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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

REVIEW ARTICLE BIOSCIENCE RESEARCH, 202219(3):1585-1592. OPEN ACCESS

A Review on heterotrophic Nitrification and aerobic Denitrification of Achromobacter sp. for Bioremediation in wastewater treatment

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Wastewater is a major environmental issue that has arisen as a result of various industrial and domestic activities. Improper management of wastewater treatment could be harmful to human health, livestock and plants. Biological treatment through the application of beneficial microorganisms could reduce this problem and act as a low-cost treatment. The isolation and identification study for potential heterotrophic nitrification-aerobic denitrifying bacteria was beneficial for eco-friendly wastewater treatment. *Achromobacter sp.*are gram-negative rod-shaped bacteria that originated from betaproteobacteria class with the ability to remove nitrate or nitrate as well as able to degrade pollutants that are available in the water. Production of high cell mass for this microbe requires extensive study to develop a suitable medium composition and cultivation strategy. Various chemical and physical factors play significant roles in cell growth during the fermentation process. Biological wastewater treatment methods are particularly important because of their substantial economic benefits, especially when combined with waste stabilization and resource recovery. This review article illustrates the use of the bioremediation process using *Achromobactersp* for remediation in wastewater treatment.

Keywords: Wastewater,*Achromobacter sp*., nitrification, denitrification, bioremediation

INTRODUCTION

In general, a wastewater treatment plant (WWTP) is a facility where the process of eliminating pollutants from wastewater is converted into effluent that may be reintroduced to the water cycle with little environmental damage. There are four types of wastewater treatment plants known such as Activated Sludge Plants (ASP), Effluent Treatment Plants (ETP), Common and Combined Effluent Treatment Plants (CEPT) and Sewage Treatment Plants (STP) (Ezugbe & Rathilal, 2020). Usually, WWTP contains high carbon content in organic form and a high level of nutrients with ammonia and nitrogen content which enhance the growth of beneficial microbes. However, this high level of ammonia and nitrogen content also could be harmful to human health as well as the environment when not treated properly. Excess reactive nitrogen not only harms human health, but it also adds to air and water pollution and can lead to the collapse of complex ecosystems (Holmes et al. 2018). Tertiary wastewater treatment techniques that assure the removal of reactive nitrogen species must be applied to avoid the negative impacts of excess reactive nitrogen in the environment.

Basically, there are four common ways in the wastewater treatment process known as physical water treatment, chemical treatment, sludge treatment and biological treatment (Paździor et al. 2018). Physical water treatment mainly involves sedimentation where insoluble particles settle down at the bottom. Then, the aeration technique is applied by circulating air through the water to provide oxygen before filtration was done to remove those insoluble contaminants. Waste water which is treated by applying chemicals is known as chemical treatment whereas sludge treatment is a solid-liquid separation process. Generally, sludge treatment involves the biological treatment where effective microorganisms were used to decompose organic and inorganic materials present in the sludge or wastewater into stable substances.

Many wastewater treatment plants use chemicals for tertiary treatment, although biological nitrogen removal procedures are far more ecologically friendly and cost-effective. It was stated that biological treatment techniques, as well as enzymatic digestion, are superior and safer methods than other physical and chemical treatments (Adholeya & Pant, 2007). The term bio alludes to life, specifically microorganisms that might possibly be employed to clean wastewater. Because of their capacity to utilise varied wastewater elements to supply energy for microbial metabolism and the building blocks for cell synthesis, several microorganisms were used to remove nutrients and hazardous substances. This metabolic activity is capable of removing impurities such as raw materials and by-products (Rajasulochana & Preethy, 2016). According to Wu et al. (2012), microbes play an important role in polluting the environment by ingesting inorganic nutrients. The microorganism cleans the contaminants by absorption, organic material consumption, and adsorption. It was said that the biofilm created by bacteria has a significant role in the removal of heavy metals, organic debris, phenol, nitrates, pentachlorophenol, trichlorophenol, sulphates, and quinolone. As a result, interest in biological treatments is growing. Biological techniques use nitrogen cycling microorganisms to remove reactive nitrogen from reactor systems by converting ammonia to nitrogen gas (Holmes et al. 2018).The goal of this review is to convey current trends in the use of microorganisms with focus on *Achromobacter sp*. in bioremediation and to offer significant information that has been identified as gaps in this topic area. It is currently a prominent study subject since microorganisms are eco-friendly and offer valuable genetic material for solving environmental problems.

