RESEARCH ARTICLE

Exploration of Alkaliphilic Actinomycetes for Amylase Production from Agroindustrial Waste

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ABSTRACT

Saline soil possessing high pH value (above 8.0) predominates alkaliphilic actinomycetes (Singh S *et al.,* 2010), which can be explored for the production of amylase from agroindustrial waste as substrate using solid state fermentation. In this study six types of agroindustrial waste such as wheat bran, rice bran, Dal mil waste, oil mil waste, vegetable waste and molasses were employed. Solid state fermentation (SSF) was carried out at various moisture content (10 to 35%) at 37° c. results indicates that alkaliphilic Actinomycetes from saline soil of vidarbha region produced maximum amylase in vegetable waste (4.45mg/ml) in presence of 30 % moisture after 96 hrs, followed by oil mil waste (4.5mg/ml) in presence of 25%moisture after 120hrs, wheat bran and rice bran (3.9mg/ml) at 25%moisture content after 120hrs and 96hrs respectively. Dal mil waste was found to produce 3.5mg/ml amylase in presence of 25% moisture after 96hrs and molasses showed very least production of amylase (2mg/ml) after 144hrs. Hence it indicates that agroindustrial wastes are good source for the production of amylase using alkaliphilic Actinomycetes.

Key words. Amylase, Actinomycetes, saline soil, Agroindustrial waste.

INTRODUCTION

Many enzymes hydrolyze starch. Among those many enzymes extracellular enzymes such as alpha amylase (endo-1,4 ,α-glucan glucohydrolase) cleaves 1,4-α–D-glucosidic linkage present in straight linear chain of amylase (*Poornima et al.,* 2005).

Fermentation method such as solid state fermentation shows high volumetric productivity, followed by increase concentration of product, very less effluent generated and very handful fermentation equipment (VC Renge *et al.,* 2012). Agroindustrial waste used in this study as solid substrate viz; wheat bran, rice bran, oil cake, Dal mill waste, vegetable waste and molasses.

With increasing emphasis on the environmental protection, the use of enzymes particularly from extrimophiles gain considerable attention during last several years. Saline soil having high pH (above 8) predominates alkaliphilic actinomycetes after fungi (Singh et al., 2010). Although amylase produced by mesohilic, thermophilic actinomycetes have been studied (Kuo and Hartman, 1966), no study of amylase production by alkaliphilic actinomycetes has been published using agroindustrial waste as a substrate. This report presents details regarding the production of amylase by alkaliphilic actinomycetes from saline soil of vidarbha region using agroindustrial waste and solid state fermentation.

MATERIAL AND METHODS

Sample collection from saline sites:
Soil samples were collected from different saline site of district Amravati of Maharashtra state. Samples were collected in new sterile zip lock bags using sterile spatula. They were taken to laboratory for pretreatment with CaCO3 under humid condition to increase the number of actinomycetes propagules in the soil samples (Deshmukh and Vidhale, 2014).

Isolation of alkaliphilic actinomycetes:
Actinomycetes isolation agar supplemented with glycerol (5ml/l) and nystatin (50µg/ml) as anti-fungal antibiotic to avoid fungal contamination was utilized for isolation. Isolation was carried out by serial dilution and pour plate technique and identified by cover slip method.

Screening of amylolytic alkaliphilic actinomycetes strain:
Isolated alkaliphilic actinomycetes strains were grown on starch supplemented agar medium and production of amylase was detected by flooding plate with grams iodine solution. Efficiency of starch hydrolysis was determined on the basis of diameter of zone of decolorization against the blue colour background (Selvam *et al.,* 2011).

Inoculum and fermentation medium
Agroindustrial waste such as Wheat bran, rice Bran, oil mil waste, molasses and vegetable waste were taken for production of Amylase. All Agroindustrial waste was collected from local area such as Dal mills, oil mills, sugar industries, and vegetable market places.

All collected five types of Agroindustrial waste was grinded to size about 2 mm in homogenizer and then sieved through 20 -40 mesh screens to obtain a particle having diameter between 0.42 to 0.85mm.

Solid state fermentation (SSF) method was used for the production of enzyme amylase.5grams of each Agroindustrial waste was weigh and hydrated with 5ml of basal salt solution [$(NH_2)_2SO_4$ 2g/l, KH_2PO_4 1g/l, $MgSO_4.7H_2O$ 0.5g/l, $ZnSO_4$ 0.1g/l)] and adjusted with moisture content from 10-35%. SSF requires moisture to be present on substrate, for actinomycetes to produce enzymes. Lower or higher presence of water in substrate may adversely affect the microbial activity (Renge *et al.,* 2012).

