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Electro-fermentative production of thermotolerant lipases by electrogenic bacteria isolated from palm oil mill effluents

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Abstract

This study aimed at assessing the effects of Bio-electrochemical system on the yield of thermostable lipase produced by *Bacillus licheniformis* S1S2, *Bacillus pulmilus* S1E23, *Bacillus* sp. S1E27, and *Aeribacillus* sp. S1E29. With physiological conditioning of the lipolytic activities, comparative production of thermostable lipase by the selected isolates was carried using submerged fermentation and electro-fermentation methods. An optimum lipase yield of 95.1± 0.003 U/ ml, 128± 0.001 U/ml, 110.7± 0.003 U/ ml and 122.7± 0.002 U/ml was produced by *B. licheniformis* S1S2, *B. pulmilus* S1E23, *Bacillus* sp. S1E27, and *Aeribacillus* sp. S1E29; respectively, under conventional fermentation process. However, under electro-fermentation, increased enzyme yields of 129.6± 0.002 U/ml, 164.9± 0.001 U/ml, 125.3± 0.002 U/ml, and 136.6± 0.001 U/ml, for same respective isolates was obtained. The biophysical characteristics of the enzymes produced showed that the thermostable lipases had optimum activity at a temperature range of 45^{o}C - 55^{o}C, pH of 7 - 9, and enhanced activity in the presence of Ca^{2+}, Mg^{2+}, and Zn^{2+}, however, Hg^{2+} caused a slight loss in the lipase activity.

Keywords: Bacteria, Lipase, Bio-electrochemical, Submerged fermentation

1. Introduction

The participation of lipases in the worldwide enzymes industry market has grown significantly, and it is believed that in the future they will acquire more importance comparable to that of the peptidases, which currently represent 25 to 40% of the industrial enzyme sales (Hasan *et al*., 2006). Lipases act on the carboxyl ester bonds present in the triacylglycerols (major constituents of fats and oils) and liberate fatty acids and glycerol (Abrunhosa *et al*., 2013). According to Hasan *et al*., (2006), they are also involved in a wide range of conversion reactions such as; esterification, inter-esterification, trans-esterification, alcoholysis, acidolysis and aminolysis in non-aqueous media. This versatility makes lipases the enzymes of choice for applications in the various industries mentioned earlier. However, most of these

lipases-utilizing industrial processes operate at temperatures exceeding 45°C, although most of lipases lose their activity above 45ºC, thereby causing a high demand on commercial thermostable lipases, as they allow enzymatic reactions to be carried out at high temperatures. In previous studies of Turner *et al*., (2007); Synowiecki, (2010), they reported that the advantage of choosing thermostable lipases is the intrinsic thermostability, which allows long time storage, reduced risk of microbial contamination, increased tolerance to; organic solvents, detergents, low and high pH. Moreover, Salihu and Alam, (2014) added that the thermostable lipases allows high rate of product formation with minimal diffusion restriction, high dissolution of hydrophobic substrates, high conversion efficiency, and increased kinetic energy of the reactants.

Despite the wide potential applications of thermostable lipases, the conventional processes for microbial production of lipases have been plagued with the challenge of low yield, although various strategies for optimizing the yields have been investigated and developed. However, these strategies have not satisfied the high demand for thermostable lipase by the various industries (Turner *et al*., 2007). Moreover, the effects of electro-inductive processes caused by microorganisms on substrate hydrolysis via enzyme catalysis have not been fully explored. On the other hand, Agler, (2011) pointed that the direct flow of electrons through microorganisms by the introduction of electrodes has been suggested to allow for the control of fermentation environments, resulting in higher yield of products, in addition to favorable microbial cell growth and density. Moscoviz *et al*., (2016); Schievano *et al*., (2016) recently developed an approach called Electro-fermentation (EF) that entails fermentative activity within bio-electrochemical systems (BESs), to add a supplementary way of controlling fermentation patterns by stimulating the metabolism of the microbial cells electrogenically. The availability of anode as an electron acceptor enables the microbes to oxidize NADH and recover NAD^+, using the electrode as an extracellular electron sink (anodic respiration). This allows stabilizing the microbial redox state and/or redirecting the metabolic carbon flow towards the product of interest (Kracke *et al*., 2015).

Under the rationale that an electrode can act as an electron sink in order to balance the intracellular energy and redox co-factors, the objective of this study was to investigate the effects of bio-electrochemical system on thermostable lipase production in some selected electroactive bacteria, compared with the conventional fermentation system.

2. Material and methods

2.1. Microorganisms

Lipolytic strains of *Bacillus licheniformis* S1S2, *Bacillus pulmilus* S1E23, *Bacillus* sp. S1E27, and *Aeribacillus* sp. S1E29 utilized in this study were obtained from the Microbial Culture Collection Center of The Department of Microbiology, University of Ibadan, Nigeria. These bacterial spp. were previously isolated from palm oil mill effluent, maintained on nutrient agar slants (NA), and sub-cultured monthly.

2.2. Screening of microorganisms for electrogenicity

Screening for electrogenicity of the bacterial isolates was carried out in a two-chambered bio-electrochemical system according to the procedure of Ajunwa *et al*., (2018), using a minimal salt medium (composed of 1.2 g/l Magnesium sulphate, 5 g/l Ammonium sulphate, 0.4 g/l Dihydrogen potassium phosphate, 1 g/l Potassium dihydrogen phosphate, and 1% v/v of olive oil). The bio-electrochemical system had a 100 cm^3 anodic and cathodic chamber volume, with 49 cm^2 surface area of graphite electrodes both the cathode and the anode. Nafion proton exchange membrane and copper wire connections were used to connect the electrodes to a digital multimeter (model DM-87, HTC Instruments®), used to measure the electric current in the circuit A 100 Ω resistor was also used to close the circuit.

2.3. Lipase production

Lipase production was carried by inoculating a loopful of each bacterium in both of the Erlenmeyer flasks (submerged fermentation), and the Bio-electrochemical chambers (electrofermentation), containing 100 ml of minimal salt medium (Ajunwa *et al*., 2018), supplemented with olive oil. This setup was incubated for 5 d at room temperature. After fermentation, the culture broths were centrifuged at 4000 rpm for 20 min., and the cell free supernatant obtained was considered to be the crude enzyme. The thermal stability of the produced lipase was determined by incubating the crude enzyme at different temperatures (30-60°C) in a water bath (Clifton 76988) for 30 min., after which the residual enzyme activity (expressed in 100% of relative activity) was investigated according to Jittima *et al*., (2011).

2.4. Estimation of lipase activity

The lipase activity was determined spectrophotometrically by a modified method of Zhera and Metin, (2015), using p-nitrophenyl-palmitate (pNPP) as a substrate. The reaction mixture consisted of 0.1 ml crude enzyme solution, 0.8 ml 50 mM Tris-HCl buffer (pH 8.0), and 0.1 ml 10 mM pNPP dissolved in ethanol. The hydrolytic reaction was carried out at 45°C for 30 min. Following incubation, approximately 0.25 ml of 0.1 M Na_2CO_3 was added to stop the reaction. The mixture was centrifuged and then the absorbance was measured at 410 nm. One unit of lipase activity was defined as the amount of enzyme that caused the release of 1 µmol of p-nitrophenol (molar absorption coefficient 17.34 mM^{-1} cm^{-1}) from pNPP in one min., under the stated experimental conditions.

