Utilization *of Opuntia ficus indica* waste for production of *Phanerochaete chrysosporium* bioprotein

A.S. Gad * and E.A. Hasan**, and A.Abd El Aziz
*Chemistry of Natural and Microbial products Dept.,NRC.,Dokki, Egypt.
**Cytology and Genetics Dept., NRC.Dokki, Egypt.
Fac.of Agric.Ein Shams Univ.Egypt.
dr_mona_zaki@yahoo.co.uk

Abstract: The highest % saccarification of the *Opuntia* waste was 75.6 obtained with 1% (w/v) NaOH treatment. *Phanerochaete chrysosporium* was the most potential fungus among the tested microorganisms ; *A. terreus* and *R. oryzae* for bioprotein production with 6.90 g protein /100 g *Opuntia* waste.*Opuntia ficus indica* peels proved to be the most suitable substrate among the other agricultural wastes, corn cob shred , and sugar cane bagasse which are used as carbon sources for *Phanerochaete chrysosporium* bioprotein production. Also ,The most optimum fermentation conditions were : 10 g *Opuntia* waste /L as carbon source using phosphate buffer for bioprotein extraction of ; 3% (v/v) inoculum size; supplementation of Modified Czapek Dox medium (MCD) with 0.3 %(w/v) CSL; the initial PH , 4; agitation speed at 150 rpm, and 75 ml medium was the most effective volume resulted in 11.97 g bioprotein /100 g *Opuntia* after 7 days of fermentation at 150 rpm and 30°C. [Journal of American Science 2010;6(8):208-216]. (ISSN: 1545-1003).

Keywords: Utilization *of Opuntia ficus india;* waste; *Phanerochaete chrysosporium* bioprotein

Introduction

Opuntia ficus indica is one of the most promissory by presenting the largest part of alimentation furnished to the animals during the drought. This increases the availability of fodder and alleviates the problems of the water supplement to these animals. It is also rich in minerals, calcium, iron and vitamin A. It presents an elevated texture of soluble carbohydrates besides presenting a high coefficient of digestibility of the dry matter and high productivity Lúcia de Fátima *et al., (*2005). Besides, improper handling of solid waste is a health hazard and causes damage to the environment. The main risks to human health arise from the breeding of disease vectors, like flies and rats. Healthy life and cleaner environment is the end result of solving these problems in such a way by utilizing the waste into valuable by-products ; Nigam *et al.*, (2009). Mycelium biomass from *Rhizopus oryzae*, can partly substitute high-quality fishmeal in diets to rainbow trout without causing any major short-term adverse effects on growth, nitrogen and amino acids digestibility. Nutrient digestibility of diets containing mycelium biomass of *R. oryzae* are in carnivorous fish and larvae ; Uysal *et al.* ,(2002) and Olsen *et al*.,(2006)). For humans, it is also considered as food additive to improve flavor, fat binding and more recently as a replacement for animal protein in the diet(Jamal *et al.*, 2007). Bioprotein can also be used as additives in certain chemical and pharmaceutical industries. Bioprotein is the protein extracted from cultivated microbial biomass that can be produced using a number of different microorganisms and such as low carbon cost, high energy sources agricultural wastes .Agro- wastes can be regarded as new sources for bioprotein production, which have a high nutritional value, do not compete with food for human consumption, economically feasible and locally available ;(Anupama and Ravindra, 2000, Uysal *et al.*, 2002).

Lignocellulosic waste is a complex mixture of cellulose, hemicellulose, lignin along with extractives.Wastes pretreatment is therefore a necessary process in order to achieve high yield. Grinding and milling are the primary physical accomplished by using acids or bases . The objectives of this study were beneficial in production of nutritional bioprotein from a cheaper carbon source.

Materials and method

Microotganisms:

Three different fungi were used ; culture of *Phanerochaete chrysosporium* ATCC 28236 obtained from Cairo MERCIN , Ein Shams University; culture of *Rhizopus oryzae obtained from Dr.M. U. Noaman,* Biochemistry Dept. and *Aspergillus terreus* obtained from Dr.A.F. Salah, Plant Pathology Dept. NRC.,Egypt.

Maintenance medium *:*

All strains were maintained by subculturing on PDA slants for 7 days then stored at 4ºC .

