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ORIGINAL ARTICLES

Enhancement of β-Glucosidase Production by Free and Immobilized *Aspergillus niger* NRC1 Cells

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ABSTRACT

Nine fungal strains have been examined with respect to their ability to produce β-glucosidase (β-GL) for potential industrial application. The fungus *Aspergillus niger* NRC1 was found to produce the highest level of β-GL (14.71 U/mg protein). Various cultural parameters of cultivation of *A. niger* NRC1 for the production of β-GL were optimized. The best conditions for maximum enzyme content were observed after 7 days of cultivation at 30 ºC. Maximal yield of β-GL was achieved with citric acid (1.7 % w/v) as carbon source, NH_4Cl (0.75 g N/l) as nitrogen source. On the other hand, addition of a mixture consists of wheat bran extract 1%, milk whey 1 % and triton 0.02 % stimulates the production by 27.31 %. High yield of β-GL (29.93 U/mg protein) was obtained by increasing the initial pH to 7.0. *A. niger* NRC1 was immobilized on several carriers by entrapment, covalent binding with cross-linking agent (glutaraldehyde) and physical binding methods. Cells immobilized on loofa sponge (*Luffa cylindrica*) by physical binding showed the highest specific activity (42.24 U/mg protein) and the highest effectiveness factor (0.97 %). β-glucosidase production and the stability of biocatalyst were investigated and the results showed that the immobilized cells were more efficient for enzyme production by repeated batch fermentation. The immobilized cells were able to retain 50.20 and 48.50 % of the initial activity after 7 and 8 batches (49 and 56 days), respectively. Moreover, the rate of enzyme production and the specific productivity remained between 4.93- 6.83 U/ml/h and 19.34- 26.79 U/g wet weight cells/h during 5 repeated batches (35 days, 840 h).

Key words: β-glucosidase, cell immobilization, batch, repeated batch.

Introduction

Lignocelluloses are abundant source of renewable biopolymer in the world (Liu *et al*., 2008). Accumulation of this biomass in large quantities every year results not only in deterioration of the environment but also in loss of potentially valuable materials that can be processed to yield energy, food, and chemicals (Yoon and Kim, 2005). β-glucosidase (β-GL) or β-glucoside glucohydrolase [EC.3.2.1.21] has been widely used in ethanol production using various cellulosic agricultural residues such as corn cob, rice straw, sugar baggasse, etc. (Dhake and Patil, 2005). The role of β-GL (cellobiase) in cellulose hydrolysis is significant because cellobiose is an inhibitor of both endo-and-exo-glucanases. It must be removed to allow efficient and complete saccharification of cellulose by increasing the rate of cellulose hydrolysis (Ghorai *et al.,*2010). The use of microbial enzymes for the hydrolysis of lignocellulosic materials is therefore widely researched because the hydrolysis products do not harm microorganisms used in fermentation (Boonmee, 2012). Filamentous fungi are preferred for commercial enzyme production because the levels of enzyme produced are higher than those obtained from yeasts and bacteria (Ahamed and Vermette, 2008). The application of the immobilized cells for biochemical processes offers many advantages, such as the ability to separate cell mass from the bulk liquid for possible reuse, enhance the stability of the enzyme by retaining its natural catalytic surrounding during immobilization, facilitating continuous operation over a prolonged period and enhanced reactor productivity (Abdel-Naby *et al.,* 2000; Sridhar and Kumar, 2010). Various techniques have been developed for cell immobilization, including physical adsorption, covalent binding with bifunctional reagents (as glutaraldhyde) to insoluble supports and entrapment in polymeric gels (Radva *et al.,* 2011). Many studies have shown that the adsorption method for immobilization of microorganisms has many advantages over other methods (Hideno *et al.,* 2007) and many synthetic materials have been used as carriers for the adsorption method. However, these materials are often very expensive and because they are non biodegradable, their disposal causes a lot of environmental problems after their use. It is therefore desirable to use renewable and biodegradable natural materials as carriers for cell immobilization (Hideno *et al.,* 2007). Cell immobilization techniques using

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biological material are ecofriendly and have many advantages over free cells or synthetic material (Hideno *et al.,* 2007). It has been reported that loofa sponge (*Luffa cylindrica*) is an excellent carrier for immobilization of microorganisms (Roble *et al.,* 2003; Saeed and Iqbal, 2006). The objective of the present investigation was to optimize the cultural parameters to enhance β-GL production by *Aspergillus. niger* NRC1. Furthermore, study was taken up with a view of optimization of the immobilization process using loofa sponge. We evaluated the use of the immobilized cells for β-GL production in batch and repeated batch compared with the free cells.

Materials and Methods

Organisms and culture maintenance:

In the present work, nine fungal strains were screened for the production of extracellular β-glucosidase. The fungal strains *Aspergillus niger* NRC1 and *A. niger* NRC2 were obtained from the culture collection of the National Research Centre (NRC), Dokki, Cairo, Egypt. *A. niger* NRRL 6411, *A. oryzae* NRRL 3487, *Penicillium funiculosum* NRRL 130, *P. funiculosum* NRRL 13038, *P. funiculosum* NRRL 13039, *P. funiculosum* NRRL 13041 and *Trichoderma viride* NRRL 13034 were provided by Northern Regional Research Laboratory (NRRL), Peoria, Illinois, USA. All fungal strains were sub-cultured every 3 weeks and stored at 4ºC in PDA slants.

