บันทึกข้อความ

ส่วนราชการ ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์ โทร.3343

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เรื่อง ขออนุมัติค่าตอบแทนการตีพิมพ์ผลงานในวารสารวิชาการ เรื่อง "Immobilization of Peroxidase from Cauliflower Stem on Ultrafiltration Membrane for Phenol Removal"

เรียน รองคณบดีฝ่ายวิจัยและบริการวิชาการ ผ่านหัวหน้าภาควิชาวิศวกรรมเคมี

อ้างถึงประกาศมหาวิทยาลัยเรื่อง"หลักเกณฑ์การจ่ายค่าตอบแทนการตีพิมพ์ผลงานในวารสารวิชาการ คณะวิศวกรรมศาสตร์ มหาวิทยาลัยอุบลราชธานี" ตามความทราบแล้วนั้น

เนื่องจากบทความวิจัยของ ผศ.ดร.กรรณิกา รัตนพงศ์เลขา เรื่อง "Immobilization of Peroxidase from Cauliflower Stem on Ultrafiltration Membrane for Phenol Removal" ได้รับการตีพิมพ์ในวารสารวิชาการระดับนานาชาติใน Applied Mechanics and Materials Vol. 879, pp 137-143, 2018 doi:10.4028/www.scientific.net/AMM.879.137 (ลิงค์เพื่อดาวน์โหลดไฟล์บทความ) ดังนั้นดิฉันจึงใคร่ขออนุมัติเบิกค่าตอบแทนการตีพิมพ์ผลงานในวารสารวิชาการเรื่องดังกล่าว ทั้งนี้ขอรับรองว่าผลงานดังกล่าวไม่ได้เป็นส่วนหนึ่งของการทำปริญญานิพนธ์ของผู้ขอ

จึงเรียนมาเพื่อโปรดพิจารณา

(ผศ.ดร.กรรณิกา รัตนพงศ์เลขา)
อาจารย์ประจำภาควิชาวิศวกรรมเคมี

เรียน รองคณบดีฝ่ายวิจัยฯ
เพื่อโปรดพิจารณา

21 มี.ค. 61

เรียน รองคณบดีฝ่ายวิจัยและบริการวิชาการ
ตามที่ ผศ.ดร.กรรณิกา รัตนพงศ์เลขา
ได้ขออนุมัติค่าตอบแทนการตีพิมพ์ผลงานวิจัย
ในการประชุมคณะกรรมการพิจารณาจริยธรรมการวิจัย
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Collaborative work 2 people:

Collaborative Person	Fraction of Academic Work
1. Karnika Ratanapongleka	70%
2. Apinya Onsarn	30%

ลงนามรับรองข้อมูล.....

(ผศ.ดร.กรรณิกา รัตนพงศ์เลขา)

ผู้รับผิดชอบบทความ (Corresponding Author)

แบบเสนอขอรับค่าตอบแทนในการตีพิมพ์วารสารวิชาการ

1. เอกสารประกอบการเสนอขอรับค่าตอบแทนในการตีพิมพ์วารสารวิชาการ

1. 1 แบบขอรับค่าตอบแทน

1. 2 หนังสือขออนุมัติค่าตอบแทน เรียน รองคณบดีฝ่ายวิจัยและบริการวิชาการผ่านหัวหน้าภาควิชา

1.3 สำเนาบทความวิจัยที่ได้รับการตีพิมพ์ในวารสารวิชาการ

1.4 รายละเอียดวารสาร

1.5 เอกสารแสดงค่า Impact factor ของวารสารที่ตีพิมพ์

2. รายละเอียดข้อมูลประกอบเสนอขอรับค่าตอบแทนในการตีพิมพ์วารสารวิชาการ

2.1 ผู้เสนอขอรับค่าตอบแทน ชื่อ-สกุล กรรณิกา รัตนพงศ์เลข

2.2 ชื่อบทความวิจัย (ภาษาไทย) .....

