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Bioremediation of Nitrite from Brackishwater Using Lignocellulosic Agricultural Waste - Bagasse

KISHORE K. KRISHNANI*, V. PARIMALA, B.P. GUPTA, I.S. AZAD and M.S.SHEKHAR

Central Institute of Brackishwater Aquaculture
75, Santhome High Road, R.A.Puram
Chennai, 600028, India

Abstract

Bagasse is a complex native lignocellulosic agricultural waste left after extraction of juice from sugar cane in sugar mills. Bagasse is commonly used as a captive boiler fuel aside from its minor use as a raw material in the paper industry and in low-value products. There remains an ever present need to convert this material to useful value added products, which is an objective of our continued research. The aim of the present study is to investigate four different materials prepared from bagasse for the removal of nitrite from shrimp farm brackishwater (salinity 27 ± 1 ppt) in laboratory condition. The experimental results showed that nitrite removal is effective using bagasse materials with the dose of 1 and 3 $\text{g}\cdot\text{L}^{-1}$. Raw bagasse fiber was found to be most effective followed by dried bagasse powder, which can be attributed to the rapid increase in bacterial counts and periphytic growth combined with ion exchange mechanism. Effect of bagasse materials on other water characteristics such as pH, salinity, alkalinity, dissolved oxygen, ammonia and phosphates has also been studied. The very low cost of lignocellulosic materials is a real advantage that renders it as a suitable alternative for the remediation of nitrite from aquaculture water.

* Corresponding author.
E-mail address: bioremediationk@yahoo.co.in

Introduction

The rearing of marine and brackishwater organisms results in metabolic wastes. Ammonia and nitrite are the most common toxicants in intensive culture systems (Colt and Armstrong 1981). Ammonia is the major end product of protein catabolism, which can be oxidized into toxic nitrite. Nitrite is an intermediate product in bacterial nitrification and denitrification processes. An imbalance in either of these processes can lead to elevated ambient nitrite concentrations. Significant amounts of nitrite enter the haemolymph in shrimps via diffusion (Chen and Chen 1992) and can oxidize haemocyanin into methaemocyanin at low pH (Jensen 1996). The toxicity of nitrite is known to be affected by water pH and it increases with increasing pH. Shrimp growth can be adversely affected by high concentrations of ammonia and nitrite and in extreme cases cause mortality (Wickins 1976). The safe level for *P. monodon* juveniles (Chen and Lei 1990) was estimated to be $3.8 \text{ mg}\cdot\text{L}^{-1} \text{ NO}_2\text{-N}$ and it has also been reported that the toxicity of nitrite-N on juveniles was less than the toxicity of total ammonia-N on juveniles during the first 96 hours of exposure. However, the toxicity of nitrite-N was similar to that of total ammonia-N after 96 hours of exposure.

Successful aqua farming requires the safe removal of this metabolic waste from aquatic environments. In regard to maintenance of healthy ecology in aquaculture ponds and prevention and control of disease infection among shrimps towards sustainable development of the aquaculture sector, the best way to achieve these is to adopt eco friendly aquaculture practices through minimization of adverse impact of the activity on the surrounding environment. This concept can only be effectively applied through bioremediation (Krishnani et al. 2002; 2003; 2004). There are certain plants that possess the natural ability to uptake inorganic pollutants. In addition, plants release exudates and enzymes that stimulate microbial activity and biochemical transformation, which subsequently bioremediate the pollutants. This process is often referred to as plant assisted bioremediation. A coastal area is rich in sea weeds and sea grasses, which serve as substrates for periphyton. Periphyton is important for various reasons: as a major contributor to carbon fixation and nutrient cycling in aquatic ecosystems; as an important source of food in aquatic systems; as an indicator of environmental change; it can improve water quality hence it can be used in waste water treatment; it can greatly increase aquaculture production. Algae and bacterial biofilm are able to form a symbiotic relationship- what

one needs, the other provides. The major advantage of biofilm formation is that the biofilms provide protection from the effects of an adverse environment. The multi species culture can provide and maintain the appropriate physical and chemical environments for growth and survival (Costerton 1995).

