



**METAL INDEPENDENT AND DETERGENT STABLE LIPASES FROM
AEROMONAS AND *PSEUDOMONAS* SPP. AND THEIR ROLE IN
BIOREMEDIATION****HANGLOO S¹, SOURIRAJAN A¹ AND DEV K^{1*}**

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ABSTRACT

Lipases have wide applications in industries because of their versatile properties. In the present study, effect of different solvents, metal ions, detergents and inhibitors on lipase activity of *Aeromonas* spp. (Sh 2, Sh 8 and Sh 12) and *Pseudomonas* spp. (Sh 13 and Sh 17) was studied. Lipase activity was enhanced by 2.1, 1.8, 1.4, 1.6, 1.3 and 2.2 folds in the presence of 0.5% acetone (*Aeromonas* sp. Sh 2), 1% acetone (*Aeromonas* sp. Sh 2 isolate), 0.5% ethanol (*Aeromonas* sp. Sh 8), 1% methanol (*Aeromonas* sp. Sh 12), 0.5% DMSO (*Pseudomonas* sp. Sh 13) and 0.5% ethanol (*Pseudomonas* sp. Sh 17) respectively. Increase in lipase activity by 1.2-1.7 folds was seen in the presence of Triton-X (0.1, 0.5%) for *Aeromonas* sp. Sh 2 isolate, *Aeromonas* sp. Sh 8 and *Pseudomonas* sp. Sh 17 isolates. Lipase activity was enhanced by 1.4 and 1.2 fold in the presence of 0.1% tween 20 for *Aeromonas* spp. Sh 2 and Sh 8 isolates respectively. EDTA (1-10 mM) increased lipase activity by 13.7-85.3% in *Aeromonas* sp. Sh 8 and *Pseudomonas* sp. Sh 17 isolates. Lipase activity was increased by 1.1-2.0 fold in the presence of calcium (0.5, 1 mM), magnesium (0.5-1.5 mM) and manganese (0.5 mM) for *Pseudomonas* sp. Sh 13 isolate and in the presence mercury (0.5 mM) for *Aeromonas* sp. Sh 8 isolate. *Aeromonas* and *Pseudomonas* spp. were also tested for reduction of BOD and COD of waste water. Highest BOD and COD reduction of 65-75% reduction was seen in paint industrial waste among paint, dairy and pharmaceutical waste by *Pseudomonas* sp. Sh 17 and Sh 13 isolates.

Keywords: Lipase, BOD, COD, *Aeromonas*, *Pseudomonas*, Industrial waste

INTRODUCTION

Bacteria produce different classes of hydrolytic enzymes such as proteases, amylases, amidases, esterases and lipases. These enzymes occupy the major share of industrial enzyme market [1]. Lipases are serine hydrolases that function at an oil water interface [2]. Owing to the multifaceted properties, lipases find usage in a wide array of industrial applications, such as food technology, detergent, biomedical sciences, pharmaceuticals, and pesticides, production of single - cell protein, cosmetics, waste disposal and biosensor modulations [3]. Some unique properties of lipase such as stability in organic solvents, being active without the aid of cofactors, broad substrate specificity, high enantioselectivity, stability at broad temperature and pH range are the reasons for the enormous biotechnological potential of microbial lipases [4,5]. In municipal and industrial wastes, lipids (fats, oils and grease) are major organic matters that cause severe environmental pollution. Biological treatment is most often found to be effective than physical and chemical treatments [6]. Due to clean and friendly application of microbial enzymes and stringent environmental regulations, enzymatic treatment technique has gained more attention [7]. In bioremediation for

waste disposal, lipase biotechnology is a new avenue to be explored.

Metal cations play important roles in the structure and function of enzymes, particularly Ca^{2+} and some of the lipases are strictly calcium dependent [5]. An extracellular alkaline lipase from a thermophilic isolate *Bacillus coagulans* BTS-3 showed enhancement in lipase activity in the presence of K^+ , Fe^{3+} , Hg^{2+} and Mg^{2+} ions, while Al^{3+} , Co^{2+} , Mn^{2+} , and Zn^{2+} ions were inhibitory to lipase activity. Sodium ions (Na^+) did not affect the lipase activity [8]. For lipases stability in organic solvents is desirable for synthesis reactions [9]. Lipase inhibitors find application in pharmacology and have been used in the study of structural and mechanistic properties of lipases [5]. *Pseudomonas aeruginosa* KKA-5 lipase was highly stable in the presence of solvents (methanol and ethanol) and inhibited by acetone [10]. Ethylene diamine tetraacetic acid (EDTA 70 mM) significantly (~ 88%) and Phenyl methyl sulfonyl fluoride (PMSF, 70 mM) completely inhibited lipase activity of an extracellular alkaline metallo lipase purified from *Bacillus licheniformis* MTCC 6824 [11]. Ionic and non ionic detergents might play an important role in enzymatic properties of lipases. The *Burkholderia cepacia* RGP-10 lipase retained >80%

activity in the presence of detergents Triton X-100, sodium cholate and sodium taurocholate [13]. One of the more notable thermostable lipase isolated from a *Bacillus* strain A30-1 (ATCC 53841) retained 100% of the original activity after incubation at 75 °C for 30 min [14].

Huge variation of lipases in different applications, make the availability of lipases with specific characteristics a limiting factor. Thus, to search for new lipases with different characteristics continues to be important research topic [15]. Wastewaters from industries are rich in biodegradable organic molecules, nutrients, fats and proteins that have a low biodegradability coefficient. If not treated, they can cause gross pollution of land and water with their high biological oxygen demand (BOD) and chemical oxygen demand (COD). Thus the aim of the study was to optimize the reaction conditions and study the application of *Aeromonas* and *Pseudomonas* spp. lipase in reduction of BOD and COD.

MATERIALS AND METHODS

Optimization of reaction conditions for lipase production by bacterial isolates

Aeromonas spp. (Sh 2, Sh 8 and Sh 12) and *Pseudomonas* spp. (Sh 13 and Sh 17) isolated from paint industrial waste were grown in their respective optimized media as described [16]. Optimized media for

Aeromonas sp. Sh 2 isolate consisted of minimal salt media (M9) containing glucose (0.2%) as carbon source and peptone as nitrogen source (0.25%). *Aeromonas* sp. Sh 8 isolate was grown in M9 medium containing glucose (0.2%) as carbon source and yeast extract (0.25%) as nitrogen source. Growth medium for *Aeromonas* sp. Sh 12 isolate was also M9 medium containing glucose (0.2%) as carbon source, but casein hydrolysate (0.25%) as nitrogen source. For *Pseudomonas* spp. Sh 13 and Sh 17 nutrient broth was used as optimized medium for growth. All the cultures were incubated at 37 °C for 36 hours. Cultures were centrifuged at 10,000 xg and cell free spent medium was used as source of lipase. Lipase assay was performed with 10 µg of protein and using p-nitrophenyl palmitate as substrate as described [16]. Protein was estimated by Bradford method [17].

Effect of solvents and detergents on lipase activity of *Aeromonas* and *Pseudomonas* species

Effect of solvents on lipase activity of *Aeromonas* spp. (Sh 2, Sh 8 and Sh 12), *Pseudomonas* spp. (Sh13 and Sh 17) isolates was determined by supplementing isopropanol, methanol, ethanol, dimethylsulphoxide (DMSO), toluene, hexane, acetonitrile, acetone and phenol (0.5 and 1%) in the lipase reaction mixture.

Detergents such as tween 20, tween 80, sodium dodecyl sulphate (SDS) and triton X-100 (0.1, 0.5 and 1%) were supplemented in the lipase assay mixture to study effect of detergents on lipase activity. The relative lipase activity was calculated with respect to control, without supplementation of solvents and detergents and considered as 100% lipase activity.

