Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1096 - 1106
Received 05 March 2021; Accepted 01 April 2021.

Treatment of Palm Oil Mill Effluent by Thermotolerant Cellulase and Xylanase - Producing Fungi

Hatsalinda Binma-Ae[1], Haleemah Saek[2], Darunee Kayeeyu[3]

[1,2,3]Department of Microbiology, Faculty of Science Technology and Agriculture, Yala Rajabhat University, Muang, Yala, Thailand
Email: [1]haslinda.b@yru.ac.th

ABSTRACT

Fungi strain,CD, HD, GD, RH, RA, RS andPD, whichproduce cellulase and xylanase for palm oil mill effluent treatment were isolated from dungs and agriculture waste. Studies on the propertiesof thermotolerantfungi at room temperature to 60°C was evaluated. The result showed that all isolated strains were found to be thermotolerant fungi that have a growth temperature at 50°C and require optimum temperature as 45°C. Strain CD1, HD1, RH1, RA2, RS1 andPD1were classified as *Aspergillus* sp. while GD1was classified as *Scytalidium* sp. The enzyme activity of thermotolerant fungi revealed that PD1 produced the highest activities of carboxymethyl cellulase (CMCase) (678.14U/ml) and xylanase (2369.12 U/ml). The activities of carboxymethyl cellulase (CMCase) and xylanase exhibited 80.14 and 244.54U/ml of protein, respectively. This study also demonstrated the effect of treatment efficiency in palm oil mill effluent. The result showed that *Aspergillus* sp. PD1 is the best strain that have the ability to reduce BOD and COD value of 31.02 % and 49.05% after the treatment, respectively. Therefore, the thermotolerant enzyme-producing fungi are potentially useful to remove organic in palm oil mill effluenttreatment.

Keywords
Thermotolerant Fungi, Cellulase, Xylanase, Organic Removal, Palm Oil Mill Effluent

INTRODUCTION

The world's markets of oil palmin over the past 4 years has grown by 5.1 % are used as raw materials for foodstuffs and energy (biodiesel) industryand has mainly been cultivated in Indonesia, Malaysia and Thailand [1]. In Thailand, 85 % of oil palm plantations and crude palm oil extraction mills are in the south of the country. The dramatically increase in demand for the plantation area was around 10% with an average annual growth rate of 11 % from 1981 to 2000 and 9 % from 2001 to 2010 [2]. Nowadays,Thai government has set out the Oil Palm and Oil Palm Industries Development Strategy 2015-2026 to expand the oil palm plantation areas by another 3 million rai which increase yields per rai from 3.2 tonnes to 3.5 tonnes and raises the proportion of oil in the fresh palm to 20% in Thailandincluded three southern border provinces (Pattani,Yala and Narathiwat) [1]. Standard or wet process is commonly employed for palm oil extraction in the palm oil mill that resulted in the generation of large quantity of a liquid waste, average 2.5 tonnes of palm oil mill effluent (POME) are generated for every ton of crude palm oil production [3]. POME had acidic pH (pH 4.2-4.5) and contained high organic matter (45.2-143.9g/L COD), solids (17-71.5g/L total solids (TS), 8.5-34.2g/L suspended solids (SS)), oil&grease (2.3-10.6g/L) but low nitrogen (0.083-0.92 g/L) [4,5,6,7]. These characteristics of POME arevery high of organic matter and cause serious environmentalproblems. POME has generally been treated by chemical, physical and biological. Chemical method using coagulation, precipitation and ion exchange, which was foundmore effective than the biological method using bacteria, yeast and fugal. Biological treatment of POME to reduce the wastewater strength is one potential method that can be adopted to alleviate the pollution problem faced by both the palm oil industry and eco-friendly. Recently many researchers have resorted to the use of microorganisms to the treat POME, especially,fungi. The fungi have the ability to convert dissolved and

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1096 - 1106
Received 05 March 2021; Accepted 01 April 2021.

suspended organic matter into a mycelium that is high in protein contentwhich can be readily recovered by simple filtration or screening **[**8**].** There are any fungi, such as *Trichodermviride*, *T. harzianum*, *T. virens*,*Myceliophtora thermophila*,*Geotricum candidium*, *Penicillium digitatum*, *P. italicum*,*Aspergillus* sp, *A. niger*, *A. terreus*, *Mucor racemosus*, *Rhizopus stolonifera*were used for treatment of POME **[**6,8,9,10**].** In addition, to mitigation the environmental problem caused by POME, the organic matter would be better utilized for the production of some valuable product such as enzyme cellulase and xylanase **[**11**].** For instance, *Phanerochaete chrysosporium*, *Aspergillus* sp.,*A. terreus*, *T. reesei*, *Penicillium* sp. and *Rhizopus* sp. presence cellulase activity **[**12,13**].** Otherwise, in a wet process, fresh fruit bunch was sterilized **(**at 120-130°C, 40 lb/in^2 for 45 min**) [**14**]** and the freshly discharged POME from the factory has a temperature of 85-90°C **[**15**].** For this reason, this study focuses on the fungi that able to survival and growth in wide and variedextreme environments**.** The fungi that grow only within the temperature range of a minimum below 20°C to maximum near 50°C are considered to be thermotolerantfungi **[**16**].** This work aims to investigate the aerobic treatment of the POME using the thermotolerant fungi for production of cellulase and xylanase to remove organic matter**.**

