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Maximization of chitosan production by *Aspergillus niger* on different culture conditions

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ABSTRACT

The optimal environmental and nutritional conditions for chitosan production by *A.niger* strains (A4 and A11) were studied. However, molasses and corn steep liquor as carbon and nitrogen sources were used for maximization chitosan production. Results showed that the highest records of biomass, chitin and chitosan produced by both strains were observed when sucrose and urea were used at a rate of 75 g/l, 1.7 g/l as a sole carbon and nitrogen sources, respectively. Increasing the incubation temperature led to gradual increase in biomass and chitosan yield up to 28 °C for both strains. The highest values of chitosan production were obtained when both strains of *A.niger* were incubated at pH 4.5 for 7 days then decreased thereafter. Moreover, increasing of corn steep liquor as nitrogen source to reach their maximum chitosan production at 150 g/l. Results indicated that the two *Aspergillus* strains produced higher amounts of chitosan when cane molasses was used as a carbon source rather than beet molasses. However, maximum values of chitosan were observed when mixture of cane and beet molasses was used as a sole carbon source.

Key words: Chitosan, *Aspergillus niger*, environmental conditions, nutritional conditions, molasses and corn steep liquor.

INTRODUCTION

Chitosan is a copolymer of D- glucoseamine and N- acetyl-D- glucoseamine units, derived from the deacetylation of chitin in the presence of hot alkaline. This polymer can be used as a suitable functional material because it processes desirable properties such as; biocompatibility, biodegradability, non-toxicity and adsorption of fats. The mycelia of several fungi specially *Aspergillus* and *Rhizopus* genera have been considered as possible sources of chitin and chitosan due to their presence in the cell walls (Suntornsuk *et al*, 2002).

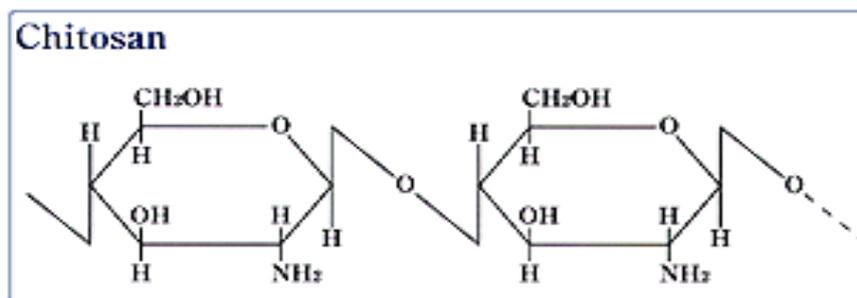


Fig 1. Structure of chitosan.

Chitosan is found as a supporting material in many aquatic organisms; insects; terrestrial crustaceans; nematodes and fungi (New *et al*, 2010). Chitosan and its derivatives can be variously used as a permeability control agent, an adhesive, a paper sizing agent, a fining agent, flocculating and chelating agents, an antimicrobial compound and a chromatographic support (McGahren *et al*, 1984; Crestini *et al*, 1996 and Shahidi *et al*, 1999). It is also used to immobilize enzymes or to deliver drugs to their target (Muzzarelli, 1977). Chitosan is a safe material that has an antifungal activity against many plant pathogens (El-Mougy *et al*, 2002). The accumulation of these agro-industrial residues causes major environmental pollution problems, due to the high content of starch and sugars, these residues can be potentially used for the growth of many microorganisms.



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Therefore, this work aimed to investigate the influence of environmental and nutritional conditions for chitosan production by *Aspergillus niger* strains.

MATERIALS AND METHODS

Fungal strains

Aspergillus niger strains (A4 and A11) used in this study was isolated and identified in Agric. Botany Department(Microbiology branch) Fac . Agric. Moshtohor ,Benha Univ.

Inoculum preparation

Spores of *Aspergillus niger* strains (A4 and A11) were suspended by adding 10 ml of sterilized distilled water and spores suspension was counted using a haemocytometer and adjusted to 10^4 spore / ml.

