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Water pollution remediation in Kazakhstan: evaluating bacterial consortiums for organic pollutant decomposition

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ABSTRACT

Wastewater treatment is one of the key problems that has to be solved by environmental biotechnology. Wastewater bioremediation is one of the most efficient and safest methods to replenish water resources. The aim of this study is to investigate the potential of using bacterial consortiums for reducing the organic load in wastewater, specifically focusing on water samples collected from three water bodies in central and northern Kazakhstan, which are known for their high levels of organic pollution. This study utilized bacterial strains from a microorganism collection to create consortiums. These consortiums were used to treat wastewater from polluted water bodies in Kazakhstan, focusing on parameters like COD, BOD5, ammonia, and phosphate. The methodology involved culturing strains, collecting water samples, and analyzing various parameters. Statistical analysis was performed to assess the results. The study found that two bacterial consortiums, 7BLB and 7BLPA, were the most effective in reducing COD, ammonia, and ammonia nitrogen in wastewater. The consortium 6BLP was highly effective at reducing phosphate levels, surpassing acceptable standards. Hydrogen levels met regulatory requirements in all cases. The study recommends further investigation of these consortiums' impact on other water quality indicators and suggests conducting field experiments in natural water ponds.

Key words: biofilm, biological oxygen demand, bioremediation, chemical oxygen demand, eutrophication

HIGHLIGHTS

- The biological method of water treatment is the safest and one of the most effective methods of water purification from organic and mineral substances.
- The use of several biological forms – free-floating, fouling substrates, and forming a surface biofilm – can significantly increase the efficiency of utilization and neutralization of excess and toxic organic substances.

GRAPHICAL ABSTRACT

INTRODUCTION

Wastewater treatment is one of the key problems that has to be solved by environmental biotechnology. The annual consumption of clean water on Earth is 3,300–250 km^3. The agriculture sector consumes most water, followed by chemical production and energy sectors and heavy industry. After its use, most of the water is returned in the form of wastewater and enters the water basins. According to the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan (2023), out of 69 rivers in Kazakhstan, only nine can be classified as 'clean water'. The remaining six rivers are polluted to varying degrees; a similar situation is observed with lakes and water storage ponds, and only two of them have been recognized as clean. The main sources of pollution are waste from the mining industry, as well as run-off from agricultural land and livestock enterprises. Solving the problem of the most efficient and high-quality wastewater treatment contributes to a more rational consumption and re-use of household, municipal, and industrial water. The biological method of water treatment is the safest and one of the most effective methods of water purification from organic and mineral substances that is carried out with the help of biological agents – bacteria, cyanobacteria, and fungi (Karches 2012; Karches & Buzás 2023). Proper selection of microorganisms and their communities makes it possible to purify wastewater from a wide range of pollutants: oils, petroleum industry hydrocarbons, ammonia, aldehydes, protein substances, acids, alkalis, and ions of heavy metal. Currently, the metabolic capabilities of known microorganisms are widely used, which is reflected in publications of recent years (Korostynska *et al.* 2012; Zhukova 2012; Yaqin & Rozi 2020; Ali *et al.* 2022; Khan *et al.* 2022).

Two main methods of biological wastewater treatment are described in the literature: changing the physicochemical parameters of the environment in order to activate the microflora of activated sludge (Galperina 2011; Buhari *et al.* 2022). For example, Buhari *et al.* (2022) describe an efficient way to remove ammonia from wastewater by means of separating isolates from the activated sludge of water ponds. Another direction is the introduction of artificially grouped consortiums of microorganisms (Khan *et al.* 2022). The possibilities of using bacterial communities in the form of biofilms are also being actively explored (Tolker-Nielsen *et al.* 2020). John *et al.* (2020) investigated the consortium effectiveness of two species of *Bacillus* and *Pseudomonas* to reduce ammonia, nitrate, and nitrite in the wastewater from aquaculture. Jokhakar & Dudhagara (2022) studied the effectiveness of using bacterial consortiums containing species of the genera *Bacillus*, *Pseudomonas,* and other bacterial species for the decomposition of organophosphorus compounds and the utilization of phosphates in wastewater. Abbew *et al.* (2022) demonstrate the importance of nitrifiers in consortiums that decompose organic-rich water due to the accumulation of nitrites and free nitrous acid during the oxidation of protein compounds.

The research by Hashem *et al.* (2021) tackles a different environmental concern, specifically the pollution generated by conventional wet-salting methods used in tanneries, resulting in chloride-containing wastewater. To address this issue, a salt-free 'green method' involving the use of *Sphagneticola trilobata* leaf paste for goatskin preservation is proposed. The study compares this innovative approach with traditional wet-salting methods over a 28-day period. Various parameters, including moisture, nitrogen, hydrothermal stability, and bacterial growth, are examined and compared between the two methods. The results demonstrate that the 'green method' not only meets standard requirements for leather quality but also significantly reduces pollution loads, including chloride, total dissolved solids, biochemical oxygen demand (BOD), and chemical oxygen demand (COD).

The use of several biological forms – free-floating, fouling substrates, and forming a surface biofilm – can significantly increase the efficiency of utilization and neutralization of excess and toxic organic substances. This study is devoted to the selection of the optimal composition of consortium based on bacteria cultivated under artificial conditions and the study of its ability to decompose the most common organic pollutants in waste and natural waters. The composition of the consortium of microorganisms depends on the type and degree of the water pond eutrophication, as well as the presence of specific pollutants. In the previous works of authors, the effectiveness of consortiums of aerobic bacteria 7BLB and 5LB in relation to wastewater treatment with indicators below 10 mg/mg is shown. However, many natural water ponds have increased eutrophication, especially those with increased anthropogenic load, located close to industrial or livestock complexes.

