chemosensors

MDPI

Review

Luminescent Analysis of ATP: Modern Objects and Processes for Sensing

Elena Efremenko, Olga Senko, Nikolay Stepanov, Olga Maslova, Galina Yu. Lomakina and Natalia Ugarova *

Faculty of Chemistry, Lomonosov Moscow State University, Lenin Hills 1/3, Moscow 119991, Russia
* Correspondence: nugarova@gmail.com; Tel.: +7-(495)-939-2660; Fax: +7-(495)-939-5417

Abstract: Bioluminescent analysis of adenosine triphosphate (ATP) concentrations is now acquiring new applications in the form of objects and processes in which it can be effectively used for sensing. A quick analysis of biological objects and systems for which the level of ATP concentrations is one of the main parameters, and a forecast of the development of various situations in such biosystems under industrial production conditions or the ecological state of the environment, confirmed by various results of analytical control of other parameters, turns out to be simple and effective. Sanitary control, quality control of purified water, microbial analysis in the food industry, maintenance of drugs and estimation of their quality, and monitoring of the metabolic state of biocatalysts used in various biotechnological processes are between the main trends of recent applications of bioluminescent ATP-assay. Additionally, the new areas of ATP sensing are developed, and the following topics are their creation of synthetic microbial consortia, their introduction as new biocatalysts to biodegradation of pesticides, suppression of methane accumulation in model urban land fields, control of dangerous development of biocorrosive processes, design of chemical-biocatalytic hybrid processes, creation of effective antimicrobial dressing and protective tissue materials, etc. These aspects are the subject of this review.

Keywords: bioluminescence; ATP; firefly luciferase; detection; biocatalysis; consortia; inhibition

Citation: Efremenko, E.; Senko, O.; Stepanov, N.; Maslova, O.; Lomakina, G.Y.; Ugarova, N. Luminescent Analysis of ATP: Modern Objects and Processes for Sensing. *Chemosensors* **2022**, *10*, 493. https://doi.org/10.3390/chemosensors10110493

Academic Editors: Zheng Zhao and Zijie Qiu

Received: 29 October 2022
Accepted: 18 November 2022
Published: 21 November 2022

1. Introduction

Interest in the bioluminescent method for the determination of adenosine triphosphate (ATP) has not decreased for several decades [1], since ATP is the main carrier and universal source of energy for different biochemical processes occurring in all living cells. Generally, synthesis and hydrolysis of ATP is used by living organisms to provide energy for various biochemical transformations and physiological processes, therefore, changes in the concentration of ATP in cells confirm the level of their viability and metabolic activity and indicate the presence of factors in the microenvironment of cells that can have a negative impact on them. At the same time, there are various approaches to determining the concentration of ATP, but the most sensitive, fast and specific is the method of ATP-metry based on the use of the bioluminescent luciferin-luciferase system of fireflies [2,3].

Modern studies using bioluminescent ATP sensing are interesting for the understanding of main trends and possibilities of using this highly sensitive analysis. In many cases it appeared that bioluminescent ATP-chemosensing is continued to be used for research in the fields of sanitation, biomedicine, toxicology, in solving environmental problems, developing and using environmental technologies, antimicrobials and food products, chemical and biological protective agents and anticorrosive agents, new and effective biocatalysts and biotechnological processes, cell storage facilities, and differentiated cell analysis (Table 1 [4–51]).

At the same time, it should be noted that there are completely new studies using bioluminescent control of ATP in complex reaction media with the participation of naturaly and artificially composed consortia of microorganisms that catalyze, including large-scale

processes (accumulation of landfill gases, methanogenic destruction of agricultural and petrochemical waste, toxic pesticides) [52–54]. The use of ATP-metry as a method of to express an adequate and integral assessment of the state of complex biocatalytic systems allows us not only to study, but also to select factors that most effectively regulate the properties of the objects under study, leading the processes to the desired characteristics.

It is important to note that the authors of the ongoing research in the field of bioluminescent ATP-metry noted and investigated the possibility that the influence of certain chemicals (detergents, antibiotics, preservatives, salts, etc.) introduced into reaction media with microorganisms could not only affect the level of intracellular ATP concentration as determined by the luciferin-luciferase reaction but also have an inhibitory effect on luciferase itself and the enzymatic reaction catalyzed by it [16,34,36].

The purpose of this review is to consider the main modern areas of application of bioluminescent ATP analysis to identify the presence and study of certain living objects and complex biocatalytic systems, as well as processes that attract the most attention for their further development and application in practice. At the same time, special attention is paid in the review to the facts of the revealed influence of certain factors on the analysis of ATP itself, in particular, on the activity of luciferase, which may be important for users of this method. The review has been prepared by researchers who are actively involved in the using of this bioluminescent ATP-assay, including on the results of their own various experiments, presenting them in comparison with the characteristics of media, biocatalysts and processes controlled in parallel by other analytical methods.

Table 1. Main recent directions of bioluminescent ATP-based chemosensing developments.

The Application of Bioluminescent ATP Sensing [Reference]	Main Objects for ATP Detection	Comments
Sanitarian Control and Hygiene Monitoring in Public Places		
ATP control in evaluating the effectiveness of hygiene interventions aimed at preventing viral spreading in the workplaces [4]	54 selected office surfaces for the analysis of MS2 bacterial virus spreading inside enclosed spaces	ATP measurements confirmed the spreading and contamination of a half of the tested hands and surfaces in the offices by the virus MS-2 within 4 h. There was no direct correlation between ATP measurements and viral concentrations. The application of some disincentive means (hand sanitizers, facial tissues, disinfecting wipes) reduces virus spreading, although ATP does not measure viruses. The results demonstrated the possible use of ATP control for preventing viral spreading.
Estimation of microbial contamination of hospital surfaces [5]	*Staphylococcus aureus*, *Enterobacteriaceae*, *Enterococci*, *Pseudomonas spp.* cells in the samples from the surfaces of nurse's computer touch screens, surgical lighting, instrument tables, operating tables	ATP-bioluminescence-assay is used as a tool to measure the efficiency of cleaning procedures also in environments with very low microbial counts.
Identification of microorganisms in hospital water sources [6]	27 water samples from faucets and water purifiers at hospitals	A positive correlation between the ATP sensing data and microbiological studies were shown in 85.2% of studies. However, the presence of urine and blood in hospital water, as well as detergents and antimicrobials, led to an incomplete correlation between the data obtained by two methods on the presence of bacterial contamination.
Monitoring of the cleanliness of medical devices (surgical instruments) in a sterilization service [7]	Total microbial pollution	The bioluminescent ATP chemosensing, showed that automatic washing of instruments leads to almost 2 times less residual microbial contamination than their manual washing.

Table 1. *Cont.*

The Application of Bioluminescent ATP Sensing [Reference]	Main Objects for ATP Detection	Comments
Control assay of the manual cleaning of reusable surgical instruments [8]	140 surgical instruments of 12 different types before and after cleaning procedures	ATP bioluminescent method allows validating the manual cleaning procedure, to confirm that multi-stage disinfection procedures of various surgical instruments guarantee the 99.9% purity from cells of microorganisms.
Disinfection with hybrid hydrogen peroxide (HHP) fogging in a critical care setting [9]	*Staphylococcus aureus, Pseudomonas aeruginosa* with resistance to methicillin	ATP bioluminescent sensing and microbial traditional method showed that HHP fogging provides up to 98% of bacterial removal from various surfaces in hospital rooms without use of UV light.
Examining the amount of bio-burden on frequent touch points (FTP) in patient areas to estimate changes in the efficacy of post-discharge cleaning [10]	Microbial cells in 11 hospitals in the special places: toilet flush, bathroom tap, inside bathroom door handle, patient call button, over bed tray table, bed rails	The efficacy of post-discharge cleaning of FTP was maximum 72%.
Examination of contamination presence on endoscope surfaces [11]	*Stenotrophomonas maltophilia, Klebsiaella pneumonia* synthesizing carbapenamase, *P. aeruginosa,* synthesizing AmpC beta-lactamase	The 42 flexible endoscopes were examined and 9% of them were found to be contaminated. The results were confirmed by microbiological and ATP assays. The cleanness of all tested endoscopes was verified after repeated treatment.
Spread of aerosol and splatter during dental treatments [12]	Total contamination of masks, goggles of patients and dentists, operators bodies, before and after dental treatments	ATP bioluminescence is used for rapid estimation of cleanliness of various surfaces in dental clinics and helps to reduce health risks for both patients and dentists.
Quantitative ATP-based assay revealing presence of microorganisms violating sterility [13]	*Aspergillus brasiliensis*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Propionibacterium acnes*	The following ranking of different methods used for control by their sensitivity to sterility was established (in descending order): (1) method of bioluminescent ATP analysis enhanced by adenylate kinase application; (2) microbiological method; (3) regular bioluminescent ATP-assay.
Assessment of the correspondence between the results of ATP and microbiological analyses [14]	Any objects present in hospitals or nursing home settings and contaminated with pathogenic microorganisms or parasites, in contact with which there is a risk of infection present	The opportunity of using of ATP-assay for an assessment of cleanliness in health care facilities was demonstrated.
Using ATP analysis to monitor hygiene in long-term care facilities [15]	*Clostridioides difficile* as a source of frequent healthcare-associated infection (diarrheal illness) in long-term care facilities	ATP-analysis of high-touch surfaces in 11 facilities was performed. ATP analysis revealed the presence of this compound on the surfaces of the handrails of the patient's bed, tables and chairs in the patients' rest rooms, which confirmed the insufficient level of hygiene.
	Food industry	
Insurance of adequate cleanliness in food industry by using the ATP rapid hygiene-monitoring tests in combination with ADP and AMP determination [16]	Different foods (meat, seafood, dairy, nuts, fruits, vegetables, fermented foods); stainless steel exposed to raw meat	Approach based on detection of total adenylate (ATP + Adenosine diphosphate (ADP) + Adenosine monophosphate (AMP)) was applied in the determination of food contamination. The ATP bioluminescent assay was combined with 2 enzymes, pyruvate kinase and pyruvate phosphate dikinase, converting ADP into ATP and recycling AMP into ATP, respectively. Considerable inhibiting effect (up to 93%) on the ATP analysis was revealed in presence of residual concentrations of disinfectants (sodium hypochlorite, ethanol, and benzalkonium chloride).

Table 1. *Cont.*

The Application of Bioluminescent ATP Sensing [Reference]	Main Objects for ATP Detection	Comments
Estimation of ultrasound action on both Gram-negative and Gram-positive microorganisms as a potential non-thermal sterilization technology in food industry [17]	*Esherichia coli*, *Staphylococcus aureus*	The significant ATP decrease was estimated after ultrasound treatment for both Gram-negative (*E. coli*) and Gram-positive (*S. aureus)* cells. However, the Gram-negative bacteria were more sensitive when the initial concentrations of both cell types taken for the investigation were similar (8.24–8.34) log CFU/mL.
Sanitary control of the cleaning efficiency of various surfaces in the food industry [18]	Swabs at predetermined sites of tofu production facility	ATP-assay revealed the areas of the production environment needing additional sanitary treatments. Microbiological analysis confirmed results of ATP analysis and additionally disclosed the presence of aerobic microorganisms, lactic acid bacteria, yeasts and molds.
Evaluation of efficiency of cleanliness of food contact surfaces [19]	*Escherichia coli*, *Staphylococcus aureus* (microbial contamination of food contact surfaces before and after treatments by sanitizers)	ATP-bioluminescence allows revealing contamination and the degree of cleanliness of food contact surfaces with efficiency better than conventional methods.
Estimation of hygiene in the cutting rooms of poultry slaughterhouse [20]	*Staphylococcus aureus*, *Escherichia coli* on the surfaces of stainless steel surfaces, polyurethane belts and polyethylene boards	ATP chemosensing approach allows detecting in seconds extremely low levels of contamination. The combination of hot water (50 °C) with detergents brought the best results in the treatment of various surfaces contacting with food products.
Estimation of water safety and the sanitary-hygienic state of surfaces of household objects [21]	Microbial contaminants on the surfaces of wooden cutting boards, kitchen knives, food containers, food containers, glassware (plates), glassware (wineglass)	Low ATP concentrations were observed on the internal surface of new (plastic) food containers. The ATP-assay gave immediate information about the contaminations of both surfaces of food production equipment and liquids.
Maintenance of drug and medications quality		
Development of fiber materials with pronounced chemical-biological protection [22]	*Bacillus subtilis*, *E.coli*	ATP chemosensing helps to make a screening of the most effective types of fiber materials protecting against the action of organophosphorus neurotoxins and bacterial cells.
Revealing of levofloxacin resistance [23]	*E.coli, K. pneumoniae, Enterobacter cloacae, Morganella morganii, Salmonella enterica*, *Staphylococcus aureus, S. capitis,S. epidermidis, Streptococcus pyogenes, S. mitis, S. agalactiae, Enterococcus faecium*	ATP-based analysis for 6 h gave information usable to assess the presence of a certain level of antibiotic resistance or the absence of such. This approach is important for decision-making in blood sepsis.
Detection and investigation of resistance to vancomycin [24]	*Clostridium difficile*	ATP chemosensory helps to overcome the use of microbiological method for determining the minimum bactericidal concentration for vancomycin under anaerobic conditions leading to slow growth of *Clostridium* cells.
Screening of platelet inhibitors [25]	Platelets from blood of 3-month-old Male Sprague Dawley rats	The ATP-chemosensory allows monitoring activation of platelets by staurosporin and their inhibition by Ly294002.
Monitoring of autophagy in real time in yeast cells [26]	*Saccharomyces cerevisiae*	Screening of potential drugs in a library of small molecules that can modulate or reduce dysfunctional autophagy developed as a cellular response to tarvation.

