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Microbial population dynamics at effluent treatment plants

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The requirements for treated wastewater are becoming increasingly more stringent, and therefore the improved efficiency of biological treatment processes is indispensable at industrial effluent treatment plants (ETPs). Microorganisms such as bacteria play an important role in the natural cycling of materials and particularly in the decomposition of organic wastes. The knowledge of the interactions among these microbial populations needs to be harnessed for optimum evaluation and functioning of effluent treatment plants. Modern molecular techniques have revolutionized the methods of assessing these microbial populations. The combination of the results of these microbial assessments along with the on-site parameters at ETPs would favor an efficient treatment. In this review, the various approaches and importance of correlating the microbial population dynamics and treatment of wastewater at industrial ETPs has been elaborated.

Introduction

In nature as well as in a wastewater treatment plant, organisms are the key players in keeping our water clean.[1,2] In both natural and engineered treatment systems, microorganisms such as bacteria, fungi, protozoa and crustaceans play an essential role in the conversion of organic waste to more stable and less polluting substances.[3,4] They form a kind of food chain wherein bacteria, fungi and algae consume the inorganic and organic substances in wastes. These are in turn consumed by protozoa and nematodes which are later consumed by rotifers.

Recently, microbial-based treatment systems for the degradation of organic matter have gained importance[5,6] since biological treatment has several significant advantages over chemical or physical technologies. Biological systems for wastewater treatment are more efficient (because of the high surface-to-volume ratio), they have lower construction and operating costs (systems can operate at ambient temperatures), they are more robust and they do not need replacement once loaded. As with any technology, science is the foundation, and engineering makes sure that the technology works as designed. Wastewater treatment involves mechanical, physical, biological, and chemical methods. Currently a lot of energy is spent in providing the dissolved oxygen to the generic microbes involved in treatment plants, without getting the desired results.[7,8] Though it is known that biodegradation occurs due to biomass, very little attention is given to control the types of bacteria that dominate the treatment system. Such bacteria are always present in the environment and given the right conditions of food availability, temperature and other environmental factors, they grow and multiply.[9–11] Besides amount, the type and functionality of bacteria is also very important in an effluent treatment plant (ETP).[12,13] Molecular methods like polymerase chain reaction for target genes appears to allow rapid detection of these bacteria in wastewater by providing levels of sensitivity and specificity difficult to achieve with traditional culture-based assays, which often take days to perform.[14–16] Development and application of these new molecular biological methods to rapidly screen the most appropriate sludge samples for the active degradative microbial population has led to the monitoring of microbial composition and activity during operation of the ETPs.[17–19] The bacteria selected are then enriched in batch cultures with the aim of obtaining pure cultures for further physiological studies.[20–22]

A number of research activities are going on in the field of biological treatment of municipal and industrial wastewater. They are focused partly on the diversity and activity of bacteria involved in the biological removal of nitrogen, phosphorus and environmental pollutants and partly on microorganisms important for sludge–liquid separation by flocculation, settling and dewatering in activated sludge systems. These are further identified by physiological and molecular methods. The overall aim is to solve problems in treatment plants, to optimize existing procedures and to investigate whether new methods or technologies can be implemented to treat various types of wastewater. This review focuses on investigations and the importance of population dynamics of cultivated and non-cultivated bacteria, on the strength of which the treatment plant works efficiently without any upsets.

Effluent treatment plants (ETPs)

Background

Evidence of water treatment dates far back in history. Ancient Egyptian inscriptions describe a wide variety of water purification processes including boiling water, exposing it to sunlight, filtering it through charcoal, or just letting it settle in jugs. At the beginning of the 20th century, a few cities and industries began to recognize that the discharge of wastewater directly into streams was detrimental, and this led to the construction of wastewater-treatment facilities. Owing to socio-economic weaknesses of the early 20th century, few municipalities and industries provided wastewater treatment. During the 1950s and 1960s, the US government encouraged the prevention of pollution by providing funds for the construction of municipal waste-treatment plants, water-pollution research, and technical training and assistance. New processes were developed to treat sewage, analyze wastewater and evaluate the effects of pollution on the environment. In spite of these efforts however, expanding population and economic growth continued to exacerbate water pollution. In response to the need to make a coordinated effort to protect the environment, the National Environmental Policy Act (NEPA) was signed on January 1,

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1970. In the same year, the Environmental Protection Agency (EPA) was created to bring under one roof all pollution-control programs related to air, water and solid wastes.