Nitrogen cycling during wastewater treatment

In the atmosphere, approximately 78% by volume consists of nitrogen gas. However, not all of this nitrogen compound is available to be consumed directly. Therefore, effective microorganisms are needed to convert that nitrogen into functional nitrogen form which is consumable for other organisms in sustaining their life (Augustyn et al. 2020). Basically, the nitrogen cycle can be defined as a biogeochemical process through which nitrogen is converted into many forms usually carried out by a series of microbial transformations. It involves several processes such as nitrogen fixation, nitrogen assimilation, ammonification, nitrification, and denitrification (Augustyn et al. 2020).

Nitrification is the oxidation of ammonia to nitrite and nitrate by specialised nitrifying bacteria, which are divided into two groups: ammonia oxidising bacteria (AOB) and nitrate oxidising bacteria (NOB) (Rao et al. 2017). Nitrosomonas and Nitrosolobus are examples of AOB microbes (nitrose referring to the formation of a nitroso functional group). These two strains may convert ammonia to nitrite by utilising two enzyme systems: ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO). Nitrospira, Nitrobacter, and Nitrococcus, on the other hand, used an enzyme complex system to further oxidise the generated nitrite to nitrate (Forrez et al. 2011).

Heterotrophic nitrification–aerobic denitrification

Heterotrophic nitrification and aerobic denitrification are two novel nitrogen removal processes that are gaining popularity. Because heterotrophic nitrification and aerobic denitrification microorganisms (HNADMs) may carry out nitrification and denitrification concurrently under aerobic conditions, NH4-N can be aerobically transformed to nitrogenous gas (Khanichaidecha et al. 2018). This shows that HNADMs have a high potential for wastewater pollution treatment since they can remove both nitrogen and phosphorus.

HNADMs may be an alternative to typical wastewater treatment, despite significant limitations. Traditional wastewater treatment methods rely on autotrophic nitrifying bacteria (ANB) and heterotrophic denitrifying bacteria (HDB) (HDB). ANB's sluggish growth and sensitivity to the environment limited its employment in nitrogen removal treatment (Sun et al. 2016; Huang et al. 2017), whereas HDB can only denitrify in anoxic circumstances (Luo et al. 2016). As HNADMs may develop fast under aerobic conditions, their use in wastewater treatment is advantageous in attaining effective simultaneous nitrification and denitrification (Chen et al. 2016). Furthermore, several investigations have discovered that HNADMs can maintain nitrification and denitrification under harsh circumstances (low pH, salty wastewater, low temperature) (Huang et al. 2020a; 2020b). According to Chen et al. (2016), HNADMs are widely distributed and have been found in many environmental conditions. HNADMs bacteria are widely found in soil, sludge and wastewater. Table1 shows the list of bacteria that could perform heterotrophic nitrification-aerobic denitrification simultaneously in its specific isolation source.

Table 1: Different types of microorganisms that could perform heterotrophic nitrification-aerobic denitrification simultaneously in its specific isolation source

Microorganisms	Isolation sources	References
Achromobacter sp. A14	Sludge sample from Shi Bian Yu reservoir (Shanxi Province, China).	Su et al. 2018
Achromobacterxylosoxidans S18	Small-scale slaughterhouse wastewater	Kundu et al. 2018
Achromobacter sp. GAD3	Landfill leachate treatment system	Chen & Ni, 2011
Agrobacterium sp. LAD9		
Comamonas sp. GAD4		
Chryseobacterium sp. R31	Abattoir Wastewater	Kundu et al. 2012
Alcaligenes faecalis C16	Coking wastewater treatment plant	Liu et al. 2018
Cupriavidus sp. S1	Coking wastewater	Sun et al. 2016
Pseudomonas mendocina TJPU04	Activated sludge from Yunnan Third Wastewater Treatment Plant	He et al. 2019
Pseudomonas stutzeri KTB	Activated sludge from West Sewage Treatment Plant, Wenzhou, China	Zhou et al. 2014
*Vibrio diabolicus*SF16	Marine sediments	Duan et al. 2015

Characteristic of *achromobacter sp*.

Generally, *Achromobacter sp*. are gram-negative rod-shaped bacteria that originated from betaproteobacteria class. Genus *Achromobacter sp*. are obligately aerobic which are motile with the presence of peritrichous flagella. *Achromobacter sp.* are oxidase-positive, catalase-positive, citrate utilization-positive, urease-negative and indole-negative.

