AkiNik

Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

ISSN 2320-7078
JEZS 2013; 1 (5): 43-47

Received 20-08-2013
Accepted: 29-08-2013

Moufid Yassine
Associate professor, Dept of Food Biotechnology, School of Pharmacy, Tishreen University. Latakia, Syria

Izdihar Amar
Assistant professor, Dept of Marine biology at HIMR, Tishreen University

Rabee Raya
MS. Student in Marine biology at HIMR, Tishreen University

Correspondence:
Moufid Yassine
Associate professor, Dept of Food Biotechnology Pharmacy school, Tishreen University. Latakia, Syria.
E-mail: moufeedy@hotmail.com

Enrichment of the Protein Content of Biomass Derived from Coastal Remains of Marine Organisms to be used as Poultry Fodder

Moufid Yassine, Izdihar Amar, Rabee Raya

ABSTRACT

The research concentrates on utilization of remains of marine organisms accumulated on sea shore to be used as a culture medium for fermentation in purpose of obtaining biomass –rich in protein using strains of microorganisms Saccharomyces cerevisiea, Aspergillus niger and Geotrichum candidum comparatively protein content was evaluated before and after acidic hydrolysis fermentation optimum conditions of temperature and pH primary, concentration the remains and the duration of incubation were determined .Findings show that the protein was directly proportional to duration of incubation which would take days, in which protein content were (28.52, 25,13, 20.46%) of dry matter using *Aspergillus niger, Saccharomyces cerevisiea and Geotrichum candidum*, respectively, while protein content was 3.44% of dry matter of the initial sample, further more fodder mixture was applied to groups of poultry and protein source feed (soybean) in a commercial fodder was replaced with the resulted biomass and the daily gain Average was 18.70g and feed conversion was 2.05 for the studied chicks of ages ranged 3-16 days.

Keywords: Saccharomyces Cerevisiea, Aspergillus Niger, Geotrichum Candidum, Biomass, Fodder, Coastal Remains of Marine Organisms

1. Introduction

The single-cell protein is a potential source of protein for the shortage of food due to the rapid increasing of population, despite animal protein the best types of proteins for its essential amino acids of most types now it is competed by microbial protein known as single cell protein (scp) with higher nutritive value [1], microorganisms can grow on a poor nutritional media such as agricultural wastes and Agro-industrial wastes and other different methods of processing in order to provide protein biomass which enhances the nutritional supplements and achieves food stability , the most important features of protein biomass is wide spectrum usage materials and microorganism on one hand , different methodologies adopted for this purpose as well as the qualified capability to convert the substrate and high productivity, due to the rapid rate of microorganism growth and to independence of climate changes on other hand [2] microbial production of single cell protein makes microorganisms and derivatives partially or wholly as a source of food and they are currently recommended to be used as animal fodder, this unconventional protein derived from various residues is widely marketed [3] Microorganisms derived biomass is a relatively richer amino acids comparison to other conventional protein sources like wheat. Moreover it was found that fish feed by biomass produced from fungi and yeasts showed immure against microbial infections that may decimated fish forms [4] yeast *Saccharomyces cerevisiea* is considered the most vital source of single cell protein because of its easy harvesting, bigger cell size with lower content of nucleic acids and bioactive mixture of essential amino acids This would be a promising strategy in the third world and developing countries where are suffering from malnutrition [6] *Geotrichum candidum* is important biotechnologically due to its assimilation of carbon of several sources such as (galactose and glucose and fructose, mannose) further it has been found that *G. candidum* is active in producing of lipase through microbial fermentation processes [7] biotechnological application were exploited to produce much of vital compounds

