Novel application of immobilized *Bacillus* cells for biotreatment of furfural-laden wastewater

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ABSTRACT

Immobilization of growing bacterial cells was considered as an innovative technique and very effective in dealing with major environmental challenges for bioremediation of organics-loaded wastewater. This study was undertaken to investigate the aerobic biodegradation of furfural in aqueous solution by using immobilized *Bacillus* cells in spouted bed bioreactor. *Bacillus* cells were individually immobilized in the matrices of sodium alginate, guar-gum, and agar-agar; each was cross linked in polyvinyl alcohol. Immobilized cells exhibited efficient furfural degradation in several successive reuse batches up to 3 cycles without losing their degradation activity which could provide economic advantages when used in industrial-scale applications. Results of batch experiments in fluidized spouted bed bioreactor demonstrated that complete removal of furfural using immobilized *Bacillus* cells in the first and second cycles was achieved after 450 and 480 min, respectively. The removal efficiency of furfural was 100%, 100%, and 95% for the 1st, 2nd, and 3rd, cycles, respectively. Unlike the free cells, the potentiality of the immobilized cells is that they could be reused without reduction in their ability to degrade hazardous furfural. Additional advantage of immobilized cells compared to free cells, is their ability to tolerate high concentrations of furfural.

Keywords: Biodegradation, *Bacillus* sp., Immobilized cells, Furfural, and Spouted bed bioreactor.

1. INTRODUCTION

Biological treatment is drawing attention due to the potential of complete mineralization of the toxic compounds from aqueous solutions while producing innocuous end products, maintaining concentrations of pollutants below the toxic limit, cost competitive, there is no secondary problems (toxic by-products) in the effluent and no chemicals are involved [1,2]. Many hazardous compounds contained in industrial wastewater are poorly removed in conventional biological processes due to their toxicity, recalcitrance and inhibition. Furthermore, they also have adverse impact on the composition and activities of microorganism communities in free cells (FC) system [3, 4].Thus, most of these compounds pass through conventional wastewater treatment facilities unaltered. The previous drawbacks can be partly overcome by immobilization of bacterial cells [5]. Furfural, or 2-Furaldeyde, probably more known as furfural with a chemical formula of $C_5H_4O_2$, is a viscous, colorless liquid with a pleasant pungent aromatic (almond-like) odor upon exposure to air; it turns dark brown or black. It has properties similar to those of benzaldehyde [6]. Furfural is an organic compound derived from a variety of agricultural byproducts, including corncobs, oat, wheat bran and sawdust. Furfural is present in many food items as a natural product or as a contaminant. It has a wide variety of other usages such in the manufacture of pesticides and an ingredient of resins and together with phenol, acetone, or urea to make solid resins. Furfural is a toxic compound which causes several health problems for humans. Furthermore, it has a few exposure routes for entering the human body, including oral, dermal and nasal. The liver, kidney and lung are frequently observed target organs for furfural and furan compounds that may be damaged if the exposure continues; these compounds may cause tumors and mutations. The lowest concentration has been observed in the brain [7, 8]. Furfural is readily absorbed via the inhalation and dermal exposure routes. The risk characterization of furfural in the aquatic environment, depending on the toxicity in fish, indicates that several activities locally give predicted environmental concentration (PEC) values above 1mg/L [9]. Furfural leakage causes not only pollution problems in the environment, but also considerable economic losses. Regarding its effects on health and from the parsimonious point of view, there has been a growing interest for the removal or recycle of furfural from wastewater [8].

Several methods were applied for the removal of furfural from wastewater including but not limited to advanced oxidation technologies using UV/H_2O_2 [9], electro-Fenton and Fe(II)-activated peroxydisulfate (PDS) processes, ultrasound technique [10], and aerobic biotreatment using free bacterial cells. On the other hand, immobilized cells have the potential to degrade toxic refractory compounds faster than free cells in conventional treatment systems since high densities of specialized microorganisms are used in immobilized cells (IC) systems. The (IC) technique not only simplifies separation and recovery of microbial cells but also makes the application reusable which reduces the overall cost [11]. Currently, different kinds of

immobilization have found wide applications not only in the field of biotechnology, but also in environmental pollution control, scientific endeavors, food industry, pharmaceutical and biosensor industries. The success of immobilization method is mainly due to very mild conditions under which it is performed and it is a fast, simple and cost-effective technique. Fig. 1 presents the scheme of simplest imagination of immobilization technique.