Effect of carbon sources

Karimi's medium (Karimi *et al*, 2007) was modified by replacing the carbon source and used for growing *Aspergillus niger* strains. Different carbon sources i.e., glucose, fructose, sucrose, maltose, lactose and manitol as a sole carbon source at concentration of 5% for Karimi's medium. One hundred ml of the modified media were inoculated by *Aspergillus niger* strains using spore suspension containing 10^4 spore / ml .The flasks were incubated at 28°C for 7 days using surface method.

Karimi's medium was modified by replacing the initial concentration of sucrose at the same concentration 5% with different other concentrations of sucrose i.e. 2.5, 5, 7.5 and 10%. Flasks containing 100 ml of the best media were inoculated with fungal strain using spore suspension containing 10^4 spore / ml . The flasks were incubated at 28°C for 7 days using surface method.

Effect of nitrogen sources

One hundred ml of modified Karimi's medium was dispensed in 500 ml Erlenmeyer flasks with different nitrogen sources i.e. peptone, urea, ammonium nitrate, ammonium sulphate and potassium nitrate. The media were inoculated using suitable spore suspension containing 10^4 spore /ml .The flasks were incubated at 28°C for 7 days using surface method.

Effect of incubation temperature

Flasks containing 100 ml of modified Karimi's medium supplemented with the optimum carbon and nitrogen sources . Karimi's medium was inoculated with *A. niger* strains using suitable spore suspension 10^4 spore/ml . Inoculated media were initially adjusted at different temperature values i.e. 22, 24, 26, 28 and 30°C. The flasks were incubated for 7 days using surface method.

Effect of different incubation periods

The culture media were incubated for different periods i.e. 3, 5, 7, 9 and 11 days at the optimum temperature according to the method described by Ghonaimy(2010). The media were inoculated as mentioned before and then incubated under different periods.

Effect of different pH values

Karimi's medium was initially adjusted at different pH values namely, 3, 3.5, 4, 4.5 and 5 according to the method described by Ghonaimy (2010). The media were inoculated and incubated as described before.

Effect of corn steep liquor as a nitrogen source on chitosan production

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One hundred ml of the Karimi's medium was dispensed in 500 ml Erlenmeyer flasks and supplemented with sucrose as a carbon source. Different amounts of corn steep liquor i.e. 25, 50, 75, 100, 125, 150 and 175 g/l were added as a nitrogen source and then Flasks were inoculated with spore suspension containing 10^4 spore/ml for both strains of *A. niger*. The flasks were incubated at 28°C for 7 days using surface method.

Effect of molasses as a carbon source on chitosan production

One hundred ml of prepared medium was supplemented with different kinds of molasses as a carbon source namely, cane molasses, beet molasses and mixture of cane and beet molasses (40:60) substituted with equal dose of sucrose in the and corn steep liquor was added as a nitrogen source (150g/l). Media were initially adjusted at pH 4 and inoculated with *A. niger* strains as mentioned before. After the incubation period at 28 °C for 7 days, fungal mycelia were harvested and washed with distilled water, then dried at 60°C to a constant weight. Chitosan was extracted from dried fungal mycelia according to the method described by **Synowiecki and Al-Khateeb (1997)**

RESULTS AND DISCUSSION

Factors affecting chitosan production by *A. niger* strains:

1- carbon sources

Data in **Fig.2** showed that *A. niger* A4 gave higher records of chitosan production compared to A11 strains with all different investigated carbon sources. The highest records of chitosan were observed when sucrose was used in fermentation medium as a sole carbon source followed by fructose. The highest amounts of dry chitosan production using sucrose were 505.8 and 440 mg/l for *A. niger*A4 and A11 strains , respectively.