Cultivation media:

For inoculum preparation, spores from *A. niger* NRC1 were produced on PDA slants for 5 days at 30ºC. One ml of spore suspension ($6x10^{10}$ spores) was then inoculated in 250 ml-Erlenmeyer flasks containing 50 ml production medium. The pre-culture medium and production medium (with the same compositions) were prepared (g or ml /l) from a mixture of 3.5 g $(NH_4)_2SO_4$, 3.0 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g $CaCl_2$, 0.2 ml tween 80. Each flask contained 2.5 g corn cobs and 2.5 g wheat bran and the pH was adjusted to 5.0. The flasks were shaken at 150 rpm and 30 ºC for different incubation periods (5, 7 and 11 days). The culture was filtered through cloth to remove the mycelia and the crude supernatants used for β-GL activity.

Effect of environmental variables on the enzyme production:

Effect of incubation period on the production of β-GL enzyme was examined at various incubation times (5-11 days). Effect of incubation temperature on β-GL production was investigated at different temperature ranged from 25ºC to 45ºC at 150 rpm for 7 days. The affinity of the organism to utilize different carbon sources was examined by replacing corn cobs (2.5 g/flask) and wheat bran (2.5 g/flask) in the aforementioned basal medium with tested carbon sources (on equal carbon basis). Similarly, $(NH_4)_2SO_4$ was replaced with various nitrogen sources on equal nitrogen basis (0.75 g nitrogen/l). The effect of some additives on β-GL production was also studied using different concentrations. The starting pH of the medium was varied between 3.5 and 9.0 to investigate its effect on enzyme production.

Immobilization procedures:

All the immobilization processes were performed under aseptic conditions. In separate experiments, the vegetative cells (0.255 g weight wet) obtained from 50 ml culture after 24 h were collected by centrifugation (4000 rpm, 15 min) in a refrigerated centrifuge. Then, the weight wet cells were suspended in 5 ml sterile distilled water and used for the cell immobilization experiments.

1- Entrapment:

The wet cells suspension obtained from 50 ml culture of *A. niger* NRC1 (0.225 g weight wet) were mixed with 5 ml of sodium alginate solution, in separate experiments. The final concentration was kept at 1, 2, and 3 % (w/v). The beads diameters ~ 2-3 mm were obtained by dropping mixture (through a sterile 5 ml pipette) into sterile $CaCl_2$ (1 %). The beads from 10 ml gel were washed with sterile H_2O (to remove the unbounded cells) and used for inoculation of 50 ml of the production medium (Abdel-Naby *et al.,* 2000).

2- Covalent binding with cross-linking:

According to El-Tanash *et al.,* (2011), 0.5 g of each tested carrier (loofa sponge, sponge, stainless steel and wool) approximately 3x3 mm fragments was treated with 10 ml of 1% glutaraldehyde overnight. The treated carriers were collected by centrifugation and washed with sterile water to remove the excess glutaraldehyde.

Then the wet cells suspension obtained from 50 ml culture medium (0.225 g weight wet) was incubated with each carrier for 2 h. The carriers were collected by centrifugation and washed with sterile water and transferred to 50 ml production medium.

3- Physical adsorption:

The wet cells suspension obtained from 50 ml culture medium (0.225 g suspended) was mixed with 0.5 g each carrier ~3x3 mm fragments for 2 h. The unbound cells were removed by washing with sterile H_2O and bound cells were used for inoculating of 50 ml production medium (Woodward, 1985).

Production of β-glucosidase by immobilized cells:

1. Batch experiment:

Batch experiment of the immobilized cells (by physical adsorption on loofa sponge) was performed in 250-ml Erlenmeyer flasks each containing 50 ml of sterile production medium. The flasks were inoculated with the calculated amount of beads (loofa sponge immobilized cells). Fermentation was conducted at 30 ºC for 7 days in a rotary shaker regulated at 150 rpm. The cultivation medium was filtered off and the clear filtrate was taken for enzyme assay.

2. Repeated batch experiment:

The experimental set up was similar to that of batch cultures. Every 7 days, immobilized cells beads were removed, washed with sterile H_2O and re-cultivated into fresh medium. The process was repeated for several batches till the activity dropped significantly.

Enzymatic assay:

β-glucosidase activity was determined according to Abdel-Naby (1999) using 0.4 % (w/v) cellobiose solution in citrate phosphate buffer (0.05 M, pH 4.8). Unless otherwise stated the reaction mixture in a total volume of 1.5 ml was composed of 1 ml cellobiose solution and 0.5 ml of appropriately diluted enzyme. The reaction mixture was incubated for 30 min in a water bath at 50 ºC. Then the reaction was stopped by heating in a boiling water bath for 5 min. The glucose released was determined by glucose oxidase kits. The estimations were based on a glucose standard curve. One unit of enzyme activity (U) was defined as the amount of enzyme that librates 1µ mole of glucose per min under the assay conditions.