Wastewater treatment systems

The total quantity of water used by the domestic and industrial sectors is much less than that consumed for irrigation. However the wastewater from these two sectors has a greater concentration of pollutants and is released at specific discharge points. Thus the wastewater generated from domestic and industrial sectors is a more potent pollutant. Nowadays it is mandatory for every industry to comply with the prescribed wastewater disposal norms. Therefore most industries set up wastewater treatment systems comprising preliminary treatment, primary treatment and secondary treatment. Preliminary wastewater treatment generally includes screening and grit removal. The objective of primary treatment is to physically remove suspended solids from wastewater by settling (primary sedimentation) or flotation. The purpose of secondary treatment is biological removal of dissolved organic matter from wastewater.[23,24] This involves disintegrating the organic matter into water, carbon dioxide and bacterial cells (sludge). These organisms and other solids are then separated from the wastewater through settling (secondary sedimentation). Before discharge to the receiving water-body, the water is disinfected by chlorination, ozonation or other means. Solids (sludge) removed from primary and secondary treatment are settled and removed for further treatment. These solids are normally processed in three steps: digestion, dewatering and disposal. The digestion stage removes organic matter and stabilizes the sludge. Dewatering brings down the water content of sludge and also decreases its volume. Final disposal can be land application or incineration.

It is not possible for each and every industry to treat their effluents as per the prescribed pollution control norms due to limited resources in terms of technology, land, finance and manpower. Only large-scale industries can afford to have their own infrastructure for treatment of their effluent water. Nowadays this problem is tackled by providing a common infrastructure for collection, treatment and disposal of the effluents from dense industrial clusters inhabited by small-scale units. Such 'Common Effluent Treatment Plants' (CETPs) have solved the ultimate objective of control of pollution from small-scale units. Unlike an ETP that receives an influent with more or less the same characteristics and contaminants,[25] a CETP receives a diverse load of influents from varied sources and hence is difficult to operate and maintain. Due to the increase in production of industrial effluents most CETPs are challenged by hydraulic and shock loads.

Biological treatment in ETPs

A. Based on microbial mechanisms

Wastewater flowing out of primary treatment contains a large amount of organic matter. Such substances are a source of food for microorganisms. Microorganisms through a plentitude of metabolic pathways can supply energy for cell maintenance and growth.[26,27] The potential to utilize pollutants in the effluents as carbon sources for metabolism varies enormously among microorganisms.[28,29] Numerous microbial mechanisms for degradation of pollutants have been discovered and research continues for newer ones. The most rapid and complete degradation of pollutants present in the ETPs is brought about under aerobic conditions through catabolic pathways.[30–32] Correspondingly, anaerobic, nitrifying–denitrifying[33] and methanogenic bacteria that carry out the detoxification of effluent contaminants have been discovered from various ETPs. These bacteria are capable of degrading complex chemical mixtures including aromatic, nitroaromatic and cyclic compounds. The activated sludge process is the most popular biological treatment process for both industrial and municipal wastewater treatment. A study for evaluating the biomass activity in such systems has become a regular feature these days.[19,34]

B. Based on engineering parameters/modules

Secondary treatment options employed on-site in ETPs include diverse biological reactors that are able to convert pollutants in the wastewater to carbon dioxide and water. The most common technologies involving these biological processes include trickling filters, rotating biological contactor (RBC), aerated lagoons and activated sludge process that are discussed further in brief below.

(i) Trickling filter. A trickling filter consists of distributor arms that spray liquid wastewater over a bed of rocks or other media. The space between the rocks/media allows air to circulate easily so that aerobic conditions can be maintained. A layer of biological slime that decomposes the waste trickling through the bed covers the individual rocks/media in the bed. This slime consists mainly of bacteria, but it also includes fungi, algae, protozoa, and rotifers. Classification of trickling filters is usually based on organic and hydraulic loadings. Depending on this loading the microbial population varies with time. Generally the higher the loading, the lower the treatment (lower BOD removal). The higher the organic loading, the faster the biomass growth to a point.