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(ภาษาอังกฤษ) Immobilization of Peroxidase from Cauliflower Stem on Ultrafiltration Membrane for Phenol Removal

2.3 รายละเอียดของวารสาร

ชื่อวารสาร Applied Mechanics and Materials

อยู่ในฐานข้อมูล ○ ISI กรณี ISI ช่วยระบุ impact factor:..... ○ SCOPUS ✓ SJR

○ TCI กลุ่มที่ 1 ○ TCI กลุ่มที่ 2

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2.4 สถานะในบทความวิจัยเป็น

✓ ชื่อแรก (first author) ✓ ผู้รับผิดชอบบทความ (corresponding author)

○ ผู้มีส่วนร่วมในบทความ

2.5 การมีส่วนร่วมในบทความของนักศึกษา

○ ใช้ขอจบการศึกษา ✓ ไม่ใช้ขอจบการศึกษา

การรับรองสัดส่วนผลงานทางวิชาการ กรุณากรอกข้อมูลตามแบบฟอร์มนี้ตามความเป็นจริง และรักษาไว้ซึ่ง **จรรยาบรรณ** และขอรับรองว่า บทความนี้ไม่เป็นส่วนหนึ่งของวิทยานิพนธ์ของผู้เสนอ

ลำดับที่	ชื่อ-สกุล	สัดส่วนผลงานทางวิชาการ (%)	ลงนามรับรองข้อมูล
1	Karnika Ratanapongleka	70%	
2	Apinya Onsarn	30%	
3			
4			

หมายเหตุ: กรณีผู้เสนอขอรับค่าตอบแทนเป็นชื่อแรก หรือ ผู้รับผิดชอบบทความสามารถรับรองแทนผู้เขียนร่วมได้

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Immobilization of Peroxidase from Cauliflower Stem on Ultrafiltration Membrane for Phenol Removal

Karnika Ratanapongleka[1,a*], Apinya Onsarn[1,b]

[1]Department of Chemical Engineering, Faculty of Engineering, Ubon Ratchathani University, Ubonratchathani 34190, Thailand

[a]karnika.r@ubu.ac.th, [b]apinya.on.58@ubu.ac.th

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Abstract. The aim of this work is to investigate phenol removal by immobilized peroxidase extracted from cauliflower stem. Peroxidase was partially purified by membrane filtration and diafiltration. Almost four-fold increase in the measured activity of partially purified peroxidase was obtained. The enzyme was then immobilized on to the surface of regenerated cellulose ultrafiltration membrane (molecular weight cut-off 30 kDa) using a dead-end filtration unit. Three different immobilization methods (physical adsorption, cross-linking and covalent-bonding using glutaraldehyde as a membrane activator) were tested. The immobilization and enzymatic reaction efficiency were evaluated in terms of the immobilization yield, the enzyme leakage from the system, the phenol removal and the permeate flux. Results showed that the immobilization methods did not much affect the permeate flux of the membrane. The peroxidase immobilization by covalent-bonding on regenerated cellulose membrane produced the highest immobilization yield and the lowest enzyme leakage. The immobilized enzymatic reaction efficiency on phenol removal was 100% at operational time 60 min and reduced to 96.4% at 600 min.

Introduction

Phenol can be found in wastewater from a variety of industries such as manufacture of petrochemicals, refineries, coal processing, paint, plastics, wood preservatives, pesticides, pulp and paper manufacturing, and metal casting [1,2]. Phenol-containing wastewater could not be discharged into the environment without treatment since phenol is considered to be hazardous and persistent pollutants as they can affect the aquatic life even at a very low concentration. In addition, human exposure to phenol through ingestion, contact, or inhalation causes serious health hazards, interfering with the endocrine system and possible leading to carcinogenesis [3,4]. Due to its toxicity the Environmental Protection Agency has limited a water purification standard of less than 1 ppb of phenol in surface waters [2].

