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# SCREENING OF NUTRIENTS FOR THE PRODUCTION OF MYCO-COAGULANT FROM *Lentinus Squarrosulus* FOR WATER TREATMENT

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**ABSTRACT**

**Chemical coagulants have been continuously used for the treatment of water turbidity and suspended solids despite their harmful consequences on living beings. These problems obliged scientists in various parts of the world to investigate eco-friendly novel technologies. In this regard, a new Myco-coagulant is successfully produced by a group of researchers at the Bioenvironmental Engineering Research Centre of International Islamic University Malaysia. The present study assessed the nutrients level required to culture a previously isolated fungus from river water known as *Lentinus squarrosulus*. Synthetic water using kaolin suspension was prepared and used to study the removal of turbidity by the cultured Myco-coagulant using the flocculation mechanisms. Thus, the paper reported the experimental findings on nutrient screening and optimization of the suitable growth conditions and production of the fungus Myco-coagulant. The growth media that showed positive effects on the fungus growth according to Plackett–Burman design were yeast extract (11.2%), malt extract (6.0%), inoculum size (3.5%), and glucose (3.0%). However, agitation speed (-12.17%) had the most negative influence on Myco-coagulant growth, followed by urea (-7.67). Other growth conditions with negative effects includes; pH (-5.8%), culture time (-5.5%), and $CaCl_2$ (-5.0%). Nevertheless, yeast extract and agitation speed were selected as the major parameters for fungus growth optimization. In conclusion, the submerged fermentation of *Lentinus squarrosulus* using yeast extract as a nutrient demonstrates a better yield than malt extract. Moreover, urea and $CaCl_2$ could be excluded from the nutrient composition because of their insignificant contribution to fungal growth.**

**KEY WORDS :** *Lentinus squarrosulus*, Wastewater, Plackett-Burman Design, Turbidity removal.

## INTRODUCTION

Water security and clean water supply are some of the main issues encountered in developing nations. River water has been the most inclusive world source of freshwater for domestic, agricultural, and industrial usage. Though, high turbidity caused by fine colloidal particles in water is a typical problem relating to freshwater quality that makes it unpleasant for human consumption and other applications (Abdullah *et al.,* 2017; Robert *et al.,* 2016). Generally, water treatment cost rises with an increase in the turbidity level of the source water. The most specific source of high turbidity in rivers is storm runoff during high rainfall. As such, the rivers in the tropical region are more vulnerable to excessive turbidity levels due to soil erosion induced by heavy downpours.

Malaysia, which is a tropical country, is one of the countries affected by high river water turbidity. The utilization of inexpensive, efficient, and eco-friendly coagulants like *Lentinus squarrosulus* becomes essential in reducing the river water turbidity (Salehizadeh and Shojaosadati, 2001). The *Lentinus squarrosulus* fungus has the potential for the biodegradation of harmful environmental effluents (Adenipekun and Isikhuemhen, 2008). Similarly, *Lentinus squarrosulus* based biocoagulant shows better flocculating properties than the most commonly used chemical coagulant 'alum' (Jebun, *et al.,* 2016). Therefore, the *Lentinus squarrosulus* fungus can be adequately sourced as a low-cost biocoagulant precursor because it flourishes well on dead leaves and barks. The proximity analysis shows that the dried weight of the fungus contains of 60.65% of carbohydrate, 22.82% of crude protein, 7.52% of ash, 6.29% crude fat, and 2.76% of moisture content (Nwanze *et al.,* 2005).

Basically, six strains of the *Lentinus squarrosulus* fungi were isolated from river water (Sungai Pusu), and the strain (RWF-5) was selected for the current research due to its remarkable flocculation property. The study was to evaluate significant nutrients required for the growth of *Lentinus squarrosulus* Fungus in a submerged medium. Also, the optimum nutrients desired to provide maximum flocculation activity (FA) for the removal of turbidity from synthetic wastewater prepared from Kaolin suspension was determined.

## MATERIALS AND METHODS

Culture conditions affect Myco-coagulant production, and the conditions must be optimized for maximum coagulant production. The biocoagulant growth and yield are directly related to some significant parameters that might include; composition of the nutrients, temperature, pH, agitation speed, and the culture time. The materials and methods used are outlined sequentially in this section.