Several studies discovered that various types of *Achromobacter sp.*were isolated from wastewater. Most of them have the ability to remove nitrate or nitrate as well as able to degrade pollutants that are available in the water. A heterotrophic strain ACM-1 with similarities to *Achromobacter xylosoxidans* was isolated from Pangasius sp. fish farms in a research. Under aerobic circumstances, the strain demonstrated the capacity to convert ammonium to nitrate. This proved that *Achromobacter xylosoxidans* ACM-1 can remove ammonium in batch cultures and might be used as a biological nitrifier in aquaculture farms (Basha et al. 2018).

Aside from that, several research have shown the ability to do nitrification and denitrification concurrently under aerobic circumstances. Previously, new Achromobacter sp. strains capable of heterotrophic nitrification-aerobic denitrification, such as Achromobacter sp. GAD3, were recovered from the landfill leachate treatment system (Chen & Ni, 2011). Also identified from pharmaceutical wastewater was Achromobacter sp. JL9 (Liang et al. 2019), while *Achromobacter xylosoxidans* S18 was isolated from small-scale slaughterhouse wastewater (Kundu et al. 2012).

According to a recent study, *Achromobacter* sp. A-8, which was isolated from petroleum-contaminated wastewater, is capable of bioremediating petroleum-contaminated settings (Deng et al. 2020). Another study revealed that *Achromobacter* sp. C-1 isolated from a hydrocarbon-contaminated location may degrade raw coking effluent (Gracioso et al. 2018). This concludes that *Achromobacter sp.* could be a potential strain in the bioremediation process which are cost-effective and act as a promising solution for the treatment of wastewater.

Factors affecting the cell growth

There are many different factors affecting the cell growth of microorganisms and in particular for *Achromobacter sp.* Chemical factors such as medium compositions including carbon sources and nitrogen sources play a vital role in cell growth during the fermentation process. Physical factors such as aeration rate, agitation rate, pH and temperature playsignificant role in the maximal production of cells.

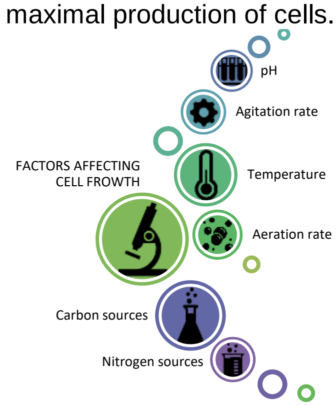

Figure 1: Chemical and physical factors affecting the cell growth

Effects of carbon source

According to a new study, the bacteria Achromobacter sp. A-8, which was isolated from petroleum-contaminated wastewater, may bioremediate petroleum-contaminated environments (Deng et al. 2020). Another investigation found that an isolate of Achromobacter sp. C-1 from a hydrocarbon-contaminated site may degrade raw coking effluent (Gracioso et al. 2018).

The addition of organic carbon in the heterotrophic nitrification process boosts ammonium removal and cell development substantially (Ren et al. 2014). Carbon sources include xylose, glucose, sucrose, lactose, fructose, and maltose, and each of them can influence *Achromobacter* sp. growth. Various criteria, such as the microorganism's metabolism, biomass production per unit of substrate, cost, source availability, and the kind of ultimate product, all impact the choice of an appropriate C source (Allikian et al. 2019).The kind of carbon source used is determined by the microorganism's species, as various species require different types of carbon sources to survive, and the denitrification capacity of the same strain varies when the carbon sources are different (Ren et al. 2014). This is due to the fact that various microbes have varied optimum carbon sources, which have variable redox potentials (Li et al. 2015).

Haloi and Medhi (2019) employed glucose, sucrose, kerosene oil, n-hexadecane, n-tridecane, and n-pentadecane as hydrophilic and hydrophobic carbon sources in a recent research. It demonstrates that the optimal carbon source for the development of Achromobacter sp. TMB 1 was glucose (Haloi&Medhi, 2019). Dextrose, fructose, glucose, starch, sucrose, maltose, and vegetable oil were all used by *Achromobacter xylosoxidans* GSMR13B in another investigation. For cell growth and biosurfactant generation, glycerol was chosen as the optimal carbon source (Reddy et al. 2018).Carbon sources such as glucose and sucrose, which are easily accessible on the market, are relatively costly. As a result, in a study published by Unuofin et al. (2019), agro-industrial residues such as mandarin peelings, grape stalks, maize stover, and wheat bran were used as a substitute for cheaper carbon sources in improving the development of *Achromobacter xylosoxidans* HWN16. Mandarin peelings were thought to be the greatest natural carbon source for *Achromobacter xylosoxidans* HWN16 cell growth and laccase synthesis (Unuofin et al. 2019).