Table 1. *Cont.*

The Application of Bioluminescent ATP Sensing [Reference]	Main Objects for ATP Detection	Comments
Estimation of oxidative stress in cells under the action of drugs [27]	Human liver slice cultures	ATP-sensing provides determination of drugs (dantrolene, cyclosporine A) provoking strong oxidative stress in mitochondria of liver cells.
Maintenance of drug metabolism and transport functions in human precision cut liver slices [28]	Human liver slices	ATP-chemosensing helps to control viability of liver cells in various media for their exposition in presence of different drugs for a long period of time.
Toxicological and pharmacological studies [29]	*Caenorhabditis elegans*	Bioluminescent monitoring confirms the mitochondrial depletion of ATP by pentachlorophenol.
Investigation of mitochondrial thermogenesis homeostasis in brown adipose tissue [30]	Brown adipocytes	ATP-chemosensing allows clarification of mechanisms participating in regulation of mitochondrial biogenesis.
Studying of ATP-release mechanism and over-activation of macrophages by extracellular ATP as a marker of sepsis development [31]	Murine peritoneal macrophages	ATP-sensing confirms the secretion of inflammatory cytokines in an autocrine manner during sepsis. Active ATP release from inflammatory cells was registered due to vesicular exocytosis.
Estimation of Bacille Calmette–Guerine (BCG) vaccine viability [32]	*Mycobacterium bovis* BCG vaccine	Bioluminescent ATP sensing allows reducing the duration of determination of BCG vaccine viability (quality) from 28 days (necessary for microbial methods) to 1 h.
	Water quality	
Presence of different metal ions in urban wastewater [33]	Samples of wastewater effluent with *E.coli*, *Pseudomonas spp.* and pesticides before and after ozonation	ATP cellular depletion was registered after wastewater disinfection by metals ions and O_3.
Control of biofouling in seawater reverse osmosis systems [34]	Water samples	The new reagents for ATP-assay in seawater was developed allowing monitoring of bacterial growth potential in seawater desalination plants.
Monitoring of ballast water treatment [35]	Samples of ballast water with microorganisms and larger planktonic organisms	ATP control discloses the presence of plankton and bacteria in ballast water treatment system.
Estimation of amounts of microalgae biomass in aquatic systems [36]	*Thalassiosira weissflogii*	The per-cell ATP concentration is a relatively constant parameter of cells used for estimation of microalgae biomass amounts.
Quantitative determination of ATP in situ in submarine environments [37]	Seawater samples taken at a depth of 3000 m	Determination of low enough ATP concentrations (5×10^{-12}–5×10^{-11} M) in the seawater around submarines.
Rapid onboard testing of living microorganisms in shipboard ballast water [38]	Plankton cells (diatom species (*Chaetoceros* simplex, *Skeletonema costatum*), dinoflagellate, *Raphidophyceae*, *Chrysophyceae* species)	ATP-assay helps to estimate the plankton cells in shipboard ballast water and to make a decision about its possible discharge.
	Biotechnology and biocatalysis	
Method to study litter decomposition [39]	Samples of leaf litter in streams	Method has been successfully applied to organic matter collected in technical and environmental systems.
Monitoring of ATP level in different cells to estimate their physiological status [40]	Bacteria, fungi, algae, plant tissue, and human cell lines	ATP-assay provides the information about viability and metabolic activity of various cells after their exposition at ultralow temperatures and under cryostress conditions.

Table 1. *Cont.*

The Application of Bioluminescent ATP Sensing [Reference]	Main Objects for ATP Detection	Comments
Estimate resistance to biocorrosion of new sufur-copolymer concrete composites [41]	Bacterial cells adsorbed on the sulfur polymer concrete composites	The ATP-assay controls the growth of bacteria on composites, containing sulfur.
Testing of viability of the parasitic nematode [42]	Parasitic gastrointestinal nematode *Haemonchus contortus*	Viability evaluation of adult worms causing synegemosis by bioluminescent measuring the concentration of ATP is quantitative.
Regulation of ATP homeostasis during cell dormancy and revealing critical factors regulating the energy metabolism in cyanobacterial cells [43]	*Synechocystis sp.* PCC 6803 (*Synechocystis*)	Bioluminescent ATP-measurements helps the investigation of life cycles of cyanobacterial strains.
Assessment of ATP in soil nematode [44]	*Caenorhabditis elegans*	Bioluminescent method of ATP determination was specially developed for the eukaryotic object.
Investigation of metabolism during nitrogen starvation and resuscitation in cyanobacteria [45]	*Synechocystis sp.* PCC 6803	The use of bioluminescent ATP-measurements makes it possible to study chlorosis in cyanobacteria and use them as a unique model system for studying subtle interaction of carbohydrate oxidation, CO_2 fixation and photosynthesis.
Investigation of effect of polyethylene microplastics on activated sludge used for water treatment [46]	Anaerobic sludge for urban wastewater treatment	ATP control provides information about influence of microplastics accumulated in the cells of sludge on its viability and characteristics of wastewater processing.
Other (building reconstruction, painting, archaeological samples, etc.)		
Control of characteristics of bacterial and fungal communities colonizing the surfaces of outdoor sculptures [47]	Swabbing the surfaces of the sculptures	ATP-assay evaluates biological contamination of selected areas of sculptures and gives the information about potential for deterioration of the artworks.
Searching for extraterrestrial life [48]	Simulations of Mars soil and *Escherichia coli*	ATP-assay can be applied for the detection of living cells (like *E. coli*) in the soil of Mars.
Rapid screening of organic and microbial contaminants on deteriorated mural paintings [49]	Total microbial contamination of the seventeenth-century wall paintings in the nave of the old Church	Possible implementation of the ATP bioluminescence method in an integrated system of wall painting conservation is discussed. Poor correlation was established between microbial counts and surface ATP levels.
Assessment of cleaning techniques and its effectiveness for controlling biodeterioration fungi on wall paintings [50]	*Arachnomyces* spp., *Parengyodontium album*, *Beauveria bassiana*, *Scopulariopsis* brumptii	The efficiency of mechanical and chemical methods used to clean and control the microbial biodeterioration on wall paintings was estimated based on the measured decreasing of ATP concentrations.
Control of biodegradation of textiles, stone, and wooden artifacts [51]	*Coniochaeta hoffmannii*, *Acremonium charticola*, *Cylindrotrichum clavatus*	ATP-assay showed the efficiency of used oils causing a significant decrease in the viability of fungus mycelia present in archaeological wood.

2. Main Modern Trends in the Use of Bioluminescent ATP Determinations

An analysis of a fairly wide list of studies related to various fields of application of bioluminescent ATP analysis published over the past 5 years (Table 1 [4–51]) showed that in some cases, the basic needs for using this analytical method remained similar to those that developed and were popular in previous years [1]. At the same time, the main areas of recent applications of bioluminescent ATP-metry include sanitary control, quality control of purified water, microbial analysis in the food industry, maintenance of drugs and estimation of medicines' quality, and monitoring of the state of biocatalysts used in various biotechnological processes (Table 1).

This method of analysis's application to detect the presence of various microorganisms is interesting and diverse, and it should be noted in the field of sanitary control of various objects. Here, ATP sensing is used to analyze various surfaces in hospitals and places intended for short-term stay of patients [4,5,9,10,14,15], surfaces of instruments used for examination and treatments of patients [7,8,11,12], and also as tools and places for slicing and cooking of food products [19,20], where sanitation is important for human health. The express analysis in real time and sensitivity of the bioluminescent determination of ATP, as well as the amount of total information obtained, make it possible to assess the degree of contamination by microbial cells from different points of examination, identify the main sites of cell pollutions and monitor the effectiveness of methods and reagents used for sanitary treatment. Comparing the method of bioluminescent ATP determination with microbiological methods of cell counting, the authors often note the effectiveness of the first approach [13,18,32]. It should be noted here that in some cases, the comparison of the results of ATP-metry and classical methods of microbiology do not always coincide [6,16] due to the fact that detergents and disinfectants used for sanitary treatment can partially inhibit the catalytic activity of luciferase, which forms the basis of the reagent used in bioluminescent ATP- assay. It appeared that residues of blood and urine present in samples from hospitals [6] also can change the luciferase reaction. Further, special attention will be paid to this issue, since the chemicals that may be present in the analyzed samples and affect the activity of luciferase, as the practice of ongoing research shows, may be different.

In addition, bioluminescent ATP-assay is used as a tool for evaluating newly developed sterilization technologies [17], since the high sensitivity of the method ensures the detection of even a small number of microbial cells [2,37].

Assessment of water quality using ATP-metry methods proved to be required in a variety of areas, including assessment of the effectiveness of wastewater treatment, counting hospital origin [6,33], the successful operation of seawater system used for simultaneous desalination and removal of microbial cells [34], and ballast water treatment systems of ships [35,38].

A special place in the studies of water samples using bioluminescent ATP-metry is occupied by experiments on the analysis of microalgae and plankton amounts, including Arctic water systems [36,37]. The results of these studies allow us not only to predict the expected pollution of ballast water on ships, but also provide information of an ecological nature, namely, on the development of certain microalgae in the waters of the seas and, consequently, the existence of conditions or factors affecting the level of their reproduction (increase or decrease in the concentration of cells).

Interestingly, in some cases, this method allows one to establish extremely interesting facts. In particular, when using the marine diatom microalgae *Thalassiosira weissflogii* cultivated under various conditions, it was found that the specific level of ATP per cell can remain relatively constant, despite changes in cell growth rates in the periodic cultivation regime by about 10 times [37].

It is also interesting that this method of bioluminescent ATP-assay has been modified to detect plankton, which in addition to microorganisms, includes larger inhabitants of marine waters ($\geq$50 microns) [35]. Modification of sample preparation for the application of the analytical method consisted in additional grinding of all sample components.

The use of bioluminescent ATP-assay in processes directly related to the study of the activity of microorganisms makes it very easy and efficient to assess the metabolic state of cells and react as quickly as possible in case of such a need [42–46]. It does not matter in which environment these cells are located. It is relevant to use the discussed method in collections of microorganisms to control the physiological state of a wide variety of cells [40,55].

It is likely that the use of bioluminescent analysis of ATP may prove promising in experiments aimed at searching for living organisms in space. In particular, the soil from Mars did not show a negative effect on the activity of luciferase in determining its artificial contamination by *E.coli* cells [48].

It is attractive to use the bioluminescent ATP-assay method in the development of new antimicrobial drugs, including those based on the combination of antibiotics or antimicrobial polypeptides with enzymes that are capable of quenching the quorum of various microorganisms (Gram-positive, Gram-negative bacterial cells, and yeast) [22,56–59]. In practical terms, the use of bioluminescent ATP-assay in the development of new porous materials with the functions of protection from the effects of chemical (organophosphorus neurotoxins, and mycotoxins) and biological agents (bacterial cells) seems to be a new and promising direction of the analytical method development.

The results obtained with ATP-metry are valuable for the subsequent development of the functional fibrous materials, since isolation of remaining living cells of microorganisms from tissue materials with detoxifying and decontaminating protective properties for further their analyzing by traditional microbiological methods is extremely difficult, whereas ATP extraction is possible and convenient [42–46]. In addition, the dependence of the residual concentration of intracellular ATP in some samples on the concentration of an antimicrobial drug used for treatment of cells makes it possible to establish the minimum inhibitory concentration of the substance or the resistance of microorganisms to this substance [23,24,29].

Determination of the resistance of some surfaces or coating materials to microbial contamination by using bioluminescent ATP-assay is also relevant in the development of new composite materials [41], during restoration works [47,49,50], and in the preservation of archaeological finds [51]. When assessing the susceptibility of various historical monuments to microbial damage, the authors, using bioluminescent ATP sensing, try to conduct environmental monitoring, establishing a relationship between the outbreak of microorganisms and the relevant environmental factors [49–51].

In general, it can be noted that the applications of bioluminescent ATP-metry can be very diverse and are expected to be associated not only with the presence/absence of microorganisms in some samples. In particular, studies with blood and liver cells seem to be quite voluminous in terms of the number of experiments conducted [25,27,28,30,31]. That is also a very positive area of ATP-metry application developed recently based on fundamental interest in the role of ATP in relation to the development of diseases.

The main task of all these studies remains the necessary sampling, ensuring the completeness of ATP extraction from cells, and rational consideration of factors affecting the accuracy and sensitivity of the determination, in particular, consideration of the possible inhibitory effects of various substances on the main participant in the determination of ATP (luciferase).