### Media Preparation

Firstly, the Potato Dextrose Agar (PDA) plate for fungi cultivation was prepared by homogenizing 39g of PDA powder and added into a litre of distilled water (Jebun *et al.,* 2016). The mixture was then autoclaved at 121 °C and 15 kPa pressure for a period of 15min. Afterward, the autoclaved liquid agar mixture was allowed to cool to a warm temperature before it was then poured into the Petri dishes for solidification. The solidified plates were sealed with a Laboratory-grade parafilm and stored at 4 °C for subsequent use.

### Production of Fungal Biomass

In the previous study, the wild strain of *Lentinus squarrosulus* fungus was collected from the Sungai pusu river and isolated at the Bioenvironmental Engineering Laboratory in IIUM (Jebun *et al.,* 2015). The pure culture of the fungus was obtained from the Lab and used in this experiment. A small incision with 1cm x 1cm dimension was made on a fungus pure culture plate using a sterilized sharp scalpel. Then, the fungus subculture was prepared by transferring the fungus mycelial incision onto a new fresh PDA plate, sealed with a parafilm, and incubated at 30 °C for 10–12 days. The lid was maintained on top of the agar along the incubation process.

### Experimental Methods and Analysis

The response surface methodology (RSM) is widely used for experimental conditions screening, characterization, and optimization (Bari *et al.,* 2009). In the present study, the Plackett-Burman design of the RSM was utilized and screened the growth media of the *Lentinus squarrosulus* in response to its flocculation activity. Table 1 illustrates the screening design medium components and process conditions for the production of myco-coagulant from *Lentinus squarrosulus*.

As a result of screening 11 independent variables of growth media, the design provided 12 experimental runs that were conducted in triplicate, and the average turbidity removal (%) was recorded as the design response (Table 2).

Therefore, to effectively manage the most significant factors, certain variables were eliminated because of their negligible influence on turbidity removal. The main effect for each factor (Table 1) is calculated based on Equation 1 to investigate the influence of the parameter on the production of Myco-coagulant.

$$\frac{\Sigma \text{ possitive effect} - \Sigma \text{ negative effect}}{12} \quad .. (1)$$

### Evaluation of flocculation Activity

The pH of the culture, the fungal supernatant volume, and the biomass weight were determined and recorded after five days of the fermentation process. The flocculation activity (FA) was tested

**Table 1.** The effect of medium components conditions

| Factors | Total (+) | Total (–) | Avg (+) | Avg (–) | Main (fx) |
|---|---|---|---|---|---|
| A: Yeast Extract | 384 | 250 | 32.0 | 20.8 | 11.2 |
| B: Malt Extract | 353 | 281 | 29.4 | 23.4 | 6.0 |
| C: Urea | 271 | 363 | 22.6 | 30.3 | -7.7 |
| D: Glucose | 335 | 299 | 27.9 | 24.9 | 3.0 |
| E: $CaCl_2$ | 287 | 347 | 23.9 | 28.9 | -5.0 |
| F: NaCl | 323 | 311 | 26.9 | 25.9 | 1.0 |
| G: pH | 282 | 352 | 23.5 | 29.3 | -5.8 |
| H: Temperature | 325 | 309 | 27.1 | 25.8 | 1.3 |
| J: Agitation Speed | 244 | 390 | 20.3 | 32.5 | -12.2 |
| K: Culture period | 284 | 350 | 23.7 | 29.2 | -5.5 |
| L: Inoculum size | 338 | 296 | 28.2 | 24.7 | 3.5 |

using both the supernatant and the fungus biomass. The flocculating activity was calculated according to Equation 2.

$$\text{Turbidity removal} = \left(\frac{A-B}{A}\right) \times 100 \qquad .. (2)$$

where, A the initial turbidity of kaolin suspension directly after preparation and B the final turbidity of kaolin suspension after the settling period, measured in Nephelometric Turbidity Unit (NTU).

## RESULTS AND DISCUSSION

The cultured fungi strains (RWF-1 to RWF-6) that were initially isolated from the River Pusu can be seen in Figure 1. The external appearance of RWF-1 and RWF-3 colonies appeared velvety and having a greenish powder aspect. However, the RWF-2 and RWF-4 colonies have net-like texture, and their surface colony colour was initially white but turned greyish while growing and finally turned to black at full maturity. But, the RWF-5 and RWF-6 colonies demonstrate white colour and texture similar to that of cotton. Comprehensive details on filamentous fungi have been reported in the literature (Alsohaili and Bani-Hasan, 2018; Iheanacho *et al.*, 2014; Pinto *et al.*, 2012).

