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THE ROLE OF MICROORGANISM IMMOBILIZATION IN THE BIOTECHNOLOGY OF NITROGEN COMPOUND REMOVAL

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In recent years, technologies employing immobilized microorganisms have demonstrated significant potential for improving wastewater treatment methods, offering substantial advantages.

Aim. To analyze current biotechnologies for nitrogen compound removal from wastewater and their modifications incorporating immobilized microorganisms, substantiating the necessity of implementing immobilization methods to enhance wastewater treatment technologies.

Methods. The study employed structural-logical and bibliosemantic analysis to examine the role of immobilization in improving nitrogen compound removal technologies. Publications from Web of Science, Scopus, PubMed, and Google Scholar databases were analyzed.

Results. Current approaches to nitrogen compound removal from wastewater were analyzed, including MLE, A2/O, UCT, and ANAMMOX technologies combined with immobilized microorganisms. It was established that immobilization enhances treatment efficiency through process stability, reduced energy consumption, and system compactness. Key factors requiring further investigation include the optimization of carriers, their materials, and application conditions to ensure maximum system performance.

Conclusions. Microorganism immobilization effectively enhances the stability, productivity, and energy efficiency of nitrogen compound removal technologies; however, further research is required to optimize carriers.

Key words: biotechnology, microorganisms, nitrogen compounds, immobilization, wastewater, nitrification, denitrification, microorganism carriers.

Nitrogen is one of the key elements regulating the biological activity of aquatic environments, with its primary sources in wastewater being proteins, organic compounds, and urea, which transform into ammonium through hydrolysis and ammonification [1]. However, exceeding permissible concentrations can lead to the eutrophication of natural water bodies, characterized by excessive growth of green algae and aquatic vegetation, decreased dissolved oxygen levels, and, consequently, the extinction of aquatic fauna [2]. Therefore, the removal of nitrogen compounds has been a significant focus in the science and practice of wastewater treatment for many years. To date, numerous technologies and methods for advanced removal of organic and inorganic nitrogen have been developed, including physical and physicochemical methods (ion exchange [3], adsorption [4], wave desorption [5], reverse osmosis [6], nanofiltration [7]), chemical methods (precipitation [8], oxidation (chlorination) [9]), and biotechnologies

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(nitrification-denitrification [10], ANAMMOX [11], partial nitrification [12], bioelectrochemical oxidation [13]).

Physical methods are effective in removing both organic substances and nitrogen compounds but are expensive and require the disposal of used materials [14]. Chemical methods offer ease of operation and high nitrogen removal efficiency; however, their significant drawbacks include labor intensity and the high cost of reagents, some of which require further disposal [15]. Biological methods are the most widespread due to their versatility, providing high efficiency for a wide range of wastewater volumes and compositions. However, their main disadvantages include energy dependency, sensitivity to process conditions, and the need for constant maintenance and process control [16, 17].

Among biological methods for advanced nitrogen removal, biotechnologies using immobilized microorganisms are particularly noteworthy. Immobilization significantly enhances the efficiency of biological treatment by increasing biomass concentration on carrier surfaces, improving process stability, especially under variable loads, and enabling the creation of compact treatment systems suitable even for small wastewater volumes (e.g., private homes and cottages) [18–20].

Additionally, they improve the efficiency of industrial wastewater treatment from organic substances and nitrogen compounds [21]. The efficiency of wastewater treatment technologies employing immobilization depends mainly on the type of carrier, as its configuration, structure, and material influence biofilm formation. These factors determine biofilm density, thickness, microbial distribution, and the presence of aerobic, anaerobic, and low-flow zones, which directly affect the intensity of nitrification and denitrification processes.

Nitrogen Removal Technologies

The majority of modern nitrogen compound removal technologies are based on the processes of nitrification and denitrification [22]. Denitrification is a multistage process of reducing nitrates to free nitrogen (N_2), carried out by heterotrophic bacteria such as *Bacillus*, *Pseudomonas*, and *Staphylococcus*, among others [23, 24]. This process requires anoxic conditions and the presence of an organic carbon source, which serves as an electron donor for the growth of heterotrophic bacteria, such as ethanol, methanol, acetate, or glucose [25]. In contrast, nitrification is a two-step process of oxidizing ammonium nitrogen to nitrates through nitrites, occurring under aerobic conditions. The primary representatives involved include *Nitrosomonas* and *Nitrosococcus*, capable of oxidizing ammonium, and *Nitrobacter* and *Nitrospira*, which oxidize nitrites [26].

