Optimization of Xanthan Gum Production by *Xanthomonas Campestris* Isolated from Cabbage Leaves Sold in Keffi, Nigeria

MD Makut[1*], NA Danladi[2] and IK Ekeleme[3]

[1,3]Department of Microbiology, Faculty of Natural and Applied Science, Nasarawa State University, Keffi,Nasarawa State, Nigeria. P.M.B 1021 Keffi

[2]National biotechnology development and research agency, Umaru Musa Yaradua express way, Abuja, Nigeria

DOI: https://doi.org/10.51584/IJRIAS.2024.908058

Received: 08 August; Accepted: 13 August 2024; Published: 17 September 2024

ABSTRACT

Xanthan is a water-soluble hetero-exopolysaccharide produced by the plant pathogenic bacterium *Xanthomonas campestris.* This investigation aimed at production from *Xanthomonas campestris* isolated from cabbage leaves sold in Keffi and Jos, Nigeria. Standard microbiological methods were employed for isolation and identification of the *Xanthomonas campestris* cabbage leaves. The yields of xanthan gum produced by the various isolates of the *Xanthomonas campestris* were determined using ethanol to precipitate the gum. The occurrence of of *Xanthomonas campestris* was 26.0 % and the isolation rate of from Keffi was 24.0 % and from Jos was 12.0 %. The screening xanthan gum showed that two species of the *Xanthomonas campestris* isolated was able to produce xanthan gum isolate with code Xjc2 produced 11.0 ± 0.03 g/l and Xkc5 produced 0.6 ± 0.00 g/l. On effect of pH the highest xanthan gum was produced by Xkc5 at pH 6.0 (1.09g/l) with biomass of (2.78g/l) and the lowest was at pH 4.0 (0.44g/l) and biomass (0.74g/l) While Xjc5 produced highest at pH 5.5 (1.12g/l) and biomass (3.78 g/l) and lowest was at pH 4.0 (0.34g/l) and biomass (0.78g/l). On effect of temperature the highest xanthan gum produced by Xkc5 was recorded at 30^{o}C (1.11 g/l) and biomass (3.88 g/l) and the lowest was at 45 ^{o}C (0.37 g/l) and biomass (1.65g/l) while Xjc2 produced highest at 30^{o}C (1.91 g/l) and biomass (4.97 g/l) 45 ^{o}C (0.41 g/l) and biomass (1.52g/l). The highest xanthan gum was produced by Xkc5 was observed at 80% concentration of (1.00g/l) and biomass of (4.04g/l) and the least was at 20% concentration (0.39g/l) and biomass (0.92g/l) while Xjc2 produced highest at 80% concentration (1.09g/l) and biomass of (3.89g/l) and the lowest was at 20% concentration (0.47g/l) and biomass of (1.22g/l). The highest xanthan gum was produced by Xkc2 was observed after 120 hours (1.38g/l) and biomass (3.38g/l) and the lowest was after 24 hours (0.35g/l) and biomass (0.98g/l) while Xjc2 produced highest after 144 hours (1.41g/l) and biomass (4.12 g/l) and lowest was after24 hours (0.24g/l) and biomass (1.20 g/l). The *Xanthomonas campestris* isolated from cabbage leaves sold in Keffi and Jos revealed great ability in production of xanthan gum.

Keywords= *Xanthomonas campestris,* xanthan gum, temperature, biomass and production

INTRODUCTION

Xanthan is a water-soluble hetero-exopolysaccharide produced by the plant pathogenic bacterium *Xanthomonas campestris* (*X. campestris*) [1]. This organism belongs to the genus *Xanthomonas* and family *Pseudomonas*. It is a single rod 0.4±0.7mm wide and 0.7±1.8mm long. It is motile, Gram negative and has single flagella. The bacterium cannot denitrify and test positive to catalase and negative to oxidase respectively [2]. The colonies are smooth, viscid and yellowish in colour. *X. campestris* grow on standard laboratory media and different strains have been observed in batch and continuous fermentation [1,3]. Xanthan gum has shown a myriad application ranges such as thickening, emulsifying, suspending and stabilizing agent in the food, cosmetics, paint, pharmaceutical, paper, textile and oil industries [4]. Production of Xanthan gum is constantly increasing because of its numerous demands and applications. It is estimated that the global production of xanthan gum is over 80.000 tonnes worth $400 million per year [5].

