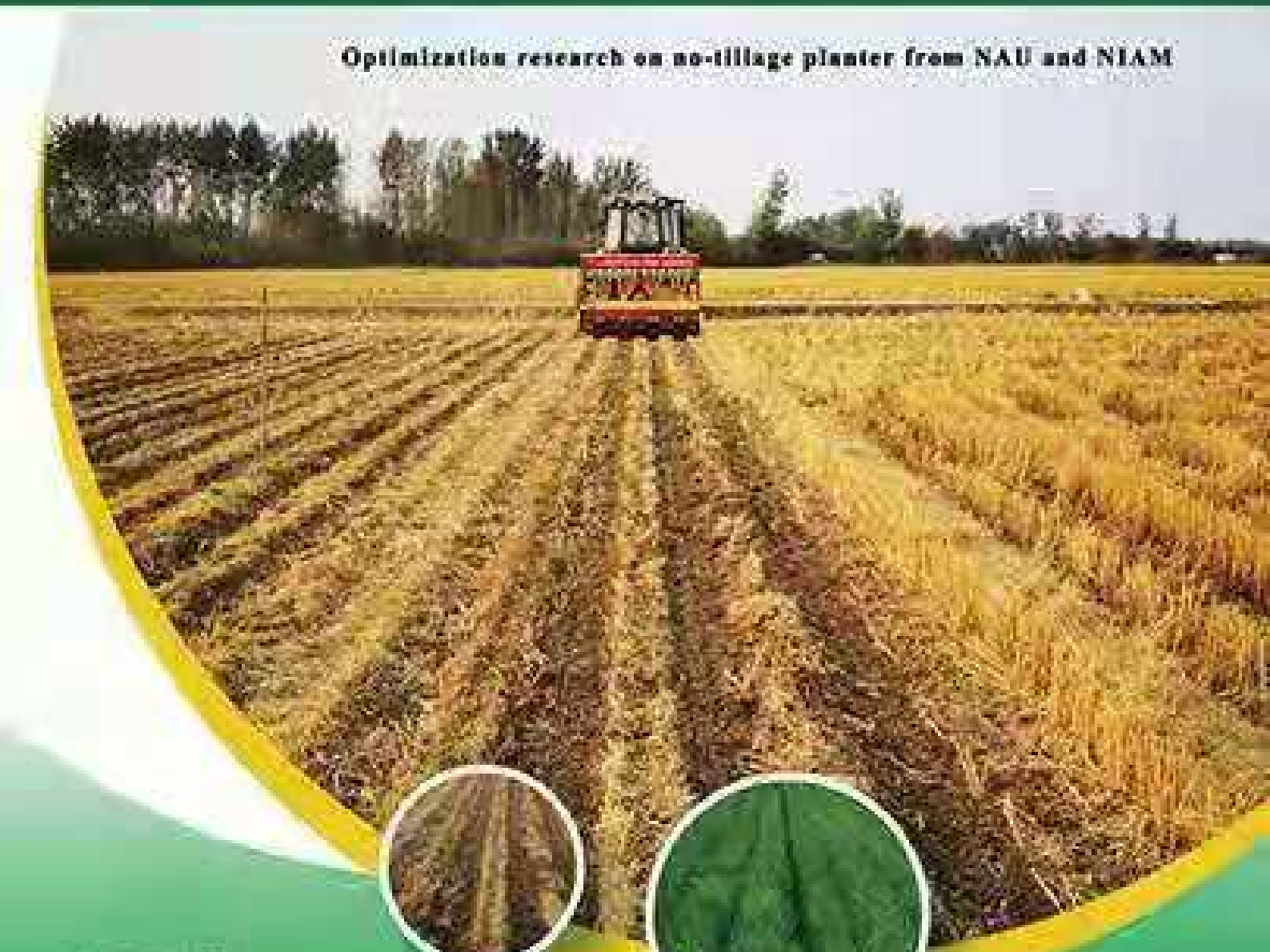
ISSN 1934-6344
eISSN 1934-6352

IJABE

3

International Journal of Agricultural and Biological Engineering



Optimization research on no-tillage planter from NAU and NIAM

Vol. 14 No.3
2021

International Journal of Agricultural and Biological Engineering

COUNTRY

China

Universities and research institutions in China

SUBJECT AREA AND CATEGORY

Agricultural and Biological Sciences
Agricultural and Biological Sciences (miscellaneous)

Engineering
Engineering (miscellaneous)

PUBLISHER

Chinese Society of Agricultural Engineering

H-INDEX

31

PUBLICATION TYPE

Journals

ISSN

19346344, 19346352

COVERAGE

2008-2020

INFORMATION

Homepage

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SCOPE

International Journal of Agricultural and Biological Engineering (IJABE, https://www.ijabe.org) is a peer reviewed open access international journal. IJABE, started in 2008, is a joint publication co-sponsored by US-based Association of Agricultural, Biological and Food Engineers (AOCABFE) and China-based Chinese Society of Agricultural Engineering (CSAE). The ISSN 1934-6344 and eISSN 1934-6352 numbers for both print and online IJABE have been registered in US. Now, Int. J. Agric. & Biol. Eng (IJABE) is published in both online and print version by Chinese Academy of Agricultural Engineering.

