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Potential Bioremediation of Waste Corn Oil by Extracellular Lipase Enzyme from *Monascus purpureus* W7

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Recent awareness of the direct disposal of waste cooking oil to the sewer system enhanced the development of eco-friendly approaches of spent lipids treatments. Among many, the bioremediation of waste cooking oil by the hydrolytic action of their natural biocatalysts, lipases, was proposed. Therefore, in this study the potential bioremediation of waste corn oil using extracellular lipases produced by indigenous fungal isolates was evaluated under their optimum and most stable operation conditions. Subsequently, the production of the extracellular lipase that showed the highest degree of waste oil hydrolysis produced by *Monascus purpureus* W7 was optimized and its potential use in bioremediation of waste corn oil was restudied. Approximately the 3 folds enhancement in the extracellular enzyme production that recorded after stepwise optimization of the enzyme production reflected as 3.7 times enhancement in the waste corn oil hydrolysis with 87.6% hydrolysis degree. The recent results supported the employment of the extracellular lipase from *Monascus purpureus* W7 for the bioremediation of used cooking oil.

Keywords: Extracellular lipases, indigenous fungi, Waste corn oil, Bioremediation

INTRODUCTION

Waste lipids are continuously produced worldwide in huge amounts by houses, restaurants, food industry and other means (Okino-Delgado et al., 2017). At the same time, the direct dumping of the spent fat, oil and grease (FOG) from the source points into the sewer system is still the most common disposal practice in many countries. As a growing concern to municipalities, recent alertness of fat, oil and grease disposable practices and its connected environmental issues were pointed out to increase public awareness about this issue (Yau et al., 2018; Wallace et al., 2017). On the other hand, the calls for the development of an international FOG management programs for effective and proper handling of FOG wastes stressed on considering them as valuable resources rather than just wastes (Wallace et al., 2017). In common, these management programs emphasized on direct sink disposal prevention, proper collection, treatment, recycling and valorization of waste lipids as their main outlines.

Research developments pertaining to eco-friendly approaches of spent lipids treatment, recycling and valorization concentrate largely on their use as feedstocks for either biodiesel production (Pinotti et al., 2018; Vescovi et al., 2016) or for making a derived fuel (Chang et al., 2018). Further investigations were elaborated on using microbial pretreatment of domestic FOG wastewater through oil biodegradation using bacteria and fungi for obtaining less toxicity and amendment of their biodegradability (Affandi et al., 2014; Wu et al., 2009; Takeno et al., 2005). In addition, it was used as nutritional ingredient in

several microbial cultures to produce biotechnological valuable materials such as lipid-rich biomasses, bioplastics, rhamnolipid biosurfactant, biolubricants, carotene and methane (Papanikolaou et al., 2011; Nascimento et al., 2018; Kamilah et al., 2018; Ozdal et al., 2017; Chowdhury et al., 2013; Nanou et al., 2017; Liu et al., 2018). Further appealing applications for waste cooking oil recently stated, was being a modifier and for the rejuvenation of aged asphalt (Singh-Ackbarali et al., 2017; Zhang et al., 2017).

However, the bioremediation of waste cooking oil by the hydrolytic action of their natural biocatalysts, lipases, still another promising approach to be intensified. The application of enzymatic hydrolysis of FOG in wastewater as function of lipases were early reported in sewage disposal plants in United States (Rosa et al., 2006). Lipases (EC 3.1.1.3) are hydrolytic enzymes that naturally breakdown lipids into free fatty acids and glycerol both of which are highly valuable products utilized in several oleochemical industries (Preczeski et al., 2018). The generated fatty acids and crude glycerol from the lipolytic hydrolysis of waste lipids successfully used as lubricant and biodiesel feedstock options (Chowdhury et al., 2016; Vescovi et al., 2016; Xu et al., 2016). On the other hand, crude glycerol produced as a by-product from enzymatic hydrolysis of waste cooking oil was used as a C-source in several microbial cultures for lactic acid (Yuwa-amornpitak and Chookietwatana, 2018) 1,3 and 1,2-propanediol, ethanol, single cell oil, biosurfactants, organic acids and polyols production (Dobson et al., 2012; Nicol et al., 2012). However, several commercially available lipases or produced by isolated microorganisms were specifically explored as catalysts in the hydrolysis of various waste lipids under different conditions. As each kind of waste lipids has its own chemical and physical properties, the search for a suitable biocatalyst for each is still ongoing field of research (Allimoun et al., 2015: Al-Limoun et al., 2018). To date, not much information is available on lipases produced by *Monascus purpureus*. Therefore, in the present investigation, production and potential of extracellular lipases from potent lipolytic indigenous *Monascus purpureus* W7 in comparison to other fungal isolates were evaluated as biocatalysts in enzymatic bioremediation of waste corn oil.

MATERIALS AND METHODS

Fungal strains and extracellular lipase production media

Through primary screening of 15 soil samples collected from olive oil mills in Al-Karak province, south of Jordan, 34 fungal strains were isolated and purified on PDA plates. The isolated fungal strains were further screened for extracellular lipase production in broth cultures containing olive oil or Tween 80 as enzyme inducers. The basal culture media (pH 7.0) composed of 0.5% (w/v) starch, 1.0% (w/v) yeast extract, 0.3% (w/v) Tween 80 or 1.0% (w/v) olive oil and 0.5% (w/v) NaCl. The media were inoculated with 1.0 mL of standard inocula of 10^4 spore suspension and incubated at 30°C and 150 rpm agitation speed in shaker incubator (Incu-Shaker 10L, Benchmark, Germany) for 96 hrs. Among them, the fungal strains coded as W7, W5H, WX and W5D that produced the highest extracellular lipases were chosen for further evaluation of their extracellular lipases as potential biocatalyst in used corn oil hydrolysis. The identities of the four potent isolates were revealed using internal transcribed spacer (ITS) sequencing (GENWIZ, USA). The obtained DNA sequences were blasted in NCBI gene library for sequence similarity analysis and then the sequences were submitted to NCBI and accession numbers were obtained.

Enzyme assay

Lipase activities were determined in the fungal culture filtrates spectrophotometrically as described by Winkler and Stuckmann (1979). The assay reaction solution was prepared by dissolving the enzyme substrate, 0.5 mM *p*-nitrophenyl laurate, in 10 mL of dimethyl sulfooxide (DMSO). Then the *p*-nitrophenyl laurate-DMSO solution was emulsified in 90 mL of emulsification solution composed of 50 mM phosphate buffer pH 7.0 (unless otherwise stated), 0.1% (w/v) polyvinyl alcohol (PVA) and 0.4% (w/v) Triton X-100. To determine the enzyme activities in the culture filtrates 1.0 mL of the crude enzyme was added to 3.0 mL of the reaction mixture. The assay solution-crude enzyme mixture was then statically incubated for 15 min at 30°C (unless otherwise applied). The color changes was measured at 410 nm (SPUV-19, SCO TECH, Germany) and compared with the *p*-nitrophenol standard curve. One unit (U) of lipase activity was defined as the amount of the

enzyme that released 1µmol of *p*-nitrophenol per min.

Temperature/pH optima and enzyme stability

In order to determine the optimum temperature of the extracellular lipases produced by W7, W5H, WX and W5D fungal isolates 1.0 mL of the crude lipase filtrates was added to the assay reaction mixture (pH 7.0), and the reaction was allowed to proceed for 15 min at 25, 30, 35, 40, 45 and 50°C. To determine the optimum pH of the enzyme mixture, different pHs of the assay solutions were used (3.0, 4.0, 5.0, 6.0, 7.0 and 8.0) and incubated for 15 min at the optimum incubation temperature of each fungal strain. Stability of extracellular lipolytic enzymes of the four fungal isolates were investigated at their respective optimal incubation temperature and pH of each. Fifty mL of the crude extracellular lipase filtrates from each isolate adjusted to the respective optimum pH using 1.0 N solution of HCl and NaOH and incubated at the respective optimum temperature for 12 hrs. Three mL samples were taken every 2 hrs to determine the residual activities of the extracellular lipases as compared to the control (at zero time).

