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Mass Spectrometric Analysis of A Bioflocculant Produced from Chicken Waste

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Abstract : This work embodies use of a High performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (HPLC - MS QTOF) for the determination of molecular mass of the constituent moieties in a bioflocculant produced through *Aspergillus flavus'* fermentation of chicken viscera. The time-of-flight mass spectrometry was conducted with HPLC MS-MS QTOF G6560A, UTM in dual Ion Electrospray Ionization (ESI). The experiment was conducted at gas flow rate 12 L/min, gas temperature 350°C, skimmer cone potential 30 V, fragmentor potential 125 V, Nozzle Voltage 1000 V, mass range 100–3,000 m/z using 0.1% formic acid and Acetonitrile as the mobile phase. The chromatogram obtained shows many spectra of low and high-level molecular weight constituents with some prominent peaks that appeared in the range of 2.23 to 6.62 minutes having molecular mass of between 180.06 to 678.27 Da. The most profuse peaks were found at mass of 678.27 Da (2.90 min), 646.02 Da (2.92min) and 692.83 Da (3.59min) in the negative ESI indicating high existence of the negative ionized groups in the bioflocculant. The results also showed the bioflocculant to be composed of few positively charged compounds with +4 and +3 charges. LC- MS QTOF offers an attractive technique for the fast and specific mass measurement of the bioflocculant constituent.

Keywords: Chromatography, Bioflocculant, Chicken Waste, Molecular Mass

1. Introduction

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Bioflocculant is an extracellular biopolymeric substance that have capability to flocculate suspended particles. They can be secreted by microorganisms most especially fungi and bacteria. They have advantages of being biodegradable and sustainable as well as lack of secondary pollutant accumulation [1]. These biopolymeric substances can be used as safe flocculating agents that can effectively and sustainably replace the chemical flocculating agents. The chemical flocculants have been shown to lead to accumulation of secondary pollutants and associated health conditions such as being carcinogenic and causing Alzheimer's disease [2] due to their non-biodegradability. However, the production of bioflocculant remain at laboratory stage due to exorbitant production costs and varying production procedures as well as low flocculation efficiencies [3]. Many researchers [1, 2, 4-8] have produced bioflocculant from different microorganisms and substrates including waste and wastewater as reviewed in the work of Mohammed and Wan Dagang [9].

Characterization of every new bioflocculant at both physical and molecular level is important for understanding the flocculation behaviour of the bioflocculant. This is because the flocculation activity of a bioflocculant is a function of it physical and chemical composition as well the molecular weight. There are many existing techniques for molecular weight and chemical compositional analysis including chromatography and however, the major need of effective chromatographic mass spectrometric analysis of a substance is knowing their mass, retention time and disintegration pattern [10]. These could be challenging due to lack of immediate reference standards for newly discovered of substances. Primary information about metabolites can be collected through metabolic experimentations or chemical hydrolysis of the metabolites especially if their structure possesses the needed moiety [11]. However, access to the pure substances generally yields better outcomes when it comes to acquisition of clean spectra, consistent retention times and rudimentary data authentication.

HPLC MS QTOF is one of the result yielding techniques in terms of molecular analysis of substances because it allows quantitative and qualitative analysis of samples as well confirmation of constituent identities even at trace amount [12]. The present study uses Agilent Technologies' HPLC MS-MS QTOF to characterize our novel bioflocculant produced from chicken viscera and reported in our previous study [6] to further understand the flocculation mechanism of the bioflocculant.

2. Materials and Methods

2.1 Bioflocculant production

The bioflocculant production was achieved by cultivating A. flavus on hydrolysate of chicken viscera (containing 5.40% w/w protein, 3.20% w/w sugar, 5.86% w/w carbon, 1.27% w/w nitrogen, 0.83% w/w sulphur and 10 % w/w hydrogen prepared according to the methods described in our previous study [13]. The production was carried out at optimized culture conditions (pH 7, Temperature 35°C, 4% A. flavus inoculum, 150 rpm and 48hours incubation period) adopted in our previous study [6].

