BACTERIAL AND YEAST STRAIN SELECTION FOR TREATMENT OF GOOSE DOWN WASHING WASTEWATER

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Abstract: 15 strains bacteria and 3 strains yeasts are investigated for their ability to treat goose down washing wastewater. Bacterial strains 2, 3.1LBG, 3.2 LBG, 3.3LBG, 3.4LBG, 3.1.MRS, 3.2MRS, 3.3MRS, 3.5MRS, 3.7MRS, 4, 5, 7, 8 and yeast strain Y are isolated from soil, strain Micrococus 2 – from raw dried meat products. Yeast strains C1 and C2 are provided from yeast strains' collection. Bacterial strains 2, 3.4LBG and 3.7LBG are non-spore forming long rods, while strains 3.1LBG, 3.2 LBG, 3.3LBG, 3.1.MRS, 3.2MRS, 3.3MRS, 3.5MRS, 4, 5, 7, 8 - non-spore forming short rods.
Analyses are carried out with preliminary flocculated and filtered goose down washing wastewater. It was found that yeast strains show higher purification degree of goose down washing wastewater than bacterial strains. Short rods treat wastewater with 27, 24 % more effectively that long rods. From yeast strains C1 has highest purification degree – 28,53 %, and from bacterial strains – MRS 3.3 – 25,58%.
Keywords: bacteria, yeasts, washing wastewater, purification degree

1. Introduction

During washing poultry feather highly contaminated with organic substances wastewater, which involve the risk of environmental contamination, is received. It contains large amounts of fats and proteins [1], which are presented in form of finely dispersed difficult to separate emulsion. Their removal is possible if wastewater is subjected to prior treatment with flocculants and subsequent flotation process [2-3]. These two processes act predominantly on suspended pollutants, but do not affect the solutes [4]. Removing the last ones is possible using biological treatment methods. Most of them are based on the biochemical degradation of pollutants by the action of specially adapted to the particular wastewater bacterial and yeast strains [5-7].

The aim of this work is the selection of bacterial and yeast strains for the treatment of preliminary flocculated and filtered goose down washing wastewater.

2. Materials and methods

2.1. Microorganisms

In this work are used 15 strains of bacteria and three strains of yeasts. Bacterial strains 2, 3.1LBG, 3.2LBG, 3.3LBG, 3.4LBG, 3.1MRS, 3.2MRS, 3.3MRS, 3.5MRs, 3.7MRS, 4, 5, 7, 8 and yeast strain Y are isolated from soil. Bacterial strain *Micrococcus* 2 is isolated from raw-dried meat products. Yeast strains C1 and C2 are provided by the microorganisms' collection of the Department "Microbiology" at University of Food Technology, Plovdiv, Bulgaria. Through microscopic observations it is found that bacterial strains 2, 3.4 LBG and 3.7MRS are non-sporeforming long rods with rounded ends, while strains 3.1LBG, 3.2LBG, 3.3LBG, 3.1MRS, 3.2MRS, 3.3MRS, 3.5MRS, 4, 5 , 7 and 8 are non-sporeforming short rods with rounded ends.

2.2. Nutrient mediums

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

2.2.2. **Luria – Bertany glucose agar medium (LBG agar) 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.

2.2.3. **Malt extract medium (ME) with composition:** malt extract (Kamenitza, Bulgaria) in 1:1 ratio with tap water (vol/vol). pH=6,5 – 7,0. The medium is sterilized for 25 minutes at 121º C.

2.2.4. **Malt agar medium (MA) with composition:** malt extract (Kamenitza, Bulgaria) in 1:1 ratio with tap water (vol/vol), agar – 20 g. pH=6,5 – 7,0. The medium is sterilized for 25 minutes at 121º C.

2.2.5. Lactobacillus **medium on the formulation of de Man, Rugosa and Sharpe (MRS) with composition** (g/dm^3): peptone (Scharlau) – 10 g, meat extract (Scharlau) – 5 g, yeast extract (Scharlau) – 5 g, glucose (Scharlau) – 20 g, K_2HPO_4 – 2 g, diammonium hydrogen cictrate – 2 g, $NaOOCCH_3$ – 5 g, $MgSO_4$ – 0,1 g, $MnSO_4$ – 0,05 g. pH 6,5 ± 0,2. The medium is sterilized for 25 minutes at 121º C.

2.2.6. Lactobacillus **agar medium on the formulation of de Man, Rugosa and Sharpe (MRS agar) with composition** (g/dm^3): peptone (Scharlau) – 10 g, meat extract (Scharlau) – 5 g, yeast extract (Scharlau) – 5 g, glucose (Scharlau) – 20 g, K_2HPO_4 – 2 g, diammonium hydrogen cictrate – 2 g, $NaOOCCH_3$ – 5 g, $MgSO_4$ – 0,1 g, $MnSO_4$ – 0,05 g, agar – 20 g. pH 6,5 ± 0,2. The medium is sterilized for 25 minutes at 121º C.

