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Rating fixed film nitrifying biofilters used in recirculating aquaculture systems

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Abstract

Predicting the performance of biofilters is an engineering challenge that is critical to both designers and managers. The task is complicated by the wide variety of water quality expectations and environmental conditions displayed by a recirculating aquaculture system (RAS). A myriad of biofilters designs have been generated reflecting approaches of engineers attempting to maximize specific surface area and oxygen transfer within the context of a biofilm management strategy. A rating strategy is presented for biofilters to facilitate the identification of appropriate matches between biofiltration formats and RAS applications. As a foundation, a previously proposed RAS classification system based upon salinity, temperature and trophic levels is upgraded to create 17 systems classifications. A biofilter classification system identifies seven combinations of trophic level and pH which should be sufficient to serve the RAS demands. Temperature and salinity are neglected as a means of simplifying the approach. An experimental methodology based upon chemical feeds is proposed to represent the steady-state RAS performance of the biofilters. Data is summarized by linear analysis of filter performance for concentration ranges below 1.0 g TAN m^{-3} and simple averaging is proposed for higher trophic levels. Input from the aquacultural engineering community and RAS aquaculturists is required to further refine the approach prior to endorsement.

Keywords: Biofilters; Nitrification; Recirculating aquaculture

1. Introduction

Proper selection and sizing of biofilters are critical to both the technical and economic success of a recirculating aquaculture system (RAS). These critical decisions are complexed by the wide variety of biofilters available and the variability of conditions under which a given biofiltration platform is expected to perform. Nitrifying bacteria are sensitive to water quality and management strategies. High levels of ammonia, or more commonly nitrite, undermine commercial production objectives as the toxic impacts are manifested through impaired growth or chronic diseases (Manthe et al., 1985; Cheng et al., 2004; Svobodova et al., 2005).

This paper presents a rational foundation for an industry-wide approach ranking fixed film biofilters

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used for organic carbon, ammonia and nitrite control in recirculating systems. The approach is formulated with a secondary objective of providing performance criteria that will aid in the selection and sizing for differing types of recirculating applications. A brief review of basic nitrifying bioreactor configurations, used in support of recirculating aquaculture systems, are presented followed by the kinetic basis for establishing first a RAS classification then a fixed film bioreactor classification system. The paper closes with the development of an experimental protocol that can be used to implement the ranking system.

2. Fixed film bioreactors

The recirculating aquaculture industry has tended to utilize fixed film bioreactors rather than suspended growth systems. The fixed film reactors are more stable than suspended growth systems, and arguably, a more appropriate technology for an industry where good water quality is a secondary objective to the production of large quantities of fish or crustaceans. In a fixed film biological process, the dissolved or colloidal wastes are transported by diffusion into the biofilm, which coats a filter media. Rock, shells, sand and plastic are commonly used to support these bacterial films. The biofilm can be viewed as a bacterial habitat that endures wide varieties of flow and quality regimes while maintaining its inherent ability to process wastes (Wheaton, 1977).

Fixed film bioreactors are not without their own limitations. The biofilm itself is defined by the rapidly reproducing heterotrophs while the filters' performance is usually determined by a much more sensitive subpopulation of chemoautrophs, the nitrifiers (Zhang and Bishop, 1994). Opinions on how to best overcome diffusional limitations on nutrient supply or means of controlling excessive biofilm development have resulted in the propagation of a confusing myriad of fixed film formats as evidenced by the variety of filters that appear in studies (Rogers and Klemetson, 1985; Miller and Libey, 1986; Westerman et al., 1996; Greiner and Timmons, 1998; Singh et al., 1999; Hall, 1999). Fixed film biofilters can be organized into four fundamental blocks distinguished by the strategy used to provide oxygen, and by their means of handling biofilm growth (Fig. 1).

The "emergent" filters are designed to maximize oxygen transfer as water cascades directly over the media. In the case of the tricking filter the cascade is achieved by water falling over the media (Twarowska et al., 1997; Greiner and Timmons, 1998; Lekang and Kleppe, 2000; Eding and Kamstra, 2001; Sandu et al., 2002; Shnel et al., 2002), whereas, rotating biological contactors create the same effect by rotating the media in and out of the water (DeLosReyes and Lawson, 1996). The media is fixed and biofilm accumulations

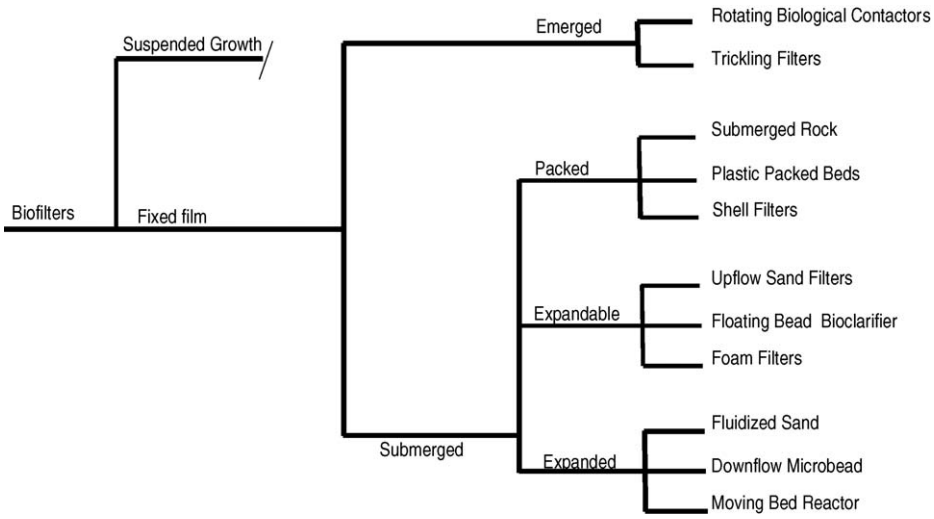

Fig. 1. RAS biofilters can be clustered into four basic blocks that display similar characteristics.

