported that rotational water velocity of 0.5-2.0 times fish body length per second is optimal to maintain fish health, muscle tone, and respiration. For driving solids to the central drain, the velocity should be greater than approximately 15-30 cm/sec (Burrows and Chenoweth, 1970; Mäkinen et al., 1988). The rotational velocity in the present experiment was set at around 20 cm/sec at circumference and adjusted accordingly later.

2.2.2. Solids removal compartments

2.2.2.1. Solids generation, characteristics and impacts

The solids in aquaculture systems are mainly composed of uneaten feed particles, feces, and bacteria consortia. Decomposition of these solids can lead to consumption of oxygen and release of inorganic nutrients. The impacts identified also include direct damage to fish gills and mechanical clogging of biofilters (Chapman et al., 1987; Liao and Mayo, 1974; Spotte, 1979). So, removing solids from the culture water becomes another key factor, along with TAN removal, determining the success or failure of a recirculating system. Also, many environmental regulatory agencies have considered the solids load as one of the main criteria for evaluating aquaculture systems.

The quantity of solids produced in a RAS can be evaluated by considering the feces production, uneaten feed, and bacterial biomass. Some investigations have been done for several species (Liao and Mayo, 1974; Malone et al., 1990; Wimberley, 1990) and an average value of around 30% of the feed applied is commonly recognized. Since no detailed information about the feces excretion of Korean rockfish is available, the total solids production is assumed to be 30% of the feed applied here.

The generated solids can be divided into two categories: settable solids and non-settable solids. In present experiment system, solids concentration in culture tank water is controlled by the combinations of dual-drain system, sedimentation basin, foam fractionator, and biofilters.

2.2.2.2. Dual-drain system

The structure of the central drain system plays a key role in controlling the solids removal, especially the separation of solids within culture tank. Dual-drain system has the potential to improve solids removal within recirculating systems (Losordo et al., 1995) and enable the majority of solids to be removed in a relatively small flow exiting tank through one outlet while the majority of flow with low solids concentration through another outlet (Summerfelt et al., 2000). A modified dual drain system was constructed with two concentric pipes (Fig. 19). The inner pipe had a diameter of 30 mm and a height of 70 cm and was connected to the outside standpipe. This inner pipe was connected to low-solids outlet. The outer pipe had a diameter of 50 mm and a height of 20 cm. Six vertical perforated slots (1.5 cm by 15 cm) were evenly spaced along the pipe. Slots were used rather than round holes since slots are easier to wash, provide greater open area, and do not clog as readily as round holes (Pankratz, 1995; Piper et al., 1982; Sedgwick, 1985). The high solids outlet was connected to the sedimentation basin.

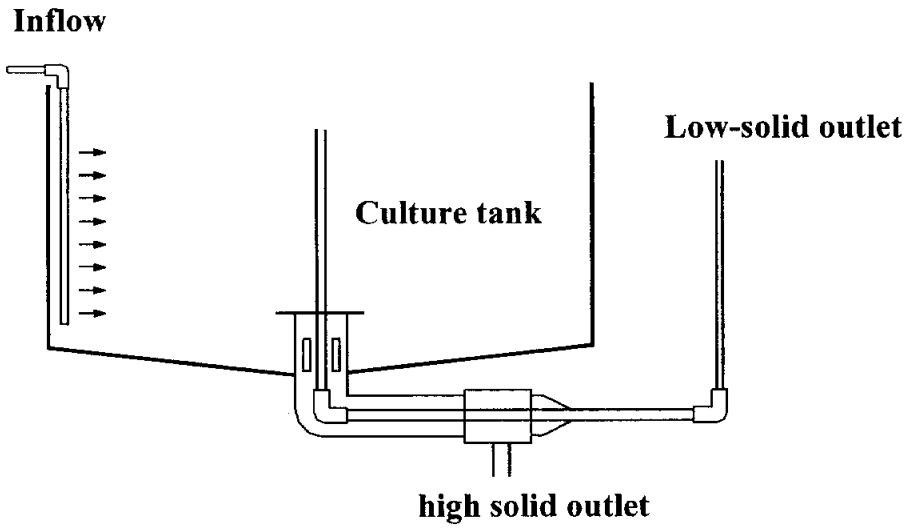

Fig. 19. Schematic diagram of the culture tank and dual-drain system.

Recently, settled solids have been reportedly concentrated in 5-20% of the total flow that leaves the bottom center drain of circular culture tanks when remainder of the flow leaving the tank is withdraw through pipes above the bottom drain or part-way up the tank's side wall (Eikebrokk and Ulgenes, 1993; Lunde et al., 1997; Mäkinen, et al., 1988; Timmons and Summerfelt, 1997). The turnover rate of the culture tank is set at 1 hour, which equals to total flow rate of 600 L/hour. So, the higher solids outlet rates should be around 30-120 L/hour.

2.2.2.3. Sedimentation basin

Selection of the best treatment system for solids removal is difficult given the variety of processes available, and the lack of a uniform methodology for evaluation of the effectiveness, and of an accounting of economic and practical consideration. Many systems have been developed for removing solids from aquaculture systems including sedimentation basins, microscreens, sand filters, hydrocyclones, constructed wetland; foam fractionators, etc. (Chen et al., 1993c; Henderson and Bromage, 1988; Schwartz and Boyd, 1995; Summerfelt et al., 1999; Wheaton, 1977). Among them, sedimentation is the simplest one. Sedimentation basins require little energy input, are cheap for construction and are easy for management. So, they have been the most common solids removal methods used not only in the municipal wastewater treatment system but also in many aquaculture systems (Jones et al., 2001; Shnel et al., 2002). Stechey and Trudell (1990a) found that properly designed and operated settling systems could achieve solids removal efficiencies approaching 90%. However, sedimentations are usually believed to be not effective for fine suspended solids removal (Chen et al., 1994a). Another disadvantage is that the low HLR required for effective solids removal.

For compensation of the turbulence caused by scour in the sedimentation

basin, it is better to increase the surface area of sedimentation basin. Also, the inflow stream should be introduced evenly to the cross-section of the sedimentation basin. Weir was used at the inflow section to ensure the even distribution of inlet water and also to reduce the inlet velocity (Fig. 20). Similarly, another weir was used before the outflow pipe to assure a uniform discharge rate and baffles were used inside the sedimentation basin to enhance settling of solids.

The key factor for designing sedimentation basins is the volumetric flow rate per unit surface area of the basin and the most important is that the flow rate through the sedimentation basin should be slow enough to ensure the settling of solids. Stechey and Trudell (1990b) found that the appropriate overflow rate for design of the sedimentation basin in intensive salmonid aquaculture typically ranges between 40-80 $m^3/m^2 \cdot$ day, and at this overflow rate, approximately 65%-85% of TSS would be removed. McLaughlin (1981) and Mudrak (1981) reported that greater than 80% of total solids were removed at designed overflow rate of between 21.4 and 120 $m^3/m^2 \cdot$ day. Parjala (1984) recommended that a maximum horizontal flow rate of 2-5 cm/sec in aquaculture sedimentation basins. So, rectangular basin with flow direction along the long side was used here to ensure the settling of solid and reduce the scour effect. A relatively low overflow rate of around 5 $m^3/m^2 \cdot$day is selected as the designed value since in present experiment the sedimentation basin not only serves for solids removal but also serves for digestion of organic matters, as well as when considering the relatively small-dimension of the sedimentation basin would be used in present design. Volumetric flow rate through the basin is set at around 90 L/hour, which is in the range of 30-120 L/hour for high solids outlet flow calculated above. Though the sedimentation of solids is independent of basin depth, very shallow basins would induce excessive scour. The height of basin is set at 60 cm, which is similar as used by Shnel et al. (2002). The surface area of the sedimentation basin was calculated as follows

(Chen et al., 1994a):

$$A = \frac{Q}{V} = \frac{100 \times 24 \div 1000}{5} = 0.48\ m^2$$

Where: A, surface area of the basin; V, overflow rate; Q, volumetric flow rate through the basin.

Dimension of the sedimentation basin is eventually set at 1.2 m long, 0.4 m wide with a depth of 0.6 m.

2.2.2.4. Foam fractionator

Sedimentation basin is not efficient for fine solids removal and aeration is a necessity in intensive fish culture system, so foam fractionator is used here mainly for fine solid removal. The dimension and other detailed information of this foam fractionator are as described in the second part of this thesis.

Foam fractionator was evaluated at fixed HRT of 2 minutes, 3 different SAV of 0.743, 1.114, and 1.486 cm/min, and 4 foam overflow heights of 1, 3, 5, and 7 cm. The performances of the foam fractionator at different HRTs were not evaluated in present experiment. Weeks et al. (1992) already found that water flow rate through the foam fractionator column did not have significant effect on solids removal in freshwater aquaculture system.

2.2.3. Biofiltration

2.2.3.1. Ammonia production in RAS

Ammonia production in recirculating aquaculture systems mainly related to the ammonia excretion rate of the cultured species. Also, the feeding strategy, feed composition, and temperature etc. all exert some effects.

Meade (1985) reviewed information on ammonia production in different

fish species and found ranges of 20-78.5 g/kg feed. Three out of the five cited production rates by Meade (1985) were between 31-37.4 g/kg feed • day. Kim and Chin (1995) did some researches on the ammonia excretion rates of juvenile Koran rockfish but their data are not applicable due to the great difference in body weight, 3 g in their study versus around 200 g in present experiment. According to the literatures cited and values commonly used (Wheaton et al., 1994b), in case lack of the accurate data on ammonia excretion rate of the cultured species, TAN production of 30 g /kg feed is used here. The whole system was designed to support a daily feed loading rate of 0.4 kg/day. The daily TAN production would be around 12 g.