The aim of this study is to assess the potential of bacterial consortiums in mitigating organic pollution in wastewater. Specifically, the research focuses on water samples collected from three heavily polluted water bodies in central and northern Kazakhstan. The tasks included selecting a compatible consortium, measuring initial contamination levels, incubating samples with the chosen consortia, and assessing the impact by measuring indicators after incubation. The novelty of this study lies in its comprehensive approach to wastewater treatment using bacterial consortiums. While previous research has explored individual aspects of wastewater remediation, this study uniquely combines the selection of a compatible bacterial consortium with the measurement of various contamination indicators before and after incubation. The necessity for this study arises from the pressing need to improve wastewater treatment processes and address environmental pollution challenges.

This study consists of a methodological framework through which the findings of the study were identified. Also, the authors have analyzed the sources on the topic of this article. The conclusions of the article emphasize all the results obtained and give an understanding of further perspectives within this field of research.

MATERIALS AND METHODS

In this study, bacterial strains in the form of pure cultures from the Republican Collection of Microorganisms were used to create consortiums.

The following strains of bacteria were used: *Bacillus subtilis BM-2/17*, *Bacillus amyloliquefaciens ES-1*, *Lactobacillus bulgaricus BM-3/17*, *Lactobacillus fermentum 9 LB*, *Lactobacillus acidophilus BM-5/17*, *Lactobacillus casei BM-4/17*, *Lactococcus lactis* subsp. *cremoris BM-1/17*– as part of the '7BLB' consortium; *L. bulgaricus BM-3/17*, *L. fermentum 9 LB*, *L. acidophilus BM-5/17*, *L. casei BM-4/17*, *L. lactis* subsp. *cremoris BM-1/17*– as part of the 5LB consortium. These two consortiums showed the best indicators of wastewater treatment in the previous studies, and therefore they were chosen for further studies. The new consortia were selected based on the species from the same genera – *Bacillus* and *Lactobacillus*. The consortia that did not demonstrate sufficiently high cleaning performance were modified. The following species were used as additional ones: *Acinetobacter calcoaceticus*, accumulating phosphates and heavy metals, *Pseudomonas putida* – denitrifier, and *Alcaligenes denitrificans*, which denitrify and accumulate heavy metals.

All strains were obtained from the Republican Collection of Microorganisms. The following bacterial strains were used: *A. calcoaceticus* B-RKM 0186, *Pseudomonas fluorescens* B-RKM 0196, *P. putida* B-RKM 0060, and *A. denitrificans* B-RKM 0195.

Thus, two more consortia had the following composition – 7BLPA: *B. subtilis*, *Bacillus migulanus*, *A. calcoaceticus*, *P. putida*, *Bacillus coagulans*, *L. bulgaricus*, *L. fermentum* and consortium 6BLP: *B. subtilis*, *B. migulanus*, *A. denitrificans*, *B. coagulans*, *L. bulgaricus*, *L. fermentum*.

To accumulate the cultures' biomass, the separate incubation was carried out at 37 °C within 24 h. All strains were incubated in nutrient media prepared according to the Thermo Fisher Scientific requirements.

Samples taken from lakes were characterized by increased anthropogenic load and eutrophication. The samples include the Maybalyk Lake (Yesil district of Astana city) and the Zerenda Lake (Zerendi district of Akmola region), as well as the Samarkand water storage pond located on the Nura River (Temirtau city of Karaganda region). From each water pond, three water samples were taken at different sampling points. These water samples were selected as one of the most polluted, according to the report of the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan (2023). There are many industrial enterprises in the areas of these water ponds, and pollutants enter the water ponds in the form of untreated waste from industrial waters, as well as infiltrate with groundwater. Livestock farms and domestic wastewater are also the sources of organic substances in water ponds.

The COD value of the studied wastewater was determined by means of bichromate analysis. Silver sulfate was used as a catalyst. In the resulting samples, the coefficients of COD were measured after delivery to the laboratory. In addition, the following indicators were studied: indicators of BOD5, the content of ammonia compounds, ammonia, phosphates, and hydrogen sulfide (Method for performing measurements of chemical oxygen demand (COD) in samples of natural and waste waters by the titrimetric method. CV 3.01.17-01 A 2005).

The COD measurement was done by the bichromate method. For this, 2 ml of the test water was added to the flask, 1 ml of potassium dichromate with a concentration of 0.025 mol/l and 3 ml of a solution of silver sulfate in concentrated sulfuric acid were added. The content was boiled for 2 h. After cooling, 5 ml of distilled water was added to the flask and cooled again. Three drops of ferroin solution were added to the flask and the residue of unreacted potassium dichromate was titrated with Mohr's salt solution until the bluish-green color changed to red-brown. Simultaneously, an experiment with a blank sample containing 2 ml of distilled water was conducted. The COD value was calculated by the following formula:

$$X = \frac{8 \times (V_{\mathrm{MK}} - V_{\mathrm{M}}) \times C_{\mathrm{M}} \times 1,000}{V} \quad (1)$$

where V_{MK} is the volume of Mohr's salt solution, mol/l equivalent; VM is the Mohr's salt solution used for titration of the water sample, ml; C_{M} is the concentration of Mohr's salt solution, mol/l; V is the volume of the water sample, ml; 8 is the mass of milligram equivalent of oxygen.

If the COD value exceeded 8 mg/ml, the sample was diluted and re-measured. The results were presented as $X \pm$ standard deviation (Method for performing measurements of COD in samples of natural and waste waters by the titrimetric method. CV 3.01.17-01 A 2005). Determination of BOD was carried out in water samples incubated at 20 °C within days. Before determination, the samples were sedimented for 2 h, the supernatant liquid was taken and the determination was carried out in it. It was conducted as a control for the solution pH to be in the range of 6–9. Before the measurements, the sample was homogenized and saturated with air. Then, the pH level was measured using a laboratory ionomer. To suppress nitrifying bacteria, 1 ml of thiourea solution (1:1,000) per 1 ml of the solution was added. The samples were diluted with distilled water, and the dilution coefficient was calculated using the following formula:

$$N = \frac{C_{\mathrm{COD}}}{2 \times K} \quad (2)$$

where C_{COD} is the COD value in the sample, mg/l; 2 is the coefficient that sets 50% level of BOD from COD; and K is the expected sufficient oxygen concentration in the sample after incubation, mg/l.

