

# Microbial Biofilm Reactor for Sustained Waste Water Treatment and Reuse



Shaon Ray Chaudhuri

**Abstract** Freshwater scarcity is a global problem that pertains to the ever-increasing population, contamination of freshwater by wastewater generated from different anthropogenic sources as well as misuse of freshwater for secondary (non-potable) applications. Later two issues could be addressed through the appropriate implementation of Microbial Technology in an eco-friendly way. The application will involve proper selection of the wastewater sources (for microbial isolation), their pollutant identification, selection of tailor made bacterial consortium/ isolates for treatment of the wastewater and converting the waste into reusable by-product. This is the current trend used for pilot scale wastewater treatment using biofilm bioreactors. This article talks about the few successful case studies implemented for different types of wastewater treatment (municipal/Agricultural runoff, petrochemical, tannery/mining industry and milk processing plant wastewater) in biofilm reactors that could run for years after being installed in the pilot scale. The processes are faster, sludge free and, in most cases, ensure complete reuse of treated water, hence preventing wastage of freshwater for non-potable applications. Biofilm based system makes them resistant to external perturbation, stable with enhanced efficiency. Through this approach, eco-friendly processes of wastewater treatment could be made self-sustainable.

**Keywords** Biofilm reactors · Tailor-made consortium · Wastewater · Biofertilizer · Non-potable application · Sludge free system

## 1 Introductory Background

Seventy-five percent of our planet, Earth, is covered with water, of which only 2.5% is suitable for consumption (freshwater). The population is rapidly growing, demanding more freshwater. However, our freshwater reserves are dwindling, with 40% of the world population (in 50 countries) threatened to face water scarcity by 2025 as per the United Nations report. The freshwater consumption in the United States per month

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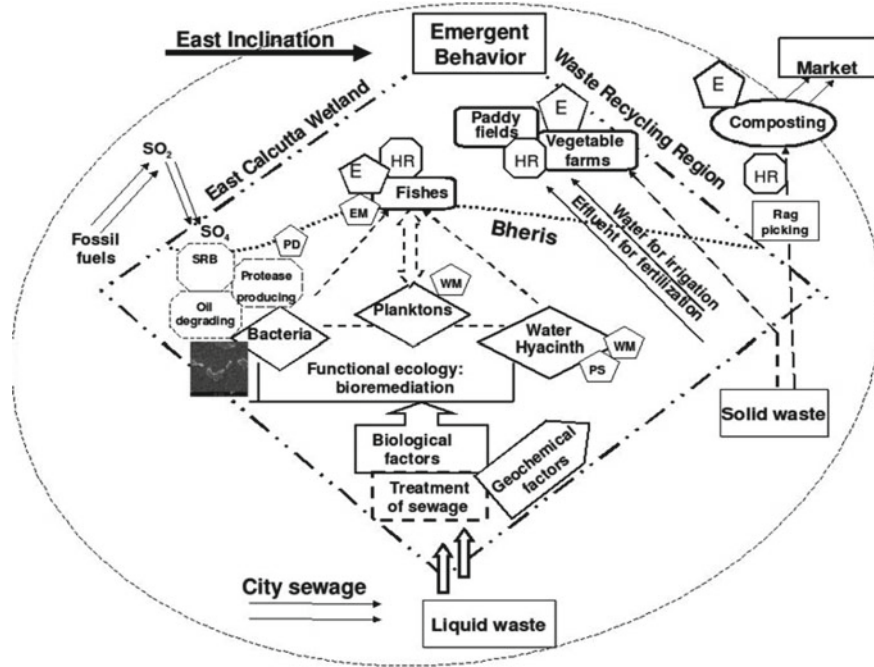
is about  $1.486 \times 10^{10} \text{ m}^3$ . On average, about  $10^6 \text{ m}^3$  of freshwater is used per day worldwide. While a portion of it is for potable purposes, the rest is wasted for non-potable application (that can be carried out using adequately treated wastewater). The major share of water consumption every day is used in agriculture (75 to 90%), which does not require fresh water. Freshwater is used directly for drinking, personal cleaning and indirectly (virtual water) through the products we use in our day to day lives. The lion's share of the virtual water (as proposed by Professor Tony Allen) goes for the food production (about  $3.5 \text{ m}^3/\text{day}/\text{person}$ ), be it the grains ( $1.12 \text{ m}^3/\text{kg}$ ), chocolate ( $24 \text{ m}^3/\text{kg}$ ), or the meat ( $15.4 \text{ m}^3/\text{kg}$ ) to name a few (The World Counts 2021). More than a billion people worldwide (mostly in developing countries) lack access to safe potable water.

The principal concern in front of mankind across the globe is ensuring the security of food, water, and energy. The limited freshwater resource is either misused for non-drinking purposes or contaminated due to anthropogenic reasons. To fulfil the demand for freshwater, it is essential to use freshwater judiciously and prevent its contamination through proper management. Hence, the wastewater could become valuable if adequately treated to prevent pollution and reused in the secondary application. Considering the amount of wastewater generated, the available treatment facility is substantially compromised (both in terms of quantity/capacity and quality of treatment) (Kaur et al. 2012). The quantity of wastewater is expected to rise with time, demanding more effluent treatment plant (ETP) installation. The existing treatment systems are affordable mostly by the large installations due to the cost involved in setting up and running the ETP (CPCB 2005a). In the absence of such facilities, the untreated or partially treated wastewater either pollutes the water bodies (Trivedy et al. 2001) due to discharge or is used for agriculture leading to employment generation, livelihood support from the sale of the produce but with a constant concern of occupational and environmental health hazard. About 49% of the annual groundwater recharged is used for irrigation, while 4.2% is used for the rest (domestic and industrial) of the activities (CGWB 2011), which is expected to rise to 6.7% by 2025. In addition, freshwater availability gradually decreases with a simultaneous increase in greywater generation (Bhardwaj 2005), calling for the rapid development of suitable water management technologies.