2.5. Lipase production in electro-fermentation and submerged fermentation systems

The standard physiological conditions used for the lipase production by the electrogenic bacteria were temperature of 37^{o}C, pH 7, incubation time for 72 h, and 1% v/v of olive oil. However, the effects of different incubation periods, substrate types, concentrations and different catholytes on lipase production were investigated is briefly described below. Determination of the effect of different incubation periods on enzyme production, involved the inoculation of the bacterial strains under different fermentation set-ups at varied incubation times (24, 48 and 72h) before the assay. For evaluating the effects of different lipid substrates on enzyme production, several lipid sources such as; shea butter oil, olive oil, castor oil and coconut oil, were added individually to the production medium at a concentration of 1% (v/v), under both of the conventional fermentation and electro-fermentation, and the lipase activity was quantified for each lipid substrate after incubation for 72h. To determine the effect of different lipid concentrations on enzyme production, about 0.5 %v/v, 1 %v/v, and 1.5 %v/v of the best functional lipid sources were added individually before the assay to the electro-fermentation and conventional fermentation production media, and then incubated for 72h. Finally, the effect of catholyte types on lipase activity was evaluated by comparing two catholytes (0.1 M Potassium permanganate and 0.1 M potassium ferricyanide) in the cathodic chamber.

2.6. Partial characterization of the produced lipase

Partial characterization of the produced lipase by the bacterial strains was carried out by investigating the effect of different incubation temperatures, different pH and metal ions on the activity of the crude lipase enzyme. To determine the effect of temperature on enzyme activity, the assay was carried out in the two fermentation systems for different incubation temperatures ranging from 30^{o}C-60^{o}C for 72h incubation time. The effect of different pH values on enzyme activity was determined by incubating the enzyme (at 37^{o}C for 72h) with p-nitrophenylpalmitate substrate prepared in sodium acetate, potassium phosphate and Tris-HCl buffers, in the pH ranges of 3-10. The effects of different metal ions on activity of thermostable lipase enzyme was conducted by utilizing various metal salts (i.e. $ZnCl_2$, $MgCl_2$,

$CaCl_2$ and $HgCl_2$), which were added individually to the crude enzyme at concentrations of 5 mM and 10 mM, and incubated at the optimum temperature for 30 min. The relative lipase enzyme activity was compared to that of the control enzyme without metal salts.

2.7. Statistical analysis

Results obtained were subjected to analysis of variance using ANOVA, and separation of means was carried out by Duncan's multiple range tests according to Duncan, (1955).

3. Results and Discussion

3.1. Lipolytic activity and electrogenicity of the bacterial isolates

The lipolytic activity of enzymes produced by the bacterial strains is presented in Table (1). These bacteria were further investigated for electrongenicity based on their abilities to elicit electrogenic voltages, where the voltage was quantified as open circuit voltages (OCV), and closed circuit voltage (CCV) (Ajunwa *et al*., 2018). Results showed that all the bacteria investigated were electrogenic (electrogenic voltage >182 mV). *Bacillus* spp. as electroactive bacteria have been previously reported by Shankar *et al*., (2014), who investigated the electrogenic potentials of *Bacillus* spp. fed with cellulose for bioelectricity generation, as well as simultaneous cellulose hydrolysis. Moreover, these authors further reported that *Bacillus* spp. can be electroactive even in electrochemical systems that does not contain electrogenic mediator. According to Yoganathan and Ganesh, (2015), this might be attributed to the presence of electrochemically active redox molecules on their outer membranes that transfer electrons to the external materials. The rate of extracellular electron transfer by the electroactive strains was found to increase with time, with the highest electrogenic voltage of 415± 0.1 produced by *B. pumilus* after 120h, similar trends were observed for *B. licheniformis* and *Aeribacillus* sp. with electrogenicity of 362.5± 0.0 and 211.5± 1.5, respectively. For *Bacillus* spp., an electrogenic voltage of 407.0 ± 3.0 was recorded at 72 h (Table1). These current results are similar to the previous reports of Yoganathan and Ganesh, (2015), who measured the electrogenic voltage elicited by *B. subtilis* and *B. megaterium* over a period of 240h and found that it increased with time. As suggested by Yu *et al*., (2017), the change in the electrogenic voltage over time may be due to the changes in gene expression of relevant membrane proteins such as membrane-bound dehydrogenases, which are involved in mediated electron transfer.

3.2. Thermostability of lipase produced by the electrogenic bacteria

Results of thermal stability of the lipase enzyme produced by the electrogenic bacterial strains are presented in Fig. (1). The results showed that all these strains retained more than 70% of enzyme activity after 30 min. of incubation at 50^{o}C, with 100%, 80.7%, 78.1% and 70.5% residual activities for *Aeribacillus* sp., *B. pumilus*, *Bacillus* sp. and *B. licheniformis*, respectively (Fig. 1). These thermal stabilities are similar to those reported for lipase produced by *B. pumilus* isolated from an oil contaminated region in India (Kumar *et al*., 2012). Variations in the thermostability of different enzymes under the same conditions could be attributed to the type and the number of amino acid residues participating in secondary interactions, hydrogen bond formation and hydrophobicity profile of the various enzymes. Furthermore, Salihu and Alam, (2014) reported that non-covalent interactions result in the formation of salt-bridge which contributes in stabilization of the enzymes at elevated temperatures, by entropically restricting the flexibility of the lipase enzyme active site. Thermostable lipases are needed in several biocatalytic reactions, and some advantages of their catalysis at higher temperatures include; high rate of product formation with minimal diffusional restriction, high dissolution of hydrophobic substrates, high conversion efficiency, increased kinetic energy of reactants, and limited chance of contamination (Fariha *et al*., 2006).

