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Growth of periphyton on different plastic materials in freshwater medium

***Bikash C. Mohapatra, Hrushikesha Sahu, Sudeep K. Mahanta, Sacharita Lenka, K. Anantharaja and Pallipuram Jayasankar**

ICAR - Central Institute of Freshwater Aquaculture, Bhubaneswar - 751002, Odisha, India

ABSTRACT

Experiments were conducted in laboratory to observe periphytic growth on four types of plastic sheets, such as polyethylene (PE), polypropylene (PP), fiber reinforced plastic (FRP) and acrylic placed inside the glass aquaria filled with fertilized freshwater for 45 days. Observations were made in every 15th day for growth of periphyton both qualitatively and quantitatively on the plastic sheets, and physico-chemical properties of aquaria water were recorded during periphyton samplings. Significant difference ($P<0.05$) in periphyton quantity per unit area of the plastic sheets was found among the treatments and the volume from FRP sheet was higher (7.10±0.26 ml/0.1 m^2) than the polyethylene (4.43±0.35 ml/0.1 m^2), polypropylene (3.35±0.20 ml/0.1 m^2) and acrylic sheet (2.32±0.31 ml/0.1 m^2). Total 38 periphytic microalgae (Chlorophyceae– 4 types, Cyanophyceae – 11 types, Bacillariophyceae – 15 types and Desmidiaceae – 8 types) were recorded from these sheets. Polyethylene sheet had more types of Cyanophyceae and Bacillariophyceae attached on to it and the acrylic had fewer types. On all sheets Bacillariophyceae (Diatoms) had developed more in numbers and Navicula was the dominant type. FRP sheets can be used as a substrate in aquaculture system for periphytic growth on them, which can be utilized by fishes as natural food.

Keywords: Algae, Plastic sheets, Periphyton, Water quality

INTRODUCTION

In pond ecosystem, periphyton is conspicuous component of the littoral zone comprising of algae, fungi, bacteria and protozoa. It grows mainly on the organic and inorganic substrates as a thin coating inside the water. Ecologically, periphyton plays an important role in nutrient cycling and biological productivity in aquatic systems [1]. Periphyton is sensitive towards some environmental conditions, which can be detected by changes in species composition, cell density and ash free dry mass (AFDM), chlorophyll and enzyme activity, *e.g.*, alkaline and acid phosphatase. Hence, it has often been used as an indicator of water quality and ecological functioning in both freshwater and marine environments [2], [3], [4] and [5].

Periphyton growth on substrates not only serves as the natural food and productivity in fish ponds, but also it improves water quality by utilizing nutrients from the medium [6], [7], [8] and [9]. Periphyton based aquaculture lowers the production cost and it is a better fish culture practice for the poor farmers [10] and [11]. Algae associated in periphytic mass requires substrates for attachment, which are virtually absent in many fish ponds. According to Van Dam *et al.*, 2002 [12], the use of periphyton in aquaculture is mainly by the herbivorous fish. Organic and inorganic fertilizers, and supplementary feed are added in semi-intensive type aquaculture systems, whereas, the intensive systems are based predominantly on quantity and quality of feeds. From the total nitrogen added to the ponds as fertilizer, only 5–15% is harvested as fish biomass [13] and [14]. It is well documented that the fish farming is possible in periphyton-based aquaculture [15].

Some workers have compared the natural and artificial substrates for periphyton growth, and reported that more periphytic growth found on the natural substrates [16], [17], [18], [19] and [20]. The taxa present in natural samples were nearly similar to the artificial substrates [16]. No work has been done on the periphyton growth on different types of plastic sheets in freshwater system or ponds. Hence, the present study was undertaken to observe the periphyton growth on different types of plastic materials, such as polyethylene (PE), polypropylene (PP), fibre reinforced plastic (FRP) and acrylic in freshwater medium. From this study the plastic material suitable for periphyton growth can be recommended for periphyton-based aquaculture system.