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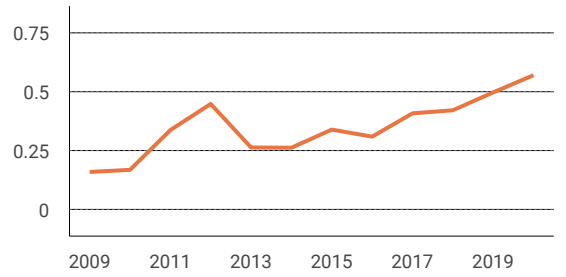

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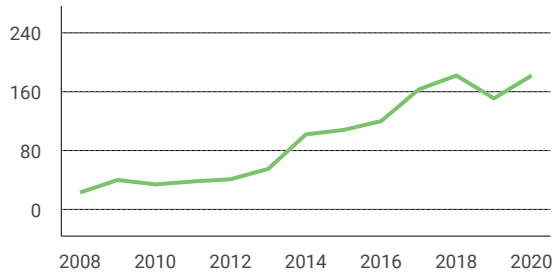

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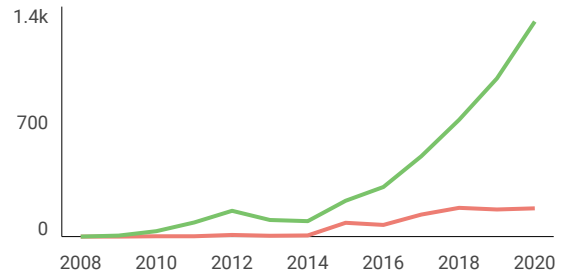

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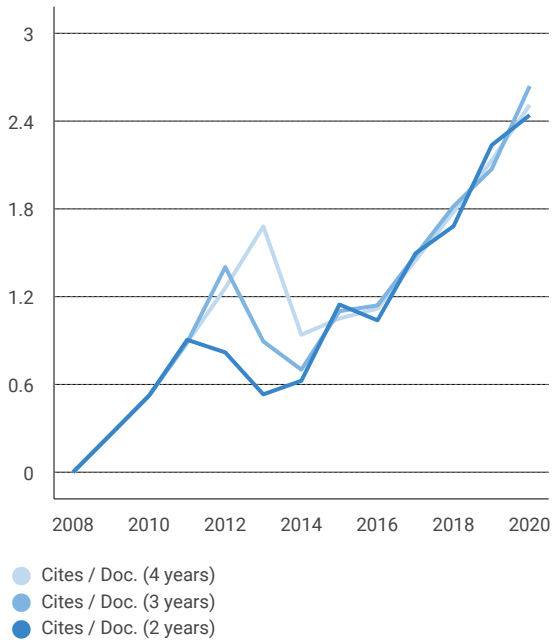

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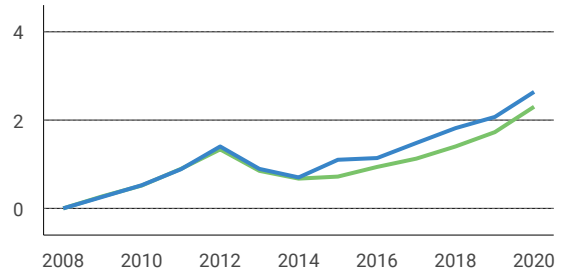

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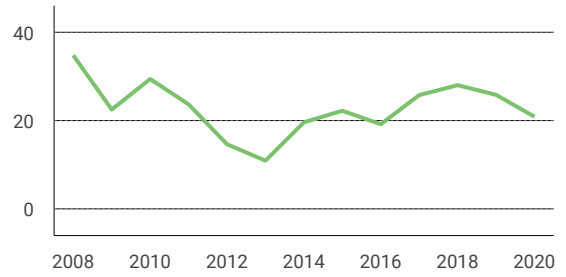

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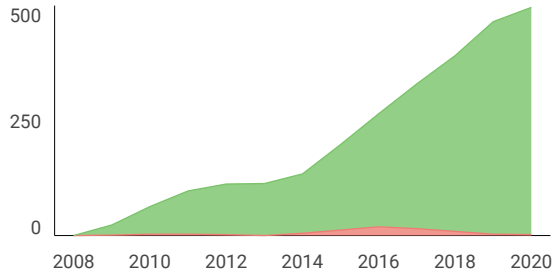

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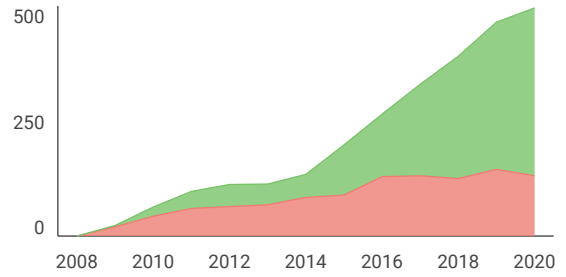

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IJABE International Journal of Agricultural and Biological Engineering

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Editorial Policies

Focus and Scope

International Journal of Agricultural and Biological Engineering (IJABE, https://www.ijabe.org) is a peer reviewed open access international journal. IJABE, started in 2008, is a joint publication co-sponsored by US-based Association of Agricultural, Biological and Food Engineers (AOCABFE) and China-based Chinese Society of Agricultural Engineering (CSAE). The ISSN 1934-6344 and eISSN 1934-6352 numbers for both print and online IJABE have been registered in US. Now, Int. J. Agric. & Biol. Eng (IJABE) is published in both online and print version by Chinese Academy of Agricultural Engineering.

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Openly accessible IJABE provides wider sharing of knowledge and the acceleration of research, and is thus in the best interest of authors, potential readers, and increases the availability, accessibility, visibility and impact of the papers, and the journal as a whole. IJABE aims at promoting agricultural and biological engineering, increasing academic prosperity, and fostering international academic exchange and cooperation.

This journal provides a platform for sharing the latest high-quality research concerning the agricultural, food and biological engineering and the application of these engineering techniques in all areas of agriculture. The journal features works of great significance, originality, and relevance in all the concerned areas. Our readers are from the international scientific community and may include educators, policy makers, agricultural engineers and scientists, and interested members of the public around the world.

Up to August 08, 2016, over 3000 submissions were received from over 30 countries, and 9 volumes with 38 issues and over 450 papers were published. The website of IJABE attracts over one million visits from over 90 countries around the world. Many papers published in IJABE were cited by other prestigious journals. The total registered users in IJABE database reached over 10 000 up to date.

