The Role of Microorganisms in Wastewater Treatment

DOI: https://doi.org/10.63345/ijrmp.v15.i11.IJRMP1126007

Ms. Aarthi Pradhan

Naran Lala College of Professional and Applied Sciences, Navsari

Veer Narmad South Gujarat University, Surat

ORCID: https://orcid.org/0009-0007-4925-9813

Abstract : In biological wastewater treatment microorganisms oxidise organic matter, convert nitrogen compounds and accumulate phosphorus. This paper reviews the roles of the main microbial groups in treatment and presents laboratory experiment in which the levels of chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), suspended solids, ammonia nitrogen and total phosphorus removed during wastewater treatment by aeration, activated sludge and activated sludge with additional Bacillus consortium were compared. It is shown that removal in activated sludge treatment is significantly higher for all parameters ($p < 0.001$) and that addition of Bacillus consortium further improves removal in particular for phosphorus. Therefore, managing microbial communities is crucial for designing and running treatment plants.

Keywords: wastewater treatment; activated sludge; microorganisms; nitrification; denitrification; bioaugmentation; COD removal

Introduction

Untreated wastewater from households, industries and agriculture contains organic matter, nutrients, suspended solids and pathogens that cause harm to water bodies and human health. Biological wastewater treatment plants are designed to remove these pollutants before discharge. Although screening, gritting, settling and disinfection are part of the process, biological treatment with microorganisms is the key step in modern processes as microorganisms utilise pollutants as food and energy and produce harmless end-products and biomass that can be separated from the water (Metcalf & Eddy, 2014).

The activated sludge treatment process was developed more than a century ago and it remains the most popular method of biological treatment. In this process, wastewater is mixed with a suspension of microbial flocs and then flocs are separated and partially returned to the tank. Composition and activity of the microbial flocs determine the performance of the process. The flocs are formed by bacteria, protozoa and fungi surrounded by extracellular polymers. Biological wastewater treatment has been considered as a typical system for microbial ecology because of high density, diversity of communities and engineering conditions (Daims et al., 2006).

Different groups of microorganisms perform different tasks: heterotrophic bacteria oxidise organic carbon, autotrophic nitrifying bacteria convert ammonia to nitrate in the presence of oxygen, while denitrifying bacteria reduce nitrate to nitrogen gas in anoxic

conditions. Phosphate is accumulated by bacteria in the excess amount. Protozoa graze bacteria and improve quality of the effluent and filamentous organisms affect floc formation. Understanding these processes is important for design of treatment plants and solution of operating problems.

Two purposes of this paper are to review the functions of the main microbial groups in biological wastewater treatment and to compare experimentally the removal efficiency of selected parameters in treatment by aeration, activated sludge and addition of microbial consortium to activated sludge in controlled laboratory conditions.

Literature Review

Detailed description of the microbiology of activated sludge has been given in specialist literature (Seviour & Nielsen, 2010). Process of carbon removal has been described by modelling growth of heterotrophic biomass on biodegradable organic matter. Mathematical models such as Activated Sludge Models became the usual tool to describe interaction of carbon, nitrogen and

© 2026. This article is licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0).

phosphorus removal processes (Henze et al., 2000). Wagner and Loy (2002) presented review of composition and functions of bacterial communities in sewage treatment systems and demonstrated that molecular methods revealed more diverse communities than culture-based methods.

Removal of nitrogen compounds has been studied extensively. Nitrification is a process performed by ammonia-oxidizing and nitrite-oxidizing bacteria in two steps, and it is sensitive to temperature, pH, dissolved oxygen and toxicity of the wastewater (Gerardi, 2002). Discovery of Nitrospira bacteria capable of performing complete oxidation of ammonia to nitrate in single cell – comammox – changed the concept of nitrifying communities (Daims et al., 2015). Denitrification is a respiratory process when bacteria use nitrate and nitrite as electron acceptors instead of oxygen and produce nitrogen gas with nitric and nitrous oxides as intermediates (Zumft, 1997). Anaerobic ammonium oxidation (anammox) bacteria can directly convert ammonium and nitrite to nitrogen gas providing way to achieve this without oxygen and organic carbon (Kuenen, 2008).

Phosphorus removal by enhanced biological removal depends on accumulation of phosphorus as polyphosphate by special bacteria. The microbiology and biochemistry of the process has been described by Mino et al. (1998), including roles of storage polymers and competition between phosphate accumulating and other organisms. These studies explained why phosphorus removal is usually more variable than carbon removal.