Potential application in waste corn oil hydrolysis

The potential of the crude filtrates of the four selected isolates in waste corn oil hydrolysis were evaluated in oil/water two-phase systems. The two-phase systems of waste corn oil hydrolysis experiments composed of 2 grams of filtered waste corn oil and 20 ml of buffered enzyme filtrates (10 U/mL lipolytic enzyme activities). The hydrolysis experiments were conducted at the respective optimum pH and temperature of each isolate in temperature controlled chamber (Incu-Shaker 10L, Benchmark, Germany) for eight hrs. The two-phase reaction mixtures were stirred at 300 rpm using multi-position magnetic stirrer (Super-Nuova Multi-Place, Thermo Scientific, USA). After eight hrs. the hydrolysis degree of spent corn oil was determined by titration of the reaction mixture with 0.2 M potassium hydroxide. Blank experiments were prepared and conducted as explained earlier except that the buffered enzyme filtrates were replaced by buffered fresh media. In another experiment, a time course (2 hrs intervals for 8 hrs) of waste corn oil hydrolysis after optimization of enzyme production by *Monascus purpureus* W7 isolate was conducted. The hydrolysis reaction mixture was prepared and the experiment was conducted as described before except that the enzyme loading in the hydrolysis reaction mixture was 40 U/mL.

Optimization of extracellular lipase production from *Monascus purpureus* W7 isolate

Optimization of the extracellular lipase production from *Monascus purpureus* W7 fungal isolate performed by stepwise variation of the culture conditions and the basal culture media composition. The influence of various inoculum sizes (10^3, 10^4, 10^5 and 10^6 spore suspensions), different initial pHs (4.0, 5.0, 6.0, 7.0 and 8.0) of the culture medium and different incubation temperatures (27, 30, 33 and 36°C) on the enzyme production were evaluated. Determination of the peak time for the maximum enzyme production through a time course profile was also conducted. The effects of different additional carbon sources (sugars, sugar alcohols and organic acids), different enzyme inducers (aloe oil, olive oil, soy oil, almond oil, sesame oil, argan oil, castor oil, linseed oil, corn oil, Tween 80 and Tween 20), different organic and inorganic nitrogen sources, NaCl concentration and variety of chloride containing minerals on enzyme production were also studied.

Waste corn oil hydrolysis degree

The used corn oil hydrolysis degree was determined by titration of the hydrolysis reaction mixture, containing 2.0 g oil samples, with 0.2 M potassium hydroxide. To each sample, 15 mL of diethyl ether-ethanol mixture (1:1 v:v) was added. Small amount of phenolphthalein as pH indicator was dissolved directly in the diethyl ether-ethanol mixture. The sample was then titrated against 0.2 M potassium hydroxide using digital titration system (Digitrate, Jencons, UK). The amount of 0.2 M potassium hydroxide required to neutralize the free fatty acid was noted and presented as acid value (AV). A blank titration was done with a sample where the enzyme filtrate was replaced with buffered fresh media. AV calculated using the following equation:

$$AV = \frac{56.1\ X\ CKOH\ X\ Vtitra}{m}$$

Where CKOH is the concentration of KOH (M), Vtitra is the amount of titration (mL), m is the amount of oil sample (g). Meanwhile, the hydrolysis degree (X%) of the used corn oil was calculated from the acid value (AV) and the saponification value (SV) of corn oil using the following equation:

$$X\% = \left(\frac{AV}{SV}\right) X\ 100\%$$

Statistical analysis

Results were expressed as the means of three independent determinations. Standard deviations, indicated by error bars, for each of the experimental results were calculated using Microsoft Excel software (2010). Statistical analysis was done with Student's *t*-test using Microsoft Excel software (2010) where Asterisks (*) indicate significant differences ($P \leq 0.05$).

RESULTS AND DISCUSSION

Fungal strains and extracellular lipase production

Thirty-four fungal isolates were studied based on their ability of lipase production in fixed growth conditions. From these, four isolates were able to produce lipase enzyme at concentrations of more than 10 U/mL were selected and used in the following experiments (Table 1). The enzyme system required different enzyme inducers for maximal activity. Compared with the controls, Tween 80 and olive oil enhanced the enzyme activity from W5H, WX and W5D coded strains by different extents while the control had no activity. Meanwhile, the addition of Tween 80 was shown to induce the lipase activity from W7 strain, whereas the addition of olive oil led to the less induction in activity. The four selected isolates were identified; (Table 1) based on the internal transcribed spacer (ITS) and using sequencing similarity analysis (NCBI) as *Monascus purpureus, Tritirachium oryzae, Ochroconis sp.* and *Aspergillus nidulans.*

Table 1; Fungal strains identity and extracellular lipase production from the four potent fungal isolates in selection media containing Tween 80 and olive oil as enzyme inducers.

Strain Code	Fungal Identification	Accession no.	Lipase Production (U/ml) with Tween 80 as inducer	Lipase Production (U/ml) with Olive Oil as inducer
W7	***Monascus purpureus***	MK026964	13.8 ± 0.15	1.87 ± 0.13
W5H	***Tritirachium oryzae***	MK028996	14.1 ± 0.26	12.1 ± 0.26
WX	***Ochroconis sp.***	MH969430	12.5 ± 0.37	10.5 ± 0.37
W5D	***Aspergillus nidulans***	MK026965	9.1 ± 0.33	13.7 ± 0.47

Enzyme production values are means ± SD (n=3)

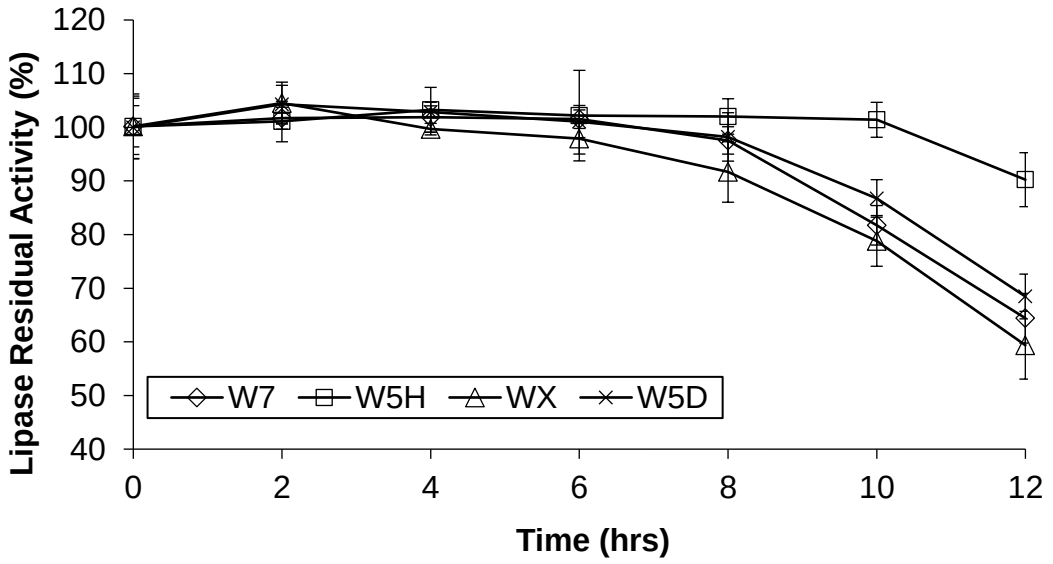

Figure 1; pH and thermal stability of the extracellular lipases produced by the four potent fungal isolates. Values are means ± SD (n=3).

Temperature and pH optima and stability

The extracellular lipases produced by the selected isolates were further characterized to explore their optimum pH and temperature as well as their thermal stability. The results revealed by Table 2 and Figure 1 summarizes the experimental findings of the optimum pH, temperature and thermal stability, respectively. As can be seen from Table 2, the extracellular lipases produced by three isolates optimally hydrolyze substrates at acidic pH 5, while only *Aspergillus nidulans* W5D was being at pH 6. Meanwhile, the optimum temperature for the extracellular lipases produced by *Tritirachium oryzae* W5H and *Ochroconis* sp. WX was 35^{o}C whereas for those produced by *Monascus purpureus* W7 and *Aspergillus nidulans* W5D was at 40 and 30^{o}C, respectively. Figure 1 shows the stability of the extracellular lipases produced by the potent isolates at their optimum pH and temperature of each. The results indicated that the four enzymes were remained stable at their optimum pH and temperature up to 8 hours incubation period with slight reduction (2-9%) in enzyme activity from *Monascus purpureus* W7, *Ochroconis* sp. WX and *Aspergillus nidulans* W5D. Further incubation of the tested enzymes for longer time caused reduction in their activity except the enzyme activity produced by *Tritirachium oryzae* W5H that maintained its original activity up to 10 hours.