Aspergillus niger, is utilized for many applications such as production of single-cell vitamins (B1,B2,B3) citric acid and extracellular enzymes besides , *Aspergillus niger* used as transformation host in the biotechnological industry *A. niger* based treatment of residue has led to increase in ash percentage absorbed material coefficient of compound digestion and raw protein content but it led to a decrease in raw fibers [8] Akintomide et al. 2012 conducted in vitro study to obtained single-cell protein from potato peels and to evaluate protein yield which (16.78%, 21.30%) with *S. cerevisiea A. niger* respectively [9] (Abalaka et al .,2011) obtained the highest content of protein from maize waste with percentage of (22.55% 20%.) using *Saccharomyces cerevisiea*, and *Candida tropicalis* respectively [10] .Also (Coman, et al. 2012) produced scp after treating paper waste with fungus *G. candidum* incubated for 10 days at pH 5.0 [11] similarly in other trail protein content became higher in waste of fruits treated with *A.oryzae* and Rhizopus oligosporus using (NH4) 2 HPO4 as a source for nitrogen [12] fermentation resulted biomass using *A. niger* was utilized as poultry fodder they found that this biomass enhanced digestibility and assimilation of nutrients hence poultry gained weight there after further more positive influence of fermentation products in the gastrointestinal health improvement by lowering the pH of gastric chicken and suppresses the activity of microbial pathogens and to improve the structure of the digestive tract mucosa [13] Abalaka and Daniyan,(2010) tested the quality of fermented food and their influence on weight gain of poultry they demonstrated a direct correlation between protein content of fodder and weight gain the higher protein content the fodder had the higher weight could be gained [14] utilization of zoo and phytoorganisms to produce fodder or as raw materials in various industries is based on their local availability biochemical structural nutritional value and energy as well as ratio of organic mass to dry weight which range in microalga from 30 - 80% as fiber and structural components In some Asian countries, marine organism were collected from the shores after a stormy day those organism were washed, and dried to be used as animal fodder [15].Approximately 7000 marine nautical product were isolated from marine organisms 25% of which were from marine alga 33% from sponges 24% from marine invertebrates [16].

In this research we focus on treatment of some of the accumulated remains marine organism on Latakia seashore using microorganisms grow these remains subjected to processing to be formulated as culture medium. This research was carried out in the labs of Higher Institute of Marine Research and the College of Pharmacy at of Tishreen University

2. Material & Methods

2.1. Chemicals and reagents:

Hexane HCL, sulfuric acid Boric acid, Methylene blue, phenol red, anhydrous sulfate sodium, distilled water, Czapek Dox's aga, potato dextrose agar PDA, Agar Nutrient

2.2 Biomaterials

Remains of marine organisms thrown on Latakia sea shore where collected from to sites of sports complex and meridian hotel these remains include marine algae and zoo benthos such as lobsters sponge and mollusks these samples were grinded and their content of protein, carbohydrates and fat were determined, on other hands the samples were hydrolyzed in acid to be utilized as a culture medium for fermentation in purpose for obtaining biomass rich in protein using strains of microorganism isolated from their natural sources Figs (1)

Fig 1: Reveals sampling site along Syrian coast

2.3 Microorganisms

Strains of microorganism were isolated from their natural sources and they were *A. niger* from molded onions G. *candidum* from fermented yogurt and previously prepared cultures of *Saccharomyces cerevisiea* suspension of isolated strains were obtained from prepared cultured, media inoculated with those strain and incubated for 5 days at temperature of 28 °C. 1m of the obtained suspension was taken and number of spores was determined using (NEUBAUER chamber), suspension was add to prepared culture medium at (10^{6} cell/ ml) figs 2 ,3, 4 illustrated the studied culture .

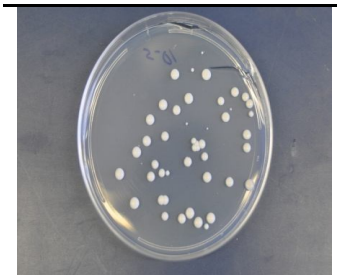

Fig 2: *Saccharomyces cerevisiea* on Agar Nutrient

Fig 3: *Geotrichum candidum* on PDA

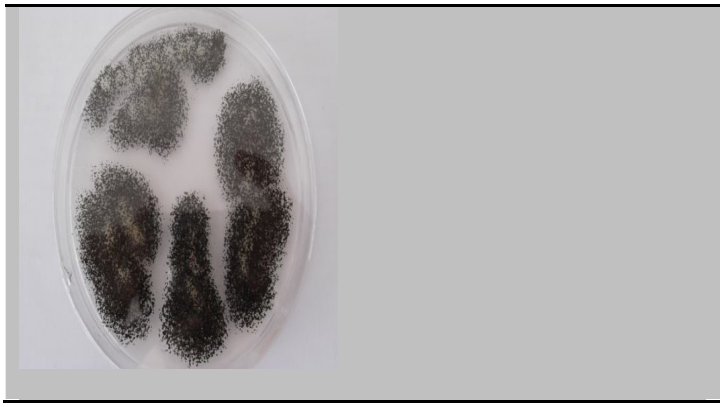

Fig 4: *Aspergillus niger* on Czapek Dox's agar

2.4 Instruments and Equipment

Kjeldahl apparatus to determine protein (Buchi Digest system K-437), Autoclave (OT4oL Nuve steamArT) , Shaker incubator (InfoRs), incubator for fungi (Napco), isolation chamber for fungus

(DLabTEh),microscope(motic) , moisture determination operates (Precisa) Analytical balance ((Precisa - XB22oA) desiccation oven (JANAT instRuEMents), bath water(K.F.T LaB.EouiPMENT), Neubauer counting chamber fridge, Erlenmeyers, beakers, test-tubes, volumetric funnels, filter paper, aluminum foil, petri dish, bases bursar, thermometer, pipette, porcelain evaporating dish, separator funnel, mortar cotton, wire loop.

