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Improvement in Immobilization of Bread Yeasts by Sol-gel Method Combined with Functionalized Nanoporous Silica (LUS-1)

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ABSTRACT

In this work, the effect of immobilization of bread yeast (Saccharomyces cerevisiae) by sol-gel technique combined with functionalized nanoporous silica was investigated in different weight ratios of silica containing materials (TMOS: LUS-1). The activities of immobilized yeast in days after immobilization were examined. The results showed immobilization maintain the yeast life for a longer time. The functionalization by C18 functional group improved the environmental conditions for yeast life. These results indicate that the immobilization technique in the gel matrix and porous solid is a good system to develop industrial fermentations.

Keyword: Nanoporous silica, Sol-gel, Bread yeast, LUS-1, Fermentation, Immobilization, Mesopore.

1. INTRODUCTION

Cells and Enzymes are immobilized by different methods including absorption, covalent linkage, entrapment, cross linking and microencapsulation [1]. Producing ethanol through consuming glucose is one of the most significant applications of yeast. Due to excluding yeasts through removing the ethanol, it is needed to stabilize the yeasts leading to decrease the costs of separation steps [2]. Immobilization of cells in a silica Matrix shows different advantages including increased metabolic activity, protection of environmental stresses and toxicities, increased plasmid stability and application as a cellular storage systems in postponement of reactions [3]. Saccharomyces Cerevisiae (SC), a type of yeast bread, was utilized in this research. In order to immobilizing the yeast, entrapping technique by sol-gel method was used. Sol-gel method provides the

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possibility of applying porous inorganic matrixes having plenty advantages rather than polymeric matrixes. Utilizing the sol-gel process commonly is accompanied with using metal alcoxides. The steps of Sol-gel process includes solution formation, gelation, drying and agglomeration [4].

Pope and co-workers investigated the immobilization of SC into tetramethylortosilicate (TMOS) gel. One day after of immobilization, the yeast did not show any activity [5]. Fennouh et al immobilized the Escherichia coli bacteria inside silica matrixes by use of entrapping method [6]. Nassif et al investigated the immobilization of Escherichia coli into TMOS and after two weeks the yeast activity were reduced and after four weeks it was deactivated [7]. Desimon et al compared the resistance of free and immobilized SC in exposure to ethanol [8]. Yu et al immobilized Moraxella cell into a gel [9].

The term "nanoporous materials" indicates the materials with pore diameters less than 100 nm [10]. LUS-1 is a type of silica with amorphous walls classified in nanoporous materials. The synthesis of this material was reported by Benneviot and Badiei in 2001 at Laval University [11]. Alvaro et al used nanoporous silica to immobilize the Lipas Enzyme [12]. Jang and et al in 2006 immobilized Tripsin Enzyme on SBA-15 (a type of mesoporous silica) with and without functionalized by thiol group [13]. As the immobilization into nanoporous silica leads to protection of yeast from unwanted environmental factors and in other side the functional group on surface of nanoporous material, help to remove the unsuitable materials such as ethanol.

In this work, the effect of immobilization of bread yeast (Saccharomyces cerevisiae) by sol-gel technique combined with functionalized nanoporous silica was investigated in different weight ratios of silica containing materials (TMOS: LUS-1). The activity of immobilized yeasts were examined through the measurement of produced CO_2 by consumption of glucose in days after immobilization and a sample able to maintain the activity of yeast after one month were determined. In comparison to the other methods which were used before including Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC), this method is more practical and convenient [15].

2. MATERIALS AND METHODS

2.1. Materials

SiO_2, cetyl-trimethylammonium bromide (CTMABr), Hydrochloric acid (37%), Sulfuric acid (98%), Tetramethylortosilicate (TMOS 98%), Trichlorooctadecylsilane, Ethanol, Toluene, Yeast Extract Bioconnection and D(+)-Glucose-Monohydrate were purchased from Merck Company. Bread Yeast was purchased from Fala Company. P-toluenesulfonicacid monohydrate (TSOH) obtained from Aldrich.

2.1.1. Characterizations

In order to characterize the functional groups on nanoporous materials IR-spectrometer model Equinox 55Bruker were applied. The morphology and shapes of synthesized materials were investigated by SEM device model Zeiss DSM 960A 15kW voltage. Pore diameter, surface area and adsorption-desorption isotherms were measured at 77 K using a BELSORP-miniII porosimeter. BET (Brunauer-Emmett-Teller) equation was performed to calculate specific surface area and BJH (Barret, Joyner and Halenda). The low angle X-ray scattering spectrum was recorded by model X'Pert Pro MPD diffractometer (40 mA, 40 kV) at room temperature with Cu Kα radiation within a range of 2θ of 0.6 - 10 degree.