3. Investigation of Factors Inhibiting the Bioluminescent ATP Analysis

Discussing the effective methods of ATP extraction from microbial cells, the importance of certain conditions for the procedure realization should be mentioned: rapid cell lysis, complete release of ATP and irreversible inactivation of enzymes hydrolyzing ATP, absence of inhibition of the luciferase reaction [2,39]. However, substances that can have an inhibitory effect on luciferase are often present in media with cells to be analyzed using bioluminescent ATP-assay (Table 2).

Table 2. The effects of various compounds present in reaction medium of bioluminescent ATP assay on the reaction.

Investigated Inhibitors [Reference]	Effects
Disinfectants (sodium hypochlorite, ethanol, and benzalkonium chloride) [16]	Up to 93% inhibition of luciferase
Salts in seawater [34]	Inhibition of the luciferase reaction
NaCl [60]	Up to 79% inhibition of luciferase activity at 140 mM NaCl
Extractants (Triton X-100 and dodecyltrimethylammonium bromide) [36]	Strong inhibition of the luciferin–luciferase reaction
Potassium humate [61]	Decrease of bioluminescence at concentration 0.5 g/L
Isoflavonoids [62]	High level of luciferase inhibition
Divalent ions of various metals [63]	Luminescence decrease in presence of Hg^{2+}
Heavy metal salts [64]	Inhibition of luciferase by Pb salts
Chalcone compounds [65]	Lucifeare inhibition by compound 3i (IC_{50}—0.2 μM)
Inhibitors of biocorrosive processes [66]	Inhibition by Kathon (Inhibition constant Ki—0.81 g/L)

The use of a bioluminescent method for determining ATP to monitor the growth potential of bacteria in seawater demonstrated [34] that the high ionic strength of seawater causes significant inhibition of the luciferase reaction. To measure the concentration of ATP, it was necessary to dilute seawater with demineralized water to avoid the effect of salts on the enzyme. However, when the initial sample was diluted, the concentration of bacteria in it also significantly decreased. In addition, bacterial cells, as studies have shown, can be destroyed due to a jump in osmotic pressure when the analyzed samples were diluted. In order to improve the methodology for determining the intracellular ATP concentration in bacteria growing in seawater, the effect of the pH of seawater and the concentration of iron on the luciferase reaction was also studied. The maximum luminescence signal was observed at the pH 8 of seawater and decreased by 40% and 60% at pH 7 and 8.5, respectively. Similarly, the luminescence signal decreased with an increase in the concentration of iron in seawater from 0.1 to 10 mg/L by 62%. The limit of direct detection of ATP in seawater was 0.3 ng/L (equivalent to 1200 cells/mL).

Mutant forms of luciferase resistant to inhibition by sodium chloride were specially obtained in other work [60]. These enzymes at 140 mM NaCl maintained their activity at 67 and 79% of the initial level, respectively, whereas the enzyme without mutations had only 44% of its initial activity. By directed mutagenesis, a double mutant luciferase CR was obtained; its luminescence in the presence of NaCl was preserved by 95% compared with luminescence in the absence of an inhibitor.

During the extraction of ATP from the cells of the *Thalassiosira weissflogii* microalgae, the inhibitory effect of Triton X-100 and dodecyltrimethylammonium bromide on the luciferin-luciferase reaction was established. A way out of the situation was found: ATP extraction from the cells of these microalgae was carried out with boiled ultra-pure water to prevent the inhibition of the bioluminescent reaction [36].

Interestingly, luciferase itself, as it turned out, can be used as a test reagent, the activity of which can decrease as a result of inhibition by a number of substances [61]. In experiments supposing analysis of ATP in soil extracts, the negative effect of various humic substances (HS) in concentrations from 1 to 10 g/L was initially evaluated using bioluminescent enzymatic reactions catalyzed by firefly luciferase (Figure 1). As it turned out, at concentrations of more than 1 g/L, all HS used in the study had an inhibitory effect on luciferase. At the same time, potassium humate (CHP) and its naphthoquinone-containing derivative showed the greatest negative effect at concentrations of 0.5 g/L.

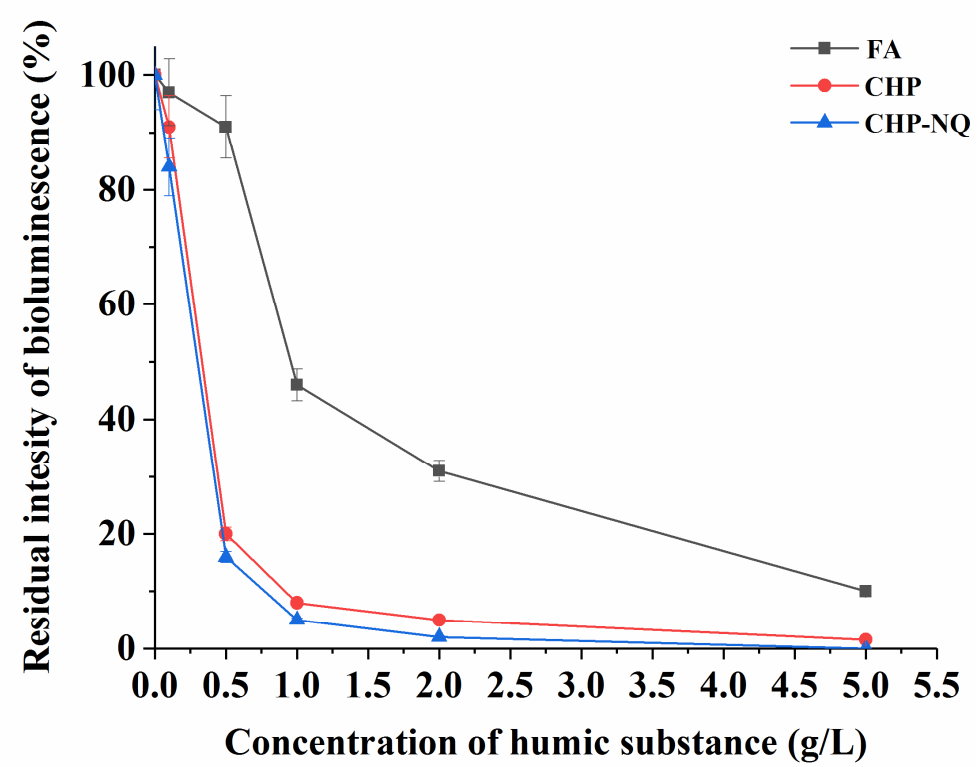

Figure 1. Decrease in the intensity of bioluminescence of firefly luciferase with an increase in the concentration of HS: FA—fulvic acids; CHP—potassium humate; CHP-NQ—potassium humate modified with naphthoquinone.

It is obvious that firefly luciferase is sensitive to inhibition by various compounds, and this can lead to distortion of the results in in vitro cell-based assays. In particular, a high degree of inhibition of the enzyme by seven isoflavonoids was shown, among which were: daidzein, genistein, glycitein, prunetin, biohanin A, calicosin, and formonetin [62].

The effect of divalent ions on the bioluminescence of luciferase of fireflies has been established and it has been shown that an increase in the concentration of Mn^{2+}, Ca^{2+} or Mg^{2+} ions does not affect the quantum yield and color of radiation, while the presence of Zn^{2+}, Cd^{2+}, Fe^{2+}, Ni^{2+} and Co^{2+} ions cause a bathochromic shift, and luminescence decreased sharply in the presence of Hg^{2+} [63].

Similar results were obtained when the effect of heavy metals on the degree of inhibition of bioluminescence of firefly luciferase was studied. Metal salts of Pb, Zn, Cu and Fe were used in concentrations from 0.5 to 2 mg/L, and it was found that the bioluminescence inhibition coefficient increases with increasing molecular weight of the compounds investigated [64].

The effect of a number of chalcone compounds used in the development of drugs on the intensity of bioluminescence of firefly luciferase revealed the strongest inhibitory effect on enzymatic activity from compound 3i (Table 2) and exceeded the effectiveness by 10 times in comparison with positive control resveratrol (IC_{50}—2.33 μM) and other L-luciferin inhibitors (IC_{50}—115 μM), dehydroluciferin (IC_{50}—2.3 μM) and dehydroluciferyl coenzyme A (IC_{50}—5 μM). The study of enzymatic kinetics showed that compound 3i is a competitive inhibitor against the aminoluciferin as one substrate and uncompetitive against ATP as other substrate. In addition, compound 3i has demonstrated excellent selectivity as a promising bioluminescent quenching agent in a simulated reporter analysis [65].

In the works on the production of inhibitors of biocorrosive processes with biocidal activity in the oil industry, a bioluminescent method was used to determine the concentration of intracellular ATP to suppress the growth of biofilms [3]. First of all, the effect of these substances on the luciferase reaction underlying the bioluminescent method for determin-

ing ATP was investigated. It is known that near the luciferin-binding site of luciferase there are SH-groups that can interact with many compounds, as a result of which modification of SH-groups occurs and complete or partial inactivation of the enzyme observes which can significantly affect the rate of the bioluminescent reaction. The influence of corrosion inhibitors such as Kathon, Khazar, Nitro-1, Nitro-2, Kaspiy-2, Kaspiy-4 on the luciferase reaction, as well as various derivatives of vegetable oils, most of which are used in the oil industry as chemical corrosion inhibitors, was studied [3,66]. To determine the kinetic parameters of the interaction of inhibitors with luciferase, their concentrations from 0.1 to 3 g/L were used and it was found that all substances inhibited the luciferase reaction. The inhibitor Kathon showed the greatest inhibitory activity (Table 2).

Thus, the current practice of using luciferase-containing reagents for the determination of ATP by the bioluminescent method continues to show that it is necessary to take into account each time the possible notable negative influence of various substances present in the analyzed samples on the enzymatic bioluminescent reaction and generation of distorted results.

4. Application of Luminescent ATP-Assay in the Development of Hybrid Catalysis and Synthetic Biology in the Form of Control and Management of the Characteristics of Processes with Natural and Artificial Consortia of Microorganisms

When developing new technologies that contribute to the achievement of sustainable development goals, strategies focused on processes that combine chemical and biological catalysts [67,68], as well as allowing the biotransformation of waste of different nature to be combined in one process, attract increasing attention.

It is known that various components of reaction media (substrates, intermediates or/and accumulated end products) can have an inhibitory effect on the metabolic activity of cells of microorganisms involved in these reactions as biocatalysts. This is one of the main reasons limiting the practical implementation of hybrid chemical-biocatalytic processes with an initial chemical stage [69]. It is possible to solve this problem by switching from the use of suspended cells to biocatalysts in immobilized form. The advantages of immobilized cells, in addition to their long period of semi-inactivation due to quorum stabilization, include the possibility of successful multiple reuse [70].

In the biotransformation of complex and combined wastes from agriculture and industry, as well as potentially toxic substances for living organisms, the formation and use of complex synthetic microbial consortia of microorganisms that are well studied and have the necessary target activity is considered as a promising approach to improving the efficiency of the process [53–55]. Cell immobilization can bring additional stabilization to such consortia [67–69]. It should be noted that the development and application of artificial consortia is considered today as one of the relevant directions in the development of synthetic biology [52,71] (Table 3).

When developing and implementing new processes related to hybrid catalysis and synthetic biology, analytical express methods, in particular, bioluminescent ATP-assay, are promising and even unique in essence tools that can be recommended for use at different stages of work with these cellular biosystems, having both complex compositions and forms of use [3,53]. The active use of bioluminescent ATP-assay in this case provides the necessary level of analysis of biosystems, when traditional methods of microbiology cannot do the same in any way, since it is almost impossible to isolate cells without changing their viability level from the carrier matrices used for immobilization.

Table 3. Application of bioluminescent ATP-assay for the study and directed regulation of biocatalytic processes using artificial consortia of microorganisms.

Consortium [Reference]	Process	Aim of Bioluminescent ATP Application
Immobilized cells of anaerobic sludge, *D.vulgaris*, bacteria of genus *Rhodococcus* [53,54]	Hybrid process of oil desulfurization and methanogenesis	Screening of biocatalysts for the ability to function under process conditions, formation of a synthetic consortium, selection of optimal conditions for the biocatalytic stage, evaluation of reactivation efficiency and storage duration of biocatalysts
Synthetic immobilized consortium of *Pseudomonas putida* and *Rhodococcus ruber* cells [55]	Destruction of organophosphorus pesticides	Selection of biocatalyst composition (ratio and proportion of biomass)
Synthetic consortium with immobilized fungi of genus *Rhizopus*/*Aspergillus* and microalgae *Chlorella vulgaris* [3]	Wastewater treatment, including those containing organophosphorus pesticides	Monitoring the duration of use of biocatalysts, control the accumulation of microalgae biomass in the cultural media

4.1. Bioluminescent ATP-Sensing to Assess the Metabolic Activity of Anaerobic Consortium Used for Biotransformation of Sulfur-Containing Extracts as Waste of Pre-Oxidized Crude Oil and Oil Fractions

Combined chemical-biocatalytic desulfurization of hydrocarbon raw materials is an example of a new hybrid process and a possible innovation for the oil and gas sector enterprises, which can potentially significantly increase the environmental friendliness and resource conservation of the oil refining processes, as well as ensure rapid extraction of sulfur from oil fractions with the subsequent biotransformation of the resulting liquid organic waste to non-toxic products [69]. This hybrid process is three-stage and implies: (1) chemical oxidation of sulfur-containing organic compounds in oil with environmentally friendly and affordable hydrogen peroxide [72]; (2) subsequent extraction of oxidized forms of sulfur-containing compounds from raw materials by extraction [72]; (3) anaerobic biotransformation of effluents in the form of sulfur-containing extracts to obtain biogas in the process of methanogenic conversion using synthetic anaerobic consortia [53,54].

