PSEUDOMONAS SP. AND *AEROMONAS SP.* SELECTION FOR TREATMENT OF GOOSE DOWN WASHING WASTEWATER

Aleksander SLAVOV[1], Zapryana DENKOVA[2], Mima HADJIKINOVA[1], Nikolai MIHALKOV[3]

[1]*Department "Environmental engineering" University of Food Technologies, Plovdiv, Bulgaria,*
aleksander_slavov@yahoo.com , mimi_uht@abv.bg
[2]*Department "Microbiology" University of Food Technologies, Plovdiv, Bulgaria, zdenkova@abv.bg*
[3]*"Water projects" Ltd, Plovdiv, Bulgaria*
vodniproekti@abv.bg
*Corresponding author
Received 28 August 2012, accepted 4 September 2012

Abstract: *10 bacterial strains, isolated from municipal wastewater and active sludge that belong to Aeromonas sp. (3) and Pseudomonas sp. (7), were investigated. Strains were analyzed for their ability to treat pre-centrifuged and diluted in different ratios goose down washing wastewater.*
It is shown that Aeromonas sp. exhibit higher purification degree of goose down washing wastewater compared to the strains of Pseudomonas sp. only at a dilution of 1:1, and the highest purification degree is determined for strains Aeromonas 2 and Aeromonas 2AS with 64,94% each. At a dilution of goose down washing wastewater 1:5 the highest purification degree is at a strain Pseudomonas 1 – 86,49%, and at a dilution of goose down washing wastewater 1:10 Pseudomonas 3AS – 87,27 %.

Keywords: *Aeromonas sp., Pseudomonas sp., goose down washing wastewater, purification degree*

1. Introduction

From poultry processing as by-product large quantities feathers are produced - 7% of live weight [1]. Utilization of feathers include using of various technologies – composting, in food industry after hydrolysis of keratin [2, 3, 4, 5, 6], as adsorbent of heavy metals [7], filling material for clothes and bags [8], in composite wood MDF [9]. Obtaining quality feather include its preliminary treatment with NaCl and HCl solutions, followed by washing it with detergents and, if necessary - discoloration. As a result large quantities of wastewater with high pollution levels are produced.
Biological methods have application in wastewater treatment from food industry, and many of them are based on the specific action of particularly selected bacterial strains.
The aim of this work is the selection of *Aeromonas sp.* and *Pseudomonas sp.* strains for treatment of goose down washing wastewater.

2.Materials and methods

2.1.Microorganisms

In this work are used 10 strains of bacteria. Bacterial strains *Aeromonas sp. Aeromonas* 2 are isolated from municipal wastewater, while *Aeromonas* 1AS, *Aeromonas* 2AS – from activated sludge. Bacterial strains *Pseudomonas sp. Pseudomonas* 1, *Pseudomonas* 3, *Pseudomonas* 4 were isolated from municipal wastewater, while *Pseudomonas* 1AS, *Pseudomonas* 2AS, *Pseudomonas* 3AS, *Pseudomonas* 4AS – from activated sludge.

2.2.Nutrient mediums

***2.2.1.Luria – Bertany glucose agar medium (LBG) with composition (g/dm^3)*:** triptone (Difco) – 10 g, yeast extract – 5 g, NaCl – 10 g, glucose (Scharlau) – 10 g; agar – 20 g. pH=7,5. The medium is sterilized for 25 minutes at 121º C.

***2.2.2.Glutamate-starch-phenol red agar medium (GSP) with composition (g/dm^3)*:** sodium glutamate - 10 g, starch (soluble) - 20 g, KH_2PO_4 - 2 g, $MgSO_4$, Phenol - red - 0,36 g, agar - 12 g. pH 7,2 ± 0,2. All ingredients are dissolved and medium is sterilized for 15 min at 121° C. Cool to 45-50° C and aseptically add to it 100 IU/ml penicillin G, and if necessary, 10 μg/ml pymaricyn. The resulting composition is mixed thoroughly and dispensed in sterile petri dishes.

2.3.Wastewater: In the experiments goose down washing wastewater to slaughterhouse is used. Wastewater is pre-centrifuged at 3000 min^{-1} for 10 min to remove insoluble substances and fat and diluted with non-sterile distilled water in ratios 1:1, 1:5 and 1:10.