Xanthan gum production has been shown to be halt by many factors such as bacteria species type and

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

environmental factors including dissolved oxygen level, media composition, temperature, pH and incubation time among others [6]. A cost reduction in xanthan gum production can be achieved by using inexpensive sources such as molasses, cheese whey, starch, kitchen waste, glycerol, coconut shell, passion fruit peel, corn straw and cobs and jackfruit seed powder [7]. These materials have been used as a carbon source in submerged or solid state fermentations. Also, the type and concentration of nitrogen source affects xanthan gum production. Especially, organic nitrogen sources have been found to be better than inorganic nitrogen sources for xanthan production [8]. Peptone, yeast extract, corn steep liquor and ram horn peptone have been used as organic nitrogen source in xanthan gum fermentations. Therefore, there is a need for cheap and available organic nitrogen sources [8,5]. This study focused on Xanthan gum production using *Xanthomonas campestris* isolated from Cabbage leaves sold in Keffi and Jos market, Nigeria

METHODS

2.1 Study Area

This study was carried out in Keffi and Jos markets. Keffi lies between longitude 8-5 ^{0}S and latitude 7 ^{0}N and above the sea level of latitude 630 m. Keffi is approximately 53km away from the Federal Capital Territory, Abuja and 133 km away from the state capital Lafia [9].

2.2 Sample Collection

Cabbages were collected from local vegetables market in Keffi and from Jos market. Those showing the yellow necrotic lesions was selected for the present study and transported to Nasarawa State University Keffi, Department of Microbiology Laboratory for analysis.

2.3 Isolation of *Xanthomonas campestris*

Isolation of *Xanthamonas campestris* was carried out following a method described by Makut *et al*. [4]. Briefly, 1g of cabbage leaves sample with yellow necrotic lesions will be submerged in sterile distilled water and ten-fold serial dilution was done by transferring 1 ml of the water into a test tube containing 9 ml of sterile distilled water. This step was repeated ten times to obtain a dilution factor of 10-7. From each of the last test tubes, 0.2 ml was taken and spread on Malt, Yeast (YM) medium containing (w/v) 0.3% yeast extract, 0.3% malt extract, 0.5% bacteriological peptone, 1.0% glucose, and 2.0% agar plates and will be incubated at 35°C for 24 hours.

2.3 Gram staining

The Gram-reaction of each isolate was determined following the staining procedure of Selvi *et al.* [10]. First, thinly spread bacterial smear was prepared on a clean slide, dried in air and fixed by heating. The dried smear was flooded with crystal violet solution for one minute and washed in tap water for few seconds. It was again flooded with iodine solution for one minute and washed and blot-dried. It was decolorized with 95% ethyl alcohol by applying drop by drop until no more colour flows from the smear and washed and blot dried. Finally, slides were counter stained for about 10 seconds with safranin, washed and examined under microscope using oil immersion objective. Isolates that appeared pink, Gram negative bacteria were subjected for further tests.

2.4 Biochemical characteristics

Various biochemical tests were performed for the identification of the isolates like, methyl red test, indole production test, urease test, citrate utilization test, starch hydrolysis, milk Proteolysis, gelatin liquefaction and tween 80-hydrolysis test [6].

2.5 Preparation of Fermentation Media

Banana and Plantain peels was collected, sun-dried and homogenized into powder form using clean grinding machine and sieve.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

2.5.1 Preparation of Inoculum

Inoculants of the selected strains of *Xanthomonas campestris* was cultured into yeast malt broth (YM) medium and incubated at 28ºC for 24 hours as described by Soudi *et al*. [11].

