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Research Report

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Title of Research Report

Anti-bacterial Forma-ridden *rouxii* Chitogels

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Anti-bacterial Forma-ridden *rouxii* Chitogels

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Abstract

Anti-bacterial Forma-ridden rouxii Chitogels *are green, innovative formaldehyde remover that remove toxic formaldehyde emitted by furniture made of particle board emitting formaldehyde,* ***rouxii Chitogels*** *are hydrogels of chitosan of Hermetia illucens, black soldier fly* ***BSF*** *obtained using chitin deacetylase from Mucor rouxii via* **GVFM**. (Ghani, 2018) *The incorporation of chitin deacetylase from Mucor rouxii in the innovative* **GVFM** *could produce* ***Anti-bacterial Forma-ridden rouxii Chitogels (71.0% yield at 50°C)*** *and eliminate the use of highly corrosive conc. NaOH in deacetylation with a yield of* 71.0 at 50°C. *(cf. 41.5% from 17.6M NaOH).*

Urea formaldehyde ***UF*** *is the most widely used thermoset glue in the wood composite board industry.* ***UF*** *emits formaldehyde* (Kelleci, 2022) *which is classified as one of the group I carcinogens by the International Agency for Research on Cancer (IARC). With a 1–part per billion (ppb) increase in the daily concentration of formaldehyde, mortality risks in nonaccidental, circulatory, and respiratory diseases increased by 0.36%, 0.36% and 0.41% respectively* (Ban, 2022). ***Chitogels*** *and* ***rouxii Chitogels*** *remove formaldehyde (****Forma****)* ***HCHO*** *via condensation to give non-polluting water only.* ***Chitogels*** *are also anti-bacterial as chitosan present is anti-bacterial.* ***Chitogels*** *and* ***rouxii Chitogels*** *are biosourced and biodegradable as they can be derived from shells of* ***BSF****.*

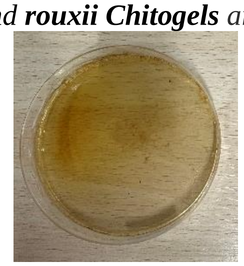

Figure 2 Anti-bacterial forma-ridden Chitogels of black soldier fly **BSF** (using 16.7M NaOH)

Figure 3 Anti-bacterial forma-ridden *rouxii* Chitogels of black soldier fly **BSF**

Figure 4 Anti-bacterial forma-ridden *rouxii* Chitogels as filter in an air purifier

Figure 5 Anti-bacterial forma-ridden *rouxii* Chitogels as formaldehyde remover in new furniture of drawers emitting about 0.5mg/m³ **HCHO** (exceeding safety limit of 0.125 mg/m³)

Figure 6 Anti-bacterial forma-ridden Chitogels as formaldehyde remover in a newly renovated Lecture Theatre emitting about 0.30mg/m³ **HCHO** (exceeding safety limit of 0.125 mg/m³)

Anti-bacterial forma-ridden rouxii Chitogels *and* ***Chitogels*** *showed the highest percentage of removal of HCHO from 1:100* ***HCHO*** *(by mass) of 93.0% and 87.8% when compared with some commercial formaldehyde removers (cf. wall paint HCHO-busters: 53.0%; activated Charcoal 35.5%; 3M* ***HCHO*** *remover 26.2%). Besides the concentration of* ***HCHO*** *left were 0.06mg/m³ and 0.105mg/m³ (within safety limits of 0.125mg/m³) after 24 hours at 19.9°C.* (EUR13216EN, 1990). *When applied as filter in an air purifier, the Percentage of* ***HCHO*** *removed using* ***rouxii Chitogels*** *as filter was 68.0% at 19.9°C which outperformed that of filter only of 56.3% by 20.8%. The conc. of* ***HCHO*** *remained was 0.125mg/m³ when using filter with* ***rouxii Chitogels*** *19.9°C which was within the safety limit of HCHO of 0.125 mg/ m³. When applied as formaldehyde remover in a furniture of drawers,* ***rouxii Chitogels*** *could remove 5.40mg/g* ***HCHO*** *effectively in 117 days at 31°C or below. The concentration of* ***HCHO*** *could reach as low as 0.23 mg/m³ in 3 days at 22°C or below. More than 45.0% of* ***HCHO*** *was removed from the drawers (0.0578m³) in 117 days. When applied in a newly renovated Lecture Theatre,* ***Chitogels*** *removed* ***HCHO*** *effectively as the concentration of* ***HCHO*** *(from 0.37 mg/m³) reached 0.12 mg/m³ (within safety limit 0.125 mg/m³)*

*in 10 mins at 29^{o}C. Condensation was confirmed by FTIR as DD% of **Chitogels** decreased from 59.7% to 51.1% and that of **rouxii Chitogels** decreased from 61.1% to 49.9% (due to formation of **-N=C between -NH_2** in **Chitogels** and **-C=O** in formaldehyde).*