2.2.7. **Soya – caseine broth medium (SCB) with composition (g/dm^3):** triptone (Difco) – 17 g, soya peptone (Scharlau) – 3 g, NaCl – 5 g, K_2HPO_4 – 2,5 g, glucose (Scharlau) – 2,5 g. pH=7,3 ± 0,2. The medium is sterilized for 25 minutes at 121º C.

2.2.8. **Soya – caseine agar medium (SCA) with composition (g/dm^3):** triptone (Difco) – 17 g, soya peptone (Scharlau) – 3 g, NaCl – 5 g, K_2HPO_4 – 2,5 g, glucose (Scharlau) – 2,5 g, agar – 20 g. pH=7,3 ± 0,2. The medium is sterilized for 25 minutes at 121º C.

2.2.9. **Medium for *Micrococcus varians* and *Staphylococcus saprophyticus* (SMS) with composition** (g/dm^3): yeast extract (Scharlau) – 5 g, meat extract (Scharlau) – 1 g, peptone (Scharlau) – 10 g, NaCl – 5 g, glucose (Scharlau) – 10 g, K_2HPO_4 – 0,5 g. pH 6,8 ± 0,2. The medium is sterilized for 25 minutes at 121º C.

2.2.10. **Agar medium for *Micrococcus varians* and *Staphylococcus saprophyticus* (SMS agar) with composition** (g/dm^3): yeast extract (Scharlau) – 5 g, meat extract (Scharlau) – 1 g, peptone (Scharlau) – 10 g, NaCl – 5 g, glucose (Scharlau) – 10 g, K_2HPO_4 – 0,5 g, agar – 20 g. pH 6,8 ± 0,2. The medium is sterilized for 25 minutes at 121º C.

2.3. Wastewater:

In the experiments goose down washing wastewater to slaughterhouse is used. Wastewater is treated prior to addition of 15 cm^3 0,1% solution of flocculant HENGFLOC 82 412 (Beijing Hengju)/100 cm^3 wastewater. The composition is stirred and allowed to stand for 20 min. After the reaction time wastewater is centrifuged at 3000 min^{-1} for 10 min and filtered through a glass filter to separate the flocculated impurities. The resulting clear filtrate of the effluent is used for further analysis.

2.4. Cultivation and storage of microorganisms.

Bacterial and yeast strains are grown on different media, respectively for bacterial strains 2, 3.1LBG, 3.2LBG, 3.3LBG, 3.4LBG – LBG; for bacterial strains 3.1MRS, 3.2MRS, 3.3MRS, 3.5MRS, 3.7MRS – MRS; for bacterial strains 4, 5, 7 and 8 – SCB; for bacterial strain *Micrococcus* 2 – SMS; for yeast strains Y, C1 и C2 – ME at 30° ± 2° C in thermostat for 48 h and are stored in refrigerator at 4° ± 2° C for 3 weeks.

2.5. Analytical methods.

2.5.1. Development of isolated bacterial and yeast strains in pre-treated with flocculants and filtered wastewater.

2.5.2. With biomass from developed on mediums LBG agar, MRS agar, SMS agar, SCA at 30° ± 2° C for 48 h strains of bacteria and yeasts developed on medium MA 5 cm^3 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 bacteria strains 2, 3.1LBG, 3.2LBG, 3.3LBG, 3.4LBG, 3.1MRS, 3.2MRS, 3.3MRS, 3.5MRS, 3.7MRS, 4, 5, 7, 8 and *Micrococcus* 2 and yeast strains Y D, C1 and C2 to break down pollutants in pre-flocculated and filtered wastewater are investigated. Summarized results are presented in Figure 1 and Figure 2. Experimental data in Figure 1 show an average level of treatment for all strains of bacteria and yeasts, while Figure 2 – average purification degree of bacterial strains.

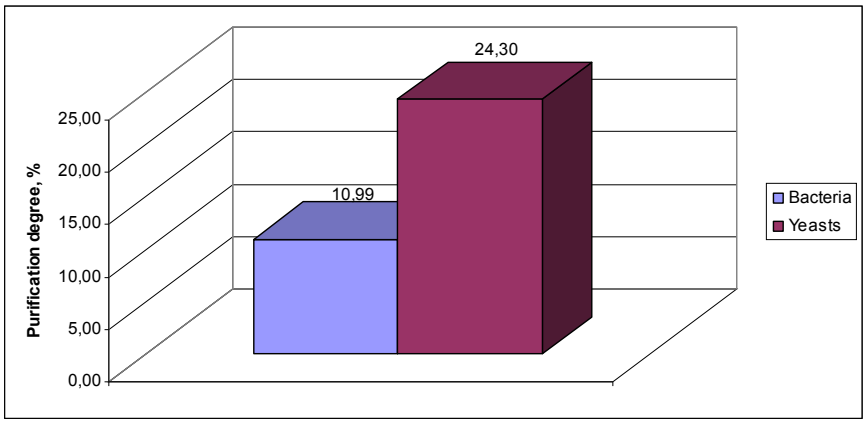

Fig. 1. Average purification degree of preliminary flocculated and filtered goose down washing wastewater with bacterial and yeast strains.