The biochemical properties of two possibly cold active extracellular lipases produced by *Monascus purpureus* M7 (Lip2) and *Monascus ruber* M7 (Lip10) were reported (Kang et al., 2017; Guo et al., 2016). In agreement to our results, both lipases exhibited maximum activity at 40^{o}C. In contrast to our results, the reported pH optima for Lip2 and Lip10 of *Monascus purpureus* M7 and *Monascus ruber* M7, respectively was at alkaline pH, 8.0. On the other hand, themostability studies of Lip2 and Lip10 showed that both enzymes were stable for 1.5 hour at 40^{o}C. Similarly, the optimum temperatures of the extracellular lipases produced by two strains of *Aspergillus nidulans* was reported to be at 40^{o}C (Peña-Montes et al., 2009; Mayordomo et al., 2000). Meanwhile, the optimum pH of the same two strains was 6.5 (Mayordomo et al., 2000) and 7-9 (Peña-Montes et al., 2009). Both enzymes displayed reduced thermostability at temperatures higher than 20 ^{o}C (Mayordomo et al., 2000) and 30-40^{o}C (Peña-Montes et al., 2009). However, there is no previous information of investigations on *Tritirachium oryzae* and *Ochroconis* sp to make a comparison between the extracellular lipases of same species to show if there is a variation of the enzyme characteristics.

Table 2; pH and temperature optima of the extracellular lipases produced by the four potent fungal isolates.

Strain	Optimum pH	Optimum Temperature
***Monascus purpureus* W7**	5.0	40^{o}C
***Tritirachium oryzae* W5H**	5.0	35^{o}C
***Ochroconis* sp. WX**	5.0	35^{o}C
***Aspergillus nidulans* W5D**	6.0	30^{o}C

Potential application in waste corn oil hydrolysis

The bioremediability of waste corn oil by the activity of four extracellular lipases were confirmed in this study by hydrolyzing the waste corn oil with varied degrees (Figure 2). The highest degree of hydrolysis was recorded for lipase from *Monascus purpureus* (23.7%) as compared with the enzymes from other tested fungal isolates. The quality of hydrolysis level was poor, however, probably due to low concentration of excreted enzyme. This is likely true as long as the enzymes are generally stable and experiments were conducted under optimum conditions. Besides to the enzyme loading, which is one of the most important limiting factors to obtain high degree of oil hydrolysis, other factors such as the oil loading, mixing speed and the oil/water ratio, may play a role. Therefore, to improve waste corn oil hydrolysis process, in the following experiments a stepwise optimization of the enzyme production from the isolate *Monascus purpureus* W7 was performed.

Optimization of extracellular lipase production from *Monascus purpureus* W7

In the optimization experiments, cultural and growth parameters were varied in order to enhance the extracellular lipase production from *Monascus purpureus* W7. As illustrated in Figure 3, significantly higher enzyme production, 18.9 U/mL, was obtained from the dispersed mycelia when the freshly inoculum size of 10^5 spore suspension was applied as starting inoculum. Meanwhile, spore suspensions lower or higher than 10^5 significantly resulted in less enzyme production. Inoculum size, age, type and

inoculation methodology were reported as significant variables that affect enzyme production particularly from fungi (Colla et al., 2016; Iftikhar et al., 2015). The initial inoculum concentration in association with rotation speed applied can influence the morphology, clumps or dispersed, of the growing mycelia and hence lipase production (Colla et al., 2016).

Interpretation of the results of the effect of initial pH of the culture media (Figure 4) on the enzyme production revealed that the highest production of extracellular lipase (28.0 U/mL) and biomass accumulation obtained when initial pH of culture media was adjusted to 6.0. Significant reduction in enzyme activity were recorded when the initial pH were varied, from either lower or higher than 6.0. Generally, lipase production from various fungal species showed different initial pHs ranging from acidic to neutral pH (Padhiar et la., 2011; Lanka et al., 2015; Colla et al., 2016; Xia et al., 2011). It was reported that, initial pH of the culture medium of fungi strongly affect extracellular enzyme loading and their excretion across the cell membranes in species dependent manner (Paranthaman et al., 2009; Sandhya et al., 2005).

The extracellular enzyme activity and biomass amount were optimally achieved at a 33^{o}C incubation temperature (Figure 5). The lipase production increased with elevation the incubation temperature until 33^{o}C beyond which the increase in temperature to 36^{o}C led to the lowering in the enzyme activity concomitant with the increase in the biomass. Temperature clearly had a strong impact on the production of lipases by fungi (Lima et al., 2003), as the mesophilic temperature produced the best conditions for their production. It appears that lipase production from fungi could occur at room temperature, with 33^{o}C being the optimum temperature for its activity with some exceptions in different strains being above or lower this range of temperature. However, temporal studies by Sreelatha et al. (2017) revealed that the variation in lipase production as function of temperature could be not only species but also strains dependent.

The maximal enzyme production and biomass accumulation from *Monascus purpureus* W7 was attained at 120 hrs time point beyond which the enzyme production was significantly reduced (Figure 6). Even though a comparable accumulation of fungal biomass was seen at 144 hrs, the reduction in the enzyme activity after 120 hrs of incubation time could be due to the production of proteolytic enzymes as the culture being worn out with a time.

The effect of different non-lipidic carbon sources supplied to the basal culture media containing Tween 80 on the production of lipase and biomass production by W7 fungal isolate, was examined (Table 3). All carbon sources supplied, did not further enhance the lipase activity as compared with the control (41.2 U/mL). The hydrolysis repression by fructose, mannose, sucrose, lactose, glycerol, sorbitol, and mannitol was occurred although the cell biomass increased; this might be a result of catabolite repression by these sugars (Khleifat, 2006, Khleifat et al., 2006; Shawabkeh et al., 2007). Even though, dextrin and starch supported higher biomass accumulation and non-significant increase in enzyme production (40-44 U/mL) as compared with the control the next experiments were conducted with no added carbon sources. Similar results was reported by Eltaweel et al. (2005), they stated that, the addition of various carbon sources to the basal fermentation medium of *Bacillus* sp. strain 42, containing olive oil, did not stimulate lipase enzyme production.

Table 3; Influence of additional carbon sources on extracellular lipase production from *Monascus purpureus* W7 isolate.

Carbon Source	Enzyme Production (U/mL)	Biomass (g/L)
Control	41.2 ± 1.51	4.98
Succinic acid	18.1 ± 2.53 *	0.58
Trisodium citrate	26.1 ± 1.71 *	1.13
Potassium acetate	22.6 ± 2.49 *	1.94
Glucose	34.1 ± 1.88 *	4.84
Fructose	41.3 ± 2.13	5.79
Mannose	30.7 ± 2.01 *	6.67
Sucrose	40.5 ± 1.59	6.55
Lactose	30.9 ± 1.96 *	6.85
Dextrine	44.1 ± 2.61	6.56
Starch	42.4 ± 0.55	5.96
Glycerol	21.2 ± 1.32 *	6.68
Sorbitol	34.5 ± 1.47 *	6.21
Mannitol	23.5 ± 2.53 *	6.41

Values are means ± SD (n=3). Asterisks indicate significant differences from the control experiment ($P \leq 0.05$).

The results in Table 4 showed no further stimulation in the production of extracellular lipase, where maximal enzyme production (40.53 ± 0.65 U / mL) was obtained with Tween 80 as an enzyme inducer already considered as essential constituent of culture medium. Therefore, the replacement of Tween 80 by other different

possible lipase enhancers did not achieve any increase in enzyme production. At the same time, adding different concentrations of Tween 80 (volume/weight) did not further increase the activity above 41 U / mL and three concentrations 0.3, 0.6 and 0.9% led to the insignificant variations between their maximum activities 39-41 U / mL (Figure 7). Therefore, the concentration chosen in the next experiments was 0.3% (w/v). It has been suggested that the cell permeability occurred by Tween-80 could be a key factor leading to the increased levels of extracellular lipase production (Kumar and Gupta, 2008).

The effect of different organic and inorganic nitrogen sources on the enzyme production were also evaluated (Table 5). No inorganic nitrogen sources showed any improvements in enzyme activity compared with a control of culture media that was deprived of additional nitrogen source supplementations (Table 5). All organic nitrogen sources led to production of enzyme activity except casein, which exhibited zero activity.