are usually managed through the process of sloughing. The sloughing process demands a relatively high porosity (as well as high interstitial distances) so released biofilm can fall free of the media. Thus, to avoid biofouling problems, emergent filters are associated with media displaying relatively low specific surface areas. Biodrums, a variant of the RBC utilizes a media held loosely in an enclosed rotating drum (Wortman and Wheaton, 1991) to enhance biofilm removal capabilities while maintaining the secondary benefits of aeration and CO_2 stripping provided by the cascading water displayed by all the emergent designs (Hall, 1999).

Submerged filters presume that sufficient oxygen can be transported with waters circulated through the filter. This presumption is normally assured by use of high recirculation rates, internal recycling, or through oxygen enrichment of influent waters. These filters are distinguished by the strategies used to manage their biofilm accumulations. There are three fundamental types of submerged filters.

The first category utilizes static ''packed'' beds that provide no active means of biofilm or solids removal. Submerged rock filters and submerged packed beds depend entirely upon endogenous respiration to control biofilm accumulations (Manthe et al., 1988). Water flow may be from top to bottom (downflow) or from bottom up (upflow). These filters are used mainly in lightly loaded systems such as display aquaria or crab shedding systems. They have, however, also been utilized in a variety of formats for recirculating shrimp production systems that are more heavily loaded (Davis and Arnold, 1998; Tseng et al., 1998). They are inexpensive and work well until overfeeding and solids accumulation in the packed bed causes excessive bacterial growth, which limits water penetration. Some variants utilize blown air or draining to mitigate the solids accumulation issue.

The second category of submerged beds employs static beds that are intermittently ''expandable''. Biofilm grown while the bed is static is periodically removed by the process of abrasion as the individual medium elements move randomly rubbing or striking each other. Motion in the bed is induced by hydraulic, pneumatic or mechanical forces. The aggression of the backwashing mechanism and backwash frequency are employed to control the biofilm growth (Sastry et al., 1999; Golz et al., 1999; Tijhuis et al., 1996). The ability to clean the media allows the use of a much smaller media than used in the packed bed formats. These approaches have been limited to coarse sands or larger beads that have the mass capable of generating the momentum necessary during a cleaning event of short duration. The inability to maximize specific surface area is offset by the management flexibility provided by the backwashing strategy and these filters usually show the ability to be used concurrently as solid capture devices (Cooley, 1979). Custom bead designs can also be used to overcome the specific surface area shortcomings (Beecher et al., 1997). Sponge filters are typically small but a widely recognized variant of this strategy. Backwashing in this case is provided by manual compression of the media.

The third category of submerged filters abandons the static bed in favor to a medium that is in constant motion. ''Expanded'' filters continually abrade biofilm by a hydraulic or pneumatic means. The rate of biofilm loss is usually determined by the media selection. These units can utilize granular media with extremely high specific surface areas, although the finest media are limited in their abrasion capabilities and thus find limited use in eutrophic applications. Fluidized filters are the oldest of this class. Fluidized beds can incorporate diverse material types (granite, anthracite, activated carbon) and a wide variety of particulate diameters as a means of adjusting the abrasion requirements to the trophic level of the application (Heinen et al., 1996). These fluidized beds are typically expanded by hydraulic means although a number of units have been augmented by pneumatic means. A second subclass of filters employs a limited degree of media movement to maintain the hydraulic conductivity of the media. Microbead filters employ this strategy to achieve conversion across a variety of trophic levels (Greiner and Timmons, 1998). These filters use a small floating polystyrene bead in a downflow mode to take advantage of high specific area characteristics of the media. Moving bead reactors show promise as they operate across a wide range of substrate concentration, display excellent TAN conversion rates, and can operate in a low-head environment (Zhu and Chen, 1999). This emerging technology employs aeration to control biofilm development in a larger media that mitigates excessive abrasion by providing protected areas for biofilm

development (Odegaard et al., 1994). Seo et al. (2001) describes a similar approach that employs immobilized nitrifying bacteria.

This attempt to summarize the distinguishing features of common biofiltration designs (Table 1) is misleading in the sense that an almost endless variety populates each class, though they differ in hull configurations, media characteristics and circulation strategies. The performance capabilities are also broad with small design features controlling performance.

