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Studies on yeasts and yeast-like microorganisms in the denitrification unit biocenosis

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It was found that *Candida famata, Hansenula californica and Rhodotorula rubra* occurred in reactor UASB-type biocenosis in the course of denitrification carried out in the presence of lactic acid as a carbon source. The role of those species in nitrogen removal process was discussed with respect to their physiology.

INTRODUCTION

Investigations carried out by Grabińska-Łoniewska (in press) have proved that yeasts and yeast-like microorganisms are besides bacteria the main group of organisms in the denitrification unit biocenosis. Considering their physiology, the role of those microorganisms in nitrogen removal process may include: a – assimilation of nitrate and b – fixation of elementary nitrogen arised during denitrification. From the ecological point of view it is interesting that interactions between yeasts and bacteria take place during their growth in denitrification unit. As it has been shown, that the majority of bacteria, in pure cultures, were able to carry out denitrification only in the presence of yeast extract or simple organic compounds (Grabińska-Łoniewska, in press). In these studies denitrification unit biocenosis was fed with a mineral medium supplemented with only one of the following compounds: methanol, acetic acid or lactic acid. Thus, the source of succouring substrates for denitrifying bacteria could be only the autolized cells of other microorganisms in biocenosis, mainly yeasts and yeast-like ones.

This paper, as well as the earlier ones (Sláviková, Grabińska-Łoniewska, 1986 (1988)) attempt to contribute to the explanation of

these problems. It concerns the identification of yeasts and yeast-like microorganisms in the reactor UASB – type biocenosis in the course of denitrification carried out in the presence of lactic acid as a carbon source. The role of these microorganisms in nitrogen removal process will be considered regarding to their physiological characteristics.

MATERIALS AND METHODS

The microorganisms were isolated by Grabińska-Łoniewska in X1984-V1985 from the biocenosis populated denitrifying unit (reactor UASB-type), situated in Warsaw Technical University. The concentration of lactic acid in a feed medium was 2500 mg $\cdot$ l^{-1}. Medium composition, unit operation, as well as and isolation and identification techniques were described in previous papers (Grabińska-Łoniewska et al. 1985; Slávíková, Grabińska-Łoniewska, 1986 (1988). Classification was carried out according to Kočkova-Kratochvilová (1984), Kočková-Kratochvilová Slávíková (1985), Kreger-van Rij (1984) Lodder (1970).

RESULTS AND DISCUSSION

In the denitrification carried out at C:N ratio = 1,67 and at unit nitrate loading (L_v) ranging from 220 to 890 mg $N\text{-}NO_3$ l^{-1} d^{-1}, 12 yeast strains were isolated from the biocenosis. They identified as *Candida famata* (5 strains), *Hansenula californica* (4 strains) *and Rhodotorula rubra* (3 strains). At the studied L_v range. *Candida famata* and *Hansenula californica* dominated in mycoflora. They constituted 35-62% and 36-50% respectively of total fungi number. It has been found that frequency of the occurrence of *Candida famata* in biocenosis decreased with the increasing unit nitrate loading (L_v). *Rhodotorula rubra* could be considered as a concomitant microorganism. Representatives of this this species were responsible for 10-16% of total fungi number. They only were observed in the biocenosis 670 at a loads of and 890 mg $N\text{-}NO_3$ l^{-1} d^{-1}. The description of the cell and culture morphology, as well as physiological characteristics of the isolated strains is given below. Fermentation and assimilation of various sugars and nitrogen compounds is summarized in Table 1.

Candida famata (Harrison) Meyer et Yarrow – strains Ml 6 D4, Ml 7 D6, Ml 17 D6 (CCY 26-9-13)*, Ml 17 D7 and Ml 22 D6 (CCY 26-9-14).

The cells are spherical to ovoid (Fig. 1*A*). During the growth in liquid medium a sediment is formed. The giant colonies are greyish-white, semidull, soft and

* a numbers of strains in Czechoslovak Collection of Yeasts (CCY).