Thermostability of *Aeromonas* and *Pseudomonas* species lipases at 60°C

Lipase from *Aeromonas* spp. (Sh 2, Sh 8 and Sh 12), *Pseudomonas* spp. (Sh 13 and Sh 17) isolates was pre incubated at 60 °C for 6 hours. Samples were drawn at regular intervals of 1 hour and lipase assays were performed. Lipase without pre incubation served as control and was considered 100% lipase activity. Residual activity was calculated with respect to control.

Effect of metal ions and inhibitors on lipase activity of *Aeromonas* and *Pseudomonas* species

To evaluate the effect of metal ions on lipase activity of *Aeromonas* spp. (Sh 2, Sh 8 and Sh 12), *Pseudomonas* spp. (Sh 13 and Sh 17) isolates, reaction mixture was incubated with different salts of metal ions (Ni^{2+} , Ca^{2+} , Mn^{2+} , Mg^{2+} , Cu^{2+} , Hg^{2+} , Fe^{2+}) at 0.5, 1, 1.5, 2 and 2.5 mM for 10 min. Lipase assays were carried in the presence of PMSF and EDTA at 1, 5 and 10 mM to study effect of inhibitors on lipase activity.

Lipase activity calculated without addition of metals ions and inhibitors was taken as control and set as 100%. Relative activity was calculated with respect to control.

Determination of BOD and COD

Waste water samples were collected from different industries such as dairy, paint and pharmaceutical industry. BOD was determined as per the procedure described by Helrich [18].

For waste sample, BOD bottles containing 10 ml waste water were filled with dilution water and for blank BOD bottle was completely filled with dilution water alone. For seeded waste water, BOD bottles containing 10 ml of waste water sample and seeded with 1% overnight grown cultures ($\text{O.D}_{600} = 1$) of *Aeromonas* spp. (Sh 2, Sh 8 and Sh 12) and *Pseudomonas* spp. (Sh 13 and Sh 17) was filled with dilution water. One of the BOD bottles from waste sample, blank and seeded waste sample were analysed immediately and other bottles were incubated at 20°C in a BOD incubator and analysed after 5 days. Volume of sodium thiosulphate for blank, waste sample and seeded waste sample was used to calculate BOD. BOD of waste water from dairy, paint and pharmaceutical industry calculated after 5 days was taken as control and set as 100%. BOD of seeded waste water was also calculated and plotted with respect to control.

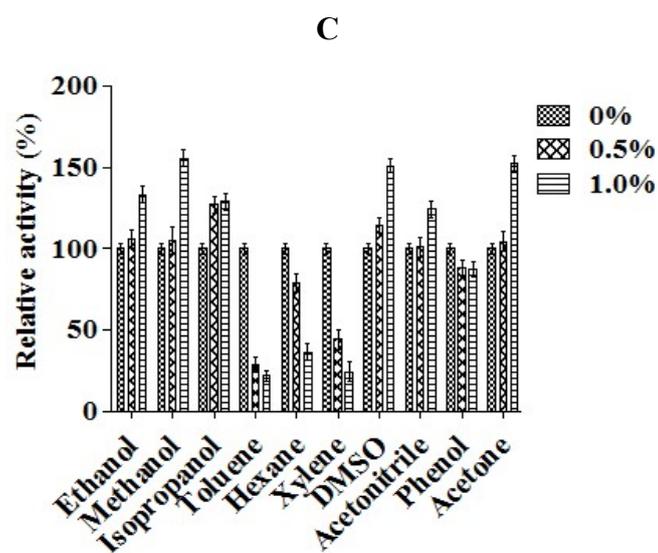
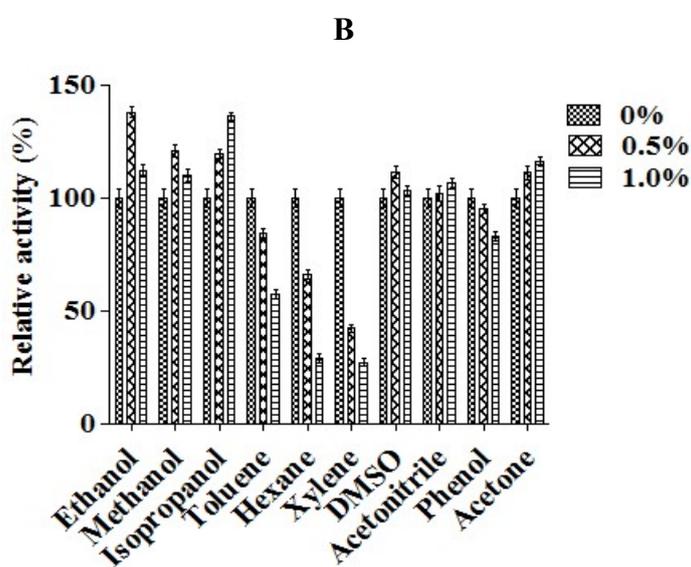
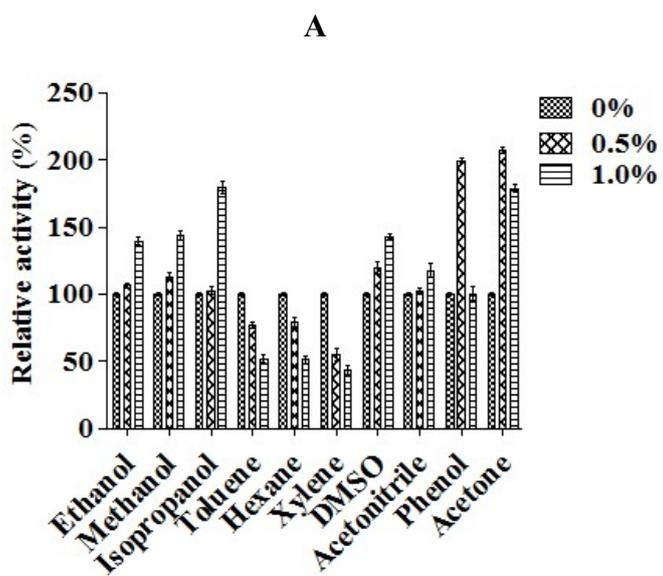
COD was also determined as per the procedure described by Helrich [18]. To 100 ml waste water, 1% inoculum ($O.D_{600}=1$) of each *Aeromonas* spp. (Sh 2, Sh 8 and Sh 12) and *Pseudomonas* spp. (Sh 13 and Sh17) grown in NB medium for 18 hours was added and incubated at 37 °C for 5 days. Volume of ferrous ammonium sulphate added for blank, waste sample and seeded waste sample was used to calculate COD. COD of waste sample was taken as control and set as 100%. COD of seeded waste sample was calculated with respect to control.