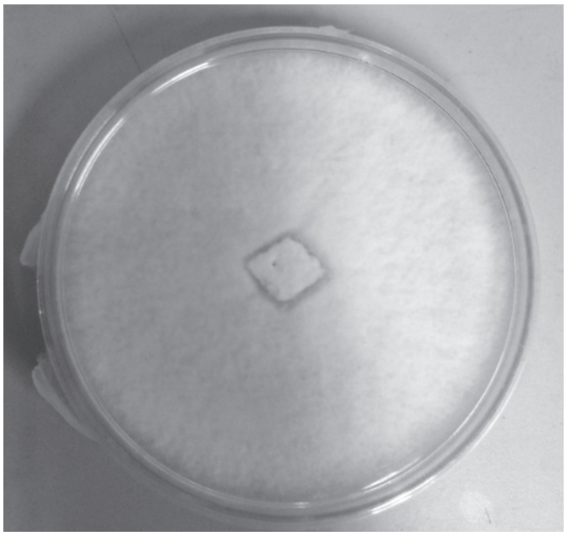

**Fig. 1.** Cultured *Lentinus squarrosulus*

The fungus nutritional broth was prepared by mixing malt extract, yeast extract, urea, glucose, NaCl, and $CaCl_2$ within the range of 0 to 1% (w/v). Then, the broth pH was adjusted within 5-8 by adding 0.1 M of NaOH or 0.1 M of HCl. Subsequently, 100ml of the broth was transferred into a 250 ml Erlenmeyer flask and secured with a sterile cotton ball and aluminum foil. Lastly, the broth was autoclaved at 121 °C for 15 minutes and allowed to cool to room temperature before use. Once the broth reaches room temperature, 3 ml mycelial suspension is transferred into the flask at a 3% ratio using an aseptic technique and fermented in a shaking incubator. The fermentation conditions such as; temperature (25– 35 °C), agitation speed (150–250 rpm), time (5–8 days), and inoculum size (2–4 %) were adjusted according to Plackett-Burman design. Figure 2 displayed the absolute growth of the fungal biomass in the liquid nutritional media.

As shown in Table 2, Run 8 provided the highest flocculating activity when the fermentation factors were at the highest design points; agitation speed (250 rpm), temperature (35 °C), culture time (8 days), and inoculum size (4%). A similar response of flocculating activity of 81% was obtained by Run 5 when glucose and $CaCl_2$ are not included in the media. Thus, the increase of glucose and sodium chloride by 1% (w/w) had no significant increase in the metabolite activity as well as urea and calcium chloride addition might also affect the fungal growth and subsequently reduced the metabolite activity of the fungus and flocculation of the kaolin

**Table 2.** Fungus growth variables and the respective responses developed using Plackett-Burman design

| Run | Yeast extract (%) | Malt extract (%) | Urea (%) | Glucose (%) | $CaCl_2$ (%) | NaCl (%) | pH | Temp (°C) | Agitation (rpm) | Culture period (day) | Inoculum size (%) | Response: FA (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 0 | 1 | 1 | 1 | 0 | 5 | 25 | 150 | 5 | 4 | 52 |
| 2 | 1 | 0 | 1 | 1 | 0 | 1 | 7 | 35 | 150 | 5 | 2 | 55 |
| 3 | 0 | 0 | 1 | 0 | 1 | 1 | 5 | 35 | 250 | 8 | 2 | 21 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 25 | 150 | 5 | 2 | 49 |
| 5 | 1 | 1 | 0 | 1 | 1 | 1 | 5 | 25 | 150 | 8 | 2 | 81 |
| 6 | 0 | 0 | 0 | 1 | 0 | 1 | 7 | 25 | 250 | 8 | 4 | 45 |
| 7 | 0 | 1 | 0 | 1 | 1 | 0 | 7 | 35 | 250 | 5 | 2 | 41 |
| 8 | 1 | 1 | 0 | 0 | 0 | 1 | 5 | 35 | 250 | 8 | 4 | 88 |
| 9 | 1 | 1 | 1 | 0 | 0 | 0 | 7 | 25 | 250 | 8 | 2 | 49 |
| 10 | 1 | 0 | 0 | 0 | 1 | 0 | 7 | 35 | 150 | 5 | 4 | 59 |
| 11 | 0 | 1 | 1 | 0 | 1 | 1 | 7 | 25 | 150 | 5 | 4 | 33 |
| 12 | 0 | 1 | 1 | 1 | 0 | 0 | 5 | 35 | 150 | 5 | 4 | 61 |

suspension (Ahmad *et al.*, 2013).

