ABOUT IJBMBR

The International Journal for Biotechnology and Molecular Biology Research (IJBMBR) (ISSN 2141-2154) is published Monthly (one volume per year) by Academic Journals.

International Journal for Biotechnology and Molecular Biology Research (IJBMBR) provides rapid publication (monthly) of articles in all areas of the subject such as Green energy from chemicals and bio-wastes, Studies in the graft copolymerization of acrylonitrile onto cassava starch by ceric ion induced initiation, Antimutagenic activity of aqueous extract of Momordica charantia, Ethnomedicinal plants and other natural products with anti-HIV active compounds and their putative modes of action etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in IJBMBR are peer-reviewed.

Contact Us

Editorial Office: ijbmbr@academicjournals.org
Help Desk: helpdesk@academicjournals.org
Website: http://academicjournals.org/IJBMBR
Submit manuscript online http://ms.academicjournals.me/
## Editors

**Prof Atagana, Harrison**  
*Institute for Science and Technology Education*  
*University of South Africa*

**Prof. UC Banerjee**  
*Department of Pharmaceutical Technology*  
*(Biotechnology)*  
*National Institute of Pharmaceutical Education and Research*  
*Punjab, INDIA*

**Dr. Y. Omid**  
*Faculty of Pharmacy,*  
*Research Center for Pharmaceutical Nanotechnology,*  
*School of Advanced Biomedical Sciences,*  
*Tabriz University of Medical Sciences,*  
*Tabriz, Iran.*

**Prof. Mohamed E. Wagih**  
*University of New Brunswick (UNB-SJ),*  
*Saint John College, NB,*  
*E2L 4L5, Canada*

**Dr. Sripada M. Udupa**  
*ICARDA-INRA Cooperative Research Project,*  
*International Center for Agricultural Research in the*  
*Dry Areas (ICARDA), B.P. 6299,*  
*Rabat Instituts, Rabat,*  
*Morocco*

**Dr. Amjad Masood Husaini**  
*Sher-e-Kashmir University of Agricultural Sciences & Technology*  
*Bholochipora, Dr. Ali Jan Road,*  
*Nowshera, Srinagar, J&K-190011, India*

**Dr. Om Prakash Gupta**  
*Directorate of Wheat Research (ICAR)*  
*Post Box-158, Agrasain Marg, Karnal-132001,*  
*Haryana, India*

## Editorial Board

**Dr. Amro Hanora**  
*Suez Canal University, Department of Microbiology*  
*and Immunology,*  
*Faculty of Pharmacy, Suez Canal University,*  
*Box 41522 Ismailia, Egypt*

**Dr. C. Rajasekaran**  
*VIT University*  
*School of Bio-Sciences & Technology (SBST)*

**Dr. Yasar Karadag**  
*Gaziosmanpasa University*  
*Faculty of Agriculture,*  
*Department of Field Crops, Tokat-Turkey*

**Dr. Ahmet Tutus**  
*KSU (Kahramanmaraş Sutcu Imam University)*  
*Faculty of Forestry,*  
*Department of Forest Industrial Engineering,*  
*Kahramanmaraş 46100 Turkey*

**Dr. Vinod Joshi**  
*Desert Medicine Research Centre,*  
*Indian Council of Medical Research*  
*New Pali Road, Jodhpur, India*

**Dr. Eshrat Gharaei Fathabad**  
*K.M.18 Khazarabad road,*  
*Sari, Mazandaran, Iran*

**Dr. Shashideep Singhal**  
*121 Dekalb Ave, Brooklyn,*  
*NY 11201, New York, USA*

**Dr. Masayoshi Yamaguchi**  
*101 Woodruff Circle, 1305 WMRB,*  
*Atlanta, Georgia 30322-0001, USA*

**Dr. Okonko Iheanyi Omezuruike**  
*Department of Virology,*  
*Faculty of Basic Medical Sciences,*  
*College of Medicine,*  
*University College Hospital,*  
*Ibadan, Nigeria*

**Dr. S. M. Shahid**  
*University of Karachi,*  
*Karachi-75270, Pakistan*
Dr. Chethan Kumar M  
*Post Graduate Departments of Bio-technology and Biochemistry, Ooty Road, Mysore - 570 025, Karnataka, India*

Dr. M. Sattari  
*Rice Research Ins. of Iran Iran*

Dr. Zaved Ahmed Khan  
*VIT University India*

Dr. Subbiah Poopathi  
*Vector Control Research Centre, Indian Council of Medical Research (Ministry of Health & Family Welfare, Govt. of India) Medical Complex, Indira Nagar India*

Dr. Reyazul Rouf Mir  
*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru - 502 324, Greater Hyderabad, India*

Dr. Prasanna Kumar S  
*Virginia Commonwealth University, USA*

Dr. Naseem Ahmad  
*Plant Biotechnology Laboratory Department of Botany Aligarh Muslim University Aligarh- 202 002, (UP) India*

Dr. Zhen-Xing Tang  
*Food Bioengineering institute, Hangzhou Wahaha Co. Ltd, Hangzhou, Zhejiang, China*

Dr. Jayanthi Abraham  
*VIT (Vellore Institute of Technology) University, Tamilnadu, India*

Dr. Gobianand Kuppannan  
*National Institute of Animal Science South Korea*

Dr. R. Harikrishnan  
*Jeju National University South Korea*

Dr. Asit Ranjan Ghosh  
*Vellore Institute of Technology (VIT) University, School of Bio Sciences & Technology, Medical Biotechnology Division, Vellore-632014, India*

Dr. Kamal Dev  
*Shoolini University of Biotechnology and Management Sciences (SUBMS) India*

Dr. Wichian Sittiprapaporn  
*Mahasarakham University Thailand*

Dr. Vijai Kumar Gupta  
*Molecular Glycobiochemistry Group, Department of Biochemistry, School of Natural Sciences, National University of Ireland, Galway, Ireland*

Dr. Jeffy George  
*Department of Microbiology and Immunology F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences 4301 Jones Bridge Road, Bethesda, MD 20814 USA.*

Dr. Gyanendra Singh  
*Stanley S. Scott Cancer Center, School of Medicine, Louisiana State University Health Sciences Center New Orleans, LA 70112, USA.*

Dr. Anupreet Kour  
*1620 Chevy Chase Dr. Champaign, IL 61821 USA.*
Dr. Arun Sharma
Institute for Plant Genomics and Biotechnology (IPGB)
Borlaug Center,
TAMU 2123
Texas A&M University
College Station, TX 77843
USA.

Dr. Mohsen Asker
Microbial Biotechnology Dept.
National Research Centre
Cairo,
Egypt.

Dr. Elijah Miinda Ateka
Department of Horticulture,
Jomo Kenyatta University of Agriculture and Technology (JKUAT)
Kenya.

Dr. Jozélio Freire De Carvalho
Faculdade de Medicina Da USP, Reumatologia
Av. Dr. Arnaldo, 455 - 3º andar – Sala 3133.
São Paulo - SP
Brazil

Dr. Premendra Dhar Dwivedi
Food Toxicology Division
Industrial Institute of Toxicology Research,
Post Box No: 80, Mahatma Gandhi Marg,
Lucknow 226001,
India

Dr. Muhammad Abd El-Moez El-Saadani
Universities and Research Center District,
New Borg El-Arab,
P.O.Box: 21934 Alexandria,
Egypt.

Dr. Donald J. Ferguson
Advanced Orthodontic Training Program,
Nicolas & Asp University College
Dubai,
UAE

Dr. Kalyan Goswami
Department of Biochemistry & JB Tropical Disease Research Centre,
Mahatma Gandhi Institute of Medical Sciences,
Sevagram, Wardha-442102

Dr. A.K. Handa
National Research Centre for Agroforestry,
Gwalior Road, JHANSI-284003 UP
India.

Dr. Amjad M.Husaini
Metabolic Engineering & Biotechnology Laboratory
Division of Plant Breeding & Genetics
Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir
J&K-191121,
India

Dr. Vinod Joshi
Laboratory of Virology & Molecular Biology,
Desert Medicine Research Centre,
Pali Road, Jodhpur-342 005,
India

Dr. T. Kalaivani
D/O S. Thiagarajan
B-43, Rajaram Nagar,
Salem - 636 007,
Tamil Nadu, India

Dr. Priya Kalia
Orthopaedic Research Unit,
Department of Surgery,
Cambridge University, Cambridge,
UK

Dr. Patricia Khashayar
Tehran University of Medical Sciences
Endocrinology and Metabolism Research Center
Shariati Hospital

Dr. Zaringhalam Moghadam
Shahid Beheshti Medical University (M.C)
Tehran,
Iran

Dr. Okeke Ikechukwu Linus
Department of Surgery, University of Ibadan
Nigeria.

