

## MICROBIOLOGICAL AND BIOCHEMICAL CHARACTERISATION OF DAIRY AND BREWERY WASTEWATER MICROBIOTA

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### Abstract

*This research was conducted to study the wastewater microbiota and to identify some new active strains adapted to the wastewater physical - chemical conditions, for using them as specialised inoculum in wastewater treatment on test models, in laboratory bioreactors or pilot plants. Thus, the dairy and brewery industry wastewater's microorganisms were isolated in pure cultures and were analyzed regarding the biochemical properties. The biodegradation essay revealed the potential of the isolated cultures to metabolize organic compounds, similar to those which determine the pollution of food industry wastewaters such as starch, casein, basic carbohydrates and lactic acid. There were identified strains able to produce a fast biodegradation of the organic compounds. Epifluorescence microscopy revealed the microbiota variety in the food industry wastewaters, with Gram negative bacteria as predominant microorganisms.*

**Keywords:** wastewater microbiota, dairy and brewery industry, biodegradability, microbiological and biochemical characterization.

### Rezumat

*Scopul acestei activități de cercetare a fost studiul microbiotei apelor uzate reziduale și identificarea unor tulpini noi adaptate la condițiile fizico - chimice din aceste ape, în vederea obținerii de culturi adaptate pentru epurare și utilizarea acestora pentru testare pe sisteme model, în condiții de laborator (bioreactor) sau în stația pilot de epurare biologică aerobă a apelor reziduale. Astfel, s-au izolat în culturi pure și s-au analizat din punct de vedere al proprietăților biochimice, microorganisme componente ale microbiotei apelor reziduale din industria laptelui și a berii. S-a studiat potențialul culturilor izolate de a metaboliza compuși organici, similari cu cei care determină poluarea organică a apelor reziduale din industria alimentară (amidon, cazeină, glucide simple și acid lactic). Au fost identificate tulpini capabile să biodegradeze rapid compușii organici. Cu ajutorul microscopului cu epifluorescență, s-a pus în evidență o microbiotă diversă, predominante fiind bacteriile Gram negative.*

**Cuvinte cheie:** microbiota apelor reziduale, industria laptelui și a berii, biodegradabilitate, caracterizare microbiologică și biochimică.

### 1. Introduction

Water is one of the basic life compounds and that is why it is very important to know its quality. Although Romania has only 1700 m<sup>3</sup> water consumption per year per inhabitant, it has a very rich water resource taking the 21<sup>st</sup> place in Europe (according to the United States statistics) (Banu, 1998).

The main pollutant derived from the industrial wastewaters are organic and inorganic substances, solved or in suspension, with different harmfulness degree (Banu, 1998).

The natural and the zymogene microorganisms (which come in waters by pollution) constitute a diverse microbiota adapted to the different physical - chemical wastewater conditions, being very important for biodegradation (Dawson, 2005).

The wastewater microbiota depends on the waters origin. Usually there is a large amount of heterotrophic microorganisms which belong to the following species: *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter*, *Streptococcus faecalis*, *Escherichia coli* etc. The yeasts belonging to the genus *Saccharomyces*, *Candida*, *Cryptococcus*, are frequently found in wastewaters (Madigan *et al.*, 2000, Carawan and Hansen, 1979).

For the environment protection and for the health and security of biocenosis, in the context of Romania's integration in UE, it is imperative the alignment of our country to the demands of the environment protection policy, meaning also the demands those regarding the treatment - through different methods - of the wastewater derived from industry and other fields. In this way, the food industry wastewaters are very important.

They are characterized by a high amount of organic compounds correlated with a high amount of microorganisms adapted for biodegradation of substances found in the environment.

To get an efficient biological wastewater treatment it is very important to know the wastewater microbiota composition and the biochemical properties correlated to the origin of pollutants, as well as the optimum metabolic activity and the physical - chemical conditions (Janczukowicz *et al.*, 2007).

The aim of this study is to evaluate the dairy and brewery wastewater microbiota and its biochemical activities, in order to obtain pure cultures adapted for wastewater treatment and to use them on model and real systems of biological treatment. The data will be used to study the biological treatment processes.

## 2. Materials and Methods

### 2.1. Water sampling

A sampler with a piston, flexible tube and plastic recipients of 150 ml volume were used for the wastewater sampling. Before sampling, the recipients were disinfected with ethylic alcohol.

