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Application of zeolites for biological treatment processes of solid wastes and wastewaters - A review

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ABSTRACT

This review reports the use of zeolites in biological processes such as anaerobic digestion, nitrification, denitrification and composting, review that has not been proposed yet. It was found that aerobic processes (activated sludge, nitrification, Anammox) use zeolites as ion-exchanger and biomass carriers in order to improve the seattlebility, the biomass growth on zeolite surface and the phosphorous removal. In the case of anaerobic digestion and composting, zeolites are mainly used with the aim of retaining inhibitors such as ammonia and heavy metals through ion-exchange. The inclusion of zeolite effect on mathematical models applied in biological processes is still an area that should be improved, including also the life cycle analysis of the processes that include zeolites. At the same time, the

application of zeolites at industrial or full-scale is still very scarce in anaerobic digestion, being more common in nitrogen removal processes.

Keywords: activated sludge; anaerobic digestion; composting; nitrogen removal; zeolites

1.- Introduction

Zeolites are crystalline substances with pores of molecular dimensions that permit the passage of molecules below a certain size. Zeolites are mainly constituted by Al, O, and metals including Ti, Sn, Zn, and others.

The first reference for the term zeolite was made in 1756 by Axel Fredrik Cronstedt (Bingre et al., 2018). This author observed that the rapid heating of the material produced large amounts of steam from water that had been previously adsorbed by the material. Based on this property, he called the material *zeolites*, from the Greek ζέω (zéō), meaning "to boil", and λίθος (líthos), meaning "stone" (Breck, 1973). Zeolites have a porous structure that can accommodate a wide variety of cations, such as Na^+, K^+, Ca^{2+}, Mg^{2+} and others. These positive ions are rather loosely held and can be readily exchanged for others in a contact solution.

As of 2016 the world's annual production of natural zeolites was approximately 3 million tons. The main producers are: China (2 million tons), South Korea (210,000 t), Japan (150,000 t), Jordan (140,000 t), Turkey (100,000 t), Slovakia (85,000 t), the United States (59,000 t) and Cuba (56,500 t) (Bernhardt & Reilly, 2019). The ready availability of zeolite-rich rock at low cost along with the shortage of competing minerals and rocks are probably the most important factors for its large-scale use. Zeolite can also be fabricated or modified in order to improve its characteristics. Indeed, Koshy & Singh, (2016) presented a review with the applications to water treatment of zeolite made from fly ash. In this sense, the availability of those kinds of wastes (fly ash) can also be an advantage to get zeolite with better properties for different applications.

The use of zeolites for different applications in industrial (Collins et al., 2020), waste to energy processes (Nizami et al., 2016) and water and wastewater treatments (Reeve & Fallowfield, 2018) has been reviewed. Furthermore, the effect of zeolite on anaerobic digestion also has been reviewed (Montalvo et al., 2012; Romero-Guiza et al., 2016). Nevertheless, a wider review involving not only the biological anaerobic digestion but also other biological processes such as nitrification, denitrification, Anammox and composting has not been proposed. Zeolite properties (ion exchange capacity, big superficial area, porosity) have been also exploited in different biological processes in order to improve different bioreactor configurations or decreasing inhibition effects.

Thus, the present work has the objective of review the most relevant uses and applications of zeolites in biological processes for waste treatments, including a brief analysis of the main mechanisms proposed for explaining the improvement in each type of process.

2.- Zeolite utilization in anaerobic digestion processes

Montalvo et al. (2012) published a comprehensive literature review containing the most relevant uses and applications of zeolites in anaerobic digestion processes. They presented results related to the utilization of zeolites for the immobilization of microorganisms in different kinds of high-rate reactor configurations. Because the effect of zeolite on anaerobic digestion was reviewed by Montalvo et al. (2012), the present review will focus only in papers published after 2012.

The majority of the applications of zeolite are related to wastes rich in ammonia nitrogen, as it is shown in Table 1S in supplementary data. It is known that zeolite works as ionic exchanger of NH_4^+, situation that to allow the decreasing of ammonia nitrogen that inhibit the anaerobic digestion process (Montalvo et al., 2012). Indeed, some works had used modified zeolites in order to increase the removal of ammonia nitrogen through ion exchange (Lin et al., 2013) and improve the anaerobic process of wastes rich in ammonia. Furthermore, the process has been studied in both, batch and continuous processes. In batch process, the main goal has been to determine the effect and range of zeolite concentrations on the process, while in continuous processes the goal has been to use the zeolite as porous-structure microbial carrier and ion-exchanger simultaneously. Table 1S also shows the wide range of improvement in the methane production. An increase as high as 1050% has been recently reported (Sudibyo et al., 2018), while the lowest improvement percentage was 4% (Dutta et al., 2014). In terms of COD removal, there was also a dispersion of results, although the enhancement range falls between 1 and 100%. In addition, the diameter of zeolite studied in anaerobic digestion normally was lower than 2 mm, with a couple of works that used a zeolite with higher diameters. Specifically, Wang et al., (2019a) used a diameter in the range of 8.0-10.0 mm in a batch system for treating manure.

In batch systems, several works have been reported, but only the most relevant ones will be analyzed in this section. Nordell et al., (2013) using a mixture of slaughterhouse waste and industrial/food waste assessed the effect of zeolite on the long chain fatty acids (LCFA) inhibition. Interestingly, this study showed that the positive effect was not due to ammonium adsorption, cation exchange or colonization of the zeolite particles, but due to the adsorption of LCFA to the zeolite particles. Wang et al., (2015) also studied a co-digestion of *Phragmites australis*, feces and kitchen waste, indicated that clinoptilolite (one main kind of zeolite) improved both methane generation and COD reduction. In this case, the improving of anaerobic digestion was attributed not only to ammonia adsorption, but also to the adsorption of Ca^{+2} and Mg^{+2}, which allow enhancing the microbial utilization of these cations during the anaerobic co-digestion. The ion exchange capacity of zeolite to decrease the ammonia inhibition was also studied by Poirier et al. (2017), but focusing on the microbial communities. The results indicated clearly that 10 g/L of zeolite improved the methane production in presence of ammonia concentrations as high as 19 g/L, mainly because zeolites enhanced or preserved the growth of microbial populations that were either dominant under non-inhibiting conditions, specifically *Methanosarcina* and *Methanobacteriums*. Later, Poirier et al., (2018) studied the use of different support media (activated carbon (AC) and zeolite) on microbial community dynamics during anaerobic digestion of biowaste in presence of 1.3 g/L of phenol. The zeolite allowed a decrease of lag-phase in comparison to the system without carriers and enhanced the implantation of Operational Taxonomic Units (OTUs) assigned to bacterial phylum *Cloacimonetes*. Nevertheless, the AC had better performances in comparison to zeolite. An interesting study about the use of zeolite for the reduction of antibiotic resistance genes (ARGs) during mesophilic (mAD) and thermophilic digestion (tAD) of swine manure was carried out by Zhang et al., (2018). This work founded that zeolite can effectively reduce the ARGs based in the possibility that zeolite could have passivated heavy metals, which availed the ARGs reduction. As can be seen, in batch systems the main mechanism of improvement when the zeolite is added is ion-exchange but not only of ammonia, but also of cations and long chain fatty acids.