Dynamic aspects of communities are important for stability of operation. Ju and Zhang (2015) traced the bacterial communities in a full scale plant over time and found that communities assemble by deterministic and stochastic events, and there are temporal patterns of communities correlated with operating conditions. It means that the microbial communities respond to changes in the plant and can be managed using them. Bioaugmentation – addition of specific microorganisms for the purpose of improving performance – has been applied for difficult pollutants and for faster start-up, although the results have varied depending on organism, dosing and conditions (Herrero & Stuckey, 2015).

This literature review showed that there are two gaps in the existing knowledge. Reviews discuss functions of the groups, but there are few experimental studies comparing contribution of microbial activity and aeration/physical processes to pollutant removal. Results of bioaugmentation of municipal wastewater treatment systems are conflicting. This paper tries to fill these gaps by performing small experiment.

Research Methodology

Wastewater and reactors

Municipal wastewater was collected as grab samples from the inlet of a sewage treatment plant after screening and grit removal, and used within two hours. Main characteristics of the wastewater are given in Table 1. Batch reactors of 2 L working volume were prepared in temperature-controlled room at 28 ± 1 °C and aerated with diffused air to provide dissolved oxygen above 2 mg/L.

Table 1. Characteristics of the raw wastewater (mean ± SD of six samples)

Parameter	Value
pH	7.3 ± 0.2
COD (mg/L)	486 ± 32
BOD_5 (mg/L)	224 ± 18
Total suspended solids (mg/L)	262 ± 21
Ammonia nitrogen (mg/L)	38.4 ± 3.1
Total phosphorus (mg/L)	6.6 ± 0.5

Treatments

Comparison was done for three treatments, each in six replicates. In the control, wastewater was aerated without any added biomass to measure the effect of stripping, adsorption and settling. In the activated sludge treatment, return activated sludge from a functional plant was added to reach the concentration of mixed liquor suspended solids 2,500 mg/L. Third treatment consisted in addition of consortium of Bacillus subtilis, Bacillus licheniformis and Bacillus megaterium (10^6 cells per mL) to activated sludge. The reactors were operated for 24 hours.

Measurements

Samples were taken at 0, 3, 6, 12 and 24 hours. COD, BOD_5, total suspended solids, ammonia nitrogen and total phosphorus were measured by the methods described in Standard Methods for the Examination of Water and Wastewater (APHA, 2017). Removal efficiency was calculated as the percent reduction from the initial concentration. Heterotrophic plate count was determined on nutrient agar and presented as log

© 2026. This article is licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0).

colony-forming units per mL. Temperature, pH and dissolved oxygen were continuously monitored.

Statistical analysis

Results are presented as mean ± SD of six reactors. Removal efficiency was compared for the three treatments by one-way analysis of variance, with Tukey-Kramer test for pairwise comparisons. Effect size is presented as η^2. P value below 0.05 was considered significant.

Microbial groups and their roles

Main microbial groups in biological wastewater treatment are summarised in Table 2 and presented schematically in Figure 1.

Table 2. Main microbial groups in wastewater treatment

Group	Examples	Function	Conditions
Heterotrophic bacteria	Pseudomonas, Bacillus, Zoogloea	Oxidise organic carbon; form flocs	Aerobic
Nitrifiers	Nitrosomonas, Nitrobacter, Nitrospira	Oxidise ammonia to nitrite and nitrate	Aerobic; sensitive to pH and toxins
Denitrifiers	Pseudomonas, Paracoccus, Thauera	Reduce nitrate to nitrogen gas	Anoxic; need organic carbon
Phosphate-accumulating bacteria	Candidatus Accumulibacter	Store phosphate as polyphosphate	Alternating anaerobic and aerobic
Protozoa and metazoa	Ciliates, rotifers	Graze on bacteria; clarify effluent	Aerobic
Filamentous bacteria and fungi	Sphaerotilus, Microthrix	Form floc backbone; excess causes bulking	Aerobic
Anaerobic consortia	Hydrolytic bacteria, methanogens	Convert sludge and organics to methane	Anaerobic

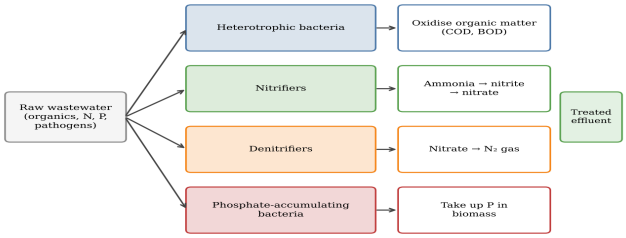


Figure 1. Roles of the main microbial groups in the removal of pollutants.