MATERIALS AND METHODS

1. Study location and materials

The study was conducted in duplicate sets in the laboratory of All India Coordinated Research Project on Plasticulture Engineering & Technology (AICRP on PET), centre at ICAR - Central Institute of Freshwater Aquaculture, Bhubaneswar, Odisha (*latitude* 8° 43' 39.1" N and *longitude* 77° 42' 51) during 15 March 2013 to 14 April 2013, and 1 May 2013 to 14 June 2013. Four different types of plastic sheets such as polyethylene (PE), polypropylene (PP), fiber reinforced plastic (FRP) and acrylic were used for periphytic growth on them. Sheets experimented were with length 40 cm and width 25 cm. Thicknesses of the plastic sheets were polyethylene: 3.1 mm, polypropylene: 3.04 mm, FRP: 3.0 mm and acrylic: 3.02 mm.

2. *Experiment period*

Each set of experiment was conducted in triplicate with different plastics sheets, such as polyethylene (PE), polypropylene (PP), fiber reinforced plastic (FRP) and acrylic placed in glass jars of size 30 cm (L) x 30 cm (B) x 37.5 cm (H), filled with 30 L of water (10 L pond water and 20 L tap water). The pond water was used to act as the inoculum for the experiment as in the experiment reported by Cavalcante *et al*., 2010 [21]. The water of the each jar was fertilized by adding urea 2.0 g and single super phosphate 3.0 g. The fertilizer dose of the water of the jar was derived from the dose of the fertilizers applied to the fish culture ponds [22]. The plastic sheets were inserted inside the jars in slanting position facing towards the glass windows of the laboratory and towards the east for sunlight in the morning hours. Only one side of the sheet received sunlight through the glass windows. One set of experiment was conducted for 45 days (15 March 2013 to 14 April 2013) and the second set was repeated for 45 days during 1 May 2013 to 14 June 2013.

3. Periphyton collection and analysis

Periphyton was collected on 15th, 30th and 45th day from the portion of the plastic sheets submerged inside the jar water facing towards east direction for sunlight. 10 cm x 10 cm area from each sheet was scrubbed by a stainless steel blade [23] and collected in to an enamel tray. Then the collected periphytic mass along with water for each sheet was transferred from enamel tray to a graduated test tube and allowed for settlement for 24 hours. It was treated with 4% formalin for preservation and kept under refrigeration. After 24 hours, the supernatant was decanted from the test tube and the settled periphytic volume for each sheet was quantified and recorded. From the total settled volume of periphytic mass, 1.0 ml was taken and diluted it to 10^{-3} times with distilled water and poured in to the Sedgwick grafter cell [24] for genera identification under microscope.

4. *Collection of water samples and analysis*

The water samples from the jars were collected on 15th, 30th, and 45th day and essential water quality parameters like temperature, pH, conductivity (µS/cm), alkalinity (mg/L), hardness (mg/L), dissolved oxygen (mg/L), carbon dioxide (mg/L), ammonia (mg/L), nitrite (mg/L) and nitrate (mg/L) were analyzed following APHA [25] laboratory methods.

5. Statistical analysis

Data were analyzed by using SAS 9.1.3 for Windows. Duncan's Multiple Range Test (DMRT) was performed at 95% significance level to compare the treatment means.

RESULTS AND DISCUSSION

There were 38 different periphytic microalgae grew on different plastic sheets immersed in freshwater medium (Table - 1). During experiment, the group Cyanophyceae: green algae (*Crucigenia, Ankistrodesmus, Botryococcus, Cladophora, Microspora, Pediastrum, Protococcus, Scenedesmus, Spirogyra, Sorastrum, Kirchneriella*); Desmidiaceae: desmids (*Closterium, Desmidium, Gonatozygon, Mesotaenium, Micrasterias, Netrium, Spirotaenia, Penium*); Bacillariophyceae: diatoms (*Cocconeis, Diatoma, Epithelia, Fragilaria, Frustulia, Melosira, Navicula, Nitzschia, Stephanodiscus, Stauroneis, Synedra, Eunotia, Tabellaria, Cyclotella, Amphora*) and Chlorophyceae: blue-green algae (*Anabaena, Nostoc, Rivularia, Merismopedia*) were observed in all the four plastic sheets. Diatoms were found more on plastic sheets, followed by green algae, desmids and blue-green algae. On polyethylene sheet the numbers of genera found were blue green algae (2), green algae (10), desmids (5) and diatoms (13); On FRP sheet blue green algae (2), green algae (2), desmids (2) and diatoms (11); on polypropylene sheet blue-green algae (1), green algae (3), desmids (4) and diatoms (10); and on acrylic sheet blue green algae (1), green algae (5), desmids (4) and diatoms (8). From the result it was found that on all plastic sheets the Bacillariophyceae (Diatoms) grew more in number compared to other groups.