Protein determination:

Protein content was estimated by the method of Lowry *et al*. (1951).

All the values presented in graphs and tables are the means of the three replications and standard deviations have been added in figures.

Results and Discussion

Screening for β-glucosidase producer:

Some fungal strains (*Aspergillus*, *Penicillium* and *Trichoderma*) have been examined with respect to their ability to produce β-GL for potential industrial application (Fig.1). Of the nine strains examined, *Aspergillus niger* NRC1 was found to produce the greatest amount of β-GL (16.18 U/ml) with the highest specific activity (14.71 U/ mg protein) after 7 days which was higher than other values described in the literature. Lemo *et al.* (2002) and Leite *et al.* (2008) reported that the maximum production of β-GL (0.5 and 1.3 U/ ml) was obtained by *Aureobasidium sp*. and *A. pullulans*, respectively. The time required for β-GL production by *A. oryzae* was reported to be 14 days (Riou *et al.,* 1998) and that for *P. purpurogenum* was 4 days (Dhake and Patil, 2005). Cellulase production has been described for many *Aspergillus* species (Abdel-Naby *et al.,* 1999; Wang *et al.,* 2006; Gao *et al.,* 2008).

Effect of environmental variables on enzyme production:

1- Effect of temperature on enzyme production:

Under the different culture temperature (25-45 ºC), the β-GL activity was detected (data not shown). The highest value of β-GL production occurred at 30 ºC (14.71 U/mg protein) which suggests that the temperature is a critical factor of β-GL production. Similar result obtained by Dhake and Patil (2005) on the production of β-GL by *P. purpurogenum*. Joo *et al.* (2010) suggested that the maximum β-GL production by *P. pinophilum* KMJ601was achieved at 32 ºC.

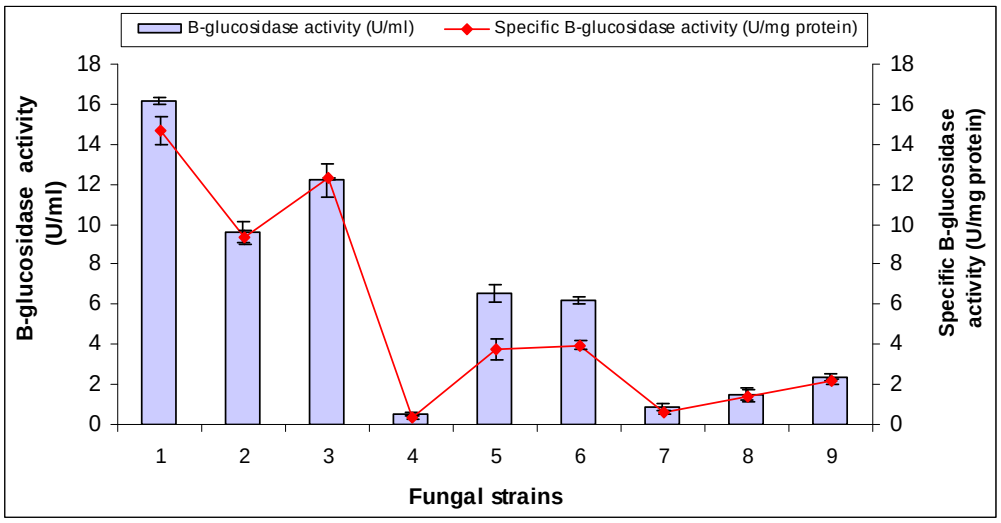


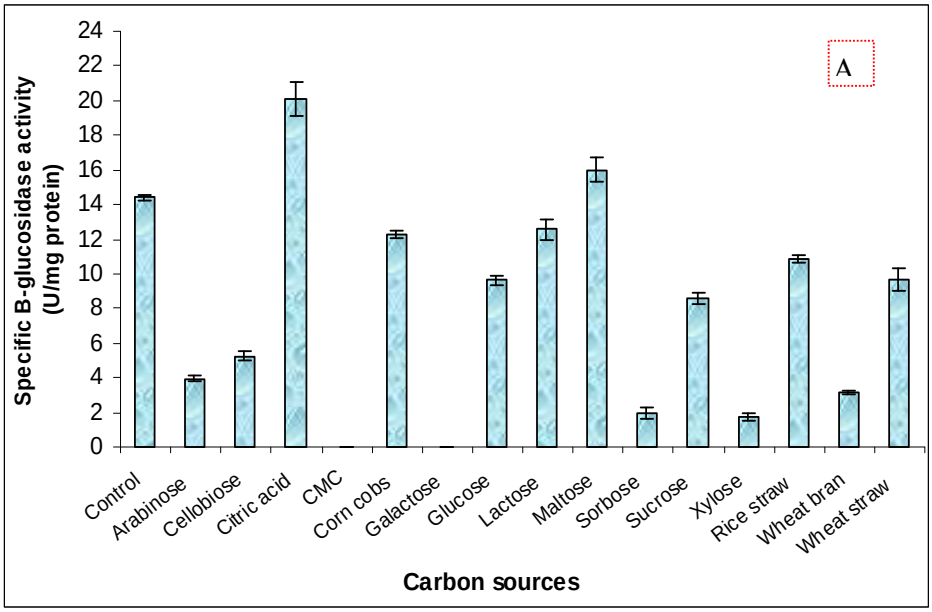
Fig. 1: Survey of some fungal strains for β-glucosidase production after 7 days whereas: (1) *A. niger* NRC1, (2) *A. niger* NRC 2, (3) *A. niger* NRRL 6411, (4) *A. oryzae* NRRL 3487, (5) *P.funiculosum* NRRL 130, (6) *P. funiculosum* NRRL 13038, (7)*P. funiculosum* NRRL 13039, (8) *P. funiculosum* NRRL 13041, (9) *T. viride* NRRL 13034.