Dr. Rajesh Kumar Patel
Centre for Analysis and Learning in Livestock and Food (CALF)
National Dairy Development Board (NDDB)
Anand- 388 001 (Gujarat)
INDIA
Dr. Pooja Ralli-Jain  
Department of Pathology and Laboratory Medicine  
University of California Irvine, Irvine, California, U.S.A.

Dr. Meltem Sesli  
College of Tobacco Expertise, Turkish Republic, Celal Bayar University 45210, Akhisar, Manisa, Turkey

Dr. Reda H. Sammour  
Tanta University, Faculty of Science, Tanta, Egypt

Dr. Seyed Soheil Saeedi Saravi  
Mazandaran University of Medical sciences, Sari, Iran

Dr. R. Senthil Kumar  
St. Matthew’s University, School of Medicine  
Grand Cayman  
Cayman Islands

Dr. Mohammad Reza Shakibaie  
Kerman University of Medical Sciences, Kerman, Iran

Dr. Srividya Shivakumar  
Dept of Microbiology, CPGS, Jain university, Bangalore

Dr. Shashideep Singhal  
The Brooklyn Hospital Center  
NewYork-Presbyterian Healthcare System  
Brooklyn, NY.

Dr. Sripada M. Udupa  
International Center for Agricultural Research in the Dry Areas (ICARDA), B.P. 6299, Rabat Instituts, Rabat, Morocco.

Dr. Wei Wu  
Institute for Biocomplexity and Informatics  
Department of Bio Science  
The University of Calgary  
Canada

Dr. Xiao-Bing Zhang  
Molecular Regeneration Laboratory, MC1528B  
11234 Anderson Street  
Loma Linda, CA  92350

Prof. Dr. Ozfer Yesilada  
Inonu University  
Faculty of Arts and Sciences  
Department of Biology  
44280 Malatya  
Turkey

Dr. Edson Boasquevisque  
Universidade do Estado do Rio de Janeiro- UERJ  
Av 28 de setembro, 87, fundos (LMMC-IBRAG). Vila Isabel, city: Rio de Janeiro/ RJ  
Brasil

Dr. Abhilash M.  
The Oxford College of Engineering  
Hosur Road, Bangalore - 560068

Dr. Nasar Uddin Ahmed  
Department of Genetics and Plant Breeding  
Patuakhali Science and Technology University  
Dumki, Patuakhali-8602  
Bangladesh

Dr. Mervat Morsy EL- Gendy  
Chemistry of Natural and Microbial Products Department,  
National Research Center, Dokki, Cairo, Egypt

Dr. Gjomrakch Aliev  
Health Science and Healthcare Administration Program,  
University of Atlanta, Atlanta, Georgia, USA

Dr. Muhammad Asgher  
Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan

Dr. Anand Bharatkumar  
Parul Institute of Pharmacy, Limda, Waghodia, Vadodara
Dr. Chinmoy Kumar Bose,
Netaji Subhash Chandra Bose Cancer Research Institute
16A, Park Lane, Park Street, Kolkata 700 016, India.

Dr. Mousumi Debnath
Jaipur Engineering College and Research Centre (JECRC) Department of Biotechnology,
Shri Ram ki Nangal, Via Vatika, Tonk Road, Jaipur-303905, India

Dr. Dolan C. Saha
Dept. of Biochemistry and Molecular Biology, Faculty of Medicine, University of Calgary, Canada

Dr. Ramasamy Harikrishnan
Department of Aquatic Biomedical Sciences
School of Marine Biomedical Science
College of Ocean Sciences
Jeju National University
Jeju city, Jeju 690 756, South Korea

Dr. Abdul Haque
Health
Biotechnology division, nibge, Faisalabad, Pakistan

Dr. Kuvalekar Aniket Arun
Interactive Research School for Health Affairs (IRHSA), Bharati Vidyapeeth University, Pune, Maharashtra, India

Dr. Asit Ranjan Ghosh
School of Bio Science & Technology, Division of Medical Biotechnology, Vellore Institute of Technology (VIT) University, Vellore-632014, India

Dr. Prasanna Kumar Santhekadur
Department of Human and Molecular Genetics, Virginia Commonwealth University Richmond, VA

Dr. Majid Sattari
Rice Research Institute of Iran
Iran

Dr. Mihael Cristin Ichim
National Institute Research and Development for Biological Sciences / “Stejarul” Research Centre for Biological Sciences
Alexandru cel Bun St., 6, Piatra Neamt, 610004, Romania

Dr. Sailas Benjamin
Enzyme Technology Laboratory
Biotechnology Division
Department of Botany
University of Calicut
Kerala - 673 635 India

Dr. Sreeramanan Subramaniam
School of Biological Sciences, Universiti Sains Malaysia (USM), Minden Heights, 11800, Penang, Malaysia

Dr. Vijai Kumar Gupta,
Department of Biochemistry, NUI, Galway, Ireland

Dr. Vitor Engrácia Valenti
Universidade Federal de São Paulo
Rua Napoleão de Barros, 715, Térreso São Paulo, SP Brazil.

Dr. Ravindra Pogaku
School of Engineering and IT
Universiti Malaysia Sabah
88999 Kota Kinabalu
Sabah, Malaysia

Dr. Ahmed Eid Abdel-Hamid Eweis Fazary
School of Pharmacy, College of Medicine, National Taiwan University, Taipei 100, Taiwan.

Dr. Mohammad Hashemi
Dept. of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
Dr. Hesham, Abd El-Latif  
Genetics Department,  
Assiut University, Assiut 71516,  
Egypt.

Prof. Jia-ying Xin  
College of Food Engineering  
Harbin University of Commerce  
138 Tongda Road  
Daoli District  
Harbin 150076, Heilongjiang  
P.R.China

Dr. Kabir Mohammad Humayun  
Plant Molecular Biotech Lab  
Department of Medical Biotechnology  
College of Biomedical Science  
Kangwon National University  
Kangwon-do, Chuncheon, 200-701  
South Korea

Dr. Kalpesh Gaur  
Geetanjali College of Pharmaceutical Studies Manwa Khera,  
Udaipur- 313002. Rajasthan,  
India

Dr. Meganathan, Kannan  
Center for Biologics Evaluation and Research (CBER),  
U.S. Food and Drug Administration (FDA),  
Bldg. NIH 29A, Room 2C-10,  
8800 Rockville Pike,  
Bethesda, MD 20892.  
USA.

Assist. Prof. Ali Karadeniz  
Department of Physiology,  
Faculty of Veterinary Medicine,  
University of Ataturk 25240 ERZURUM  
Turkey

Dr. Matthew Kostek  
Department of Kinesiology  
University of Connecticut  
Storrs CT

Dr. Tansu Kucuk  
Gulhane School of Medicine  
Department of Obstetrics and Gynecology  
Etilk 06018 Ankara,  
Turkey

Dr. Kuo-Sheng Hung  
Department of Neurosurgery  
Taipei Medical University - Wan Fang Medical Center  
111 Section 3, Hsing-Long Rd,  
Taipei 116,  
Taiwan

Dr. V. Manju  
Department of Biochemistry,  
Periyar University,  
Salem -11.

Dr. Mbagwu Ferdinand Nkem  
Department of Plant science and Biotechnology,  
Faculty of Science,  
Imo State University  
Nigeria.

Dr. Anand Pithadia  
Parul Institute of Pharmacy  
Vadodara, Gujarat,  
India

Dr. Radhakrishnan Ramaraj  
Department of Internal Medicine  
University of Arizona  
Tucson 85724  
AZ

Dr. M. Rasool  
School of Bio Sciences and Technology,  
VIT University,  
Vellore-632104, Tamil Nadu,  
India

Dr. Reda A.I. Abou-Shanab  
Genetic Engineering & Biotechnology Research Institute (GEBRI)  
Mubarak City for Scientific Research and Technology Applications  
New Burg El-Arab City, Universities and Research Institutes  
Zone, P.O. 21934, Alexandria,  
Egypt.

