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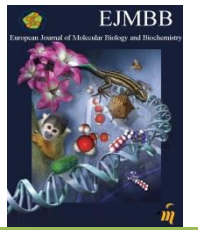

ISOLATION OF AMYLASE PRODUCING BACTERIA AND OPTIMIZATION OF GROWTH CONDITIONS

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ABSTRACT
Starch degrading bacteria isolated from polluted water samples and screened the isolates by starch agar base plate method. More potent bacteria were identified as *Micrococcus sp* VR. By morphological and biochemical characters based on Bergey's manual determinative bacteriology and DNA sequencing. Effect of bio parameters on enzyme production and enzyme activity by Dinitrosalicylic acid method were studied by adjusting the incubation temperature at 27^0C and 37^0C, incubation time (24, 48, 72hrs), production medium pH range from 6 to 8 (6, 6.2, 6.4, 6.6, 6.8, 7, 7.2, 7.4, 7.6, 7.8 and 8) and different concentration of starch (0.5, 1, 1.5, 2 & 2.5%) and optimize growth conditions. Maximum amylase activity of VR was 0.9942 IU/L at 1% of starch, 37^0C, and 24 hr of incubation and at pH 7.2.

INTRODUCTION

Amylases are among the most important enzymes and are of great significance in present-day biotechnology. Amylases can be derived from several sources such as humans, animals, plants and microbes [1]. Microbes producing amylase enzyme are among the most important hydrolytic enzymes and their application requires unique properties [2]. Several physical and chemical parameters like pH, temperature, incubation time, carbon source and nitrogen source etc. affect the enzyme production and the enzyme activity [3].

Microbial amylases have found huge applications in the starch saccharification, in the textile, food, brewing, baking, detergent, and paper industries [4]. Gelatinization and liquefaction of the starch are the key processes involved in the manufacture of dextrose and syrups from native starch [5].

Micrococcus is a gram positive bacterial cell that grows in the tetrad arrangement and has optimum growth temperatures range from 25 to 35^0C, is a strictly aerobic organism. *Micrococcus is* generally thought to be a saprotropic or commensal organism, though it can be an opportunistic pathogen. Present study, is to carry out the amylase production optimization of pH, incubation temperature, culture incubation time and 5 concentrations of starch were studied using *Micrococcus sp* (VR), isolated from polluted water. The test organism was identified based Bergey's manual determinative bacteriology.

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MATERIALS AND METHOD

Sample collection and isolation of microorganism

Polluted water samples were collected from different places of Ernakulam district and aseptically transfer to the laboratory immediately. The isolation of organisms were done by using serial dilution method. Dominant 6 colonies were sub cultured to get pure colonies.

Screening of Amylolytic Bacteria

Amylolytic organisms were screened by qualitative plate assay. Isolated organisms were separately inoculated on starch agar base plates [Starch (20g), Peptone (5g), Beef extract (3g), Nacl (3g), Agar (15g)] and incubated at 37^0C for 48 hours. After 48 hours, plates with bacterial colonies were flooded with Iodine solution. Amylolytic strains hydrolyse the starch present in the culture medium and produce zone of degradation. Colonies

showing clear zone around them were picked out and more powerful colonies were selected for studies.

Identification

VR was identified based on morphological, biochemical and physiological characteristics according to Bergey's manual of determinative bacteriology.

Enzyme Preparation

Prepare starch broth medium VR was inoculated, after 24hr of incubation time, medium was centrifuged at 4000rpm for 15 min at 4^0C. Cell pellets were discarded and resultant supernatant was used as the crude enzyme for various enzyme assay.

Optimization of Bioparameters

Effect of incubation temperature, pH, concentration of starch and incubation time on enzyme production and enzyme activity was studied by adjusting the incubation temperature at 27^0C and 37^0C and production medium pH range from 6 to 8(i.e., 6, 6.2, 6.4, 6.6, 6.8, 7, 7.2, 7.4, 7.6, 7.8 and 8) in 5 different concentration of starch (0.5, 1, 1.5, 2 & 2.5%) and at 24, 48, 72hrs of incubation time.

Amylase Assay by DNSA Method

Amylase assay was done by using a dinitrosalicylic acid (DNSA) reagent [6]. Enzyme activity expressed in 1U/L, one unit of amylase activity was defined as the amount of amylase that liberates 1.0 mg of glucose per minute under assay conditions. (i.e., 1 unit of amylase activity was defined as the amount of amylase which is required to catalyze the liberation of reducing sugar equivalent to one micro gram of glucose per minute under the assay condition).