2.5 Fermentation medium

70 gram of coastal wastes (remains) digested in sulfuric acid 2% were added to 1000 ml of distilled water containing 2 gram of sodium nitrate, 1 g of potassium mono phosphate, 0.5 gram of hydrous magnesium sulfate, 0.5 gram of potassium chloride and 0.01 gram of hydrous ferrous sulfate.

The mixture was stirred vigorously and distributed to 250 ml Erlenmeyers each of them contained fermentation medium previously autoclaved at 121 c for 1s minutes then left to cool and inoculated with starter 10^{6} to be placed in a shaker in cubature at a speed of 125 rpm for 15 days. The initial PH was adjusted to 6.5.

2.6 Determination of Protein: content in biomass protein content was determined using method (Akintomide *et al*., 2012)

2.7 Utilization of new mixed fodder for poultry feeding protein source in commercial fodder was replaced with soybean at a parentage of 100% of resulted biomass. Daily gain average and factor of feed conversion were determined. The experiment was applied to chicks of (Hubbard classic) at ages ranged from 3-16 days.

3. Results and discussion

3.1 Biochemical analyses of coastal remains (wastes): moisture content of the studied samples was determined and it reached 20.1%. Findings of chemical analyses of the studied samples were as follows: saccharides 42.37% protein 3.44 fat 0.3891.

3.2 Fermentation and Culturing:

Culturing was done on two stages; the first was without chemical processing of coastal remains (without hydrolysis) the second stage was conducted after acidic hydrolysis of remains using sulfuric acid at a concentration of 2%. The fermentation media were autoclaved and then inoculated with spores. Finally they were incubated for 15 days respecting conditions mentioned previously.

3.2.1 Evaluation of the resulted protein:

Using Kjeldahl method the percentages of protein were evaluated at different in trials of 3, 7, 15 days of incubation. Comparison of protein content was done between media with acidic hydrolyzed wastes and those without acidic hydrolyzed wastes as explained in table 1 and figure (5)

Table (1) the influence of time intervals on producing protein using microorganism before and after acidic hydrolyzed (g /100g)

Protein content after 15 days		Protein content after 7 days		Protein content after 3 days		Protein content at the beginning of experiment	Culture
After acidic hydrolyzed	Before acidic hydrolyzed	After acidic hydrolyzed	Before acidic hydrolyzed	After acidic hydrolyzed	Before acidic hydrolyzed		
20.46	10.53	12.80	6.22	7.15	3.55	3.44	*G. candidum*
25.13	10.70	14.58	7.42	6.43	4.81	3.44	*S. cerevisiae*
28.52	14.38	16.12	9.79	7.55	5.34	3.44	*A. niger*

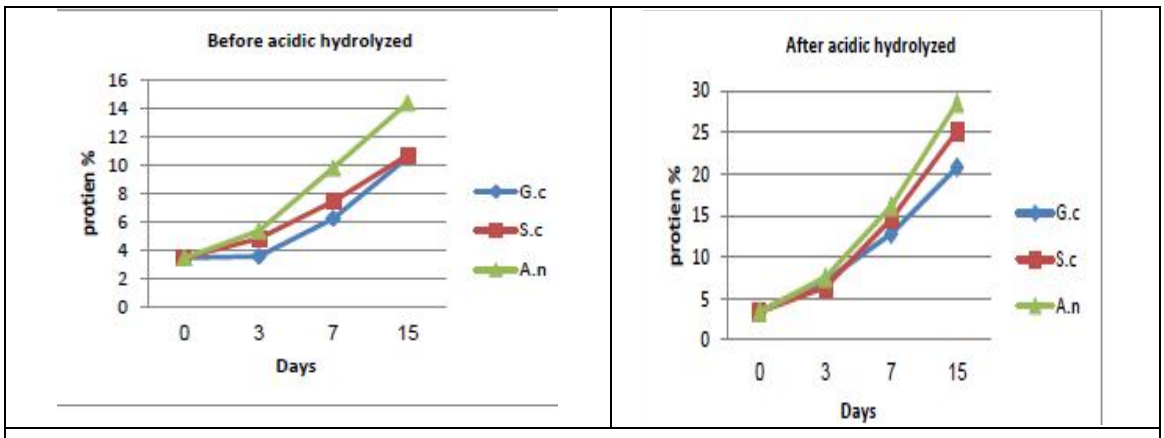

Fig 5: The Influence of time Intervals on Producing Protein using microorganism before and after acidic hydrolyzed (g /100g)