The use of immobilized biocatalysts proved to be justified and expedient at the biocatalytic stage of the hybrid desulfurization process to preserve the viability and metabolic activity of cells.

Bioluminescent ATP-metry was successfully tested to assess the viability of biocatalysts at different stages during the development of the biocatalytic biotransformation of sulfur-containing wastewater obtained as a result of chemical oxidative desulfurization of oil and its fractions [3,73]: screening of promising bacterial strains with hydrolytic and acetoclastic activity; formation of synthetic microbial consortia and obtaining of immobilized biocatalysts; selection of optimal parameters for biocatalytic stage in hybrid process; determination of appropriate periods for biocatalyst application and reactivation; and long-term storage of cells under different conditions.

During the screening of biocatalysts capable of maintaining a high level of viability during biotransformation of oxidized sulfur forms that can appear in the composition of liquid hydrocarbon raw materials after its oxidation with hydrogen oxide, media containing benzothiophene sulfone (BTO_2) and/or dibenzothiophene sulfone ($DBTO_2$) were tested as model compounds. As a result of the analysis of the concentrations of intracellular ATP in the cells of these biocatalysts, it was found that the best viability indicators corresponded precisely to the immobilized variants of microbial consortia. Of the three tested natural anaerobic consortia, only one (Anaerobic sludge III) had the best characteristics in the process of desulfurization of model fuels of different compositions, which was selected for further studies [73].

In order to select the most optimal variant of the extractant (with the best extraction efficiency in relation to oxidized sulfur forms and with the least toxicity in relation to living cells), the influence of pre-selected potential candidates (ethanol, isopropanol, dimethyl-

formamide (DMFA), acetonitrile, and *N*-methylpyrrolidone) on cell viability of anaerobic sludge III was evaluated using ATP-metry [72].

It was found that after exposure of anaerobic sludge for 24 h in media containing the tested extagents with dissolved oxidized sulfur compounds, the concentration of intracellular ATP decreased most significantly when acetonitrile, DMFA, and *N*-methylpyrrolidone were added to the sludge compared to the control (based on an aqueous medium). In the presence of ethanol and isopropanol, a slight positive change in the level of ATP was observed in comparison with the control [73]. Immobilized cells, as expected, had a higher residual level of intracellular ATP in comparison with suspension cells. In general, the changes in the concentration of intracellular ATP observed over 24 h indicated that ethanol and isopropanol are the most appropriate of the whole range of potential extractants for the hybrid biotechnological process under development involving anaerobic biocatalysts in the form of active sludge [72]. Further, it was found that hydrolysates of microalgae *Chlorella vulgaris* biomass remaining after lipid extraction, as well as glucose, are a co-substrate suitable for biogas production in the presence of oxidized forms of organic sulfur-containing compounds. During the biotransformation of BTO_2 both with wastewater containing residual glucose concentrations and with biomass hydrolysis of microalgae *Chlorella vulgaris* cells, the concentration of intracellular ATP decreased in all studied biocatalysts. At the same time, the degree of influence of BTO_2 on various anaerobic cells was different. The maximum negative effect of the presence of sulfone in the medium was noted for the cells of sulfate-reducing bacteria *Desulfovibrio vulgaris*. At the same time, the presence of BTO_2 at a concentration of 0.15 mM had practically no negative effect on the viability of *Clostridium acetobutilycum* cells [70].

It was also noted in these studies that the observed decrease in the specific concentration of intracellular ATP is not a limiting factor for the further use of the studied biocatalysts and their subsequent introduction into other artificial consortia. The decrease in intracellular ATP concentration even in anaerobes under experimental conditions could be observed as a result of some limitations in nutrients present in the medium. That idea was confirmed by the data from the control (medium without BTO_2) since a decrease in the intracellular ATP concentration was also revealed there.

Bioluminescent ATP-assay was successfully tested during the establishment of the operating range of concentrations of oxidized sulfur forms on the example of $DBTO_2$, which can be introduced into a reactor with an artificially formed, mobilized consortium of the following composition: anaerobic sludge—80%, *C. acetobutylicum* B1787—10%, *D.vulgaris* B4053—10% [74]. Analysis of the results obtained showed that immobilized *D. vulgaris* cells are the most resistant to the inhibitory effects of $DBTO_2$ (Figure 2).

Thus, it was found, due to use of ATP-assay, that the effect of $DBTO_2$ [74] and BTO_2 [70] on sulfate-reducing cells is different. At a concentration of 2.5 mM $DBTO_2$, no significant decrease in the concentration of intracellular ATP was observed in all participants of the immobilized anaerobic consortium, therefore, for this sulfone, this concentration was considered acceptable as the initial one for injection into the reactor with a biocatalyst. Similar experiments were carried out to select the initial concentrations of the substrates (different sulfones and their mixtures obtained after sulfur oxidizing in real wastewater samples) or the degree of their dilutions before loading in the reactor, based on the analysis of the integral change in the concentration of ATP in cells.

The concentration of intracellular ATP was analyzed in order to monitor the physiological state of cells in the composition of synthetic biocatalytic consortia in the process of their use for biotransformation of sulfur-containing extracts of oil fractions in effluents The following fractions were used: straight-run gasoline fraction Naphtha, straight-run diesel fraction, straight-run diesel fraction, non-hydrotreated vacuum gas oil, gas condensate, crude oil. At the same time, the composition of the synthetic consortium was enhanced by the introduction of oil–degraded cells of genus *Rhodococcus* [54].

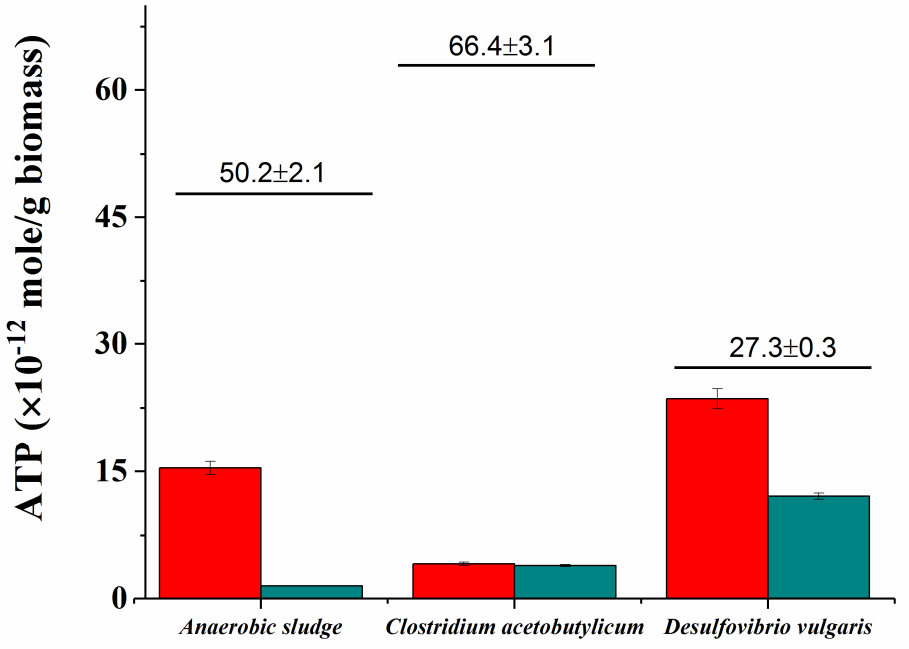

Figure 2. Assessment of the energy status by intracellular ATP concentration in the components of artificial anaerobic consortium in the methanogenesis of biomass of *Chlorella vulgaris* microalgae for 10 days in the absence (■) and presence (■) of $DBTO_2$ in the cultural medium. The lines indicate the initial levels of intracellular ATP concentrations of corresponding cells.

During the use of artificially formed consortia in immobilized form, various changes in the concentration of intracellular ATP relative to the initial level of this parameter in cells were observed. However, in none of the studied biocatalysts did this indicator decreased to levels indicating the loss of vitality by cells (the concentration of ATP decreased up to several times, and not by several orders of magnitude, which is usually observed during cell death) [53].

It was found that during the bioconversion stage of mixed substrates in the methane tank, including oxidized sulfur-containing extracts of petroleum fractions, with a decrease in intracellular ATP concentration by 15% or more, as well as with observed changes in the values of other parameters, the most effective action was the introduction of additives into the medium in the form of organic substrates. As it was shown, hydrolysates of various renewable raw materials (wheat straw, waste biomass of microalgae grown during wastewater treatment, etc.) can be used as such additives. In the presence of such co-substrates, stabilization of the metabolic activity of biocatalysts was observed, which manifested itself in the establishment of a certain constant level of intracellular ATP [70].

During the further development of the hybrid process of desulfurization of oil fractions, the main attention was paid to further spreading of the co-substrates' spectrum. For this purpose, even such a large-tonnage agricultural waste as chicken manure was used in experiments for anaerobic biotransformation. In parallel with this, the composition of the synthetic anaerobic consortium was also improved [54]. As a result of such actions and the consistent use of a methane tank and a DEAMOX (Denitrifying Ammonium Oxidation) reactor for denitrifying ammonium oxidation, a successful joint bioconversion of a vacuum gasoil extract containing high sulfur content (5.4 g COD/L), hydrolysates of microalgae and chicken manure was carried out (mass ratio of hydrolysates was 9:1, 1 g COD/L). At the same time, at all stages of this multi-stage process, the activity of biocatalysts was periodically monitored by assessing the concentration of intracellular ATP.

The possibility of multiple (at least three working cycles') use of the same biocatalysts in the process of joint bioconversion of extracts of petroleum fractions and co-substrates in the form of hydrolysates of microalgae and chicken manure was shown. The most significant decrease in the concentration of intracellular ATP was detected for immobilized

Rhodococcus opacus cells, which were part of one of the tested synthetic consortia, which contained 80% anaerobic sludge, 10% *R. opacus* cells and 10% *Desulfovibrio desulfuricans* cells. To restore the metabolic activity of immobilized artificial anaerobic consortia, they were placed for 12 days in a nutrient medium optimized for restoring their functionality, after which the viability and catalytic activity of cells were partially or completely restored [54].

The greatest degree of decrease in the concentration of ATP at the end of the working cycle was noted for the cells of oil-degrading *Rhodococcus* bacteria and *C. acetobutilycum* cells. An approach to restoring their catalytic activity (reactivation) was proposed and successfully implemented. In particular, after separation from the working medium, the biocatalysts were transferred to the appropriate nutrient media with the optimal composition for cells and exposed for 60–100 h there. After this procedure, the energy status of the cells was completely restored, corresponding to their high metabolic activity [3]. Thus, an algorithm was formulated for restoring the energy status of cells: transferring cells to a specially selected synthetic nutrient media and exposing cells in it until a high level of intracellular ATP concentration was reached. The intracellular ATP concentration in the cells during their use in hybrid process was about 10^{-12}–10^{-11} mole/g.

Based on the analysis of data on the concentration of intracellular ATP, it was shown that the procedure of immobilization of cells into poly (vinyl alcohol) cryogel granules used for the formation of biocatalysts makes it possible to successfully store artificial anaerobic consortia for a long time at −21 °C without significant loss of their activity [3,73]. It is looks like a nature-like type of immobilization and storage regime for cells. Cells immobilized in this way can be stored frozen for a long time (several years), which may be in demand for the implementation of an industrial process requiring a stock of active biocatalysts for the treatment of sulfur-containing wastewater.

Thus, using the bioluminescent method of analysis of intracellular ATP in the course of experimental studies, it was possible to identify a number of main factors that determine the effectiveness of the entire hybrid process as a whole. These factors are associated with a cascade of sequential reactions and the chemical and microbial catalysts used in them, which are taken at certain concentrations and used under certain conditions for the implementation of catalytic transformations. It turned out that the most significant factors are: the composition of the biocatalysts used; the nature of the extractant used to extract polar oxidized organic sulfur compounds; the sulfur compounds themselves present in petroleum raw materials; the environment for methanogenesis, in which, in fact, in parallel with the accumulation of biogas, the reductive transformation of oxidized sulfur compounds occurs. At the same time, it turned out that the substrate used to maintain effective methanogenesis and the accumulation of biogas with maximum methane content can be variable and interchangeable, representing a waste of food or agricultural production [3]. The bioluminescent ATP-assay confirmed that the immobilized biocatalysts showed better viability compared to suspension cultures in all comparative experiments, therefore, for the practical implementation of the hybrid process with the initial chemical stage, the use of biocatalysts in an immobilized form can be recommended.

