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Lactobacillus casei cell immobilisation on mineral carriers for continuous lactic acid production

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ABSTRACT

The first purpose of the study was to evaluate the influence of incubation parameters for efficiency of bacterial adhesion on surface of three solid, mineral porous carriers. Second aim of the study was the use of the immobilized *Lactobacillus casei* cells, in the production of lactic acid and selection, best carriers for continuous production using column bioreactor. The results showed that bacterial adhesion was different depending on the incubation conditions. In all tested samples, the highest efficiency of bacterial adhesion achieved after incubation at 30 °C without shaking which was related to bacterial viability. The results showed that, the highest efficiency of lactic acid production by free cells has been obtained at 30 °C after 48h – 110% (w/w) (lactic acid yield from consumed glucose). This results have been confirmed by further tests which have shown that in stationary culture, the highest production of lactic acid was reached by immobilised bacteria cells on pumice and kermiste after 48 hours at 30 °C.

***Keywords*:** immobilisation, lactic acid, adsorption, mineral carriers, biofilm, continuous production, *Lactobacillus casei*

1. INTRODUCTION

Lactic acid is organic acid with a wide variety of industrial application. In food industry it is used as acidulant, antimicrobial agent and preservative. It is also used in production of

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polylactic acid polymers [1]. Microbial lactic acid production process is one of the most commonly studied. The efficiency of conventional lactic acid production process is limited by the relatively low concentration of bacteria cells in the reactor. In biotechnological processes, immobilisation is designed to protect the cells against infections and accidental changes in process conditions [2,3].

Immobilisation also offers high concentration of bacterial cells [4-8,19]. Due to the enormous advantages of using immobilised organisms, increased interest of implementation of these systems in biotechnological processes is observed. Immobilisation of bacteria on a solid, porous matrices using adhesion is a natural process, often occurring in nature. This method of immobilisation is relatively fast and simple, widely used in many industrial processes.

It involves passive/natural immobilisation usually occurring in bioreactors. Rigid, inorganic carriers seem to be an interesting alternative to the traditionally used hydrogel systems such as alginate/calcium beads or microcapsules. In the case of microorganisms one of the most important interactions between the carrier and the cell is the ability of immobilised bacterial cells for proper growth of the surface on matrix [9]. Their high mechanical strength enables the use of immobilisation in a continuous fermentation processes.

The first purpose of the study was to evaluate the influence of incubation parameters for efficiency of bacterial adhesion on surface of three solid, mineral porous carriers. Second aim of the study was the use of the immobilised *Lactobacillus casei* cells, in the production of lactic acid and selection, best carriers for continuous production using column bioreactor.

2. MATERIALS AND METHODS

2. 1. Materials

2. 1. 1. Bacteria

The culture of lactic acid bacterium Lactobacillus casei was obtained from the collection of Faculty of Food Science and Nutrition, Department of Biotechnology and Food Microbiology Poznań University of Life Sciences (Poland). Culture condition parameters were also specified by Poznań University of Life Sciences.

2. 1. 2. Carriers

Three types of porous, mineral carriers for bacterial immobilisation were used:

volcanic rock- pumice stone, fraction 2-4 mm (Sigma-Aldrich, USA),
sintered foam glass - Poraver, fraction 2-4 mm (kind gift from Dennert, Germany)
clay - keramsite, fraction 2-4 mm (Liapor, Germany).

2. 1. 3. Medium composition

Bacteria were pre-grown in MRS broth for Lactobacillus (Biocorp, Poland) with addition of agar (Merck, Germany). Process of lactic acid production was carried out in MRS broth. The MRS broth was prepared according to the Biocorp protocol.

2. 2. Methods

2. 2. 1. Preparation of carrier for immobilisation

Mineral, porous carriers were transferred to the falcon test tubes (3 mL of each carrier per one test tube) and sterilized in autoclave at 121 °C; with 2.2 bar pressure for about 15 minutes. There were three test tubes for each carrier.

2. 2. 2. Immobilisation process

For the immobilisation of bacteria L. casei were pre-grown on MRS agar medium for 48h at 30 °C. After incubation the biomass was suspended in sterile 0.85% NaCl solution. Then suspended biomass was added to sterile flask with MRS broth, and stirred on magnetic stirrer (DragonLab, China) for 15 minutes at 150 rpm. After stirring, 30 mL of medium with bacteria culture (1.67×10^5 CFU/mL) was added to sterile falcon tests tubes with 3 mL of mineral carrier (3 falcons for each carrier - 1 falcon per day). L. casei strain was immobilised for 72 hours on mineral carrier in four different variants of incubation:

- Shaken bacterial culture at 30 ºC - 250 rpm (Ika, Germany),
- Static bacteria culture at 30 ºC
- Shaken bacteria culture at 37 ºC - 250 rpm
- Static bacteria culture at 37 ºC.

2. 2. 3. Bacterial adhesion on porous carriers

Adhesion was determined each day during 72 h of the process. After incubation, carriers were removed from falcon test tubes and gently rinsed with sterile distilled water (20 mL dH_2O per 1 mL of carrier). Then carriers were suspended in 50 mL of distilled water and homogenised in laboratory blender, BagMixer (Interscience, UK) for 5 minutes with blending speed 10 strokes per second. From each suspension serial decimal dilutions were made. Cell concentration was expressed as colony-forming units (CFU) per mL and determined by plating on MRS broth with agar. CFU were counted after 48 hours incubation at 30 ºC. Six repetition for each sample were made. Results are presented as mean values with standard deviation. Concentration of bacteria cells in medium broth of each falcon test tube was also investigated.

