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OPTIMIZATION THE COMPOSITION OF THE MICROBIAL GROWTH MEDIUM FOR THE PRODUCTION OF *B*- GALACTOSIDASE BY YEASTS

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ABSTRACT

The study was undertaken to evaluated the effect of some carbon and nitrogen sources as well as some cheap and locally agricultural or industrial wastes i.e., sugar cane bagasse and permeate on *β*- galactosidase production by *Kluyveromyces lactis*, No.(1) and *Pichia kudriavzevii* No.(13). Results revealed that, in general galactose was the best carbon source followed by lactose, maltose and sucrose, for both two tested strains. The media containing galactose at concentration of 13% gave the highest enzymatic activity being 801.57 and 769.48 (nmol/ml/min.) for *Kluyveromyces lactis*, No.(1) and *Pichia kudriavzevii* No.(13), respectively. In addition, results indicated that, in general, yeast extract was the best nitrogen source followed by peptone and malt extract, for the both two tested strains. A concentration of 0.5 % gave the highest enzyme yield for *Kluyveromyces lactis* No. (1), while at 1.5% for *Pichia Kudriavzevii* No. (13). Also, results indicated that the maximum values of enzyme production were in the control medium which containing 0.1% $NH_4H_2PO_4$ as well as $(NH_4)_2HPO_4$ for the both investigated yeast strains. Also, results indicated that, 7.0 % concentration of sugar cane bagasse was the most suitable level of enzyme production (317.86 and 351.24 nmol/ml/min.) being about 39.25% and 44.35% of that produced in the control medium for *Kluyveromyces lactis* No.(1) and *Pichia Kudriavzevii* No.(13), respectively. Concerning, the permeate medium, the concentration of 70% gave the highest activity of enzymem was about 82.18 % and 81.63% of the control medium for *Kluyveromyces lactis* No.(1) and *Pichia Kudriavzevii* No.(13) respectively.

Key Words: *β*- galactosidase, *Kluyveromyces lactis, Pichia kudriavzevii* , carbon and nitrogen sources, agro-Industrial wastes.

INTRODUCTION

β-galactosidase (EC.3.2.1.23) is an enzyme that hydrolysis lactose into its monosaccharides, glucose and galactose (**Hussain *et al*., 1995** and **Matioli *et al*., 2003).** The enzyme is widely distributed in biological systems such as plant and animal as well as in microorganisms such as bacteria, yeast and fungi (**Shukla** &**Wierzbicki 1975; Zadow, 1992; Soares *et al*., 2001** and **Husain, 2010).** Compared to the plant and animal sources of the enzyme,

microorganisms produce enzyme at higher yields and thus lower the price of commercial ***β***-galactosidase production **(Santos *et al.*, 1998**). The commercial ***β***-galactosidase is generally extracted from yeasts (*Kluyveromyces lactis* and *Kluyveromyces fragilis*) and fungi (*Aspergillus niger* and *Aspergillus oryzae*) (**Harju, 1987; Sarabana, 1996; Dağbağlı** and **Göksungur, 2008** and **AL-Jazairi *et al*., 2014).**

The enzyme, has different biotechnological applications. It plays an important role to avoid the problems of lactose intolerance by individuals who are deficient in lactose and to synthesis di-, tri- or higher galacto-oligosaccharides (GOS) which is known as prebiotic food ingredient (**Zarate & Lopez-Leiva, 1990** and **Artolozaga *et al*., 1998).** It has been also, to prevent the environment pollution caused by cheese whey **(Mlichova** and **Rosenberg ,2006).**

The activity of enzyme is influenced by the type of strains and the growth medium composition (**Sonawat *et al.*, 1981**; **Chen *et al*., 1992; Fiedurck & Szczodrak, 1994;** and **Am-alam** & **khanongnuch, 2015**). The microorganisms can utilize a wide variety of organic matter as a source of carbon and nitrogen. This capability makes it possible to benefit from cheap agricultural or industrial organic wastes in growing suitable strains with high enzyme synthesis (**Bales** & **Castillo, 1979; Furlan *et al*., 2001; Perini *et al*., 2013** and **Orrego** & **Klotz-Ceberio, 2022)**

Therefore, this current study was an attempt to throw some light on some cultural factors affecting the productivity of *β*-galactosidase such as carbon and nitrogen sources by local yeast strains. The waste materials e.g., sugar can bagasse and permeate cause a serious problem to raise the resent environmental pollution. Therefore, the study was aimed to find suitable and simple method for the bioconversion of these wastes for the production of *β*-galactosidase.

