International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 9, Issue 5 -2022

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.05.008

Screening and characterization of a bacterial amylase from some decaying roadside fruit samples in Kaduna township, Nigeria

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Abstract

Amylases have been isolated from various sources owing to their importance in many industrial applications. This current work is also another attempt to further explore cheaper and readily available microbial amylase with simultaneous aim of ridding the environment of some agro wastes which could constitute hazard to the environment. A total of four bacterial isolates from five decaying fruit samples were identified using simple pour plate technique of microbial culturing. They were tested for their ability to produce amylases and isolates with high enzyme activity were pooled together for characterization studies. An optimum temperature and pH of 55°C and 4 were respectively recorded for this amylase. While incubation period shows that enzyme activity was higher after 96 hours of incubation with enzyme been stable between 40- 65°C. Substrate concentration studies on enzyme activity gave a Km of 0.02M and Vmax of 7.5µmole/ml/min respectively. Other parameters reported all point to the characteristics of this amylase as possessing a potential for future application in many industrial processes and saving a lot of foreign exchange for a country like Nigeria.

Keywords: Amylase, Bacteria, Fruits, Industry, Nigeria

Introduction

Amylases are enzymes that degrade starch and related polymers tosimple sugars like glucose or products characteristics of individual amylolytic compounds. They arefound virtually in every form of life. These enzymes are also found in the human saliva where they start the chemical process of starch digestion and as such foods containing large amounts of starch but little sugar, such as rice and potatoes, may acquire a slightly sweet taste as they are chewed because amylases degrade their starch into sugar in the mouth [1]. Amylases from pancreas and salivary glands hydrolyze dietary starch into disaccharides and trisaccharides which are converted by other enzymes to glucose supplying the body with energy. Amylases can be divided into endo and exo sub types. The endo-amylases catalyze hydrolysis in a random manner within the

Starch molecule. This causes the formation of linear and branched oligosaccharides of various chain lengths. While the exo-amylases hydrolyze the substrates from the non- reducing end, resulting in successively shorter end products [2].

Amylase production varies from one microbe to another even among the same genus, species, and strain. The level of amylase production also varies depending on the microbe's origin, where strains isolated from starch- or amylose-rich environments naturally produce higher yields of enzyme. Other factors like pH, temperature, carbon and nitrogen also play vital roles in the rate of amylase production. Microbes can be optimized to produce efficient amylases that are stable at stringent conditions. Such improvements reduce contamination by background proteins and minimize reaction time leading to less energy expenditure in the reaction process [3].Among microbial species that secrete amylases, bacterial ones are easier to extract and cheaper than other microbial sources[4,5].

The cost of amylase production and procurement by developing countries like Nigeria is high due to importation and customs duties. There is need therefore for local sources of these enzymes. Littered around some major cities of Northern Nigeria are decaying and decomposing fruits from road side vendors who do not have the capacity to store unsold stock. Such kind of fruits constitute a major source of environmental pollution in major cities across Africa. Interestingly most bacterial amylases could easily be extracted from this kind of perishing fruits. An effective and efficient amylase production from those agro waste will significantly reduce environmental pollution and enhance economic opportunities for the teeming unemployed youths. In Nigeria, local production of the enzyme will save millions of naira that is spent annually on importation. It is in the light of this therefore that more studies involving the isolation and improvement of novel strains are needed to pave the way towards creating clones with higher yields. The goal of this study was to isolate and characterize amylases from bacteria in decaying abandoned fruit samples from roadside vendors in Kaduna metropolis of Nigeria and study the processes that can be used to increase yield.

Materials and Methods

Study Area and Sample Collection

Samples of decaying fruit samples (Banana, Apple, Carrot, Cucumber, and Water melon) were collected in containers under sterile conditions from different locations of the city where different decaying fruit samples are usually dumped.

Isolation of bacteria

One gram of the freshly collected mashed samples were each mixed with 9.0 ml of sterile distilled water in sterile test tubes, serial dilutions then followed. 0.5ml of 10^{-5} dilution was pipetted into a sterile petri dish and overlaid with 20ml of nutrient agar. This was incubated at 37°C for 24 hours. Many colonies were observed and each sub-cultured until a pure culture was obtained. Pure isolates were maintained on nutrient agar slant and stored at 4°C for further studies.

