

# The Impact of Different Aeration Conditions on Whey Bioutilization\*

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The aim of this paper was to investigate the impact of increased pressure in the bioreactor and oxygen concentration increase in the inlet stream by enrichment of the air with pure oxygen on the yield and productivity of *Kluyveromyces fragilis* yeast and parallel lactose utilization and reduction of organic load of a medium simulating dairy waste (whey). On the basis of biomass yield and productivity and the degree of COD reduction, the optimum range of oxygen concentrations in the media was estimated.

The experiments were performed for a wide range of levels of dissolved oxygen in the media. The air supplied to the bioreactor was enriched with pure oxygen to increase the dissolved oxygen concentration in the liquid phase. The culture was also grown in the bioreactor at the pressure equal to 0.35 MPa.

The highest biomass yield was obtained in the process in which the air supplied to the bioreactor was not enriched with oxygen, and the lowest dissolved oxygen concentration in the culture medium was 3.2 mg dm<sup>-3</sup>, which corresponded to about 40 % of saturation at aeration carried out at atmospheric pressure (without the enrichment with oxygen). In this case, high experimental yield coefficient constituted about 90 % of the theoretical yield and maximum COD reduction of about 80 % was observed.

Development of modern technologies of dairy industry causes that increased amount of whey is produced. Whey is a by-product in the production of rennet cheese, curd cheese, homogenized cheese, and casein. Whey is valuable raw material for production of proteins and vitamins obtained in the presence of different microorganisms which in the form of biomass can be separated or dried [1]. In Poland only 15 % to 18 % of the produced whey is processed, while the rest is discharged to sewage and then to water reservoirs [2]. A reason of this is the low content of lactose (about 4 %) due to which the process of whey utilization is not fully profitable if environmental problems are not taken into account. As a waste, it is a serious environmental hazard (COD of about 50 g dm<sup>-3</sup>). On the other hand, whey processing into other products leads to purification of industrial water discharged to sewage accompanied by 95 % reduction of organic compounds.

The main component of the dairy waste loading is lactose. One of the microorganisms, which utilizes this substrate, is the yeast *Kluyveromyces fragilis*. Installations, in which this yeast biomass is cultivated

on an industrial scale, have been operated for many years [3]. The strains of *Kluyveromyces* belong to aerobic organisms, however, at limited oxygen availability some of them are able to ferment lactose. In typical aerobic cultures high concentrations of yeast cells are obtained and oxygen is usually the main factor which limits the process of their cultivation [4].

Oxygen transfer rate (OTR) in tank bioreactors is most often improved by increasing the speed of stirrer rotations, which results in an increase of the volumetric coefficient of oxygen transfer in the liquid phase,  $k_{La}$ . Another method used to increase the value of OTR is to increase the oxygen concentration in the gas supplied to the bioreactor (through enrichment of the inlet air stream with pure oxygen), or to heighten the pressure in the bioreactor. As a result, the equilibrium partial oxygen pressure in the medium grows. There are, however, some limitations in the above methods: a change in the stirring intensity may result in high power demand and/or mechanical destruction of microbial cells, and excessive oxygen concentration is toxic to microorganisms. This phenomenon is known as an oxygen stress [4]. Displacement of four electrons

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in the respiratory chain causes that the safe products are formed (two water molecules), but partial reduction leads to the formation of hazardous compounds. A potentially destructive factor is first of all the peroxide anion ( $O_2^-$ ) formed by the transfer of one electron to oxygen. As a result of peroxide anion protonation, a hydroperoxide radical ( $HO_2^-$ ) is formed, which can react spontaneously with another peroxide anion resulting in the formation of hydrogen peroxide ( $H_2O_2$ ) [5]. These hazardous compounds can cause destruction of enzymes, nucleic acids, and lipids. To neutralize the active radicals the aerobic organisms use enzymes (peroxide dismutase, catalase, peroxidase), which catalyze their transformation into the safe products.

The aim of this study is to investigate the impact of increased pressure in the bioreactor and oxygen concentration increase in the inlet stream by enrichment of the air with pure oxygen on the yield and productivity of *K. fragilis* yeast and parallel lactose utilization and reduction of organic load of model dairy waste (whey).

## EXPERIMENTAL

The aerobic process of yeast cultivation on model whey was carried out using the yeast of *Kluyveromyces fragilis* species LPCK0027. The yeast studied represented example of a so-called aerobically respiring yeast strain which grows exclusively according to an oxidative metabolism when sufficiently aerated. The strain was stored on agar slants with YPG medium at 4°C. The semi-synthetic culture medium with a composition simulating dairy waste (whey) contained lactose – about 40 g dm<sup>-3</sup>,  $\rho((NH_4)_2SO_4) = 4.6$  g dm<sup>-3</sup>,  $\rho((NH_4)_2HPO_4) = 2.5$  g dm<sup>-3</sup>, yeast extract – 3.0 g dm<sup>-3</sup>, and peptone – 5.0 g dm<sup>-3</sup> ( $n(C)/n(N) = 8.74$ ). The 24 h cultivation carried out in shaken flasks with a culture medium (at 30°C) constituted an inoculum which was supplied to the bioreactor in the amount of 5 %.