MATERIALS AND METHODS

Isolation, identification and maintenance of thermotolerant *fungi*

The thermotolerant fungal strains wereisolated from cow dung **(**CD**)**, horse dung **(**HD**)**, goat dung **(**GD**)**, rice husk **(**RH**)**,rice husk ashes **(**RA**)**, rubber sawdust **(**RS**)** and plant debris **(**PD**)** in three southern border provinces of Yala, Pattani,and Narathiwat**.** Yeast starch agar **(**YpSs**).** The YpSs medium containing0**.**4 **%** Yeast extract, 0**.**1 **%** K_2HPO_4, 0**.**05 **%** $MgSO_4 \cdot 7H_2O$, 1**.**5 **%** starch and 2**%** agar **[**16, 17, 18**].**The incubation temperature of fungi for primary isolation was 45°C and monitoredfungal growth for 5 days**.** Single colonies on the plates were isolated and purified by transferring in several times**.** The purity of the isolated fungus was checked by the pattern of fungal growth on the plateat optimum temperature for fungal growth and confirmed by microscopic examination of the cultureat 40×magnification using light microscope**.** Fungi were identified on the basis of their colonial and morphological characteristics **[** 16,18**].** After ensuring the purity, fungi were subcultured onYpSs agarslants and allowed to grow for 5-7 days at 45°C and subsequently stored at 4°C as stockcultures**.**

Optimum fungal growth temperature

The isolated fungiwerecultured on YpSs agarat room temperature **(**28±2**)**, 45, 50, 55, 60 and 65°C for 7 days and measured the growth zone of colony daily**.**

Qualitative and quantitative assay of cellulaseand xylanase

Agar plate assay

A modified substrate agar assay was used to assay carboxymethyl cellulase activity **[**19**]** and xylanase**[**17**]** qualitatively with the clear zone of Congo red dye**.** The purified colonies were selected and grown on CMC agar and xylan agar**.** The CMC agar medium was having the following compositions**:** carboxymethyl cellulose sodium salt, 1g/l; KH_2PO_4, 2g/l; NH_4NO_3, 2**.**0g/l; $MgSO \cdot 7H_2O_4$, 0**.**2g/l; yeast extract, 0**.**2g/land agar, **15**g/l**.** The xylan agar medium has the following compositions**:** Birchwood xylan, 2g/l; $MgSO_4 \cdot 7H_2O$, **0.2**g/l;KH_2PO_4, 0**.**4g/l;KCl, 0**.**2g/l;NH_4NO_3, 5g/l;$FeSO_4 \cdot 7HO_2$, 0**.**01g/l;$ZnSO_4$,0**.**01g/l;$MnSO_4$,0**.**01g/l; and agar, 15g/l**.** After plating, plates were incubated at their optimum temperature for 5 days**.**Then, the plates were

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1096 - 1106
Received 05 March 2021; Accepted 01 April 2021.

stained with 1 **%** Congo red for 10-15minfollowed bydestaining with 1 M NaCl solution to remove unbound dye, and then observed of yellow zone of hydrolysis around the colonies**.** The cellulose hydrolytic activities were recorded by measuring zone of diameter hydrolysis in millimeters**.**

Carboxymethyl cellulase (CMCase) and xylanase activity

All the test thermotolerant fungi were screened for production of carboxymethyl cellulase **(**CMCase**)**and xylanase on YpSs medium at the optimum fungal growth temperature**.** Spore suspension of each strain was prepared by adding 10 ml of 0**.**1**%** Tween 80 onto YpSs slant of 5 days old culture of theeach strains**.** The concentration was adjusted to reach 2**.**4 X 10^6 spore/ml in YpSs under shaking condition that were constant at 200 rpm for 5 days at their optimum temperature**.** Samples were taken every 24 h to determine for CMCase activity **[**20**]**, xylanase activity **[**21**]** and protein measurement **[**22**].** The isolated fungus which giving the highest CMCase and xylanase activity efficiency were selected for further studies**.**

Determination of palm oil mill effluent (POME) characteristics

The POME was taken from Cooperative estate bacho, in Mueang district, Narathiwat province, Thailand and kept at -20°C until used**.** The samples physicochemical parameters, such ascolor, pH,biochemical oxygen demand **(**BOD**)**, chemical oxygen demand **(**COD**)**, total solids **(**TS**)**, suspended solids **(**SS**)**, oil&grease **[**23**]**, totalnitrogen **(**by total Kjeldahl nitrogen method**)** and total phosphorus **(**by DIN 38402 A51 method**) [**24**]** were determined**.**

Treatment of thermotolerant CMCase and xylanase-producing fungi in POME

Spore suspension were prepared by adding 10ml of 0**.**1**%** Tween 80 onto YpSs slant of 5 days old culture of the isolated fungus at the optimum fungal growth temperature**.** The concentration was adjusted to reach 2**.**4 x 10^6 spore/ml**.** Starter culture **(**10**%** v/v**)** of each fungal isolate was inoculated into 500 ml Erlenmeyer flasks containing 100 ml each of aseptic POME under shake-flask condition **(**200 rpm**)** for 6 days at their optimum temperature**.** Samples were taken every 24h to determine thecolor, pH, BOD, COD, SS, TS and oil&grease**.**