(ii) Rotating biological contactor (RBC). An RBC consists of a series of closed spaced, circular plastic disks. About 40% of each disk is submerged in a tank containing the wastewater to be treated. The microbial film that grows on the surface of the disks moves into and out of the wastewater as the RBC rotates. While the microorganisms are submerged in the wastewater, they consume organic matter; while they are rotated out of the wastewater, they are supplied with needed oxygen from the air.

(iii) Lagoons/oxidation-stabilization ponds. Lagoons are large, shallow earth basins that are used for treatment of wastewater by natural processes involving the use of both algae and bacteria.[35] Aeration is provided in some cases to enhance the organic removal efficiency, but anaerobic systems are also in use. The technology of high-performance aerated lagoons has much in common with that of activated sludge. Furthermore, with modification or with the addition of low-tech process units, they can be designed to nitrify. Unlike activated sludge plants where suspended activated sludge eliminates the dissolved contaminant from the wastewater, the active biomass is essentially as a fixed biological film at the bottom of the lagoon.

(iv) Membrane bioreactors. Membrane bioreactors are systems integrating biological degradation of waste products with membrane filtration. Filtration enables complete removal of suspended solids and control of the solid retention time (SRT)[36] and hydraulic retention time (HRT) is possible ensuring optimum control of microbial population and flexibility in operation.[37] The membrane modules integrated into the treatment system not only retain biomass but also prevent the escape of enzymes and soluble oxidants creating a more active biological mixture capable of degrading a wider range of carbon sources.[38] The biomass retained on the membrane due to extensive biological acclimatization to the pollutants, is able to withstand fluctuations in nutrient concentrations in the influent. The membrane is cleaned by frequent permeate back-pulsing and occasional chemical backwashing. Anaerobic compartments are incorporated to facilitate degradation by anaerobic organisms.[39]

(v) Activated sludge (AS). The activated sludge process is the most popular biological treatment process for both industry and municipal wastewater treatment.[40,41] The key unit in an activated sludge process is an aeration tank, in which microorganisms are mixed with incoming wastewater.[42] To maintain aerobic conditions, air is pumped into the tank and the mixture is kept thoroughly agitated. A portion of these solids is returned to the aeration tank to maintain the proper bacterial population, while the remainder is processed and disposed off. The activated sludge comprises a complex microbiological community. Bacteria are the most common of the organisms and are responsible for removal of about 85–90% of the BOD remaining after primary treatment. Sequencing batch reactors have become a common modification of activated sludge process, operated in fill and draw mode.[43] The hallmark of these reactors is its inherent flexibility of cyclic phasing, providing different operating modes.[34,43]

(vi) Upflow anaerobic sludge bed (UASB). Anaerobic wastewater treatment is widely used all over the world.[44,45] It needs lower energy input and gives less surplus sludge as compared to aerobic wastewater treatment.[46–50] The Upflow Anaerobic Sludge Bed (UASB) bioreactor is the favorite anaerobic treatment system used. This system uses anaerobic microorganisms to process high concentration organic wastewater.[51] The microbiological process produces and recovers methane gas and carbon dioxide from organic substances for reuse as fuel.

Tracking the bacterial population

Significant advances have been made in the microbiological and biochemical methods related to activated sludge.[52–54] The latest techniques provide a finer resolution in the study of microbial populations in ETPs which was hitherto unachievable by physiological techniques like plate counts, chemical oxygen demand (COD), biological oxygen demand (BOD) or respirometric analysis. Reports on sludge population optimization,[55] biodegradability testing of refinery wastewater by respirometry[56] advocate that stringent environmental requirements necessitate that the quality of effluent be detected in terms of specific compounds in order to follow sludge capacity.

