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INTEGRATED BIOTECHNOLOGICAL SYSTEMS FOR THE REMOVAL OF HEAVY METALS FROM WASTEWATER

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Aim. To analyze the transformation of industrial wastewater treatment towards integrated biotechnological complexes based on circular economy principles, focusing on synergistic mechanisms of simultaneous biogas production and heavy metal (Pb, Zn, Cr, Ni, Cu) utilization.

Methods. A systematic review of scientific literature (2015–2025) was conducted using Scopus, Web of Science, PubMed, and Google Scholar databases. Traditional physicochemical methods were compared with biotechnological approaches, including anaerobic digestion, biosorption, bioaccumulation, and bioelectrochemical systems.

Results. Traditional treatment methods are energy-intensive and generate toxic secondary waste. In contrast, anaerobic digestion enables energy recovery from high-strength wastewater, with optimization strategies (substrate pretreatment, micronutrient addition, enhanced electron transfer) increasing methane yield by 20–40%. Biotechnological metal removal mechanisms include biosorption, bioaccumulation, Cr(VI) bioreduction to Cr(III), bioleaching, and bioprecipitation. Advanced developments include metal-resistant microbial consortia, hybrid carrier materials (activated carbon with iron nanoparticles), "green" nanoparticle synthesis (92.5% Cu^{2+} removal), and digital twin technologies for adaptive process control.

Conclusions. Biotechnological methods transform waste into resources by integrating biogas production with metal recycling. Key future directions include synthetic biology, metagenomics, and digitalization of biotechnological process control.

Key words: biotechnology, industrial water treatment, microorganisms, biogas, anaerobic fermentation, recycling.

The global industry faces a triple challenge: the need to radically improve water efficiency, reduce its carbon footprint, and minimize its environmental impact. Traditional wastewater treatment systems, generally focused on meeting discharge standards, have evolved into significant energy consumers and generators of toxic waste, such as sludge enriched with heavy metals, creating a cycle of secondary environmental problems [1, 2]. This linear approach contradicts the fundamental principles of sustainable development and the circular economy. At the same time, industrial effluents, especially those generated by the food, pulp and paper, chemical, and electroplating industries, contain significant potential in the form of organic compounds with energy value, as well as inorganic components, particularly metals. The systematic loss of these resources is economically irrational. Modern biotechnology, enhanced by advances in synthetic biology, nanoscience, and artificial intelligence, offers a fundamentally new concept: the transformation of conventional treatment plants into bioprocessing facilities. In this innovative model, biological processes not only degrade pollutants but also catalyze

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their conversion into useful products, embodying the principle of 'waste equals resources' [3].

The purpose of this review was to conduct a comprehensive analysis of the technological transformation of industrial wastewater treatment systems towards a circular biotechnological model, with an emphasis on researching the synergistic mechanisms of parallel biogas production and heavy metal utilization, as well as identifying promising areas for the development of integrated biosystems for resource-efficient water treatment.

The object of the study was modern biotechnological systems for the comprehensive treatment of industrial wastewater, including anaerobic digestion, biosorption, bioaccumulation, biotransformation, and bioelectrochemical technologies, as well as their integration into technological chains.

The relevance of the topic lies in the study of promising methods for the disposal of heavy metals (Pb, Zn, Cr, Ni, Cu), as well as the possibility of integrating them into general industrial wastewater treatment processes. This problem is particularly important due to the active hostilities on the territory of Ukraine, one of the negative consequences of which is an increase in the concentrations of these chemical elements in water and soil.

The subject of the study is the scientific principles, technological solutions, and efficiency of integrated biosystems that ensure synergistic interaction among the removal of organic pollutants and heavy metals, energy recovery in the form of biogas, and the recycling of material resources, in particular metals, from industrial wastewater.

To achieve this goal, the following tasks are set in the work:

- to analyze the structural limitations and shortcomings of traditional physical-mechanical, chemical, and physical-chemical methods of industrial wastewater treatment used in modern industry;
- to characterize the main types of biotechnological treatment processes, their advantages and limitations, and conduct a comparative analysis with traditional approaches using tabular data;
- to investigate the mechanisms of anaerobic fermentation and strategies for optimizing this process to maximize biogas yield, including substrate pretreatment, micronutrient supplementation, acceleration of interspecies electron transfer, and the concept of anaerobic biorefineries;
- to systematize knowledge of biotechnological methods for heavy metal disposal, such as biosorption, bioaccumulation, biotransformation, and bioprecipitation, and to consider mechanisms for integrating them into treatment systems to achieve metal recycling.