MATERIALS AND METHODS

Microorganisms

The cultures of *Kluyveromyces lactis* No.(1) and *Pichia Kudriavzevii* No.(13) were previously isolated and identified based on morphological and phenotypically characteristics by 18s rDNA gene sequence analysis by **Mahmoud *et al.*, (2025)** . The both strains were maintained on yeast malt agar slants **(Dağbağlı** and **Göksungur (2008)**, stored at 4^{o}C and sub cultured monthly.

Source of agro-industrial wastes

1. Sugar cane bagasse

It was obtained from local sugar cane bagasse juice shops at Fayoum Governorate, Egypt. It was air dried, crushed and then ground in a hammer mill grinding machine and finely powdered (4 mm particle size) before used **Ferreira *et al.*, (2018).**

2. Permeate

It was obtained from Department of Dairy Science, Faculty of Agricultural, Fayoum University, Egypt, which produced from Ultra-high filtration cheese manufacture and concentrated by evaporation in a rotary evaporator at 50ºC and 40 rpm (**Orrego** and **Klotz-Ceberio (2022).** The organic carbon content of sugar cane bagasse and permeate was determined at Soil and Water Institute, Agricultural Research Center, Giza, Egypt.

Fermentation experiments

Preparation of inoculum

After incubation period of 24 hrs cultures of the tested strains on yeast malt agar slants were suspended in sterilized distilled water. A 1.0 ml. of culture was aseptically transferred to 250 ml erlenmeyer flasks, each containing 100 ml of the medium which containing 2% lactose and 0.5% yeast extract (**Sarabana (1996)**. Flasks were incubated on a rotary shaker at 120 rpm and 30^0C for 24 hrs.

Fermentation

Ten ml of the inoculum were aseptically inoculated in 250 ml erlenmeyer flasks containing 100 ml of the fermentation medium which contained (g/l) lactose 30.0, yeast extract 1.0, K_2HPO_4 2.0, $(NH_4)H_2PO4$ 1.o, $(NH_4)HPO_4$ 1.0 and $MSO_4.7H_2O$ 2.0 (**Dağbağlı** and **Göksungur ,2008).** pH of the medium was adjusted at 5.0 and incubated on a rotary shaker at 30^0C , 120 rpm for 48 hrs. At the end of the experiment, the enzymatic activity was estimated.

Factors affecting the production of *B*-galactosidase enzyme

In order to study the effect of different factors on *β*-galactosidase production, fermentation medium was modified to be suitable for studying the respective factors as follows:

a- Effect of various concentrations of some carbon sources

The effect of different carbon sources on enzyme production namely lactose, galactose, sucrose and maltose at concentrations ranging from 5.0 to 15% were studied.

b- Effect of various concentrations of some nitrogen sources

1- organic nitrogen sources

Different organic nitrogen sources, namely, yeast extract, peptone and malt extract at concentrations ranging from 0.5 to 2.5% were studied.

2. Inorganic nitrogen sources

Various inorganic nitrogen sources, namely $NH_4H_2PO_4$ as well as $(NH_4)_2HPO_4$ at four increasing concentrations ranging from 0.25% to 1.0% were studied.

Agro-Industrial wastes used for production of *β*- galactosidase

Due to the fact, any waste product containing in concentration of 3% or more can be fermented (**Abd EL-Bakey, 1974**). Several agro-industrial wastes can serve as raw materials for the *β*-galactosidase synthesis of yeasts (**Perini *et al.*, 2013; Raol *et al.*, 2015; Bosso *et al.*, 2019; Khonngam** & **Salakkam**

2019; Orrego and Klotz-Ceberio 2022). Sugar cane bagasse and permeate were selected as a carbon source for production of *β*- galactosidase. In addition, an experiment was designed to study the effect of various concentrations of these wastes that produce the highest *β*- galactosidase enzyme by both tested yeast strains.

Therefore, sugar cane bagasse at concentration ranging from 3.0 to 11.0%, as well as permeate at concentration ranging from 50 to 90 % which is equivalent ranging from 1.69 to 6.56% carbon were added to the fermentation medium and substituted lactose.

Determination of enzyme activity

At the end of incubation period the yeast cells were harvested by centrifugation at 4200 rpm at 4ºC for 20 min and the supernatant was discarded. 0.2 g of cell was re-suspended in 25 ml of 0.2 M potassium phosphate buffer at pH 6.8 (**El-Diwany *et al.*, 1994).** The cell suspensions were sonicated on ice in glass tubes using a Pulse 150 Sonic Power Sonicator (150 W, 30 sec with 30 sec cooling periods) for 4 min. Then, they were centrifuged at 4ºc m 4200 rpm for 20 min and the supernatant was used for measuring *β*- galactosidase activity (**Song** and **Jacques , 1997).** 750µl of substrate solution Nitrophenyl-β-D-galactopyranoside (ONPG) (0.01 moles L-1) was prepared in a phosphate buffer at pH 6.8 and 250 µl of crude enzyme extract were added in a test tube. 2ml of phosphate buffer (pH 6.8, 0.2 M) was added to the test tube and then incubated at 37ºC for 70 minutes. The reaction was quenched by adding 250 µl Na_2CO (0.5moles L-I). Reaction progress was determined spectrophotometrically at 420 nm against the blank (750 µl ONPG, 2250 µl phosphate buffer at pH 6.8) (**Fernandes *et al.*,(2002**) and **Dahal *et al.*, 2020).** The enzyme activity was calculated follows:

$$\textbf{Enzyme activity} = \frac{\text{OD } 420 \times \text{Reaction volume}}{0.0045 \times \text{Enzyme volume} \times \text{Time}}$$

RESULTS AND DISCUSSIONS

Effect of different carbon sources

The experiments were designed to find the best carbon source and its concentration which produce the highest amount of ***β***–galactosidase by *Kluyveromyces lactis* No. (1) and *Pichia Kudriavzevii* No.(13). Different carbon sources namely; lactose, galactose, sucrose and maltose were individually tested as the sole source of carbon in the fermentation medium.

The results presented in Table (1) and graphically illustrated by Figs. (1 and 2) show that, in general galactose was the best carbon source followed by lactose, maltose and sucrose for the both two tested strains. These results are in agreement with the studies made by **Selim** and **El-Diwany, (1985)** and **Sarabana, (1996)** who found that, galactose was a good carbon source for *β*–D-galactosidase synthesis in each of *Saccharomyces fragilis, Kluveromyces lactis* and *Kluveromyces fragilis.* While **Khaled *et al.*, (2016**) found that, lactose is the

best carbon source for *Kulyveromyces marxianus* DIV13-247 (589U/mg) followed by galactose (257U/mg) , xylose (208U/mg), arabinose (164U/mg), saccharose (128U/mg), maltose (124U/mg) and furactose (121U/mg).

Table (1) Effect of various concentrations of some carbon sources on production of *β*–galactosidase by tested yeast strains.

Carbon Conc. (%)	*Kluyveromyces lactis* No.(1)				*Pichia Kudriavzevii* No.(13)			
	B–galactosidase activity (nmol/ ml/ min.)							
	Lactose	Galactose	Sucrose	Maltose	Lactose	Galactose	Sucrose	Maltose
Control	186.39	186.39	186.39	186.39	174..07	174..07	174..07	174..07
5.0	210.32	265.32	25.29	64.46	191.02	245.16	25.01	69.23
7.0	229.24	283.85	30.99	99.56	205.14	260.09	28.89	98.51
9.0	250.38	390.06	61.98	104.22	226.17	364.99	56.39	115.42
11.0	413.75	496.61	91.89	240.56	398.81	479.35	90.67	220.79
13.0	791.35	801.57	101.82	370.37	749.37	769.48	99.98	320.22
15.0	179.75	295.63	105.03	231.97	160.13	162.94	107.66	113.58

*The enzymatic activity of the control medium was considered as reference (100%) activity for the media tested.

Therefore, It could be concluded that, the type of the carbon affect the enzyme production by its induction and repression effects which differ from one substrate to another for a particular enzyme, (**Kheiralla *et al.*, 1994** and **Khaled *et al.*, (2016).**

Accordingly, to the concentration of these substrates also, affected the amounts of *β*- galactosidase production which indicated that the enzyme yield increased with increase of substrate concentration to a certain level after which the activity was gradually decreased. This may be attributed to the substrate inhibition or repression. This conclusion is in agreement with **Afolabi *et al.*, (2022).**

Also, the results indicated that, the media containing galactose at concentration of 13% gave the highest enzymatic activity being 801.57 and 769.48 (nmol/ml/min) which presented 430.05 and 442.05% of that produced in the control medium for *Kluyveromyces lactis* No.(1) and *Pichia Kudriavzevii* No.(13) respectively.