2. 2. 4. Comparison of lactic acid production by immobilised L. casei

After comparison of bacteria adhesion on porous carriers, one variant of incubation has been chosen. After that, bacteria have been once again immobilised on chosen carriers, incubated in best variant and compared in terms of lactic acid production for 7 days. Two samples, in which the highest amount of lactic acid was reached, has been chosen for further studies.

Total lactic acid content was determined by HPLC (Knauer, Germany) using a Aminex HPX-87H organic acid analysis column and RI detector (Smartline S2300, Knauer, Germany). The injection volume of the sample or external standard was 10 µL. Quantitative determinations of lactic acid and glucose were based on method of the external standard and calculated using the integrated computer program (Eurochrom, Knauer, Germany).

Concentration of used external standards was: 10 g/L; 5 g/L; 2.5 g/L; 1.25g/L and 0.625 g/L. The column, maintained at 25 °C, was eluted with 5 mM H_2SO_4 at a flow rate of 0.6 mL/min, samples ran for 30 minutes. The retention time of lactic acid under these conditions was 13.35 min.

Lactic acid productivity was calculated as grams of lactic acid per litre liquid volume produced per day [g/Lh].

Lactic acid production yield was expressed as grams of lactic acid produced, to amount of grams consumed glucose (w/w)×100%. Conversion of glucose was calculated by subtracting final concentration from initial.

2. 2. 5. Selection of best temperature parameter for lactic acid production process

Bacteria cells of L. casei were pre-grown on MRS agar medium for 48h at 30 °C. After incubation the biomass was suspended in sterile 0.85% NaCl solution (OD 600 nm 1, 27~5 McF). Then suspended biomass was added to sterile flask with MRS broth (in a ratio of 1:10) and stirred on magnetic stirrer (DragonLab, China) for 15 minutes at 150 rpm. After stirring 50 mL of medium with bacteria culture was added to two sterile 100 mL glass flask and incubated at temperature: 30 °C and 37 °C for 96 hours. Each day, samples were taken for chromatography analysis and cell viability. Concentration in broth was determined by plating method on MRS agar.

2. 2. 6. SEM

Before and after immobilisation, microscopic analysis were performed using a microscope Vega 3 LMU (Tescan) scanning electron microscope (SEM). The tests were necessary for examine the porous structure of carriers and to confirm adhesion of L.casei cells on surface of the carriers. Analysis was performed at room temperature with tungsten filament, and an accelerating voltage of 20 kV was used to capture SEM images for both of the pure carriers samples and immobilised carriers. All specimens were viewed from the top.

2. 2. 7. Continuous production of lactic acid by immobilised L. casei on keramsite and pumice stone

Two most appropriate carriers were used in continues production of lactic acid. L. casei (2.60×10^8 CFU) was immobilised on pumice and keramiste for 48 hours. After immobilisation carriers were aseptically transferred to column XK 26/20 (GE Healthcare Life Sciences, USA) connected to peristaltic pump (MRC, Israel). Both column packed with carriers (working volume for each column – 40 mL) filled with sterile MRS broth and incubated for 8 hours without flow (Fig. 1).

The temperature in the column were kept at 30 °C using thermo jacket connected to thermostat (Julabo, Poland). After incubation in column bioreactor continuous lactic acid process was started with flow rate 0.2 mL/min with dilution rate = 0.3 h^{-1} and HRT 3,33h. Each day samples for chromatography and viability of the cells were taken.

