SENRA Academic Publishers, Burnaby, British Columbia
Vol. 4, No. 2, pp. 1207-1216, June 2010
ISSN: 1715-9997

Canadian Journal of pure&applied sciences

ENZYMATIC SURFACE HYDROLYSIS OF POLY(ETHYLENETEREPHTHALATE) BY LIPASE ENZYME AND ITS PRODUCTION

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ABSTRACT

In this study, hydrolysis of the surface of poly(ethyleneterephthalate) (PET) fabric by *Bacillus* isolate 5W and 6C lipases was confirmed by improved hydrophilic property which tested by wetability and staining with basic dyes, scanning electron microscope, and FTIR measurements. Lipase treated PET fabrics showed good physical and mechanical properties. It was observed that the retained strength of lipase treated PET was 96-98% and pilling and static charges were reduced. *Bacillus* isolate 5W was chosen for economic production of PET surface modifying lipase enzyme in 14 agro-industrial by-products. The most suitable substrate for the highest lipase production was cotton seed meal at 6% and initial pH 7.5 (90U/ml). Effect of volume of the medium in the flask, inoculums size and incubation period were investigated and 25 ml of the medium, 25 x 10^6 CFU/ml, and 24h, respectively were the optimal. Addition of peptone to cotton seed meal medium enhanced the lipase production 22.5% (130.7 U/ml). The results of this article focuses on production of lipase enzyme that hydrolyze PET under feasible and economic conditions for replacing harsh chemical processes currently used for hydrophilization.

Keywords: Lipase production, Poly(ethyleneterephthalate), surface modification, *Bacillus*, agro-industrial by-products.

INTRODUCTION

In the last few years there has been increasing interest in enzymatic surface modification of poly(ethyleneterephthalate) (PET), a synthetic polymer which widely used in the textile industry with an annual production of 36 million tons (Alisch-Mark *et al*., 2004; Fischer-Colbrie *et al*., 2004; Vertommen *et al*., 2005; Alisch-Mark *et al*., 2006; Heumann *et al*., 2006; Kim and Song, 2006; Silva *et al*., 2007; Guebitz and Cavaco-Paulo, 2008). PET produced from purified terephthalic acid or alternatively dimethyl terephthalate and ethylene glycol. PET has become very successful within the fashion industry due to its chemical resistance, wrinkle resistance and quick-drying properties. However it has a moisture regain of 0.4% (measured at 20°C and 65% relative humidity), this means that PET is extremely hydrophobic. Therefore, the use of PET in textile applications such as sportswear, underwear and bedding is restricted due to a relatively low level of comfort, as moisture is not absorbed nor drawn away from the skin. Additionally, PET exhibits static problems such as cling during wear and difficulty in cutting and sewing.

Consequently, increasing its hydrophilicity is essential for many applications ranging from textiles to medical and electronics (Guebitz and Cavaco-Paulo, 2008). Alkaline treatment is used to increase hydrophilicity of PET based textile materials (Shalaby *et al*., 2007). However, it leads to the deterioration of the fiber properties such as formation of pit-like structure as a result of high weight loss and reduction of fiber strength (Shalaby *et al*., 2007).

Recent studies clearly indicate that the modification of synthetic and natural polymers with enzymes is an environmentally friendly alternative to chemical methods using harsh conditions. Potential of microbial enzymes for the targeted surface functionalization of synthetic fibers has recently been assessed (Fischer-Colbrie *et al*., 2003). The major advantages of enzymes in polymer modification compared to the chemical methods are milder reaction conditions and easier control, environmental friendlier process, and specific non-destructive transformations on polymer surfaces (Matama *et al*., 2006).

Enzymes like lipases (EC. 3.1.1) and cutinase (EC. 3.1.1.74) may potentially hydrolyze the surface ester bonds of PET resulting in superficial formation of hydroxyl and carboxyl groups (Killis *et al*., 2001; Gubitz and Cavaco-Paulo, 2003; Silva and Cavaco-Paulo, 2004; Hasan *et al*., 2006). Due to the size of enzymes and the insoluble nature of PET fiber in an aqueous medium, the enzymes are merely active at the surface so that the bulk characteristics of fibers remain unchanged.

Recently attempts to isolate microorganisms that produce lipase enzyme which used for surface modification of textile gain a special interest.

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The purpose of the present study is selection of a novel *Bacillus* isolate that is capable of producing lipase enzyme that has the ability to modify PET surface without losing its strength. In addition, production of this enzyme by using industrial by-products as cost effective method for feasible application in textile industry. The results of this work support the research for potential utilization of lipase as a hydrolytic source for modification of PET.