The agitation speed and the inoculum size of Run 5 were low compared to Run 8. It could be possible considering that the fungal metabolites increases with the inoculum size up to an amount of 2 g/kg (Benluvankar *et al.*, 2015). Higher inoculum to substrate ratio would reduce the enzyme yield since the mycelium had rapidly consumed most of the substrate for their growth and essentially decreasing enzyme synthesis (Carlile, Watkinson and Gooday, 2001).

Generally, a decrease in protease amount was observed with higher inoculum concentration, which could be due to the fast depletion of nutrients since biomass is far greater than the available nutrients (Sandhya *et al.*, 2005). Moreover, a higher inoculum concentration could also result in improper distribution of oxygen in the culture that might result in cell replication shortfall (Rahman *et al.*, 2005). Accordingly, urea, glucose, and calcium chloride were the most desirable nutrient composition of the media to be excluded because of their insignificant contribution to fungal growth.


**Fig. 2.** *Lentinus squarrosulus* growth in liquid media

Therefore, yeast extract and agitation speed are the two parameters selected by the Plackett–Burman design (Table 2) as significant factors for Myco-coagulant production with optimum flocculation activity (Figure 3).

The addition of 1% yeast extract in the fermentation media had provided a positive effect on the fungus growth by 11.17% because yeast extract had been used as a nitrogen source to support some wooden fungal growth (Ahmad *et al.*, 2013). In another study, 0.3% yeast extract was used in the culture media to grow *Sphingomona spaucimobilis* for gellan gum production (Banik *et al.*, 2007). Generally, the increase in the yeast extract of the *Lentinus squarrosulus* growth media was found to boost the mycelium biomass production (Ahmad *et al.*, 2013).

Similarly, the media temperature has a significant influence on Myco-coagulant fermentation. Previously, it was reported that the optimum temperature for the growth of *Aspergillus terrreus* for protease production was 38 °C (Wu, Mohammad, Jahim and Anuar, 2009). However, a lower temperature of 32 °C has shown to be adequate to culture *Aspergillus flavus* optimally (Malathi and Chakraborty, 1991).

The agitation speed exhibits the highest negative effect on Myco-coagulant production. However, maintaining the process at a lower rate of 150 rpm

