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Microbial Fouling in a Water Treatment Plant and Its Control Using Biocides

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Water treatment plants (WTP) are vital in the food, pharmaceutical and chemical process industries. This investigation describes the dense microbial fouling by microbes and organic compounds in a WTP of a heavy water producing industrial unit. On-site observations showed severe algal and bacterial growth in the various units of the WTP which are open to the atmosphere and very dense fungal fouling in the closed vacuum degasser unit. Digital and microscopic images showed that the microbial fouling problem was primarily due to a fungus. Microbiological analysis showed a count of ~10^5 cfu mL^{-1} in various sections of the WTP. On the contrary, slime/biofilm scrapings had very high bacterial populations (>10^9 cfu cm^{-2}). High organic carbon values in the system (5.0 to 19.5 ppm) had supported the growth of the fouling fungus in various sections of the WTP along with bacteria. Chlorination was found to be inadequate in controlling the biofouling problem. Consequently chlorine dioxide was tested and found to be a better biocide in controlling the bacterial population. A 2.0% Sodium-2-pyridinethiol-1-oxide solution had completely inhibited the fouling fungus. The paper discusses the importance of fungal adaptation in an industrial unit and highlights the biodeterioration of various sections of the WTP unit.

Key words : Demineralization plant / Slime / Fungus / Chlorine-dioxide / Sodium -2-Pyridinethiol-1-oxide.

INTRODUCTION

Water treatment systems consist of many different components such as demineralization units, reverse osmosis, ultra-filtration and nano-filtration systems as well as of various pumping units that are interconnected (Howe and Clark, 2002). These various systems and subsystems have many things in common, like manifolds feeding raw water to many components and allowing passage of water from them. There are also applications for this same filtration process in other industries. The purification of water consists of physical and chemical processes. The Clean-In-Place systems installed in food, dairy, and pharmaceutical industries are meant to clean out the piping and components between production batches. The use of heat exchangers in many different areas from marine vessels to food production factories is another common example (Fan et al., 2001). Similarly, generating high quality water is of relevance in heavy water production. Microbial fouling remains a major problem in power, chemical and processing industries in spite of improvements in the quality of water treatment technology. Fouling of water treatment plants (WTPs) brings multiple adverse effects to the downstream process as well as to the system sustainability (Lin et al., 2006). The microbial fouling phenomenon is driven by the quality of the WTP inlet water (i.e., feed water).

The microbial inventory is of primary concern in the source water quality and the downstream treatment stages. In reality, biofouling cannot always be fully eradicated by pretreatment of the feed water because of the small growth generation times and adaptation capabilities of microorganisms (Zhang et al., 2011). Therefore, source water quality is a key factor in the progression of

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fouling in a WTP. The intensity of fouling depends upon the abundance and nature of the bacteria species present. Despite the availability of relatively good pretreatment procedures, source water of different qualities may show diversity in the mechanisms and kinetics of biofouling. In addition to the general composition of the feed water, the fouling trend is also dependent upon the chemical constituents, as they can potentially influence the progression of the fouling phenomena. Furthermore, the presence and diversity of microorganisms in the feed water might also play a role in the microbial fouling profile and biofilm formation of in a unit (Rao et al., 2009).

Generally, microorganisms exist predominantly as multicellular communities called biofilms attached to surfaces (Allison and Gilbert, 1992; Costerton et al., 1995). In the initial phase of biofilm development, ambient water quality and nutrient chemistry at the solid/liquid interface influence the settlement of primary colonizers. The exopolymeric matrix of the biofilm provides structural stability, and anionic constituents (such as nitrate, phosphate, silicate) from bulk water make the biofilm milieu a nutrient niche (Rao et al., 1997; Rao, 2003). Biofilm formation in industrial cooling circuits poses serious problems, wherein they reduce heat transfer efficiency resulting in a significant pressure drop in the condensers as well as impairing the performance of ancillary systems (Bott, 1995; Rao et al., 2009).

Heavy water (D_2O) production plants need good quality water with very low dissolved oxygen concentration for heavy water production. The Heavy Water Plant, Kota, India, is an indigenous production unit, using the bi-thermal H_2O-H_2S exchange process. The feed water used for heavy water production has to be well treated for efficient performance of the ion exchange columns and thereafter for the distillation process in the exchange unit. A proper disinfection of the feed water should be done at the entry point of the WTP, as well as at plausible locations to prevent biofouling. This step has been seriously compromised over the years at the site of the present study. As a consequence, the microbial fouling problem started and grew to unmanageable levels.

The microbial fouling problem in the vacuum degasser unit of this WTP was first observed in the year 2009, subsequently becoming aggravated in a couple of years. The microfouling growth had decreased the water flow from the vacuum degasser to the exchange unit, thereby affecting the operation of the plant. When the plant was shut down for maintenance, the vacuum degasser unit was opened for inspection. Very dense microbial fouling was observed on the stainless steel matrix. After the preliminary inspection, digital images of the fouled stainless steel matrix were recorded. Later the matrix was cleaned manually by lancing (cleaning with a water jet), assembled again and treated with sodium hydroxide and fitted for operation.

In the present investigation the severity of the microfouling problem of the WTP unit is described in detail, the critical determinants have been studied and a plausible microbial fouling control method is suggested. The following are the objectives of the study: a) complete (chemical and microbial) analysis of the feed water and total organic carbon analyses of WTP sub units, b) confocal laser scanning and epifluorescence microscopy study of biofilm and slime samples, c) phylogenetic analyses of microbial fouling organisms, d) biocidal assay with chlorine dioxide vis-a-vis chlorine of the WTP water samples, and e) control of the fouling fungus by Sodium-2-pyridinethiol-1-oxide.

MATERIALS & METHODS

Description of the System

The schematic of the WTP unit of the heavy water plant is illustrated in Fig.1. Feed water (600 m^3 h^{-1}) is drawn from the lake (Ranapratap Sagar) and the water passes through the clariflocculator tank to the sand beds, where the water is chlorinated for microbial control. The water then goes to the activated charcoal bed and forms the feed water to the cation resin bed followed by atmospheric degasser. The water then goes to the anion exchange resin bed and then is pumped to a storage tank before it enters the vacuum degasser unit. In the initial years of operation the vacuum degasser unit was filled with cylindrical pall rings, with the main purpose of the vacuum degasser unit to keep the dissolved oxygen level as low as possible. To further reduce the dissolved oxygen levels of the processed water to the exchange unit, the cylindrical pall rings were replaced with a stainless steel matrix [SS-316 steel was used, which is of an austenitic grade with the following % composition: carbon: 0.08, chromium: 16.00, manganese: 2.00, molybdenum: 2.00, nickel: 10.00, iron: balance]. Before the water enters the exchange unit for heavy water production, nitrogen gas is purged to further remove the dissolved oxygen.