MATERIALS AND METHODS

MATERIALS

p-nitrophenyl-acetate (*P*-NPA) was from Sigma, sodium dihydrogen phosphate, disodium hydrogen phosphate, non-ionic detergent Hostpal CVL-EL from Clarient, glacial acetic acid purchased from El-Naser Pharmaceutical Chemical Company.

Basic dyes (C.I Basic Red 18 and C.I Basic Yellow 28) kindly supplied by European Colour Plc, Stockport, England, however, Methylene Blue was from Merck. All other used chemicals were of analytical grade.

Polyester 100% fabric was provided by Hosam Textile Company, Bourge El-Arab, Alexandria, Egypt. Specification of woven fabric is 22 ends/inch, 20 picks/inch and 177g/m^2 fabric weight.

Agro-industrial byproducts were from Oil Extraction Unit and Animal Nutrition Department at National Research Center. These Agro-industrial byproducts were sugar beet pulp, potato peels, orange peels, pea peels, beans peels, banana peels, carrots pomace, jojoba meal, cress meal coconut meal, linen meal, sesame meal, wheat germ meal, and cotton seed meal.

METHODS

Screening of lypolytic bacilli

Ninety one *Bacillus* isolates were screened for lipase production by plating on tributyrin agar medium. These isolates were isolated from underground sand of underground spring, sands carried by winds during spring season in Cairo, sands and plant roots from the Egyptian desert, and sugar cane roots from upper Egypt. The bacterial isolates producing maximum clear zone were selected, subcultured, and stored on nutrient yeast agar slants at 4°C.

Media used

Nutrient yeast broth (NYB) contains per liter, 5g peptone, 3g beef extract and 5g yeast extract. For solidification, 20g agar is added.

Tributyrin agar medium (TBA) contains per liter, 5g peptone, 3g yeast extract, 10ml tributyrin, and 20g agar.

Agro-industrial by-products media contained 60g of agro-industrial wastes in 1 liter tap water.

Growth conditions and Preparation of cell free extracts

The isolates were grown in 25ml NYB medium in Erlenmeyer conical flask (250ml) for 3 days at 30°C and 150rpm on an orbital shaker. The cultures were harvested by centrifugation at 10000 x g for 10min. The supernatant was examined for lipase activity.

Lipase activity assay

Lipase activity was assayed according to Meghwanshi *et al*. (2006) and Chen *et al*. (2007) with some modifications. Lipase activity was determined using *p*-nitrophenyl acetate (*p*-NPA) as substrate. The substrate solution was prepared by dissolving *p*-NPA in isopropanol. The mixture of 880µl of 50mM phosphate buffer (pH 8), 20µl of substrate solution and 100µl of suitably diluted enzyme solution was incubated at the tested temperature for 30min. The reaction was terminated by the addition of 0.2ml of 100mM $CaCl_2$ solution (at 0°C) and keeping it on ice. The reaction mixture was centrifuged to clarify the solution and the absorbance of the yellow color of the supernatant was read at 410nm.

One unit of enzyme activity was defined as a 0.1 increase in OD_{410} under the standard assay conditions.

Enzymatic treatments of textile

Enzymatic treatments of textile were according to (O 'Neil, 2007). All fabric samples were washed to remove the impurities. The fabric was washed with 10g/l hostapal at 65°C for 1 h, followed by rinsing several times with tap water then the fabric was washed in aqueous solution contain 2g/l sodium carbonate for 1h at 65 °C, followed by rinsing several times with tap water. Finally, the fabrics gently squeezed and air dried. Two sets of experiments were performed. One gram of the fabric was incubated in a glass vessel containing a solution of 0.05 M sodium phosphate buffer (pH 8) using the same amount of crude enzyme (4%), the liquor ratio was 50:1. One set of experiment was incubated at 50°C for 8 h and the other set was incubated at 37°C for 24h on an orbital shaker at 150rpm. After enzymatic treatments, all samples were washed several times with tap water then with 2g/l sodium carbonate for 1h at 50°C followed by washing with distilled water for 1h at 50°C. Finally, the samples were washed with running tap water for 5 min and allowed to dry in open air.

Staining the fabrics

The staining was carried out below the glass temperature (Tg) of PET (at 50°C for 90 min) as described by O 'Neil (2007). After enzymatic treatments, the fabric samples were stained together in the same sealed glass vessel (500ml) on shaking water bath at proper pH. The dyeing was performed with 0.5 % shade owf, liquor ratio 50:1 and at 200rpm agitation. After dyeing process, samples

were washed with aqueous solution containing 2g/l non ionic detergent at 55°C for 1h then, washed with tap water and dried in oven at 50°C for 6h.

