CELLULASE AND XYLANASE-PRODUCING *Trichoderma asperellum* AND *Rhizopus oryzae* FOR EFFECTIVE SACCHARIFICATION OF OIL PALM FROND LEAVES BY SOLID-STATE FERMENTATION

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ABSTRACT

Cellulase, xylanase and pectinase contribute almost 20% to the world enzyme market. The growing demand for cellulases and xylanases in lignocellulosic degradation and reutilization has instigated the need for their improved production at a low cost. This study, therefore, evaluated oil palm frond leaves (OPFL) as a cheap and sustainable growth substrate for two novel fungi species to produce cellulase and xylanase under solid-state fermentation (SSF). Morphology, 18S rRNA, phylogeny and BIOLOG® analyses identified the cellulase and xylanase-producing fungal strains as *Trichoderma asperellum* UC1 and *Rhizopus oryzae* UC2. While UC2 is robust and fast-growing, its enzyme production rate is slower and sustained; in contrast, strain UC1 showed a higher production rate of the same enzymes. Using the one variable at a time (OVAT) method, optimised fermentation parameters for strain UC1 (30 °C, 60-80 % moisture content, 2.5×10^6 spores/g inoculum size, 6.0-12.0 pH) and strain UC2 (30 °C, 40 % moisture content, 2.0×10^8 spores/g inoculum size, 6.0-12.0 pH) resulted in a corresponding 2.7, 2.6, 1.1, 1.7 (strain UC1) and a 2.3, 3.3, 1.2 and 1.0 (UC2)-fold increase in CMCase, FPase, β-glucosidase and xylanase maximum activities. Cellulases and xylanase were produced within a broad pH range between pH 4.0−12.0. Proteome analysis using SDS-PAGE, of the enzyme complexes from *in situ* hydrolysis of raw OPFL under SSF by strain UC1 and UC2 revealed existence of four endo-β-1,4-xylanases and endoglucanases, as well as one exoglucanase and β-glucosidase each for strain UC1 and one endo-β-1,4-xylanase, endoglucanase, exoglucanase as well as three β-glucosidases for strain UC2. Compositional and structural analysis (FESEM) of OPFL before and after *in situ* hydrolysis confirmed their degradation, that resulted in 31.16 % and 75.5 % hydrolysis efficiency for strain UC1 and UC2 enzymes. Furthermore, the enzyme complexes from both strains showed thermophilic and acidophilic characteristics at 50−60 °C and pH 3.0−5.0. Glucose (16.87 and 26.74 mg/g) and fructose (18.09 and 50.83 mg/g) were among the dominant fermentable sugar products from the hydrolysis of OPFL, aside from cellobiose (105.92 and 58.31mg/g) and xylose (1.08 and 1.44 mg/g), by strain UC1 and UC2 respectively. Thermal and pH stability tests for their enzyme cocktails revealed half-lives for UC1 CMCase, FPase, β-glucosidase and xylanase to be 15.18, 4.06, 17.47, 15.16 h at 60 °C, as well as 64.59, 25.14, 68.59 and 19.20 h at pH 4.0; UC2 - 5.13, 1.48, 18.81, 9.23 h when incubated at 60 °C and 27.55, 12.23, 18.26, 4.43 h at pH 4.0. Optimisation using response surface methodology resulted in maximum activities of CMCase (126.87 U/g), FPase (85.53 U/g) and xylanase (215.42 U/g) under optimised SSF conditions (30 °C, 2.0×10^7 spores/g, 75 % moisture content, pH 6.0) and β-glucosidase (131.76 U/g) at 32 °C, 2.0×10^7 spores/g, 50 % moisture content at pH 12.0. Enzymatic saccharification on ultrasonicated OPFL yielded 1240 mg/g of total reducing sugar as well as 56.21, 72.68 and 43.83 mg/g of glucose, xylose and cellobiose. The enzymes also enhanced the clarification of orange juice and rising of dough by 82−88 % and 1.7−2.0-fold. Based on the findings, it was apparent that *T. asperellum* UC1 and *R. oryzae* UC2 are robust producers of cellulolytic and xylanolytic enzymes using OPFL as the main SSF substrate for the production of large quantities of reducing sugars.