In India, the STPs are located on major river banks (CPCB 2005b), operating on the principle of Oxidation Pond/Activated Sludge/Up-flow Anaerobic Sludge Blanket/Waste Stabilization Ponds. The latter is considered the most suitable for developing nations with limited population and technological expertise (Shuval et al. 1986). Smaller industries in the same location often set up common ETP by implementing methods in isolation or different combinations (dissolved air floatation/dual media filter/activated carbon filter/sand filtration/tank stabilization/flash mixer/clarifier/flocculation/secondary clarifiers/Sludge drying beds). The cost for ETP set up for treating the entire volume of greywater would be of a different order of magnitude on the higher side (Kumar 2003). The step of ETP operation needing further attention is solid sewage handling which involves labour, energy, time and proper disposal. The lack of direct economic return and heavy investment is why local civic bodies have little interest in ETP/STP operation.

The large volume of wastewater and sewage is often sold for irrigation in places with a lack of alternative water sources (Bhamoriya 2004). This wastewater, due to its high N and P content, enhances the production of paddy, wheat, range of vegetables, flowers, fruits and fodder per unit land when supplemented with 50 or 75% of the prescribed quantity of chemical fertilizer (Strauss and Blumenthal 1990; Minhas and Samra 2004). The wastewater is also used for fish feed production during wastewater fed aquaculture at East Kolkata Wetland, India (Ray Chaudhuri et al. 2008). These activities are carried out in large chunks of land in countries like India (Sengupta 2008), generating employment (Minhas and Samra 2004) and nutritious food (Ray Chaudhuri et al. 2008; Ray Chaudhuri et al. 2007; Pradhan et al. 2008) while utilizing the plant growth nutrients in the wastewater and preventing the use of freshwater for non-potable application. However, there always remains a concern of environmental deterioration and health risks for the workers and the consumers of the produce (Satyawali and Balakrishnan 2008; Minhas et al. 2006; Tripathi et al. 2011). The integrated approach of utilizing wastewater for aquaculture (Ray Chaudhuri et al. 2008) and then using the treated water for agriculture (Ray Chaudhuri et al. 2008) resulted in safer produce which serves about one third of the population of the metropolitan (Kolkata) (Ray Chaudhuri et al. 2012). The science behind the purification of the wastewater has been investigated at length (Ray Chaudhuri and Thakur 2006; Adarsh et al. 2007; Chowdhury et al. 2008, 2011; Ray Chaudhuri et al. 2008; Yadav et al. 2010; Nasipuri et al. 2010; Chowdhury 2010; Mishra 2010; Nasipuri 2011) for understanding the ongoing process. An emergent behaviour was observed in the entire operation (Mishra 2010), a key feature of a complex system (Mukherjee et al. 2010). Based on this understanding, the system has been successfully replicated in Bangladesh (Khanam 2016; Khanam et al. 2016). The complexity of the ongoing practice at East Kolkata Wetland which treats the entire sewage of Kolkata generating a portion of its fish and vegetable demand along with ensuring environmental protection and employment, is represented in Fig. 1.

In India, in collaboration with the urban local bodies, the State Government is responsible for setting up and maintaining the sewage treatment plants. The funding for setting up the treatment plant and incentives for taking adequate measures for pollution reduction is also in place with contribution from the Central government. The statutory power for monitoring the operation/performance lies with the State Environmental Protection Agency (Pollution Control Board) as per the Water Act 1974, with adequate provision for using the treated water for irrigation. Different by-products are recovered during wastewater treatment (Satyawali and Balakrishnan 2008; Pant and Adholeya 2007). However, the notion of biological treatment alone being insufficient for optimum pollutant removal persists (Pant and Adholeya 2007), calling for elaborate ETP design. Conventional wastewater treatment involves physical, chemical and biological unit operations. The physical unit operations include screening, comminution, flow equalization, sedimentation, floatation, granular medium filtration. The chemical unit operation mostly involves chemical precipitation, adsorption, disinfection, dechlorination. The biological unit operation includes activated sludge, aeration lagoon, trickling filters, rotating biological contractors, pond stabilization, anaerobic digestion or biological nutrient removal



**Fig. 1** The emergent behaviour observed in the ongoing integrated resource recovery system operating at East Kolkata Wetland where the city sewage is treated through indigenously designed wastewater fed aquaculture practice followed by using the sediment/silt and the treated water (with > 99% reduction in coliform count) for agricultural practices. It generates employment and revenue while protecting the environment. It involves close interaction among the microbes and plants in the region leading to detoxification and a perfect understanding of the aquaculture practice, which converts the nutrients in wastewater into fish feed (phytoplankton/zooplankton) to eliminate the expenditure of fish feed addition during fish cultivation. The abbreviations used in the figure are as follows: PD means Product Development, EM is Environment Management, WM stands for Waste Management, E is the abbreviated form of Economy Generation, and HR indicates Human Resource. Conceptualized by Prof Indranil Mukherjee and Dr Shaon Ray Chaudhuri while drawn by Dr Madhusmita Mishra from the West Bengal University of Technology (now renamed Maulana Abul Kalam Azad University of Technology West Bengal India)

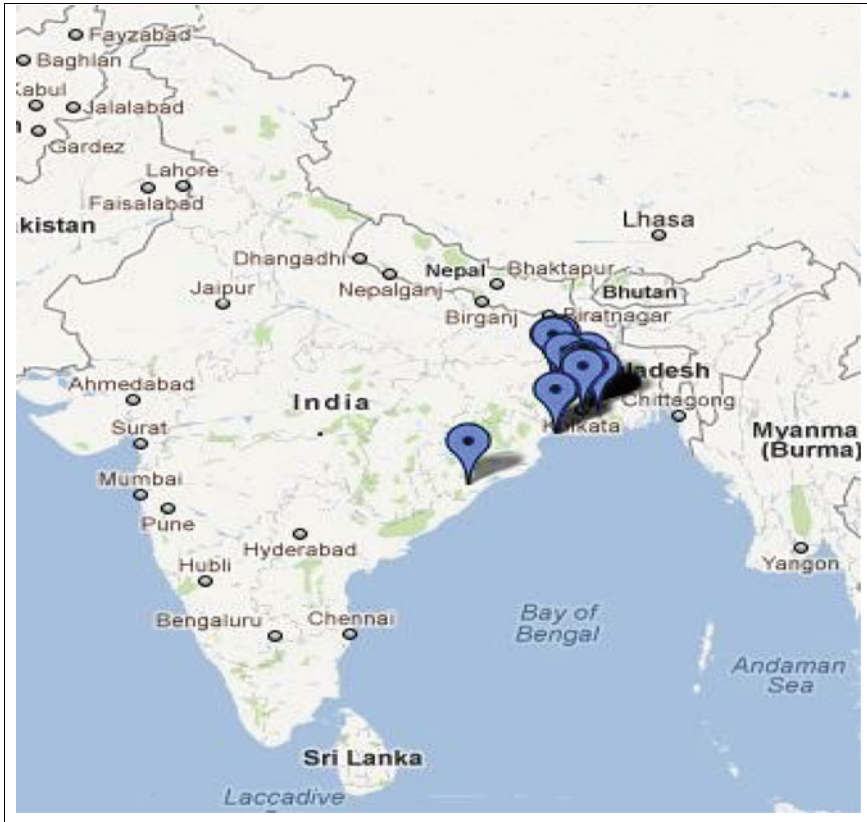
(United Nations 2003). The treatment steps are selected based on the wastewater composition and volume. The different unit operations selected are used in combination to get the desired purification of the wastewater. Algal wastewater treatment and nutrient removal through constructed wetlands are economically viable options. Still, they need a large area for water treatment due to the prolonged hydraulic retention time with minimum energy requirement (Kadlec and Wallace 2009; Kadlec 2009). Land availability is a major problem in developing countries. Hence, a strong need is felt to develop rapid, eco-friendly biological processes with adequate scientific and technological intervention. The need based development of wastewater treatment technology is evident from ancient times (Chakraborty et al. 2018).