Dr. MR. Pravin Babarao Suruse  
Department of Pharmaceutics  
Sharad Pawar College of Pharmacy  
Wanadongri, Hingna Road  
Nagpur- 441 110. (M. S.)
Dr. Jan Woraratanadharm  
*GenPhar, Inc.,*
*Mount Pleasant, SC*

Dr. Serap Yalin  
*Mersin University Pharmacy Faculty*
*Department of Biochemistry, Mersin*
*Turkey*

Dr. YongYong Shi  
*Bio-X Center,*
*Shanghai Jiao Tong University,*
*Hao Ran Building, 1954 Hua Shan Road,*
*Shanghai 200030,*
*PR China*

Dr. Jyotdeep Kaur  
*Department of Biochemistry,*
*Post Graduate Institute of Medical Education and Research (PGIMER),*
*Chandigarh*

Dr. Rajkumar  
*Dept. Of Radiation Biosciences,*
*Institute of Nuclear Medicine and Allied Sciences*
*Brig. S.K. Mazumdar Road, Timarpur,*
*Delhi 110054*
*India*

Dr. Meera Sumanth  
*Visveswarapura Institute of Pharmaceutical Sciences,*
*22nd Main, 24th Cross, B.S.K II stage,*
*Bangalore-560070*
*Karnataka,*
*India.*

Dr. Jai S. Ghosh  
*Department of Microbiology,*
*Shivaji University,*
*Kolhapur 416004,*
*India*

Prof. Dr. Alaa H. Al-Charrakh  
*Babylon University, College of Medicine.*
*Dept. of Microbiology*
*Hilla, Iraq*
Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The cover letter should include the corresponding author’s full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author’s surname, as an attachment.

Article Types

Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.

Decisions will be made as rapidly as possible, and the Journal strives to return reviewers’ comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the AJMR to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors’ full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited. Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard Abbreviations should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer’s name and address. Subheadings should be used. Methods in general use need not be described in detail.
**Results** should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors’ experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

**The Discussion** should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

**The Acknowledgments** of people, grants, funds, etc should be brief.

**Tables** should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

**Figure legends** should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

**References:** In the text, a reference identified by means of an author’s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author’s name should be mentioned, followed by ‘et al.’ In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ‘a’ and ‘b’ after the date to distinguish the works.

Examples:

- Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)

References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:


**Short Communications**

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.
**Fees and Charges:** Authors are required to pay a $650 handling fee. Publication of an article in the International Journal for Biotechnology and Molecular Biology Research is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

**Copyright: © 2016, Academic Journals.**

All rights Reserved. In accessing this journal, you agree that you will access the contents for your own personal use but not for any commercial use. Any use and or copies of this Journal in whole or in part must include the customary bibliographic citation, including author attribution, date and article title.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

**Disclaimer of Warranties**

In no event shall Academic Journals be liable for any special, incidental, indirect, or consequential damages of any kind arising out of or in connection with the use of the articles or other material derived from the IJMBR, whether or not advised of the possibility of damage, and on any theory of liability.

This publication is provided "as is" without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability, fitness for a particular purpose, or non-infringement. Descriptions of, or references to, products or publications does not imply endorsement of that product or publication. While every effort is made by Academic Journals to see that no inaccurate or misleading data, opinion or statements appear in this publication, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the responsibility of the contributor or advertiser concerned. Academic Journals makes no warranty of any kind, either express or implied, regarding the quality, accuracy, availability, or validity of the data or information in this publication or of any other publication to which it may be linked.
Effect of the initial pH on the performance characteristics of the deproteinization process of galactose supplemented shrimp shells by Aspergillus niger in a solid state drum bioreactor
Abdel Ghaly and Nesreen Mahmoud
Full Length Research Paper

Effect of the initial pH on the performance characteristics of the deproteinization process of galactose supplemented shrimp shells by *Aspergillus niger* in a solid state drum bioreactor

Abdel Ghaly\(^1\)* and Nesreen Mahmoud\(^2\)

\(^1\)Department of Process Engineering and Applied Science, Faculty of Engineering, Dalhousie University, Halifax, Nova Scotia, Canada.
\(^2\)Department of Agricultural Engineering, Faculty of Agriculture, Cairo University, Giza, Egypt.

Received 2 April, 2015; Accepted 14 September, 2015

The aim of the research was to study the effect of adjusting the initial pH of shrimp shell (8.64 vs. 7.00) on the ability of the fungus *Aspergillus niger* to carry out deproteinization process of galactose supplemented shrimp shells and to investigate the performance characteristics of the deproteinization process. The results showed that the initial pH of the shells has a significant effect on the temperature, carbon dioxide emission, moisture content, galactose, proteinase activity, protein, chitin and final appearance of shells. The temperatures of the shrimp shells and the exhaust gas declined during the lag period as the heat losses from the bioreactor were higher than the heat generated by microbial activities in the bioreactor. They started to rise during the exponential growth when the heat generation by metabolic activity exceeded the heat losses, reaching maximum values of 37.5 and 30.6°C after 60 h and of 29.1 and 27.2°C after 72 h for the runs with the initial pH of 8.64 and 7.0, respectively. The carbon dioxide increased with time reaching maximum values of 0.49 and 0.22% and then declined reaching 0.06 and 0.08% by the end of the experiment for the runs with initial pH of 8.64 and 7.00, respectively. A strong correlation between the concentration of carbon dioxide in the exhaust gas and the temperature of the shrimp shells was observed. The initial moisture content of 60% fell below 25.5 and 21.5% and the galactose concentration decreased from the initial value of 20 to 1.48% and 6.17% by the end of the deproteinization process for the runs with initial pH of 8.64 and 7.00, respectively. Although the protease activity increased by 3.3-6.3 fold, the reduction in the protein concentration did not correspond to the increase in the protease activity due to the high pH of the shrimp shells. The chitin concentration increased from an initial value of 16.56 to final values of 22.68 and 21.35 for the initial pH of 8.64 and 7.00, respectively. The spent shrimp shells obtained from the run with the initial pH of 8.64 were wet and had a pale pink-orange color with some tan patches, whereas the spent shrimp shells obtained from the with the initial pH of 8.64 appeared dry and had a gray-black color due to the presence of *A. niger* spores. The use of lactic acid to lower the pH of shrimp shells inhibited the growth of *A. niger* and protease production and activity and enhanced sporulation. The existence of the pink-orange color was an indication of the presence of pigments, which were not utilized during the fermentation process.

Key words: Shrimp shells, *Aspergillus niger*, deproteinization, protein, protease, chitin, galactose, temperature, pH, moisture content.
INTRODUCTION

Chitin is a polymer of nitrogen containing polysaccharide \((C_9H_{13}O_7N_\text{X})_n\) which render it a tough protective covering or structural support in certain organisms. It is found in plants and shell fish and it makes up the cell walls of fungi and exoskeleton of insects. Chitin and its derivatives are versatile environmentally friendly modern materials that have been used in virtually every segment of the economy because of their wide range of properties. The applications for chitin and its derivatives include water treatment, pulp and paper, biomedical and therapeutic devices, cosmetics, biotechnology, agriculture, food science and membrane technology (Abdullah, 1995; Kumar, 2000; Sashiwa and Alia, 2004; Pillai et al., 2009).

Chitin present in the crustacean waste is associated with proteins, minerals (mainly calcium carbonate) and pigments. The traditional method of chitin extraction from crustacean waste involves the use of strong acid (HCl) for demineralization and strong alkali (NaOH) for deproteinization (Zakaria et al., 1998; Bustos et al., 1999; Ghanem et al., 2004). The disadvantages of this method are: (a) it yields chitin with variable physiochemical properties, (b) it results in a wastewater that creates costly disposal problems, (c) it wastes other valuable components such as protein and pigments present in the waste material and (d) it is very costly. Therefore, a less expensive environmentally friendly method for chitin extraction from crustacean waste is needed.

Several authors reported on biological deproteinization of shrimp shells using proteolytic microorganisms (Wang and Chio, 1998; Teng et al., 2001; Ghaly and Mahmoud, 2015) or purified proteolytic enzymes (Wang and Chio, 1998; Mahmoud and Ghaly, 2015) and organic acids for demineralization (Hall and Reid, 1994; Beane et al., 2005). However, the use of purified enzymes is not as effective as living microbes. The presence of microorganisms provides a constant and gradual increase of protease throughout the fermentation (Teng et al., 2001). Thus, biological chitin extraction using microorganisms appears to be a good alternative to the harsh chemical extraction because: (a) it is considered less expensive as compared to the chemical method, (b) it results in an effluent that is less harmful to the environment and (c) it preserves the natural state of the biopolymer.