Many treatments have been introduced to remove the phenol from wastewaters [5]. Enzymatic treatment has been proposed by many studies as a potential alternative due to the efficient, easily handle, specific target and low cost degradation of the pollutants. Peroxidases (EC 1.11.1.7) are oxidoreductases enzyme produced by plants, microorganisms and animals [6]. Peroxidases catalyze the reduction of peroxide and the oxidation of variety of organic and inorganic compounds. For example, horseradish peroxidase, soybean peroxidase and turnip peroxidase have been used for bioremediation of wastewaters contaminated with phenols, aromatic amines and phenolic compounds. Lignin peroxidase and manganese peroxidase have been observed to remove aromatic phenols and aromatic dyes [6]. Many researches on detoxification of wastewater contaminated with phenol and phenolic compounds have used horseradish peroxidase. However, because of its high cost, the alternative sources of peroxidases such as soybean, turnip and bitter have been suggested. Cauliflower is one of the alternatives that performs the highest peroxidase activity compared to other rich sources such as horseradish [7]. In this study, peroxidase from cauliflower stem was chosen since these stems were a waste product and could be easily obtained in local market.

Since low stability, high cost associated with isolation and purification and poor reusability of the enzyme has limited its application. In order to enhance the utility of the enzyme in treatment

process, many efforts have been focused on the preparation of enzyme in immobilized form including a variety of support materials and methods of immobilization [8].

The use of enzyme-immobilized ultrafiltration (UF) membrane offers a number of advantages including easy control, straightforward scaling, reduce cost, high volume capacities, possible performing a reaction simultaneously with a separation function, multiplying biochemical chain reaction and lower susceptibility to process disturbances [1]. Chemical methods such as covalent attachment and cross-linking used for enzyme immobilization onto UF membrane generally enhance structural rigidity and stability of the enzyme. However, the effects of chemical modification on enzyme properties are hard to speculate since these methods probably have the disadvantage of denaturing the original enzyme during binding process. Physical adsorption is another interesting method because of simplicity, less expensive and reusability of membrane after inactivation of immobilized enzyme [9]. Therefore, identification of suitable peroxidase immobilized method on the UF membrane is an importance.

Although several works have reported about using peroxidase for phenol removal [5,8,10,11], there is little information concenring the study of ultrafiltration for phenol removal and particularly in combining enzyme with ultrafiltration. Then the research finding will be of special interest to the phenol removal regarding the use of different methods for peroxidase immobilization on the ultrafiltration membrane. The objective of this work is to study phenol removal by UF membrane-bound peroxidase with different immobilization methods (physical adsorption, cross-linking and covalent-bonding immobilizations). Peroxidase extracted from cauliflower stem was partial purification before it was employed. To achieve a proper immobilized methods, the immobilization and enzymatic reaction efficiency were evaluated in terms of immobilization yield, enzyme leakage, phenol removal and permeate flux.

Materials and Methods

Materials. Bovine Serum Albumin (BSA), glutaraldehyde, hydrogen peroxide, citric acid, sodium hydroxide and Coomassie Brilliant Blue G250 were purchased from Fluka (UK). Phenol, guaiacol, 4-aminoantipyrine and potassium ferriccyanide were obtained from Sigma-Aldrich (UK). All chemicals were of analytical grade and used without further purification. Phenol solution was prepared using deionized water.

Experimental System. The experiments were carried out in a stirred cell module (Amicon 8400, Millipore, USA). The module was used with a regenerated cellulose (41.8 cm^2 membrane surface area, molecular weight cut off of 30 kDa). The stirring speed was controlled at 100 rpm. Nitrogen gas was filled into the cell to keep a constant pressure. The permeate passed through the membrane and it was collected in a beaker which was placed on an analytical balance (Model BL-2200H, Shimadzu, Japan). The amount of permeate collected was weighed in order to determine the permeate flux. Before being use in each experiment, a new membrane UF sheet was initially placed in the stirred cell unit. The membrane was then cleaned and pre-compacted with deionized water.

Peroxidase Extraction and Partial Purification. Cauliflower stems used in this work were obtained from local market in Ubonratchathani, Thailand. The stems were thoroughly washed with tap water and cut into pieces. Then they were passed through juice extractor. The juices were then centrifuged at 8000 rpm for 15 min. The clear supernatant (crude extract) was collected and subjected to further partial purification.