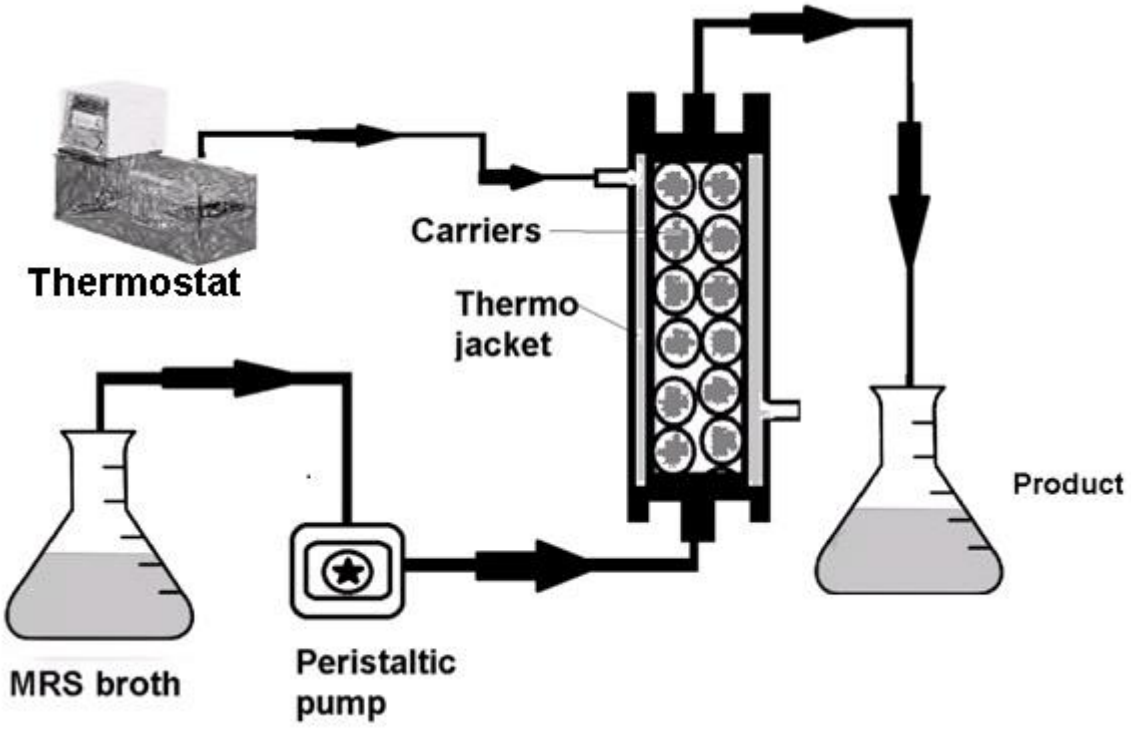

Fig. 1. Experimental set-up

2. 2. 8. Statistic evaluation

The data were analysed with Statistica 10 with ANOVA test. Differences with P values < 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

Bacterial adhesion is a very complex process, which is affected by many factors, including physical condition of the medium, presences of the nutrients, and factors including some characteristics of the bacteria strain, the physical and chemical nature of the target material surface [10]. It has direct influence on the effectiveness of fermentation processes. It is important to obtain a high concentration of microorganisms on the surface "interphase" of the porous carriers. The tests which were done using scanning electron microscope (SEM) confirmed that volcanic rock (pumice stone), foamed glass (Poraver), and clay (keramsite) are porous materials and can be used as carriers during immobilisation experiments. The study proved that temperature and shaking had an influence on concentration of *L. casei* on a surface of the carrier. It has also been proved that one of the most important factors that influence on bacterial accumulation is a type of mineral carrier, its properties and composition which was confirmed by Hrenovic et al. [11].

Authors have studied the effect of mineral carrier composition on bacteria immobilisation. They showed that the key feature which determined the immobilisation of *Acinetobacter junii* was the type of carrier and the higher amount of immobilised cells was

reached for the Mg-exchanged carriers. The type of carrier and also its shape were very important for Wang et al. [12]. Authors did not use mineral carriers but also immobilised living cells on surface of chosen carrier. They designed matrix for immobilisation from stainless steel wire mesh, laminated with cotton cloth. Each matrix unit made by these authors had an asterisk shape with six blades at 60° angular interval. Five different matrix sizes (diameters: 1; 1.5; 2; 2.5 and 3 cm; all with a 0.5 cm height) were studied for their effects on cell immobilisation and lactic acid production. As they showed, increasing the matrix diameter generally also increased cell growth and lactic acid production, with the highest lactic acid production (49.5 g/L) reached when the matrix diameter was 2 cm. The microbial cells grew as a biofilm on the larger matrices (2; 2.5 and 3 cm in diameter) and reached a thickness of 0.1–0.2 cm during 3 days. In contrast, for the smaller matrices with 1 and 1.5 cm diameter, the cells on the matrix grew into large clumps, which limited cell growth and lactic acid production due to mass transfer limitation.