Then, the samples were incubated in hermetically sealed flasks packed to the rafters. The samples were left for incubation within 5 days at 20 °C (Method for determination of biochemical oxygen demand after 5 days of incubation (BOD5) in samples of drinking, natural and waste water by amperometric method. NDP 10.1:2:3.131-2016 2016). After incubation, a measurement was made in a blank sample containing water-diluent. This was followed by a measurement made using an oximeter, by immersing the sensor into the flask (with constant stirring in a mixer). BOD value was calculated using the formula:

$$\mathrm{BOD}_5 = ((X_1 - X_2) - (X_3 - X_4)) \times N \quad (3)$$

where X_1 is the mass fraction of dissolved oxygen in diluted water before incubation; X_2 is the mass concentration of dissolved oxygen in the diluted sample of analyzed water after incubation; X_3 is the mass fraction of oxygen in a blank sample before incubation; X_4 is the mass fraction of oxygen in a blank sample after incubation; and N is the degree of dilution.

The measurements of ammonia nitrogen, ammonia, and phosphate were made according to the Standard Methods for the Examination of Water and Wastewater (1999). The measurement of all parameters was conducted immediately after the delivery of samples to the laboratory – a day, as well as 5 days after incubation. The pH level was measured using a laboratory ionomer according to the instruction manual. Data for three samples from each water pond were combined and presented in the form of an average value $\pm$ with a standard deviation. The statistical significance of data was carried out using T-test, and data were considered significant at $p \leq 0.05$.

RESULTS

Water samples from each pond were separately studied for the composition of organic impurities immediately after being transferred to the laboratory. The COD value was quite high in all the studied water samples, which indicates a high level of pollution by oxidizable organic substances. The Maybalyk Lake exhibited the highest COD, measuring at 44.2 ± 0.8 mg/ml, while Zerenda recorded a COD of 38.1 ± 0.55 mg/ml. The Samarkand water storage pond had a COD of 29.5 ± 0.49 mg/ml. These values indicate a rather high level of eutrophic load in these water bodies. The assessment of biological oxygen demand indicated significant activity among the examined consortia.

The production of microbial consortium variants such as 7BLB, 5LB, 7BLPA, and 6BLP involves a rigorous process that includes the careful selection of microbial strains based on their metabolic capabilities, comprehensive strain characterization, assessment of compatibility among strains, optimization of growth conditions, and extensive laboratory testing to validate their efficiency in degrading or removing target pollutants. Error analysis and reproducibility assessments are essential to ensure reliable results. Once optimized, the consortium undergoes scale-up for practical application and field testing to evaluate its performance in real-world scenarios. Quality control measures are implemented to maintain consistency and purity, and compliance with relevant regulations is considered. This multifaceted approach ensures the development of effective and environmental friendly microbial solutions tailored to specific applications, such as wastewater treatment and pollutant remediation.

The 7BLB consortium displayed the highest activity across all water sample variations, consistent with previous research by the authors. In the case of samples from the Maybalyk Lake, the BOD for this consortium was 21.3 ± 0.35 mg/ml, whereas in samples from the Zerenda Lake, the BOD was measured as 19.8 ± 0.6 mg/ml, and in samples from the Samarkand water storage pond, it registered at 15.9 ± 0.86 mg/ml (Table 1).

BOD of this consortium for samples from the Maybalyk Lake was 21.3 ± 0.35 mg/ml; in samples from the Zerenda Lake – 19.8 ± 0.6 mg/ml; from the Samarkand water storage pond – 15.9 ± 0.86 mg/ml (Figure 1). The new variant of the 7BLPA consortium was in second place in terms of efficiency of the organic substrates' oxidation; it was ahead of the other two variants of consortiums. In samples from the Maybalyk Lake, BOD amounted to 16 ± 0.49 mg/ml; from the Zerenda

Table 1 | COD and BOD values after incubation with the studied bacterial consortiums

Consortium	Water pond	COD, mg/ml	BOD_5, mg/ml	COD/BOD
7BLB	Maybalyk	44.20	21.30	1.92
	Zerenda	38.1	19.80	1.86
	Samarkand	29.50	15.90	4.17
5LB	Maybalyk	44.20	10.60	3.10
	Zerenda	38.1	12.30	3.47
	Samarkand	29.50	8.50	2.76
7BLPA	Maybalyk	44.20	16.00	2.63
	Zerenda	38.1	14.50	2.34
	Samarkand	29.50	12.6	5.89
6BLP	Maybalyk	44.20	7.50	3.99
	Zerenda	38.1	9.55	4.28
	Samarkand	29.50	6.9	2.08