The approach for innovative rapid sludge free microbial wastewater treatment process development using microbial formulations has been established by the Microbial Technology Group, India, for different types of wastewater. The criterion for developing the microbial formulation involves the steps mentioned in the subsequent lines. The wastewater composition is analyzed to assess the pollutants to be treated (quantitative and qualitative assessment) as the first step towards consortium development. Microbial isolation is carried out using inoculum from environmental sites, selected based on two criteria, sites that receive point sources of the pollutant but do not show pollutant toxicity during quantitative estimation. The absence of elevated pollutant levels indicates the presence of potent bioremediants. The next step is selecting the medium for isolation of the microbes/consortium based on the literature. The microbial growth medium (based on the literature survey) for cultivating bacteria for bioremediation of the identified pollutant (in the wastewater) is used for strain/consortium enrichment. The pH and growth temperature are determined, keeping in mind these parameters in the environmental samples used for microbial isolation. The process of tailor made consortium development for sludge free wastewater treatment for different types of wastewater treatment are detailed in the subsequent sections.

## **2 Municipal/Agricultural Runoff Treatment Using Selectively Developed Consortium**

The common pollutants present in municipal sewage and agricultural runoff are nitrates and phosphates. The origin of these is from the surface runoff (natural process of leaching from the soil, synthetic fertilizers leaching during agriculture), food products, animal excreta and manure, human excreta, detergents used for cleaning, leaching of industrial effluents like dairy effluent (farmyard runoff as well as milk processing plant runoff). The conventional sewage treatment process is an elaborate 240 h process using energy and labour (Saha et al. 2018). The essential plant growth nutrients in the wastewater are lost during the process. These include nitrates, ammonia and phosphate. While nitrogen fertilizer (urea) production needs immense energy, the phosphate reserves are rapidly depleted. The current trend is to recover these nutrients and reuse them for safe agricultural use. It is evident from the ongoing phosphate recovery practices (Ulrich and Frossard 2014; Chipako and Randall 2020; Alemayehu et al. 2020) from wastewater and human excreta. As mentioned earlier, wastewater based agricultural practices have a major concern of contaminating the produce, if used untreated, causing environmental hazards. The treatment of the generated wastewater needs immense expenditure for setting up the sewage treatment plant and hiring trained manpower for the labour intense operation, which is often not affordable by the concerned authority. There is a huge gap between the amount of wastewater generated and the available treatment facility. So there remains a need for the development of a rapid eco-friendly process.

The work started with screening for nitrate and phosphate reducing bacteria from different environmental origins. Thirty-two different sites were selected based on the point sources of pollutants received by them (DebRoy et al. 2012). Various kinds of water bodies like rivers, natural and artificial water bodies, marine coast, hot spring, rhizosphere of plants, algal mats and agricultural sites had been selected for sample collection (Fig. 2). In addition, microbial biofilms from nitrate containing wastewater treatment plants were also selected for bacterial isolation (Mishra et al. 2014). Next, the water samples were analyzed for their physicochemical properties. The sites that



**Fig. 2** Environmental screening for isolation of efficient microbes for wastewater treatment. The 1st three rows show site identification based on receiving point sources of nitrate and phosphate. The 4th row shows representative sites of sample collection from Mayurakshi (Tilpara barrage), Ultadanga over bridge, hot spring Agnikundu at Bakreshwar, wastewater canal next to Captain Bheri. The 5th row consists of images of the water plants whose rhizosphere were used to isolate microbes, namely Bulrush, Aquatic grass, water lily and water hyacinth. The 6th row consists of the enrichment techniques used like hay enrichment, air bubbling technique, Winogradsky column and plating techniques showing streaking of pure isolates on enriched medium with different concentrations (2000 mg/L) of nitrate and phosphate. The 7th row represents light microscopic images of the representative isolates



Fig. 2 (continued)

received point sources of nitrate and phosphate but did not show high concentrations of those pollutants were selected to isolate the bacteria for consortium development. The logic behind the selection was the assumption that there might be the presence of efficient pollutant reducers at those sites and hence a low level of pollutants inspite of receiving point sources. The purpose of the study was to sequester the essential nutrients during wastewater treatment. Microbes like *Beggiatoa*, *Thiomargarita* and *Thioploca* were known for nitrate accumulation (Mussmann et al. 2007; Otte et al. 1999; Schulz et al. 1999), while phosphate accumulation was well documented in domain bacteria (DebRoy et al. 2013a). The enrichment techniques were adopted based on the criterion for the enrichment of the nitrate accumulators. The growth medium for selective enrichment of nitrate and phosphate accumulators was based on literature survey. The samples used for cultivating the bacterial isolates from the selected sites, namely water, sediment, plants rhizosphere and algal mats, were