An agricultural country generates considerable amount of agricultural waste material such as bagasse, which is a natural highly fibrous lignocellulosic byproduct of sugar cane. It is reported that around 8 million tons of dry bagasse was produced in India in the year 2001 (Khan et al. 2004). Bulking agents such as bagasse not only improve soil porosity, but also provide a carbon source, due to its high carbohydrate content (Pandey et al. 2000). Bagasse is a biodegradable substrate, which harbours higher periphytic biomass unlike nonbio degradable ones. This could be because biodegradable substrates provide a better surface structure for periphytic species to attach to, or they may leach nutrients beneficial for the growth of periphyton, predominantly consisting of bacteria (Krishnani et al. 2004). Periphyton has more than one role in aquaculture. It improves fish/shrimp production and water quality, thus enhancing the efficiency of aquaculture systems. Due to numerous carboxylic groups present in polysaccharide and ion exchange, this natural adsorbent is able to remove some of the heavy metals (Reddad et al. 2002; Krishnani et al. 2004; Krishnani and Ayyappan 2006). In addition, this material has been converted into an anion exchanger for the removal of nitrate (Orlando et al. 2002). However, reports on bagasse assisted bioremediation of shrimp culture water are scanty. In the present paper, a study has been carried out, probably for the first time, to investigate the use of four different kinds of materials from bagasse in the removal of nitrite from brackishwater.

Materials and Methods

Materials

Four different types of materials were prepared from bagasse

- i. Raw bagasse fiber (RBF): This was dried in oven at 50°C in order to get constant moisture content.
- ii. Dried bagasse fiber (DBF): Raw bagasse fiber (RBF) was thoroughly washed with water and then dried in oven.

- iii. Dried bagasse powder (OBP): Dried material (DBF) was powdered.
- iv. Charred material (CBP): Dried material (DBF) was charred at 250°C and then powdered.

Procedure

The salinity of brackishwater collected from a shrimp farm for the experiment was 27 ± 1 ppt. Initial pH, alkalinity, dissolved oxygen, total ammonia-nitrogen (TAN), nitrite-N and phosphate were 7.85, 75 as CaCO_3 $\text{mg}\cdot\text{L}^{-1}$, $7.2 \text{ mg}\cdot\text{L}^{-1}$, $0.125 \text{ mg}\cdot\text{L}^{-1}$, $0.92 \text{ mg}\cdot\text{L}^{-1}$ and $0.10 \text{ mg}\cdot\text{L}^{-1}$, respectively. An appropriate amount of potassium nitrite (E. Merck) was added separately in brackishwater to attain various initial nitrite concentrations of 1.524, 2.015, 2.514 and $5.045 \text{ mg}\cdot\text{L}^{-1}$. For each experimental run, 10 litres of brackishwater were taken in aquarium jars containing soil base and were treated only once with four different kinds of bagasse with the dose of 1, 3 and $6 \text{ g}\cdot\text{L}^{-1}$ with a separate set of controls. In all the treatments and control an intermittent stirring every after 24 hours was done using sterile glass rod and no aeration was given throughout the experiment as initial DO of brackishwater was $7.2 \text{ mg}\cdot\text{L}^{-1}$. This was done in order to prevent nitrite loss, if any, due to aeration alone. Further, water samples were analyzed for measurement of nitrite concentrations at daily intervals for a period of 6 days. In all the treatments and controls, before sampling of water for analysis, brackishwater in jars was stirred homogenously using sterile glass rod and then allowed to settle for 15 minutes.

The following experiments were also conducted to know the effect of indigenous micro-organisms on nitrite removal; (i). brackishwater was treated with bagasse fibre with the dose of $6 \text{ g}\cdot\text{L}^{-1}$ and then treated water samples were collected in 1 l glass jars at different time intervals from 24 to 432 h. An appropriate amount of potassium nitrite was added in brackishwater to attain various nitrite concentrations ranging from 0.424 to $1.01 \text{ mg}\cdot\text{L}^{-1}$ to study the effect of aged brackishwater treated with bagasse on nitrite removal. Further, water samples were analyzed for measurement of nitrite concentrations at different time intervals from 24 to 96 h. (ii). sterile brackishwater (SBW) and non sterile brackishwater (BW) were treated with sterile RBF (SRBF) and non sterile RBF (RBF) with the dose of $1 \text{ mg}\cdot\text{L}^{-1}$.

Water was collected from the soil-water interface from the treatment jar, in which the superficial soil was also mixed. The water and soil suspension was mixed, sedimented and passed through a clean sterile

coarse filter paper. The clean filtrate was passed through a sterile 0.25 μ membrane filter. The membrane was used vortexed thoroughly in sterile phosphate buffered saline (PBS, pH 7.2). The volume of PBS was adjusted to the original water volume using fresh sterile PBS and saved for further work.

The above suspension was used for developing the biofilm, wherein 15% nutrient alkaline peptone medium was used. RBF and DBF were used as substrates. The flasks with the nutrient medium and the substrates were sterilized and inoculated with 5 ml of the prepared jar water suspension. Biofilm development was facilitated by keeping the flasks over a mechanical rotor for half an hour at hourly intervals. Aseptic samples were drawn from the flasks on a daily basis up to four days.