2.2 Purification of the bioflocculant

The pure bioflocculant was obtained from crude culture broth using the methods described by Salehizadeh [5] and Xiong [14]. Briefly, culture broth was centrifuged to obtain a bioflocculant rich supernatant, absolute chilled ethanol (95%) was added to the supernantant at a ratio of 2:1 ethanol - supernatant and refrigerated at 4°C overnight to allow for precipitation of the bioflocculant from the culture supernantant. The mixture was subsequently spinned at 10,000 rpm, 20 minute and 4°C. The resultant residue was liquified in deionized water at ratio 1:2 (v/v). This process was carried out twice before the pure bioflocculant obtained was lyophilized and dried in a vacuum

2.3 Bioflocculant sample preparation for mass spectrophotometric analysis

The lyophilized and vacuum dried bioflocculant samples were hydrolysed using 4 mL of 2 M trifluoroacetic acid (TFA) at 105 ◦ C for 2 hours. The lingering TFA was expunged from the sample using methanol. The trimethylsilyl (TMS) derivative of hydrolysate was prepared by adding 1 mL pyridine, 0.4 mL hexamethyldisilane and 0.2 mL trimethylchlorosilane and heated for half an hour at 80 ◦ C. The sample was allowed to cool before it was filtered with a 0.45-µm membrane. The 50 µl of filtrate obtained was injected into high performance liquid chromatography tandem mass spectrometry of quadrupole time of flight (HPLC MS-MS QTOF G6560A) for spectrophotometric analysis.

2.4 Mass Spectrometric Analysis

To obtained the molecular weight of the bioflocculant, mass spectrometric analysis of the bioflocculant was conducted using Agilent Technologies' HPLC MS-MS QTOF G6560A with dual Ion Electrospray Ionization (ESI) ionizations. The experimental conditions were gas flow of 12 L/min, gas temperature 350°C, skimmer cone potential 30 V, fragmentor potential 125 V, Nozzle Voltage 1000 V, mass range 100–3,000 m/z, for both negative and positive-ion ionization. Sampling of the sample into the spectrometer was carried out with an Agilent Technologies Series Column Compartment model G1316C. The mobile phase included A (0.1% formic acid in water) and B (Acetonitrile). Samples were introduced to the column using an Agilent Technologies Series HiP Sampler G4226A with 10.00 µL injection volume with elution of the bioflocculants by an Agilent Technologies model G4220A binary Pump at flow rate of 0.2 mL/min. The gradient (solution B) was 95%, 15min; 95%, 20 min; 5%, 35 min. All chemicals used were degassed on an Agilent Technologies 1260 µ-degasser.

3. Results and Discussions

3.1 Mass Spectrometric Analysis of the Bioflocculant

The LC-MS QTOF chromatogram of the purified bioflocculant indicated many spectra at low and high-level molecular weight constituents for both positive and negative ESI scan (**Figure**) with some prominent peaks indicating that the bioflocculant was heterogenous in nature similar to Achromobacter xylosoxidans exopolysaccharide bioflocculant reported by Subudhi, Bisht [15]. The prominent peaks which appeared in the range of 2.23 to 6.62 minutes have molecular mass between 180.06 to 678.27 Da (Error! Reference source not found.). The chromatogram displays no distinctive pattern of distribution however most abundant peak were found at mass of 678.27 Da (2.90 min), 646.02 Da (2.92min) and 692.83 Da (3.59min) through the negative ESI mode which indicates high existence of the negative ionized groups in the bioflocculant. This collaborated our earlier report [16] that the bioflocculant was a negatively charged one that needed cationization especially with Ca2+ and Mg2+ to be able to flocculate a negatively charged Kaolin particles and wastewater samples

Table1: Molecular mass of the bioflocculant components (LC-MS QTOF)

Retention Time (Min)	Molecular mass (Da)	Ion	ESI
2.23	429.88	6	-ve
2.24	565.94	3	-ve
2.34	340.10	4	-ve
2.61	180.06	4	+ve
2.66	221.09	3	+ve
2.70	221.09	3	+ve
2.90	678.27	4	-ve
2.92	646.02	3	-ve
3.42	452.22	3	+ve
3.44	303.15	2	+ve

Retention Time (Min)	Molecular mass (Da)	Ion	ESI
3.45	302.42	4	+ve
3.48	328.34	2	+ve
3.49	387.52	4	+ve
3.55	398.53	8	+ve
3.59	692.83	7	+ve
3.72	268.15	3	-ve
3.94	310.12	4	-ve
4.62	472.18	4	-ve
4.70	260.14	3	+ve
4.73	260.14	6	-ve
5.06	222.10	3	+ve
6.01	294.12	4	+ve
6.28	335.15	3	+ve
6.62	425.41	4	+ve

The presence of negatively charged groups also explain the need for cationization of the bioflocculant to achieve high flocculation efficiency. The flocculation efficiency of the bioflocculant could be attributed to synergetic behaviour among these major constituents of the bioflocculant. According to Salehizadeh and Shojaosadati [17], the bioflocculants with high molecular weight are characterized with more absorption points that can trap much particles to allow for large floc formation. These authors also indicated that greater ionization of the peptides containing EPS had apparent effect on their biological properties.