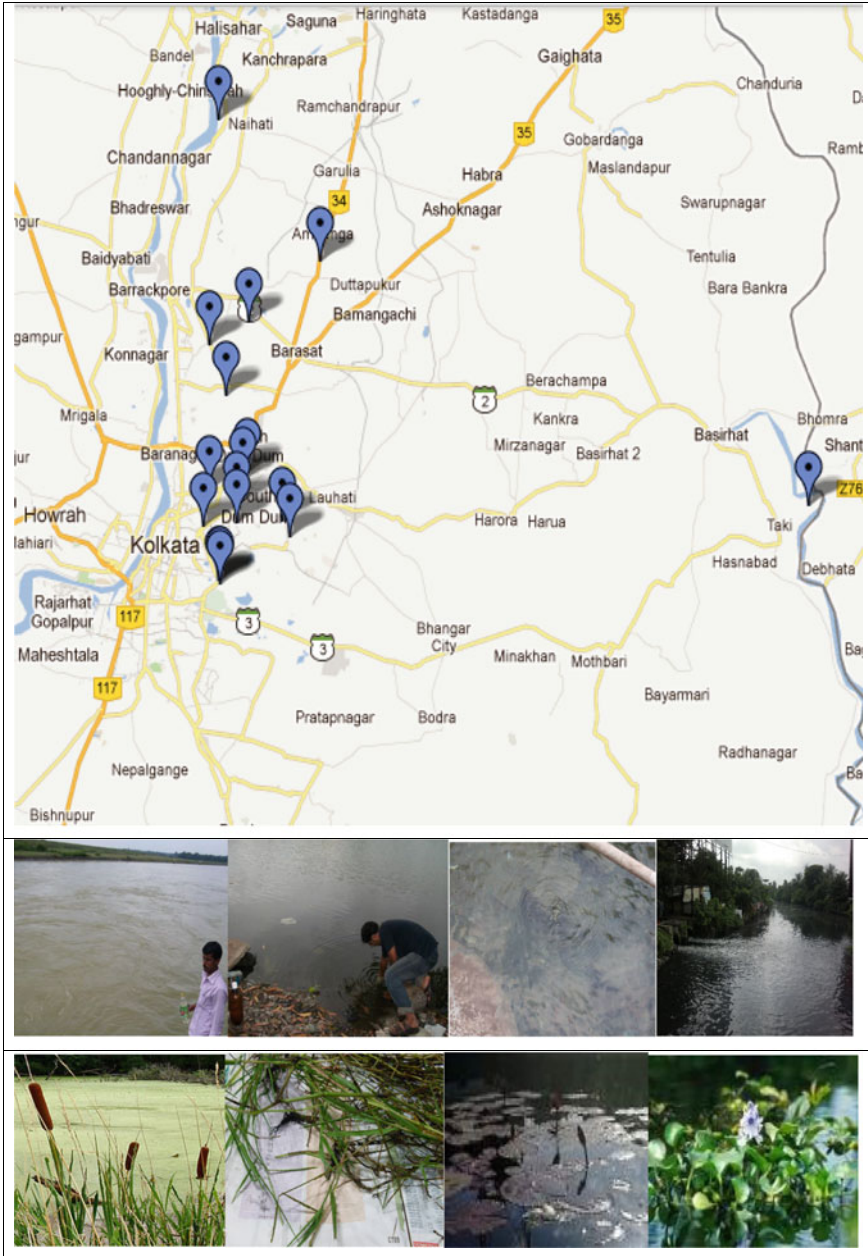
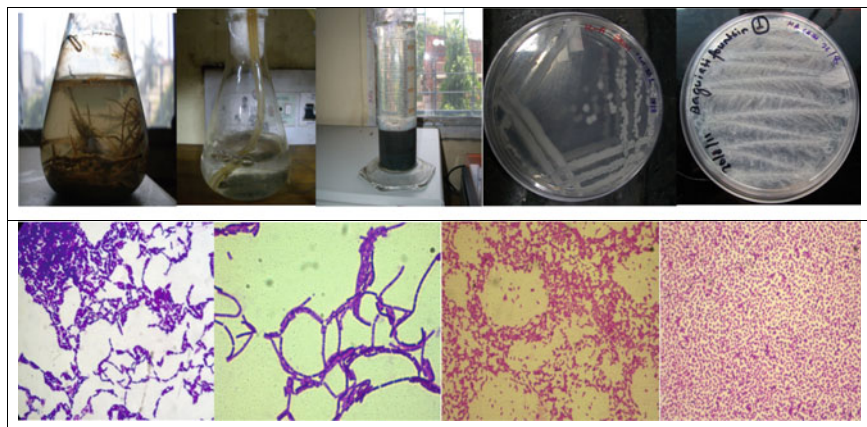


Fig. 2 (continued)





**Fig. 2** (continued)

carried aseptically at room temperature for further processing, as shown below in Fig. 2.

From a total of 32 sites with different types of samples (water, sediment, algal slime, rhizosphere of plants) from each site, about 130 different isolates were purified on nitrate broth alone. On the assumption (based on the literature survey) that the nitrate accumulators might be filamentous, preliminary screening of the pure isolates was done to select the isolates that were bacilli or filamentous in shape. The selected 18 pure isolates and the other 6 isolates of the environmental origin from the laboratory stock were further selected for nitrate and phosphate removal from the medium. The isolates with maximum simultaneous nitrate and phosphate removing ability were selected to test the nitrate accumulating ability through cell lysis, followed by nitrate concentration assessment in the intracellular sap through the method of (Cataldo et al. 1975). Only one isolate (*Bacillus* sp. MCC0008) was found to accumulate nitrate intracellularly up to 1278.66–1302.122 ppm/gm of cell pellet wet weight when grown in nitrate broth with 1000 ppm of nitrate within 2 h of growth (Ray Chaudhuri et al. 2016a). Through Dissimilatory Nitrate Reduction to Ammonium and using nitrogenase enzyme, *Baggiatoa* is reported to accumulate 20,000 times more nitrate inside the vacuoles than the surrounding environment (Teske et al. 2006; Ruuskanen 2014). This strain was selected for consortium development.