The substrate was taken into sterile centrifuge tubes and washed once with sterile PBS before suspending in a known volume of fresh PBS. The substrate was vortexed thoroughly for 2-3 min to dislodge the biofilm cells. This supernatant was plated on to Zobell's Marine Agar to determine the total plate count using spread plate method. The count was expressed as cfu per gram of substrate. For determination of total plate count in brackishwater, the sample from treatment jars were also subjected to spread plate method.

In order to get an image of the biofilm, the wet bagasse fibres (RBF and DBF) were placed on clean glass slides and observed as wet mounts under the microscope (Nikon) and the microphotographs were taken using a digital camera (Nikon Cool Pix 4500).

Analysis

Nitrite in brackishwater was analysed at daily intervals following a standard method (Strickland and Parsons 1972) using UV-Visible spectrophotometer (Hitachi-U-2000). Other parameters such as pH, salinity, alkalinity, dissolved oxygen (DO), total ammonia nitrogen, phosphates and total plate counts in brackishwater were analyzed following standard methods (Strickland and Parsons 1972; Clesceri et al. 1989).

Statistical analysis

The data was statistically analyzed using 5 (duration) x 4 (materials) Factorial Completely Randomized design with two replications for each dose. Duncan's multiple range test was applied to identify significant

differences between main effects and interaction effects. M-STATC statistical software was employed to perform statistical analysis.

Results

Determination of effective amount of bagasse for nitrite removal

The effect of four different materials from bagasse with the dose of $1 \text{ g}\cdot\text{L}^{-1}$, $3 \text{ g}\cdot\text{L}^{-1}$ and $6 \text{ g}\cdot\text{L}^{-1}$ on the removal of $0.92 \text{ mg}\cdot\text{L}^{-1}$ nitrite is presented in figure 1 (a, b and c). This shows that raw bagasse fiber (RBF) is most effective in nitrite removal followed by dried bagasse powder (OBP) as they removed initial nitrite concentrations of $0.92 \text{ mg}\cdot\text{L}^{-1}$ to the extent of 100% in 24 h. In the treatment with DBF and CBP with the dose of $1 \text{ g}\cdot\text{L}^{-1}$, percent nitrite removal were 9% and 5% in 24 h, 15% and 29% in 48 h, 35% and 83% in 96 h and 58% and 99% in 120 h, respectively. In the treatment with DBF and CBP with the dose of $3 \text{ g}\cdot\text{L}^{-1}$, percent removal was only 12% in 24 h. There was a further decline in nitrite concentration and percent nitrite removal were 24% and 42% in 48 h and 91% and 100% in 96 h, respectively. In the treatment with the dose of $6 \text{ g}\cdot\text{L}^{-1}$, DBF and CBP decreased nitrite concentration to the extent of 55% and 13% in 24 h and 84% and 78% in 48 h respectively, while 100% removal was achieved in 96 h.

Effect of initial nitrite concentration on nitrite removal using RBF

The effect of initial nitrite concentrations at 0.535, 0.920, 1.524, 2.015, 2.514 and $5.045 \text{ mg}\cdot\text{L}^{-1}$ on the nitrite removal with RBF with the dose of $1 \text{ g}\cdot\text{L}^{-1}$ is shown in figure 2(a), the % nitrite removal appears to be decreasing from these initial concentrations to nil (100%), nil (100%), 0.458 (70%), 0.8463 (58%), 1.383 (45%) and $3.128 \text{ mg}\cdot\text{L}^{-1}$ (38%) in 24 h, respectively. In the case of 1.524 and $2.015 \text{ mg}\cdot\text{L}^{-1}$ initial concentrations, 100% nitrite removal was achieved in 48 h. Results shows that percent nitrite removal was found to decrease with an increase in initial nitrite concentration.