The biomass concentration was determined by gravimetric (drying at 65°C for 24 h and at 105°C to constant mass) and spectrophotometric methods [6]. Lactose concentration was determined by the anthrone reagent method [7] and by HPLC (Mini-Stork-500 pump, RI detector from Knauer, Berlin, Germany) using Aminex column HPX-87C (Bio-Rad Laboratories, USA) at temperature 85°C, with elution with deionized water at 0.5 cm<sup>3</sup> min<sup>-1</sup>. COD was determined by the dichromate method in the Hach apparatus [8].

Experimental investigations of aerobic batch processes were carried out in the bioreactor BIostat ED (B. Braun, Germany) with working volume 16 dm<sup>3</sup>. The geometrical characterization and characterization of hydrodynamic parameters of this apparatus is given elsewhere [9]. The experiments were carried out at a constant temperature (30°C), pH 4.5 (con-

trolled by means of 1 M-NaOH solution), and a constant mixing rate during the whole process. In subsequent cultures the agitation rate increased in the range from 200 min<sup>-1</sup> to 500 min<sup>-1</sup> in order to obtain higher OTR values. Aeration rate ranged from 0.5 min<sup>-1</sup> to 1.5 min<sup>-1</sup>. The air supplied to the bioreactor was enriched with pure oxygen to increase the dissolved oxygen concentration in the liquid phase ( $\rho(O_2)/(mg\ O_2\ dm^{-3})$ ). To measure the concentration of CO<sub>2</sub> and O<sub>2</sub> in outlet gases, gas analyzers (Analyser Series 1400/4995, Servomex, UK) were used. The culture was grown in the bioreactor under pressure equal to 0.35 MPa.

On the basis of separate experiments carried out in water in the tested bioreactor, the dependence of volumetric oxygen transfer coefficient in the liquid phase,  $k_La$ , on aeration rate, rotations frequency of the stirrer, and pressure in the apparatus was determined. The value of  $k_La$  was determined by the dynamic method, using pure nitrogen to displace oxygen from the solution and air to its saturation assuming ideal mixing in liquid and gas phases. In calculations dynamic characteristics of oxygen probe were taken into account. The obtained correlation has the form

$$k_La = 4.9 \times 10^{-8} Q^{1.24} n^{1.88} p^{0.19} \quad (1)$$

where  $k_La$  is the oxygen transfer coefficient (h<sup>-1</sup>),  $Q$  the gas flow rate (dm<sup>3</sup> min<sup>-1</sup>),  $n$  the stirring speed (min<sup>-1</sup>),  $p$  the overpressure in the bioreactor (MPa).

The rate of oxygen dissolution in the liquid (OTR (mg O<sub>2</sub> dm<sup>-3</sup> h<sup>-1</sup>)) was calculated from the equation

$$OTR = k_La(C_L^* - C_L) \quad (2)$$

where  $C_L$  is the real concentration of oxygen dissolved in the liquid (mg dm<sup>-3</sup>), indicated by the oxygen electrode during the process. The boundary equilibrium oxygen concentration in the liquid phase,  $C_L^*$  (mg dm<sup>-3</sup>), had different values in different cultures, depending on pressure in the apparatus and oxygen content in the inlet gas. The effect of medium composition on oxygen solubility in the liquid phase was considered according to the method described elsewhere [10, 11].

The above equation enables the calculation of a real, instantaneous rate of oxygen dissolution in the liquid, at the moment when the measurement is performed. The values of OTR given in Table 1 refer to this stage of the process in which oxygen uptake by microorganisms was the highest, *i.e.* at the lowest dissolved oxygen concentration,  $C_L$ . Such conditions lasted for about 10 h of the cultivation until the transition from the intensive biomass growth to accumulation stage occurred.