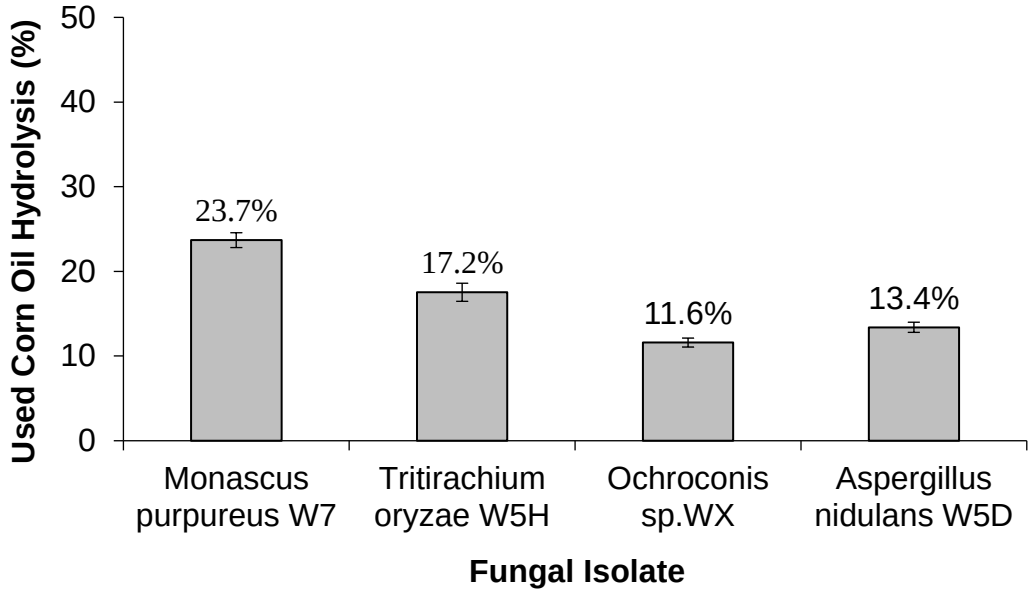

Figure 2 ; Potential bioremediation of the extracellular lipases produced by the four potent indigenous fungal isolates in used corn oil hydrolysis. Values are means ± SD (n=3).

Table 4; Effects of different enzyme inducers on extracellular lipase production from *Monascus purpureus* W7 isolate.

Inducers	Enzyme Production (U/mL)	Biomass (g/L)
Control	ND	2.17
Aloe oil	2.33 ± 0.83 *	6.75
Olive oil	2.47 ± 0.66 *	5.48
Tween 80	39.5 ± 0.65	4.75
Soy oil	2.02 ± 0.61 *	5.95
Tween 20	5.58 ± 0.93 *	2.94
Almond oil	2.80 ± 0.57 *	5.35
Sesame oil	1.51 ± 0.33 *	5.48
Argan oil	8.42 ± 0.49 *	6.18
Castor oil	10.7 ± 0.86 *	4.71
Linseed oil	ND	4.34
Corn oil	1.04 ± 0.37 *	4.79

Values are means ± SD (n=3). Asterisks indicate significant differences from the experiment with the highest enzyme production ($P \leq 0.05$). ND indicates not detectable.

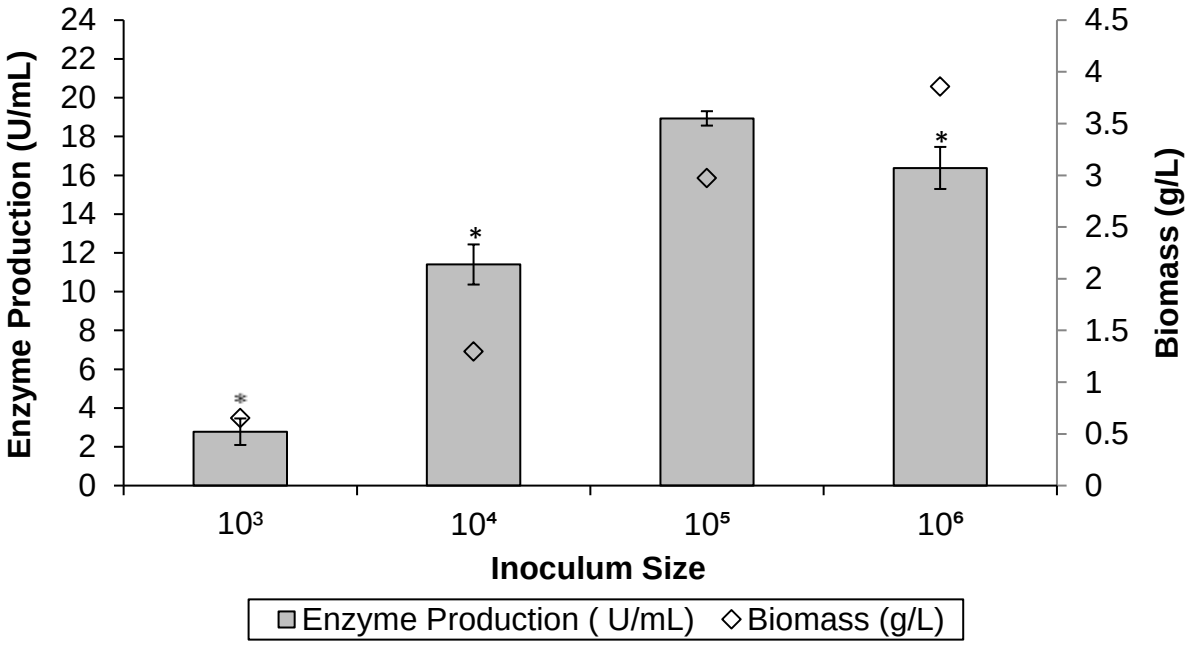

Figure 3; Effect of inoculum size on extracellular lipase production from *Monascus purpureus* W7 isolate. Values are means ± SD (n=3). Asterisks indicate significant differences from the experiment with the highest lipase production (P ≤ 0.05).

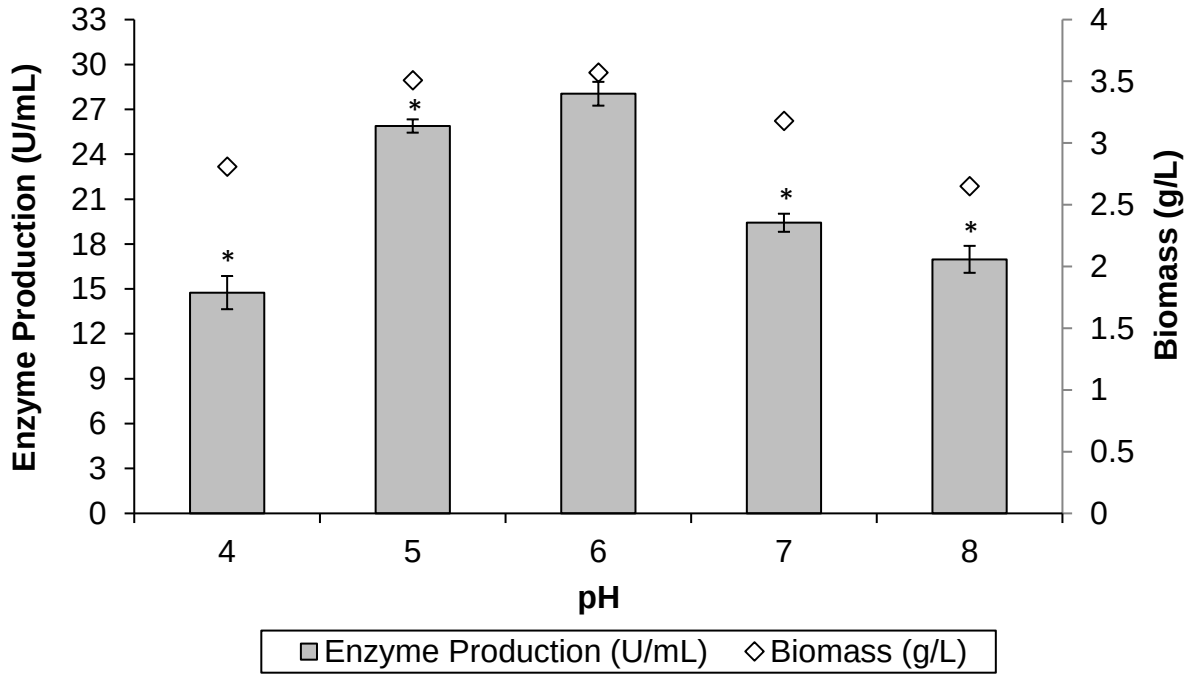

Figure 4; Effect of production medium initial pH on extracellular lipase production from *Monascus purpureus* W7 isolate. Values are means ± SD (n=3). Asterisks indicate significant differences from the experiment with the highest lipase production (*P* ≤ 0.05).