Inoculum preparation:

Spores were harvested from a week old slants in five ml of sterile distilled water. 0.5 ml of spore suspension was added to fifty ml broth with pretreated substrate based medium in 250 ml conical flasks. The inoculated flasks were incubated at rotary shaker 100 rpm for 30°C for 7 days. (Anupama and Ravindra, 2000).

Preparation of lignocellulosic substrates

-Fresh *Opuntia* peels were collected from local markets during summer season stored in a refrigerator until use. The fresh *Opuntia* skins were washed and cut into smaller pieces (1-2 cm) before use;Olivera *et al.,(*2001).

-Corn cob shred: Obtained from Sugar and Starch plant, Mustrud , Egypt,washed, dried in an oven at 60-100°C to a constant weight. The dried substrate was ground, sieved ,before use.

-The sugar cane bagasse: The sugarcane dust was obtained from a market . The sugarcane dust was washed, dried at a temperature of 60 °C for 96 h, was grind and sieved before use.

lignocellulosic waste acid treatment:

Five grams of fine carbon sources; corn cob , *Opuntia* and bagasse were suspended in 50ml of 1%(v/v) HCL for I hr in a boiling water bath .The treated materials was washed until the wash was neutral; (Gupta *et al*.,(1972).

lignocellulosic waste a lkali treatmrnt:

suspending 25 g of different substrates in 500 of 0.5 % NaOH solution (0.1 g NaOH/g substrate)in 1 liter conical flask boiling for 1 h in a water bath ; Choudhury *et al.*(1980). The treated material was washed until the supernatant was alkali free as checked by pH meter

Fermentation medium:

0. 25 g of an accurately weighed treated lignocellulosic waste(corn cob , sugar cane bagasse and *Opunti*a peels) were placed in a 250 ml conical flask containing fifty mls of modified Czapek Dox medium (MCD medium) in order to obtain the biomass. MCD medium contained the following ingredients (g/100 ml): K_2HPO_4, 0.12; $MgSO_4$, 0.06; $FeSO_4$, 0.05; KCl, 0.02; $NaNO_3$, 0.3 and sucrose,3, Anupama and Ravindra (2001), adjusted to pH 4, autoclaved at 121°C for 15 min. Inoculated with 1 %(v/v)from 7 days old slant ,then incubated on a rotary shaker at 100 rpm and 30°C for seven days harvested after fermentation. |

Culture Harvesting and biomass concentration :

The cultures were harvested by filtering the biomass through a weighed Whatman no. 1 filter paper, the fungal biomass washed with distilled water to remove nitrate adhered to the mycelium .The filter papers along with the biomass were dried at 60°C for 48 h to constant weight,APHA (1989), left in desiccators(Cardoso,1981), ground in a mortar to a very fine powder and kept desiccated. The biomass means the mycelium together with unfermented substrate residue and then analyzed for their protein content .

Chemical analysis:

The amount of total sugar in the homogenized *Opuntia* peel samples was determined. For estimation **of total sugars**, one g of the substrate was suspended in 60 ml of distilled water. This was kept at an ambient temperature for12 h for the extraction of sugars that are measured .by the phenol-sulfuric acid method with glucose as a standard (Dupois *et al.*,1965). The moisture content of the sample was determined according to methods (A.O.A.C., 1980) and total protein by Lowry *et al.*, (1951

Extraction buffer of microbial protein:

0.25 g of fermented moldy biomass was soaked with 50 mL of **phosphate buffer**, pH(6.9) and stirred for 30 min. The extract was collected by filtration. The temperature during the course of extraction was maintained at 4°C. The supernatant obtained was used for estimating protein content. The extraction process was repeated five times, all the extractants were transferred to a flask, and the final volume was made up to 100 mL with distilled water; Olivera *et al.*,(2001).

Total protein : Protein determination was measured according to Lowry *et al.* (1951) method using folin-phenol reagent and bovine serum albumin standard .All Spectrophotometer reading was recorded at 660 after 20 minutes.

Determination of total reducing sugar:

Reducing sugars were determined according to Nelson (1944).

Culture conditions

Effect of carbon source

Different carbon sources were used; *Opuntia* waste ,bagasses and corn cob in concentration of 0.5% (w/v).