For the partial purification of peroxidase, membrane filtration consisted of microfiltration and ultrafiltration was applied. Microfiltration was done using hollow fiber membrane with MiniKros® Sampler Filter Modules (Spectrum Lab). Obtained permeate was feed for subsequent concentration using ultrafiltration with molecular weight cut-off 30 kDa. Finally, partially purified peroxidase (retentate) was stored at 4 ^0C for further immobilization step. The amounts of protein and peroxidase activity in these solutions were measured.

Peroxidase Immobilization on the Ultrafiltration Membrane. In order to acheive high immobilization efficiency and effective phenol removal, three different methods of peroxidase immobilization on the ultrafiltration membrane were examined. The quatity of enzyme (11.8 U of peroxidase) introduced to UF sheet for each method was equal.

Method 1: Physical adsorption. After a new membrane was cleaned and pre-compacted with deionized water, the membrane was immersed for 60 min at 4 ^{0}C in the peroxidase solution. The membrane-bound peroxidase was washed with deionized water and 0.1 M phosphate buffer under a pressure of 0.1 bar to remove the unbound enzymes. The washings were done until no protein or enzyme activity could be detected in the washing solution.

Method 2: Cross-linking. The membrane was immersed for 60 min at 4 ^{0}C in glutaraldehyde solution (0.1%v/v) containing peroxidase at the same amount. The membrane-bound peroxidase was washed with deionized water, 0.1 M NaCl and 0.1 M phosphate buffer under a pressure of 0.1 bar to remove the unbound enzymes. The washings were done until no protein or enzyme activity could be detected in the washing solution.

Method 3: Covalent-bonding. The membrane was activated using 0.1%v/v glutaraldehyde for 60 min. After further washings with deionized water, 0.1 M NaCl and 0.1 M phosphate buffer, the membrane was immersed for 60 min at 4 ^{0}C in the peroxidase solution. Coupling of the enzyme to glutaraldehyde occurred through the amine groups of the enzyme and the aldehyde groups appeared on the membrane surface. The membrane-bound peroxidase was washed with deionized water and 0.1 M phosphate buffer under a pressure of 0.1 bar to remove the unbound enzymes. The washings were done until no protein or enzyme activity could be detected in the washing solution.

The amount of immobilized enzyme on the UF membrane was calculated by subtracting the amount of the enzyme recovered in the solution at the end of immobilization and into the washing solution from the amount of enzyme initially used for the immobilization.

Phenol Removal. All properties of peroxidase immobilization on the UF membrane were evaluated with phenol. The removal experiments were conducted by employing parameters of pH solution 7.0, at room temperature, 1 mM of hydrogen peroxide and 1 mg/L of phenol concentration. The phenol solution was fed into the dead-end filtration module at a operating pressure 0.1 bar. Samples were drawn from permeate and analyzed for residual phenol concentration. Meanwhile, the permeate flux was measured by direct measurement of permeates flow in terms of volume per unit area per unit time and the results were performed in normalized flux. All experiments were done in triplicate at room temperature.

The residual concentration of phenol in the permeate was determined colorimetrically using 4-aminoantipyrine and potassium ferriccyanide as described previously [12]. The increase in absorbance was measured at 510 nm using a UV-VIS spectrophotometer. The absorbance values were transformed into phenol concentration by creating a phenol standard curve.

Enzymatic Activity and Protein Assays. Peroxidase activity was determined by monitoring the change in absorbance at 436 nm using guaiacol as the substrate and H_2O_2 as the hydrogen donor [13]. The substrate mixture contained 18 mM guaiacol, 0.05% hydrogen peroxide and 0.1M phosphate buffer. The reaction cuvette contained 2.99 ml substrate mixture and 0.1 ml enzyme extract. The enzyme activity was expressed in U/ml.

Protein concentration was assayed using Bradford method [14] based on the color change of Coomassie Brilliant Blue G250 in response to binding with proteins. BSA was used as the protein standard to construct the calibration curve.

Results and Discussions

Partial Purification of Peroxidase from Cauliflower Stem. Peroxidase extracted from cauliflower stem was partially purified using microfiltration followed by ultrafiltration. The results are presented in Table 1. The first step crude peroxidase was microfiltration through 0.2 μm membrane, which resulted in remove larger particles and fiber residues. After microfiltration, light green colored enzyme solution was ultrafiltration in which the enzyme was concentrated and

diafiltered in order to reduce ballast protein amount. Increase in the specific activity of the peroxidase was observed and almost four-fold purification of the crude peroxidase was obtained in the final step.