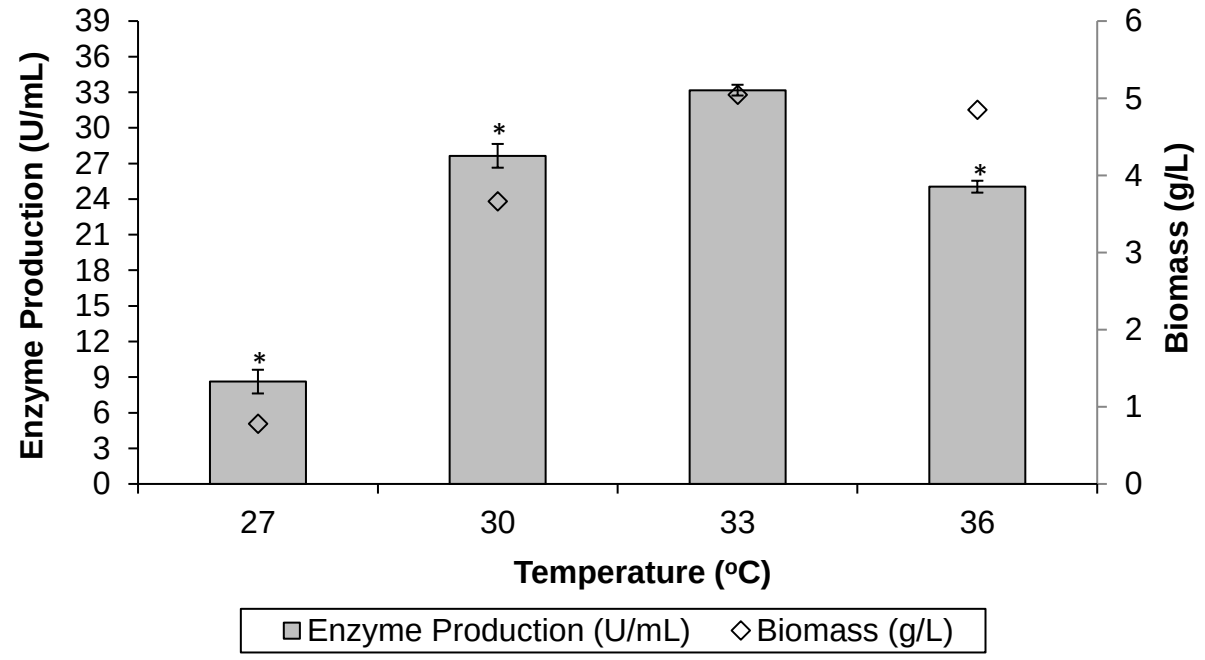

Figure 5; Effect of incubation temperature on extracellular lipase production from *Monascus purpureus* W7 isolate. Values are means ± SD (n=3). Asterisks indicate significant differences from the experiment with the highest lipase production (*P* ≤ 0.05).

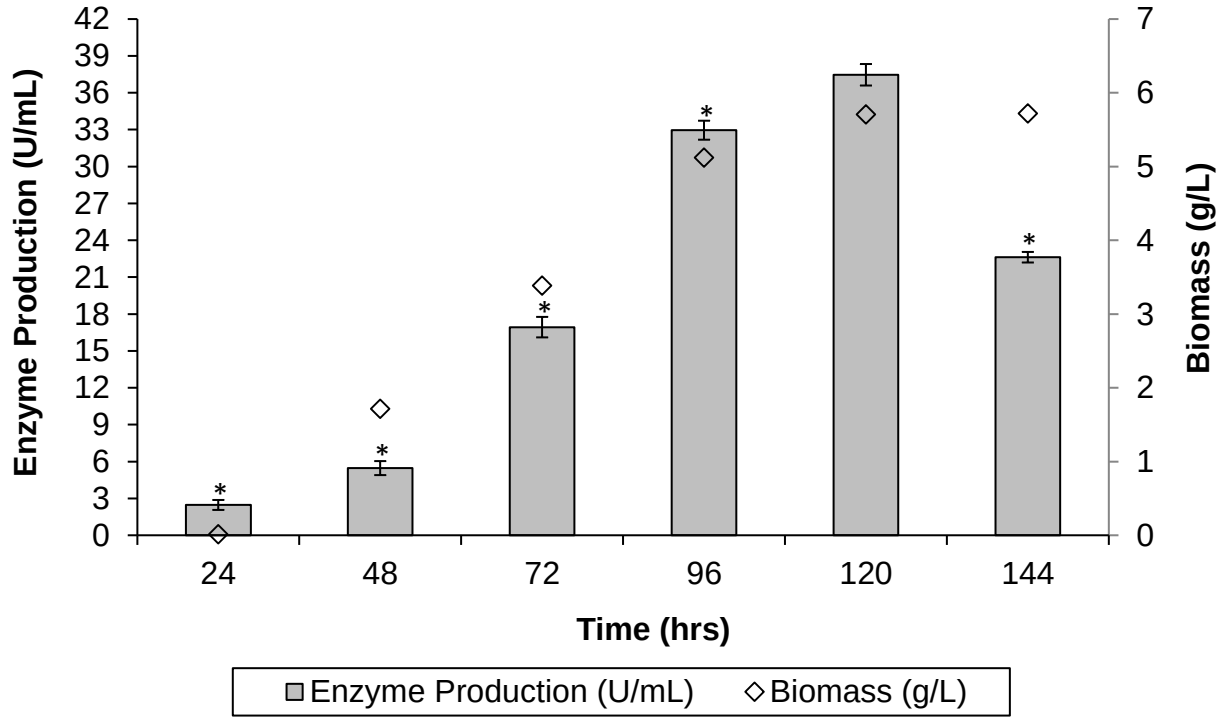

Figure 6; Time profile of extracellular lipase production from *Monascus purpureus* W7 isolate. Values are means ± SD (n=3). Asterisks indicate significant differences from the experiment with the highest lipase production (*P* ≤ 0.05).

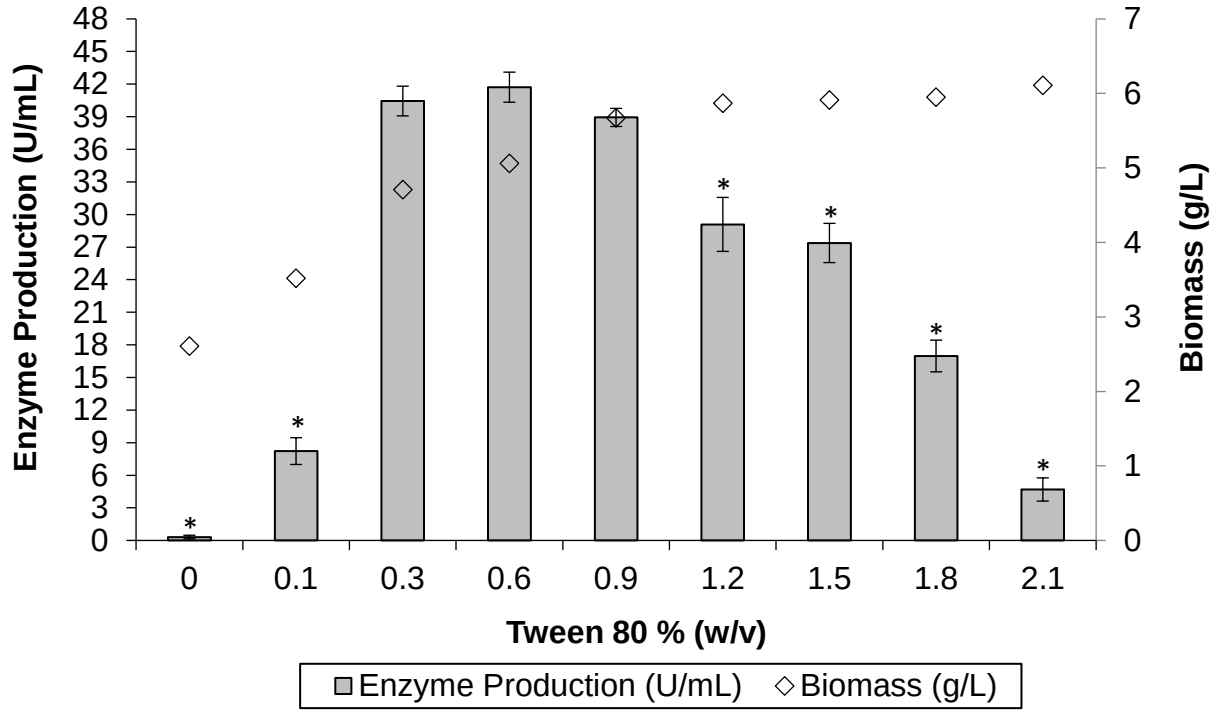

Figure 7; Effect of different concentrations of Tween 80 on extracellular lipase production from *Monascus purpureus* W7 isolate. Values are means ± SD (n=3). Asterisks indicate significant differences from the experiment with the highest lipase production (*P* ≤ 0.05).