Among the methods of wastewater treatment with advanced nitrogen compound removal, a number of technological approaches can be distinguished, among which the Ludzak-Ettinger process, the A2/O system, the Bardenpho technology, the UCT (University of Cape Town) process, and Anammox serve as the foundation for the development of most modern technologies. One of the earliest modifications was proposed by James Barnard with the adaptation of the classical Ludzack-Ettinger Process, also known as MLE (Modified Ludzack-Ettinger) or A/O (Anoxic-Oxic) [27]. Its most common configuration is shown in Fig. 1.

The technology includes two separate reactors — an anoxic reactor and an aerobic reactor. The key feature of this technology is the use of readily biodegradable organic matter from untreated wastewater as a carbon source for denitrifiers. Simultaneously, internal recirculation from the second reactor to the first ensures a sufficient supply of nitrates and nitrites in the anoxic zone due to

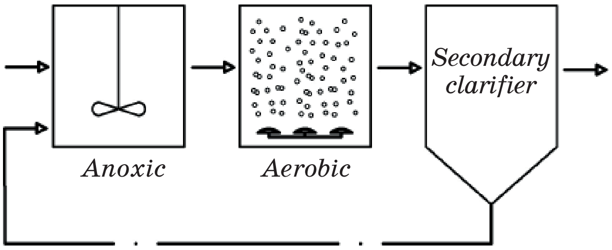

Fig. 1. **Modified Ludzack-Ettinger Process (MLE)**

the oxidation of ammonium compounds in the second reactor [28].

Over time, this technology has undergone numerous improvements and modifications, including the optimization of technological parameters [29–31] and the addition of technology blocks to address the issue of incomplete nitrate removal. These modifications include the addition of zones with intermittent aeration [32] or the reduction of excess sludge generation using OSA (Oxic-Settling-Anaerobic) technology [33].

The efficiency of removing both nitrogen compounds and organic matter can be enhanced by increasing the concentration of activated sludge microorganisms to 2000–4000 mg/L MLVSS, with a high nitrification rate of 6.5–12 mg/L/h, which is almost twice the rate observed for conventional activated sludge (4 mg/L/h). [34]. For instance, in a modified MLE reactor using immobilized microorganisms on carriers made of polyvinyl alcohol and polyethylene glycol, an ammonium nitrogen removal efficiency of 99% was achieved [35].

This modification of the technology was implemented in Ukraine in the project for upgrading the wastewater treatment plant in the city of Kolomyia, Ivano-Frankivsk region. The aeration tank was equipped with both anoxic and aerobic zones utilizing immobilized microorganisms on inert carriers to enhance the capacity of the treatment facilities, improve sludge sedimentation properties, and more [36].

The Anaerobic-Anoxic-Oxic Process (A2/O) is a modification of the MLE process proposed by Marshall Spector [37]. Its distinguishing feature is the inclusion of an additional unit — a preliminary anaerobic reactor.

Due to the additional zone, this treatment plant configuration enables the simultaneous implementation of denitrification and phosphorus removal processes [38]. However, today this technology is rarely developed in its original form. Instead, its modification — A2/O-MBR — has gained widespread adoption, incorporating anaerobic and anoxic reactors in combination with a membrane aerobic bioreactor [39]. The use of a reactor with immobilized microorganisms enables the removal efficiency of ammonium and total nitrogen to reach 99% and 82–89%, respectively, at initial concentrations of 70±10 mg/dm^3 and 80±10 mg/dm^3 and an MLSS concentration of 5600–6450 mg/dm^3. At the same time, phosphorus compound removal reaches 75% [40, 41].

Further improvements in the efficiency of the A2/O-MBR technology focus on optimizing operational parameters — such as the C/N ratio, oxygen levels, and recirculation rates [42] — as well as combining it with other treatment technologies [41]. For instance, integrating A2/O-MBR with Biological Aerated Filter (BAF) technology and ozonation not only effectively removes biogenic compounds but also reduces chromium and humic acid-like substances in wastewater [41].