Table 1. Lipolytic and electrogenic activities of the bacterial spp. isolated from palm oil mill effluent

Microorganisms	Lipolytic activities	Electrogenic activities					
	Zone size (mm)	Time (h)/ Electrogenic voltage (mV)					
		0	24	48	72	96	120
B. licheniformis SIS2	2.1±0.12^{c}	0	76.5±0.5^{c}	250.5±0.5^{c}	275.0±0.0^{c}	282.0±0.0^{c}	362.5±0.0^{b}
B. pumilus SIE23	2.1±0.14^{c}	0	336±3.0^{a}	341±0.5^{b}	373±4.0^{b}	369±0.5^{a}	415±0.1^{a}
Bacillus sp. S1E27	2.9±0.11^{b}	0	0	352.5±0.5^{a}	407.0±3.0^{a}	294.0±0.1^{b}	229.0±2.0^{c}
Aeribacillus sp. SIE29	3.2±0.12^{a}	0	169.0±1.0^{b}	200.0±1.0^{d}	200.0±0.0^{d}	200.0±0.0^{d}	211.5±1.5^{d}

Data are means of three replicates, ±Standard deviation of means. Values followed by the same letters are not significantly different by Duncan's multiple range tests ($P \leq 0.05$)

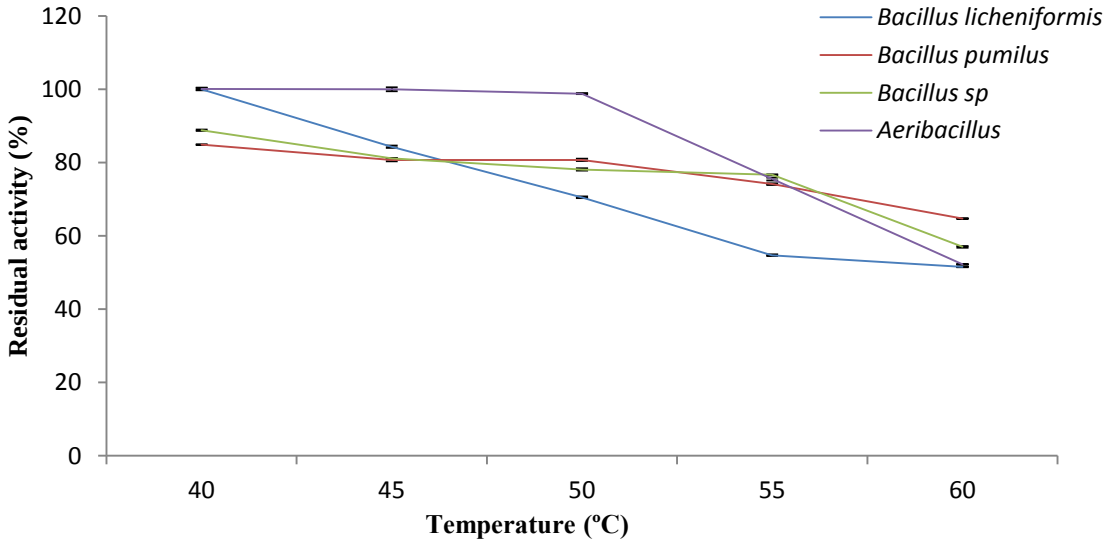


Fig. 1. Thermal stability of Lipase produced by electrogenic bacteria isolated from Palm oil mill effluent. Data are given as means of three replicates.

3.3. Time course of thermostable lipase production in submerged and bio-electrochemical fermentation systems

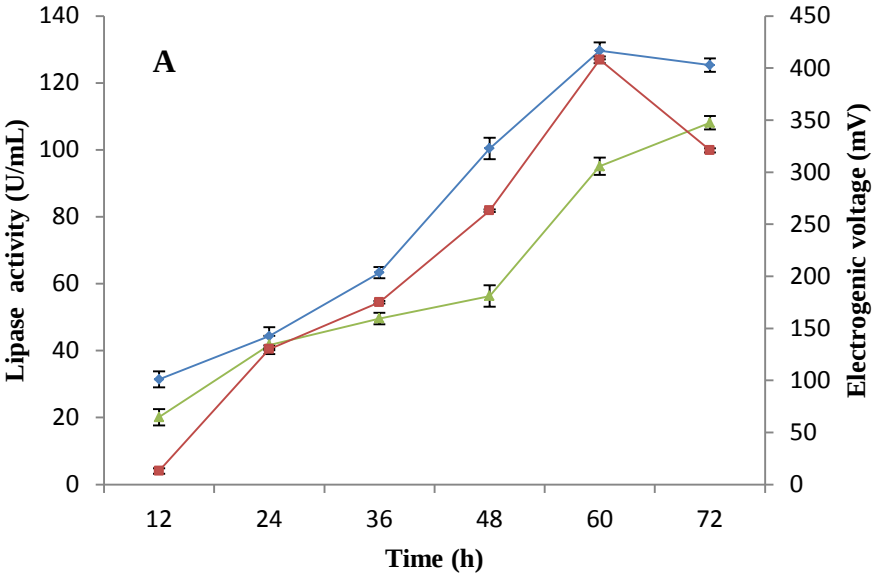
Thermostable lipase production in submerged and electrochemical systems was monitored with respect to time. Furthermore, extracellular electron transfer was also monitored and the results are presented in Fig. (2). Results revealed that the rate of extracellular electron transfer as determined by electrogenic voltage elicited was found to increase with enzyme production for all the tested strains over a period of 72. The optimum time for thermostable lipase production corresponded with the highest electrogenicity values for all the tested strains. The optimum lipase activity for *B. licheniformis* S1S2, *B. pumilus* S1E23, *Bacillus* sp. S1E27, and *Aeribacillus* sp. S1E29 was found to be 129.6± 0.002 U/ ml, 164.9± 0.001 U/ ml, 125.3± 0.002 U/ ml, and 136.6± 0.001 U/ ml, respectively under electrochemical system. On the other hand, the optimum activity observed under the conventional system (control) was 95.1± 0.003 U/ ml, 128± 0.001 U/ ml, 110.7± 0.003 U/ ml and 122.7± 0.002 U/ ml, respectively (Fig. 2). Although lipase production was higher under the electrochemical system than that of the conventional one; however, the lipase production level under conventional system was found to increase after 60h for all the strains (Fig. 2). This is in accordance with the previous results of Lai *et al*., (2016), which showed that the bioconversion rate of glucose increased by 36 % in the presence of an anode that served as an electron sink, and this also positively correlated with the rate of electrons produced.

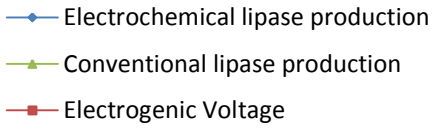
3.4. Effects of different lipid sources on lipase production