Materials and Methods

This review was based on a systematic analysis, generalisation and synthesis of data published in international scientific journals. Literature sources were searched using the scientometric databases Scopus, Web of Science, PubMed, Google Scholar, as well as the electronic archives of leading publishers (Elsevier, Springer, Wiley, MDPI). The search mainly covered the period 2015–2025 to ensure data relevance, including foundational works from earlier years that are crucial to understanding the basic processes.

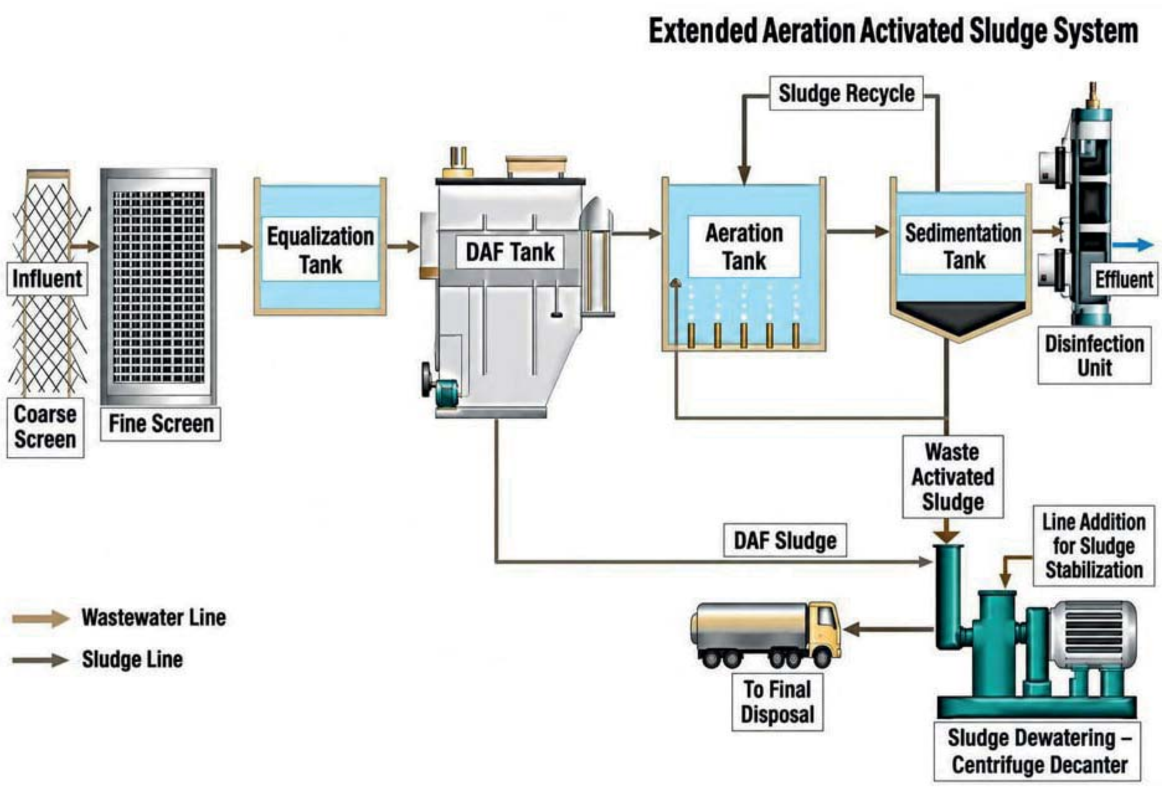
The search query was formed using combinations of keywords in English and Ukrainian: industrial wastewater, biotechnology, anaerobic digestion, biogas, heavy metals, biosorption, bioaccumulation, bioremediation, circular economy, resource recovery, bioelectrochemical systems, microbial consortia, biorrefinery, industrial wastewater, biotechnology, anaerobic digestion, biogas, heavy metals, bioremediation.

The criteria for including publications in the analysis were: peer-reviewed status, availability of experimental data or a thorough theoretical analysis, relevance to the research topic, and clarity of the presentation of the methodology and results.

Results

First of all, conventional industrial wastewater treatment trains (Figure) rely on a sequence of physical-mechanical, chemical, and physicochemical unit operations, each designed to target a distinct class of contaminants. Although these techniques have reached a high level of engineering maturity, they still exhibit several systemic and often interlinked limitations.