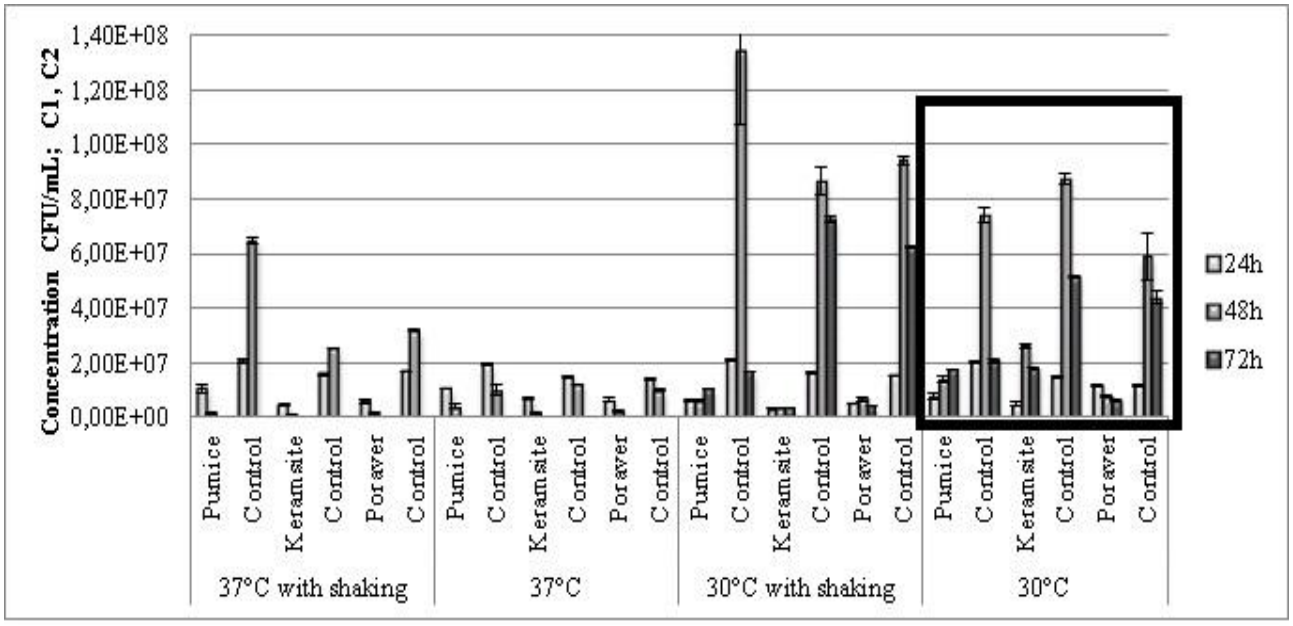

Fig. 2. Concentration of bacterial cells immobilised on mineral carriers during experimental period

The results of this study proved that all carriers used for immobilisation do not interact negatively on *L. casei*. The initial number of *L. casei* cells in first experiment was about 1.67×10^5 CFU/mL. The results showed that accumulation of bacterial cells in control samples with shaken pumice stone after first 24h of incubation increased to 2.06×10^7 CFU/mL at 37 °C and 2.08×10^7 CFU/mL at 30 °C (Fig. 2). Similar situation was observed in control samples with shaken keramsite where concentration of bacteria cells in broth increased to 1.59×10^7 CFU/mL at 37 °C and 1.62×10^7 CFU/mL at 30°C (Fig. 2). The number of bacteria in a sample with shaken poraver was $1.67x10^7$ CFU/mL for process incubated at 37 °C and 1.52×10^7 CFU/mL at 30 °C (Fig. 2). The results of tests showed that concentration of bacterial cells was comparable in all samples after first 24 hours of incubation which can be a

proof that temperature had no significant impact on bacterial concentration in broth. Significant differences were visible after 48 hours where the highest concentration of *L. casei* in the broth with mineral carriers in the shaken systems was observed. The important notice is that amount of bacterial cells for samples incubated at 30 °C was higher than at 37 °C. Higher amount of bacteria cells in broth than on a porous surface of carrier may be considered as evidence that adhesion is a unstable and weak process but it shows that presence of carrier in culture broth effects positively on bacteria growth.

Adhesion on pumice stone

The study has shown that adhesion of microbial cells was higher for samples without shaking, it has also been shown that strain of *L. casei* that had been used in the studies prefers lower temperatures. If the process of immobilisation was lead at 37 °C without shaking, the higher number of *L. casei* cells on the surface of pumice in a short time was observed (Fig. 2). However after more than 24 hours of incubation, numbers of immobilised cells reduced at both configuration about 87.5% in samples with shaking and about 61.2% in a samples without shaking. If the process of immobilisation was lead at 30 °C, it needed more time to obtain the high concentration of bacterial cells on pumice surface but viability of bacteria was higher than viability of bacteria immobilised at 37 °C. Significant is that, the highest efficiency of immobilisation $2{,}8\times10^{7}\pm9.6\times10^{4}$ CFU/mL was obtained after 72 hours at 30 °C in samples without shaking. SEM analysis confirmed presents of bacteria cells on carrier (Fig. 3). All results of bacteria concentration on pumice are presented in Table 1. However statistical evaluations showed that temperature was insignificant factor for static culture concentration but significant differences between obtained results had been observed for shaken culture. Also time of incubation was insignificant factor for static culture but for shaken culture it was significant. ANOVA test also confirmed that there were significant differences between concentrations of bacteria cells for static and shaken culture at 30 °C for first two days. Shaking was insignificant parameter at 37 °C.

Table 1. Concentration of bacterial cells immobilised on mineral carriers during experimental period (± standard deviation)

	37 °C with shaking						37 °C					
	PUMICE		KERAMSITE		PORAVER		PUMICE		KERAMSITE		PORAVER	
	Pumice	Control	Keramsite	Control	Poraver	Control	Pumice	Control	Keramsite	Control	Poraver	Control
24h	1.04×10^{7}	2.06×10^{7}	4.51×10^{6}	1.59×10^{7}	5.73×10^{6}	1.67×10^{7}	1.05×10^{7}	1.93×10^{7}	6.82×10^{6}	1.47×10^{7}	6.42×10^{6}	1.41×10^{7}