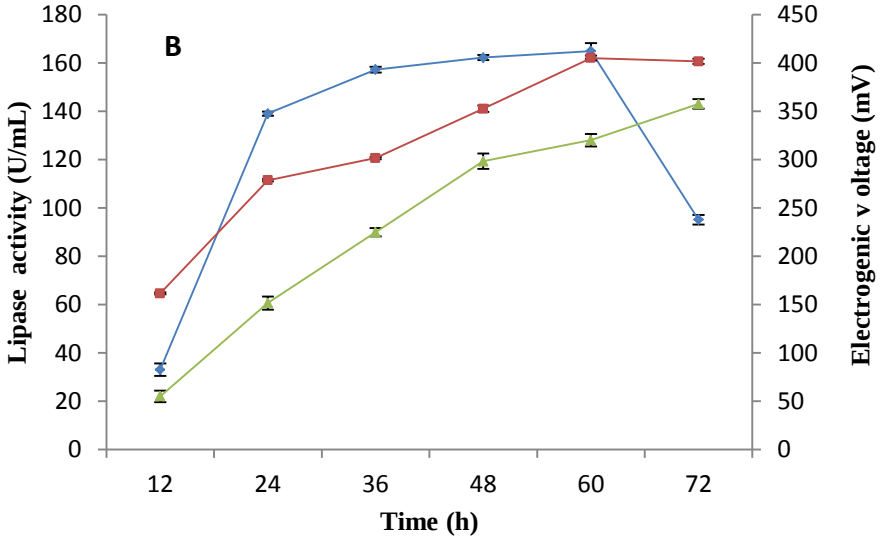
Different lipid substrates such as; coconut oil, shea butter oil, castor oil, and olive oil were used to induce thermostable lipase production by each of the four strains. The best lipid source that supported the production of thermostable lipase was the shea butter oil with average yield of 151.9± 0.001 U/ ml, 136.6± 0.003 U/ ml, 147.6± 0.001 U/ ml, for *B. licheniformis* S1S2, *Bacillus sp*. S1E27, and *Aeribacillus* sp. S1E29, respectively (Fig. 3). This was closely followed by coconut oil and then olive oil, while castor oil caused the lowest lipase yield in the three strains. These results differ from the report of Heravi *et al*., (2008), who recorded that olive oil supported the highest lipase production by *Bacillus* sp. PD12. However, current lipase production by *B. pumilus* S1E23 was best supported by olive oil as a lipid substrate giving a yield of 164.9± 0.001 U/ ml, while castor oil caused the least enzyme yield (Fig. 3). Furthermore, lipase yield is generally higher in the electro-fermentation system compared to the yield of the conventional fermentation system (Fig. 3). Similarly, Alabras *et al*., (2017) reported that the existence of oil in the bacterial medium stimulates the bacteria to produce lipase to utilize the oil as a nutrient source.

3.5. Effects of different substrate concentrations on lipase enzyme production

The shea butter oil substrate that best supported lipase production by each bacterial strain determined earlier in Fig. (3) was used at varying concentrations of; 0.5% v/v, 1% v/v, and 1.5% v/v, to determine its effect on lipase production in both conventional and electrochemical fermentation systems. Lipase production was concentration under both fermentative conditions; however, lipase yield by the four strains in the electrochemical system was higher compared to that in the conventional system at the three tested concentrations (Fig. 4). Lipase yield in *B. licheniformis* S1S2, *B. pumilus* S1E23, *Bacillus* sp. S1E27, and *Aeribacillus* sp. S1E29 under conventional fermentation conditions was 99.4 U/ ml, 102.4 U/ ml, 86.5 U/ ml and 119.2 U/ ml, respectively. On the other hand, the enzyme yield recorded in the electrochemical system was; 170.8 U/ ml, 165.8 U/ ml, 162.4 U/ ml and 187.4 U/ ml, respectively (Fig 4). Similarly, Alabras *et al*., (20017) reported an increase in lipase enzyme production by *Geobacillus stearothermophilus* with increasing lipid concentration up to 2% (v/v).


A
Lipase activity (U/mL)
Electrogenic voltage (mV)
Time (h)
0
20
40
60
80
100
120
140
0
50
100
150
200
250
300
350
400
450
12
24
36
48
60
72


Electrochemical lipase production
Conventional lipase production
Electrogenic Voltage


B
Lipase activity (U/mL)
Electrogenic v oltage (mV)
Time (h)
0
20
40
60
80
100
120
140
160
180
0
50
100
150
200
250
300
350
400
450
12
24
36
48
60
72