Initially the 5g each agroindustrial waste hydrated with basal salt solution, after sterilization in autoclave for 15 minute at 15 lb/inch^2 was inoculated with 7 days old 1% inoculum culture of actinomycetes in conical flask and incubated at 37˚C for 7 days.

Enzyme estimation:
After every 24 hrs of incubation, to each fermented substrate adjusted at different moisture percent, 20 ml of 0.1 M phosphate buffer (pH7) was added. Then it was vigorously shaken in rotary shaker for 15 min at 120 rpm. The mixture was filtered through cheese cloth and centrifuged at 8000 rpm at 4°C for 15 min. The supernatant was filtered through whatman's filter paper and filtrate was used as crude enzyme (Suganthi et al., 2011; George *et al.,* 1945). Enzyme was assayed by DNS method (Bernfeld, 1955).

DNS (3,5-dintrosalicyclic acid) Reagents: DNS colour reagent contained 1g DNS dissolved in 50ml of Rochelle salt (sodium potassium tartarate tetrahydrate) in small lots, solution became milky yellow in colour. when 20ml of 2M NaOH was added ,colour changes from milky yellow to solution to transparent orange yellow colour at last final volume was made up to 100 ml by double distilled water.
Buffer: sodium phosphate buffer 0.02 M (pH, 6.9) with 0.006M sodium chloride, 2N sodium hydroxide.

Starch solution: 1% soluble starch solution was prepared by dissolving 1.0g soluble starch in 100ml 0.02 N sodium phosphate buffer (pH, 6.9).This solution was Solubilized by heating and simultaneous stirring for 15 min. After cooling solution was retained to its original volume (100ml) by addition of distilled water.

Maltose stock solution: Prepared by dissolving 180mg (MW 360.3) in 100ml reagent grade water in a volumetric flask.

Enzyme assay by SSF: Amylase activity was measure by Spectrophotometric assay. The activity of amylase was assayed by 0.5 ml crude enzyme with 0.5 ml soluble starch (1%w/v) prepared in 0.0M sodium phosphate buffer. After incubation at 37°for 30 min the reaction was stopped by addition of 2ml of 3,5-dinitrosalycylic acid reagent and absorbance was measured at 540 nm by UV/Vis spectrophotometer. Maltose released was determined from the standard curve. One unit (U) is defined as amount of enzyme which releases 1µmole of reducing end groups of glucose per minute in 0.02M sodium phosphate buffer (pH, 6.9) with 1%soluble starch as substrate at 37°C.

RESULTS AND DISCUSSION

In present study, 68 alkaliphilic actinomycetes were isolated from saline soil. Morphological identification was done by cover slip method (Selvam *et al.,* 2011).

Screenings of amylase were carried out on starch agar plate assay method. The strain of alkaliphilic actinomycetes showing maximum zone of clearance against dark blue background was chosen for production of amylase from Agroindustrial waste. (Karanwal *et al.,* 2013)

Screened amylolytic alkaliphilic actinomycetes strain was used to produce amylase from agroindustrial waste by SSF. The fermentation medium was centrifuged. The supernatant was filtered and used as crude enzyme for estimation of amylase activity (Renge *et al.,* 2012)

Estimation of amylase was carried out by spectrophotometer. The maximum amylase activity shown by alkaliphilic actinomycetes strain at different humidity (10 to 35%) are shown in Table 1, and figures 1 to 6.

From table and figures, results indicates that alkaliphilic Actinomycetes from saline soil of vidarbha region produced maximum amylase in vegetable waste (4.45mg/ml) in presence of 30 % moisture after 96 hrs, followed by oil mil waste (4.5mg/ml) in presence of 25%moisture after 120hrs, wheat bran and rice bran (3.9mg/ml) at 25%moisture content after 120hrs and 96hrs respectively. Dal mil waste was found to produced 3.5mg/ml amylase in presence of 25% moisture after 96hrs and molasses showed very least production of amylase (2mg/ml) after 144hrs.

Table 1: Production of amylase by alkaliphilic actinomycetes at 37˚C incubation temperature and different moisture content by SSF.