2- Effect of different carbon sources:

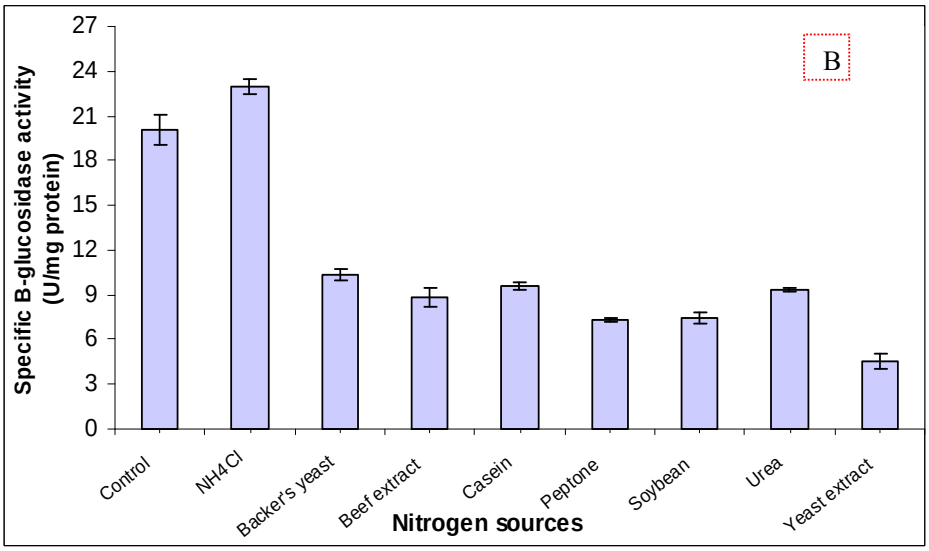
Biotechnological process is likely to base on crude enzymes, so it is important to increase their activities in the culture supernatants by selecting the best carbon sources and optimizing its concentrations (Liu *et al.,* 2008). From Fig. 2A, it can be observed that there is a significant difference in the production in the presence of different carbon sources. Citric acid yielded the maximum β-GL production (20.08 U/mg protein). On the other side, the production of β-GL was inhibited by 34.87 % in the presence of the glucose. This result is agreed with that obtained by Issam *et al.* (2003) on the production of β-GL by *Sclerotinia sclerotorum*. Low level of cellulolytic enzymes in the presence of glucose could be attributed to repression of synthesis of cellulolytic enzymes involved in the utilization of cellulose by easily metabolisable carbon glucose that was demonstrated in many organisms (Narasimha *et al.,* 2006). Using different concentrations of the selected carbon source (citric acid), β-GL production was measured (data not shown). Maximum β-GL production (20.08 U/mg protein) was obtained at 1.7 % (w/v) concentration. The decreased in β-GL production with the highest citric acid concentration was probably owing to osmotic effects (Rajaka *et al.,* 2004). Joo *et al.* (2010) reported that 5 % rice straw enhanced β-GL production by *P. pinophilum* KMJ601 to be 19 U/ mg protein.

3- Effect of different nitrogen sources:

Organism requires nitrogen for the synthesis of compounds like protein, nitrogenous compounds, vitamins and nucleic acid. Mainly ammonium sulphate is use as a nitrogen source in the medium. Among organic and inorganic nitrogen sources (on equivalent nitrogen basis 0.75 g N/l) ammonium chloride enhanced the β-GL production by 4.14 %. As presented in Fig.2B enzyme activity was higher with inorganic nitrogen compared with organic sources. Gao *et al.* (2008) suggested that on testing different nitrogen sources, the organic nitrogen sources enhanced enzyme production by *A. terreus* M11 when compared with inorganic sources. Yeast extract gave the lowest β-GL production (4.53 U/mg protein). In contrast, Gao *et al.* (2008) and Liu *et al.* (2008) reported that yeast extract was the best nitrogen source for β-GL synthesis by *A. terreus* M11 and *P. decumbens* L-06. Furthermore, the concentration of NH_4Cl was optimized (data not shown). The maximum β-GL production was obtained at 0.75 g N/l from NH_4Cl (23.17 U/mg protein). Above or below this concentration enzyme production was decreased.