Further, the bioflocculant was composed of assembly of vastly ionized compounds, most with +4 and +3 charges. Fewer compounds had charge of +2, +6 and +12 as indicated in **Error! Reference source not found.**. This indicates that the bioflocculant was a highly charged EPS and thus, further explains its bioflocculation efficiency. ESI is an appropriate crossing point for mass spectrometry of polar and heat sensitive substances. The volatile additives such as formic acid used as mobile phase in this investigation can deprotonate the acidic groups in negatively charged state and vice versa for positively charged state [18]. The gas phase proton shift effect has been implicated for the creation of the ions. In the instance of the proton transmission mechanism, it was suggested that the signal response does not build up in the presence of additives in the mobile phase, and protonated molecules can be detected using alkali containing mobile phases [15].

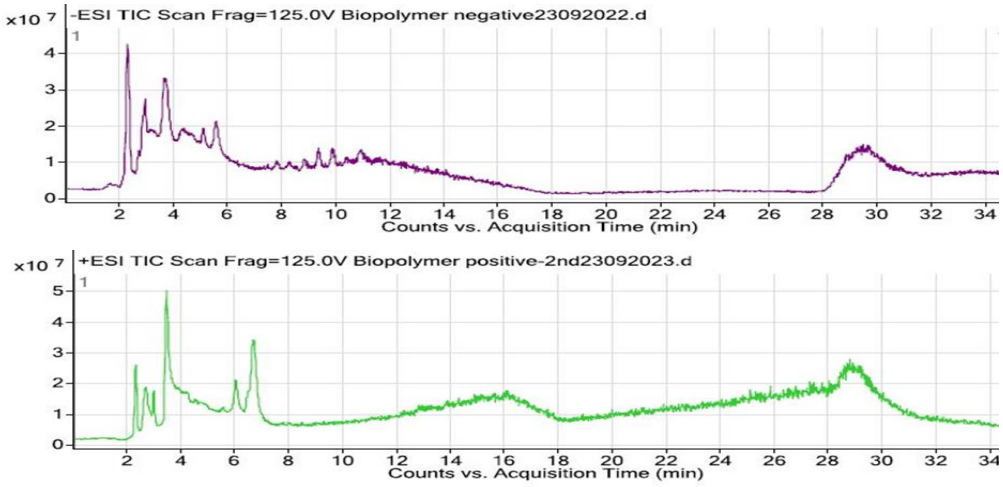

Figure 1: LC-MS QTOF positive and negative ESI spectrogram of the bioflocculant

4. Conclusion

The mass spectrometric analysis of the purified bioflocculant revealed many spectra at low and high-level molecular weight constituents (180.06 to 678.27 Da) with varying ionization indicating that the bioflocculant was heterogenous in nature. The presence of abundant peaks in the negative ESI indicates existence of the negative ionized moieties in the bioflocculant and need for hybridization of the bioflocculant with cations for effective flocculation. These composition and characteristics contributed to the flocculation capacity of the bioflocculant.

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References

[1]. Siddharth, T., P. Sridhar, V. Vinila, and R. D. Tyagi, Environmental applications of microbial extracellular polymeric substance (EPS): a review. Journal of Environmental Management 2021: https://doi.org/10.1016/j.jenvman.2021.112307

[2]. El-Gaayda, J., Titchou, F. E., Oukhrib, R., Yap, P. S., Liu, T., Hamdani, M and Akbour, R. A. (). Natural flocculants for the treatment of wastewaters containing dyes or heavy metals: a state-of-the-art review. Journal of Environmental Chemical Engineering, 2021. 9(5), https://doi.org/10.1002/tcr.202000153

[3]. Mohammed, J.N. and W.R.Z.W. Dagang, Role of Cationization in Bioflocculant Efficiency: A Review. Environmental Processes, 2019. 6(2): p. 355-376.

[4]. Pu, S.-y., L.-l. Qin, J.-p. Che, B.-r. Zhang, and M. Xu, Preparation and Application of a Novel Bioflocculant by Two Strains of Rhizopus Sp. Using Potato Starch Wastewater as Nutrilite. Bioresource technology, 2014. 162: p. 184-191.