RESULTS

In the present research work, the amylase producing bacteria were isolated from polluted water samples and more enzyme producer from screening was used for following studies. The study organism was identified as *Micrococcus sp* based on morphological, biochemical and physiological characters according to Bergey's manual determinative bacteriology (Table 1).

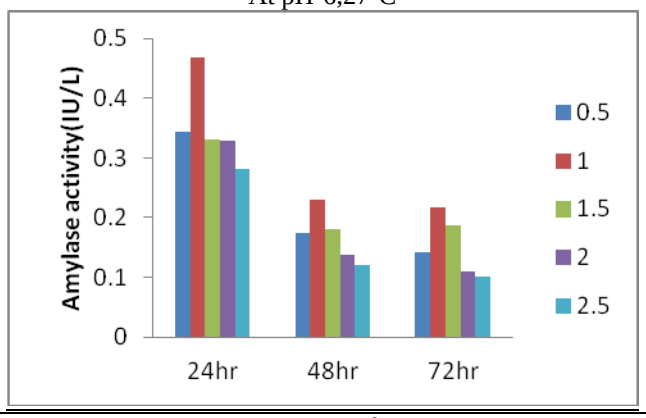
From the optimization studies of VR, higher production of an enzyme was in pH between 6.8-7.4 and lesser production of enzymes in other low and high pH and has suitable temperature of 37^0C. Other parameters for higher enzyme production were 24hr of incubation time and 1% of starch concentration. The optimum parameters for maximum production of amylase enzyme from VR at 24hr of incubation time,1% of starch concentration , pH 6.8-7.4 and 37^0C of incubation temperature (Figure1).

The test organism was identified based on the Bergey's Manual of Determinative Bacteriology.

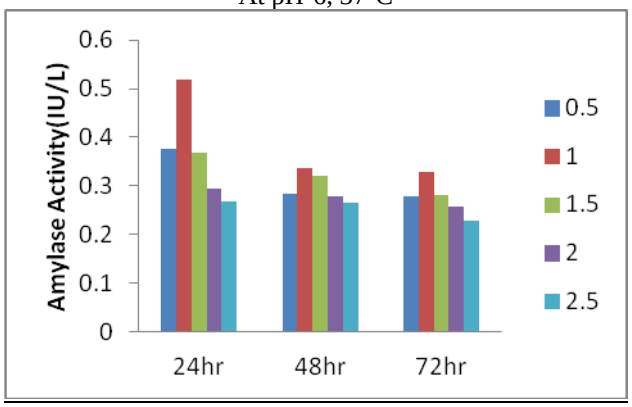
Table 1. Biochemical characteristics of VR

SI no	Biochemical test	Results
1	Grams reaction	+
2	Shape	Cocci,tetrad arrangement
3	Aerobic growth	+
4	Growth at 45-55^{0}C	-
5	Mac Conkey growth	+,NLF
6	Motility	-
7	Endospore	-
8	Capsule	+
9	Starch hydrolysis	+
10	Gelatin hydrolysis	-
11	Glucose fermentation	+
12	Lactose fermentation	+
13	Sucrose fermentation	+
14	Mannitol fermentation	-
15	H_2S production	-
16	Indole test	-
17	Methyl red	-
18	VP	-
19	Citrate	-
20	Catalase	+
21	Oxidase	+
22	Urease	-
23	6.5%NaCl	+
24	Pigmentation	Orange
Identification		*Micrococcus sp*

