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MEDIA OPTIMIZATION FOR LIPASE PRODUCTION BY ASPERGILLUS FLAVUS USING FISHERY WASTE

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ABSTRACT

Aspergillus flavus is a saprotrophic fungus capable of producing lipase. The production of lipase by Aspergillus flavus in solid state fermentation was studied using fishery waste as the nitrogen medium. In this study, different media components such as carbon concentration, nitrogen concentration and lipid concentration were optimized. Moreover, the physico- chemical parameters such as pH and temperature were also optimized. Sardine, tuna, shrimp and squid wastes were used as nitrogen source along with carbon and lipid. Sardine waste has been proved to yield high lipase activity with 73.4 U/ml when compared to other fish wastes. Various carbon and lipid concentrations were analyzed from that lactose and gingelly oil possess maximum

lipase activity of 21.3 U/ml and 35 U/ml respectively. The pH and temperature optima for lipase were 7 and 40°c respectively. Based on the results lactose, sardine fish waste and gingelly oil were found to be the best medium combination for lipase production.

Keywords - *asppergillusflavus*, fishwaste, lipase, solid substrate fermentation.

1. INTRODUCTION

Fish processing industries in India generates 2 million metric tons of fishery waste (Nurdiouna and Mazlina,2009). Solid waste which represents 20-60% of the initial raw material contains various kinds of residues (whole waste fish, fish head, guts etc) (Awarenet 2004). Solid waste includes heads, viscera, frames and skin proved to be a great source of 58% protein dry matter and fat (19% D.M) and it contain trace amounts of minerals such as nitrogen ,phosphorus, magnesium, sodium, potassium, calcium , iron, zinc , ammonium, manganese and copper (Ramakrishna v.et al.2013). These minerals supports the growth of microbes as they act as cofactors for various metabolic activities. Myristic, palmitic and stearic acids are the important saturated acids present in fish . Fish oil with higher level of polyunsaturated fatty acids is helpful for human health (Kim, B. S., & Hou, C. T.2006). However, these solid waste are not utilized properly and impose threat to the environment by causing infectious diseases to the human beings. Hence, the fish waste with nutritional value may be used for the production of industrially important enzymes.

A large number of micro organisms including bacteria, yeast, fungi produce different groups of enzymes protease, lipase, etc having high commercial interest food processing, detergent, textile, etc (Undercofler et al.1958). Microbial synthesis of enzymes has been reported to be influenced by various factors such as carbon sources, nitrogen sources, and operating parameters (temperature, pH, etc) (Forest and Moss 1987; Gupta et al. 2004; Jacop and Prema 2006; Palaniyappan et al.2009). The diversified utilization of sardine fish wastes as potential media for microbial lipase production is expected to deliver an attractive and promising strategy for large scale lipase enzyme production .In commercial practice, the optimization of medium composition is done to maintain the *Aspergillus flavus* nutrients. The medium has been established for the best production of lipase from *Aspergillus flavus* .

Lipases are currently receiving much attention involving selected micro organisms especially from fungi, bacteria and yeast (Sharma et al. 2001). Lipases are a class of enzymes which catalyse the hydrolysis of long chain triglycerides and are of considerable commercial interest in various industrial applications (detergent, food, flavour industry, biocatalytic resolution of pharmaceuticals, esters and amino acid derivatives, fine chemicals, agrochemicals, use as biosensor, bioremediation, cosmetics, perfumery, etc.) (Hasan et al. 2006).

Solid state fermentation process can be done in absence or near absence of free water under controlled conditions. Most of the industrial enzymes are produced from this fermentation process. It has more advantages such as high volumetric productivity, low energy consumption, natural habitat for bacteria and fungal culture, physical support, suitable value addition and cost effective.

2. MATERIALS AND METHODS

SAMPLE COLLECTION

Fish waste samples such as sardine fish waste, tuna fish waste, shrimp wastes and squid waste were collected from fish processing industries in and around Tuticorin.

PREPARATION OF MEDIA

Potato dextrose agar media was used to culture *Aspergillus flavus.* Apporoximately, 5g of Potato dextrose agar (PDA) powder was suspended in to 100ml of distilled water. Then, the medium was stirred until completely dissolved and undergo sterilization by autoclaving for 15 minutes at 121°c. After that, the medium was cooled to ambient temperature before poured into conical flask. The media was then stored at 4°c for further use.

DEVELOPMENT OF *ASPERGILLUS FLAVUS*

Aspergillus flavus was purchased from kamni biotech laboratory, Nagerkoil Tamilnadu, India. The culture was grown in Potato dextrose agar (PDA) slant at 28°c for 7days. Subcultures were maintained for further use.