Since phosphate removal is through polyphosphate accumulation in bacteria, the maximum phosphate removers (assessed through Daniges (Deniges 1920)) were selected for consortium development. The well characterized (DebRoy et al. 2013a, b, c; Ray Chaudhuri et al. 2016a) three selected isolates combined in definite proportion were used for simultaneous nitrate and phosphate removal from raw sewage canal water with agricultural runoff. The single unit operation process with minimal carbon source addition was scalable with little energy requirement. It was scaled upto 2.64 m<sup>3</sup>/day processing capacity with about 55 to 61% phosphate and 93% nitrate removal within 2 h (hydraulic retention time) with an associated Chemical

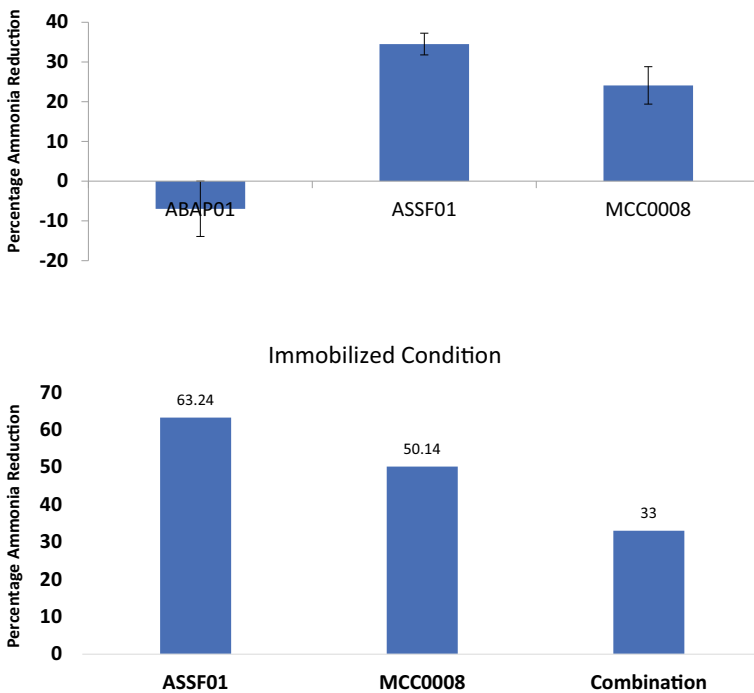
Oxygen Demand (240 mg/L) reduction of 91.8% and Biological Oxygen Demand (167.7 mg/L) reduction of 97.4% (Saha et al. 2018). Analysis of the different soluble and gaseous by-products formed during the metabolic pathway indicated the majority of the nitrogen to be accumulated as nitrate intracellularly in this low C/N ratio while showed higher ammonia production under high C/N ratio (nitrate broth). The finding was at par with available literature (Yoo et al. 1999). The bioreactor could run continuously for more than 200 days with little sludge generation before the system was dismantled. This is by far the fastest sewage treatment process (Ray Chaudhuri et al. 2017a). The accumulated nitrate and phosphate within the biomass could promote plant growth with the nutrient quality enhancement of mung bean seeds (Ray Chaudhuri and Thakur 2013). The treated wastewater with neutral pH (hence no phosphate precipitation) (Saha et al. 2018) was suitable for agriculture and aquaculture.

Adequate biotechnological intervention developed a single unit biofilm-based process for treating the sewage within 2 h without sludge formation using a tailor-made bacterial consortium from environmental origin suitable for secondary application. It reduces about 90% CO<sub>2</sub> equivalent gas emission due to a drastic reduction in energy consumption. In addition, it also saves investment on land for the installation of the ETP. Complete nitrate removal was reported from enriched (C/N) wastewater (Hao et al. 2013), but the current process could achieve similar results from low C/N rich wastewater within a very short time (Saha et al. 2018). Adopting this process can fill the gap between the amount of wastewater generated and the available processing capacity. It could also be introduced in existing ETP resulting in an enhanced treatment capacity.

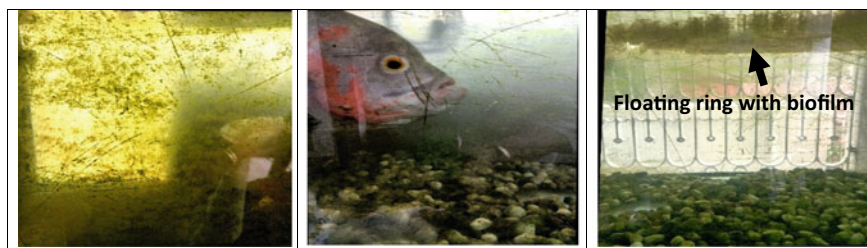
### **3 Aquaculture Wastewater Treatment Using Single Unit Bacterial Biofilm System**

The aquaculture industry is important for a countries economy. It uses a large volume of fresh water and generates a copious amount of wastewater. The system is extremely sensitive to elevated nitrogen concentration. The toxicity level for aquaculture is much lower than those permitted by Environmental Protection Agency for all other environmental purposes. The source of pollution is from the unutilized fish feed and the fish excreta (Cho and Bureau 1997; Axler et al. 1996). The major toxicity is due to ammonia (above 0.5 mg/L) and nitrite (above 0.02 mg/L), leading to reduced excretion, blood and tissue toxicity, lethargy, and finally, the mortality of fishes. The amount of organic waste produced is about 2.5 times fish production (Ackefors and Enell 1994). To avoid fish mortality, large volumes of freshwater are used for diluting the pollutants. This approach is becoming non-viable due to the fast depleting reserves of freshwater, necessitating the development of rapid, efficient, economic and eco-friendly aquaculture wastewater treatment processes (Goldburg and Triplett 1997; Porrello et al. 2003). Yet another contaminant released into the wastewater from aquaculture practice is phosphate (Piedrahita 2003), which must be removed

simultaneously. The existing treatment process is laborious to meet the strict guidelines according to the Water Resource Act and FAO (Moccia et al. 1997), making it unadoptable for the smaller agencies inspite of the extensive environmental damage caused by the discharge of untreated effluent (Doupe et al. 1999; Boyd et al. 2001). Innovative (but expensive) methods have been developed for minimizing pollution during fish cultivation (Mayer and McLean 1995). Biological treatment of aquaculture wastewater has been reported both under suspended (Barman et al. 2016; Lyles et al. 2008) as well as immobilized (Fei et al. 2019; Gogoi et al. 2021a, b) conditions within 7 days, 4 days, 24 h and 14 h (Gogoi et al. 2021a, b), respectively. The biofilm based system was found to show higher efficiency using a pure isolate of *Bacillus albus* AFFS01 from the sludge of aquaculture pond instead of a consortium developed using efficient ammonia removers (Fig. 3). It is not essential that consortium will always work better during environmental application. The efficiency of performance will depend on the interaction among the isolates.