Although IJABE is a newly-launched journal, it has been growing quickly and steadily. So far, online and print IJABE has been covered and indexed by SCIE, JCR, Current Contents, Biological Abstracts, BIOSIS Preview, Ei Compendex, CA, INSPEC, Indian NAAS Journal Scoring, Abstract Journals (VINITI RAN), Agricola, AGRIS, Scopus, CSA, OCLC, ProQuest, Index Copernicus, Google scholar, CAB International, CAB ABSTRACTS Full Text Select, Colorado Alliance of Research Libraries-Open Access Digital Library, EBSCOhost --- Academic Search Complete, Academic Search R&D, Academic Source Complete, and Food Science Source, Socolar, China Educational Publications Import & Export Corporation. All the papers published in IJABE can be searchable via the Thomson ISI Web of Science-All Databases. IJABE is also the Abstracting & Indexing Sources for AgBiotech News and Information (Active) (Print), Genetics Abstracts (Active) (Print), Index Veterinarius (Active) (Print), Pig News & Information (Active) (Print), Pollution Abstracts (Active) (Print), Postharvest News and Information (Active) (Print), Review of Aromatic and Medicinal Plants (Active) (Print), Virology and AIDS Abstracts (Active) (Print), Wheat, Barley and Triticale Abstracts (Active) (Print). And it has been listed on the world's biggest online OA journals platform: Directory of Open Access Journals (DOAJ), Open J-Gate, and also Ulrich's Periodicals Directory.

Most importantly, received its first impact factor of 1.007, according to 2016 update of its Journal Citation Reports®(JCR) released by The Intellectual Property (IP) Science Business of Thomson Reuters on June 13, 2016. Based on the Journal Impact Factor(JIF) values, IJABE ranks 7th out of 14 SCI-indexed journals in the category of agricultural engineering in the world, locating in Q2 Quartile. According to the latest statistics from Thomson Reuters Web of Science All Databases Citation Report, till July 9 2016 (Data on June 28 2015 in comparison), 474(355) items including articles, news, editorials and cover stories published in IJABE were found, and the sum of times cited reached 1144 (735). Average citation per item is 2.41 (2.07), and h-index is 12 (10).

Please note that you can help to maintain and achieve a greater success by Publishing your high-quality research papers in IJABE, Reviewing papers for IJABE if requested, and Citing related papers published in IJABE as much as possible in your own publications (short for supporting IJABE via PRC), and also by encouraging your colleagues, students and friends to do so. You are invited to submit your next high quality manuscript (in particular review papers) to IJABE.

IJABE covers but is not limited to the following 10 technical disciplines:

1 Applied Science, Engineering and Technology (ASET)
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3 Biosystems, Biological and Ecological Engineering(BBEE)
4 Power and Machinery Systems (PMS)
5 Natural Resources and Environmental Systems (NRES)
6 Information Technologies, Control Systems and Sensors (ITCSS)
7 Renewable Energy and Material System (REMS)
8 Agro-product & Food Processing Systems (AFPS)
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Section Policies

Editorials

Editors

- Wang Yingkuan

☑ Open Submissions ☑ Indexed ☐ Peer Reviewed

Invited Review/Research Article

Only papers invited by editors can be included in this section.

☑ Open Submissions ☑ Indexed ☑ Peer Reviewed

Applied Science, Engineering and Technology

Applications of physical and mathematical techniques to fundamental investigations and emerging areas within the physical and life sciences; How to use engineering methods and the principles of biology to make the most of natural resources and to solve problems related to agriculture, aquaculture, forest, and environment. Major areas of emphasis are in applied physics, engineering sciences, engineering basics, biomaterial sciences, Biology, biological engineering fundamentals, bioprocessing, biological kinetics, biosystem modeling, and bioelectronics, chemistry, chemical engineering, heat/mass transport, and other types of engineering, hydrology and ecological engineering, technology for improving environmental quality, engineering for sustainability.

☑ Open Submissions ☑ Indexed ☑ Peer Reviewed

Animal, Plant and Facility Systems

Encompasses environment within structures for animal housing, plant production, and commodities storage; environmental air quality; climatic and meteorological data for engineering design, Environment of animal and plant structures, design and management of environmental control systems for animal production facilities; analysis, design, and construction of agricultural structures, imposed loads on structures, and materials of construction, climate control technologies (heating, cooling, and dehumidification), and control strategies for plant production under protective structures, i.e.: greenhouses, plant growth chambers, plant factories, and high tunnels; environment air quality; facilities & systems for human and animal housing, agricultural waste management, pollution source and emission, and animal care; Beef and dairy facilities and systems; milk handling equipment and systems for on-farm processing of milk and milk products; Swine housing, manure management and environmental control strategies for improved production efficiency and enhanced animal well-being and welfare ; poultry housing including environmental control strategies for improved production efficiency and enhanced animal well-being ; animal welfare and care; Electrical Code for Agriculture -development of practical electrical codes and standards for safety in electrical wiring systems used in agricultural production and processing. Plant growth LED lighting, Electric fence controller.

☑ Open Submissions ☑ Indexed ☑ Peer Reviewed

Power and Machinery Systems

Design and manufacturing, Mechanization and automation, Man-machine system interaction, Precision agriculture and emerging technologies, application of agricultural fans,standby electric power; control and/or communications network systems for mobile agricultural equipment; electronics as applied to animal identification and tracking; wireless communications in agricultural applications; agricultural machinery common tests; testing procedures for positioning & guidance systems in agricultural applications; agricultural tractor and self-propelled implement cabs used during the application of pesticides; safety and comfort of the operator(s) of agricultural machinery; agricultural equipment braking, agricultural loaders; mobile agricultural equipment lighting and marking; engineering technology for agricultural pest control and fertilizer application; ground application of liquid materials; agricultural aviation, aerial application of dry and liquid materials; development, evaluation and implementation of engineering technology for agricultural pest control and fertilizer application by ground application of dry materials. computer modeling of spray application; engineering technologies for agricultural harvest and grain harvesting systems, mechanical aspects of harvesting, handling, storing and processing of forages and biomass and derivatives there from; all engineering aspects of cotton production, harvesting, handling and processing; soil-machine and soil-plant dynamics; design of components for agricultural tractors and self-propelled machines; all engineering aspects of specialty crop production, post-harvest operations, storage and distribution; engineering for all turf and landscape equipment systems. Tractor & Implement Hydraulics, Tractor Implement Interface/PTO-implement and implement to implement mechanical interfaces, PTOs, and general hitching issues; Cultural Practices Equipment- engineering technology for tillage, planting, seeding, and other cultural practice equipment for field crop production. Agricultural Machinery Management & Logistics- efficient and profitable production systems. Precision Agriculture-development, evaluation, and adoption of technologies and systems for use in precision agriculture. Agricultural Equipment Automation-Collects and disseminates technical information on the automation of agricultural equipment.