On Figure 1 is clearly shown that yeast strains exhibit higher purification degree of pre-flocculated and filtered goose down washing wastewater with 13.31% in comparison with bacterial strains.

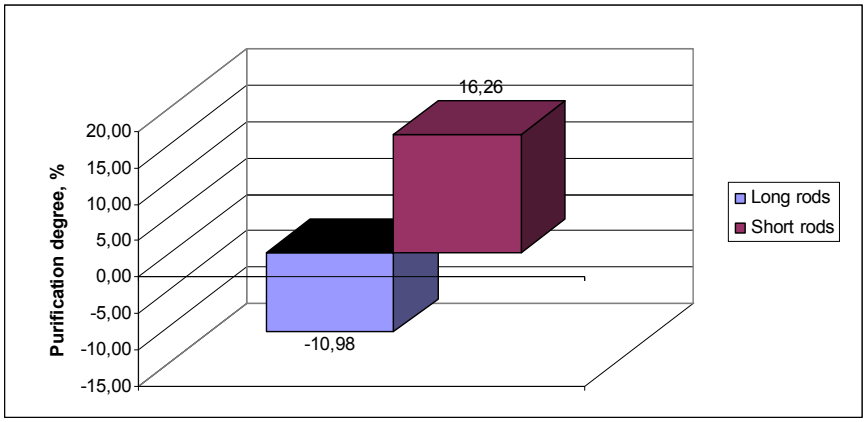

Fig. 2. Average purification degree of preliminary flocculated and filtered goose down washing wastewater with bacterial strains long rods and short rods.

In comparison of bacterial strains short rods treat better preliminary flocculated and filtered goose down washing wastewater better than the long rods (Figure 2) with 27,24%.

Comparative characteristics between bacterial and yeast strains for the purification degree of preliminary flocculated and filtered goose down washing wastewater. Results from these investigations are presented in Figure 3.

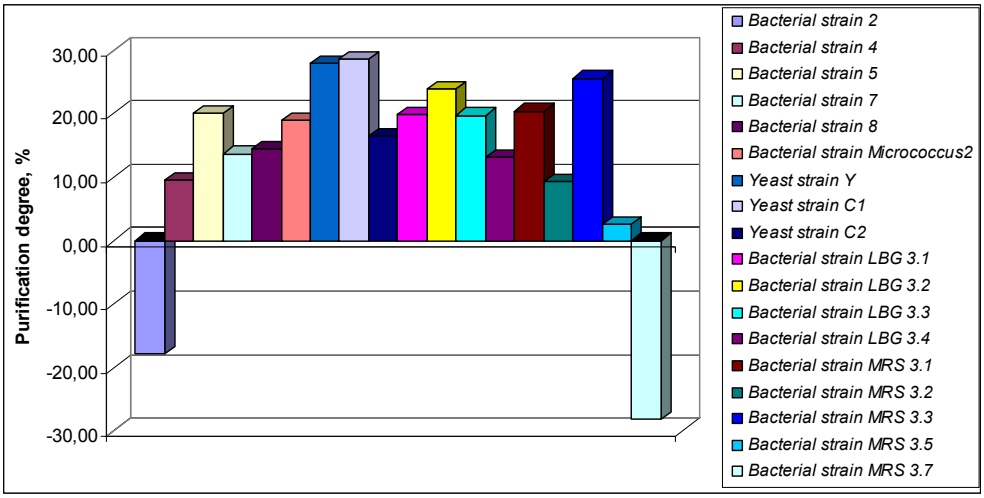

Fig. 3. Purification degree of preliminary flocculated and filtered goose down washing wastewater with bacterial and yeast strains.

The experimental data shown in Figure 3 shows that with purification degree of preliminary flocculated and filtered goose down washing wastewater over 20% are yeast strains - Y and C1 and strains of bacteria - 5, 3.2LBG, 3.1MRS and 3.3MRS. Yeast strain with the highest purification degree is strain C1 with purification degree – 28,53%, followed by yeast strain Y – 27,89%. At bacterial strains the highest purification degree has bacterial strain 3.3MRS – 25,58%, followed by 3.2LBG – 23,86% and 3.1MRS – 20,42%. With the lowest purification degree is bacterial strain 3.7MRS, followed by bacterial strain 2, which give negative values.

4. Conclusion.

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

1. Investigated yeast strains exhibit higher purification degree of wastewater compared to the strains of bacteria;

2. Bacterial strains short rods treat more effectively effluent compared to the strains of bacteria long rods with 27.24%;

3. The highest purification degree is determined in yeast strains C1 and Y 28,53 % and 27,89 %, respectively. Bacterial strains with the highest purification degree is strain 3.3MRS – 25,58 %, followed by 3.2LBG – 23,86 % and 3.1MRS – 20,42 %.

5. References.

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