This study aimed to investigate for the first time the potential of immobilized *Bacillus* cells individually immobilized in immobilized in the matrices of sodium alginate (SA), guar-gum (GG), and agar-agar (AA) each was cross linked in polyvinyl alcohol (PVA) for furfural degradation in a special type of fluidized bed bioreactor named spouted bed bioreactor.

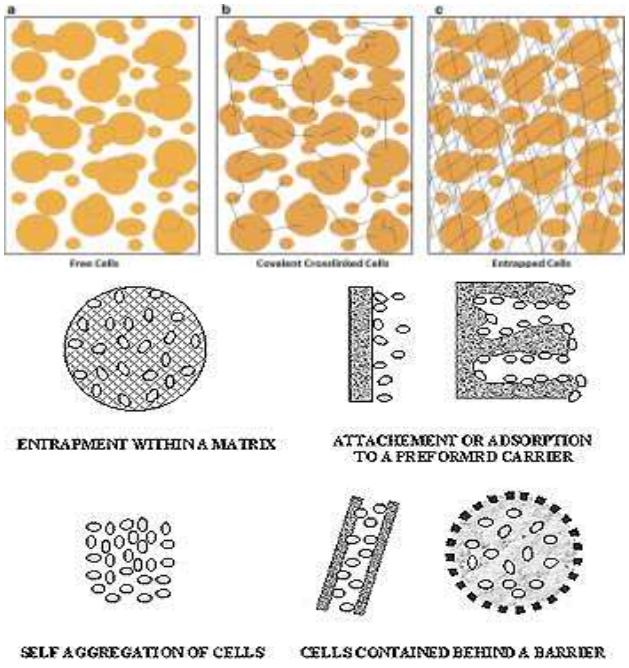

Fig. 1 Scheme of immobilization technique

2. MATERIALS AND METHODS

Bio-carrier materials

The materials that used as bio-carriers in this study included natural polymers of guar gum (GG), agar agar (AA), and sodium alginate cross-linked with polyvinyl alcohol (PVA) to improve and increase the mechanical stability of beads. Natural polymeric carriers are abundant but they are less stable in wastewater than synthetic polymers. Table1 presents the types and sources of the utilized polymers.

Immobilization protocol

Six grams of PVA and 2 grams of GG were dissolved in 100 ml distilled water and blended by a magnetic stirrer at 70 °C for 30 min. After the mixture cooled to room temperature, it was inoculated with 5 ml of biomass cells and was stirred by a magnetic stirrer for 15 min. The obtained solution was poured into sterile micro-plates to form beads with immobilized cells. Keeping them in the freezer for 12 h and then thawing them. The freezing-thawing procedure was repeated for 3 times to improve the stability of beads [12]. Generally, polymer gel of GG is more brittle than alginate gels. Sodium alginate (SA) solution was prepared in sterilized distilled water and combined with PVA. To the PVA-SA slurry, biomass inoculum (5 ml) was added and stirred for 10 minutes to get a uniform mixture taking into consideration that no bubbles were entrapped inside. The slurry was poured in sterile hypodermic syringe. The alginate solution was dropped into ice cold mixture of calcium chloride (4 %) and boric acid (6%). Beads were formed in $CaCl_2$ solution that was incubated overnight for curing. The cured beads were washed with sterile distilled water 3 to 4 times. When the beads were not being used, they were preserved in 0.9 % sodium chloride in the refrigerator [12]. (For more photos of beads see Appendix A). Generally, polymer gels including; Agar-Agar, Gum Arabic, and Guar Gum are more brittle than alginate gels. To improve and increase the mechanical stability of the beads, PVA was cross-linked with these gel polymers [13]. A 2% solution of agar-agar was prepared with 0.9 % PVA powder. The biomass inoculum (5ml) was added to the PVA-agar mixture, shaken well for few seconds (without forming entrapped bubbles), poured into 2 sterile micro-plates and allowed to solidity in the freezer and then thawed. The beads were washed with distilled water 3 to 4 times [12]. Fig. 2 presents samples of the prepared beads.