Effect of sterile and nonsterile RBF on nitrite removal from brackishwater

The effect of sterile and nonsterile RBF at $1 \text{ g}\cdot\text{L}^{-1}$ on nitrite removal from brackishwater is shown in figure 2(b). This indicates that nitrite

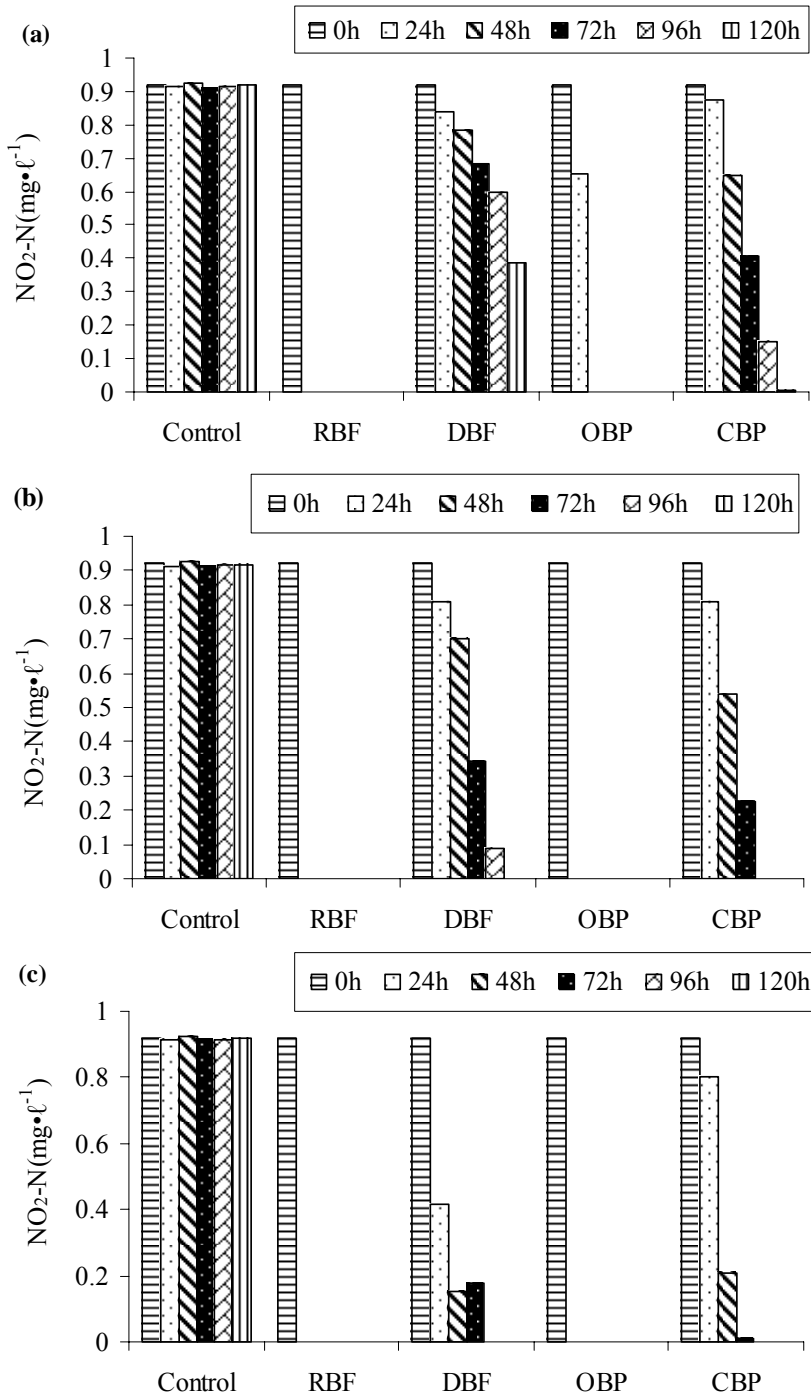


Fig. 1. Effect of bagasse products on the nitrite removal (a). 1 g·L⁻¹ (b). 3 g·L⁻¹ (c). 6 g·L⁻¹