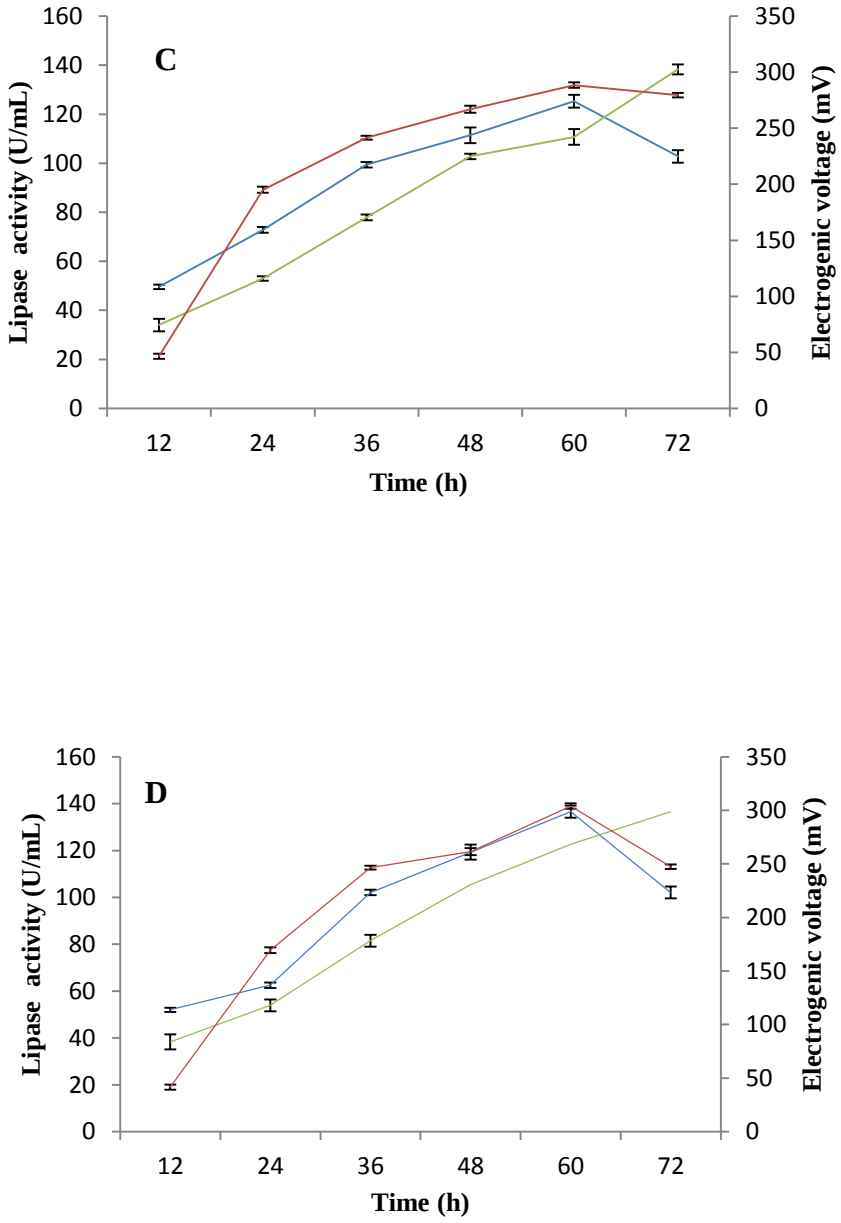
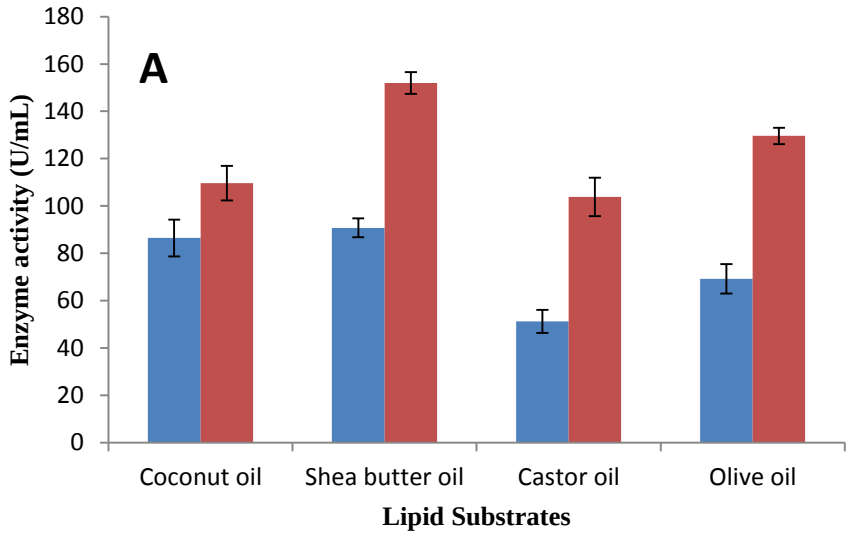
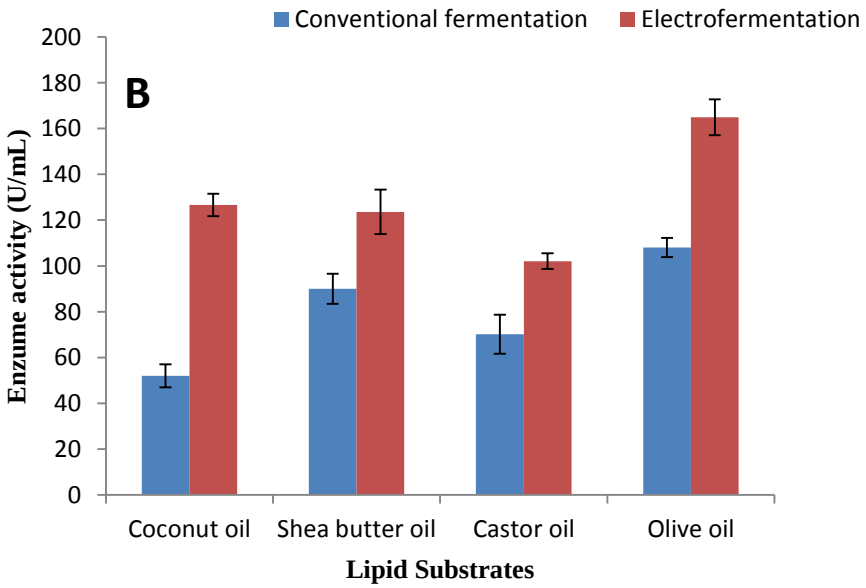
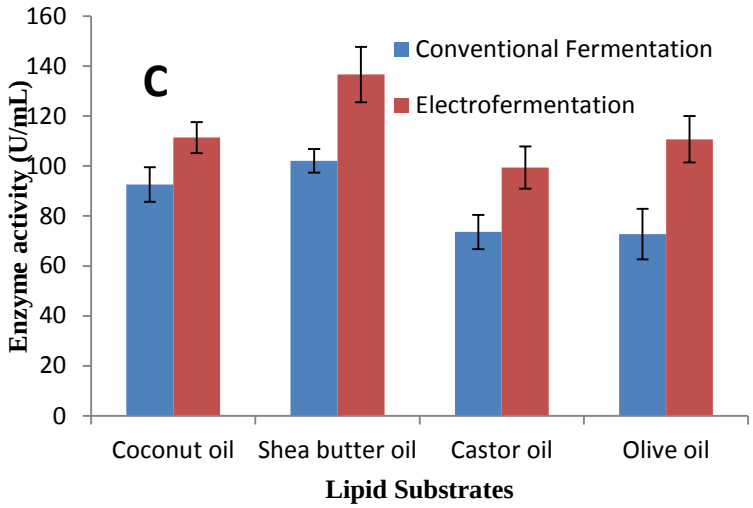


Fig. 2. Time course of lipase production by *B. licheniformis* S1S2 (A), *B. pumilus* S1E23 (B), *Bacillus* sp. S1E27 (C), and *Aeribacillus* sp. S1E29 (D).
Data are given as means of three replicates; Error bars represent the standard deviations.


A
Enzyme activity (U/mL)
180
160
140
120
100
80
60
40
20
0
Coconut oil
Shea butter oil
Castor oil
Olive oil
Lipid Substrates


Conventional fermentation
Electrofermentation
B
Enzume activity (U/mL)
200
180
160
140
120
100
80
60
40
20
0
Coconut oil
Shea butter oil
Castor oil
Olive oil
Lipid Substrates