Fig. 2 Schematic diagram of the pilot-plant

Table 1 Polymers used as bio-carriers in the study

Name	Abbreviation & Chemical formula	Source	E. Code*
Polyvinyl alcohol	PVA $(C_2H_4O)n$	raw material of vinylon	_____ _
Guar Gum	GG	Obtained from non-marine botanical resources	E412
Sodium Alginate	SA	algal polysaccharides derivatives (Marine-Seaweed algae)	E401
Agar-Agar	AA		E406

*(E. Number): is a code for a substance that can be used as food additives within European Union & Switzerland.

Experimental system configuration and set up

The experimental system consisted of a specially designed fluidized bed bioreactor, known as spouted bed bioreactor (SBBR) made of Perspex column (inner diameter 50 mm, height 70 cm) with 45° conical base. The SBBR was outfitted with a Perspex jacket (inner diameter 80 mm) for temperature control. A water bath was designed to continuously circulate the water at a desired temperature of 30°C. The water bath consisted of 6 Liter-cylindrical Perspex tank, occupied with heater and water pump to circulate the water into the reactor jacket. The aqueous solutions were fed to the SBBR via a peristaltic pump (Type: Thomas 3386 Mini variable speed pump). In order to provide an intense mixing and maintain aerobic environment into the spouted bed bioreactor, air was injected from the bottom of the reactor by an air pump through a 6 mm-orifice. A flow meter was provided to control the air flow into the system. The experimental setup is given in Fig. 3. The spouted bed bioreactor operated in an up flow co-current air/water mode at a temperature of 30°C. The experimental work was accomplished in a batch mode. The system was used to assess the effects of the detention time, furfural initial concentration, free versus immobilized cells, and number of effective biodegradation cycles.

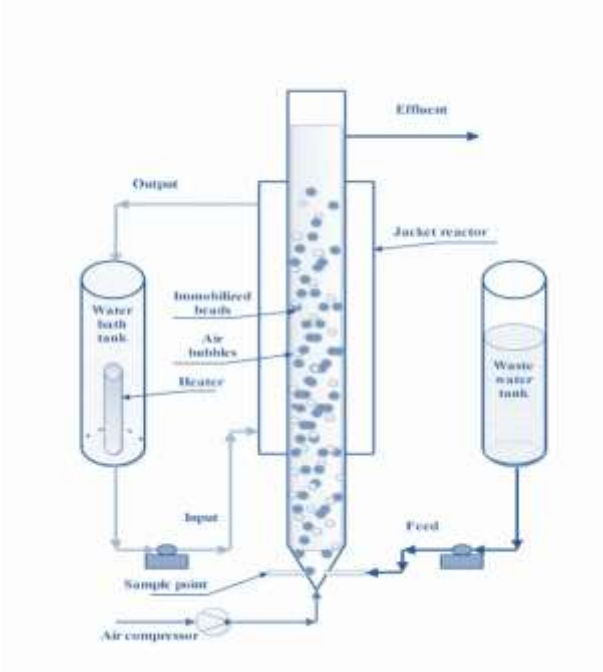

Fig.3 Samples of the prepared beads

Analytical analysis and methodologies

Furfural concentrations in aqueous samples were determined by T80 UV-VIS Spectrophotometer at 278 nm. The concentration of the free biomass cells (FCs) was measured using the volatile suspended solids (VSS) measurement according to the procedure reported in the *Standard Methods* [14].

3. RESULTS AND DISCUSSION

Assessment of effective biomass concentration

Prior to preparing the immobilized cells beads, a preliminary set of experiments were conducted using free cultural cells to investigate the effect of inoculum concentration on the pollutants biodegradation. Results revealed that the concentration of inoculums had a notable impact on the degradation of furfural as given in Fig.4.

Results indicated that complete biodegradation of furfural was equally obtained at concentrations 5% and 7% v/v (inoculum/aqueous phase). Accordingly, 5% v/v was considered as the optimum inoculum concentration which was further applied in the subsequent experiments. Anyhow, although increasing the biomass concentration leads to excess binding sites available in the bead, the initial inoculum volume in the bead should not be in excess.

During the process of biodegradation, the excessive growth of bacterial cells in the bead due to the degradation of organic substrate can cause the saturation of binding sites, and then decreasing the performance of the beads with immobilized cells [15, 16].