Screening of potent amylase producing bacteria by starch hydrolysis test

Bacterial isolates were screened for amylase production by starch hydrolysis test on agar plates [7]. The isolates were streaked on the starch agar plate and incubated at 37°C for 48 hours. After incubation the plates were checked for amylase production by addition of gram's iodine solution dropwise. Amylase activity was indicated by the clear zone of starch hydrolysis surrounding the colony. Major bacteria present in the fruits were identified using the usual laboratory procedures which included Gram's staining, catalase, oxidase, citrate, motility, sugar fermentation tests, spore staining etc.

Crude Amylase production from bacterial Isolates

Each isolate was grown in semi-synthetic medium containing 1%w/v soluble starch and 1.0% bacteriological peptone (6%, $MgSO_4 \cdot 7H_2O$ 0.1%

and KCl 0.5%). The culture supernatant was obtained by centrifugation at 6000rpm for 10min (this serves as the source of the crude enzyme). Isolates with high yields of amylase enzyme were then pooled together for the enzyme assay.

Amylase Assay

Amylase activity determination was done by the amount of sugar given out using dinitrosalicylic acid(DNSA) method. This was performed by incubating a mixture of 2ml of each enzyme source and 2% soluble starch dissolved in citrate phosphate buffer (0.01 M) at pH 6.5 and 45°C for 20 minutes. The reaction was stopped was by adding 2ml of 1N HCl followed by boiling with for 10 minutes. Thereafter some drops of iodine were added to each of the experimental tes ttubes and allowed to stand for 5 minutes. Reducing sugar released was measured using a spectrophotometer (Jenway 6305 UV/visible) at 670 nm. Concentration was determined from a standard curve under same condition using glucose.

Calculation of Enzyme Activity: U/ml = μg of glucose/ml of enzyme x incubation time [8].

Characterization of Amylase

Effect of incubation time on Amylase activity

Effect of incubation time on enzyme activity was carried out by stopping the reaction in the test tubes periodically at every 24 hours up to 144 hours [8] and assaying for amylase activity as described earlier.

Effect of pH on Amylase Activity

The effect of pH on the activity of the crude enzyme was performed by incubating 0.2 ml of the crude enzyme with 1 ml of amylase substrate preparation in different citrate phosphate buffer ranging from pH between 2.0 - 8.0.

Effect of Temperature on Amylase Activity

The effect of temperature on enzyme was determined at 20˚C, 30˚C, 40˚C, 50˚C, 60˚C, 70˚C, and80˚C, respectively [9].

Effect of Heating Duration on Amylase Activity

Samples of enzyme preparations were heated at 70˚C for 5 minutes, 10˚C, for 15 minutes, 20˚Cfor 25˚ minutes, 30˚C and 35˚C minutes respectively in order to determine heating time effect on amylase activity [9]

Effect of Substrate (Starch) Concentration on Amylase Activity

Concentrations ranging from 0.8%, 0.7%, 0.6%, 0.5 %,0.3%, 0.2%, and 0.1% of starch solution were used as substratesin 0.2 M citrate phosphate buffer (pH 6.0)and their effect on enzyme activity were studied subsequently [9].

Results and Discussion

Four organisms were extracted in total and identified from the mashed flesh of the fruits as presented in Table 1 and Table 2. All the identified organisms belong to the Bacillus family of bacteria. Isolates screened were those that tested positive to starch hydrolysis and the activity enzyme was assayed after every 24 hours (on each day of production).

Table 1: Biochemical identification of bacterial isolates from different decaying fruits

S/N	FRUIT WHERE ISOLATE CAME FROM	OXIDASE	METHYL RED	CATALASE	INDOLE	COAGULASE	CITRATE	STARCH HYDROLYSIS	GLUCOSE	LACTOSE	SUCROSE	FRUCTOSE	MALTOSE	SUSPECTED ORGANISM
1	APPLE 1	-	+	+	-	-	+	+	+ -	- -	- -	- -	- -	
2	APPLE 2	-	+	+	-	-	+	+	- -	- -	- -	- -	- -	
3	APPLE 3	-	+	+	-	-	+	+	- -	+ -	- -	- -	- -	
4	BANANA 1	-	+	+	-	+	+	+	- -	+ -	+ -	+ -	- -	
5	BANANA 2	-	+	+	-	+	+	-	+ -	- -	+ -	- -	- -	
6	BANANA 3	-	+	+	-	-	-	+	+ -	- -	+ -	+ +	- -	
7	CARROT 1	-	+	+	-	-	+	+	+ +	- -	+ -	- -	+ -	
8	CARROT 2	-	+	+	-	+	+	+	- -	- -	- -	- -	- -	
9	CARROT 3	-	+	-	-	-	+	+	- -	+ -	- -	- -	- -	
10	W/MELON 1	-	+	+	-	+	+	+	+ -	- -	+ -	+ +	- +	
11	W/MELON 2	-	+	+	-	+	+	+	+ -	- -	+ -	- -	+ +	
12	W/MELON 3	-	+	+	-	-	+	+	+ +	- +	+ +	+ -	- +	
13	CUCUMBER 1	-	+	+	-	-	+	-	- -	- -	+ -	- -	- -	
14	CUCUMBER 2	-	+	+	-	+	+	+	+ -	+ -	+ -	+ +	+ -	
15	CUCUMBER 3	-	+	+	-	+	+	+	+ +	+ +	+ +	+ +	+ +	