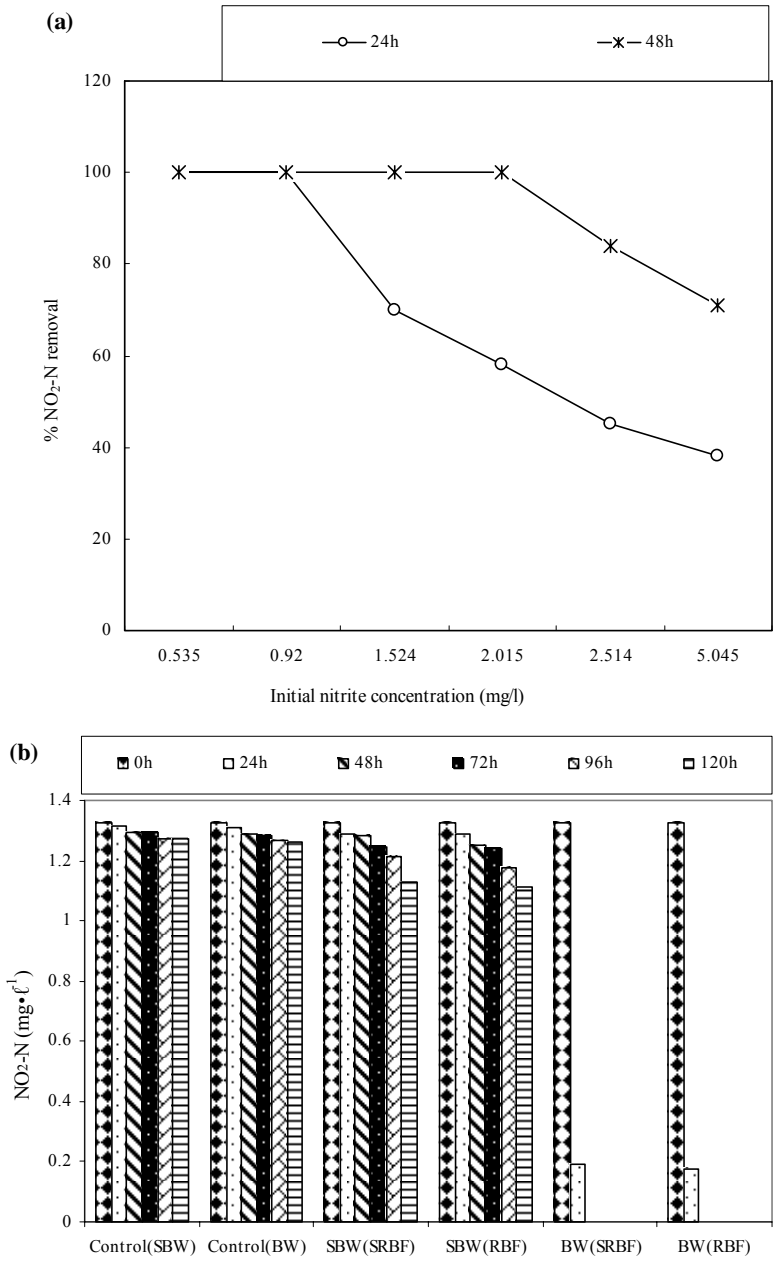


Fig. 2. (a). Effect on initial nitrite concentration and (b). sterile brackishwater on nitrite removal using RBF (1 g·L⁻¹). (BW: Brackishwater, SBW: Sterile brackishwater, SRBF: Sterile RBF)

removal is highest with nonsterile brackishwater treated with nonsterile RBF (RBF) and sterile RBF (SRBF), which remove initial nitrite concentration of $1.326 \text{ mg}\cdot\text{L}^{-1}$ to 100% in 48 hours, whereas in the case of sterile brackishwater (SBW) treated with RBF and SRBF, nitrite removal was comparatively very less and it was in the range of 15-16% in 120h.

Effect of aged water treated with bagasse on nitrite removal

Effect of 24 h, 72 h, 96 h and 432 h aged water on nitrite removal is presented in Fig. 3. This shows that 24 h aged brackishwater has removed initial nitrite concentration of $0.454 \text{ mg}\cdot\text{L}^{-1}$ to the extent of 100% within 24 h (Fig. 3a), whereas in the treatment with 72 h aged water for the initial nitrite concentration of $0.424 \text{ mg}\cdot\text{L}^{-1}$, percent nitrite removal were 54% and 94% in 24 h and 48 h respectively (Fig. 3b). In the case of treatment with 96h aged water with the initial nitrite concentration of $1.01 \text{ mg}\cdot\text{L}^{-1}$, 40%, 63% and 94% nitrite removal were achieved in 24 h, 48 h and 96 h respectively (Fig. 3c). In the case of treatment with 432 h aged water with initial nitrite concentration of $0.532 \text{ mg}\cdot\text{L}^{-1}$, nitrite removal were 40%, 67% and 100% in 24 h, 48 h and 96 h respectively (Fig. 3d).