After the examination of the plant plans the identification and the examination of the wastewater evacuation points followed. The sampling was performed in the outlet canal placed in the production unit yard (in the case of brewery industry) and inside the production unit (in the case

of dairy industry), before the wastewater being overflowed in the municipal collecting system. In both of them, the wastewater samples came from two different sources.

In the brewery plant, the wastewater samples came from the general sewage which collects the wastewater from fermentation, filtration and packing units.

In the dairy plant, the wastewater samples came from cheese production and general sewerage.

The wastewater samples were collected at the beginning of January and at the ending of February being kept at 0..4°C and analysed in the Wastewater Treatment Lab of Food Science and Engineering Faculty, *Dunarea de Jos* University of Galati.

### 2.2. Wastewater microbiological valuation

Wastewater microorganisms were isolated by Koch method based on the cell dilution and spreading on the agar medium. From the individual colonies resulted on the medium, bacteria, yeasts and moulds, pure cultures were obtained by inoculation in test tubes with sloping medium surface. The optimal conditions used were those recommended for the microorganism cultivation, namely the cultivation on MEA (malt extract agar) at temperatures of 25...28°C, for 3–5 days (yeasts and moulds), and the cultivation on PCA (plate count agar) medium at temperature of 37°C, for 48 hours (bacteria).

The pure cultures were encoded and kept as stock cultures for biochemical evaluation (Table 1).

A morphological evaluation through the examination of cultural characters of the colonies and by microscopic analysis of cells Gram stained by using epifluorescence and phase contrast microscope Olympus 4BX was performed for the isolated strains.

### 2.3. Biochemical properties evaluation for the isolated cultures from the wastewater microbiota

To distinguish the cell growth on the different carbon and nitrogen sources, on plate agar media the isolated pure culture cells were inoculated by pricking the solid media surface with different unique carbon and nitrogen sources as follows:

*The basal medium used for the bacteria growth (code BC):*

NaCl.....	0.5%
NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub> .....	0.1%

MgSO <sub>4</sub> .....	0.02%
K <sub>2</sub> HPO <sub>4</sub> .....	0.1%
Agar .....	2.0%

Supplemented with:

- Carbon sources: 1% maltose, 1% lactose, 1% starch;
- Nitrogen source: 1% casein

*The basal medium used for yeast multiplication (code Dj):*

MgSO <sub>4</sub> · 7H <sub>2</sub> O .....	0.07%
NaCl .....	0.05%
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.74%
K <sub>2</sub> HPO <sub>4</sub> .....	0.013%
KH <sub>2</sub> PO <sub>4</sub> .....	0.1%
Agar .....	2.0%

Supplemented with:

- Carbon sources: 1% maltose, 1% lactose

*The basic medium used for the mould growth (Czapek salts, code Cza):*

NaNO <sub>3</sub> .....	0.2%
K <sub>2</sub> HPO <sub>4</sub> .....	0.1%
MgSO <sub>4</sub> · 7H <sub>2</sub> O .....	0.05%
KCl .....	0.05%
FeSO <sub>4</sub> · 4H <sub>2</sub> O .....	0.001%
Agar .....	2.0%

Supplemented with:

- Carbon sources: 1% starch, 1% lactic acid

The capacity of microorganisms to metabolise different carbon and nitrogen compounds was monitored through diameter colony determination by cell growth on the specific medium, supplied with maltose, lactose, starch or lactic acid as carbon sources, and casein as nitrogen source. For starch and casein biodegradation potential assay, the determination of the biodegradation potential was

possible through a hydrolysis index evaluation, based on ratio between diameter of hydrolyse zone of substrate around the active colonies and colony diameter. All experiments were performed in duplicates and the data plotted represent average values and standard deviations.

### 3. Results and Discussion

#### 3.1. Microbiota diversity in the food industry wastewaters

Both the dairy and the brewery wastewaters contain a high concentration of biodegradable organic compounds which represent a favourable environment for the growth of microorganisms.

In order to identify the type of microorganisms, after the cultural examination of the pure cultures obtained, there were performed microscopic and macroscopic observations.

Bacteria are the microorganisms which predominate in wastewaters. In the dairy wastewater, the yeast and mould culture diversity and the bacteria form a complex community in correlation with the carbon and organic nitrogen compounds (lactose, lactic acid, casein etc).