Effects of nitrogen source

Nitrogen is required for cell development and the creation of a variety of metabolic products. Organic and inorganic nitrogen sources are the two types of nitrogen sources that may be found in nature. Yeast extract, soybean meal, peptone, beef extract, maize steep liquor, rice protein hydrolysate, and casein hydrolysates are all organic nitrogen sources. Ammonium salts such as urea, sodium nitrate, ammonium nitrate, ammonium phosphate, ammonium chloride, ammonium acetate, and ammonium sulphate are examples of inorganic nitrogen sources. The study on medium optimization of *Achromobacterxylosoxidans* HWN16 employed nitrogenous sources such as sodium nitrate, potassium nitrate, ammonium nitrate, L-asparagine, yeast extract, and tryptone. According to the findings, sodium nitrate was the best nitrogen source for growing a high cell mass in *Achromobacter xylosoxidans* HWN16.

In a separate investigation, nitrogen sources such as yeast extract, urea, peptone, ammonium chloride, potassium nitrate, and ammonium sulphate were employed to determine the optimal growth of *Achromobacter* sp. strain TERI-IASST N. Organic nitrogen sources as urea, yeast extract, and peptone significantly boosted the cell development of strain TERI-IASST N. The highest cell mass was obtained with urea (84%) followed by yeast extract (79%) and peptone (71%) whereas cell mass was obtained with inorganic nitrogen sources ammonium chloride, potassium nitrate, and ammonium sulphate were 67, 65, and 62 percent, respectively. The inclusion of vitamins and trace elements in organic nitrogen sources may contribute to higher cell mass production.

The growth of bacteria is also affected by the concentration of nitrogen sources. A high nitrogen concentration promotes aggressive cell development, increased growth substrate synthesis, and quicker cell synthesis. Ammonium sulphate was shown to be the optimal nitrogen source for optimum development of *Achromobacter* sp. LH-1, according to Zeng et al (2020). When *Achromobacter* sp. LH-1 was grown in a medium with a high nitrogen content (4−6 g/L NH_4NO_3), it produced the most cell growth.

Effect of aeration

Dissolved oxygen (DO) is an important factor in the denitrification and ammonia oxidation (nitrification) processes, particularly in aerobic microorganisms, where it represses or induces several primary and secondary metabolism enzyme systems, thereby activating oxidative reactions for nutrient assimilation and energy build-up for cell formation (Geets et al. 2006). Heterotrophic nitrification and aerobic denitrification microorganisms (HNADMs) may undertake denitrification in aerobic circumstances, unlike heterotrophic denitrifying bacteria (HDB) (Zhao et al. 2012; Ren et al. 2014). Various HNADMs are most likely to have different optimum DO for denitrification, as can be observed. HNADMs have a high capacity for adaptability. Denitrification efficiency will steadily decline as DO rises.

Generally, the effect of oxygen limitation during submerged cultivation could affect cell growth, cell mass production, and metabolisms functionality (Baez &Shiloach, 2014). It was reported that the species of the genus *Achromobacter*are strictly aerobic rods. However, they also have the ability to dissimilatory denitrify under anaerobic conditions depending on the availability of nitrate or nitrite reductase enzymes formed in the bacteria (Busse& Stolz, 2006). Also, nitrifiers have been shown to live in anaerobic environments, such as in fish-pond sediments (Diab et al. 1992) and in the anaerobic hypolimnion of wastewater reservoirs (Abeliovich, 1987). According to Diab et al. (1992), nitrifying bacteria may survive anaerobic circumstances by either adjusting their metabolism to a very low rate, resulting in resting cells, or switching from a nitrifying to a denitrifying activity.