☑ Open Submissions ☑ Indexed ☑ Peer Reviewed

Natural Resources and Environmental Systems

☑ Open Submissions ☑ Indexed ☑ Peer Reviewed

Information Technology, Sensors and Control Systems

Artificial intelligence, Advanced sensing technology, Biosensors and system control, Computer aided systems, Application of GIS, GPS, and RS in agriculture; Computational Methods, Simulations & Applications-development and application of computational methods and simulations for addressing current issues in agricultural and biological engineering; Biosensors- biosensor technologies and their applications in bioprocessing, food processing, food safety and quality, bioenvironmental and agricultural systems; Machine Vision-development and application of robotics, machine vision, image processing, and pattern recognition technology; Mechatronics & Biorobotics- development and application of mechatronics, robotics and automation with respect to agriculture, food and biological systems; Electromagnetics & Spectroscopy-Maintains contact with scientific developments in sonic, ultrasonics, electromagnetics, spectroscopy, and particle radiation for applications in engineering for agriculture and biological systems; Instrumentation & Controls- instrumentation and controls for application in engineering research and production; Emerging Information Systems-Development and application of information technologies, database management systems, information retrieval, electronic publishing, software development, internet applications, networking, machine learning, data analytics, cloud-based computing and hand-held/wireless device applications.

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Biosystems, Biological and Ecological Engineering

Biosystems Engineering is a field of engineering which integrates engineering science and design with applied biological, environmental and agricultural sciences. It represents an evolution of the Agricultural Engineering discipline applied to all living organisms not including biomedical applications. Therefore, Biosystems Engineering is the branch of engineering that applies engineering sciences to solve problems involving biological systems.

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Renewable Energy and Material Systems

Innovative energy sources, Renewable energy technologies, Biomass production, handling, and utilization, Energy efficiency and conservation, and solar energy technology related to agriculture. Techno-economic Feasibility and Sustainability- technical and economic feasibility and sustainability issues for energy projects; Renewable Power Generation-alternative energy sources and conversion systems for renewable heat and power generation; Biomass Energy & Industrial Byproducts-development, analysis, implementation, and dissemination of bio-based energy, fuels

and products; Solid Biofuels- Thermally Treated Solid Biofuels; Electrical Utilization & Energy Application - electrotechnology related to agriculture energy efficiency, electrical wiring systems, and electrical utility programs; application of agricultural lighting systems. Renewable materials. Biomass and feedstocks utilization: bioconversion of agro-industrial residues; biological waste treatment ; Thermochemical conversion of biomass: combustion, pyrolysis, gasification, catalysis.

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Agro-product and Food Processing Systems

May include post-harvest handling and storage, healthy food technology, processing engineering (food and biological), Imaging and sensing technology, food safety and security, biomaterials and bio-chemicals, crop handling, storage, drying, quality, processing equipment and methods. Produce primary commodities, and subsequently handle, process, transport, market and distribute food and other agro-based products to consumers; domestic and global agro-food value chain; production, processing and inspection of solely food products such as grain, dairy, coffee, fruit, vegetables.

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Structures and Bio-environmental Engineering

Encompasses environment within structures for animal housing, plant production, and commodities storage; environmental air quality; climatic and meteorological data for engineering design, Environment of animal and plant structures, design and management of environmental control systems for animal production facilities; analysis, design, and construction of agricultural structures, imposed loads on structures, and materials of construction, climate control technologies (heating, cooling, and dehumidification), and control strategies for plant production under protective structures, i.e.: greenhouses, plant growth chambers, plant factories, and high tunnels; environment air quality; facilities & systems for human and animal housing, agricultural waste management, pollution source and emission, and animal care; Beef and dairy facilities and systems; milk handling equipment and systems for on-farm processing of milk and milk products; Swine housing, manure management and environmental control strategies for improved production efficiency and enhanced animal well-being and welfare ; poultry housing including environmental control strategies for improved production efficiency and enhanced animal well-being ; animal welfare and care; Electrical Code for Agriculture -development of practical electrical codes and standards for safety in electrical wiring systems used in agricultural production and processing. Plant growth LED lighting, Electric fence controller.

☑ Open Submissions ☑ Indexed ☑ Peer Reviewed

Safety, Health and Ergonomics

All engineering aspects of ergonomic human safety and health for users of equipment systems and facilities in agriculture; agricultural safety and health, food security and safety, life cycle analysis

☑ Open Submissions ☑ Indexed ☑ Peer Reviewed

Overview Articles

Overview, either research review or literature review papers submitted by authors voluntarily, can be included in this section.

☑ Open Submissions ☑ Indexed ☑ Peer Reviewed

Expert Forum

Editors

- Wang Yingkuan

☑ Open Submissions ☑ Indexed ☐ Peer Reviewed

Science News

Editors

- Wang Yingkuan

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Special Column of Orchard Information System

This special column is only used to publish cooperated papers from an international conference. The regular submission should not go to this section.

Editors

- Wang Yingkuan

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Color Insert Pages

Color pages of figures, maps and other graphs from papers will be printed in order to be more clear and listed in this section.

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Special Section of SWAT-related Submissions

IJABE publishes a special issue or special column of SWAT papers presented in SWAT Conferences or free submissions.

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AUS SEAG 2013 Special Issue

We will publish a special issue online only after getting all the papers ready, which come from SEAG 2013 conference proceedings.

