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ORIGINAL ARTICLE

A review of wastewater bacterial bio oxidation: mechanisms, reactions, and behaviors

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ABSTRACT – The most overlooked biological process is the oxidation process, due to the poor technical understanding of bioremediation and the many unexplained biological mechanisms. As a matter of fact, wastewater companies favor the physical and chemical approaches over the biological means to ensure process performance. Biological oxidation provides environmentally friendly treatment with limited operational requirements. It has a tremendous ability to metabolize ammonia, H_2S, phosphate, and ferrous iron under the right conditions. Bacterial communities attack these wastewater components through using oxygen as a strong electron acceptor, resulting in simplified forms of wastewater components (e.g., ammonia to nitrogen gas). This manuscript reviews in details the mechanisms of wastewater bacteria against various pollutants, with the possible reactions and behaviors. It also presents the benefits of utilizing wastewater microorganisms against different pollutants.

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INTRODUCTION

Discharge and dumping of chemically contaminated wastewater, including dyes, pesticides, solvents, and heavy metals threats our freshwater [1]. Scientific understanding of the wastewater impact has evolved with good credibility to prove several a number of severe damages triggered by contaminated water. Various quantities of contaminants have been found in very remote locations from their discharge station [2]. As a result, a scientific approach has begun to understand and assess the ability of different industries to produce profits without harm [3–4].

It is important to define the relationship between the sort of bacteria and the obtainable performance because the bacterial metabolism controls the biotreatment efficacy. Most biotreatments are derived from engineering understanding, and the bacterial dynamics are largely neglected. A major approach in wastewater treatments (WWTs) will be developed once the microbial dynamics are considered. Furthermore, environmental conditions are the main factors that have revealed the changes in bacterial communities [5–7].

Wastewater bacteria

The amount of heterotrophic and autotrophic bacteria varies in each wastewater on the basis of the treatment system and the sort of wastewater used [8]. Betaproteobacteria is considered to be the most densely populated organism in municipal wastewater, while Phylum Proteobacteria is the predominant microorganism, and *Bacteroidetes*, *Acidobacteria* and *Chloroflexi* are subdominant [9–10]. *Tetrasphaera, Trichococcus, Candidatus Microthrix, Rhodoferax, Rhodobacter, Hyphomicrobium,* p-55-a5, P2CN44 and B45 bacteria are the most frequently identified, according to the evaluation of 20 wastewater treatment plants [11]. Approximately 6.3%-7.4% of wastewater microorganisms are *Ascomycota*, while *Euryarcheota* is recognized as predominant with 1.5% of total wastewater microorganisms [12]. The wastewater remediation always favors some microorganism classes over the rest, according to the molecular studies. The availability of molecular data may support the scientific understanding of wastewater bioremediation (e.g., anaerobic ammonium oxidation (anammox), nitrification). Oxidation ditches, and membrane bioreactors (MBR) have been found to have fewer microscopic species than anaerobic/anoxic/oxic (A2O) and anaerobic/oxic (AO) processes [9,13]. In addition, low diversity of microorganisms in MBR could be due to the low availability of biodegradable organics and long solid retention time (SRT) [9].

Much of the available data on wastewater treatment is focused on scientific study [14]. The aerobic granulation method is a strong example among biological treatments due to the high population of microorganisms such as *Thauera* sp., *Aquabacterium* sp. and *Flavobacterium* sp. These microorganisms support their attachment by creating extracellular polymer substance (EPS), and proceed the denitrification process with slow evolution [15–17]. Certain microbial groups such as filamentous can be encouraged to expand under ample capital of energy-rich substrates such as fructose and glucose [18]. Domestic WWTs have been found to have a higher diversity of ammonia oxidizing bacteria (AOB) than municipal WWTs, and the major bacteria in the nitrification cycle are Nitrosomonas sp. [19–20]. Another great example of bioremediation is the sequencing batch reactor (SBR) that generates high biodiversity and population through its pretty

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decent engineering. Bioremediation failure in WWTs is due to poor engineering understanding of biological treatment through providing unsatisfactory circumstances such as high range of chemical inhibitors, low DO, extreme pH, and low temperatures [21].