ABSTRAK

Selulase, xilanase dan pektinase menyumbang hampir 20% kepada pasaran enzim dunia. Permintaan yang tinggi terhadap selulase dan xilanase dalam degradasi dan pengggunaan semula lignoselulosa telah mendorong kepada keperluan bagi penghasilannya yang lebih baik pada kos yang rendah. Oleh itu, kajian ini telah mengenalpasti daun pelepah kelapa sawit (OPFL) sebagai substrat pertumbuhan yang murah dan mudah didapati untuk dua spesies kulat bagi menghasilkan selulase dan xilanase di bawah fermentasi bentuk pepejal (SSF). Analisa morfologi, 18S rRNA, filogeni dan BIOLOG® mengenalpasti strain kulat penghasil selulase dan xilanase sebagai *Trichoderma asperellum* UC1 dan *Rhizopus oryzae* UC2. Walaupun UC2 merupakan strain yang lasak dan tumbuh cepat, kadar pengeluaran enzimnya lebih lambat dan lama; sebaliknya, strain UC1 menunjukkan pengeluaran yang lebih tinggi untuk enzim yang sama. Menggunakan kaedah satu pemboleh ubah pada satu waktu (OVAT), parameter fermentasi yang optimum untuk strain UC1 (30 °C, 60−80 %, kadar kelembapan, 2.5×10^6 spora/g berat inokulum, pH 6.0−12.0) dan strain UC2 (30 °C, 40 % kadar kelembapan, 2.0×10^8 spora/g berat inokulum, pH 6.0-12.0) menghasilkan 2.7, 2.6, 1.1, 1.7 (strain UC1) dan masing-masing 2.3, 3.3, 1.2, dan 1.0 (UC2) kali ganda peningkatan aktiviti maksimum CMCase, FPase, β-glukosidase dan xilanase. Selain itu, selulase dan xilanase dihasilkan dalam sela pH yang luas iaitu antara pH 4.0−12.0. Analisis protein SDS-PAGE ke atas kompleks enzim mendapati dari hidrolisis *in situ* OPFL mentah oleh strain UC1 dan UC2 di bawah SSF menunjukkan kehadiran empat endo-β-1,4-xilanase dan endoglukanase, serta satu exoglukanase dan β-glukosidase untuk strain UC1 dan satu endo-β-1,4-xylanase, endoglukanase, exoglukanase serta tiga β-glukosidase untuk strain UC2. Analisis komposisi dan struktur (FESEM) OPFL sebelum dan selepas hidrolisis *in situ* mengesahkan degradasi tersebut menghasilkan 31.16 % dan 75.5 % efisiensi hidrolisis untuk strain UC1 dan UC2. Selain itu, kompleks enzim dari kedua-dua strain menunjukkan ciri-ciri termofilik dan asidofilik pada suhu 50−60 °C dan pH 3.0−5.0. Glukosa (16.87 dan 26.74 mg/g) dan fruktosa (18.09 dan 50.83 mg/g) adalah di antara produk gula fermentasi dominan dari hidrolisis OPFL, selain dari selobiosa (105.92 dan 58.31 mg/g) dan xylosa (1.08 dan 1.44 mg/g) oleh strain UC1 dan UC2. Ujian stabiliti termal dan pH untuk koktail enzim mendedahkan separuh-hayat untuk UC1 CMCase, FPase, β-glukosidase dan xilanase adalah 15.18, 4.06, 17.47, 15.16 jam pada 60 °C, serta 64.59, 25.14, 68.59 dan 19.20 jam pada pH 4.0; UC2 - 5.13, 1.48, 18.81, 9.23 jam apabila dieram pada 60 °C dan 27.55, 12.23, 18.26, 4.43 jam pada pH 4.0. Pengoptimuman menggunakan kaedah respon permukaan menghasilkan aktiviti maksimum CMCase (126.87 U/g), FPase (85.53 U/g) dan xilanase (215.42 U/g) di bawah keadaan SSF optimum (30 °C, 2.0×10^7 spora/g, 75 % kadar kelembapan, pH 6.0) dan β-glukosidase (131.76 U/g) pada 32 °C, 2.0×10^7 spora/g, 50 % kadar kelembapan pada pH 12.0. Sakarifikasi enzimatik ke atas OPFL ultrasonikasi menghasilkan 1240 mg/g jumlah gula penurunan serta 56.21, 72.68 dan 43.83 mg/g glukosa, xilosa dan selobiosa. Enzim-enzim turut meningkatkan klarifikasi jus oren dan kenaikan doh sebanyak 82−88 % dan 1.7−2.0 kali ganda. Berdasarkan penemuan tersebut, jelas menunjukkan *T. asperellum* UC1 and *R. oryzae* UC2 adalah pengeluar enzim selulolitik dan xilanolitik yang kuat dengan menggunakan OPFL sebagai substrat utama SSF bagi menghasilkan gula penurunan dalam jumlah yang besar.