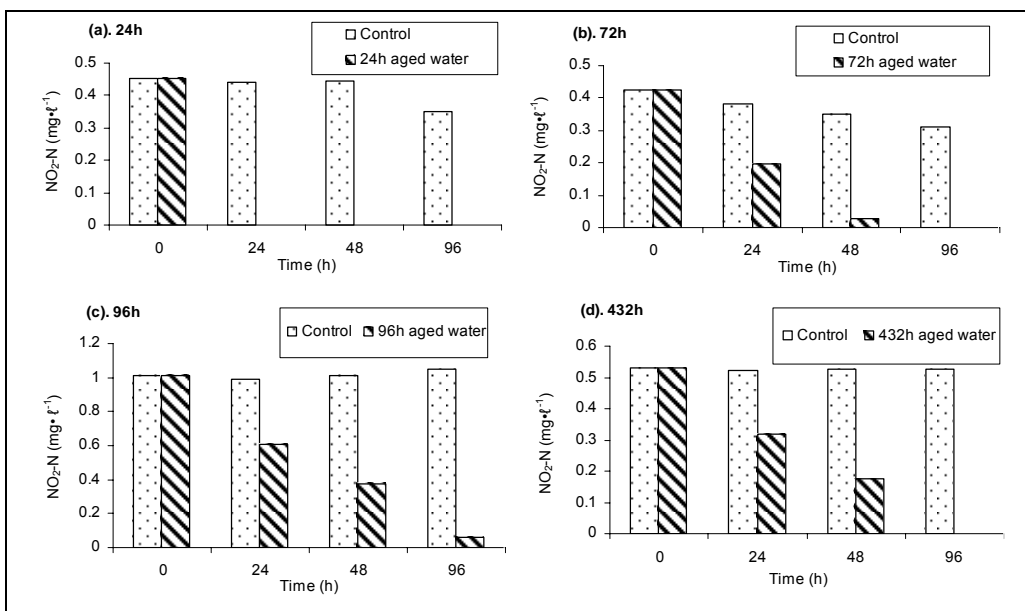


Fig. 3. Effect of aged water on nitrite removal

Effect of bagasse on other water quality parameters

During the course of the experiment, other water quality parameters such as salinity and alkalinity did not show much changes with the treatments using bagasse materials and they ranged from 26-28 ppt and 65-75 as $\text{CaCO}_3 \text{ mg}\cdot\text{L}^{-1}$ respectively. The RBF, DBF, OBP and CBP with a dose of $1 \text{ g}\cdot\text{L}^{-1}$ decreased pH from 7.85 to 7.11, 7.67, 7.36 and 7.54, DO from 7.2 to 1.6, 4.0, 2.2 and $4.8 \text{ mg}\cdot\text{L}^{-1}$, and ammonia from $0.125 \text{ mg}\cdot\text{L}^{-1}$ to nil, 0.024, nil and $0.112 \text{ mg}\cdot\text{L}^{-1}$ within 24 h respectively. These materials with the dose of $1 \text{ g}\cdot\text{L}^{-1}$ have increased phosphates from $0.1 \text{ mg}\cdot\text{L}^{-1}$ to 0.257, 0.118, 0.348 and $0.269 \text{ mg}\cdot\text{L}^{-1}$ in 120 h respectively. The total plate counts (TPC) of bacteria in brackishwater was found to be highest with the treatment with RBF ($110 \times 10^4 \text{ cfu}\cdot\text{ml}^{-1}$) followed by OBP ($64 \times 10^4 \text{ cfu}\cdot\text{ml}^{-1}$) as compared to control ($8 \times 10^4 \text{ cfu}\cdot\text{ml}^{-1}$).

Figure 4

Images of biofilms formed over bagasse fibers (RBF and DBF) are shown in figure 4 and results of the growth kinetics of the biofilm of the microbial consortia from the brackish-water grown on selected substrates are depicted in figure 5. The results reveal a clear and significant difference between two substrates in supporting the biofilm mode of growth of the consortia. The DBF supported lesser biofilm population compared to the RBF in the 15% nutrient medium.