Microbiological Studies

Microbiological analysis of the water samples and scraped biofilm/slime from the various sections of the WTP were done using standard methods (Holt et al., 1984). Water samples were collected from different sections of the WTP and for biofilm samples, biofilm from 5 X 2 cm areas on the conduit walls was scraped and suspended in 10 mL of 0.85% normal saline and serially diluted. The diluted samples were vortexed and a 0.1 mL aliquot of the serially diluted sample was plated in various culture media, and the total culturable bacteria were detected using nutrient agar medium

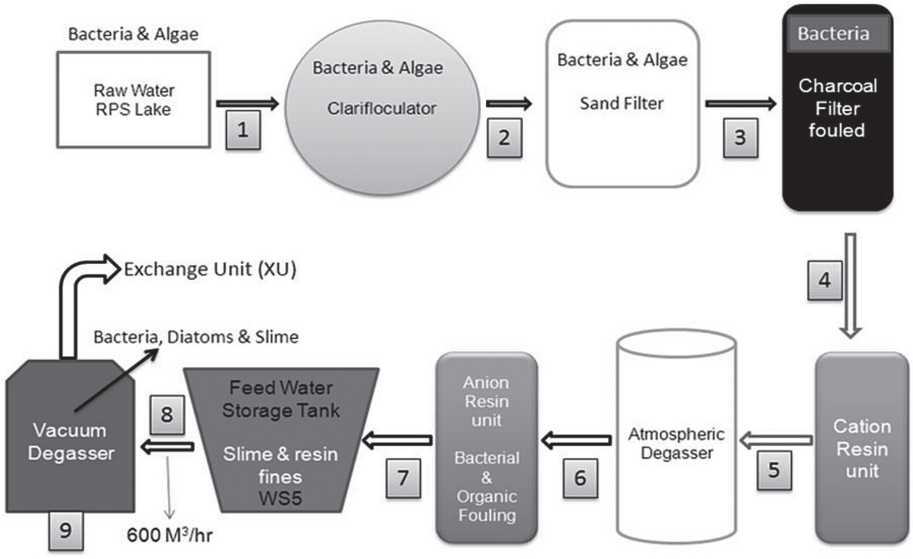

FIG. 1. Schematic details of the water treatment plant of the heavy water production unit. The probable microbial and organic fouling observed in different sections is also indicated.

enriched with glucose (0.1%). *Pseudomonas* bacteria counts were made using King's B medium (Bergan, 1981). Sulphate reducing bacteria were counted using Postgate medium (Rao et al., 2009). The bacterial counts were represented as colony forming units per milliliter (cfu mL^{-1}) when plated on agar medium. For the isolation of fungi, potato dextrose agar seeded with ampicillin (100 µg mL^{-1}) was used, and the petri plates incubated at 30℃ for 72 h. The vacuum degasser slime samples were analyzed three times, for consistency in the microbial isolation. Algae and chlorophyll contents were not analyzed.

Epifluorescence direct counting method (AODC-acridine orange direct counting) was used to assay the total bacterial population in the scraped biofilm and the slime samples (Rao et al., 1998), and the bacterial count was represented as cells cm^{-2}. The sand filter slurry, activated charcoal sample and slime from the vacuum degasser unit stainless steel mesh were also stained with acridine orange and observed with a confocal scanning laser microscope (CSLM), Model: TCS-SP2-AOBS, equipped with a DM IRE 2 inverted microscope (Leica Microsystems, Hessen, Wetzlar, Germany).

Fungal DNA extraction and 18S rRNA gene amplification

Fungal genomic DNA was extracted using a modified method of Ferrer et al. (2001). Two-day old fungal growth suspension from potato dextrose broth was transferred to a 1.5 mL Eppendorf tube and centrifuged at 8000 rpm for 10 min. The supernatant was discarded and the pellet frozen at -20℃ for 1 h. The mycelial tissue was homogenized with the aid of a sterile micro pestle. A 0.5 mL of extraction buffer (50 mM Tris-HCl pH 8.0, 50 mM EDTA pH 8.0, 3% SDS, and 1% 2-mercapto-ethanol) was added to the homogenate, mixed well and incubated at 65℃ in a water bath for 1 h. The lysate was extracted with equal volumes of phenol: chloroform: isoamyl alcohol (25:24:1), and the upper aqueous phase was transferred to a fresh Eppendorf tube. To the aqueous phase (~350 µL), 65 µL of 3M sodium acetate and 75 µL of 1M sodium chloride were added and incubated at 4℃ for 30 min. DNA was recovered by isopropanol precipitation and washed with 70% ethanol. The resulting DNA was air dried and redissolved in 50 µL of 1X TE buffer and stored at -20℃. The extracted genomic DNA from the pure fungal isolate was used as a template for PCR. The universal primers used for fungal 18S rRNA gene amplification were ITS1 (5' TCC GTA GGT GAA CCT GCG G 3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3') (The ILECO, Chennai). The reaction conditions were as follows: initial denaturation at 95℃ for 5 min, followed by 30 cycles of 30 seconds denaturation at 94℃, 1 min primer annealing at 59℃ and 1 min extension at 72℃. The amplification was completed by a final extension step at 72℃ for 5 min (Ferrer et al., 2001).

Bacterial DNA extraction and 16S rRNA gene amplification

A 1.5 mL aliquot of overnight grown bacterial culture in nutrient broth was centrifuged at 8000 rpm for 5 min. The supernatant was decanted and DNA was extracted

from the pellet using the Qiagen DNA isolation kit (QIAamp DNA Mini Kit) according to the manufacturer's protocol. Full-length 16S rRNA gene amplification was carried out on the extracted DNA from the pure bacterial isolate using 8F (5' AGR GTT TGA TCC TGG CTC AG 3') and 1492R (5' GCY TAC CTT GTT ACG ACT T 3') primers. The reaction conditions were carried out as per the following procedure: 5 min initial denaturation of DNA at 95℃, followed by 30 cycles of 1 min denaturation at 94℃, 1 min primer annealing at 59℃ and 2 min extension at 72℃. Amplification was completed by a final extension step at 72℃ for 5 min (Weisburg et al., 1991).

DNA sequencing and phylogeny

The PCR amplified full length DNA from both, the fungus (~540 bp) and the bacteria (~1500 bp) were confirmed in a 1.5% agarose gel and purified using the Hipur A kit (Himedia, India) as specified by the manufacturer's protocol and custom sequenced using the facility at Eurofins Genomics Private Limited, Bengaluru, India. The obtained gene sequences were then submitted to a BLAST search to obtain the best matching sequences. For phylogenetic analyses, reference sequences required for comparison were downloaded from the Genbank database (http://www.ncbi.nlm.nih.gov/Genbank). Sequences were aligned with the aid of the multiple sequence alignment program CLUSTAL W. The aligned sequences were then checked for gaps manually, and saved in the molecular evolutionary genetics analysis (MEGA) format using the MEGA 5 software. To obtain the confidence values, the original data set was resampled 1000 times using the bootstrap analysis method and used directly for constructing the phylogenetic tree. The phylogenetic trees were constructed using the neighbor-joining method. All these analyses were performed with the aid of Mega 5 software (Tamura et al., 2011).