Substrate concentration

Varying concentration of alkali treated *Opuntia ficus indica* esubstrate 0. 5-1.5 % (w/v)were investigated.

Effect of initial pH: (MCD) medium was adjusted to different pH values before autoclaving.

Effect of agitation speed:
The fermentation medium were shaked at different speed ranging from 50 – 200 rpm at 30°C.

Effect of nitrogen supplementation:
0.3 % (w/v) ammonium sulphate ,peptone, yeast extract and CSL were supplemented instead of sodium nitate in the(MCD) broth.

CSL(corn steep liquor) obtained from Sugar and Starch company, Mustrud, Egypt .

Effect of aeration:
To study the effect of air /medium ratio on bioprotein production ,different volumes of cultivation medium in 250 ml Erlenmyer flask were used.

Calculations:
-%saccharification =Amount of reducing sugars(mg/ml)/Amount of substrate(mg/ml) x 0.9 x100; Dey *et al.*, (1992).

Protein recovery(g/l) = protein content(%) x biomass -(g/l).

Protein recovery (g/100g) = protein recovery (g/l) /g substrate used / L(.Ebrahim,1998)

Results and Discussion

Ligbocellulosic waste Treatment with 1% NaOH :
On using NaOH pretreated,corn cob, sugar cane bagasse and *Opuntia* wastes saccarification reached 75.6 % from *Opuntia* skin waste as a potential, low cost and high content of carbohydrate make it more suitable compared to other wastes ; 63.9 and 56.16 % for sugar cane bagasse and corn cob waste ,respectively . Corn cob is a very hard lignocellulosic waste of corn industry that must be deliginfy as it cannot be utilized efficiently by microbes without pretreatment for single cell protein *with P. chrysosporiu*m;Asad *et al.*,(2000) .

Table(1). % Saccarification of carbon sources after treatment with 1% Na OH .

Waste	Total reducing sugar concn(mg/ml)	Saccarification(%
Opuntia ficus indica	4.2	75.6
Sugar cane bagasse	3.55	63.9
Corn cob	3.12	56.16

Ligbocellulosic waste treatment with 1% HCL:
As it could be seen from Table 2. 64.8 % saccharification could be obtained with *Opuntia* skin with HCL treatment compared to 45.9 % and 44.1 % for sugar cane bagasse and corn cob wastes .Such low cost agro-cultural lignocellulosic wastes must be treated by physical/chemical methods to liberate cellulose from lignins. Since cellulose in lignin-hemicellulose-cellulose complex is not accessible to enzymatic hydrolysis ,Asad *et al.* (2000), from table (1,2) it is obvious that alkali treatment is more effective in preparing *Opuntia* skin for fermentation, so, this pretreatment will be applied in the next experiments. Many pre-treatment methods have been reported which vary from alkali or acid treatment, steam explosion or even x-ray radiation; Rodriguez-Vazquez *et al*.(1992).

Table(2). % Saccarification of carbon sources after treatment with 1% HCl.

Waste	Total reducing sugar concn(mg/ml)	Saccarification(%)
Opuntia ficus indica	3.60	64.8
Sugar cane bagasse	2.55	45.90
Corn cob	2.45	44.1

Evaluation of potential microorganism:
As could be indicated from table 3 ,the total protein % production by each strain differ from the other. As many fungal species are used as a protein-rich food. They provide the B-complex group of vitamins and thy also show a low level of nucleic acid content. Biomass obtained from *P. chrysosporium* has been found to contain most of the essential amino acids (Balagopalan and Padmaja, 2000). However, as stated by Jamal (2007);the fermentation time for maximum production of bioprotein was different for every microorganism, *A .niger* and *P. chrysosporium* showed highest concentration on sixth day.Also, As it could be seen from table 3, *P. chrysosporium* is of importance due to the highest bioprotein production 6.90 g/100g substrate together with the highest biomass 5.95g/l compared to the other microorganisms. . Other strains gives 3.374, 1.016 g protein /l00g *Opuntia* for *A.terreus* and *R.oryzae,* respectively. The protein from microorganisms is cheap and competitive with other protein sources. It may have good nutritive value depending, however upon their amino acid composition. It is necessary to use microorganisms which is non toxic to the animal. Most organisms used in direct single cell protein are fungi as *Aspergillus , Fusarium and Trichoderma*

Table (3). Screening on microbial bioprotein production using *Opuntia ficus indica peels waste.*

Microogan ism	Bio ma ss (g/l)	Total protei n(%)	Total protei n recov ery(g/ l)	recovery protein(g/ 100gsubst rate)
P. chrysospor ium	5.9 5	5.8	34.51	6.90
A. Terreus	4.8 2	3.5	16.87	3.374
R.oryzae	4.6 2	2.2	10.16 4	1.016

Initia1 substrate concentration is 5 g/ l broth.