Types of waste	Moisture (%)	Production of amylase (mg/ml) at different incubation period in hours.						Mean ± SD Value
		24	48	72	96	120	144	
1.Wheat bran	10	0.60	1.00	1.00	1.25	1.40	1.00	1.04±0.27
	15	0.95	1.20	1.30	1.85	2.10	1.35	1.45±0.43
	20	1.05	1.35	1.35	1.75	2.15	2.90	1.75±0.67
	25	1.25	1.45	1.65	2.45	3.90	3.30	2.33±1.07
	30	1.15	1.45	1.60	2.20	2.60	2.50	1.91±0.59
	35	1.05	1.30	1.55	2.10	2.35	2.25	1.76±0.54
2. Rice bran	10	0.70	0.90	0.50	1.00	0.80	0.70	0.76±0.17
	15	0.40	0.50	0.70	0.90	0.70	0.60	0.63±0.17
	20	0.90	1.20	1.30	1.90	1.80	1.50	1.43±0.37
	25	1.80	2.00	2.80	3.90	3.40	2.90	2.8±0.80
	30	1.40	1.60	2.60	3.20	3.00	2.70	2.41±0.74
	35	1.20	1.40	2.10	2.95	2.20	1.90	1.95±0.62

Table 1: Continued…

Types of waste	Moisture (%)	Production of amylase (mg/ml) at different incubation period in hours.						Mean ± SD Value
		24	48	72	96	120	144	
3. Dal mill waste	10	0.35	0.40	0.55	0.60	0.650	0.40	0.49±0.12
	15	0.65	0.40	0.60	0.55	0.45	0.30	0.49±0.13
	20	1.00	1.40	1.25	1.30	1.35	1.20	1.25±0.14
	25	1.20	1.35	1.95	3.50	3.45	3.00	2.40±1.04
	30	2.60	2.10	2.40	2.45	3.00	2.90	2.57±0.33
	35	2.00	1.90	1.50	1.60	1.75	1.50	1.70±0.21
4. Oil mill waste	10	0.90	1.00	1.20	1.40	1.60	2.00	1.35±0.40
	15	1.10	1.20	1.10	1.15	1.20	1.25	1.16±0.06
	20	2.00	2.1	2.55	2.65	3.00	3.10	2.56±0.45
	25	2.25	2.65	3.10	3.90	4.50	.90	2.88±1.27
	30	2.10	2.55	2.75	3.10	2.70	2.20	2.56±0.37
	35	0.95	1.15	2.10	2.15	2.00	2.25	1.76±0.56
5. Vegetable waste	10	0.60	0.40	0.50	0.90	1.00	1.00	0.73±0.26
	15	0.90	0.95	1.00	1.20	1.25	2.15	1.24±0.46
	20	1.15	1.25	1.35	1.30	1.25	3.90	1.7±1.07
	25	2.15	2.35	2.95	3.95	4.25	3.95	3.26±0.90
	30	2.25	2.45	3.15	4.45	4.20	4.00	3.41±0.93
	35	2.00	1.50	2.15	2.25	3.10	3.00	2.33±0.61
6. Molasses	10	0.30	0.98	1.00	1.10	1.00	0.85	0.87±0.29
	15	0.45	0.65	0.95	1.00	0.95	0.70	0.78±0.21
	20	0.55	0.60	0.90	1.20	1.00	0.75	0.83±0.24
	25	0.65	0.65	0.95	1.25	1.00	0.65	0.85±0.24
	30	0.75	0.95	1.10	1.30	1.25	0.95	1.05±0.20
	35	0.45	0.35	0.55	1.15	1.10	0.85	0.74±0.34

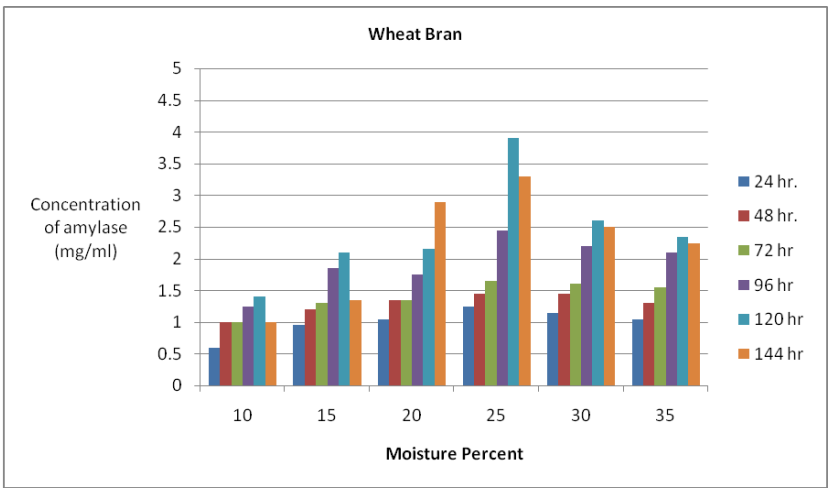

Figure 1: Wheat bran: Production of amylase by alkaliphilic actinomycetes at 37˚C incubation temperature and different moisture content by SSF.