Bacteria inhabiting activated sludge have been studied extensively using molecular tools since culturing of the sample and sequencing of DNA in a few cases is not required. In recent years attempts have been made to investigate bacterial community structures in activated sludge by direct detection methods, *i.e.* non-culture-dependent.[57–60] The most frequently used polymerase chain reaction (PCR) method is an ingenious tool for molecular biology and it is so sensitive that in practice 25–30 cycles of PCR result in an approximately 10^6 fold increase in the amount of template DNA.[61] 16S rRNA gene sequencing followed by database searching is frequently used as a preliminary tool for bacterial identification and for taxonomic studies.[62] Additionally the 16S rDNA gene is also employed for the denaturing gradient electrophoresis (DGGE)[63] and amplified ribosomal DNA restriction analysis (ARDRA)[64,65] for further delineation at the genotypic level. Similarly, the random amplified polymorphic DNA (RAPD) analysis approach has offered a means of comparing different reactor communities and for monitoring population changes in a single reactor with time[66] For differentiation among various strains, the restriction fragment length polymorphism (RFLP) technique has become the most popular.[67]

Hiraishi and group characterized a bacterial population in an anaerobic–aerobic activated sludge system on the basis of respiratory quinone profiles.[68] Microbial consortia in municipal activated sludge have been analyzed using *in situ* hybridization techniques.[69] Likewise, assessment of activated sludge microbial community in biological wastewater treatment plants using patterns of fatty acid isopropyl esters (FAPEs) has been estimated.[70] Watanabe *et al.* have described the identification of a functionally important population in phenol-digesting activated sludge with antisera raised against isolated bacterial strains.[52] On the contrary, community structures were determined in a more direct manner by isolation of the total protein content of the samples which alleviated the need to culture bacteria.[71] Community dynamics in bioreactors were also evaluated using fluorescence PCR single strand conformation polymorphism (SSCP) analysis.[24] Besides community analyses, issues such as type and abundance of specific organisms[18,22,72] and detection of the presence of genes mediating specific functions[73,74] without subculturing the microbial population have also been addressed at the molecular level. The ability of molecular methods to provide high-level information not attainable by conventional methods comes about because they directly identify the genetic information contained by the microorganisms.[75,76]

Isolation and characterization in terms of degradation of microorganisms from an acclimated activated sludge have been reported.[4,17,21,72,77] Studies for evaluating the biomass activity in ETPs have today become a regular feature.[16,19] The recognition that a number of bacteria cannot be grown easily on standard laboratory media has led to the development of techniques that enable cultivation-independent analyses.[15,78–81] Many organisms are not cultivable or not cultivated (because it is time consuming). However, by studying 16S rDNA these organisms can be identified and monitored.

Specific staining methods like fluorescence *in situ* hybridization (FISH) with rRNA-targeted probes permit detection and location of microorganisms directly in their natural habitats. FISH (Fluorescent *In Situ* Hybridization) has been recently applied as a molecular monitoring tool,[82] linking the microscopy and the molecular precision of phylogenetic identification. Despite the different modes of operation only minor differences in the bacterial composition were detected by FISH analysis based on the various probes.[83] Fluorescent *in situ* hybridization (FISH) using 16S and 23S rRNA-targeted probes together with construction of an archaeal 16S ribosomal DNA (rDNA) clone library was used to characterize the microbial populations of an anaerobic baffled reactor successfully treating industrial dye waste.[84] Real time PCR is being investigated as a molecular tool for studying the evolution of bulking events in activated sludge.[85] Microarrays that allow monitoring complex systems might accurately predict such events by early detection of the causing organisms. The microarray technique is also convenient for detection of a large number of organisms, making it the preferred tool for analysis of complex samples.

Microbial community analysis

Activated sludge reactors contain highly dynamic microbial communities, which obscure the understanding of the inherent microbial population. The stability of the bacterial communities in activated sludge systems depends not only on operational parameters like hydraulic retention time, food to microorganism ratio (F/M) but also on the eukaryotic community.[86] Molecular methods alter and ameliorate the understanding of this phenomenon drastically.[87–89] Purohit *et al.* have described a method that facilitates the extraction of PCR-compatible DNA from different activated sludge samples.[57] Reports have shown that bacterial communities in identically operated activated sludge reactors became considerably different over time, even though they initiated from a common community.[90] Conversely, during comparison of diversity in activated sludge plants, the contribution of influent bacteria in the microbial communities of full scale activated sludge systems to the functioning of the system could not be inferred.[91] The microbial community structure was characterized using fluorescent, ribosomal RNA targeted oligonucleotide probes specific for Bacteria, Archaea and phylogenetically defined groups of methanogens and sulfate-reducing bacteria in anaerobic wastewater reactors.[92] A molecular approach was used to evaluate the effect of nonylphenol ethoxylate surfactants on the bacterial diversity in lab-scale activated sludge reactors.[93] These new methodologies altogether have simplified the studies for microbial community analyses at ETPs.