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Perspective and Insight

Perspective articles are intended to provide a forum for authors to discuss models and ideas from a personal viewpoint. They are more forward looking and/or speculative than Reviews and may take a narrower field of view. They may be opinionated but should remain balanced and are intended to stimulate discussion and new experimental approaches.

Perspectives follow the same formatting guidelines as Reviews. Both are peer-reviewed and edited substantially by IJABE's editors in consultation with the author.

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Special Column

IJABE publishes a special issue or special column .

☑ Open Submissions ☑ Indexed ☑ Peer Reviewed

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All manuscripts will be critically reviewed by the editor and invited referees within 2 months. All manuscripts submitted to IJABE are peer-reviewed according to the following procedure:

Initial review: A Division Editor evaluates all manuscripts sent his/her division to determine if submitted manuscripts are appropriate for consideration by IJABE. Manuscripts that do not meet the minimum criteria are returned to the authors within one week of receipt. This is in the best interest of the authors who could then decide to fix the problems or to submit the manuscript to a more appropriate venue, avoiding delay caused by a lengthy review process that would nonetheless lead to rejection.

Peer review: Manuscripts passing the initial review are assigned to an Associate Editor, who selects and invites two reviewers based on their expertise in the particular field. A manuscript is reviewed by at least two reviewers. Reviewers are asked to evaluate the manuscript based on

- Material is original and timely
- Writing is clear
- Study methods are appropriate
- Data are valid
- Conclusions are reasonable and supported by the data
- Information is important
- Topic has general engineering interest and practical significance

To facilitate timely publication, reviewers are asked to complete their reviews within one month. If the two reviewers have very different opinions on the manuscript, the Associate Editor or Division Editor's review will weigh in. After collecting the referees' reports, the Associate Editor makes a recommendation on the acceptability of the manuscript to the respective Division Editor.

Recommendation: Based on the reviewers' comments and the Associate Editor's recommendation, the Division Editor makes a final decision on the acceptability of the manuscript, and communicates to the authors the decision, along with reviewers' reports. The decision can be:

- accept as is
- minor revision
- major revision
- reject

A revised manuscript should be re-submitted within six months of the decision. It will usually be returned to the original reviewers for evaluation.

A rejection decision is made typically because the manuscript does not meet the criteria outlined above such as originality, importance to the field, cross-discipline interest, or sound methodology.

If the Division Editor and Associate Editor have conflicting opinions on a manuscript, they will seek consultation with the Editor in Chief who may make the final decision.

Honest and Polite
After each round of the review, review reports will be sent to the author(s) and all reviewers of the manuscript under consideration. It is important for a reviewer to be honest but not offensive when providing comments. Review reports with opinions expressed in a kind and constructive way will persuade the authors of the merit of the review more effectively.

Writing the Review
The purpose of the review is to provide the editors with an expert's opinions on the quality of the manuscript under consideration. A good review report should identify both the strengths and the weaknesses of the paper, and should also provide constructive and specific comments on how to improve the paper. If the reviewer believes that the paper is not suitable for publication in IJABE, the review report should provide brief but sufficient information that enables the author(s) to understand the reasons for the decision.

Publication Frequency

Two issues in 2008. First issue in August and second in December in 2008.

Quarterly from 2009 to 2013.

Currently, IJABE is Biomonthly from 2014.

It may be Monthly in the near future.

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IJABE JIF=1.349, ranking 6 out of 13, locating Q2 according to JCR2018.

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IJABE International Journal of Agricultural and Biological Engineering

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Chitinase activity potential and identification of chitinolytic bacteria isolated of swimmer crab's cell

Rieny Sulistijowati, . Sudin, Rita Marsuci Harmain

Abstract

This study aimed at investigating the chitinase enzyme activity produced by chitinolytic bacteria from the skin of blue swimmer crab (Portunus pelagicus) and identification of the genus isolate. This study consists of two stages: firstly, the qualitative and quantitative activity of the chitinase enzyme; and secondly, biochemical identification of the bacteria. The quantitative chitinase enzyme activity is measured using the UV-Vis spectrophotometer UV-Vis at the wavelength at 660 nm. The chitinase enzyme is obtained from the isolation of chitinolytic bacteria cultured within a media to grow solid chitin, which contains colloidal chitin substrate as chitinase inductor at the temperature of 30°C. The highest chitinolytic activity is obtained from the 24 h supernatant culture, with a value of enzyme activity at 0.149 U/mL. Macroscopic and microscopic identification showed that the chitinolytic bacteria isolate R1, whereas the biochemical cell shows the characteristics of the genus Pseudomonas.
Keywords: biodegradable, chitinase, spectrophotometer, Portunus pelagicus, Pseudomonas
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Chitinase activity potential and identification of chitinolytic bacteria isolated of swimmer crab's cell

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Abstract: This study aimed at investigating the chitinase enzyme activity produced by chitinolytic bacteria from the skin of blue swimmer crab (*Portunus pelagicus*) and identification of the genus isolate. This study consists of two stages: firstly, the qualitative and quantitative activity of the chitinase enzyme; and secondly, biochemical identification of the bacteria. The quantitative chitinase enzyme activity is measured using the UV-Vis spectrophotometer UV-Vis at the wavelength at 660 nm. The chitinase enzyme is obtained from the isolation of chitinolytic bacteria cultured within a media to grow solid chitin, which contains colloidal chitin substrate as chitinase inductor at the temperature of 30 ℃. The highest chitinolytic activity is obtained from the 24 h supernatant culture, with a value of enzyme activity at 0.149 U/mL. Macroscopic and microscopic identification showed that the chitinolytic bacteria isolate R1, whereas the biochemical cell shows the characteristics of the genus *Pseudomonas.*

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1 Introduction

The earth chitin is among the most abundant biomass present. Chitinase plays an important role in the decomposition of chitin and potentially in the utilization of chitin as a renewable resource. The implementation of biotechnology toward chitin, which keeps progressing, is the utilization of enzymes from microorganisms for biodegradation. In biodegradation, an enzyme derived from microorganisms breaks large molecule or chitin polymer into utilizable products. In general, types of an enzyme that degraded the chitin are chitinase enzyme[1].