4.2. Bioluminescent ATP-Assay in the Development of Artificial Bacterial Consortium Degrading Organophosphorus Pesticides

Another example of the successful application of bioluminescent ATP-metry is the assessment of cell viability in synthetic consortia designed for the degradation of chemically synthesized and widely used in agriculture organophosphorus pesticides (OPP), which consists of the biodegradation of residues of these substances in aqueous media. The reason for the interest in such processes is simple: the production and use of OPP in the world is not decreasing, since the problem of preserving the yields of various crops remains in the world today. Undecomposed residues of these chemical compounds accumulate in the soil and also enter water systems, and further along the food chains, they have a negative impact on living organisms [75]. One of the tasks associated with the need to find solutions to the OPP problem involves the development of effective nature-like biocatalytic systems

capable of biodegradation of OPP in aquatic environments, which is due to the possible rapid migration and spread of these pollutants together with water resources over large areas, including through atmospheric phenomena.

An artificial immobilized biocatalyst (IBC) for biodegradation of a number of OPP in the composition of agricultural wastewater was developed on the basis of an artificial consortium consisting of cells of bacteria *Pseudomonas esterophilus* and *Rhodococcus rubber*. The possibility of its repeated use for these purposes was evaluated [52]. This assessment was carried out by determining the concentration of intracellular ATP in the cells of a synthetic consortium. At the same time, the energy status was assessed not for each individual culture that makes up the artificial consortium, but for the total actual level of ATP in the samples of polymer granules of the biocatalyst prepared on the basis of these two microorganisms, which were co-immobilized by inclusion in a porous carrier. The metabolic activity of this complex biosystem introduced into porous support was monitored by the intracellular ATP-assay. It was not possible to isolate the cells from the polymer matrix without cell death and using the microbial methods for analysis. The ATP measurements gave the general information about various negative effects of different factors influencing cells in the immobilized consortium (Table 4). This information allowed an adequate assessment of the situation with the metabolic state of the immobilized biocatalyst as a whole [52]. This information coming from bioluminescent ATP-assay was especially useful in reuse of IBC.

Table 4. Degradation of paraoxon (0.15 mM) and changings of intracellular ATP concentration in bacterial cells participating in artificial immobilized consortium functioning in multiple working cycles.

Working Cycle	**1st**			**2nd**			**3rd**		
Cell concentration in IBC (%)	10	20	30	10	20	30	10	20	30
Paraoxon degradation (%)	97	97	97	58	79	92	35	67	88
ATP (nmole/g IBC)	4	14	24	4	9	20	0.7	5	14

It was found that the main tendency to decrease the level of ATP in cells of studied IBC, the metabolic state of which was controlled during the degradation of paraoxon in several consecutive working cycles, suggests that this pesticide, used by cells as the only carbon source, is not the best substrate for them. Therefore, additional co-substrates should be introduced in the culture medium with such a consortium to provide better conditions for pesticide degradation by the cells.

It was found that cells loaded at 30% biomass concentration into IBC polymer granules are characterized by the highest levels of intracellular ATP. This means that such a highly cell-concentrated biosystem has a more stable metabolic activity than other studied variants. It retained metabolic activity during the decomposition of paraoxon even when IBC was repeatedly used to carry out this process (Table 4). In this regard, the consortium of the specified composition with the used concentration of cells in the biocatalyst granules can be considered successful for the purification of aqueous media from OPP, and the method of bioluminescent ATP-metry proved to be indispensable for assessing the state of the microbial consortium and making decisions about the possibility and appropriateness of its reuse.

4.3. Application of Bioluminescent ATP-Assay in the Development of Wastewater Treatment Processes Using an Artificial Consortium Based on Microalgae Chlorella Vulgaris and Fungous Genus Rhizopus or Aspergillus

It is known that the world practice gives preference to biological methods of wastewater treatment [76]. Carrying out such processes in aerobic conditions with the use of aerobic activated sludge is usually accompanied by the accumulation of a significant amount of sludge biomass, which should be considered as a renewable source of raw materials. Among the carbon-containing or nitrogen-containing compounds usually present in vari-

ous wastewater, there may be some substances that have a negative effect or even provoke a possible toxic effect on microorganisms used in water purification (pharmaceuticals, pesticides, detergents, etc.). Since then, discussing the possibility of intensifying and increasing the efficiency and stability of the functioning of water purification processes, the prospects of using microbial cells in an immobilized form should be noted. This is a nature-like approach, because under natural conditions, cells themselves tend to form biofilms, that is, to make self-immobilization in order to increase the chance of their survival under unfavorable conditions of existence [73].

Based on this approach, consortium consisting of mycelial fungus *Rhisopus oryzae* cells immobilized in cryogel of poly (vinyl alcohol) and capable of decomposing both organic substances [3] and chemically complex xenobiotics, for example, organophosphorus compounds [77], were used in combination with immobilized microalgae *Chlorella vulgaris* inoculum for water purification (Figure 3). The microalgae cells provided rapid accumulation of suspension cells in the medium with high efficiency of water purification. The stable functioning of the immobilized cells was monitored using bioluminescent ATP.

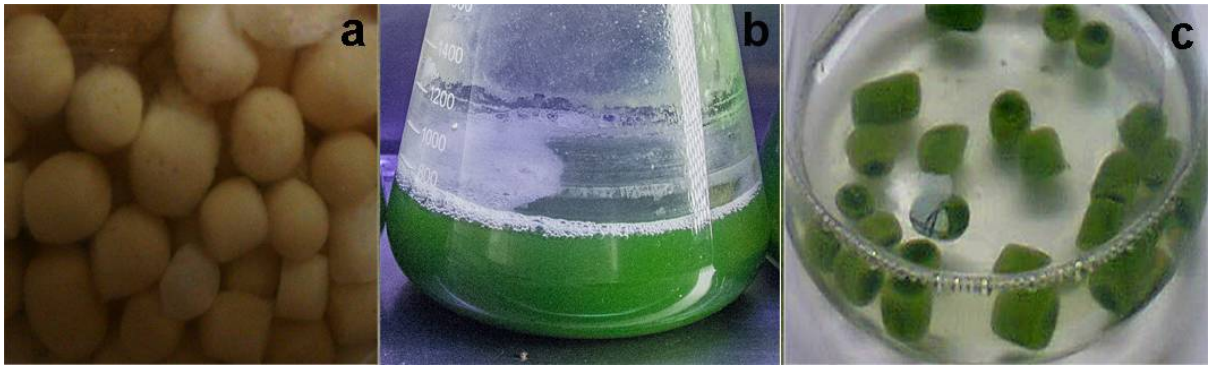

Figure 3. Photos of immobilized mycelial fungi of *R. oryzae* (**a**), and accumulating suspension of *C.vulgaris* cells (**b**) in the medium with the immobilized mycelial fungous cells, when the immobilized inoculum of microalgae *C. vulgaris* cells (**c**) are loaded into the same broth.

It is interesting that the immobilized mycelium of fungi secreting a whole spectrum of hydrolytic enzymes acts as a sorbent for suspended microalgae cells, which simplifies their "harvesting". The introduction of granules with immobilized inoculum of *Chlorella vulgaris* microalgae cells into the medium with immobilized fungus makes it possible to create a high concentration of evenly distributed cells in the reactor volume, which contributes to the rapid accumulation of the suspension "daughter" generation of *Chlorella* cells in the medium [55]. Microalgae cells produced oxygen, which was necessary for the effective functioning of the immobilized mycelial fungi. Among other things, microalgae could use organic metabolites of mycelial fungi as a source of heterotrophic nutrition. Finally, the interesting nature-like microbial system was formed in these experiments.

It should be emphasized that bioluminescent ATP analysis can be of great importance in assessing the effectiveness and finding optimal solutions for the organization of wastewater treatment processes being developed using such consortia based on immobilized cells of mycelial fungi and microalgae. Within the co-cultivation of such different cells being in the Quorum state (cells present in a form of high-density populations) in one reactor it is important to monitor the viability of both biosystem participants.

Previously, we have implemented similar processes for wastewater treatment of food industry enterprises, where consortium of microalgae *C. vulgaris* and mycelial fungi *R. oryzae* was developed and applied [3,73], and for treatment of aqueous media containing organophosphorus pesticides, where consortium of microalgae *C. vulgaris* and mycelial fungi *Aspergillus niger* were used [77]. With the help of bioluminescent ATP-assay in these processes, the viability of the main participants of the consortia was permanently monitored: the intracellular ATP concentration of immobilized mycelium and immobilized

microalgae inoculate was (1–9) $\times 10^{-7}$ mol/g dry biomass and (0.5–8.5) $\times 10^{-7}$ mol/g dry biomass, respectively. These data made it possible to estimate the vital activity of the mycelial fungi, the sorption of microalgae cells on mycelium, and appropriate duration of intermittent use of inoculum. In addition, the concentration of intracellular ATP in the medium was evaluated (it was (1–9) $\times 10^{-11}$ mol/mL), as an indicator of the presence of suspended, unsorbed cells on the mycelium surface.

Thus, the well-known classical method of bioluminescent ATP-assay has allowed "evolving" approaches to water purification together with the development of biocatalytic systems, expanding the range of their target application and allowing the creation and use of nature-like solutions.

4.4. The Use of Bioluminescent ATP-Assay in the Development of Strategies Reducing Methane Emissions as Result of Activity of Microbial Methanogenic Consortia

Today, landfill gas with a predominance of CH_4 in its composition occurs in landfills as a result of the metabolic activity of methanogenic natural microbial consortia formed spontaneously in vivo. Decreasing the concentration of greenhouse gases in the atmosphere and regulating their emissions, as well as reducing fire-hazardous situations in landfills where methane is released, is considered today as an important environmental task [78]. In the course of developing strategies to reduce most methane emissions, various chemical or biochemical agents that affect or alter the metabolic activity of cells that are part of methanogenic compounds, are used [61,79,80].

The effect of the use of various substances on the metabolic activity of cells is estimated by the change in the amount of released alkaline gas and by the concentration of CH_4 in its composition. Monitoring of landfill gas and determination of methane in it in real landfill conditions is difficult and requires expensive equipment.

The process of methanogenesis is quite long, but the search for methods that allow us to quickly and adequately assess the impact of various measures taken and reagents introduced to reduce the effectiveness (productivity) of methanogenesis is extremely relevant. Since anaerobic consortia functioning under various conditions have a specific micro-biological profile, it is difficult to determine which cells of microorganisms involved in the formation of biogas are inhibited under the influence of certain substances.

Despite the taxonomic diversity due to the substrates and conditions of consortium formation, several microorganisms can be identified that occur in the prevailing majority of cases. Stable participants are bacteria of the genera *Pseudomonas*, *Clostridium*, *Enterobacter*, *Bacillus*, *Lactobacillus*, *Desulfovibrio*, and *Flavobacterium* and archaea of the genera *Methanosarcina*, *Methanobacterium*, *Methanobrevibacter* and *Methanosaeta* (Figure 4). It is quite obvious that the palette of participants in methanogenic communities is very wide. Therefore, the selection of substances and their combinations for suppression of methanogenesis carried out by natural very complex consortia is interesting and important for investigation.

The results of assessing the effect of various methanogenesis inhibitors on the metabolic activity of natural methanogenic consortia were compared by the following way: the concentration of intracellular ATP was analyzed by the bioluminescent method concurrently with the determination of characteristics traditionally used to assess the methanogenic process (the efficiency of the process and the CH_4 content in the accumulating biogas) (Table 5).

The use of natural anaerobic consortia in the methanogenic process conducted in presence of 1 to 10 g/L FA showed that such additives stimulate the accumulation of biogas, and the CH_4 content in biogas remains high enough (30–46%). At the same time, the cells retain a high concentration of intracellular ATP [81].

The presence of fulvic acids modified with naphthoquinone (FA-HQ) in the methanogenic medium also has no significant negative effect on the energy status of bacterial cells in methanogenic consortia. At the same time, there was a slight decrease in the efficiency of biogas accumulation. The proportion of CH_4 in the composition of biogas decreased with an increase in the 5 g/L concentration of FA-HQ by 10% compared to 1 g/L, and a further increase in the concentration to 10 g/L led to an increase in the proportion of CH_4 by the same

10% [61]. A high concentration of intracellular ATP was preserved in cells at all concentrations of FA-HQ, including 10 g/L. Thus, the level of ATP concentration coincided with the main effects observed with other characteristics of the process.