According to a survey conducted on 14 WWTs, approximately 95% of the activated sludge composition comprises of *Myxococcales, Planctomycetales, Clostridiales, Verrucomicrobiales, Xanthomonadales, Rhizobiales, Burkholderiales, Rhodocyclales, Anaerolineales,* and *Sphingobacteriales* [10]. Microscopic species differ based on the geographical place, and circumstances like temperature. For instance, *Chloroflexi, Actinobacteria*, and *Betaproteobacteria* comprise 50% of the total microbial population regardless of temperature, while *Alphaproteobacteria*, and *Saprospiraceae* as a whole are affected by temperature levels [22]. In the same way, filamentous bacteria have been detected varying by type of WWT, and their geographical location. Usually, wastewater contains a small amount of filamentous bacteria that enable the formation of a microbial matrix. Despite this, an excessive number of filamentous bacteria leads to sludge bulking. Wastewater often involves different filamentous groups, such as *Microthrix parvicella*, Type 1863 *Acinetobacter*, *Mycobacterium fortuitum*, and *Nostocola limicola* I and II, which make up 1.86%-8.99% of the total population of wastewater microorganisms [23].

The usual activity in wastewater is the development of an immobilized multilayer matrix of microorganisms on solid surfaces utilizing hydrophobic interactions, covalent bonds, and electrostatic interactions known as biofilm formation [16]. These microbial communities include EPS, cell wall components, *cilia*, and *fimbriae*. In addition, the fine engineering design of the biofilm structure provides oxygen penetration through determined depth, mass diffusion, and genetic transfer through multiple channels and pores, leading to toxic compound resistance, metabolic activity, and bacterial growth [24–25]. EPS compositions are based on cell lysis, material shedding from the cell surface, and material absorption from the surrounding area. A researcher stated that the structure of EPS differs between biofilms and activated sludge in the same environment [26]. Free nitrous acid and ammonia in WWTs are the key causes for the microbial complex formation of biofilm bacteria to protect against harmful effects [27]. EPS structure provides a diffusion mechanism that reduces the nearby toxic level. The constitute of EPS is about 80-95% organic biomass, while the rest is bacteria [28].

Bacterial Bio Oxidation

Bio oxidation is the most environmentally sustainable approach for the removal of phosphorus (P), nitrogen (N) and organic matters from wastewater. In order to avoid aquatic eutrophication, the bio oxidation mechanism is important for wastewater. In fact, many WWTs rely on microbial activities for the removal of pollutants. Aerobic systems have been found to be unable to minimize P and N, but have high rates of organic carbon removal. A conventional WWT system was used to deliver different amounts of dissolved oxygen to trigger all bacterial groups for simultaneous nitrification, denitrification and phosphate removal (SNDPR) [29–30]. Ammonium oxidizing bacteria (e.g., *Nitrosomonas*) and nitrite oxidizing bacteria (e.g., *Nitrospira*) [complete ammonia oxidizing bacteria (comammox)] convert ammonia to nitrite through aerobic conditions through a nitrification process [31]. Anoxic conditions are then used to convert nitrite to N_2 by the denitrification process. At the same time, there are different microbial groups to treat polyP known as polyphosphate (polyP) accumulating organisms (PAOs) [32]. During anaerobic conditions, PAO uses polyP at the intracellular as food, accumulates phosphate from wastewater, and seizes polyP in cells under aerobic conditions.

A researcher examined bioremediation and found that inorganic chemicals were used as energy source for aerobic bacteria (nitrification) under aerobic circumstances, followed by heterotrophic and anaerobic bacteria producing biomass through anaerobic or anoxic conditions (denitrification) [33]. Xue et al. [34] confirmed that dissolved oxygen (DO) determines the overall efficacy of biodegradation and relates to first-order biodegradation constants. Some elements and compounds, on the other hand, resist biodegradation at any DO concentration [33]. Shortly, AOB (ammonia oxidizing bacteria), and NOB (nitrite oxidizing bacteria) are heterotrophs, while PNA (partial nitritation annamox) is thought to be heterotrophic. Figure 1 shows the full ammonia conversion cycle by PNA [35].