In the case of continuous processes, several types of reactors were studied, from classical fixed-bed reactor to expanded granular sludge bed (EGSB). Mustafa et al., (2014) studied the anaerobic digestion of primary sludge (PS) and thickened waste activated sludge

(TWAS) using an anaerobic fluidized bed bioreactor (AnFBR) employing zeolite particles as microorganisms carrier. Even though this work did not compare the AnFBR with other kinds of carrier, Mustafa et al. (2014) indicated that this technology is cheaper than conventional anaerobic digester, at least at laboratory scale. Andalib et al., (2014) used also an AnFBR for treating thin stillage (a by-product from the corn ethanol industry) as well as primary sludge from municipal wastewater treatment. They found that the AnFBR using zeolite achieved 88% COD removal efficiency at an OLR as high as 29 kg COD/m^3 d, indicating its superiority over conventional suspended-growth anaerobic technologies for readily biodegradable high SS wastes. Zheng et al., (2015) using a fixed-bed reactor constructed with zeolite that was fixed in three different kinds of polymer materials including chlorinated polyethylene (CPE), polymer foaming sponge (PFS) and porous nylon (PN), treated ammonium-rich swine wastes with ammonia concentrations as high as 7.51 g/L. These researchers found that synergy of ammonia adsorption and microorganism's immobilization by CPE fixed zeolite system contributed to the enhanced anaerobic digestion efficiency up to 350%. Zhang et al., (2016a) used a fixed bed reactor with zeolite as carrier combined with intermittent illumination in order to improve the anaerobic digestion of substrate rich in ammonia. The result showed that the illuminated fixed-bed bioreactor presented the greatest methane concentration (70%), methane yield and quantity of methanogens comparing with no-bed bioreactor. The illumination in the bioreactor allowed a higher biomass growth in comparison to a dark bioreactor, situation that improved the anaerobic digestion. The study of hydrodynamic in an anaerobic expanded granular sludge bed reactor (EGSB) using natural zeolite as biomass carrier was presented by Perez-Perez et al., (2017). The authors found that the real hydraulic retention time (HRTr) was increased with both increased zeolite bed height and increased upflow velocity. The short-circuit results for 5 cm of zeolite bed and 6, 8 and 10 m/h upflow velocity were 0.3, 0.24 and 0.19 respectively, demonstrating the feasibility of using zeolite for a proper hydrodynamic environment to operate the EGSB reactor. Perez-Perez et al., (2018) studied the treatment of synthetic swine wastewater using an expanded granular sludge bed (EGSB) reactor modified with zeolite. This study founded that at an OLR higher than 30 kg COD/m^3 d, the COD removal efficiency in the presence of zeolite was 78% with significant differences in comparison with the control reactor, with the archaeal community being more sensitive to the organic load increases than the bacterial community. Furthermore, the kinetics of the process in presence of zeolite was also faster than in the system without zeolite. Recently, Chen et al., (2019b) using an anaerobic fluidized-bed bioreactor (AFBR) connected in series with an anaerobic fluidized membrane bioreactor (AFMBR) with natural zeolites as carriers, studied the biotreatment of a campus domestic wastewater. The results showed that the AFBR-AFMBR system operated adequately with a short HRT at ambient temperature removing both COD and SS efficiently to the levels lower than the discharging limits.

Regarding the modeling of the effect of zeolite on methane production in batch systems, the modified Gompertz model or similar models were evaluated (Huilinir et al., 2014; Li et al., 2019; Li et al., 2017; Poirier et al., 2018; Poirier et al., 2017; Wang et al., 2015; Wijesinghe et al., 2018; Wijesinghe et al., 2019; Zheng et al., 2017). In these models, the parameters that increased when zeolite is present were the potential methane production and the specific biogas production rate. Kinetic analysis of COD removal have been also proposed (Andalib et al., 2014; Chen et al., 2019b; Mustafa et al., 2014; Nikolaeva et al., 2013). In these models, the parameter that is positively affected by zeolites is the degradation rate, specifically the first-order kinetic constant. Few works have taken into account and simultaneously modeled the methane generation and COD removal (Purnomo et al., 2017; Sudibyo et al., 2018). There are also studies applying response surface methodology in order to optimize the zeolite doses on the anaerobic digester (Jimenez et al., 2015; Liu et al., 2015). Nevertheless, these types of models were only applied in the range of zeolite doses and wastes studied and it cannot be applied to other conditions. In this sense, none models have attempt to describe the effect of zeolite mechanistically and trying to simulate both gas and liquid phases.

As can be seen, zeolite can help in the majority of the cases to a better development and performance of anaerobic digestion. The main mechanism exploited for this improvement is the ion-exchange capacity of zeolite in order to reduce mainly ammonia from the liquid phase. Furthermore, the high porosity of zeolite can also improve the development of biofilms that also improves the performance of anaerobic bioreactors.

3.- Zeolite utilization in aerobic processes for organic removal from wastewaters

The use of zeolites in biological wastewater treatments has been also extended to aerobic processes. In this case, zeolite is used to help to improve the nitrogen removal and the settling properties of sludge, as well as the phosphorous removal through P-accumulating bacteria immobilization. Indeed, the first application of zeolite in aerobic processes was assessed by Carrondo et al., (1980), evaluating the effect of zeolite-A on the performance of an activated sludge. They found that zeolite-A allows increasing the total volatile suspended solids in the aeration tank and an increase in the ability of the sludge to settle, favoring the further dewatering of sewage sludge. A mechanism for understanding the better settleablity in presence of zeolite was proposed by Wei et al. (2013), who indicated that the powder zeolite with its high porosity and large specific surface area provided a stable environment for the growth of attached bacteria, which could replace the three dimensional structures built up by filamentous bacteria. Subsequently, the microbial aggregates would be finally shaped by hydrodynamic shear force to stabilize a certain structured community. Thus, the zeolite decreases the risk of filamentous bulking and shortens the formation time of aerobic granular sludge effectively.