Control contain (wheat bran + corn cobs)



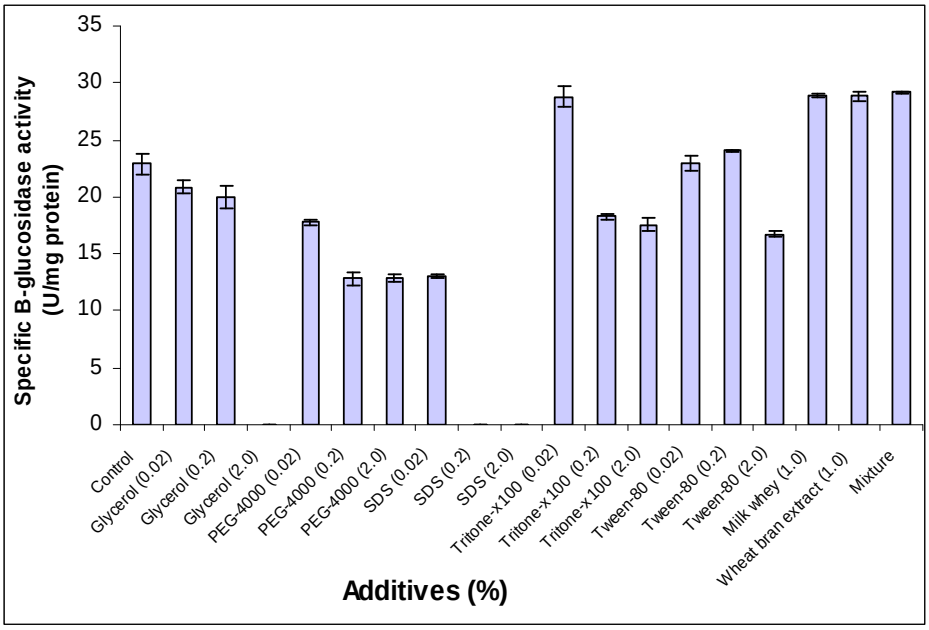
Control contain $(NH_4)_2SO_4$

Fig. 2: Effect of carbon sources (A) and nitrogen sources (B) on the production of *A. niger* NRC1 β-glucosidase enzyme.

4- Effect of additives on the β-GL production:

Addition of different additives in the cultivation medium has an impact on β-GL production as shown in Fig 3. Addition of Sodium Dodecyl Sulphate (SDS) at different concentrations inhibited β-GL production. Similar observation was recorded by Dhake and Patil (2005) on addition of SDS to the production medium of β-GL which inhibited the growth of by *P. purpurogenum* hence no enzyme production was observed. On the other side, Poly Ethylene Glycol (PEG-4000) inhibited β-GL production by 22.43 to 44.15 %. Pardo (1996) reported that PEG increased the production of cellulase system by *Nectria catalinensis*. In separate experiments, Triton x-100 (0.02 %), milk whey (1 %) and wheat bran extract (1 %) improved β-GL production by 25.44, 26.18 and 25.74 %, respectively. Surfactant like Tween and Triton could improve the enzyme production by increasing permeability of the cell membrane, allowing more rapid secretion of enzymes, which in turn lead to higher enzyme synthesis (Ahamed and Vermette, 2008). Moreover, Liu *et al.* (2008) explained the effect of surfactant in the permeability of the cell membrane by affect zymophore microenvironment by changing the ionic

strength, chelating metal ion, and also by other methods or could change the permeability of the cell membrane, which resulted in the imbalance of enzyme environment and the effect of cell growth and enzyme production. On the other hand, a mixture of wheat bran extract (1 %), milk whey (1 %) and Triton x-100 (0.02 %) caused increases in β-GL production by 27.31 %. It is interesting to use industrial waste (milk whey and wheat bran) to improve the β-GL production as inexpensive, safe and available additives.



Control contain Tween-80 (0.02%). Mixture contain wheat bran extract (1.0 %) + milk whey (1.0 %) + triton-x 100 (0.02 %)

Fig. 3: Effect of some additives on the production of *A. niger* NRC1 β-glucosidase enzyme.

5- Effect of varying pH on the β-GL production:

The influence of culture medium pH on the β-GL production was studied for values in the range from 3.5 to 9.0 (Fig. 4). As the pH of culture media increased, the β-GL production increased up to pH 7.0 enhancing the production by 2.61 %. The same experiment was also performed by Liu *et al.* (2008) for *P. decumbens* L-06 by varying initial pH, where pH 8.0 was found to be optimum as initial pH for the β-GL production. On the contrary, Joo *et al.* (2010) reported that pH 3.5 was the suitable value for the maximum β-GL production by *P. pinophilum* KMJ601.

Optimum culture condition:

A. niger NRC1 was cultured in the optimal conditions, the β-GL activity was determined. The highest value of β-GL activity (29.93 U/mg protein) obtained after 7 days at temperature 30 ºC and initial pH 7.0. Compared with the initial culture conditions the specific activity of β-GL was increased by 103.47 % fold.