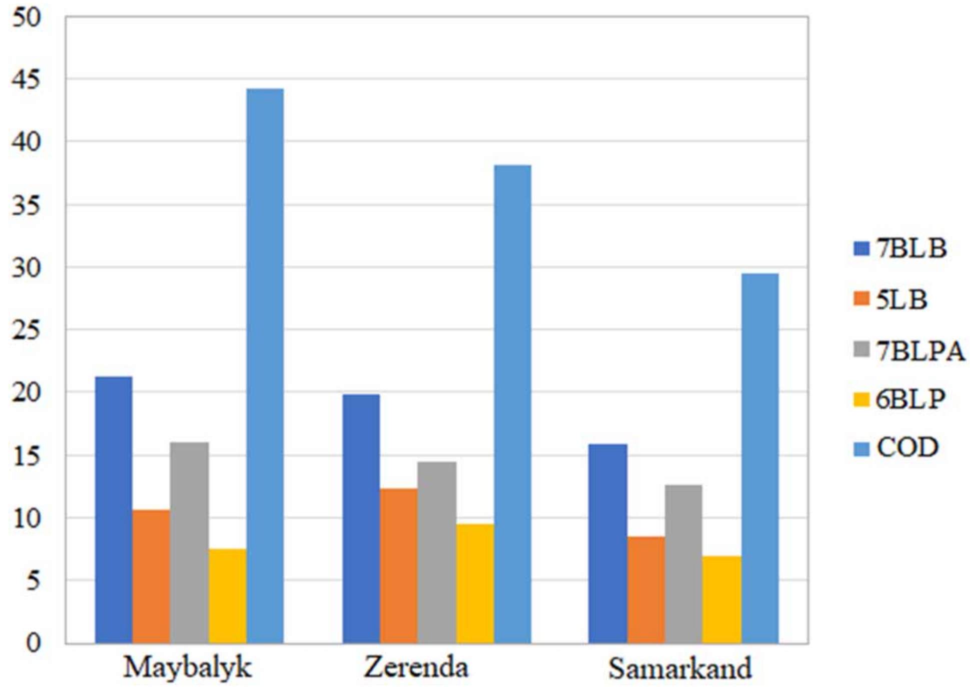

Figure 1 | COD and BOD_5 values in water obtained from different water ponds.

Lake – 14.5 ± 0.81 mg/ml; from the Samarkand water storage pond – 12.6 ± 0.2 mg/ml. The 5LB consortium took third place in terms of activity; BOD of this consortium for samples from the Maybalyk Lake amounted to 10.6 ± 0.55 mg/ml; in samples from the Zerenda Lake – 12.3 ± 0.45 mg/ml; from the Samarkand water storage pond – 8.5 ± 0.55 mg/ml. The 6BLP consortiums demonstrated the lowest oxidation rates. In samples from the Maybalyk Lake, BOD amounted to 7.5 ± 0.2 mg/ml; from the Zerenda Lake – 9.55 ± 0.23 mg/ml; from the Samarkand water storage pond – 12.6 ± 0.29 mg/ml. It follows from the results that different consortia of bacteria are optimal for water from different water ponds. This may be a consequence of the preference for certain substrates and the nature of pollution of the water ponds.

The ratio of COD to BOD demonstrates the degree of availability of organic substrates for biological oxidation. This indicator should not exceed 2.5 – exceeding this threshold indicates the presence of a large number of carbon-containing impurities that are inaccessible for biodegradation. These indicators were optimal for the 7BLB consortium for the samples from all the studied water ponds: 2.08 ± 0.2 – for samples from the Maybalyk Lake, 1.92 ± 0.15 – for samples from the Zerenda Lake, and 1.86 ± 0.2 – for samples from the Samarkand water storage pons. The ratio of indicators of COD to BOD for 7BLRA consortium was as follows: the Maybalyk Lake – 2.76 ± 0.17; the Zerenda Lake – 2.63 ± 0.2; the Samarkand water storage pond – 2.34 ± 0.17. The indicators of the 5LB consortium somewhat exceeded the optimal values: the Maybalyk Lake – 4.17 ± 0.23; the Zerenda Lake – 2.63 ± 0.17; the Samarkand water storage pond – 3.47 ± 0.2 (Figure 2). In the samples incubated with the 6BLP consortium, all indicators exceeded a factor of 4, i.e., the degree of water biodegradation was extremely low. On comparing the results between the samples obtained from different water ponds, it is possible to notice that there is a difference in the level of efficiency of bacterial decomposition of contaminants. Thus, the cleaning indicators in samples from the Zerenda Lake for the 7BLB and 7BLRA consortia were the closest to optimal ones, and these indicators were not statistically significant, whereas for the consortia 5LB and 6BLP, the differences were statistically significant ($p \leq 0.05$). The indicators of the Samarkand water storage pond were approaching the indicators of water from the Zerenda Lake. This indicates that the water pond is polluted with more readily available organic pollutants of economic and household type, while the water of the Maybalyk Lake is subjected to a greater run-off of industrial water.

The main parameters of impurities measured in wastewater are shown in Table 2. The ammonia nitrogen is an indicator of the presence of other forms and compounds of nitrogen in wastewater (dissolved nitrogen, nitrite ion, and nitrate). The measurement of ammonia nitrogen showed that its lowest content was in the samples incubated with the 7BLRA consortium,

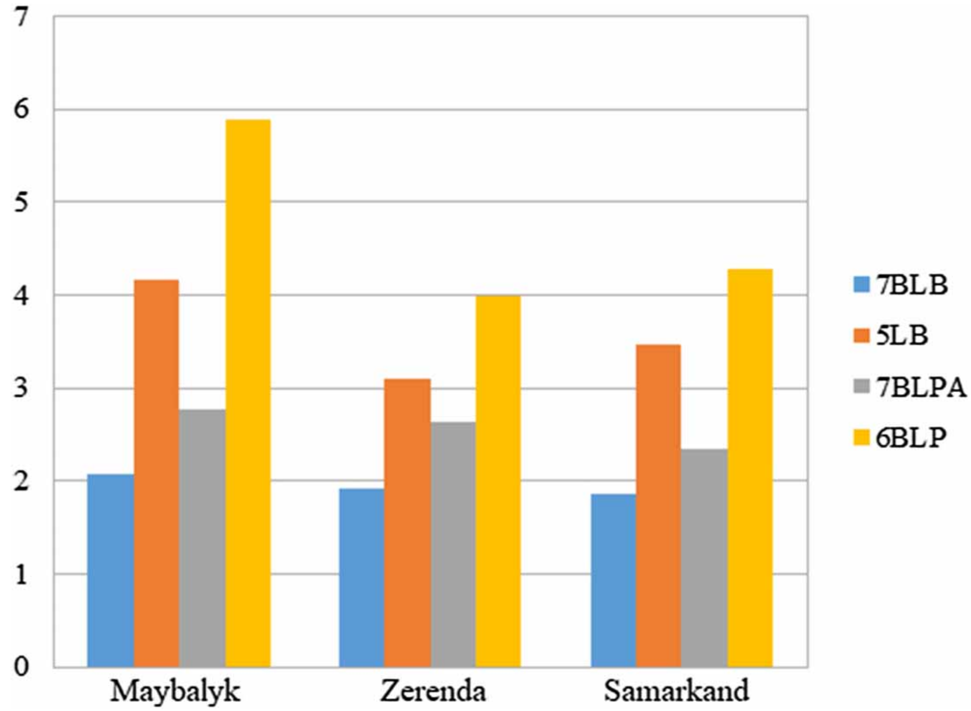

Figure 2 | The ratio of COD to BOD_5 in water obtained from different water ponds.