Microorganisms that degraded chitin, in general, are those derived from bacteria group. Chitinase enzyme produced by chitinolytic bacteria has the potential to degrade chitin due to the existence of the chitinase enzyme, which enables the conversion of abundantly available chitin into usable products. The bacteria that produce chitinase enzyme or chitinolytic bacteria can be found within the habitat that contains a high level of chitin, such as in the cell of the blue swimmer crab. Blue swimmer crab's cell (*Portunus pelagicus*) can be obtained from the processing waste or fresh. Chitinase enzyme application can be informed of enzymatic production of chitin. The chitin can be produced enzymatically and chemically. The enzymatic method uses enzymes or bacteria for deproteinization by adding enzyme or by the involvement of chitinase to degrade chitin. Meanwhile, the chemical process is through demineralization by adding acid or alkali, such as HCl and NaOH[2].

Arbia et al.[3] isolate chitinolytic bacteria to produce several bacteria, one of which was *Pseudomonas aeruginosa* bacteria isolated from crab's cell. The production of chitinase enzyme from *Aspergillus niger* utilized the blue swimmer crab's waste as inducer[4]. Protease produced by *Pseudomonas aeruginosa* K-187, the highest protease activity was as high as 21.2 U/mL, 10-fold that (2.2 U/mL) obtained before optimization[5]. In common with all enzymes, external factors such as temperature, pH and type of media are important for the activity, catalytic efficiency, stability and proper functioning of proteases[6]. Chitinase activity of isolates chitinolytic bacteria can degrade different chitins.

The needs for the chitin derivatives are increasing. Thus, researches on chitinase enzyme activity through the isolation of bacteria from the blue swimmer crab is needed. Two isolates chitinolytic bacteria from the skin of blue swimmer crab were observed primarily in Katialada village of Kwandang sub-district of North Gorontalo Regency, Gorontalo Province of Indonesia. The results obtained from the purification process following 48 h incubation in the temperature of 30 ℃ shows a clear zone that formed in the colony of the bacteria[7]. However, specific zones are only found in white and light brown-colored bacteria.

Furthermore, these two isolates are macroscopically and microscopically identified. The R1 isolate shows a white-colored colony; meanwhile, the R2 isolate shows a light brown-colored colony. Meanwhile, from the shape/form and elevation of the colony, there are no differences between isolates R1 and R2, both colonies have a circular shape and convex elevation. The result of gram staining in these two chitinolytic bacteria isolates R1 and R2 shows the gram-negative result. This is signified by changes of color of these two isolates into the red after the gram staining. The study shows that CI 11 of the R1 isolate has the largest Chitinolytic Index by one, the chitinolytic index shows the ability of the microbes to degrade chitin. The more enzyme produced, the wider the clear zone produced as more chitin is degraded.

Received date: 2019-12-25 **Accepted date:** 2020-06-29

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This is due to the type of bacteria growth pattern and enzyme activity needs to be known to have a good degrading ability. Therefore, this study aimed at testing the produced chitinase activity and identification chitinase producer bacteria of blue swimmer crab.

2 Methods and materials

2.1 Station and laboratory

Isolate R1 was obtained from fresh blue swimmer crab's cells which came from the crabs' farmer in Katialada village of Kwandang sub-district of North Gorontalo Regency, Gorontalo Province of Indonesia. The identification biochemical test of the chitinolytic bacteria were carried out at the Fish Quarantine Station Laboratory Quality Control and Fisheries Product Security Class I Gorontalo Province of Indonesia, and the chitinolytic bacteria enzyme activity test are carried out at the Pharmaceutical Laboratory of Universitas Negeri Gorontalo.

2.2 Materials

The tools used in this study were test tube, inoculum needle, vortex, measuring cup, beaker glass, Erlenmeyer, centrifuge, centrifuge tube, shaker, pH meter, micropipette, micro tip, stirrer, magnetic stabilizer, petri dish, oven, crooked spoon, Bunsen lamp, incubator, laminar air, analytical scales, UV-vis spectrophotometer, and microscope.

Materials used in this study were R1 isolated of blue swimmer crab's cell, chitin, chitin colloidal (chitin, condensed HCl, NaOH, distillate water), chitin agar (chitin colloidal, KH_2PO_4, $MgSO_4$ $7H_2O$, yeast extract, agar, distillate water), Luria broth (yeast extract, tryptone water, NaCl, distillate water), nutrient agar, aluminum foil, alcohol, crystal violet, iodine solution, glucose, sucrose, lactose, maltose, mannitol, triple sugar iron agar, motility indole ornithine, oxidative/fermentative, methyl red-Voges Proskauer, methyl-red, and sterile liquid paraffin.

2.3 Quantitative activity of chitinase enzyme test

One dose of inoculum was added into 100 mL of production medium (similar composition to a solid medium, but without agar) then incubated in the temperature of 30 ℃ and centrifuged in the speed of 170 r/min. Every three hours, 2 mL of cell culture was sampled for 33 h. Then centrifuged in the temperature of 4 ℃ using 10.000 r/min speed for 10 min, the formed supernatant was the raw extract of chitinase enzyme. The absorbance is then measured using spectrophotometer UV/Vis in wavelength of 660 nm[8], the sample was carried out twice repetitions.