After a decade from the first study of zeolite application to activated sludge, Suh et al., (1994) proposed the immobilization of *Pseudomonas fluorescens* on zeolite and applied to a modified activated sludge-type reactor to mimic the actual activated sludge system of a coke-plant wastewater treatment system. This work demonstrated that the rate of cyanide degradation was enhanced four times when cells were immobilized on zeolite. However, the researchers reported problems on the continuous process caused by cell lysis; this point must also be considered in order to compare the operational cost and efficiency with conventional systems. Lee et al., (2001) studied the addition of alum and zeolite in a submerged membrane bioreactor (MBR) not only to reduce membrane fouling but also to increase the removal of nitrogen and phosphorus. According to these authors, the addition of natural zeolite greatly enhanced the membrane permeability due to the formation of rigid floc that had lower specific resistance than that of the control activated sludge floc. The rigid structure of the zeolite added to the sludge might reduce the membrane fouling by forming a less compressible cake layer. It was supported by the fact that the specific resistance of sludge when zeolite particles were added was reduced to one fourth than that of control activated sludge in this report. Furthermore, the nitrification efficiency was over 95% even at N-shock loading due to the ion-exchange capacity of zeolite. Finally, the authors indicated that zeolite had a much more positive effect on the MBR in comparison to the alum. Hrenovic et al., (2003) studied the phosphorous removal in an aerobic process and the effect of different zeolite concentrations on the process. This work obtained higher phosphorus removal efficiency when zeolites were added to the system, indicating that the addition of 5 g/L of zeolites in the aerobic phase improved phosphorus removal in systems both with synthetic wastewater and fresh municipal wastewater. The explanation for the increasing of P removal in presence of zeolite was the larger surface area of zeolite particles available for bio-sorption with P-accumulating bacteria and not the adsorption of P on zeolite. In addition, in the reactors with zeolite addition the COD, ammonium and nitrate values decreased significantly when compared to the control. Finally, the zeolite improved the activated sludge settling values, without changes in the pH values of the medium. Damayanti et al., (2011) studied three biofouling reducers (BFRs) in hybrid membrane bioreactors (MBR) treating palm oil mill effluent (POME), namely powdered active carbon (PAC), zeolite (Ze), and *Moringa oleifera* (Mo). These authors indicated that PAC obtained the best results as BFR, although zeolite also had very good characteristic for using as BFR, situation that agreed with the study of Lee et al., (2001). This result was also obtained by (Yuniarto et al., 2013), which compared the PAC and zeolite for treatment of diluted palm oil mill effluent (POME). The positive effect of zeolite powder on MBR was also reported by Gkotsis & Zouboulis, (2019) who used synthetic wastewater that simulated a municipal wastewater. This study demonstrated that the addition of zeolite contributed mainly to the alleviation of irreversible fouling, leaving the reversible fouling practically unaffected, while the addition of bio-carriers had the opposite effect. Hrenovic et al., (2011) proposed the immobilization of phosphate (P)-accumulating bacteria *Acinetobacter junii* onto natural zeolitized tuff (NZ) in order to improve the P removal. This work demonstrated that the immobilization of P-accumulating bacteria A. *junii* onto NZ can be performed in raw, non-sterile wastewater which passed through the primary treatment. The use of zeolite in this case allows a good micro-environment for the development of this kind of bacteria, improving the level of pure microorganism survival. Tarjanyi-Szikora et al., (2013) studied three types of biomass carriers in an activated sludge for municipal wastewater treatment. This study showed that although the zeolite had good characteristic for absorption of methylene blue and a very good performance for phosphorous removal, its presence in a batch system treating municipal wastewater was not good enough for COD and nitrogen removal in comparison to a normal activated sludge. Ivankovic et al., (2013) studied how a bioparticle (particle comprising natural zeolitized tuff with a developed biofilm of the phosphate-accumulating bacterial species, *Acinetobacter junii*, on the surface) can resist extreme environmental conditions, such as pH fluctuations, high concentrations of toxicants or grazing of protozoa. This study demonstrated that the bioparticles are able to resist extremes of pH, disinfectant (BAC) and the grazing of protozoa. It is important to note that this study was performed only in a batch system and using 24 h of exposition to the extreme conditions. It is still necessary to get information in continuous process. Andraka et al., (2017) and Ospanov et al., (2016) proposed the use of a kind of filter inside of an aerobic tank for improving the performance of the activated sludge. These researchers indicated than there was higher efficiency in the hybrid installation (with biofilm attached to natural zeolite carrier), especially in removal of nitrogen compounds, than in the standard installation with activated sludge. Finally, Wang et al., (2019b) proposed the use of zeolite, polyelectrolyte and Fe(II) Persulfate Oxidation in order to improve the dewaterability of sludge. This study demonstrated that the combination of $Fe(II)/S_2O_8^{2-}$ and zeolites had a significant effect on enhancing WAS dewaterability. According to this study, the presence of zeolites provided channels/cavities for water escaping by forming a layer of the porous medium composed of zeolites between filtration cloth and WAS flocs, thus promoting the dewaterability. Recently, Wang et al., (2019c) used zeolite in order to improve the microalgal-bacterial system for producing biomass that is fed with wastewater coming from hydrothermal liquefaction (HTLWW) in a membrane photobioreactor (MPBR). This type of wastewater presents a high toxicity and instability, reducing biomass accumulation and further limit biocrude oil production. In this study it was demonstrated that zeolites act as adsorbents relieving the NH_4^+ concentration and provide favorite habitats to form biofilms, which promote the tolerance of microorganisms to the high HTLWW concentrations and increased the biomass production.

According to this review, zeolite improves the nitrogen removal in aerobic systems, the settling properties of sludge and the retention of biomass capable of phosphorous removal. The positive changes on settling sludge properties were also used for improving the performance of membrane bioreactors, in order to decrease the fouling of the membrane.