CALCULATION OF LIPASE ACTIVITY

One unit of enzyme is defined as no of µmol of fatty acid released per ml of sample.

$$\mu\text{mol fatty acid/ml subsample} = \frac{*(\text{ml NaOH for sample} - \text{ml NaOH for blank}) \times N \times 1000]}{5}$$

Where N is the normality of the NaOH titrant used (0.05 in this case) & 5ml is volume of reaction mixture used.

PRODUCTION OF LIPASE BY SSF :

The media optimization experiment was started by cultivating the fungi in enrichment media containing different nitrogen sources (sardine, shrimp, tuna and squid) , different carbon sources (glucose, fructose, sucrose, lactose and maltose) and different lipid sources (coconut oil, gingelly oil, palm oil, cod liver oil and castor oil) . Each sample was taken in 250 ml flask, then the culture was inoculated in to each flask. The culture flask were incubated at 25°c for 7 days.

After incubation 100 ml of distilled water was added to each culture flask and left it 1hr. The culture containing enzyme was filtered through what man filter paper no.1. The filtrate was used for further titrimetric determination of lipase assay. **[**Praphan Pinsirodom and Kirk L. Parkin, 2001.]

PROTEIN WAS ESTIMATION BY USING LOWRYS METHOD.

EFFECT OF pH

Optimum pH for lipase activity was determined by using different pH buffers (pH4 and pH5 for acetic acid and sodium acetate, pH6 for citric acid and sodium citrate and pH7 and pH8 for NaH_2Po_4 and Na_2HPo_4) respectively.

EFFECT OF TEMPERATURE:

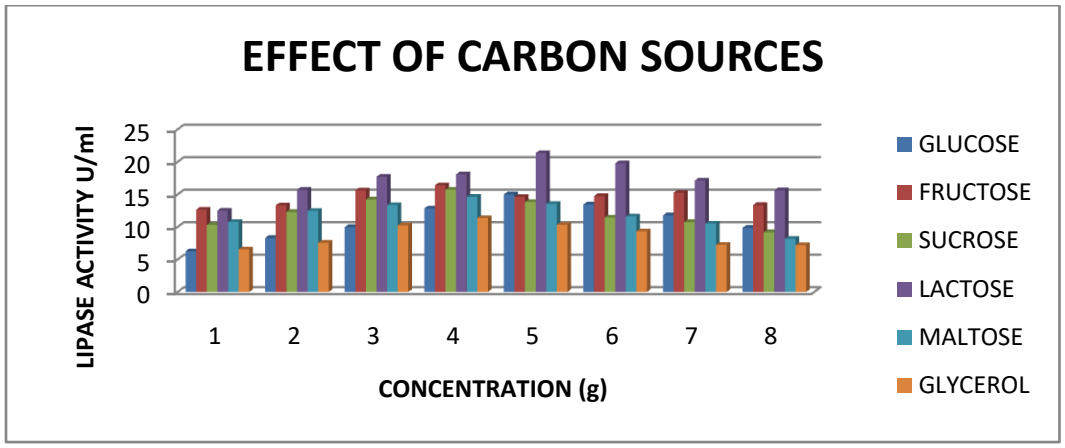
The effect of temperature on lipase activity was studied by incubating the medium containing nitrogen source, carbon source and phosphate buffer at various temperature (30, 35, 40, 45, 50 and 55°c) respectively.

3. RESULTS

EFFECT OF CARBON SOURCES ON LIPASE PRODUTION:

The effect of carbon source for lipase production in different concentration was shown in fig 1. Among the carbon sources lactose was yielded high lipase activity of 21.3U/ml was observed. The lowest lipase activity (10.3U/ml) was observed in glycerol.

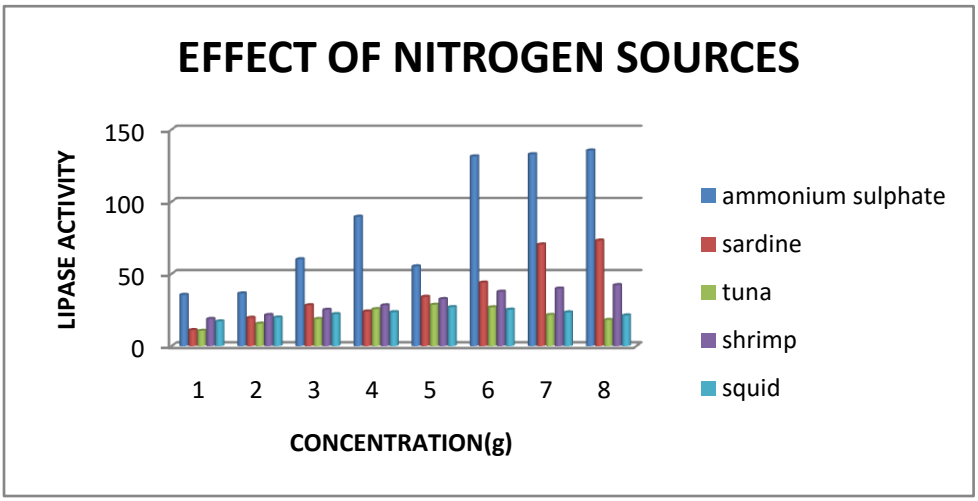
FIGURE:1. EFFECT OF CARBON SOURCES



EFFECT OF NITROGEN SOURCES ON LIPASE PRODUCTION:

The effect of nitrogen sources for lipase production was given in fig 2. Among the nitrogen sources sardine fish waste has high yielded of lipase activity of 73.4U/ml. However, tuna waste has shown lowest lipase activities of 18.1U/ml.

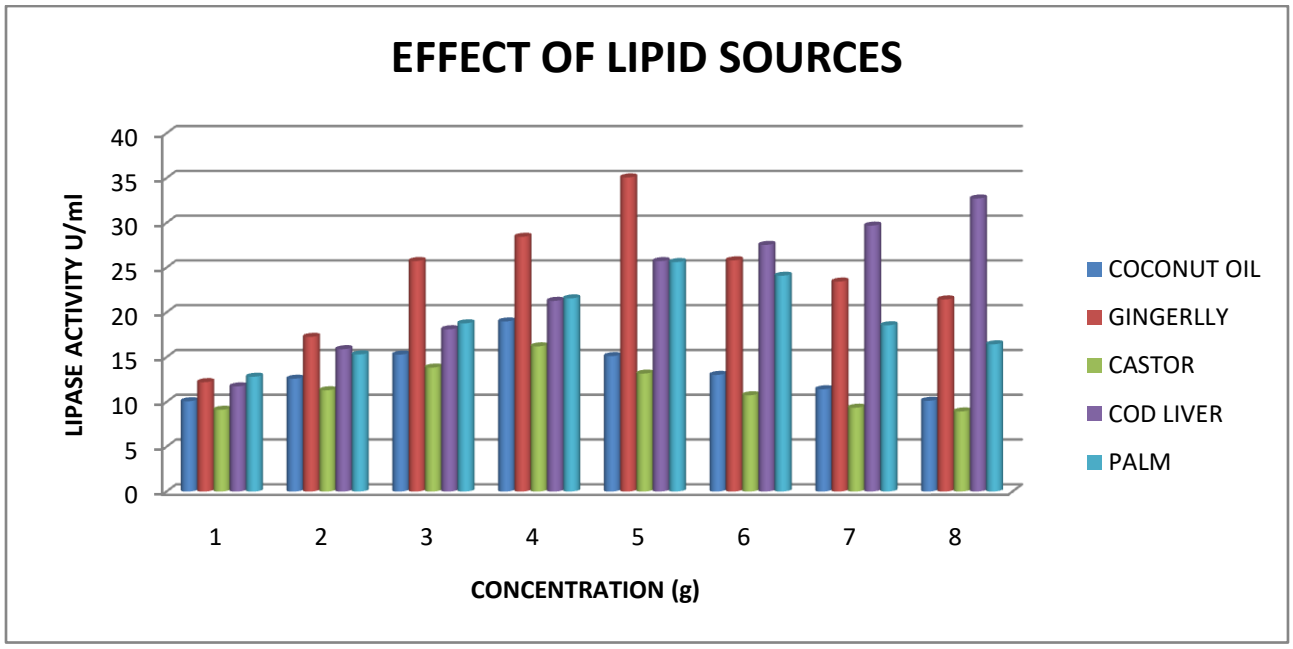
FIGURE: 2. EFFECT OF NITROGEN SOURCES



EFFECT OF LIPIDS SOURCES ON LIPASE PRODUCTION:

The effect of lipid source for lipase production was shown in fig 3. Among the sources, gingerly oil has yielded high lipase activity of 35U/ml. However, castor oil lowest lipase activities of 13.1U/ml.