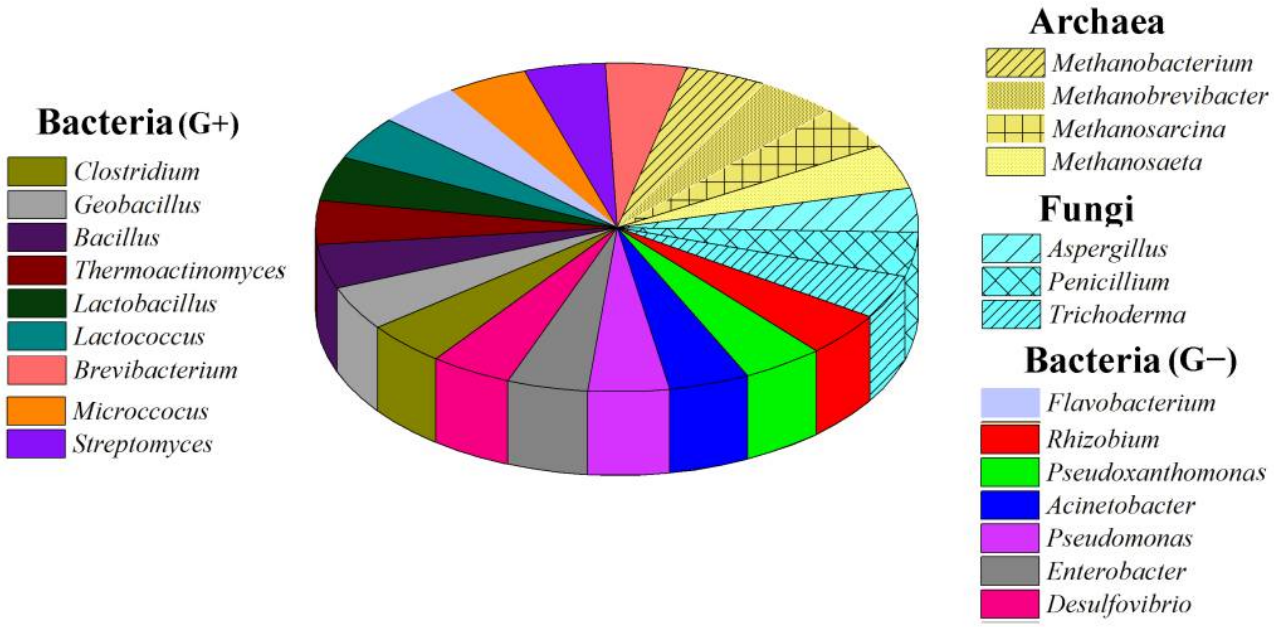

Figure 4. A palette of the most common microorganisms present in anaerobic consortia producing biogas, the action of which is a target for suppression by reagents.

Table 5. Effect of various suppressors on the action characteristics of natural methanogenic consortia (biogas production, CH_4 content, and intracellular ATP concentration).

Suppressor	Concentration (g/L)	Efficiency of Biogas Production (%)	CH_4 Content in Biogas (%)	ATP ($\times 10^{-11}$ mole/mL)
FA	1.0	88.3 ± 2.7	46.4 ± 1.4	30.2 ± 1.5
	5.0	91.4 ± 2.7	40.4 ± 1.2	22.6 ± 1.2
	10.0	97.2 ± 2.9	32.2 ± 1.0	18.7 ± 0.9
FA-HQ *	1.0	60 ± 2.6	28.6 ± 0.8	19.2 ± 1.0
	5.0	46 ± 2.7	18.4 ± 0.6	16.4 ± 0.8
	10.0	42 ± 2.9	28.1 ± 0.8	10.6 ± 0.6
CHP	1.0	68.4 ± 2.1	47.4 ± 1.4	6.8 ± 0.5
	5.0	57.2 ± 1.7	42.1 ± 1.3	3.2 ± 0.3
	10.0	48.3 ± 1.5	35.2 ± 1.1	1.5 ± 0.1
CHP-NQ	5.0	72.0 ± 2.1	37.4 ± 1.1	4.5 ± 0.3
	10.0	58.5 ± 1.8	13.3 ± 0.4	2.2 ± 0.1
$FeSO_4$	5.0	75.4 ± 2.3	36.3 ± 1.1	4.9 ± 0.3
$K_2S_2O_8$	5.0	64.8 ± 1.9	5.3 ± 0.2	3.5 ± 0.2
Bacitracin	0.2	48.7 ± 1.5	27.6 ± 0.8	1.5 ± 0.1
His_6-OPH **	0.002	81.8 ± 2.5	42.3 ± 1.3	7.1 ± 0.5
$K_2S_2O_8$ + His_6-OPH +Bacitracin	5.0/0.004/0.1	42.2 ± 1.3	1.3 ± 0.0	4.0 ± 0.2

* FA-HQ—fulvic acids modified with naphthoquinone; ** His_6-OPH—hexahistidine-containing organophosphorus hydrolase.

With the introduction of CHP at concentrations of 1–10 g/L, the efficiency of methanogenesis decreased, and the concentration of ATP did the same, however, the proportion of

CH_4 in biogas remained high, which indicated that archaea, the main producers of methane, were not affected by these inhibitors.

The introduction of CHP-NQ revealed a similar pattern in the change in the concentration of intracellular ATP of cells that are part of methanogenic natural consortia, as in the case of natural CHP. The bioluminescent ATP-assay confirmed the suppressive effect of CHP derivatives modified with naphthoquinone (CHP-NQ) on the cell metabolism of anaerobic consortia. The decrease in ATP level inside of cells became more obvious with increased substances' concentration up to 10 g/L in the cultivation medium. Together with ATP, the efficiency of the accumulation of biogas and CH_4 in its composition decreased.

The presence of iron sulfate in the medium did not significantly affect the characteristics of methanogenesis and the concentration of ATP in cells, although it is known that the formation of methane is strongly suppressed under conditions of iron reduction [80].

In the presence of $K_2S_2O_8$, it was possible to achieve a significant effect of reducing the proportion of CH_4 in the composition of the accumulated biogas, while the process of biogas accumulation itself was very intensive. This indicated that the introduction of this additive mainly affected the metabolic processes of methanogenic archaea and did not affect the metabolism of other microorganisms that are part of the anaerobic consortium, which mainly produced carbon dioxide [80].

There was a decrease in the efficiency of biogas production and intracellular ATP concentration when bacitracin was injected into the medium (0.2 g/L), but the proportion of CH_4 in the biogas composition remained high (27%). This indicated a greater activity of this antibiotic against those microorganisms that catalyze biochemical reactions preceding the action of archaea and the accumulation of methane in multistage methanogenesis [80].

The introduction of enzyme His_6-OPH did not affect the metabolic activity of the methanogenic consortium. However, when this enzyme was combined with the antibiotic bacitracin and $K_2S_2O_8$, taken in low enough concentrations, a maximum decrease in the proportion of CH_4 in biogas was observed. At the same time, it turned out that the level of ATP in the cells decreased significantly under the action of this compounds' mixture, although in general, cells retained their viability [80].

Generally, the bioluminescent method for determination of intracellular ATP made it possible to supplement the overall picture of the obtained characteristics of methanogenesis occurring under the influence of various suppressors, as well as to fairly clearly assess the effect of the introduction of various substances on the effectiveness of the methanogenesis process carried out by such complex systems as natural methanogenic consortia.

5. Conclusions

Timely and rapid bioluminescent analysis of ATP in various samples with verified interpretation of obtained results by other methods becomes an important lever in the development of various scientific directions (Figure 5), in the management of biochemical and biocatalytic processes and makes it possible to simplify scientific and practical research. In some cases ATP-assay even makes the impossible investigation possible, in particular, in the analysis of cells in media (in complex consortia, in tissues, in polymer matrices, etc.), from which cells cannot be isolated for control by traditional methods without damaging them.

The information provided in this review confirms this. At the same time, the list of directions of using bioluminescent ATP-assay, considered in this review, is certainly not exhaustive [82], and it should be noted that today, in addition to applied research, theoretical studies are actively developing, in particular, aimed at clarifying the role of ATP as protein-solvating agent, while maintaining high concentrations of proteins in their active forms without aggregation [83]. This basic knowledge can provide a new approach to the prevention of many diseases due to the understanding that ATP performs an additional important function in living organisms, which can be taken into account in further research with ATP-assay. The combination of luciferases possesing improved characteristics [84–86] with the opportunity to use portable light detectors compatible with smartphones [87] can

form a perspective for the further development of bioluminescent ATP-assay and its active application in everyday life.

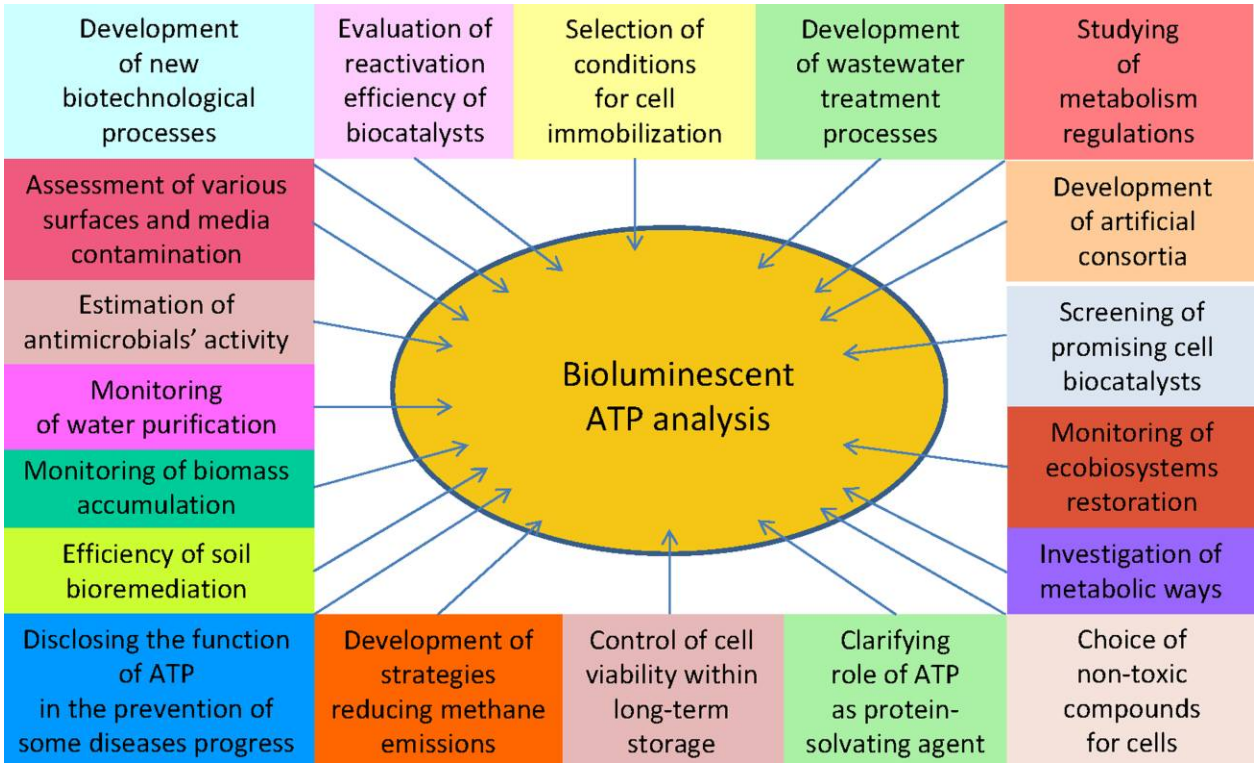

Figure 5. Generalizing scheme of recent applications of bioluminescent ATP-assay.

Author Contributions: Conceptualization, E.E.; methodology, E.E., O.S., N.S., O.M. and G.Y.L.; investigation, E.E., O.S., N.S., O.M., G.Y.L. and N.U.; data curation E.E.; writing—original draft preparation, E.E., O.S., N.S., O.M., G.Y.L. and N.U.; writing—review and editing, E.E., and N.U.; visualization, O.S., N.S. and O.M.; supervision, E.E.; project administration, N.U. All authors have read and agreed to the published version of the manuscript.

Funding: The research was funded by State Task of Lomonosov Moscow State University (121041500039-8).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: This research was performed according to the Development program of the Interdisciplinary Scientific and Educational School of Lomonosov Moscow State University—"The future of the planet and global environmental change".