Production of β-GL enzyme by immobilized cells:

1. Batch culture:

The ability of *A. niger* NRC1 cells to produce β-GL enzyme using different immobilization methods and carriers to immobilized the same amount of cells was investigated. As shown in Table 1, the activity (U/ml) of the immobilized cells was lower than the corresponding amount of free cells. The key parameter for selecting the suitable method and carrier for cell immobilization is the effectiveness factor (EF) which is the ratio of the enzyme activity of the immobilized cells to the same amount of the free cells. The highest EF with the highest activity (0.97 % and 19.01 U/ml) was obtained by physical binding of *A. niger* NRC1 cells to loofa sponge (*Luffa cylindrica*) followed by covalent binding method on loofa sponge (0.95 % and 18.55 U/ ml). On the contrary, physical binding to sponge gave the lowest EF with the lowest activity (0.14 % and 2.68 U/ ml). Kumakura *et al.,* (1989) reported that cellulase production by immobilized *Trichoderma reesei* cells was lower

than that obtained by free cells. Similar observation has been reported for the production of other enzymes by immobilized cells (Abdel-Naby *et al.,* 2000; Singh *et al.,* 2004; Ahmed and Abdel-Fattah, 2010). The EF of the immobilized cells would always be less than one because the immobilized cells represent a heterogeneous catalysis fermentation in which the activity or rather synthesis of primary or secondary metabolites is dependent upon the external and internal mass transport and adequate oxygen supply (Jamuna and Ramakrishna, 1992). In comparison with other carriers used for cell immobilization, loofa sponge is a lignocellulosic material with very high specific pore volume, stable over wide range of pH, can be autoclaved many times without any visible change in the shape, structure and texture, cheap, safe, available and suitable for industrial applications. Therefore, *A. niger* NRC1 cells immobilized on loofa sponge were used throughout the further investigation.

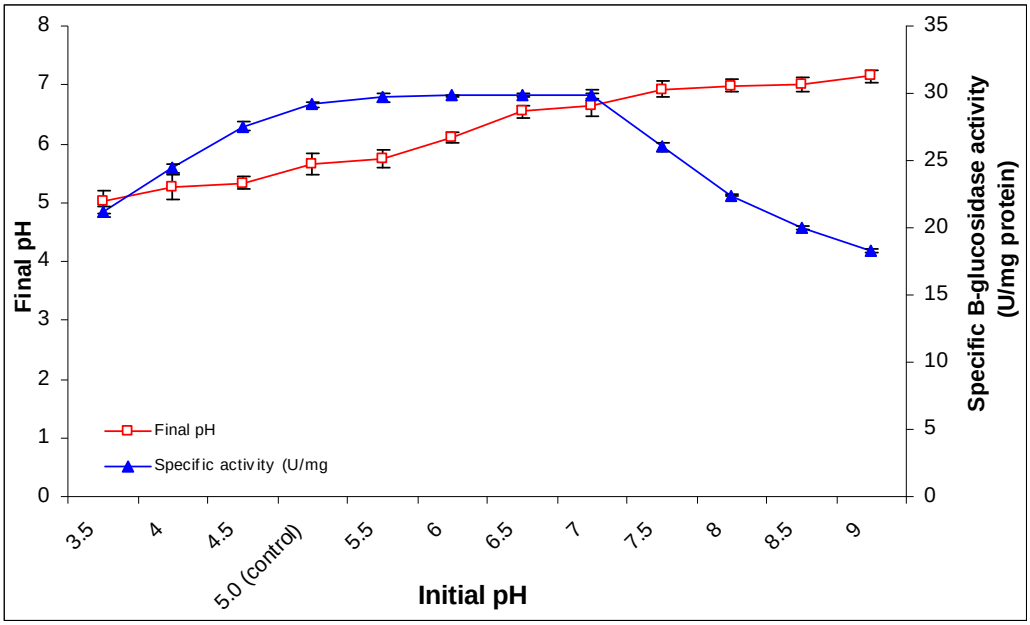


Fig. 4: Effect of initial pH on the production of *A. niger* NRC1 β-glucosidase enzyme.

Table 1: Immobilization of *A. niger* NRC1 cells for β-glucosidase production.

Immobilization method and carrier	Activity (U/ml)	Relative activity (%)	Protein content (mg/ml)	Specific activity (U/mg protein)	Specific productivity (U/g wet cells/h)	Effectiveness factor
Free cells	19.50	100	0.59	33.05	22.76	1
Entrapment						
Na alginate (1%)	10.52	53.95	0.4	26.30	12.28	0.54
Na alginate (2%)	15.16	77.74	0.46	32.96	17.69	0.78
Na alginate (3%)	11.41	58.51	0.42	27.17	13.32	0.59
Covalent binding						
Loofa sponge	18.55	95.13	0.54	34.35	21.65	0.95
Sponge	13.73	70.40	0.44	31.20	16.02	0.70
Stainless Steel	5.00	25.64	0.42	11.90	5.84	0.26
Wool	0.0	0.0	0.0	0.0	0.0	0.0
Physical binding						
Loofa sponge	19.01	97.49	0.45	42.24	22.19	0.97
Sponge	2.68	13.74	0.42	6.38	3.13	0.14
Stainless Steel	0.0	0.0	0.0	0.0	0.0	0.0
Wool	5.18	26.56	0.44	11.77	6.05	0.27

Effect of carrier concentration:

Different concentrations of loofa sponge (0.25-1.25 g/ flask) were investigated to immobilize the same amount of cells (Fig. 5A). The highest enzyme yield (22.95 U/ml) with the highest specific productivity (26.79 U/g/h) was obtained at high carrier concentration (1 g/flask). This result might be due to the increase in the surface area of the carrier. Kumakura *et al.* (1989) found that the filter paper activity of the immobilized *Trichoderma reeesei* cells with fabric carrier at higher concentration was higher than that of the lower

concentration. On the other hand, the use of lower concentration (0.25 g/flask) resulted in about 25.75 % drop of the enzyme yield recorded at 1 g/ flask.