Production of *P. chrysosporium* bioprotein using different carbon sources :

The proximate composition of *Opuntia w*aste used was as follows : 0.95% total protein, moisture 85%, and 55.25*%.* total carbohydrates of its weight.

The term single cell protein (SCP) refers to dead, dry cells of micro-organisms such as yeast, bacteria, fungi and algae which grow on different carbon sources. As it could be seen from table 4, *P.chrysosporium* protein recovery reached 7.28 g/l00g .Hence, *Opuntia* waste could be used as one of less expensive means of increasing the protein quality such as cassava and wheat flour ;Ghaly *et al.,* (2004) than sugar cane bagasse and corb cob which produce 2.570 and 2.351 g protein /l00 g substrate ,respectively . Other potential substrates for SCP include citrus wastes, sulphite waste liquor, sewage ,molasses, animal manure, whey, starch and wheat flour, Jamal *et al.,* (2008).. etc.

Table (4). *P.chrysosporium* bioprotein on using *different carbon sources* .

Waste	Biomass (g/l)	Total protein (%)	Protein recovery (g/l)	Protein recovery (g/100g substrate)
Opuntia cactus	5.6	6.5	36.4	7.28
Sugar cane bagasse	4.59	5. 6	25.704	2.570
Corn cob shred	4.61	5. 1	23.511	2.351

Initia1 substrate concentration is 5 g/ l broth.

Effect of carbon source concentration on *P.chrysosporium* bioprotein production*:*

The biomass values on using different concentrations of *Opuntia* skin is shown in table (5) . It could be stated that reduced substrate, result in greater protein yield , and the more oxygen required for the oxidation of the substrate. 1%(w/v) substrate concentration result in 8.557g bioprotein /100 g *Opuntia* waste so, it will be used in the next experiments . 2%(w/v) substrate result in 4.870g biprotein/100g waste, this may be due to exhaustion of nutrients required in fermentation medium other than the opuntia waste.

One of the main advantages of SCP compared to other types of protein is the small doubling time of cells. Due to this property, the productivity of protein form micro-organisms is greater than that of traditional proteins. For the assessment of the nutritional value of SCP, factors such as nutrient composition, amino acid profile, vitamin and nucleic acid content as well as palatability .

Table (5). Effect of varying concentration of *Opuntia ficus indica* skins on *P. chrysosporium* protein production.

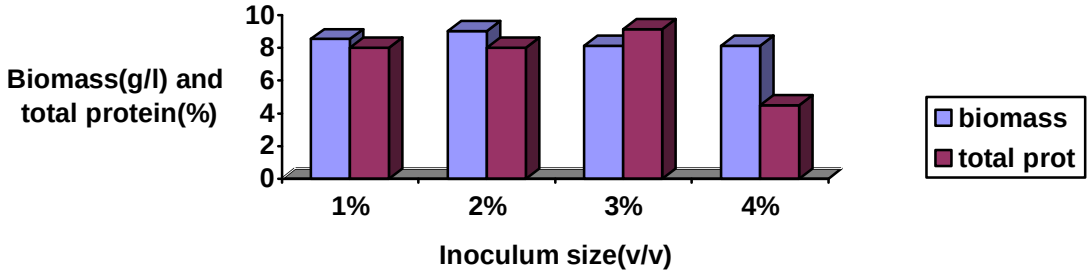
* (substrate concentration %(w/v)	Biomass (g/l)	Total protein(	Protein recovery (g/l).	Protein recovery(g/100g substrate).
0.5	6..6	5.9	38.94	7.792
1.0	8.15	10.5	85.575	8.557
1.5	14.4	5.6	80.64	5.376
2.0	19.1	5.1	97.41	4.870

Initia1 substrate concentration is 5,10,15,20 g/ l broth.