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Amodio, E.; Dino, C. Use of ATP bioluminescence for assessing the cleanliness of hospital surfaces: A review of the published literature (1990–2012). *J. Infect Public Health* **2014**, *7*, 92–98. [CrossRef] [PubMed]
2. Lomakina, G.Y.; Modestova, Y.A.; Ugarova, N.N. Bioluminescence assay for cell viability. *Biochemistry* **2015**, *80*, 701–713. [CrossRef] [PubMed]
3. Efremenko, E.N.; Ugarova, N.N.; Lomakina, G.Y.; Senko, O.V.; Stepanov, N.A.; Maslova, O.V.; Aslanly, A.G.; Lyagin, I.V. *Bioluminescent ATP-Metry: Practical Aspects*; Scientific Library: Moscow, Russia, 2022; 376p, ISBN 978-5-907497-77-1. [CrossRef]

4. Sifuentes, L.Y.; Fankem, S.L.; Reynolds, K.; Tamimi, A.H.; Gerba, C.P.; Koenig, D. Use of ATP readings to predict a successful hygiene intervention in the workplace to reduce the spread of viruses on fomites. *Food Environ. Virol.* **2017**, *9*, 14–19. [CrossRef] [PubMed]
5. Sanna, T.; Dallolio, L.; Raggi, A.; Mazzetti, M.; Lorusso, G.; Zanni, A.; Farruggia, P.; Leoni, E. ATP bioluminescence assay for evaluating cleaning practices in operating theatres: Applicability and limitations. *BMC Infect. Dis.* **2018**, *18*, 583. [CrossRef]
6. Arroyo, M.G.; Ferreira, A.M.; Frota, O.P.; Rigotti, M.A.; de Andrade, D.; Brizzotti, N.S.; Peresi, J.T.M.; Castilho, E.M.; de Almeida, M.T.G. Effectiveness of ATP bioluminescence assay for presumptive identification of microorganisms in hospital water sources. *BMC Infect. Dis.* **2017**, *17*, 458. [CrossRef]
7. Veiga-Malta, I. Preventing healthcare-associated infections by monitoring the cleanliness of medical devices and other critical points in a sterilization service. *Biomed. Instrum. Technol.* **2016**, *50*, 45–52. [CrossRef]
8. Masia, M.D.; Dettori, M.; Deriu, G.M.; Bellu, S.; Arcadu, L.; Azara, A.; Piana, A.; Palmieri, A.; Arghittu, A.; Castiglia, P. ATP bioluminescence for assessing the efficacy of the manual cleaning procedure during the reprocessing of reusable surgical instruments. *Healthcare* **2021**, *9*, 352. [CrossRef]
9. Khandelwal, A.; Lapolla, B.; Bair, T.; Grinstead, F.; Hislop, M.; Greene, C.; Bigham, M.T. Enhanced disinfection with hybrid hydrogen peroxide fogging in a critical care setting. *BMC Infect Dis.* **2022**, *22*, 758. [CrossRef]
10. Mitchell, B.G.; McGhie, A.; Whiteley, G.; Farrington, A.; Hall, L.; Halton, K.; White, N.M. Evaluating bio-burden of frequently touched surfaces using adenosine triphosphate bioluminescence (ATP): Results from the researching effective approaches to cleaning in hospitals (REACH) trial. *Infect Dis. Health* **2020**, *25*, 168–174. [CrossRef]
11. Gedik, H.; Günay, L.; Şahin, E.C.; Sharifzade, M. The improvement of endoscope reprocessing with ATP-bioluminescence tool. *Ann. Med. Health. Sci. Res.* **2018**, *8*, 6–9.
12. Watanabe, A.; Tamaki, N.; Yokota, K.; Matsuyama, M.; Kokeguchi, S. Use of ATP bioluminescence to survey the spread of aerosol and splatter during dental treatments. *J. Hosp. Infect.* **2018**, *99*, 303–305. [CrossRef] [PubMed]
13. Yefimov, S.V. Sterility test: Comparison of the sensitivity of rapid microbiological methods based on ATP bioluminescence with compendial plate count method. Establishment of a scale of sensitivity-Transition from qualitative microbiological method to quantitative method. *Int. J. Chem. Pharm. Sci.* **2022**, *13*, 9–15.
14. van Arkel, A.; Willemsen, I.; Kluytmans, J. The correlation between ATP measurement and microbial contamination of inanimate surfaces. *Antimicrob. Resist. Infect. Control* **2021**, *10*, 16. [CrossRef] [PubMed]
15. Cannon, J.L.; Park, G.W.; Anderson, B.; Leone, C.; Chao, M.; Vinjé, J.; Fraser, A.M. Hygienic monitoring in long-term care facilities using ATP, crAssphage, and human noroviruses to direct environmental surface cleaning. *Am. J. Infect Control* **2022**, *50*, 289–294. [CrossRef] [PubMed]
16. Bakke, M.; Suzuki, S. Development of a novel hygiene monitoring system based on the detection of total adenylate (ATP + ADP + AMP). *J. Food Prot.* **2018**, *81*, 729–737. [CrossRef] [PubMed]
17. Liao, X.; Li, J.; Suo, Y.; Chen, S.; Ye, X.; Liu, D.; Ding, T. Multiple action sites of ultrasound on *Escherichia coli* and *Staphylococcus aureus*. *Food Sci. Hum. Wellness* **2018**, *7*, 102–109. [CrossRef]
18. Sogin, J.H.; Lopez-Velasco, G.; Yordem, B.; Lingle, C.K.; David, J.M.; Çobo, M.; Worobo, R.W. Implementation of ATP and microbial indicator testing for hygiene monitoring in a Tofu production facility improves product quality and hygienic conditions of food contact surfaces: A case study. *Appl. Environ. Microbiol.* **2021**, *87*, e02278-20. [CrossRef]
19. Altemimi, A.B.; Alhelfi, N.; Ali, A.A.; Pasqualone, A.; Fidan, H.; Abedelmaksoud, T.G.; Giuffrè, A.M.; Ibrahim, S.A. Evaluation of baseline cleanliness of food contact surfaces in Basrah Governorate restaurants using ATP-bioluminescence to assess the effectiveness of HACCP application in Iraq. *Ital. J. Food Sci.* **2022**, *34*, 66–90. [CrossRef]
20. Rodrigues, L.B.; dos Santos, L.R.; Rizzo, N.N.; Ferreira, D.; de Oliveira, A.P.; Levandowski, R.; do Nascimento, V.P. ATP-bioluminescence and conventional microbiology for hygiene evaluation of cutting room surfaces in poultry slaughterhouse. *Acta Sci. Vet.* **2018**, *46*, 6. [CrossRef]
21. Volovyk, T.; Yegorova, A.; Kylymenchuk, O.; Okhotska, M.; Kyshenia, A.; Khareba, V. ATP monitoring as an express method to determine contamination of objects. *Food Sci. Technol.* **2019**, *13*, 112–117. [CrossRef]
22. Lyagin, I.; Stepanov, N.; Frolov, G.; Efremenko, E. Combined modification of fiber materials by enzymes and metal nanoparticles for chemical and biological protection. *Int. J. Mol. Sci.* **2022**, *23*, 1359. [CrossRef] [PubMed]
23. Matsui, A.; Niimi, H.; Uchiho, Y.; Kawabe, S.; Noda, H.; Kitajima, I. A rapid ATP bioluminescence-based test for detecting levofloxacin resistance starting from positive blood culture bottles. *Sci. Rep.* **2019**, *9*, 13565. [CrossRef]
24. Jarrad, A.M.; Blaskovich, M.A.T.; Prasetyoputri, A.; Karoli, T.; Hansford, K.A.; Cooper, M.A. Detection and investigation of eagle effect resistance to vancomycin in *Clostridium* difficile with an ATP-bioluminescence assay. *Front. Microbiol.* **2018**, *9*, 1420. [CrossRef] [PubMed]
25. Wang, L.; Li, Y.; Guo, R.; Li, S.; Chang, A.; Zhu, Z.; Tu, P. Optimized bioluminescence analysis of adenosine triphosphate (ATP) released by platelets and its application in the high throughput screening of platelet inhibitors. *PLoS ONE* **2019**, *14*, e0223096. [CrossRef] [PubMed]
26. Mishra, P.; Rai, S.; Manjithaya, R. A novel dual luciferase based high throughput assay to monitor autophagy in real time in yeast *S. cerevisiae*. *Biochem. Biophys. Rep.* **2017**, *11*, 138–146. [CrossRef]
27. Vickers, A.E.M.; Ulyanov, A.V.; Fisher, R.L. Liver effects of clinical drugs differentiated in human liver Slices. *Int. J. Mol. Sci.* **2017**, *18*, 574. [CrossRef]

28. Starokozhko, V.; Vatakuti, S.; Schievink, B.; Merema, M.T.; Asplund, A.; Synnergren, J.; Aspegren, A.; Groothuis, G.M.M. Maintenance of drug metabolism and transport functions in human precision cut liver slices during prolonged incubation for 5 days. *Arch. Toxicol.* **2017**, *91*, 2079–2092. [CrossRef] [PubMed]
29. Markovich, Z.R.; Hartman, J.H.; Ryde, I.T.; Hershberger, K.A.; Joyce, A.S.; Ferguson, P.L.; Meyer, J.N. Mild pentachlorophenol-mediated uncoupling of mitochondria depletes ATP but does not cause an oxidized redox state or dopaminergic neurodegeneration in *Caenorhabditis elegans*. *Curr. Res. Toxicol.* **2022**, *3*, 100084. [CrossRef]
30. Kato, H.; Okabe, K.; Miyake, M.; Hattori, K.; Fukaya, T.; Tanimoto, K.; Beini, S.; Mizuguchi, M.; Torii, S.; Arakawa, S.; et al. ER-resident sensor PERK is essential for mitochondrial thermogenesis in brown adipose tissue. *Life Sci. Alliance* **2020**, *3*, e201900576. [CrossRef]
31. Dosch, M.; Zindel, J.; Jebbawi, F.; Melin, N.; Sanchez-Taltavull, D.; Stroka, D.; Candinas, D.; Beldi, G. Connexin-43-dependent ATP release mediates macrophage activation during sepsis. *eLife* **2019**, *8*, e42670. [CrossRef]
32. Ugarova, N.N.; Lomakina, G.Y.; Perevyshina, T.A.; Otrashevskaya, E.V.; Chernikov, S.V. Controlling BCG Vaccine's cell viability in the process of its production by an bioluminescent ATP assay. *Mosc. Univ. Chem. Bull.* **2019**, *74*, 191–197. [CrossRef]
33. Malvestiti, J.A.; Cruz-Alcalde, A.; Lopez-Vinent, N.; Dantas, R.F.; Sans, C. Catalytic ozonation by metal ions for municipal wastewater disinfection and simulataneous micropollutants removal. *Appl. Catal. B* **2019**, *259*, 118104. [CrossRef]
34. Abushaban, A.; Mangal, M.N.; Salinas-Rodriguez, S.G.; Nnebuo, C.; Mondal, S.; Goueli, S.A.; Schippers, J.C.; Kennedy, M.D. Direct measurement of ATP in seawater and application of ATP to monitor bacterial growth potential in SWRO pre-treatment systems. *Desalin. Water Treat.* **2017**, *99*, 91–101. [CrossRef]
35. Curto, A.L.; Stehouwer, P.; Gianoli, C.; Schneider, G.; Raymond, M.; Bonamin, V. Ballast water compliance monitoring: A new application for ATP. *J. Sea Res.* **2018**, *133*, 124–133. [CrossRef]
36. Bochdansky, A.B.; Stouffer, A.N.; Washington, N.N. Adenosine triphosphate (ATP) as a metric of microbial biomass in aquatic systems: New simplified protocols, laboratory validation, and a reflection on data from the literature. *Limnol. Oceanogr. Methods* **2021**, *19*, 115–131. [CrossRef]
37. Fukuba, T.; Noguchi, T.; Okamura, K.; Fujii, T. Adenosine triphosphate measurement in deep sea using a microfluidic device. *Micromachines* **2018**, *9*, 370. [CrossRef]
38. Hyun, B.; Cha, H.G.; Lee, N.; Yum, S.; Baek, S.H.; Shin, K. Development of an ATP assay for rapid onboard testing to detect living microorganisms in ballast water. *J. Sea Res.* **2018**, *133*, 73–80. [CrossRef]
39. Abelho, M. ATP as a measure of microbial biomass. In *Methods to Study Litter Decomposition (A Practical Guide)*, 2nd ed.; Barlocher, F., Gessner, M.O., Graca, M.A.S., Eds.; Springer Nature Switzerland AG: Gewerbesrasse, Switzerland, 2020; pp. 291–299. ISBN 978-3-030-30514-7. [CrossRef]
40. Bajerski, F.; Stock, J.; Hanf, B.; Darienko, T.; Heine-Dobbernack, E.; Lorenz, M.; Naujox, L.; Keller, E.R.J.; Schumacher, H.M.; Friedl, T.; et al. ATP content and cell viability as indicators for cryostress across the diversity of life. *Front. Physiol.* **2018**, *9*, 921. [CrossRef]
41. Gutarowska, B.; Kotynia, R.; Bieliński, D.; Anyszka, R.; Wręczycki, J.; Piotrowska, M.; Koziróg, A.; Berłowska, J.; Dziugan, P. New sulfur organic polymer-concrete composites containing waste materials: Mechanical characteristics and resistance to biocorrosion. *Materials* **2019**, *12*, 2602. [CrossRef]
42. Nguyen, L.; Zajíčková, M.; Mašátová, E.; Matouskova, P.; Skálová, L. The ATP bioluminescence assay: A new application and optimization for viability testing in the parasitic nematode *Haemonchus contortus*. *Vet. Res.* **2021**, *52*, 124. [CrossRef]
43. Doello, S.; Burkhardt, M.; Forchhammer, K. The essential role of sodium bioenergetics and ATP homeostasis in the developmental transitions of a cyanobacterium. *Curr. Biol.* **2021**, *31*, 1606–1615. [CrossRef] [PubMed]
44. Palikaras, K.; Tavernarakis, N. Intracellular assessment of ATP levels in *Caenorhabditis elegans*. *BIO-PROTOCOL* **2017**, *6*, e2048. [CrossRef] [PubMed]
45. Doello, S.; Klotz, A.; Makowka, A.; Gutekunst, K.; Forchhammer, K. A specific glycogen mobilization strategy enables rapid awakening of dormant cyanobacteria from chlorosis. *Plant Physiol.* **2018**, *177*, 594–603. [CrossRef] [PubMed]
46. Alvim, C.B.; Castelluccio, S.; Ferrer-Polonio, E.; Bes-Piá, M.; Mendoza-Roca, J.; Fernández-Navarro, J.; Alonso, J.; Amorós, I. Effect of polyethylene microplastics on activated sludge process—Accumulation in the sludge and influence on the process and on biomass characteristics. *Process Saf. Environ. Prot.* **2021**, *148*, 536–547. [CrossRef]
47. Silva, N.C.; Madureira, A.R.; Pintado, M.; Moreira, P.R. Biocontamination and diversity of epilithic bacteria and fungi colonising outdoor stone and mortar sculptures. *Appl. Microbiol. Biotechnol.* **2022**, *106*, 3811–3828. [CrossRef]
48. Enya, K.; Sasaki, S. Laboratory experiment of ATP measurement using Mars soil simulant: As a method for extraterrestrial life detection. *Anal. Sci.* **2022**, *38*, 725–730. [CrossRef]
49. Unković, N.; Ljaljević Grbić, M.; Stupar, M.; Vukojević, J.; Subakov-Simić, G.; Jelikić, A.; Stanojević, D. ATP bioluminescence method: Tool for rapid screening of organic and microbial contaminants on deteriorated mural paintings. *Nat. Prod. Res.* **2019**, *33*, 1061–1069. [CrossRef]
50. He, D.; Wu, F.; Ma, W.; Gu, J.-D.; Xu, R.; Hu, J.; Yue, Y.; Ma, Q.; Wang, W.; Li, S.-W. Assessment of cleaning techniques and its effectiveness for controlling biodeterioration fungi on wall paintings of Maijishan Grottoes. *Int. Biodeterior. Biodegrad.* **2022**, *171*, 105406. [CrossRef]