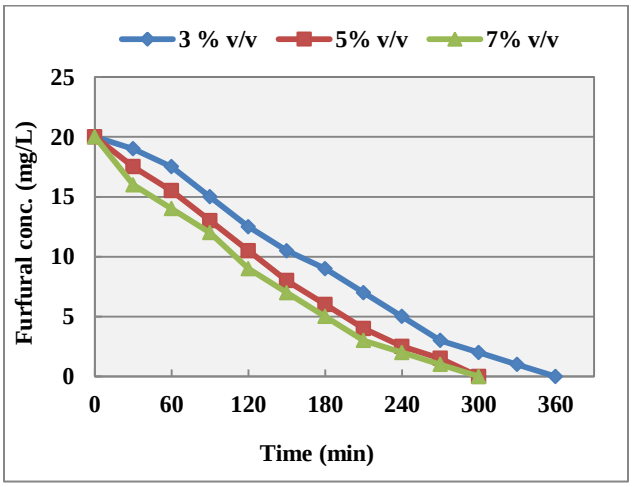

Fig. 4 Effect of inoculum volume on furfural degradation by pure cultures *Bacillus* at 20 mg/L initial concentration of furfural

Effect of beads concentration on biodegradation rate

The amount of beads (immobilized cells) in the bioreactor plays an important role in the biodegradation process. The effect of beads amounts (v/v) on the biodegradation rate of furfural is given in Fig.5. It is well observed that higher degradation rates were obtained using 25 % (v/v). Increasing the biodegradation with increasing the beads concentration was well expected since the concentration of the beads in the bioreactor can be related directly to the amount of the bacterial biomass immobilized in these beads. However, higher concentrations of beads in the bioreactor have declined the biodegradation rate (data not shown). This could be attributed to the fact that high concentration of beads may hinder their movement in the solution mixing and consequently, reduce the biodegradation rate. On the other and from an economic point of view, selecting the optimum concentration of beads is very important with

respect to biocatalysts and the carrier materials consumption at an industrial scale design for efficient biotreatment.

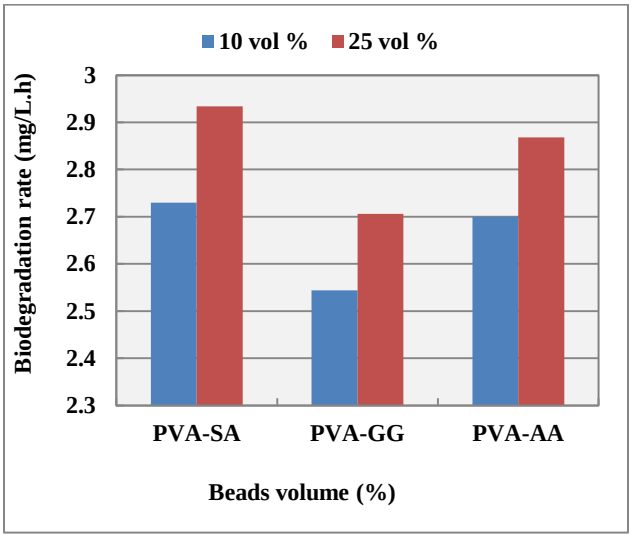

Fig. 5 Profiles of furfural degradation by immobilized *Bacillus* cells in PVA-SA, PVA-GG, PVA-AA.

On the other hand, results indicated that the higher biodegradation rate of furfural was obtained by using PVA-SA as the bio-carrier. This can be attributed to fact that PVA-SA have a loose structure and could bind the bacterial cells more firmly than the other bio-carriers matrices; PVA-GG and PVA-AA.