2.2. Methods

2.2.1. Synthesis of LUS-1

LUS-1 was synthesized according to the literature [16], with a molar ratio of SiO_2: 0.054 CTMABr: 0.054 TSOH: 80 H_2O. Prepared LUS-1 was washed with a solution of HCl (2 M) and ethanol, with a ratio of 1:9 for 2 hours, and then was filtered off under vacuum and dried in an oven overnight.

2.2.2. Functionalization of LUS-1

Acid washed LUS-1 was functionalized with Trichlorooctadecylsilane. 1 g LUS-1 with 30 mL dried Toluene and 1.18 mL Trichlorooctadecylsilane (diluted in 5 mL Toluene) were refluxed for 3 hrs at 150°C. Then it filtered off and washed with Toluene [14].

Table 1: *Texture properties of samples.*

	LUS-1	LUS-1+C_{18}	0. 5(LUS1+C_{18})+Sc
a_s (m^2/g)	900	85	236
Total pore Volume (cm^3/g)	0.303	0.112	0.301
D_p (nm)	2.4	2.3	2.7

2.2.3. Immobilization of bread yeast on functionalized LUS-1

Immobilization of yeast was performed through sol-gel method. 5 gel samples were prepared by adding different amounts of functionalized LUS-1 to 22.5 µL of HCl 0.01 M and 1.5 mL TMOS into ice-water mixture and adding 0.375 g yeast extract to 1.5 mL water. Mixed materials were stirred for 20 minutes and left to room temperature to produce gel [10]. During stirring, the Si-O-R bonds were created. Hydrolysis process was catalyzed by HCl. The synthesized samples were kept in a refrigerator on 4°C. In order to investigate pore size effect on immobilization of yeast different ratios of different silica materials were utilized. The silica amounts of TMOS were measured and then LUS-1 was added in weight ratios of 1:1, 0.75, 0.5, 0.375 and 0.3125.

2.2.4. Measurement of immobilized yeast activity

To investigate the remained yeast activity, the amount of produced carbon dioxide resulted in glucose consumption by yeasts were measured. The yeasts consume the Glucose through following reaction.

$$\text{Glucose } (C_6H_{12}O_6) \rightarrow 2 \text{ Ethanol } (C_2H_5OH) + 2\ CO_2$$

A mixture of 1.5 g Glucose and 0.375 g yeast extract were dissolved in 75 mL deionized water and were added to gel. Two 100 mL Erlenmeyer flasks containing 50 mL H_2SO_4 0.1 M in one and Gel (feremantor) in another were joint through a tube. The lid of flasks were closed and sealed with parafilm to isolate the system. The produced CO_2 in gel container were excluded and leaded to H_2SO_4 container. Thus the weight in gel container reduced frequently. Its weight was measure every 15 minutes. The reduced weight is equal to produced CO_2 [15].

Also, above experiment were utilized for functionalized LUS-1 and Free yeast.

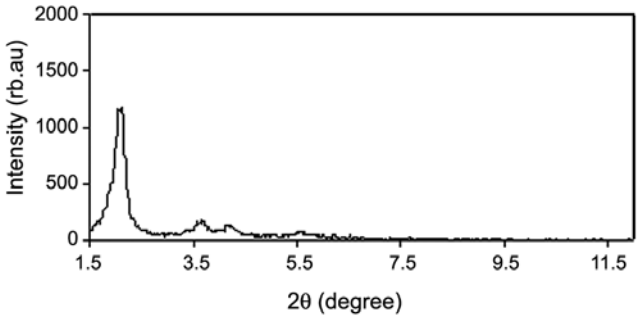

Figure 1: *Low angel X-Ray Diffraction pattern of the functionalized LUS-1.*

3. RESULTS AND DISCUSSION

Figure 1 shows the functionalized LUS-1 X-ray diffraction pattern. Three well-known and characteristic XRD peaks at 2θ = 2.33°, 3.90°, 4.49°, which are due to diffraction peaks of (100), (110), and (200), are at-