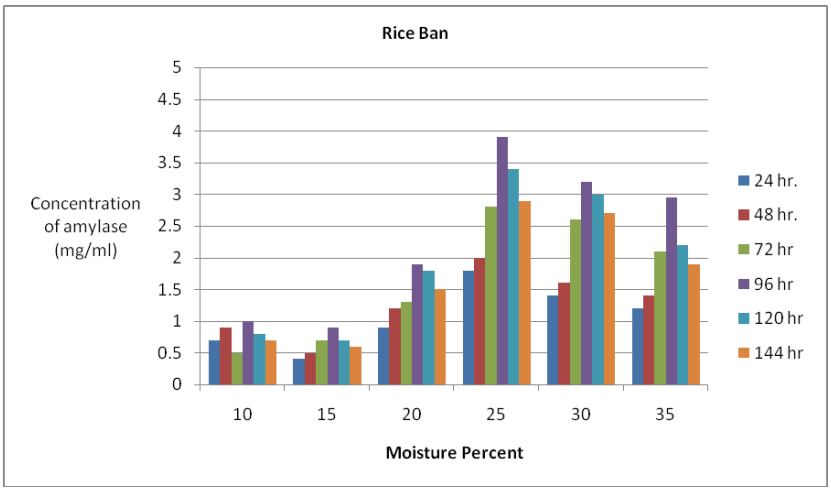

Figure 2: Rice bran: Production of amylase by alkaliphilic actinomycetes at 37°C incubation temperature and different moisture content by SSF.

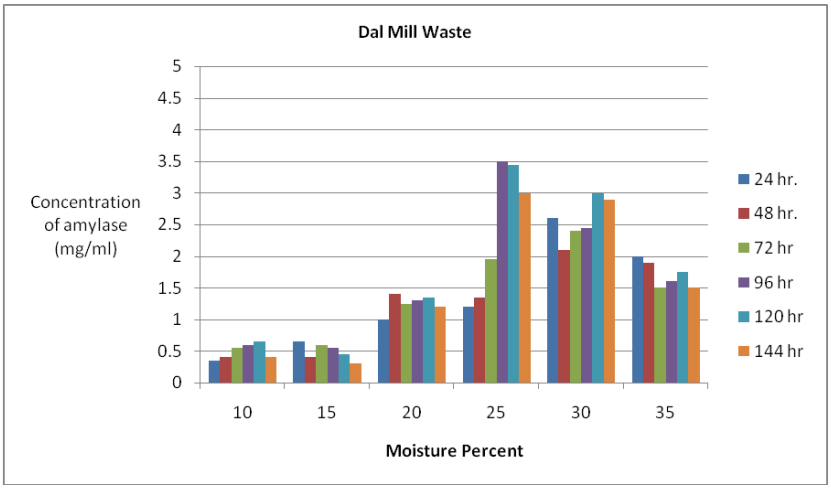

Figure 3: Dal mill waste: Production of amylase by alkaliphilic actinomycetes at 37°C incubation temperature and different moisture content by SSF.

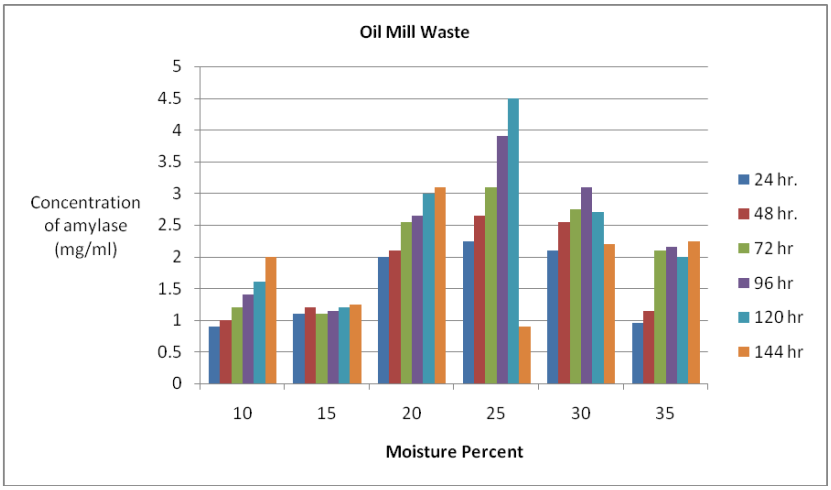

Figure 4: Oil mill waste: Production of amylase by alkaliphilic actinomycetes at 37°C incubation temperature and different moisture content by SSF.