RESULT

Solvent tolerant and detergent stable lipases from *Aeromonas* and *Pseudomonas* species

Aeromonas and *Pseudomonas* spp. lipases were tested for lipase activity in the presence of solvents (ethanol, methanol, acetone, isopropanol, DMSO, acetonitrile, phenol, toluene and xylene) at 0.5 and 1%. Lipase activity was enhanced by 2.1, 1.8, 1.4, 1.6, 1.3 and 2.2 folds in the presence of 0.5% acetone (*Aeromonas* sp. Sh 2 isolate), 1% acetone (*Aeromonas* sp. Sh 2 isolate), 0.5% ethanol (*Aeromonas* sp. Sh 8 isolate), 1% methanol (*Aeromonas* sp. Sh 12 isolate), 0.5% DMSO (*Pseudomonas* sp. Sh 13 isolate) and 0.5% ethanol (*Pseudomonas*

sp. Sh 17 isolate) respectively. Lipase activity of *Aeromonas* sp. Sh 2 isolate was increased (2.4-107.7%) in the presence of ethanol, methanol, isopropanol, DMSO, acetonitrile, phenol and acetone. Lipase activity of *Aeromonas* spp. Sh 8 and Sh 12 isolates was increased (1.1-55.6 %) in the presence of ethanol, methanol, isopropanol, DMSO, acetonitrile and acetone. Increase in lipase activity by 0.3-29.3% was observed for *Pseudomonas* sp. Sh 13 isolate in the presence of methanol, isopropanol and DMSO (0.5%), while 1.0-23.8% for *Pseudomonas* sp. Sh 17 isolate in the presence of ethanol, methanol, acetonitrile (1%) and isopropanol (0.5%). On the other hand, lipase activity decreased (20.5-56.8%) in the presence of toluene, hexane and xylene for *Aeromonas* sp. Sh 2 isolate and 4.8-78% in the presence of toluene, hexane, xylene and acetone for *Aeromonas* spp. Sh 8 and Sh 12 isolates. Lipase activity decreased significantly (9.2-99.8%) in the presence of ethanol, hexane, toluene, DMSO, xylene, phenol and acetone for *Pseudomonas* sp. Sh 13 isolate, while decreased by 5.2-68.2 % in the presence of isopropanol (1%), hexane, DMSO, acetone, toluene, xylene and phenol for *Pseudomonas* sp. Sh 17 isolate (Fig.1 A-E).



D

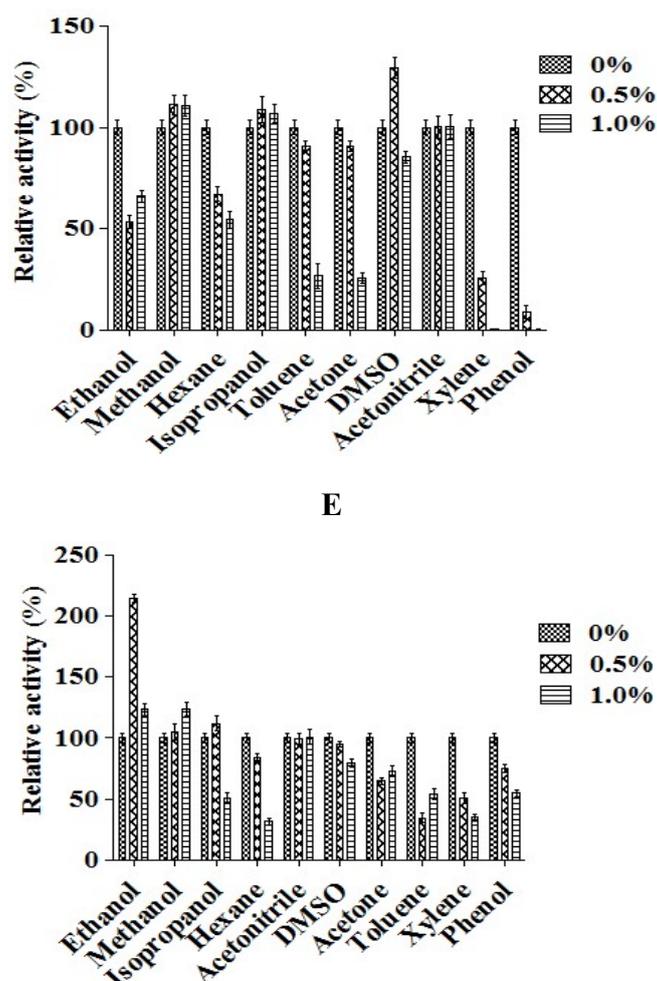
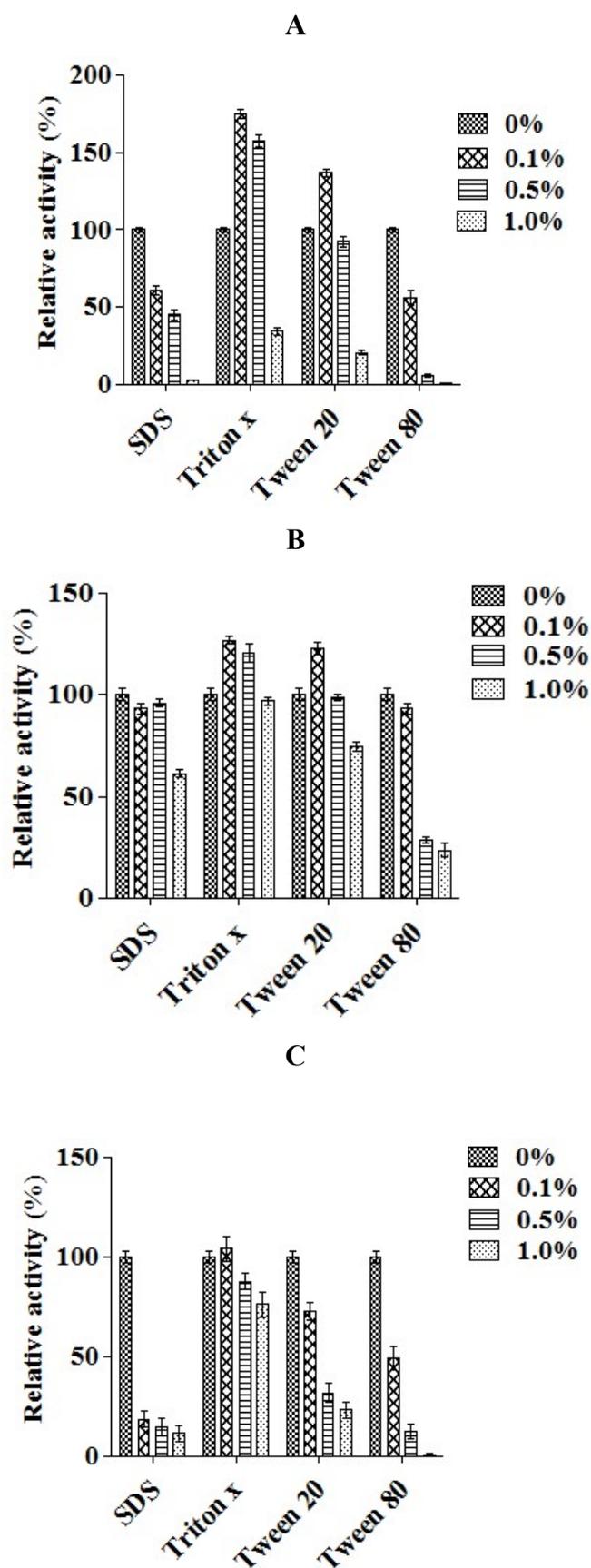


Figure 1: Effect of solvents on the lipase activity of *Aeromonas* and *Pseudomonas* species: Lipase assays were carried out in the absence and presence of different solvents (0.5 and 1%). Reaction without solvents was set as control and was considered as 100 % lipase activity. The relative lipase activity of *Aeromonas* sp. Sh 2 (A), Sh 8 (B) and Sh 12 (C), *Pseudomonas* sp. Sh 13 (D) and Sh 17 (E) isolates was plotted against the indicated concentrations of solvents. Data of three independent experiments was plotted with standard deviation.