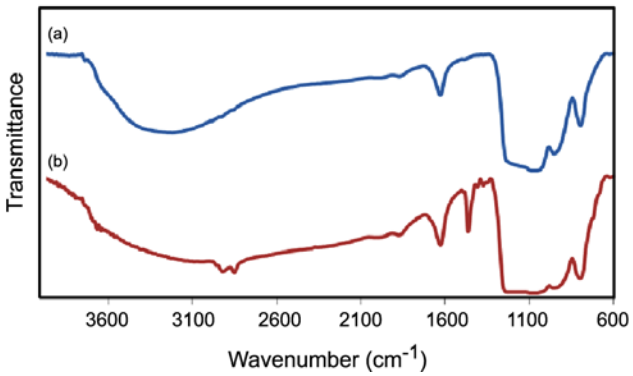

Figure 2: *IR Spectrophotometer: (a) LUS-1 (b) functionalized LUS-1.*

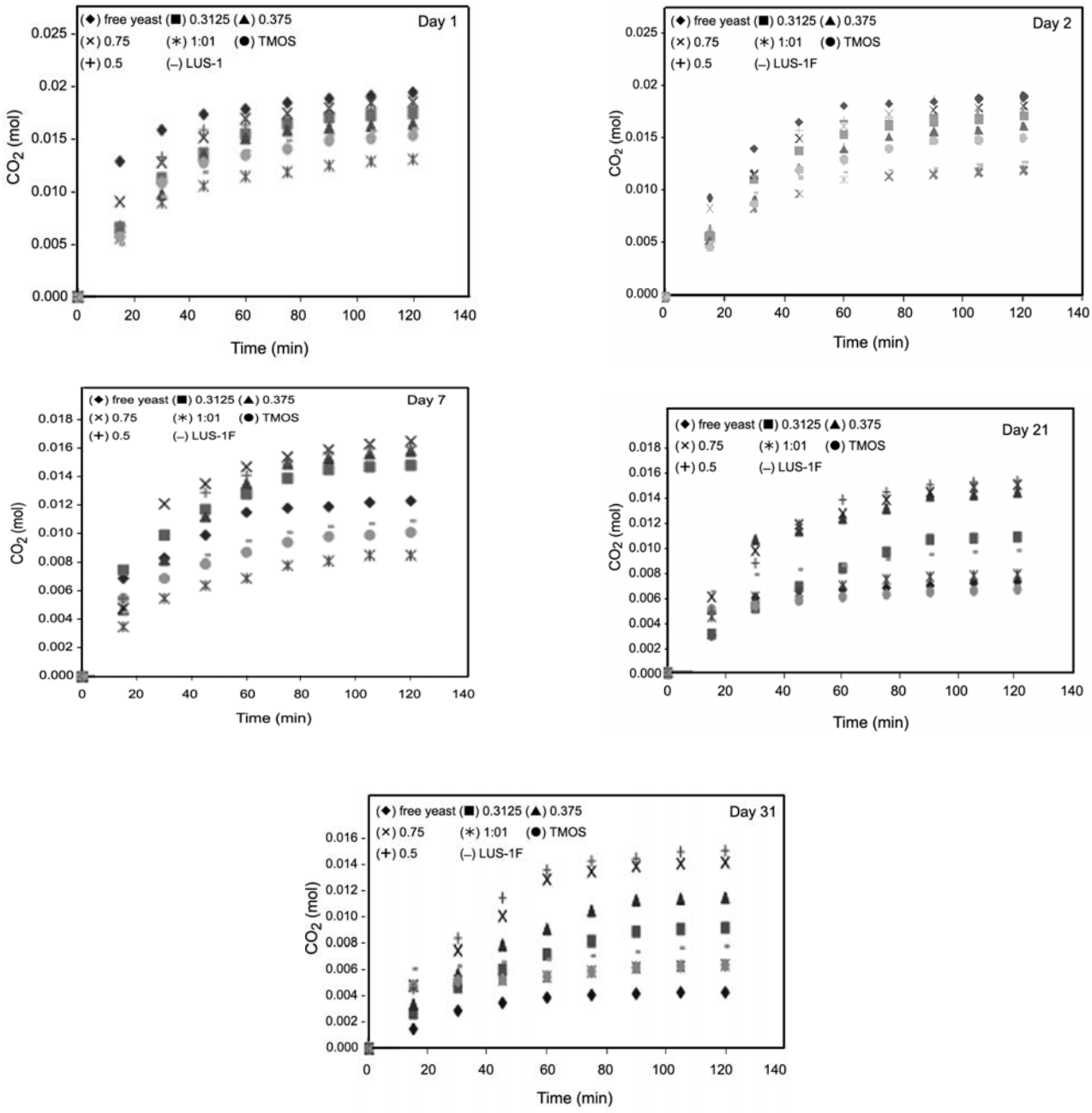

Figure 3: *Producing CO_2 from free and immobilized yeasts at days 1, 2, 7, 21 and 31.*

tributed to hexagonal P6mm symmetry for mesoporous structures [17, 18]. It clearly confirmed that functionalization have not changed the hexagonal structure of LUS-1.

LUS-1, functionalized LUS-1and gel samples (TMOS/LUS-1: 1:0.5) were investigated by nitrogen adsorption-desorption analysis. Data for pore diameters and surface area are provided on Table 1.

Surface area and total pore volume in functionalized LUS-1 in comparison to LUS-1 illustrated a significant decrease clarifying that the functional group are on surface of silica and in some parts pore blocking happened. In gel sample, surface area and total pore volume in comparison to functionalized one is increased indicating the functionalized LUS were placed inside gel structure and resulted data was attributed to pores of gel.