2.2.3.2. Nitrification biofilter

Ammonia excreted by fish or produced through degradation of organic matter in aquaculture system must be removed since ammonia is highly toxic to fish. Styrofoam bead filter, which was tested previously in simulated seawater system and showed reasonable TAN removal efficiency, was used for nitirfication in present system because of the low price and easy management.

Though ammonia production is not constant, experience has shown that an average hourly ammonia loading calculated based on daily ammonia loading is adequate to determine potential for ammonia concentrations become lethal in a closed system (Wheaton et al., 1994b). So, the effect of peak TAN concentrations was neglected here when designing the nitrification biofilters. The volume of the media needed for removal of TAN produced at highest feed loading rate of 0.4 kg feed/day can be calculated as:

$$\text{Volume of media} = \frac{12\text{g/day}}{284\text{g/m}^3 \cdot \text{day}} = 0.042\ \text{m}^3.$$

The volume of biofilter media needed is about 42 L. So, two styrofoam bead filters were used here, each containing 20 L media, instead of one to

minimize the risk of failure of biofilter. The dimension of biofilter and other detailed information are same as described in the previous study.

2.2.3.3. Denitrification sand filter

Closed recirculating aquaculture systems where there is no significant primary productivity exhibit increasing nitrate concentrations over time. Hrubec et al. (1996) found that prolonged exposure to elevated levels of nitrate may decrease the immune response, induce hematological and biochemical changes which are indicative of a pathologic response, and may increase mortality. Another problem associated with high nitrate concentrations in fish culture system is the potential of formation of nitrite in oxygen-poor zones. Discharge of effluent water is an additional problem related to nitrate-rich fish culture systems.

Most of denitrification reactors employed in aquaculture systems are based on immobilization of denitrifiers on media and supply of suitable carbon source. Another, cheaper carbon sources for denitrification is the organic carbon produced in the fish culture units by fermentation process (Aboutboul et al., 1995; van Rijn et al., 1995). The potential use of this carbon sources for nitrate and phosphorus removal have been studied in freshwater systems (Arbiv and van Rijn, 1995; Barak and van Rijn, 2000a,b,c; Barak et al., 2003; Shnel et al., 2002). Here in present experiment, a combination of sedimentation basin for organic matter degradation and sand filter for nitrate removal using the organics produced in the sedimentation basin was studied.

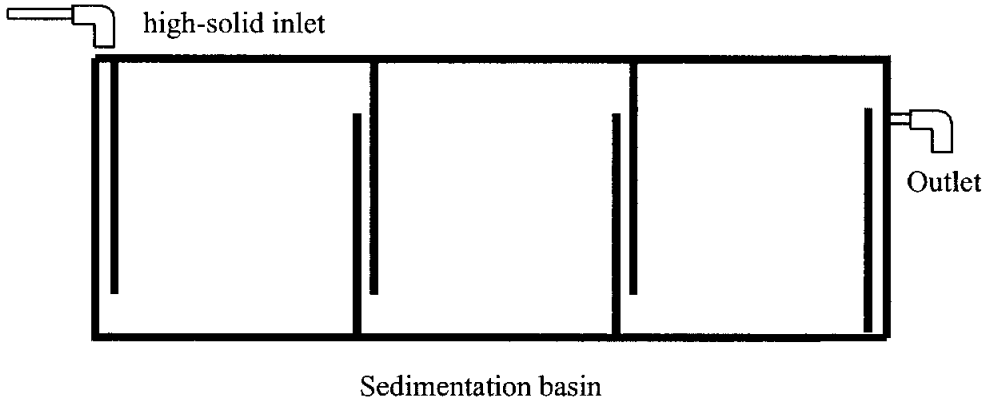

Fig. 20. Schematic diagram of the sedimentation basin.

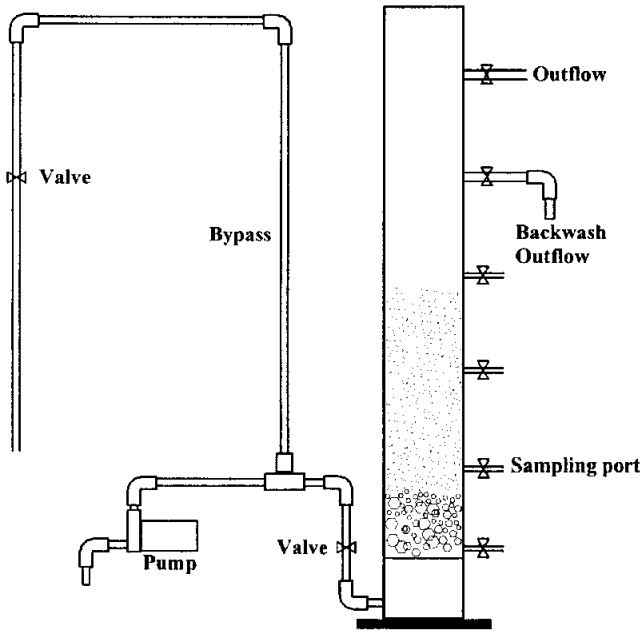

Fig.21. Schematic diagram of the denitrification sand biofilter.

Since no detailed information about the use of this denitrification biofilters in seawater recirculating system is available, a relatively large dimension of the denitrification sand filter was used when compared with that used by Shnel et al. (2002). The volume of sand filter was 35 L (height, 200 cm; diameter, 15 cm) and contained 10 L of sand (Fig. 21). Size distribution of sand was in the range of 0.5-1.5 mm. The filter column was made of PVC pipe with five sampling ports along the column and one grating was placed inside to hold the media. The sand filter was initially designed to operate in upflow, fluidized mode using a bypass to control the flow rate through the sand filter.

2.2.4. Aeration and degasification

Totally two air blowers were used, one for foam fractiontor and the other one for aeration in the water sump and fish culture tank. No special degasification device was employed because the dimension of the system was small and aeration was provided.

2.3. Experimental procedure

This whole system was conditioned for 2 months before stocking by continuous feeding synthetic wastewater. For nitrification styrofoam bead filters, synthetic wastewater was supplied to the culture tank to stimulate nitrification. Sodium nitrate and acetic acid were added to the sedimentation basin to stimulate denitrification. At this period, sedimentation basin was separated from the system. Twenty-kg Korean rockfish (130 fish) with an average fish weight of 153.8 g was stocked to the culture tank on November 13, 2002. The water temperature were maintained within the range of 16-19℃ by thermostatic heating system. All the fish were harvested on February 28, 2003.

2.4. Feed composition, feeding, and fish growth

Commercial formulated feed was used. The composition was as follows: 40% protein; 10 % fat; 18% ash; 1% calcium; 0.8% phosphorus; 2.3% moisture. Fish were fed at 0900 and 1600 h, 7 days a week, by hand. Daily feeding rate is around 1-1.5% of total fish weight. Fish growth was determined by monthly-interval weight determination over the experimental period. Any dead fish, if found, was taken out and weighed.

2.5. Sampling and analysis

Water samples were taken once every 3 days from inlet and the outlet water of each compartment. Also, foam condensates and culture tank w ater were sampled and analyzed.

TAN, NO_2-N, NO_3-N, and PO_4-P were measured using the methods described by Strickland and Parsons (1972). DO was measured with DO meter (KDO 5151, KRK Co.) and pH was measured with pH meter (Oregon, model 720 A). Total alkalinity was measured by the titration method (Grasshoff et al., 1999). Protein analysis was conducted according to Lowry et al. (1951). TSS was measured according to APHA (1995).

3. Results

3.1. Water quality parameters

During the experimental period, pH values fluctuated between 7.3-7.9 (Fig. 22). Relatively sharp drop of pH was experienced in the first 2 months. So,

sodium bicarbonate was added to the system twice a week to prevent pH to drop to too low levels. Then, the pH decreased at low rates and sodium bicarbonate was added randomly whenever the pH values were found below 7.6. The decreased demands of sodium bicarbonate for maintaining pH levels higher than 7.6 were due to the elevated pH values in the outlet water of sedimentation basin and sand filter. DO concentrations in culture tank water were maintained above 5.5 mg/L during the experimental period (Fig. 22). Temperature fluctuated between 16.3 and 19.2℃.

TAN concentrations were below 1 mg/L in the first 2-months and showed a declining trend. However, TAN concentrations increased thereafter to around 1 mg/L (Fig. 23). Nitrite nitrogen concentrations were within the range of 1-2 mg/L for most sampling days (Fig. 24) while NO_3-N concentrations increased constantly within the first month and then fluctuated around 45 mg/L (Fig. 25). Phosphate (PO_4-P) increased to around 31 mg/L and then fluctuated between 25-31 mg/L thereafter (Fig. 26).

The differences between inlet and outlet TAN concentrations in the various treatment compartments through the experimental period are showed in Fig. 27. It can be seen that TAN was removed in bead filters and sand filter. TAN was removed in the sedimentation basin in the initial experimental period but produced later, especially in the last several weeks. Removal of nitrite was found in sand filter during the experimental period (Fig. 28). Basically, nitrite was removed in bead filters while it was either removed or produced in the sedimentation basin. Nitrate was produced in bead filters and removed in sand filter and sedimentation basin (Fig. 29). A relatively high nitrate removal was found in sand filter in the last experimental period.