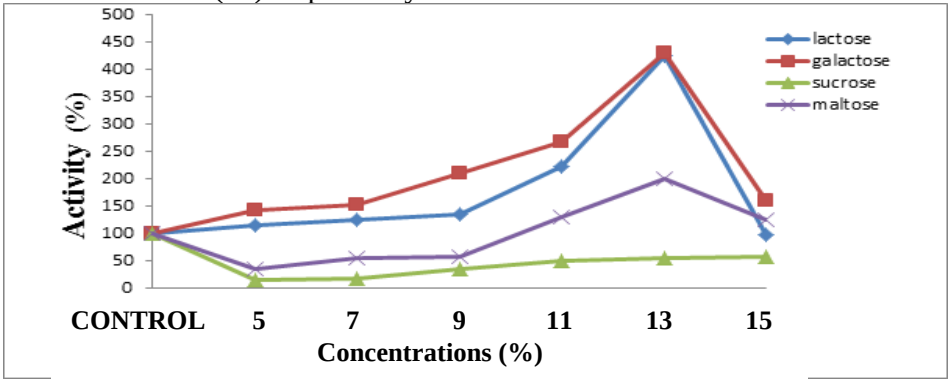

Fig (1): Effect of various concentrations of some carbon sources on the production of *β*-galactosidase by *Kluyveromyces lactis* No.(1).

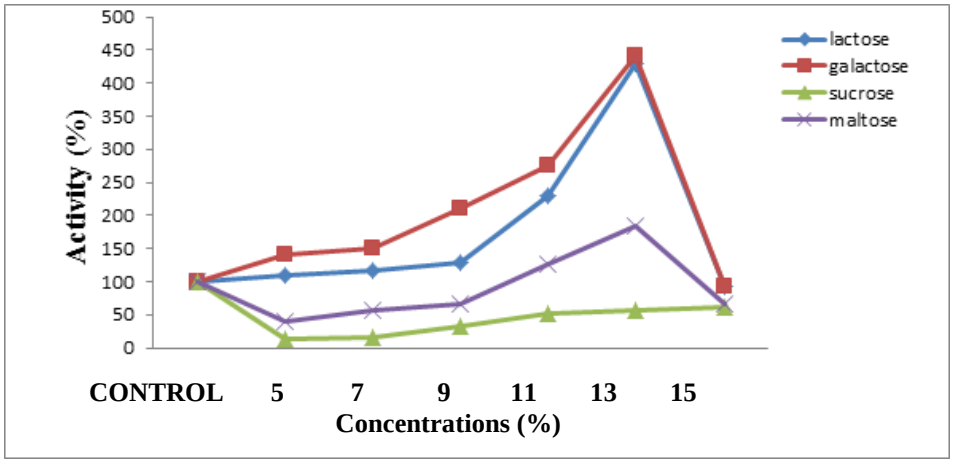

Fig (2): Effect of various concentrations of some carbon sources on the production of *β*-galactosidase by *Pichia Kudriavzevii* No.(13).

Effect of different nitrogen sources:

Organic nitrogen sources:

Different organic nitrogen sources were tested at increasing concentrations ranging from 0.5 to 2.5 % for detect the most suitable source and the best concentration for the highest production of *β*-galactosidase enzyme by the both tested yeast strains.

The results presented in Table (2) and graphically illustrated by Figs (3 and 4) show that, in general yeast extract was the best nitrogen source followed by peptone and malt extract for the both two tested strains. These results are compatible with those reported by **Manera *et al.*, (2008).** On the contrary, **Rao** and **Dutta (1977)**, mentioned that malt extract was highly stimulatory the *Kluyveromyces marxianus* DIV13-247 to produce ***β*** -galactosidase.

Table (2) Effect of various concentrations of some organic nitrogen sources on production of *β*–galactosidase by tested yeast strains.

Concentation (%)	*Kluyveromyces lactis* No.(1)			*Pichia Kudriavzevii* No.(13)		
	β–galactosidase activit (nmol/ml/min.)					
	Yeast extract	Peptone	Malt extract	Yeast extract	Peptone	Malt extract
Control	798.99	798.99	798.99	768.01	768.01	768.01
0.5	810.19	695.57	695.57	772.43	501.23	500.81
1.5	804.27	724.01	720.46	796.23	698.24	653.53
2.0	800.98	232.29	686.23	783.56	221.97	491.95
2.5	703.18	201.41	680.17	714.52	195.89	486.98

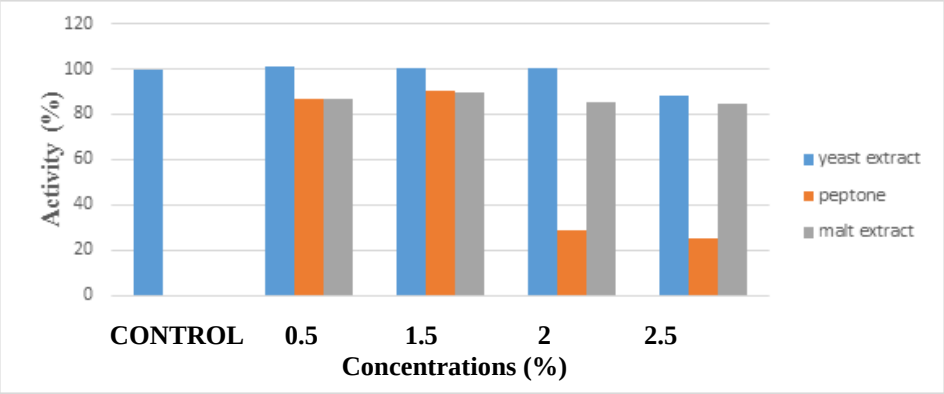

Fig. (3): Effect of different organic nitrogen source on the production of *β* –galactosidase by *Kluyveromyces lactis* No.(1).