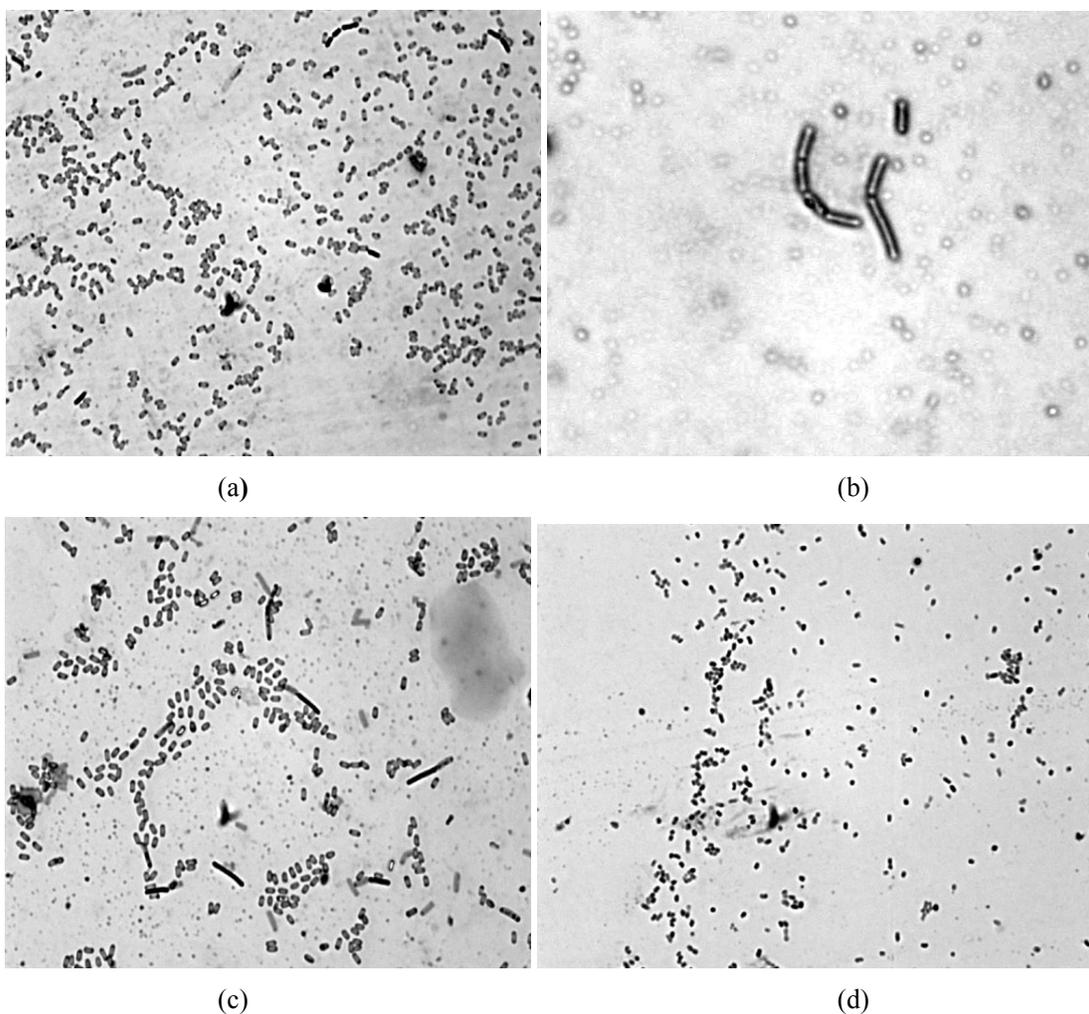
After the microscopic examination correlated with the cultural characters, there were identified Gram positive and Gram negative bacteria as *Bacillus* sp. and *Pseudomonas* sp., yeast species which belong to the *Saccharomyces*, *Torulopsis* and *Kluyveromyces* genus and moulds which belong to the *Aspergillus* and *Geotrichum* genus. The morphology of the cells is presented in Figures 1, 2 and 3.