**Fig. 3** Top graph—Percentage of ammonia reduction by three strains of genus *Bacillus* sp ABAP01 (Ammonia producer), ASSF01 and MCC0008 under the suspended condition of growth in nitrate broth. Bottom graph—Percentage ammonia reduction by two *Bacillus* strains ASSF01 and MCC0008 and their consortium (1:1). The graph shows a better ammonia reduction under an immobilized state by the pure isolates ASSF01. It also reveals an antagonistic interaction between ASSF01 and MCC0008



**Fig. 4** Wall of the aquarium in absence and presence of the immobilized bacterial biofilm as floating rings on the surface of the water after one month of operation

The immobilized cells in the bioreactor could work efficiently for more than 20 months treating real effluent with maintained efficiency. The novel, sludge free microbial biofilm based approach developed by the Microbial Technology Group for treatment of aquaculture effluent tested at 15.43 L/day capacity at the laboratory scale in continuous mode (Gogoi et al. 2021a, b) was also found to be effective for refinery effluent treatment. Aquaculture effluent treatment is also required at a relatively smaller scale in the aquariums, which periodically involves labour for cleaning and freshwater replacement. The immobilized bacterial biofilm on raschig rings was found to drastically reduce algal growth on the aquarium walls (254 L) even after one month of operation without additional energy expenditure inspite of uneaten fish feed and sufficient sunlight, indicating efficient ammonia, nitrate and phosphate removal from the wastewater. This could alleviate the need for water replacement and clean regularly. The pictorial representation of the algal deposition on the walls of technology deployed and non-deployed aquarium, treated under identical conditions after one month of deployment in running aquarium is provided in Fig. 4.

#### **4 Milk Processing Plant Wastewater Treatment Using Selectively Developed Consortium**

The dairy industry produces 1.5 to 10 L of wastewater per litre of milk processed with composition dependent on the upstream operation. This kind of wastewater is C/N rich and, unless treated adequately, leads to the environmental deterioration (Biswas et al. 2019). Literature states that microbes lead to ammonification under a high C/N ratio instead of denitrification (Yoo et al. 1999). Since dairy wastewater is enriched and in large volume, biotransformation was attempted instead of bioremediation into ammonia-rich liquid fertilizer as ammonia is preferred over nitrate for plant growth (Nasholm et al. 2009). Through this approach, the plant growth nutrients (nitrate, nitrite, ammonia, phosphate, potassium, magnesium) in dairy wastewater could be completely reused along with the entire volume of treated water which could cut down on the loss of freshwater for agriculture. This would not

only cut down on the wastage of freshwater but will also prevent environmental pollution caused due to chemical fertilizer leaching. It would drastically reduce chemical fertilizer production, hence saving energy expenditure (used during urea production) and finite resources like rock phosphate. Unlike the application of untreated dairy wastewater, the treated wastewater would not harm the soil as the concentration of protein and lipids will be low, hence the associated Chemical Oxygen demand and Biological Oxygen Demand would be substantially reduced during the treatment. Based on the literature, the growth medium for cultivating the microbes for bioconversion was selected. The microbes known to be involved in ammonification, and preferably lacking denitrification, were selected for assessing their growth media and growth conditions. The activated sludge of Mother Dairy ETP at Dankuni, West Bengal, India, was used for bacterial strain isolation as per standard culture based procedure (Biswas et al. 2019). These organisms, along with other environmental isolates with nitrate, protein, phosphate reducing properties, were also considered for consortium development (Halder et al. 2020). The consortium with the highest ammonia producing ability was used for biofilm based reactor development. The performance of the consortium was enhanced under immobilized conditions. It is known that the cell density of the organism is higher in a biofilm, and so is the extracellular polymeric substances secreted by the cells. These factors lead to enhanced pollutant removal as a concentration gradient is created within the bulk liquid. The biofilm provides a higher actual hydraulic retention time as the pollutant diffuses from the bulk liquid through the extracellular polymeric substances to the cells in the biofilm. To understand the actual reason for performance enhancement in the current case, the consortium was tested for assessing its doubling time under the immobilized condition as per standard procedure (Gogoi et al. 2021b). The doubling time of the consortium in suspension culture was 78 min 56 s while the same under immobilized conditions was 17 min 10 s. The doubling time was determined as per the method reported by (Gogoi et al. 2021b). The determination of doubling time was done by allowing growth medium to pass through packed bed column with immobilized consortium at different flow rates (2.2 ml/min, 3.3 ml/min and 8.8 ml/min) under ambient condition.

The column was eluted with 25 times the bed volume with the bacterial growth medium. The fraction eluted were checked for scattering at 600 nm. The data was plotted with the elution time on the x axis while the optical density on the y axis. The data showed a doubling time of 17.6 min (at 2.2 ml/min), 17.16 min (3.3 ml/min) and 17.1 min (8.8 ml/min) for the different flow rates (Fig. 5). The average doubling time was 17.28 min, closest to 17.16 min at a flow rate of 3.3 ml/min. Hence the doubling time was taken as 17.16 min, equivalent to 17 min and 10 s. The doubling time was calculated by assessing the difference between the two peaks. It is assumed that the flow rate should be such that the eluted cells (after the cell divides) do not divide within the column. So every time that the cells divide, a peak is observed. Hence, the doubling time will be the gap between two subsequent peaks. The peak height increased with a slower flow rate, hence a longer retention time. It reflects the relative abundance of the cells getting eluted at a particular time.