***Chitogels** and **rouxii Chitogels** are anti-bacterial and showed significant anti-bacterial effect towards oral bacterial. [no. of bacterial colonies of **Chitogels** (60, 11 w.r.t. dilution 10^{-3}, 10^{-4}) cf. oral bacterial (control: 232, 52) and **rouxii Chitogels** (0, 0 w.r.t. dilution 10^{0}, 10^{-2}) cf. oral bacterial (control: 58,1)] After condensation with formaldehyde, **Chitogels** of **BSF** kept showing anti-bacterial effect towards oral bacteria. [no. of bacterial colonies: **Chitogels** (0,1 w.r.t. dilution 10^{-3}, 10^{-4}) cf. oral bacterial (control: 232, 52)] and **rouxii Chitogels** (0, 1 w.r.t. dilution 10^{0}, 10^{-2}) cf. oral bacterial (control: 58, 1) **Chitogels** and **rouxii Chitogels** are bio-degradable as they took 38 days and 14 days to be fully bio-degraded in soil.*

***Chitogels** and **rouxii Chitogels** are non-allergic as surface protein allergens were absent, so the marketing of **Anti-bacterial forma-ridden rouxii Chitogels** should be eligible for marketing.*

Keywords: anti-bacterial, formaldehyde, condensation, chitosan, *Hermetia illucens*, black soldier fly, urea formaldehyde, *Mucor rouxii*, chitin deacetylase

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Declaration

This project was submitted to World Science, Environment and Engineering Competition 2024, Hong Kong Chemistry Olympiad for Secondary Schools 2024 and Hong Kong Student Science Project Competition 2024.

Commitments on Academic Honesty and Integrity

We hereby declare that we

1. are fully committed to the principle of honesty, integrity and fair play throughout the competition.
2. actually perform the research work ourselves and thus truly understand the content of the work.
3. observe the common standard of academic integrity adopted by most journals and degree theses.
4. have declared all the assistance and contribution we have received from any personnel, agency, institution, etc. for the research work.
5. undertake to avoid getting in touch with assessment panel members in a way that may lead to direct or indirect conflict of interest.
6. undertake to avoid any interaction with assessment panel members that would undermine the neutrality of the panel member and fairness of the assessment process.
7. observe the safety regulations of the laboratory(ies) where the we conduct the experiment(s), if applicable.
8. observe all rules and regulations of the competition.
9. agree that the decision of YHSA(Asia) is final in all matters related to the competition.

We understand and agree that failure to honour the above commitments may lead to disqualification from the competition and/or removal of reward, if applicable; that any unethical deeds, if found, will be disclosed to the school principal of team member(s) and relevant parties if deemed necessary; and that the decision of YHSA(Asia) is final and no appeal will be accepted.

(Signatures of full team below)

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Noted and endorsed by

(signature) Wong Wai Keung

Name of school principal: Dr Wai Keung WONG

Content

1. Theory

1.1 Investigation of the feasibility of using hydrogel of black soldier fly *(Hermetia illucens*) **BSF** in making **Anti-bacterial forma-ridden *rouxii* Chitogels**

Anti-bacterial urea **forma**-ridden **Chitogels** are hydrogels of black soldier fly **BSF** that could remove formaldehyde emitted from furniture made of particle board (**HCHO** reduced to safety limit of 0.125 mg/m^3) (Robert, 2011) as formaldehyde is removed by chitosan of **BSF** via condensation, so they are newly invented formaldehyde remover. Structural changes are confirmed by determination of the change in structure of black soldier fly *(Hermetia illucens)* **BSF** hydrogel before and after condensation with formaldehyde using FTIR.

1.1.1 Black soldier fly **BSF** *Hermetia illucens*

The productivity of chitin obtained from the larvae of black soldier flies ***H. illucens*** was found to be approximately 5.00 ± 0.002%. These chitins were converted to chitosan by chemical treatment with a yield of about 93 ± 0.16% (Lee, 2022). The deacetylation degree of the extracted B**SF** chitin and chitosan were 33.4 and 61.6%, respectively (Jayanegara, 2020). The application of black soldier fly (**BSF**), *Hermetia illucens* based technology to process organic wastes produces feed materials (protein, fat), biodiesel, chitin and biofertilizer which presents a practical option for organic waste management. **BSF** organic wastes recycling is creating new economic opportunities for the industrial sector and entrepreneurs as it is a sustainable and cost-effective process that promotes resource recovery, and generates valuable products (Rehman, 2022). The entire life cycle of the black soldier fly is approximately 38 days (**Figure 1.1.1**). Larvae hatch from eggs, transition through five larval instars, pupate and then emerge as adult flies (Hall, 2002). When incorporating **BSF** into your composting process, you can harvest the compost in as little as 21 days. (retrieved from https://www.insectschool.com/uncategorized/understanding-the-life-cycle-of-the-black-soldier-fly/) The supply of shells of black soldier fly B**SF** is apparently continuous and environmentally sustainable.

https://www.insectschool.com/uncategorized/understanding-the-life-cycle-of-the-black-soldier-fly/

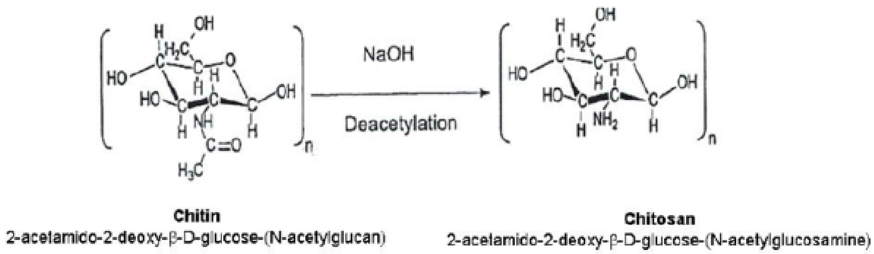
Figure 1.1.1 The life cycle of the black soldier flyer