Table 1: Partial Purification of Peroxidase.

Purification step	Total protein [mg]	Total activity [U]	Specific activity [U mg^{-1}]	Purification [fold]
Crude enzyme	120,195.6	1815.0	0.0151	1
Microfiltration	59,808.0	1120.0	0.0187	1.24
Ultrafiltration and Diafiltration	18,897.6	1132.8	0.06	3.97

Immobilization of Peroxidase and Phenol Removal. Since enzyme-membrane interactions play an important role in maintaining the activity of the enzyme on the UF membrane, three different enzyme immobilized methods consisted of physical adsorption, cross-linking and covalent-bonding are selected to study in this work. In order to find out the best immobilized methods for obtaining the highest enzyme-immobilized membrane activity, immobilization yield, enzyme leakage, phenol removal and permeate flux were investigated.

The percentages of immobilization yield from different enzyme immobilization methods were compared in Fig. 1(a). The minimum immobilization yield was observed from physical adsorption method (77.40±0.52%). Since physical adsorption methods are characterized by weaker, monocovalent interactions such as hydrogen bonds, hydrophobic interactions, van der Waals forces, affinity binding, ionic binding of the enzyme with the support [13]. Then the adsorption bonding is too weak to keep the enzyme fixed to the membrane and is prone to leaching of the enzyme. Furthermore, the properties of the regenerated cellulose membrane used in this study generally provide a very low adsorption capacity. In case of cross-linking method, it was performed by formation of intermolecular cross-linkages between the enzyme molecules by glutaraldehyde. The size of crosslinked enzyme molecules becomes bigger resulting in more enzyme accumlation on the membrane surface. While covalent-bonding method, the enzyme was bound covalentely to the membrane using glutaraldehyde as a membrane activator. This approach normally provides the strongest enzyme/membrane interaction. The immobilization yield obtained from cross-linking was close to covalent-bonding methods (90.38±0.79% and 95.47±0.62%, respectively).

The leakage of immobilized enzyme from the membrane is one of the important considerations. The measurement of enzyme activity in the collected volume permeate stream during operating time was examined. The presence of enzyme activity in the stream confirmed that there was a leakage of the enzyme. The effect of enzyme leakages on enzyme immobilized methods is shown in Fig. 1(b). The maximum percentage of the enzyme leakage was found in physical adsorption while the covalent-bonding methods showed the lowest loosing of the enzyme. As can be seen, covalent bonds provided powerful link between the peroxidase and the membrane and enhanced enzyme attachment. Compared with covalent-bonding immobilization methods, the physical adsorption and cross-linking methods allowed the enzyme deposited on the membrane surface and the interaction between enzyme-membrane was not strong as covalent bonding. The applied pressure during operation would probably support a diffusion of the enzyme into the pores resulting in higher enzyme leakage.