Total organic carbon analysis

The total organic carbon of the various samples was analyzed using a SHIMADZU TOC-VCSH model with a catalytically-aided platinum combustion reactor and non-dispersive infrared (NDIR) detector (for CO_2 measurement) with the ASI-V auto-sampler unit. Absorption specific to infrared light, 2350 cm^{-1} measures the CO_2 gas flow over time through the detector. Glassware and sample containers used for total organic carbon analyses were scrupulously cleaned using nitric acid to remove organic contamination. Total organic carbon was obtained by subtracting the measured inorganic carbon from the total carbon, which is the sum of organic carbon and inorganic carbon.

Total organic carbon = Total Carbon – Inorganic Carbon.

Biocide assay

Chlorine and chlorine dioxide were used to study the biocidal efficacy on water samples collected from the different sections of the WTP. A sodium hypochlorite solution was used to generate hypochlorous acid. The total residual oxidants of chlorine was measured by the N, N-diethyl-p-phenylenediamine (DPD) method (Cooper et al., 1982) using a Hach colorimeter™ II (Hach, USA). In this method, the DPD amine is oxidized by chlorine to form two oxidation products. At a nearly neutral pH, the primary oxidation product is a semi quinoid cationic compound known as a Würster dye, with a magenta color. The Würster dye can be measured spectro-photometrically at wavelengths ranging from 490 to 555 nm. Chlorine dioxide (ClO_2) was generated by using a 1:1 mixture of solution A and B of commercial formulation (Scotmas, UK), which generates 50,000 ppm of stabilized ClO_2, and the concentration of ClO_2 was determined by the DPD method. Suitable dilutions of the stock solution were made to generate 1-3 ppm ClO_2. Finally 1 ppm free residual oxidant (ClO_2) was used to test the water samples of the WTP. Water samples after biocide treatment were assayed for total culturable bacteria at 15, 30 and 60 min intervals.

Effect of antifungal agent Sodium-2-pyridinethiol-1-oxide

Commercial anti-fungal agent Sodium-2-pyridinethiol-1-oxide 40% (also known as Sodium omadine) was used as a biocide. Various biocide dilutions ranging from 0.001% to 4% were tested against the pure culture of the fungus and bacteria. Fungal biomass was collected from the potato dextrose agar plate using a Tween 80 dipped sterile cotton swab. A 100 µL aliquot of fungal suspension was inoculated in all the flasks (containing 300 mL of sterile half strength potato dextrose broth) and incubated at room temperature. The biomass was suspended in one mL of sterile 1X phosphate buffered saline (pH 8.0) and vortexed to make a uniform suspension, which was further centrifuged at 8000 rpm for 3 min to remove heavy particles. 100 µL of this suspension was added to one mL of sterile phosphate buffered saline containing different concentrations of Sodium-2-pyridinethiol-1-oxide and incubated for 60 min at room temperature. After incubation, the suspension was centrifuged at 14000 rpm for 3 min and pellets were re-suspended in 1 mL of sterile 1X phosphate buffered saline. This step was repeated twice to remove all residual Sodium-2-pyridinethiol-1-oxide, and finally 100 µL aliquots were plated on potato dextrose agar plates and incubated for 72 h. Control tubes were also processed along with the test samples,

except for the step involving the addition of Sodium-2-pyridinethiol-1-oxide. Biocidal activity in *Pseudomonas* bacteria culture was also tested by using the same concentrations of Sodium-2-pyridinethiol-1-oxide. All the biocidal studies were carried out in triplicate to check for reproducibility.

RESULTS

Removal and cleaning of microbial fouling biomass

The fouling growth, observed in the vacuum degasser unit, was in layers (thickness ranged from 2-5 cm) and had completely clogged the stainless steel assembly (Fig.S1). The fouling biomass was tested for its dissolution in various chemicals, and the list of the chemicals used and the observations are given in the supplementary data (Table S1). The biomass was soluble in 10% sodium hydroxide solution in one hour contact time. After treating the vacuum degasser unit with 10% NaOH, the stainless steel matrix assembly was dismantled and the complete removal of the fouling biomass was made possible by lancing.

The vacuum degasser stainless steel matrix assembly was cleaned as per the following sequence: a) antibacterial treatment using sodium hypochlorite solution b) rinsing in freshwater, c) soaking in sodium hydroxide solution and d) flushing with water, i.e. lancing, and e) reassembling the stainless steel matrix and rinsing with fresh demineralized water.

The schematic illustration of the WTP unit of the heavy water production unit is shown in Fig.1. The types of microorganisms involved in the fouling process in the various sections of the WTP are also indicated. During the study period, the dissolved oxygen values ranged from 40-800 ppb. The pure demineralized water obtained from the WTP finally goes to the exchange unit where the heavy water is produced.

Water quality data

The quality data of the feed water to the WTP are presented in Table 1. All the analyzed parameters showed that the feed water was soft with a conductivity value of 285 μS cm^{-1}. The total organic carbon content of the feed water was high (~18 ppm). The total culturable bacteria count was ~10^5 cfu mL^{-1}. Table 2 describes the total culturable bacteria counts from water samples of different sections of the WTP unit. The bacterial count of feed water after 48 h of incubation was 2.1 x 10^5 cfu mL^{-1}. Water samples from the activated charcoal bed, sand filter and clariflocculator outlet had a bacterial count of 8.4 x 10^4, 1.8 x 10^5 and 4.1 x 10^5 cfu mL^{-1} respectively. There was no growth of bacteria in the atmospheric degasser outlet sample. However, the vacuum degasser unit outlet had a significant bacterial population (1.9 x 10^3 cfu mL^{-1}). The vacuum degasser unit slime had a bacteria count >10^9 cfu cm^{-2}. Sulphate reducing bacteria colonies were observed only in the feed water and in slime sample, when the samples were grown on sulphate reducing bacteria specific medium.

Table 3 details the total organic carbon analysis of the

TABLE 1. The feed water quality of the WTP of the heavy water production unit

S. No	Parameter	Value
1	pH	8.3
2	Conductivity ($\mu\Omega^{-1}$)	285
3	Total dissolved Solid (ppm)	195
4	Turbidity (NTU)	1
5	Silica (ppm as $CaCO_3$)	5.5
6	Total hardness (ppm as $CaCO_3$)	90
7	Calcium hardness (ppm as $CaCO_3$)	55
8	Magnesium hardness (ppm as $CaCO_3$)	34
9	Total alkalinity (ppm as $CaCO_3$)	90
10	Chloride (ppm as $CaCO_3$)	27
11	Sulphate (ppm as $CaCO_3$)	12
12	Chemical oxygen demand (COD, ppm)	5
13	Total Organic Carbon (ppm)	18
14	Total culturable bacteria (cfu mL^{-1})	~10^5
15	Sulphate reducing bacteria (cfu mL^{-1})	100

TABLE 2. Total culturable bacteria count from various water samples of WTP and biofilm scrapings

Samples	Total Culturable Bacteria Count (cfu mL^{-1})	Sulphate Reducing Bacteria Count (cfu mL^{-1})
Feed water	2.1 X 10^5	100
Feed water after chlorination	7.6 X 10^3	11
Clariflocculator Outlet	4.1 X 10^5	12
Sand Filter Outlet	1.8 X 10^5	20
Activated Charcoal Filter Outlet	8.4 X 10^4	NG
Atmospheric Degasser Inlet	NG	NG
Atmospheric Degasser Outlet	1.4 X 10^2	NG
Vacuum degasser Inlet	7.6 X 10^2	NG
Vacuum degasser Outlet	1.9 X 10^3	NG
Vacuum degasser Slime (cells cm^{-2})	10^9	100
AODC Biofilm counts (cells cm^{-2})	10^6	—

NG = no growth; AODC = Acridine orange direct count.