4.- Zeolite utilization in nitrogen removal processes

In general, biological nitrogen removal processes consist in the transformation of different nitrogen species into gaseous nitrogen that is discharged to the atmosphere without major environmental risk (Gong et al., 2012). This transformation can be done in several ways, being the classic process the nitrification-denitrification in series. In nitrification process, the nitrogen as ammonium is oxidized by aerobic autotrophic bacteria called ammonia oxidizing bacteria (AOB) to nitrite. Then, another type of autotrophic bacteria called nitrite oxidizing bacteria (NOB) oxidizes nitrite to nitrate. Both processes are aerobic, being the aeration the main problem in terms of process cost. Due to nitrate and nitrite are both toxics, they must be eliminated from water. Biological denitrification can do that through the reduction of nitrate to nitrite and then to N_2, which is an innocuous compound. This process required an electron donor which could be organic matter for heterotrophic denitrification or inorganic compounds such as sulfide, thiosulfate, Fe or gas H_2 for autotrophic denitrification. There is another process, called Partial Nitrification, where ammonium is oxidized partially to NO_2^-, in order to reduce the oxygen consumption in the aerobic process and organic matter (or inorganic matter) in denitrification. During the last 20 years, a new process called ANAMMOX (Anaerobic Ammonium Oxidation) has been installed as a good alternative for saving energy. Anammox process together to partial nitrification have been developed not only at lab and pilot scale, but also at industrial scale (Grismer & Collison, 2017). All these processes have tested zeolite as improver of biological activity, either as carrier or ionic exchange, achieving good results.

4.1.- Nitrification, Partial nitrification and ANAMMOX processes

In nitrification process, zeolite has been used as ion exchange material, due to its ability to remove ammonium ions from wastewater, together to its capacity as biofilm carrier. In this sense, it has been proposed that nitrifying biomass can regenerate the zeolite capacity of ion exchange. This mechanism called "bioregeneration" was originally proposed by Semmens & Goodrich (1977) and later developed by several researchers (Jung et al., 2004; Park et al., 2002; Park et al., 2003b). According to this mechanism, the microorganisms are attached on the surface of zeolite or are entrapped in the powdered zeolite particles forming a zeolite-floc. This zeolite-floc adsorbed ammonium nitrogen during an anoxic fill-phase. At the same time, denitrification can occur if electron donors are present. In the aerobic step, the adsorbed ammonium nitrogen is released into the liquid phase due to the chemical equilibrium, and transformed to nitrite and nitrate by AOB and NOB. Because of these two processes (release of ammonium and biological reaction), the zeolite is completely regenerated. This regeneration using biological nitrification is called "bioregeneration" (Aponte-Morales et al., 2018; Jung et al., 2004). The same principle was applied later by Aponte-Morales et al., (2018) using chabazite. A scheme of this proposed mechanism is shown in Figure 1.

During the last 20 years, several works about the effect of zeolite on nitrifiying, simultaneous nitrification-denitrification or Anammox have been performed. In general, the application of zeolite improved the nitrification and Anammox processes, with increases of ammonia removal between 6% and 1100% (See Table 2S in supplementary data).

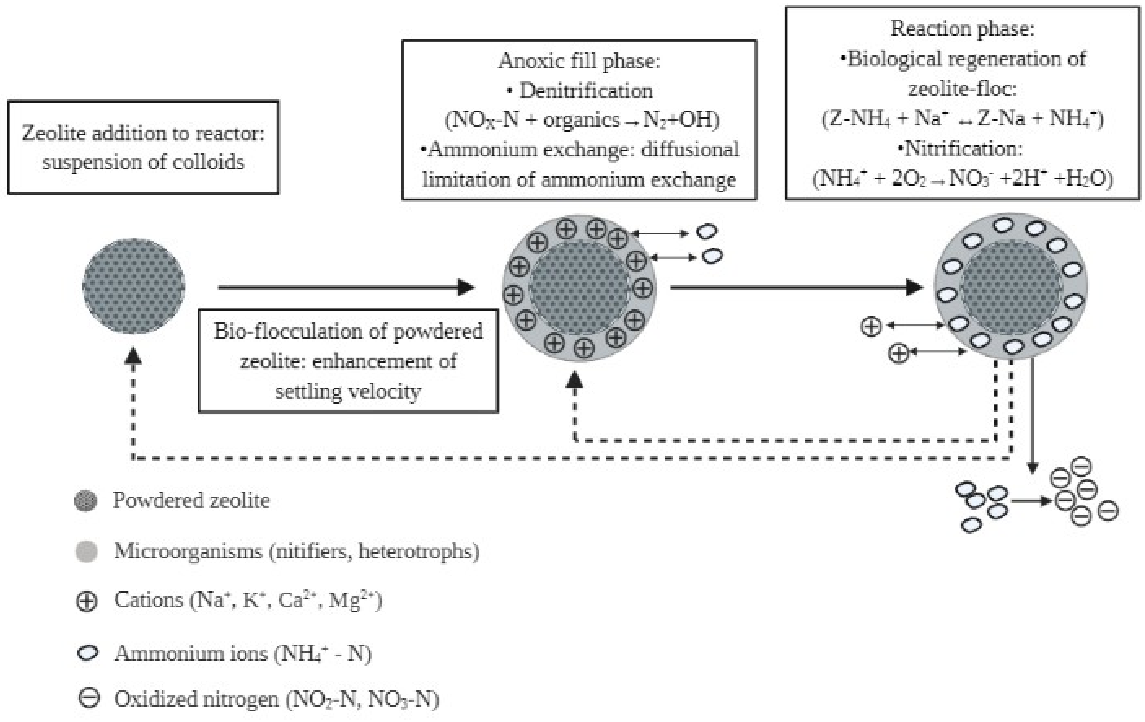

Figure 1: Mechanism proposed as "bioregeneration" of zeolite in nitrifying bioreactors. Scheme modified from Jung et al. (2004).