KEY

A= Apple, B= Banana, CA= Carrot, CU=Cucumber, WM= Water Melon and Apple, += positive, - = negative, ++ =presence of color change and gas production, + - = presence of color change and absence of gas production, - - = Absence of color change nor gas production, - + = Absence of color change and prsence of gas production

Table 2: Frequency of isolation of bacteria from different fruit samples

Isolate	Fruit Source	Number of Isolate	Frequency of Isolation (%)
B. *cereus*	Apple	2	10.4
	Banana	1	6.2
	Carrot	1	4.1
	Cucumber	3	2.0
	Water Melon	4	1.4
B. *anthracis*	Apple	5	12.4
	Banana	1	6.1
	Carrot	1	3.0
	Cucumber	3	2.5
	Water Melon	2	0.8
B. *subtillis*	Apple	3	9.6
	Banana	2	7.8
	Carrot	1	5.3
	Cucumber	2	3.5
	Water Melon	6	1.9
B. *olivae*	Apple	2	6.5
	Banana	2	4.2
	Carrot	3	3.1
	Cucumber	1	2.8
	Water Melon	5	1.7

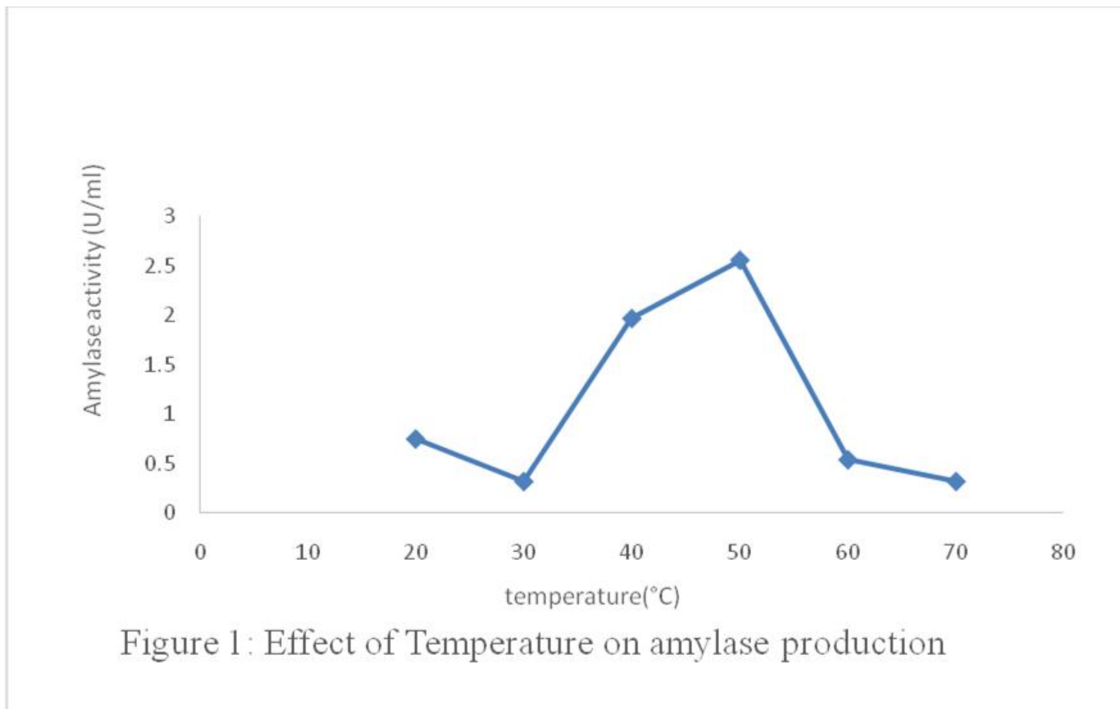

Figure 1: Effect of Temperature on amylase production

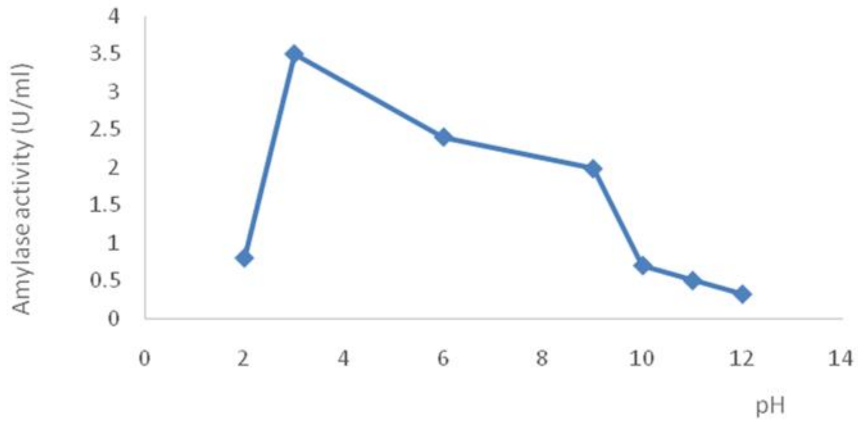

Figure 2: Effect of pH on amylase production

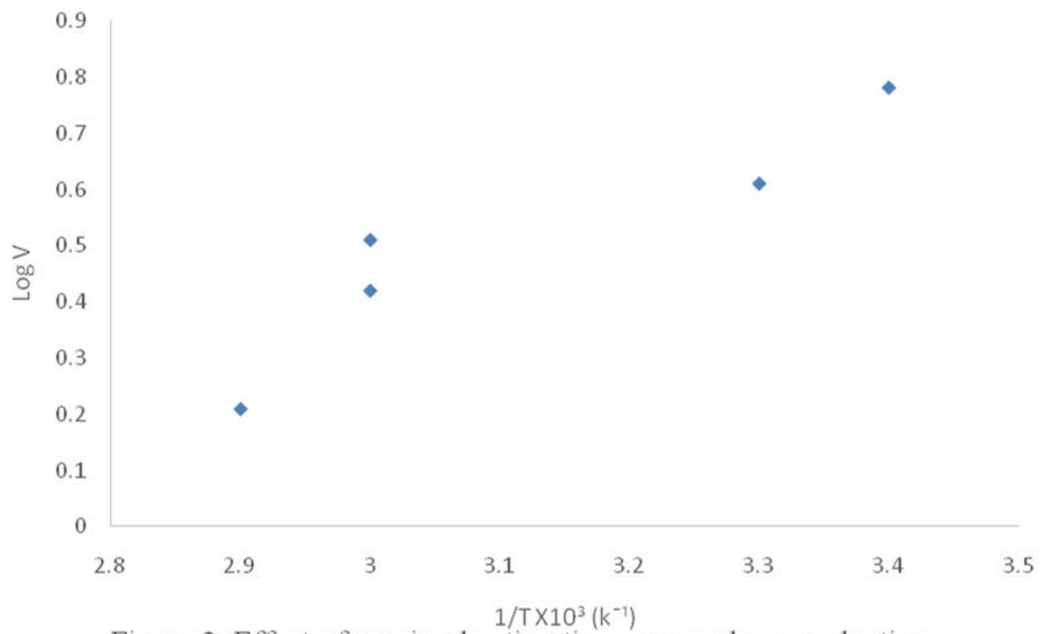

Figure 3: Effect of varying heating time on amylase production

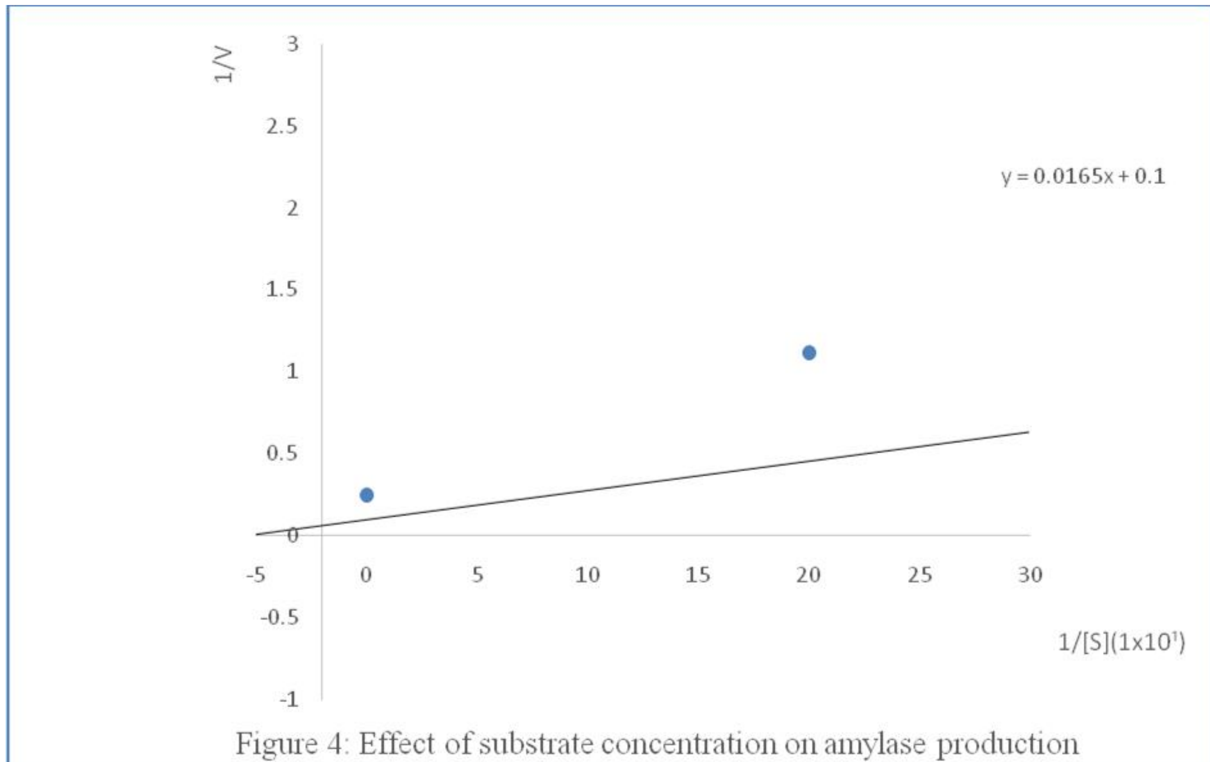

Figure 4: Effect of substrate concentration on amylase production

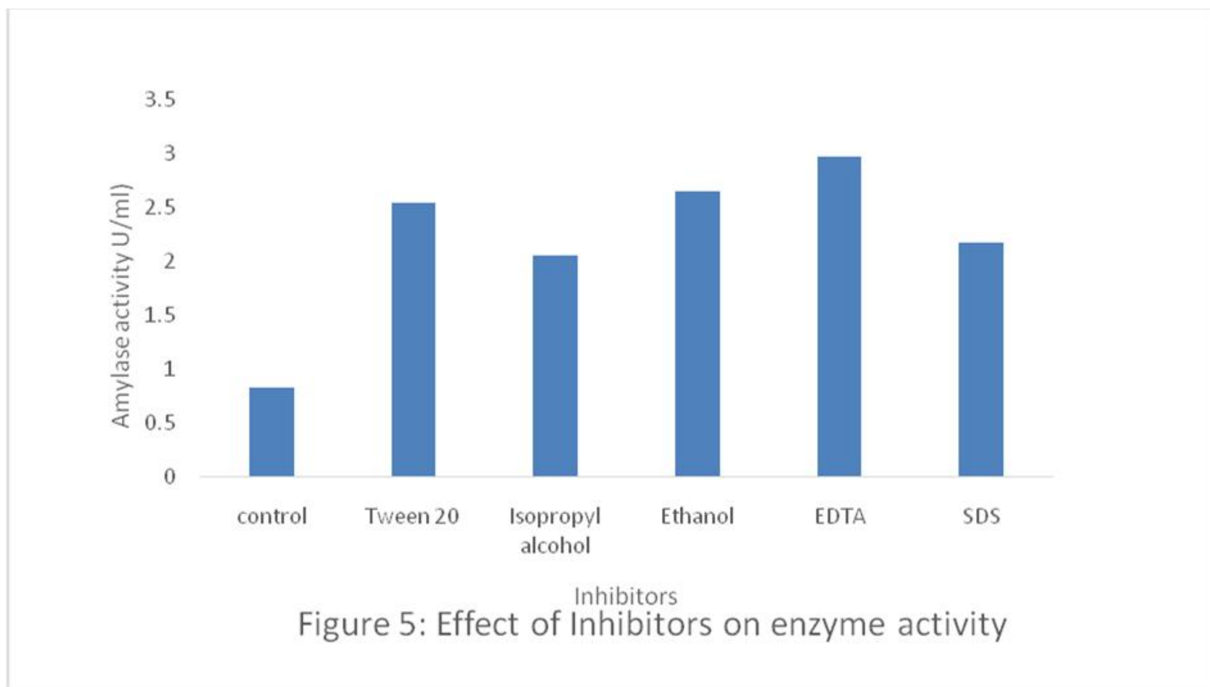

Figure 5: Effect of Inhibitors on enzyme activity