Table 2 | Effect of bacterial consortiums on NH_3-N, NH_4, PO_4, and pH levels in water ponds

Consortium	Water pond	NH_3-N mg/ml 0 day	NH_3-N mg/ml 5 days	NH_4 mg/ml 0 day	NH_4 mg/ml 5 days	PO_4 mg/ml 0 day	PO_4 mg/ml 5 days	pH up to 0 day	pH after 5 days
7BLB	Maybalyk	1.55	0.70	4.90	2.50	5.84	3.28	8.0	6.20
	Zerenda	1.2	0.45	4.57	2. 9	4.78	2.36	8.9	7.00
	Samarkand	0.79	0.20	4.30	2.3	5.67	3.00	8.1	6.90
5LB	Maybalyk	1.55	0.99	4.90	3.07	5.84	4.50	8.0	6.40
	Zerenda	1.2	0.90	4.57	3.70	4.78	3.50	8.9	5.90
	Samarkand	0.79	0.48	4.30	3.10	5.67	2.90	8.1	6.90
7BLPA	Maybalyk	1.55	0.55	4.90	1.50	5.84	2.50	8.0	7.00
	Zerenda	1.2	0.50	4.57	1.10	4.78	2.30	8.9	8.30
	Samarkand	0.79	0.50	4.30	2.10	5.67	1.90	8.1	6.90
6BLP	Maybalyk	1.55	0.91	4.90	2.80	5.84	3.00	8.0	6.00
	Zerenda	1.2	0.60	4.57	2.90	4.78	3.30	8.9	6.40
	Samarkand	0.79	0.38	4.30	2.78	5.67	2.7	8.1	7.1
Standard values		**1**		**2**		**3.5**		**6–9**	

and the following indicators were obtained: the Maybalyk Lake – 0.55 $\pm$ 0.07 mg/ml; the Zerenda Lake – 0.50 $\pm$ 0.02 mg/ml; the Samarkand water storage pond – 0.50 $\pm$ 0 mg/ml 2. Similar values were obtained during incubation with the 7BLPA consortium: the Maybalyk Lake – 0.70 $\pm$ 0.05 mg/ml; the Zerenda Lake – 0.45 $\pm$ 0.07 mg/ml; the Samarkand water storage pond – 0.20 $\pm$ 0.02 mg/ml. In the other two variants of the strains, the values were higher, reaching 0.99 $\pm$ 0.05 mg/ml in some samples. Data on the content of ammonia nitrogen correlated with the content of ammonia. The lowest values were found after incubation with the strain 7BLPA: the Maybalyk Lake – 1.50 $\pm$ 0.06 mg/ml; the Zerenda Lake – 1.10 $\pm$ 0.02 mg/ml; the Samarkand water storage pond – 2.10 $\pm$ 0.03 mg/ml. After exposure of the 7BLB consortium, the following ammonia values were recorded: the Maybalyk Lake – 2.50 $\pm$ 0.05 mg/ml; the Zerenda Lake – 2.9 $\pm$ 0.04 mg/ml; the

Samarkand water storage pond – 2.3 ± 0.02 mg/ml. In the other two strains, the value of this indicator was about 3 mg/ml (Table 2). The 7BLB consortium demonstrated the highest activity.

The content of the phosphate ion in samples incubated with all the studied strains was within the permissible levels – 3.5 mg/ml. However, in water samples treated with the strain 7BLRA, the value of phosphates was lower than in all variants of the experiment: the Maybalyk Lake – 2.9 ± 0.5 mg/ml; the Zerenda Lake – 2.3 ± 0.5 mg/ml; the Samarkand water storage pond – 1.9 ± 0.3 mg/ml. This is probably due to the presence of phosphate-reducing bacterium *A. calcoaceticus*. The 7BLB and 6BLP consortia showed comparable results in terms of phosphate content. After incubation with the strain 7BLB, the following values were obtained: the Maybalyk Lake – 3.28 ± 0.4 mg/ml; the Zerenda Lake – 2.36 ± 0.3 mg/ml; the Samarkand water storage pond – 3 ± 0.5 mg/ml. During incubation with the strain 6BLP, the content of phosphates was as follows: the Maybalyk Lake – 3 ± 0.5 mg/ml; the Zerenda Lake – 3.3 ± 0.5 mg/ml; and the Samarkand water storage pond – 2.27 ± 0.5 mg/ml. The indicators after incubation with the 5LB strain were shown to be slightly higher: the Maybalyk Lake – 3.07 ± 0.45 mg/ml; the Zerenda Lake – 3.7 ± 0.55 mg/ml; the Samarkand water storage pond – 3.1 ± 0.5 mg/ml.

The pH value was also within the established sanitary norms (Order of the Minister of National Economy of the Republic of Kazakhstan No. 209. 'On approval of the Sanitary Rules 'Sanitary and epidemiological requirements for water sources, places of water intake for domestic and drinking purposes, domestic and drinking water supply and places of cultural and domestic water use and safety of water bodies' 2015) and there was a trend of its dependence on the ammonia content in the sample; its increased content led to a pH shift to a more alkaline side. The iron content was equivalent in all samples obtained from the same water pond, and it did not change significantly depending on the processing by one or another consortium. The average values of Fe content in water ponds were as follows: the Maybalyk Lake – 0.44 ± 0.02 mg/ml; the Zerenda Lake – 0.37 ± 0.02 mg/ml; and the Samarkand water storage pond – 0.42 ± 0.02 mg/ml.