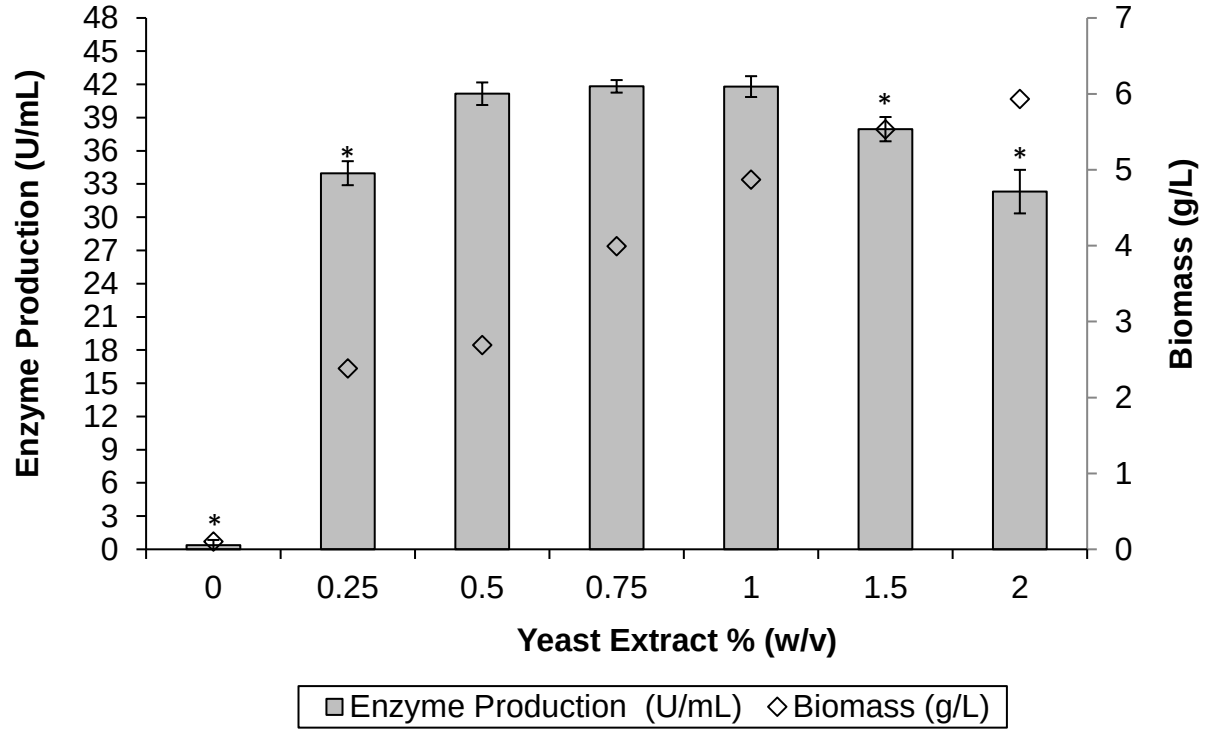

Figure 8; Effect of different concentrations of yeast extract on extracellular lipase production from *Monascus purpureus* W7 isolate. Values are means ± SD (n=3). Asterisks indicate significant differences from the experiment with the highest lipase production (*P* ≤ 0.05).

The highest enzyme production (41.7 U/mL) was recorded with yeast extract which originally present in the basal culture media therefore no enhancement in the overall production of the enzyme was recorded. Only beef extract and tryptone poorly stimulated biomass although the enzyme activity was repressed as compared with yeast extract. Therefore, the lowest concentration of yeast extract led to the highest enzyme production was 0.5% and was approximating the effect of utilized higher concentrations of yeast extract (0.75 and 1.0%) with insignificant differences (Figure 8). Thus, in the course of next experiments the 0.5% concentration of yeast extract was employed. Similar results were reported by Colla et al. (2016) for yeast extract which caused highest lipase production by *Aspergillus niger* and *Aspergillus flavus*, a result is associated with composition nature of yeast extract, beside of being nitrogen source, it is carbon chain content and vitamin B complex as co-enzymes, required for the aerobic metabolism. The high protein and peptide content of yeast extract that require proteases secretion to utilize them and simultaneously degrade lipase enzyme can be considered a possible reason for the low activities of extracellular lipase in the culture filtrate at high concentrations of yeast extract.

Table 5; Effects of different nitrogen sources on extracellular lipase production from *Monascus purpureus* W7 isolate.

Nitrogen Sources	Enzyme Production (U/mL)	Biomass (g/L)
Control	ND	0.45
Yeast extract	41.77 ± 0.94	4.88
Beef extract	33.81 ± 1.39 *	5.12
Malt extract	33.33 ± 1.48 *	2.88
Tryptone	29.97 ± 0.87 *	5.09
Peptone	31.95 ± 0.66 *	3.85
Casein	ND	0.33
$NaNO_3$	ND	0.17
NH_4Cl	ND	0.21
NH_4NO_3	ND	0.18
Urea	ND	0.37

Values are means ± SD (n=3). Asterisks indicate significant differences from the experiment with the highest enzyme production ($P \leq 0.05$). ND indicates not detectable.

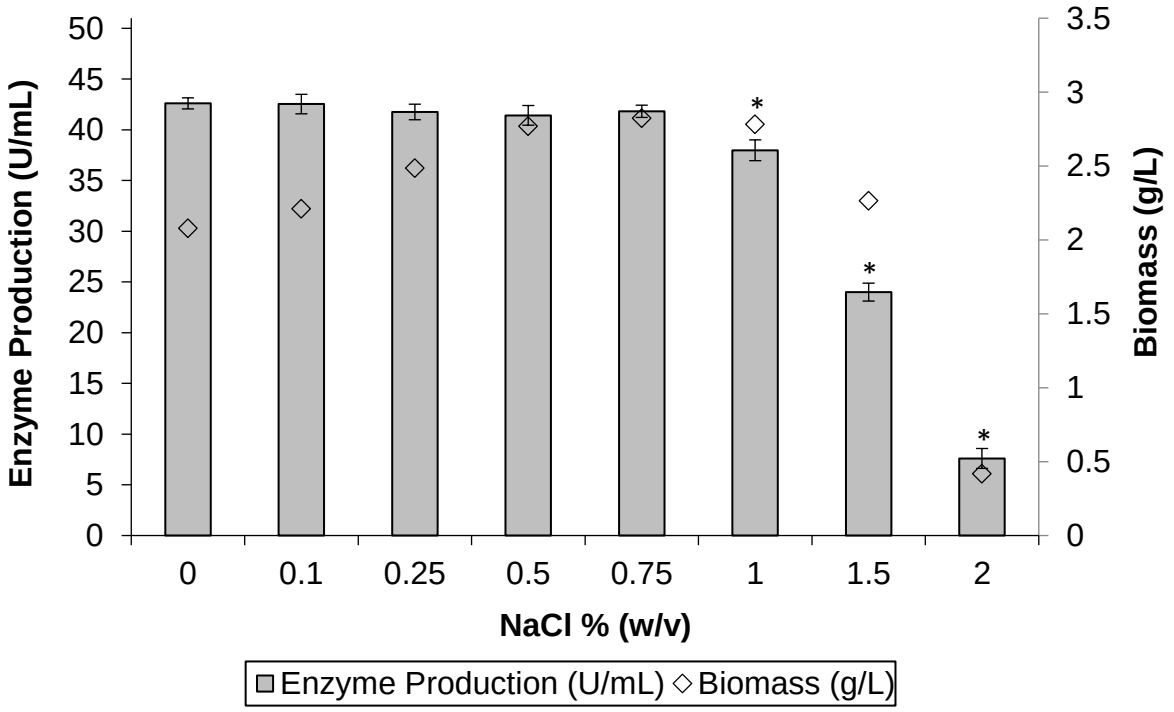

Figure 9; Effect of different concentrations of NaCl on extracellular lipase production from *Monascus purpureus* W7 isolate. Values are means ± SD (n=3). Asterisks indicate significant differences from the experiment with the highest lipase production ($P \leq 0.05$).