51. Antonelli, F.; Bartolini, M.; Plissonnier, M.L.; Esposito, A.; Galotta, G.; Ricci, S.; Davidde Petriaggi, B.; Pedone, C.; Di Giovanni, A.; Piazza, S.; et al. Essential oils as alternative biocides for the preservation of waterlogged archaeological wood. *Microorganisms* **2020**, *8*, 2015. [CrossRef]
52. Efremenko, E.; Stepanov, N.; Maslova, O.; Senko, O.; Aslanli, A.; Lyagin, I. "Unity and struggle of opposites" as a basis for the functioning of synthetic bacterial immobilized consortium that continuously degrades organophosphorus pesticides. *Microorganisms* **2022**, *10*, 1394. [CrossRef]
53. Maslova, O.; Senko, O.; Stepanov, N.; Gladchenko, M.; Gaydamaka, S.; Akopyan, A.; Polikarpova, P.; Lysenko, S.; Anisimov, A.; Efremenko, E. Formation and use of anaerobic consortia for the biotransformation of sulfur-containing extracts from pre-oxidized crude oil and oil fractions. *Bioresour. Technol.* **2021**, *319*, 124248. [CrossRef] [PubMed]
54. Maslova, O.; Senko, O.; Stepanov, N.; Gladchenko, M.; Gaydamaka, S.; Akopyan, A.; Eseva, E.; Anisimov, A.; Efremenko, E. Sulfur containing mixed wastes in anaerobic processing by new immobilized synthetic consortia. *Bioresour. Technol.* **2022**, *362*, 127794. [CrossRef] [PubMed]
55. Senko, O.; Stepanov, N.; Maslova, O.; Efremenko, E. "Nature-like" cryoimmobilization of phototrophic microorganisms: New opportunities for their long-term storage and sustainable use. *Sustainability* **2022**, *14*, 661. [CrossRef]
56. Aslanli, A.; Domnin, M.; Stepanov, N.; Efremenko, E. "Universal" antimicrobial combination of bacitracin and His_6-OPH with lactonase activity, acting against various bacterial and yeast cells. *Int. J. Mol. Sci.* **2022**, *23*, 9400. [CrossRef] [PubMed]
57. Lyagin, I.; Maslova, O.; Stepanov, N.; Presnov, D.; Efremenko, E. Assessment of composite with fibers as a support for antibacterial nanomaterials: A case study of bacterial cellulose, polylactide and usual textile. *Fibers* **2022**, *10*, 70. [CrossRef]
58. Aslanli, A.; Lyagin, I.; Stepanov, N.; Presnov, D.; Efremenko, E. Bacterial cellulose containing combinations of antimicrobial peptides with various QQ enzymes as a prototype of an "enhanced antibacterial" dressing: In silico and in vitro data. *Pharmaceutic* **2020**, *12*, 1155. [CrossRef] [PubMed]
59. Frolov, G.; Lyagin, I.; Senko, O.; Stepanov, N.; Pogorelsky, I.; Efremenko, E. Metal nanoparticles for improving bactericide functionality of usual fibers. *Nanomaterials* **2020**, *10*, 1724. [CrossRef]
60. Yawata, S.; Noda, K.; Shimomura, A.; Kuroda, A. Mutant firefly luciferase enzymes resistant to the inhibition by sodium chloride. *Biotechnol. Lett.* **2021**, *43*, 1585–1594. [CrossRef]
61. Efremenko, E.; Senko, O.; Stepanov, N.; Mareev, N.; Volikov, A.; Perminova, I. Suppression of methane generation during methanogenesis by chemically modified humic compounds. *Antioxidants* **2020**, *9*, 1140. [CrossRef]
62. Kenda, M.; Vegelj, J.; Herlah, B.; Perdih, A.; Mladěnka, P.; Sollner Dolenc, M. Evaluation of firefly and *Renilla* luciferase inhibition in reporter-gene assays: A case of isoflavonoids. *Int. J. Mol. Sci.* **2021**, *22*, 6927. [CrossRef]
63. Kotlobay, A.A.; Kaskova, Z.M.; Yampolsky, I.V. Palette of luciferases: Natural biotools for new applications in biomedicine. *Acta Nat.* **2020**, *12*, 15–27. [CrossRef]
64. Ratnawulan, R.; Fauzi, A.; Arma, A.S. Effects of heavy metals on intensity and coefficient of inhibition of firefly bioluminescence. *J. Phys. Conf. Ser.* **2019**, *1185*, 012016. [CrossRef]
65. Zhang, H.; Su, J.; Lin, Y.; Bai, H.; Liu, J.; Chen, H.; Du, L.; Gu, L.; Li, M. Inhibiting firefly bioluminescence by chalcones. *Anal. Chem.* **2017**, *89*, 6099–6105. [CrossRef]
66. Ismayilov, I.T.; Stepanov, N.A.; Efremenko, E.N.; Abbasov, V.M. Evaluation of biocidal properties of vegetable oil-based corrosion inhibitors using bioluminescent enzymatic method. *Mosc. Univ. Chem. Bull.* **2015**, *70*, 197–201. [CrossRef]
67. Clayton, A.D.; Labes, R.; Blacker, A.J. Combination of chemocatalysis and biocatalysis in flow. *Curr. Opin. Green Sustain. Chem.* **2020**, *26*, 100378. [CrossRef]
68. Heuson, E.; Dumeignil, F. The various levels of integration of chemo- and bio-catalysis towards hybrid catalysis. *Catal. Sci. Technol.* **2020**, *10*, 7082–7100. [CrossRef]
69. Maslova, O.; Senko, O.; Akopyan, A.; Lysenko, S.; Anisimov, A.; Efremenko, E. Nanocatalysts for oxidative desulfurization of liquid fuel: Modern solutions and the perspectives of application in hybrid chemical-biocatalytic processes. *Catalysts* **2021**, *11*, 1131. [CrossRef]
70. Efremenko, E.N. *Immobilized Cells: Biocatalysts and Processes*; RIOR: Moscow, Russia, 2018; p. 524. ISBN 978-5-369-02004-3. [CrossRef]
71. Che, S.; Men, Y. Synthetic microbial consortia for biosynthesis and biodegradation: Promises and challenges. *J. Ind. Microbiol. Biotechnol.* **2019**, *46*, 1343–1358. [CrossRef]
72. Akopyan, A.V.; Polikarpova, P.D.; Anisimov, A.V.; Lysenko, S.V.; Maslova, O.V.; Senko, O.V.; Efremenko, E.N. Oxidation of dibenzothiophene with the subsequent bioconversion of sulfone. *Theor. Found. Chem. Eng.* **2021**, *55*, 778–785. [CrossRef]
73. Senko, O.; Maslova, O.; Gladchenko, M.; Gaydamaka, S.; Efremenko, E. Biogas production from biomass of microalgae *Chlorella vulgaris* in the presence of benzothiophene sulfone. *IOP Conf. Ser. Mater. Sci. Eng.* **2019**, *525*, 012089. [CrossRef]
74. Senko, O.V.; Maslova, O.V.; Efremenko, E.N. Optimization potential of anaerobic biocatalytic processes using intracellular ATP concentration as the main criterion for decision making. *IOP Conf. Ser. Mater. Sci. Eng.* **2020**, *848*, 012080. [CrossRef]
75. Fu, H.; Tan, P.; Wang, R.; Li, S.; Liu, H.; Yang, Y.; Wu, Z. Advances in organophosphorus pesticides pollution: Current status and challenges in ecotoxicological, sustainable agriculture, and degradation strategies. *J. Hazard. Mater.* **2022**, *424*, 127494. [CrossRef]
76. Saeed, M.U.; Hussain, N.; Sumrin, A.; Shahbaz, A.; Noor, S.; Bilal, M.; Aleya, L.; Iqbal, H.M. Microbial bioremediation strategies with wastewater treatment potentialities–A review. *Sci. Total Environ.* **2021**, *818*, 151754. [CrossRef] [PubMed]

77. Varfolomeev, S.D.; Efremenko, E.N. *Organophosphorus Neurotoxins*; RIOR: Moscow, Russia, 2020; p. 380. ISBN 978-5-369-02026-5. [CrossRef]
78. He, P.; Chen, J.; Shao, L.; Zhang, H.; Lu, F. In-situ neutralize methane emission from landfills in loess regions using leachate. *Sci. China Technol. Sci.* **2021**, *64*, 1500–1512. [CrossRef]
79. Habtewold, J.; Gordon, R.; Voroney, P.; Sokolov, V.; VanderZaag, A.; Wagner-Riddle, C.; Dunfield, K. Sodium persulfate and potassium permanganate inhibit methanogens and methanogenesis in stored liquid dairy manure. *J. Environ. Qual.* **2018**, *47*, 786–794. [CrossRef]
80. Efremenko, E.; Stepanov, N.; Senko, O.; Maslova, O.; Volikov, A.; Zhirkova, A.; Perminova, I. Strategies for variable regulation of methanogenesis efficiency and velocity. *Appl. Microbiol. Biotechnol.* **2022**, *106*, 6833–6845. [CrossRef]
81. Stepanov, N.; Senko, O.; Perminova, I.; Efremenko, E. A new approach to assess the effect of various humic compounds on the metabolic activity of cells participating in methanogenesis. *Sustainability* **2019**, *11*, e3158. [CrossRef]
82. Syed, A.J.; Anderson, J.C. Applications of bioluminescence in biotechnology and beyond. *Chem. Soc. Rev.* **2021**, *50*, 5668–5705. [CrossRef]
83. Greiner, J.V.; Glonek, T. Intracellular ATP concentration and implication for cellular evolution. *Biology* **2021**, *10*, 1166. [CrossRef]
84. Calabretta, M.M.; Gregucci, D.; Martínez-Pérez-Cejuela, H.; Michelini, E. A Luciferase mutant with improved brightness and stability for whole-cell bioluminescent biosensors and in vitro biosensing. *Biosensors* **2022**, *12*, 742. [CrossRef]
85. Carvalho, M.C.; Tomazini, A.; Amaral, D.T.; Murakami, M.T.; Viviani, V.R. Luciferase isozymes from the *Brazilian aspisoma lineatum* (Lampyridae) firefly: Origin of efficient pH-sensitive lantern luciferases from fat body pH-insensitive ancestors. *Photochem. Photobiol. Sci.* **2020**, *19*, 1750–1764. [CrossRef] [PubMed]
86. Viviani, V.R.; Silva, J.R.; Amaral, D.T.; Bevilaqua, V.R.; Abdalla, F.C.; Branchini, B.R.; Johnson, C.H. A new brilliantly blue-emitting luciferin-luciferase system from Orfelia fultoni and Keroplatinae (Diptera). *Sci. Rep.* **2020**, *10*, 9608. [CrossRef] [PubMed]
87. Calabretta, M.M.; Lopreside, A.; Montali, L.; Zangheri, M.; Evangelisti, L.; D'Elia, M.; Michelini, E. Portable light detectors for bioluminescence biosensing applications: A comprehensive review from the analytical chemist's perspective. *Anal. Chim. Acta* **2022**, *1200*, 339583. [CrossRef] [PubMed]