Wetability test
A water drop test was applied to lipase treated fabric and control samples according to AATCC standard method.

Colour Measurement
The K⁄S percentage increments after the enzymatic treatments and staining of the fabric samples were measured in order to detect differences in reactive groups formed on the surface according to Matama *et al*. (2006) and Silva *et al*. (2007). The colourimetric data K⁄S of the dyed samples was collected using Mini Scan XE integrated with Hunter lab universal software at maximum absorption, as an average of three readings. The relative K⁄S of enzymatic treated samples was calculated as follow:

$$\% \text{ Relative K/S} = \{(K/S)_{treated} - (K/S)_{control}\}/(K/S)_{control} * 100$$

Scanning electron microscope (SEM)
Changes of the fiber surface morphology due to enzymatic treatment were followed by SEM model JEOL JXA-84OH Electron Prope Microanalyzer, Japan operating at 19 KV. A thin coating film (~10 nm) of gold was deposited onto the samples, before examination by SEM.

FTIR measurements
To investigate the effect of enzymatic treatment on structure of the PET fabrics surface, the fabrics were analyzed by FTIR spectroscopy using Nicolet 380 spectrophotometer in the spectral range 4000-650 cm^{-1}, with a resolution of 4 cm^{-1} and a number of scans of 128. Fabrics were measured by "Smart Performer ATR" unit accessory with zinc selenide crystal.

Transfer printing
Transfer printing was done by using two disperse dyes, C.I Disperse blue 56 and C.I Disperse Red 60.

Weight loss
Weight loss, was evaluated according to the following equation

$$\text{Weight loss } \% = (W_1 - W_2)/W_1 * 100$$

Where W_1, W_2 are the weight before and weight after enzymatic hydrolysis, respectively.

Oily Stain resistance
Oily stain release was measured according to AATCC Test method 130-1992.

Pilling resistance
Pilling resistance of PET fabrics was measured according to ASTM 4966/4970 (2002) using Martindale Abrasion and Piling Testers (M235 Martindale SDL ATLAB, England).

Air permeability
Air permeability was measured according to ASTM D 737 (96).

Static charge
Static charge was measured using electricity collect type Potentiometer Type KS- 525 KASUGA, DENKJ, Inc., Tokyo, Japan.

Tensile strength
Tensile strength of PET fabrics was measured according to ASTM D3822-01 by INSTRON, USA. Tensile strength results are the arithmetic means of three tests per sample.

Morphological and biochemical identification of isolated *Bacillus*
Bacillus isolate which produce high lipase enzyme, and showed good PET surface modification was identified using standard morphological and biochemical procedures according to Gordan (1974), Claus and Berkeley (1986) and Stukus (1997).

Optimization of the medium conditions for lipase production
1. A group of locally available agro-industrial by-products were tested as possible media for lipase production.
2. Effect of the best agro-industrial byproduct concentrations on lipase production was studied.
3. Effect of initial pH of the medium was studied by buffering the medium with 0.05M phosphate buffer at pH values ranging between 5.5 and 9.0.
4. Effect of aeration level was tested by varying the volume of the medium in the experimental flasks between 6-100ml.
5. Effect of inoculum size was studied by varying the inoculum size between 5 $x10^6$ and $25x10^7$ CFU/ml.
6. Effect of incubation period was investigated by incubation for varying periods up to five days.
7. Effect of supplementation of the medium with additional nutrient sources at 2% final concentration was studied.

RESULTS AND DISCUSSION

Screening of *Bacillus* isolates for lipase production.
Thirty one of the tested *Bacillus* isolates showed high lypolytic activity as clear zone around their colonies on TBA medium.

Quantitative screening for lipase activity by *P*-NPA showed that 19 *Bacillus* isolates (2U, 1W, 2W, 5W, 6C, 8C, 12C, 14C, 11P, 16P, 18P, 19P, 20P, 21P, 22P, 36P, 38P, 41P and 45P) were the most lypolytic isolates at 37°C. Most of them showed promising activities at 50°C (data not shown).

The literature reported that several *Bacillus* species isolated from several diverse environments produced lipase enzyme (Sharma *et al*., 2001; Kumar *et al*., 2005; Hasan *et al*., 2006; Deive *et al*., 2007).