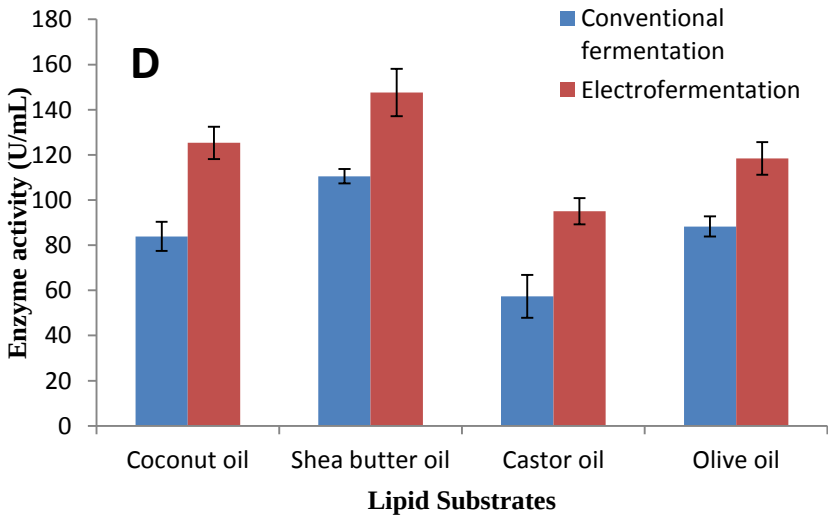
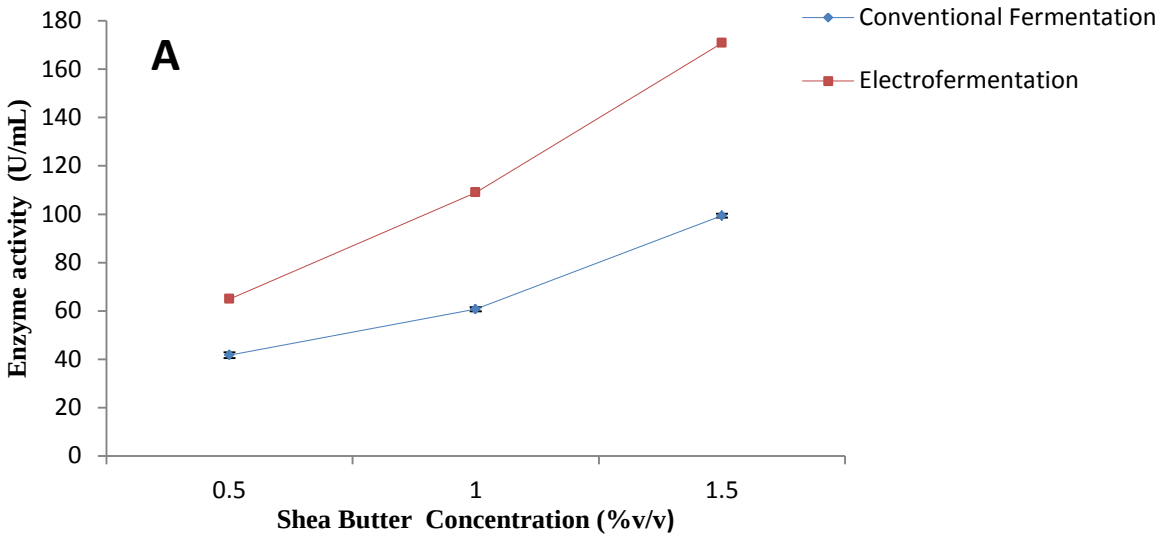
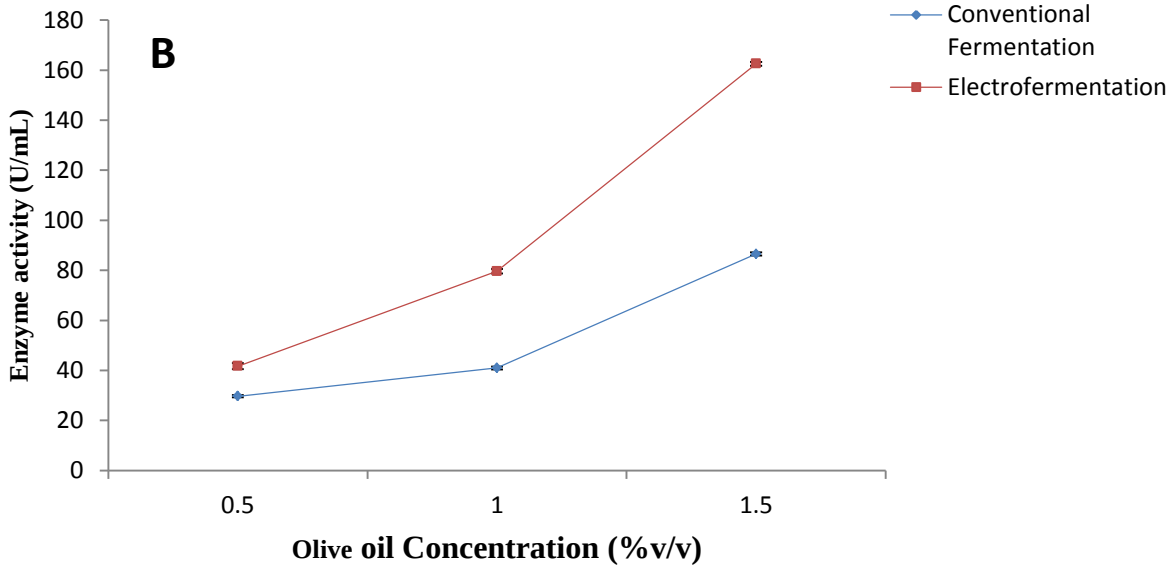


Fig. 3. Effects of different lipid substrates on lipase production by *B. licheniformis* S1S2 (A), *B. pumilus* S1E23 (B), *Bacillus* sp. S1E27 (C), and *Aeribacillus* sp. S1E29 (D). Data are given as means of three replicates; Error bars represent the standard deviations.


A
Conventional Fermentation
Electrofermentation
Enzyme activity (U/mL)
180
160
140
120
100
80
60
40
20
0
0.5
1
1.5
Shea Butter Concentration (%v/v)


B
Conventional Fermentation
Electrofermentation
Enzyme activity (U/mL)
180
160
140
120
100
80
60
40
20
0
0.5
1
1.5
Olive oil Concentration (%v/v)