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LIST OF ABBREVIATIONS

Adj. R^2	-	Adjusted coefficient of determination
ANOVA	-	Analysis of Variance
AOAC	-	Association of Official Analytical Chemists.
BBD	-	Box-Behnken Design
BLAST	-	Basic Local Alignment Search Tool
BSA	-	Bovine Serum Albumin
CAZy	-	Carbohydrate-active enzymes
CBH	-	Cellobiohydrolase
CBMs	-	Carbohydrate-binding module
CCD	-	Central composite design
CMC	-	Carboxymethylcellulose
CMCase	-	Carboxymethylcellulase
COPD	-	Chronic obstructive pulmonary disorder
DNA	-	Deoxyribonucleic acid
DNS	-	3,5-Dinitrosalicylic acid
EFB	-	Empty fruit bunch
EI	-	Enzymatic index
FESEM	-	Field emission scanning electron microscopy
FPase	-	Filterpaperase
GH	-	Glycosyl hydrolase
HPLC	-	High performance liquid chromatography
IUPAC	-	International union of pure and applied chemistry
LCB	-	Lignocellulosic biomass
LCW	-	Lignocellulosic waste
MEGA 6	-	Molecular Evolutionary Genetics Analysis Software
MF	-	Mesocarp fibre
OPB	-	Oil palm biomass
OPF	-	Oil palm frond
OPFL	-	Oil palm frond leaves
OPT	-	Oil palm trunk

OVAT	-	One-variable-at-a-time
OPEFB	-	Oil palm empty fruit bunch
PAHs	-	Polyaromatic hydrocarbons
PDA	-	Potato dextrose agar
PCR	-	Polymerase Chain Reaction
PDB	-	Potato dextrose broth
PKS	-	Palm kernel shell
ρNP	-	P-nitrophenol
ρNPG	-	P-nitrophenyl-β, D-glucopyranoside
POME	-	Palm oil mill effluent
R^2	-	Coefficient of determination
RID	-	Refractive index detector
RSM	-	Response surface methodology
SDS-PAGE	-	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SEM	-	Scanning Electron Microscopy
SSF	-	Solid state liquid
TRS	-	Total reducing sugar
TSP	-	Total soluble protein
UV-Vis	-	Ultraviolet-Visible
18S rRNA	-	18 Subunit Ribosomal Deoxyribonucleic Acid

LIST OF SYMBOLS

%	-	Percentage
°C	-	Celsius
d	-	Day
Ca	-	Calcium
h	-	Hour
kg	-	Kilogram
min	-	Minute
mL	-	Millilitre
mM	-	Millimolar
NaCl	-	Sodium Chloride
NaOH	-	Sodium Hydroxide
H_2O	-	Water
CO_2	-	Carbon (IV) oxide
HCl	-	Hydrochloric acid
K_2HPO_4	-	Dipotassium potassium
KH_2PO4	-	Monopotassium phosphate
$CaCl_2.2H_2O$	-	Calcium chloride dihydrate
$MgCl_2.6H_2O$	-	Magnesium chloride hexahydrate
$MgSO_4.7H_2O$	-	Magnesium sulphate heptahydrate
$(NH_4)_2SO_4$	-	Ammonium sulphate
kDa	-	Kilodalton
nm	-	Nanometer
rpm	-	Revolution per minute
s	-	Second
v/v	-	Volume percentage per 100 mL volume
w/v	-	Weight per volume percentage
µmol	-	Micromole
g	-	Gram
kg	-	Kilogram
U/g	-	Unit per gram

U/mL	-	Unit per mL

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The environmental inconvenience of post-harvest agricultural lignocellulosic biomass left behind to decompose naturally is a predicament faced by many nations throughout the globe (Fritsch et al., 2017). This is because the practice of passive biomass dumping is not just an eye sore to the local community but also an environmentally unfriendly practice. Certain nations resort to using methods of open burning and chemical treatments to eliminate post-harvest biomass from the environment, but in turn, created other problems such as acid rain and emissions of greenhouse gases that further exacerbated ecological pollution (Kumar et al., 2015; Zhang et al., 2017). The released gases are often toxic including carbon IV oxide, methane, nitrous oxide, polyaromatic hydrocarbons (PAHs), as well as fluorinated gases (Blasing, 2016), all of which ultimately contribute to the phenomenon of global warming.

