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FORMULATION AND EVALUATION OF AN AUTOMATIC DISHWASHING DETERGENT CONTAINING T1 LIPASE

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfilment of the Requirements for the Degree of Master of Science

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FORMULATION AND EVALUATION OF AN AUTOMATIC DISHWASHING DETERGENT CONTAINING T1 LIPASE

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IZUDDIN B. ABDUL RAHMAN

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Chairman: Professor Raja Noor Zaliha Raja Abd Rahman, PhD
Institute: Bioscience

Due to the concerns on the environment, the detergent industry is required to take more environmentally friendly approaches in formulating detergents, which include using biodegradable chemicals and enzymes. As a result, the use of enzymes in detergent formulation has become a must, and enzymes of many types and functions are being developed. Consequently, a recombinant enzyme called T1 lipase (E.C. 3.1.1.3), which has been well-studied and successfully produced, was `evaluated as a detergent enzyme for automatic dishwashing detergent (ADD) formulation.

T1 lipase was mass produced at shake flask scale by growing the *Escherichia coli* BL21 expression host, which carried the T1 gene and harvesting the crude T1 lipase from the bacterial cells through sonication. The enzymatic activity was assayed, and the presence of the enzyme was confirmed using sodium dodecyl sulfate

polyacrylamide gel electrophoresis (SDS-PAGE). The crude enzyme was not purified to its homogeneity, but the presence of the host proteins was assumed not to give any significant effect to the subsequent results of this study, which was proven by the absence of the lipases, amylases, and proteases from the host organism.

The crude T1 lipase in its free form was then checked for compatibility with the detergent components by conducting stability tests and checking its residual enzymatic activity. T1 lipase was mostly stable in nonionic surfactants, especially those that are made of polyhydric alcohols. T1 lipase was also stable in a mixture of sodium carbonate and glycine, which yielded pH 9.25 close to T1 lipase optimum pH of 9.0. However, sodium carbonate alone destabilized T1 lipase possibly due to the interaction between carbonates and Ca2+. These results indicated that polyhydric alcohols and glycine had stabilizing effects on T1 lipase.

Next, the crude T1 lipase was spray-dried with the addition of wall materials, such as gum arabic and maltodextrin. A blend consisting of gum arabic/maltodextrin/T1 lipase supernatant with a ratio of 6:12:3 produced products that were mostly spherical when viewed under an electron microscope, and the encapsulated T1 lipase yielded a three-fold increase in enzymatic activity compared to the free T1 lipase. The spraydried product was also more soluble due to the hydrophilic nature of the wall materials.

Subsequently, the crude T1 lipase was evaluated as an auxiliary component for ADD formulations. The dishwashing performance of the formulated ADDs was evaluated in term of percent soil removed using the Leenert's Improved Detergency Tester. The dishwashing performance of the formulated detergent was positively affected by the increase in temperature but negatively affected by the presence of hard water, specifically Ca2+ and Mg2+. The presence of hard water reduced the efficiency of the formulated ADDs in removing soil by an average of 68%. However, T1 lipase was not negatively affected by the presence of hard water, and this enzyme was enhanced by the presence of polyacrylates. Moreover, the presence of Ca2+ improved the structural integrity of T1 lipase, especially at elevated temperature.

Ultimately, an ADD formulation dubbed as ―U1‖ was produced based on the results of this study. Overall, the addition of T1 lipase to the ADD formulations indicated an improved dishwashing performance of up to 17% compared with the absence of T1 lipase, especially in the presence of hard water. Furthermore, this study will give more insight on lipase as detergent enzyme, specifically in automatic dishwashing.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

FORMULASI DAN PENILAIAN DETERGEN UNTUK MESIN PENCUCI PINGGAN MANGKUK AUTOMATIK YANG MENGANDUNGI LIPASE T1

Oleh

IZUDDIN B. ABDUL RAHMAN

Januari 2013

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Disebabkan oleh keprihatinan terhadap alam sekitar, industri detergen dikehendaki untuk mengambil pendekatan yang lebih mesra alam dalam merumuskan detergen, termasuk menggunakan bahan kimia terbiodegradasi dan enzim. Kesannya, penggunaan enzim dalam formulasi detergen telah menjadi satu kemestian, dan enzim dari pelbagai jenis dan fungsi sedang dibangunkan. Dengan itu, enzim rekombinan dipanggil lipase T1 (E.C. 3.1.1.3), yang telah dikaji secara mendalam dan berjaya dihasilkan, telah dinilai sebagai enzim detergen untuk formulasi detergen untuk pencuci pinggan mangkuk automatik (ADD).