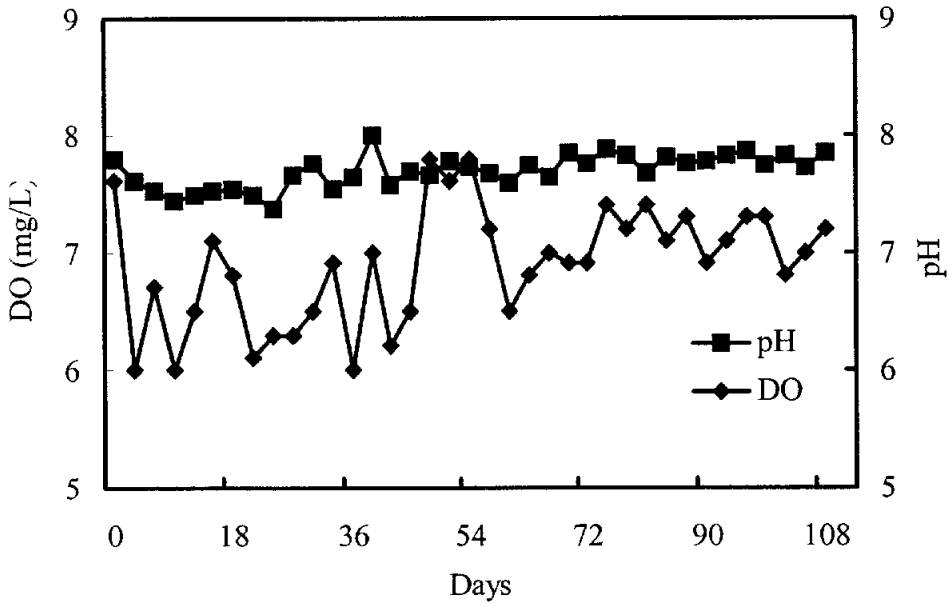

Fig. 22. Change of DO concentration and pH values in culture tank water during the experimental period.

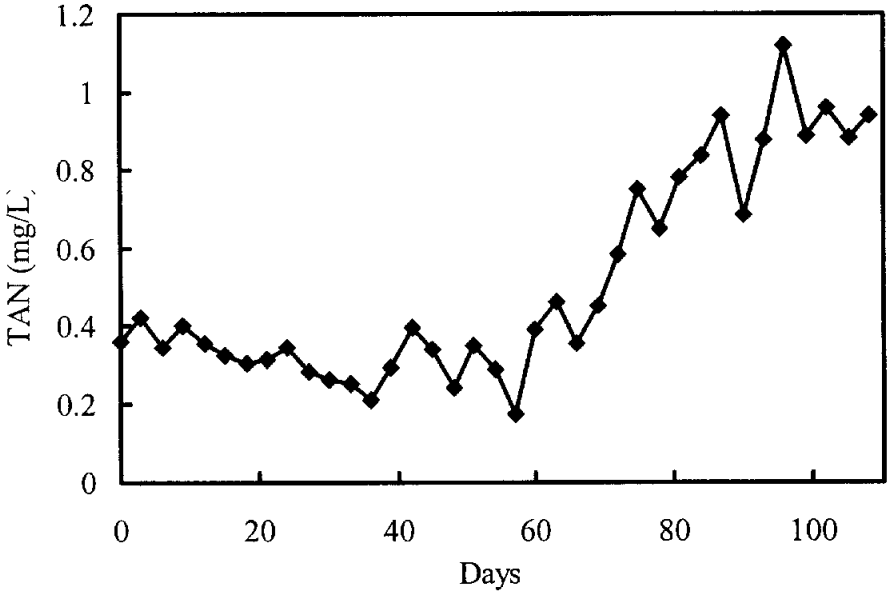

Fig. 23. Change of TAN concentrations in culture tank water during the experimental period.

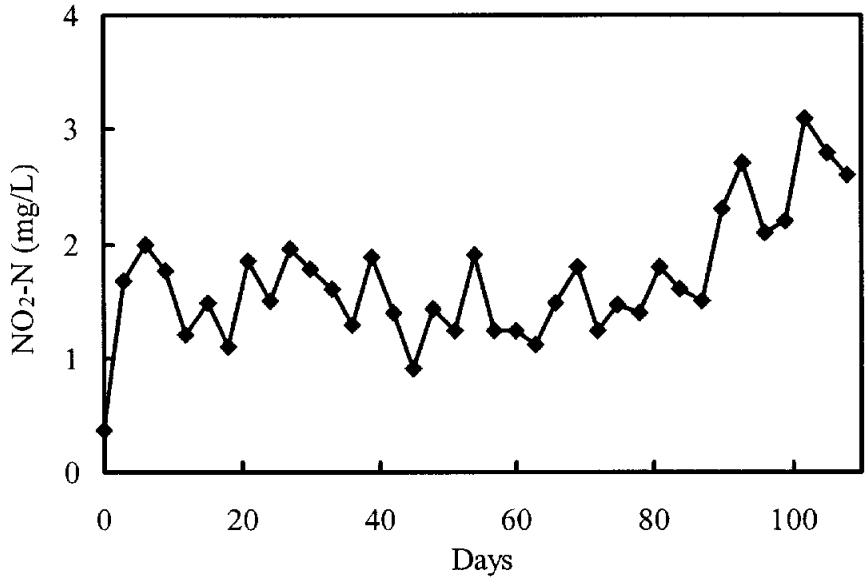

Fig. 24. Change of NO_2-N concentrations in culture tank water during the experimental period.

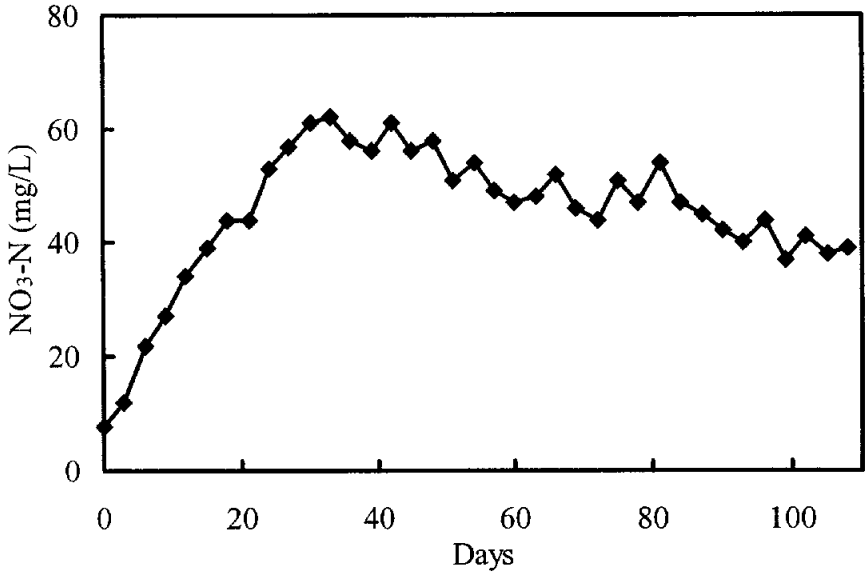

Fig. 25. Change of NO_3-N in culture tank water during the experimental period.

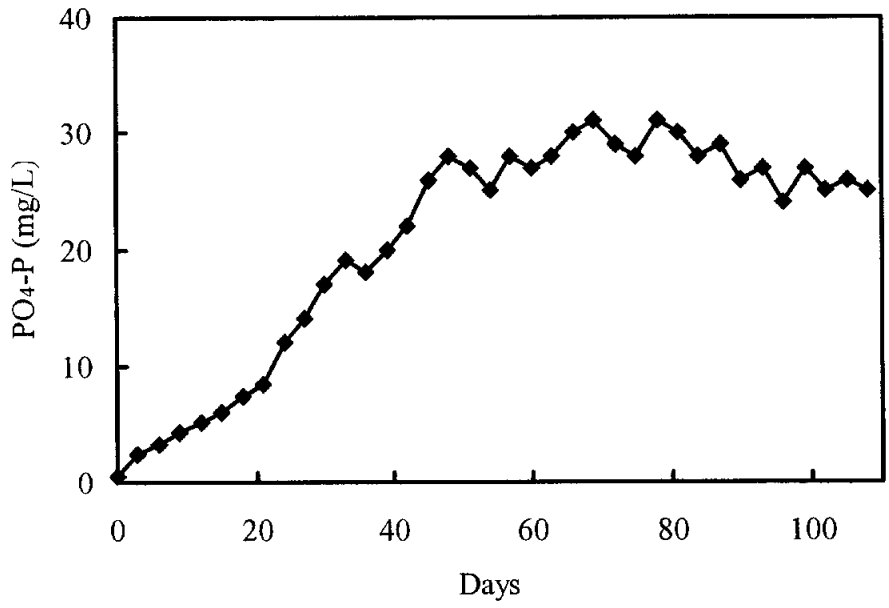

Fig. 26. Change of PO_4-P concentrations in culture tank water during the experimental period.