The effect of detergents such as SDS, Triton-X 100, tween 20 and tween 80 at 0.1, 0.5 and 1% was studied in a reaction mixture containing lipase. Lipase activity of *Aeromonas* sp. Sh 2 isolate was enhanced by 1.7 and 1.6 folds in the presence of 0.1 and 0.5% Triton -X respectively. Lipase activity was enhanced by 1.3 (*Aeromonas* sp. Sh 8) and 1.2 (*Pseudomonas* sp. Sh 17) folds in the presence of 0.1% Triton- X 100 and 1.4 and 1.2 fold in the presence of 0.1% tween

20 for *Aeromonas* spp. Sh 2 and Sh 8 isolates. *Pseudomonas* sp. Sh 13 isolate showed 17.2-92.2% decrease in lipase activity in the presence of all detergents tested. Lipase activity of *Aeromonas* spp. Sh 2 and Sh 8 and *Pseudomonas* sp. Sh 17 isolates decreased by 1.3-99.2% in the presence of SDS, triton X 100 (1%), tween 20 (0.5,1%) and tween 80. Lipase activity decreased specifically by 12.5-99.4% in the presence of SDS, tween 20, tween 80 and

Triton-X (0.5, 1%) for *Aeromonas* sp. Sh 12 isolates (Fig. 2 A-E).



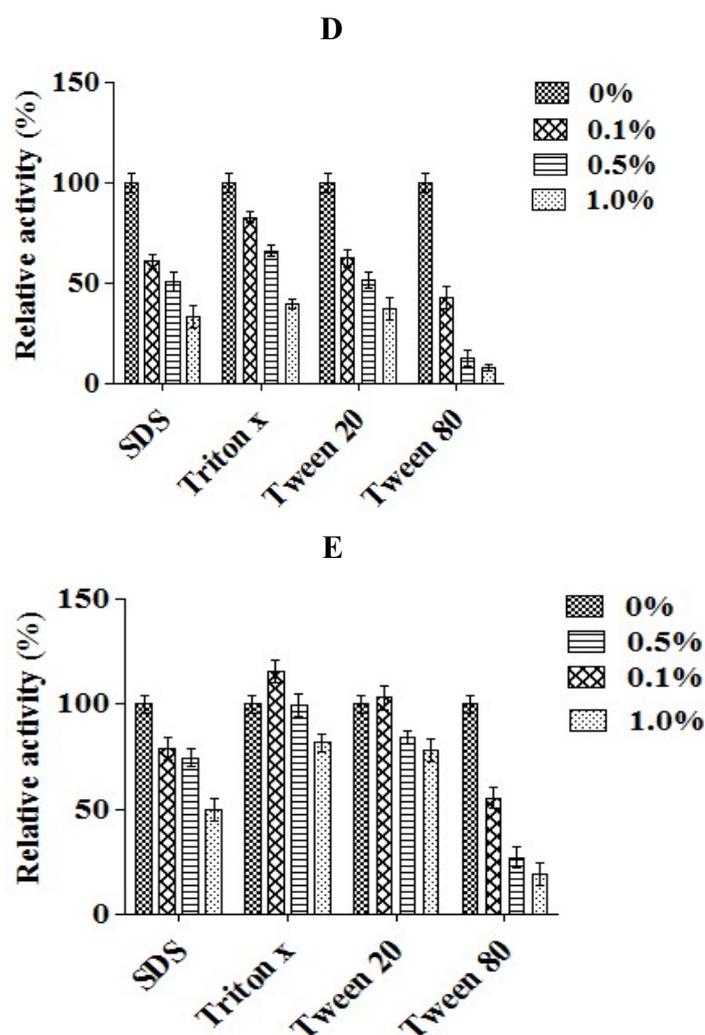


Figure 2: Effect of detergents on lipase activity of *Aeromonas* and *Pseudomonas* species: Lipase reaction was supplemented with detergents (SDS, Triton-X 100, tween 20 and tween 80) and incubated for 10 minutes at 37°C and lipase assays was performed. Reaction without detergents served as control and was considered as 100% lipase activity. The relative lipase activity was plotted against the various concentrations of detergents as indicated for *Aeromonas* spp. Sh 2 (A), Sh 8 (B) and Sh 12 (C) isolates, and *Pseudomonas* spp. Sh 13 (D) and Sh 17 (E) isolates. Data of three independent experiments was plotted with standard deviation.

Thermostability of lipase from *Aeromonas* and *Pseudomonas* species at 60 °C

Lipase activity was decreased by 32.9-95.2% for *Aeromonas* spp. (Sh 2, Sh 8 and Sh 12) and *Pseudomonas* spp. (Sh 13 and Sh 17) after 1, 2, 3, 4 and 5 hours of incubation at 60 °C. Only 0.25-8.2% lipase

activity was retained by all the *Aeromonas* and *Pseudomonas* species after 6 hours of incubation.

Aeromonas sp. Sh 2, *Pseudomonas* sp. Sh13 and Sh 17 had $T_{1/2}$ of 1 hour 30 min and *Aeromonas* sp. Sh 8 and Sh 12 had $T_{1/2}$ of 2 hours 30 min and 2 hours respectively (Fig. 3).