Regionally, the undesirable effects of pollution due to the open burning of agricultural biomass have impacted populations in the Southeast region of Asia *viz.* Indonesia, Malaysia, South Thailand, Brunei and the South Philippines (Thepnuan et al., 2019). Frequent occurrences of the 'hazy season' in these regions are testaments of the gravity of such practice. Worryingly, large scale open burning consequently releases large amounts of tiny particulates (diameter < 2.5 μm, $PM_{2.5}$) as well as highly toxic and carcinogenic dioxins into the atmosphere (Thepnuan et al., 2019; Weidemann et al., 2016). These substances are harmful to all living beings particularly humans. Premature deaths of as many as 3.3 million people annually have been linked to open burning worldwide, most of which were in Asia (Beelen et al., 2015; Ostro, 2016). In eastern USA, Europe and Russia, agricultural emissions make up the largest relative contribution to $PM_{2.5}$ (Lelieveld et al., 2015). Apart from causing the depletion

of the ozone layer, it is one of the main causal agents to the escalation of respiratory diseases among humans. Studies have shown that short, as well as long term exposure of human beings to such hazards could adversely impact human health (Lelieveld et al., 2015). Among the reported increased incidences of respiratory diseases include acute bronchial asthma, chronic bronchitis, chronic obstructive pulmonary disease (COPD) and other acute respiratory infections (Xing et al., 2016). Other frequently used methods for pretreatment and removal of lignocellulosic biomass, for instance, physico-chemical, chemical and biological, are far from satisfactory and yield unsustainable results.

It's evidently clear that the current practice of many nations to get rid of excess agricultural biomass is unhealthy, unsustainable as well as wasteful. Not only that, such practice does not harness the full potential of the biopolymeric components *viz.* lignocellulose (cellulose, hemicellulose and lignin) in the various agricultural biomass. In this context, the study believes that these plant wastes are good sources of renewable plant organic resource (Saini et al., 2015) e.g. carbohydrate polymers (cellulose and hemicellulose) and phenolic polymer (lignin). These renewable organic carbon sources can be broken down into smaller subunits i.e. simple sugars, useful as platform chemicals for manufacturing other functional materials. In fact, cellulose (30–50% of total dry matter) is a glucose polymer formed by the basic building block of glucose-glucose dimers called cellobiose, linked by β–1,4 glycosidic bonds. In contrary, hemicellulose (20–40% of total dry matter) is constructed of relatively shorter polymer chains of highly branched five-carbon (C5) polymer and six-carbon (C6) sugars and finally, lignin (15–25% of total dry matter), a polyphenolic constituent of plants, which make up the largest non-carbohydrate fraction of lignocellulose (Chandel et al., 2018; Ravindran & Jaiswal, 2016). These freely available and renewable sugar polymers make ideal and cost-effective effective carbon source for various applications.

For effective harvest and utilisation of sugars from complex carbohydrates, bioprospecting for exceptional microorganisms producing cellulases capable of 'benignly' degrading such multifaceted plant composite without the liberation of harmful substance, may prove useful and more practical. Not only that, the obtained sugar products can be used as platform chemicals to produce other value-added

products. Specifically, focusing on isolating mesophilic (20−45 ˚C) cellulose and xylanase-producing fungi is a feasibly cleaner as well as more cost-effective approach to make full use of unwanted agricultural biomass. The high growth rate of fungi can effectively enhance metabolism and decomposition of the carbohydrate polymers (cellulose and hemicellulose) as well as phenolic polymers (lignin) into their basic subunits, even at ambient temperatures (Garg, 2016; Hooker et al., 2018; Shirkavand et al., 2016).