1.1.2 Removal of calcium carbonate by demineralization of carbonates using 2M HNO_3

$CaCO_3(s) + 2H^+(aq) \rightarrow Ca^{2+}(aq) + CO_2(g) + H_2O(l)$

1.1.3 Deproteinization through deacetylation of chitin to chitosan

Deproteinization can be done using NaOH room temperature (RT), 363 and 393 K, hydroxide concentration (2.0 or 10.0 mol dm3) and time (3 and 24 h) on shrimp chitin deacetylation (Pires, 2014).
Chitin is extracted from crab shell from which chitosan is made by N-deacetylation
https://www.researchgate.net/figure/Conversion-of-chitin-to-chitosan-by-deacetylation_fig1_285543611



Conversion of chitin to chitosan by deacetylation

https://www.researchgate.net/figure/Conversion-of-chitin-to-chitosan-by-deacetylation_fig1_285543611

Figure 1.1.3 Conversion of chitin to chitosan by deacetylation

1.1.4 Determination of Degree acetylation DA% in chitin and degree of deacetylation DD% in chitosan using FTIR

The following FTIR Spectrum Two.

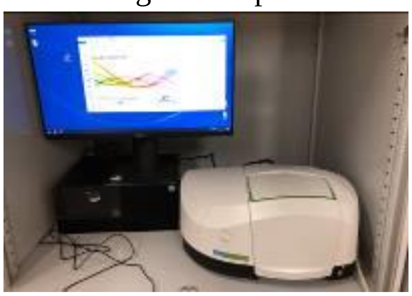


Figure 1.1.4.1 FTIR Spectrum Two

Spectrum Two FTIR spectrometers feature:

Standard, high-performance, room-temperature $LiTaO_3$ (lithium tantalate) MIR (mid infra-red) detector with a SNR (signal to noice ratio) of 9,300:1
Optional temperature-stabilized, high-performance DTGS (deuterated triglycine sulfate) MIR detector with a SNR of 14,500:1. Ideal for low-light, high throughput applications
Standard optical system with KBr windows for data collection over a spectral range of 8,300 – 350 cm^{-1} at a best resolution of 0.5 cm^{-1}
Figure 1.1.4.2 Features of FTIR Spectrum II

Same as in the NMR spectroscopy, also in the FTIR spectroscopy, several procedures and equations are described in literature for calculation of degree of deacetylation. (Biskup, 2012)

R. Czechowska-Biskup, D. Jarosińska, B. Rokita, P. Ulański, J. M. Rosiak

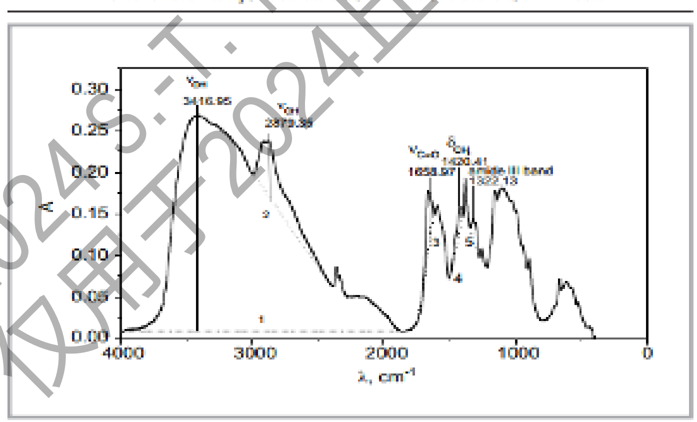


Figure 11. Rereferences bands and corresponding baselines, based on Duarte et al. [21] (1 - 3) and Brugnerotto et al. [22] (4 - 5) for FTIR spectrum of chitosan sample S2.

Figure 1.1.4.2 Reference bands and corresponding baseline

Figure 11. *Rereferences bands and corresponding baselines, based on Duarte et al. [21] (1 - 3) and Brugaretto et al. [22] (4 - 5) for FTIR spectrum of chitosan sample S2.*

$$DA\ [\%] = \frac{A_{1655}}{A_{3450}} \times 100/1{,}33\ [17, 23] \qquad (15)$$

$$DA\ [\%] = \frac{A_{1655}}{A_{2870}} \times 100/1{,}33\ [23] \qquad (16)$$

$$DA\ [\%] = \frac{A_{1655}}{A_{3450}} \times 115\ [17] \qquad (17)$$

$$DA\ [\%] = \left(\frac{A_{1320}}{A_{1420}} - 0{,}03822\right)/0{,}03133\ [22] \qquad (18)$$

where: A_{3450}, A_{2870}, A_{1655}, A_{1420}, A_{1320}, are values of absorbance from baseline 1, 2, 3, 4, 5 to maximum, respectively. In ***Figure 11***, on the basis IR spectrum of chitosan S2, baseline settings and individual bands ascribed for characteristic groups in chitosan are presented.

