# Biodegradation of organic compounds in wastewater

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**Abstract.** Biodegradation is a sustainable and efficient method for removing organic pollutants from the aquatic environment. We studied the biological purification of aqueous solutions from betaine organic matter under the action of bacterial strains of the genus *Pseudomonas* and determined the rate of decomposition in the presence of chloride ions and heavy metal cations. The bacteria showed lower activity in the presence of salts of heavy metals and performed more efficiently in the presence of chloride ions. Almost complete degradation of organic matter was observed on the 21st day. Thus, these strains of microorganisms can be used as decomposers of organic betaine compounds.

## 1 Introduction

Industrial enterprises have a negative impact on ecosystems if they do not have good wastewater treatment systems [1–4].

Environmental requirements for the content of pollutants in industrial wastewater are becoming tougher every year. This is done to prevent the accumulation of toxic substances in the environment and, therefore, avert an ecological disaster. Microbiological methods are best for water purification from organic pollutants. Compared to other methods, they are of low cost, provide complete purification, and have minimal harmful effects on the ecosystem [5].

Wastewater from the food industry is contaminated with complex organic substances, inorganic electrolytes, colloidal particles of organic and inorganic origin, and toxic components of detergents. Enterprises that do not have their own wastewater treatment system throw all these components into the sewer without prior cleaning.

Thus, industrial wastewater is a complex toxic multicomponent mixture that needs to be specially treated before being discharged into sewer systems or tanks. Such treatment can be done by using modern facilities or introducing highly efficient technology.

Contaminated water from dairy plants contains various triglycerides, animal proteins, carbohydrates, mineral components, solids in suspension, chlorides, phosphorus, nitrogen compounds, cationic and amphoteric surfactants, and synthetic detergent components (strong alkalis, acids) [6].

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Washing and disinfecting organic substances are used to prevent pathogenic microflora from entering the food system [7]. However, they are toxic when released into the natural environment and can remain there unchanged for a long time [8].

The biological method is the safest and most effective method for degrading organic matter. However, some substances in multicomponent systems can inhibit enzymatic reactions and suppress the vital activity of microorganisms [9].

Therefore, it is important to select particular strains of microorganisms with high biodestructive action against certain components. A properly selected consortium can biodegrade toxic organic components in wastewater without posing a risk to the environment.

Swati and Indu [10] used *Pseudomonas aeruginosa* bacteria for the biodegradation of polycyclic organic compounds. Panigrahy *et al.* [11] described using *Pseudomonas putida, Arthrobacter Chlorophenolicus, Pseudomonas aeruginosa,* and *Pseudomonas fluorescens* for the biological removal of phenolic pollutants from the environment.

In a study by Liu *et al.* [12], the bacteria *Agrobacterium rhizogenes* decomposed up to 98% of terbutylazine in 39 hours. The scientists identified six metabolites and proposed three new pathways for strain development, including dealkylation, deamination-hydroxylation, and ring-opening reactions. Risk assessment showed that most degradation products could be significantly less harmful than terbutylazine.

Egorova *et al.* [13] studied *Achromobacter* and *Brevundimonas* strains capable of biodegrading hexachlorocyclohexane. The first step in its biodegradation is to break the carbon–chlorine bond catalyzed by dehydrochlorinase.

The best studied are the destructive properties of bacteria of the genus *Pseudomonas*. These microorganisms can oxidize various organic compounds in wastewater [14].

Bacteria of the genus *Pseudomonas* predominate in microbial consortia. In our study, they showed the highest biodegradation characteristics in relation to cocamidopropyl betaine in a pure two-component solution. We identified fragments that resulted from the destruction of this organic matter (Fig. 1). Further, N,N-dimethylglycine decomposed into the anion of acetic acid, ammonia, nitrogen oxides, as well as carbonic and N-allammonium forms [8].

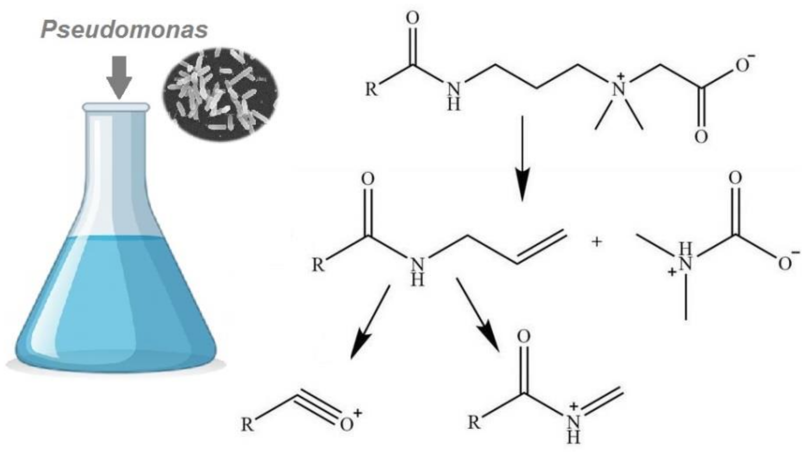


**Fig. 1.** Destruction of the organic betaine substance.

We aimed to study the biological purification of aqueous solutions from betaine organic matter under the action of bacterial strains of the genus *Pseudomonas*.