Lipase enzymes produced by the above 19 *Bacillus* isolates were examined for hydrolysis of PET fabrics.

Wetability of lipase treated PET fabrics

The results of wetability of PET fabrics treated with lipase of *Bacillus* isolates 2U, 5W, 6C, 16P and 22P at 37°C for 24h were more effective than at 50°C for 8h as proved by the decrement of the time for water drop absorption as shown in table 1. This revealed that they are promising lipase producers for improving surface wetability of PET fabrics. These results indicated that the amount of hydrophilic groups (OH and COOH) at the surface of PET increased as a result of lipase treatment. Hsieh and Cram (1998) reported that, the surface wetability of enzyme treated substrates is associated with hydrolytic action rate.

Cationic Dye Binding

Significant increase in K/S values were observed for stained lipase treated PET fabrics. The results supported that the enzymatic treatment increased adhesion of cationic compounds probably by creating carboxyl groups on the surface of the treated fabrics. The staining degree of lipase treated PET fabrics varies according to the basic dye used which may be attributed to the size of dye molecule and the pore size created on the fabric surface as a result of enzymatic hydrolysis. PET fabrics treated with lipase enzyme produced by *Bacillus* isolates 5W and 6C at 37°C for 24 h showed promising increase in K/S percentage after staining by Methylene Blue, C.I. Basic Yellow 28 and C.I. Basic Red 18 as shown in table 2.

Transfer Printing

The impact of lipase treatment on transfer printing of PET using two different disperse dyes, C.I Disperse Blue 56 and C.I Disperse Red 60 was shown in table 3. The results revealed that a significant improvement of color strength of printed modified PET fabrics. Lipase enzyme produced by *Bacillus* isolate 5W enhanced the relative color strength to large extent.

Physical and Mechanical properties

Table 4 illustrates the effect of lipase treatment on some physical and mechanical properties of PET fabrics. Based on these data, it was observed that breaking strength of PET decreased with treatment of the fabrics with lipase enzymes produced by *Bacillus* isolates 5W and 6C to a limited extent and 96-98% of the strength of the fabric was retained. The decrease in breaking strength may be due to the formation of pits on the surface which probably

Table 1. Wetability of PET fabrics treated with lipase produced by *Bacillus* isolates

Bacillus isolate	Lipase activity (U/ml) at		Wetting time (min) of lipase treated PET at 37 °C for 24 h	Wetting time (min) of lipase treated PET at 50°C for 8 h
	37°C	50°C		
2U	39.6	29.4	0.22	1.6
5W	39.8	32.4	0.23	1.2
6C	19.2	21.0	0.28	3.8
16P	30.7	21.2	0.43	5.37
22P	30.2	20.9	0.33	2.37
Control (buffer)	-	-	6.3	3.3

Table 2. Relative K/S of dyed PET fabric treated with lipase enzyme produced by *Bacillus* isolates.

Lipase produced by *Bacillus* isolate	% Relative K/S of PET treated with lipase enzyme at 37 °C for 24h or 50°C for 8h and stained by					
	Methylene blue λ_{max} 650 pH 9.5		C.I. Basic yellow 28 λ_{max} 460 pH 4.5		C.I. Basic Red 18 λ_{max} 520 pH 4.5	
	37°C for 24 h	50°C for 8 h	37°C for 24 h	50°C for 8 h	37°C for 24 h	50°C for 8 h
2U	-13.6	5.7	18.4	3.16	6.3	-2.1
5W	28	9.49	18.4	17.6	84	-27.3
6C	18.4	5.13	65.9	18.35	56.3	32.87
16P	12.4	3.8	22.09	-24.05	12.8	2.8
22P	-3.23	5.13	1.58	7.6	27.3	5.87

-ve value means that K/S of control is more than K/S of treated samples.

act as weak points when the fabric is elongated under stress. In addition, the amount of static charge decreased as a result of lipase treatment of PET. This may be attributed to the creation of hydroxyl and carboxyl groups consequently enhanced the moisture regain and the amount of static charge decreased. Improvement of pilling resistance and dissipation of electrostatic charge soil release resistance were noticed. Lipase pretreatment of PET also reduced the susceptibility of the fabric to stain by corn oil in comparison to the control. Presumably enzymatic hydrolysis of PET and formation of hydroxyl and carboxyl groups decrease hydrophobicity and attraction for oily soil thereby enabling the easier removal of the corn oil stain.