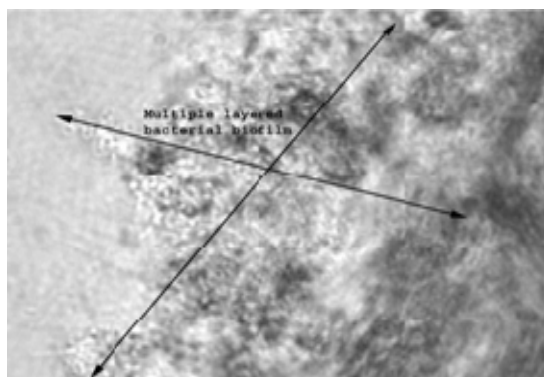


Figure 5

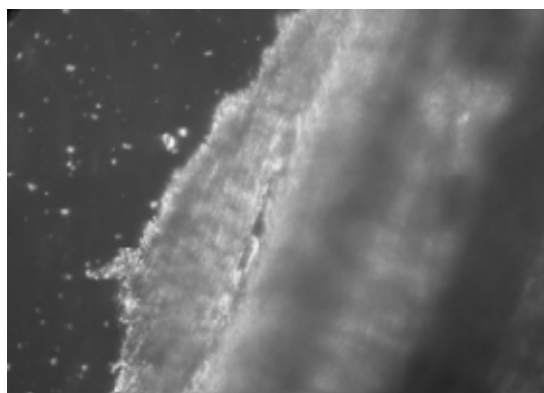


Fig. 4. Images of biofilms formed over RBF and DBF in brackishwater

Determination of effective amount of bagasse by statistical analysis

Results of the statistical analysis of the data are given in [table 1](#). This shows that there was a significant difference among efficacies of all the four materials with a dose of $1 \text{ g}\cdot\text{L}^{-1}$ for nitrite removal. However, there was no significant difference between RBF and OBP with doses of $3 \text{ g}\cdot\text{L}^{-1}$ and $6 \text{ g}\cdot\text{L}^{-1}$, probably due to the combined effect of rapid increase in bacterial counts and ion exchange mechanism. It was observed that in the treatment with RBF in all three doses and OBP with a dose of 3 and $6 \text{ g}\cdot\text{L}^{-1}$, there was no significant effect of duration on nitrite removal and maximum nitrite removal was achieved within 24 h. In the case of DBF and CBP with a dose of 1 and $3 \text{ g}\cdot\text{L}^{-1}$, nitrite removal was significantly different with respect to duration and maximum removal was achieved in 96 h. Analysis indicated that nitrite removal was highest with RBF with a dose of $1 \text{ g}\cdot\text{L}^{-1}$ and OBP with a dose of $3 \text{ g}\cdot\text{L}^{-1}$ in 24 h, whereas in the case of DBF and CBP, maximum nitrite removal was achieved with a dose of $6 \text{ g}\cdot\text{L}^{-1}$ by 96 h.

Discussion

In the present study, decrease in dissolved oxygen (DO) concentration as observed mainly for the treatment with RBF and OBP indicates high oxygen consumption by the microbial population. This is consistent with the previous study ([Visscher and Duerr Eirik 1991](#)). The effective removal of nitrite with RBF and OBP can mainly be attributed to increase in bacterial counts and periphytic growth combined with PO_4^- ion exchange mechanism.

Fish production can be significantly increased by the introduction of biodegradable substrates- sugar cane bagasse and paddy straw into culture systems ([Mridula et al. 2003](#)), wherein the reduction in DO and total ammonia content in bagasse based treatment has also been observed. It seems that the increase in fish production is partly a result of the additional food that the periphyton provides and it was estimated that autotrophic productivity could be doubled by providing a substrate area similar to the pond water surface area ([Mridula et al. 2003](#)). Significant declines in DO concentration as observed for the bagasse based treatments in the present study, indicate high oxygen consumption by the autotrophic microbial population.

Table 1. Comparison of efficacies of four different materials from bagasse in the removal of nitrite from brackishwater.

(a). Effect of materials

Bagasse	Mean ^a		
	Nitrite (0.92 mg•L ⁻¹)		
	1 g•L ⁻¹	3 g•L ⁻¹	6 g•L ⁻¹
RBF	0.1840E	0.1840D	0.1840C
DBF	0.7654A	0.5780A	0.3332B
OBP	0.3144C	0.1840D	0.1840C
CBP	0.6004B	0.4986B	0.3886A

(b). Interaction effect of materials and duration

Bagasse	Hours	Mean ^a		
		Nitrite (0.92 mg•L ⁻¹)A		
		1 g•L ⁻¹	3 g•L ⁻¹	6 g•L ⁻¹
RBF	24h	0.00001J	0.00001I	0.00001F
	48h	0.00001J	0.00001I	0.00001F
	72h	0.00001J	0.00001I	0.00001F
	96h	0.00001J	0.00001I	0.00001F
DBF	24h	0.8390B	0.8080B	0.4150C
	48h	0.7830C	0.7000C	0.1510E
	72h	0.6850D	0.3450E	0.1800DE
	96h	0.6000E	0.0860H	0.00001F
OBP	24h	0.6520D	0.00001I	0.00001F
	48h	0.00001J	0.00001I	0.00001F
	72h	0.00001J	0.00001I	0.00001F
	96h	0.00001J	0.00001I	0.00001F
CBP	24h	0.8740B	0.8090B	0.8040B
	48h	0.6500D	0.5380D	0.2070D
	72h	0.4050F	0.2240G	0.0120F
	96h	0.1530H	0.0020I	0.00001F

^aMeans in the vertical row with different superscripts are significantly different ($p \leq 0.05$).