2.4 Biochemical test

The biochemical test was carried out to identify and classify bacteria into their group of taxonomy. The principle of this biochemical test is that if the bacteria are cultured in several media, the bacteria show macroscopic differences in their growth[9,10]. Carbohydrate fermentation test was to find out the bacteria's ability in fermenting carbohydrate by preparing the carbohydrate broth which consists of glucose, sucrose, maltose, and mannitol; MR-VP (methyl red-Voges Proskauer) test was to inoculate bacteria into a medium, which incubated in the temperature of 30 ℃ for 24 h by adding methyl red reagent and KOH, to observe the bacteria ability in producing the mix acid and acetylenes; Citrate test was inoculating bacteria into a medium and incubated in the temperature of 30 ℃ for 24 h by adding bromothymol blue (BTB) reagent, then the ability of the bacteria to use citrate as the only source of carbon was observed; H_2S test was to inoculate the bacteria into sulfide indole motility (SIM) which incubated for 24-48 h in the temperature of 30 ℃, then the ability of the bacteria to produce H_2S which signified by the existence of black sediment was observed; Oxidation/fermentation (O/F) test was inoculating the bacteria into the O/F medium, which incubated for 24 h in the temperature of 30 ℃, then observe the ability of the bacteria to use carbohydrate through fermentation or oxidation; TSIA (triple sugar iron agar) test was inoculating bacteria into TSIA media, which incubated for 24-48 h in the temperature of 30 ℃, then the ability of the bacteria in fermenting glucose, lactose, and sucrose was observed; Indole test was inoculating bacteria into indole media, which incubated for 24 h in the temperature of 30 ℃, then the ability of bacteria in degrading the tryptophan amino acid in the medium was observed.

3 Results and discussion

3.1 Quantitative activity of the chitinolytic bacteria enzyme

The activity of the chitinolytic bacteria enzyme is essential to be known to find out the ability of the bacteria to produce the enzyme in 33 h with an interval of 3 h. As shown in Figure 1, there were several increasing and decreasing stages in chitinase activity. The first inclination happened during the incubation time of 0 to 12 h. The second increase of the enzyme activity showed that the substrate was starting to be hydrolyzed to produce the chitinase enzyme. Hence, bacteria could digest nutrition. Patil et al.[11] found that bacteria produce extracellular chitinase to take on nutrition. Following this inclination, there is the first declining phase on the 15th to the 18th hour of incubation. The decrease of this enzyme activity is due to other compounds (aside from N-Acetyl glucosamine) that triggers the decrease of enzyme production.

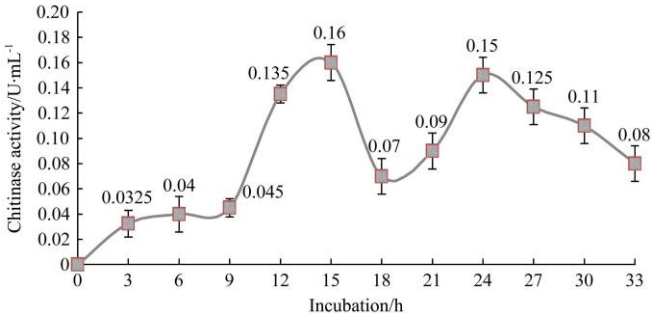

Figure 1 Chitinase activity curve from *Pseudomonas*

This phenomenon is due to the existence of other chitin-degrading enzymes produced by the bacteria. Colloidal chitin also can be hydrolyzed by deacetylating chitin produced by chitosan and chitosanase which produce chitobiose[12]. Following this declining phase, the chitinase activity climbs up in the incubation time of the 21st hour to its highest chitinase activity can be obtained from the supernatant culture in the incubation of time of the 24th hour, which stated with the value of enzyme activity of 0.149 U/mL. One unit of chitinase enzyme activity is defined as several enzymes needed to release 1 mmol NAG/min. This result is different from the result of the enzymes activity test carried out by Purkan et al.[8] who found that the highest enzyme activity was in the 18th hour of incubation time, which stated with the value of enzyme activity of 0.3850 U/mL. Moreover the ability of the bacteria to produce chitinase highly varied. Factors such as different types of bacteria, the growth rate of each isolate in the medium, temperature, pH or laboratory treatment during the experiment can be factors that influence variation in the produced enzyme activity[13,14]. Chitinase activity was 0.213 and 0.219 U/mL respectively of PBK 2 and SA 1.2 isolates from shrimp waste. Based on 16S rDNA sequences, isolate of PBK 2 was identified as *Acinetobacter*

johnsonii 3-1, whereas SA 1.2 was identified as *Bacillus amyloliquefaciens* GR53 with 99.78% similarity[15].

The rebound of enzyme activity shows that there is more of the substrate being hydrolyzed. The chitinase enzyme activity is steadily increasing until it reaches optimum incubation time. Following the reach of this optimum incubation time, the enzyme activity decreases due to the accumulation of hydrolyzed products, which can further inhibit the enzyme activity. This is characterized by the decrease of enzyme activity on the incubation time of hour 27 to hour 33. The decrease of chitinase enzyme activity after the optimum incubation time is due to the changes in the state of the enzyme ion and the state of substrate ion which caused denaturation of enzyme which followed by the loose of enzyme catalytic activity[12]. Besides, there are also change in the tertiary structure of the enzyme due to denaturation, which made the hydrophobic amino acid group within the enzyme come into contact with water, thus, the solubility of the enzyme weakens. The decrease of chitinase solubility causes a gradual decrease in enzyme activity.

Chitinolytic bacteria isolates showed unstable chitinase activity (fluctuate). This may be due to the isolate that produces the chitinase at the beginning of its growth[13]. In line with the utilization of nutrition for growth, it is also suspected that chitinase is also used by bacteria as a source of protein, thus its chitinase activity decreases.

The decrease of enzyme activity can also be caused by factors such as temperature, pH, substrate and biomass during treatment in the laboratory. The temperature has two main influences on the reaction and the denaturation. The influence of reaction toward the enzyme is that the increase of temperature will accelerate the reaction process, while the decrease in the temperature will cause the reaction to slow down. When the temperature reaches a certain limit, it will cause denaturation. Besides, when the pH of the environment is too acid or base, enzyme denaturation can also happen. Reaction speed catalyzed by the enzyme is highly influenced by substrate concentration. In the low level of substrate concentration, reaction speed by catalyzed by the enzyme can also be very low. In reverse, reaction speed will increase along with the increase of substrate concentrate up to certain points that is the maximum reaction speed limit. When this saturated point of the enzyme has been reached, it will not function properly. Lastly, the number of bacteria inoculum (biomass) inserted into the media also strongly influence the enzyme activity.