Scanning Electron Microscope

The physical modification of lipase treated PET fabrics was examined by SEM. As shown in figure 1, a significant modification of the fiber surface was observed. The surface of PET fabrics treated with buffer was smooth however, the enzymatic treated fabrics gained heterogeneous appearance such as cracks, pits, pores, and rough surfaces.

FTIR measurements

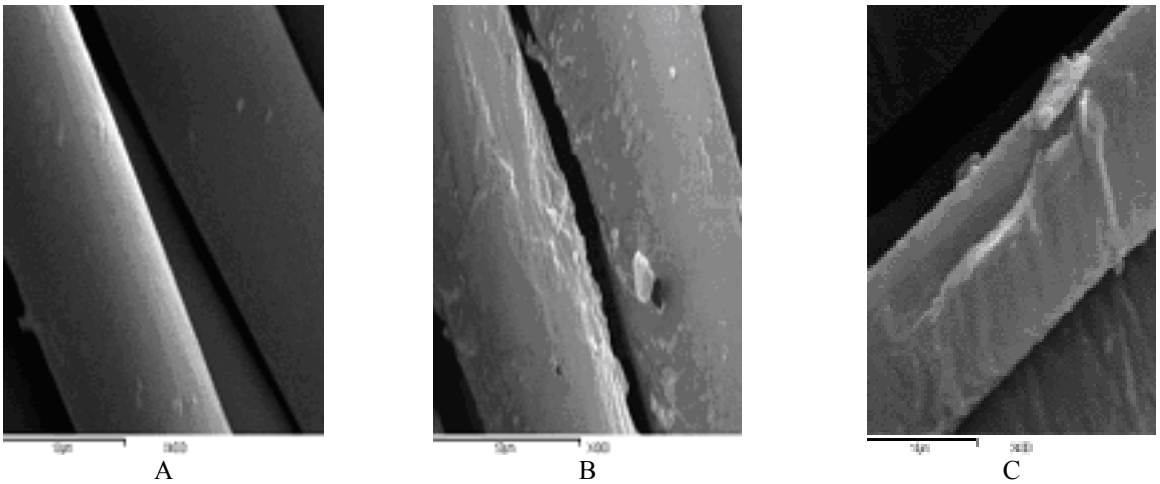
Infrared spectra for PET fabric samples are shown in figure 2. Different spectra were obtained for control (denaturated enzyme) and lipase treated samples. There is intensity differences in some bands between lipase treated and untreated fabrics. The selected region used for comparison is from 1350-1150 cm^{-1}, where the peak of C-O is located at 1241cm^{-1}. The spectra reveal an increase in the absorbance of C-O vibration for the lipase treated samples as compared to the control. This may be attributed to the formation of carboxyl groups (COOH) due to lipase hydrolysis of the ester linkages in PET producing polar carboxyl and hydroxyl groups.

Table 3. Effect of Lipase treatment of PET fabric on transfer printing.

Lipase produced by *Bacillus* isolate	Relative K/S		% Increase in K/S	
	C.I Disperse Blue 56	C.I Disperse Red 60	C.I Disperse Blue 56	C.I Disperse Red 60
5W	0.82	4.331	148.5	305.5
6C	0.4	1.1242	21.2	5.24
Control (buffer)	0.33	1.068	-	-

Table 4. Effect of lipase treatment of PET fabric on physical and mechanical properties.

Lipase produced by *Bacillus* isolate	Weight loss (%)	Breaking strength (Kg)	Retained strength (%)	Elongation (%)	Static charge (KV)	Pilling resistance	Soil release
5W	0.35	108	95.57	24.1	6.6	3-4	3
6C	0.3	111	98.2	26.1	7.66	3-4	2-3
Control (buffer)	0.31	113	100	23.3	8	2	1



A B C

Fig.1. SEM of PET fabric treated with buffer solution (A) and lipases of *Bacillus* isolate 5W and 6C (B and C, respectively).

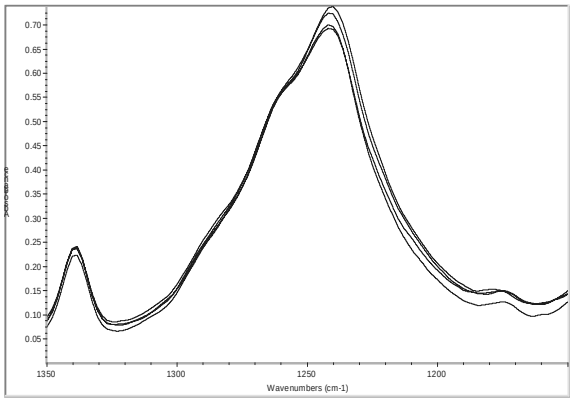


Fig. 2. FTIR spectra of PET fabric treated with (from bottom to top) denaturated enzyme of *Bacillus* isolate 5W, denaturated enzyme of *Bacillus* isolate 6C, lipase enzyme of *Bacillus* isolate 6C and lipase enzyme of *Bacillus* isolate 5W showing increase in the absorbance of the C-O vibrations (1241 cm^{-1}) as a result of lipase treatment.