RESULTS AND DISCUSSION

Isolation of thermotolerant fungi

Thermotolerant fungi from cow dung **(**CD**)**, horse dung **(**HD**)**, goat dung **(** GD**)**,rice husk **(**RH**)**, rice husk ashes **(**RA**)**, rubber sawdust **(**RS**)** and plant debris **(**PD**)** on YpSs at 45°C for 5 days were used in this study**.** In this experiment, **Table 1**shows the distribution of thermotolerant fungi of different habitats**.** A total of 20 collected samples were thermotolerant fungi isolated from dungs and agriculture waste including strainCD **(**2**)**, HD **(**2**)**, GD **(**2**)**, RH **(**3**)**, RA **(**3**)**, RS **(**6**)** and PD **(**2**).** The thermotolerant fungi have a growth temperature range of a minimum below 20°C to maximum near 50°C **[** 16**].** The various strains of thermotolerant fungi were able to grow in different habitats**.** The 68 thermophilic and thermotolerant fungi were isolated from various heated environments in which 15from stored seeds, 12 from soils, 11 from compost, 10 from wheat straw, 9 from wood chips, 5 from litter, and 3 from decaying organic matter and 3 from bird nests at 45°C for 2-7 days **[**17**].** A total of 13 and 11 fungal species were detected in the compost and air sampler at 1**.**5 m height in three large industrial composting facilities at 50°C for 7 days **[**25**].** In addition, the several of environments is an outstanding habitat for growth of thermotolerant fungi such as from soil, decaying organic matter and sugarcane piles**(27** of

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1096 - 1106
Received 05 March 2021; Accepted 01 April 2021.

fungal strains)at 45°C [26],from composting soils (9 of fungal strains) at 50°C for 3 days [27],from composting soils of local habitat in India (14 of fungal strains) at 45-50°C for 7-10 days [28] and from decaying vegetable and soil sample (2of fungal strains)at 45-55°C [29].

Optimum temperature for fungal growth

Strain CD1, CD2, HD1, HD2, GD1, GD2, RH1, RH2, RH3, RA1, RA2, RA3, RS1,RS2, RS3, RS4, RS5, RS6,PD1 and PD2werecultured on YpSs at room temperature (28±2), 45, 50, 55, 60 and 65°C for 7 days and measured growth zone of colonydaily and record the qualitatively (+, -) **(Table 2).** The result in **Table 2**showed that allstrains were found to be thermotolerant fungi that have a growth temperature at 50°C and require the optimum temperature as 45°C**.** A total of 20 collected samples werethermotolerant fungi becauseable to grow at 28°C and 45°Cfollowing the definition described by Cooney and Emerson (1964) [16]. Therefore, the optimum temperature for cultivation of all strains was 45°C that this temperature was used for further studies**.**

Table 1 Distribution of thermotolerant fungi and qualitative assay of carboxymethyl cellulase and xylanase activity in different samples

No.	Sample	No. of fungi isolated	Strain	The average diameter ofhydrolysis zone (cm)		The quantitative assay (U/mL)	
				cellulase	xylanase	CMCase	xylanase
1.	Cow dung (CD)	2	CD1	3.20 ± 0.18^{C}	4.65 ± 0.45^{C}	567.13 ± 0.23^{C}	1566.43 ± 0.13^{C}
			CD2	-	-	-	-
2.	Horse dung (HD)	2	HD1	3.85 ± 0.15^{B}	6.05 ± 0.13^{B}	650.43 ± 0.56^{B}	1969.12 ± 0.24^{B}
			HD2	-	-	-	-
3.	Goat dung (GD)	2	GD1	3.32 ± 0.16^{C}	4.81 ± 0.22^{C}	577.11 ± 0.18^{C}	1697.86 ± 0.21^{C}
			GD2	-	-	-	-
4.	Rice husk (RH)	3	RH1	3.52 ± 0.35^{C}	5.03 ± 0.05^{C}	597.33 ± 0.12^{C}	1807.59 ± 0.21^{C}
			RH2	-	-	-	-
			RH3	-	-	-	-
5.	Rice husk ashes (RA)	3	RA1	-	-	-	-
			RA2	2.45 ± 0.03^{D}	3.55 ± 0.17^{D}	497.56 ± 0.13^{D}	1344.58 ± 0.27^{D}
			RA3	-	-	-	-
6.	Rubber sawdust (RS)	6	RS1	3.45 ± 0.52^{C}	4.93 ± 0.02^{C}	589.27 ± 0.23^{C}	1797.86 ± 0.05^{C}
			RS2	-	-	-	-
			RS3	-	-	-	-
			RS4	-	-	-	-
			RS5	-	-	-	-
			RS6	-	-	-	-
7.	Plant debris (PD)	2	PD1	4.93 ± 0.23^{A}	7.93 ± 0.12^{A}	678.14 ± 0.45^{A}	2369.12 ± 0.51^{A}
			PD2	-	-	-	-

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1096 - 1106
Received 05 March 2021; Accepted 01 April 2021.

The data in columns was presented as mean values with standard deviations, with not significance threshold $p>0.05$

- = no activityof enzyme

Table 2Thermotolerant fungi and optimum growth temperature of20 fungal strains on YpSs agarplatesincubated for 7 days.