2.5.2 Biomass Fermentation and Xanthan Gum Production by *Xanthomonas Campestris*

The batch fermentation was carried out as described by Makut *et al.,* [4], with slight modifications. Banana and plantain peels starch hydrolysate [banana peel starch (20 g/L), plantain peel starch (20 g/L), NH4Cl 0.4 g, KH2PO4 0.1 g, MgSO4, 7H2O 0.025 g] and will be taken in 250 ml conical flasks. The flasks were plugged with cotton and autoclaved at 15 psi for 15 min. The sterilized flasks were inoculated with 5.0 ml of the inoculum under aseptic conditions. Sterilized ferrocyanide (200 ppm free ions concentration) was added to each flask. The flasks were placed in a shaker incubated at different temperature. All the experiments were run parallel in duplicates.

2.5.3 Optimization of conditions for Xanthan Gum production Carbon Sources

The fermentation medium was formulated for production of Xanthan gum using different carbon sources as follows:

M1 [20 g of banana peels starch medium NH4Cl 0.4 g, KH2PO4 0.1 g, MgSO4, 7H2O 0.025 g and water to liter, the medium (200 ml) was poured into each Erlenmeyer flask 250 ml and 5 ml of the standardize inoculum was inoculated into different fermentation medium (v/v)], M2 [20 g of plantain peels starch medium NH4Cl 0.4 g, KH2PO4 0.1 g, MgSO4, 7H2O 0.025 g and water to liter, the medium (200 ml) was poured into each Erlenmeyer flask 250 ml and 5 ml of the standardize inoculum was inoculated into different fermentation medium (v/v)] as described by Makut *et al*., [4]

2.5.4 Temperature

The optimization of temperature was carried out following a method described by Gazal, [12] at different temperature ranges, 28ºC, 32ºC, 35°C, 37ºC for 3 day.

2.5.5 pH

The optimization of pH was carried out following a method described by Makut *et al*. [4] at different pH ranges such as 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 using 1M HCl.

2.5.6 Time

The optimization of fermentation was carried out following a method described by Makut *et al*. [4] at different time interval such as 24 hours, 48 hours, 72 hours, 96 hours and 120 hours.

2.5.7 Extraction of Pellet Supernatant for Quantification of the Xanthan Gum

After the fermentation at optimized conditions for the biomass and xanthan gum production, all flasks were centrifuged for extraction of biomass and xanthan gum. The media was centrifuged at 1000 rpm for 15 min for the pellets and supernatant. Pellets were suspended in deionized water for washing and recentrifuged at 4000 rpm for 10 min. To precipitate the biomass, it was collected in pre-weighed plate with aluminium paper and dried in the oven at 60°C for two hours and weighed to determine the dry mass per liter medium [12,4]

2.5.8 Quantification of Xanthan Gum

The supernatant that was collected after extraction of biomass was mixed with 2 to 3 volumes of ethanol and continually shake to precipitate the xanthan gum. The obtained precipitate will be separated by centrifugation at 6000 rpm for 15 min. The collected residue was transferred into a pre-weighed micro-centrifuge tube. The tubes were kept in hot air oven for drying at 60°C for 20 hours. The microcentrifuge tube was cooled at room temperature then the dry weight was determined. The concentration of xanthan gum was obtained by calculating

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

the dry weight of Xanthan gum per liter medium [6].

2.5.9 Statistical Analyses

The data obtained in this study were analysed by used of one-way Analysis of Variance (ANOVA) using Smith Statistical Package version 2.8 and the level of significance were determine at 5% probability (i.e at p= 0.05)

RESULTS

3.1.1 Cultural, morphological and biochemical identification of the isolates

The cultural, morphological and biochemical characteristics of *Xanthomonas* species isolated from infected cabbage leaves are as given in Table 1, shows that the organisms appear yellowish in color, gram negative, rod shape and biochemical reactions namely: Starch hydrolysis: positive, methyl red: negative, gelatin liquefaction: positive, Milk proteolysis: positive, Urease production: positive, citrate utilization: negative, indole production: negative as shown.