The first publication about the effect of zeolite on nitrification was presented by Nishimura et al., (1996), who proposed a system with fluidized-bed reactors in series using in the first one activated carbon (BAC) as biofilm carrier and natural zeolite as biofilm carrier in the second one (BZ). The BAC removed the organic matter from the wastewater, while BZ removed ammonia. This study indicated that ammonia oxidation increased linearly with the ammonia load rate up to values of 4 mg N/g zeolite d, value considered better than that achieved with other supports used until this date. Yang (1997) also studied a fluidized bed reactor with natural zeolite in order to clarify if other processes such as stripping or ion exchange can also be involved in nitrifying bioreactors. They demonstrated that concentrations of ammonium lower than 20 mg N/L are removed mainly by air stripping, while concentrations superior to 50 mg N/L promoted both ion exchange and biological nitrification. Nevertheless, according to this study the air stripping is always present in fluidized systems. Jung et al., (1999) used powdered natural zeolite in SBR in order to determine its characteristics as improver of the nitrification. According to their results, the zeolite is able to entrap quickly into the microbial floc within 3 days of operation in the sequencing batch reactors. Furthermore, the settling velocity of the bio-flocculated zeolite was 2-3 times higher than that reported for the system without zeolite. However, this study did not report an improving on the nitrification rate or efficiency when zeolite was added in the bioreactor, situation attributed to the exchanged ammonium that might be released into the liquid phase where ammonium was oxidized by nitrifying biomass. Later, Pak et al., (2002) using a fixed-bed reactor, indicated that biofilter with zeolite has a better performance that the control bioreactor that used activated carbon as media. They showed that *Nitrobacter*, a kind of NOB biomass, growth much more than in the control reactor, indicating that zeolite is a very good medium for developing NOB. Park et al., (2002) evaluated the effect of zeolite (clinoptilolite) on nitrification in an activated sludge. They determined that the use of zeolite improved the nitrification rate when the C/N ratio increased in comparison to a classic activated sludge. Furthermore, even though the formation of biofilm on zeolite surface, they observed that the ion exchange capacity of zeolite did not decrease, being it able to adsorb the ammonia. This situation, together to the increase of biomass concentration in bioreactor with zeolite, could explain the better performance of the system when higher organic matter concentrations were tested. Also, using a SBR, He et al., (2007b) showed several advantages of the use of zeolite in this type of reactors. Additionally to the higher ammonia removal in the reactor with zeolite, these authors indicated that the reactor with zeolite could remove also phosphorous and organic matter faster than the control reactor, improving also the settling property that can inhabit the bulking. Similar results were also reported by Wei et al., (2010) and Jung et al., (2004). He et al., (2007a) using now a fixed-bed reactor demonstrated that the biofilter with zeolite has a stronger adaptability to NH_3-N shock load and low temperature compared to expanded clay biofilter. Studying also the biomass in the biofilm, these authors claimed that the amounts of heterobacteria and nitrobacteria in two biofilters indicated that a more favorable environment for nitrifying bacteria was provided in the biofilter with natural zeolite due to its ion exchange capacity. Similar conclusions were reported by Chang et al., (2009), who studied the textile wastewater treatment using a biological aerated filter with zeolite as media. Qiu et al., (2010) studied three biomass carriers (ceramic, zeolite and carbonate media) for nitrification in fixed-bed reactors. It was determined that the fixed-bed reactor with zeolite had the best nitrification performance with also a smaller bed volume necessary for simultaneously COD, SS and nitrogen removal. Mery et al., (2012) studied in batch reactors the influence of zeolite particle sizes on microorganism adherence to zeolite and the identification of microbial populations. They concluded that a particle size of 1.0 mm is the most suitable for using in fixed or fluidized beds, due to the highest adherence of microorganisms and adsorption equilibrium. The microbial populations of the nitrifying reactors in this work were very similar for all the sizes studied, being the dominant microbial population the Gammaproteobacteria, making up 80% of the total communities found. Betaproteobacteria, making up 12% approx. of the total, were also identified. Li et al., (2013) studied the partial nitrification in fixed bed reactors using natural and modified zeolite. The results showed that modified zeolite should be more beneficial to the specific immobilization of AOB than natural zeolite. Furthermore, this work indicated that the community structure of AOB shifted during partial nitrification and immobilization, but *Nitrosomonas*-like group remained predominant. Rezaei & Mehrnia, (2014) investigated the effect of zeolite (clinoptilolite) on the performance of a membrane bioreactor (MBR). The main idea of adding zeolite to a MBR was to improve the sludge characteristics in terms of settleability and thereby reduce membrane fouling. This work indicated that the presence of zeolite in the system results in particle size growth, soluble microbial product reduction and SVI elevation which have a direct role in membrane fouling and inhibit transmembrane pressure (TMP) extension when flux is increased. Therefore, the zeolite not only slows down the developing trend of TMP, but also the permeation in MBR with zeolite was always higher than in control MBR and the fouling rate of MBR with zeolite was always lower than control MBR at all fluxes. Thus, the presence of clinoptilolite provides the possibility of using MBRs at higher fluxes and develops the functional range of MBRs. Guerrero et al., (2016) using a SBR with step-feed strategy, studied the effect of zeolite on the nitrification-denitrification process. This work demonstrated that the incorporation of zeolite in the system helded higher concentration of biomass in the reactor, reducing the start-up to 21 days and improving 11.31 % removal kinetics. Tao et al., (2016) compared the performance of two upflow fixed bed reactors, one packed with natural zeolite and the other one with ceramic. This work showed that, in this case, the zeolite did not improve the biological nitrogen removal, being the biofilter with ceramic which had a much better performance. The explanation should be related to the low performance of biological nitrification, situation that did not increase the nitrite and nitrate concentrations preventing the denitrification. A similar situation was reported by Zhang et al., (2016b), who compared three different carriers in a natural ventilation trickling filters. This work indicated that zeolite and ceramic could have problems with mass transfer from the bulk liquid, because the large biomass can restrain mass transfer of nutrients and oxygen, and then can decrease the biofilm activity. It is important to note that these two works used systems with low oxygen concentrations. This fact could have a stronger effect on biofilm formation on zeolite in comparison to other types of carriers. Nevertheless, Cheng et al., (2018) could probe that zeolite can be used in systems with passive aeration, at least at lab scale. However, this design generates a biofilm potentially artificially enriched in ammonium oxidizing bacteria (AOB) which cannot be reproduced in large scale operation, being necessary further studies in order to determine if this methodology could be used. Yang et al., (2017) studied the partial nitrification in fixed-bed reactors with zeolite (ZBAF) as carriers. This research indicated that ZBAF was feasible for stable partial nitrification and excellent nitrite accumulation for ammonium wastewater based on free ammonia inhibition, which indicated that ZBAF was highly potential in the treatment of ammonium wastewater. Aponte-Morales et al., (2018) studied the use of chabazite, a type of natural zeolite, in nitrification of centrate from anaerobic digestion of swine waste. This work also proposed a model in order to take into account several processes for explaining why zeolite improved the nitrification. The model proposed included ion-exchange of ammonia, and sodium at the chabazite surface, surface diffusion of adsorbed NH_4^+ within the chabazite grains, sequential nitrification of aqueous NH_4^+ to nitrite and nitrate and inhibition of nitritation and nitratation rates by NH_4^+. It is important to note that this work was the first in proposing a mathematical model that included the bioregeneration of zeolite in the biological nitrification process. Furthermore, they determined that a chabazite dose of 150 g/L decreased the FA concentration below the inhibitory level and increased the nitrification rate from 0.16 to 0.36 mg N/g VSS h. Chen et al., (2018) studied the partial nitrification in a fixed-bed reactor with zeolite as carrier followed by a SBR for denitrification. This work demonstrated that it was possible to have a stable partial nitrification in the reactor with zeolite, due to the FA inhibition based on ammonium adsorption and desorption equilibrium might be the main factor for nitrite accumulation. Huilinir et al., (2018) used zeolite in order to improve the resistance of nitrifying biomass to two inhibitors: organic matter and sulfide. Using batch system, this study showed that zeolite allowed a better performance of nitrification in presence of organic matter and sulfide, with ammonia removal rate and nitrate production rate increases of 51% and 38%, respectively, in relation to the control system, with the best results achieved at a zeolite concentration of 15 g/L. Interestingly, the improvement of nitrification in this case cannot be related to biofilm formation on zeolite, because the zeolite was added at the beginning of each assay. Then, zeolite could also work as "catalyst" of nitrification process, beyond its use as biofilm carrier. Chen et al., (2019c) studied the influence of salinity (NaCl) on partial nitrification in a zeolite biological aerated filter (ZBAF), showing that NaCl affected negatively both ammonium oxidation and nitrite accumulation. In this case, the zeolite allowed that ZBAF didn't crash during the whole operational periods, reflecting that ZBAF was considerably potential for ammonium-rich saline wastewater treatment. Feng et al., (2019) proposed the partial nitrification-Annamox process for high-strength ammonium iron oxide red wastewater. In this case, zeolite was used as carrier in two-stage ZBAF, which could achieve the partial nitrification of high-strength ammonium with Na_2CO_3 as alkalinity donor and saved about 40% of the alkalinity cost relative to the common $NaHCO_3$. Furthermore, high throughput sequencing-based approaches showed that *Nitrosomoadaceae* (AOB) was dominance in two-stage ZBAF, without detecting NOB presence in these bioreactors. Huiliñir et al., (2020) proposed the use of natural zeolite as biofilm carrier in sequencing moving bed biofilm reactor in order to study the nitrification process in presence of organic matter and sulfide. This study showed that the system with zeolite improved the performance of a nitrifying system, keeping the efficiency of TAN and COD removals higher than 90% for both inhibitory conditions. In addition, the presence of zeolite allowed the development of a robust biofilm, with diameters between 400 to 600 μm, situation that also improved the settling properties of the biomass.