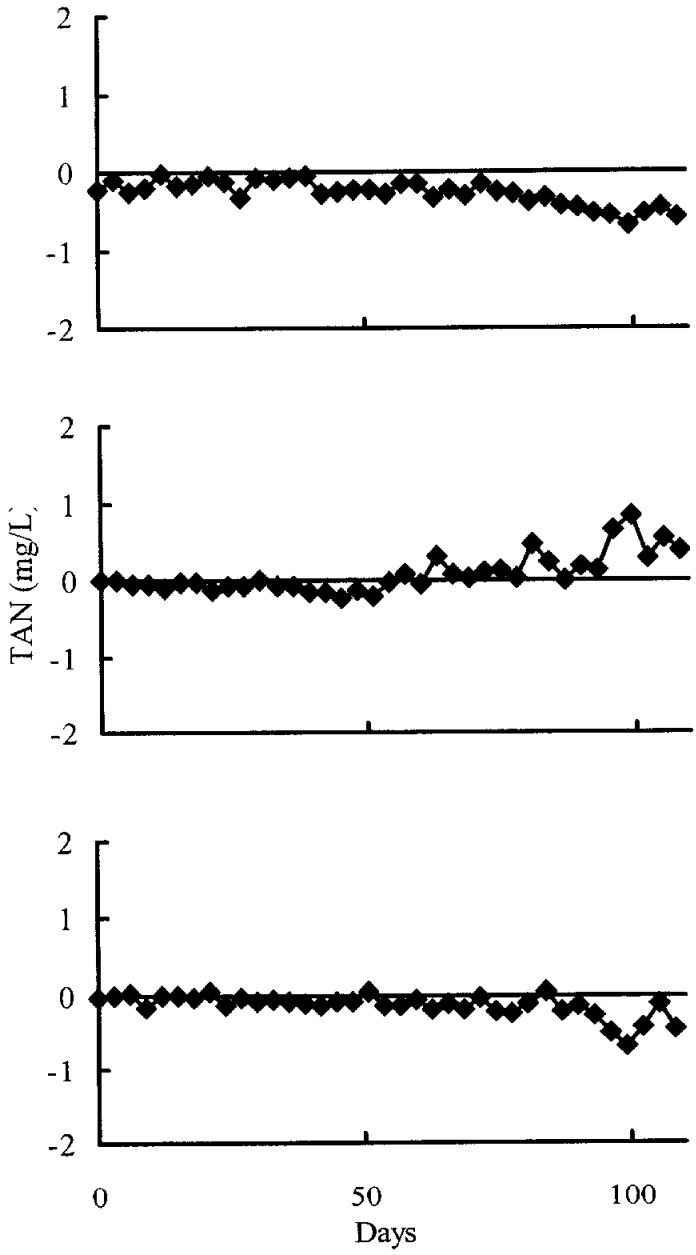

Fig. 27. Average production (positive values) and removal (negative values) of TAN in the different treatment compartments. (Upper, styrofoam bead filters; Middle, sedimentation basin; Lower, sand filter).

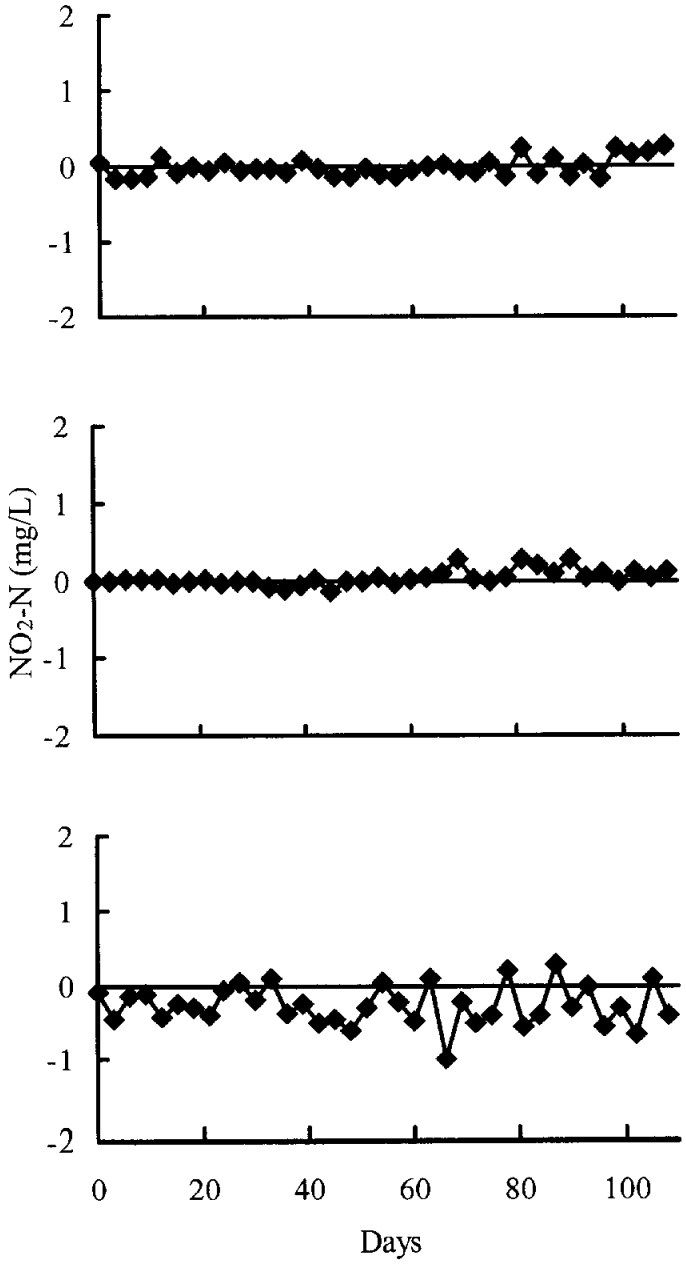

Fig. 28. Average production (positive values) and removal (negative values) of NO_2-N in the different treatment compartments. (Upper, styrofoam bead filters; Middle, sedimentation basin; Lower, sand filter).

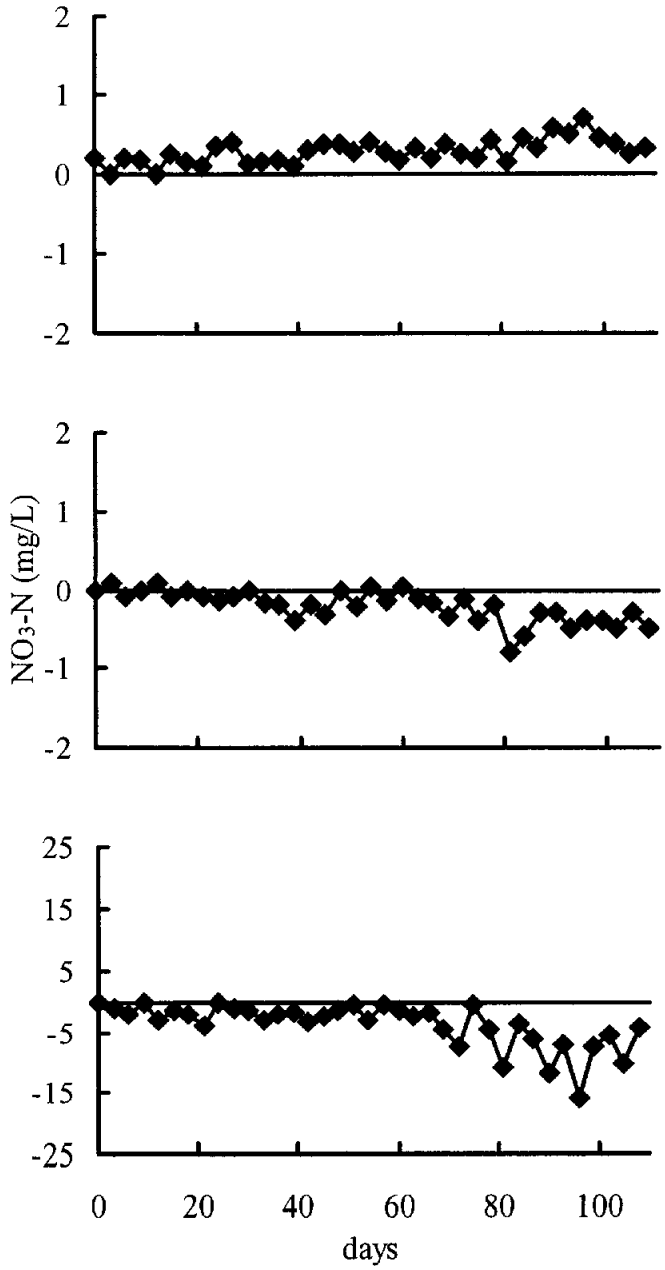

Fig. 29. Averaged production (positive values) and removal (negative values) of NO_3-N in the different treatment compartments. (Upper, styrofoam bead filters; Middle, sedimentation basin; Lower, sand filter).

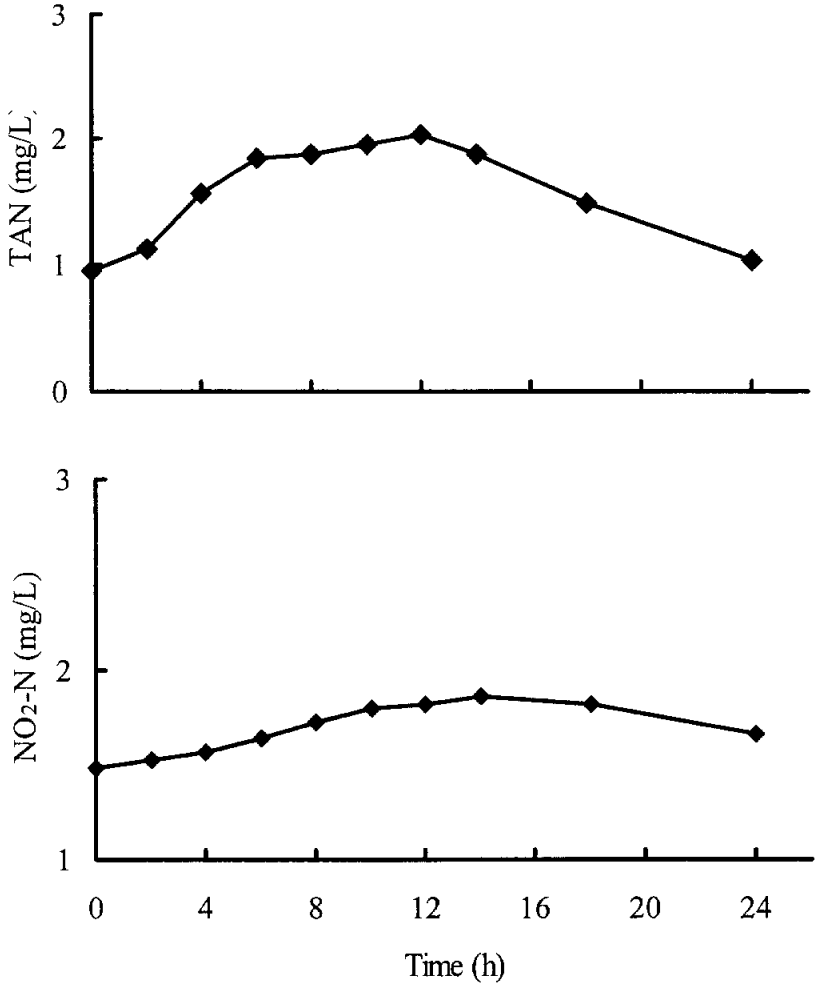

Fig. 30. Diurnal change of TAN and NO_2-N concentrations in culture tank water measured on day 87.