3.1.2 Occurrence of Xanthomonas species from infected leaves

The occurrence of *Xanthomonas* species is as given in Table 2. The highest occurrence was observed from cabbages leaves sold from Jos (28.0%) and the lowest was from cabbages leaves from Keffi (20.0%)

3.1.3 Screening for xanthan gum production

Screening for Xanthan gum production is as shown in Table 3. Isolates from different infected leaves showed varying ability in Xanthan gum production isolate with code (Xkc2 – Xk6) are from Keffi and isolate Xkcs produced 0.6g/l. While isolates with code (Xjc1-Xjc3) were from Jos and isolate Xjc2 produced 11.0g/l and Xjc6 produced 0.4g/l respectively.

Table 1: cultural morphological and biochemical Identification of the isolates

				Biochemical characteristics								
Isolates Codes	Morphological characteristics colony characters appearance shape color	Gram Staining	Cell morphology	Sh	Gl	Mp	Up	Tl	Cit	**MR**	ind	inference
Xcf1	Not transparence Cocci cream/milkish	-	Rod shape	+	+	+	+	+	-	-	-	*Xanthomonas* sp
Xcf2	Not transparence Cocci cream/milkish	-		+	-	+	+	+	-	+	-	*Xanthomonas* sp
Xcf3	Not transparence Cocci cream/milkish	-	Rod shape	+	+	+	+	+	-	-	-	
Xcf4	Not transparence Cocci cream/milkish	-		+	+	+	+	+	-	-	-	*Xanthomonas* sp
Xjc1	Not transparence Cocci cream/milkish	-	Rod shape	+	+	+	-	+	-	-	+	
Xjc3	Not transparence Cocci cream/milkish	-	Rod shape	+	+	-	+	+	-	-	-	
Xjc4	Not transparence Cocci cream/milkish	-	Rod shape	+	+	+	-	+	+	-	-	*Xanthomonas* sp

Key: X1-8 = infected leaves; SH= Starch hydrolysis, GI= Gelatin liquefaction, MP=Milk proteolysis, UP =

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

Urease production, TI Tween 80 lipolysis, CIT= Citrate utilization test

Table 2 Occurrence of *Xanthomonas* species from Cabbages leaves in different location

Location	Number sample	No. (%) isolated
Cabbages leaves in Keffi	25	5 (20.0)
Cabbages leaves from Jos	25	7(28.0)
Total	50	13 (26.0)

Table 3 Screening for xanthan gum production by isolates

Isolates codes	Xanthan gum (g/l)
Xkc2	0. .0 ± 0.00
Xkc3	0.0 ± 0.00
Xkc4	0.0 ± 0.00
Xkc5	0.6 ± 0.00
Xkc6	0.0 ± 0.00
Xjc1	0.0 ± 0.00
Xjc2	11.0 ± 0.03
Xjc3	0.0 ± 0.00
Xjc4	0.0 ± 0.00
Xjc5	0.0 ± 0.00
Xjc6	0.0 ± 0.00

Key: Xkc2 – Cabbages sold in Keffi; Xjc1 – Xjc6 = Cabbages leaves from Jos

3.1.4 Effect of pH on xanthan gum production by Xanthomonas spp

Effect of pH on xanthan gum production using *Xanthomonas* spp isolated from infected cabbages leaves sold in Keffi and Jos is as shown in Table 4. The highest xanthan gum produced with isolate Xkc5 was at pH 6.0 (1.09g/l) and biomass of (2.78g/l) followed by pH 5.5 (0.98 g/l) and biomass of (2.24 g/l), at pH 5.0 the xanthan gum produced (0.87 g/l) and biomass (1.42 g/l), at pH 6.5 the xanthan gum produced (0.88g/l) and biomass of (1.89 g/l), at pH 4.5 the xanthan gum produced (0.65g/l) and biomass of (0.88g/l), and the lowest was after pH 4.0 (0.44g/l) and biomass of (0.74g/l). While Xjc5 produced highest at pH 5.5 (1.12g/l) and biomass of (3.78 g/l) followed by at pH 5.0 the xanthan gum produced (1.10 g/l) and biomass (2.99 g/l), at pH 6.5 the xanthan gum produced (0.81g/l) and biomass of (1.94 g/l),), at pH 6.5 the xanthan gum produced (0.62 g/l) and biomass of (1.02 g/l), at pH 4.5 the xanthan gum produced (0.41 g/l) and biomass of (0.91 g/l), and the lowest was after pH 4.0 (0.34g/l) and biomass of (0.78g/l) respectively.