Production of lipase by *Bacillus* isolate 5W on agro-industrial by-products.

Bacillus isolate 5W produced higher lipase activity and PET fabrics treated with this lipase showed good wetability, staining, physical and mechanical properties. These results supported by SEM and FTIR studies, which proved the modification of PET surfaces. Therefore, this isolate was selected for further studies. It was identified according to the morphological and biochemical tests as *Bacillus subtilis.*

Fourteen agro-industrial residues were studied for production of lipase by *Bacillus* isolate 5W without pretreatment under submerged fermentation conditions. As shown in table 5 nine substrates enhanced the lipase production by the tested isolate as or more than NYB medium. The most efficient media were cotton seed meal, banana peels, wheat germ meal, and cress meal. The maximum production (52.8U/ml) was obtained with cotton seed meal (CSM) medium. Concentrations of CSM was tested and maximum lipase yield was at 6%.

Haba *et al*. (2000) used frying oils wastes for production of lipase by 47 strains of bacteria and yeasts. They reported that the genera *Pseudomonas*, *Bacillus*, *Candida*, *Rhodococcus* and *Staphylococcus* grew on the waste oil and produced high lypolytic activity. Rohit *et al*. (2001) and Mohan *et al*. (2008) reported that high levels of lipase activity were obtained by *Bacillus* strains grown in olive oil as a medium. Ertugrul *et al*. (2007) found that 20% whey with 1% triolein gave the highest lipase activity by *Bacillus* sp. Souissi *et al*. (2009) produced lipase by *Staphylococcus simulans* grown on sardinella hydrolyzates and peptone. Lin *et al*. (1996) reported an extracellular alkaline lipase produced by *Pseudomonas alcaligenes* F-111 in a medium that contained soybean meal, peptone and yeast extract. Kumar *et al*. (2005) produced lipase by *Bacillus coagulans* BTS-3 with refined mustard oil, peptone and yeast extract.

Table 5. Production of lipase by *Bacillus* isolate 5W on agro-industrial by-products.

Industrial by-products	Final pH	Lipase activity (U/ml)
Sugar beet pulp	6.16	5.25
Potato peels	7.25	24.6
Orange peels	5.74	0.0
Pea peels	7.47	36.0
Bean peels	6.25	30.8
Banana peels	6.65	44.6
Carrot pomace	7.65	33.1
Jojoba meal	7.32	17.8
Cress meal	8.4	40.1
Coconut meal	5.94	5.25
Linen meal	8.32	32.4
Sesame meal	7.25	31.4
Wheat germ meal	8.16	43.1
Cotton seed meal	8.41	52.8
NYB	8.18	34.5

Effect of initial pH of the medium

Effect of initial pH of the medium on lipase production by *Bacillus* isolate 5W is shown in figure 3. There is an increase in the lipase production with increasing the pH between 5.5 and 7.5. Increasing the pH of the medium more than 7.5 has destructive effect on the lipase production by the tested organism. These results are in consistence with that reported by Achamma *et al*. (2003) and Deive *et al*. (2007). They found that lipase production by *Bacillus* sp. and *Pseudomonas aeruginosa* MB was the maximum at neutral pH. However Ertugrul *et al*. (2007) reported that the optimal pH for lipase production by *Bacillus* sp was 6. Although bacteria prefer pH around 7 for best lipase production, maximum activity at higher pH values were also reported in the literature (Kumar *et al*., 2005).

Effect of aeration level on lipase production

The enzyme levels formed at different aeration levels under shaking conditions are shown in figure 4. The maximum lipase production (91.2U/ml) was at 25ml medium in 250ml flask (10% of the flask volume).

Effect of inoculum size

Lipase activity of *Bacillus* isolate 5W reached its maximum value (109.3 U/ml) upon using approximately $25x10^6$ CFU/ml as shown in figure 5. However, further increase in inoculum size lead to a dramatic decrease in lipase production.