Figure 1.1.4.3 (Biskup, 2012)

1.2 Comparing the efficiency of removal of **HCHO** of hydrogel of black soldier fly (*Hermetia illucens) and other commercial formaldehyde removers*

1.2.1 Condensation of chitosan and formaldehyde i.e. removal of formaldehyde using hydrogel of chitosan of black soldier fly **BSF**

Condensation reaction of a carbonyl group with an amino group is the key reaction in many biological and enzymic processes. (Dermawan, 2020). Melamine, polyvinyl alcohol, and adipic acid dihydrazide could be added to urea-formaldehyde (**UF**) resin for reducing the free formaldehyde as modifiers. (Liu, Free formaldehyde reduction in urea-formaldehyde resin adhesive: Modifier addition effect and physicochemical property characterization, 2020) https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Supplemental_Modules_%28Organic_Chemistry%29/Aldehydes_and_Ketones/Reactivity_of_Aldehydes_and_Ketones/Reaction_with_Primary_Amines_to_form_Imines

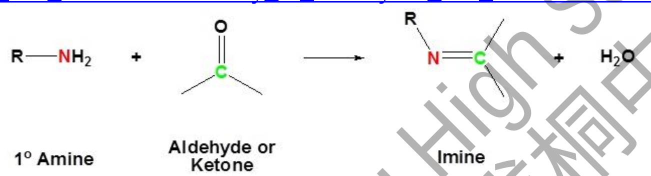


Figure 1.2.1 Condensation of two molecules of glucose

1.2.2 Technologies used in commercial formaldehyde removers

Table 1.2.2 Comparison of formaldehyde removal options

Methods	Technology	Concentration	Operating Temp.	Operating Cost
Recovery	Adsorption	Low, High	Ambient	Acceptable
	Membrane	High	Ambient	High cost of material
	Condensation	High	Ambient and cryogenic	High cost of energy
Destruction	Thermal Oxidation	High	~815 °C	High cost of Energy
	Catalysis	Low, High	Ambient, 200~500 °C	Acceptable
	Photo catalysis	Low, High	Ambient	Costly dopants required
	Non-thermal plasma w/wo catalyst	Low	Ambient	High cost of system assembly
	Biological/Botanical filtration	Low	-	-

https://www.researchgate.net/publication/360646327/figure/tbl1/AS:1160525400547329@1653702225104/Comparison-of-formaldehyde-removal-options.png

Traditional condensation method to remove formaldehyde is effectively but requires a high operating cost to recover formaldehyde by lowering of temperature and applying a high temperature.

1.3 Investigation of the anti-bacterial effect of hydrogel of black soldier fly (*Hermetia illucens*) before and after condensation with **HCHO**.

Chitosan is a versatile material with proved anti-bacterial activity. Three antibacterial mechanisms have been proposed:
i) the ionic surface interaction resulting in wall cell leakage;
ii) the inhibition of the mRNA and protein synthesis via the penetration of chitosan into the nuclei of the microorganisms; and
iii) the formation of an external barrier, chelating metals and provoking the suppression of essential nutrients to microbial growth. It is likely that all events occur simultaneously but at different intensities. (Goy, 2009)

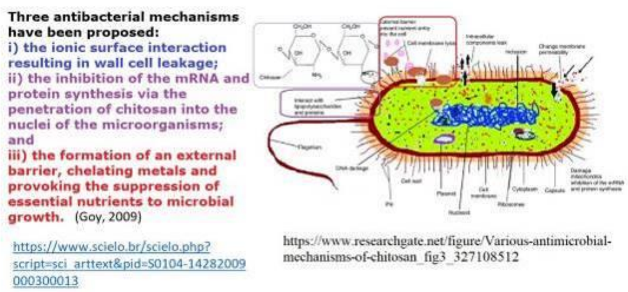


Figure 1.3 Various anti-bacterial mechanisms of chitosan

1.4 Applying **Chitogels** and ***rouxii*** **Chitogels** as formaldehyde remover on-the-spot

The guideline value for formaldehyde in the non-industrial indoor environment is 0.15 mg/m^3.
This guideline is intended to protect the general population (excluding the hypersusceptible) from sensory irritation caused by formaldehyde. The limit value for occupational environments is 1 ppm (1.2mg/m^3) for old productions and 0,3 ppm (0.36 mg/m^3) for new ones. From 1992 on all productions will have to comply with 0.36 mg/m^3. Furniture can only be circulated if the wood-based materials used for its construction meet the above-mentioned specifications or the whole piece of furniture passes the chamber test successfully, not giving rise to more than 0.12 mg/m^3 (0.1 ppm) formaldehyde in the air. (retrieved from file:///C:/Users/teacher/Downloads/EUR%2013216%20EN.pdf)

1.5 Biodegradability of chitosan

Chitosan can be considered as an interesting biosourced and biodegradable alternative, despite its low water resistance. (Mati-Baouche, 2019)

1.6 Cross-reactive allergens risks of *Hermetia illucens*, black soldier fly **BSF**

Cross-reactive allergens, tropomyosin and arginine kinase, were detected in some BSFL samples. (Bessa, 2021) As shells of black soldier fly BSF would be used in **Chitogels**, 3M Clean-Trace ALLTEC60 which detects as little as 3 µg of protein on surfaces and is validated for a range of allergenic proteins, including egg, milk, gluten, soy, peanut, almond and buckwheat, would be used to ensure that the use of **Chitogels** would not cause allergic problem.