3.1.4 Effect of temperature on xanthan gum production by Xanthomonas spp

The effect of temperature on xanthan gum production using *Xanthomonas* spp isolated is as shown in Table 5. The highest xanthan gum produced by Xkc5 was recorded at 30^{o}C (1.11 g/l) and biomass of (3.88 g/l) followed

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

by 35ºC hours (0.85 g/l) and biomass of (3.97g/l), at 25ºC the xanthan gum produced (0.68g/l) and biomass (2.19g/l), at 40 ºC the xanthan gum produced (0.55g/l) and biomass of (0.55 g/l), and the lowest was at 45 ºC (0.37 g/l) and biomass of (1.65g/l) while Xjc2 produced highest at 30ºC (1.91 g/l) and biomass of (4.97 g/l) followed by 35ºC hours (1.08 g/l) and biomass of (4.71 g/l), at 25ºC the xanthan gum produced (0.84 g/l) and biomass (3.15 g/l), at 40 ºC the xanthan gum produced (0.70 g/l) and biomass of (1.25 g/l), and the lowest was at 45 ºC (0.41 g/l) and biomass of (1.52g/l) as shown in Table 5

Table 4 Effect of pH on xanthan gum production by *Xanthomonas* spp

pH	Xkc5		Xjc2	
	Biomass (g/l)	Xanthan gum yield (g/l)	Biomass (g/l)	Xanthan gum yield (g/l)
4.0	0.74 ± 0.12	0.44 ± 0.66	0.78 ± 0.02	0.34 ± 0.02
4.5	0.88 ± 0.21	0.65 ± 0.31	0.91 ± 0.11	0.41 ± 0.25
5.0	1.42 ± 0.25	0.87 ± 0.24	2.99 ± 0.31	1.10 ± 0.10
5.5	2.24 ± 0.60	0.98 ± 0.18	3.78 ± 0.14	1.12 ± 0.15
6.0	2.78 ± 0.14	1.09 ± 0.51	1.94 ± 0.60	0.81 ± 0.11
6.5	1.89 ± 0.31	0.84 ± 0.32	1.02 ± 0.25	0.62 ± 0.14

Key: Xkc5 = Cabbages sold in Keffi, Xjc2 = Cabbages leaves from Jos

Table 5 Effect of temperature on xanthan gum production by *Xanthomonas* spp

Temperature	Xkc5		Xjc2	
	Biomass (g/l)	Xanthan gum yield (g/l)	Biomass (g/l)	Xanthan gum yield (g/l)
25ºC	2.19 ± 0.25	0.68 ± 0.14	3.15 ± 1.01	0.84 ± 0.12
30ºC	3.88 ± 0.11	1.11 ± 0.21	4.97 ± 0.51	1.91 ± 0.01
35ºC	3.97 ± 0.01	0.85 ± 0.07	4.71 ± 0.13	1.08 ± 0.11
40ºC	1.70 ± 0.18	0.55 ± 0.32	1.25 ± 0.14	0.70 ± 0.14
45ºC	0.65± 0.02	0.37 ± 0.50	1.52 ± 0.18	0.41 ± 0.11

Key: Xkc5 = Cabbages sold in Keffi, Xjc2 = Cabbages leaves from Jos

3.1.6 Effect of substrate concentration on xanthan gum production by *Xanthomonas* spp

The effect of substrate concentration on xanthan gum production using *Xanthomonas* spp is as shown in Table 6. The highest xanthan gum was produced by Xkc5 was observed at 80% concentration of (1.00g/l) and biomass of (4.04g/l) followed by 100% concentration (0.89g/l) and biomass of (3.18g/l), at 60% the xanthan gum produced (0.72g/l) and biomass (2.69g/l), at 40% the xanthan gum produced (0.48g/l) and biomass of (1.87g/l), and the lowest was 20% concentration (0.39g/l) and biomass of (0.92g/l) while Xjc2 produced highest at 80% concentration of (1.09g/l) and biomass of (3.89g/l) followed by 60% concentration (0.97g/l) and biomass of (3.18g/l), at 100% the xanthan gum produced (0.72g/l) and biomass (2.89g/l), at 40% the xanthan gum produced (0.62g/l) and biomass of (1.93g/l), and the lowest was 20% concentration (0.47g/l) and biomass of (1.22g/l) as

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

shown in Table 6.