Aspergillus niger is an important microorganism that has been used in different industrial applications including production of organic acids such as citric and gluconic acids (Ruijer et al., 1999; Ali et al., 2002; Auta et al., 2014) and extracellular enzymes such as pectinase, amylase, protease, maltase, lactase, catalase, proteinase, trehalase, tannase, diptelatase, polypetelatase, lipase, cellulose, amidase, glucose oxidase, glucose dihydrogenase, urease, insulase, melibase and zymase (Naidu and Panda, 2003; Mahmoud and Ghaly, 2015). A. niger is also considered a source of chitin as it contains up to 42.0% chitin of the dry weight of the fungal cell wall; the cell wall weight is 20-40% of the total dry cell weight (Ghaly and Mahmoud, 2015). Therefore, A. niger has a great potential for deproteinization of the crustacean waste in a solid state fermentation as the proteolytic enzymes released from the fungi can deproteinize the shells and the chitin in the cell wall of the fungi can be considered an additional source of chitin (Teng et al., 2001).

Northern pink shrimp \((Pandalus borealis)\) is commonly fished in the North Atlantic both on the East Coast of Canada and the West Coast of Norway. \(Pandalus borealis\) has a mean length of 22-25 mm at maturity (Hansen and Aschan, 2000). The total landing of Northern shrimp in Eastern Canada in 2013 was 185974 tonnes (DFO, 2015). Based on the processing, removal of these shrimp (80% of its original weight), the total amount of waste produced in 2013 was 148000 tonnes most of which was dumped in the ocean or landfills.

The aim of this study was to evaluate the performance of a solid state deproteinization of shrimp shells supplemented with galactose as a carbon source by the fungus \(A. niger\) under different pH regimes. The specific objectives of the research were: (a) to study the effect of adjusting the initial pH of shrimp shells (7.00 vs. 8.64) on the ability of the fungus \(A. niger\) to produce the protease enzyme and carry out the deproteinization process of shrimp shells and (b) to investigate the changes in the kinetic parameters (pH, moisture content, galactose content, protease activity, protein content and chitin content) of the deproteinization process and the final shells appearance.

MATERIALS AND METHODS

Experimental apparatus

The slid state deproteinization system (Figure 1) consisted of a main frame, three drum bioreactors, an aeration system and a data acquisition system. The main frame was made of two polyvinyl chloride (PVC) rectangular sheets (13 mm thick) and two hexagon stainless steel sheets (3 mm thick). One of the PVC sheets (560 x 460 mm) was used as a base and the other one (560 x 380 mm) was fixed vertically on the base. The two hexagon stainless steel sheets were fixed to the two PVC sheets by means of stainless steel screws.

The main frame held the drum bioreactors, the pressure regulator, the flow meters, the inlet air and exhaust gas
Figure 1. Experimental setup.

1. Laboratory air supply  2. Pressure regulator  3. Humidifier  4. Air filter
manifold, tubing and sampling ports, the thermocouple wires, the mixing motor along with the transmission system and the switch.

Three 1.8 L drum bioreactors with mixing motors and transmission system were used. Each drum bioreactor (Figure 2) consisted of a removable inner stainless steel mesh (aperture of 1.5 mm) which was used as a lining for an outer stainless steel horizontally rotating basket of 88 mm diameter and 292 mm length. One stainless steel plate, with a drilled hole for sampling, was used to close one end of the rotating basket. The other end was left opened for charging and cleaning purposes and was designed so that it can be recessed and secured into a rotating disc after charging the reactor. An outer casing made from a Plexiglas cylinder of 12.5 mm diameter was installed for each bioreactor. The Plexiglas cylinder was recessed and secured into the main frame from one end by six stainless steel screws. The other end of the Plexiglas cylinder was covered by a removable circular Plexiglas plate and was recessed and secured by six stainless steel screws and wing nuts. A rubber gasket lining (O-ring, 2.5 mm thick) was used at both ends of the Plexiglas cylinder to provide an air tight seal. A hole was drilled through the cylinder wall for the release of the exhaust gas. The rotating discs were connected to a motor (Synchronous Motor, 20-34245G-24007, Xerox127P1292/B,
Air was supplied continuously at the required flow rate inside each drum bioreactor from the laboratory air supply. The air passed first through a pressure regulator (ARO, Model no. 129125-510, Bryan, Ohio, USA) in order to regulate the air pressure around 5 kPa and then through a 1 L humidifier which contained 0.75 L sterilized distilled water kept at room temperature. The humidified air was passed through a bacterial filter and then through a flow meter (No. 60648, Cole-Parmer Instrument Co., Illinois, USA) and finally introduced into the bioreactor through a small perforated stainless steel tube that ran along the center of the basket. The aeration tube was fixed through the center of the rotating disc and remains stationary while the basket is rotating. The air inlet sampling port was placed right after the bacterial filter whereas the three exhaust gas sampling ports were located on the exhaust tubes; each was made of a rubber septum. The three exhaust gas tubes were connected to a manifold and the exhaust gas was bubbled through a small container of water in order to create a slight gas pressure in the bioreactors.

Eleven T-type thermocouples (Thermo Electric Ltd., Brampton, Ontario, Canada) were used to measure the temperature during the course of the fermentation process. Two thermocouples were threaded through the aeration tube of each bioreactor and used to measure the temperature of the material inside the bioreactor. The other five thermocouples were used to measure the ambient temperature (1), inlet air temperature (1) and exhaust gas temperature of each bioreactor (3). The temperature data were monitored and stored using a data acquisition system which consisted of a master unit (Multiscan 1200, Omega, and Stamford, CT), a thermocouple/volt scanning card (MTC/24, Omega, Stamford, Connecticut, USA), a software (Tempview, Omega, Stamford, Connecticut, USA) and a personal computer.

Microorganisms

The fungus *A. niger* (Figure 3) was chosen for this study because of its ability to produce acid protease and the presence of chitin in its cell wall. The genus *Aspergillus* is characterized by a well-developed foot cell at the base of the conidiophore. The colony consists of colorless mycelium from which conidiophora arise. The spores develop the black color and the powdery appearance of the colony surface as sown in Figure 3.
A. niger (ATCC 16513) was obtained from the American Type Culture Collection (Rockville, Maryland). The freeze dried culture was revived in 6 mL of 0.1% sterilized peptone solution, which was prepared by dissolving 1 g Becto-Peptone (Difco, Detroit, Michigan, USA) in 1 L deionized-distilled water and then sterilized in an autoclave (Model No. STM-E, Market Forge Sterilmatic, New York, USA) at 121°C and 103.4 kPa for 30 min. The rehydrated culture was kept in the peptone solution in a capped test tube for 24 h at room temperature (21°C). 1 mL of the rehydrated A. niger was transferred to each of three test tubes containing 9 mL potato dextrose broth (PDB), which contained infusion from 200 g potatoes (4 g/L) and 20 g/L Bacto dextrose. The test tubes were kept tightly capped for 48 h at room temperature (21°C) and then stored in the fridge at 4°C and subcultured when needed. A spore stock suspension was obtained by growing the fungus on Czapek’s agar (which contained 30.00 g/L saccharose, 2.00 g/L Bacto dextrose and 15.0 g/L agar) at room temperature (25°C) for 4 days. The conidia were harvested from the surface by adding sterilized deionized distilled water containing 0.01% (v/v) Tween 80 (dissolved by preparing 0.1 mL Tween 80 in 1 L distilled deionized water and then autoclaved at 121°C and 103.4 kPa for 30 min) and gently scrape the surface with a sterile spatula. The spore concentration was determined as follows: the rehydrated culture was revived in 6 mL of 0.1% Tween 80 and then placed in an oven (Isotemp Oven, Model 655F, Fisher Scientific, Montreal, Quebec, Canada) at 105°C for 24 h. The aluminiun dish along with the dried sample was then placed in a desiccator to cool down and then weighed. The moisture content was determined as follows:

$$MC = \frac{W_{ws} - W_{ds}}{W_{ws}} \times 100$$  \hspace{1cm} (1)

Where: MC is the moisture content (%); Wws is the weight of the wet sample (g); Wds is the weight of the dry sample (g).

**Shrimp shells**

The shells of the Northern Pink Shrimp (Pandalus borealis) were obtained from a shell processing plant in Mulgrave, owned by Ocean Nutrition Ltd. of Bedford, Nova Scotia. The shrimp shells were stored at about -25°C in the Biotechnology Laboratory till needed. The shrimp shells were autoclaved (Model No. STM-E, Market Forge Sterilmatic, New York, USA) at 121°C and 103.4 kPa for 45 min before use. Table 1 shows some of the characteristics of the shrimp.