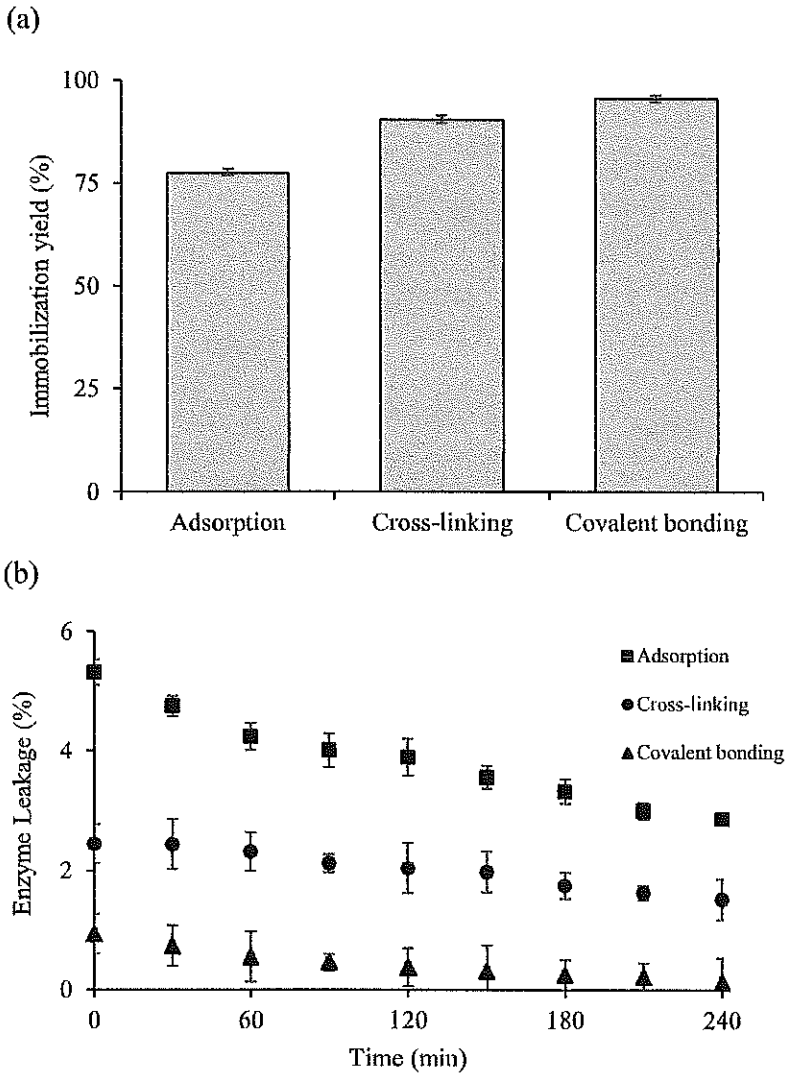

Fig. 1: Comparison of immobilized methods (a) Immobilization yield (b) Enzyme leakage.

To prove that the covalent-bonding method was suitable for peroxidase immobilized UF membrane in addition to terms of immobilization yield and the enzyme leakage, the immobilized enzyme activity on phenol removal is displayed in Fig. 2. Three different enzyme immobilization methods were used in the dead-end mode of operation to remove initial phenol concentration of 1 mg/l at pH 7.0, operating pressure 0.1 bar and 25°C. The reaction was conducted for 600 min. During the first 60 reaction minutes, the phenol removal efficiency from using covalent-bonding enzyme increased quickly and reached to 100%. Then the removal efficiency reduced to 96.4% at 600 min. For cross-linking method, the obtained efficiency was close to covalent-bonding method while the maximum phenol removal from physical adsorption was only 76% at 150 min. After 150 min, the removal efficiency gradually decreased. The gradual decrease in removal efficiency obtained from three immobilization methods might be related to enzyme inactivation, conformational changes of the enzyme, the inhibition of enzyme activity from the accumulation of substrate or reaction products or the leakage of the enzyme from the system.

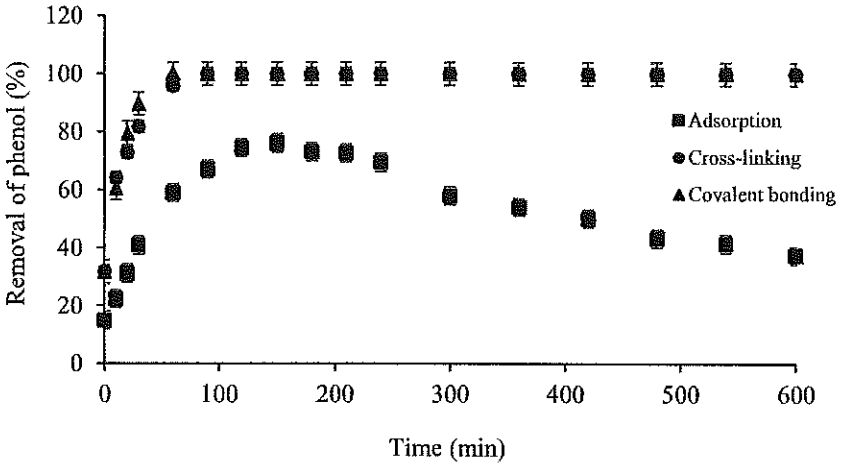

Fig. 2: The effect of immobilized methods on phenol removal.