Mechanical and *physicochemical* separation techniques include sedimentation, flotation, filtration, and membrane-based processes. They are very efficient at extracting suspended solids, oil, and grease. However, the most advanced variants–nanofiltration and reverse osmosis–which can retain even dissolved ionic species, demand considerable amounts of energy. Research



Schematic diagram of wastewater treatment at an industrial wastewater treatment plant [4]

indicates that desalinating one cubic meter of water by reverse osmosis requires between 3 and 10 kWh of electricity [3]. In addition, these membrane processes unavoidably generate a concentrated reject stream that can represent up to 25–30% of the initial feed volume; disposing of this concentrate is a difficult and expensive challenge, especially when the stream is highly saline or contains toxic substances [5].

Chemical methods such as coagulation, flocculation, neutralization, and oxidation (including ozonation) are the principal means of removing colloids, phosphates, heavy metals, and recalcitrant organic substances. These techniques work by dosing chemicals — for instance, iron or aluminum salts, bases, acids, or oxidizing agents — which trigger the precipitation of a solid phase (sludge) that is subsequently removed. This approach suffers from several systemic drawbacks: substantial operational expenses driven by the continuous consumption of costly chemicals; the generation of large volumes of sludge that frequently qualifies as hazardous waste because it concentrates heavy metals and persistent organic pollutants, necessitating disposal in dedicated landfills [6]; and the potential for secondary contamination, exemplified by the production of carcinogenic trihalomethanes during chlorination or elevated sulphate levels when conventional coagulants are employed [7].

Evaporation, *crystallization*, and *incineration* are used to treat industrial effluents with high contaminant concentrations. Despite achieving maximum removal efficiency, these methods are characterized by the largest carbon footprint due to their enormous energy consumption. In particular, incineration can be accompanied by emissions of dioxins, nitrogen oxides, and Sulfur, which require the integration of complex and expensive gas-cleaning systems [8].

Thus, traditional methods, while solving the immediate task of water purification, paradoxically create global problems: they increase energy dependence, generate toxic waste, and lead to irreversible losses of potential resources. This situation creates a critical need for alternative, regenerative approaches.

On the other hand, *biological purification* relies on the metabolic activity of consortia of microorganisms, including bacteria, archaea, fungi, algae, and macrophytes, to biotransform pollutants.

Biological treatment technologies cover a spectrum of processes. Aerobic configurations–including activated sludge, biofilters, and membrane bioreactors — rely on microorganisms that, in the presence of dissolved oxygen, convert organic pollutants into carbon dioxide and water, while also achieving nitrogen removal via nitrification–denitrification and phosphorus uptake. These approaches perform well on wastewaters with low to moderate organic loads, yet they demand considerable energy for mechanical aeration. In contrast, anaerobic systems, such as methane tanks, UASB (Upflow Anaerobic Sludge Blanket), and IC reactors, operate under oxygen-free conditions. Within them, specialized syntrophic microbial consortia progressively degrade complex organic substrates into methane–making up 50-75 % of the biogas — and carbon dioxide. This route is particularly important for treating the high-strength effluents characteristic of the food and forestry sectors, because it eliminates the need for aeration energy and actually yields usable energy in the form of biogas [9].

Biosorption and *bioaccumulation* employ living or dead biomass (algae, fungi, bacteria, or agricultural residues) to passively sequester heavy metals via functional groups present on cell walls [9]. Phytoremediation, frequently applied as a polishing step, exploits the capacity of aquatic plants such as duckweed or water hyacinth to extract heavy metals and nutrients [12].

The fundamental advantage of biotechnologies is their focus on resource recovery rather than simple destruction. They are characterized by low energy consumption, especially in anaerobic systems, and can even demonstrate a positive energy balance [13]. These processes are generally more selective and environmentally friendly, as they do not produce secondary toxic products, except for some undesirable compounds, such as excess hydrogen sulfide.

The main limitations of biotechnological methods include the sensitivity of microbial consortia to toxic shocks from high concentrations of heavy metals, pesticides, or antibiotics. The speed of biological processes is usually lower than that of chemical methods. Effective operation requires skilled maintenance and a deep understanding of microbiological principles. In addition, productivity is highly dependent on temperature conditions, with an optimum range of 25–35 °C.

A comparative overview of the main groups of industrial wastewater treatment methods is presented in Table 1, illustrating the fundamental differences between linear and circular treatment concepts.