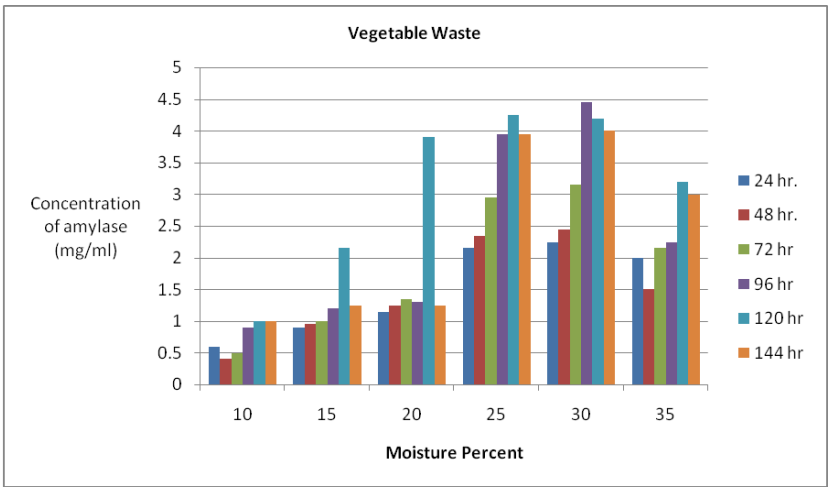

Figure 5: Vegetable waste: Production of amylase by alkaliphilic actinomycetes at 37˚C incubation temperature and different moisture content by SSF.

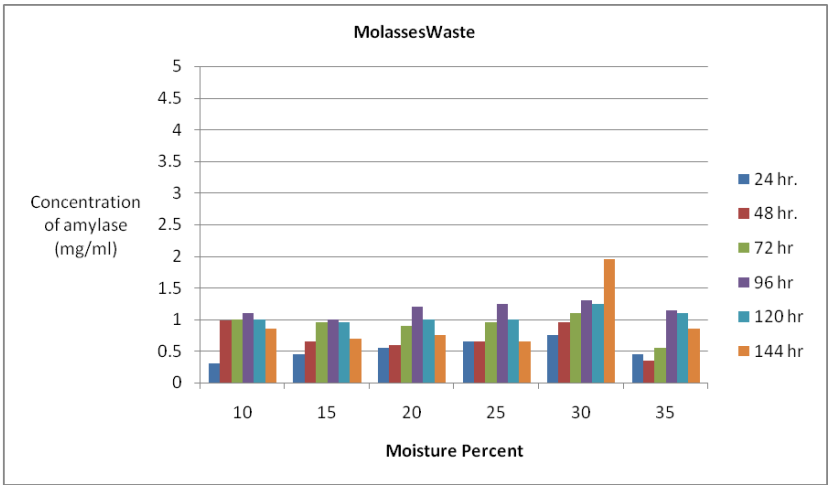

Figure 6: Molasses: Production of amylase by alkaliphilic actinomycetes at 37˚C incubation temperature and different moisture content by SSF.

Vidyalakshmi *et al.* (2009) studied production of extracellular amylase by bacillus spp. in submerged fermentation. The production of enzyme was maximum about at 10 hours after incubation at 35˚C and pH 7.

Poornima *et al.* (2008) suggested that actinomycetes strain can effectively be used in large scale production of α-amylase for commercial purpose, after testing and ascertaining the strains capability in large scale fermentation. Hence it indicates that agroindustrial waste are good source for the production of amylase using alkaliphilic Actinomycetes.

CONCLUSION

The high level of enzyme production using agro industrial waste is significant due to cheap nature of these sources. The findings are quite attractive, as only few actinomycetes, particularly alkaliphilic ones, have so far been explored for their enzymatic potential.

Conflicts of interest: The authors stated that no conflicts of interest.

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