The cellulase enzyme system is divided into three main sub-groups: endoglucanases (EG), exoglucanases (cellobiohydrolases, CBH) and β-glucosidase (BGL), which belong to the EC 3.2.1.X class, while xylanase is a single-component enzyme. Cellulases include endo-β-(1, 4)-glucanases (EC 3.2.1.4), Exo--β-(1, 4)-glucanase (EC 3.2.1.91 and β-glucosidase (EC 3.2.1 21) (Kickenweiz et al., 2018; Shewale, 1982). Xylanase (endo-β-1,4-D-xylanohydrolase; EC 3.2.1.8) is an enzyme that catalyse the hydrolysis of β-1,4-D-xylosidic bonds in xylan, the major component of hemicellulose in plant cell walls. Highly prolific fungi producing such enzymes have been reported for the *Trichoderma* and *Aspergillus* species. *Trichoderma asperellum* and *Rhizopus oryzae*, being a traditional bio-control species and food fermenter respectively, have been biotechnologically explored for their enzyme-producing abilities. This quality seems responsible for their exceptional environmental expedience stretching from saprotrophy to biotrophy (Kwon et al., 2014; Wang et al., 2015). The study also believes the synergistic breakdown of agricultural biomass by the aforementioned fungal enzymes can be further enhanced using solid-state fermentation (SSF). The use of SSF is advantageous as the method: can ease enzyme recovery, cost effective, yields high concentrations of products and produces less effluent, thus less polluting (Behera & Ray, 2016). However, this process is presently faced with some limitations, especially in large scale applications, for instance, the build-up of heat, limited oxygen transfer, limited pH control, mass and heat transfer. Others include challenges include the accurate measurement of microbial growth and kinetics (Manan and Webb, 2017). These fungal enzymes synergistically catalyse the complete hydrolysis of plant biomass into their basic sugar components (Behera & Ray, 2016; Ryu & Mandels, 1980) or mineralization to H_2O and CO_2 (Metreveli et al.,

2017) whose products can be used for manufacturing other important compounds (Alrumman, 2016).

1.2 Problem Statement

Considering the unsustainable methods used to dispose or reutilise unwanted agricultural biomass, (Hassan et al., 2018), the high cost of lignocellulolytic enzymes due to the lack of sufficient and prolific fungal producers of cellulases and xylanases (da Silva et al., 2018), alongside limitations in current SSF technique to obtain large quantities of fungal enzymes, the quest for greener and cleaner alternative strategies to alleviate such issue merits global attention. Newly developed strategies should enable mankind to fully harvest and utilise the renewable organic carbon locked within the various lignocellulosic agricultural biomass worldwide. While lignocellulosic biomass is a renewable and abundant resource with great potential for bioconversion to value-added by-products, such an endeavour remained economically unfavourable due to the prohibitively high production costs of commercial cellulases and hemicellulases, essential for converting lignocellulosic biomass into valuable products such as fermentable sugars, biofuel etc., as well as the lack of robust cellulolytic and xylanolytic microbes (especially fungi) to produce these efficient enzymes (Saritha et al., 2015).

Herein, the study proposes a strategy to bioprospect for cellulase and xylanase-producing mesophilic fungal strains that can efficaciously degrade the carbohydrate polymers (cellulose and xylan) and subsequently permit the harvesting of valuable sugar components. This study was focused on reducing the high production cost of producing fungal cellulase and xylanase by capitalizing on cheap and abundant renewable materials i.e. oil palm frond leaves (OPFL) (without the petioles) biomass as the substrate for SSF to cultivate the new isolated fungal strains to yield high quantities of cellulases. OPFL was chosen as Malaysia is the second largest producer of oil palm in the world, constantly generating large masses of oil palm wastes from pruning, replanting and milling activities (Awalludin et al., 2015; Loh, 2017).

The strategy of fermenting OPFL *via* the SSF technique may prove useful in maximising the usefulness of the biomass by harvesting their sugar components and for cultivation of beneficial fungi for their enzyme cocktail, as well as alleviating presence of surplus biomass in the environment. It is hypothesised that the use of fungal cellulase and xylanase may be a cleaner and more efficient means to degrade OPFL without contaminating the environment, as such enzymes catalyse more specifically than chemical processes (Souza, 2014). Moreover, the use of OPFL as the sole carbon source to cultivate novel fungal strains isolates to produce three cellulase enzymes (endoglucanase, exoglucanase, β-glucosidase) and xylanase under SSF is not available**.** This study also intends to develop an optimised protocol for the production of these enzymes using the abundantly generated OPFL waste, which could be employed in its cleanup at the oil palm plantations and the use of its basic sugar products for production of value-added products such as bioethanol and compost. Moreover, ultrasonication (acoustic bombardment) was chosen for the OPFL pretreatment as it is a cleaner method to increase the surface area of cellulose and xylan components in the cell wall. This was to allow effective binding and hydrolysis of the fungal cellulases and xylanases, thereby yielding a more effective degradation of the biomaterial into valuable sugar products.