2.4.Cultivation and storage of microorganisms.

Isolated strains are grown in LBG agar medium at 30º C in thermostat for 48 h and stored in a refrigerator at 4º ± 2° C for 2 weeks.

2.5.Analytical methods.

2.5.1.Isolation of Aeromonas sp. u Pseudomonas sp. strains.

Isolation of strains of *Aeromonas sp*. and *Pseudomonas sp*. was carried out by seeding samples of municipal wastewater, and active sludge on GSP agar medium and their subsequent development at 30° ± 2° C in a thermostat for 24 h. Colored in yellow colonies belong to *Aeromonas sp*., while red relate to *Pseudomonas sp*.

2.5.2.*Development of isolated strains of bacteria in the diluted wastewater*.

With biomass from developed on LBG agar medium at 30° ± 2° C for 48 h bacterial strains 5 cm^3 of diluted wastewater is inoculated. Wastewater samples with investigated cultures are cultivated for 72 h at 30° ± 2° C.

2.5.3.*Purification degree wastewater determination using the permanganate oxidizability method.*

Developed for 72 h at 30° ± 2° C in wastewater cultures are centrifuged at 3000 min^{-1} for 10 min. The resulting supernatant is analyzed for permanganate oxidizability in accordance with standard BS 17.1.4.16-79.

100 cm^3 sample or lower volume brought to 100 cm^3 with distilled water is placed in a 250-300 cm^3 flask, which has added pumice. 5 cm^3 H_2SO_4 (1+2) and 20 cm^3 0,01 N $KMnO_4$ solution are added. The composition is heated so as to boil for no more than 5 min and boiling for 10 min. To the hot solution 20 cm^3 0,01 N solution of HOOC-COOH are added. Hot discoloured solution is titrated with 0,01 N $KMnO_4$ solution. The temperature of the solution during titration should not be below 80° C. If the boiling solution is discolored or brown precipitates are formed, the determination is repeated with a smaller sample size.

During titration should not spend more than 12 cm^3, and not less than 4 cm^3 of $KMnO_4$ solution for diluted sample. Similarly, a blank is done with wastewater. Oxidizability (X) in mg/dm^3 oxygen is given by the formula:

$$X = \frac{(a-b).N.8000}{V},$$

where

a – V_{KMnO4}, spent for titration of the sample, cm^3;

b – V_{KMnO4}, spent for titration of blank, cm^3;

N – exact normality of $KMnO_4$ solution;

V – volume of sample taken for analysis, cm^3.
Similarly oxidizability of a control sample of analyzed wastewater at the corresponding dilution of 1:1, 1:5 and 1:10 is determined.
Purification degree PD, in %, is determined using the formula:

$$PD = \frac{Xc - Xs}{Xs} \cdot 100,\%$$, where

Xc – oxidizability of control sample, mg/dm^3 oxygen;
Xs – oxidizability of sample, mg/dm^3 oxygen.

3. Results and discussion.

In a set of experiments the ability of isolated *Aeromonas sp*. and *Pseudomonas sp*. strains to break down pollutants in washing goose down wastewater, diluted with distilled water in different ratios, is investigated. Summarized results are presented in Fig. 1, Fig. 2 and Fig. 3. Experimental data show an average level of treatment for all strains of *Aeromonas sp*. and *Pseudomonas sp*. at wastewater dilution 1:1, 1:5 and 1:10, respectively.

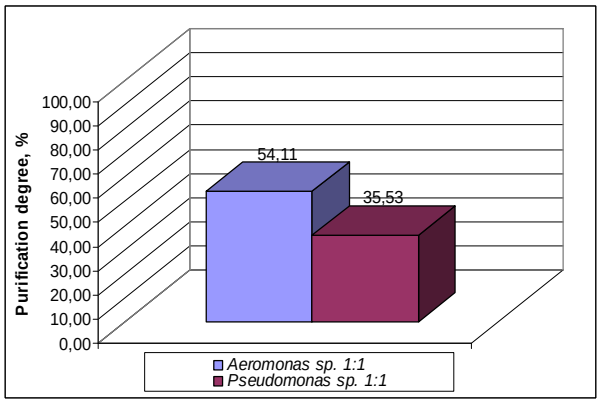

Figure 1. Average purification degree of goose down washing wastewater, diluted in ratio 1:1 with *Aeromonas sp*. and *Pseudomonas sp*.