This approach to modifying treatment technologies for enhanced nitrogen removal is becoming increasingly popular as a simple way to boost treatment efficiency by increasing the biomass of activated sludge microorganisms [43]. For example, the modified University of Cape Town (UCT) technology, similar to the A2/O process, includes an additional anaerobic reactor at the beginning, where recirculated activated sludge from the first anoxic zone is introduced (Fig. 3).

The first anaerobic reactor is designed for advanced denitrification, while the first anoxic reactor is dedicated to phosphorus removal without the inhibitory effects of

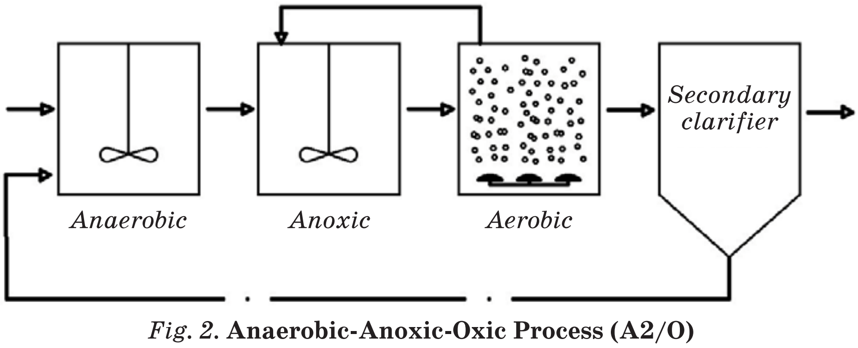

Fig. 2. **Anaerobic-Anoxic-Oxic Process (A2/O)**

nitrates. The process then continues as in the MLE technology [44].

Today, an alternative variant — UCT-MBR — has gained significant traction, featuring an additional MBR reactor instead of a secondary clarifier [45]. This modification allows for high treatment efficiency within just four months of operation (COD > 94%, total nitrogen 89–92%, total phosphorus 80–92%). Considering the fluctuation range of initial concentrations: COD = 160–910 mg/dm^3, total nitrogen = 24–123 mg/dm^3, total phosphorus = 1.3–7.4 mg/dm^3. [46].

Meanwhile, incorporating stationary carriers in the aerobic reactor of the UCT technology, alongside the MBR reactor, not only enhances the removal of organic and nitrogen compounds but also reduces N_2O emissions. However, this technology is more prone to membrane fouling [47].

Apart from the technologies based on nitrification-denitrification processes, an alternative approach involves technologies utilizing the ANAMMOX process (Anaerobic Ammonium Oxidation). The bacteria capable of performing this process, *Candidatus* "*Brocadia anammoxidans*" and *Candidatus* "*Kuenenia stuttgartiansis*", belong to the *Planctomycetes* family [48].

In the ANAMMOX process, nitrite and ammonium are used to produce gaseous nitrogen via NO and N_2H_4 as intermediates. In this process, nitrite acts as the electron acceptor, making the correct nitrite-to-ammonium nitrogen ratio one of the most critical factors for successful process implementation [49]. Additionally, to avoid competition between heterotrophic denitrifiers and ANAMMOX bacteria, it is necessary to maintain a low C/N ratio, a low temperature, and high-quality pretreatment to remove organic pollutants. Certain compounds, such as methanol, can completely inhibit the ANAMMOX process [49].

Thus, partial denitrification/ANAMMOX (hereafter PD/A) is the simplest and most common variant of this technology. In this case, partial denitrification ensures a stable supply of nitrites as the primary electron acceptor [50]. Today, there is a wide variety of reactor configurations using this technology: single-stage up-flow reactors (UASB) [51, 52], SBR (Sequencing Batch Reactor) [53], and MBR (Membrane Bioreactor) [54].

The use of carriers in the PD/A technology significantly enhances the stability of the system, creates favorable conditions for microbial symbiosis, and reduces the startup duration of the system [55]. Moreover, depending on the material used for the carriers, the mass transfer process within the biofilm may vary, and the mechanical strength of the carrier may improve [56].

The use of up-flow reactors such as UASB can also be combined with immobilization carriers. For instance, an up-flow dual-layer gel reactor (UDGR) operating in intermittent aeration mode achieves total nitrogen removal of up to 90%, complete ammonia removal, and a high nitrogen removal rate at low nitrogen loading rates (≤140 mg/dm^3 of ammonium nitrogen at the inlet) [57].