TABLE 3. Total organic carbon analysis of water samples collected from different sections of the WTP

S. No	Sample Details	Sample Dilution	Total Carbon [TC] (ppm)	Inorganic Carbon [IC] (ppm)	Total organic Carbon [TOC] (ppm)
1	Feed water	1	21.20	3.00	18.20
2	Feed water (After Chlorination)	1	16.50	2.90	13.60
3	Clariflocculator outlet	1	22.10	3.60	18.50
4	Sand filter outlet	1	22.20	2.50	19.70
5	Activated charcoal bed outlet	1	21.20	4.40	16.80
6	Atmospheric Degasser inlet	1	21.00	3.00	18.00
7	Atmospheric Degasser outlet	1	10.00	2.60	7.40
8	Vacuum Degasser inlet	1	9.20	2.90	6.30
9	Vacuum Degasser outlet	1	8.00	3.00	5.00

TC = Total Carbon; IC = Inorganic Carbon; TOC = Total Organic Carbon

water samples of different sections of the WTP. The feed water had a total organic carbon content of 18 ppm, but after chlorination the total organic carbon level reduced to 13.6 ppm. The total organic carbon values of the activated charcoal bed, clariflocculator outlet, atmospheric degasser inlet and sand filter outlet were in the range of 16.8 to 19.7 ppm. The atmospheric degasser outlet sample showed a significant reduction in total organic carbon value (7.4 ppm). In the vacuum degasser inlet and outlet there was no reduction in total organic carbon concentration. Inorganic carbon values of the water samples did not show any significant variation.

Microscopic study of microbial fouling phenomena

The microbial fouling density of the vacuum degasser unit stainless steel matrix is illustrated in Fig.2 (a-b): (a) shows a cleaned stainless steel matrix and (b) shows the completely fouled vacuum degasser matrix. The image of the fouled vacuum degasser unit through the manhole is given in the supplementary data (Fig. S1). After cleaning and one year of operation, the vacuum degasser unit was opened and inspected. Microbial fouling was noticed at the periphery of the vacuum degasser steel assembly and in the center the biomass was almost absent (Fig.S2-a). This variation could be due to the water flow dynamics in the vacuum degasser unit. Fig.S2 (b) shows fouling of vacuum degasser stainless steel mesh as well as carbon steel corrosion deposits.

Fig.3 (a-f), shows a few CSLM images of a variety of microorganisms in the fouling biomass. The microbiota ranged from micro algae, filamentous forms, bacterial cells and fungal mycelia in different sections of the WTP. Filamentous forms and rod shaped bacteria could be seen. Fig.4 shows the stereo zoom microscope image of microbial slime on vacuum degasser stainless steel mesh with resin particles.

The microbial fouling phenomena of sand bed slurry

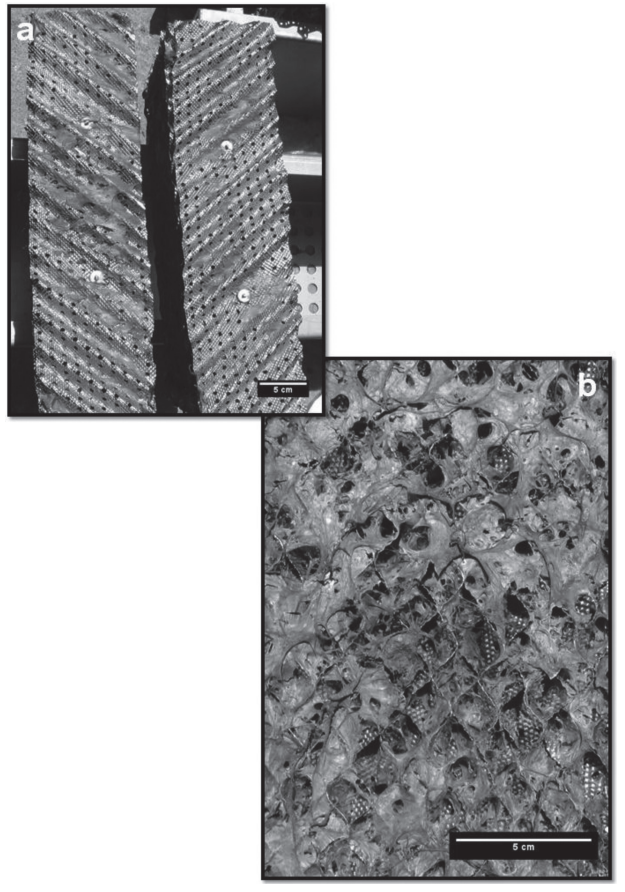

FIG. 2. Illustration of the biofouling problem in the vacuum degasser unit of water treatment plant. a) Digital camera images of a stainless steel matrix sheet of the vacuum degasser unit; b) A densely fouled segment of the vacuum degasser stainless steel matrix.

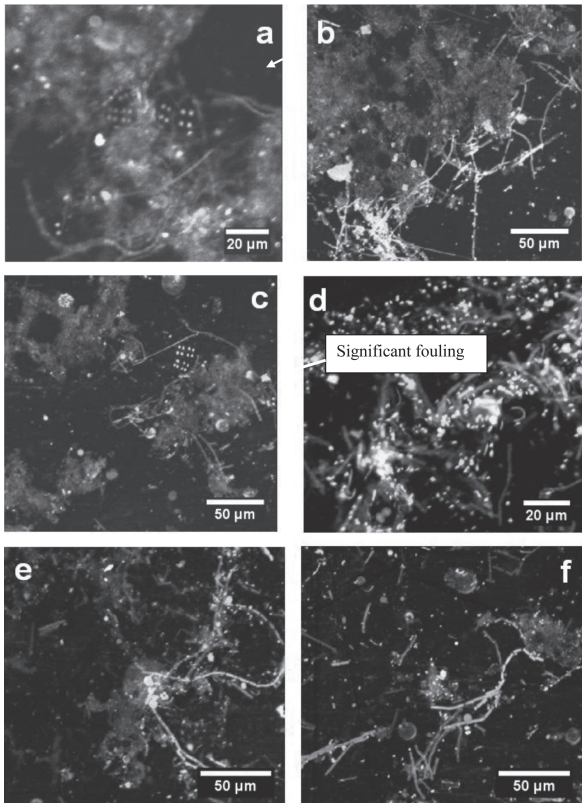

FIG. 3. Multiple CSLM images showing microbial fouling phenomena recorded from samples collected from the fouled water treatment plant. The microbiota ranged from micro-algae, filamentous forms, different bacterial cells and fungal mycelia.