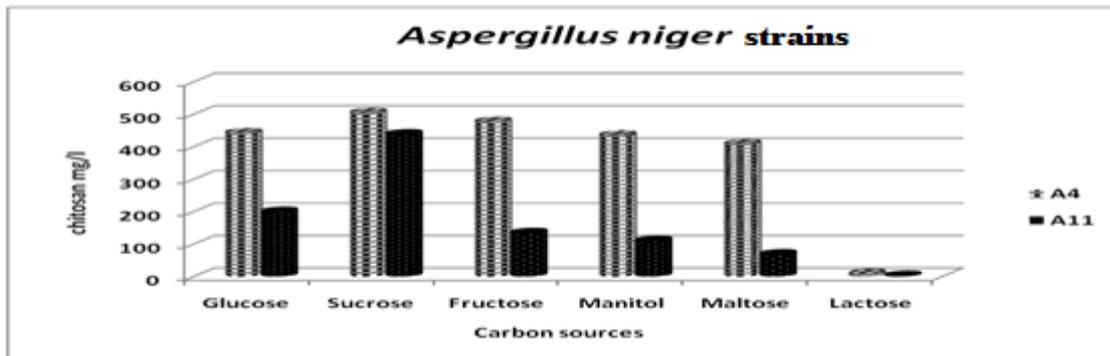


Fig 2.Chitosan production by *A. niger* strains using different carbon sources

This result is in agreement with that found by **Kubicek and Rohr (1989)** who stated that sucrose is preferable to glucose as *A. niger* has a potent extracellular mycelium bound invertase that is active at low pH and rapidly hydrolyzes sucrose. Superiority of sucrose over glucose and fructose was demonstrated by **Xu et al (1989)**. It is worthily to mention that the lowest records of chitosan production by both fungal strains of *A. niger*A4 and A11 (10 and 4 mg/l, respectively) were obtained when lactose was used in the fermentation medium as a sole carbon source. **Wang et al (2006)** studied chitosan production by *Aspergillus niger* on the culture medium and found that the sucrose was the best carbon source.

2- Sucrose concentrations

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Data in Fig.3 showed that both strains of *A. niger* A4 and A11 produced considerable amounts of chitosan at different concentrations of sucrose.*A.niger*A4 strain gave higher records of chitosan production compared to A11 strains at different concentrations of sucrose.

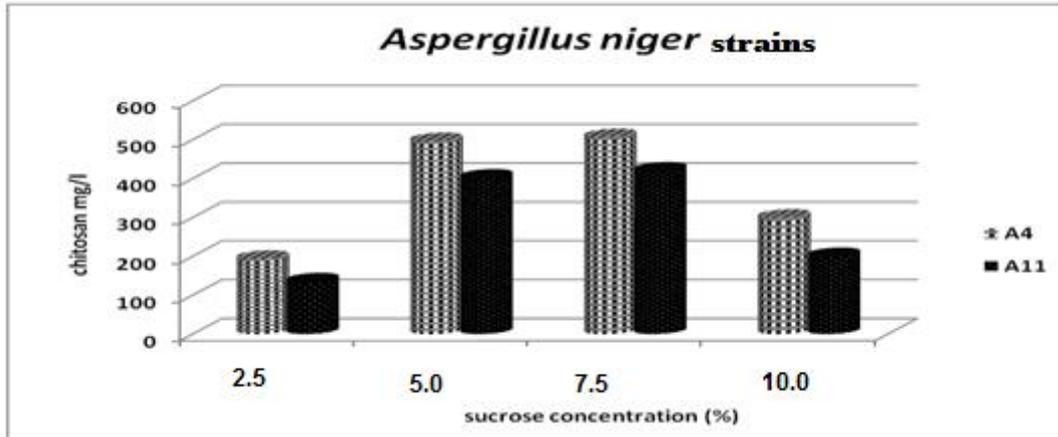


Fig 3.Chitosan production by *A. niger* strains using different sucrose concentrations

Data showed that chitosan production by the two *A.niger* strains was increased with the increasing of sucrose concentrations to reach their maximum values at 7.5% and decreased thereafter. Meanwhile, the lowest records of chitosan were obtained at 2.5% sucrose concentration. The highest amounts of dry chitosan production at 7.5 % were 506 and420 mg/l for *A. niger* A4 and A11 strains, respectively. However, the lowest amounts of dry chitosan production were observed at 2.5% sucrose concentration since the values were193 and 135 mg/l for *A. niger*A4 and A11 strains, respectively.

3- Nitrogen sources

Data in Fig.4 emphasized that *Aspergillus niger* A4 strain gave higher records of chitosan production compared to A11 strain at different nitrogen sources .