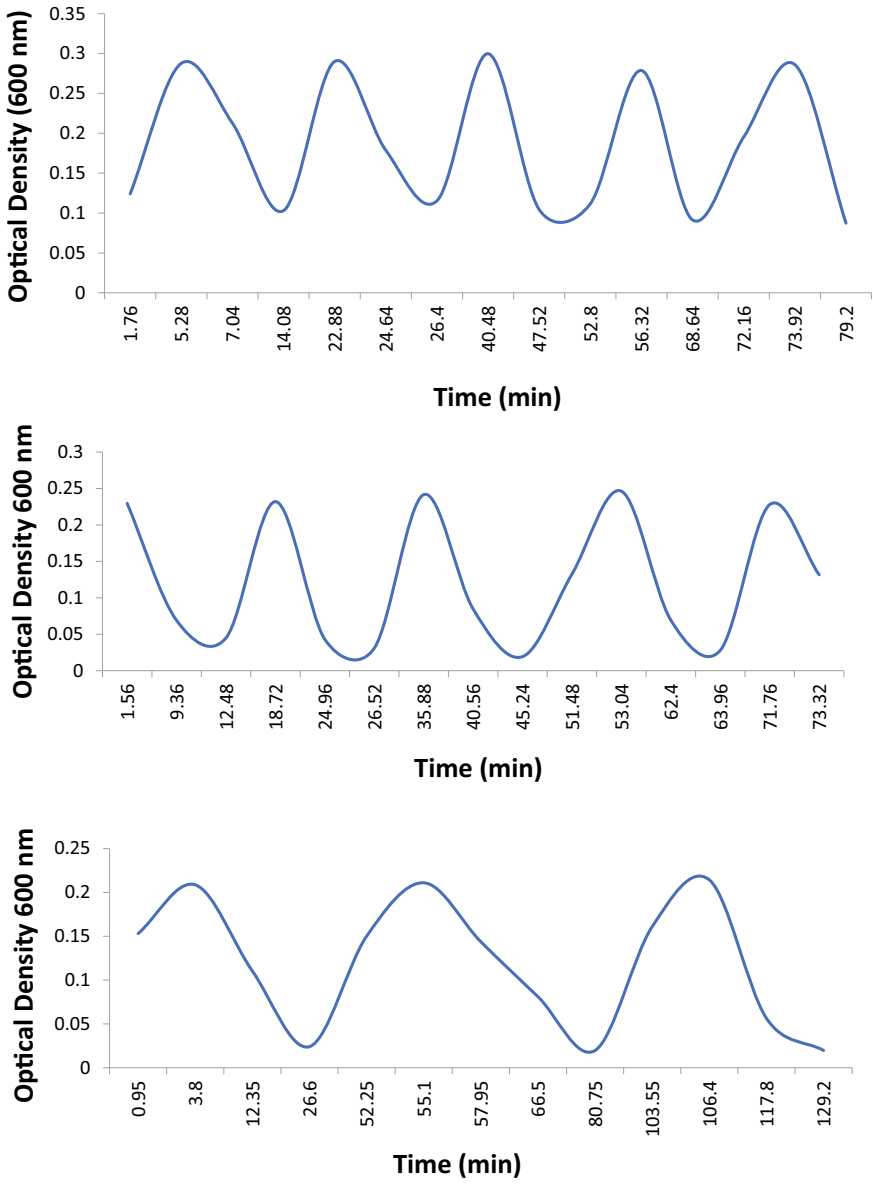


Fig. 5 Doubling time of the consortium revealed at a flow rate of 2.2, 3.3 and 8.8 ml

## 5 Selection of Microbial Growth Medium for Conversion

The consortium inside a packed bed biofilm reactor could convert the dairy wastewater into liquid biofertilizer within 16 h (Halder et al. 2020) with associated reduction of nitrate, phosphate, protein, lipid, chemical oxygen demand and biological oxygen demand and production of ammonia. The process was scaled up to 11 m<sup>3</sup>/day capacity with sustained efficiency (Gogoi et al. 2021c). The reactor starts performing from the first day of operation. Further analysis of the process revealed the desired reduction and production to occur within 4 h, making it's the fastest dairy wastewater conversion process. It enhanced yield of the economic crop at par with chemical fertilizer or higher than that as in the case of Mung bean, Black gram, Field pea, Maize, sugar cane, Ramie, Sorghum sudangrass, Aloe vera, lemongrass. It also sustained the cultivation of Cassava, Colocasia, Yam, Elephant foot yam and sweet potato. In the case of potato and yam bean, the yield was lower than chemical fertilizer grown produce but higher than convention biofertilizer based growth. But in all cases of the tuber crops, the carbohydrate content of the tubers was substantially less than chemical fertilizer grown plants. As per cited literature, it is expected due to the high nitrogen content of the fertilizer. Hence, this liquid biofertilizer not only alleviates the bottleneck of compromised production of the crop, but also produces diet tuber suitable for the diabetic and obese population. Using this tailored made microbial process, milk processing plant wastewater can be economically treated with more production of healthy, economic crop from a given area of land. A single unit operation with minimal energy expenditure, cuts down on the CO<sub>2</sub> equivalent gas emission from the operation of the plant by approximately 90%. Through this approach, the milk processing plant wastewater treatment can be converted into an economically affordable, eco-friendly, self-sustainable system that runs for years after charging the reactor. However, such a system could work only for the rural dairies with large farmlands in their vicinity. In the case of urban dairies, the transportation of the large volumes of liquid fertilizer from the point of production to the point of use might become a problem (economically not sustainable). Concentrating the fertilizer will result in loss of water, hence the replacement of freshwater for irrigation would not be possible anymore. Yet another treatment has been developed for the urban dairies. A bacterial consortium with 8 bacterial isolates mostly from the activated sludge of dairy ETP was found to efficiently bioremediate the milk processing plant wastewater within 20 h of hydraulic retention time to near discharge level (as per Central Pollution Control Board Norms). The phosphate and the ammonia concentration was just above the discharge level. The treated water was further treated for 48 h using a biofilm based microalgae bacterial mixed consortium. The nutrient was completely removed, making the treated water suitable for secondary application. It was accompanied by a significant increase in the biomass yield, lipid and carbohydrate content of the dried biomass compared to non-dairy wastewater treated samples. The conventional treatment takes about 105 to 120 h, while this process took 68 h with substantially reduced energy requirement. Hence, the urban dairies could adopt this two-unit operation with reduced energy and space requirement for effluent treatment with eco-friendly by-product generation (Biswas et al. 2021).