*The enzymatic activity of the control medium was considered as reference (100%) activity for the media tested.

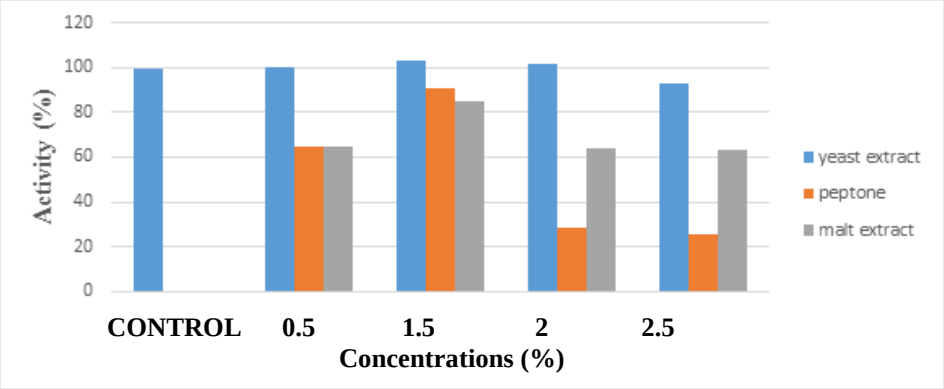

Fig. (4): Effect of different organic nitrogen source on the production of *β* – galactosidase by *Pichia Kudriavzevii* No.(13)

*The enzymatic activity of the control medium was considered as reference (100%) activity for the media tested.

Concerning to yeast extract, it was found that, the highest enzyme activity was obtained at concentration of 0.5 % for *Kluyveromyces lactis* No.(1). While at 1.5% for *Pichia Kudriavzevii* No.(13). Under or above this level showed decrease in the enzyme production. However, at higher concentrations up to 2.5%, the enzyme yield was decreased by 13.30 %, 10.63% for *Kluyveromyces lactis* No. (1) and *Pichia Kudriavzevii* No. (13) respectively. These results are in harmony with these obtained by **Sarabana (1996)**, who found that, the highest *β*-galactosidase production by *Kluveromyces fragilis* was in the presence of 0.5% yeast extract. On the other hand, **El-Sawah *et al.*, (1991)**, found that, the concentration of 0.1 % (w/v) yeast extract added to 12% sweet dry whey

medium was the most appreciable for the maximal production of *β*-galactosidase by *Kluveromyces fragilis*. While the concentration of 0.15% (w/v) yeast extract added to 10% whey medium was the most for the maximal production of the enzyme by *Kluveromyce lactis.*

Within the peptone media, the medium containing 1.5% peptone gave the highest enzymatic activity for both tested yeast strains. At this concentration the enzymatic activity about 90.61 % and 90.91% from that produced in the control medium for *Kluveromyce lactis* No.(1*)* and *Pichia Kudriavzevii* No.(13) respectively. At higher concentrations a lower enzymatic activity was obtained. The concentrations 2.0 and 2.5% showed a marked decrease in enzymatic only being 29.07, 25.21, 28.90 and 25.51 % for *Kluveromyce lactis* No.(1) and *Pichia Kudriavzevi* No.(13) respectively. The decrease in the enzyme yield corresponding may be due to unfavourable C/N ratio resulted from the increase in the nitrogen source.

In addition, results presented in Table (2) as well as Figs (3 & 4) clear, in general, that all media containing malt extract gave a lower yield of enzymatic activity than control for the both tested strains. The same finding was obtained by **Afolabi *et al*., (2022).**

Within the malt extract media, the medium containing 1.5% gave the relatively highest enzyme activity only 90.17% and 85.09% from that the control medium of strains *Kluveromyce lactis* No.(1) and *Pichia Kudriavzevii* No.(13) respectively. With the increase in the concentration of malt extract, the enzymatic activity gradually decreased.

Inorganic nitrogen sources:

Results presented in Table (3) show that, the maximum values of enzyme production was in the control medium which contains 0.1% $NH_4H_2PO_4$ as well as $(NH_4)_2HPO_4$ for both investigated yeast strains.

Table (3) Effect of different concentrations of inorganic nitrogen sources on the production of β–galactosidase by tested yeast strains.