3.2. Diurnal change of TAN and NO_2-N in culture tank water

Diurnal changes of TAN and NO_2-N concentrations in culture tank water were depicted in Fig. 30. Dramatic fluctuations of TAN concentrations were noted, reaching the highest levels at around 4 hours after feeding. NO_2-N concentrations also increased following feeding and slight NO_2-N accumulation was noted.

3.3. Fish growth performance and water consumption

Table 11 summarized the main growth characteristics of Korean rockfish *Sebastes schlegeli* over 107-day culture period. During this period, initial density was 33.3 kg/m^3 and final density was 51.7 kg/m^3 on culture tank volume basis. On whole system water basis, the initial density was 18 kg/m^3 and final density was 28.2 kg/m^3. Average daily feed addition over the experimental period was 247 g with the maximum addition of 340 g (Fig. 34). The overall feed conversion ratio (FCR) was 2.4 and survival was 90.7%.

Water losses in the system were caused by evaporation, losses associated with fish weighing and disease treatment procedures and flushing of dual drain system. On daily basis, water addition was 3.4% of the total water volume in the system. Water consumption was 373 liter for production of 1 kg fish and this value was high.

3.4. Nitrification characteristics of bead filters

TAN conversion rate increased in the styrofoam bead filters with increase of inlet TAN concentrations (Fig. 32). The data present in this figure were collected during the experimental period, which means that temperature, pH, and other water quality parameters fluctuated. TAN concentrations in the inlet

water for the most sampling days during the experimental periods did not exceed 2 mg/L. Therefore, the maximum conversion rates were not obtained as they occur at higher inlet TAN concentrations as found in previous experiment. Areal TAN conversion rates (ATR) can be described as ATR=47.62TAN+5.6615.

Nitrite removal was plotted against TAN removal in Fig. 33. It can be seen that the higher the TAN removal, the more nitrite accumulates. The estimated threshold point where TAN removal equals nitrite removal was around TAN removal value of 0.33 mg/L. Beyond this TAN removal value, TAN removal exceeded nitrite oxidation and would cause increment of nitrite concentrations in the outlet water.

Table 11. Performance parameters of the recirculating system

Parameters	Value
Growth period (days)	107
Initial average fish weight (g)	153.8
Final average fish weight (g)	263
Total biomass produced (kg)	11
FCR	2.4
Survival (%)	90.7
Average daily water exchange (% of total water volume)	3.4
Specific water consumption (L/kg fish produced)	373

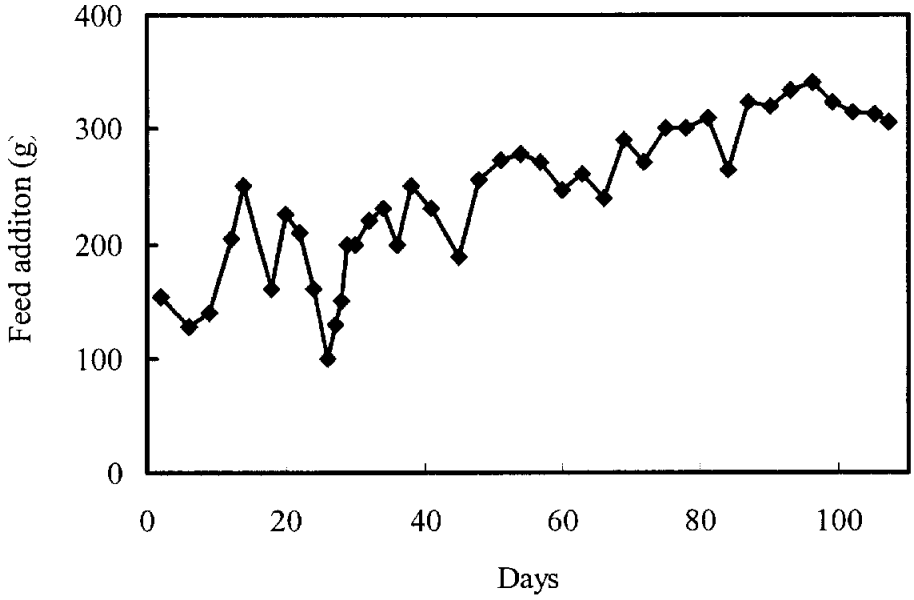

Fig. 31. Average daily feed input to the experimental system during the experimental period.

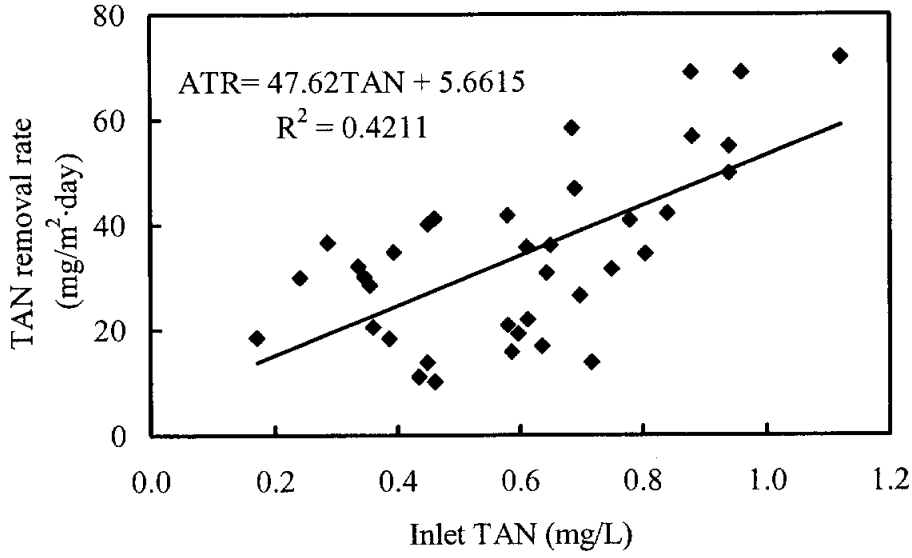

Fig. 32. TAN conversion rates of styrofoam bead filters at various in TAN concentrations in the recirculating system.

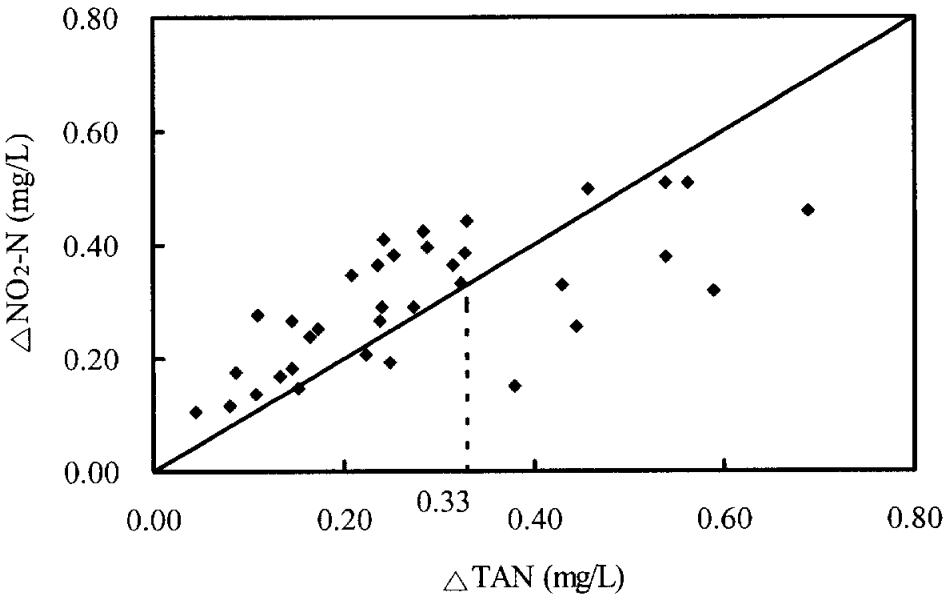

Fig. 33. NO_2-N removal by the styrofoam bead filters as a function of TAN removal. The drawn line indicates the point where nitrite removal equals TAN removal. Dashed line indicates the threshold value above which nitrite would accumulate.