Although bio oxidation is considered to be an eco-friendly process between other treatments, it emits CH_4 through anaerobic digestion, CO_2 through respiration, and N_2O through nitrification and the denitrification [36]. A small volume of N_2O emissions is equivalent to a large carbon footprint, and its impact is 265 times greater than CO_2, where the lifetime of N_2O gas is 114 years in the atmosphere [37]. In fact, 26% of global warming is caused by N_2O pollution [38]. Heterotrophic denitrification, denitrification of nitrifiers, and oxidation of hydroxylamine (NH_2OH) are the main sources of N_2O emissions (see Figure 2) [39]. Figure 2 demonstrates the process of production of N_2O by AOB (i.e., nitrifiers' denitrification, and NH_2OH oxidation), and heterotrophic denitrifiers (i.e., heterotrophic denitrification) [39].

A wastewater treatment with low temperatures, high concentration of NO_2^-, and low concentration of DO are the key factors behind the N_2O release from the biological cycle. At these conditions, the AOB variety overcomes the NOB population, especially during low DO ranges. Chen et al. [40] found that the emission of N_2O was due to the oxic phase of AOB. Converting the anaerobic phase to idle conditions will reduce 42% of N_2O emissions due to the consumption of N_2O by heterotrophic denitrification. Ahn et al. [41]confirmed, through their empirical study, that NO and N_2O were high ranges at low DO levels. When DO levels rise from 1 to 4.5 mg/l, N_2O decreases from 6% to 2.2%, according to Pijuan et al. [42].

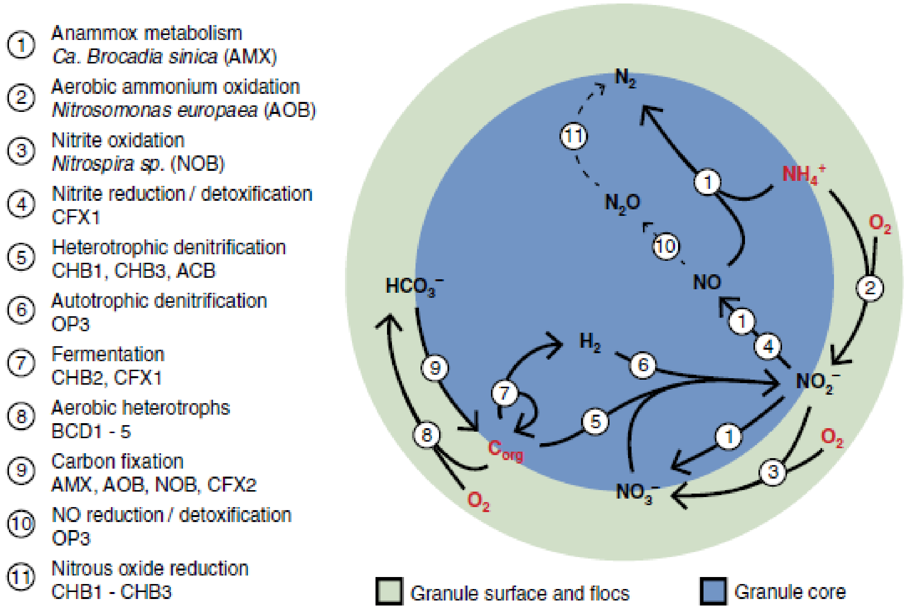

Figure 1. Illustration of PNA reaction including ammonia [35].