[5]. Kurniawan, S. B., Siti Rozaimah S. A., Muhammad F. I., Nor Sakinah M. S., Nur'Izzati I., Hassimi A. H., Ahmad R. O. and Ipung F. P. Challenges and opportunities of biocoagulant/bioflocculant application for drinking water and wastewater treatment and its potential for sludge recovery. International journal of environmental research and public health 17, 24 (2020): https://www.mdpi.com/1660-4601/17/24/9312

[6]. Mohammed, J.N. and W.R.Z. Wan Dagang, Culture Optimization for Production and Characterization of Bioflocculant by Aspergillus Flavus Grown on Chicken Viscera Hydrolysate. World Journal of Microbiology and Biotechnology, 2019. 35(8): p. 1-19.

[7]. Kurniawan, S.B., S.R.S. Abdullah, A.R. Othman, I.F. Purwanti, M.F. Imron, A. Ahmad, and H.A. Hasan, Isolation and Characterisation of Bioflocculant-Producing Bacteria from Aquaculture Effluent and Its Performance in Treating High Turbid Water. Journal of Water Process Engineering, 2021. 42: p. 102194.

[8]. Chen, S., Y. Zheng, C. Han, H. Liu, Y. Chen, J. Zhou, and S. Su, Production of a Bioflocculant Using Old Polyester Fibre as a Fermentation Feedstock and Its Use in Treatment of Polyester Alkali-Peeling Wastewater. Journal of Environmental Chemical Engineering, 2021. 9(4): p. 105455.

[9]. Mohammed, J.N. and W.R.Z. Wan Dagang, Implications for Industrial Application of Bioflocculant Demand Alternatives to Conventional Media: Waste as a Substitute. Water Science and Technology, 2019. 80(10): p. 1807-1822.

[10]. Allen, D.R. and B.C. McWhinney, Quadrupole Time-of-Flight Mass Spectrometry: A Paradigm Shift in Toxicology Screening Applications. The Clinical Biochemist Reviews, 2019. 40(3): p. 135.

[11]. Sudol, P.E., K.M. Pierce, S.E. Prebihalo, K.J. Skogerboe, B.W. Wright, and R.E. Synovec, Development of Gas Chromatographic Pattern Recognition and Classification Tools for Compliance and Forensic Analyses of Fuels: A Review. Anal. Chim. Acta, 2020. 1132: p. 157-186.

[12]. Ling, Y., Z. Lei, X. Li, X. Yan, S. Wang, W. Yang, . . . J. Yu, Characterization and Identification of the Chemical Constituents of Gynostemma Pentaphyllum Using High Performance Liquid Chromatography–Electrospray Ionization–Quadrupole Time-of-Flight Tandem Mass Spectrometry (Hplc-Esi-Qtof-Ms/Ms). Analytical Letters, 2020. 53(5): p. 760-773.

[13]. Mohammed, J.N. and W.R.Z.W. Dagang, Development of a New Culture Medium for Bioflocculant Production Using Chicken Viscera. MethodsX, 2019. 6: p. 1467-1472.

[14]. Xiong, Y., Y. Wang, Y. Yu, Q. Li, H. Wang, R. Chen, and N. He, Production and Characterization of a Novel Bioflocculant from Bacillus Licheniformis. Appl. Environ. Microbiol., 2010. 76(9): p. 2778-2782.

[15]. Subudhi, S., V. Bisht, N. Batta, M. Pathak, A. Devi, and B. Lal, Purification and Characterization of Exopolysaccharide Bioflocculant Produced by Heavy Metal Resistant Achromobacter Xylosoxidans. Carbohydrate polymers, 2016. 137: p. 441-451.

[16]. Mohammed, J.N. and W.R.Z.W. Dagang, Effect of Cation on Efficiency of Aspegillus Flavus Bioflocculant Produced from Chicken Viscera Hydrolysate. Indian Journal of Public Health Research & Development, 2019. 10(11).

[17]. Salehizadeh, H. and S. Shojaosadati, Extracellular Biopolymeric Flocculants: Recent Trends and Biotechnological Importance. Biotechnology advances, 2001. 19(5): p. 371-385.

[18]. Gao, Q., U. Nilsson, L.L. Ilag, and C. Leck, Monosaccharide Compositional Analysis of Marine Polysaccharides by Hydrophilic Interaction Liquid Chromatography-Tandem Mass Spectrometry. Anal. Bioanal. Chem., 2011. 399(7): p. 2517-2529.