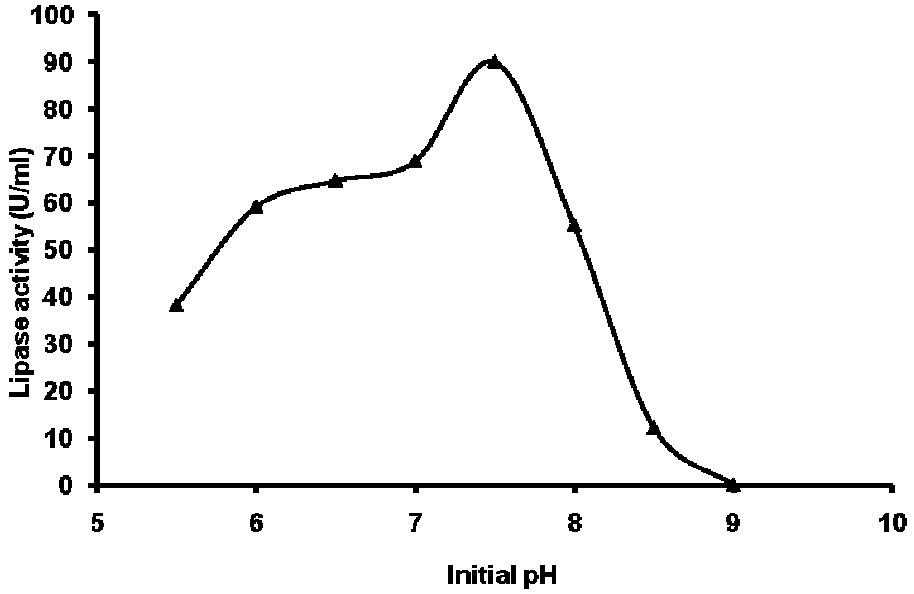


Fig. 3. Effect of initial pH of the medium on lipase production by *Bacillus* isolate 5W.

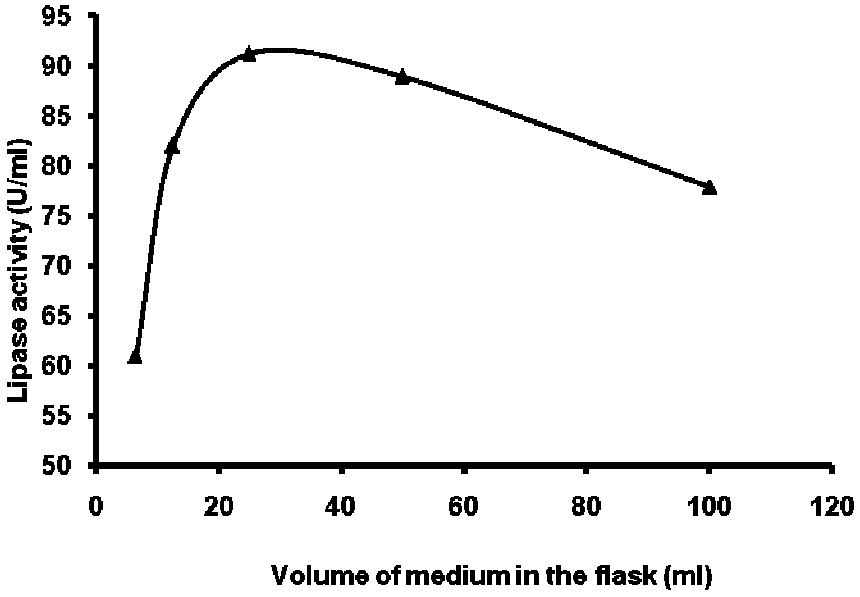


Fig. 4. Effect of aeration level on lipase production by *Bacillus* isolate 5W.

Effect of incubation period

Lipase production by *Bacillus* isolate 5W grown for extended incubation periods up to 96h. The highest lipase production was obtained after 24h of incubation and no significant variations could be observed in activity with the increment of the incubation period up to 96h. Kumar *et al*. (2005) and Ertugrul *et al*. (2007) reported that the time course for the maximum intracellular lipase production by *Bacillus* was 48 and 63h, respectively.