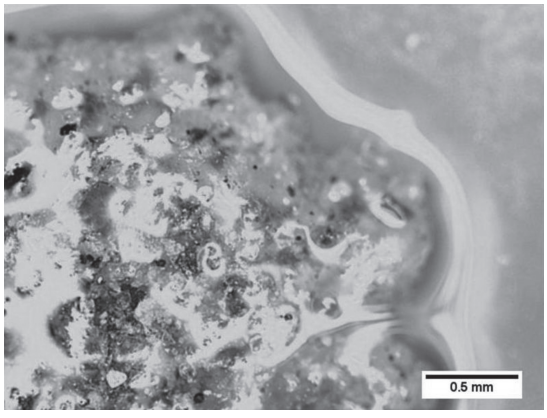

FIG. 4. Digital image showing fouling by microbial slime of the vacuum degasser stainless steel mesh with resin particles enmeshed in the deposits.

and activated charcoal filter sample are shown in supplementary data (Fig.S3). Anion resin size analysis showed that the average diameter of the resin beads is 0.54 (±0.15 mm). The diameter range was large 0.216-1.536 mm, the circularity of the beads was 0.75

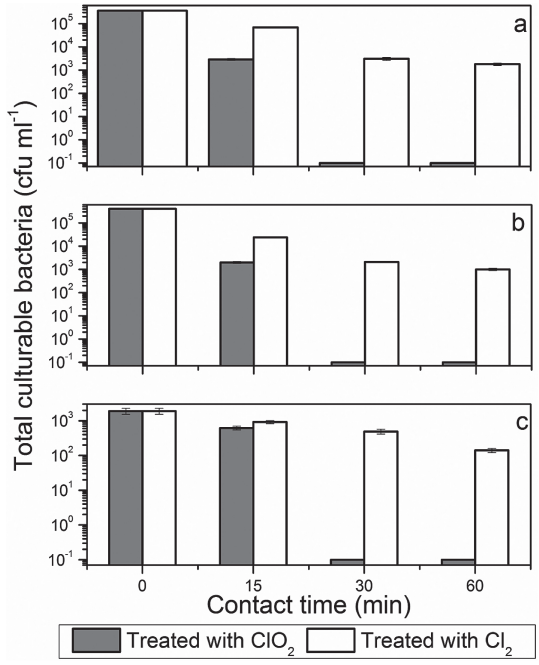

FIG. 5. Biocidal action of ClO_2 and Cl_2 on total culturable bacteria of the WTP water samples. a) Feed water, b) Clariflocculator outlet, c) Vacuum degasser outlet. Biocide concentration of 1 ppm free residual oxidants (i.e., ClO_2 or Cl_2) was used for testing. Error bars are the standard deviation values of the triplicate experimental data.

units. The fouled anion resin sample showed the presence of filamentous bacteria and slime (data not shown).

Evaluation of biocides

Fig.5 explains the biocidal efficacy of ClO_2 and Cl_2 on the water samples of the three main sections of the WTP. Fig.5a shows two orders of magnitude reduction within 15 min of contact time in the feed water total culturable bacteria count with respect to ClO_2, and the total culturable bacteria count was nil after 30 and 60 min of contact time. However, Cl_2 showed only a marginal reduction at 15 min contact time, and later at 30 and 60 min contact time the total count was reduced by two orders of magnitude.

Fig.5b illustrates the biocidal assay of clariflocculator water samples, wherein a 2-log reduction in the total cultural bacteria count within 15 min of contact time was noticed in the case of ClO_2. Consequently, the total count was nil by 30 min. Chlorine showed an almost similar trend as was observed in the case of feed water sample.

Fig.5c showed a difference in the biocidal activity in the vacuum degasser outlet. No significant reduction was noticed with ClO_2 and Cl_2 at 15 min contact time but ClO_2 showed complete reduction in total culturable bacteria at 30 and 60 min contact time. The total cultur-

able bacteria count of the vacuum degasser outlet control sample was ~10^3 cfu mL^{-1} and neither ClO_2 nor Cl_2 could show significant biocidal activity at 15 min contact time in spite of the low bacterial count.

In a separate study, a 2% solution of Sodium-2-pyridinethiol-1-oxide completely inhibited the growth of the fouling fungus *Gibberella moniliformis*. Surprisingly, the slime bacteria *Pseudomonas* sp, was not inhibited by Sodium-2-pyridinethiol-1-oxide even up to 2% concentration (see supplementary data Table S2).

3.5 Molecular characterization of the microfouling organisms

The phylogenetic tree and molecular identification of the slime microbiota are detailed in the supplementary data (Fig.S4). Four morphologically different bacterial isolates were observed in the initial isolation; however, on subsequent sampling only one bacterial isolate, which was more predominant, was characterized. Only one fungal isolate was obtained in potato dextrose agar medium. The fungus was characterized based on the 18S rRNA gene sequence and identified as *Gibberella moniliformis*. The bacterial strain was identified using the 16S rRNA gene sequence as *Pseudomonas* sp. The GenBank sequence database under the accession numbers; fungus (JQ811767, *Gibberella moniliformis* strain KOTA-1) and bacteria (JQ811768, *Pseudomonas* sp. RB001) were deposited. Figure S4 also provides the details of the partial DNA sequences of the two isolates which have caused the microfouling. The microscopic images of the fungus growth on the potato dextrose agar media are provided in the supplementary data (Fig.S5).

DISCUSSION

Water distribution system failures do occur despite the best design in industries, and such systems warrant constant monitoring and treatment. A successful water treatment program in an industry depends on the properties of the available water supply. Water used for industrial purposes, contains dissolved ions, gases, organic matter, micronutrients, microorganisms and macroorganisms (Venkateswarlu, 1990). Thus, a thorough monitoring of all the above parameters is required for effective utilization of the water for industrial purposes (Flemming, 2002; Rao et al., 2009). The data thus obtained, can be used as baseline information, which is to be rigorously adhered to, during the operation of the plant. A few basic water quality parameters (e.g., pH, conductivity, hardness, total organic carbon, bacterial counts) are required to be monitored throughout the operational phase of the plant.

During the course of this investigation, the microscopic observations of the WTP samples have provided useful information on the variety of microbiota. There were filamentous bacteria, fungal mycelia and other bacteria enmeshed in the slime/biofilm samples. The CSLM images of the slime deposits collected from the WTP showed many bacterial and microalgal morphotypes, indicating high microbial diversity (Fig.3). The stainless steel mesh of the vacuum degasser unit was covered with microbial slime, which predominantly consisted of *Pseudomonas* sp., and the fungus *Gibberella moniliformis* and resin fines. The slime growth on the stainless steel matrix had completely impaired the very purpose of its deployment in the vacuum degasser section (i.e., reducing dissolved oxygen levels). During maintenance it was also observed that the produce water storage tank (W5, Fig.1) had a large accumulation of organic sludge in the bottom with slime and resin fines.