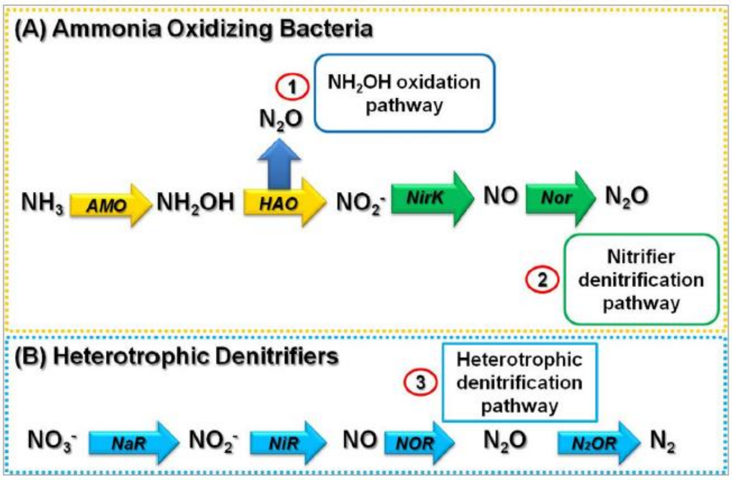

Figure 2. Illustration of processes for N_2O generation [38].

Ammonia bio oxidation

High levels of ammonia concentrations can turn water into a toxic medium for aquatic life, because it makes a high demand for oxygen, resulting in eutrophication. Ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) population are determined by the influent concentration of ammonia. AOA conducts an autotrophic oxidation cycle in WWTs. For example, the AOB population in industrial WWTs is higher than AOA due to high ammonia availability, whereas the AOA abundance is higher than AOB in municipal WWTs [43]. However, a well-removal process can be achieved even under a low population of autotrophic nitrifiers.

Wang et al. [44] and Ma et al. [45] observed that heterotrophic nitrifiers have been developed from the *Azoarcus*, *Paracoccus*, *Thauera*, and *Comamonas* genera for the remediation of rich ammonium coking wastewater by activated sludge reactors. Nitrifiers and denitrifiers often request a variety of substrates for their production. In the process of rotating biological contactors (RBC), it was found that the population of nitrifers increased at low abundance of denitrifers (*Azoarcus*, *Thauera*, *Paracoccus*, and *Rhodanobacter* genera) [46]. It was observed that the AOB resided on the flocs and the outer surfaces of granules, while the anammox bacteria remained in the inner zone [47]. AOB partially oxidizes ammonia to NO_2^-, while the remaining NH_4^+ is oxidized by anammox using NO_2^- as an electron acceptor [38].

The DNA testing for denitrifiers showed that most of the AOBs belong to *Nitrosospira* sp., and *Nitrosomonas* sp., and share various similarities [12]. Denitrifier functions are influenced by the same circumstances as DO, N, organic load, and temperature with varying outputs. It has long been belived that oxic conditions are suitable mediums for ammonia oxidation, but Broda has suspected the presence of chemolithoautotrophic anammox bacteria for two decades on the basis of thermodynamic calculations [48]. The electron acceptor was nitrite in the ammonia oxidation process [36], [49].

AOB delivers nitritation by converting ammonium to nitrite, then the NOB group delivers nitratation by converting nitrite to nitrate. Nitrification concept applies to both nitritation and nitratation, whereas AOB, and NOB groups are called nitrifiers [38]. Often the process of nitrification is known as the method of using NO_2^- to convert NH_4^+ to NO_3^- by microorganisms of AOB and NOB. Hydroxylamine (NH_2OH) is usually produced as an intermediate product when NH_4^+ is oxidized with one of O_2 atoms [38]. The hydroxylamine oxidoreductase then oxidizes the intermediate to NO_2^- [50]. The process of decomposition of the intermediate produces N_2O. Sometimes, N_2O can be produced by the denitrification process at excessive concentrations of nitrite, where N_2O, and N_2 are formed by the reduction of NO_2 [51-52].

Oxygen is required for the bioremediation process to be used as an electron acceptor, and as a substrate for the production of hydroxylamine by NH_4 oxidation. 4.2 g of oxygen is required for each gram of ammonium nitrogen nitrified by the nitrification process [53]. Nitrite may be used as an electron acceptor in the case of low DO concentration.

A broken reaction causes serious ecological risks, such as foam formation, methemoglobinemia, toxic nitrate and/or nitrite, and the death of fish [54]. Nitrification and denitrification were studied in a number of WWTs, such as aeration tanks, moving bed biofilm reactor (MBBR), and rotating disk reactor [55–57]. Figure 3 displays the oxidation process.