No.	Strains	Temperature (°C)					
		Room temperature (28±2)	45	50	55	60	65
1.	CD1	++	+++	+	-	-	-
2.	CD2	++	+++	+	-	-	-
3.	HD1	++	+++	+	-	-	-
4.	HD2	++	+++	+	-	-	-
5.	GD1	++	+++	+	-	-	-
6.	GD2	++	+++	+	-	-	-
7.	RH1	++	+++	+	-	-	-
8.	RH2	++	+++	+	-	-	-
9.	RH3	++	+++	+	-	-	-
10.	RA1	++	+++	+	-	-	-
11.	RA2	++	++	+	-	-	-
12.	RA3	++	+++	+	-	-	-
13.	RS1	++	+++	+	-	-	-
14.	RS2	++	+++	+	-	-	-
15.	RS3	++	+++	+	-	-	-
16.	RS4	++	+++	+	-	-	-
17.	RS5	++	+++	+	-	-	-
18.	RS6	++	+++	+	-	-	-
19.	PD1	++	+++	+	-	-	-
20.	PD2	++	+++	+	-	-	-

Remarks +++ = very good growth **(**> 4 cm**)** ++ = medium growth **(**2**.**1-4 cm**)**
+ = little growth **(**0**.**5-2 cm**)** - = no growth

Production of* CMCase and xylanase *by the isolatedfungal strains

The aim of this study is to test CMCase and xylanase produced by thermotolerant fungi, subsequently characterize to find the interesting properties for potential application ofpalm oil mill effluent **(**POME**)** treatment in further experiment**.** Since the thermotolerant fungi may be used as viable the organic matter **(**75**.**2-96**.**2g/L COD**)** in POME that could causeenvironmental problems **[**4**].** Lignocellulosic biomass which is produced from the oil palm industries includes oil palm trunks **(**OPT**)**, oil palm fronds **(**OPF**)**, palm shells, palm pressed fibers **(**PPF**)**, empty fruit bunches **(**EFB**)** and POME**.** Lignocellulosic biomass refers to plant material that is mainly composed of 45-55 **%** cellulose, 25-35**%** hemicellulose and20-30**%** lignin **[**30**].** All strains of thermotolerant fungiwere analyzed the qualitative and quantitative of extracellular cellulase and xylanase using enzyme diffusion technique**.** The modified substrate agar plate assay was used to determine the qualitative of enzyme activity by observing clear zone of Congo red dye**. Table 1** shows the diameter ofhydrolysis zone on enzyme production from purified colonies of CD1, CD2, HD1, HD2, GD1, GD2, RH1, RH2, RH3, RA1, RA2, RA3, RS1, RS2, RS3, RS4, RS5, RS6, PD1 and PD2fungal strains**.** Strains CD1, HD1, GD1, RH1, RA2, RS1 and PD1showed the

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1096 - 1106
Received 05 March 2021; Accepted 01 April 2021.

activity of cellulase and xylanase, these productions being observed zones of enzymatic hydrolysis**.**The maximum diameter of the zoneswas 4**.**93±0**.**23cm on CMC agar plates,and 7**.**93±0**.**12 cm on xylan agar plates **(**PD1**) (Figure 1A)** while2**.**45±0**.**03 mm and 3**.**55±0**.**17 mm were minimum diameter of the zones presented on CMC agar, and xylan agar plates **(**RA2**)**, respectively**.** In contrast,strainsCD2, HD2, GD2, RH2, RH3, RA1, RA3, RS2, RS3, RS4, RS5, RS6 and PD2 had nohydrolysis zone of cellulase and xylanase **(Figure1B).** Out of these 22 test fungi from 68 isolated thermotolerant fungi that collected samples of soils, stored seeds, and decomposing organic matter in India showed the xylanase activity by forming the cleared zone **(**4-29 mm**) [**17**].**

The results of quantitative evaluation of CMCaseand xylanase activity on YpSs medium at the optimum fungal growth temperature are showed in **Table 1.** The results exhibited that seven thermotolerant fungalcan produce CMCaseand xylanase including CD1, HD1, GD1, RH1, RA2, RS1 and PD1**.** CMCase and xylanase productions ranging from 497**.**56 to 678**.**14 and 1344**.**38 to 2369**.**12 U/mL, respectively, were observed**.** The strain PD1 produced the highest CMCase and xylanase activity **(**678**.**14 and 2369**.**12 U/mL**)** and specific CMCase activity **(**80**.**14 and 244**.**54 U/mg protein**)** at 6 days cultivation **(Figure 2).** In addition, HD1 produced the high CMCase and xylanase activity with 650**.**43 and 1969**.**12 U/mL and specific CMCase activity with 72**.**14 and 226**.**63 U/mg protein at 6 days cultivation**.** Conversely, strains RA2 that isolated from rubber ashes produced the lowerCMCase and xylanase activity **(**497**.**56 and 1344**.**38 U/mL and specific CMCase activity with 61**.**17 and 172**.**58 U/mg protein**)** at 6 days cultivation**.** Since the temperature relationship affects the growth of fungal isolates **[**17**].** As a result, the cultivation at room temperature **(**28±2**)** and 45°C may be unviable optimum growth for enzyme production **(Table 2).** Therefore, the strain PD1 and HD1cultivation at 45°Cwas the optimum condition for production of CMCase and xylanaseand it also used for further studies**.**