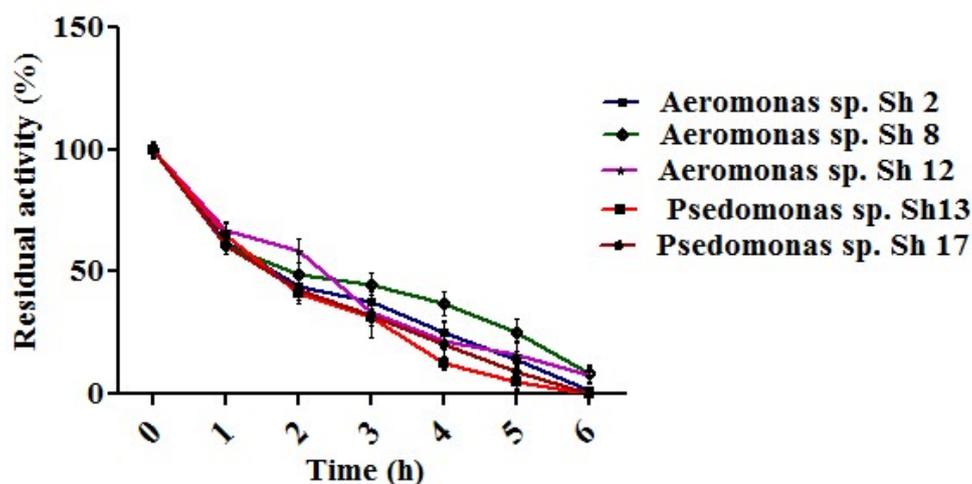


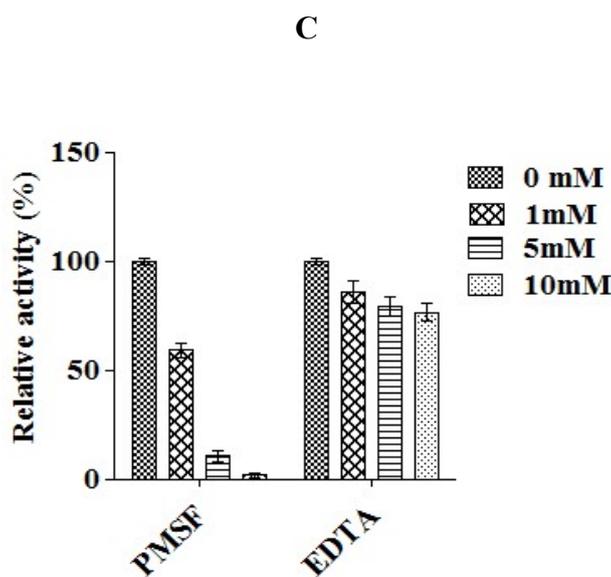
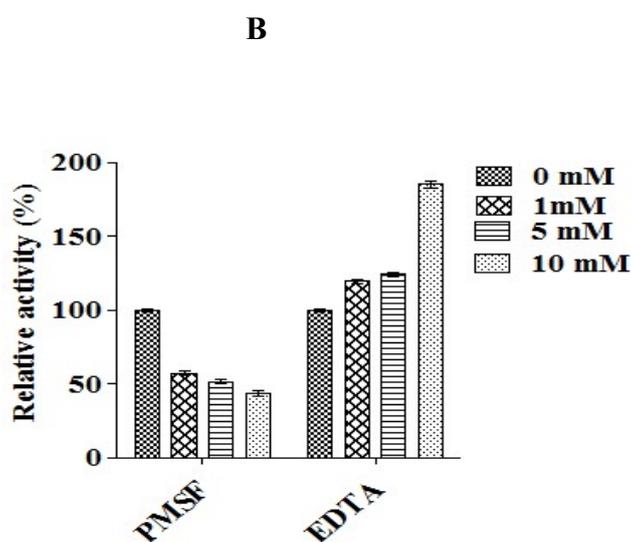
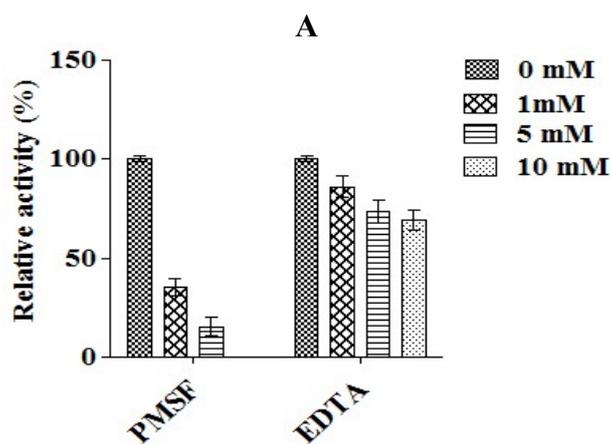
Figure 3: Thermal stability of lipase from *Aeromonas* and *Pseudomonas* species: Lipase reaction mixture was preincubated at 60°C for 6 hours and lipase activity was determined at regular interval of 1 hour for 6 hours. Sample at 0 hour (before incubating at 60°C) served as control and was considered as 100% lipase activity. The residual activity of *Aeromonas* spp. Sh 2, Sh 8 and Sh 12 and *Pseudomonas* spp. Sh 13 and Sh 17 isolates was plotted against different time intervals as indicated. Data of three independent experiments was plotted with standard deviation.

Stability of lipase in presence of metal ions and effect of inhibitors (PMSF and EDTA) on lipase activity of bacterial isolates

PMSF is a serine protease inhibitor and EDTA is a metal ion inhibitor. In order to test whether lipase from *Aeromonas* and *Pseudomonas* spp. are inhibited by serine protease or metal ion inhibitor, lipase assay was carried in the presence of PMSF and EDTA at 1, 5 and 10 mM. Complete inhibition of lipase activity was observed at 5 mM PMSF for *Pseudomonas* spp. Sh 13 and at 10 mM PMSF for *Pseudomonas* sp. Sh 17 and *Aeromonas* sp. Sh 2 isolates. Only 1.8% lipase activity was retained by *Aeromonas* sp. Sh 12 isolate at 10 mM PMSF. Lipase activity was decreased by

29.1 and 46.7% at 1 mM PMSF for *Pseudomonas* spp. Sh 17 and Sh 13 isolates respectively. Lipase activity of *Aeromonas* spp. Sh 2 and Sh 12 isolates was inhibited by 40.8-89.5% at 1 and 5 mM PMSF and 42.7-56.4% at 1, 5, 10 mM PMSF for *Aeromonas* sp. Sh 8 isolate.

Lipase activity was also inhibited by 7.4-31% with the increase in concentration of EDTA from 1- 10 mM for *Aeromonas* spp. Sh 2 and Sh 12 isolates and *Pseudomonas* sp. Sh 13 isolate. On the other hand, lipase activity was increased by 13.7-85.3% in the presence of EDTA (1, 5, 10 mM) for *Aeromonas* sp. Sh 8 and *Pseudomonas* sp. Sh 17 isolates (Fig. 4 A-E).



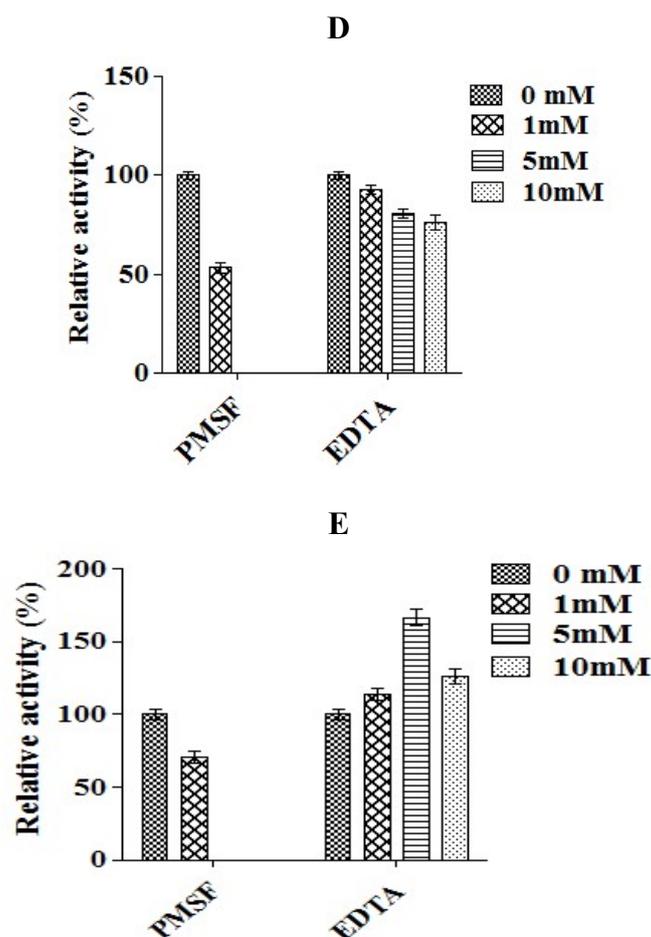
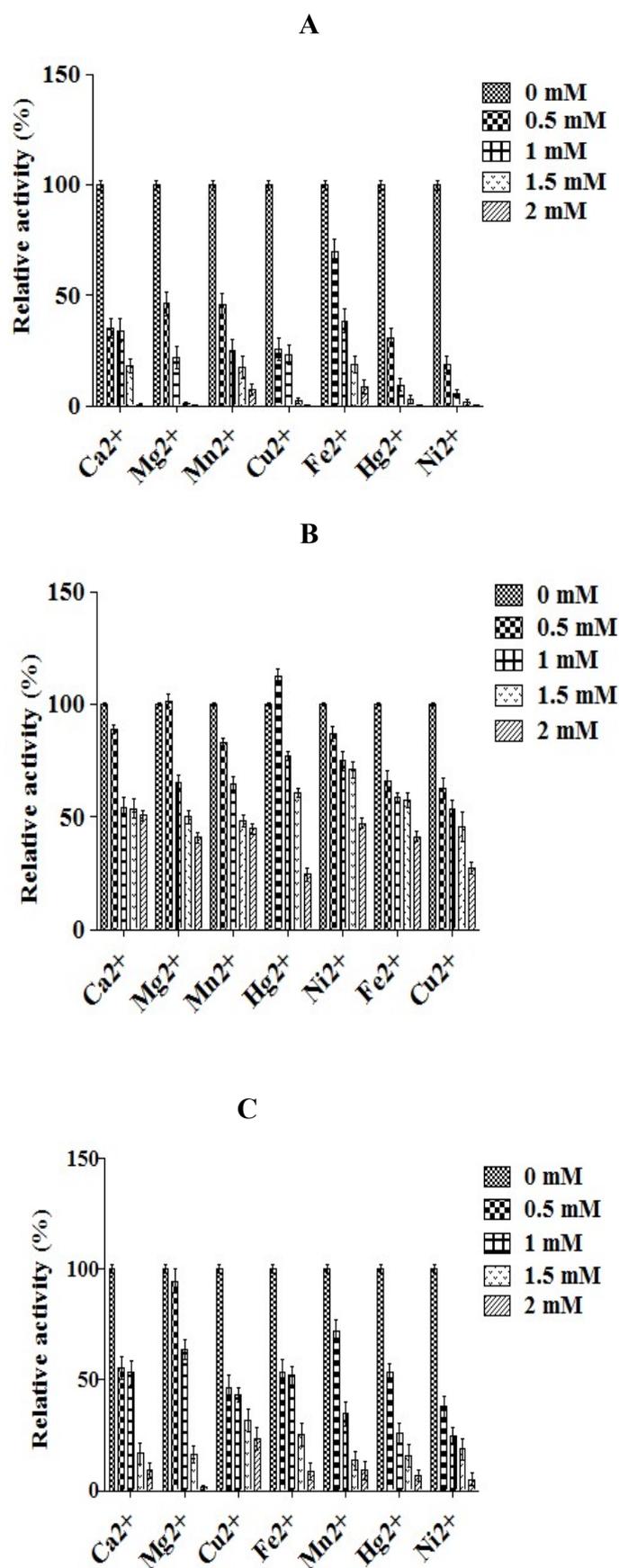