1.3 Aim of Research

The research was aimed in using the cellulase and xylanase-producing fungal isolates for effective production of cellulase and xylanase using OPFL as a cost-effective fermentative substrate and in turn be degraded to its basic sugar subunits.

1.4 Research Objectives

To achieve the aim the following objectives were set:

1. To isolate and identify cellulase and xylanase-producing fungi.

2. To optimise parameters for cellulase and xylanase production under SSF for *in situ* hydrolysis of OPFL.

3. To characterise the physicochemical properties of the two fungal enzyme cocktails produced under SSF.

4. To statistically optimise the production of cellulase and xylanase for the enzymatic saccharification of OPFL.

1.5 Scopes of Study

Several strains of fungi were isolated from a decaying oil palm empty fruit bunch and qualitatively screened for efficient cellulase and xylanase production. Screening was carried out on carboxymethyl cellulose (CMC)-agar and xylan-agar plates. Two fungal strains were selected for further identification through morphological, molecular (18S rRNA sequencing) and biochemical methods (BIOLOG, Gen II), and was subsequently identified as *Trichoderma asperellum* UC1 and *Rhizopus oryzae* UC2. Production and extraction of cellulase and xylanase by the fungal strains using ground OPFL as substrate was done under SSF. This was followed by assay for endoglucanase (CMCase), exoglucanase (FPase), β-glucosidase and xylanase activity at 24 h interval over 7 days.

Next, chemical analysis of OPFL was done to establish the chemical composition of the major constituents of the plant. This was followed by optimisation of SSF parameters (fermentation temperature, pH, inoculum size, initial moisture content) for improved production of cellulase and xylanase using the one-variable-at-a-time (OVAT) method for the two isolates. Subsequently, *in situ* saccharification of raw OPFL under SSF was done using the two fungal strains *Trichoderma asperellum* UC1 and *Rhizopus oryzae* UC2, individually. The next step involved the determination of total reducing sugar and individual monosaccharide sugars using the 3, 5-dinitrosalicylic acid (DNS) and high-performance liquid chromatography (HPLC) methods.

The crude enzyme cocktails extracted from the SSF batches were then subjected to physicochemical characterisation of the enzymes of interest. The tests performed were to ascertain the effects of pH and temperature on enzyme stability and the effects of pH and temperature on activities of enzymes. Qualitative proteome analysis and determination of total soluble protein in the crude enzyme cocktails were undertaken using sodium dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Lowry-Folin method, respectively. Furthermore, composition and structural analysis of OPFL biomass before and after SSF using field emission scanning electron microscopy (FESEM) was done to assess the morphological changes due to depolymerization of the structural components. This helped with the subsequent determination of hydrolysis efficiency of OPFL by the enzymes after *in situ* saccharification using both fungal isolates.

Finally, statistical optimisation of SSF parameters (fermentation temperature, pH, inoculum size, initial moisture content) to obtain polynomial models that could reliably predict the best SSF conditions was done. The response measured was for optimum activity of the fungal enzymes, and this part of the study aims to observe the effects of independent and dependent variables on the measured response. The Response surface methodology software was used in the optimisation work. Based on the initial findings, the statistical optimisation was done to specifically improve CMCase, FPase and xylanase production for strain *Trichoderma asperellum* UC1, while β-glucosidase activity was optimised for strain *Rhizopus oryzae* UC2 only. Afterwards, synergistic enzymatic hydrolysis of ultra-sonicated OPFL using crude enzyme cocktail mixture from strain UC1 and UC2 was undertaken. This was followed by assessment of hydrolysed total reducing sugar and individual monosaccharide sugars using the DNS and HPLC methods, respectively. Biotechnological applications of the crude enzymes produced by the isolated fungi in the clarification of fresh orange juice and in dough rising were determined.

1.6 Significance of Study

The SSF protocol developed in this study is a cleaner and more efficient means to remove OPFL wastes that are abundantly generated in all oil palm plantations in Malaysia. The strategy highlighted here can also complement existing strategies to utilise oil palm wastes at large. Most importantly, it offers a more sustainable way of sustaining a greener way of life while converting “Wastes into Wealth”.

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