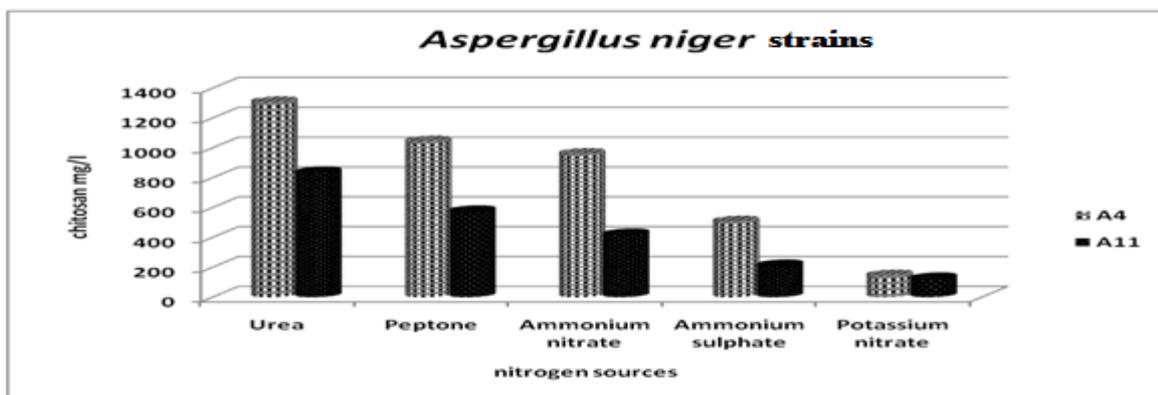


Fig4. Chitosan production by *A. niger* strains using different nitrogen sources

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Urea gave higher records of chitosan production by both strains being 1305.3 and 835.2 mg/l, respectively. On the other hand, it is worthily to mention that the lowest records of chitosan production by both strains were obtained when potassium nitrite was used in the cultural medium as a sole nitrogen source. The amounts of produced chitosan were 144.4 and 125.5 mg/l for strain A4 and A11 strain , respectively. These results are in agreement with those obtained by **Maghsoodi et al (2009)**who examined the ability of *A.niger* for chitosan production in corn residue medium supplemented with urea as a nitrogen sole source, they found that urea at 0.65% gave the highest chitosan yield. **Wang et al (2008)** used different nitrogen sources such as urea, sodium nitrite, ammonium sulphate, yeast extract and corn steep liquor for chitosan production by *Absidia coerulea*. It was found that urea was the best nitrogen source and gave 5.24g chitosan/kg substrate.

4 - Incubation temperature

Data in **Fig .5** clearly indicated that increasing incubation temperature led to gradual increase in chitosan yield up to 28°C for both strains of *A.niger*. Chitosan yield ranged from 294 to 1220mg/l for strain A4 and A11 strain, respectively. Therefore, *A. niger* A4 strain gave higher values of chitosan compared to *A. niger* A11 strain at all the tested incubation temperature.

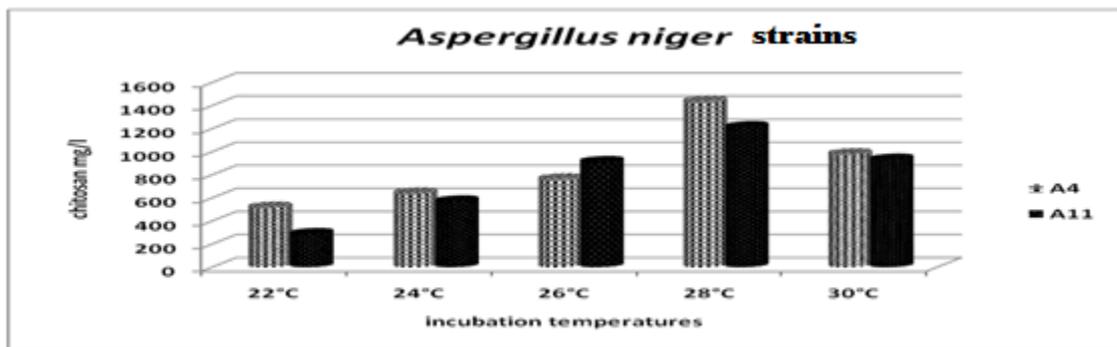


Fig 5: Chitosan production by *A. niger* strains different incubation temperatures

Data emphasized that the chitosan production amounts by *A.niger* A4 strain and A11 strain were higher at 30°C incubation temperature compared to either 24 °C or 26 °C.