Conclusions

Based on the conducted literature review, immobilization is an effective tool for improving existing biotechnologies for nitrogen compound removal from wastewater. It ensures system stability, enhanced efficiency, reactor volume savings, consistent performance under varying conditions, as well as reduced energy consumption and sludge production. The application of immobilization

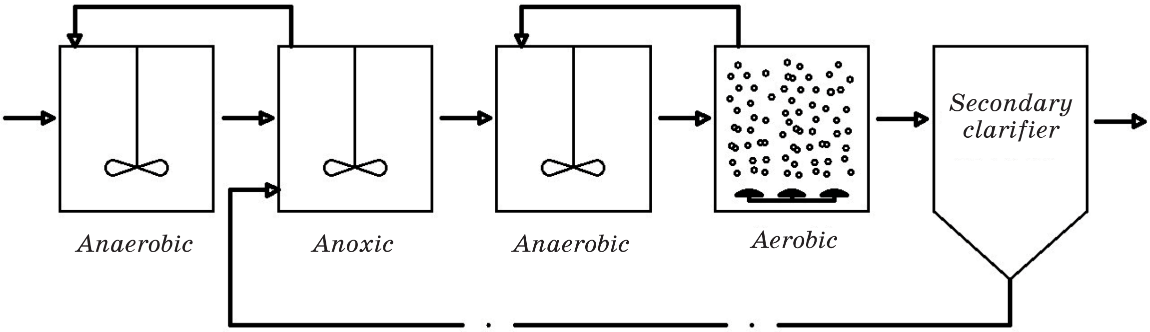

Fig. 3. **Modified UCT Technology**

in both classical and modern technologies (MLE, A2/O, UCT, ANAMMOX) contributes to improved performance of wastewater treatment technologies in each case. However, there is currently no universally optimal solution for the selection of carriers, the conditions for their rational application, or the ultimate capabilities of their use, ances of Adsorption for Removing Nitrate and Phosphate from Waste Water. *J. Water Process Eng.* 2022, 49:103159.

Author Contributions

LS: definition of the research direction, setting the aim and tasks of scientific research, discussion of the results and formulation of conclusions. HA: data collection processing, result interpretation, article writing.

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Conflict of Interest

There were no conflicts of interest.

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РОЛЬ ІММОБІЛІЗАЦІЇ МІКРООРГАНІЗМІВ У БІОТЕХНОЛОГІЇ ВИДАЛЕННЯ СПОЛУК НІТРОГЕНУ

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В останні роки, технології з використанням іммобілізованих мікроорганізмів демонструють значний потенціал для вдосконалення процесів очищення стічних вод, надаючи значні переваги.

Мета. Аналіз сучасних біотехнологій видалення сполук нітрогену зі стічних вод та їх модифікацій із використанням іммобілізованих мікроорганізмів, обґрунтування необхідності впровадження методів іммобілізації для вдосконалення технологій очищення стічних вод.

Методи. У дослідженні використано структурно-логічний та бібліосемантичний аналіз для вивчення ролі іммобілізації у вдосконаленні технологій видалення сполук нітрогену. Проаналізовано публікації з баз даних Web of Science, Scopus, PubMed та Google Scholar.

Результати. Проаналізовано сучасні підходи до видалення сполук нітрогену зі стічних вод, зокрема технології MLE, A2/O, UCT та ANAMMOX, у поєднанні з використанням іммобілізованих мікроорганізмів. Встановлено, що іммобілізація підвищує ефективність очищення завдяки стабільності процесів, зниженню енерговитрат та компактності систем. Виявлено, що ключовими чинниками, які потребують подальшого дослідження, є оптимізація носіїв, їх матеріалів та умов застосування для забезпечення максимальної ефективності роботи систем.

Висновки. Встановлено, що іммобілізація мікроорганізмів ефективно підвищує стабільність, продуктивність і енергоефективність технологій видалення сполук нітрогену, проте потребує подальших досліджень щодо оптимізації носіїв.

Ключові слова: біотехнологія, мікроорганізми, сполуки нітрогену, іммобілізація, стічні води, нітрифікація, денітрифікація, носії мікроорганізмів.