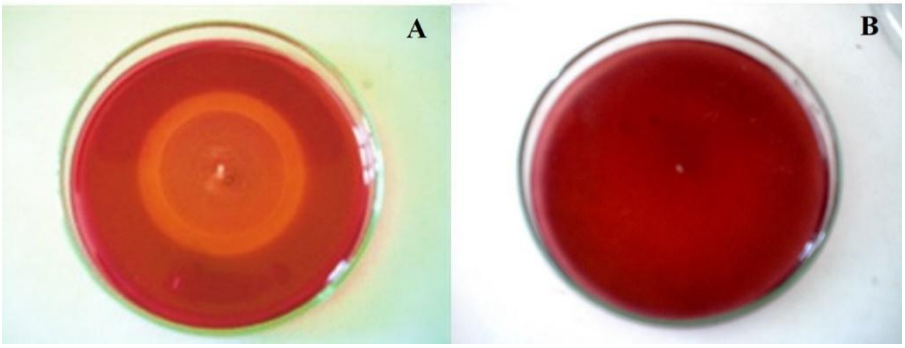

Figure 1 Plate presencehydrolysis zone of crude enzymexylanasein substrate-agar: **plate** (**A**) **presencethe highest xylan hydrolysis of PD1isolated from Plant debrisand** (**B**) **absence thexylan hydrolysis of PD2isolated from Plant debris**.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1096 - 1106
Received 05 March 2021; Accepted 01 April 2021.

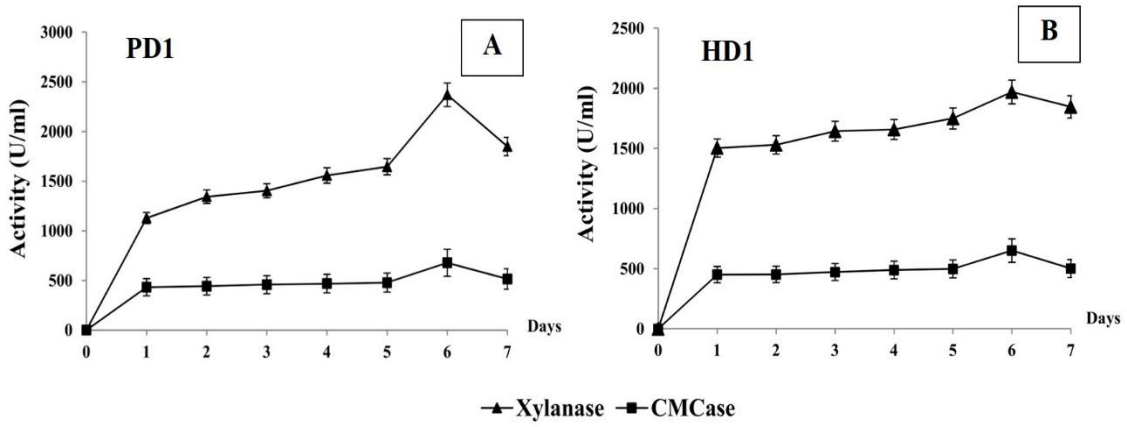

Figure 2 Comparison of enzymes production in YpSs medium by strain PD1 (A) and HD1 (B).

Morphological characterization of thermotolerant *cellulase and xylanase-producing fungi*

The CD1, HD1, GD1, RH1, RA2, RS1 and PD1 isolated thermotolerant fungi that produced *cellulase and xylanase* were identified on the basis of their colonial and morphological characteristics**. [**16,17,31,32**].** The result shows the CD1, HD1, RH1, RA2, RS1 and PD1 were identified as *Aspergillus* sp**. (Figure 3)** while GD1 was *Scytalidium* sp**.** There are several thermotolerant*Aspergillus* sp**.** in the different habitats are grown in the range of26-48°C such as *A. carneus, A. flavus, A. fumigatus, A. niger, A. terreus, A. ficuum*and *A. versicolor***[**31,33,34,35,36,37**].** Moreover, some strains of *Aspergillus* sp**.** were found to produce xylanase in wheat bran contain *A. fumigatus* and*A. terreus*at 45°C for 7 days **[**17**].** The thermotolerant *A. terreus*was cultivated on wheat bran, solka floc and pectin showed the xylanase activity of 171, 33 and 0**.**2, respectively **[**38**].** The *Scytalidium thermophilum* which refer to a synonym of *Humicola insolens* collected from the dung of different herbivore animal; cow and sheep dung were reported to associate with 20**%** of frequency and 3**.**2**%** abundance of *Scytalidium* sp**.** in this sample **[**36**].** *Scytalidium* sp**.**GD1in this study was obtained from dung that similar result withprevious studies **[**36**].** In addition, this strain was able to grow and produce xylanase at 45°C for 7 days in wheat bran as a carbon source **[**17**].**

In this case, *Aspergillus* sp**.** PD1 and *Aspergillus* sp**.** HD1 gave the high CMCase and xylanase production at 45°C**.** Thus, the strains *Aspergillus* sp**.** PD1 and *Aspergillus* sp**.** HD1were chosen for the next experiments**.**

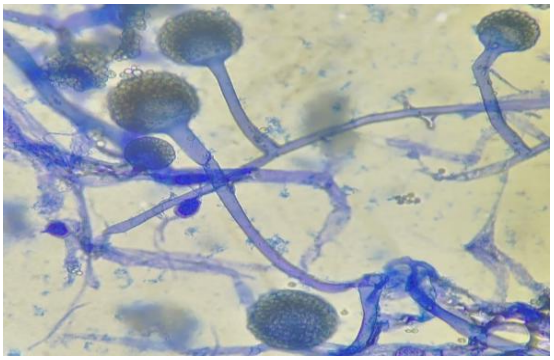

Figure 3Microscopic image showing morphological of thermotolerant fungi:***Aspergillus*sp. PD1**

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1096 - 1106
Received 05 March 2021; Accepted 01 April 2021.