It is noteworthy that with increasing dilution increases the degree of purification as in *Aeromonas sp*., and in *Pseudomonas sp*. Exceptions are strains *Aeromonas sp*. with the degree of dilution 1:5 (Fig. 2).

Strains *Aeromonas sp*. purify wastewater better than the strains *Pseudomonas sp*. when diluted 1:1 with 18,58% (Fig. 1), while at 1:5 and 1:10 dilutions of the strains *Pseudomonas sp*. show a higher level of treatment - with 34,86% (Fig. 2) and 29,73% (Fig. 3).

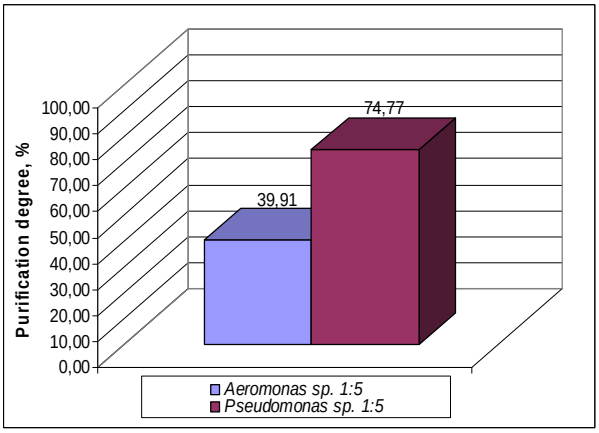

Figure 2. Average purification degree of goose down washing wastewater, diluted in ratio 1:5 with *Aeromonas sp*. and *Pseudomonas sp*.

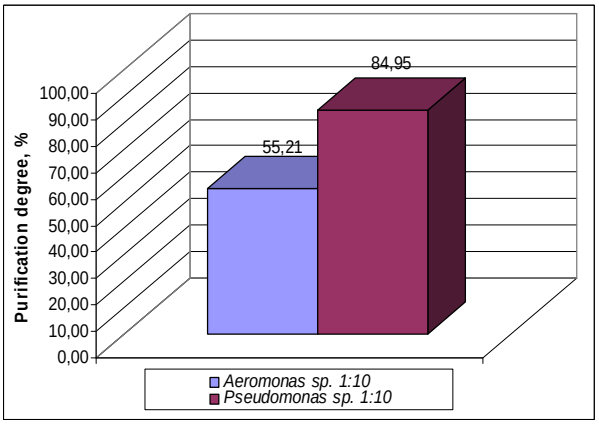

Figure 3. Average purification degree of goose down washing wastewater, diluted in ratio 1:10 with *Aeromonas sp*. and *Pseudomonas sp*.

A comparative characteristic between strains of *Aeromonas sp*. and *Pseudomonas sp*. for the purification degree of goose down wastewater at different dilution ratios is done. Results from these studies are presented in Figure 4, Figure 5 and Figure 6. The experimental data represented in Figure 4 show that with purification degree over 50% are strains

Aeromonas 2, *Aeromonas* 2AS, *Pseudomonas* 3, *Pseudomonas* 2AS and the highest is the degree at *Aeromonas* 2 and *Aeromonas* 2AS – 64,94%. Strain *Pseudomonas* 3 exceeds the other three *Pseudomonas sp*. with the purification degree 59, 09 %.