## 6 Petrochemical Wastewater Treatment Using Selectively Developed Consortium

A similar approach has also been adopted to develop a tailor-made bacterial consortium for petrochemical wastewater treatment. Bacteria isolated from the sludge and the effluent of different compartments of the ETP at a petrochemical industry terminal at Vishakhapatnam, India, were screened based on culture dependent method. The well characterized isolates were mixed in a definite proportion to develop the consortium. The consortium was immobilized to enhance the biological oxygen demand removal within a stipulated period. The immobilized consortium in a biofilm reactor was tested for its efficiency in pollutant removal in terms of reducing biological oxygen demand at a laboratory scale. Based on its performance, the system was scaled up to 12 m<sup>3</sup>/day capacity at the ETP of the petrochemical receiving terminal. The consortium with 31 isolates could remove the pollutant (as evident from the reduction in the biological oxygen demand) at the industrial scale within 18 h of hydraulic retention time from a moving bed biofilm reactor. After installation (which took about 10 days to become fully operational), the system could run for nearly 5 years without any breakdown at industrial site, making the industry Central Pollution Control Board compliant. The treated water could replace the purchase of freshwater for landscaping and firefighting storage to a substantial extent. This is another successful example of tailor-made consortium based sludge free wastewater treatment (Ray Chaudhuri et al. 2020; Biswas 2021).

The above examples establish that the innovative process of wastewater treatment using tailor-made consortium works for different kinds of wastewater with improved efficiency in an eco-friendly manner without sludge generation at different scales of operation with sustained performance. However, the consortium development could also be achieved by selecting the consortium directly from the environmental origin instead of combining individual isolates. Some examples are given below.

## 7 Enriched Consortium Based Treatment of Agricultural Runoff and Tannery/mining Effluent

As stated above, literature-based selection of growth medium and culture conditions was followed to enrich the consortium. To remove nitrate and phosphate from the agricultural runoff, nitrate broth was selected, and soil from East Kolkata Wetland (old dumping ground converted to agricultural land) was used as inoculum. The ongoing activity at the location ensured access to nitrate and phosphate. In contrast, the healthy growth of vegetables and fish in the location indicated a lack of nitrate and phosphate toxicity, hence the presence of strong bioremediants. The nitrate broth's anaerobic condition (nitrogen atmosphere) gave efficient consortium enrichment. In other experiments, biomass from nitrate reducing reactor was used as inoculum to develop a consortium under the aerobic condition in nitrate broth. The consortium



individually could remove nitrate and phosphate simultaneously from the solution. When the consortium was applied in a definite combination to the soil, it could prevent nitrate leaching from soil between 8 to 11 cm depth of soil (root zone). This nutrient was made available for the plants grown on that soil, resulting in promoting plant growth with yield enhancement (Ray Chaudhuri et al. 2016a, 2017b; Banerjee 2018). Hence the adverse effect of nutrient leaching during agriculture could be prevented while making the soil nitrate available to the plants over an extended period. The soil fertility was retained even after the cultivation was over.

Soluble sulphate reduction is required from wastewater from the tannery, mining industry, acid rain, to name a few. A similar approach was used for developing soluble sulphate reducing consortium in sulphate reducing bacteria specific medium using inoculum from wastewater fed aquaculture pond, waste dumping ground receiving the cities runoff (containing acid rain), mining effluent, raw sewage canal water, tannery effluent, hot springs to name a few. Multiple consortia were enriched and stabilized through repeated rounds of cultivation (Nasipuri 2011). Their efficiencies were compared (Nasipuri et al. 2010), and the most efficient consortium was further used for scale up operation (Ray Chaudhuri et al. 2016b), which revealed it to be the most efficient bio remedial system for soluble sulphate removal within 4 h of hydraulic retention time, showing similar efficiency for simulated wastewater, tannery effluent and mining effluent (Nasipuri 2011). Bioreactor design and minimal medium composition optimization made its adoption viable for pilot scale operation (Chanda et al. 2020).

This chapter details the criteria for a bacterial consortium based sludge free wastewater treatment.

## 8 Conclusion

Microbial technology can lead to efficient, economic, eco-friendly process development. The treatment systems developed are rapid, less energy consuming, stable with the generation of treated water suitable for the non-potable purpose. A comparative advantage of the developed wastewater treatment technologies reported in this chapter is tabulated (Table 1) below at a glance which is self-explanatory and summarizes the chapter.

**Table 1** Wastewater treatment technologies developed by the Microbial Technology Group, India

Criterion	Dairy wastewater	Petrochemical wastewater	Municipal wastewater/ Agricultural runoff	Ammonia rich wastewater	Tannery/Mining effluent
Patent status	201,731,003,023 dt 27th January 2017	202,031,011,766 dt 18th March 2020	1179/KOL/2013 dt16 Oct 2013 <b>351564</b> granted on 13th Nov 2020 (India); <b>1005753</b> granted on 24th Oct 2017 (Bangladesh)	202131002964 dt 21st January 2021	789/KOL/2011 dt June 10th, 2011; <b>US8398856B2</b> ; <b>US20120312743A1</b> <b>341914</b> granted on 17th July 2020 (Indian)
Space requirement	25% of conventional	Similar	45% of conventional	50% of conventional	45% of conventional
Current HRT/ Conventional HRT	16 h/120 h	18 h/24 h (only biological)	2 h/240 h	14 h/24 h	4 h/12 h
Energy Requirement	10%	Less than conventional	20%	20%	20%
CO <sub>2</sub> emission	90% less	Less than conventional	80%	80%	80%
Product	Liquid Biofertilizer	Irrigable water	Irrigable/ aquaculture suitable water	Irrigable/ aquaculture suitable water	For discharge
Microbial inoculum (actively functions)	1 time (more than 3 years)	1 time (more than 4 years)	1 time (more than 1.5 years)	1 time (more than 1.5 years)	1 time (more than 1.5 years)
Scale of operation	11 m <sup>3</sup> /day in 2 dairy farms	12 m <sup>3</sup> /day at EIPL Vishakhapatnam	2.64 m <sup>3</sup> /day at laboratory scale	0.041 m <sup>3</sup> /day at laboratory scale	2.64 m <sup>3</sup> /day at laboratory scale

(continued)

**Table 1** (continued)

Criterion	Dairy wastewater	Petrochemical wastewater	Municipal wastewater/ Agricultural runoff	Ammonia rich wastewater	Tannery/Mining effluent
Scum Production	Scum free	Scum free	Scum free	Scum free	Largely scum free
Funding for Technology Development	BIRAC under the BIG scheme	MHRD under the FAST scheme	ICAR under National Fund Scheme	-	DAE under BRNS scheme
Recognition/Award	Visitor's Award 2019 Regional Climate Launchpad 2019	NASI-Reliance Industries Platinum Jubilee Award 2020	DST Lockheed Martin India Innovation Growth Program 2014	-	-

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