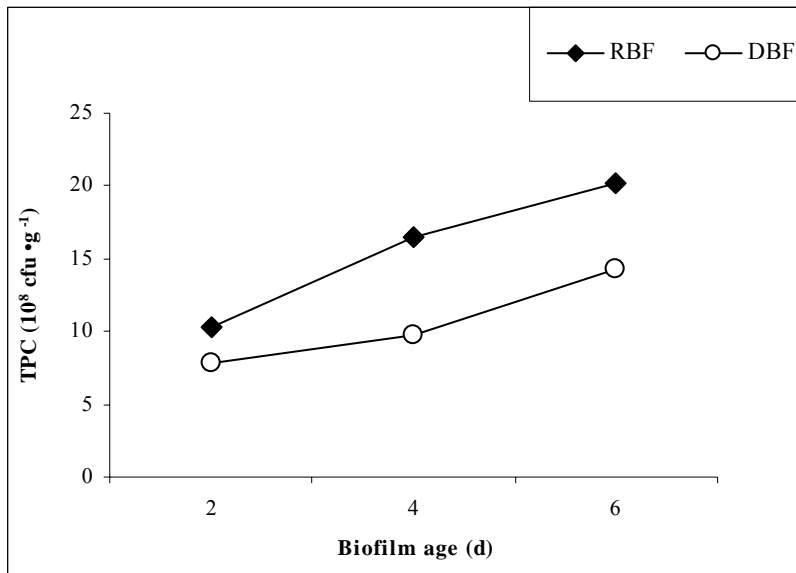


Fig. 5. Growth kinetics of biofilm bacteria on two different substrates grown in 15% peptone water

Azim et al. (2001) observed that periphyton improved water quality in aquaculture system by increasing nitrification, which uses substantial amounts of oxygen and hydrogen carbonates, reducing hardness and buffering capacity. The nitrifying bacteria as well as heterotrophic bacteria present in the system form biofilms on all surfaces in the system (Stickney 1993). It is believed that attachment of bacteria to form a biofilm is dependent on the type of substrate. Nitrifying bacteria are delicate organisms and extremely susceptible to a variety of inhibitors. They are extremely slow growing, which have a very narrow pH tolerance, preferring a range between 7.5 – 8.6 and need a minimum of 1 mg/l of dissolved oxygen. The nitrifying organisms are aerobic and have a high requirement for oxygen and their source of carbon is bicarbonate ion. In the present study, decrease in pH may be due to the consumption of bicarbonate ions by nitrifying organisms.

Bagasse forms a potential base for feeds when applied to extensive shrimp cultures (Freeman et al. 1992; Bombeo-Tuburan et al. 1993) and has no adverse impact on the water quality (Visscher and Duerr Eirik 1991). This has also been used as a substrate in a polyculture farm trial, which yielded significantly higher production of fresh water fishes (Keshavanath et al. 2001). Umesh et al. (1999) obtained 45-50% higher

production of common carp, tilapia and rohu in concrete tanks through biofilm formation with sugarcane bagasse as substrate. Ramesh et al. (1999) compared dried sugarcane bagasse, paddy straw and water hyacinth (*Eichhornia* spp.) leaves as substrates and observed the best growth of common carp using bagasse through biofilm formation. Biofilm populations are generally resistant to environmental stresses (Costerton et al. 1999), these are likely to be stable through time. There are many other reports (Pandey et al. 2000) on the use of lignocellulosic materials for enhancing the growth of microorganisms and they suggested that bagasse could be used as a source of carbon in bioprocess techniques. In the present study, bagasse enhances the periphytic bacterial growth, which may also be responsible for the removal of nitrite.

Conclusion

Studies on the use of four different materials from lignocellulosic agricultural waste-bagasse for bioremediation of nitrite from brackishwater were carried out and the following conclusions were drawn:

1. The present study shows that bagasse can be used for the effective removal of nitrite from brackishwater. Raw bagasse fiber (RBF) and dried bagasse powder (OBP) with a dose of $1-3 \text{ g}\cdot\text{L}^{-1}$ are efficient materials in the maximum removal of nitrite within 24 h, whereas, charred bagasse powder (CBP) was the least effective.
2. The removal of nitrite was found to depend on the bagasse material, dose, duration and initial nitrite concentration. Results show that percent nitrite removal was found to decrease with an increase in initial nitrite concentration.
3. The effective removal of nitrite can mainly be attributed to increases in bacterial periphytic growth combined with PO_4^- ion exchange mechanism.
4. These results may be of value in developing sound remediation strategies for water contaminated with toxic nitrite. Furthermore, use of cheaper substrates like sugarcane bagasse may offer an ecofriendly approach and successful studies on this material could be beneficial for the treatment of culture water / wastewater in aquaculture systems.

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