Initially, when bacteria were characterized by selective medium isolation and Gram staining at the plant site, four different types of bacterial colonies were observed in the slime scrapings. However, the slime forming *Pseudomonas* sp., was predominant. The other three bacterial strains were not of significance since they were not isolated upon repeated sampling.

The slime had a bacterial population of ~10^9 cfu cm^{-2}, while the sulphate-reducing bacteria count was <100 cfu cm^{-2}. The sulphate reducing bacteria cells could have thrived due to microbial fouling, organic compounds and anoxic conditions at the slime-metal interface. The absence of sulphate-reducing bacteria in other sections of the WTP shows that there were no favourable anaerobic zones for their growth. The significant absence of bacteria in the atmospheric degasser inlet could have been due to the low pH (~3.0) at the sampling point. This indicates that bacteria generally do not grow at a very high flow rate under acidic conditions.

During the course of this study the repeated isolation of the fungus (JQ811767, *Gibberella moniliformis* strain KOTA-1) and bacteria (JQ811768, *Pseudomonas* sp. RB001) from the vacuum degasser slime was a significant observation. During the course of this study the repeated isolation of the fungus (JQ811767, *Gibberella moniliformis* strain KOTA-1) and bacteria (JQ811768, *Pseudomonas* sp. RB001) from the vacuum degasser slime is a significant observation. In microbial systems, cells belonging to the same taxa have been documented partaking in cooperative interactions such as dispersal and construction of biofilms. The opportunistic bacterium *Pseudomonas* generally uses quorum sensing to coordinate the formation of biofilms (Sauer et al., 2002). According to Czaran and Hoekstra (2009), there is an important link between quorum sensing and anaerobiosis that can significantly influence biofilm formation.

Generally a cooperative interaction benefits only one recipient; however, in the present case the selective interaction of *Pseudomonas* bacteria and the *Gibberella* fungus was on the basis of mutual benefit which is unique and remarkable. According to classical evolutionary theory, an organism in an idyllic situation will only behave in ways that maximize its own fitness. Therefore, the origin of cooperative interactions, or actions by individuals that result in other individuals receiving fitness benefits, seems counterintuitive.

In a relevant context, bacterial mycophagy (Leveau and Preston, 2008) which is a trophic behavior, where bacteria actively obtain nutrients from living fungi can be considered. Such cooperative scenarios are not mutually exclusive when both microorganisms are benefitted under conditions of nutrient limitation. Typically, such approaches have been very valuable by offering new insight into the complexity of bacterial/fungal interactions. The mycophagous phenotype of collimonads was recently explained as an adaptation to life under conditions of limited nutrient availability (Leveau et al., 2010). Correspondingly, the vacuum degasser system which is anoxic and nutrient-limited could have resulted in the unique bacteria/fungi interaction, which resulted in severe microbial fouling.

Ion exchange resin beds of most WTPs are generally fouled due to organic compounds and bacteria (Anupkumar et al., 2001). Most types of ion exchange resins can become contaminated with suspended solids and dissolved organic carbon in the feed water. Precipitation of organic compounds can occur as a result of changes in pH, resulting in insoluble salts in the demineralization process. It should be emphasized that regular cleaning can prevent accumulation of fouling organisms and extend the resin life. Good operating practices should ensure that the ion exchange units are regularly treated with sufficient regenerant as per standard procedure.

Organic fouling of resins can be due to humic and fulvic acids found in water. These organic species, because of their relatively large molecular weights, become trapped within the resin matrix and impair resin performance. The symptoms of organic fouling include long rinsing time, poor exchange capacity and, in the case of strong base resins, higher silica leakage. In the present study, both anion and cation resins were fouled, resulting in loss of resin capacity and premature replacement of resin beds. Besides, resin fines were also seen enmeshed in the slime of the vacuum degasser unit, which served as the carbon source and indirectly supported the propagation of biofouling.

The levels of dissolved organic carbon in industrial water vary widely from site to site. It also depends on operational parameters and the quality of the make-up water. Dissolved organic carbon concentrations in water support microbial growth and result in fouling of WTPs, resin beds etc, (Anupkumar et al., 2001). Organic compounds are introduced into the process system from the feed water, and also due to biogrowth in the distribution system. Total organic carbon can also be used as a process monitoring parameter to check the performance of ion exchange units in a demineralization plant. The high levels of total organic carbon (~19 ppm) observed in the present study could have supported the luxurious growth of microbes in the WTP system.

Thus, the microbial infestation resulted in microbial fouling problems in the different sections of the WTP. Therefore, appropriate control strategies need to be taken to reduce the organic compounds in the feed water, which would be a long term solution for fouling control. An earlier official report based on various investigations on the WTP at the heavy water plant, recommended the use of macro-porous resin for removal of organic compounds. Cross-linked macro-porous ion-exchange resins have high crush strength and are useful in the removal of colloidal silica, iron, aluminum, organic compounds and other metal hydroxides from water.

Biofilms/biofouling in industrial water systems are usually controlled by chemical treatment using biocides and synthetic bio-dispersants (Meesters et al., 2003; Simões et al., 2010). In the present investigation chlorination was found to be inadequate in controlling the microbial fouling problem of the WTP. This could be due to the low production (<20%) of the biocidal molecule hypochlorous acid at the pH of the feed water (pH 8.3) and its consumption in chlorine-organic reactions. Hence, a stronger biocide ClO_2 was tested for biocidal efficacy. According to Richardson et al. (1994), the ClO_2 bactericidal activity is not generally affected in the pH range of 6.0 to 10.0. Since the WTP needs large volumes of water every hour, there is little contact time with an oxidant to control microbial fouling. Thus, a biocide like chlorine with a much smaller amount of total residual oxidants cannot disinfect the water or control microbial fouling.

Comparing the oxidation strength and capacity of Cl_2 and ClO_2, it was concluded that ClO_2 was a more effective biocide even at low concentrations (Aieta and Berg, 1986). Under normal circumstances, ClO_2 does not hydrolyze in water and thus has a high oxidation potential. The disadvantage is its cost of production, which must be done on-site, and its decomposition in sunlight. The studies carried out with the two biocides have clearly showed that ClO_2 is a better choice (Fig.5). Furthermore, as an important observation ClO_2 biocidal action was noticed after 15 min. contact time at 1.0 ppm residual. Contrastingly, ozone is also a highly oxidizing agent which can significantly reduce the organic load of water and bacteria (Swaraj et al., 2013). However,

ozone is not a feasible option because of the high capital and operating cost to treat large volumes of water. Furthermore, any traces of ozone in the exchange unit where heavy water is produced will adversely affect the production.