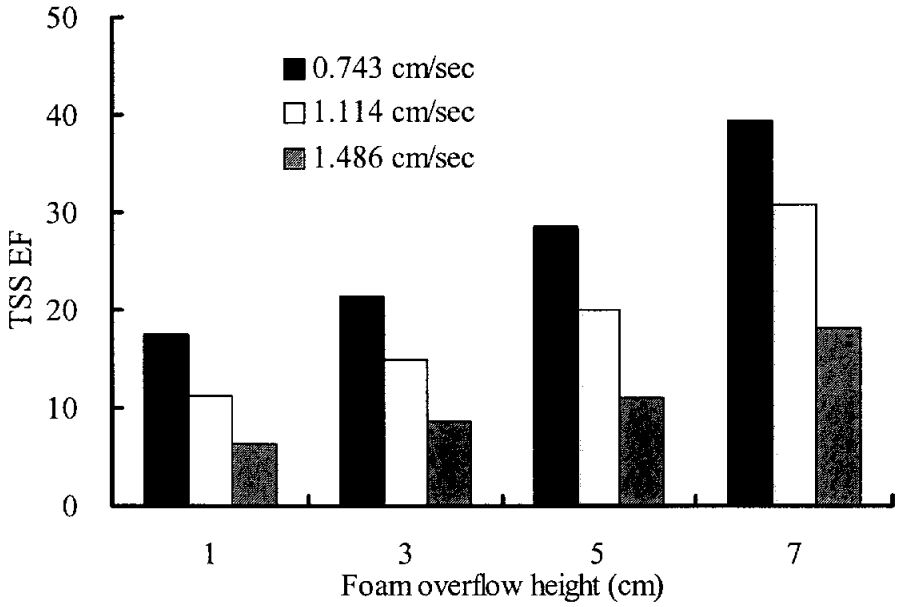

Fig. 34. Effects of foam overflow heights and superficial air velocities on TSS enrichments in foam condensates as indicated by enrichment factor (EF).

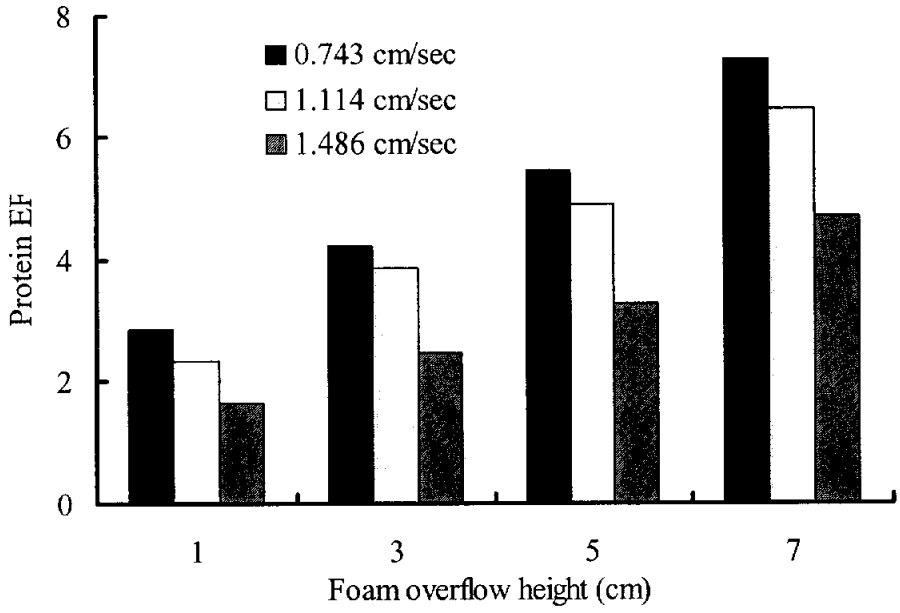

Fig. 35. Effects of foam overflow heights and superficial air velocities on protein enrichment in foam condensates as indicated by enrichment factor (EF).

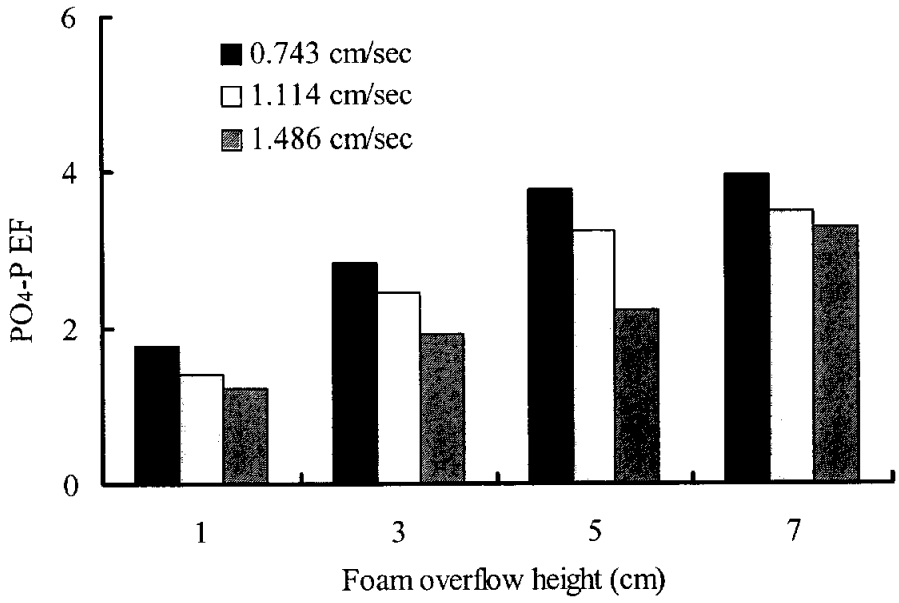

Fig. 36. Effects of foam overflow heights and superficial air velocities on PO_4-P enrichment in foam condensates as indicated by enrichment factor (EF).

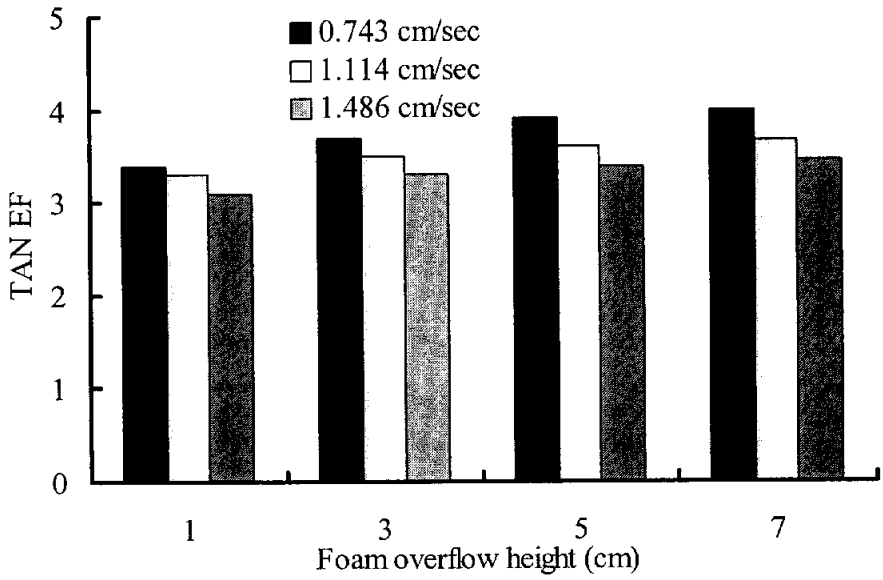

Fig. 37. Effects of foam overflow heights and superficial air velocities on TAN enrichment in foam condensates as indicated by enrichment factor (EF).

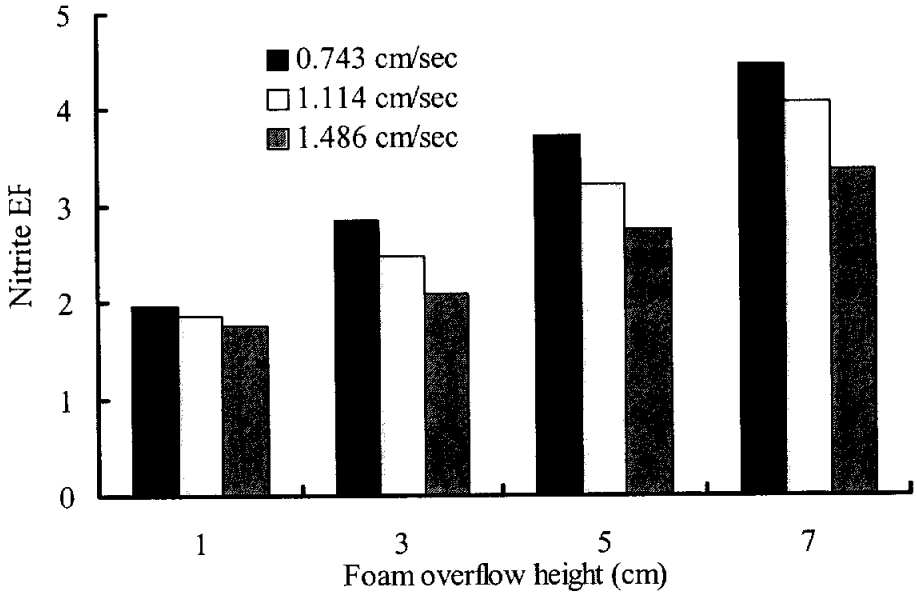

Fig. 38. Effects of foam overflow height and superficial air velocities on NO_2-N enrichment in foam condensates as indicated by enrichment factor (EF).

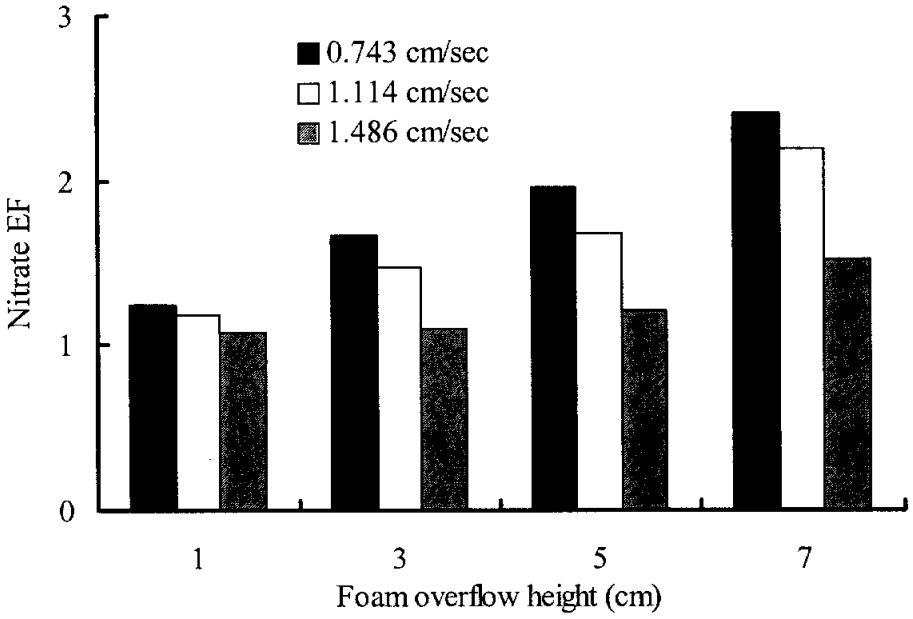

Fig. 39. Effects of foam overflow heights and superficial air velocities on NO_3-N enrichment in foam condensates as indicated by enrichment factor (EF).