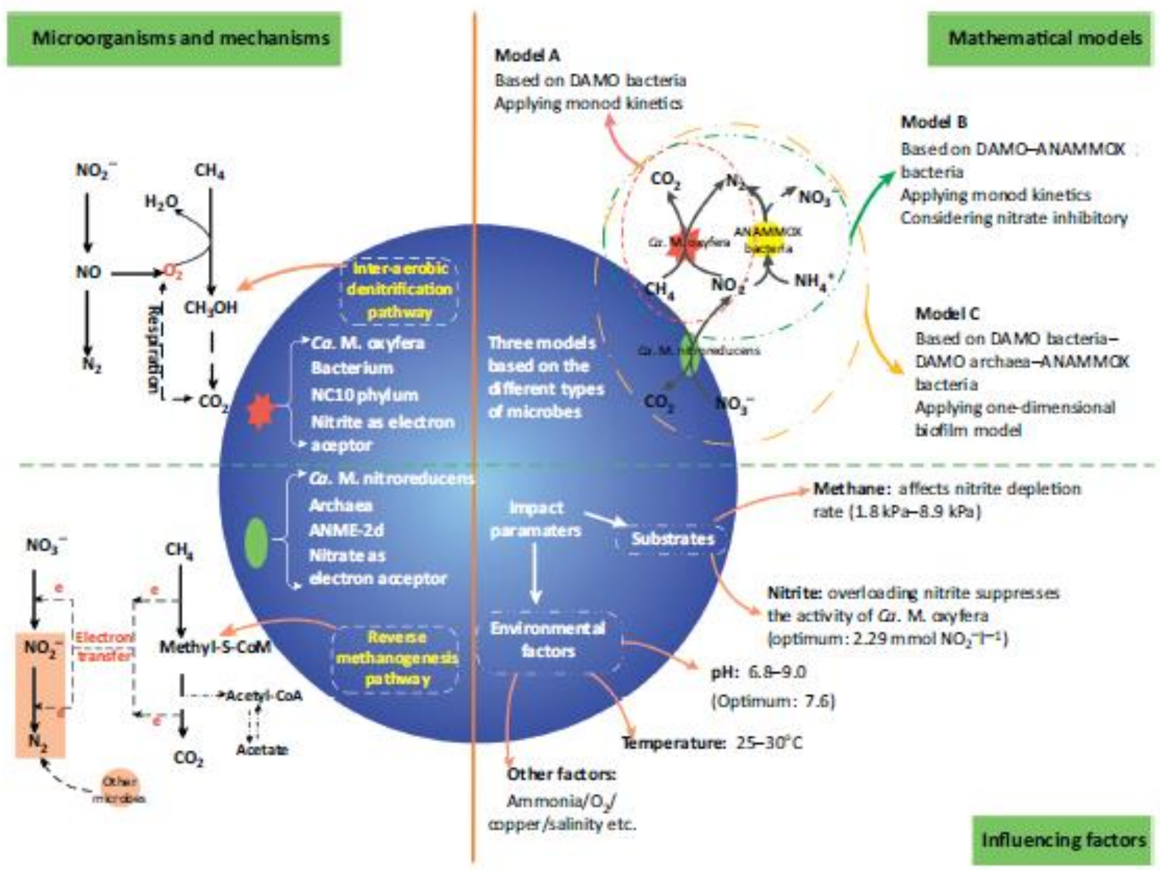

Figure 3. Overview of the oxidation process [58].

The TKN concentration is a crucial parameter to present the achievement of nitrification. Reactions that have occurred through the nitrification and denitrification processes are outlined in equations 1, 2, 3, and 4. These conventional reactions have been identified for a long time [49].