It's obvious from table 1 and figure (5) that protein content in increased slowly with time fungus and yeasts unable to meet their needs from remains. this was due to complexes of saccharide which are only found in marine organism but not in terrestrial organism these compounds cannot be utilized by yeast and fungus for growth process ,the highest yield of protein was recorded before hydrolyzed when treated with fungus *A. niger* and it reached 14.38 g/ 100g of dry weight the activity of yeast *S. cerevisiea* and fungus *G.* candidum was less than as, shown in fig 5 after acidic hydrolyzed protein percentages were noticeably higher than recorded before hydrolyzed on the other hand it was observed that primary treatment through acidic hydrolyzed of remains using sulfuric acid 2 % increased the fermented metabolized saccharide of remains consumed by fungus and yeasts as a source of carbon necessary to growth hence biomass is formed besides obvious increases in protein content was occurred in association with hydrolysis compared to without hydrolysis it was demonstrated that *A. niger* the most active for proportional increases in producing protein over the experiment period .the maximum content was recorded at value of 28.52 g/ 100g after 15 days *S. cerevisiea* appeared to be second most efficient with content of 25.13 g/ 100g followed by fungus *G.* candidum with least content of 20.46 g/ 100g

3.3 Feeding a Group of Poultries with Modified Mixture of Fodder

Protein source in commercial fodder was completely replaced with the mixture resulted of processing of fermented costal remains of acidic hydrolysis and culturing the studied microorganism then this mixture was applied to a group poultries of (**Hubbard Classic**) species 2 chicks of each experiment were raised at ages range from 3-16 days at typical farm condition of temperature pasteurized water suitable moisture. In addition a group of factors were determent as follows

Weight gain per chick. (g) = weight in the end of experiment – initial weight

Feed conversion = quantity of taken dried fodder / gain in chick weight daily gain Average = final weight - initial weight / experiment duration

Table 2: Explains components of commercial fodder in 1 kg of pellet granular fabricated fodder

Material	Weight /g	Material	Weight/g
Maize	534	Chechen vitamin	1
Soymeal	420	Mineral salts	1
Soy oil	5	Methionin	1.8
Calcium diphosphate	20	Cholin	1.2
Calcium carbonates	10.6	Licin	0.8
Salt	3	Antifungal	1
		Anticoccidia	0.6

Table 3: Impacts of modified fodder mixture and commercial fodder on chicks

Coefficients	Grop1 fed on modified fodder mixture	Grop 2 fed on commercial fodder
Weight gain (g)	243.14	326.53
daily gain Average	18,70	25.11
Feed conversion Average	2,05	1,76

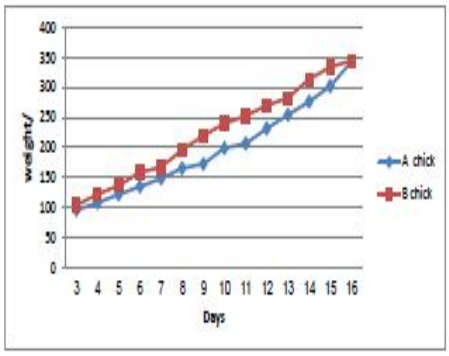

Fig 6: daily weight gain g/day of two chicks fed on experimental modified fodder mixture

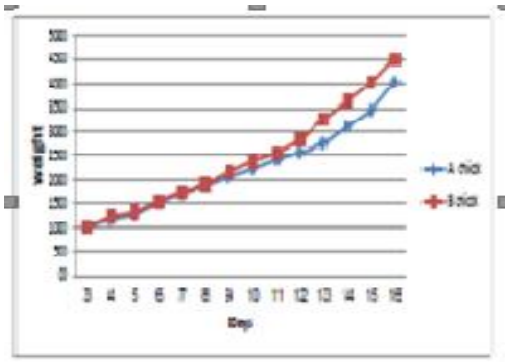

Fig 7: daily weight gain g/day of two chicks fed on commercial fodder

5. Conclusion

Highest percentage of protein was recorder when costal remains of marine processed with *A. niger* after 15 days reaching 28.52 g/ 100g of dry matter comparing to protein content of 3.44% of dry matter in initial material.