Table – 1: Types of algae recorded from different plastic sheets immersed in freshwater for different periods

		15 Days				30 Days				45 Days			
		PE	**PP**	**FRP**	**Acrylic**	**PE**	**PP**	**FRP**	**Acrylic**	**PE**	**PP**	**FRP**	**Acrylic**
	Green algae (Cyanophyceae)												
1	*Crucigenia*	-	-	-	-	+	+	-	-	-	-	-	-
2	*Ankistrodesmus*	-	-	-	+	+	-	-	-	+	-	-	-
3	*Botryococcus*	-	-	-	-	+	+	-	-	+	+	-	-
4	*Cladophora*	-	-	-	-	-	+	-	-	-	+	-	-
5	*Microspora*	+	+	-	+	-	-	-	-	-	-	-	-
6	*Pediastrum*	-	-	-	-	-	-	+	-	-	-	+	-
7	*Protococcus*	-	-	+	+	+	-	+	-	-	-	+	-
8	*Scenedesmus*	-	-	+	+	+	-	+	-	-	-	+	-
9	*Spirogyra*	+	+	-	-	-	-	-	-	-	-	-	-
10	*Sorastrum*	-	-	-	-	-	-	-	-	+	-	-	-
11	*Kirchneriella*	-	-	-	+	-	-	-	-	-	-	-	-
	Desmids (Desmidiaceae)												
12	*Closterium*	+	-	-	-	-	-	-	+	-	-	-	+
13	*Desmidium*	-	-	-	-	-	+	-	-	-	+	-	-
14	*Gonatozygon*	+	+	-	-	-	+	-	+	-	+	-	+
15	*Mesotaenium*	-	-	-	-	+	+	+	-	+	-	+	-
16	*Micrasterias*	-	-	-	-	+	-	-	-	+	-	-	-
17	*Netrium*	-	-	-	-	-	-	-	+	-	-	+	-
18	*Spirotaenia*	-	-	-	-	-	-	+	-	-	-	-	-
19	*Penium*	-	-	-	-	+	+	-	+	+	+	-	+
	Diatoms (Bacillariophyceae)												
20	*Cocconeis*	-	-	-	-	+	-	-	-	+	-	-	-
21	*Diatoma*	+	+	+	+	+	+	+	+	+	+	+	+
22	*Epithelia*	+	-	-	-	-	-	-	-	-	-	-	-
23	*Fragilaria*	+	+	-	+	+	+	+	-	+	+	+	-
24	*Frustulia*	+	-	+	-	-	-	-	+	-	-	-	+
25	*Melosira*	+	+	-	+	+	+	+	+	+	+	+	+
26	*Navicula*	+	+	+	+	+	+	+	+	+	+	+	+
27	*Nitzschia*	+	+	-	+	+	-	-	-	-	-	-	-
28	*Stephanodiscus*	-	-	-	-	+	-	-	-	+	-	+	-
29	*Stauroneis*	-	-	+	-	-	-	-	-	-	-	-	-
30	*Synedra*	+	+	+	+	+	+	+	+	+	-	+	+
31	*Eunotia*	+	+	-	-	+	-	-	-	+	-	-	-
32	*Tabellaria*	+	+	+	-	+	-	-	-	+	-	+	-
33	*Cyclotella*	+	+	+	-	+	+	-	+	-	+	-	-
34	*Amphora*	-	-	-	-	-	-	-	-	-	-	+	-
	Blue – Green Algae (Chlorophyceae)												
35	*Anabaena*	-	-	-	-	-	-	+	-	-	-	-	-
36	*Nostoc*	-	-	-	-	+	-	-	-	+	-	-	-
37	*Rivularia*	-	-	-	-	+	+	-	-	+	+	-	-
38	*Merismopedia*	-	-	-	+	-	-	+	-	-	-	+	-

Polyethylene (PE), Polypropylene (PP), Fiber reinforced plastic (FRP)