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + 2H_2O \quad (1)$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad (2)$$

$$2NO_3^- + 10H^+ + 10e^- \rightarrow N_2 + 2OH^- + 4H_2O \quad (3)$$

$$2NO_2^- + 6H^+ + 6e^- \rightarrow N_2 + 2OH^- + 2H_2O \quad (4)$$

As far as denitrification is concerned, 2.47 g of methanol is needed for each nitrate nitrogen gram, in case methanol is used as an electron donor [54]. The right electron donor is methane because it is readily accessible and cheap for denitrification, but it is slowly consumed [59]. A large amount of organic carbon is also required to provide a complete process. Ammonium oxidation can be occurred under anoxic conditions using organic compounds and hydrogen as an electron donors, according to Schmidt and Bock (1997) [60], [61]. Nitrite is the perfect electron acceptor for the oxidation process (see equations 5 and 6).

$$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+ \quad (5)$$

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (6)$$

Approximately 10% of the feed ammonia and nitrite have been converted to NO_3^- under anaerobic conditions, and the main product is still N_2. Equation 7 describes the total balance of nitrogen from the feed to the last product via the nitrification [62].

$$NH_4^+ + 1.31NO_2^- + 0.0425CO_2 \rightarrow 1.045N_2 + 0.22NO_3^- + 1.87H_2O + 0.09OH^- + 0.0425CH_2O \text{ (Biomass)} \quad (7)$$

The overall balance of anammox cultures was estimated as shown in equation 8.

$$NH_4^+ + 1.31NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (8)$$

Phosphorus removal

Polyphosphate accumulating organisms (PAOs) are used in WWTs to remove phosphate through aerobic or anaerobic conditions. PAOs are capable of eliminating phosphate by using nitrate or nitrite as electron acceptors [63]. *Accumulibacter* sp., and *Tetrasphaera* sp. are examples on the PAOs. *Accumulibacter* sp. consume volatile fatty acids and store polyhydroxyacids (PHAs) while *Tetrasphaera* sp. take amino acids and do not store PHAs. 3-10% of the population is *Accumulibacter* sp., while 30-35% is *Tetrasphaera* sp. [64]. The carbon to phosphate ratio determines the abundance of *Accumulibacter* sp., and *Tetrasphaera* sp. [65]. *Dechloromonas* is another group of PAOs which is capable of nitrite/nitrate reduction, PHA storage, polyphosphate, and acetate acceptance [66]. *Candidatus Accumulibacter phosphatis* is the most known group of PAOs belonging to *Rhodocyclaceae* family of the Betaproteobacteria class. Figure 4 shows an examination result from full scale oxidation WWT in Japan of finding the working microorganisms on P removal.

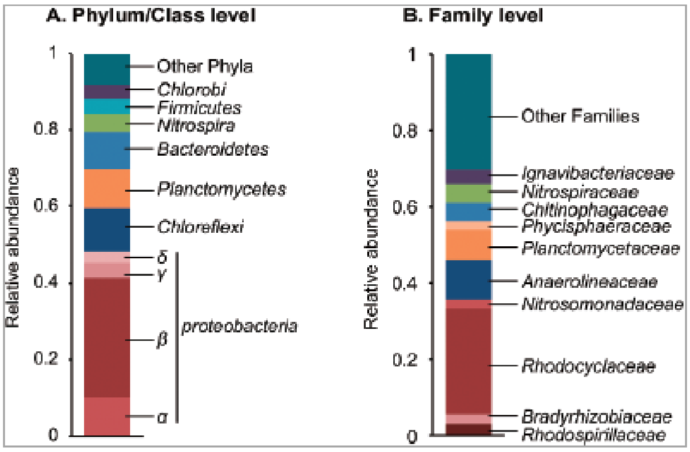

Figure 4. Distribution of Phylogenetic's gene clones in WWT oxidation ditch [67].

H_2S oxidation

The common species for sulfide removal are purple sulfur bacteria, colorless sulfur bacteria, and phototrophic sulfur bacteria. As wastewater has a high level of nitrates under denitrifying conditions, the sulfur bacteria use nitrates as an electron acceptor. The oxidation process of H_2S produces sulphate and sulfur. Sulfide is then oxidized by reducing nitrate into nitrite by the sulfide oxidizing bacteria (SOB). The sulfide oxidation reaction is zero-order. In the event of incomplete oxidation of sulfide, the solution becomes white due to the excess amount of the intermediate element of the reaction known as sulfur. The suggested incomplete reaction is shown in equation 9.