3.1.8 Effect of fermentation time on xanthan gum production by *Xanthomonas* spp

The effect of fermentation time on xanthan gum production using *Xanthomonas* spp isolated is as shown in Table 7. The highest xanthan gum was produced by Xkc2 was observed after 120 hours (1.38g/l) and biomass of (3.38g/l) followed by 144 hours (1.36g/l) and biomass of (3.62g/l), at 96 hours the xanthan gum produced (1.05g/l) and biomass of (1.88g/l), at 72 hours the xanthan gum produced was (1.00g/l) and biomass of (1.12g/l), at 48 hours the xanthan gum produced (0.89g/l) and biomass (1.01g/l) and the lowest was after 24 hours (0.35g/l) and biomass of (0.98g/l) while Xjc2 produced highest after 144 hours (1.41g/l) and biomass of (4.12 g/l) followed by 120 hours (1.31g/l) and biomass of (2.12 g/l), at 96 hours the xanthan gum produced (0.91 g/l) and biomass of (1.97 g/l), at 72 hours the xanthan gum produced was (0.73 g/l) and biomass of (1.42g/l), at 48 hours the xanthan gum produced (0.44 g/l) and biomass (1.22 g/l) and the lowest was after 24 hours (0.24g/l) and biomass of (1.20 g/l) as shown in Table 7.

Table 6 Effect of substrate concentration on xanthan gum production by *Xanthomonas* spp

	Xkc5		Xjc2	
Concentration	Biomass (g/l)	Xanthan gum (g/l)	Biomass (g/l)	Xanthan gum (g/l)
20%	0.92 ± 0.12	0.39 ± 0.12	1.22 ± 0.12	0.47 ± 0.16
40%	1.87 ± 0.04	0.48 ± 0.22	1.93 ± 0.04	0.62 ± 0.12
60%	2.69 ± 0.02	0.72 ± 0.14	3.18 ± 0.36	0.97 ± 0.21
80%	4.04 ± 0.14	1.00 ± 0.02	3.89 ± 0.14	1.09 ± 0.14
100%	3.18 ± 0.36	0.89 ± 0.11	2.89 ± 0.02	0.72 ± 0.15

Key: Xkc1 = Cabbages sold in Keffi, Xjc2 = Cabbages leaves from Jos

Table 7 Effect of fermentation time on xanthan gum production by *Xanthomonas* spp

Time	Xck5		XJc2	
	Biomass (g/l)	Xanthan gum yield (g/l)	Biomass (g/l)	Xanthan gum yield (g/l)
24 hrs	0.98 ± 0.32	0.35 ± 0.12	1.20 ± 1.21	0.24 ± 0.02
48 hrs	1.01 ± 0.97	0.89 ± 0.10	122± 1.13	0.44 ± 0.25
72 hrs	1.12 ± 0.88	1.00 ± 0.11	1.42 ± 1.41	0.73 ± 0.14
96 hrs	1.88 ± 0.60	1.05 ± 0.12	1.97 ± 1.11	0.91 ± 0.31
120 hrs	3.38 ± 1.01	1.38 ± 0.01	2.12 ± 1.22	1.31 ± 0.25
144 hrs	3.62 ± 0.91	1.36 ± 0.01	4.12 ± 1.31	1.41 ± 0.11

Key: Xkc1 = Cabbages sold in Keffi, Xjc2 = Cabbages leaves from Jos

DISCUSSION OF FINDINGS

In industrial production of xanthan gum, conditions of production of the products must be carefully controlled

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VIII August 2024

for optimal yield of the product of interest. These conditions include temperature, pH, fermentation time and substrate concentration [13]. Many authors have carried out studies on conditions that is best in industrial production of many organic products [14.15].