Figure 4: Effect of Serine protease inhibitor (PMSF) and metal ion chelator (EDTA) on lipase activity of *Aeromonas* and *Pseudomonas* species: Lipase assay was performed by supplementing 1-10 mM PMSF and EDTA to lipase reaction mixture. Reaction without inhibitors served as control and was set as 100% lipase activity. The relative lipase activity was plotted against the various concentrations of inhibitors as indicated for *Aeromonas* spp. Sh 2 (A), Sh 8 (B) and Sh 12 (C) isolates, *Pseudomonas* spp. Sh 13 (D) and Sh 17 (E) isolates. Data of three independent experiments was plotted with standard deviation.

Lipase assay was carried out by incubating reaction mixture with salts of metal ions (Ni^{2+} , Ca^{2+} , Mn^{2+} , Mg^{2+} , Cu^{2+} , Hg^{2+} , Fe^{2+}) and determining residual lipase activity. Lipase activity was increased by 1.3-2.0 fold in the presence of Ca^{2+} (0.5, 1 mM), Mg^{2+} (0.5-1.5 mM) and Mn^{2+} (0.5 mM) for *Pseudomonas* sp. Sh 13 isolate. Lipase activity of *Aeromonas* sp. Sh 8 isolate was increased by 1.6 % and 12.4 % at 0.5 mM concentration of Mg^{2+} and Hg^{2+} respectively, while lipase activity of *Pseudomonas* sp. Sh 17 isolate was

increased by 3.1% in presence of 0.5 mM Mg^{2+} . Lipase activity of *Aeromonas* sp. Sh 2, Sh 12 and *Pseudomonas* sp. Sh 17 isolates was inhibited by 5.6-99.8% in presence of all metal ions tested, except at 0.5 mM concentration of Mg^{2+} for *Pseudomonas* sp. Sh 17 isolate. Lipase activity decreased specifically by 10.8-75.5% in the presence of Ca^{2+} , Mg^{2+} (1-2 mM), Mn^{2+} , Hg^{2+} , Ni^{2+} and Cu^{2+} for *Aeromonas* sp. Sh 8 isolate and 11.5-93.6 % in presence of Cu^{2+} , Fe^{2+} , Hg^{2+} , Ni^{2+} , Mg^{2+} (2 mM), Mn^{2+} (1-2 mM), Ca^{2+} (1.5, 2

mM) for *Pseudomonas* Sh 13 isolate (Fig. 5 A-E).



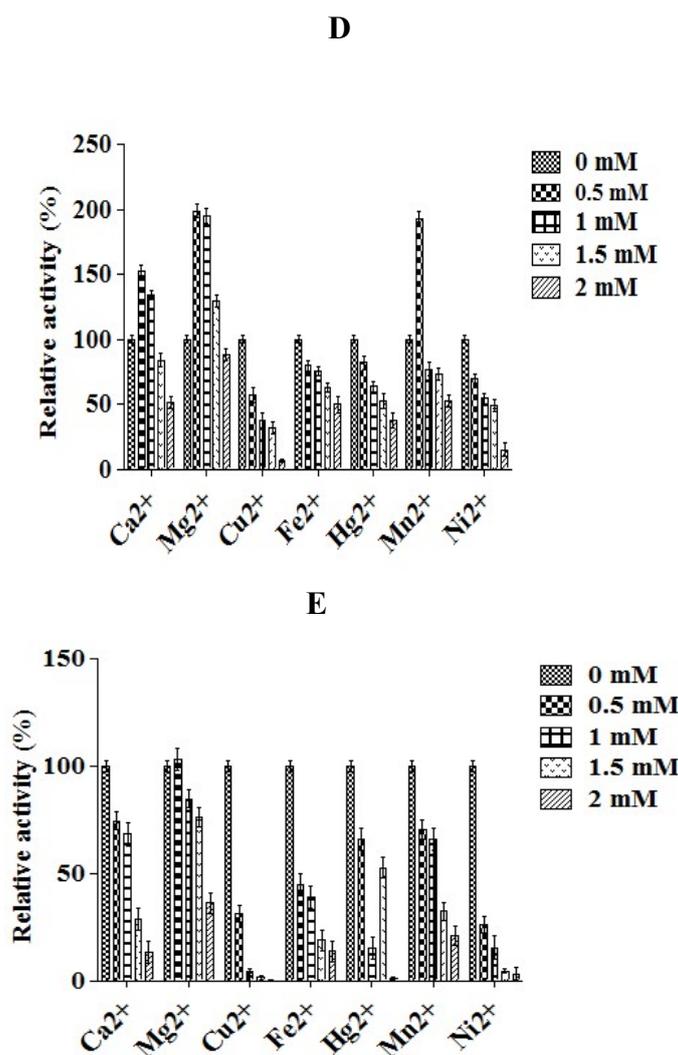


Figure 5: Effect of metal ions on the lipase activity of *Aeromonas* and *Pseudomonas* species: Lipase assays were carried by supplementing reaction mixture with salts of metal ions (Ni^{2+} , Ca^{2+} , Mn^{2+} , Mg^{2+} , Cu^{2+} , Hg^{2+} , Fe^{2+}). Reaction without metal ions served as control and considered as 100% lipase activity. The relative lipase activity of *Aeromonas* spp. Sh 2 (A), Sh 8 (B) and Sh 12 (C), *Pseudomonas* spp. Sh 13 (D) and Sh 17 (E) isolates was plotted against the various concentrations of metal ions as indicated. Data of three independent experiments was plotted with standard deviation.