$$NO_3^- + HS^- \rightarrow S^0 + NO_2^- + OH^- \quad (9)$$

Sublette and Sylvester (1987) reported a practical oxidation reaction by converting 40%-60% of the sulfide fed to sulphate [68]. Possible complete reactions are presented in equation 10 and 11.

$$HS^- + 1/2O_2 \rightarrow S^0 + OH^- \quad (10)$$

$$NO_3^- + H^+ + 2e^- \rightarrow NO_2^- + OH^- \quad (11)$$

Iron oxidation

Ferrous iron acts as an electron donor under anaerobic conditions, or as an additional electron donor in the presence of acetate. In addition, iron is the most suitable metal for the redox process [16]. Iron-reducing bacteria also treat ferric iron in sediments and soils under anoxic conditions [69]. Light can be used as an energy source with O_2 for phototrophic bacteria to carry out biological oxidation by taking the electron from the iron as an electron donor and giving it to nitrate as an electron acceptor [70]. Equation 12 shows the possible oxidation reaction.

$$10\ FeCO_3 + 2\ NO_3^- + 24\ H_2O \rightarrow 10\ Fe(OH)_3 + N_2 + 10\ HCO_3^- + 8H^+ \quad (12)$$

Successful experiments on the use of ferrous iron to reduce nitrous oxide and nitrite have been reported [71-72]. Finally, the work in the present review has led the authors to illustrate the complete bio oxidation of ammonia, phosphate and polyphosphate, and H_2S (see Figure 5).