## 2 Materials and methods

### 2.1 Bacterial lines

We used the following strains of *Pseudomonas* bacteria from the Russian National Collection of Industrial Microorganisms (the Bioresource Centre of the Kurchatov Institute): *Pseudomonas fluorescens* TR (B-4881), *Pseudomonas putida* TP-19 (B-6582), *Pseudomonas mendocina* 2S (B-4710), and *Pseudomonas oleovorans* TF4-1L (B-8621).

Culture suspensions were prepared with a final concentration of 107–108 CFU/mL in 0.9% NaCl solution. The number of colonies was determined by the turbidity of cell suspensions within 0.5 McFarland units (1×108 CFU/ml) using a DEN-1 densitometer.

The microorganisms were cultivated in a chamber at 37 °C on a peptone nutrient medium for 24 hours.

### 2.2 Model wastewater

We prepared three model wastewater solutions. The first solution contained only organic matter (betaine surfactant) at a concentration of 2% (wt.). The second solution contained sodium chloride at a concentration of 1 g/100 ml. The third system contained metal salts (copper sulfate and zinc sulfate) with a total content of metal ions of 1 g/100 ml.

### 2.3 Biodegradation of the organic compound

Culture liquids with the bacterial cells under study were added to 1000 ml solutions.

Each system was tested in five repetitions. The components were bio-oxidated in an LSI-3016A/LSI-3016R shaker-incubator (Daihan Labtech, South Korea) under isothermal conditions at 37°C. The data were processed in MS Excel.

### 2.4 Water solutions analysis

Samples of the model aqueous solutions were collected for analysis on a daily basis. Prior to the determination of wastewater parameters, they were pre-treated in a CV-50 centrifuge (ELMI, Latvia).

The total chlorine content in water in the form of free chloride ion was determined by the ionometric method with an ion-selective electrode. Heavy metal ions were measured by potentiometry from ion-exchange electrodes on an Anion-4110 device (Russia). The concentrations were established by calibration curves when determining the known concentrations of metal ions in the calibration solutions.

The rate of surfactant biodegradation was determined by measuring the equilibrium concentration of organic matter in the solution. For this, we measured the optical density at 659 nm against the sodium phosphate buffer in the presence of the eriochromic black T indicator, which forms a complex compound with betaine surfactant [15].

## 3 Results and Discussion

The residual amounts of organic matter for each of the studied strains are shown in Fig. 2.

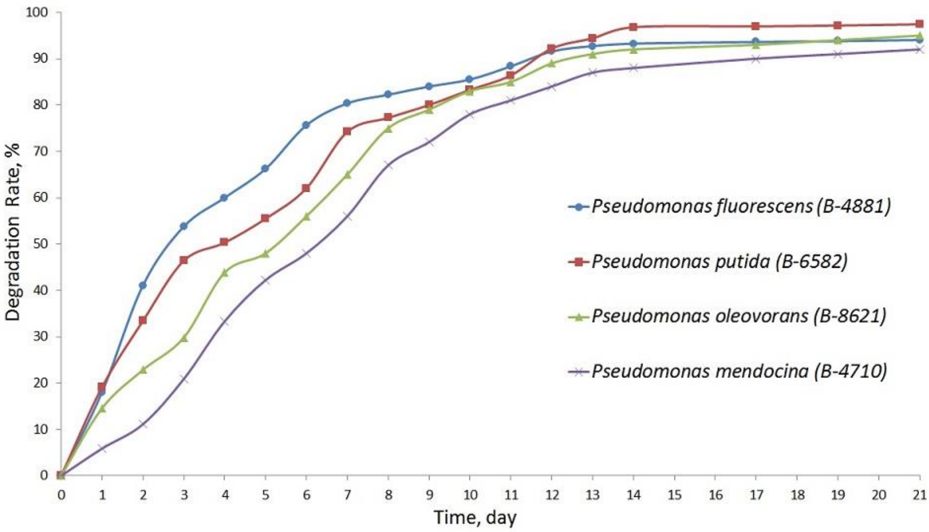


**Fig. 2.** The rate of betaine degradation versus time.

The bacterial strains decreased the concentrations of the organic pollutant with different intensity. In the system with *Pseudomonas mendocina* (B-4710), the degradation rate was 30% lower during a period of 7 days. This means that these bacteria had a long period of adaptation to the new toxic substance. In contrast, the system with *Pseudomonas fluorescens* (B-4881) showed a sharper and more intense decrease in the pollutant.

In our previous work [8] on the kinetics of biodegradation of betaine organic substances, they showed effective biodegradation in bicomponent systems. Changes in the rate of equilibrium were observed when additional contaminants were introduced into the system (Fig. 3).