**Experimental protocol**

The effect of initial pH (8.64 and 7.00) on the deproteinization process of shrimp shells was studied. The experimental conditions of the deproteinization process are shown in Table 2. The sugar solution was prepared by dissolving 29 g galactose in 1 L deionized distilled water and then autoclaved (Model No. STM-E, Market Forge Sterilmatic, New York, USA) at 121°C and 103.4 kPa for 30 min. Each reactor was loaded up to 75% of its capacity (200 g shells based on dry weight). An inoculum concentration of 1 × 107 spores per 1 g shrimp shell waste was used. The initial moisture content of the shrimp shells was adjusted to 60% with the addition of sugar and spores solutions and the material was mixed thoroughly. Lactic acid was used to adjust the pH to 7. Air was introduced inside each reactor at a flow rate of 5 VMM (volume air in mL per shell waste mass in grams per minute). The experiment ran for 6 days. At the start of the experiment, the reactors were rotated (1 rpm) continuously for 30 min and then intermittently for 15 min every hour.

**Experimental analyses**

The particle size distribution, moisture content, pH, galactose concentration, ammonium nitrogen, total Kjeldahl nitrogen, protein and chitin were performed on the shrimp shells. During the course of the fermentation period, shrimp shell samples of 10 g each were collected every 12 h and analyzed for moisture content, pH, galactose concentration, protease activity, ammonium nitrogen, total Kjeldahl nitrogen and protein. Exhaust gas samples were also taken every 12 h and analyzed for carbon dioxide concentration. The bulk temperature was monitored and recorded every 10 min. The deproteinized shells were analyzed for chitin and appearance.

**Particle size distribution**

A known weight of shrimp shells were sieved for 30 min using a sieve shaker (Model RX-86, Fisher Scientific, Montreal, Quebec, Canada) with different sieve sizes (6.300, 4.000, 2.000, 0.850, 0.300, 0.180, 0.075 mm aperture size). Each particle size fraction obtained was weighed and the percentage from the total weight was calculated. Table 3 show the particle size distribution of the shrimp shells used in this study.

**Moisture contents**

A known weight of shrimp shells sample was placed in a preweighed aluminum dish. The dish and content were weighed and then placed in an oven (Isotemp Oven, Model 655F, Fisher Scientific, Montreal, Quebec, Canada) at 105°C for 24 h. The moisture content was determined as follows:

$$MC = \frac{W_{ws} - W_{ds}}{W_{ws}} \times 100$$  \hspace{1cm} (1)

Where: MC is the moisture content (%); Wws is the weight of the wet sample (g); Wds is the weight of the dry sample (g).

**pH**

20 mL of deionized distilled water was added to one gram of shrimp shell sample and kept at room temperature (24°C) for 30 min with frequent stirring using a stir plate (Thermix® Sturrer Model 120MR, Fisher Scientific, Montreal, Quebec, Canada). The sample was then measured using a pH meter (Model 805MP, Fisher Scientific, Montreal, Quebec, Canada).

**Ammonium nitrogen**

Samples were washed thoroughly several times with deionized distilled water until the wash water was clear and then dried in an oven (Isotemp Oven, Model 655F, Fisher Scientific, Montreal, Quebec, Canada) at 60°C till constant weight. The dried shells were ground using a small conventional grinder (Hamilton Beach, Markham, Ontario, Canada). Ammonium nitrogen (NH₄-N) of dry ground samples was determined directly using Kjeldahl system (KJELTEC AUTO 1030 Analyzer, Fisher Scientific, Montreal, Quebec, Canada).

**Total Kjeldahl nitrogen**

Samples were washed thoroughly several times with deionized distilled water until the wash water was clear and then dried in an oven (Isotemp Oven, Model 655F, Fisher Scientific, Montreal, Quebec, Canada) at 60°C till constant weight. The dried shells were...
ground using a small conventional grinder (Hamilton Beach, Markham, Ontario, Canada) and then digested by heating the sample with concentrated sulfuric acid and Kjeltabs (which contained 3.5 g K₂SO₄ and 0.0035 Se) for 45 min. The K₂SO₄ promotes the oxidation of organic matter and conversion of organic nitrogen to ammonium nitrogen by increasing the temperature of the digest (420°C). Se is a catalyst which increases the rate of oxidation of organic matter by sulfuric acid. 5 mL sulfuric acid with 1 kjeltab per 0.2 g dry weight sample was used in this study. The total Kjeldahl nitrogen (TKN) of the digested samples was as determined using Kjeldahl system (KJELTEC AUTO 1030 Analyzer, Fisher Scientific, Montreal, Quebec, Canada).

### Protein

The protein content of the samples was determined using the following equations which are based on the fact that the protein contains about 16% nitrogen:

\[
\text{(Org.-N)}_s = \text{TKN}_s - (\text{NH}_4\text{-N})_s \quad (2)
\]

\[
\text{PR}_c = \left[ (\text{Org.-N})_s - (\text{Org.-N})_c \right] \times 6.25 \quad (3)
\]

Where: PRₖ is protein content (mg/kg); (Org.-N)ₖ is organic nitrogen of the recovered chitin (mg/kg); (Org.-N)ₖ is organic nitrogen of the sample (mg/kg); TKNₖ is total Kjeldahl nitrogen of the sample (mg/kg); (NH₄-N)ₖ is ammonium nitrogen of the sample (mg/kg).

### Galactose concentration

20 mL of deionized distilled water was added to one gram of fermented shrimp shell sample and kept at room temperature (24°C) for 30 min with frequent stirring using a stir plate (Thermix® Stirrer Model 120MR, Fisher Scientific, Montreal, Quebec, Canada). The extract was then filtered under suction using coarse porosity filter paper (Reeve Angel Grade 202, Whatman Inc., Clifton, New Jersey).
Table 2. Experimental conditions of the shrimp shells deproteinization process.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Studied Parameter</td>
<td>Initial pH</td>
</tr>
<tr>
<td>Load</td>
<td>75% of the Bioreactor Volume</td>
</tr>
<tr>
<td>Initial Moisture Content</td>
<td>60%</td>
</tr>
<tr>
<td>Inoculum Size</td>
<td>1 x 10^7 Spores/g Shell</td>
</tr>
<tr>
<td>Aeration</td>
<td>5 VMM</td>
</tr>
<tr>
<td>Agitation</td>
<td>Intermittent (15 min/h)</td>
</tr>
<tr>
<td>Particle Size</td>
<td>Intact Shrimp Shells (0.075-6.30 mm)</td>
</tr>
<tr>
<td>Autoclaving</td>
<td>Galactose</td>
</tr>
<tr>
<td>Initial Concentration</td>
<td>Autoclaved Shrimp Shells</td>
</tr>
<tr>
<td>Initial pH</td>
<td>7.00 and 8.64</td>
</tr>
</tbody>
</table>

Table 3. Particle size distribution of the shrimp shells.

<table>
<thead>
<tr>
<th>Size (mm)</th>
<th>Under (%)</th>
<th>Above (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.300</td>
<td>98.11</td>
<td>1.89</td>
</tr>
<tr>
<td>4.000</td>
<td>88.97</td>
<td>9.13</td>
</tr>
<tr>
<td>2.000</td>
<td>59.33</td>
<td>29.67</td>
</tr>
<tr>
<td>0.850</td>
<td>35.74</td>
<td>64.23</td>
</tr>
<tr>
<td>0.300</td>
<td>11.25</td>
<td>90.75</td>
</tr>
<tr>
<td>0.180</td>
<td>3.96</td>
<td>96.04</td>
</tr>
<tr>
<td>0.075</td>
<td>0.92</td>
<td>99.08</td>
</tr>
<tr>
<td>&lt; 0.075</td>
<td>0.24</td>
<td>99.78</td>
</tr>
</tbody>
</table>

Protease activity

Protease produced by A. niger was first extracted from the samples (1 g each) using 20 mL deionized distill water and kept at room temperature for 30 min with continuous stirring using a stir plate (Thermix® Stirrer Model 120MR, Fisher Scientific, Montreal, Quebec, Canada). The extract was then filtered under suction using coarse porosity filter paper (Reeve Angel Grade 202, Whatman Inc., Clifton, New Jersey, USA) and the supernatant was used for the assay of enzyme. Protease activity was measured using Protease Colorimetric Detection Kit (Product Code PC0100, Sigma, Saint Louis, Missouri, USA). The assay was based on using a casein substrate, which is cleaved by the protease to trichloroacetic acid soluble peptides. The formed peptides contain tyrosine and tryptophan residues, which react with the Folin and Ciocalteu’s reagent causing color change, which can be estimated calorimetrically at 560 nm using a microplate reader (µQuant, Bio-Tek Instruments, Inc., Winooski, Vermont, USA).