Next, anaerobic digestion, carried out in high-rate reactors such as UASB and EGSB, is the most important biotechnology for recovering energy from high-strength organic wastewater [14]. The multi-stage

Table 1. **Comparative analysis of traditional and biotechnological methods of industrial wastewater treatment**

Criterion	Traditional physical-chemical/ chemical methods	Biotechnological methods
Energy balance	Mainly energy-consuming (especially membrane and thermal)	Energy-independent operation or even energy recovery (anaerobic systems) is possible
By-products	Toxic sludge, concentrated brines, chemical by-products (trihalomethanes, etc.)	Biogas (CH_4, CO_2), stabilized biomass, biopolymers, and CO_2. Solid waste volumes are minimal
Effectiveness against heavy metals	High, but with the formation of toxic sludge (precipitation, ion exchange)	Medium to high due to biosorption, bioaccumulation, and bioremediation. Potential for metal recycling
Operating costs	High (reagents, energy, sludge disposal)	Lower, because the ‘reagent’ is microorganisms. Income from biogas/ resources can cover costs
Environmental footprint	High due to CO_2 emissions from energy/reagent production and toxic waste	Low, nature-like technologies promote decarbonisation
Flexibility to changes in effluent composition	Low, requires readjustment of reagent doses	Medium/high, microbial communities can adapt, but it takes time
Main objective	Achievement of discharge standards	Cleansing and restoration of energy and materials

synergistic process–hydrolysis, acidogenesis, acetogenesis and methanogenesis–is often constrained by slow hydrolysis of resistant polymers, the accumulation of volatile fatty acids that can acidify the reactor, a critical dependence on trace elements (nickel, cobalt, molybdenum, iron) for methanogenic enzyme activity [15], and the presence of inhibitors such as heavy metals, ammonium, hydrogen sulfide and antibiotics [16]. Current optimisation strategies address these bottlenecks: substrate pretreatment (e.g., ultrasonic treatment raising methane yield by 20–25% [17]); targeted micronutrient supplementation with bioavailable chelated metals, which can increase gas yield by 15–30% and restore stability after disruption, as shown by cobalt and nickel addition to biofuel wastewater systems [18]; acceleration of direct interspecies electron transfer via conductive materials like granular activated carbon, graphene or biogenic magnetite nanoparticles, with one study reporting a 40% increase in methane yield [19]; and advanced process control using the ADM1 kinetic model combined with online sensors for pH, volatile fatty acids and gas pressure to predict and prevent stress [20]. Within a modern anaerobic biorefinery concept, raw biogas is purified to biomethane (>95% methane) suitable for gas grid injection or use as a clean transport fuel [21]; the CO_2 released can be captured and used to cultivate microalgae as additional biomass [22]. The nutrient-rich digestate can be converted into high-quality biofertilizers or serve as feedstock for bioplastics such as polyhydroxyalkanoates [23].

Because heavy metals (cadmium, lead, mercury, chromium, copper, nickel, zinc) are not biodegradable, biotechnological approaches focus on altering their chemical form or physically separating them for safe disposal or recycling. The main mechanisms include biosorption, a rapid passive binding of metal ions to functional groups (carboxyl, hydroxyl, amino, phosphate) on living or dead biomass–algae such as *Sargassum* and *Chlorella*, fungi like *Aspergillus niger*, bacterial cells, and low-cost agricultural waste [24]; bioaccumulation, a slower energy-dependent uptake into cells where metals are sequestered by metal thiolates or polyphosphates, achieving high intracellular concentrations [25]; biotransformation, exemplified by the microbial reduction of toxic, soluble Cr(VI) to the less mobile and less toxic Cr(III) by bacteria like *Shewanella oneidensis* or *Pseudomonas aeruginosa* under anaerobic conditions [26]; bacterial leaching, in which acidophilic chemolithotrophs such as *Acidithiobacillus ferrooxidans* and *A. thiooxidans* oxidise iron and sulfur to generate sulfuric acid and ferric ions that dissolve metals from solid matrices for subsequent hydrometallurgical recovery [27]; and bioprecipitation, where sulfate-reducing bacteria precipitate metals as highly stable sulfides (CuS, ZnS) or urease-active bacteria form carbonates, enabling straightforward mechanical separation [28].

Table 2 provides general examples of specialized microbial consortia used to treat industrial wastewater.