The use of zeolite for improving the Annamox process has also been studied. Grismer & Collison, (2017) published a literature review about zeolite utilization in Anammox processes. According to this review, single-reactor zeolite-Anammox systems successfully remove nitrogen at a wide temperature range and varying wastewater strength. On the other hand, as a fixed-media bed system, the zeolite-anammox reactors could be affected by pore clogging and a pre-treatment for solid removal must be previously applied. Earlier, Yapsakli et al., (2017) proposed a biofilter with zeolite as carrier in order to study the Anammox process. This work indicated that the high efficiencies obtained even at NO_2^- N:NH_4^+ ratio far from stoichiometry could be feasible because the sorption of surplus NH_4-N by zeolite particles in case that ammonium rich influent came in excess with respect to Anammox stoichiometry. Similarly, when ammonium-poor influent is fed to the reactor, ammonium desorption took place due to shifts in ion-exchange equilibrium and deficient amount were supplied by previously adsorbed NH_4-N. Thus, zeolite system acts as a buffer system to generate a stable effluent of nitrogen.

Zeolite has also been used in wetlands, in order to improve or increase the nitrifying biomass activity and the ammonium removal through ion-exchange. Wen et al., (2012) used zeolite for reducing the seasonal fluctuation and enhancing the efficiency of nitrogen removal in vertical flow-horizontal subsurface flow (VFHSF) constructed wetlands. This work demonstrated that the use of zeolite enhanced the nitrogen removal in a 50% in comparison to a VFHSF without zeolite, and also reported a bioregeneration of 91% of zeolite, due to ammonia adsorbed by zeolite during the cold seasons was desorbed, and then nitrified in warm seasons. Chen et al., (2017) investigated nitrogen removal and its relationship with the nitrogen-cycle genes and microorganisms in the horizontal subsurface flow constructed wetlands. They used four substrates (oyster shell, zeolite, medical stone and ceramic) in order to compare them. As results, the study determined that the constructed wetland with zeolite as substrate was the best for nitrogen removal. Han et al., (2019a) studied a pilot-scale zoning biozeolite-based tidal flow constructed wetland (TFCW) to treat anaerobically digested dispersed swine wastewater. The system could remove COD, NH_4^+-N and TN by 73.79%, 72.99% and 70.71%, respectively, even at low temperatures (16 °C). A theory of dynamic process of rapid-adsorption and bioregeneration in biozeolite for NH_4^+-N removal was proposed. Nitrifiers (*Nitrosospira*, *Rhizomicrobium*, etc.), denitrifiers (*Ottowia*, *Thauera*, *Rhodanobacteria*, etc.) and organic matter degraders (*Saccharibacteria*, etc.) dominated in bio-zeolite layer (ZL), which demonstrated occurrence of simultaneous nitrification and denitrification and explained why most of COD, NH_4^+-N and TN were removed in the zeolite layer. Later, Han et al., (2019b) also studied a CW partially saturated for treat high-strength ammonium wastewater using zeolite as substrate. This study also showed that, due to the "rapid-adsorption bioregeneration" process that could reach dynamically stable, the biozeolite-based CW could achieve persistent nitrogen removal on treating decentralized swine wastewater. Because of sites of zeolites were not all occupied, the CW could operate with the fluctuant influent water quality and under low temperatures to some extent.

All these studies showed that, in general, the zeolite improved the performance of nitrification, partial nitrification and Anammox processes. Depending of the type of bioreactor, the higher ammonia removal has been explained by ion-exchange capacity and biofilm formation on zeolite. Furthermore, the zeolite could also improve the settling properties of biomass, situation that was also exploited in the case of MBR for decreasing the fouling of membrane.