Culturable and unculturable population

Physiological studies have indicated that the number of cultivable bacteria is 5–15% of the total number of bacteria in activated sludge.[15,94,95] Juang and Hwu identified and compared the predominant agar-plated bacteria in the activated sludge of two popular wastewater treatment systems, the sequencing batch reactor (SBR) and the continuous-flow stirred tank reactor (CFSTR).[96]

The activated sludge forms a complex ecosystem due to high biomass density, low humic acid content and the presence of bacterial aggregate flocs.[97] Hence, the microbial studies on such systems have been biased essentially by the unculturability of many microorganisms and the lack of sensitivity of traditional microbiological methods. The extraction of nucleic acids from activated sludge has been reported by various groups.[14,72,98–100] These studies paved the way for studying the unculturable bacterial population. Alternatively, fecal contamination may go undetected if indicator bacteria are in a viable but non-culturable state. Development of a protocol that would obviate the need to culture organisms for detection could remedy shortcomings of traditional techniques by allowing rapid, sensitive, and specific identification of pathogens of concern rather than indicator organisms.

Time dependent selection of consortia through natural process

The intrinsic property of sludge to evolve continuously with respect to influent load provides it with diverse catabolic capacities at different time intervals. A combination of PCR, hybridization, 16S rDNA diversity and physiological analysis has been emphasized for detection of the degradative capacity of different sludge to demonstrate variation in bacterial population over a period of time in response to change in conditions of the ETP.[19] Fernandez *et al.*[101] studied the population dynamics in a functionally stable methanogenic reactor over a period of 605 days. A submerged membrane bioreactor (SMBR) and a conventional activated sludge system (CAS) were compared in parallel over a period of 210 days on treating synthetic ammonia-bearing inorganic wastewater under similar conditions.[23] Rapid shifts in the species composition of the bacterial population were observed in an anaerobic digester running under constant environmental conditions over a period of two years.[24] This was undertaken to show the available diverse sludge characteristics. Respirometric methods offered other possibilities to follow changes in sludge capacities, for both heterotrophic and autotrophic microorganisms, and wastewater composition.[102–104] Over a period of 227 days the properties of activated sludge grown in a sequencing batch reactor (SBR) operated under stable conditions were analyzed and a good correlation between settling properties and floc size distribution was obtained as well as a new dominant species being observed in the DGGE patterns.[105] It can be inferred from these studies that over a period of time the microbial population always keeps flourishing.

Bioaugmentation for desired output

Bioaugmentation of activated sludge by an indigenous 3-chloroaniline-degrading *Comamonas testosteroni* strain has been demonstrated.[106] In one study, a 2,4-DCP degrading mixed culture was bioaugmented in terms of enhancing 2,4-DCP removal and maintaining system stability under shock loading conditions.[107] Reports indicate that the performance of the constructed consortium was more reproducible than that of an undefined community, which is an essential feature for bioaugmentation treatment of industrial wastes.[108] We stress that optimizing the microbial community structure and property should be an explicit aim for the design and operation of a treatment plant. The major limitations to sludge population optimization revolve around inadequate microbiological data, specifically community structure, function and kinetic data.[109] A study on bioaugmentation with the resin acid-degrading bacterium *Zoogloea resiniphila* DhA-35 to counteract pH stress in an aerated lagoon treating pulp and paper mill effluent, suggests that it is feasible and potentially useful to use bioaugmentation with resin-acid-degrading bacteria such as DhA-35 to restore and enhance resin acid removal by aerated lagoon microbial communities.[110] The reports indicate that sludge population optimization can be considered as a new dimension for the control of biological wastewater treatment systems.