Modern treatment schemes increasingly integrate these mechanisms into unified process chains. Pre-biosorption with low-cost

Table 2. **Examples of specialized microbial consortia for industrial wastewater treatment**

Wastewater type/ contaminants	Examples of key microorganisms	Main function and mechanism
Heavy metal effluents	*Pseudomonas aeruginosa*, *Geobacter spp.*, *Rhodopseudomonas palustris*	Biosorption, bioaccumulation, and biotransformation of metals into less toxic or mobile forms [30–32]
Wastewater containing persistent organic pollutants (petroleum products, pesticides)	*Bacillus subtilis*, *Pseudomonas putida*, *Penicillium chrysogenum*	Production of specific enzymes for breaking down complex hydrocarbon chains and aromatic compounds [33]
Pharmaceutical effluents (antibiotics, xenobiotics)	Consortia based on *Klebsiella pneumoniae*, *Enterobacter cloacae*, specific fungi	Cometabolism and cooperative degradation of persistent molecules that are difficult to decompose by individual species [34]
Concentrated organic effluents (distilleries, breweries)	Consortia of *Micrococcus*, *Flavobacterium*, *Pseudomonas*, *Bacillus*	Effective anaerobic fermentation of high concentrations of organic matter with maximum biogas yield [35]

materials such as grain or wood residues can shield downstream biological units (activated sludge, anaerobic reactors) from metal toxicity. Bioelectrochemical systems, notably microbial fuel cells and electrolytic reactors, enable simultaneous organic oxidation at the anode, electricity generation, and reductive precipitation of pure metals at the cathode, thereby integrating water treatment, energy recovery, and metal extraction [36]. Cascade systems sequentially apply bioremediation, coagulation, and bioleaching: for instance, Cr(VI) is first reduced to Cr(III), removed as a sludge, and the chromium is then recovered from that sludge via bacterial leaching as a saleable product [37].

Emerging nanobiotechnology and *synthetic biology* offer powerful new tools. "Green" synthesis of iron oxide nanoparticles using heavy-metal-resistant *Aspergillus niger* cell-free extract yielded a nanosorbent that removed 92,5 % Cu^{2+}, 84,8% Mn^{2+}, 80,8% Cr^{3+}, 72,8% Fe^{3+}, and 70,3% Zn^{2+} from synthetic wastewater. Up to 90.7% Fe^{3+} and 78.8% Zn^{2+} from real effluents, providing an environmentally benign alternative to chemical nanosynthesis [38]. Metagenomics uncovers uncultured microbes with novel detoxification pathways [39], while genetic engineering can boost metal-binding metallothioneins or introduce hydrogen sulfide-producing enzymes. A modified *Escherichia coli* strain engineered to generate H_2S, for example, efficiently precipitated cadmium as CdS [40].

Recent advances in integrated systems focus on synergistically coupling biogas production with metal recovery, tackling metal toxicity through several innovations. For example, selenium addition stimulates the *in situ* formation of biogenic elemental selenium nanoparticles that bind mercury and protect methanogens [41]. In contrast, pre-adaptation of anaerobic sludge to low metal concentrations induces extracellular polymeric substances that chelate metals and shield cells [42]. Molecular profiling of treatment-plant microbial communities reveals ecological and functional dynamics essential for process stability [43]. Two metabolic separation strategies are employed—when metals dominate, a "metals first" approach uses bioelectrochemical systems or biosorption to remove toxicants before anaerobic fermentation.

In contrast, for highly organic streams, an "energy first, metals second" sequence applies, with digestion followed by bioleaching of the metal-enriched sludge for recovery [44]. Carrier engineering has yielded hybrid immobilization materials such as granular activated carbon modified with iron nanoparticles, which simultaneously support biofilm growth, enhance direct interspecies electron transfer, and sorb metals through ligand exchange and co-precipitation [45], along with biopolymer-based composites (e.g., alginate or chitosan encapsulating *Shewanella* biomass and magnetite nanoparticles) that reduce Cr(VI) and adsorb the resulting Cr(III) [46]; detailed characterization of analogous humic acid-alginate composites has also been reported [50]. Process control has advanced through the deployment of digital twins that fuse IoT sensor streams, periodic metagenomic snapshots, and machine learning algorithms to forecast biogas yields from microbial community dynamics [46–48], optimize nutrient and pH adjustments, and detect early stress signals such as volatile fatty acid accumulation or hydrogen sulfide spikes, enabling pre-emptive corrective actions [49].

Prospects for further research

Future academic work will increasingly focus on the deliberate integration of wastewater treatment, biogas production, and heavy metal removal in integrated biorefinery concepts. Research will advance through the synthetic design of multi-kingdom microbial consortia — assembling bacteria, archaea, fungi, and microalgae — to simultaneously degrade organics for biogas generation, detoxify metal-laden streams, and recover metals via tailored metabolic networks.