Chitin

The chitin content was determined based on the fact that chitin contains about 6.89% organic nitrogen (Zakaria, 1997). In order to determine the chitin nitrogen, samples were first deproteinized and demineralized.

The deproteinization process was performed using 5% (w/v) NaOH solution. One gram of ground shrimp shell sample (dry weight) along with 100 mL of NaOH solution were placed in a 250 mL wide-mouth flask and the flask was covered with a piece of tin foil and sealed with a rubber band to ensure the retention of all reacted materials. The flask was then placed in a boiling water bath for 1 h. The sample was filtered under suction through a Buchner funnel with coarse porosity filter paper (Reeve Angel Grade 202, Whatman Inc., Clifton, New Jersey, USA) and washed thoroughly with deionized distill water. The deproteinized sample was dried in an oven (Isotemp Oven, Model 655F, Fisher Scientific, Montreal, Quebec, Canada) at 60°C till constant weight. The weight of the recovered dry deproteinized sample was determined.

The denatured sample along with 50 mL of 1.0 M HCl were placed in a 250 mL wide-mouth flask and the flask was covered with a piece of tin foil and sealed with a rubber band to ensure the retention of all reacted materials. The flask was then placed in a boiling water bath at 80°C for 30 min. The mixture was then cooled and a solution of 1% solution of 1% NaOH was added to the sample and the mixture was carefully stirred using a magnetic stirrer. The mixture was then filtered using a coarse porosity filter paper (Reeve Angel Grade 202, Whatman Inc., Clifton, New Jersey, USA) and the supernatant was used for the assay of enzyme. The assay was based on using a casein substrate, which is cleaved by the protease to trichloroacetic acid soluble peptides. The formed peptides contain tyrosine and tryptophan residues, which react with the Folin and Ciocalteu’s reagent causing color change, which can be estimated calorimetrically at 560 nm using a microplate reader (µQuant, Bio-Tek Instruments, Inc., Winooski, Vermont, USA).

Chitin

The chitin content was determined based on the fact that chitin contains about 6.89% organic nitrogen (Zakaria, 1997). In order to determine the chitin nitrogen, samples were first deproteinized and demineralized.

The deproteinization process was performed using 5% (w/v) NaOH solution. One gram of ground shrimp shell sample (dry weight) along with 100 mL of NaOH solution were placed in a 250 mL wide-mouth flask and the flask was covered with a piece of tin foil and sealed with a rubber band to ensure the retention of all reacted materials. The flask was then placed in a boiling water bath for 1 h. The sample was filtered under suction through a Buchner funnel with coarse porosity filter paper (Reeve Angel Grade 202, Whatman Inc., Clifton, New Jersey, USA) and washed thoroughly with deionized distill water. The deproteinized sample was dried in an oven (Isotemp Oven, Model 655F, Fisher Scientific, Montreal, Quebec, Canada) at 60°C till constant weight. The weight of the recovered dry deproteinized sample was determined.

The deproteinized sample along with 50 mL of 1.0 M HCl were placed in a 250 mL wide-mouth flask and the flask was covered with a piece of tin foil and sealed with a rubber band to ensure the retention of all reacted materials. The flask was then placed in a boiling water bath at 80°C for 30 min. The mixture was then cooled and a solution of 1% solution of 1% NaOH was added to the sample and the mixture was carefully stirred using a magnetic stirrer. The mixture was then filtered using a coarse porosity filter paper (Reeve Angel Grade 202, Whatman Inc., Clifton, New Jersey, USA) and the supernatant was used for the assay of enzyme. The assay was based on using a casein substrate, which is cleaved by the protease to trichloroacetic acid soluble peptides. The formed peptides contain tyrosine and tryptophan residues, which react with the Folin and Ciocalteu’s reagent causing color change, which can be estimated calorimetrically at 560 nm using a microplate reader (µQuant, Bio-Tek Instruments, Inc., Winooski, Vermont, USA).
the dry deproteinized-demineralized sample and the chitin content was then calculated as follows:

\[
(Org.-N)_c = [(TKN_c - (NH_4-N)_c) \times W_c/W_s]
\]

\[
CH_c = (Org.-N)_c \times 14.51
\]

Where: \(CH_c\) is chitin content (mg/kg); \((Org.-N)_c\) is organic nitrogen of the recovered chitin; \(TKN_c\) is total Kjeldahl nitrogen of the recovered chitin (mg/kg); \((NH_4-N)_c\) is ammonium nitrogen of the recovered chitin (mg/kg); \(W_c\) is weight of recovered chitin (g); \(W_s\) is weight of sample (g).

**Ash content**

The dried shrimp shells were analyzed for their ash content. A known weight of the material was placed in a preweighed aluminum dish. The dish and content were weighed and then placed in a muffle furnace (Isotemp® Muffle Furnace model 186A, Fisher Scientific, Montreal, Quebec, Canada) at 700°C for 2 h. The dish and content was taken from the muffle furnace, placed in a desiccator to cool down and then weighed. The ash content was determined as follows:

\[
AC = \frac{W_{ds} - W_a}{W_{ds}} \times 100
\]

Where AC is the ash content (%); \(W_s\) is the weight of the ash (g).

**Minerals**

The dried shrimp shells were analyzed for their minerals content. Quantitative trace element analyses (magnesium, calcium, manganese, potassium, sodium, iron, silicon, aluminum, titanium and copper) were performed on the ash using an Atomic Absorption Spectrophotometer (SpectrAA 55B, Varion, Mulgrave, Victoria, Australia) in the Minerals Engineering Center, Dalhousie University, Halifax, Nova Scotia. For magnesium, calcium, manganese, potassium, sodium, iron and copper analyses, the samples were first digested with hydrochloric, nitric, hydrofluoric and perchloric acids (30, 10, 10 and 5 mL/g sample, respectively) in a closed vessel at a temperature of 100°C and then the elements were determined by flame atomic absorption with detection limit of 1 ppm. For silicon, aluminum and titanium analyses, 1 g of the sample was fused with a flux of lithium metaborate and lithium tetraborate and leached with 1:9 nitric acid. Sulfur was determined with Leco Sulfur analyzer along with Leco Induction Furnace (Leco Corporation St Joseph, Michigan, USA). Phosphorus was determined as \(P_2O_5\) by a colorimetric method using spectrophotometer with micro flow-thru system (Spectronic 100, Bausch & Lomb Incorporation, Rochester, New York, USA) at 430 nm.

**Visualization of shrimp shells**

The shrimp shells were visually inspected at the end of the deproteinization with the naked eye as well as under the incident light stereomicroscope (Carl Zeiss Stemi SV8, Carl Zeiss Canada Ltd., Toronto, Ontario, Canada) at a magnification of 60x. The stereoscope was equipped with a cold light source (SCOHTT KL 1500, SCHOTT North America Inc., New York, USA) and a single chip CCD color video camera (Sony DXC-101, Sony of Canada Ltd., Toronto, Ontario, Canada).

**RESULTS AND DISCUSSION**

The effect of initial pH on various kinetic parameters (temperature, pH, moisture content, galactose utilization, carbon dioxide evolution, protease activity, residual protein and chitin concentration) of the deproteinization process was investigated.

**Temperature**

Figure 5 shows the changes in the temperatures of the shrimp shell bed and the exhaust gas during the course of deproteinization as affected by the initial pH. The values are the average of three replicates. The coefficient...
of variation ranged from 0.29 to 4.97. The fermentation reaction of shrimp shells is exothermic in nature in which heat is generated giving rise to the temperature of the medium. The fungus utilized organic matter for synthesis of new microbial cells, product formation and energy generation. The heat stored in the bioreactor is the net of metabolic heat produced minus the heat lost (by conduction through the shrimp shells and the bioreactor walls, by convection with the exhaust gas and latent heat through water evaporation from the shrimp shells).

In this study, the temperatures of the shrimp shells and the exhaust gas declined at the beginning of the fermentation process (lag period) as the heat losses from the bioreactor due to water evaporation (latent heat) and the cooling effect of the inlet air were higher than the heat generated in the bioreactor by microorganisms.