These results are in accordance with those obtained by **Ghonaimy, (2010)**who cultured *A. niger* strains at 28°C for chitosan production. Also,**Long and Qing (2007)** produced chitosan from *A. niger* incubated at 28°C.While, **Maghsoodi and Yaghmaei (2010)** cultured *Aspergillus* strains at 30°C for chitosan production.

5 - Incubation periods

Data in **Fig.6** showed that the values of chitosan production by *both* strains were gradually increased with increasing the incubation period to reach their maximum values at 7 days and decreased thereafter.

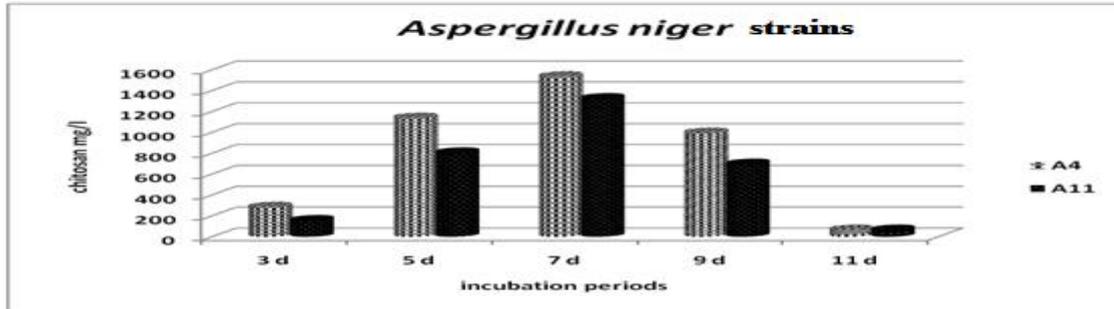


Fig 6: Chitosan production by *A. niger* strains using different incubation periods

Data recorded showed that *A. niger* A4 gave the highest values being 1540 mg / l of chitosan when grown for 7 days. This value was 1540 mg/l. Similarly, the optimal period for chitosan production of strain A11 was after 7 days being 1330 mg/l, respectively .On the other hand, the lowest records of chitosan production by both strains were observed at 11th day of incubation period. The lowest amount of dry chitosan production was 70 mg/l for both strains.

These results are in harmony with those obtained by **Pochanavanich and Suntornsuk (2002)** who reported that 6 days was the most suitable incubation period for maximum chitosan production from *A. niger*. Also, **Ghonaimy (2010)** reported that the growth rate of *A. niger* gradually increased by increasing the incubation period reaching the maximum value at the 7th day and the highest amount of chitosan was 0.78g/l.

6 - pH values

Data presented in **Fig.7** showed that strain A4 gave the highest values of chitosan at pH 4.5 being 1888 mg/l, while strain A11 reached maximum chitosan production at pH 4 being 1398 mg/l.

On the other hand, the lowest records of chitosan was recorded at pH3. However, the lowest amounts of chitosan production were 658 and 345 mg/l for strains A4 and A11 , respectively. Moreover, the increase of pH over 4.5 decreased the values of the previous parameters.