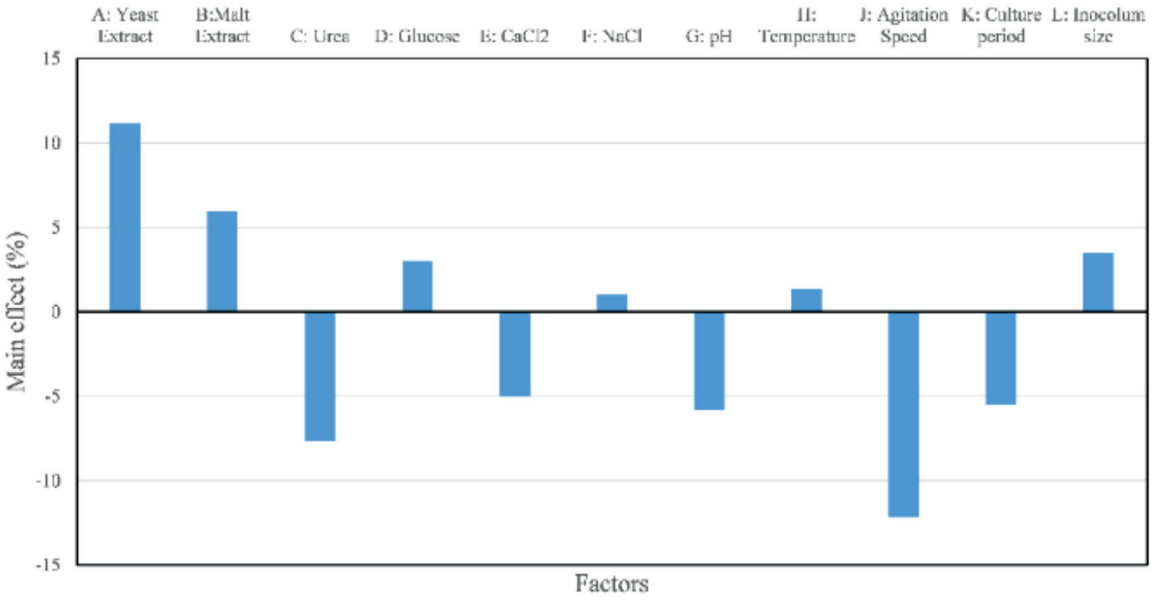


**Fig. 3.** Effect of each factor in respect to the medium

enhances the flocculating activity of the metabolite. The advantage of proper aeration and nutrients distribution in the growth media at a higher speed was remarkable. However, the flocculating activity of the Myco-coagulant drastically reduces due to cell damage caused by shear forces at a higher stirring speed (Ibrahim, 2015). Besides, shear stress has become the major factor that affects cell morphology and secondary metabolite in filamentous organisms cultivation (Xia *et al.*, 2014). It was reported in the literature that moderate shearing stress due to stirring at 120 rpm has an insignificant impact on the immobilized beads that causes rupturing or denaturalization of extracellular enzymes (Shide *et al.*, 2004).

As shown in Table 2, the acidic condition caused a negative effect on Myco-coagulant flocculating activity. Usually, *Lentinus squarrosulus* has proved to grow in a neutral pH better (Tripathi, 2011). Accordingly, the inoculum size of 2%, fermented for five days, provides an optimum growth of *Lentinus squarrosulus.*

## CONCLUSION

In conclusion, the present research demonstrated that nutrient type and agitation speed were the most significant factors that affect the liquid fermentation *of Lentinus squarrosulus* in shake flasks. Furthermore, the result shows that the addition of 1% yeast extract and malt had positive effects on the flocculating activity of the metabolite produced. Though, yeast extract contributed a better flocculating activity than malt extract. Other nutrients such as urea, glucose, sodium chloride, and calcium chloride have an insignificant effect on Myco-coagulant growth. Therefore, the growth of *Lentinus squarrosulus* in a yeast extract liquid media can be affected by agitation speed, temperature, the culture period, pH, and inoculum.

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## REFERENCES

Abdullah, A. M., Hamidah, H. and Alam, M. Z. 2017. Research progress in bioflocculants from bacteria. International Food Research Journal.

Adenipekun, C. O. and Isikhuemhen, O. S. 2008. Bioremediation of engine oil polluted soil by the tropical white rot fungus, *Lentinus squarrosulus* Mont. (Singer). *Pakistan Journal of Biological Sciences.* https://doi.org/10.3923/pjbs.2008.1634.1637.

Ahmad, R., Al-Shorgani, N. K. N., Hamid, A. A., Wan Yusoff, W. M. and Daud, F. 2013. Optimization of medium components using response surface methodology (RSM) for mycelium biomass and

exopolysaccharide production by <i> *Lentinus squarrosulus* & lt;/i> *Advances in Bioscience and Biotechnology*. https://doi.org/10.4236/abb.2013.412144.

Alsohaili, S. A. and Bani-Hasan, B. M. 2018. Morphological and molecular identification of fungi isolated from different environmental sources in the northern eastern Desert of Jordan. *Jordan Journal of Biological Sciences.*

Banik, R. M., Santhiagu, A. and Upadhyay, S. N. 2007. Optimization of nutrients for gellan gum production by Sphingomonaspaucimobilis ATCC-31461 in molasses based medium using response surface methodology. *Bioresource Technology.* https://doi.org/10.1016/j.biortech.2006.03.012.

Bari, M. N., Alam, M. Z., Muyibi, S. A., Jamal, P. and Abdullah-Al-Mamun. 2009. Improvement of production of citric acid from oil palm empty fruit bunches: Optimization of media by statistical experimental designs. *Bioresource Technology.* https://doi.org/10.1016/j.biortech.2009.01.005.

Benluvankar, V., Jebapriya, G. R. and Gnanadoss, J. J. 2015. Protease Production By *Penicillium* sp. Lcj228 Under Solid State Fermentation Using Groundnut Oilcake As Substrate. *International Journal of Life Science & Pharma Research.*

Carlile, M. J., Watkinson, S. C. and Gooday, G. W. 2001. *The Fungi.* 2nd. Science Direct, Academic Press, San Diego.