**Table 1.** The different strains of microorganisms identified in the dairy and brewery wastewater microbiota

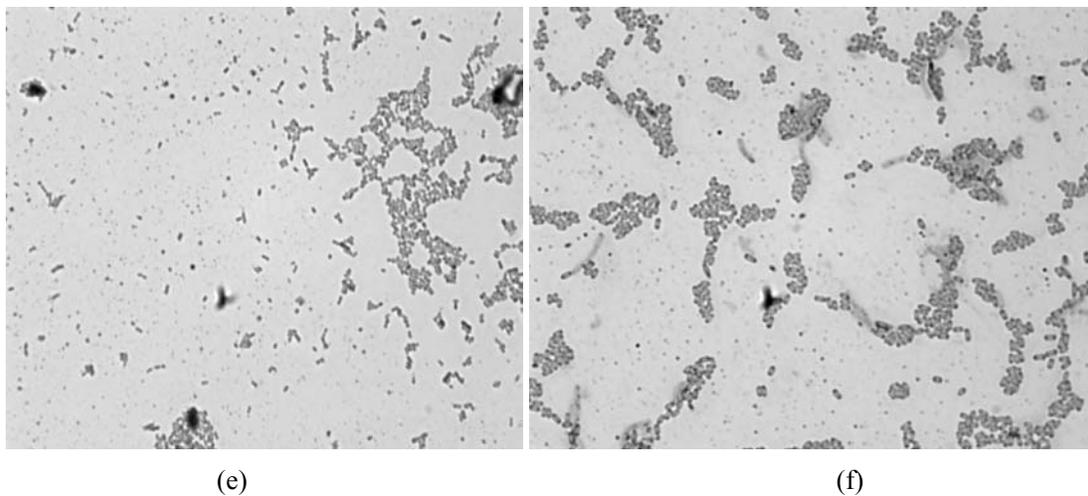
The source		Bacteria	Yeasts	Moulds
Brewery (B)	Source 1 (S <sub>1</sub> )	I BS <sub>1</sub> Bc1		II BS <sub>1</sub> Fg1
		I BS <sub>1</sub> Bc2		II BS <sub>1</sub> Fg2
		I BS <sub>1</sub> Bc3		
		I BS <sub>1</sub> Bc4		
		I BS <sub>1</sub> Bc5		
		I BS <sub>1</sub> Bc6		
		II BS <sub>1</sub> Bc1		
	Source 2 (S <sub>2</sub> )	I BS <sub>2</sub> Bc1	I BS <sub>2</sub> Dj1	
		I BS <sub>2</sub> Bc2	II BS <sub>2</sub> Dj1	
		II BS <sub>2</sub> Bc1		