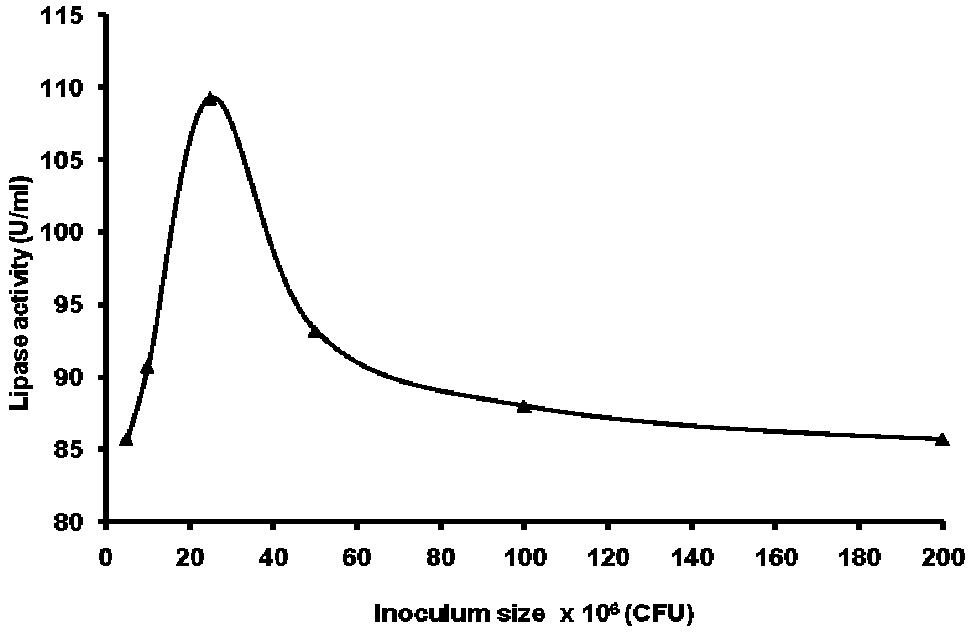


Fig. 5. Effect of inoculum size on lipase production by *Bacillus* isolate 5W.

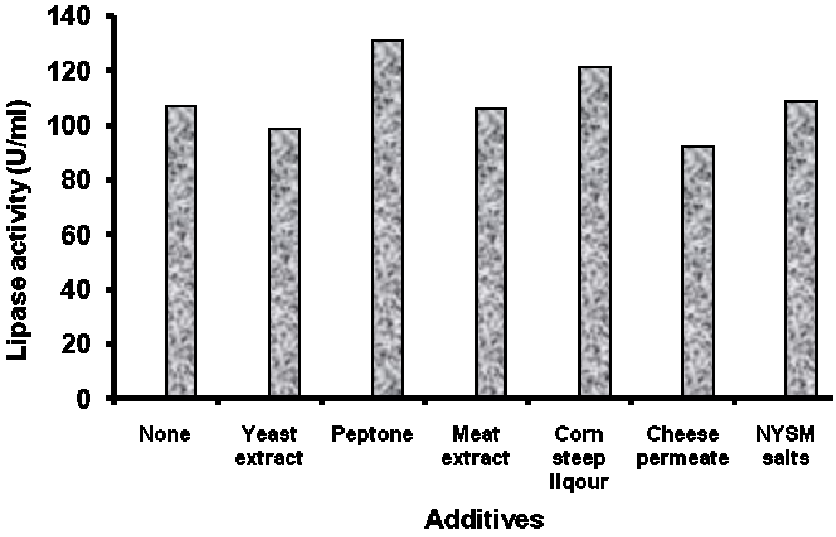


Fig. 6. Effect of additives on the lipase production by *Bacillus* isolate 5W.

Effect of supplementation of CSM with additional nutritional sources

Selected nutritional sources were incorporated as supplements to CSM medium for further enrichment of the growth medium. As shown in figure 6. Peptone and corn steep liquor at 2% enhanced the lipase production about 22.5% (130.7 U/ml) and 9% (121.1U/ml), respectively. Lin *et al*. (1996); Kumar *et al*. (2005) and Souissi *et al*. (2009) added peptone to their media for production of lipase by *Pseudomonas alcaligenes* F-111, *Bacillus coagulans* BTS-3 and *Staphylococcus simulans*, respectively. Corn steep liquor was an optimal nitrogen

source for intracellular and extracellular lipase production by the fungus *Rhizopium oryzae* as reported by Essamri *et al*. (1998) and Hiol *et al*. (2000), respectively.

Effect of metal ions

A group of metal ions (Ca^{2+}, Mg^{2+}, Na^{+}, Co^{2+}, Cu^{2+}, Fe^{2+}, K^{+}, Mn^{2+}, Mo^{2+} , and Zn^{2+}) were added to the medium separately as lipase enhancer but they did not enhance the lipase production with exception of Zn^{2+} which enhanced its production by 8%.

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Received: April 3, 2010; Accepted: May 26, 2010