After 24 h, the temperature of the shrimp shells started to rise as the heat generation by metabolic activity
Figure 6. Typical batch growth curve.

exceeded the heat losses. However, the temperature profiles for the runs with the initial pH of 8.64 and 7.00 were different for both the shrimp shells and exhaust gas. For the shrimp shells, temperature peak of 37.5 and 30.6°C were noticed after 60 and 72 h for the runs with the initial pH of 8.64 and 7.0, respectively. Similarly, the temperatures of exhaust gas reached maximum values of 29.1 and 27.2°C after 60 and 72 h for the runs with initial pH of 8.64 and 7.0, respectively. Ghaly and Mahmoud (2015) and Mahmoud and Ghaly (2015) reported similar temperature profiles during solid-state fermentation of shrimp shells. Pandey (2003) reported that temperatures in the middle of the bed can reach about 20°C higher than the temperature of the inlet air. Saucedo-Castañeda et al. (1990) reported an axial temperature gradient of 0.17°C/cm and a radial temperature gradient of 5°C/cm in the bioreactor during the fermentation of cassava using A. niger. In the current study, the peak temperature of the shells material was 13.5 and 6.6°C higher than that of the inlet air temperature for the runs with initial pH of 8.64 and 7.00, respectively. There were no temperature gradients in the radial or axial direction because of mixing.

A typical microbial batch growth curve (Figure 6) includes: (a) lag phase, during which microbial population remains unchanged and adaptation to the surrounding environment takes place, (b) exponential growth phase, during which the specific growth rate is constant (c) deceleration growth phase, during which growth decelerates either because of the depletion of an essential nutrient or the accumulation of an inhibitory product, (d) stationary phase, during which the net growth rate is zero as a result of the balancing of reproduction rate by an equivalent death rate and (e) death phase, during which cells die faster than new cells are produced. Zakaria (1997) stated that temperature is one of the most significant parameters affecting the growth and activities of microorganisms in solid-state fermentation. Ghaly et al. (2003) and Mahmoud and Ghaly (2015) showed that the microbial growth and the temperature curves are similar in shape and the temperature curve can be used to determine microbial kinetic parameters.

The growth kinetic parameters shown in Table 4 were determined from the temperature data. The length of the lag period was 12 and 18 h, the exponential growth phase was 48 and 60 h, the stationary phase was 4 and 24 h and the maximum temperatures of 37.5 and 30.6°C were attained after 60 and 72 h of deproteinization for the runs with initial pH of shrimp shells of 8.64 and 7.00, respectively. Decreasing the initial pH from 8.64 to 7.00 decreased the specific growth rate of A. niger from 0.022 to 0.008 h⁻¹.

The significant reduction in the specific growth phase for initial pH of 7.00 could be the result of the inhibitory effect of the lactic acid used to lower the initial pH of the shrimp shells.

pH

Figure 7 shows the change in the pH of shrimp shells during the course of deproteinization as affected by the initial pH. The values are the average of three replicates. The coefficient of variation ranged from 0.49 to 3.62%.
Table 4. Growth kinetic parameters calculated from the temperate curve.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.64</td>
</tr>
<tr>
<td>Lag phase (h)</td>
<td>12</td>
</tr>
<tr>
<td>Exponential phase (h)</td>
<td>48</td>
</tr>
<tr>
<td>Specific growth rate (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.022</td>
</tr>
<tr>
<td>Stationary phase (h)</td>
<td>4</td>
</tr>
<tr>
<td>Initial temperature (°C)</td>
<td>21.8</td>
</tr>
<tr>
<td>Maximum temperature (°C)</td>
<td>37.5</td>
</tr>
<tr>
<td>Time of maximum temperature (h)</td>
<td>60</td>
</tr>
</tbody>
</table>

Figure 7. Effect of the initial pH on the pH of deproteinized shrimp shells.

For the run with the initial pH of 8.64, the pH of the shrimp shells decreased during the first 60 h reaching 7.34, then increased reaching 8.06 at the 83 h and finally declined to 7.86 by the end of the deproteinization process (120 h). For the run with the initial pH of 7.00, the pH of the shrimp shells increased during the first 36 h reaching 7.73, then decreased reaching 6.88 at the 48 h, then increased to 7.3 at the 60 h and finally declined to 6.75 by the end of the deproteinization process (120 h).

Zakaria et al. (1998) reported a drop in the pH to a value of 5 over the first 48 h of lactic acid fermentation of scampi waste after which the pH increased reaching a final value of 6.6 as a result of the buffering capacity of the solubilized calcium. Beaney et al. (2005) reported rapid decrease in pH to 3.5 over 7 days during lactic acid fermentation of prawn shells as a result of metabolic lactic acid production.

The decrease in the pH of the shrimp shells observed in this study was due to the production of acid protease while the increase in the pH of the shrimp shells was due to the buffering capacity of the calcium carbonate released from the shrimp shells as well as the production of ammonium nitrogen as reported by Yang and Lin (1998) and Ghaly and Mahmoud (2015).

Moisture content

Figure 8 shows the change in the moisture content of the shrimp shells during the course of deproteinization as affected by the initial pH. The values are the average of

metabolites accumulation resulting from D-glucose degradation. Beaney et al. (2005) reported rapid decrease in pH to 3.5 over 7 days during lactic acid fermentation of prawn shells as a result of metabolic lactic acid production.
three replicates. The coefficient of variation ranged from 0.24 to 4.53%. The net moisture content of the shrimp shell bed can be defined as follows:

\[ MC_{\text{net}} = MC_i + MC_m - MC_e \]  \hspace{1cm} (7)

Where:

- \( MC_{\text{net}} \) is the net moisture content (%);
- \( MC_i \) is the initial moisture content (%);
- \( MC_m \) is the metabolic moisture content (%);
- \( MC_e \) is the moisture lost through evaporation with the exhaust gas (%).

The moisture content of the shrimp shells declined first slowly in the first 48 h from the initial value of 60(%) and 52(%) and 47% and then sharply decreased reaching final values of 25.5(%) and 21.5% by the end of the deproteinization process for the runs with the initial pH of 8.64 and 7.00, respectively. The initial slow decrease in the moisture content was due to the initial low temperature which affected the amount of water lost by evaporation while the steep decline in the moisture content observed thereafter was due to the loss of moisture through evaporation because of the high temperature observed during the exponential growth phase. This indicated that water evaporation was higher than the metabolic water production.

The moisture content of the substrate has a significant impact on solid-state fermentation. Yang and Lin (1998) stated that low moisture content caused the substrate to be too dry for microbial growth and product formation and high moisture content caused packing of the substrate and prevention of gas exchange. Mahmoud and Ghaly (2015) reported optimum initial moisture content between 50-60% for the production of protease by solid-state fermentation of sweet potato residue supplemented with rice bran and minerals using A. niger. An initial moisture content of 70% can cause the substrate to stick together preventing gas exchange and causing low protease production whereas an initial moisture content of 40% can make the substrate to be too dry for mycelial growth causing inhibition of microbial activities and very poor protease production.

In this study, the initial moisture content of the shrimp shells was adjusted to 60%, which fell below 40% after 66 and 76 h and reached 25.5 and 21.5% by the end of the deproteinization process for the runs with initial pH of 8.64 and 7.00, respectively. This low moisture content affected fungus growth and protease production and in turn the hydrolysis of protein. To maintain the moisture of the substrate inside the bioreactor at the desired level, the exhaust gas should be passed through a condensation tower and the recovered water pumped back into the bioreactor through the aeration tube.

**Galactose utilization**

Figure 9 shows the residual galactose concentration in the shrimp shells during the course of deproteinization as affected by the initial pH. The values are the average of three replicates. The coefficient of variation ranged from 2.91 to 3.39%.

The optimum galactose concentration of 20% w/w was used in this study as recommended by Mahmoud and
Ghaly (2015). The galactose concentration decreased gradually from 20 to 1.48% and 6.17% over the course of the deproteinization process (120 h) for the runs with initial pH of 8.64 and 7.00, respectively. The high residual galactose concentration experienced by the end of the deproteinization process with initial pH of 7.00 could be the result of the inhibitory effect of lactic acid which was used to adjust the initial pH of the shrimp shells.

**Carbon dioxide evolution**

Figure 10 shows the effect of the initial pH on carbon dioxide concentration in the exhaust gas. The values are the average of three replicates. The coefficient of variation ranged from 1.99 to 5.34%.

Temperature and carbon dioxide evolution are considered strong indicators of microbial activity during solid-state fermentation (Bellon-Maurel et al., 2003). The CO\textsubscript{2} evolution is related to microbial growth and the rate of CO\textsubscript{2} evolution can be used as a measure of the rate of microbial growth (Mahmoud and Ghaly, 2015).