Effect of *P. chrysosporium* inoculum size on bioprotein production on using *Opuntia ficus indica based medium.*

As it could be seen from fig 1,the total protein content of the biomass was only 8.02 % (w/v) with 1*%(v/v) P. chrysosporium* inoculum when *Opuntia* based medium incubated for 7 days at 30°C on 100 rpm .The bioprotein was found to be increased to 9. 139% with elevation of the inoculum size to 3 (v/v)%.However, no increase in total protein was noticed with 4(v/v) %, as it gave 4.5g/100g substrate . So, 3% (v/v)will be used in the next experiments .

Fig(1).Eeffect of inoculum size on P.chrysosporium bioprotein produvction



Effect of nitrogen source on (CDM) medium for *P. chrysosporium* bio protein production:

As it could be seen from Table(6), CSL resulted in 9.639 g protein /100g *Opuntia cactus*. This was essential in supporting and enhancing the growth of *P. chrysosporium* as it gave 8.10 g biomass/l as well as to promote the production of bioprotein, this comes in agreement with Chahal *et al.* (1987) who produced maximum biomass protein (40%) from corn steep liquor as additional nitrogen source is required to support both microbial and biomass production. These results are also in accordance with those by (Nacib *et al*., 2001); that the production of lactic acid by using date juice as fermentation medium could be increased by supplementing date juice with nitrogen sources, and also Rosma and Cheong (2007) who stated that inorganic nitrogen source, are the main components in the growth of biomass and building block of proteins is due to the lack of nitrogen sources in pineapple juice. The effect of inorganic nitrogen source was reported by Chung and Muhammad (2000) to give the highest yield in cellulose production..T he importance of using carbon and nitrogen source were reported to be essential in providing a suitable growth media for *P. chrysosporium* which was used in the molasses wastewater (MWW) (Ahmadi *et al*., 2006), while there are also many reports on use of C and/or N sources for support of *P. chrysosporium* growth (Kirk *et al.*, 1978; Jansheker and Fiechter, 1983).Urea, peptone and ammonium sulphate when supplemented to the medium along with *Opuntia* peels waste gave higher protein yield compared to medium supplemented by sodium nitrate as they gave, 8.259, 6.595 and 6.099 g protein /100 *Opuntia* substrate, respectively .So ,CSL will be used in the next experiments. CSL is an expensive source of nutrient in the fermentation medium as the sole source of nitrogen ,vitamin ,growth stimulant and other nutritional requirement;Amarty and Jeffries(1994).

Table (6). Effect of nitrogen source for the production of *P. chrysosporium* bio protein *using Opuntia ficus indica* based medium.

Nitrogen source in (MCD)	Biomass(g/l)	Total protein(%)	Total protein recovery (g/l)	recovery protein (g/100g substrate)
CSL	8.10	11.9	96.39	9.639
Urea	7.12	11.6	82.59	8.259
Peptone	7.76	8.5	65.96	6.595
$(NH_4)_2SO_4$	7.82	7.8	60.99	6.099

Initia1 substrate concentration is 10 g/ l broth.

(CDM)medium Initial pH effect on *P. chrysosporium* bioprotein production on using *Opuntia ficus indic* based medium.:

On optimizing medium conditions for attaining higher production of bioprotein ,as it could be seen from Table(8) with pH 4 result in 9.256 g protein /100 g *Opuntia skins*;.Jamal *et al.*, (2009) stated that any increase in pH medium becomes inhibitory for the organism and induction of enzyme synthesis.