The values of oxygen uptake rate (OUR (mol O<sub>2</sub> h<sup>-1</sup>)) and carbon dioxide evolution rate (CER (mol CO<sub>2</sub> h<sup>-1</sup>)) were calculated on the basis of the oxygen and carbon dioxide balance in the inlet and outlet air

**Table 1.** Process Conditions of *K. fragilis* Growth

Run	Agitation rate	Air flow rate	Aerobic conditions (air)	Oxygen content at the inlet	Dissolved oxygen concentration	Oxygen transfer rate, OTR	Oxygen transfer coefficient, $k_{L,a}$
	$\text{min}^{-1}$	$\text{min}^{-1}$		vol. %	$\text{mg dm}^{-3}$	$\text{mg O}_2 \text{ dm}^{-3} \text{ h}^{-1}$	$\text{s}^{-1}$
1	200	0.5	Addition of $\text{O}_2$	60	0–22.1	920.0	0.012
2	300	1	Addition of $\text{O}_2$	45	0.8–16.5	873.7	0.059
3	200–300	0.5–1.5		22	3.2–7.8	1140.0	0.091
4	400	1	Addition of $\text{O}_2$	46	10.9–16.8	801.3	0.101
5	500	1	Addition of $\text{O}_2$	45	12.4–16.5	1278.0	0.153
6	500	1	Addition of $\text{O}_2$	46	13.4–16.8	1047.0	0.152
7	400	1	Pressure 0.35 MPa	20.9	18.9–26.9	1263.0	0.043

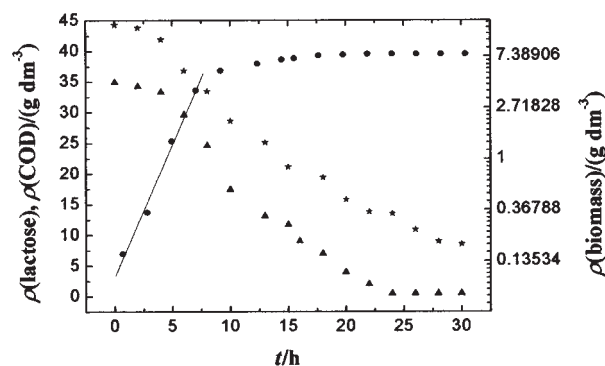
stream. The respiratory quotient (RQ) is the ratio of  $\text{CO}_2$  amount released and  $\text{O}_2$  amount consumed.

## RESULTS AND DISCUSSION

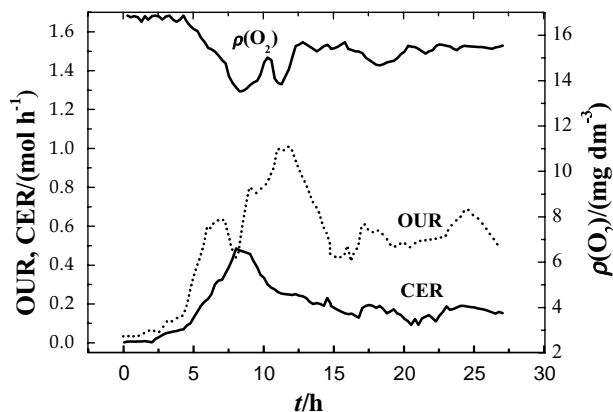
The cultivation of *K. fragilis* yeast biomass was investigated at different dissolved oxygen concentrations in the culture medium for 36 h. Typical profiles of changes of the yeast biomass concentration, used lactose amount, oxygen uptake rate (OUR),  $\text{CO}_2$  evolution rate (CER), and dissolved oxygen concentration in the culture medium are illustrated in Figs. 1 and 2. It was observed during the processes that the stage of intensive yeast growth lasted for about 10 h. Biomass increments during the accumulation stage (10–36 h of the process) were small (Fig. 1). The adaptation stage was short; it lasted about 2 h. The maximum specific growth rate,  $\mu_{\text{max}}$ , was  $0.549 \text{ h}^{-1}$ .

Results obtained during the experimental runs are compared in Table 2. The highest biomass yield per mass of lactose consumed (0.229) and the biomass productivity ( $0.281 \text{ g dm}^{-3} \text{ h}^{-1}$ ) were obtained during the experiment in which the air supplied to the bioreactor was not enriched with oxygen. In this process the dissolved oxygen concentration in the culture medium exceeded  $3.2 \text{ mg dm}^{-3}$ , which corresponded to about 40 % of saturation if the aeration under atmospheric pressure was performed (with no enrichment with oxygen), due to a change of the agitation rate and the air flow rate. The experimental value represents 87.1 % of the maximum theoretical yield (0.263) [12, 13].

It was also found that the oxygen uptake rate (OUR) and  $\text{CO}_2$  evolution rate (CER) changed according to the operation conditions applied. Fig. 2 shows the changes of these values for different concentrations of dissolved oxygen (run 6). However, the character of the changes of the respiratory quotient, irrespective to the concentration of dissolved oxygen in the medium, was similar. Changes illustrated in Fig. 3 show that during the stage of intensive growth, the RQ increased to about 1.1, and then during the stage of accumulation it was between the values of 0.2 and 0.5.



**Fig. 1.** Changes in the biomass and lactose concentration during cultivation of *K. fragilis* (run 3,  $\rho(\text{O}_2) = 3.2$  to  $7.8 \text{ mg dm}^{-3}$ ). Lactose (triangles), COD (stars), biomass (circles).