2.1. Nitrification kinetics

The nitrification capability of a biofilter is due to its biofilm. The biofilm is often described in terms of a layered structure with an inner layer formed by inert biomass near the media surface, tightly overlain by a nitrifying rich population, with heterotrophs dominating the outer layer (Zhang et al., 1995; Ohashi et al., 1996). However, these conclusions must be continually reviewed as the complex nature of biofilms become more completely understood (Bishop, 1997; de Beer et al., 1997; Schramm et al., 1996). This layering results from the relatively high reproduction rate of the heterotrophic bacteria. For example, Fdz-Polanco et al. (2000) cite heterotrophic specific growth rates of 4.8 day^{-1} versus ammonia and nitrite oxidizers specific growth rates of only 0.76 and 0.84 day^{-1}. This reproductive advantage allows the heterotrophs to position themselves in the top layers of the biofilm (Mann and Rittmann, 1992). The heterotrophic bacteria thus become an important contributing factor in the definition of rates as they generally define the biofilm thickness, and thus, the distance of diffusion (Harremoes, 1982; de Beer et al., 1997; Zhang and Bishop, 1994). The biofilters' organic loading (kg feed m^{-3} day^{-1}) and the impact of pretreatment steps, i.e. solids removal, in turn, become major modifiers of the baseline nitrification capacity of the filter (Bovendeur et al., 1990).

On occasion, the rate of nitrification can be controlled by the nitrite oxidizers. However, this condition usually occurs only under conditions of improper management or during times of transitional imbalances. Nitrite oxidizers are generally recognized as having higher oxidation rates than ammonia converters and thus low nitrite levels are typical in a balanced filter. However, the nitrite oxidizers display reproductive rates lower than the ammonia oxidizers (Metcalf Eddy, 1991; Paller, 1992). Thus, they are the last group to adjust their population to an increase in nitrogen loading and the first to display mean cell residence time problems when biofilm removal is used to control biofilm thickness. Nitrite accumulations are also the first indication of low oxygen supply to (or within) a biofilm (Manthe et al., 1985; Garrido et al., 1997). Although, it is not known whether this stems from a reduction in nitrification rates or results from nitrate reduction by secondary populations within the biofilm (Alleman and Preston, 1991). Although TAN diffusion normally defines the rate of nitrification, nitrite oxidation should not always be presumed even under the conditions of a steady loading.

Monod relationships are used to describe the relationship between the rate of substrate utilization

Table 1
A variety of strategies are used in the design of biofilters to address oxygen supply and biofilm management issues (these underlying strategies dictate the media type and thus, the specific surface area of the units)

Filter	Oxygen transfer mechanism	Biofilm management	Specific surface area
RBC	Cascading	Sloughing	Low
Trickling filter	Cascading	Sloughing	Low
Submerged rock filter	Flow transport	None	Low
Submerged shell filter	Flow transport	None	Low
Submerged packed bed	Flow transport	None	Low
Upflow sand filter	Flow transport	Backwashing	High
Floating bead bioclarifier	Flow transport	Backwashing	High
Sponge filter	Flow transport	Backwashing	High
Fluidized sand bed	Flow transport	Continual abrasion	Very high
Microbead	Flow transport	Continual abrasion	Very high
Moving bed reactor	Direct aeration	Continual abrasion	Moderate

and substrate:

$$\mathrm{VTR} = \mathrm{VTR}_{\max}\left[\frac{\mathrm{TAN}}{k_A + \mathrm{TAN}}\right] \quad (1)$$

where VTR is the volumetric TAN conversion rate (g-N m^{-3} media day^{-1}), VTR_{max} the maximum VTR sustainable (g-N m^{-3} media day^{-1}), TAN the TAN concentration of the liquid medium (g-N m^{-3}) and k_A is the apparent half saturation constant (g-N m^{-3}).

An analogous equation can be formulated for nitrite conversion:

$$\mathrm{VNR} = \mathrm{VNR}_{\max}\left[\frac{\mathrm{NO_2}}{k_N + \mathrm{NO_2}}\right] \quad (2)$$

where VNR is the volumetric NO_2-N conversion rate (g-N m^{-3} media day^{-1}), VNR_{max} the maximum VNR sustainable (g-N m^{-3} media day^{-1}), NO_2 the NO_2-N concentration of the liquid medium (g-N m^{-3}) and k_N is the apparent half saturation constant (g-N m^{-3}).

These Monod kinetics are underpinned by Michaelis-Menton enzyme kinetics (Knowles et al., 1965; Malone et al., 1993) which presume substrate limitation within the context of an enzymatic reaction. Nitrification is normally controlled by diffusion of TAN through a water boundary layer and through the biofilm itself, a phenomenon that has been shown to be well represented by Monod type kinetics (Tanaka and Dunn, 1982; Harremoes, 1982). The impacts of diffusional characteristics on nitrite conversion are not as clearly defined as nitrite is generated and converted within the biofilm (Schramm et al., 1996). This kinetic approach projects filter performance in both the linear (low substrate) and zero order (high substrate) concentration regimes. The principle shortcoming stems from the fact that neither of the fixed parameters can be realistically expected to be constant across differing organic carbon loading regimes. VTR_{max} and VNR_{max} contain a hidden term reflecting the effective nitrifying biomass in the biofilm (Malone et al., 2005). The term clearly must increase proportionally to the nitrogen loading under the steady-state conditions of typical recirculating applications. The apparent half saturation constant, k_A (k_N), can be expected to be impacted by changes in carbon loading associated with feed increases. Net diffusional characteristics change with biofilm thickening and displacement of nitrifying populations by heterotrophs (Zhang and Bishop, 1994; Ohashi et al., 1996; Zhu and Chen, 2001). Eq. (1) is easily calibrated by direct measurements of flow and concentration from operating filters (Malone and Beecher, 2000):