Further in fermentation medium acidic hydrolysis occurred portion content became higher compared to its content porter to acidic hydrolysis despite Weight gain was good.

It didn't influence the Weight gain of chicks when protein of commercial fodder was replaced with fermentation resulted biomass Remains of marine organism accumulated on coast are riches go in vain which motivate us to utilized them due to the high value of these essential material for fermentation medium they are very efficient to from biomass with high content of protein as well as to produce compound and vital enhancer at the same time utilization these remains extends from the production of biomass to disposal of these pollutants and preservation of environment and recreational areas

6. Acknowledgments
Deepest gratefulness goes to Professor Dr. Moufid Yassine for his advice and constant support.

7. Reference:

1. Adedayo, M.R., Ajiboye, E.A., Akintunde, J.K. Odaibo. A,(2011) Single Cell Proteins: As Nutritional Enhancer, Advances in Applied Science Research, , 2 (5):396-409
2. Nasseri A.T. Rasoul-Amini.S, Morowvat M.H. and Ghasemi M.H, (2011). Single Cell Protein: Production and Process. *American Journal of Food Technology, 6: 103-116*
3. Lafi .A .S .A, Al-Rawii . D. F , Khalil.S.S , Al-Assaffii .A.A (2008) Produce the single cell protein (SCP) by the liquid cultures methods from *Schanginia aegyptiaca* powder by the use *Aspergillus niger* and testing Biological , Folder Anbar University of Science Pure Vol 2, Issue 2, ISSN: 1991-8941
4. Ugalde. O, Castrillo .J (2005). Single Cell Proteins from Fungi and Yeast, Applied Mycology and Biotechnology. Agriculture and Food Production ,Vol.2,Elsever, London,pp.123-150
5. Callihan, C.D. and C.E. Dunlap,(2000) Construction of a Chemical-Microbial Pilot Plant for Production of Single-Cell Protein from Cellulosic Wastes, Report SW-24c, U.S. Environmental Protection Agency, Cincinnati, Ohio, USA,
6. Bacha .U, Nasir. M, Khalique .A, Anjum .A.A, and. Jabbar. M. A (2011) , comparative assessment of various agro-industrial wastes for *saccharomyces cerevisiae* biomass production and its quality evaluation as single cell protein , The Journal of Animal & Plant Sciences, 21(4) Page: 844-849
7. Boutrou R.,and Gueguen, T.M.(2005) Interests in Geotrichum candidum for cheese technology , *International Journal* of food Microbiology 102,120
8. Frisvad.J.C, Smedsgaard.C, Samson .R.A , Larsen.T.O And Thrane.U (2007) Fumonisin B2 Production by *Aspergillus niger*, *J. Agric. Food Chem.*, *55,* 9727–9732
9. Akintomide .M.J , Antai .S .P(2012) inorganic nitrogen supplementation and micro-fungal fermentation of white yam peels (flour) into single cell protein Journal of Microbiology, Biotechnology and Food Sciences *2 (3) 820-832*
10. Abalaka, M.E., Adeyemo, S.O., And Daniyan, S.Y.(2011) Production of Protein Rich Animal feed from Spent Sorghum (a waste material)by Solid State Fermentation using Strains of *Candida tropicalis* and *Saccharomyces cerevisiae* , Fermentation Technology and Bioengineering 1 71-76
11. Coman.G, Leustean.I, Georgescu.L,Bahrim.G (2012) Optimization of protein production by Geotrichum candidum miug 2.15 by cultivation on paper residues,using response surface methodology ,Fungal protein production , BioResources 7(4), 5290-5303
12. Yousufi.M.K (2012) to determine protein content of single cell protein produced by using various combinations of fruit wastes and two standard food fungi , International Journal of Advanced Biotechnology and Research, Vol 3, Issue 1, pp 533-536
13. Mathivanan,R., Selvaraj,P and Nanjappan,K.(2006). Fedding of Fermented Soybean Meal on Broiler Performance. International Journal of Poultry Science, 5 (9): 868 -872
14. Abalaka M.E., and Daniyan.S.Y (2010) Assessment of the Performance of Chicks Fed with Cereal Wastes Enriched with Single Cell Protein - *Candida tropicalis*, AU J.T. 13(4): 261-264 Apr
15. Torbatinejad N, Sherlock. R (2008), Comparison of feeding value of a treated sea plant,*Posidonia australis,* with lucerne, pasture and wheat *International Journal of Plant Production 2: 47-56*.
16. Kijjoa.A and Sawangwong.P, (2004) Drugs and cosmetics from the sea, Mar Drugs, 2, 73-82.