±	48h	±	24h			±	48h	±	72h
2.91×10^{5}	6.11×10^{6}	4.28×10^{4}	6.23×10^{6}	Pumice	PUMICE	1.51×10^{6}	1.30×10^{6}	3.67×10^{5}	-
2.67×10^{7}	1.34×10^{8}	3.03×10^{5}	2.08×10^{7}	Control		7.11×10^{5}	6.49×10^{7}	1.18×10^{6}	-
2.91×10^{5}	3.44×10^{6}	6.57×10^{4}	3.23×10^{6}	Keramsite	KERAMSITE	2.68×10^{5}	9.26×10^{5}	4.34×10^{5}	-
4.85×10^{6}	8.66×10^{7}	3.17×10^{5}	1.62×10^{7}	Control		4.28×10^{5}	2.54×10^{7}	6.67×10^{4}	-
8.74×10^{5}	6.78×10^{6}	1.23×10^{5}	5.03×10^{6}	Poraver	PORAVER	5.07×10^{5}	1.67×10^{6}	5.5×10^{5}	-
1.81×10^{6}	9.40×10^{7}	1.6×10^{5}	1.52×10^{7}	Control		1.49×10^{5}	3.17×10^{7}	3.67×10^{5}	-
30 °C with shaking									
1.02×10^{6}	1.41×10^{7}	1.31×10^{6}	7.73×10^{6}	Pumice	PUMICE	3.29×10^{5}	4.07×10^{6}	6.34×10^{5}	-
2.85×10^{6}	7.39×10^{7}	5.11×10^{5}	2.00×10^{7}	Control		2.98×10^{5}	1.02×10^{7}	5.98×10^{5}	-
1.11×10^{6}	2.62×10^{7}	9.02×10^{5}	4.8×10^{6}	Keramsite	KERAMSITE	4.88×10^{5}	1.39×10^{6}	5.21×10^{5}	-
1.81×10^{6}	8.76×10^{7}	4.82×10^{5}	1.47×10^{7}	Control		8.15×10^{5}	1.21×10^{7}	5.00×10^{5}	-
5.29×10^{5}	7.78×10^{6}	8.72×10^{4}	1.17×10^{7}	Poraver	PORAVER	8.15×10^{5}	2.41×10^{6}	5.0×10^{5}	-
8.71×10^{6}	5.91×10^{7}	3.96×10^{5}	1.15×10^{7}	Control		$5,7\times10^{5}$	9.92×10^{6}	3.99×10^{5}	-

72h	1.05×10^{7}	1.69×10^{7}	3.44×10^{6}	7.23×10^{7}	4.33×10^{6}	6.23×10^{7}	1.77×10^{7}	2.08×10^{7}	1.81×10^{7}	5.17×10^{7}	6.33×10^{6}	4.38×10^{7}
±	1.58×10^{6}	8.67×10^{4}	2.4×10^{4}	$1.29\text{x}10^{6}$	6.67×10^{4}	4.85×10^{5}	1.33×10^{5}	9.12×10^{5}	2×10^{5}	3.96×10^{6}	3.06×10^{5}	2.41×10^{6}

Adhesion on keramsite

The results of study showed that temperature of 37 °C enables the adhesion of a large number of *L. casei* cells on the surface of carrier. Similarly as in the case of pumice, this temperature also enables high adhesion of microorganisms in a short time in case of keramiste which was 6.8×10^{6} $\pm5.0\times10^{5}$ CFU/mL. However after 48 hours of incubation, concentration of bacterial cells reduced about 79.5% in samples with shaking and about 80.5% CFU/mL without shaking. The highest efficiency of bacterial adhesion $6.8\times10^{7} \pm 4.8\times10^{6}$ CFU/mL was obtained after 48 hours of incubation at temperature of 30 °C without shaking. Amounts of bacteria cells on porous surface in shaken cultures were the same after 48h and 72h (Tab.1) However in a static culture reduction of concentration between 48h and 72h was about 73.2%. Control samples has confirmed negative impact of temperature of 37 °C on viability of *L. casei* after 48 hours of cultivation. SEM analysis confirmed the presence of bacteria on the surface of the support (Fig. 3). All results of bacteria concentration on keramsite are presented in Table 1. ANOVA test showed that temperature was insignificant parameter for concentration of bacteria cells. It also confirmed as in the case of pumice that shaking is not significant parameter for bacteria cells concentration. Statistical evaluation showed that time of incubation is insignificant parameter for shaken culture but there were significant differences between obtained results in case of static culture.

Adhesion on poraver

The results of experiments showed that as in the case of pumice and keramsite, temperature of 37 °C also impact similarly on adhesion of *L. casei* cells on the surface of poraver and its equal 6.4×10^{6} CFU/mL. Unfortunately after 48 hours of incubation amount of bacterial cells reduced about 70.8% in samples with shaking and about 62.5% in samples without shaking. The highest concentration of bacterial adhesion on poraver has also been obtained after first 24h in samples incubated at 30 °C without shaking. After 48 hours of incubation at 30 °C the amount of bacterial cells on poraver raised about 35% for samples that were shaken, but for samples without shaking it has reduced about 33.5%. It has shown that as in the case of pumice and keramsite, even after 72 hours, adhesion of bacterial cells on poraver was higher for samples incubated at 30 °C without shaking than in samples with shaking (Tab.1). SEM analysis confirmed the presence of bacteria on the surface of the support (Fig. 3). All results of bacteria concentration on poraver are presented in Table 1. ANOVA test showed that neither time of incubation nor temperature did not affect significantly for bacterial adhesion to porous surface of poraver.