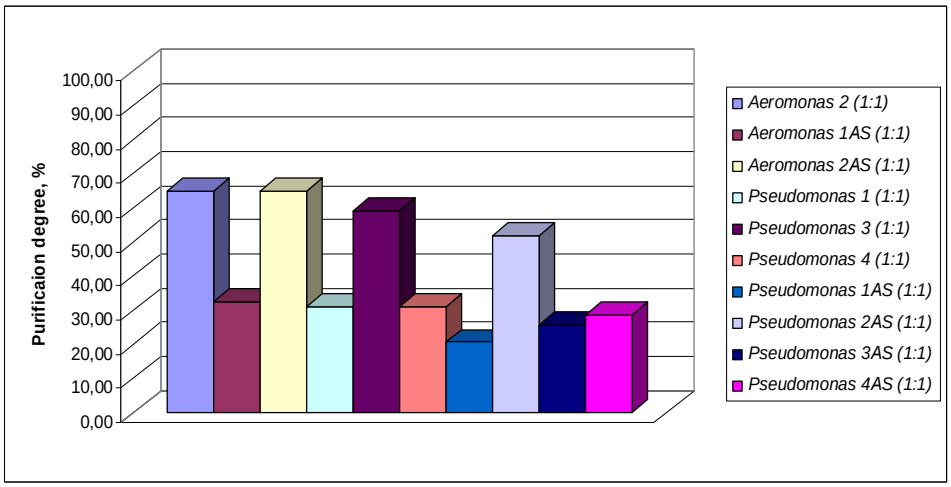

Figure 4. Purification degree of goose down washing wastewater, diluted in ratio 1:1 with *Aeromonas sp.* and *Pseudomonas sp*.

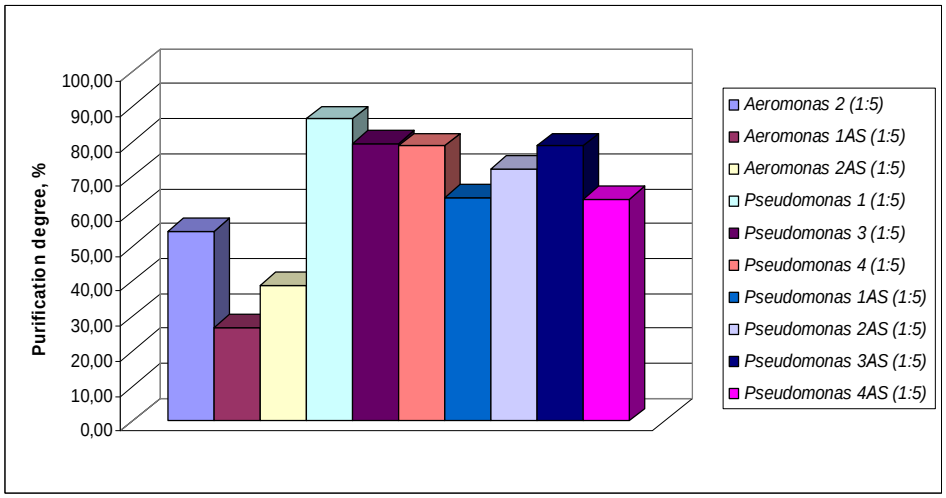

Figure 5. Purification degree of goose down washing wastewater, diluted in ratio 1:5 with *Aeromonas sp*. and *Pseudomonas sp*.

At a dilution ratio 1:5 (Figure 5) strain *Pseudomonas* 1 shows the highest degree of purification – 86,49%, followed by strain *Pseudomonas* 3 – 79,48 %. Third place is shared by strains *Pseudomonas* 4 and *Pseudomonas* 3AS - with average value 78,70 %. From *Aeromonas sp*. with the highest purification degree is strain *Aeromonas* 2 with 54,23%.

At 1:10 dilution ratio (Figure 6) the highest purification degree has strain *Pseudomonas* 3AS – 87,27%, followed by *Pseudomonas* 3 and *Pseudomonas* 1 with 87,19% and 87,01%, respectively. Purification degree of *Aeromonas sp*. strains reached only 57,95% of *Aeromonas* 2AS, followed by *Aeromonas* 2 and *Aeromonas* 1AS with 56,92% and 50,77%, respectively.