Conc. %	NH_4H_2PO4				$(NH_4)_2HPO_4$			
	Kluyveromyces lactis No.(1)		*Pichia Kudriavzevii* No.(13)		*Kluyveromyces lactis* No.(1)		*Pichia Kudriavzevii* No.(13)	
	β–galactosidase activity nmol/ml/min.	Activity* %	*β*–galactosidase activity nmol/ml/min.	Activity* %	*β*–galactosidase activity nmol/ml/min.	Activity* %	*β*–galactosidase activity nmol/ml/min.	Activity* %
Control (0.1)	810.19	100.00	796.23	100.00	810.13	100.00	778.09	100.00
0.25	795.02	98.13	756.17	94.96	789.32	97.43	751.08	96.53
0.50	790.45	97.56	745.38	93.61	764.80	94.40	746.94	96.00
0.75	763.15	94.19	701.95	88.16	745.67	93.15	694.95	89.31
1.0	691.68	85.37	673.65	84.60	740.13	91.36	672.42	86.42

*The enzymatic activity of the control medium was considered as reference (100%) activity for the media tested

However, increasing the concentrations over 0.1% of NH_4 H_2PO_4, the enzyme activity decreased by 1.87, 2.44%, 5.81% and 14.63% for *Kluyveromyces lactis* No.(1) and 5.04%, 6.39%, 11.84 % and 15.40% for *Pichia Kudriavzevii* No. (13) at concentration of 0.25, 0.5, 0.75 and 1.0% respectively.

Concerning the effect of **(**$NH_4)_2$ HPO_4 on enzyme production, results presented in Table (3) show that, the enzyme yield was obtained in the medium containing 0.25 % **(**$NH_4)_2$ HPO_4 which gave approximately the same as that produced in the control medium about 97.43% and 96.53 % of the control yield for *Kluyveromyces lactis* No.(1) and *Pichia Kudriavzevii* No.(13), respectively. Above these concentrations enzyme activity was slightly decreased. Therefore it could be concluded that, the type of the nitrogen affect the enzyme production which differ from one substrate to another. This difference may be attributed to their variable composition. **Akcan (2011)**, mentioned that, both organic and inorganic forms of nitrogen sources are metabolized in microbial cell to produce amino acids, nucleic acids, proteins and cell wall compounds.

Effect of agro-industrial wastes on production of *β*–galactosidase

The present experiments were designed to study the effect of different concentrations of sugarcane bagasse ranging from 3.0 to 11.0 % (equivalent 1.69 - 6.22% carbon) that produce the highest enzyme yield.

Results presented in Figs (5 & 6) show that, in general, all media containing sugar cane bagasse gave a lower enzymatic activity than control for the both tested yeast strains.

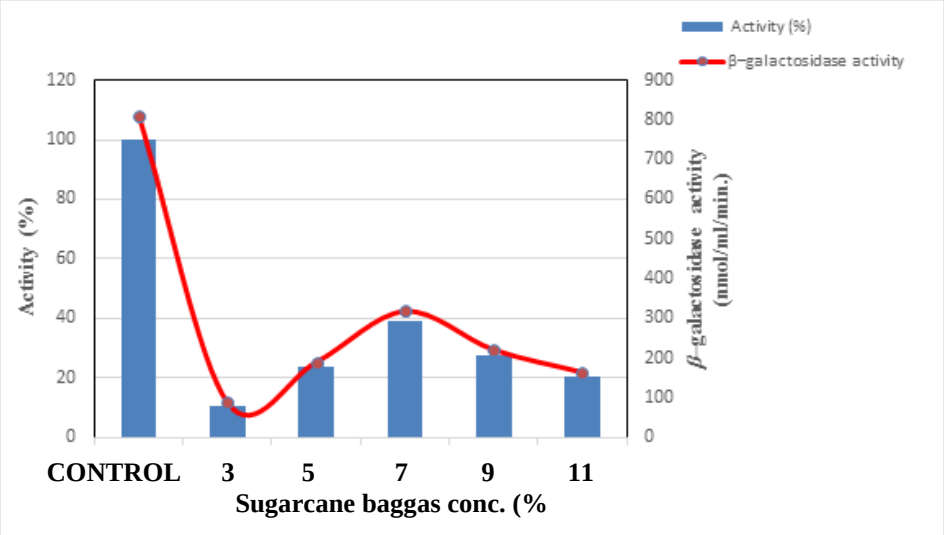

Fig (5): Effect of various concentrations of sugar cane bagasse on the production of *β*-galactosidase by *Kluyveromyces lactis* No.(1).