Bacillus species have been found to be good producers of α-amylases because amylolytic enzymes are more predominant in the bacterial kingdom as corroborated in this current work [10]. In the same vein, incubation time also affect growth of organisms in a culture medium as seen here where the biomass yield was found to decrease with an increase in incubation period. This study is in line with [11] who reported that maximum production of amylase occurred at 48 hours of incubation and begin decreasing in most of the bacterial isolates after 72 hours of incubation most likely due to early onset of death phase.

Factors like temperature play very crucial role on amylase production and this is related to the growth of the organism. Enzyme activity generally increase with temperature until an optimum is attained after which it begins to decrease at higher temperature. The amylase activity of the isolates in this study had optimum temperature of 55˚C (Figure1). This value correlates with the work of Natasa et al [12] who reported an optimum temperature of 50˚C for bacterial amylase activity. However it slightly differs from the findings of Nisa et al [13] who got maximum enzyme production at 60˚C from his work. Reduction in enzyme activity recorded at temperatures between 60˚C and above might simply be attributed to denaturation of proteins by heat. However, high enzyme activities observed at40˚C-55˚Cshows that this amylase possess some qualities for industrial application [13].

The pH of a growth medium induces morphological changes in the organisms thereby affecting enzyme secretion. The pH change observed during the growth of these organisms also affects stability of products in the medium [14]. It's generally known that amylases are stable over a wide range of pH (between 4 and 11). In this study, amylase from the samples shows optimum activity at pH 4.0 (Figure 2). This differs from[15] who reported that Bacillus species used for the production of alpha amylases by sub-merged fermentation have an optimum pH between 6.0 and 7.0 which is best for enzyme production. There was a drastic change in enzyme activity with higher pH which indicates that the enzyme loses activity as alkaline concentration is approached. This finding agrees with [15].

Effect of heating period on activity of enzyme is a function of the thermo-stability of an enzyme (Figure 3). After heating at 65˚C, the amylase lost its activity completely. The enzyme stability in this study correlates with the findings of Oyeleke and Oduwole [16] who reported that the enzyme stability of most amylases declined at temperatures above 70˚C. From this study, enzyme stability trend agrees with the behaviour of amylases from most Bacillus species. It has been widely reported that the reaction velocity of an enzyme decreases after its maximum velocity has been attained. Additional amounts of substrate added to the reaction mixture after this point actually decreased the reaction rate (figure 4). Popovic et al [17] assumed that there are so many substrate molecules competing for the active sites on the enzyme surface such that they block the active sites and prevent any other substrate molecules from occupying them [18]. Studies on the effect of inhibitors as seen in figure 5 also explains the nature of this enzyme and its susceptibility to different substances that can inhibit its activity.

Conclusion

In conclusion, this work shows that bacteria isolated from peeled endocarps of decaying fruits dumped by fruit vendors in some Nigerian urban centers are capable of producing amylases which can be exploited for large scale industrial production if conditions for optimization are properly utilized.

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How to cite this article:
Ekpa, Emmanuel, Bashir Bello Maimuna, Micheal Nancy Erika, Saleh Jamila and Adeleke Adeniran Maryam. (2022). Screening and characterization of a bacterial amylase from some decaying roadside fruit samples in Kaduna township, Nigeria. Int. J. Adv. Res. Biol. Sci. 9(5): 73-82.
DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.05.008