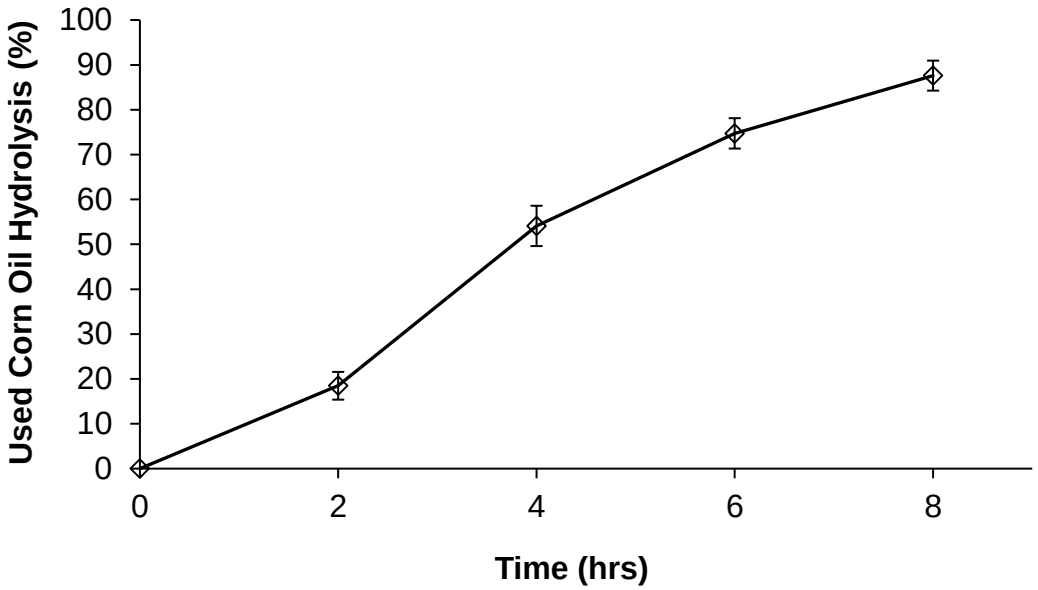

Figure 10; Time course of waste corn oil hydrolysis by *Monascus purpureus* W7 isolate extracellular lipase. Values are means ± SD (n=3).

Interestingly, the effect of NaCl concentration on the extracellular lipase production (Figure 9) shows that the absence or the presence of NaCl up to 0.75% (w/v) in the production media did affect the biomass accumulation but not the enzyme production. Non-significantly different and comparable results of extracellular lipase production was observed in the culture flasks from 0 to 0.75% (w/v) NaCl concentration with gradual increase in biomass accumulation. Meanwhile, using more than 0.75% (w/v) concentrations of NaCl, reduced the amount of enzyme activity and cell biomass. There was no stimulation in the activity of the enzyme above the established level (42.6 U/mL), which was considered a control and therefore, subsequent experiments were done with no added NaCl. Several metal chlorides for their effects on extracellular lipase production were examined (Table 6). Among the tested metal chlorides, only Cu and K although slightly but significant increase in the enzyme production was observed. The results also showed that the addition of certain metals such as Ba, Ca and Mg did not affect, significantly, the enzyme production as compared to the control experiment. Meanwhile, the presence of Fe in the culture media resulted in significant reduction in the enzyme activity.

Table 6; Effects of mineral salts on extracellular lipase production from *Monascus purpureus* W7 isolate.

Mineral Salts	Enzyme Production (U/mL)	Biomass (g/L)
Control	41.71 ± 0.52	1.97
$FeCl_2$	37.02 ± 0.44 *	2.08
KCl	46.53 ± 0.41 *	1.93
$CuCl_2$	45.86 ± 0.75 *	1.77
$BaCl_2$	42.31 ± 0.49	1.72
$CaCl_2$	43.55 ± 1.5	2.11
$MgCl_2$	43.15 ± 2.6	1.74

Values are means ± SD (n=3). Asterisks indicate significant differences from the control experiment ($P \leq 0.05$). ND indicates not detectable.

Potential application in used corn oil hydrolysis

It is well known that the biocatalysts availability and quantity of concentration is one of the major factors that affect bioremediation processes. Therefore, the optimization of enzyme production that made in stepwise manner led to getting gradually higher amount of enzyme production and consequently enhanced the bioremediation process. For instance, when comparing the early results of bioremediation processes (Figure 2), the latest results (Figure 10) obtained after the optimization processes, showed

almost 3.7-fold enhancement in the oil hydrolysis with achieving a relative degree of hydrolysis by about 87.6%. However, several reports focused on the importance of enzyme loading on bioremediation technology of the lipases using waste cooking oil as substrate (Mulinari et al., 2017;Talukder et al., 2010).

CONCLUSION

The preliminary screening experiments of soil samples for extracellular lipase producing fungi have resulted in the isolation of four potent isolates with considerable extracellular lipolytic activities. The selected isolates were identified as *Monascus purpureus*, *Tritirachium oryzae*, *Ochroconis* sp. and *Aspergillus nidulans*. Among them, the extracellular lipase produced by *Monascus purpureus* W7 isolate gave the highest degree of waste corn oil hydrolysis from which the extracellular lipase production was optimized in the subsequent experiments. After the physicochemical optimization of lipase production by *Monascus purpureus* W7 fungal isolate, approximately 3 folds enhancement in the extracellular enzyme production was recorded which eventually reflected as 3.7 times enhancement in the waste corn oil hydrolysis with 87.6% hydrolysis degree.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Muhamad O. Al-limoun is the author who conducts all experiments of the present research

REFERENCES

Affandi IE, Suratman NH, Abdullah S, Ahmad WA, Zakaria ZA, 2014. Degradation of oil and grease from high-strength industrial effluents using locally isolated aerobic biosurfactant-producing bacteria. Int Biodeterior Biodegradation 95: 33-40.

Allimoun MO, Ananzeh MR, Khleifat KM, 2015. Screening selection and optimization of extracellular methanol and ethanol tolerant lipase from *Acinetobacter* sp. K5b4. Int J Biosci 6(10): 44-56.

Al-Limoun MO, Khleifat KM, Alsharafa KY, Qaralleh HN, Alrawashdeh SA, 2018. Purification and characterization of a mesophilic organic solvent tolerant lipase produced by *Acinetobacter* sp. K5b4. Biocatal Biotransformation 37(2): 139-151.

Chang FC, Tsai MJ, Ko CH, 2018. Agricultural waste derived fuel from oil meal and waste cooking oil. Environ Sci Pollut Res Int 25(6): 5223-5230.

Chowdhury A, Mitra D, Biswas D, 2013. Biolubricant synthesis from waste cooking oil via enzymatic hydrolysis followed by chemical esterification. J Chem Technol Biotechnol 88(1): 139-144.

Chowdhury A, Sarkar D, Mitra D, 2016. Esterification of free fatty acids derived from waste cooking oil with octanol: Process optimization and kinetic modeling. Chem Eng Technol 39(4): 730-740.

Colla LM, Primaz AL, Benedetti S, Loss RA, Lima MD, Reinehr CO, Bertolin TE, Costa JAV, 2016. Surface response methodology for the optimization of lipase production under submerged fermentation by filamentous fungi. Braz J Microbiol 47(2): 461-467.

Dobson R, Gray V, Rumbold K, 2012. Microbial utilization of crude glycerol for the production of value-added products. J Ind Microbiol Biotechnol 39(2): 217-226.

Eltaweel MA, Rahman RNZ, Salleh AB, Basri M, 2005. An organic solvent-stable lipase from *Bacillus* sp. strain 42. Ann Microbiol 55(3): 187-192.

Guo H, Zhang Y, Shao Y, Chen W, Chen F, Li M, 2016. Cloning, expression and characterization of a novel cold-active and organic solvent-tolerant esterase from *Monascus ruber* M7. Extremophiles 20(4): 451-459.

Iftikhar T, Abdullah R, Iqtedar M, Kaleem A, Aftab M, Niaz M, Tabassum Sidra B, Majeed H, 2015. Production of lipases by *Alternaria* sp.

(mbl 2810) through optimization of environmental conditions using submerged fermentation technique. Inter J Biosciences 7(2): 178-186.

Kamilah H, Al-Gheethi A, Yang TA, Sudesh K, 2018. The use of palm oil-based waste cooking oil to enhance the production of Polyhydroxybutyrate [P (3HB)] by *Cupriavidus necator* H16 strain. ARAB J SCI ENG 43(7): 3453-3463.