In a study conducted by Huang et al. (2012), the concentration of dissolved oxygen (DO) was controlled at 2–4 mg/L during the aeration phase with an aeration time of 4 hours. It shows the cell mass production of *Achromobacter sp.* S-3 achieved its maximum production at 3 hr. This indicated that when aeration and oxygen supply increased as the reaction time prolongs, it would enhance the cell mass production. However, after 3h cultivation, cell growth enters the stationary phase and suppressed the cell mass production (Huang et al. 2012). Thus, it could be concluded that under aerobic condition, adequate dissolved oxygen needs to be supplied in maintaining the cell mass production. After all, exceedingthe amount of oxygen could be a decline in respiration of the cells due to increasing dissolved oxygen tension and therefore causing a lag in cell growth, cell mass production, substrate bioconversion as well as enzyme secretion (Unuofin et al. 2019). Thus, this concludes that dissolved oxygen supply needs to be monitored well as it may have a very great effect on the performance of *Achromobacter sp.* growth and its cell mass production.

Effect of agitation

In submerged fermentation, agitation speed plays an important role in cell growth and cell mass production. Agitation speeds that were higher or lower than 100 rpm could result in decreased enzyme production and less bacterial growth depending on the type of strains (Ibrahim et al. 2013). Generally, *Achromobacter sp.* grows well under agitation speed of 120-150 rpm (Unuofin et al. 2019; Basha et al. 2018; Mbagwu et al. 2018). A recent study by Unuofin et al. (2019) recorded that maximum cell growth of *Achromobacterxylosoxidans* HWN16 was achieved at a low agitation speed (100 rpm). Another study indicated the growth of *Achromobacter sp.* HZ01 gradually increased and reached its maximum cell mass production at 150 rpm, which ensures maintaining enough amount of dissolved oxygen for strain HZ01 growth (Deng et al. 2014). Above all, most researchers reported that the optimum agitation speed for *Achromobacter sp.* growth and its high cell mass production was at 150 rpm (Reddy et al. 2018; Deng et al. 2014; Subudhi et al. 2016).

Effects of pH

The effectiveness of microbial growth is usually affected by the pH condition in the culture medium. The concentration of hydrogen ions in a solution is measured by the pH. The pH value of a solution decreases as the number of hydrogen ions in the solution increases. Microbial degradation and enzymatic activity will be affected by acidic or alkaline conditions, affecting cell mass formation. The strength of nitrification is significantly associated with pH. The rate of heterotrophic nitrification is slowed by low pH. Extremely acidic and alkaline pH, according to Reddy et al. (2018), would result in limited cell mass production. Some HNADMs may develop in a pH range of 3 to 9. The pH tolerance range of HNADMs, on the other hand, fluctuates, since many strains can widen their pH tolerance range following adaption (Zeng et al. 2020).Although certain HNADMs may survive a wide range of pH values, the majority of HNADMs thrive best in neutral or mildly alkaline conditions. The ideal pH range for most HNADMs is 6-9, and pH levels that are too high or too low might inhibit their development and metabolism (Nancharaiah et al. 2018).

The ideal pH medium for the development of Achromobacter sp. was discovered by several researches to be between 5.0 and 8.0. (Liang & Hu, 2020; Unuofin et al. 2019; Jin et al. 2015; Deng et al. 2014). According to a research, the ideal pH for cell development of Achromobacter sp. L3 was between 5.0 and 7.0, with the starting pH of 7.0 yielding the highest cell mass production (Liang & Hu, 2020). Another research found that Achromobacterxylosoxidans GSMSR13B develops well from pH 6.5 to 9.0, with pH 8 being the optimal pH for large cell mass production (Reddy et al. 2018).The considerable growth of Achromobacter sp. TMB 1 was monitored in the range of 4-10 and demonstrated that the most suitable pH was between 5.5 and 7.2, according to Haloi&Medhi (2018). Several researches showed that pH 7 was the optimal pH for obtaining high cell mass production in Achromobactersp (Liang & Hu, 2020; Haloi&Medhi, 2018; Deng et al. 2014; Jin et al. 2015; Huang et al. 2012).

Effects of Temperature

Temperature is an important factor affecting microbial growth and cell mass production. Both nitrification and denitrification processes are sensitive to temperature. Su et al. (2018) indicated that temperature is one of the primary factors influencing the ability of substrates and nutrients to enter the cell. Therefore, the temperature can influence cell mass production and its functionality. Generally, nitrification and denitrification would be severely inhibited when the environment temperature is below 10°C (Rodriguez-Caballero et al. 2012). The optimal temperature range of most HNADMs is 25-37 °C. For most of HNADMs, their metabolism will be significantly inhibited under high-temperature and low-temperature conditions (Liu et al. 2019). Although temperature change will significantly affect the activity of HNADMs, they can still maintain part of the nitrification and denitrification capacity when the temperature is so high or so low. The discovery of HNADMs at different temperatures indicates that HNADMs may have been playing an important role in the nitrogen cycle in different temperature environments.