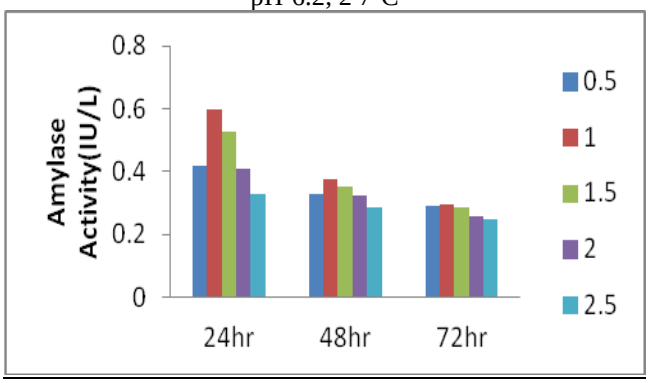
At pH-6,27^{0}C



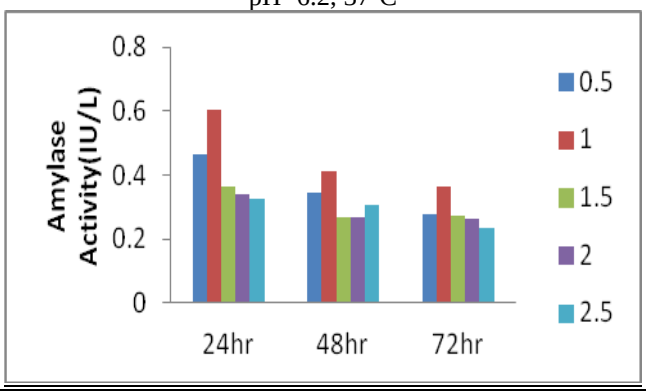
At pH-6, 37^{0}C



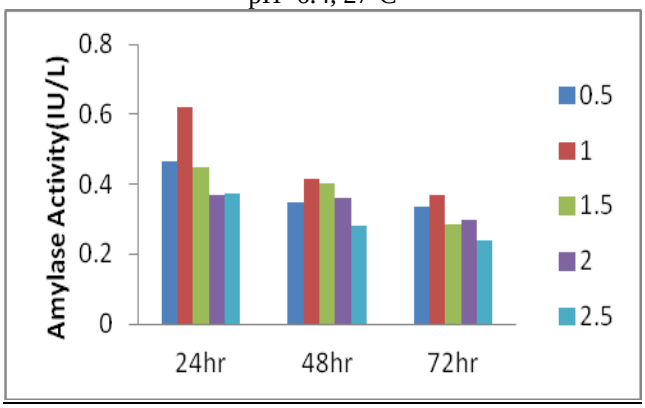
pH-6.2, 2 7^{0}C



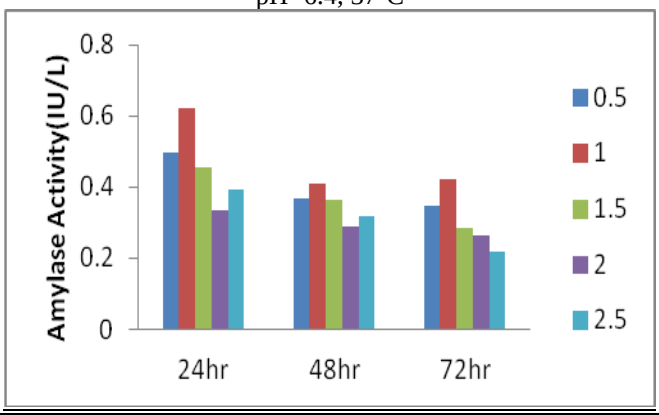
pH- 6.2, 37^{0}C



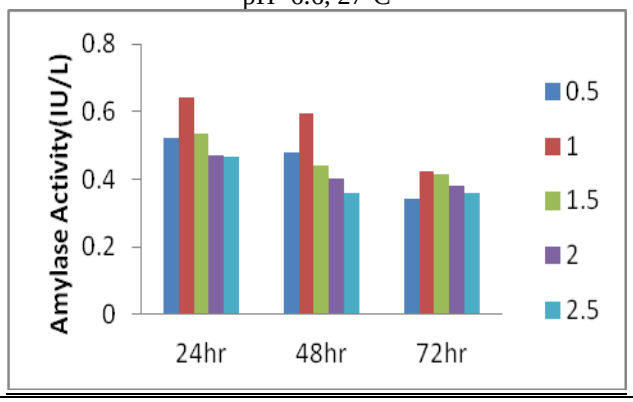
pH- 6.4, 27^{0}C



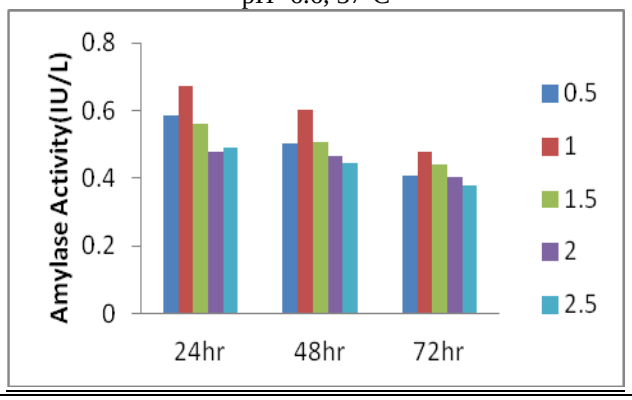
pH- 6.4, 37^{0}C



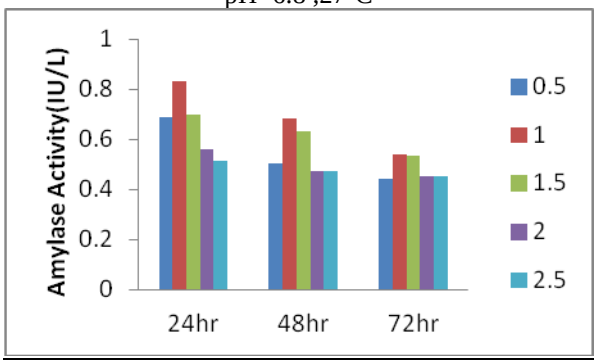
pH- 6.6, 27^{0}C



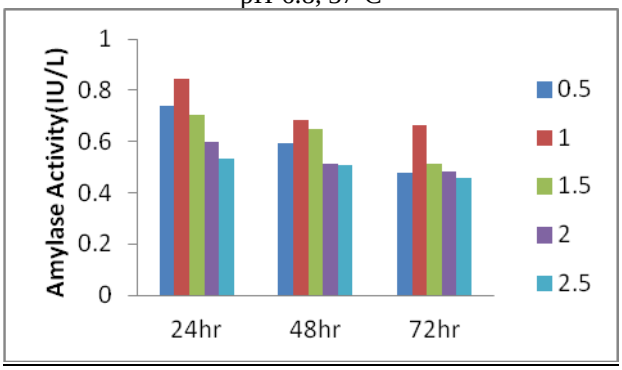
pH- 6.6, 37^{0}C



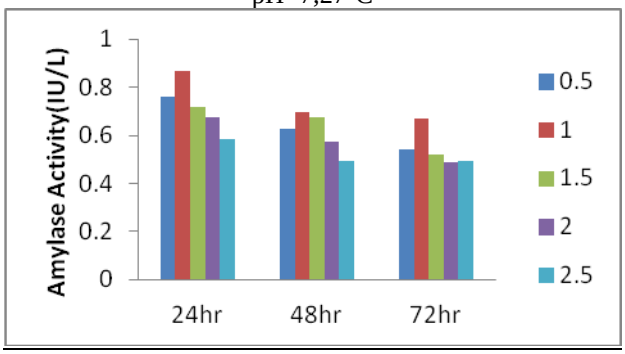
pH- 6.8 ,27^{0}C



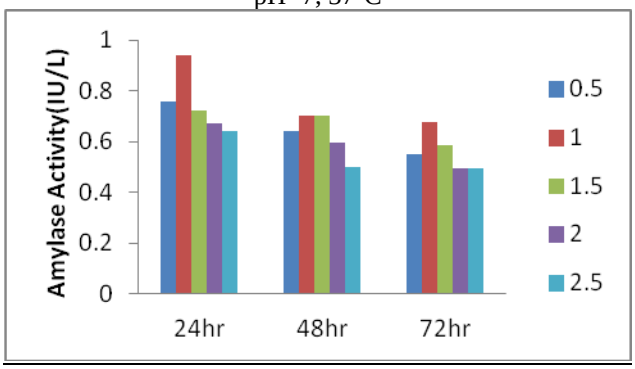
pH-6.8, 37^{0}C



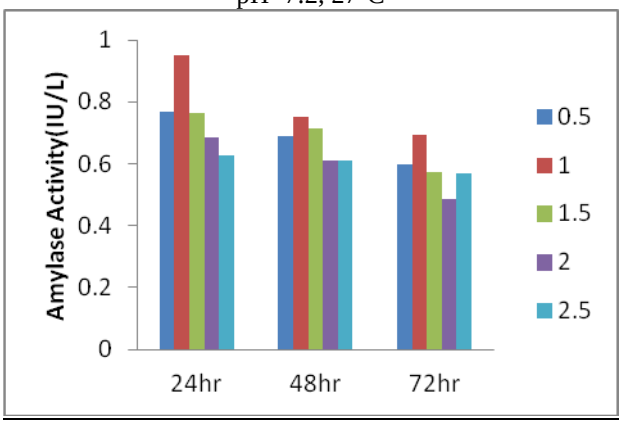