3.5. Performance of foam fractionator

TSS Enrichment factors (EFs) in the foam condensates collected when the foam fractionator was operated at different SAV and foam overflow heights are shown in Fig. 34. Great differences of TSS concentrations in the collected foam condensates were found between the different foam overflow heights treatment at the lower SAV of 0.743 cm/sec. TSS concentrations increased with increase of foam overflow heights for each treatment at different SAV. This indicates the great effect of foam overflow heights on TSS concentrations in foam condensates. The effects were greater for lower SAV treatment than those for higher SAV treatments. TSS concentrations decreased with increase of SAV at each foam overflow height. The enrichment factors ranged from 6.4 to 39.4.

The effects of different foam overflow heights and SAVs on protein concentrations in the collected foam condensates were shown in Fig. 35. Protein enrichment factors ranged from 1.6 to 7.3. These values were much lower than TSS enrichment factors. Also, protein concentrations in the foam condensates increased with increase of foam overflow heights at each SAV and higher SAV resulted in lower protein concentrations in foam condensates at each overflow height as indicated by the enrichment factors. Effects of foam overflow heights on enrichment factors were greater for lower SAV treatments than those at higher SAV treatments. Phosphate enrichment factors showed the same trends as for TSS and protein (Fig. 36). The values were within the range of 1.2-3.9.

TAN, NO_2-N, and NO_3-N enrichment factors are shown in Fig 37, 38, and 39, respectively. No great differences of TAN enrichment factors were found among all the treatments. Relatively greater differences of NO_2-N enrichment factors were found among different foam overflow heights treatments at each SAV applied. Also, the higher SAV induced lower values of NO_2-N

enrichment factors. NO_3-N enrichment showed the same trends as for nitrite.

Table 11 summarizes foam overflow rates, calculated daily removal of TSS and protein by foam fractionator. Daily removals of TAN, NO_2-N, NO_3-N and PO_4-P were not shown here due to the low values calculated and this showed that the low efficiency of foam fractionator for reduction of these variables. Foam overflow rates decreased with increase of foam overflow heights at each SAV treatment and were higher for high SAV treatments than low SAV treatments when compared at the same foam overflow height. The effects of foam overflow heights on foam overflow rate were greater for higher SAV treatments than those at lower SAV treatments. Foam overflow rates were quite higher for lower overflow height treatments than those at higher overflow height treatment and this is especially evident for higher SAV treatment. Weeks et al. (1992) found the same trends in fresh water aquaculture system. Though concentrations of TSS and protein concentrations were higher in foam condensates collected in higher overflow height treatments, the calculated daily removals of these variables were greatly affected by the foam overflow rates. Lower foam overflow height induced greater removal of these variables when compared with high foam overflow height treatments.

Table 12. Foam overflow rate (FOR) and calculated removal rates of different water quality variables at different superficial air velocity (SAV) and foam overflow height (FOH).

SAV (cm/sec)	FOH (cm)	TSS (g/day)	Protein (g/day)	FOR (ml/min)
0.743	1	5.67	0.92	6.8
0.743	3	3.07	0.60	3.0
0.743	5	1.93	0.37	1.4
0.743	7	1.29	0.19	0.6
1.114	1	7.10	1.47	9.2
1.114	3	4.73	1.23	5.0
1.114	5	3.60	0.88	3.8
1.114	7	1.79	0.37	1.6
1.486	1	10.94	2.78	36.2
1.486	3	5.01	1.44	12.4
1.486	5	3.76	1.10	7.1
1.486	7	2.15	0.56	2.5

4. Discussion

This study was to present the performance data, with respect to growth performance and water quality, of an intensive seawater recirculating system. The main purpose aimed at demonstrating the technical feasibility of Korean rockfish culture in a nearly completely closed mode and reasonable results were obtained. However, further studies are needed to evaluate the commercial feasibility of this kind of system because the small-scale system was used in present experiment. Nevertheless, present study still provides some basic information for further studies of this kind of system.

4.1. Water quality

The whole water quality parameters were within the levels commonly recommended for fish culture on most of the sampling days, except relatively high nitrite concentrations encountered. Fortunately, nitrite is not so toxic in seawater as that in freshwater. Since sampling was conducted just after the first feeding, the depicted TAN concentrations did not represent the maximum concentrations in the culture system. The diurnal changes of TAN concentrations measured on day 87 (Fig. 30) showed that maximum concentrations exceeded 2 mg/L. This indicated that the system was already operated at maximum feed load or nearly the maximum carrying capacity. Increasing in the hydraulic loading rates through the nitrifying bead filter could possibly accomplish this (Greiner and Timmons, 1998; Nijhof and Klapwijk, 1995; Shnel et al., 2002). However, this could increase the nitrite concentrations in the culture tank water as found in the previous study, so no attempts have been done about this. On the other hand, production of TAN in the sedimentation basin attributed to the elevated TAN concentrations in the last

experimental period. This presents another question in designing a recirculating system, taking into account this TAN production should be a necessity whenever using the sedimentation basin for digestion purpose. However, no detailed information about TAN production in sedimentation basin was provided till now and quantification of this TAN production needs further study.

High nitrite concentrations in the culture tank water was partially due to the possible production of nitrite in bead filters at high inlet TAN concentrations and partially due to the production of nitrite in the sedimentation basin, especially in the last experimental period.

Though fish are poorly utilizing phosphorus in feed (Avnimelech and Lacher, 1979; Green and Boyd, 1995; Schroeder et al., 1991), decline of phosphate concentrations in culture tank water indicates that phosphorus should have been recovered in the treatment compartments. Shnel et al. (2002) reported that phosphate was recovered in digestion basin and denitrification biofilter. Barak and van Rijn (2000a) found that denitrifiers were capable of phosphate uptake in excess of their metabolic requirements and phosphate removal from the water in the experiment system was mainly mediated by denitrifying organisms. Studies on a number of denitrifier isolates (Barak and van Rijn, 2000b,c) further confirmed their findings. This would be true in present experiment considering the similarity of the systems used.

4.2. Growth performance

Fish growth was lower than expected. The main reason should be the disease encountered during the experimental period. Fish were found to be heavily infested with parasites and possibly bacteria. They were treated with formalin and antibiotics. Feed conversion ratio was relatively high when compared with the common values reported.

4.3. Nitrification characteristics of bead filters

The measured maximum TAN conversion rate in the styrofoam bead filters was 72 $mg/m^2 \cdot day$. Higher TAN conversion rates could probably be obtained at higher inlet TAN concentrations as found in the previous study, TAN conversion rates increasing with increase of inlet TAN concentrations. TAN conversion rates were lower than those obtained in previous experiment when compared on an equal basis of inlet TAN concentrations. The differences could be easily recognized by comparing the equation obtained by plotting the TAN conversion rates versus inlet TAN concentrations obtained in present experiment with that obtained in previous study. Solids would affect TAN conversion rates. During the experimental period, once or twice daily backwashing is necessary to ease clogging problem or beads would be washed out of the biofilter column. Chitta (1993) observed in an experimental propeller-washed filter that the rate of TAN oxidation increased with a decrease in backwashing frequency, an aspect he attributed to the longer mean cell residence time. However, inhibition of nitrifiers may take place due to accumulation of organic solids and heterotrophic biomass. Malone et al. (1993) explained that organic loading inhibits spatial transport of nutrients to the nitrifying bacteria. TAN conversion rates showed large fluctuation when compared with those obtained in previous experiment and this indicated that nitrification could be affected largely by the operating factors of the system. Kamstra et al. (1998) also found a large fluctuation in trickling filters used on eel fish farms.

Nitrite accumulation was found to occur when TAN removal was more than 0.3 mg/L. This demonstrates that high removal rates of TAN by biofilters do not necessarily imply an overall improvement of the biofilter performance. Though nitrite accumulation was found at low ambient DO concentrations, it seems unlikely that low DO caused nitrite accumulation in present experiment

considering the high DO concentrations in the outlet water. The reasons should be as discussed as in previous experiment.

4.4. Performance of sedimentation basin and sand filter

Nitrate removal was found to occur in sedimentation basin. Since internal baffles were used, the organic matter was mainly accumulated in the inlet region of the basin. This may caused an uneven distribution of inorganic nutrients within the basin. Aboutboul et al. (1995) and Shnel et al. (2002) concluded that it is in this region where labile organic matter is degraded and volatile fatty acids are released and used for nitrate reduction by denitrifiers. The low organic matter in the sedimentation basin would have caused the low nitrate reduction at the beginning. Also, the high HRT also attributed to this low nitrate removal. Another important reason is that the time lag for developing anoxic conditions and heterotrophic bacteria within the basin. Later, the water flow rates through the sedimentation basin were further reduced and this increased nitrate removal.