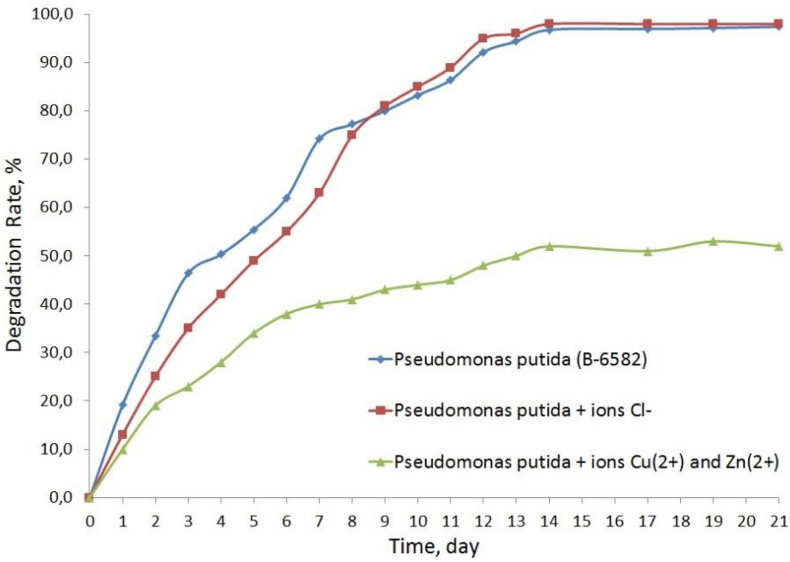


**Fig. 3.** The rate of betaine degradation versus time in the presence of additional pollutants.

In order to biodegrade toxic organic components in wastewater, it is important to select those strains of microorganisms which would be highly destructive against particular components. In addition, microbial consortia have great advantages over isolated strains due to their stronger substrate tolerance and the synergy of different bacterial species. Therefore, microbial consortia are considered more suitable for bioremediation in complex environments [16].

Bacterial strains perform differently in multicomponent and monocomponent systems. For example, a microbial consortium including the bacteria *Pseudomonas, Methylophaga, Pseudidiomarina, Thalassospira,* and *Alcanivorax* showed an excellent ability to degrade a polycyclic mixture of aromatic hydrocarbons under a wide range of conditions [17]. In particular, this consortium degraded the polycyclic mixture of phenanthrene, anthracene, fluoranthene, and pyrene (50 mg/l each) by approximately 73%, 69%, 52%, and 48%, respectively, within 21 days. The consortium showed great adaptation to a wide range of environmental conditions.

Aditya *et al.* [18] compared the performance of microalgae bacteria in wastewater treatment to create a roadmap for the practical implementation of the consortium's systems in industry. The strain *Pseudomonas fluorescens* TR (B-4881, Russian National Collection of Industrial Microorganisms) showed the highest rate of decomposition. In seven days, it decreased the organic matter by 80%. The strain *Pseudomonas putida* TP-19 (B-6582) decomposed the substance by 74% in the same period. *Pseudomonas oleovorans* TF4-1L (B-8621) destroyed the organic surfactant by 65% on the seventh day. The lowest rate of biodegradation was shown by *Pseudomonas mendocina* 2S (B-4710).

Due to their enzymes, microorganisms can decompose pollutants in a complex system more efficiently than in a two-component system. However, some substances in multicomponent systems can inhibit enzymatic reactions and suppress the activity of microorganisms.

Toxic and non-biodegradable heavy metals (Cd, Cu, Cr, and Pb) usually have an adverse effect on the biodegradation of organic pollutants by inhibiting microbial activity and inactivating microbial enzymes [16]. In our study, the microorganisms of the genus *Pseudomonas* showed lower activity in the presence of salts of heavy metals. However, chloride ions aided biodegradation and decomposed the organic matter almost completely on the 21st day.

We observed the inhibition of enzymatic degradation by metal ions Zn(2+) and Cu(2+). On the 14th day, the concentration of organic matter decreased by 50% in their presence. Without the metal ions, however, the degradation was almost 100%.

The importance of nitrogen as a substrate for microorganisms can be determined by concentrations of ammonium nitrogen, nitrites, and nitrates. Some microorganisms can completely decompose cocamidopropyl betaine in 4 days when they are co-cultivated [19]. These include *Pseudomonas sp.* FV CCM 8810 and *Rhizobium sp.* CCM 8811. The *Pseudomonas* bacterium performs primary biodegradation and destroys the alkyl radical, while the *Rhizobium* strain degrades alkylamidopropyl betaine residues. However, this process can be effective only in a medium rich in low-molecular-weight inorganic nitrogen-containing compounds. To perform fast and complete biodegradation, these microorganisms need an available source of nitrogen. In a suspension without mineral components, biodegradation lasts 29 days, which is a rather long time [20].

## 4 Conclusion

Biodegradation is a sustainable and efficient method for removing organic pollutants from the aquatic environment. For this, pure microorganisms and cultures in consortia need to be isolated with a high tolerance to toxicity.

Microorganisms have necessary enzymes to decompose pollutants both in complex and two-component systems. However, some substances in multicomponent systems can inhibit enzymatic reactions and suppress microbial activity.

The strains of microorganisms that we used in the study can decompose organic betaine compounds. However, more research is needed on the biodegradation of complex organic compounds in wastewater in the presence of other interfering pollutants.

In addition, there is a need for new kinetic models of microbial growth and degradation of pollutants with multiple substrate systems in real industrial wastewater [11].

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