Recycling of immobilized cells for biodegradation

Recycling of immobilized cells for further biodegradation of organic pollutants is one of their major advantages versus free cells. The validity of recycling immobilized cells of *Bacillus* in various matrices was determined by carrying out consecutively excessive batch experiments using recycled beads. Results revealed that immobilized cells can be efficiently reused without decline in their biodegradation capability. This could reduce expenses during operational periods as reported by Cai et al. [17]. In general, results indicated that the recycled beads were potentially effective up to 5 cycles. However, the potential of natural gums including guar gum, and agar-agar for biodegradation were slightly lower than sodium alginate for the last cycles. This could be limited by their mechanical strength and the lack of open spaces to accommodate active cell growth resulting in their rupture and cell release into the growth medium especially for long periods of use as reported by Couto [18]. The addition of polyvinyl alcohol (PVA) to natural polysaccharide- materials promotes the strength of the bio-carrier matrix. However, for all types of bio-carriers, the biodegradation rates of pollutants were slightly and gradually decreased with the consecutive cycles due to the mechanical instability of beads and gradual leakage from their porous. Results given in Fig.6 present the profiles of furfural removal by *Bacillus* sp. Complete biodegradation of furfural was achieved in reasonable time duration.

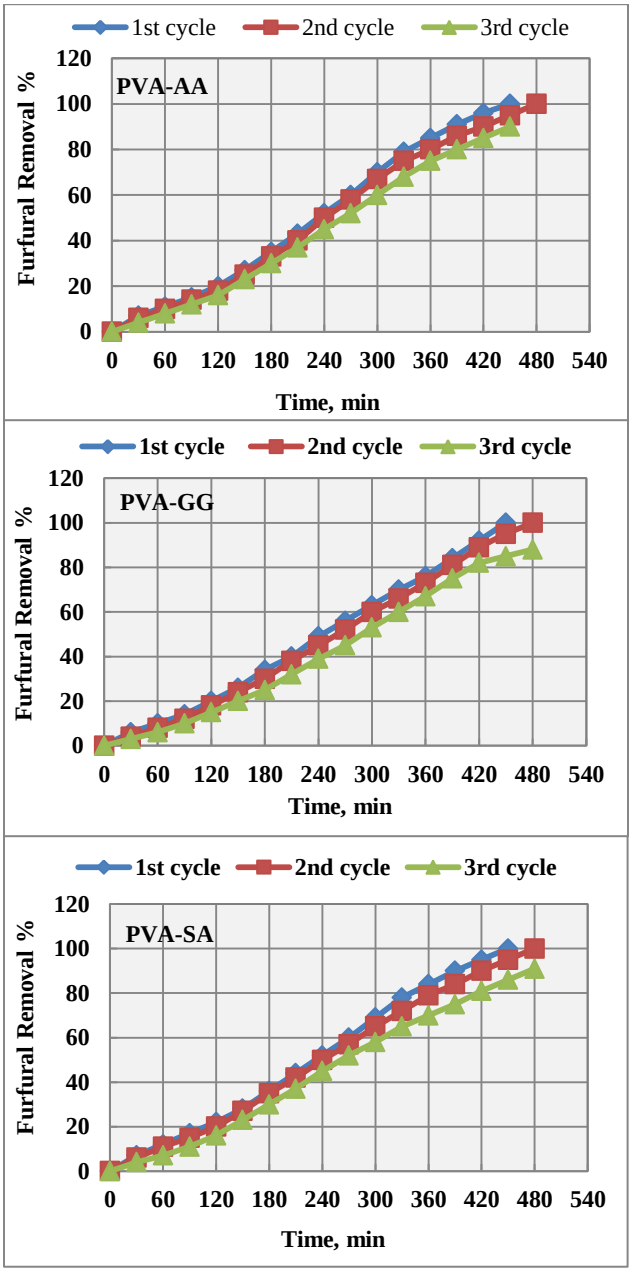

Fig.6 Furfural biodegradation performance (%) by *Bacillus* immobilized in PVA-AA, PVA-GG, and PVA-SA beads

Effect of initial concentration of organic substrate

Initial concentrations of organic pollutants play an important role in the biodegradation process, since some of them including phenol are known to have inhibitory effect on the activity of the biomass [19]. Fig.7 presents the effect of furfural initial concentration on the bacterial activity for furfural biodegradation. It is well observed that at 20 mg/L initial concentration of furfural, both immobilized cells (IC) and free cells (FC) were capable of complete biodegradation of furfural. At 50 mg/L initial concentration of furfural, the free cells were inhibited, whereby the immobilized cells maintained their ability for complete degradation of furfural even at higher concentration of furfural up to 100 mg/L.