The biocidal action of Sodium-2-pyridinethiol-1-oxide against the fungi involves disrupting the ion gradients across the cell membrane and inhibiting the nutrient transport. This results in the starvation death of the mycelia and the fungal colony; however, there are no reports on the denaturation of proteins and nucleic acids in the fungal protoplast. The pyrithione groups of compounds are known chelators of metal cations. They form complexes with the cations, which are required for the cell metabolism in the water as well in the cell milieu, resulting in essential micronutrient starvation. The disrupted configuration of the cell wall would then allow the passage of free pyrithione molecules across the cell envelope and into the cytosol. This in turn would result in the intracellular action of the biocide (Dinning et al., 1998). The strong oxidizing character and highly volatile property of ClO_2 result in the chemical disruption of the bacterial cell wall, which is the most likely action. When the cell wall is severely breached, protein and nucleic acids are released into the immediate milieu. However, in a classical study by Benarde et al (1967), it was reported that there were no characteristic peaks in the 260-280 nm region for protein and nucleic acids. They concluded that ClO_2 did not produce leakage of cell contents and apparently did not oxidize the proteinaceous material of the cytoplasm.

Comprehensive and consequent studies at the plant site for a couple of years and specific experiments in the laboratory provided valuable data, which resulted in framing the following recommendations to the WTP. The first step is to clean the clariflocculator and sand bed channels by removing the slime and algal growth by mechanical scrubbing followed by spraying with copper sulfate solution to kill the algae. High biocide dosing with a free residual oxidant of ~3.0 ppm removes biofilm growth. Both units, i.e., clariflocculator and sand bed should be covered to prevent sunlight entry. This will reduce the algal growth to some extent and also prevents photo-oxidation of ClO_2 in WTP. Since the activated charcoal beds are also fouled, they need to be replaced with new activated granular carbon to enhance functionality. Corrosion of the vacuum degasser structural material, i.e., carbon steel connectors (See Supplementary data), has to be controlled. Otherwise, the connectors should be replaced with a stainless steel frame, as iron corrosion products can potentially aid the growth of microbes such as iron bacteria and can also clog the stainless steel matrix pores. The particulate corrosion products can also accrue with the slime and result in galvanic corrosion of the WTP structural material.

In this way, water treatment should be considered mandatory and the manner of treatment has to be decided on a case-to-case basis for effective control of biofouling problems. Over the decades there have been serious efforts by many researchers to understand biofouling problems in industries and also to develop methods for its prevention. This has resulted in preserving the valuable service life of industrial distribution systems for as long as possible.

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Supplementary Data

TABLE S1. The effect of various chemicals in removing the vacuum degasser fouling deposit

S. No	Solvent	Observation
1	Acetone	Insoluble
2	Alkaline Brine	Moderately soluble
3	Ethyl Alcohol	Insoluble
4	Hydrochloric acid	Soluble
5	Methylene bis thiocynate	Insoluble
6	Oxalic acid	Moderately soluble
7	Nitric Acid	Soluble
8	Potassium permanganate	Insoluble
9	10% Phosphoric Acid	Insoluble
10	5% Soda lime	Insoluble
11	Sodium hypochlorite	Slightly soluble
12	10% Sodium hydroxide	Soluble

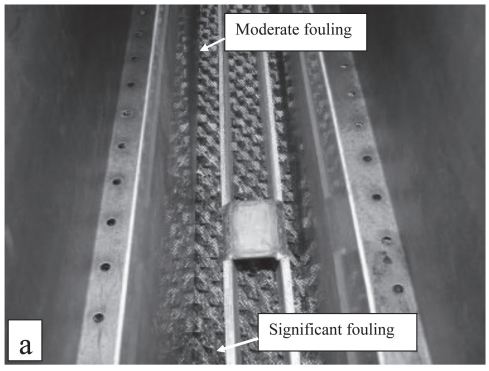
Experiments with chemical solutions to remove biofilm were carried out to monitor their effect in reducing the biofilm cohesiveness and increase biofilm detachment. Xavier et al. (2005) have studied the effect of NaOH on biofilm removal; in the various experiments without changing the velocity they showed biofilm removal in the form of cell clusters within 1 min of the introduction of dilute NaOH. This observation suggests that the chemical treatment weakened the mechanical properties of the biofilm. The reduction in biofilm cohesiveness resulted in the detachment of biofilm in less than 4 min after the NaOH was added. In the present study among the various chemicals used for removal of biofouling growth on the SS matrix, 10% NaOH was found to be more effective.

TABLE S2. Biocidal action of Sodium-2-pyridinethiol-1-oxide (SP) against the microfouling fungi and bacteria

	Fungi (*Gibberella moniliformis*)		Bacteria (*Pseudomonas* sp.)	
% SP	Contact time 30 min	Contact time 60 min	Contact time 30 min	Contact time 60 min
0.001	Growth	Growth	Growth	Growth
0.003	Growth	Growth	Growth	Growth
0.005	Growth	Growth	Growth	Growth
0.007	Growth	Growth	Growth	Growth
0.01	Growth	Growth	Growth	Growth
0.03	Growth	Growth	Growth	Growth
0.05	Growth	Growth	Growth	Growth
0.07	Growth	Growth	Growth	NA
0.1	Growth	Growth	Growth	NA
0.2	Growth	Growth	Growth	NA
0.5	Growth	Growth	Growth	NA
1.0	Growth	Inhibition	Growth	NA
2.0	Inhibition	Inhibition	Growth	NA
4.0	Inhibition	Inhibition	Less growth	NA

NA - Not analyzed

FIG. S1. Digital image showing the top portion of stainless steel matrix in the vacuum degasser unit through the manhole



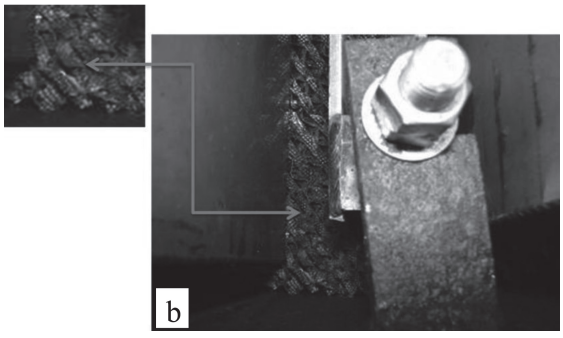
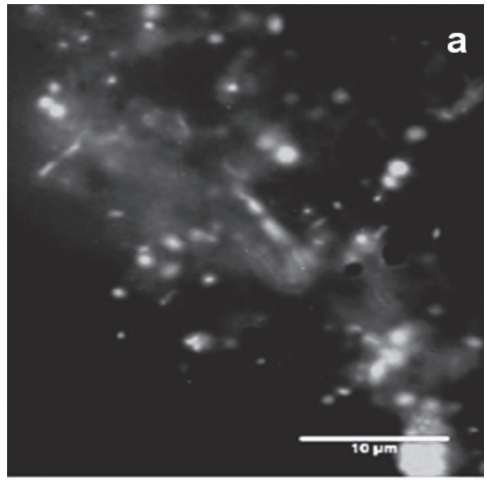


FIG. S2 (a). Digital image showing biofouling intensity of the vacuum degasser stainless steel matrix after cleaning and one year of operation. Significant biofouling was noticed at the periphery of the unit. **S2 (b)** Digital image showing fouling of vacuum degasser stainless steel mesh as well as carbon steel corrosion deposits (Scale could not be provided since the images was recorded at an angle).