pH- 7,27^{0}C



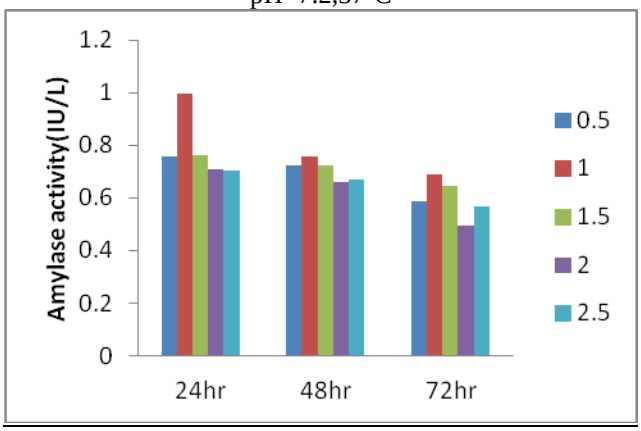
pH- 7, 37^{0}C



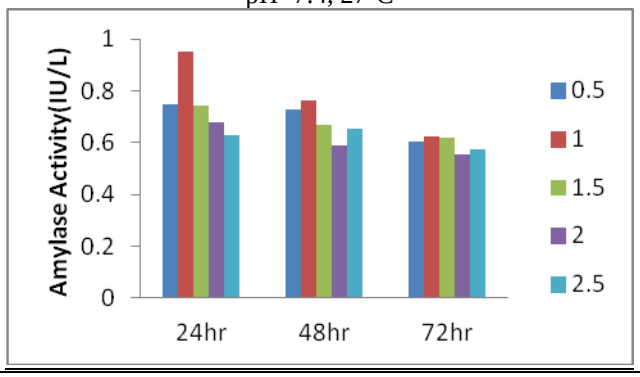
pH- 7.2, 27^{0}C



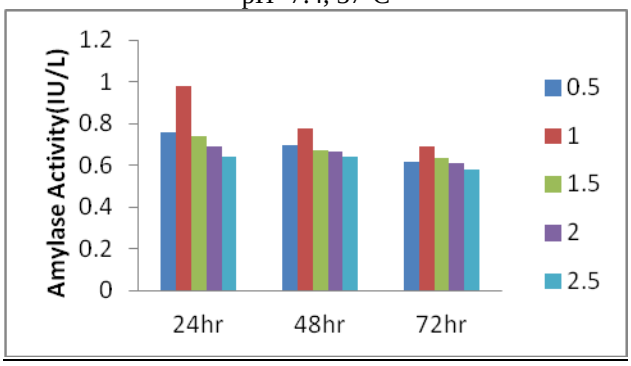
pH- 7.2,37^{0}C

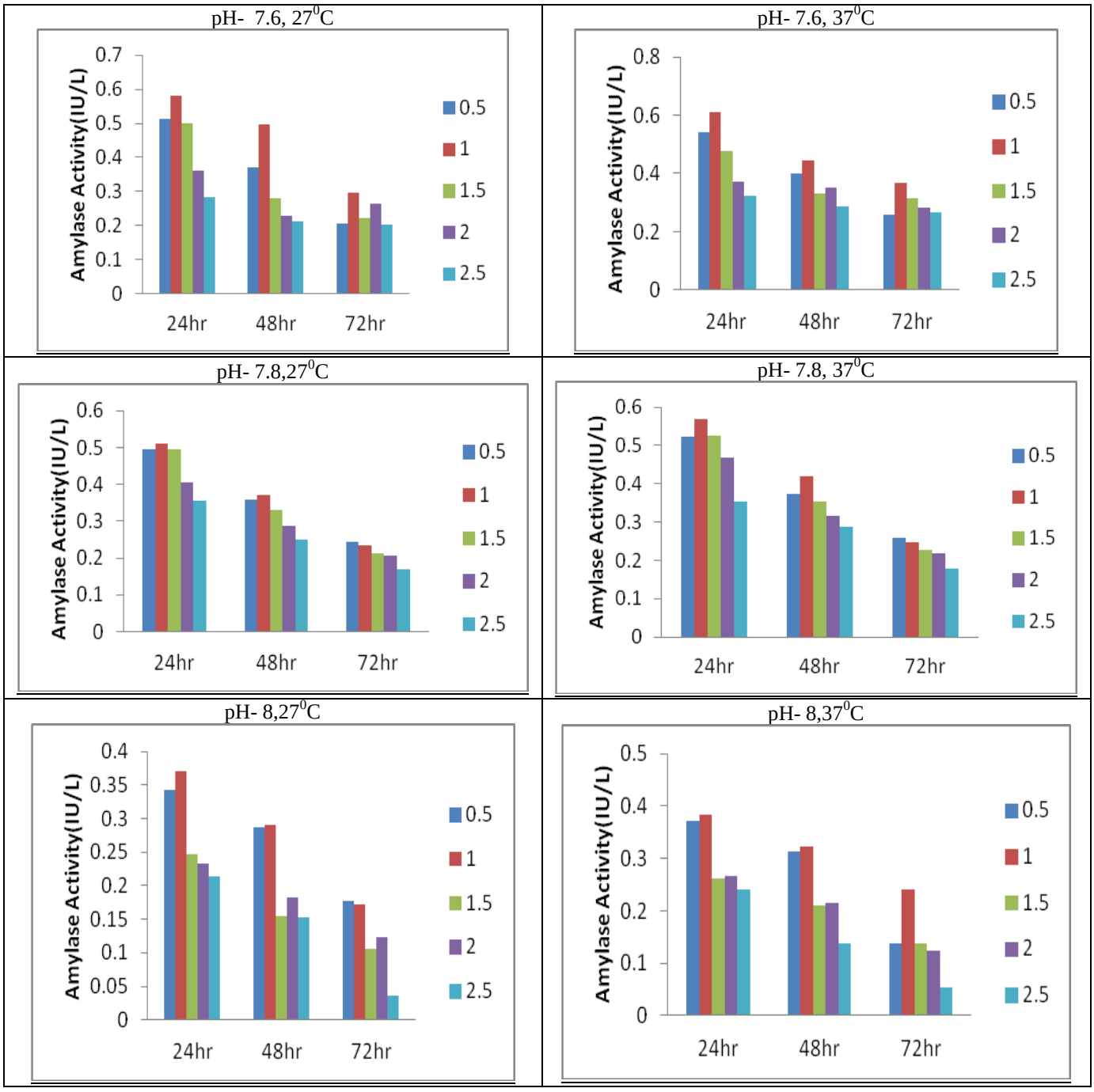


pH- 7.4, 27^{0}C



pH- 7.4, 37^{0}C





DISCUSSION

Amylolytic VR was isolated from the polluted water samples and identified as *Micrococcus sp* .Crude enzyme VR was used for various enzyme assay. From the optimization studies of an enzyme, 0.9942 IU/L was the highest activity of VR and was at the parameters such as 1% of starch, 37^{0}C of incubation temperature, 24 hr of incubation time and at pH 7.2. Similar work of