Determination of POME Characteristics

The characteristics of the POME taken from a palm oil mill were determined **(Table 3).** pH value of POME was 4**.**52which is the typical acidic pH for POME**.** The POME contains the average values of 14**.**35g/l BOD, 42**.**33g/l COD, 25**.**4g/l total solids, 10**.**6g/l suspended solids, 4**.**7g/l oil&grease,0**.**52g/l total nitrogen and 0**.**15g/l total phosphorus**.** There values were similar to those from other remarks as reported in **Table 3 [** 4,6,7**].** Thedifference in POME characteristics in various studies was due to the difference in the quality of raw material, the extraction process, the efficiency of the process and the sampling time **[**39,40**].**

Table 3Characteristics of palm oil mill effluent from Cooperative estate bacho in comparison to other palm oil mills.

Parameter	This study	1	2	3
Color	Brown	Brown	-	Brown
pH	4**.**52	4**.**2-4**.**5	4**.**2	4**.**5
BOD **(**g/l**)**	14**.**35	-	16**.**3	-
COD **(**g/l**)**	42**.**33	75**.**2-96**.**3	105	143**.**9
Total solids **(**g/l**)**	25**.**4	35-42	18**.**7	71**.**5
Suspended Solids **(**g/l**)**	10**.**6	8**.**5-12	-	34**.**2
Oil & grease **(**g/l**)**	4**.**7	8**.**3-10**.**6	7**.**5	10
Total nitrogen **(**g/l**)**	0**.**52	0**.**8-0**.**9	1**.**04	1**.**2
Total phosphorus **(**g/l**)**	0**.**15	0**.**09-0**.**12	-	0**.**5

Remark; 1**:** O-Thong *et al***. [**4**]**,2**:** Njoku*et al***. [**6**]**, 3**:** Prasertsan andBinmaeil **[**7**]**

- **:** Not Determined

Comparison on treatment efficiency of thermotolerant CMCase and xylanase - producing fungi in POME

The POME was treated by *Aspergillus* sp**.** PD1 and *Aspergillus* sp**.** HD1at their optimumtemperature of 45°C undershaked flask condition **(**200 rpm**)** for 6 days**.** *Aspergillus* sp**.** PD1 gave the highest BOD reduction **(**31**.**02**%)**, COD reduction **(**49**.**05**%)**,total solids reduction **(**28**.**13**%)** with less amount of sediment after 6 days of cultivation **(Figure 4).** In addition, *Aspergillus* sp**.** PD1 also produced the highest activities of carboxymethyl cellulase **(**CMCase**) (**623**.**25U/ml**)** and xylanase **(**2103**.**15U/ml**)** while *Aspergillus* sp**.** HD1 gave lower activitywith 580**.**19U/ml and 1723**.**86U/ml, respectively**.** Consequently, the *Aspergillus* sp**.** PD1 was thermotolerant fungi producing CMCase and xylanase property could be used to treat POME**.** A higher amount of the mycelial growth, enzyme and biopolymer production by ***R. oryzae*** ST29cultivated in POME, complemented with 0**.**025**%** fertilizer and the initial pH of 4**.**5 at 45°C for 4 dayswere found**.** This resulted also showed treatmentefficiency of 80**%** COD removal and 54**.**1 mg polymer/gbiomass **(**9**.**77g/L**)** in POME **[**7**].** To improvethe treatment efficiency in POME by *Aspergillus* sp**.** PD1 should be optimized conditions such as POME concentration on grow, nitrogen source and initial pH on growth**.**

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 1096 - 1106
Received 05 March 2021; Accepted 01 April 2021.

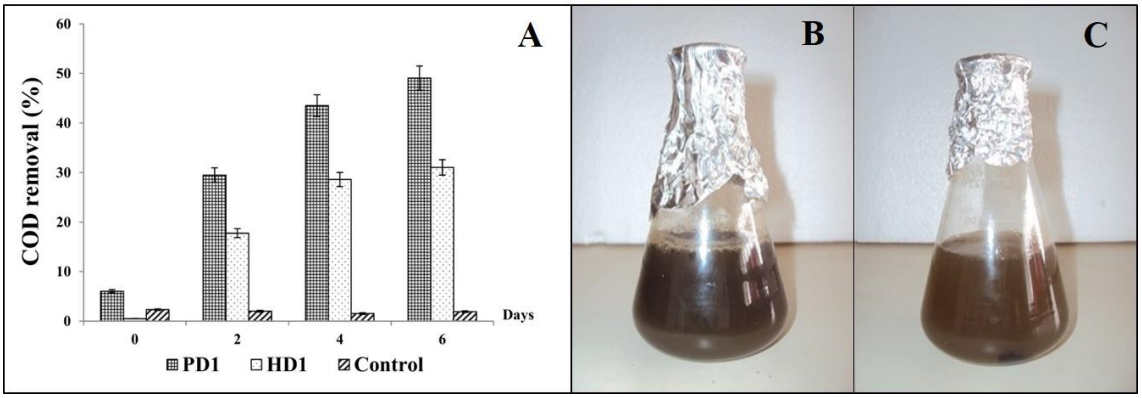

Figure 4Comparison of (**A**) **COD removal from*Aspergillus* sp**. **PD1 and *Aspergillus* sp**. **HD1and comparison of** (**B**) **before treatment and** (**C**) **after treatment from enzyme-producing fungi *Aspergillus* sp**. **PD1 cultivation in palm oil mill effluent on a shaker** (**200 rpm**) **at 45°C for 6 days**.