Based on the ratio of the content indicators of the main polluting ions before the start of incubation with consortia and after 5 days, the percentage of efficiency of water purification against this pollutant was calculated. The highest level of purification efficiency against ammonia nitrogen was demonstrated by the 7BLB and 6BP consortia in the treatment of water from the Maybalyk Lake: 74.68 and 75.48%, respectively. The purification rates against ammonia nitrogen by these consortia of waters from other water ponds were also quite high within the range of 55–67%. When analyzing the decrease in ammonia content, the best indicators were obtained using the 7BLPA consortium in water samples from the Maybalyk Lake. The following percentage decrease in ammonia content in water samples was obtained: the Maybalyk Lake – 69.39%; the Zerenda Lake – 75.93%; the Samarkand water storage pond – 51.16%. The 7BLB and 6BLP consortia became the next ones in terms of efficiency.

Regarding the reduction of phosphate concentrations, the 7BLPA consortium has performed in the best way. After incubating with the 7BLPA consortium, the following results of the percentage decrease in phosphates in water samples were obtained: the Maybalyk Lake – 40.07%; the Zerenda Lake – 39.33%; and the Samarkand water storage pond – 66.49%. In other consortia, the results were somewhat lower and comparable with each other.

The nature of polluting compounds can indicate the different efficiencies of ion removal from different water samples; apparently, not all of them are available for organic decomposition. Despite the presence of these differences, the average values for samples for each strain was calculated, considering the errors (Figure 3). According to the content of NH_3-N, the following results for strains were obtained: 7BLB – 64.01 ± 10.01%; 5LB – 33.46 ± 7.49%; 7BLPA – 53.15 ± 14.6%; and 6BLP – 67.64 ± 7.41%. The change in ammonia content was as follows: 7BLB – 46.98 ± 8.5%; 5LB – 37.35 ± 5.3%; 7BLPA – 65.49 ± 12.83%; and 6BLP – 38.25 ± 4.04%. The following results were obtained for the reduction of phosphates: 7BLB – 47.18 ± 3.4%; 5LB – 32.86 ± 13.98%; 7BLPA – 48.63 ± 15.47%; and 6BLP – 43.99 ± 11.44%. As it is seen, the average values for the decrease in phosphates are comparable, and the existing differences are not statistically significant. In general, the best performance in reducing nitrogen-containing pollutants was demonstrated by the 7BLB and 7BLPA consortia.

Regarding NH3-N reduction, the 7BLB consortium showed the highest efficiency, with an average reduction, followed by 6BLP. The 7BLPA consortium also demonstrated substantial removal, with an average reduction. In contrast, 5LB exhibited a lower average reduction. The trends in ammonia content reduction followed a similar pattern, with 7BLB and 6BLP outperforming the other consortia.

Thus, the studied water ponds had the increased eutrophic indicators, reflecting the high content of organic impurities. High COD values confirm this fact. The studied strains of bacteria were effective in reducing the organic load, and this

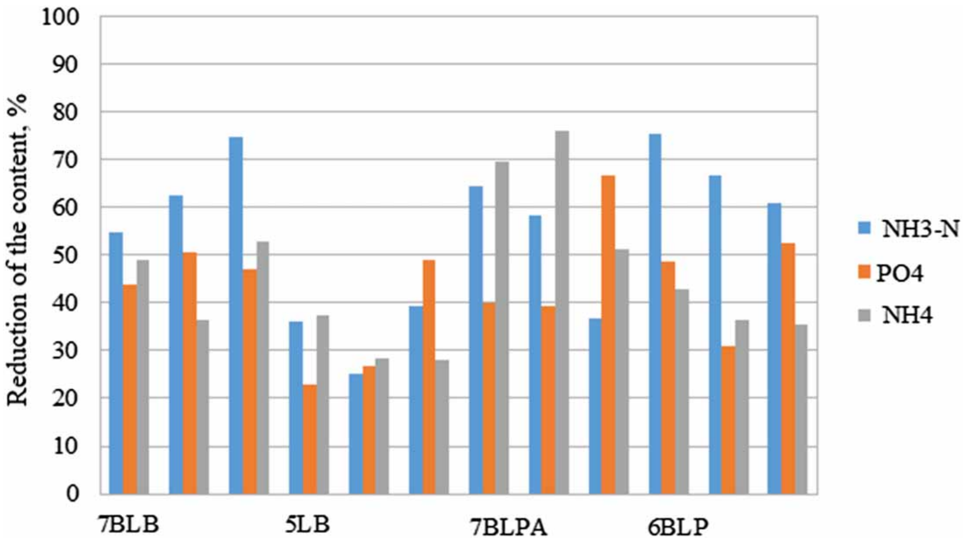

Figure 3 | Percentage of reduction in the level of basic ions in water samples under the influence of the studied consortia.

was reflected in high BOD coefficients. Based on the data obtained, it can be concluded that two of the four studied bacterial consortia (7BLB and 7BLPA) have more pronounced oxidizing and nitrogen-reducing properties. This is expressed as an increase in the ratio of BOD to COD and a decrease in nitrogenous compounds. Phosphate-reducing properties were comparable in all consortia; however, 7BLPA was very effective in cleaning water samples from the Maybalyk Lake.