α – amylase from *Bacillus marini* used as enzyme for industrial use and maximum production was found to be at optimum temperature 70^{0}C and pH 9.0 [7]. The optimum pH of VR, in between 6.8-7.4.

The highly alkaline amylase producing *Bacillus megaterium* has maximum enzyme activity obtained at 370C after 96h of incubation and the enzyme relatively stable between pH 5-pH13 and temperature ranging from 32^{0}C to 50^{0}C [8].

Similar study reported, *Bacillus licheniformis* has maximum α-amylase production at pH of 8 in iodine method, and in 3.5.dintrosalicylic acid [9]. In production of amylase using *Bacillus subtilis* and *Bacillus amyloliquefaciens*, the optimum pH for activity was 4-7 for strain *Bacillus subtilis* and 4-8 for strain *Bacillus liquefaciens* while the optimum temperature for their

activities were in the range 37-75^{0}C at 0.5% starchandin the range 85-95^{0}C at 35% starch [10].

CONCLUSION

This work concluded that *Micrococcus sp* VR was a potential producer of amylase enzyme and the optimum conditions for higher production was at pH 6.8-7.4,incubation time of 24 hrs, 37^{0}C of culture temperature and at 1% of starch. By using these culture conditions, maximum amylase may produce in mass for industrial purposes.

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