Fig. 1(a, b, c, d): Periphyton composition for different plastic sheets

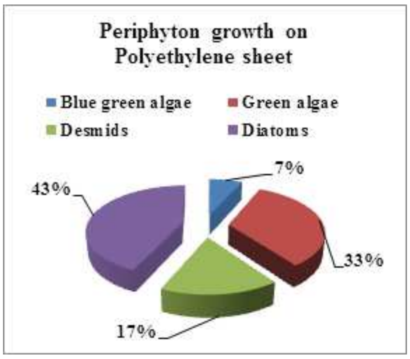


Figure 1(a)

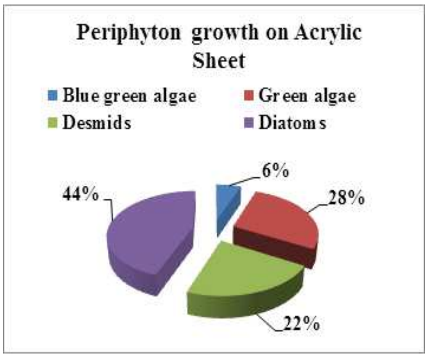


Figure 2(b)

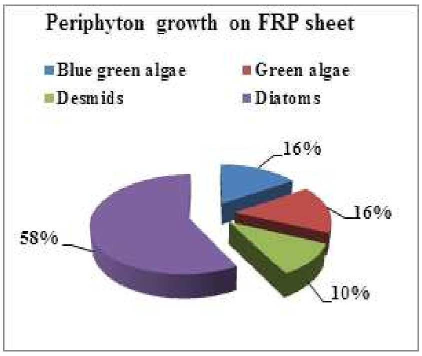


Figure 3(c)

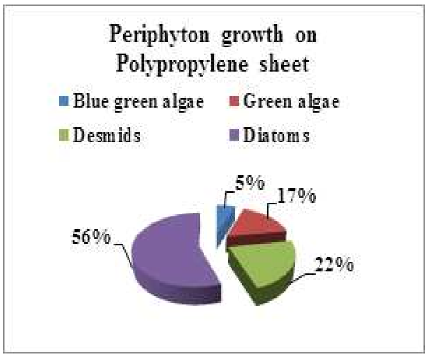


Figure 4(d)

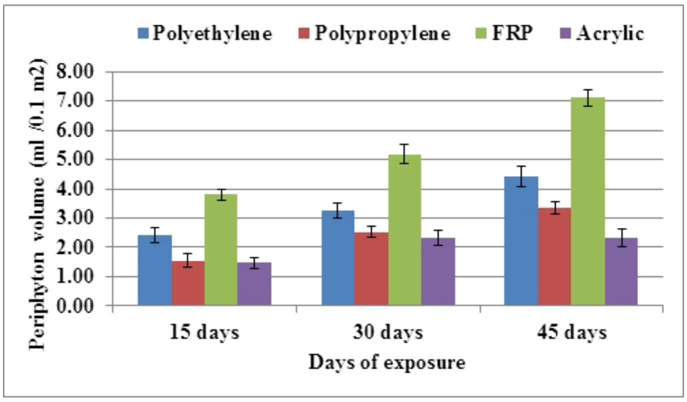
Statistical analysis was done for the sheet wise algal volume for 15, 30 and 45 days of exposure period and presented in Table - 2. For this the triplicate data for each sheet from two experimental sets were pooled. Statistically there was found significant difference (P<0.05) in periphyton volume among all the treatments of 45 days duration and the plankton volume from FRP sheet was comparatively higher (7.10±0.26 ml/0.1 m^2) than the polyethylene (4.43±0.35 ml/0.1 m^2), polypropylene (3.35±0.20 ml/0.1 m^2) and acrylic (2.32±0.31 ml/0.1 m^2). The volume of periphyton from the polyethylene and FRP sheets during 15 and 30 days sampling had significant difference (P>0.05). During 15 and 30 days sampling, the volumes of periphyton from polypropylene and acrylic did not vary significantly (P<0.05).