According to Lopes *et al.* [13], reported that the best optimal condition for production of xanthan gum was achieved at pH between 6.0 and 7.0. This is similar to the findings of this study, which also showed that the best optimum pH for the production of xanthan gum was recorded at pH 6.5 (1.18g/l yield and 2.99 g/l biomass). Many researchers [14, 16] argue that neutral pH is the optimum value for growth of *X. campestris,* as the pH lower from neutral pH to a condition close to 5 owing to acid groups present in xanthan gum production.

In some studies, temperature ranges has been evaluated in order to find the best temperature in relation to the yield and rheological characteristics of the xanthan gum. In this research, different temperatures (25℃, 30℃, 35℃, ℃ and 4℃) were evaluated for optimum or best temperature for production of xanthan gum. In this study it observed that temperature of 30℃ was the best for production of xanthan gum which the yield and biomass were 1.21g/l and 2.81g/l respectively. This finding was in agreement with similar study carried out on effect of temperature on xanthan production by other authors who reported the optimum temperature in production of xanthan gum was at temperature between 30℃ to 32 ℃ [4, 14, 17].

But studies by Barua *et al.* [18], showed that the highest yield of xanthan gum can be obtain with decreasing temperature from 30 – 25ºC or increasing from 30 – 35ºC, while Palaniraj and Jayaraman [19] reported that the optimal temperature for a high yield or production of xanthan gum was between 31 and 33ºC, this is similar to the finding of this study.

This study showed that substrate concentration effect the rate at which xanthan gum is been produced. It was observed that as the substrate concentration increases the yield of xanthan gum increases therefore if there is in sufficient substrate or source of carbon in the fermentation media the rate of xanthan gum will be low as observed in this study the highest production of xanthan gum was at the highest substrate concentration. This shows that as substrate concentration increases, the xanthan gum production also increases and it is agreement with findings of other authors findings [14,16, 4].

Fermentation time is thus an important factor that should be optimised during xanthan gum production [20]. In the present research, the xanthan gum yield and biomass gradually increased with corresponding 24-hour increase in fermentation time. This was generally the case and the optimal value was reached at 96 hours of fermentation with xanthan gum yield of 1.00g/l and biomass 1.88 g/l. This finding is in agreement with studies by Chavan and Baig [14] and Barua *et al.* [18], whose works also reveal that the optimum fermentation time for xanthan gum production and biomass was within the first 96 hours, compared to latter stages of fermentation where production is less optimum. Beyond this optimal 96-hour fermentation period, there continued to be a gradual, but much slower increase with a yield of 1.38g/l and biomass of 3.38g/l at 120 hours. After 144 hours of fermentation the xanthan gum produced (1.38g/l yield and 4.12g/l biomass) was the highest yield of xanthan gum that was produced.

CONCLUSION

Xanthomonas species isolated from infected cabbage leaves collected from Keffi markets and Jos market were able to produce different quantity of xanthan gum at different parameter studied. The best parameter for xanthan gum for temperature was 30$^{\circ C}$, and substrate concentration of 80%, fermentation time of 120 hours, and the optimum pH is 5.5 and 6.0

REFERENCES

1. Petri D. F. (2015). Xanthan gum: a versatile biopolymer for biomedical and technological polysaccharide “Xanthan gum”from Xanthomonas spp. International Journal of Current Microbiology and Applied Science. 8(05): 1019-1030.
2. Sarker, S., Sultana, N. and Aminuzzaman, F.M. (2017). Biochemical characterization of Xanthomonas axonopodis pv. malvacearum isolated from infected cotton plant and it’s in vitro sensitivity against some