According to Reddy et al. (2018), *Achromobacterxylosoxidans* GSMSR13B was a temperature-dependent strain. This is because it produced maximum cell mass when incubated at 37°C and was totally inhibited when the incubation temperature increased to 40°C. A recent study discovered that the optimum temperature for maximum cell mass of *Achromobacter sp.* L3 is at 30°C (Liang & Hu, 2020). Also, Haloi&Medhi (2018) reported that *Achromobacter sp.* TMB 1 has good growth in temperature ranging from 23 to 45°C but the optimum temperature was found to be 30-37°C and its maximum cell mass production was achieved at 30°C. The evaluation of temperature on *Achromobacter sp.* HZ01 revealed that the optimum temperature for achieving its maximum cell mass was 28 °C whereas decreasing temperature could lower the cell growth and cell mass production (Deng et al. 2014). Another study discovered that the optimal temperature for *Achromobacter* strain W-1 production was 35°C however increase in temperature would decrease its cell mass production (Jin et al. 2015). The effect of temperature on *Achromobacter sp.* S-3 was investigated in the range of 15-35°C where it shows that increase in temperature could increase the cell mass production and reached a maximum value at 30°C. Most of the findings reported that the optimum temperature for high cell mass production of *Achromobacter sp.* was at 30°C (Liang & Hu, 2020; Haloi&Medhi, 2018; Huang et al. 2012) and further increase in temperature to 35°C would cause the loss of cell viability and denaturation of the enzymes (Huang et al. 2012).

Batch cultivation in bioreactor

In achieving an efficient commercial production process, bioreactor cultivation studies are considered the best solution (Zhu et al. 2011). Fermentation processes at the bioreactor scale need strict control of parameters such as pH, temperature, carbon dioxide (CO_2), agitation, aeration/dissolved oxygen (DO), foam, and many other parameters to maximize cell growth and productivity (Chikere et al. 2016). Culture conditions play an important role on bacterial cell growth, cell mass production and cell metabolism (Shoda, 2017). Fermentation processes at the bioreactor scale could monitor those parameters such as pH, temperature, carbon dioxide (CO2), agitation, aeration/dissolved oxygen (DO), foam, and many other parameters to maximize cell growth and productivity (Tan et al. 2020; Zhu et al. 2020).

Some strains exhibit good simultaneous nitrification and denitrification under batch experimental settings, and a reactor research was conducted to increase treatment efficiency (Wang et al. 2020; Jia et al. 2020). However, they have not yet reached the level of application, owing to a lack of efficient bacterial resources and ambiguity about the metabolic features of heterotrophic nitrification and denitrification microorganisms (HNADMs) (Song et al. 2021). As a result, in order to offer support for the development of large-scale application systems, additional HNAD strains must be discovered, and the parameters controlling growth and metabolism must be investigated (Song et al. 2021).

CONCLUSION

Improper management in wastewater treatment plants appears to have a significant impact on the environment and human health. Therefore, this review has discussed suitable bioremediation by the application of *Achromobacter sp.* which has the ability to conduct heterotrophic nitrification and aerobic denitrification (HNAD) simultaneously. These HNAD bacteria are responsible for biological purification by reducing nitrogen accumulation in wastewater, eliminating of nitrate by denitrification and decreasing the eutrophication of sewage water ecosystems. It was proven that wastewater treatment through biocontrol mainly HNAD microorganisms are economical operation, eco-friendly approach and maintains resource recovery.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT

The authors would like to thank Research Management Center at UTM, Malaysia, Indah Water Sdn Bhd through grant No. R.J130000.7651.4C449 and the Ministry of Higher Education, Malaysia for the partial support through FRGS (FRGS/1/2020/TK0/UTM/02/16).

AUTHOR CONTRIBUTIONS

DJD, NZN, AMA, AAAN, LTT, SR, ZIAR, and NS were involved in the data collection and writing the manuscript. KBC and HAE reviewed the manuscript. All authors read and approved the final version.

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