CONCLUSIONS

The twenty thermotolerant fungal strains were isolated fromcow dung **(**CD**)**, horse dung **(**HD**)**, goat dung **(**GD**)**, rice husk **(**RH**)**, rice husk ashes **(**RA**)**, rubber sawdust **(**RS**)** and plant debris **(**PD**).** All isolated strains have the optimum temperature at 45°C and maximum growth at 50°C**.** The isolates of CD1, HD1, RH1, RA2, RS1 andPD1 were classified as *Aspergillus* sp**.** while GD1 was classified as *Scytalidium* sp**.** The CD1, HD1, GD1, RH1, RA2, RS1 and PD1 were tested for their enzyme activities**.***Aspergillus* sp**.**PD1showed the activities of carboxymethylcellulase **(**CMCase**) (**6**.**78**.**14U/ml**)** andxylanase **(**2369**.**12U/ml**)** outstanding at 45°C follow by *Aspergillus* sp**.** HD1**.** Therefore, the strains *Aspergillus* sp**.**PD1 and *Aspergillus* sp**.** HD1 cultivation at 45°C was the condition of CMCase and xylanase production and used for POME treatment studies**.** The optimum temperaturesused for treatment of POMEand CMCase/xylanase produced by the *Aspergillus* sp**.** PD1 found49**.**05**%** COD removalwas achieved**.**

Acknowledgements
Thank theYala Rajabhat University, Thailand, for the funding support for this study**.**

REFERENCES

[1] W Petchseechoung**.** Thailand industry outlook 2017*:* Oil palm industry**.** *Krungsri Research.* 2017**. 19**, 1-8**.**

[2] J Dallinger**.** Oil palm expansion in South East Asia*:* trends and implications for local communities and indigenous peoples ***(***Online***).*** Available at*:* http*://*www**.**forestpeoples**.**org/sites/fpp/files/publication/2011/11/chapter-1-oil-palm-development-thailand-economic-social-andenvironmentalconsiderations**.**Pdf accessed December 2020**.**

[3] YS Madaki and L Seng**.** Palm oil mill effluent ***(***POME***)*** from Malaysia palm oil mills*:* waste or resource**.** *IJSET***.** 2013**. 2**, 1138-1155**.**

[4] S O-Thong, PPrasertsan, N Intrasungkha, S Dhamwichukorn and NKÅ Birkeland**.** Optimization of simultaneous thermophilic fermentative hydrogen production and COD reduction from palm oil mill efuentby *Thermoanaerobacterium*-rich sludge**.** *Int. J. Hydrogen*

Energy. 2008. **33**, 1221–1231.

[5] C Mamimin, P Thongdumyu, A Hniman, P Prasertsan, T Imai, S O-Thong. Simultaneous thermophilichydrogen production and phenol removal from palm oil mill effluent by Thermoanaerobacterium-rich sludge. *Int. J. Hydrogen Energy.* 2012. **37**, 15598–15606.

[6] I Njoku, P N and A O. K. Use of fungi in bioremediation of palm oil mill effluent *(*POME*)*. *IJOART*. 2014. **3**, 1-6.

[7] P Prasertsan and H Binmaeil. Treatment of palm oil mill effluent by thermotolerant polymer-producing fungi. *J. Water Environ.Technol.* 2018. **16**, 127–137.

[8] MIA Karim and AQA Kamil. Biological treatment of palm mill effluent using *Trichoderma virid.Biol.Wastes.* 1989. **27**, 143-152.

[9] S Vikineswary, AJ Kthubutheen and AA Ravoof. Growthof *Trichodermaharzianum*and *Myceliophtora thermophila*inpalmoilsludge. *World J. Microbiol. Biot.* 1997. **13**, 189-194.

[10] N Jalaludin1, RA Rahman1, F Razali1, HFA Barghash and SSM Sukri. Optimization of fresh palm oil mill effluent biodegradation with *Aspergillus niger*and *Trichoderma virens. Arch. Environ. Prot.* 2016. **42**, 63–73.

[11] P Prasertsan, A H-Kittikul, A Kunghae, J Maneesri and S Oi. Optimization for xylanase and cellulose production from *Aspergillus niger* ATTC 6275 in palm oil mill wastes and its application. *World. J. Microbiol. Biotechnol.* 1997.**13**, 555-559.

[12] SS Rashid, Md.Z Alam, MIA Karim and MH Salleh. Management of palm oil mill effluent through productionof cellulases by filamentous fungi. *World J Microb Biot.* 2009. **25**, 2219–2226.

[13] **AM** Roslan, **MA Hassan, S Abd-Aziz and PL Ye.** Effect of palm oil mill effluent supplementation on cellulase production from rice straw by local fungal isolates. *Int. J. Agric. Res.*2009. **4**, 185-192.

[14] P Prasertsan and S Oi. Enzymatic saccharification of hemicellulose extracted from palm oil mill wastes. Songklanakarin *J. Sci. Technol.* 2001. **23***(*Suppl.*)*, 789-795.

[15] KL Hii, SP Yeap and MD Mashitah. Cellulase production from palm oil mill effluent in Malaysia*:* economical and technical perspectives. *Eng. Life Sci.* 2012. **12**, 7–28.

[16] DGCoony and R Emerson. *Thermophilic Fungi Defined.In:*Thermophilic Fungi. DM Whitaker, REmerson and GW Beadle *(*Ed*)*. WH Freeman and company, United States of America, 1964, P. 3-7.