DISCUSSION

The use of bacterial consortia for the treatment of wastewater and natural water ponds is a promising and actively developing area of environmental biotechnology. This is due to the high efficiency and safety of these methods. Today, almost all known data on the bacterial metabolism are used to construct their communities. Bacteria are able to decompose organic compounds, as well as bind inorganic compounds and convert them into safer forms. Much attention is paid to the study of communities based on the association of autotrophic and heterotrophic bacteria and microalgae (Fito & Alemu 2019; Madhushika *et al.* 2020; Chan *et al.* 2022). However, for water ponds with high eutrophication, it is more relevant to use consortia based on the heterotrophs that decompose organic compounds, rather than synthesize them. This study demonstrates that the use of such communities can be effective even for water ponds with high COD rates. Recognition in the authors' study of the different profiles of pollutants in water bodies is consistent with the need for the case-by-case approach discussed above. Collaborative efforts can lead to a more holistic and effective approach to water quality management, bridging the gap between the removal of organic and inorganic pollutants while taking into account the unique characteristics of each water body.

In many modern studies, it has raised the problem of wastewater bioremediation in the textile industry, due to their significant organic load. It also studied the possibility of bacterial consortia for the decomposition of bioorganic waste in the textile industry (Raza *et al.* 2022). Another similar study (also focused on the detoxification of organic waste from the textile industry) studied consortia based on the species of genera *Bacillus* and *Pseudomonas* (Madhushika *et al.* 2020). The efficacy of 24–94% has also been shown using various concentrations and incubation times (Joshi *et al.* 2022). Raza *et al.* (2022) demonstrated an effective COD reduction (about 50%) with a bacterial consortium containing *Enterobacter* sp., *Staphylococcus aureus*. Aneeba *et al.* (2022) conducted a study investigating the effectiveness of consortia consisting of *Alcaligenesfecalis* and *Bacillus paramycoides* for treating hospital wastewater. Various biochemical and physical indicators of water were investigated. It has shown a significant reduction in COD with high BOD5, a decrease in the concentration of heavy metals to acceptable standards, as well as the suitability of treated wastewater for growing various crops. In this study, it was shown that the ratio of COD to BOD depends on the composition of bacteria consortium and the nature of water pond pollution. Thus, the 7BLB consortium has shown a ratio of COD to BOD5 close to 2 during the treatment of water samples from the Maybalyk and Zerenda lakes, which is the best result. In water samples from the Samarkand water storage pond, the 6BLP consortium showed similar results in reducing COD. According to this study, the consortia of bacteria may be suitable for samples from different water ponds, by solving cleaning problems more or less effectively. The authors of this study agree with the opinion of the authors of the analyzed papers and believe that cross-comparison of the results obtained will allow comparative analysis, optimization of consortium composition, and a deeper understanding of the adaptability of bacterial consortia to different pollution levels and geographic regions.

The effectiveness of using bacterial consortia containing *B. subtilis*, *B. megaterium*, *P. fluorescens* and other bacterial species for the utilization of phosphates in wastewater has been studied. These species have the ability to decompose organophosphorus compounds to safer forms of phosphates (Jokhakar & Dudhagara 2022). A similar situation was observed in this study, because the 7BLPA consortium demonstrated the best ability of *Bacillus*, *Lactobacillus*, and *Pseudomonas* to utilize phosphates. The results of all consortia were quite high and were in the range of 40–15%, with the exception of the 7BLPA consortium in the purification of water samples from the Maybalyk Lake. This is probably due to the favorable substrate for the main phosphate reducer of the 7BLPA consortium, *A. calcoaceticus*. Abbew *et al.* (2022) emphasize the importance of using nitrifiers in consortia, decomposing the organic-rich water, due to the accumulation of nitrites and free nitrous acid, which have an antimicrobial effect, during the oxidation of protein compounds. Buhari *et al.* (2022) describe an efficient way to remove ammonia from wastewater by separating isolates from the activated sludge of water ponds. It has shown a high ability to reduce the content of ammonia and COD. This study also demonstrated the correlation of these indicators. Das *et al.* (2022) demonstrated the possibility of effective treatment of concentrated wastewater of dairy production with COD in the range of 55–72 mg/l using the bacterial consortium containing carbon-oxidizing and nitrifying bacteria. The purification efficiency in COD, with post-treatment with microalgae, amounted to about 90%. It has also shown a significant reduction in the content of ammonia nitrogen and phosphates. These studies were fundamental in the context of the authors' study of the structure of bacterial consortia. Integrating the results of these studies can lead to the development of customized treatment strategies that target multiple pollutants simultaneously, improving overall treatment efficiency and environmental sustainability.

John *et al.* (2020) demonstrated the effectiveness of a consortium containing *Bacillus cereus*, *B. amyloliquefaciens*, and *Pseudomonas stutzeri* to reduce ammonia, nitrate, and nitrite in aquaculture wastewater. At the same time, the concentration of ammonia decreased by 2 times, and it gradually oxidized to fewer toxic nitrites, and then to nitrates. This ultimately reduced the mortality of fry in aquaculture by 40%. This research provides a good model for the study of biological treatment methods in natural water ponds. In the presented study, consortia based on bacteria of the genera *Bacillus* and *Pseudomonas* also effectively reduced the ammonia content in water by 50–70%, bringing it within the normal range. A patent study by Poltavskaya *et al.* (2001) demonstrated a high nitrilase activity of bacteria of the genera *Rhodococcus* and *Alcaligenes* and they are recommended as producers of carboxylic acids based on the nitrate compounds. These data are correlated with the results of the current study, which show that consortia containing bacteria of these genera have the highest rates of denitrification. Another study showed the ability of *Alcaligenes* sp. to decompose polychlorinated cyclic organic compounds with the formation of environmentally safe neutral non-cyclic compounds (Yajima *et al.* 2020). Raza *et al.* (2022) demonstrated the efficient removal of nitrates and phosphates using a bacterial consortium containing *Enterobacter* sp. and *S. aureus*. At the same time, its efficiency increased significantly when microalgae were added to the consortium. The efficiency of phosphate removal in this case reached 90% and nitrates removal reached 60% (in this study, the denitrification activity exceeded 60%). These studies can complement each other, providing insight into the effectiveness of using bacterial consortia for wastewater treatment.