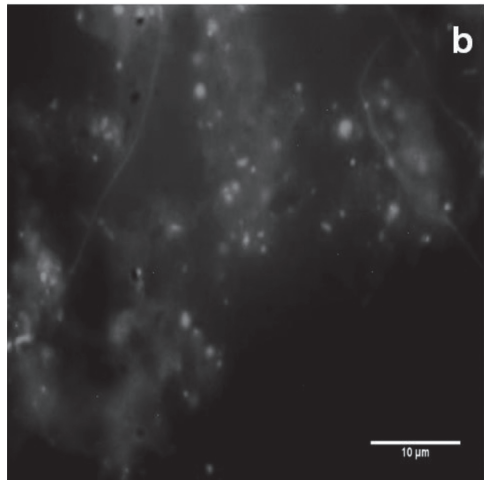


FIG. S3. Epifluorescence microscopic images of the fouled activated charcoal filter bed sample of the WTP. (a) Charcoal particles enmeshed in the microbial slime and bacterial cells. (b) Filamentous forms and slime with charcoal particles

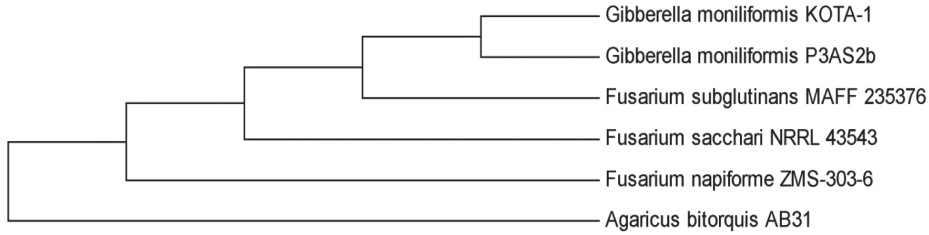


FIG. S4 (a). Phylogenetic tree based on 18S rRNA gene sequences constructed by the neighbor-joining method, showing the phylogenetic relationship between *Gibberella moniliformis* KOTA-1 and other close fungal species. *Agaricus bitorquis* was used as the fungal outgroup

***Gibberella moniliformis* strain KOTA-1 18S ribosomal RNA gene, partial sequence**

GenBank: JQ811767.1

>gi|386698314|gb|JQ811767.1| *Gibberella moniliformis* strain KOTA-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

CCAACCCCTGTGACATACCAATTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCAC
AAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGA
AGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTA
TTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCAGCTTGGTGTTGGGACTCGCGAGTCAAATCGCGTTCC
CCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAA
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CCCTCTGCTGGTTCACCTACGGAAACCTTGTTAC

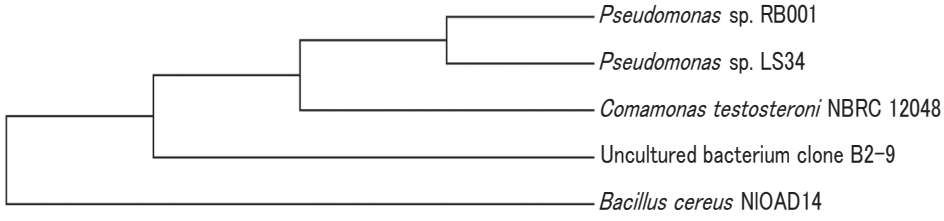

FIG. S4 (b). Phylogenetic tree based on 16S rRNA gene sequences constructed by the neighbor-joining method, showing the phylogenetic relationship between *Pseudomonas* sp. RB001 and other close bacterial species. *Bacillus cereus* was used as the bacterial outgroup. The identified sequences were deposited in the GenBank sequence database under the accession numbers; fungus (JQ811767, *Gibberella moniliformis* strain KOTA-1) and bacteria (JQ811768, *Pseudomonas* sp. RB001)

***Pseudomonas* sp. RB001 16S ribosomal RNA gene, partial sequence**

GenBank: JQ811768.1

>gi|386698315|gb|JQ811768.1| *Pseudomonas* sp. RB001 16S ribosomal RNA gene, partial sequence

CCATGCAGTCGAACGGTAACAGGTCTTCGGATGCTGACGAGTGGCGAACGGGTGAGTAATACATCGGAACGTGCCTAGTAGTG
GGGGATAACTACTCGAAAGAGTAGCTAATACCGCATGAGATCTACGGATGAAAGCAGGGGACCTTCGGGCCTTGTGCTACTAGA
GCGGCTGATGGCAGATTAGGTAGTTGGTGGGGTAAAGGCTTACCAAGCCTGCGATCTGTAGCTGGTCTGAGAGGACGACCAGC
CACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCA
GCAATGCCGCGTGCAGGATGAAGGCCCTCGGGTTGTAAACTGCTTTTGTACGGAACGAAAAGCCTGGGGCTAATATCCCCGGG
TCATGACGGTACCGTAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAA
TTACTGGGCGTAAAGCGTGCGCAGGCGGTTTTGTAAGACAGTGGTGAAATCCCCGGGCTCAACCTGGGAACTGCCATTGTGAC
TGCAAGGCTAGAGTGC

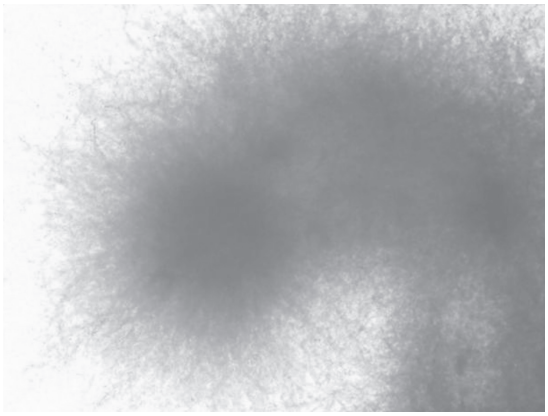

FIG. S5 (a). The Stereo-zoom microscope image of the fouling fungus *Gibberella moniliformis* (Magnification 125X)

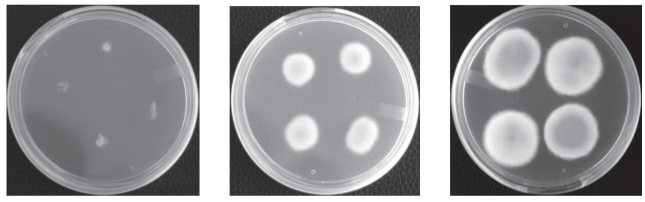

FIG. S5 (b). Growth of fungal colony on potato dextrose agar perti-plate, completely grown fungus had an orange colour in the centre of the colony. The images show an increase in the pigmentation of the fungal colony *Gibberella moniliformis* at different days of incubation