**Table 1.** The different strains of microorganisms identified in the dairy and brewery wastewater microbiota

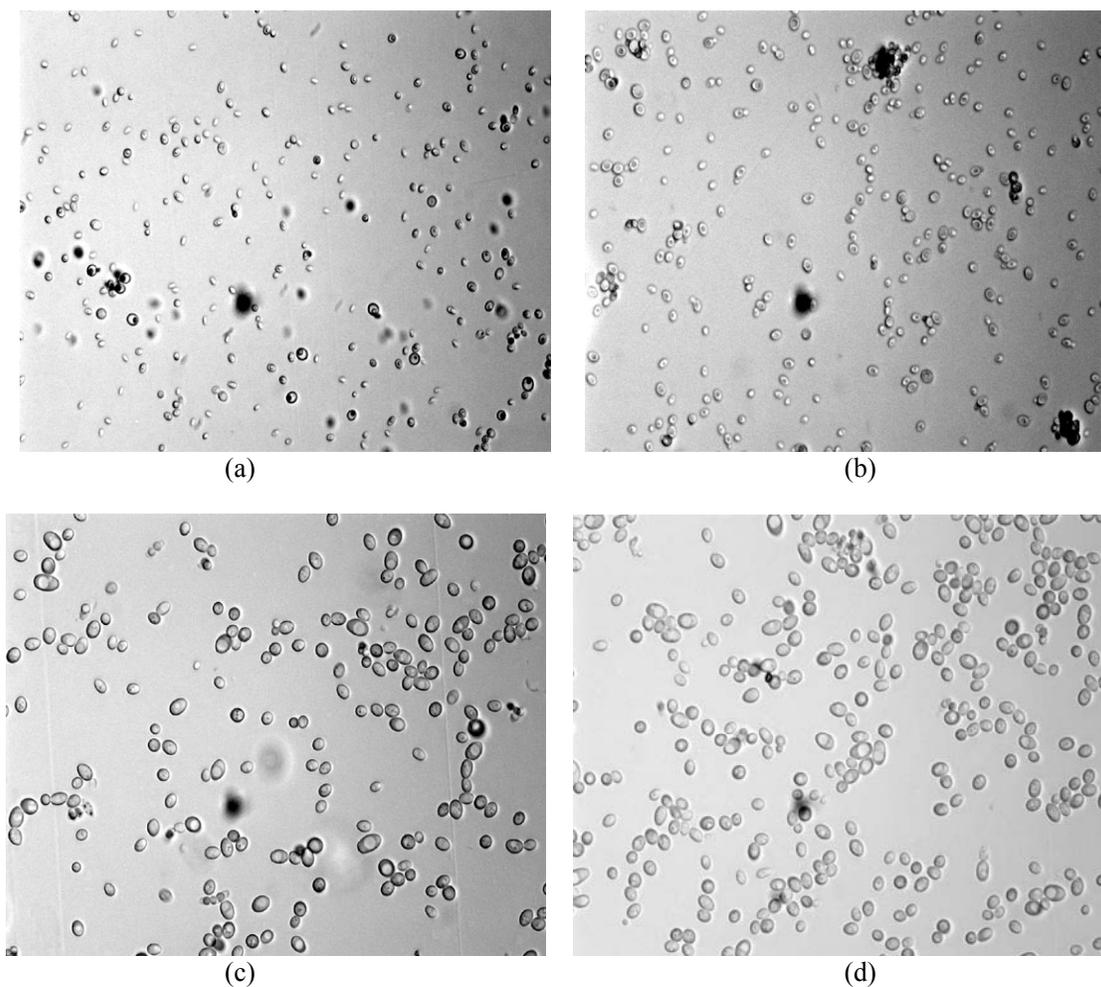
The source		Bacteria	Yeasts	Moulds
Dairy (L)	General sewerage (G)	I LGBc1		II LGFg1
		I LGBc2		II LGFg2
		II LGBc1		
		II LGBc2		
	Cheese production sewerage (B)	II LGBc3		
		I LBBc1	I LBDj1	II LBFg2
		I LBBc2	II LBDj1	II LBFg3
		II LBBc1	II LBDj3	II LBFg4
		II LBBc2	II LBDj4	
			II LBDj7	
			II LBDj8	
			II LBDj9	



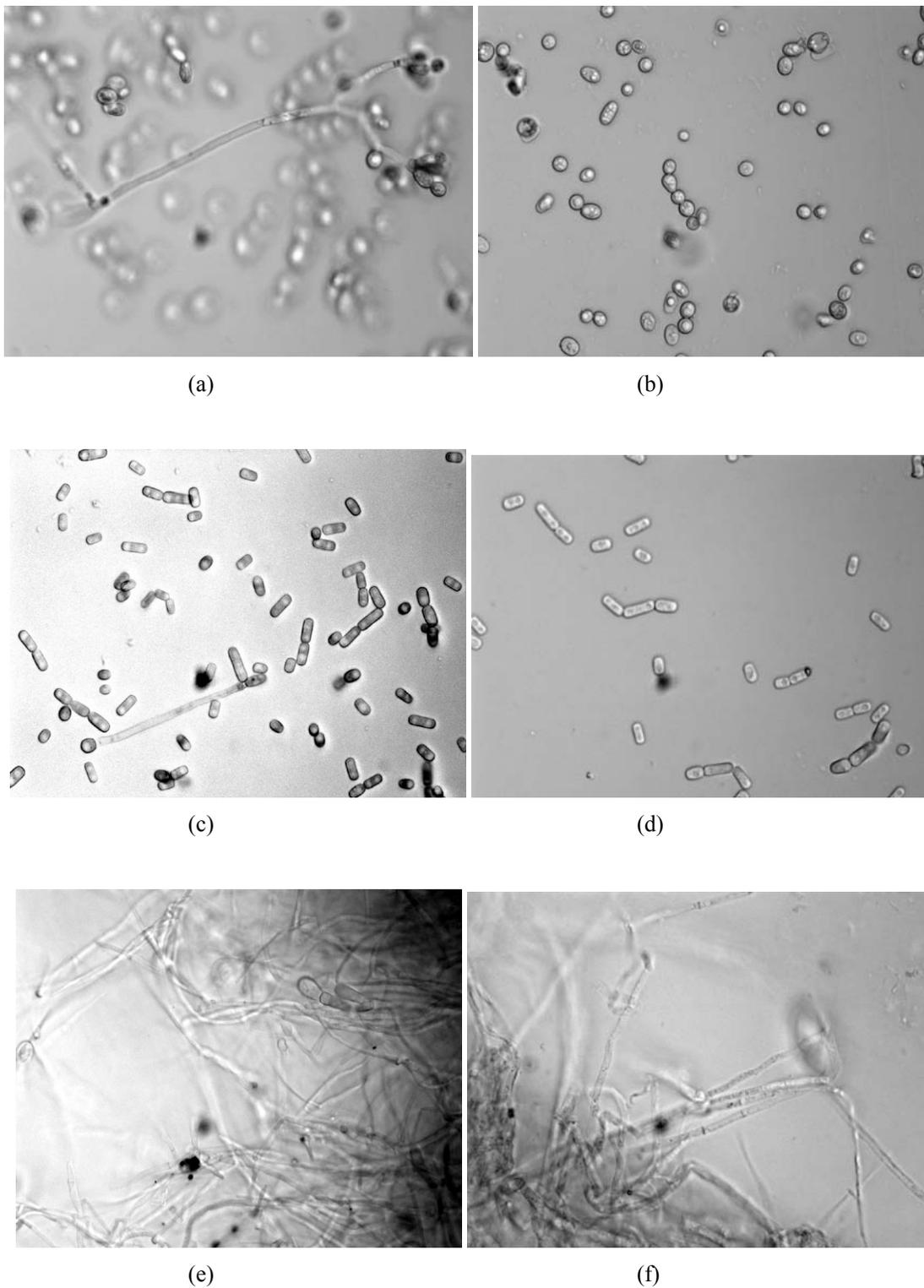
**Figure 1.** Microscopic characters of the bacteria isolated from the dairy and brewery wastewaters (a) strain coded IBS1Bc2; (b) strain coded IBS2Bc1; (c) strain coded IIBS2Bc1; (d) strain coded ILGBc1;