4.2.- Denitrification process

Denitrification process has been also studied using zeolite as improver. For this process, zeolite has been used mainly as biocarrier in order to improve the biomass growth and settling properties. The application of zeolite on denitrification has been scarcely reported and focused mainly in heterotrophic process (see supplementary data, Table 3S).

Nevertheless, in most of these works, the zeolite has exerted a positive effect on the process.

Park et al., (2003a) studied the effect of zeolite addition in an activated sludge on its enhanced denitrification capacity. This work reported that for heterotrophic denitrification in continuous flow process, the system with zeolite had a 39% higher removal than that achieved in the system without zeolite, even at lower COD/NO_3^--N ratio. The improvement was attributed to the effect of zeolite on biomass growth. Almost a decade further, Foglar & Gasparac, (2013) also used natural zeolite as biomass carrier for heterotrophic denitrification of surface water (SW). In this case, it was reported that the reactor with zeolite could effectively reduce 100 mg NO_3^--N/L present in the SW, without nitrite accumulation. Furthermore, stable and effective nitrate and COD removals were obtained even with the use of raw surface water. Montalvo et al., (2014a) studied the effect of zeolite on the heterotrophic nitrate removal, focusing on the microbiology in the batch system and on the start-up in an UASB with zeolite. This work showed that there was a high microbial diversity with a strong presence of Gammaproteobacteria (70% of the total microorganisms) in reactors with zeolite 0.5 mm in diameter, while Archaea were only detected in the reactors with zeolite 1 mm in diameter. In the continuous process, nitrate removals higher than 92% were achieved when the reactor operated at high organic loading rates (44 kg COD/(m^3 d)) and low hydraulic retention time (2.5 h) in the system with zeolite. A higher biomass growth was always observed in the reactor with zeolite. Montalvo et al., (2016a) proposed the use of zeolite on the autotrophic denitrification with sulfide as electron donor. In this case, the zeolite improved the start-up of the batch and continuous reactor (UASB), decreasing up to 50% the time required to achieve stabilization of the process. Nevertheless, there was not a clear advantage of zeolite on nitrogen removal. Hossain et al., (2019) proposed a passively aerated simultaneous nitrification and denitrification (PASND) performing biofilm to treat concentrated wastewater using zeolite as biomass carrier. This study demonstrated that the treatment of high-strength wastewater in the zeolite amended PASND biofilm reactor does not require a proportionally longer treatment time, being the anaerobic phase similar for different strength feed, while the aerobic bioregeneration times should be adjusted according to feed strength.

5.- Zeolite application in composting

In the case of solid wastes, zeolite has been used in order to improve the composting product. Unlike the wastewater treatment processes, the zeolite does not improve the kinetics of the process or the removal of organic matter, but improves the quality of the final compost. This is obtained through the retention of N in the compost by the zeolite and also retaining heavy metals from the composting. This last point is achieved through the ion-exchange between the zeolite and the solid while the composting process occurs.

The loss of ammonia, via NH_3 generation, in the composting process is a problem that not only reduces the agronomic value of the end-product, but also contributes to pollution of the environment. In this sense, there are several research works that showed an increase up to 50% in the retention of ammonium when natural zeolites are applied in the composting (Awasthi et al., 2016a; Awasthi et al., 2016b; Chan et al., 2016; Venglovsky et al., 2005; Wang & Zeng, 2018). The mechanism behind this improvement is the ion-exchange of the zeolite, situation that allows retaining the nitrogen in the solid phase. The first report about the use of zeolite on composting was done by Johnson et al., (1983), who proposed its use on composting of vegetables. Later, Witter & Lopezreal, (1988) proposed the use of zeolite for composting of sewage sludge-straw mixtures in pilot-scale composting trials and at laboratory scale. This earlier works demonstrated that zeolite was able to retain the nitrogen in the end-product and decreased the loss of ammonia to the atmosphere. At the beginning of XXI century, Zorpas et al., (2000a) studied the zeolite utilization as metal uptake in sludge with a high concentration of heavy metals, based on the capacity of natural zeolite clinoptilolite to uptake heavy metals like Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn. It was found that an important percentage of the metal not taken up by zeolites was associated with the inert residual fraction. Later, Zorpas et al., (2002) determined that heavy metals can be sufficiently removed by using 25% w/w of zeolite with particle sizes of 3.3-4.0 mm for sewage-sludge composting. The application of zeolite for co-composting of sewage sludge and food waste in order to retain heavy metals was also studied by Zorpas et al., (2000b), indicating that effectively zeolite can improve the compost quality. Koushafar et al., (2011) evaluated the effect of natural zeolites as an ingredient for the reduction in salinity and heavy metal concentration in compost. The addition of 5%, 10%, and 15% of zeolites resulted in 11.1%, 25.9% and 42.6% reduction in salinity levels, and also in an increase of zinc, iron, copper, and cadmium removal in the final product. Villasenor et al., (2011) studied the effect of zeolite addition on the composting of sludge-straw using a pilot-scale rotary drum reactor. In these experiments, the authors used three commercial natural zeolites: a mordenite and two clinoptilolites. All zeolites were capable of removing 100% of Ni, Cr, Pb, and more than 60% of Cu, Zn and Hg. Clinoptilolite was able to increase the removal efficiency of ammonia and heavy metals as its concentration increased in the compost. According to the results obtained, the authors claimed that the final compost could be applied directly to soil, or metal-polluted zeolites could be separated from the compost prior to application. Zorpas (2014) used natural zeolites (clinoptilolite) at a dose of 25% w/w in sewage sludge composting for several times in order to determine the recycling index and which mathematical model could describe this phenomenon. The final results showed that the same amount of natural zeolites could be used more than 22 times. Hence, the whole process could be more sustainable and feasible.