selected chemicals. Advances in Research. 11(4): 1 – 10.
3. Soudi, M.R., Alimadadi, N., Ghadam, P. (2011). Minimal phenotypic test for simple differentiation of Xanthomonas campestris from other yellow-pigmented bacteria isolated from soil. Iranian Journal of Microbiology. 3(2): 84 – 91.
4. Makut, M.D., Agbonkhese, P.E. and Bello, A. (2018). Production of xanthan gum using Xanthomonas campestris isolated from some plants leaves in Keffi, Nigeria. Asian Journal of Biotechnology and Bioresource Technology. 3(4): 1 – 9
5. Ozdal M and Kurbanogle EB. (2019). Use of chicken feather peptone and sugar beet molasses as low cost substrates for xanthan production by Xanthomonas campestris MO-03. Fermentation. 5: 1-9.
6. Rana, B.M. and Raval, A.A. (2019). Isolation, production and characterization of the polysaccharide "xanthan gum" from Xanthomonas spp. Int. J. Curr. Microbiol. App. Sci. 8(5): 1019 – 1030
7. Murad, H.A., Abo-Elkhair, A.G. and Azzaz, H.H. (2019). Production of xanthan gum from non-traditional substrates with perspective of the unique properties and wide industrial applications. JSMC Microbiology. 1(6): 1 – 6
8. Mabrouk, M.E.M., El-Ahwany, A.M.D., Beliah, M.M.B. and Sabry, S.A. (2013). Xanthan production by a novel mutant strain of Xanthomonas campestris: application of statistical design for optimization of process parameters. Life Science Journal. 10(1): 1660 – 1667
9. Akwa, V.L., Binbol, N.L., Samaila, K.L., and Marcus, N.D., (2007). Geographical Perspective of Nasarawa State. Onaiv Printing and Publishing Company, Keffi. pp 3
10. Selvi, V., Vijayagopal, V. and Sonia, K.S. (2015). Biochemical characterization of locally isolated strain producing xanthan gum and kinetic modelling. International Journal of Recent Scientific Research. 6(1): 2369 – 2373
11. Soudi, M.R., Alimadadi, N., Ghadam, P. (2011). Minimal phenotypic test for simple differentiation of Xanthomonas campestris from other yellow-pigmented bacteria isolated from soil. Iranian Journal of Microbiology. 3(2): 84 – 91
12. Gazal S.M.A. (2011). Genetically modified strains of Xanthomonas campestris higher xanthan producer and capable to utilize whey. Current Research in Bacteriology. 4(2):44-62
13. Lopes, D.M.B., Vinicius, L.L., Barbara, M.S., Marco, A.D.S.C.F., Egon, S. and Luiz, G.L. (2015). Xanthan gum: properties, production conditions, quality and economic prospective. Journal of Food and Nutrition Research. 54(3): 185 – 194
14. Chavan, S. and Biag, M.M.V. (2016). Relationship of biomass and xanthan gum production by Xanthomonas campestris: optimization of parameters. British Biotechnology Journal. 11(1): 1 – 8.
15. Tasleem, S., Anwar, F., Arif, M., Khan, A.N., Ali, A., Ali, S., Sumra, A.A., Qureshi, W.A. and Shah, S.A. (2020). Production of xanthan gum from Xanthomonas campestris isolated from cruciferous vegetables. Advances in Bioresearch (ABR). 11(5): 1 – 12
16. Berg T, Tesoriero L, Hailstones DL. (2005). PCR based detection of Xanthomonas by Xanthomonas campestris NRRL-B-1449 from sugar beet molasses. The International Journal of Engineering and Science. 2(5): 52-55.
17. Gilani, S.L., Najafpour, G.D., Heydrazadeh, H.D. and Zare, H. (2011). Kinetics models for xanthan gum production using Xanthomonas campestris from molasses chemical industry and chemical engineering. Quarterly. 17(2) 179 – 187
18. Barua, R., Alam, M.J., Salim, M. and Tamzida, S.A. (2016). Small scale production and characterization of xanthan gum synthesized by local isolates of Xanthomonas campestris. Indian Journal of Experimental Biology. 51(1): 151 – 155
19. Palaniraj, A. and Jayaraman, V. (2011). Production, recovery and applications of xanthan gum by Xanthomonas campestris. Journal of Food Engineering. 10(6): 1 – 12
20. Amenaghawon, N.A., Osemwengie, S.O., Omoregbe, O. and Asogwa, U.J. (2015). Application of experimental method for the optimization of xanthan gum production from pineapple peels using Xanthomonas campestris via submerged fermentation. Nigerian Journal of Technology (NIJOTECH). 34(3): 491 - 498