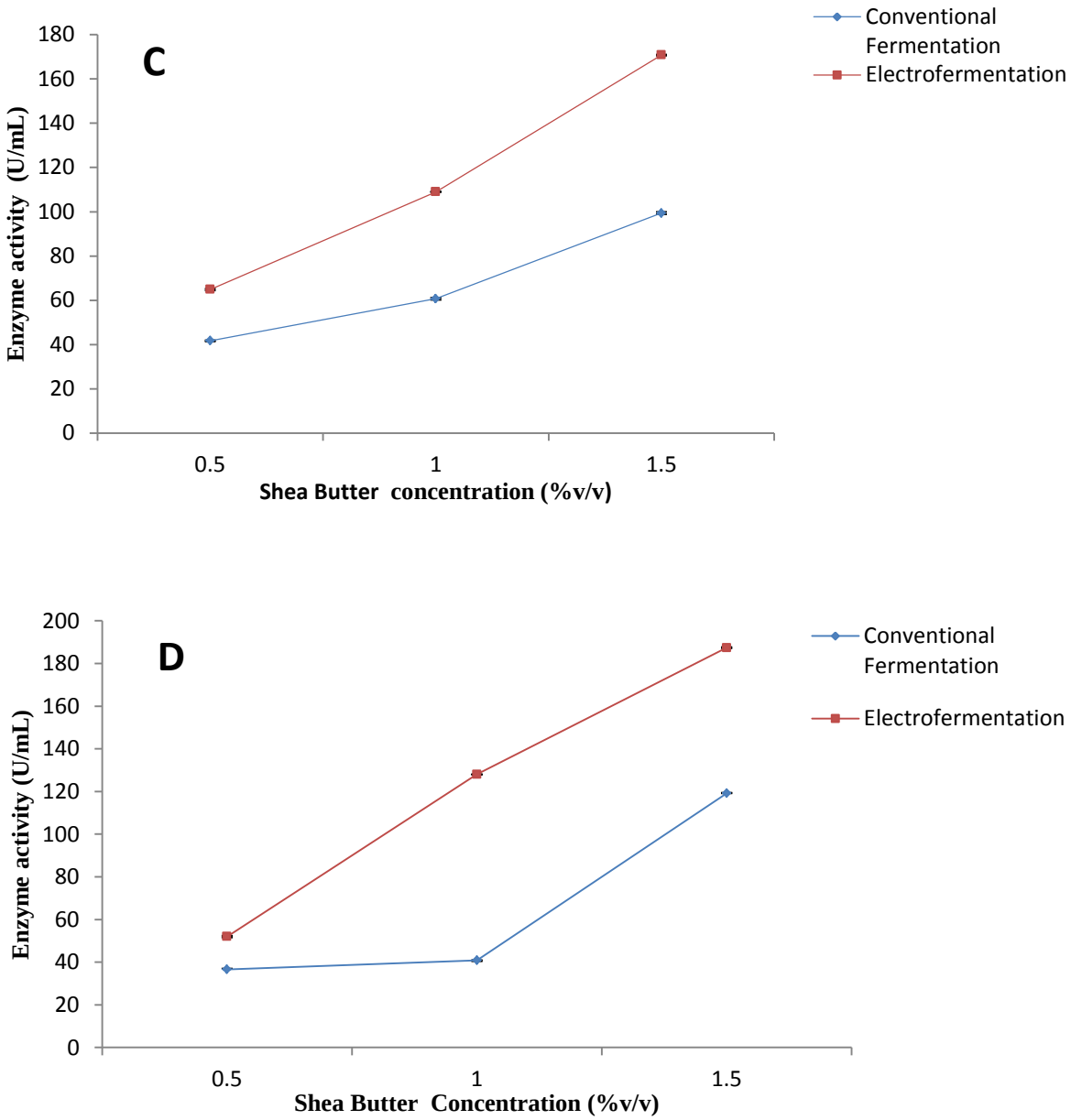


Fig. 4: Effects of different concentration of lipid substrates on lipase production by *B. licheniformis* S1S2 (A), *B. pumilus* S1E23 (B), *Bacillus* sp. S1E27 (C) and *Aeribacillus* sp. S1E29 (D). Data are given as means of three replicates.

3.6. Effects of different catholytes on current production

The catholytes used to study their effects were potassium ferricyanide and potassium permanganate at a concentration of 0.1 M, and the electron flow was measured with a multimeter after 48h. It is observed that the set up having $K_3F(CN)_6$ as an electron acceptor produced higher voltage for all the electrogenic strains. The highest voltage is produced by *B. pulmilus* S1E23 (321mV), while the least is produced by *Aeribacillus* sp. S1E29 (185mV) (Fig. 5). These results expressed potassium ferricyanide as an excellent cathodic electron acceptor.

3.7. Characterization of the crude lipase enzyme

3.7.1. Effects of temperature on lipase activity

This condition for characterizing the enzyme was carried out by incubating the 4 bacterial strains at different temperatures ranging from 30^{o}C- 60^{o}C at 5^{o}C interval, using their optimum lipid substrate for maximum enzyme activity. Although the increase in temperature caused an increase in lipase activity, however lipase from all the strains showed varying degree of activity with respect to the changes in incubation temperature. Optimum lipase activity from *B. licheniformis* S1S2, *B. pumilus* S1E23, *Bacillus* sp. S1E27, and *Aeribacillus* sp. S1E29, was shown at 45oC (263.8 U/ ml), 50oC (263.8 U/ ml), 50^{o}C (473 U/ ml), and 55^{o}C (419.8 U/ ml), respectively (Fig 6). The result obtained is similar to the report of Lima *et al*., (2004) who observed maximum activity of lipase produced by *B. megaterium* at 55^{o}C. Similarly, Faouzi *et al*., (2015) reported that lipase produced by *B. pumilus* exhibited optimum activity at 45^{o}C. The previous study of Lima *et al*., (2012) recorded that the optimum lipase activity has been found between 30-60^{o}C. Devi and Chary, (2018) attributed the higher enzyme activity observed at elevated temperatures to the increased kinetics of the enzymatic reactions. The thermostable lipases are very promising for industrial applications such as detergent formulations and other bio-transformations, since processes performed at high temperatures increase the reaction rate.

3.7.2. Effect of pH on lipase activity

The effect of pH ranges of 3-10 on the activity of the thermostable lipase was examined for the four bacterial strains. Results showed that the optimum lipase activity is observed at neutral to slightly alkaline pH (7-9) as clear in Fig. (7). Lipase produced by *B. licheniformis* S1S2, *B. pumilus* S1E23, *Bacillus* sp. S1E27, and *Aeribacillus* sp. S1E29 showed best activity at pH values of; 8 (109 U/ ml), 9 (71.9 U/ ml), 7 (82.2 U/ ml), and 9 (71.9 U/ ml), respectively. Above these pH values, loss in enzyme activity is observed (Fig. 7). Previous study of Gupta *et al*., (2004) showed that pH in the range of 6-10 is optimal for lipases produced by several bacterial species such as *Pseudomonas* sp., *Acinetobacter calcoaceticus*, *P. cepacia*. Furthermore, Guncheva and Zhiryyokova, (2011) reported that most *Bacillus* lipases are stable in neutral to moderately alkaline pH. In a previous study, Jittima *et al*., (2011) documented that lipase produced by *Aeromonas* sp. EBB-1 exhibited maximum activity towards p-nitrophenylpalmitate at pH 8. Later, Faouzi *et al*., (2105) reported optimum pH value of 8 for lipase produced by B. pumilus. In an earlier study, Pratuangdeiku and Dharmsthiti, (2000) attributed the lower catalytic enzyme activity observed at acidic pH to the loss of Ca^{2+}, which has a coordinating role at the active site of the enzyme.

3.7.3. Effects of various metal ions on lipase activity

Effects of varying concentrations (5 mM and 10 mM) of different divalent metal ions including; Ca^{2+}, Zn^{2+}, Mg^{2+}, and Hg^{2+}, on the activity of thermostable lipase was investigated, where the enzyme solution without metal ion was used as control. During this study, Ca^{2+} and Mg^{2+} and Zn^{2+} are observed to enhance the lipase activity with increasing their concentrations for all the bacterial strains, while Hg^{2+} has a slight inhibitory effect on the lipase activity.

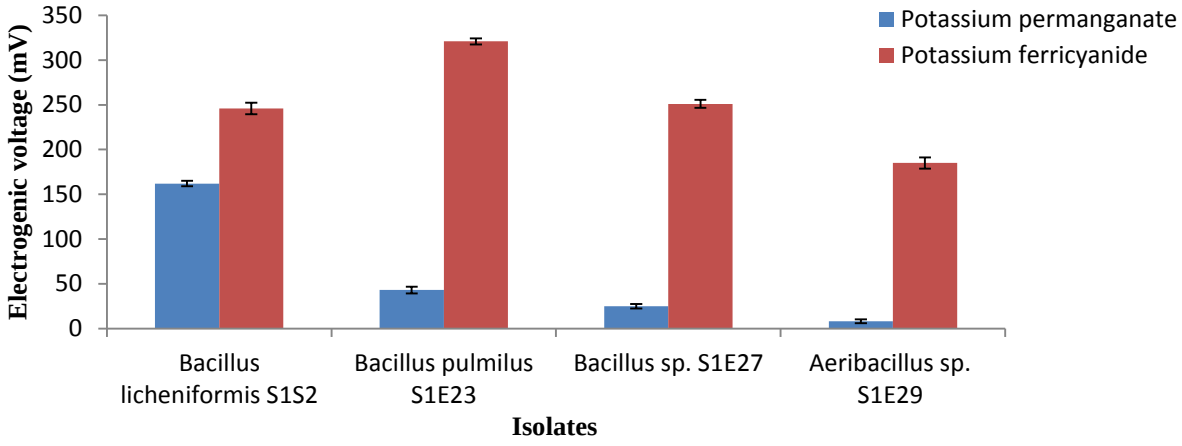


Fig. 5. Effects of different Catholytes on the performance of Bio-electrochemical system. Data are given as means of three replicates; Error bars represent the standard deviations.