[17] SAhirwar, H Soni, B P Prajapati and N Kango. Isolation and screening of thermophilic and thermotolerant fungi for production of hemicellulases from heated environments. *Mycology.* 2017. **8**, 125-134.

[18] R K Salar and K R Aneja. Thermophilic Fungi*:* Taxonomy and Biogeography. *J. Agric Technol.* 2007. **3**, 77-107.

[19] MM Uddin, Md. MH Chowdhury, S Moojumder and D Sikdar. Multiple Endo-B-1,4-glucanases Present in the Gut Fluid of a Defoliating Beetle, *Podontia quatuordecimpunctata (*Coleoptera*:* Chrysomelidae*)*. *Pakistan J Biol Sci.* 2012. 15,333-340

[20] M Mendels and J Weber. *The production of cellulase In:* Cellulase and Their Applications. RE Gould *(*Ed*)* American Chemistry Society, Waaaashington, D.C. 1969, P.391-398.

[21] LUL Tang, EKC Yu, GW Louis-size and JW Saddler. Inexpensive, rapid procedure for bulk purification of cellulase-free beta,1-4, D-xylanase for high specific activity. *Biotechnol. Bioeng.* 1987. **30**, 96-106.

[22] OH Lowry, NJ Rossebrougggh, AL Farr and RJ Randall. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 1951. **193**, 265-276.

[23] APHA, AWWA and WEF. Standard Method for the Examination of Water and Wastewater. 18th ed. N. Y. *:*American Public Health Association.1998.

[24] A.O.A.C. Official Methods of Analysis of the Association of official Analytical

Chemists15th ed. The Association of official Analytical Chemists. Inc Verginia. 1990.

[25] F Sebok, C Dobolyi, J Bobvos, S Szoboszlay, B Kriszt and D Magyar. Thermophilic fungi in air samples in surroundings of compost piles of municipal, agricultural and horticultural origin. ***Aerobiologia (Bologna).***2015. **32**, 255–263.

[26] MMS Moretti, DA Bocchini-Martins, R D Silva, A Rodrigues, LD Sette and E Gomes. Selection of thermophilic and thermotolerant fungi for the production of cellulases and xylanases under solid-state fermentation. *Braz J.*2012. **43**, 1062–1071.

[27] B S Chadha, G Harmeet, M Mandeep, HS Saini and N Singh. Phytase production by the thermophilic fungus *Rhizomucor pusillus. World J Microbiol Biotechnol.* 2004. **20**, 105–109.

[28] SK Ghatora, BS Chadha, HS Saini, MK Bhat and CB Faulds. Diversity of plant cell wall esterases in thermophilic and thermotolerant fungi. *J. Biotechnol.* 2006. **125**, 434-445.

[29] N Martin, SRD Souza, SD Silva and E Gomes. Pectinase production by fungal strains in solid – state fermentation using agro-industrial bioproduct. *Braz. Arch. Biol. Technol.* 2004. **47**, 813-819.

[30] R Deutschmann and RFH Dekker. From plant biomass to bio-based chemicals*:* latest developments in xylan research. *Biotechnol. Adv.*2012. **30**, 1627-1640.

[31] J Mouchacca. Thermotolerant fungi erroneously reported in applied research work as possessing thermophilic attributes. *World J Microb Biot.*2000. **16**, 869–880.

[32] RK Salar and KR Aneja, Thermophilic Fungi*:* Taxonomy and Biogeography. *J of Agric Technol.* 2007. **3**,77-107.

[33] H Hassouni, M Ismaili-Alaoui, I Gaime-Perraud, C Augur and S Roussos. Effect of culture media andfermentation parameters on phytase production by the thermophilic fungus *Myceliophthora thermophila* in solid state fermentation. *Micol Aplicada Int.*2006. **18**, 29-36.

[34] Berlin A, Maximenko V, Gilkes N, Saddler J. Optimization of enzyme complexes for lignocellulose hydrolysis. *Biotechnol Bioeng.* 2007. **97**, 287–296.

[35] V Bru-Adan, N We´ry, M Moletta-Denat, P Boiron, JP Delge`nes and JJ Godon. Diversity of Bacteria and Fungi in Aerosols During Screening in a Green Waste Composting Plant. *Curr Microbiol.* 2009. **59**, 326-335.

[36] B Sreelatha, AS Priya and S Girisham. Incidence of thermophilic fungi in different dung samples of warangal district of AP. *IJPBS.* 2013. **3**, 355-359.

[37] A Langarica-Fuentes, PS Handley, A Houliden, G Fox and GD Robson. An investigation of the biodiversity of thermophilic and thermotolerant fungal species in composts using culture-based and molecular techniques. *Fungal Ecol.* 2014. **11**, 132-144.

[38] P Maijala, N Kango, N Szijarto and L Viikari. Characterization of hemicellulases from thermophilic fungi. *Antonie van Leeuwenhoek.* 2012. **101**, 905–917.

[39] PF Rupani, RP Singh, H Ibrahim and N Esa. Review of current palm oil mill effluent *(*POME*)*treatment methods*:*Vermicomposting as a sustainable practice. *World. Appl. Sci.* 2010. **11**, 70-81.

[40] JD Bala, J Lalung and N Ismail. Biodegradation of palm oil mill efuent *(*POME*)* by bacterial. *Int. J. Sci. Res. Publ.* 2014. **4**,1–10.