These biological cores will be governed by autonomous digital twins that fuse real-time sensor data, metagenomic profiling, and machine learning to orchestrate treatment, methane yield optimization, and metal precipitation or biosorption in a closed-loop, self-adaptive fashion. Coupling the entire system with surplus renewable electricity from solar or wind to drive the operations of electrolytic and bioelectrochemical units will enable energy-independent treatment complexes that harmoniously deliver purified water, biomethane, and recovered metal products. Achieving this vision demands an interdisciplinary collaboration spanning microbiology, process engineering, materials chemistry, and data science to translate fundamental understanding of consortia dynamics, hybrid carrier materials, and metabolic regulation into resilient, resource-positive technologies.

Conclusions

1. Traditional physical-mechanical, chemical, and physical-chemical treatment methods, despite their technological maturity, are characterized by high energy consumption, high operating costs, and the generation of toxic secondary waste, which makes them unable to meet the requirements of the circular economy. In contrast, biotechnological methods offer a philosophy focused on transforming waste into resources that operate with a low energy balance, minimize the formation of toxic by-products, and create conditions for recycling valuable components.

2. Anaerobic digestion is a key technology for recovering energy from highly concentrated organic waste streams. Modern optimization strategies, such as substrate pretreatment, targeted supplementation of trace elements, and acceleration of direct interspecies electron transfer using conductive materials, can increase methane yield by 20–40% and ensure process stability. The concept of an anaerobic biorefinery transforms the system into a multifunctional resource-recovery hub that produces biomethane and biofertilizers.

3. Biotechnological methods for heavy metal disposal offer an environmentally safe alternative to chemical precipitation, creating opportunities for metal recycling. The integration of these mechanisms into unified technological chains, particularly through bioelectrochemical systems and cascade schemes, enables the sequential detoxification of effluents and the extraction of metals in commercial form. Advances in genomics and synthetic biology are paving the way for the creation of microorganisms with increased metal-binding efficiency.

4. Cutting-edge research shows a trend towards the creation of synergistic systems where biogas production and metal recovery reinforce each other. Key achievements include the modification of microbial consortia to increase metal resistance, the concept of metabolic stream separation, the development of hybrid carrier materials, and the use of digital twins with machine learning for adaptive control, which forms the basis for the autonomous biorefineries of the future.

Authors' Contribution

Y. Kaliuzhnyi — literature review, data analysis, writing — original draft; V. Mel'nick — methodology, writing — review and editing.

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ІНТЕГРОВАНІ БІОТЕХНОЛОГІЧНІ СИСТЕМИ ДЛЯ ВИДАЛЕННЯ ВАЖКИХ МЕТАЛІВ ЗІ СТІЧНИХ ВОД

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Мета. Проаналізувати трансформацію очищення промислових стічних вод у бік інтегрованих біотехнологічних комплексів на основі принципів циркулярної економіки, зосереджуючись на синергетичних механізмах одночасного виробництва біогазу та утилізації важких металів (Pb, Zn, Cr, Ni, Cu).

Методи. Проведено систематичний огляд наукової літератури (2015–2025 рр.) з використанням баз даних *Scopus, Web of Science, PubMed* та *Google Scholar*. Традиційні фізико-хімічні методи порівнювали з біотехнологічними підходами, включаючи анаеробне розщеплення, біосорбцію, біоакумуляцію та біоелектрохімічні системи.

Результати. Традиційні методи очищення є енергоємними та генерують токсичні вторинні відходи. На противагу цьому, анаеробне зброджування дозволяє відновлювати енергію з висококонцентрованих стічних вод, а стратегії оптимізації (попереднє оброблення субстрату, додавання мікроелементів, посилений перенос електронів) збільшують вихід метану на 20–40%. Біотехнологічні механізми видалення металів включають біосорбцію, біоакумуляцію, біовідновлення Cr(VI) до Cr(III), біовилуговування та біоосадження. Передові розробки включають металостійкі мікробні консорціуми, гібридні матеріали-носії (активоване вугілля з наночастинками заліза), синтез «зелених» наночастинок (видалення 92,5% Cu^{2+}) та технології цифрових двійників для адаптивного керування процесами.

Висновки. Біотехнологічні методи перетворюють відходи на ресурси шляхом інтеграції виробництва біогазу з переробкою металу. Ключові напрямки майбутнього включають синтетичну біологію, метагеноміку та цифровізацію управління біотехнологічними процесами.

Ключові слова: біотехнологія, промислове очищення води, мікроорганізми, біогаз, анаеробне бродіння, переробка.

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