1.7 Production of **Anti-bacterial forma-ridden *rouxii* Chitogels**

1.7.1 Incorporation of an innovative Green Vinegar Method in making **Chitogels**

Chitosan is known to be very soluble in acetic acid (Castano, 2019). It will be greener to produce **Anti-bacterial forma-ridden Chitogels** by use vinegar instead of highly corrosive 16.7M NaOH for deacetylation.

1.7.2 Incorporation of *Mucor rouxii* in an innovative Green Vinegar Fermentation Method in making ***rouxii*** **Chitogels**

1.7.2.1 Green Vinegar Fermentation Method

Chitin deacetylase, the enzyme that catalyzes the hydrolysis of acetamido groups of N-acetylglucosamine in chitin in the bio-conversion of chitin to chitosan, had been purified to homogeneity from mycelial extracts of the fungus Mucor rouxii. (Kafetzopoulos, 1992) *Incorporation of Mucor rouxii in an innovative Green Vinegar Fermentation Method **GVFM** in making **Anti-bacterial Forma-ridden rouxii Chitogels** would be a green way to remove formaldehyde in daily life.*

Chitin with DA% of about 72% or below was known to be soluble in acetic acid and slightly soluble in water. Hydrogen bonds of water-soluble chitin could be easily disrupted (Cho, 2000) *allowing enzyme such as chitin deacetylase from Mucor rouxii to gain access to the chitin-substrate more easily for enzymatic deacetylation to take place.* (Sultan, 2023) *It will be greener to produce forma -ridden rouxii* ***Chitogels*** *by use vinegar followed by chitin deacetylase from Mucor rouxii instead of highly corrosive 16.7M NaOH for deacetylation.*

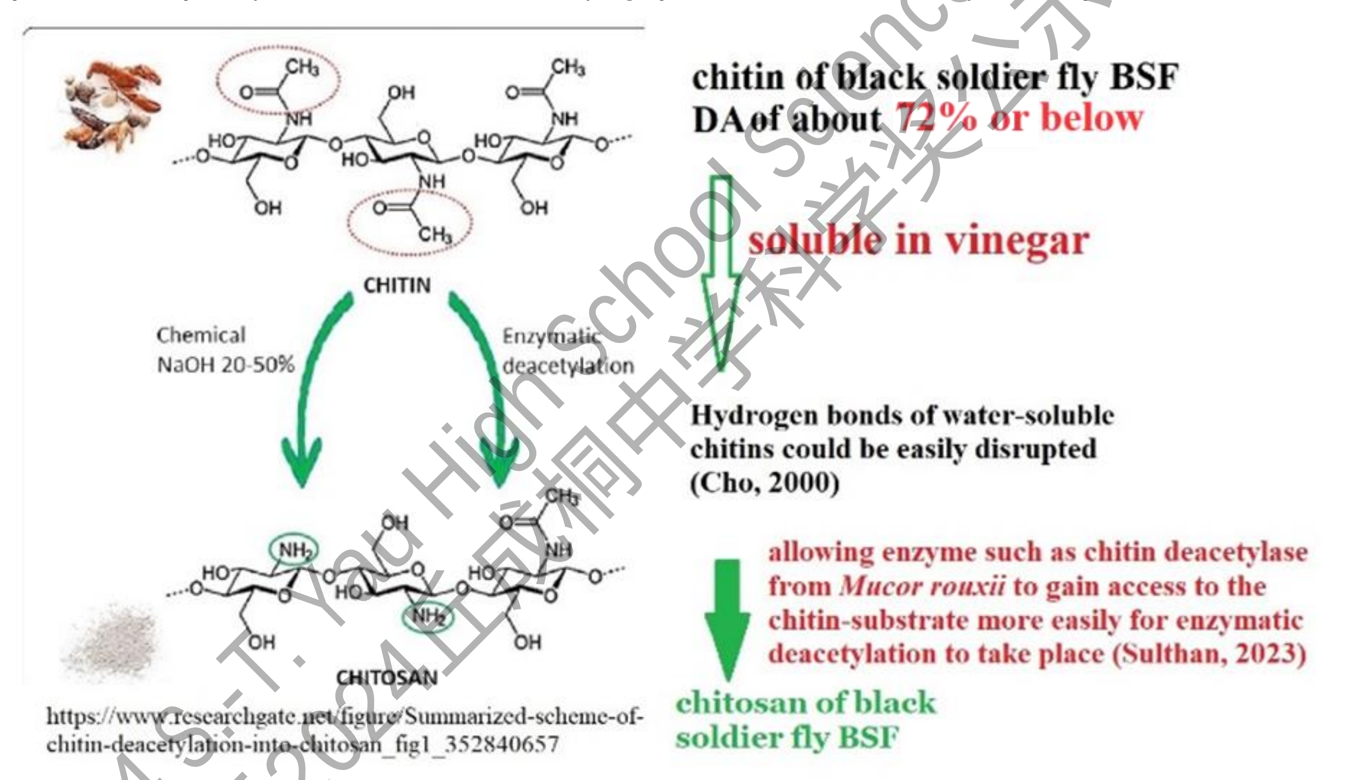


Figure 1 Schematic diagram for the mechanism of **GVFM**

1.7.2.2 *Mucor rouxii*

Chitin deacetylase, the enzyme that catalyzes the hydrolysis of acetamido groups of N-acetylglucosamine in chitin in the bio-conversion of chitin to chitosan, had been purified to homogeneity from mycelial extracts of the fungus *Mucor rouxii*. When glycol chitin (a water-soluble chitin derivative) was used as substrate, the optimum temperature for enzyme activity was determined to be -.50°C and the optimum pH was -4.5. (Kafetzopoulos, 1992)