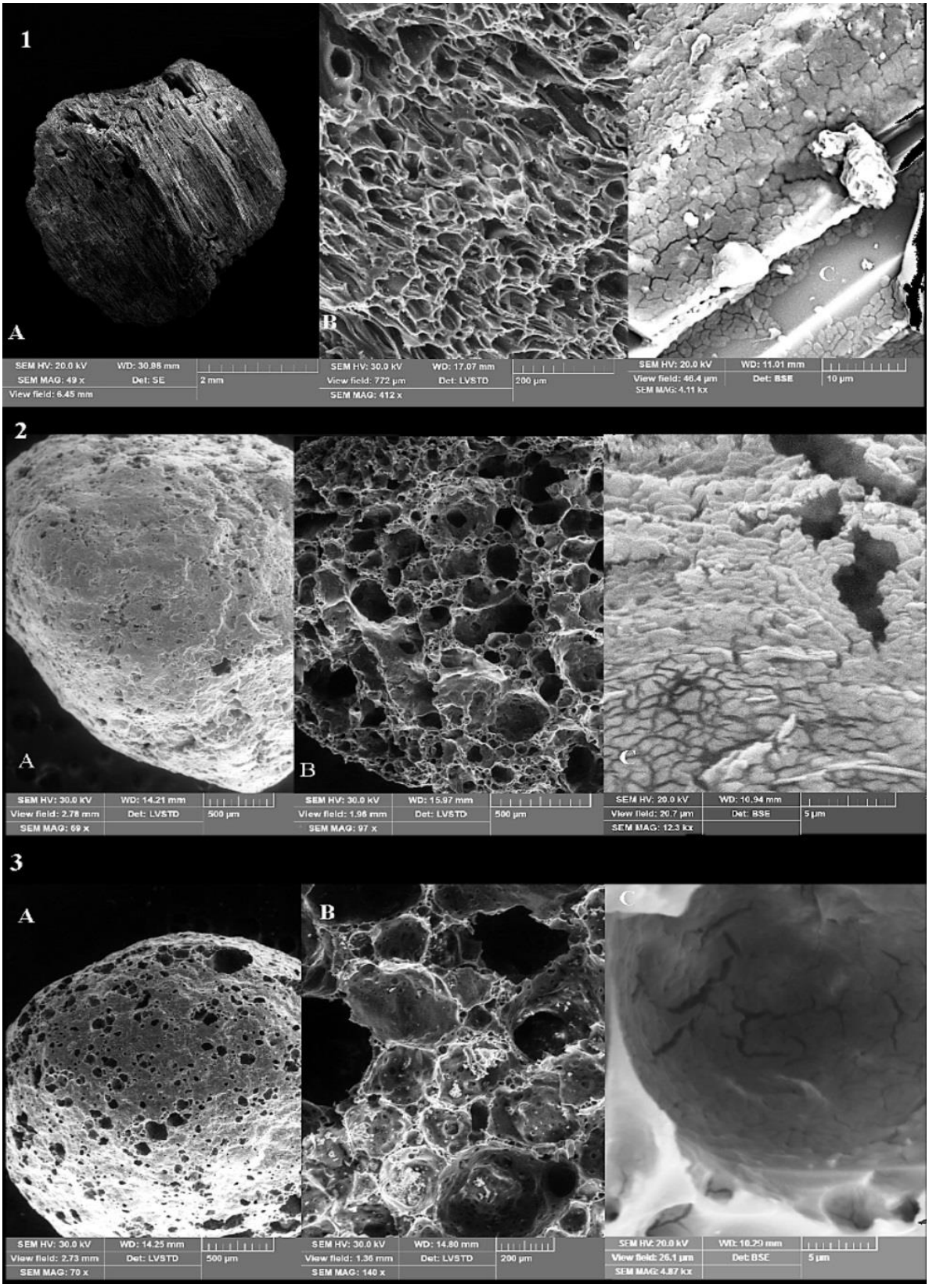

Fig. 3. SEM observations of mineral carriers and immobilised bacterial cells

The study showed that almost for all samples temperature and shaking has an insignificant impact on the efficiency of bacterial immobilisation although the highest efficiency of bacterial adhesion has been achieved after the incubation at 30°C in static culture. It has been also shown that the highest concentration of *L. casei* was obtained after 48h of incubation on porous surface of keramsite. However, obtained statistical results showed that time did not affect on concentration. The carrier which characterized the longest cultivation viability was pumice stone. Poraver was a carrier on which surface in the shortest time, high concentration of bacteria cells was observed. T

he difference in the adhesion on surface of examined supports may result from its properties like pH or external structure, that was mentioned by Munoz et al. [13]. Authors have used poraver as a carrier for immobilisation of *Pseudomonas aeruginosa* for biodegradation of hexane, but results obtained by them, were considerably lower than results reported by Kibazohi et al. [14], who used mixture of perlite and peat as biofilter in packed bioreactor. Also Guoqiang et al. [15] have mentioned that Poraver had poor adsorption characteristics for the *L. casei*, but pretreatment with the charged polymer PEI made the support a suitable adsorbent for cells. The results obtained by Hrenovic et al. [11] showed that the number of immobilised cells can decrease with the increase in particle size of the carrier, what was also confirmed by Ivankovic et al. [8].