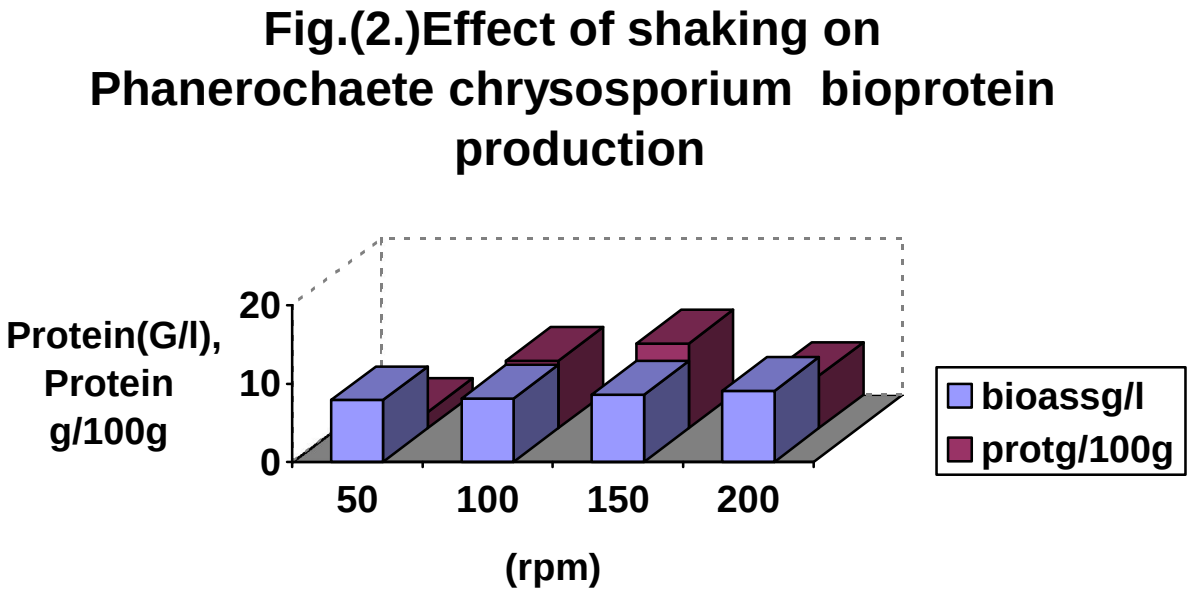
Table(7) . Effect of initial pH on bio protein production by *P. chrysosporium* grown on *Opuntia ficus indica* based medium .

Initial PH	Biomass (g/l	Total (%)protein	Protein recovery g/l	recovery protein(g/100g substrate)
2	7.4	3.3	24.42	2.442
4	8.12	11..4	92.568	9.256
6	7.12	1. 6	11.3 92	1.139
8	7.1	1 .1	7.832	0.783

Initia1 substrate concentration is 10 g/ l broth.

Effect of shaking on *P. chrysosporium* bioprotein production using *Opuntia ficus* indica based medium.

As could be seen from fig (2), with 200 rpm lower protein content 6.7 g/100g *Opuntia* waste on 7 th day of fermentation at 30°C with 1% (w/v)substrate concentration, this is in agreement with with Daugulis, (2004) who stated that , higher agitation rates results in over production of cellulose and reduced levels of microbial protein. With 150 rpm speed resulted in 10.85 g protein /100 g *Opuntia* waste while the biomass still growing to 9.11 g/l with 200 rpm.So,150 rpm will be used in the next experiment.



Effect of aeration on *P. chrysosporium* bioprotein production using *Opuntia ficus indica* based medium :

As it could be seen from Table(8),with 50 ml medium the biomass was 8.9 g/l while protein was10.546 g/100 g opuntia substrate. With 75 ml broth volume /250 ml flask capacity at pH 4, when inoculated with 3% spore suspension and incubated for 7 days at 30°C on 150 rpm , resulted in 11.97 *g P. chrysosporium* bioprotein /100 *g Opuntia cactus* waste, with 9.5 g biomass /l concentration is highest biomass concentration because it reach

maximum growth yet and were still in growth phase.The biomass should increase exponentially as the cell is growing and when only the cells enter the decline phase or death phase, biomass will decrease. With 125 ml culture volume , the reduction in protein content 3.213 g/100g opuntia waste because of the decreased aeration which may reduce the growth of the organism.

Table(8). Effect of aeration on *P. chrysosporium* bio protein production by using *Opuntia ficus indica* based medium .

culture volume(ml)	Biomass (g/)l	Total protein(%)	Total protein recovery (g/l)	recovery protein(g/100 g substrate)
50	8.9	11.85	105.465	10.546
75	9.5	12.6	119.70	11.97
100	8.5	4.12	35.02	3.502
125	7.8	4.12	32.136	3.213

Initia1 substrate concentration is 10 g/ l broth.