**Figure 1.** Microscopic characters of the bacteria isolated from the dairy and brewery wastewaters  
(e) strain coded ILGBc2; (f) strain coded IBS1Bc1



**Figure 2.** Microscopic characters of yeasts isolated from the dairy and brewery wastewaters  
(a) strain coded IBS2Dj1; (b) strain coded IILBDj7; (c,d) strain coded IILBDj8



**Figure 3.** Microscopic characters of moulds isolated from the dairy and brewery wastewaters  
(a, b) strain coded IILBFg4; (c, d) strain coded IILBFg2; (e, f) strain coded IIBS1Fg

### 3.2. Microorganism's potential to metabolize different carbon and nitrogen compounds similar to those from the dairy and brewery wastewaters

The cells belonging to the three types of microorganisms from the isolated pure cultures were inoculated on the specific media. Thus, the bacteria were tested for their capacity to metabolize starch, maltose and lactose through cultivation on BC medium supplemented with 1% starch, 1% maltose or 1% lactose. After 48 hours of incubation at 37°C, the growth of the colony was monitored through the colony diameter measurement. The diameter values and the standard deviation were calculated and plotted in the figure 4.

All bacteria strains isolated from the brewery wastewater can metabolize maltose. The strains coded IBS1Bc5, IBS1Bc6 show the best capacity to metabolize the maltose.

Bacteria's capacity to hydrolyse starch was determined by 0.1N Lugol staining agar medium after the colony growth 48 hours on 1% starch media surface. The colony diameters (Dc) and the hydrolysis zone diameters (Dhz) were measured. The substrate consumption was settled by the ration Dhz:Dc.

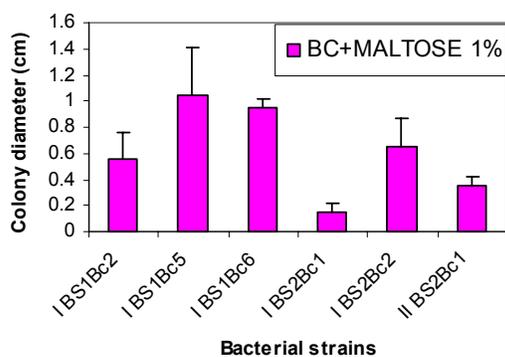


Figure 4. Brewery wastewater bacteria able to metabolize the maltose

The results can be seen in figure 4.

The bacteria cultures isolated from the brewery wastewater have the starch biodegradation capacity; thus the strain coded IBS1Bc2 has the best potential (figure 5).