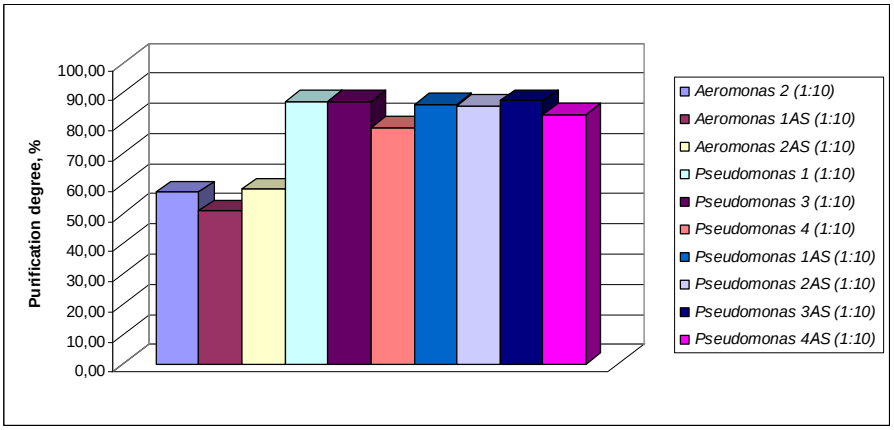

Figure 6. Purification degree of goose down washing wastewater, diluted in ratio 1:10 with *Aeromonas sp*. and *Pseudomonas sp*.

4. Conclusion.

As a result of investigated analyses on goose down washing wastewater treatment the following more important conclusions can be made:

1.The more dilution of goose down washing wastewater is done, the greater is its purification degree. Exception is made for strains *Aeromonas sp*. with dilution factor of the effluent 1:5;

2.Strains *Aeromonas sp*. exhibit higher purification degree of goose down washing wastewater compared to *Pseudomonas sp*. strains only at a dilution factor 1:1.

3.At a dilution factor of goose down washing wastewater 1:1 the highest purification degree is determined at strains *Aeromonas* 2 and *Aeromonas* 2AS with 64,94%, at dilution ratio 1:5 – at strain *Pseudomonas* 1 – 86,49% and at dilution factor 1:10 strain *Pseudomonas* 3AS – 87,27%.

5. References.

[1] LORTSCHER, L. L., G. F. SACHSEL, D. WILKELMY JR. AND R. B. FILBERT JR., Processing poultry by products in poultry slaughter plants. *U.S. department of agriculture marketing research department*, No. 181, Washington, DC, (1957)

[2] NAGAL, S. И JAIN, P. C., Feather degradation by strains of Bacillus isolated from decomposing feathers. *Brazilian journal of microbiology*, Vol. 41, 196-200, (2010)

[3] WRZESNIEWSKA-TOSIK, K. И ADAMIEC, J., Biocomposites with a content of keratin from chicken feathers. *Fibres and textiles in eastern Europe*, Vol. 15 (1), 106-112, (2007)

[4] FUJI, T. И LI, D., Preparation and properties of protein films and particles from chicken feather. *Journal of biological macromolecules*, Vol. 8 (2), 48-55, (2008)

[5] KIM, J.-D., Preliminary characterization of keratinolytic enzyme of Aspergillus flavus K-03 and its potential in biodegradation of keratin wastes, *Mycobiology*, Vol. 31 (4), 209-213, (2003)

[6] KUMAR, D. J. M., PRIYA, P., BALASUNDARI, S. N., DEVI, G. S. D. N., REBECCA, A. I. N. И KALAICHELVAN, P. T., Production and optimization of feather protein hydrolyzate from Bacillus sp. MPTK6 and its antioxidant potential. *Middle-east journal of scientific research*, Vol. 11 (7), 900-907, (2012)

[7] SUN P., LIU Z.-T., LIU Z.-W., Chemically Modified Chicken Feather as Sorbent for Removing Toxic Chromium(VI) Ions. *Industrial and engineering chemistry research*, Vol. 48 (14), 6882-6889, (2009)

[8] CHOBAN, A. И WINKLER, I., Feather-processing wastewater: composition, influence on the natural water objects and decontamination technologies. *Croatian journal of food technology, biotechnology and nutrition*, Vol. 6 (1-2), 58-62, (2011)

[9] WINANDY, J. E., MUEHL, J. H., MICALES, J. A., RAINA, A. И SCHMIDT, W., *Potential of chicken feather fibre in wood MDF composites. Proceedings paper EcoComp 2003. Queen Mary, University of London*, 1-6, (2003)