Finally, The extract obtained from nopal peel at natural pH of carbohydrate polymers from *Opuntia ficus-indica* and their physicochemical characterization indicated that they are polysaccharides .Their sugar composition indicates that all the polysaccharides obtained contain anionic moieties, galacturonic acid residues and are typical of pectin;Hatem *et al.,* (2001). It could be deduced the valuable reuse of this carbohydrate rich solid waste in minimization of costs associated with nutritional supplements in a fermentation medium is also essential for economic large-scale production, which somehow contribute to the pollution problem in the environment, either for the production of useful bioprotein or other beneficial metabolites .

Reference

Ahmadi ,M.; Vahabzadeh, F. ; Bonakdarpour B., Mehranian1, M. and Mofarrah , E.(2006).Phenolic removal in oliver mill waste water using loafah immobilized *Phanerochaete chrysosporium* . W.J. of microbiol and Biotechnol.22:119-127.

Amarty ,S, and Jeffries ,T.W.(1994).comparison od CSL wth other nutrients in the fermentation of d- xylos *by Pichia stipitis* CBS 6054.Biotechnol. Lett. 16(2). 211-214.

Anupama and Ravindra ,P. (2001).Studies on Production of Single Cell Protein by *Aspergillus niger* in Solid State Fermentation of Rice Bran. Arch. Biol. And Technol..
44(1): 79 – 88.

Anupama,and Ravindra ,P. (2000). Value-added food: Single cell protein. Biotechnology Advances, 18, 459-479.

A.O.A.C. (1980). Analysis of Association of Official Agricultureal Chemists 13th, ed. Washington, DC. USA.

APHA(1989). Standard Methods for the Examination of Water and Wastewater. 17th Edn American Public Health Association, Washington,
DC.

Asad M. J; M. Asghar; M. Yaqub ,and K.Shahzad. **(2000)**. Production of single cell protein from delignified corn cob by *Arachniotus* species.Pak.J.of Sci.;37(3-4).

Balagopalan, C., and Padmaja, G. (1988). Protein enrichment of cassava flour by solid state fermentation with *Thricoderma pseodokonigi Rifai* for cattle feed. Proc. 8th Symp. International Society for Tropical Root Crops. Bangkok, 426-432.

Chung, C.H., and Muhammad, I.I..(2007). Evaluation and Optimization of Microbial Cellulose (Nata) production using Pineapple Waste as Substrate. Dissertation. Universiti Teknologi Malaysia Institutional Repository, Malaysia.

Choudhury, N; Gray,P.P. ,and Dunn,N.W.(1980). Reducing sugar accumulation from alkali pretreated sugarcane bagasse using *Cellulomonas*.Eur. J. Appl. Microbiol.,Biotechnol., 11, 50-54.

Cardoso, M.B., and Nicoli, J.R. (1981). Single cell protein from termotolerant fungus *Phanerochaete chrysosporium* grown in vinasse. l. Production and composition. Nutrition Reports International., 24: 237-246, 1981.

Dey,D ;Hinge,J.; Shedye,A., and Rao,M.(1992). Purification o and properties of extracellular

endoxylanase from alkalalophilic thermophilic *Bascillus sp*. Can. J. Microbiol., 38,436-442.

Daugulis, Derek H. Bone (2004).Production of microbial protein from tree bark by *Phanerochaete chrysosporium*Volume 20(10**):** 1639 – 1649.

Dubois,M.;Giles,KA;Hamilton,J.K.;Rebors ,P.A.and Smith,F.(1956).Colorimeteric method for the determination of sugar and related substances.Anal.Chem.28:350-356.

Ibrahim,A.A.Shabaan(1998).Utilization of cane bagasse by product.MSc. Thesis.Fac.of Agric .Cairo Univ.

Gupta, J. K.; Das, N. B, and Supta, Y.P, (1972) .Effect of cultural conditions on eellulose formation by *Trichoderma viride*» Agr. Biol. Chem., Vol* 36, pp. 1961-1967.

Ghaly, A.E.; M. Kamal ,and L.R. Correia,(2004). Kinetic modeling of continuous submerged fermentation of cheese whey for single cell protein production. Bioresour. Technol., 96: 1143-1152.