Application of lipase in reduction of BOD and COD of waste water

BOD of waste water (dairy, paint and pharmaceutical) and seeded waste water was determined after 5 days of incubation at 20 °C. BOD of dairy, paint and pharmaceutical waste was found to be 10,034 mg/L, 2023 mg/L and 2576 mg/L respectively. Highest BOD reduction of 75.2% and 74% was seen in paint industrial

waste by *Pseudomonas* sp. Sh 17 and Sh 13 isolates respectively. In pharmaceutical waste, highest BOD reduction of 66 % and 68% was observed with *Pseudomonas* sp. Sh 13 and Sh 17 isolates respectively. In dairy waste, *Aeromonas* sp. Sh 12 and *Pseudomonas* sp. Sh 13 isolates showed highest BOD reduction by 69% and 68.4% respectively. BOD was reduced by 45.2-68 % in pharmaceutical waste, 49.3-69% in

dairy waste and 60-75.2% in paint industrial waste after 5 days of incubation with *Aeromonas* spp. Sh 2, Sh 8 and Sh 12 and *Pseudomonas* spp. Sh 13 and Sh 17 isolates (Fig. 6A).

COD of dairy, paint and pharmaceutical waste was found to be 18,134 mg/L, 5778 mg/L and 7890 mg/L respectively. COD of waste water (dairy, paint and pharmaceutical) and seeded waste water was also calculated after 5 days of incubation at 37 °C. Highest COD reduction of 76.9% and 75.4% was

observed in paint industrial waste by *Pseudomonas* sp. Sh 17 and Sh 13 isolates respectively. In dairy waste, highest COD reduction of 71.3% was observed by *Pseudomonas* sp. Sh 17 isolate. In pharmaceutical waste, *Pseudomonas* sp. Sh 13 and Sh 17 isolates showed highest COD reduction of 63.2% and 65.2%. COD was reduced by 58.6-65.2 % in pharmaceutical waste, 55-71.3 % in dairy waste and 69.6-76.9 % in paint waste after 5 days of incubation at 37 °C (Fig. 6B).

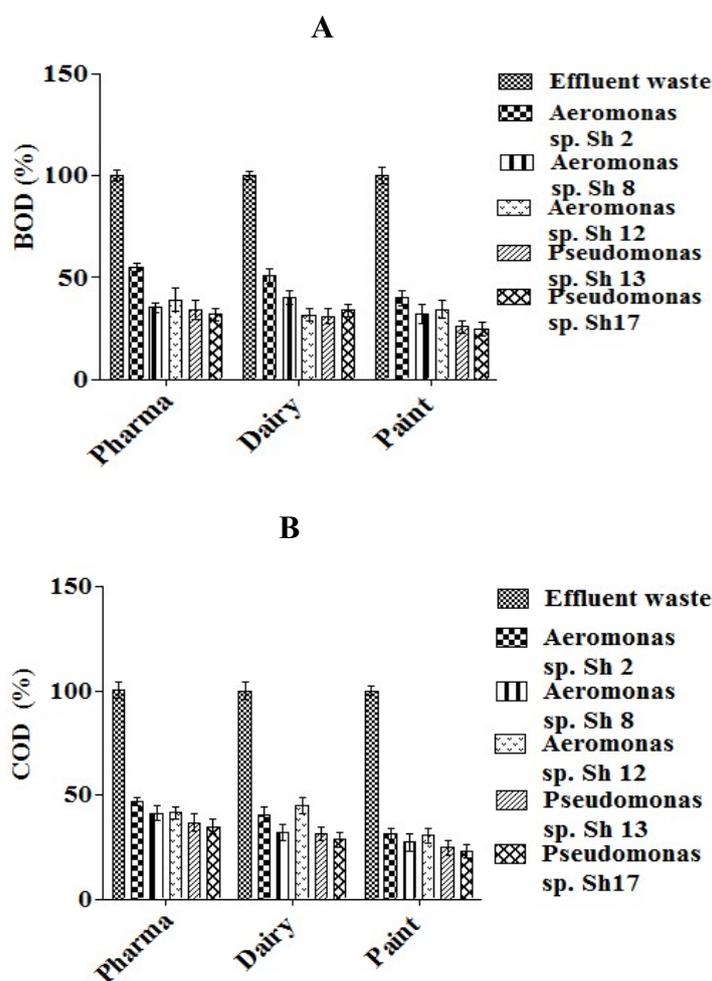


Figure 6: Treatment of effluent waste of pharma, dairy and paint industry by *Aeromonas* and *Pseudomonas* spp. : BOD values of effluents from dairy, pharmaceutical and paint industry were calculated after 5 days was set as 100% and was taken as control. BOD of effluents treated with *Aeromonas* spp. Sh 2, Sh 8 and Sh 12 and *Pseudomonas* spp. Sh 13 and Sh17 isolates after 5 days was plotted with respect to control (A). COD of industrial wastes calculated after 5 days of incubation at

37 °C was considered 100% and set as control. COD of waste incubated with *Aeromonas* spp. Sh 2, Sh 8 and Sh 12 and *Pseudomonas* spp. Sh 13 and Sh 17 isolates for 5 days was calculated with respect to control (B).

DISCUSSION

In the present study the effect of different solvents, metal ions, detergents, inhibitors on lipase activity and thermostability of novel bacteria *Aeromonas* sp. Sh 2, Sh 8 and Sh 12 and *Pseudomonas* sp. Sh 13 and Sh 17 isolated from effluent waste of paint industry was studied. Application of *Aeromonas* sp. Sh 2, Sh 8 and Sh 12 and *Pseudomonas* sp. Sh 13 and Sh 17 on reduction of BOD and COD of paint, dairy and pharmaceutical industrial waste was also investigated. Lipase activity was increased by 1.3-2.0 fold in the presence of Ca^{2+} (0.5, 1 mM), Mg^{2+} (0.5-1.5 mM) and Mn^{2+} (0.5 mM) for *Pseudomonas* sp. Sh 13 isolate. Lipase activity of *Aeromonas* sp. Sh 2, Sh 12 and *Pseudomonas* sp. Sh 17 isolates was inhibited in the presence of all metal ions tested, except at 0.5 mM Mg^{2+} for *Pseudomonas* sp. Sh 17 isolate. Lipase activity also decreased in the presence of Ca^{2+} , Mg^{2+} (1-2 mM), Mn^{2+} , Hg^{2+} , Ni^{2+} and Cu^{2+} for isolate *Aeromonas* sp. Sh 8 and in presence of Cu^{2+} , Fe^{2+} , Hg^{2+} , Ni^{2+} , Mg^{2+} (2 mM), Mn^{2+} (1-2 mM), Ca^{2+} (1.5, 2 mM) for *Pseudomonas* Sh 13 isolate. In earlier studies MgCl_2 (0.6 mM) in combination with CaCl_2 (0.4 mM) tremendously increased lipase activity to 60.92 U/ml in *Burkholderia cepacia* RGP-10 [13].

Lipase activity was enhanced by 2.1, 1.8, 1.4, 1.6, 1.3 and 2.2 folds in the presence of 0.5% acetone (*Aeromonas* sp. Sh 2 isolate), 1% acetone (*Aeromonas* sp. Sh 2 isolate), 0.5% ethanol (*Aeromonas* sp. Sh 8 isolate), 1% methanol (*Aeromonas* sp. Sh 12 isolate), 0.5% DMSO (*Pseudomonas* sp. Sh 13 isolate) and 0.5% ethanol (*Pseudomonas* sp. Sh 17 isolate) respectively. Lipase activity decreased in the presence of toluene, hexane and xylene for *Aeromonas* sp. Sh 2 isolate, in the presence of toluene, hexane, xylene and acetone for *Aeromonas* spp. Sh 8 and Sh 12 isolates. Lipase activity decreased significantly in the presence of ethanol, hexane, toluene, DMSO, xylene, phenol and acetone for *Pseudomonas* sp. Sh 13 isolate, in the presence of isopropanol (1%), hexane, DMSO, acetone, toluene, xylene and phenol for *Pseudomonas* sp. Sh 17 isolate. Previous study also reported 30 and 20 % enhancement in lipase activity in the presence of chloroform and DMSO by *Pseudomonas aeruginosa* lipase. Lipase activity decreased in the presence of acetone (5%), ethanol (10%), methanol (20%), isopropanol (35%), butanol (90%) and hexane retained 100% lipase activity [19]. Lipase activity of *Aeromonas* sp. Sh 2 isolate was enhanced by 1.6-1.7 folds in the