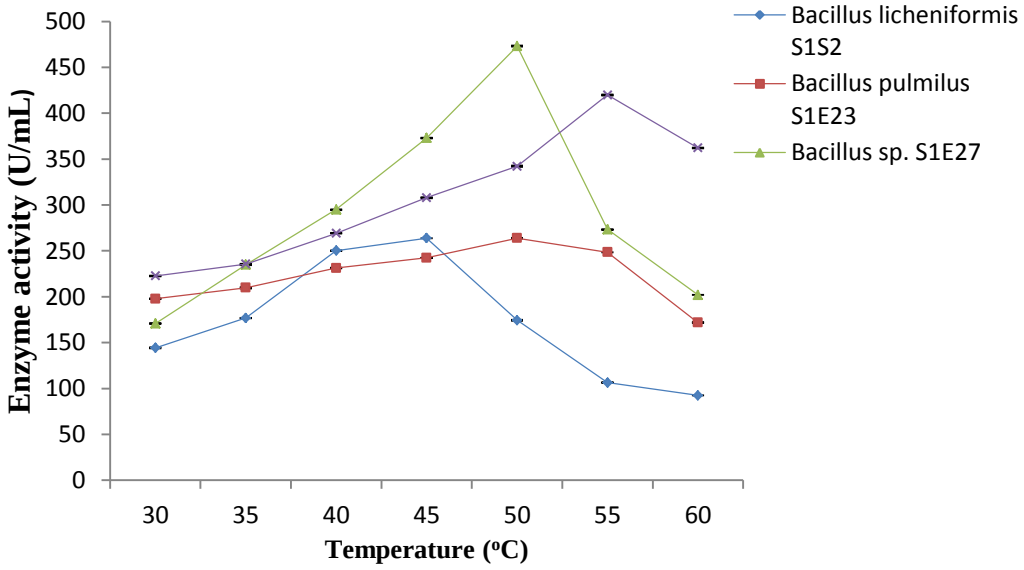


Fig. 6: Effects of different incubation temperatures on the activity of Thermostable lipase produced by the bacterial strains recovered from the soil and palm oil mill effluent. Data are given as means of three replicates.

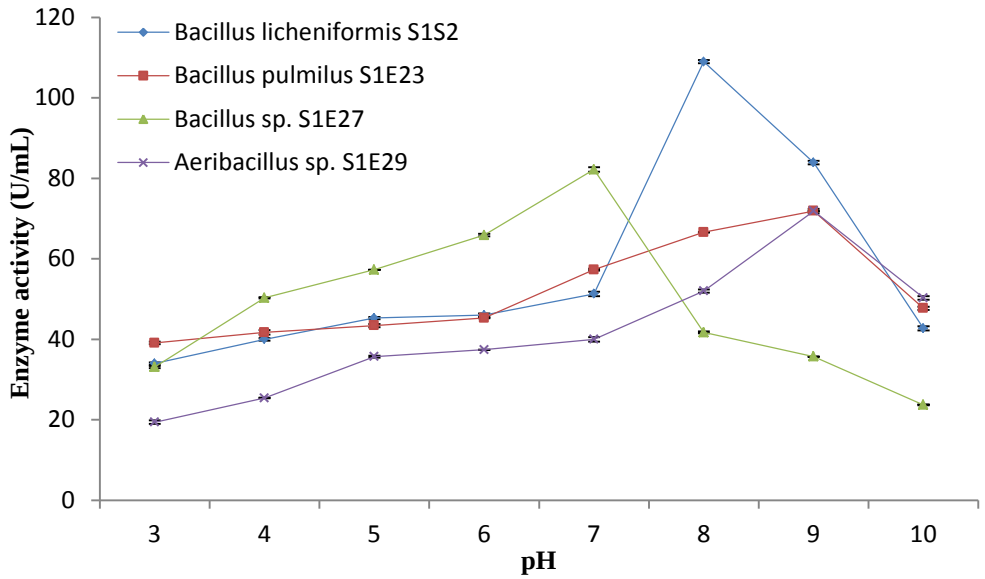


Fig. 7. Effects of different pH on the activity of Thermostable lipase produced by the bacterial strains recovered from the soil and palm oil mill effluent. Data are given as means of three replicates.

Mg^{2+} expressed the best enhancing activity on lipase produced by *B. pulmilus* S1E23, *Bacillus* sp. S1E27, and *Aeribacillus* sp. S1E29, with 156.5 %, 134.7 %, and 133.7 %, respectively. This is followed closely by Zn^{2+} and then Ca^{2+} in *B. pulmilus* S1E23 and *Bacillus* sp. S1E27. Similarly, Lima *et al*., (2012) previously reported the enhanced activity of lipase produced by *B. pumilus* in the presence of Ca^{2+}. According to Gupta *et al*., (2004), Ca^{2+} also stimulated the activity of lipase produced by *B. subtilis* 16C, *B. thermoleovorans* 1D-1, *P. aeruginosa* EF2, *Staphylococcus aureus* 226, and *Acinetobacter* sp. RAG-1. The increase in lipase activity in presence of these ions was attributed by Lima *et al*., (2012) to the vital role played by Ca^{2+} in building a stable catalytic enzyme structure, as binding of the Ca^{2+} to the internal structure of the enzyme, cause change in the solubility and behavior of the ionized fatty acids at the interfaces. In agreement with the current results, Lilie *et al*., (2005) reported that the activity of the thermo alkalophilic lipase produced by *B. thermoleovorans* CCR11 was inhibited in the presence of Hg^{2+}, while Ca^{2+} enhanced it. However, these results represent a shift from the reports of Sharma *et al*., (2001); Gururaj, (2014), who observed that the activity of lipase produced by *Bacillus* sp. RSJ-1and *Acinetobacter* sp. AU07 was strongly inhibited in the presence of Zn^{2+}.

Conclusion

The results obtained from this work shows that extracellular lipase production by electroactive bacteria can be enhanced under electrochemical conditions compared to the conventional (submerged) fermentation. This strengthens the position of electro-fermentation as a way of possibly enhancing the yield of other metabolites of an industrial interest. In addition, culture conditions such as; lipid sources, substrate concentration, and incubation period can affect the thermostable lipase production, under both of the electro-fermentation and conventional (submerged) fermentation systems. Moreover, the electrochemical parameters including the choice of catholytes can affect the overall performance of a bio-electrochemical system.

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Conflict of interest

The authors declare that there is no conflict of interests.

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