Authors studied influence of the degree of perlite expansion on immobilisation of *Acinetobacter junii*. They showed that immobilisation of *A.junii* on perlite was dependent on the particle size and the best immobilisation of bacteria was observed on semi-expended perlite. Hrenovic et al. [3] also has showed that specific surface area of carrier can increase by acid-activation. The study confirmed that bacterial adhesion to porous surface of mineral carriers was satisfying, but concentrations of bacteria cells were much higher in a broth (control) than on a carrier, what can be caused by weak bonding between cell- carrier or detachment of the successive layers of the biofilm and multiplication in the medium. To reduce elution of bacteria cells and improve binding strength between carrier and the cell surface, support surface can be treated with polymers and crosslinking substances what was mentioned by D'Souza and Melo [16].

Authors immobilised bakers yeast on jute fabric through adhesion using polyethylenimine and thus the adhesion was strong and the cells were not eluted. In contrast to our study the results obtained by Wang et al. [12] showed that the immobilisation can be very effective. The matrix composed of fibrous matrices in a honeycomb configuration provided high surface areas for cell attachment and biofilm growth. This author showed that more than 90% of inoculated spores were adsorbed onto the matrices within 6-8h and after 10h there were no suspended cells in the fermentation broth, indicating a 100% immobilisation efficiency.

Lactic acid production

Chromatography analysis and viability of cells showed that, more suitable temperature for production of lactic acid in case of *L. casei* was 30 °C (Fig. 4). Efficiency of the process (lactic acid yield from consumed glucose), that was carried out at 30 °C amounted about 110% (w/w) after 48h, and 101% (w/w) for fermentation carried out at 37 °C. Daily production of lactic acid amounted 0.32 g/Lh at 30 °C and 0.31 g/Lh at 37 °C. That is why next processes were led at 30 °C.

Sequent chromatography analysis showed that, the highest increase of amount of lactic acid was obtained after 48 hours, beside samples with poraver, in which the highest content of product was obtained after 96 hours. It has shown that samples included immobilised cells are more stable at production of lactic acid during 7 days than samples with free cells. Even though the lactic acid concentration was higher in sample with free cells (15.71 g/L), the content of acid decreases more rapidly. Similar productivity in acid production was observed by Kourkoutas et al. [17], who immobilised *L. casei* on fruit pieces and also led the process at 30°C. In this case, pieces of fruit may be an additional source of carbon, which may affect to a higher amount of lactic acid. Results showed that production of lactic acid was high till first 48 hours and after that it slowed down (Fig. 4). Neither pumice, nor keramsite does not reduce acid production.

In these two samples, a production of lactic acid was almost at the same level. In the bacterial culture immobilised on poraver, production of lactic acid was reduced for first 72 hours, after this time a production was similar. Panesar et al. [18] proved that no difference was observed for lactic acid production with agitation that is why, the effect of agitation on lactic acid production by the bacterial culture has not been studied. The different results were obtained by Wang et al. [12].

This author used asterisk-shaped fibrous matrices for bacterial cells immobilisation. His new support matrix which was inspired by honeycomb could enhance mass transfer in bioreactor for lactic acid production by *Rhizopus oryzae*. Compared to free-cell fermentation, lactic acid production increased approximately 70% (49.5 g/L vs. 29.3 g/L) and fermentation time reduced 33% (48 h vs. 72 h) in shake-flasks with 80 g/L initial glucose. In conclusion irrespective to the fact that immobilisation of bacterial cells on mineral carriers is not 100% obtained results have allowed to select two carriers for further study- pumice and keramsite.

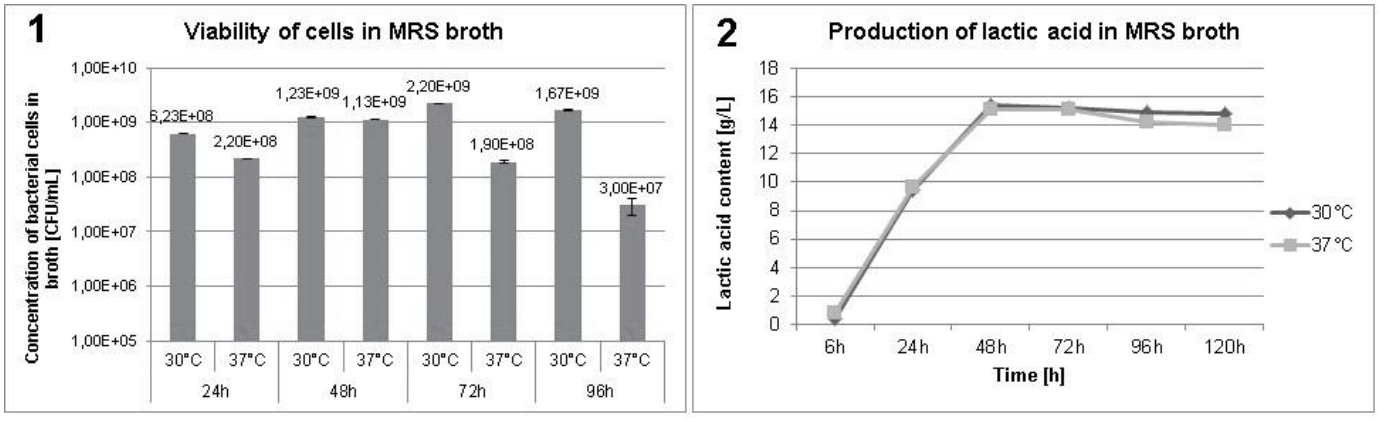

Fig. 4. Viability of cells in MRS broth and production of lactic acid in MRS broth

Continuous production of lactic acid by immobilised L. casei on keramsite and pumice

Chromatography analysis did not demonstrate significant differences in lactic acid production between cells immobilised on pumice stone and keramiste. The results indicated that after 2 days of acid production process, its amount reached 14.3 g/L for cells immobilised on pumice, and for 14 g/L for cells immobilised on keramsite. Over next days the amount of acid was similar, in both samples.