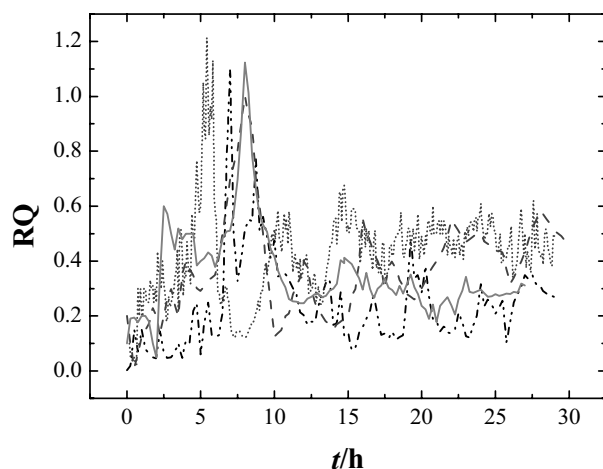


**Fig. 2.** Changes in OUR, CER and dissolved oxygen concentration in the medium,  $\rho(\text{O}_2)$ , during *K. fragilis* biomass cultivation (run 6).

The enrichment of air supplied to the bioreactor with oxygen and increase of the agitation rate caused an increase of the lowest concentration of dissolved oxygen in the medium during the run (runs 1, 2, 4–6, Table 1). The higher was the dissolved oxygen concentration, the higher was the biomass concentration. However, when this dissolved oxygen concentra-

**Table 2.** Comparison of Results of Cultivation of *K. fragilis* Biomass after 36 h of Processing

Run	Maximum specific growth rate, $\mu_{\max}$	Biomass concentration	Biomass yield	Biomass productivity	Lactose conversion	COD reduction	Experimental biomass yield/theoretical biomass yield
	$\text{h}^{-1}$	$\text{g dm}^{-3}$		$\text{g dm}^{-3} \text{h}^{-1}$	%	%	%
1	0.404	6.34	0.172	0.219	98.3	68.7	65.4
2	0.458	6.17	0.184	0.213	84.0	60.3	70.0
3	0.549	7.88	0.229	0.281	98.6	78.2	87.1
4	0.384	6.45	0.192	0.230	89.6	70.3	73.0
5	0.417	7.75	0.187	0.267	98.4	75.1	71.7
6	0.430	7.80	0.191	0.260	98.0	74.6	72.6
7	0.437	7.04	0.198	0.234	40.9	49.8	77.6

**Fig. 3.** Changes of respiratory quotient (RQ) during cultivation of *K. fragilis* carried out at different concentrations of dissolved oxygen in the medium. Experimental run 2 (dash-dotted line), 3 (dashed line), 4 (dotted line), 6 (solid line).

tion was above  $10.9 \text{ mg dm}^{-3}$  (which corresponded to about 70 % of saturation at the aeration under atmospheric pressure with no oxygen enrichment), observed increase of the biomass yield, lactose consumption, and the COD reduction was not significant. Comparing the experimental runs with and without air enrichment with oxygen, in the former case the biomass yield and its productivity decreased by about 15 %, while the lactose consumption and COD reduction were similar.

When the overpressure in the bioreactor increased up to 0.25 MPa, the concentration of oxygen dissolved in the medium ranged from  $18.9 \text{ mg dm}^{-3}$  to  $26.9 \text{ mg dm}^{-3}$ , however, no advantageous effect on the process results was reported. The biomass yield was 0.198, but the organic compound reduction was the lowest observed (process 7, Table 2). Thus, both the decrease of the concentration of dissolved oxygen in the culture medium below  $3.2 \text{ mg dm}^{-3}$ , as well as its increase above  $10.9 \text{ mg dm}^{-3}$  due to enrichment of the air supplied to the bioreactor with oxygen, or the use of increased pressure in the bioreactor had a disadvan-

tageous effect on the biomass yield, its productivity, and COD reduction.

## CONCLUSION

During the yeast cultivation on model dairy wastes using *K. fragilis*, differences in biomass yield and productivity were found depending on the degree of culture medium aeration. The highest biomass yield was obtained when the supplied air was not enriched with oxygen, and the lowest dissolved oxygen concentration in the culture medium was above  $3.2 \text{ mg dm}^{-3}$ , which corresponded to about 40 % of saturation at aeration under atmospheric pressure. In this case, experimental yield constituted about 90 % of the theoretical yield, and maximum COD reduction was about 80 %.

The enrichment of air supplied to the bioreactor with oxygen and changes in the agitation rate made it possible to obtain different concentrations of dissolved oxygen. At these conditions a decrease of biomass yield and productivity of about 15 % was reported, while the lactose consumption and COD reduction were kept on a similar level as in the process utilizing air. The application of high pressure in the apparatus did not contribute to the improvement of the cultivation process.

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