The bacterial cells were inoculated by pricking 1% casein medium. After an incubation of 48 hours at the temperature of 37°C, the active bacteria had a

point-like growth without a casein hydrolysis zone around the colonies. This suggests a poor casein catabolism.

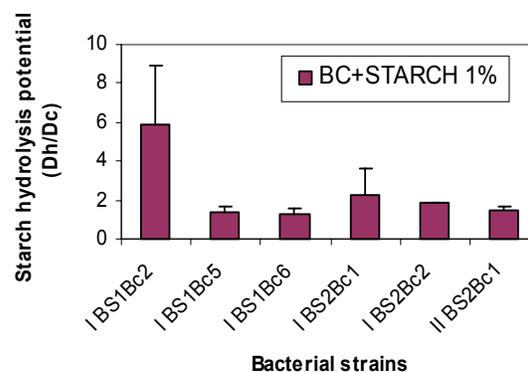


Figure 5. The brewery wastewater bacteria with starch degradation capacity

The isolated bacteria have the capacity to metabolise lactose. After an incubation of 48 hours at 37°C, on BC medium with 1% lactose as unique carbon and energy source, the tested strains showed a very good growth; the colonies reached diameters higher than 0.5 cm. The best is the strain coded IBS2Bc1 which reached the colony diameter of 1.2 cm; this indicates a very good lactose catabolism potential (figure 6).

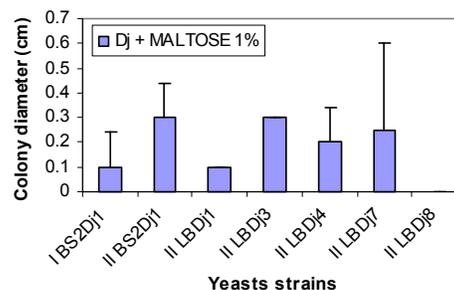


Figure 6. The brewery wastewater bacteria lactose catabolism potential

The isolated yeast strains were tested by pricking inoculation method on the basal medium with the final concentration of 1% lactose or maltose as unique carbon sources. After an incubation of 4 days at 25°C, the colony diameters were measured.

The brewery and dairy wastewater yeast strains showed poor growth ability on maltose, and the

colony diameters do not exceed the value of 0.5 cm (figure 7).

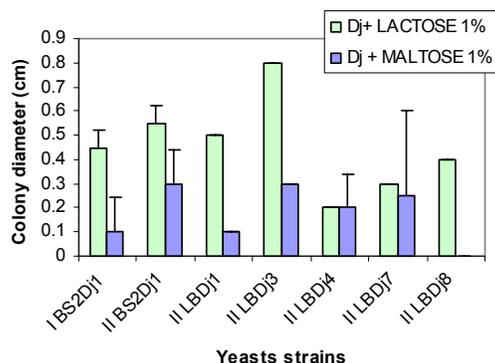


Figure 7. Food industry wastewater microbiota capacity to metabolise the simple glucides

The lactose existent in the dairy wastewaters can be metabolised by the microorganisms and turned into lactic acid, butyric acid, propionic acid and gases like carbon dioxide and hydrogen (Zara, 1999). All isolated yeast strains have the capacity to metabolise the lactose but the strain coded IILBDj3 strain shows the best potential in this way, the colony diameter reaching the value of 0.8 cm after 4 days of incubation on medium with lactose as unique carbon source.

#### 4. Conclusions

1. The brewery and dairy wastewaters present a diverse microbiota which consists of Gram negative bacteria (the predominant microorganisms), yeasts and moulds.

2. The pure cultures were morphologically characterised, based on the direct microscopic and culture examination. The microbiological tests showed the following groups of microorganisms, such as:

- bacteria belong to the genus *Bacillus*, *Pseudomonas* and *Escherichia*;
- yeasts belong to the genus *Saccharomyces*, *Kluyveromyces* and *Torulopsis*;
- moulds belong to the genus *Aspergillus* and *Geotrichum*.

3. There is a correlation between the capacity of the isolated strains to metabolize the organic compounds

and the organic compound incidence in the food industry wastewaters.

4. In accordance with the tested strain biodegradation potential, the data are useful to obtain more specialised cultures for biodegradation, in correlation to the pollutants, organic compounds from the wastewaters and the pollution degree.

5. This study offers preliminary data to identify some new active strains adapted to the wastewater physical - chemical conditions, for using them as specialised inoculum in wastewater treatment on model and natural systems.

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