The highest concentration of lactic acid was 15.048 g/L at fourth day of the process for cells immobilised on pumice. Average daily lactic acid production was 13.44 g/L for pumice and 13.09 g/L for keramsite. Average daily product productivity for fermentation with immobilised cells on pumice amounted 0.56 g/Lh with efficiency yield about 120% (w/w). Average daily product productivity for fermentation with immobilised cells on kramsite amounted 0.55 g/Lh with efficiency yield about 118% (w/w). It has showed that production of lactic acid in column bioreactor was higher than in stationary culture (Fig. 5), which was probably, associated to the depletion of nutrients.

The study of Wang et al. [12] demonstrated that immobilised-cell fermentation can be also evaluated for its long-term performance in a bubble-column bioreactor operated in a repeated batch mode for nine cycles in 36 days. The results obtained by these authors proved that the highest lactic acid production was 68.8 g/L, corresponding to a volumetric productivity of 0.72 g/Lh and 93.4% (w/w) lactic acid yield from consumed glucose. The overall yield and productivity were 77.6% and 0.57 g/Lh, respectively. Their results also showed that fermentation can be improved by increasing aeration and mixing in the bubble-column bioreactor.

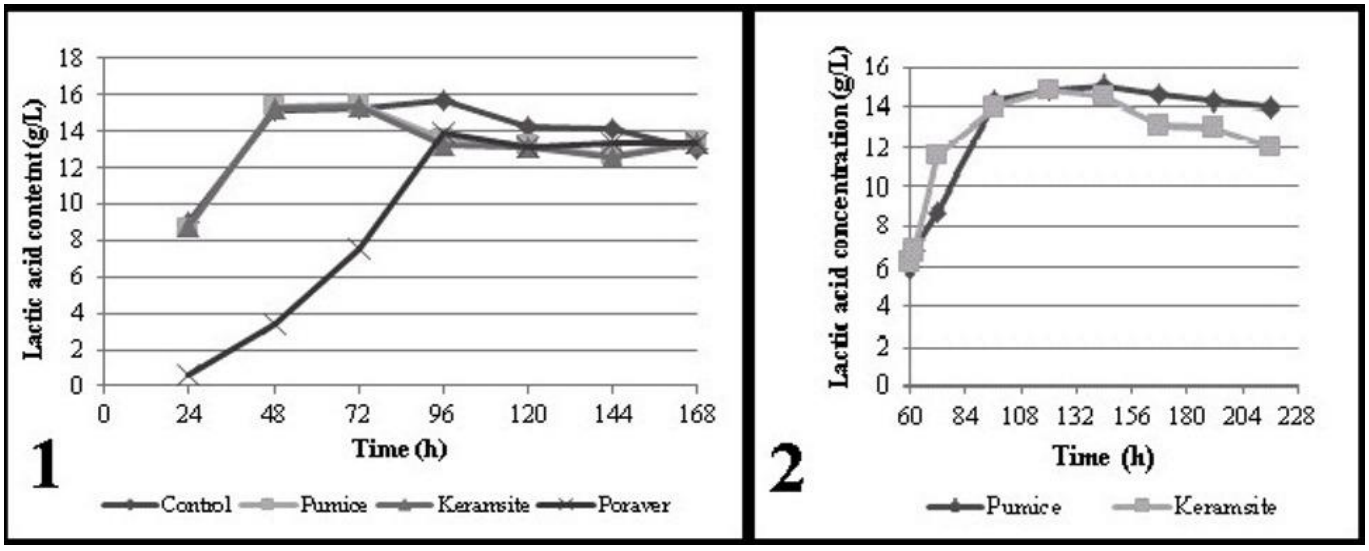

Fig. 5. Lactic acid content and lactic acid concentration

From the other hand the results obtained in this study showed that lactic acid production was quite low even lower than results obtained by Kourkoutas et al. [17], who reached after 118 hours – 15.1 g/L for strain immobilised on apple pieces and 16.7 g/L for the strain immobilised on quince pieces. Those differences can be caused due to the fact that the fruit pieces can also serve as a carbon source for the immobilised bacteria, and the fermentation process was optimised to high productivity of lactic acid, what was not the main purpose of this study.

4. CONCLUSIONS

The studies proved that temperature and agitation in case of *L. casei* strain have no affect on bacterial adhesion. However adhesion on surface of mineral carriers was higher at 30 °C without shaking than adhesion obtained on surface of mineral carriers at 37 °C with shaking. The obtained results have also shown that lactic acid production with free cells of *L. casei* was more efficient in samples that were incubate at 30° C. Also viability of bacterial cells was higher at 30 °C than viability at 37 °C which may resulted from faster glucose consumption and nutrients in broth. It has showed that production of lactic acid at column bioreactor was higher than in stationary culture, which was probably associated to the depletion of nutrients of this type bacterial culture.

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