Parameter optimization and performance of ETP based on the microbial activity

Organisms with the desired biodegrading capacity may or may not be the predominant species in an ETP and identifying such a potential in a community of microorganisms *in situ* is difficult. Special attention is paid to how it is becoming possible to relate the composition of the community of microbes present in activated sludge and the *in situ* function of individual populations there and to how such information might be used to manage and control these systems better.[111] The application of molecular methods has changed our perceptions of the identity of the filamentous bacteria causing the operational disorders of bulking and foaming, and the bacteria responsible for nitrification and denitrification and phosphorus accumulation in nutrient removal processes.[112,113] Since the implementation of the activated sludge process for treating wastewater, there has been a reliance on chemical and physical parameters to monitor the system. However, in biological nutrient removal (BNR) processes, the microorganisms responsible for some of the transformations should be used to monitor the processes with the overall goal of achieving better treatment performance. The development of *in situ* identification and rapid quantification techniques for key microorganisms involved in BNR are required to achieve this goal. One such study explored the quantification of Nitrospira, a key organism in the oxidation of nitrite to nitrate in BNR.[114]

Studies propose an integrated way of arriving at the available assimilatory capacity of activated sludge at ETP considering desired target molecules. The traditional reliance on pure culture techniques to describe microbiota is circumvented by the employment of a molecular approach. Nowadays, microbial community characterization is underway based on cloning and sequencing of 16S rRNA genes for phylogenetic analyses, to determine the nature and quantity of microbiota that constitute these ecosystems. Knowledge of the organisms naturally present can influence the design and treatment capacity of these widely used land based systems. Laboratory, intermediate and field scale systems are currently under study. Since human pathogens are known to exist in sewage effluents, their removal in wastewater infiltration systems and within the underlying soil are in need of a more fundamental understanding. The relationship between design parameters and environmental conditions, including a microbial characterization, is essential for the prevention of contamination in groundwater sources.

Use of molecular techniques, such as cloning environmental genes and fluorescent *in situ* hybridisation (FISH)[115] using oligonucleotide probes, is providing exceptional insight of microbial compositions of these environments. An improved understanding of the metabolic, physiological, structural and genetic details of these microbial processes is required. Investigations for obtaining activated sludge kinetics[102] and estimation of biodegradation parameters of pollutants on activated sludge using respirometry have been reported.[116] A study reviewed the present knowledge about the role of bacteria in relation to floc and sludge properties, and presented a new approach to investigate the identity and function of bacteria in the activated sludge flocs. The approach included identification of the important bacteria and a characterization of their physiological and functional properties. It was carried out by use of culture-independent molecular biological methods linked with other methods to study the physiology and function, maintaining a single cell resolution[117] The development of such more specific monitoring methods for activated sludge can result in a targeted steering of microbial communities, giving rise to both highly efficient wastewater treatment performance and low operational costs.

Indicator bacterial population and treatment efficiency

In a case where the reactor is sick/running inefficiently, the remedy lies in checking the microbial biomass for the indicator population, key degradative bacteria and secondary umbrella population. The degradative capacity of biomass is related to the physiology of the bacteria, which respond to all the substrates differently. Using statistical tools, the prediction of the effective combinations of the inducers to stimulate the degradative physiology directly at the industrial premises can be facilitated. Results show that a highly adapted population develops in jet-loop reactors (JLRs) treating winery effluents as compared to other bioreactors. Aerobic JLRs impose a stringent selective criterion on the composition of microbial biomass[118] The majority of pulp and paper mills now biotreat their combined effluents using activated sludge and a study showed that the pulp and paper mill primary clarifiers tested appeared to be sites of active N_2 fixation and a microbial community especially klebsiellas, are indigenous in pulp and paper mill water systems.[119] The role of *N*-acyl-l-homoserine lactones (AHLs) in microbial community dynamics in an industrial wastewater treatment system was addressed which suggested that AHLs can play a role in mediating microbial community parameters and has implications for ecosystem function and industrial wastewater treatment.[120] A study was conducted for detecting structural and functional differences in activated sludge bacterial communities originating from laboratory treatment of elementally and totally chlorine-free bleaching effluents. Differences in both metabolic potential and taxonomic structure of the microbial communities in the activated sludges were detected. It was suggested that the kinetics of the development of such differences in treatment plants and their relationships with treatment efficiency and production process conditions be evaluated further.[121] Another study analyzed raw sewage, secondary effluent, and chlorinated effluent for the detection and enumeration of four standard microorganismic indicators: standard plate count, total coliform, fecal coliform, and *Clostridium perfringens* on a weekly basis. It was found that the microbial populations present in the wastewater are very much sensitive to the wastewater temperature and, to some extent, on the wastewater flowrate.[122] Another study has suggested the use of stress proteins that may serve as sensitive and rapid indicators of toxicity which can adversely impact treatment process performance in activated sludge systems, in response to xenobiotic shock loading.[123]