Hatem, M. ; Sadok ,R; André ,D. (2001). Polysaccharides from prickly pear peel and nopals of *Opuntia ficus-indica:* extraction, characterization and polyelectrolyte behaviour. Polymer in 50,(5), : 552-560 .

Jansheker, H. ,and A. Fiechter, (1983). Lignin biosynthesis, Application and Biodegradation. In: Advances in Biochemical Engineering, Fiechter, A. (Ed.). Vol. 27, Springer Verlag, New York, ISBN: 0-387-12182-X..

Jamal, P.; Alam, M. Z.; Salleh, M. R. M., and Nadzir, M. M. (**2005**). Screening of microorganisms for citric acid production from palm oil mill effluent. Biotechnology, 4(4), 275-278.
.
Jamal, P.; Alam, M. Z., and Umi, N. (2006). Screening of potential strain to produce bioprotein from cheaper carbon source. Ist International Conferenceon Natural Resources Engineering & Technology, 24-26th Jul y, 226-232.

Jamal, P.; Alam M. Z.,and Salleh , N. U. (2008).Media optimization for bioprotein production from cheaper carbon source J. l of Engi. Sci.and Techno3, (2):124 – 130.

Jamal ,P.; Fahrurrazi T. ,M. and ,Zahangir A. ,M. (2009) Optimization of Media Composition for the Production of Bioprotein from Pineapple Skins by Liquid-State Bioconversion Journal of Applied Sciences,9 (17): 3104-3109.

Kirk, T. K.; Schultz, E.; Connors,-W. J.; Lorenz, L. F. ,and
Zeikus, J. G. (1978).Influence of Culture Parameters on Lignin Metabolism by *Phanerochaete chrysosporium*",.Arch. Microbiol. 117, 277-285.

Lowry, O.H. ;Rosebrough, N. J.; Farr, A.L. ,and Randall, R.J. (1951).Protein measurement with folin phenol reagent J. Biol. Chem., 193,112.

Lúcia de Fátima ,A.; Ariosvaldo Nunes, M.; Américo Perazzo N.; Líbia de Sousa C.O.; Flávio Luiz H. S.,(2005). Protein enrichment of cactus pear (*Opuntia ficus - indica Mill*) using *Saccharomyces cerevisiae* in solid-state fermentationBraz. arch. biol. technol. 48 .

Nancib, N.; Nancib, A.; Boudjelal, C.; Benslimane, F. Blanchard, and Boudrant, J. (2001). The effect of supplementation by different nitrogen sources on the production of lactic acid from date juice by *Lactobacillus casei subsp. Rhamnosus.* Bioresour. Technol., 78: 149-153.

Nelson, N. (1944). A photometric adaption of the Somogyi method for the determination of glucose. J. Biol. Chem.153:375-380.

Nigam, P. ,and Singh, D. (1994), Solid state(substrate) fermentation systems and their applications in biotechnology. J. Basic Microbiol., 34, 405-414.

Olsen, R. E.; Suontama, J.;Langmyhr, E.; Mundheim, H., Ringe, E.;Melle, W., Malde, M. K., and Hemre, G. L.(2006). The replacement of fish meal with Antarctic krill, *Euphausia superba* in diets for Atlantic salmon, Salmo salar. Aquacult. Nutr. 12: 280-290.

Oliveir M. A. ; Claudenice ,R.; Edson M. R. and Jorge N.(2001) Production of sungal protein by solid substrate fermentation of *Cactus cereus peruvianus* and *Opuntia ficus indica.* quím. Nova :24 (3) . **.**

Rosma A., and Cheong, M.W., (2007). Effects of nitrogen supplementation on yeast (*Candida utilis*) biomass production by using pineapple (*Ananas comosus*)waste extracted medium.Malysian J. of Microbiol.3(1): 19-26.

Rodriguez-Vazquez, R.; Villanuevaventure, G., and 317.

Riosleal**, E. (1992).** Sugar cane bagasse pith dry pretreatment for single cell protein production Biores. Tech., 39, 17-22),
.

Uysal, H.; Aydogan,M.N. and Algur,O.F. (2002). Effect of single cell protein as protein source in Drosophilla culture. Braz. J. Microbiol., 33: 314-

3/8/2010