Effect of cell loading:

The effect of biomass loading on the β-GL production by loofa immobilized *A.niger* cells was tested using the same concentration of loofa sponge (1 g/flask). The results presented in Fig 5B showed that 0.225 g weight wet cells was the suitable concentration to obtain the highest enzyme yield (22.95 U/ml) and hence, the specific productivity decreased to about 35.28 % of that obtained at a cell loading level of 0.085 g/ flask. Further increase of cell loading, however, did not significantly increase the enzyme yield. Similar results were reported by Abdel-Naby *et al.* (2000) on the production of cyclodextrin glucosyltransferase by immobilized *B. amyloliquefaciens* cells. It is worthy to note that low level of biomass may lead to rapid enzyme biosynthesis while high levels may cause diffusion limitation of nutrients.

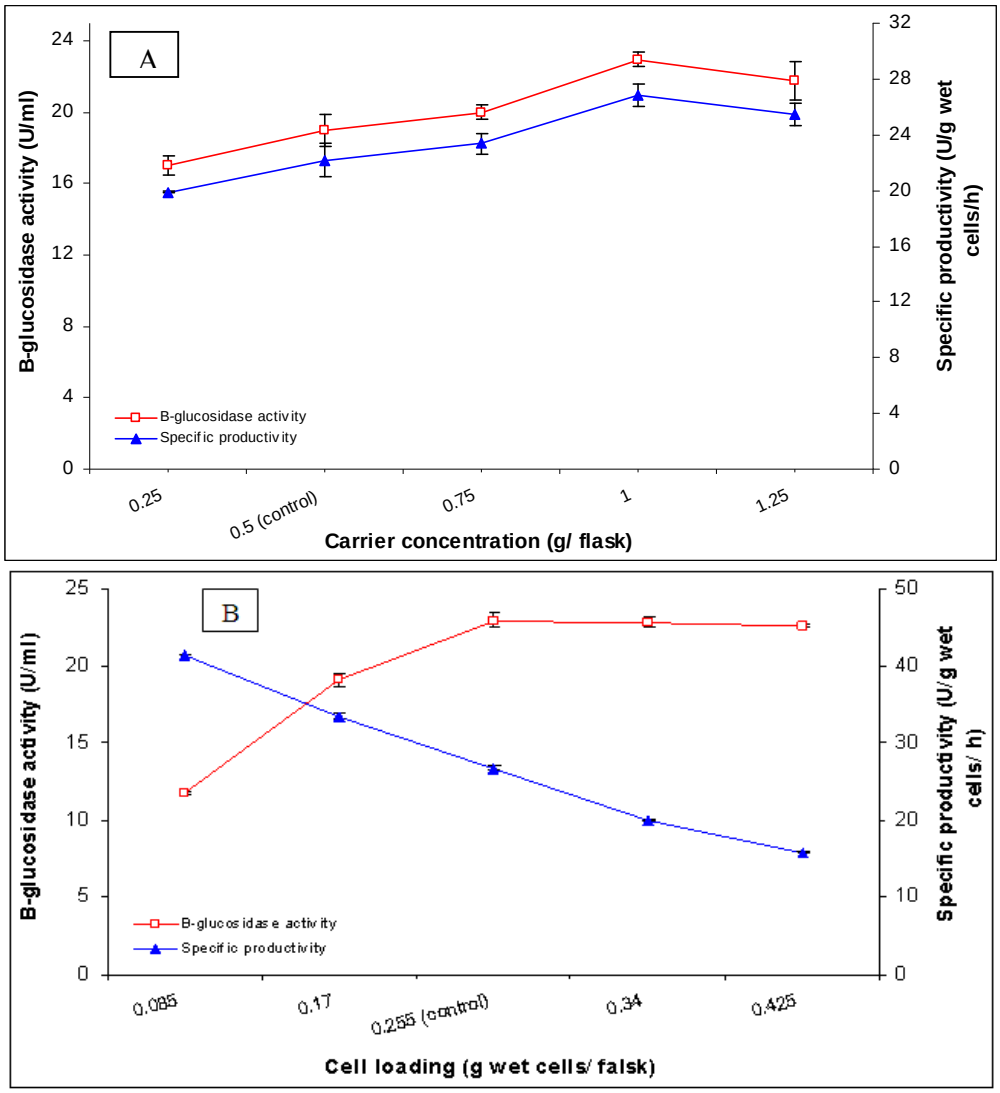


Fig. 5: Effect of carrier concentration (A) and cell loading (B) on the production of β-glucosidase enzyme by immobilized *A. niger* NRC1 cells.