Ibrahim, D. 2015. Effect of agitation speed on the morphology of *Aspergillus niger* HFD5A-1 hyphae and its pectinase production in submerged fermentation. *World Journal of Biological Chemistry.* https://doi.org/10.4331/wjbc.v6.i3.265.

Iheanacho, H. E., Njobeh, P. B., Dutton, F. M., Steenkamp, P. A., Steenkamp, L., Mthombeni, J. Q. and Makun, A. H. 2014. Morphological and molecular identification of filamentous *Aspergillus flavus* and *Aspergillus* parasiticus isolated from compound feeds in South Africa. *Food Microbiology.* https://doi.org/10.1016/j.fm.2014.05.019.

Jebun, N. Alam, M.Z., Mamun, A.A. and Raus, R. A. 2015. Screening of fungal flocculant to reduce turbidity of river water. *International Postgraduate Conference on Engineering Research.* Kuala Lumpur, Malaysia.

Jebun, N., Al-Mamun, A., Alam, M. Z. and Raus, R. B. A. 2016. Fungal flocculants to reduce turbidity of river water. *ARPN Journal of Engineering and Applied Sciences.* 11 (6) : 4094-4099.

Malathi, S. and Chakraborty, R. 1991. Production of alkaline protease by a new *Aspergillus flavus* isolate under solid-substrate fermentation conditions for use as a depilation agent. *Applied and Environmental Microbiology.* https://doi.org/10.1128/aem.57.3.712-716.1991.

Nwanze, P. I., Khan, A. U., Ameh, J. B. and Umoh, V. J. 2005. The effect of the interaction of various spawn grains with different culture medium on carpophore dry weights and stipe and pileus diameters of *Lentinus squarrosulus* (Mont.) Singer. *African Journal of Biotechnology.* https://doi.org/10.5897/AJB2005.000-3112.

Pinto, F. C. J., de Lima, D. B., Agustini, B. C., Dallagassa, C. B., Shimabukuro, M. F., Chimelli, M. and Bonfim, T. M. B. 2012. Morphological and molecular identification of filamentous fungi isolated from cosmetic powders. *Brazilian Archives of Biology and Technology.* https://doi.org/10.1590/S1516-89132012000600013.

Rahman, R. N. Z. A., Geok, L. P., Basri, M. and Salleh, A. B. 2005. Physical factors affecting the production of organic solvent-tolerant protease by *Pseudomonas aeruginosa* strain K. *Bioresource Technology.* https://doi.org/10.1016/j.biortech.2004.06.012.

Robert, E., Grippa, M., Kergoat, L., Pinet, S., Gal, L., Cochonneau, G. and Martinez, J. M. 2016. Monitoring water turbidity and surface suspended sediment concentration of the Bagre Reservoir (Burkina Faso) using MODIS and field reflectance data. *International Journal of Applied Earth Observation and Geoinformation.* https://doi.org/10.1016/j.jag.2016.06.016.

Salehizadeh, H. and Shojaosadati, S. A. 2001. Extracellular biopolymeric flocculants: Recent trends and biotechnological importance. *Biotechnology Advances.* https://doi.org/10.1016/S0734-9750(01)00071-4.

Sandhya, C., Sumantha, A., Szakacs, G. and Pandey, A. 2005. Comparative evaluation of neutral protease production by *Aspergillus oryzae* in submerged and solid-state fermentation. *Process Biochemistry.* https://doi.org/10.1016/j.procbio.2004.12.001.

Shide, E. G., Wuyep, P. A. and Nok, A. J. 2004. Studies on the degradation of wood sawdust by *Lentinus squarrosulus* (Mont.) Singer. *African Journal of Biotechnology*. https://doi.org/10.5897/AJB2004.000-2083.

Wu, T. Y., Mohammad, A. W., Jahim, J. M. and Anuar, N. 2009. A holistic approach to managing palm oil mill effluent (POME): Biotechnological advances in the sustainable reuse of POME. *Biotechnology Advances.* https://doi.org/10.1016/j.biotechadv.2008.08.005.

Xia, X., Lin, S., Xia, X. X., Cong, F. S. and Zhong, J. J. 2014. Significance of agitation-induced shear stress on mycelium morphology and lavendamycin production by engineered *Streptomyces flocculus*. *Applied Microbiology and Biotechnology.* https://doi.org/10.1007/s00253-014-5555-4.