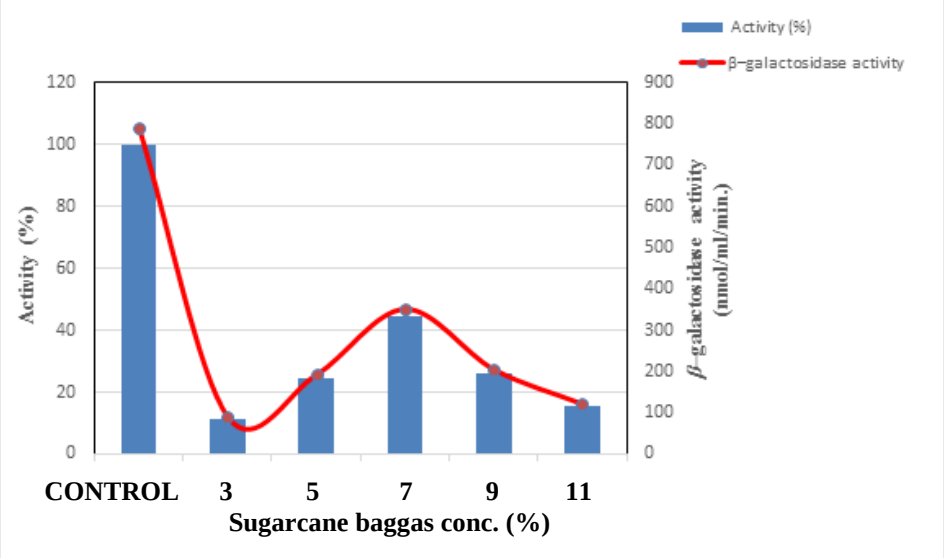

Fig (6): Effect of various concentrations of sugar cane bagasse on the production of *β*–-galactosidase by *Pichia Kudriavzevii* No.(13).

Also, results indicated that, 7.0 % concentration was the most suitable level of enzyme (317.86 and 351.24 nmol/ml/min.) being about 39.25% and 44.35% of that produced in the control medium for *Kluyveromyces lactis* No.(1) and *Pichia Kudriavzevii* No.(13) respectively. Under or above these concentrations a marked drop in enzyme production was observed. This may be due to the repression effect of accumulated catabolites **(Manera *et al.*, 2011** and **Shukla *et al*., 2014).**

2-Effect of permeate

Permeate was incorporated in the fermentation medium as the sole source of carbon. Increasing concentration from 50 to 90% (equivalent 3.65 -6.56% carbon) were tested to detected that most favorable concentration of *β*-galactosidase production.

It is obvious from results presented in Figs (7 & 8) show that in general the control medium gave a markedly higher enzyme activity than that from all fermentation media containing permeate by the two tested yeast strains.

In addition, the results indicate that, the increase in the enzyme activity was in parallel with the concentration of permeate in the medium up to 70 % (equivalent 5.10% carbon) for the both tested yeast strains. At this concentration enzymatic activity was about 82.18 % and 81.63% of the control yield for *Kluyveromyces lactis* No.(1) and *Pichia Kudriavzevii* No.(13) respectively. Above this concentration the enzymatic yield was decreased.

Finally, from aforementioned results, it could be observed also, that permeate media resulted in somewhat higher enzymatic yields as those in sugar cane bagasse.

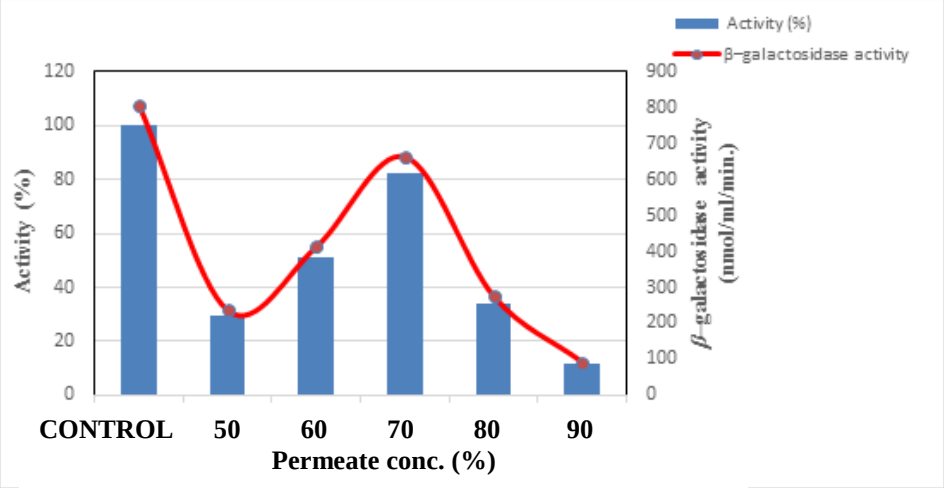

Fig (7): Effect of various concentrations of permeate on the production of *β*-galactosidase by *Kluyveromyces lactis* No.(1).