Nitrate removal in the sand filter was lower than expected, especially in the first 2 months, though the filter already was conditioned for 2 months before being cooperated into the system. The main reason should be due to the low carbon sources to mediate denitrification process. Since the water was pumped into sand filter from the outlet of sedimentation basin, organic matter already was, at least partially, consumed in sedimentation basin by denitrification process.

Sand filter was initially designed to operate in fluidized mode. But later we found this is not practical because of low organic matter and high inlet DO concentrations, around 4 mg/L at initial and 2 mg/L later. Water flow rate was reduced to only 3-15 L/hour and this improved the performance. This can be evidenced by the elevated nitrate removal in the last experimental period.

Overall, combination of sedimentation basin and denitrification sand filter worked well for nitrate reduction. This combination eliminates the requirement of external carbon sources for denitrification. However, production of ammonia within the sedimentation basin, as mentioned in previous part, would increase the volume of nitrification biofilter.

4.5. Foam fractionator

The decrease of TSS, protein, and PO_4-P concentrations in the foam condensate as a result of increase of SAV was due to the fact that at higher SAV, the foam was swept out at a faster rate, which did not allow excess water to drain from the foam. High foam overflow height increased the time needed for foam to be drained out of the fractionator column and consequently increased the time the foam had to drain water. TSS and protein concentrations were much lower in the foam condensates collected at lower foam overflow height treatments, which means that relatively dilute foam condensates were produced at low foam overflow height.

TSS enrichment factors ranged from 6.4 to 39.4. Weeks et al. (1992) found that TSS enrichment factors were within the range of 17-40 and the same trends of change of TSS concentrations in the foam condensates were found here. Chen et al. (1993b) also found an enrichment factor of more than 10 in freshwater aquaculture systems. These results showed that TSS enrichment in foam condensate could be substantial. The obtained protein enrichment factors were relatively low. This low enrichment factors indicate that not all the proteins detected by Lowry's method are surface-active. Chen et al. (1993c) reported that, on average, only 11% of the total protein detected was surfactants. Relatively high PO_4-P enrichment factors indicate that foam fractionation process could remove minerals. Hussenot et al. (1998) also found that foam fractionation apparatus was good at trapping dissolved mineral

materials such as phosphates.

TAN, NO_2-N, and NO_3-N enrichment factors were relatively low when compared with TSS and protein enrichment factors. These results showed that foam fractionation is not an effective way for removal of dissolved inorganic matters. Spotte (1979) already concluded that within the common pH ranges, foam fractionation does not reduce the level of ammonia in aquarium water. Hussenot et al. (1998) also found lower enrichment factors for TAN, NO_2-N, and NO_3-N in marine aquaculture systems. However, Suh and Lee (1997) reported higher removal efficiency of TAN and NO_2-N in a simulated aquaculture system and concluded that foam fractionation could be a very effective way for TAN and NO_2-N removal. This mainly should be due to the different operating factors used in their experiment, especially the small dimension foam fractionator column used and the high HRT and SAV applied. There are indications that small dimensional change and differences in operating parameters can have large impacts on performance (Huguenin and Colt, 1989; Know, 1971).

These results indicates that using minimal overflow heights may produce foam condensate that may only be marginally more concentrated with variables than the culture water and high overflow heights may produce extremely concentrated foam condensates, but the foam production rate may be extremely low. For practical application of foam fractionator in aquaculture systems, extremely low foam overflow height such as 1 cm height, especially when combined with high SAV, should not be used in order to minimize the volume of wastewater discharged. Nevertheless, one could select the operating factors to satisfy their desired result, as minimizing the wastewater volume or maximizing removal of variables.

Conclusion

1. Foam fractionation

Performance characteristics of foam fractionator are highly depended on the operating factors including SAV, HRT, and foam overflow height. TSS and protein removal rates increased with increase of SAV and decrease of HRT. High initial protein concentrations induced high TSS and protein removal rates. Foam condensate production decreased and concentration increased as foam overflow height increased. Protein and TSS concentrations in the foam condensates collected from the 5 consecutive trials shows that water flow rates exert little effects on overall TSS and protein removal. Though high SAV would increase the protein removal rate, but extremely high SAV may result in the formation of gas slugs (Timmons, 1994) and reduce substance removal rates but this is out of the scope of this study.

The performance of foam fractionator was evaluated in the laboratory scale seawater recirculating system. Foam overflow rates increased with increase of SAV but decreased with increase of foam overflow heights. This trend was more obvious when high SAV used. The concentrations of all the water quality variables in the foam condensates increased with increase of foam overflow height and SAV. Lower foam overflow height resulted in more dilute foam condensates. TSS, protein, and PO_4-P enrichment factors were within the range of 6.4-39.4, 1.6-7.3, and 1.2-3.9, respectively. Low values of TAN, NO_2-N, and NO_3-N enrichment factors were obtained and this indicates that foam fractionation is not an effective way for removal of dissolved inorganic nitrogen forms. The calculated maximal daily removal values for TSS and protein were 10.9 g and 1.4 g, respectively. So, foam fractionation process is very effective for solids trapping in the foam condensate. However, to the

author's observation, solids remained in the filtrate even after being filtered through 0.45-micron filter papers. So, detailed analysis of the size distribution of solids in foam condensate will be helpful for accurate evaluating which kind of solids could be removed with foam fractionator. Also, lack of the performance data of foam fractionator in seawater recircluating aquaculture system makes the interpretations of the data obtained in present experiment difficult and the practical application of the present findings to other aquaculture systems doubtful since large differences could be introduced by different managing strategies and dimensions of the foam fractionator.

2. Nitrification biofilters

All three biofilter media showed nitrification capacities. Styrofoam beads exhibited the greatest TAN conversion rates on a volumetric basis. TAN conversion rates averaged 682, 269, 79 g TAN/m^3 • day in styrofoam bead, sand and loess bead filters, respectively in trial 1. Nitrite accumulations were found in the outlet water from all three biofilters. Nitrite nitrogen removal rates averaged 384, 154, and 41 g /m^3 • day in styrofoam bead, sand and loess bead filters, respectively. Based on TAN and NO_2-N conversion rates, styrofoam bead showed the best performance among the three filter media tested. However, the accumulation of nitrite may be a problem for practical application. Decreasing water loading rate or increasing media depth may be helpful. Nevertheless, the use of synthetic water in present experiment should be questioned.

In trial 2, TAN and NO_2-N conversion rates increased with increase of HRTs. However, the improvement in biofilter performance was not linearly correlated to HLRs. At the highest HLR tested, the improvement was not as great as that at medium HLR tested. TAN conversion rates in sand filter increased with increase of HLR to 200 m^3/m^2 • day. No further increase was

observed at highest HLR due to the factor that at higher HLR, flooding on the media surface took place. HLR had significant impact on TAN conversion rates in loess bead filter up to the highest HLR tested.

TAN conversion rates were much less at organic matter loading rates of 9 and 18 kg COD/m^3 • day than those without organic carbon addition in styrofoam bead filter. The addition of glucose resulted in reduction of TAN conversion rates from 540 to 301g TAN/m^3 • day. At organic matter loading rate of 9 kg COD/m^3 • day, great reduction of TAN conversion rates were found in sand filter and loess bead filter. Clearly, organic matter can be one of the most important impacting factors on nitrification efficiency. The performance of styrofoam bead filter at different inlet TAN concentrations and fixed organic matter loading rates of 18 kg O_2/m^3 • day were tested in trial 3.

The measured maximum TAN conversion rate in the styrofoam bead filters was 72 mg/m^2 • day in the laboratory scale recirculating system. Higher TAN conversion rates could probably be obtained at higher inlet TAN concentrations. TAN conversion rates were lower than those obtained in previous experiment when compared on an equal basis of inlet TAN concentrations. Solids would affect TAN conversion rates. During the experimental period, backwashing once or twice daily is necessary to ease clogging problem or beads would be washed out of the biofilter column. This might reduce mean cell residence time. TAN conversion rates showed large fluctuation when compared with those obtained in previous experiment and this indicates that nitrification could be affected largely by the operating factors of the system.

3. Laboratory scale recirculating system

Whole water quality parameters in this laboratory scale recirculating system were within the levels commonly recommended for fish culture on most of the sampling days, except relatively high nitrite concentrations encountered.

TAN concentrations were below 2 mg/L and NO_2-N concentrations were within the range of 1-3 mg/L for most sampling days. TAN was removed in styrofoam bead filters and sand filter and removed or produced in sedimentation basin. Nitrite was removed in sand filter and basically, nitrite was removed in bead filters while it was either removed or produced in the sedimentation basin.

Diurnal changes of TAN concentrations showed that maximum TAN concentrations in culture tank water exceeded 2 mg/L. This indicates that the system was already operated at maximum feed load or nearly the maximum carrying capacity. Production of TAN in the sedimentation basin attributed to the elevated TAN concentrations in the last experimental period. This presents another question in designing a recirculating system, taking into account this TAN production should be a necessity whenever using sedimentation basin for digestion purpose. However, no detailed information of TAN production in sedimentation basin was provided till now and quantification of this TAN production needs further study.

Nitrate removal was found to occur in sedimentation basin. This process might be mediated by volatile fatty acids, which are product of decomposition of labile organic matter within the sedimentation basin. Further reduction of water flow rates through the sedimentation basin increased nitrate removal. Nitrate removal in the sand filter was lower than expected. The main reason should be due to the low carbon sources to mediate denitrificantion process. Overall, the combination of sedimentation basin and denitrification sand filter worked well for nitrate reduction.

The main purpose aimed at demonstrating the technical feasibility of Korean rockfish culture in a nearly completely closed mode. However, further studies are needed to evaluate the commercial feasibility of this system because of the small-scale system used here. Nevertheless, present study still provides some basic information for further study of this kind of system.

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