Lipase T1 telah dihasilkan secara besar-besaran pada skala kelalang makmal dengan membiak perumah penzahiran *Escherichia coli* BL21, yang membawa gen T1 dan menyediakan enzim lipase T1 yang mentah dari sel bakteria melalui sonikasi. Aktiviti

enzim telah diukur, dan kehadiran enzim telah disahkan menggunakan gel elektroforesis poliakrilamida dengan natrium dodesil sulfat (SDS-PAGE). Walaupun enzim yang mentah tidak ditulenkan, kehadiran protein perumah dianggap tidak memberi apa-apa kesan yang ketara kepada keputusan kajian ini, dan telah dibuktikan oleh ketiadaan enzim lipase, amilase, dan protease dari organisma perumah.

Lipase T1 yang mentah dalam bentuk bebas kemudiannya diperiksa keserasiannya dengan komponen-komponen detergen yang lain dengan melakukan ujian kestabilan dan memeriksa baki aktiviti yang tinggal. Lipase T1 adalah stabil dalam kebanyakan surfaktan tidak bercas, terutama yang dibuat daripada alkohol polihidrik. Lipase T1 juga stabil dalam campuran natrium karbonat dan glycine, yang menghasilkan pH 9.25 yang juga hampir dengan pH optimum lipase T1 iaitu 9.0. Walau bagaimanapun, natrium karbonat sahaja menyahstabilkan lipase T1 mungkin disebabkan oleh interaksi antara karbonat dan Ca2+. Keputusan ini menunjukkan bahawa alkohol polihidrik dan glycine memberi kesan penstabilan kepada lipase T1.

Seterusnya, lipase T1 mentah telah disembur-kering dengan penambahan bahan dinding, seperti gam arab dan maltodekstrin. Satu campuran yang terdiri daripada gam arabic/maltodextrin/supernatan lipase T1 dengan nisbah 6:12:3 telah menghasilkan produk yang kebanyakannya berbentuk sfera apabila dilihat di bawah mikroskop elektron, dan lipase T1 yang disaluti bahan dinding menghasilkan peningkatan sebanyak tiga kali ganda dalam aktiviti enzim berbanding dengan lipase T1 dalam bentuk bebas. Produk semburan-pengeringan juga lebih senang terlarut kerana sifat hidrofilik bahan dinding.

Selepas itu, lipase T1 yang mentah telah dinilai sebagai satu komponen tambahan bagi formulasi ADD. Prestasi pencucian pinggan mangkuk oleh detergen yang diformulasikan dinilai dengan mengira peratus kotoran yang berjaya ditanggalkan menggunakan Penguji Keberkesanan Pencucian Rekaan Leenert yang telah dimajukan. Prestasi pencucian pinggan mangkuk oleh detergen yang diformulasikan meningkat dengan peningkatan suhu tetapi terjejas dengan kehadiran air yang mengandungi garam, khususnya Ca^{2+} dan Mg^{2+}. Kehadiran air yang mengandungi garam ini mengurangkan kecekapan detergen yang diformulasikan dalam menanggalkan kotoran sebanyak 68% secara purata. Walau bagaimanapun, lipase T1 tidak terjejas oleh kehadiran air yang mengandungi garam, dan kecekapan enzim ini telah dipertingkatkan dengan kehadiran polyacrylates. Selain itu, kehadiran Ca^{2+} meningkatkan integriti struktur lipase T1, terutamanya pada suhu tinggi.

Akhirnya, formulasi ADD yang digelar sebagai "U1" telah dihasilkan berdasarkan keputusan kajian ini. Secara keseluruhannya, penambahan lipase T1 ke dalam formulasi ADD meningkatkan prestasi pencucian pinggan mangkuk sebanyak 17% berbanding dengan ketiadaan lipase T1, terutamanya dalam kehadiran air yang mengandungi garam. Tambahan pula, kajian ini akan memberi gambaran yang lebih jelas terhadap lipase sebagai enzim detergen, khususnya dalam pencucian pinggan mangkuk secara automatik.

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I certify that an Examination Committee has met on **date of viva voce** to conduct the final examination of **Izuddin b. Abdul Rahman** on his **Master of Science** thesis entitled "**Formulation of an automatic dishwashing detergent containing locally produced enzymes**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science Members of the Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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Date: 7 January 2013

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