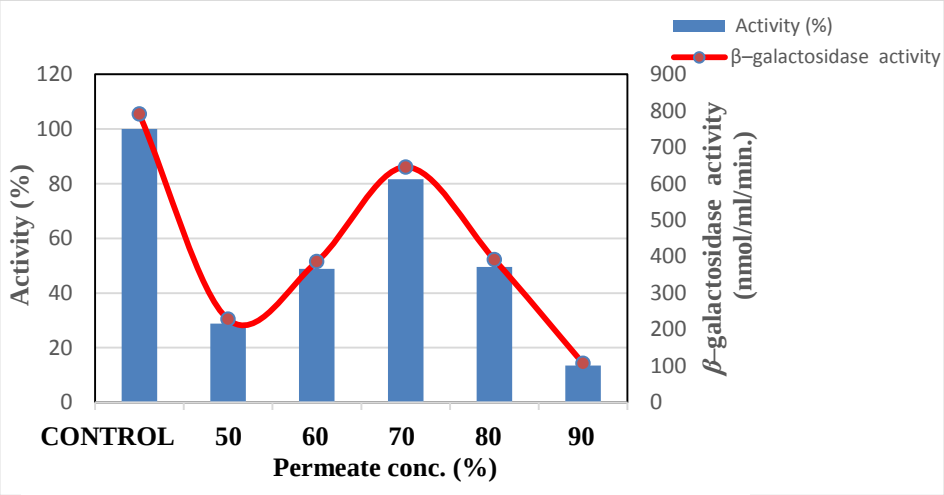

Fig (8): Effect of various concentrations of permeate on the production of *β*-galactosidase by *Pichia Kudriavzevii* No.(13).

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التركيب الامثل لبيئة النمو لانتاج انزيم بيتا – جلاكتوسيديز بواسطه الخمائر

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أُجريت هذه الدراسة لتقييم تأثير بعض مصادر الكربون والنيتروجين، بالإضافة إلى بعض المخلفات الزراعية أو الصناعية الرخيصة والمتوفرة محليًا، مثل مصاصة قصب السكر والبيرمييت، على إنتاج إنزيم بيتا–جالاكتوسيديز بواسطة سلالتين من الخمائر، وهما *Kluyveromyces lactis* No.(1) و *Pichia Kudriavzevii* No.(13). وقد أظهرت النتائج أن الجلاكتوز كان بوجه عام أفضل مصدر كربوني لإنتاج الإنزيم وذلك لكلا السلالتين، يليه اللاكتوز، ثم المالتوز، السكروز على التوالي. أعطى الجلاكتوز بتركيز 13% أعلى نشاط إنزيمي حيث كان 801.57(nmol/ml/min.) لـ *Kluyveromyces lactis* No.(1) بينما كانت 769.48 (nmol/ml/min.) لـ *Pichia Kudriavzevii* No.(13) .

كما أظهرت الدراسة أن أفضل مصدر نيتروجيني لانتاج الإنزيم كان مستخلص الخميرة، يليه الببتون ثم مستخلص المولت ، وذلك لكلا السلالتين. وكان أعلى انتاجية للانزيم عند تركيز 0.5% مستخلص الخميرة وذلك لـ *Kluyveromyces lactis* No.(1) ، و1.5% بالنسبة إلى السلاله *Pichia Kudriavzevii* No.(13) كما أوضحت النتائج أن الكنترول أعطى أعلى معدل لإنتاج الإنزيم وذلك عند دراسة تأثير كلا من $NH_4H_2PO_4$ و $(NH_4)_2HPO_4$، وذلك لكلا من السلالتين. كما أظهرت الدراسة أيضا أن تركيز 7.0% من مصاصه قصب السكر كان الأنسب لإنتاج الإنزيم، حيث بلغ النشاط الإنزيمي (.nmol/ml/min)317.86 لـ *Kluyveromyces lactis* No.(1) ، و (.nmol/ml/min) 351.24 لـ *Pichia Kudriavzevii* No.(13)، وهو ما يمثل نحو 39.25% و44.35% على التوالي بالمقارنه بالكنترول. أما بالنسبة لاستخدام البيرمييت، فقد كان التركيز الأمثل لإنتاج الإنزيم هو 70%، حيث بلغ النشاط الإنزيمي ما يعادل 82.18% و81.63% مقارنه بالكنترول لكلا من *Kluyveromyces lactis* No.(1) و *Pichia Kudriavzevii* No.(13)على التوالي.