$$\mathrm{VTR} = (\mathrm{TAN_I} - \mathrm{TAN_E})\frac{Q_r}{V_b} \quad (3)$$

where VTR is the volumetric TAN conversion rate (g-N m^{-3} media day^{-1}), Q_r the flow rate through the filter (m^3 day^{-1}), TAN_I the influent ammonia concentration (g-N m^{-3}), TAN_E the effluent ammonia concentration (g-N m^{-3}) and V_b is the total volume of media (m^3).

Likewise the volumetric nitrite-N conversion rate (VNR in g-N m^{-3} media day^{-1}) can be defined by Eq. (4):

$$\mathrm{VNR} = \mathrm{VTR} + (\mathrm{NO_{2I}} - \mathrm{NO_{2E}})\frac{Q_r}{V_b} \quad (4)$$

where NO_{2I} is the influent nitrite concentration (g-N m^{-3}) and NO_{2E} is the effluent nitrite concentration (g-N m^{-3}).

When the Monod Eqs. (1) and (2) are used in conjunction with careful calibration the relationships are useful sizing tools. Whenever it is used for design purposes it should be applied with a conservative safety factor. The sensitivity of biofilters to management and secondary factors such as the in situ nitrification should be recognized (Malone and Beecher, 2000).

3. Classification of recirculating systems

Using the TAN conversion rate as a foundation parameter for performance Malone and DeLos Reyes (1997) made an early attempt at rating biofilters. They observed that it was impossible to rate biofilters without first classifying the applications. Recognizing that the nitrification rate was the factor controlling the sizing of biofilters, they built the classification system upon factors that influenced the nitrification process. The end result was a three tiered system based upon organic loading with additional branching to address issues of salinity and temperature. A special low pH category was identified to address the needs of an ornamental fish industry that has a major focus on production of species originating in the acidic waters

draining from the rain forests of South America (Chapman et al., 1997).

The concept of trophic level, similar that used in the classification of lakes (Wetzel, 1983), was used to distinguish the level of nutrient enrichment. Production systems can be categorized as oligotrophic (severely nutrient limited), mesotrophic (moderate nutrient limitation), or eutrophic (nutrient enriched) with respect to their degree of nitrogen and biodegradable organic carbon levels. From an engineering perspective, the trophic level is normally defined by the aquaculturists based on their knowledge of the natural habitat of the target species. This selection in turn dictates the water quality conditions that the biofilter experiences, and thus, the nature of its biofilm. The trophic level terminology forms an important linkage between user expectations and biofilter performance characteristics. The trophic level analogy, however, is limited by the observation that parameters typically described in the context of the lakes trophic system, such as phosphorus and nitrate, are irrelevant. Within the context of the largely aerobic recirculating environment both can be in relatively high concentrations without significantly impacting the water column or biofilm ecology.

Although Malone and DeLos Reyes (1997) laid foundation for the current work, the industry, and thus the demands on the engineering community, have changed because of interest in marine species. These changes have identified the inability of the original classification to encompass the operations at both extremes of application in a new class of emerging marine ultra-oligotrophic larval production systems, hypereutrophic or greenwater/heterotrophic systems, and the acidic hypereutrophic systems.

Recent interest in expanding marine production has increased the demand for biosecure production systems used to produce fry and larvae for stocking (Turk et al., 1997; Watanabe et al., 1998; Malone, 2002; Otoshi et al., 2003; Pruder, 2004). The water quality expectations for these systems can fall well below the 0.3 g-N m^{-3} TAN standard set for the oligotrophic classification. A reasonable maximum standard for these systems is 0.1 g-N m^{-3} TAN. Use of the biofilters sized to the oligotrophic standard can cause serious performance problems and justify the creation of an ultra-oligotrophic classification. A number of larval growout systems use of live algae feed and some systems supporting the earliest stages operate as greenwater systems.

On the other extreme, continued production of hardy species such as tilapia (Avnimelech, 1988) and shrimp (Burford and Longmore, 2001) in greenwater or heterotrophic suspended growth systems has clearly demonstrated the ability of these organisms to the thrive at higher TAN and nitrite-N concentrations than originally allowed within the context of the eutrophic classification. These experiences are complemented by findings from freshwater production of alligators (Malone and DeLos Reyes, 1997; Malone et al., 1990) and tilapia (Lutz, 1997) in more traditional fixed film formats. Clearly, this hypereutrophic production strategy must be considered in future classifications.

Inclusion of greenwater applications in the ultra-oligotrophic and hypereutrophic categories strains the presumption that biodegradable organic carbon and nitrogen loading will be approximately proportional. The algae are largely carbon and their application to a biofilter will dramatically increase the carbon loading. This observation is partially mitigated by the fact that live algal cells can be considered refractory as they resist decay in biofilters. At the same time, the presence of algal cells clearly opens up nitrogen assimilation pathways that contrast with the pathways in heterotrophic systems. Yet, in the authors' experience, biofilters operated in eutrophic greenwater systems display carrying capacities similar to heterotrophic systems perhaps reflecting the robust nature of biofilms. The matter, however, warrants further investigation.