Table 1

Fermentation and assimilation of some sugars and assimilation of nitrogen sources

Name (number of strains) Character	*C. famata* Ml 6D4 Ml 7D6 Ml17D6 Ml17D7 Ml22D6	*Rh. rubra* Ml8D3 Ml21D8 Ml22D8	*Hansenula californica* Ml6D3 Ml8D5 Ml17D5 Ml22D5
Fermentation:			
Mal	–	–	–
Sac	–	–	–
Lac	–	–	–
Glc	–	–	+
Assimilation:			
Mal	+	+	+
Sac	+	+	+
Lac	–	–	–
Raf	+	+	–
Mlz	+	+	–
D-Xyl	+	+	+
I-Ara	–	+	–
Inl	–	–	–
Aml	–	–	–
Cel	–	+	+
Tre	+	+	+
KNO_3	–*	–*	+
Lysine	+	+	+
Tryptophane	+	+	+

Abbreviations: Glc – glucose; Mal – maltose; Sac – sucrose; Lac – lactose, Raf – raffinose; Mlz – melezitose, D-Xyl – D-xylose; L-Ara – L-arabinose; Inl – inulin; Aml – amylose; Cel – cellobiose; Tre – trehalose; – test negative; + test positive; * test positive during incubation on nitrate broth.

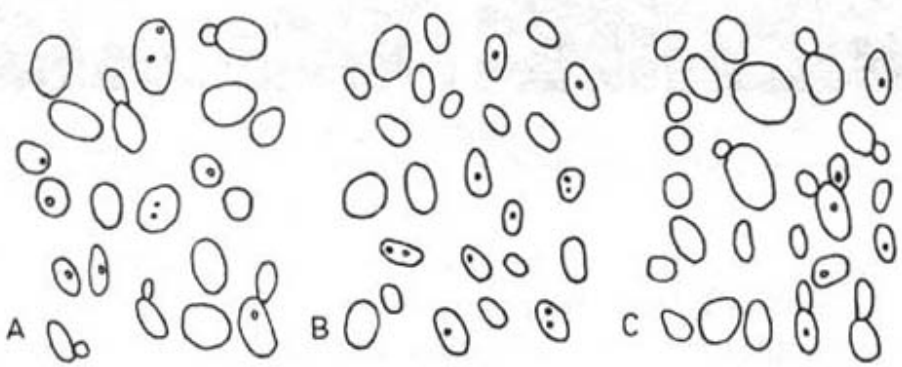

Fig. 1. Cell morphology (900 x)

A – Candida famata, strain Ml17D6; *B – Rhodotorula rubra*, strain Ml22D8; *C – Hansenula californica*, strain Ml18D5

smooth. The radial growth rate per 100 hrs at 20°C is; strains Ml 7 D6 and Ml 17 D7 – 4,63 mm, strains Ml 17 D6 and Ml 22 D6 – 4,86 mm and strain Ml 6 D4 – 4,40 mm. The pseudomycelium and sporulation are not observed. They are not able to hydrolise urea. Growth in vitamin-free medium is weak. Growth at 28 and 37°C is good while at 5 and 42°C very weak. The strains differ from standard description of *Candida famata* in negative assimilation of L-arabinose and cellobiose.

Rhodotorula rubra (Demme) Lodder – strains Ml 8 D3, Ml 21 D8 (CCY 20-7-17) and Ml 22 D8 (CCY 20-7-18).

The cells are short-ovoidal to elongate, single or in pairs (Fig. 1*B*). In a liquid medium a pink-coloured ring and a little sediment are formed. They form pink-coloured giant colonies with glistening and smooth surface, with a soft texture in the middle rugose (Fig. 2*A*). The radial growth rate per 100 hrs at 20°C is: strains Ml 8 D3 and Ml 22 D8 – 5,10 mm, strain Ml 21 D8 – 4,40 mm. The pseudomycelium is not formed by strains Ml 21 D8 and Ml 22 D8, while strain Ml 8 D3 produces it (rudimentary type). In all strains sporulation is not observed. They are able to hydrolise urea, but do not grow (or grow weekly) in vitamin-free medium. They grow well at 28 and 37°C; the growth at 5 and 42°C is weak.