Kang LJ, Meng ZT, Hu C, Zhang Y, Guo HL, Li Q, Li M, 2017. Screening, purification, and characterization of a novel organic solvent-tolerant esterase, Lip2, from *Monascus purpureus* strain M7. Extremophiles 21(2): 345-355.

Khleifat KM, 2006. Biodegradation of sodium lauryl ether sulfate (SLES) by two different bacterial consortia. Curr Microbiol 53(5): 444-448.

Khleifat KM, Abboud MM, Al-Mustafa AH, Al-Sharafa KY, 2006. Effects of carbon source and Vitreoscilla hemoglobin (VHb) on the production of β-galactosidase in *Enterobacter aerogenes*. Curr Microbiol 53(4): 277-281.

Kumar SS, Gupta R, 2008. An extracellular lipase from *Trichosporon asahii* MSR 54: Medium optimization and enantioselective deacetylation of phenyl ethyl acetate. Process Biochem 43(10): 1054-1060.

Lanka S, Pydipalli M, Latha JN, 2015. Optimization of process variables for extracellular lipase production from *Emericella nidulans* NFCCI 3643 isolated from palm oil mill effluent (POME) dump sites using OFAT method. Res J Microbiol 10(2): 38-53.

Lima VMG, Krieger N, Sarquis MIM, Mitchell DA, Ramos LP, Fontana JD, 2003. Effect of nitrogen and carbon sources on lipase production by *Penicillium aurantiogriseum*. Food Technol Biotechnol 41(2): 105-110.

Liu P, Ji J, Wu Q, Ren J, Wu G, Yu Z, Xiong J, Tian F, Zafar Y, Li X, 2018. *Klebsiella pneumoniae* sp. LZU10 degrades oil in food waste and enhances methane production from co-digestion of food waste and straw. Int Biodeterior Biodegradation 126: 28-36.

Mayordomo I, Randez-Gil F, Prieto JA, 2000. Isolation, purification, and characterization of a cold-active lipase from *Aspergillus nidulans*. J Agric Food Chem 48(1): 105-109.

Mulinari J, Venturin B, Sbardelotto M, Agnol AD, Scapini T, Camargo AF, Reichert Jr FW, 2017. Ultrasound-assisted hydrolysis of waste cooking oil catalyzed by homemade lipases. Ultrason Sonochem 35: 313-318.

Nanou K, Roukas T, Papadakis E, Kotzekidou P, 2017. Carotene production from waste cooking oil by *Blakeslea trispora* in a bubble column reactor: The role of oxidative stress. Eng Life Sci 17(7): 775-780.

Nascimento LL, Neves Nunes JM, Rodrigues PR, Druzian JI, 2018. Bioconversion of residual soybean oil into polyhydroxyalkanoates. *J.* Appl Polym Sci 135(19): 46255.

Nicol RW, Marchand K, Lubitz WD, 2012. Bioconversion of crude glycerol by fungi. Appl Microbiol Biotechnol 93(5): 1865-1875.

Okino-Delgado CH, do Prado DZ, Facanali R, Marques MMO, Nascimento AS, da Costa Fernandes CJ, Zambuzzi WF, Fleuri LF, 2017. Bioremediation of cooking oil waste using lipases from wastes. PloS one 12(10): e0186246.

Ozdal M, Gurkok S, Ozdal OG, 2017. Optimization of rhamnolipid production by *Pseudomonas aeruginosa* OG1 using waste frying oil and chicken feather peptone. 3 Biotech 7(2): 117.

Padhiar J, Das A, Bhattacharya S, 2011. Optimization of process parameters influencing the submerged fermentation of extracellular lipases from *Pseudomonas aeruginosa*, *candida albicans* and *Aspergillus flavus*. Pak J Biol Sci 14(22): 1011-1018.

Papanikolaou S, Dimou A, Fakas S, Diamantopoulou P, Philippoussis A, Galiotou-Panayotou M, Aggelis G, 2011. Biotechnological conversion of waste cooking olive oil into lipid-rich biomass using *Aspergillus* and *Penicillium* strains. J Appl Microbiol 110(5): 1138-1150.

Paranthaman R, Alagusundaram K, Indhumathi J, 2009. Production of protease from rice mill wastes by *Aspergillus niger* in solid state fermentation. World J Agric Sci 5(3): 308-312.

Peña-Montes C, Lange S, Castro-Ochoa D, Ruiz-Noria K, Cruz-García F, Schmid R, Navarro-Ocaña, Farrés A, 2009. Differences in biocatalytic behavior between two variants of StcI esterase from *Aspergillus nidulans* and its potential use in biocatalysis. J Mol Catal B Enzym 61(3-4): 225-234.

Pinotti LM, Benevides LC, Lira TS, de Oliveira JP, Cassini ST, 2018. Biodiesel production from oily residues containing high free fatty acids. Waste Biomass Valorization 9(2): 293-299.

Preczeski KP, Kamanski AB, Scapini T, Camargo AF, Modkoski TA, Rossetto V, Treichel H, 2018. Efficient and low-cost alternative of lipase concentration aiming at the application in the treatment of waste cooking oils. Bioprocess Biosyst Eng 41(6): 851-857.

Rosa DR, Cammarota MC, Freire DMG, 2006. Production and utilization of a novel solid enzymatic preparation produced by *Penicillium restrictum* in activated sludge systems treating wastewater with high levels of oil and grease. Environ Eng Sci 23(5): 814-823.

Sandhya C, Sumantha A, Szakacs G, Pandey A, 2005. Comparative evaluation of neutral protease production by *Aspergillus oryzae* in submerged and solid-state fermentation. Process Biochem 40(8): 2689-2694.

Shawabkeh R, Khleifat KM, Al-Majali I, Tarawneh K, 2007. Rate of biodegradation of phenol by *Klebsiella oxytoca* in minimal medium and nutrient broth conditions. Bioremediat J 11(1): 13-19.

Singh-Ackbarali D, Maharaj R, Mohamed N, Ramjattan-Harry V, 2017. Potential of used frying oil in paving material: solution to environmental pollution problem. Environ Sci Pollut Res Int 24(13): 12220-12226.

Sreelatha B, Rao VK, Kumar RR, Girisham S, Reddy SM, 2017. Culture conditions for the production of thermostable lipase by *Thermomyces lanuginosus*. Beni-Suef univ j basic appl sci 6(1): 87-95.

Takeno K, Yamaoka Y, Sasaki K, 2005. Treatment of oil-containing sewage wastewater using immobilized photosynthetic bacteria. World J Microbiol Biotechnol 21(8-9): 1385-1391.

Talukder MMR, Wu JC, Chua LPL, 2010. Conversion of waste cooking oil to biodiesel via enzymatic hydrolysis followed by chemical esterification. Energy Fuels 24(3): 2016-2019.

Vescovi V, Rojas MJ, Baraldo Jr A, Botta DC, Santana FAM, Costa JP, Tardioli PW, 2016. Lipase-catalyzed production of biodiesel by hydrolysis of waste cooking oil followed by esterification of free fatty acids. J Am Oil Chem Soc 93(12): 1615-1624.

Wallace T, Gibbons D, O'Dwyer M, Curran TP, 2017. International evolution of fat, oil and grease (FOG) waste management–a review. J Environ Manage 187: 424-435.

Winkler UK, Stuckmann M, 1979. Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. J Bacteriol 138(3): 663-670.

Wu L, Ge G, Wan J, 2009. Biodegradation of oil wastewater by free and immobilized *Yarrowia lipolytica* W 29. J Environ Sci (China) 21(2): 237-242.

Xia JL, Huang B, Nie ZY, Wang W, 2011. Production and characterization of alkaline extracellular lipase from newly isolated strain *Aspergillus awamori* HB-03. J CENT SOUTH UNIV T 18(5): 1425.

Xu MH, Kuan IC, Deng FY, Lee SL, Kao WC, Yu CY, 2016. Immobilization of lipase from *Candida rugosa* and its application for the synthesis of biodiesel in a two-step process. Asia-Pac J Chem Eng 11(6): 910-917.

Yau YH, Rudolph V, Lo CCM, Wu KC, 2018. Restaurant oil and grease management in Hong Kong. Environ Sci Pollut Res Int. 1-11.

Yuwa-amornpitak T, Chookietwatana K, 2018. Bioconversion of waste cooking oil glycerol from cabbage extract to lactic acid by *Rhizopus microsporus*. Braz J Microbiol 49: 178-184.

Zhang D, Chen M, Wu S, Liu J, Amirkhanian S, 2017. Analysis of the relationships between waste cooking oil qualities and rejuvenated asphalt properties. Materials 10(5): 508.