More recently, Alavi et al., (2017) evaluated the utilization of zeolites in a process of composting mixtures of vinasses, cow manure and chopped bagasse. The utilization of zeolites reduced the salinity of the final product and reduced the potassium concentration due to the adsorption of this element by zeolites. The use of zeolites also improved the quality of the final product for its use as fertilizer. Wang et al., (2017) compared the impact of biochar, zeolites and their mixture on nitrogen conservation and organic matter transformation during pig manure composting. The results indicated that adding biochar, zeolite and biochar plus zeolite it was improved the organic matter degradation and the nitrogen loss was reduced. A comparison of organic matter transformation, nitrogen conservation and compost quality indicated that the combined use of biochar and zeolites could be the most useful alternative for pig manure composting. The effect of enhancing the composting process with wood vinegar in a mixture of pig manure with wheat straw when biochar and zeolites were added was to determine the levels of greenhouse gases and ammonia emissions. The results obtained showed that the use of wood vinegar, biochar and zeolites reduced the thermophilic phase in the composting process and improved maturity compared with the control. In addition, this mixture reduced the losses in carbon and nitrogen and decreased the CO_2, CH_4 and N_2O emissions (Wang et al., 2018). The co-composting of municipal solid waste (MSW) with three different ratios of natural and Mg-modified zeolites (5%, 10%, and 15% on a wet weight basis) was studied by Taheri-Soudejani et al., (2019). They found that the use of Mg-zeolites is an environmentally friendly solution to prevent surface and groundwater pollution. The modified compost could be also used for the improvement of the 24 physicochemical properties of sandy loam soils. Zhou et al., (2019) studied the succession and metabolism functions of the bacterial communities in maize straw composting with earthworm casts and zeolite addition. The results showed that earthworm casts and zeolite addition increased temperature, decreased NH_4^+ concentration and affected the structure of the bacterial community. The amounts of *Firmicutes* and *Betaproteobacteria* increased with earthworm casts and zeolite addition at the final stage. Temperature showed a negative relationship with *Georgenia*, while NH_4^+ exhibited positive associations with *Georgenia, Devosia, Ruania* and *Mycobacterium*. Thus, earthworm casts and zeolite addition benefitted the basic species and enhanced the metabolism capacity of the bacterial community, thereby improving the quality of compost.

6.- Zeolite application at pilot and full scale in biological wastewater treatment processes

Technical results of application of zeolites at industrial scale in biological processes have been scarcely reported. In the case of anaerobic digestion, one of the questions to be considered for the scale-up of these results is the zeolite accumulation in the digester (which is the worst-case scenario). In this sense, Montalvo et al., (2006) indicated that, considering that all the zeolites added to the digester remained inside the digester, the annual volume of zeolites accumulated in the digester represents less than 1% of the volume of the digester. Nevertheless, application at industrial scale has not been reported up to now.

Applications have been reported in the case of nitrogen removal and mainly at pilot-scale. A pilot-scale system including activated sludge and bio-film attached to support media (natural zeolites) was performed in order to increase the efficiency of a municipal wastewater treatment. The pilot-scale plant was installed in the Almaty Wastewater Treatment Plant (Kazakhstan) and treated sewage after a preliminary mechanical pre-treatment. The results from the study showed a significant improvement in treatment efficiency for all examined parameters (BOD, COD, suspended solids, nitrogen compounds and phosphates) when zeolites were used (Andraka et al., 2017). A study using a pilot-plant activated sludge process of 72 m^3 volume with zeolite addition demonstrated that zeolites contributed to increase the contact-adsorption and the regeneration-stabilization process (Wu et al., 2008). Collison & Grismer, (2018) also reported the use of zeolites in an Anammox process at pilot-scale applied to a municipal wastewater treatment. In this case it was considered the application of the zeolite-Anammox process to nitrogen removal from the secondary effluent of a domestic wastewater treatment plant with final disposal to sensitive areas of the San Francisco Bay estuary. The authors claimed that the zeolite-Anammox treatment system seemed to combine the zeolite adsorption capacity to provide sites for an Anammox-nitrifier bacterial biofilm capable of effectively converting ammonium to nitrogen gas across a range of ammonium concentrations and ambient temperatures (15–30 ºC). They concluded that the zeolite-Anammox treatment system had advantages of lowered infrastructure costs, reduced energy consumption, and minimal maintenance and monitoring compared to traditional operations.

The use of zeolites at industrial scale is also available in the market through different companies that sell different bioprocesses that use the zeolite as amendment. The company Ecologix Environmental System (Ecologix Environmental System, 2018) has recently developed a plug-and-play Zeo-Clear system. The system eliminates the need for costly infrastructure, saves time and makes remote worksites, communities, camps and resorts operational, delivering an average reduction in energy costs of 20%. Collison Engineering (2019) is a company that has developed the Zeolite Anammox De-Ammonification Process. The zeolite-anammox process uses zeolites to remove ammonium by cation exchange (CEC) and also as biomass carrier. Since the bacteria assimilate or consume the NH_4^+ and regenerate the zeolites in-situ, they never become saturated. Zeolite Products (2014) is a company that produces zeolites for water and wastewater treatment with a commercial name (Zeo WT). Zeo WT is an ecological product, made exclusively from natural zeolites for use in the process of biological wastewater treatments. This company claims that a better separation of sludge and clean water, a decrease in sludge volume and a reduction in the sludge volume index are achieved when zeolites are used. In addition, a higher efficiency is also reached.

Thus, even though the use of zeolites at industrial scale has not been reported for anaerobic digestion, the use of zeolites at least at pilot scale has been reported for nitrogen removal with good results. These good results are the support for different companies in order to offer in the market different technologies that use zeolites in biological processes, which also reflects that the zeolite is a good option for nitrogen removal at industrial scale.

7.- Perspectives

According to this review, some subjects are still necessary to study in order to gain a better understanding of the effect of zeolite on biological processes of waste treatment. One aspect is the development of mathematical models that could include the effect of zeolite on biological processes, basically its use as improver of the settling properties or the kinetics of biological processes, in order to standardize and control these systems. Other aspect that should be explored is the use of zeolite in anaerobic digestion at pilot and industrial scale. Due to the evidence at lab scale of the advantage of use of zeolite in anaerobic digestion, is still necessary to explore the performance of bioreactors at least in pilot scale in order to obtain more information for possible scaling-up of the processes. The use of zeolite should also include the evaluation of a life cycle analysis of the processes, in which this material is involved.

8.- Conclusions

In this review, the use of zeolites in different biological processes was analyzed. In anaerobic digestion, nitrification, partial nitrification and Anammox processes, the use of zeolite improves the performance based on its capacity for retaining ammonia and for forming biofilms. Similar situation was observed in denitrification and phosphorous removal processes. In aerobic process for organic matter removal, the use of zeolite improves the ammonia removal, settling velocity and phosphorous removal, while in composting improves the retention of nitrogen in the product and the elimination of heavy metals through ion-exchange. The application to biological processes at full-scale is still scarce.

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