Heterotrophs remove most of carbon. They use organic compounds as carbon and energy sources, oxidise part to carbon dioxide and convert the rest into new cells, and they synthesise polymers forming flocs. Nitrifiers are slow-growing and require long sludge age for retention in the plant. Denitrifiers are facultative bacteria which are able to switch from oxygen to nitrate as the electron acceptor in the absence of oxygen. Phosphate-accumulating bacteria prefer alternation of anaerobic and aerobic conditions. The balance of these groups depends on the oxygen regime, temperature, pH, sludge age and wastewater composition.

Results

Removal of pollutants

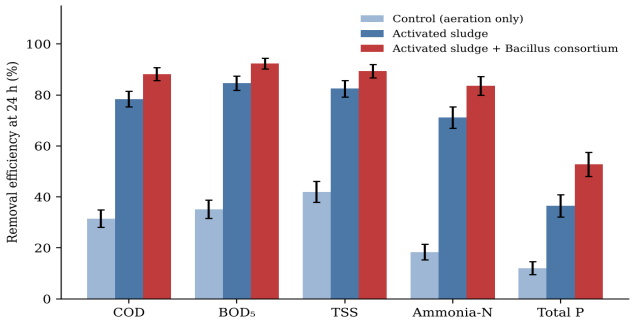
There was much higher removal in microbial treatments than in aeration alone (Table 3, Figure 2). COD removal was 31.5% in the control, 78.4% in activated sludge and 88.2% in activated sludge with consortium ($F(2, 15) = 396.5$, $p < 0.001$, $\eta^2 = 0.98$). Similar patterns were observed for BOD_5 ($F(2, 15) = 440.1$, $p < 0.001$), suspended solids ($F(2, 15) = 223.1$, $p < 0.001$), ammonia nitrogen ($F(2, 15) = 345.2$, $p < 0.001$) and total phosphorus ($F(2, 15) = 101.8$, $p < 0.001$). Tukey-Kramer test showed that activated sludge differed from the control for all parameters ($p < 0.001$), and addition of consortium produced significant further improvement over activated sludge for COD ($p = 0.001$), ammonia nitrogen ($p < 0.001$) and total phosphorus ($p < 0.001$). For BOD_5 ($p = 0.006$) and suspended solids ($p = 0.031$) improvements were smaller.

Table 3. Removal efficiency (%) after 24 hours, mean ± SD (n = 6 reactors per treatment)

© 2026. This article is licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0).

Parameter	Control	Activated sludge	Activated sludge + consortium	ANOVA F
COD	31.5 ± 4.2	78.4 ± 3.8	88.2 ± 3.1	396.5
BOD_5	35.2 ± 4.5	84.6 ± 3.5	92.3 ± 2.6	440.1
TSS	42.0 ± 5.1	82.5 ± 4.0	89.4 ± 3.3	223.1
Ammonia-N	18.4 ± 3.9	71.2 ± 5.2	83.6 ± 4.5	345.2
Total phosphorus	12.1 ± 3.2	36.5 ± 5.4	52.8 ± 5.9	101.8

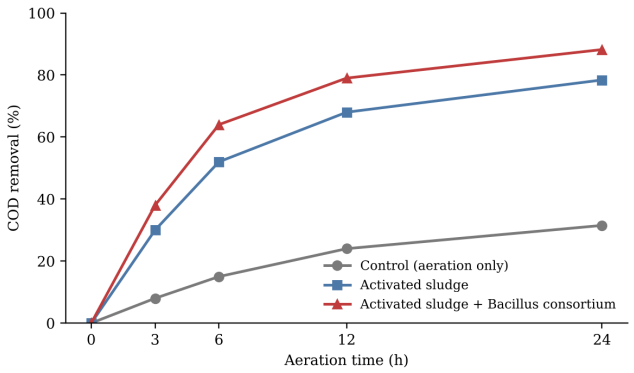
Figure 2. Removal efficiency of pollutants after 24 hours of treatment (mean with 95% confidence intervals).



Rate of COD removal

COD removal was the fastest in first three hours of biological treatment (Figure 3). At 3 hours, activated sludge removed 30% and the consortium treatment 38% of COD, compared with only 8% in the control. COD removal in the control continued slowly and reached only 31.5% at 24 hours.

Figure 3. COD removal over time in the three treatments.



Microbial counts

The number of heterotrophic bacteria after 24 hours was 5.4 log CFU/mL in the control, 7.9 in activated sludge and 8.5 with the consortium, significant difference ($F(2, 15) = 286.2$, $p < 0.001$). Higher counts correspond to removal order across the treatments.