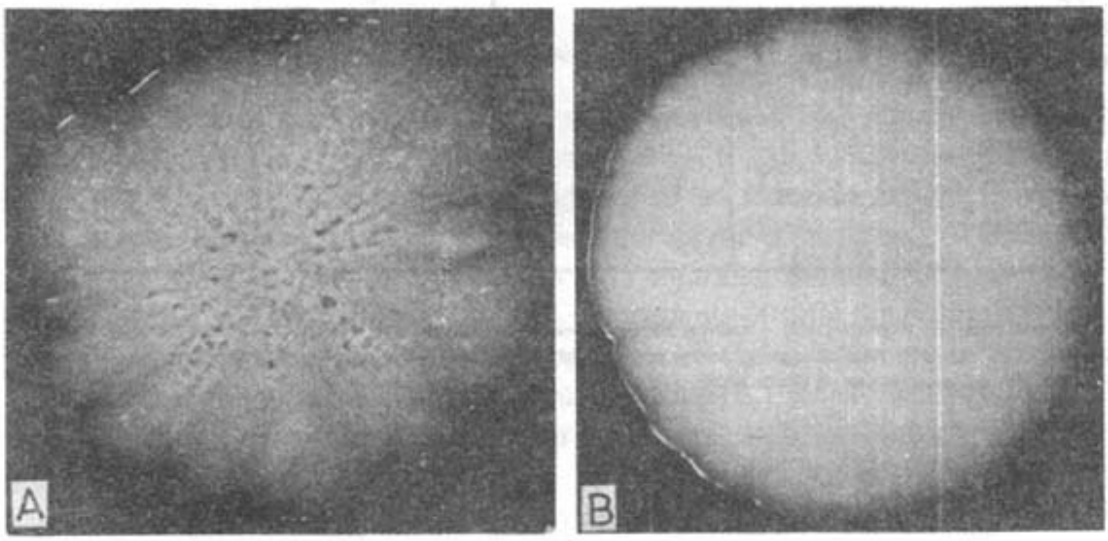

Fig. 2. Giant colonies
A – *Rhodotorula rubra*, strain Ml22D8; *B* – *Hansenula californica*, strain Ml18D5

Hansenula californica (Lodder) Wickerham – strains Ml 6 D3 (CCY 38-6-6). Ml 8 D5 (CCY 38-6-7), Ml 17 D5 and Ml 22 D5.

The cells are spheroidal to ellipsoidal, single or in pairs (Fig. 1*C*). In a culture in liquid medium a sediment is formed, no pellicle. Colonies are greyish-white, smooth and glistening (Fig. 2B), growth rate per 100 hrs at 20°C is: strains Ml 6 D3 and Ml 17 D5 – 4,63 mm, strain Ml 22 D5 – 4,40 mm, strain Ml 8 D5 –

4,86 mm. Pseudomycelium is not formed. During sporulation one to four saturn-shaped ascospores are formed. All strains do not produce urease and do not grow in vitamin-free medium. The growth at 5° C is weak, good at 28°C, they do not grow at 37 and 42°C.

The results of these studies as well investigations carried out by Grabińska-Łoniewska (in press) have shown that physiological features of the isolated yeasts and yeast-like species were diversified. *Hansenula californica* utilized both lactic acid and nitrate present in a feed medium in denitrification process. *Candida famata* and *Rhodotorula rubra* were able to assimilate nitrates but only in the presence of organic compounds and growth factors (present in nitrate broth). The growth of all species was intensified by yeast extract (0,5 g l^{-1}). This agreed with their requirement for lysine, tryptophane and vitamins for their growth. In denitrification unit these substrates could be derived only from autolized bacterial cells. It has confirmed the hypothesis that interactions took place between two main groups of organisms of the denitrification unit biocenosis, namely yeasts and bacteria.

As regards the quantity of yeasts and their species diversity, there was similarity between biocenoses fed with lactic and acetic acids. The total number of fungi in those biocenoses were (mean values): $373 \cdot 10^3$ and $580 \cdot 10^3$ cells mg^{-1}, what made 0,21 and 0,15% respectively of the total number of organisms. (Slávikova, Grabińska-Łoniewska, 1986 (1988); Grabińska-Łoniewska, in press). Both biocenoses were characterized by domination of *Candida famata* as well as by the decreasing number of representatives of this species with increasing unit nitrate loading (L_v). *Hansenula californica* and *Rhodotorula rubra* could be recognized as species specific to the biocenosis fed with lactic acid because they did not occur in biocenoses that arised in the presence of acetic acid or methanol as a carbon source. (*Hansenula californica* appeared only once, in biocenosis fed with C-deficient medium).

The results have confirmed the previous conclusions (Slávikova, Grabińska-Łoniewska, 1986 (1988)) that the direct participation of yeasts nad yeast-like microorganisms in nitrate removal process is not significant, due to their small number in biocenosis. Considering however, the strict interactions between these microorganisms and denitrifying bacteria, it could be stated that they play very important role unit biocenosis due to the intensification of growth and biochemical activity of the denitrifiers.

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BADANIA NAD DROŻDŻAMI I MIKROORGANIZMAMI DROŻDŻOPODOBNYMI WYSTĘPUJĄCYMI W BIOCENOZIE URZĄDZENIA DO DENITRYFIKACJI ŚCIEKÓW

Streszczenie

Podczas procesu denitryfikacji prowadzonego w reaktorze typu UASB w obecności kwasu mlekowego jako źródła węgla, w biocenozie wystąpiły: *Candida famata, Hansenula californica* i *Rhodotorula rubra.* Omówiono ich rolę w procesie usuwania azotu ze ścieków.