Fig. 2 presents a modification to the system classification tree presented by Malone and DeLos Reyes (1997). This classification considers addition of ultra-oligotrophic and hypereutrophic at each tier based upon existing or anticipated commercial use. The net effect is the additional two new marine ultra-oligotrophic classes (cold and warmwater) to accommodate larval production systems. Warmwater hypereutrophic classes are added to both marine and freshwater categories to reflect the growing practice of rearing animals under high substrate regimes. The European practice of operating systems at high TAN levels while suppressing toxicity through pH manipulation (Eding and Kamstra, 2001) warrants the addition of a final freshwater classification, acidic-hypereutrophic.

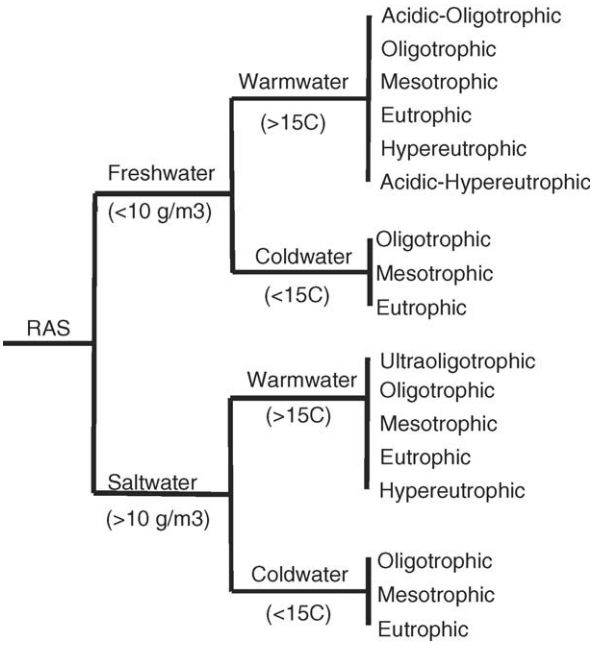

Fig. 2. The proposed recirculating system classification is based upon salinity, temperature and trophic levels.

4. Biofilter classifications

One of the primary premises in the establishment of a biofilter classification to support a comparative biofilter rating system is that each category carries with it distinctive characteristics that warrant a change in engineering design criteria from adjacent categories. Thus, the system classification must be re-examined to determine whether similar branching is required within the biofilter classification. This decision process is complicated by the profound effect that biofilter management details can have on nitrification performance. Further, it is conducted under the assumption that the majority of design guidelines will inherently have safety factors of the order of 1.5–2.

We narrow this discussion to factors impacting the performance of biofilters that are operated in a steady-state mode (on a daily time scale) with respect to their organic and nitrogen loading. This condition most closely parallels the conditions of typical recirculating applications where the loading is typically increased slowly as the stock grows. With this assumption, transitional phenomenon (shock loading, acclimation) fall from consideration. Additionally, parameters normally considered within the prevue of management (backwashing, oxygen supply, and routine pH control) are disregarded.

It is clear from the earlier discussions that water quality, specifically the concentration regime of the limiting nutrient (TAN and/or nitrite-N), is extremely important in defining the conversion capabilities of any fixed film biofilter. Thus, the nitrogen loading would seem to be an essential component. From a biofiltration perspective, the strength of this approach stems from the observation that the organic carbon loading and nitrogen loading to a biofilter is linked through the feed addition to the system. A lightly loaded biofilter, measured in terms of kilograms of feed applied per cubic meter of media per day or kg m^{-3} day^{-1} will necessarily experience a light loading with respect to carbon (thin biofilm) and nitrogen (first order kinetics). Conversely, heavily loaded systems will be categorized by thick films and potentially zero order kinetics as the heterotrophic bacteria biofilm thickness begins to dominate the process (Greiner and Timmons, 1998; Zhu and Chen, 1999). There is a close linkage between the tropic level of the system and the biofilter. The demands on the filters differ greatly as the enrichment of the systems change. The maintenance of the five trophic tiers appears warranted as a basis for a biofilter classification.

It is interesting to note at the time of the Malone and Beecher (2000) study, the trophic level classification was the only aspect of the system classification system to be reported in conjunction with biofilter sizing criteria. After nearly a decade of sizing floating bead bioclarifiers in close conjunction with both a manufacturing company and numerous end users, these authors saw no pragmatic need to generate a secondary biofilter sizing criteria based on temperature or salinity changes.

The division between marine and freshwater system seems justifiable at the system level based on the effect salinity has on the saturation level and transfer kinetics of oxygen, clarifier performance (specifically foam fractionators), and impact on carbon dioxide stripping, but, the impact on biofilter nitrification performance is not clear. No doubt, there are differences in the biofilms associated with biofilters operated in freshwater or saltwater (Nijhof and Bovendeur, 1990; Hovanec and DeLong, 1996),

but in the practical design environment those differences are mitigated by liberal safety factors, by changes in bacterial densities, or changes in operational policies. It appears that a sizing criteria dependent upon salinity is not warranted at this time. Secondary factors in this regard do exist, however; most important from a practical perspective is the extension of acclimation times for the nitrite converting bacteria dominating marine systems (Gutierrez-Wing and Malone, 2005).