Table – 2: Volume of algae quantified from 0.10 m^2 area of different plastic sheets immersed in freshwater for different periods

Days of exposure	Polyethylene (ml)	Polypropylene (ml)	FRP (ml)	Acrylic (ml)
15	$2.40±0.26^b$	$1.55±0.23^c$	$3.80±0.18^a$	$1.47±0.20^c$
30	$3.25±0.27^b$	$2.53±0.20^c$	$5.18±0.33^a$	$2.32±0.25^c$
45	$4.43±0.35^b$	$3.35±0.20^c$	$7.10±0.26^a$	$2.32±0.31^d$

Values are expressed as (Mean ±SD)
Values with same superscripts in a row do not differ significantly at P>0.05. (n=6)

Fig. 2: Periphytic volume for different plastic sheets



In the present study quantitatively the periphyton growth was found more on FRP sheet (7.10±0.26 ml/0.1 m^2) than polyethylene (4.43±0.35 ml/0.1 m^2), polypropylene (3.35±0.20 ml/0.1 m^2) and acrylic (2.32±0.31 ml/0.1 m^2). Periphyton growth comparison was made by Keshavanath *et al.*, 2012 [19] for four natural substrates such as bamboo mat, sugarcane bagasse, coconut leaf and palm leaf and they found that the coconut leaf had better periphyton growth than other tested materials. A study was undertaken by Dutta *et al.*, 2013 [20] for comparison of palm leaf and nylon net for periphyton growth and they reported better periphyton growth on palm leaf. The substrates from organic origin attract more periphytic growth on them. Stelzer and Lamberti, 2001 [26] found that diatoms are the dominant algal group, comprising 93% among total volume of algae grown on nylon mesh kept in stream. Similar result was found in the present experiment and *Navicula* (diatom) was the dominant type and abundant in all tested plastic sheets. In 2004, Komarek and Sukacova [16] studied growth of periphyton on granite placed in a river channel and found more than 40 species with diatoms as the dominant one. The taxa present in natural samples were nearly similar to the artificial substrates. In the present experiment 38 genera were found in the periphytic composition such as from Chlorophyceae (4), Bacillariophyceae (15), and Cyanophyceae (11) and Desmidiaceae (8). Bacillariophyceae was the dominant type among the groups. The present study corroborates the study of [16]. [17] and [18] compared the biodegradable and non-biodegradable substances for periphyton based aquaculture to enhance fish production. They reported about 88 genera of periphytic microalgae composed of Chlorophyceae, Bacillariophyceae and Cyanophyceae. Chlorophyceae and Cyanophyceae preferred rice stems, whereas, Bacillariophyceae preferred glass slide. In the present study total 38 genera were found and among them the Bacillariophyceae preferred plastic material for its growth and settlement in freshwater medium.

The physico-chemical parameters of water of each jar are presented in Table - 3. The electrical conductivity, CO_2, ammonia, nitrite and nitrate were increasing, whereas, pH, total hardness, total alkalinity and phosphate were decreasing during the 45 days of experimental period. Statistical analysis was conducted for the final water quality parameters of 45th day exposure. There was no significant difference (P>0.05) in nitrate, hardness, ammonia, phosphate and pH of the water in polyethylene, polypropylene, FRP and acrylic experimented jars. Significant difference (P<0.05) was found for alkalinity (76.33±13.17 mg/L and 71.00±14.52 mg/L) and nitrite (0.17±0.02 mg/L and 0.10±0.08 mg/L) of the water of FRP and polypropylene treatment jars respectively.