Modelling community characteristics

The current design and simulation models that include active biomass as the fundamental parameter remain purely hypothetical because they have not been quantitatively measured.[124–126] With the new technique of *in situ* hybridization and mathematical simulation promising tools were combined which help to understand the complex interactions in anaerobic mixed populations and the influence of reactor operational modes.[92] The wastewater treatment industry is increasingly becoming aware of the benefits of on-line optimization and control.[127] Essential for this are sufficient process knowledge and reliable on-line instrumentation. While descriptive models do exist, there is a shortage of instruments available to measure enough variables to solve for all of the model parameters. One such study aims to overcome this problem by combining recently developed titrimetric technology with traditional respirometry techniques to increase the data available for model identification.[128] In practical terms, a pH control system was used to monitor hydrogen ion production and is combined with off gas methods (used for the measurement of biomass respiration, *i.e.* oxygen uptake and carbon dioxide evolution) to characterize sludge kinetics and wastewater composition. The result is a new instrument, measuring three independent signals, which are suitable for investigation of activated sludge systems, and also other biological treatment processes. Modeling anaerobic degradation of wastewater has been described and validated further.[47] The characteristics of the bacterial community structure and population dynamics for phosphorus removal in activated sludge were investigated using laboratory-scale sequencing batch reactor (SBR) activated sludge processes fed with four different carbon sources.[129] Modelling population dynamics of denitrifying phosphorus accumulating organisms in activated sludge has also been attempted.[130] Based on observations with pure cultures and mixed cultures growing under dynamic conditions a model is proposed to describe such behaviour. Bacterial communities, when subjected to the feast–famine conditions that occur in many wastewater treatment systems, store substrates as reserve polymers. Modelling of activated sludge processes with analogous structured biomass was assessed.[131,132] These studies have analysed biological wastewater treatment processes using multicomponent gas phase mass balancing, suggesting an alternative approach for assessing wastewater treatment efficacy.

Applications of microbial activated sludge in the future

Wastewater treatment processes require careful management to ensure the protection of the water body that receives the discharge. Trained and certified treatment plant operators' measure and monitor the incoming sewage, the treatment process and the final effluent. Most of the excess sludge from a wastewater treatment plant (60%) is disposed by landfill. The production of biodegradable plastics using this sludge has been proposed as a resource utilization of excess sludge.[133] The goals of sludge treatment are to stabilize the sludge and reduce odors, remove some of the water and reduce volume, decompose some of the organic matter and reduce volume, kill disease-causing organisms and disinfect the sludge.

Understanding microbial functions in their environments and using this information in an applied manner is fundamental to this review. It is useful to identify organisms important to microbial mediated processes such as wastewater treatment, bioremediation, environmental predicaments and industrial production processes. The pollutants in domestic wastewater not only arise from domestic and food service functions, but also from some home industries and small-scale industrial units. It is practically impossible to control the varied nature of the wastewater entering the treatment plants. The operators find it very difficult to run a stable plant and discharge water conforming to tight norms. The microbiology of activated sludge has been critically reviewed here. It seems that details of microbial community structure are far from being understood. However, it is the most commonly used option for treatment of domestic and industrial wastewaters, which are being used for irrigation or ground water, recharging purposes.

Conclusion

Successful demonstration of the utility of molecular tools for biological testing of water quality up to the state-of-the art in modern microbiology, has allowed inexpensive, rapid, direct, specific detection of harmful microorganisms *via* identification of specific genetic markers. These and additional tests based on new technology would be expected to replace the archaic indirect-inference tests employing laboratory culturing of indicator bacteria, and replace tests that require days with ones that can provide measurements within hours and therefore be used more routinely for real-time monitoring of water supplies and surface and groundwater quality.

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