P. putida is being studied as a bacterium that is often found in the natural conditions of water ponds and can be a part of biofilms. Such formations have a positive effect on the ecological situation, by effectively reducing the content of bioorganic substances in the surrounding water (Tolker-Nielsen *et al.* 2020). In this study, two consortia containing this species demonstrated reasonably high rates of denitrification and also reduced phosphate to safe levels. In the previous studies (Sarmurzina *et al.* 2016, 2017; Kannappan *et al.* 2017; Tekebayeva *et al.* 2021), the antagonistic activity of bacterium of the genera *Bacillus* and *Lactobacillus* against the test pathogenic species (*Shewanella ximenensis*, *P. fluorescens*, *P. taiwanensis*, *P. aeruginosa*, *Aeromonas punctata*) was demonstrated. This makes it possible to judge the presence of antimicrobial activity in consortia based on these bacterial species. At the same time, species of the genus *Bacillus* did not show antimicrobial activity against species of the genus *Pseudomonas*, which was also demonstrated in this study. This allows using them as part of a single consortium. A comparative analysis of reviewed studies and the current paper allows us to identify universal solutions that allow simultaneous action on several pollution parameters, optimizing the composition to achieve maximum efficiency.

Eissa *et al.* (2022) centered on evaluating the microbiological quality of water within a healthcare facility's distribution network. The study uncovers variations in microbial counts at different points within the distribution system, with C4 and C13 showing notable differences in microbial density. Despite a moderate correlation between the datasets, the dendrogram analysis reveals significant clustering. The study ultimately underscores the importance of assessing the biological stability of water distribution systems within healthcare facilities and hints at its potential applicability to other types of processed water networks. The authors' study and a study by Eissa *et al.* (2022) create a more comprehensive approach to water quality management, addressing both chemical and microbial aspects.

In turn, Ho (2022) delves into the issue of microplastic contamination within wastewater treatment plant (WWTP) sludge. This study covers a broader geographic scope, reviewing scholarly papers published between 2016 and 2022. It examines how the abundance of microplastics in WWTP sludge varies across regions, influenced by population density, urbanization level, and land use. The research also underscores the challenge of reintroducing microplastics into the environment when sludge is repurposed as fertilizer, undermining the role of WWTPs as interceptors of microplastics. The synergy between the current study and the article by Ho (2022) can be used to assess the broader environmental impacts of reducing organic pollution in water bodies, thus creating a more holistic approach to pollution management.

The study addresses the use of bacterial consortia to mitigate organic pollution in water ponds, revealing promising outcomes for water quality improvement. However, it suggests unexplored avenues for research, including the influence of these consortia on various water quality indicators beyond organics and phosphates, the dynamic behavior of the microbial communities in response to changing environmental conditions, and the ecological impacts of their application. Furthermore, the study advocates for field experiments in natural water ponds to validate their effectiveness in real-world scenarios. The need for comparative assessments against existing wastewater treatment methods, an understanding of pollutant sources and characterization, and considerations of regulatory frameworks all represent important gaps in the current literature. Filling these gaps will be essential for optimizing the application of bacterial consortia in water pollution mitigation and ensuring their environmental sustainability.

CONCLUSIONS

In this study, the possibility of using bacterial consortia to reduce the organic load in wastewater is demonstrated. Using the water samples taken from three water ponds in the central and northern regions of Kazakhstan with high levels of organic pollution, the effectiveness of these consortia is studied. Water samples from the Maybalyk and Zerenda Lakes and the Samarkand water storage pond were studied. The highest efficiency of two types of consortia was shown: the previously studied 7BLB (*B. subtilis BM-2/17, B. amyloliquefaciens ES-1, L. bulgaricus BM-3/17, L. fermentum 9 LB, L. acidophilus BM-5/17, Lactobacillus casei BM-4 /17, L. lactis subsp.cremoris BM-1/17*) and 7BLPA (*B. subtilis, B. migulanus, A. calcoaceticus, P. putida, B. coagulans, L. bulgaricus, L. fermentum*). A positive effect on the reduction of COD, ammonia nitrogen, and ammonia was shown. BOD5 amounted to about 50% of the COD value. Based on the results of the ratio of COD and BOD5, it is possible to conclude that the Zerenda Lake and the Samarkand water storage pond are polluted with substances that are more readily available for biological oxidation. The Maybalyk Lake contains more non-biodegradable organic substances, which may be due to the different nature of wastewater and pollutants in the regions where the water ponds are located.

The 6BLP consortium (*B. subtilis, B. migulanus, A. denitrificans, B coagulans, L. bulgaricus,* and *L. fermentum*) had a more effective influence on reducing the phosphate levels. The indicators turned out to be lower than the acceptable standards in all samples, despite the fact that this threshold was exceeded when using other consortia. The hydrogen indicator met the regulatory requirements in all variants of consortia and samples. The data indicate the possibility of using these bacterial consortia to reduce the load of organic substances in water ponds with high eutrophication. It is also promising to study these bacterial communities in terms of their influence on other indicators of water purity (sulfates, chlorides, and heavy metals). However, it is already possible to recommend conducting field experimental studies in small natural water ponds with two selected consortia.

This study makes a significant contribution by showcasing the potential of bacterial consortia in mitigating organic pollution in wastewater, particularly in the specific regions of Kazakhstan studied. It highlights the varying efficiency of different consortia, with promising results for reducing key pollutants such as COD, ammonia, and ammonia nitrogen. The findings provide valuable insights into the nature of organic pollution in water bodies and offer a promising solution for phosphate reduction with the 6BLP consortium. Despite its limitations, including a limited geographical scope and a focus on specific parameters, the study's results hold immediate applicability for regions facing similar pollution challenges.

The scientific value of this study lies in its contribution to addressing a pressing environmental issue, its novel approach to wastewater treatment using bacterial consortia, and its empirical data-driven findings that have practical implications for improving water quality in polluted water ponds. Future research should expand its geographic reach, investigate long-term effects, and explore a wider range of water quality parameters while conducting practical field studies to validate these promising laboratory findings.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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