presence of Triton-X (0.1-0.5 %) and by 1.3 and 1.2 folds in the presence 0.1% Triton-X 100 for *Aeromonas* sp. Sh 8 isolate and *Pseudomonas* sp. Sh 17 isolate respectively. Increase in lipase activity by 1.4 and 1.2 fold was observed in the presence of 0.1% tween 20 for *Aeromonas* spp. Sh 2 and Sh 8 isolates. Lipase activity of *Aeromonas* spp. Sh 2 and Sh 8 isolates and *Pseudomonas* sp. Sh 17 isolate decreased in the presence of SDS, Triton-X (1%), tween 20 (0.5,1%) and tween 80. Lipase activity decreased in the presence of SDS, tween 20, tween 80 and Triton-X 100 (0.5, 1%) for *Aeromonas* sp. Sh 12 isolate. Similarly SDS was inhibitory, while the Triton-X and Tween-80 stimulated lipase activity in *Pseudomonas aeruginosa* KKA-5 lipase [10].

Complete inhibition of lipase activity was observed at 5 and 10 mM PMSF for *Pseudomonas* spp. Sh 13 and Sh 17 isolates and at 10 mM PMSF for *Aeromonas* sp. Sh 2 and *Aeromonas* sp. Sh 12 isolate. Lipase activity was inhibited with the increase in concentration of EDTA from 1- 10 mM for *Aeromonas* spp. Sh 2 and Sh 12 isolates and *Pseudomonas* sp. Sh 13. On the other hand, lipase activity was increased in the presence of EDTA (1, 5, 10 mM) for *Aeromonas* sp. Sh 8 isolate and *Pseudomonas* sp. Sh 17 isolate. In *Pseudomonas aeruginosa* SRT 9, lipase

activity decreased considerably in the presence of 5 mM EDTA (metal ion inhibitor) with only 36% residual activity after 30 min incubation [15]. Highest BOD reduction of 75.2% and 74% and COD reduction of 76.9% and 75.4% was seen in paint industrial waste by *Pseudomonas* sp. Sh 17 and Sh 13 isolates respectively. Previous studies have reported least BOD (112 mg/L) in palm oil, 82 mg/L in dairy, 145 mg/L in soap, 9 mg/L in domestic water effluent by *Pseudomonas aeruginosa*, where as least BOD 11 mg/L in slaughter house waste water by *Staphylococcus aureus* [7]. The decrease in COD was found to be 98% by *Acinetobacter* sp. (KUL8) and 99% by *Bacillus* sp. in bakery wastewater (KUL39) [20].

CONCLUSION

The present study showed unique properties such as metal ion, detergent and solvent tolerance of *Aeromonas* spp. (Sh 2, Sh 8 and Sh12) and *Pseudomonas* spp. (Sh 13 and Sh 17) lipase and their application in reduction of BOD and COD of industrial waste water. Thus these bacteria can find application in detergent industry, synthesis reactions, bioremediation and pollution control and make the environment cleaner and greener.

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REFERENCES

- [1] Gupta R, Gupta N, Rathi P. Bacterial lipases: an overview of production, purification and biochemical properties. *Applied Microbiology and Biotechnology*, 2004; 64: 763–781.
- [2] Gosh, PK, RK Sexana, Gupta R, Yadav RP and Davidson S. Microbial lipases production and application. *Science Progress*, 1996; 79(2):119-157.
- [3] Pandey A , Benjamin S , Soccol CR, Nigam P, Krieger N, Soccol VT. The realm of microbial lipases in biotechnology. *Biotechnology and Applied Biochemistry*, 1999; 29: 119–131.
- [4] Jaeger K-E and Reetz MT. Microbial lipases form versatile tools for biotechnology. *Trends in Biotechnology*, 1998; 16(9):396-403.
- [5] Nagar M, Dwivedi SK, Shrivastava D. A Review on industrial application in microbial lipases. *International Journal of Pharmaceutical and Research Sciences*, 2013; 2(4): 631-641.
- [6] Maygaonkar PA, Wagh PM and Permeswaran U. Biodegradation of distillery effluent by fungi. *Bioscience Discovery*, 2012; 3(2): 251 -258.
- [7] Prasad MP, Manjunath K. Comparative study on biodegradation of lipid rich wastewater using lipase producing bacterial species. *Indian Journal of biotechnology*, 2011; 10: 121-124.
- [8] Kumar S, Kikon K, Upadhyay A, Kanwar SS and Gupta R. Production, purification, and characterization of lipase from thermophilic and alkaliphilic *Bacillus coagulans* BTS-3. *Protein Expression and Purification*, 2005; 41:38–44.
- [9] Patil KJ, Chopda MZ and Mahajan RT. Lipase biodiversity. *Indian Journal of Science and Technology*, 2011; 4 (8): 971-982.
- [10] Sharon C, Furugoh S, Yamakido T, Ogawa HI, Kato Y. Purification and characterization of a lipase from *Pseudomonas aeruginosa* KKA-5 and its role in castor oil hydrolysis. *Journal of Industrial Microbiology and Biotechnology*, 1998 ; 20(5):304-307.
- [11] Chakraborty K and Raj RP. An extra-cellular alkaline metalloproteinase from *Bacillus licheniformis* MTCC 6824: Purification and biochemical

- characterization. Food Chemistry, 2008; 109:727–736.
- [12] Golani M, Hajela K and Pandey GP. Screening, Identification, Characterization and production of bacterial lipase from oil spilled soil. International Journal of Current Microbiology and Applied Sciences, 2016; 5 (3):745-763.
- [13] Rathi P, Saxena RK, Gupta R. A novel alkaline lipase from *Burkholderia cepacia* for detergent formulation. Process Biochemistry, 2001; 37:187–192.
- [14] Wang Y, Srivastava KC, Shen GJ, Wang HY. Thermostable alkaline lipase from a newly isolated thermophilic *Bacillus* strain A30-1 (ATCC 53841). Journal of Fermentation and Bioengineering, 1995; 79(5):433-8.
- [15] Borkar PS, Bodade RG, Rao SR, Khobragade C.N. Purification and characterization of extracellular lipase from a new Strain – *Pseudomonas Aeruginosa* SRT 9. Brazilian Journal of Microbiology, 2009; 40:358-366.
- [16] Hangloo S, Sourirajan A, Raina C, Chaubey A and Dev K. Isolation and characterization of Extracellular Lipase producing bacterial isolates from effluent waste of paint industry. Current Trends in Biotechnology and Pharmacy, 2016; 10 (3): 261-273.
- [17] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Analytical Biochemistry, 1976; 72(1): 248–254.
- [18] Helrich KC. Official methods of Analysis of the AOAC. Ed.15,2, Association of Official Analytical Chemists Inc., USA, 1990, pp. 314–316.
- [19] Karadzic I, Masui A, Zivkovic LI, Fujiwara N. Purification and characterization of an alkaline lipase from *Pseudomonas aeruginosa* isolated from putrid mineral cutting oil as component of metalworking fluid. Journal of Bioscience and Bioengineering, 2006; 102 (2): 82–89.
- [20] Bhumibhamon O, Kopraserstak A, Funthong S. Biotreatment of high fat and oil wastewater by lipase producing microorganisms. Kasetsart Journal: Natural Sciences, 2002; 36: 261 - 267.