In fact, even the cold/warmwater branching utilized in the system classification is difficult to justify from the biofiltration perspective. Originally assumed to be an important factor in biofilter design, reflecting its generic importance as a kinetic modifier, temperature is increasingly being viewed as a minor factor in controlling biofilter carrying capacities. Zhu and Chen (2002) found temperature had little effect on the conversion abilities of moving bed reactors. These results are supported by reports of temperature effects on operating water and wastewater filters. Payraudeau et al. (2000), for example, states that temperature-induced reductions of nitrification capacity only occurred in nitrifying activated sludge filters below 14 °C and then, only when the filters were subject to high C/N loads. They observed no practical impact of temperature in the range of 14–28 °C. Andersson et al. (2001) concluded no impact above 10 °C in their study of granular carbon filters with declining performance to 4 °C where their filters effectively shutdown. Mann et al. (1998), working with granular plastic filters, illustrated a declining trend in the range 20–10 °C, but this relationship is noisy and almost overshadowed by hydraulic and organic loading variations. These results suggest the chemoautrophic population is able to adapt, through natural selection and density, until a physical factor, such as diffusion, again limits. Without ignoring the underlying importance of temperature as a kinetic parameter, it is feasible, in the pragmatic sense, to neglect temperature impacts for the vast majority of RAS biofilter applications. Contrary results, however, do exist and the matter may warrant revisiting to determine the limits of the assumption (Wortman and Wheaton, 1991). Elimination of temperature as a design parameter does not, however, alter the demand for changes in management strategies as low temperatures can reduce the growth rate of nitrifying bacteria. Cold water systems are more susceptible to overwashing, acclimation or shock loading problems, all secondary parameters worthy of consideration when selecting a biofiltration platform.

Trophic level definitions based upon TAN concentration regime, is the most appropriate approach to the establishment of a baseline biofilter classification for the rating system. The linkage between filter conversion capabilities and limiting substrate concentration (TAN) is clearly established. The user group associates TAN concentrations with their applications often using TAN as the primary specification for system designs. TAN levels are supported by more subtle linkages that exist between nitrogen concentration and residual biochemical oxygen demand. That is to say, a given TAN specification can be used to project proximate ranges for secondary parameters, such as biochemical oxygen demand that may be of importance in defining a biofilter's nitrification performance.

Table 2 defines TAN range for each of the five trophic level classifications. This classification reflects and expansion of the approach described by Malone and DeLos Reyes (1997) which was based on three trophic levels. Alternate application terminology can be provided to facilitate presentation of the classification to the end user groups that may be uncomfortable with the more scientific trophic classification. The oligotrophic category is supplemented by an acidic category established to serve the needs of the substantial recirculating industry that exists worldwide to produce ornamental fish derived from acidic waters. An acidic-hypereutrophic category is also added.

Table 2
A six-tiered biofiltration classification is suggested to serve the needs of the recirculating aquaculture community (it is based, primarily, on trophic levels; a specialized category was established to serve the acid oligotrophic needs of the freshwater ornamental fish industry)

Class	Application	TAN/nitrite performance range (g-N m^{-3})
Ultra-oligotrophic	Larval	0.0–0.1
Acidic-oligotrophic	Ornamental	0.1–0.3
Oligotrophic	Broodstock	0.1–0.3
Mesotrophic	Fingerling	0.3–0.5
Eutrophic	Growout	0.5–1.0
Hypereutrophic	Hardy growout	1.0–5.0
Acidic-hypereutrophic	Hardy growout	1.0–20

5. Standardized fixed film capacities (SFC)

Nitrification capacity of a biofilter is complicated by the sensitivity of the nitrifying population to a variety of water quality factors (Belser, 1979). The loading history and environmental conditions to which the biofilm has been subjected needs to be given careful consideration when evaluating a filter's nitrification performance. A standardized experimental approach is proposed here to limit the impact of these secondary factors in the capacity determination.

A chemical feed used in the filter rating studies should display a fixed C/N ratio that is reflective of a biofilter load generated by feed possessing a mid-range protein content (45%) feed in a typical clarifier-biofilter configuration. Reduction in carbon loading will induce a falsely high conversion capacity (Zhu and Chen, 2001). A synthetic substrate solution containing ammonium carbonate $(NH_4)_2CO_3$, sodium bicarbonate and other necessary macro-nutrients (Table 3) should be used to achieve the ammonia-nitrogen concentrations necessary for filter evaluation. As suggested by Zhu and Chen (2001) in their studies of the effects of organic carbon on the nitrification rates of fixed film biofilters, sucrose ($C_{12}H_{22}O_{11}$) should be used as the carbon source. Zhu and Chen (2001) provide a solid case for the use of sucrose as the carbon source as it relates well with COD and BOD_5 measurements that can be directly related to feeding rates. Early experimental work conducted under this protocol should attempt to verify that the sucrose feed generates a heterotrophic response reflective of commercial operation.