The use of beneficial microorganisms such as *Mucor rousii* can produce different and unique flavors in the process of food fermentation. which improve the taste of fermented foods and give them a variety of distinct flavors. (Zhang, 2023)

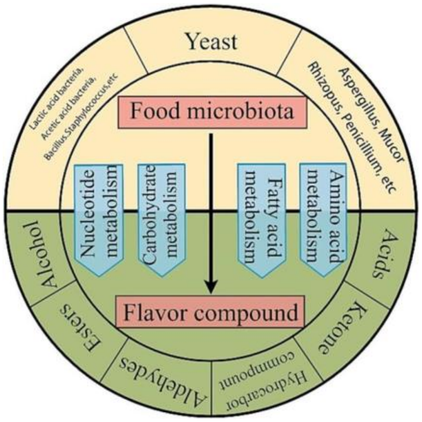


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Figure 1.7.2.2.1 Summary of microbial metabolism and accumulation of flavor substances. (Zhang, 2023)

Table 1.7.2.2.2 Mucor as a flavor-producing microorganism. (Zhang, 2023)

Microorganism	Characteristic	Major strains	Flavor components	Reference
Mucor	Mycelium developed, colony texture is loose, wadding, composed of many branching mycelium. Mycelium is generally white, without septum, and without pseudoroot.	*Mucor mucedo, Mucor rouxii, Mucor racemose, Mucor actinomyces,* etc.	2-octenal, 1-octene-3-ol, 2-methylphenol, etc.	(Chen, 2023) (Morin, 2017)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10534219/table/t0010/?report=objectonly

2. Methodology

 Shells of *Hermetia illucens,* black soldier fly (**BSF**)	 Chitin of **BSF**	 Chitosan of **BSF**	 *Mucor rouxii*	 Air purifier
 **Rubstik (<0.5g/kg formaldehyde)**	 Urea formaldehyde	 Wall paint Formaldehyde-buster	 3M Formaldehyde remover	 Activated charcoal

2.1 Production of **Chitogels** (hydrogel) and ***rouxii*** **Chitogels** of chitosan of black soldier fly **BSF** and the investigation of the structural changes using FTIR of hydrogel of chitosan of black soldier fly **BSF**

2.1.1 Production of **Chitogels** (hydrogel) and ***rouxii*** **Chitogels** of chitosan of black soldier fly **BSF**

2.1.1.1 Production of **Chitogels** (hydrogel) **BSF**

1. Shells of **BSF** were demineralized to remove $CaCO_3$ using 2M nitric acid.

Figure 2.1.1.1.1 Demineralization of shell of **BSF**

2. Chitin of **BSF** was deacetylated (deproteinated) to chitosan using 16.7M NaOH at 80ºC for 6 hours.

Figure 2.1.1.1.2 Deacetylation of chitin of **BSF** to chitosan

3. Hydrogel of chitosan of **BSF** was obtained by dissolving chitosan in vinegar.

Figure 2.1.1.1.3 Dissolving chitosan in vinegar

2.1.1.2 Production of different **Chitogels** of **BSF**

2.1.1.2.1 Production of **Chitogels** using traditional method of using highly corrosive 16.7M NaOH

1. 12.5g chitin of black soldier fly **BSF** was added to excess 16.7M NaOH and heated to 80ºC for 4 hours.

2. **Chitogel** (Hydrogel) was obtained by adding excess vinegar to the chitosan formed.

2.1.1.2.2 Production of **Chitogels** using Green Vinegar Method

1. 12.5g chitin of black soldier fly **BSF** was added to 87.5 cm^3 vinegar and stirred for 15 mins. **Chitogels** were formed.

2.1.1.2.3 Production of ***rouxii*** **Chitogels** of **BSF** using Green Vinegar Fermentation Method

1. 12.5g chitin of black soldier fly **BSF** was added to 87.5 cm^3 vinegar and stirred for 15 mins.
2. 0.5g *Mucor rouxii* was added to the mixture and embedded in an incubator at 37°C for 2 days to obtain ***rouxii*** **Chitogels** (hydrogel) of chitosan of **BSF**.