In this study, the carbon dioxide increased with time reaching 0.49 and 0.22% at 60 and 78 h and declined reaching 0.06 and 0.08% by the end of the experiment for the runs with initial pH of 8.64 and 7.00, respectively. The lactic acid used to adjust the initial pH of the shrimp shells may have inhibited the microbial activities and as a result the production of carbon dioxide. A strong correlation between the concentration of carbon dioxide in the exhaust gas and the temperature of the shrimp shells was obtained (Figure 11).

**Protease activity**

Figure 12 shows the effect of the initial pH on protease activity during the course of deproteinization. The values are presented in units per gram dry shrimp shell waste and are the average of three replicates. The coefficient of variation ranged from 2.92 to 5.84%.

Fish proteins are complex molecules consisting of chains of amino acids linked together by peptide bonds. Proteases are proteins structured in such a way that allow them to act as catalysts in the breakage of peptide bonds through a process called hydrolysis according to the following equation:

\[
\text{Fish protein} \xrightarrow{\text{Proteases}} \text{Polypeptides + Peptides + Amino acids} \quad (8) 
\]

The degree to which a microorganism will hydrolyze a protein substrate depends on its capacity to produce proteases and the stability of such protease under the reaction conditions.

The results obtained from this study revealed the ability of *A. niger* to produce extracellular proteases that resulted in deproteinization of shrimp shells to a certain degree. The protease activity increased from 0.67 to 4.21 units/g dry shrimp shell and from 0.71 to 2.34 units/g dry shrimp shell over the course of the deproteinization process (120 h) for the runs with initial pH of 8.64 and 7.00, respectively. The protease production was greater during the exponential growth phase and was low during the stationary phase.

Ashour et al. (1996) reported that protease yield from...
the fungus *A. niger* during cheese whey fermentation increased with incubation period and reached a maximum value after 6 days. Teng et al. (2001) reported protease activities in the range of 0.8 ± 0.3 to 6.8 ± 0.4 units (one unit activity was defined as 1 µM of tyrosine produced in 1 min) for 17 *A. niger* strains after 5 days of incubation of the spores at a medium pH of 7.4.

The protease of *A. niger* is an acid resistant protease with optimum pH of 2.3. Thus, lowering the initial pH of the shrimp shells is expected to enhance the deproteinization process by: (a) achieving the optimum pH for fungal growth and protease production, (b) achieving the optimum pH for the proteolytic activity and (c) preventing the risk of the growth of undesirable microorganisms.
However, the use of lactic acid to lower the pH of shrimp shells in the current study inhibited the growth of *A. niger* and the activity of acid protease and the absence of lactic acid resulted in 1.8 fold increase in the protease activity.

**Protein concentration**

Figure 13 shows the effect of initial pH on residual protein in the shrimp shells during the deproteinization process. The values are presented based on the dry weight of the samples and are the average of three replicates. The coefficient of variation ranged from 3.91 to 6.82%.

The results showed that protein concentration of the shrimp shells decreased with time as a result of protein break by the proteolytic enzymes produced by *A. niger*. The protein concentration decreased from an initial value of 30.84% to final values of 21.64 and 25.30% over 120 h of deproteinization for the runs with initial pH values of 8.64 and 7.00, respectively. This resulted in protein removal efficiency of 29.7 and 17.9% for the runs with initial pH values of 8.64 and 7.00, respectively. Zakaria et al. (1998) used lactic acid fermentation for chitin recovery from scampi waste and reported that about 77.5% of the original protein was solubilized during 5 days. Beaney et al. (2005) reported 50% decrease of the original protein concentration in prawn shell waste using lactic acid fermentation and stated that complete deproteinization through a purely biotechnological process seems hard to achieve. The low deproteinization efficiency observed in this study could be due to: (a) high pH of the shrimp shells that might have interfered with protease synthesis and/or activity, (b) protein denaturation that might have happen during shrimp shells autoclaving, (c) high temperature and/or low moisture content noticed during the deproteinization process that might have affected the production and/or activity of protease and (d) the large particles size of shrimp shells used in the study. Diniz and Martin (1997) stated that the extent of hydrolytic degradation of protein depends on pH, temperature, extend of native protein denaturation, concentration and specificity of the enzyme, composition and the molecular weight distribution of the peptides in the protein and presence of inhibitory substances.

**Chitin concentration**

Figure 14 shows the effect of initial pH on chitin content of the shrimp shells during the deproteinization process. The values are presented based on the dry weight of the samples and are the average of three replicates. The coefficient of variation ranged from 3.11 to 5.72%.

The shrimp shells used in this study contained 31.73% minerals. The chitin concentration was determined for the deproteinized samples without demineralization. The chitin concentration increased from an initial value of 16.56 to final values of 22.68 and 21.35 for the runs with initial pH of 8.64 and 7.00, respectively. Zakaria et al. (1998) reported an increase in the concentration of chitin from 12.05 to 17.48% as a result of lactic acid fermentation of scampi waste. Cira et al. (2002) reported increases in chitin concentration from 11.4-13.1 to 20.3-23.2% as a result of lactic acid fermentation of shrimp waste. Beaney et al. (2005) reported chitin concentrations of 67.9-72.3% after lactic
Ghaly and Mahmoud

Figure 13. Effect of the initial pH on residual protein.

Figure 14. Effect of the initial pH on chitin concentration.

acid fermentation of prawn shells. The low increase in the chitin concentration observed in this study is due to the low deproteinization efficiency.

Visualization of shrimp shells

Figure 15 shows the appearance of the shell waste at the end of the deproteinization process. The spent shrimp shells obtained from the run with an initial pH of 8.64 were wet and had a pale pink-orange color with some tan patches whereas the spent shrimp shells obtained from the run with an initial pH of 7.00 appeared dry and had a gray-black color due to the presence of A. niger spores. The use of lactic acid to lower the pH inhibited the growth of A. niger and enhanced sporulation. The existence of the pink-orange color was an indication of the presence of pigments, which were not utilized during
Conclusions

The temperatures of the shrimp shells and the exhaust gas declined during the lag phase as the heat losses from the bioreactor due to water evaporation and the cooling effect of the inlet air were higher than the heat generated in the bioreactor by microbial activities. Once the microbial exponential growth phase was initiated, the temperature of the shrimp shells and exhaust gas started to rise as the heat generation by metabolic activity exceeded the heat losses. The temperature attained with high pH was much higher than that attained with the low pH. A strong correlation between the concentration of carbon dioxide in the exhaust gas and the temperature of the shrimp shells was observed. The initial moisture content of 60% fell below 40 which have negatively affected fungus growth and protease production and in turn the hydrolysis of protein. In order to maintain the desired moisture content in the bioreactor, the exhaust gas should be passed through a condensation tower and the recovered water pumped back into the bioreactor through the aeration tube. The initial decrease in the pH was due to the production of acid protease while the subsequent increase in the pH was due to the buffering capacity of the calcium carbonate released from the shrimp and/or the production of ammonium. The reduction in the protein concentration did not correspond to the increase in the protease activity and the high pH of the shrimp shells is believed to be the main reason for the low efficiency of the deproteinization process since the optimum pH of acid protease production by A. niger is 2-3. Low pH favors microbial growth and acid protease production, creates a however, lower pH using lactic acid which inhibit the microbial growth and protease production. The spent shrimp shells obtained from the run with high pH of 8.64 were wet and had a pale pink-orange color with some tan patches whereas the spent...
shrimp shells obtained from the run with low pH appeared dry and had a gray-black color due to the presence of *A. niger* spores. The use of lactic acid to lower the pH inhibited the growth of *A. niger* and enhanced sporulation. The existence of the pink-orange color was an indication of the presence of pigments, which were not utilized during the fermentation process.

**Conflict of Interests**

The authors have not declared any conflict of interests.

**ACKNOWLEDGEMENTS**

The research was funded by the National Science and Engineering Council (NSERC) of Canada. The support of Dalhousie University and Cairo University is highly appreciated.

**REFERENCES**


International Journal for Biotechnology and Molecular Biology Research

Related Journals Published by Academic Journals

- African Journal of Environmental Science & Technology
- Biotechnology & Molecular Biology Reviews
- African Journal of Biochemistry Research
- African Journal of Microbiology Research
- Journal of Cell and Animal Biology
- African Journal of Biotechnology
- Scientific Research and Essays