2. Repeated batch culture:

Batch culture is a common mode for commercial fermentation in addition to fermentation time, however the production cycle also includes turnaround time (needed for sterilization, inoculation, turnaround etc.). This

lead to a reduction in overall productivity and addition to production costs (Ahmed and Abdel-Fattah, 2010). The results of repeated batch β-GL production by immobilized *A. niger* NRC1 cells were shown in Fig. 6. During the first and second batches (7 and 14 days), the relative β-GL activity obtained using cells immobilized on loofa sponge was 100 %. The results indicated that loofa immobilized cells retained their ability to produce β-GL consistently for 7 batches (49 days, 1176 h) with 50.2 % residual activity. After that, the activity gradually decreased to reach 8.87 U/ml after 11 batches (77 days, 1848 h) with 38.65 % relative activity. The decrease in activity with increase cycle number might be due to degradation in loofa sponge and the cells leaked out of loofa which leading to an increase in the amount of freely suspended cells and lowered the enzyme production. In contrast, Hideno *et al.* (2007) reported that only two repeated batches of *Trichoderma reeesei* QM9414 immobilized cells on loofa sponge, were possible to produce cellulase enzyme. The kinetic parameters of the immobilized cells in repeated batch (Table 2) indicated that the rate of enzyme production and the specific productivity remained between 4.93- 6.83 U/h and 19.34- 26.79 U/g wet weight cells/h during 5 repeated batches (35 days, 840 h).

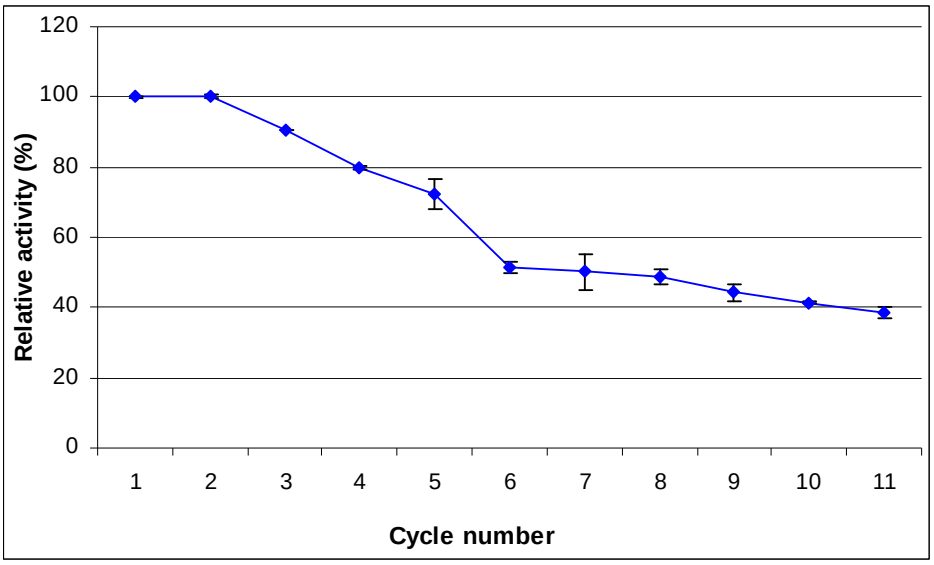


Fig. 6: Repeated batch for β-glucosidase production by immobilized *A. niger* NRC1 cells.

Table 2: Kinatic parameters for repeated batch process production of β-glucosidase by immobilized *A. niger* NRC1cells.

Cycle number	Activity (U/flask)	Rate of enzyme production (U/ml/h)	Specific productivity (U/g wet cells/h)	Relative productivity (%)
1	1147.5	6.83	26.79	100.00
2	1147.5	6.83	26.79	100.00
3	1038.0	6.18	24.23	90.44
4	913.5	5.44	21.32	79.58
5	828.5	4.93	19.34	72.19
6	590.0	3.51	13.77	51.39
7	576.0	3.43	13.45	50.21
8	565.5	3.37	13.3	49.66
9	509.5	3.03	11.89	44.38
10	474.5	2.82	11.08	41.36
11	443.5	2.64	10.35	38.63

Concluion:

This study suggests that *A. niger* NRC1 was able to grow and produce β-GL enzyme. Addition of some agriculture and industrial waste like wheat bran and milk whey enhanced the production of β-GL by 25.74 and 26.18 %, respectively. Optimized media increased the production by 103.47 % fold in compared with the initial culture conditions. It is interesting to not that loofa sponge is an excellent carrier for immobilization of *A. niger* NRC1 cells. The results indicated that loofa immobilized cells retained their ability to produce β-GL consistently for 7 batches (49 days) with 50.2 % residual activity. Moreover, the rate of enzyme production and the specific productivity remained between 4.93- 6.83 U/h and 19.34- 26.79 U/g wet weight cells/h during 5 repeated batches (35 days). Future experiments will be required to further define the continuous β-GL production by loofa sponge immobilized cells.

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