Table 4. Heterotrophic bacterial count after 24 hours (log CFU/mL, mean ± SD, n = 6)

Treatment	Count
Control (aeration only)	5.4 ± 0.3
Activated sludge	7.9 ± 0.2
Activated sludge + Bacillus consortium	8.5 ± 0.2

Discussion

The experiment shows what part of pollutant removal is due to microbial activity. Aeration alone removed one third of the COD and one fifth of ammonia, which could be attributed to stripping, adsorption to particles and the activity of the native microorganisms in the wastewater itself. Addition of activated sludge increased COD removal up to 78.4% and ammonia removal up to 71.2%, which confirms the view that the dense and active flocs are the key players in the process (Metcalf & Eddy, 2014; Seviour & Nielsen, 2010).

Improvement in removal with the Bacillus consortium suggests that bioaugmentation can increase performance, as was reported sometimes (Herrero & Stuckey, 2015). The largest improvement was in phosphorus and ammonia, the parameters dependent on specialised and slow-growing groups, and the smallest in BOD_5 and suspended solids, where activated sludge performed well. Bacillus species could contribute to COD removal with their hydrolytic enzymes and stimulate growth of the flocs, and the higher bacterial counts confirm it. The improvement in phosphorus removal should be treated with caution, because enhanced biological removal usually requires alternation of anaerobic and aerobic phases (Mino et al., 1998), and in the present experiment it reflects uptake in growing biomass.

These results are also important for the operation of full-scale plants, since the performance of the process depends on the microbial communities. The finding that the communities react on the environmental factors and change with time (Ju & Zhang, 2015) implies that regular monitoring of the biology, including microscopic study of the flocs and nitrification activity measurements, is useful additional technique to

© 2026. This article is licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0).

chemical analyses. Novel discoveries such as comammox bacteria and anammox may change the design of nitrogen removal in the future (Daims et al., 2015; Kuenen, 2008).

Limitations

The experiment was conducted with batch reactors for 24 hours, which differs from full-scale continuous plants, and effects of long-term operation, sludge age and settling were not examined. Only one wastewater type, one temperature and one consortium were tested. Community composition was determined by plate count, which captures only part of the community, and molecular methods would give a better picture. Anaerobic and anoxic stages necessary for denitrification and biological phosphorus removal were not included.

Conclusion

Microorganisms are the main agents of pollutant removal in biological wastewater treatment. In the present study, activated sludge removed significantly more COD, BOD_5, suspended solids, ammonia and phosphorus than aeration alone, and addition of a Bacillus consortium resulted in additional improvement, especially in case of ammonia and phosphorus. Thus, performance of a plant depends on maintaining healthy and diverse microbial communities under appropriate conditions. Future research should test bioaugmentation in continuous systems, include anaerobic and anoxic stages and use molecular methods to trace the communities.

References

1. APHA, AWWA, & WEF. (2017). Standard methods for the examination of water and wastewater (23rd ed.). American Public Health Association.
2. Daims, H., Lebedeva, E. V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., et al. (2015). Complete nitrification by Nitrospira bacteria. Nature, 528, 504–509.
3. Daims, H., Taylor, M. W., & Wagner, M. (2006). Wastewater treatment: A model system for microbial ecology. Trends in Biotechnology, 24(11), 483–489.
4. Gerardi, M. H. (2002). Nitrification and denitrification in the activated sludge process. Wiley.
5. Henze, M., Gujer, W., Mino, T., & van Loosdrecht, M. (2000). Activated sludge models ASM1, ASM2, ASM2d and ASM3. IWA Publishing.
6. Herrero, M., & Stuckey, D. C. (2015). Bioaugmentation and its application in wastewater treatment: A review. Chemosphere, 140, 119–128.
7. Ju, F., & Zhang, T. (2015). Assembly of Bacteria and Dynamics in Time of Activated Sludge at Full-Scale Municipal Wastewater Treatment Plant. The ISME Journal, 9, 683-695.
8. Kuenen, J. G. (2008). Anammox bacteria: From discovery to application. Nature Reviews Microbiology, 6, 320-326.
9. Metcalf & Eddy, Inc. (2014). Wastewater engineering: Treatment and resource recovery (5th ed.). McGraw-Hill Education.
10. Mino, T., van Loosdrecht, M. C. M., & Heijnen, J. J. (1998). Microbiology and Biochemistry of the Enhanced Biological Phosphate Removal Process. Water Research, 32(11), 3193-3207.
11. Seviour, R., & Nielsen, P. H. (Eds.). (2010). Microbial Ecology of Activated Sludge. IWA Publishing.
12. Wagner, M., & Loy, A. (2002). Bacterial community composition and function in sewage treatment systems. Current Opinion in Biotechnology, 13(3), 218-227.
13. Zumft, W. G. (1997). Cell biology and molecular basis of denitrification. Microbiology and Molecular Biology Reviews, 61(4), 533-616.

© 2026. This article is licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0).