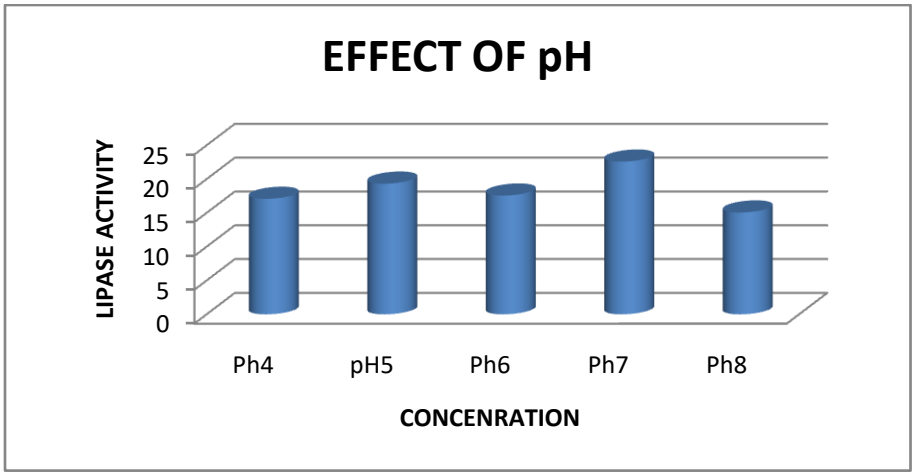
FIGURE:3. EFFECT OF LIPID SOURCES



EFFECT OF pH ON LIPASE PRODUTION ACTIVITY

Lipase activity has been found to be optimum at pH 7 (22.5U/ml). The lipase activity has been decreased slightly to 17 U/ml, 19.3 U/ml and 17.5 U/ml at pH 4,5 and 6 respectively fig 4. Beyond pH 7, lipase activity has been found to decreased moderately.

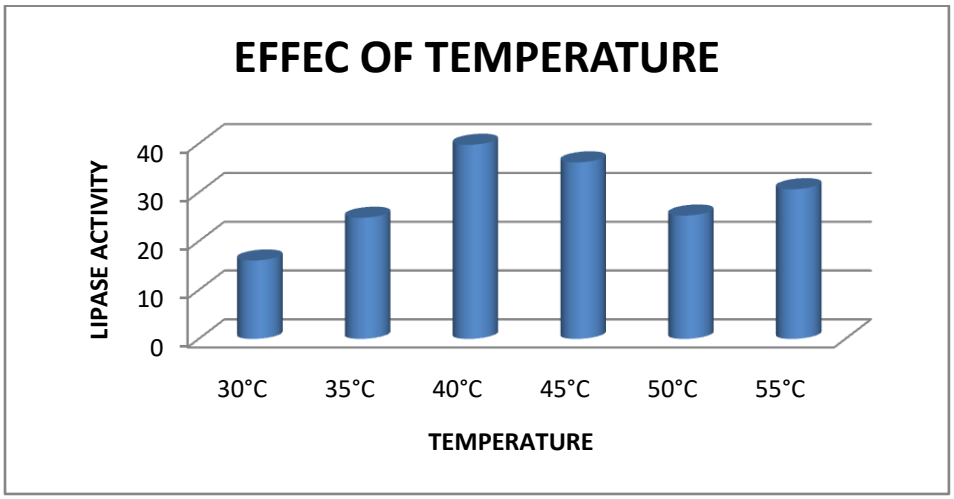
FIGURE: 4. EFFECT OF pH



EFFECT OF TEMPERATURE ON LIPASE PRODUCTION

lipase activity has been shown lower enzyme activity of 16U/ml at 30°c and higher enzyme activity of 39.7U/ml at 40°c fig 5. Above 40°c, lipase activity has been found to be decreased slightly.

FIGURE: 5. EFFECT OF TEMPERATURE



4. DISCUSSION:

Solid Substrate Fermentation is an economic mode of fermentation process employed in the production of industrially important enzymes. In this study, SSF was adopted for the production of lipase enzyme by *Aspergillus flavus.* Simple carbon sources like glucose, fructose, sucrose, lactose, maltose and glycerol might influence enzyme production. In a study, fructose was the best carbon source for highest lipase production by *Rhizopus homothallicus* (Rodriguez et al. (2006). Moreover, Ruchi et al. (2008) reported that lipase production by *Pseudomonas aeruginosa* was specially higher on meat derived on nitrogen sources. Bora and Kalita (2002) found that lipase production by Bacillus sp. DH 4 was high in media supplied with vegetable oils. This study was also supported by the positive effect of gingelly oil , coconut oil and castor oil on lipase production by *Rhizopus sp.* BTNT-2 (Bapiraju et al. 2005). The media components viz. lactose, sardine fish waste and gingelly oil has been found to be the best carbon, nitrogen and lipids source respectively. Moreover, lipase enzyme produced from *Aspergillus flavus* has been found to be active at pH (7) and temperature (40°c). However, Lipases with highest activity at pH 7 were also reported in *Staphylococcus epidermidis* (Joseph et al. 2006) and *Candida antarctica* (Pfeffer et al. 2006) and Bacillus megaterium AKG-1 (Sekhon et al. 2005), Acinetobacter sp. DYL129 (Kim et al. 2008) and Fusarium oxysporum (Prazeres et al. 2006) exhibited optimum lipase activity at the temperature range of 50–55°C.

5.CONCLUSION

The media components optimized in this study will be applied to utilize the under used fishery wastes and also helps in the production of lipase enzyme at industrial level.

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