3.2 Biochemical test

The biochemical test toward the characteristics of chitinolytic bacteria was carried out by fermenting bacteria in the various sources of nutrition as shown in Figure 2. The biochemical test result of chitinolytic bacteria is presented in Table 1.

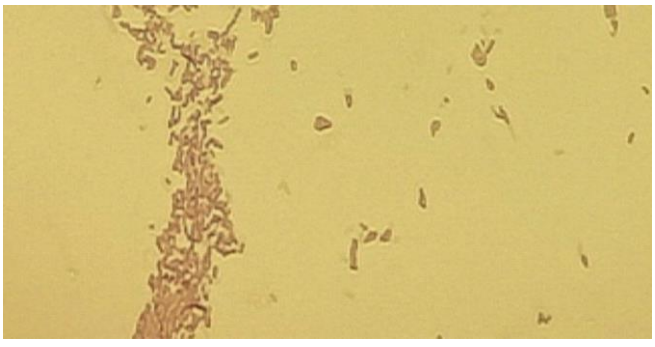

Figure 2 Isolate R1 (10×100 magnification)

Table 1 Biochemical characteristics of chitinolytic bacteria isolate R1

No	Test	Results
1	Glucose fermentation	Negative
2	Sucrose fermentation	Negative
3	Lactose fermentation	Negative
4	Maltose fermentation	Negative
5	Mannitol fermentation	Negative
6	Citrate use	Negative
7	Sulfide indole motility	Negative
8	Triple sugar iron agar	Alkaline/Alkaline
9	Methyl red reaction	Negative
10	Voges Proskauer reaction	Negative
11	Indole production	Negative
12	Oxidase/Fermentative action	Negative

Fermentation test in several types of carbohydrate (glucose, maltose, sucrose, mannitol, lactose) shows that all fermentation reaction is negative. This is characterized by the unchanging red color of the carbohydrate media. When the color of the medium in the carbohydrate test turns into yellow, it means that the colony forms acid from that carbohydrate[10].

A citrate test was carried out to find out the ability of the chitinolytic bacteria isolates to utilize citrate as the only source of carbon and energy. When a microorganism can use citrate, there will be an increase of pH and change in the color of the media into blue color. In this study, the citrate test reveals that the chitinolytic bacteria cannot utilize citrate as the only source of carbon. This is shown by the unchanging green color of the media, which means that the test result is negative. Positive test results in the citrate test are shown when the color changes from green to blue[16].

Further, the H_2S test result in SIM is negative. This negative result is reached when microorganism has no ability to hydrolyzed heavy metal within the media. H_2S is produced by several types of microorganisms, which can break or degrade amino acid within the sulfur (S). The existence of H_2S can be observed by adding several crystals of heavy metals into the media.

The reaction observable in the TSIA test shows a red color, which means that there is no change of color in both vertical and slight agar. This indicates that the bacteria are unable to ferment sugar. In the vertical agar if the bacteria can ferment glucose, the color of the media will change from red to yellow[17]. Whereas in slight agar, if the bacteria can ferment lactose and sucrose, the color of the media will change into yellow, meanwhile, when there is no fermentation process of lactose and sucrose, the color will not change.

Methyl red test also reveals a negative result. This is shown by the unchanging color of the media which does not change into yellow even after the addition of methyl red reagent. The red colour signifies the positive test result, and if the color of the broth is yellow, then the result of the test is negative[16]. Similarly, the Voges Proskauer test also shows a negative result. This is evident after the addition of the KOH solution; the color does not change. The Voges Proskauer test will be stated as positive when there is a form of acid, which signifies by the changes of medium color into pink after the KOH solution is added. Meanwhile, the indole test also shows a negative result. This result is obtained after the reagent Kovac is added, which signify by the formation of a yellow ring. The existence of indole is detected by Kovac reagent and the formation of a red ring.

The objective of the oxidizing fermentative test is to find out the oxidation and fermentation characteristics of bacteria toward glucose. Based on the result of the study on the O/F test, it does not show either oxidation or fermentation. This is evident when the media, either without liquid paraffin or without paraffin at all, do not change color. Bacteria are said to be fermentative when both inoculated media change color into yellow. Bacteria are oxidative when tube sealed with no paraffin changes color into yellow and the tube sealed with paraffin does not change color[18].

The morphology test result (macroscopic and microscopic tests) of chitinolytic bacteria have bar cell and gram-negative[9]. The biochemical test of the chitinolytic bacteria consists of carbohydrate, citrate, sulfide indole motility, triple sugar iron agar, MR-VP, indole and O/F test should obtain negative results as indicators of a genus of Pseudomonas. Several studies to determine chitinolytic bacteria from the genus of Pseudomonas[4] that utilized blue swimmer crab's waste as an inducer to the production of chitinase enzyme from *Aspergillus niger*. A study by Arbia et al.[3] isolated chitinolytic bacteria to produce several bacteria, one of which was *Pseudomonas aeruginosa* bacteria isolated from crab's cell. Genus *Pseudomonas* generally has bar cell shape and gram-negative. A study by Wang et al.[19] isolated *Pseudomonas aeruginosa* K-187 known produced two bifungtional chitinase and lysozyme. Thompson et al.[20] found that *Pseudomonas aeruginosa s*train 385 produced chitinase antigen.

4 Conclusions

This study concludes chitinolytic bacteria isolated from blue swimmer crab's cell (*Portunus pelagicus*) genus of Pseudomonas. The highest chitinase activity was obtained from the supernatant culture in the 24th hour, in which enzyme activity value was 0.149 U/mL as a good potential to degradable chitin ability.

Acknowledgements

The authors would like to thank Fish Quarantine Station Laboratory Quality Control and Fisheries Product Security Class I Gorontalo Province of Indonesia, and Pharmaceutical Laboratory of Universitas Negeri Gorontalo support facility for this research.

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