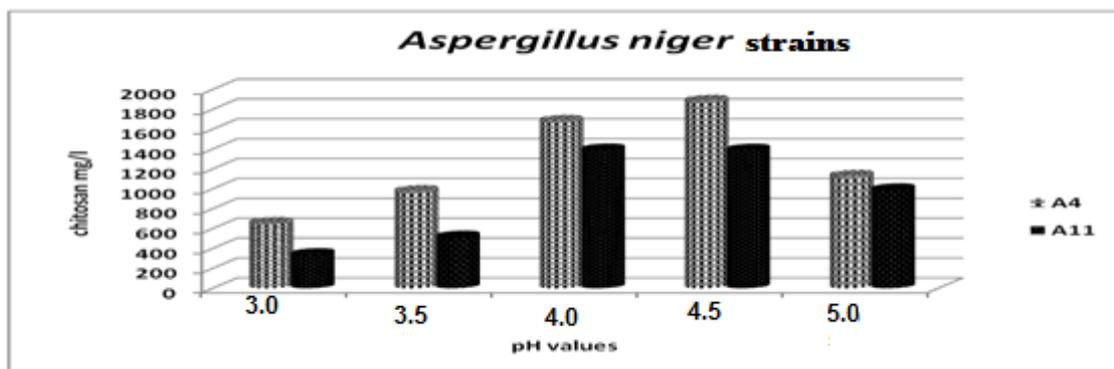


Fig 7.Chitosan production by *A. niger* strains using different pH values

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Hu *et al* (2004) reported that pH 4.5 was found to be the optimum for chitosan production by most fungal strains such as *Aspergillus* spp. In addition, Ghonaimy (2010) found that the increasing of pH up to 4 led to increase chitosan yield produced by *A. niger*. While, increasing pH up to 6 caused decreasing of chitosan production.

Chitosan production using corn steep liquor as a natural nitrogen source

Data in Fig.8 showed that the highest amounts of chitosan production at 150 g/l corn steep liquor were 2580 and 1900 mg/l for strains A4 and A11, respectively. Whereas, the lowest amount of chitosan production which recorded at 25 g/l corn steep liquor were 1020 and 1000 mg/l for A4 and A11 strains, respectively.

This result is logic since increasing corn steep liquor amount added to the medium increased nitrogen and also vitamins and amino acids which subsequently increased biomass of the fungi and chitosan yield (Valeria *et al*, 2012)

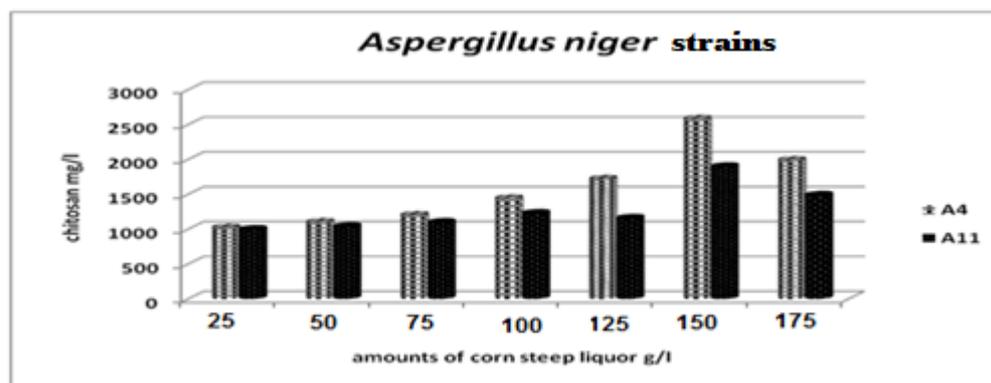


Fig.8: Chitosan production using corn steep liquor as a natural nitrogen source

These results are in harmony with those obtained with Long and Qing (2007) who cultured *A. niger* on a synthetic medium contain 4% corn steep liquor as a nitrogen source. While, Hao and Jin (2009) cultured *A. niger* at 3% corn steep liquor.

Chitosan production using molasses as a natural carbon source

The two *A.niger* strains produced higher amounts of chitosan when cane molasses was used as a carbon source rather than beet molasses(Fig.9). Moreover, maximum values of chitosan were observed when mixture of cane and beet molasses were 3000 and 2200 mg/l for strains A4 and A11, respectively. While, the lowest amounts of dry chitosan production when beet molasses was used were 600 and 570 mg/l for *A. niger* A4 and A11 strains, respectively.

This result is in agreement with that obtained by Seyis and Subasioglu (2009) who stated that in various fermentations, molasses is used as carbon source, due to its rich sucrose and other saccharide content. It is a suitable raw material for many processes, due to high concentration of organic matter.