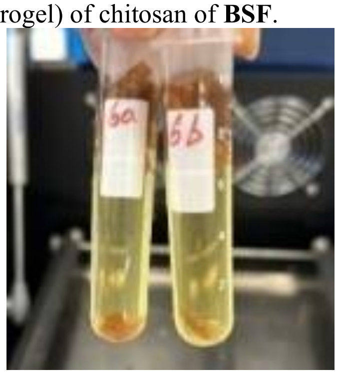


Figure 2.1.1.2.2.1 production of **Chitogels** from chitin of **BSF** using the Green Vinegar Method

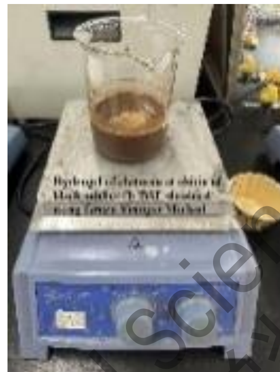


Figure 2.1.1.2.2.2 stirring chitin of **BSF** in vinegar using magnetic stirrer

Figure 2.1.1.2.3 production of ***rouxii*** **Chitogels** by incubating chitin of **BSF** with *Mucor rouxii* at 37°C in vinegar (Green Vinegar Fermentation Method)

2.1.1.2.4 Investigation of the percentage yield of ***rouxii*** **Chitogels** from chitin **BSF** using **GVFM** at different temperatures

1. 0.1g of chitin BSF was added to 10.0cm^3 vinegar.
2. 0.05g *Mucor rouxii* was added to the mixture in an incubator at 30°C, 40°C, 50°C and 60°C for 1 day to obtain ***rouxii*** **Chitogels** (hydrogel) of chitosan of **BSF**.
3. Mass of ***rouxii*** **Chitogels** obtained was weighed to obtain the percentage yield.

Figure 2.1.1.2.4 incubator

2.1.2 Investigation of the optimum ratio of mass of hydrogel of chitosan of black soldier fly **BSF** to volume of vinegar

1. Different volumes of vinegar were added to 0.25g chitosan of **BSF**.
2. Mass of dried hydrogel was weighed.
3. The optimum ratio of mass of hydrogel of chitosan of black soldier fly **BSF** to volume of vinegar corresponded to the maximum mass of dried hydrogel obtained.

Figure 2.1.2 Dried hydrogel with different ratio of mass of hydrogel to volume of vinegar

2.1.3 Investigation of the structure changes using FTIR Spectrum II of **Chitogels** (hydrogel) and ***rouxii*** **Chitogels** of chitosan of black soldier fly **BSF** before and after condensation with **HCHO**

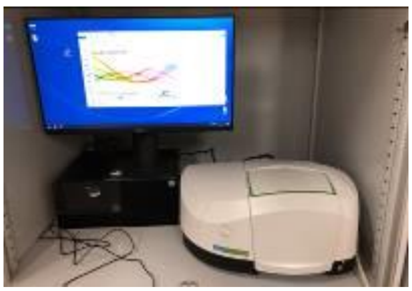


Figure 2.1.3 FTIR Spectrum Two

Spectrum Two FTIR spectrometers feature:

Standard, high-performance, room-temperature $LiTaO_3$ (lithium tantalate) MIR (mid infra-red) detector with a SNR (signal to noice ratio) of 9,300:1
Optional temperature-stabilized, high-performance DTGS (deuterated triglycine sulfate) MIR detector with a SNR of 14,500:1. Ideal for low-light, high throughput applications
Standard optical system with KBr windows for data collection over a spectral range of 8,300 – 350 cm^{-1} at a best resolution of 0.5 cm^{-1}
Figure 1.1.2.4.2 Features of FTIR Spectrum II

1. FTIR spectrum was obtained using FTIR Spectrum II.
2. DA% and DD% were obtained.

$$DA\% = \left(\frac{A_{1655}}{A_{3450}} \times \frac{1}{1.33} \times 100\%\right) \qquad DD[\%] = 100\% - \left(\frac{A_{1655}}{A_{3450}} \times \frac{1}{1.33} \times 100\%\right)$$

A absorbance in FTIR spectrum

2.2 Comparing the efficiency of removal of **HCHO** of hydrogel of black soldier fly (*Hermetia illucens)* and commercial formaldehyde removers

2.2.1 Investigating the efficiency of removal of **HCHO** of hydrogel of black soldier fly (*Hermetia illucens)* **BSF**

2.2.1.1 Investigating the efficiency of removal of **HCHO** of hydrogel of black soldier fly (*Hermetia illucens)* **BSF**

1. 0.2g hydrogel of **BSF** was put into a sealed container (300 ml) with 1.000 cm^3 1:20 by mass of formaldehyde respectively.
2. The concentration of **HCHO** remained was measured using a formaldehyde meter after 24hrs.
3. The above were repeated for Urea formaldehyde and Rubstik (<0.5g/kg formaldehyde)

In 1:20 by mass of formaldehyde

Figure 2.2.1.1 Measurement of concentration of **HCHO** removed by hydrogel of **BSF**

in urea formaldehyde

Figure 2.2.1.2 Measurement of concentration of **HCHO** removed by hydrogel of **BSF**

in Rubstik (<0.5g/kg formaldehyde)

Figure 2.2.1.3 Measurement of concentration of **HCHO** removed by hydrogel of **BSF**

2.2.2 Comparing the efficiency of removal of HCHO of **Chitogels** and ***rouxii*** **Chitogels** of BSF and other commercial formaldehyde removers

2 1g **Chitogels** (hydrogel) of **BSF** was put into a sealed container (300 ml) with 1.000 cm^3 1:100 by mass of formaldehyde respectively.