The FTIR spectra (Figure 2) of LUS-1 based material exhibit well defined peaks due to silica supports including a very strong band at 1110-1010 cm^{-1} rep-

resenting stretching vibration of Si-O-Si, a very broad band in the range of 3700-3200 cm^{-1} and a strong peak in the range of 955-830 attributed to surface hydroxyl groups, and SiO_2 vibrations may be assigned to the bands at 1080, 960 and 801 cm^{-1}. Vibratios of H_2O physisorbed onto the surface of silica appears at around 1645 cm^{-1} in spectra of all LUS-1 based material. Functional group, Cetyloctadecylsilane, on surface of LUS-1 was characterized by IR spectrophotometer (Figure 2). Two Significant bands in wavenumbers of 2851 cm^{-1} and 1462 cm^{-1} are related to stretching vibration of C-H bonds.

Vital activity of immobilized yeasts and free yeasts were investigated at 0, 1, 2, 7, 21 days and one month after immobilization (Figure 3). According to different behaviour of gels illustrated in plots, Gels in comparisons to each other show different behaiviours. In all samples by spending time the activity is reduced. The activity of free yeasts was maximum in first days and it decreased significantly after time spending. In comparison to free yeasts, this reduction of activity in immobilized ones was less. Descending rate of cell life was followed by a smaller slope. Because of internal and external penetration limits, the immobilized yeasts show less reduction of activity. In order to achieve the yeasts, the substra should pass through mass transfer resistances such as boundary layer of water (as an external one), porous maintaining lattice (as an internal one). This fact reduces the amount of yeilds in first day (which is usually in its maximum level).

Because of environmental effects and inappropriate conditions, the number of yeast was decreased. The trapped cells in Sol-gel matrix show better yield. Since Ethanol interefer with Fermentation ability

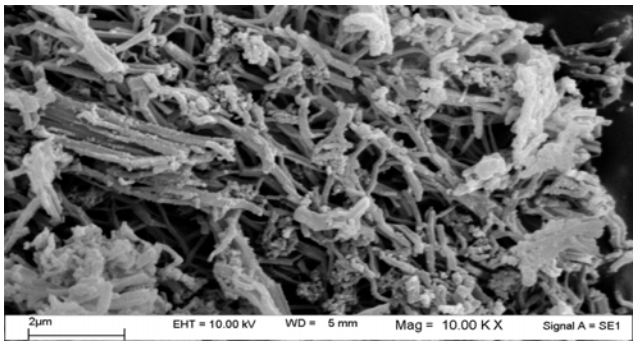

Figure 4: *SEM image of the functionalized LUS-1.*

growth rate of SC and compete to Glucose transfer which lead to slow and incomplete fermentation. As LUS-1 possess outstanding chemical, thermal and mechanical stability is able to act as a microprotective environment leading to avoid ethanols of prohibiting on yeast activity.

According to the plots, the gel with TMOS: LUS-1 ratio of 1:0.5 provides the best conditions for yeast's life. Although at first days all yeasts show same activities, 21 days after immobilization the gel with 1:0.5 ratio maintains life and activity of yeasts for more time rather than the other ratios. So this ratio of TMSO and LUS-1 is selected as an optimum ratio.

By comparing immobilized yeast's activity on functionalized LUS-1 and LUS-1, the activity of yeasts on functionalized LUS-1 was more protected. The morphology of LUS-1 in Figure 5 shows its bush-like structure. By functionalizing LUS-1, the functional groups are placed into pores and LUS-1 scaffold. Since the diameters of yeasts are greater than pores, the yeasts were trapped into LUS-1 scaffold and immobilized. By consuming Glucose, CO_2 and H_2O are produced. CO_2 is excluded through pores of LUS-1 and remained H_2O improves the yeasts lifetime. Bond between functional groups and silica increase the hydrophobic, because the hydrophobic molecules are nonpolar and show trend to similar molecules. In other side, H_2O molecules create hydrogen bonding and increase the moisture of gel. Nonpolar molecules, like CO_2, are not able to create bonds and exclude through the pores, thus the toxicity for yeasts' life are reduced.

4. CONCLUSIONS

The effect of immobilization of bread yeast (Saccharomyces cerevisiae) by sol-gel technique combined with functionalized nanoporous silica was investigated in different weight ratios of silica containing materials (TMOS: LUS-1). The activities of immobilized yeast in days after immobilization were examined. The results showed immobilization maintain the yeast life for a longer time. The functionalization by C18 functional group improved the environmental conditions for yeast life. These results indicate that the immobilization technique in the gel matrix and porous solid is a

good system to develop industrial fermentations. The easy separation of the final product and the biocatalyst reutilization was significant results.

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