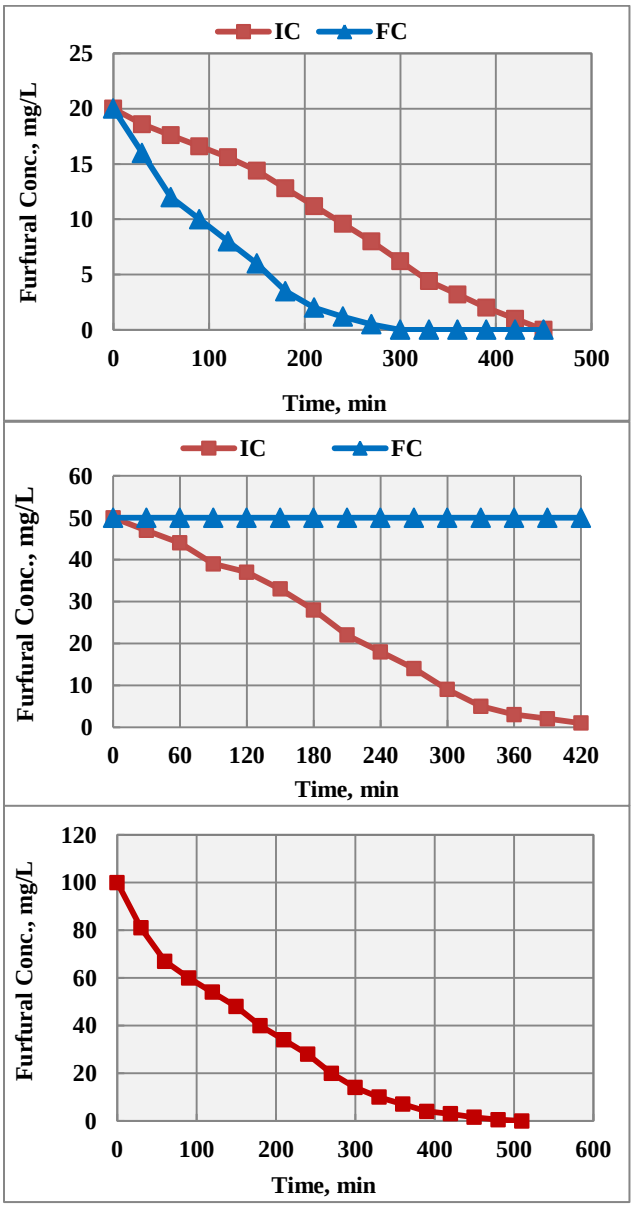

Fig.7 Comparison of *Bacillus* bacterium activity for furfural biodegradation using free cells (FC) and immobilized cells (IC) in PVA-SA beads.

In general, it is obvious that immobilized cells are more efficient than free cells for organics biodegradation. The merit of immobilization is due to the high surface area available for biomass accumulation which resulted in a high biomass concentration of 20-25 gVSS/L compared to 6-10 gVSS/L for isolated bacteria. In addition, the biotreatment systems using immobilized cells (IC) are more stable to shock loadings caused by sudden high concentrations of refractory organic compounds which may occurs from accidental spills. Additional observation which is worth to be mentioned is that pre-adoption or pre-acclimation to certain pollutant is not necessary for immobilized cells.

Use inactive beads (blank beads) for furfural removal

In order to assess if there is any abiotic process such as adsorption might be associated with the biodegradation of furfural by immobilized *Bacillus* cells, control experiment was performed using blank beads without biomass. Results revealed that no change in furfural concentration was observed during 24h, indicating the absence of any abiotic process. This finding was in a good agreement with the observation reported by Khattar et al. [20] who examined the effects of using agar-blank beads on Cr removal from aqueous solute

4. CONCLUSION

The biodegradation of furfural using *Bacillus* cells individually immobilized in PVA-GG, PVA-AA, and PVA-SA matrices was evaluated in a spouted bed bioreactor. Results revealed that complete removal of furfural can be obtained after 480 min using immobilized *Bacillus* cells at the first cycle. Excessive cycles up to 3 cycles were investigated and the experimental results indicated the potential efficiency of the immobilized cells to be recycled for complete removal of furfural at different time durations. Also, results demonstrated that the cells concentration affected the biodegradation rate.

5. AKNOWLEDGMENT

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6. REFERENCES

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