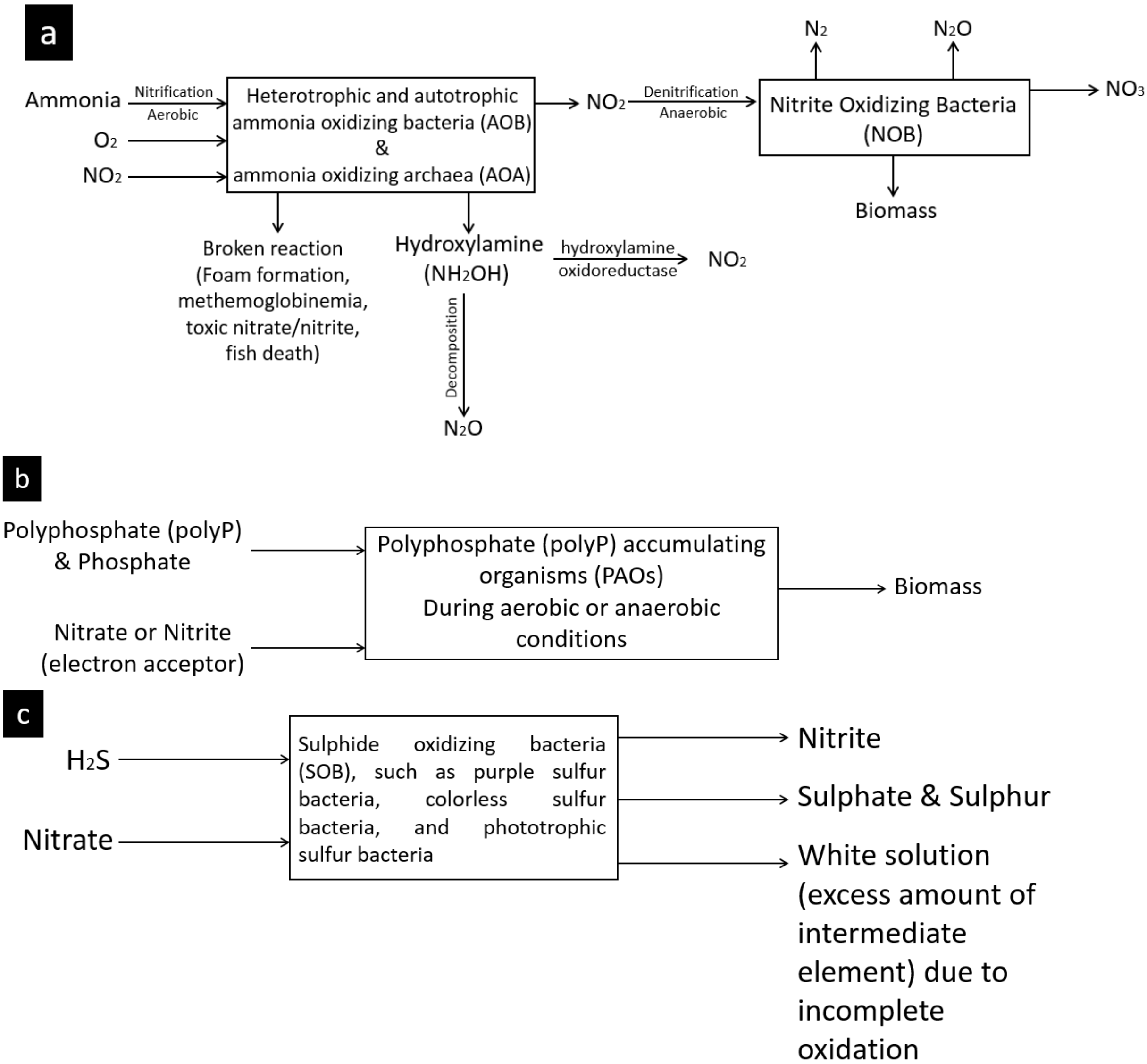

Figure 5. Flowchart of bio oxidation. (a) Ammonia bio oxidation; (b) Phosphate and polyphosphate bio oxidation; (c) H_2S bio oxidation.

Antibiotics-resistant bacteria

Ineffective degradation leads to the growth and development of antibiotic-resistant bacteria (ARB) in the environment [49]. The main home for the evolution of ARB is wastewater, which is a perfect culture for the production of antibiotic resistance genes (ARGs). As a result, the mineralization processes of wastewater pollutants by microbes are quite important to reveal the evolution of ARGs.

During various wastewater treatments, the ARB population and diversity were found to be the highest in the wastewater influent, followed by treatment effluent, anaerobic digestion sludge, and activated sludge, depending on the concentration of wastewater pollutants. Sludge treatment is usually unable to reduce the population and growth of ARBs, while other wastewater treatments are likely to result in more than 99% removal [73]. For this end, a disinfection process is needed to ensure the removal of ARBs. However, some methods may cause reactivation and regeneration of ARBs, such as chlorination [74]. In addition, the concentration and type of antibiotics within the influent determines the effluent bacterial diversity. For instance, bacteria from *Clostridia* and *Bacilli* classes, *Firmicutes*, and phyla Proteobacteria predominated effluents after treating penicillin-containing wastewater [75].

It has also been reported that the population of *Epsilonproteobacteria* grows proportionally with the presence of triclosan, quinolones, sulfonamides, penicillin, and tetracyclines, while these components have a negative affect on the growth of *Firmicutes*, and *Gammaproteobacteria* [76]. Another study showed a high diversity and abundance of different genes during the treatment of tetracycline wastewater, such as *Rhodobacter*, *Novosphingobium*, *Paracoccus*, *Longilinea*, *Azonexus*, *Hyphomicrobium*, *Prosthecobacter*, *Armatimonas*, and *Sulfuritalea* which led to a reduction in non-tetracycline ARGs [77].

Generally, dye-containing wastewater reflects sunlight from penetrating inside the reactor. Thus, multiple types of microbes will be idled, due to the depletion of sunlight. As a result, the discharge of polluted wastewater threatens our healthy life, and the ecosystem by increasing severe risks, and serious damages.

CONCLUSION

The disposal of wastewater continues to contaminate waterways, notwithstanding the rules and legislations for the discharge of effluents. Scientists have been developing the biological oxidation data, but it is slowly occurring. For the time being, adequate and reliable knowledge on bioremediation is available to structure a technique that utilises wastewater microbes in maximum effort to bio decompose suspended contaminants. The oxidation process map incorporates much of the existing evidence and demonstrates a significant contribution to the advancement of current understanding. One of the interesting points in this research is that bacterial communities and families do teamwork during bioremediation. The operational circumstances must therefore satisfy the dominant or most of the bacteria used.

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