The experimental configuration illustrated in Fig. 3 is suggested for chemical addition. The stock nutrient feed should be continuously fed into a sump that is supported by a recirculating loop containing the biofilter under evaluation. To assure complete mixing of the substrate the sump volume should be at a minimum, five times the maximum recycle flow rate. The continuous chemical substrate is supplemented by an independent flushing flow of dechlorinated tap water (alkalinity adjusted) to assure a whole system turnover every 10 days. Setting the total system hydraulic retention time (HRT) to 10 days assures that potential problems associated with accumulation of dosed chemicals and or salts are avoided. Heating or chilling elements can also be added to the sump as required to maintain the temperature within 0.5 °C of the targeted 20 °C.

Carbonate levels are adjusted to the specified level in the flushing flow by the addition of sodium bicarbonate or hydrochloric acid. The pH of the sump should be adjusted, if necessary, by diffusing low levels of carbon dioxide or conversely, by purging carbon dioxide with blown air (Thomasson, 1991). An aqueous carbon dioxide level of 10 g m^{-3} can be expected to hold the pH at 7.5 for the standard classifications (alkalinity = 150 g-$CaCO_3$ m^{-3}) and at 6.3 for the acidic classes (alkalinity = 10 g-$CaCO_3$ m^{-3}). This strategy depends on the carbonate buffering system rather than the phosphate buffers in the chemical feed thus mimicking pH behavior in recirculating systems (Loyless and Malone, 1997). The strategy also allows the potential benefits of secondary gas exchange, which occurs in aerated or emergent filters, to be appropriately reflected in the results. A phosphate buffer solution used by Ohashi et al. (1996) in their studies of C/N ratios and multi-species biofilms was not adequate to keep the environmental conditions in their studies near pH 7 and consequently a NaOH solution was introduced for pH management. Sanni and Forsberg (1996) and Hargreaves et al. (2000) provide further background on dynamic pH control for salt and freshwater systems, respectively.

All filters should be brought to full initial acclimation prior to data collection. At the target temperature it is presumed at least 30 days is required and will be evidenced by stable TAN and nitrite levels appropriate to the level of loading (Manthe et al., 1985; Hovanec et al., 1998; Seo et al., 2001). Initial acclimation is known to be problematic for both

Table 3
Composition of synthetic substrate as an ammonia feed source in biofilter nitrification studies (adopted from Zhu and Chen, 2001 and Liu and Capdeville, 1994)

Ingredient	Composition
$(NH_4)_2CO_3$ (g)	1247
$NaHCO_3$ (g)	3500
$MgSO_47H_20$ (g)	36
Na_2HPO_4 (g)	159
KH_2PO_4 (g)	153
$FeCl_36H_2O$ (g)	5
Water (L)	140

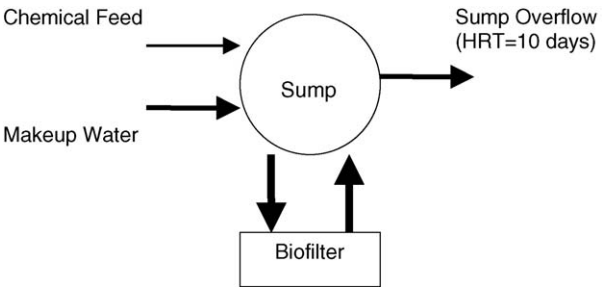

Fig. 3. Substrate transfers in the experimental recirculating aquaculture systems for filter performance studies.

marine and coldwater systems. Full acclimation of a filter can require 2–3 months (Mann and Rittmann, 1992) with chemical dosing or heating strategies used to accelerate the process (Manthe and Malone, 1987). Three samples should be collected over a 24 h period for 3 days to establish a mean response of the filter for the categories subject to linear regression. Collection of four samples over 4 days is indicated for the hypereutrophic categories. Temporal trending of data should be considered evidence of non-steady-state conditions. A period of no less than a week should be reserved for acclimation following adjustment of the nutrient feed rate or the C/N ratio. Evaluations should be conducted through stepwise increases in substrate concentration since the filter's loss of capacity as it approaches a new steady-state is much slower than its rise (Boller et al., 1997).

5.1. Filter analysis

In the development of design equations for biofilters, generally the approach has been empirical, relating filter influent and effluent ammonia concentrations as a function of flow rates; media surface area, dissolved oxygen, temperature, pH, feed loading rates and fish biomass (Wheaton, 1977; Lawson, 1994; Timmons et al., 2001). A purely empirical approach allows design engineers to specify a particular filter design for a given biomass load, system configuration, and other constraints (water availability, oxygen supply, budget). Under the SFC approach, a set of criteria will eventually become available for sizing and utilizing biofilters for specific applications as presented in Fig. 2. In Table 4, suggested sample collection locations and acceptable ranges for the standardized fixed film biofilter carrying capacity determinations are presented. Chemical oxygen demand is suggested as an appropriate measure for biodegradable organic carbon under the conditions of the test. This selection is dependent upon the source of carbon used and the maintenance of a HRT at 10 days as the buildup of refractory organic compounds can cause a divergence between COD and the more appropriate, but burdensome, BOD measurements (Christensen et al., 2000). Initial studies conducted under this protocol should verify that the COD and BOD divergence is minor. Refractory organics accumulation can be reduced by decreasing the HRT.