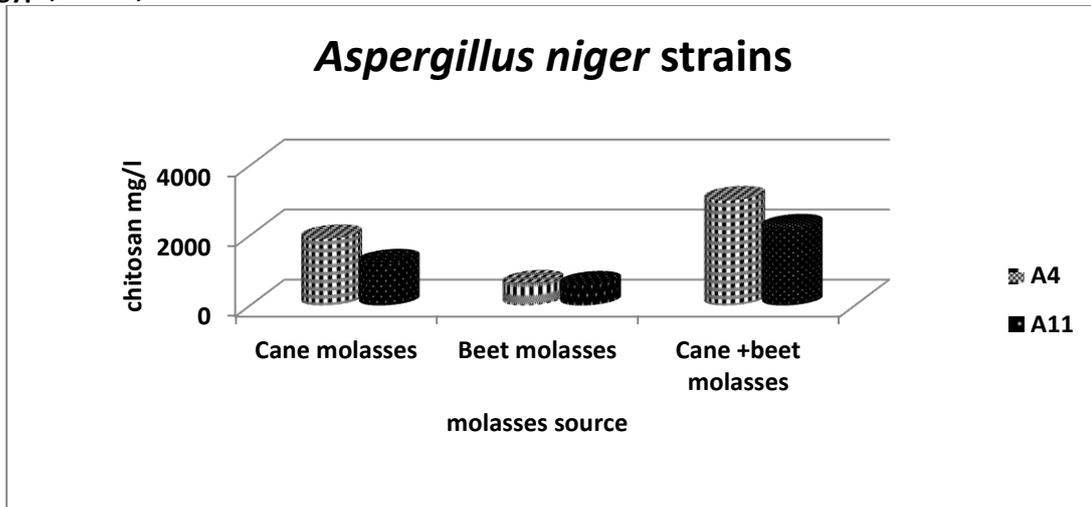


Fig. 9: Chitosan production using molasses as a natural carbon source

CONCLUSION AND RECOMENDATION

In view of the obtained results, it is clear that the chitosan can be produced by several *A.niger* strains. The optimal carbon source is mixture of cane and beet molasses. While, corn steep liquor is the best nitrogen source compared to sucrose and urea, respectively. The optimum of incubation temperature, incubation periods and pH values for chitosan production were 28°C and for 7 days and 4.5, respectively.

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تعظيم إنتاج الشيتوزان بواسطة سلالات من فطر أسبرجلس نيجر تحت ظروف مزرعية مختلفة

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تم إجراء هذه الدراسة لتحديد أفضل الظروف المثلى لإنتاج الشيتوزان بواسطة سلالات من فطر أسبرجلس نيجر ومقارنتها مع بعض المخلفات مثل المولاس وسائل منقوع الذرة. وقد أوضحت النتائج أن أعلى كمية من الكتلة الحيوية والشيتين والشيتوزان كانت عند استخدام السكروز بمعدل 75 جم / لتر كمصدر وحيد للكربون . أوضحت النتائج أن أعلى كمية من الشيتوزان المنتج بواسطة سلالاتي الأسبرجلس نيجر كانت عند استخدام اليوريا بمعدل 1,7 جم / لتر كمصدر وحيد للنيتروجين. وأشارت النتائج إلى أنه بزيادة درجة حرارة التحضين يزيد محصول الكتلة الحيوية والشيتوزان حتى درجة حرارة 28 °م لكلا السلالتين. وأيضاً أوضحت النتائج أن إنتاج الشيتوزان بواسطة سلالاتي الأسبرجلس نيجر يزداد بزيادة فترة التحضين حتى 7 أيام ثم تنخفض بعد ذلك. كما أن كمية الشيتوزان المنتج بواسطة سلالاتي الأسبرجلس نيجر ازدادت مع زيادة تركيز أيون الأيدروجين حتى 4,5 . وعند استخدام منقوع الذرة كمصدر للنيتروجين أوضحت النتائج أن الشيتوزان المنتج بواسطة سلالاتي الأسبرجلس نيجر يزداد بزيادة كمية منقوع الذرة حتى 150 جم / لتر . ولقد أظهرت النتائج أن كلا السلالتين أنتجت كميات كبيرة من الشيتوزان عند استخدام مولاس القصب مقارنة بالكميات المنتجة عند استخدام مولاس البنجر ولكن أنتجت أعلى كمية من الشيتوزان عند استخدام خليط بينهما وذلك بالنسبة لكلا من السلالتين.