Table – 3: Physico-chemical parameters of water during experimental period

Water parameters	Days of exposure	Polyethylene	Poly Propylene	FRP	Acrylic
pH	1	7.05±0.17	7.05±0.17	7.05±0.17	7.05±0.17
	15	6.97±0.16	6.83±0.18	6.87±0.19	7.09±0.19
	30	6.54±0.13	6.51±0.26	6.47±0.32	6.34±0.36
	45	6.12±0.38^{a}	5.88±0.48^{a}	6.04±0.49^{a}	5.78±0.29^{a}
EC (µS/cm)	1	495.10±0.00	495.10±0.00	495.10±0.00	495.10±0.00
	15	459.40±35.19	504.42±18.09	534.13±46.96	481.62±23.03
	30	542.60±41.69	531.67±30.74	536.10±27.72	531.45±19.20
	45	538.65±11.83^{b}	527.45±23.20^{b}	530.25±12.33^{b}	564.78±23.23^{a}
Alkalinity (mg/L)	1	104.67±7.34	104.67±7.34	104.67±7.34	104.67±7.34
	15	100.67±8.64	102.00±6.93	100.33±10.39	101.33±4.68
	30	86.67±17.24	92.67±17.24	85.33±14.01	86.00±13.02
	45	73.33±11.57^{ab}	71.00±14.52^{b}	76.33±13.17^{a}	72.67±11.22^{ab}
Hardness (mg/L)	1	133.00±14.24	133.00±14.24	133.00±14.24	133.00±14.24
	15	125.33±10.48	117.33±13.00	122.00±21.84	117.33±10.33
	30	117.33±12.24	107.67±15.62	108.67±18.27	105.67±15.20
	45	99.33±5.32^{a}	101.33±16.13^{a}	100.67±16.43^{a}	104.67±20.50^{a}
CO2 (mg/L)	1	10.00±2.19	10.00±2.19	10.00±2.19	10.00±2.19
	15	10.50±1.76	11.17±1.83	12.67±2.42	12.00±1.79
	30	13.67±1.51	12.33±2.34	14.00±1.26	14.00±1.26
	45	15.67±1.51^{ab}	14.67±2.07^{b}	16.33±1.51^{ab}	16.67±1.03^{a}
Ammonia (mg/L)	1	0.11±0.08	0.11±0.08	0.11±0.08	0.11±0.08
	15	0.19±0.17	0.20±0.16	0.26±0.28	0.23±0.20
	30	0.88±0.38	0.51±0.45	0.62±0.51	0.98±0.10
	45	1.16±0.06^{a}	1.08±0.14^{a}	1.10±0.07^{a}	1.18±0.08^{a}
Phosphate (mg/L)	1	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01
	15	0.05±0.01	0.05±0.01	0.05±0.00	0.05±0.01
	30	0.03±0.02	0.04±0.01	0.04±0.00	0.04±0.00
	45	0.04±0.04^{a}	0.03±0.02^{a}	0.04±0.03^{a}	0.04±0.02^{a}
Nitrite (mg/L)	1	0.08±0.02	0.08±0.02	0.08±0.02	0.08±0.02
	15	0.30±0.40	0.07±0.04	0.11±0.02	0.09±0.08
	30	0.13±0.02	0.10±0.08	0.12±0.06	0.12±0.02
	45	0.14±0.04^{ab}	0.10±0.08^{b}	0.17±0.02^{a}	0.15±0.02^{ab}
Nitrate (mg/L)	1	0.06±0.01	0.06±0.01	0.06±0.01	0.06±0.01
	15	0.06±0.03	0.05±0.02	0.08±0.01	0.05±0.03
	30	0.10±0.03	0.12±0.04	0.14±0.02	0.10±0.02
	45	0.16±0.02^{a}	0.16±0.02^{a}	0.15±0.02^{a}	0.14±0.02^{a}

Values are expressed as (Mean ±SD)
Values with same superscripts in the row for 45 days do not differ significantly at P>0.05. (n=6)

Growth of algae is directly proportional to pH, when pH is acidic algal growth increases; whereas, in alkaline pH, it decreases [27], [28] and [29]. The pH is an important factor for regulating periphyton growth and species composition. In the present experiment algal growth increased in acidic pH. Its increase had direct bearing to the decrease of alkalinity and hardness.

Van Dam *et al*., 2002 [12] had suggested that for increasing fish production in the periphyton-based aquaculture systems, nutrient levels are to be manipulated, or substrates are to be used for increase in surface area for periphyton growth. The periphyton based aquaculture with artificial substrates enhance fish production for poor fish farmers due to its low cost technology [30] and [5]. Several workers stated that in culture ponds artificial substrates for periphyton growth for use as fish food had increased fish production [31] and [32]. Periphyton based culture of Indian Major Carps had shown increased production of fish compared to that of production without substrate based system [33], [34], [35], [36], [37]. Increasing the nutrient levels and using substrates that help to periphyton growth seem to be possible solution to enhance fish production in periphyton-based pond aquaculture system [4] and [38]. From the present study, it is evident that the FRP sheets can be used in freshwater aquaculture system for periphytic growth on them.

CONCLUSION

Periphyton based aquaculture is a novel technology for the economically backward fish farmers. The present experiment for periphyton growth on artificial substrates has shown that the FRP sheet has the potential for better periphyton growth on them than the polyethylene (PE), polypropylene (PP) and acrylic in freshwater medium. Periphyton grown on the sheet is an important natural food source for the fishes in aquaculture system.

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