Table 4
Sample collection locations and acceptable ranges for the suggested standardized fixed film biofilter carrying capacity determinations

Parameter	Collection location (acceptable ranges)		Comment
	Sump	Filter effluent	
Temperature	20 ± 0.5 °C	20 ± 0.5 °C	
Dissolved oxygen	>5.0 g m^{-3}	>3.0 g m^{-3}	Maintain by aeration of sump
Alkalinity standard (Acidic)	150 g-$CaCO_3$ m^{-3} (10 g-$CaCO_3$ m^{-3})		Adjust in makeup water
pH standard (Acidic)	7.7 ± 0.1 (6.3 ± 0.1)		Control by aqueous CO_2 manipulation in sump
TAN			Match to category
Nitrite-N			
COD			Loading controlled by chemical feed
Recycle flow			Set by biofilter
Makeup flow	HRT = 10 days		HRT based upon whole system

Table 5
Suggested sampling and statistical analysis for standardized fixed film biofilter performance determinations

Class	Sampling range	Statistical analysis
Ultra-oligotrophic (0.0–0.1 g-N m^{-3})	3 per day for 3 days ($N = 9$)	Linear analysis
Oligotrohic (0.1–0.3 g-N m^{-3})	3 per day for 3 days ($N = 9$)	Linear analysis
Mesotrophic (0.3–0.5 g-N m^{-3})	3 per day for 3 days ($N = 9$)	Linear analysis
Eutrophic (0.5–1.0 g-N m^{-3})	3 per day for 3 days ($N = 9$)	Linear analysis
Hypereutrophic (1.0–5.0 g-N m^{-3})	4 per day for 4 days ($N = 16$)	Averaging

Criteria is proposed for data reduction and performance evaluation (Table 5). The parameter that will be used for estimating the unit's capacity is the volumetric nitrification rate, VTR. In all cases, it is proposed that this determination be based upon standardized experimental approach using chemical substrate feed. In the case of the two oligotrophic, the mesotrophic and the eutrophic regimes, data will be used as a basis for a linear regression under the assumption of a zero intercept. The resulting slope is multiplied by the mean concentration in the class to obtain a mean conversion capability in terms of g-N m^{-3} day^{-1}. The hypereutrophic classes, being much more subject to variability due to organic loading, will have their mean performance estimated directly from a mean calculated from observed conversion rates (g-N m^{-3} day^{-1}) at evenly distributed points across the range.

For the linear regression, a plot of the relationship between ammonia concentration (TAN, filter TAN inflow) and removal rate (VTR) is used to fit the direct linear model that can be expressed in the following equation as:

$$\mathrm{VTR} = m\mathrm{TAN} + b \tag{5}$$

where the parameter m is the slope of the line relating VTR and TAN while the parameter b is the y-intercept of the equation. As stated earlier in the case of the two oligotrophic, mesotrophic, and eutrophic regimes, data used as a basis for a linear regression will be under the assumption of a zero intercept (Malone et al., 2005). Consequently, the value of b in Eq. (5) is zero and a new slope, τ, is used for the remainder of the analysis with the linear relationship between VTR and TAN computed as:

$$\mathrm{VTR} = \tau\mathrm{TAN} \tag{6}$$

where τ is the normalized conversion capacity relating VTR and TAN. The units of τ are g TAN converted per m^3 of filter media per day per g TAN m^{-3} or, for simplicity day^{-1}.

6. Summary

A recirculation system classification that considers trophic level, salinity, temperature and pH was simplified by the level of commercial activity to define 17 distinctive system categories that may demand unique engineering sizing criteria when the aeration, carbon dioxide stripping, circulation, solids capture and/or biofiltration demands of the system are considered. A flexible seven tier biofiltration classification system based on trophic level is devised to serve the RAS community. A standardized experimental approach that uses a C/N balanced chemical feed solution is proposed for use with an assessment of steady-state TAN and nitrite-N conversion capabilities. A statistical approach based upon linear regression and simple averaging is suggested to provide estimation of conversion capacities that can be used to support comparative analysis and design activities. A specialized assessment may be required for low pH oligotrophic systems.

7. Recommendations

Assumptions made as a means of simplifying the fixed film classification need to be re-examined and/or verified by additional experimental work. Specific attention should be given to the limits of the assumptions that temperature and salinity are not major factors in the determination of carrying capacities.

The experimental and analysis associated with the proposed standardized biofilm capacities should be

implemented by researchers with the intent of providing a demonstration of the approach and/or as a means of refinement. Specific issues that would appear to warrant further investigation include the following:

1. The adequacy of the proposed chemical feed to supply trace elements under basic and acidic operational regimes.
2. The applicability of ratings generated under the SFC (heterotrophic) approach to fed greenwater (photoautrophic) systems.
3. The applicability of C/N ratios, controlled by SFC chemical feed solution, needs to be verified by experiments to assure that the sucrose dose is inducing heterotrophic activity within the biofilm that is consistent with a 45% protein feed.
4. The SFC assumption, with regards to lack of temperature effects, needs to be investigated experimentally and the feasibility of using an Arhenius type equation as a means of extending the temperature range of the SFC should be established.

A classification and rating strategy is only valuable if it is endorsed and ultimately adopted by the community that it serves. Review and refinement of the proposals put forth here should be considered by the Aquacultural Engineering Society.

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