For consideration in terms of flux (Fig. 3), the immobilization methods did not much affect the flux decline. The flux decline occurred in physical adsorption method was comparable to covalent-bonding method. Generally, the flux decline happens because of the fouling or solute being retained by the membrane and the solvent passing the membrane. The solute will accumulate to form a layer at the membrane interface with a relatively high concentration. As seen in Fig. 2, although the efficiency of phenol removal obtained from cross-linked enzyme was close to covalent-bonding, the observed flux decline from cross-linking was much lower. Since the size of the enzyme molecules become bigger after cross-linking. These immobilized enzymes sticked densely on the membrane surface. In addition to catalytic enzyme activity on phenol removal, the phenol molecules could be accumulated on the layer of cross-linked enzyme resulting in flux decline.

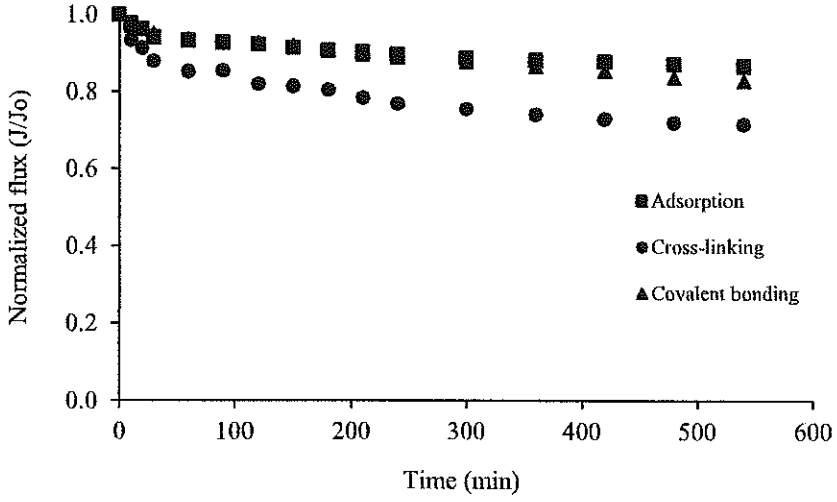

Fig. 3: The effect of immobilized methods on normalized flux.

Conclusions

It can be concluded that the immobilization methods of peroxidase extracted from cauliflower stem on UF membrane affected the catalytic behaviors of the enzyme. The suitable immobilization method was covalent-bonding using glutaraldehyde as a membrane activator. The maximum immobilization yield was observed at 95.47±0.62% and the enzyme leakage was less than 3% of the initial immobilized enzyme. The removal of phenol (1 mg/L) using peroxidase immobilized membrane was conducted in the dead-end mode of operation. The results showed that the immobilized peroxidase had a good potential to remove phenol (100% in 60 min). Further investigations on the factors affecting immobilized enzyme activity and the toxicity after the phenol transformation are needed to evaluate.

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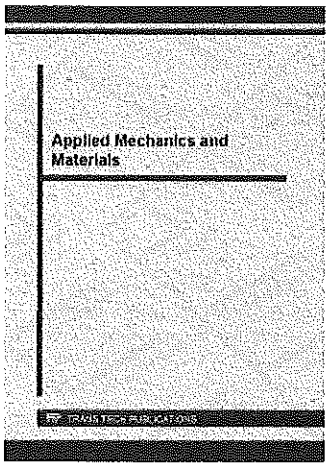

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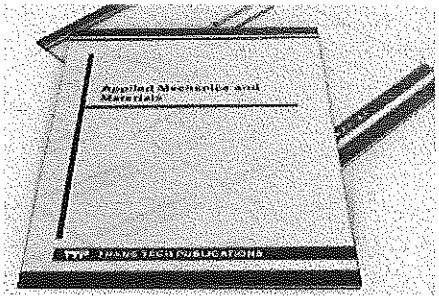

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