3 The concentration of **HCHO** remained was measured using a formaldehyde meter after 24 hrs.

4 The above were repeated for ***rouxii*** **Chitogels** and other commercial formaldehyde removers.

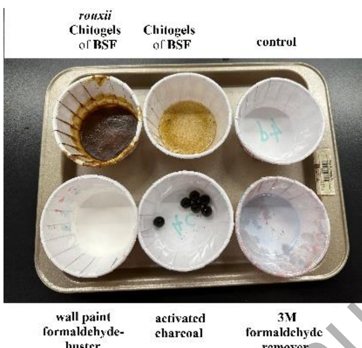


Figure 2.2.2.1 Samples of formaldehyde removers

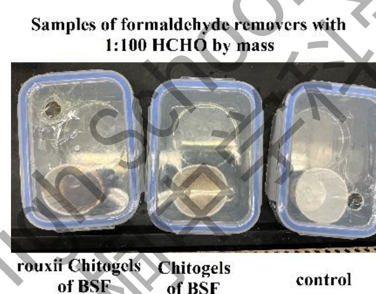


Figure 2.2.2.2 Formaldehyde removers with 1:100 **HCHO** by mass

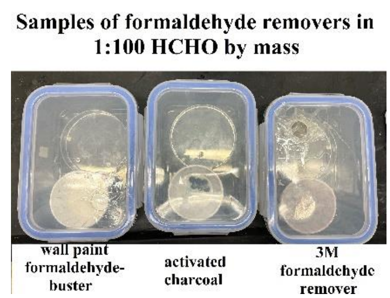


Figure 2.2.2.3 Formaldehyde removers with 1:100 **HCHO** by mass

2.3 Investigation of the anti-bacterial effect of hydrogel of black soldier fly **BSF** (*Hermetia illucens*) before and after condensation with **HCHO**. (Avila, 2009)

1. **Chitogels** (Hydrogels) of **BSF** with and without condensation with **HCHO** were soaked in water with oral bacterial obtained from drinking bottles for one night. (Avila, 2009)
2. Serial dilution was done.
3. 100 µL of the diluted solution were spread onto agar plates and incubated overnight.

4. Number of bacterial colonies were counted.

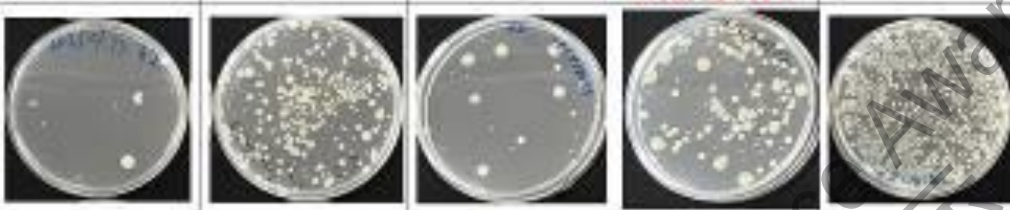
	black soldier fly BSF hydrogel	black soldier fly BSF roasted hydrogel	black soldier fly BSF hydrogel after condensation with HCHO	black soldier fly BSF roasted hydrogel after condensation with HCHO	oral bacteria only
dilution factor 10^{-1}					
No. of bacterial colonies	19	325	21	148	2450

Figure 2.3 Number of oral bacterial colonies formed in drinking water with oral bacteria soaked with hydrogels

Afterwards agar plates were NOT opened and used agar plates were autoclaved.

2.4 Investigating the removal efficiency of **HCHO** of **Chitogels** and ***rouxii*** **Chitogels** on-the-spot

2.4.1 Investigating the removal efficiency of **HCHO** of **Chitogels** and applying **Chitogels** on-the-spot in a newly renovated Lecture Theatre

1. **Chitogels** of **BSF** were put into bell jars.
2. Concentration of **HCHO** was measured using formaldehyde meter at different time.
1. **Chitogels** of black soldier fly **BSF** were put into bell jars (placed in a newly renovated Lecture Theatre overnight) to absorb and condensed with **HCHO**.
2. Readings of concentration of **HCHO** were recorded using formaldehyde meters.

Figure 2.4.1.1 a newly renovated Lecture Theatre

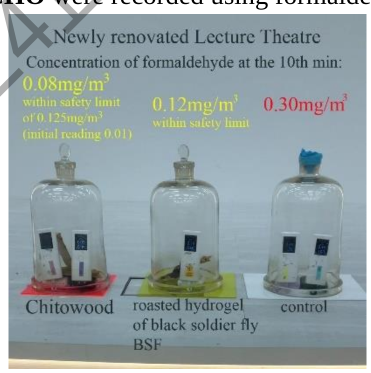


Figure 2.4.1.2 Formaldehyde meter reading at the 10^{th} min at 29°C

2.4.2 Investigating the removal efficiency of **HCHO** of ***rouxii*** **Chitogels** on-the-spot as filter in an air purifier

1. 1g of ***rouxii*** **Chitogels** of **BSF** were used as filter in an air purifier.
2. The air purifier with ***rouxii*** **Chitogels** as filter was put into a 9.3L air-tight box with 0.5g Rubstik (0.5/kg **HCHO**) and was on for 30 mins.
3. Readings of concentration of **HCHO** were recorded using formaldehyde meters.

4. Repeat using filter only.

Figure 2.4.2 ***rouxii*** **Chitogels** as filter in an air purifier in a 9.3L air-tight box with 0.5g Rubstik 0.5g/kg HCHO)

2.4.3 Investigating the removal efficiency of **HCHO** of ***rouxii*** **Chitogels** on-the-spot as filter in an air purifier

1. 1g of ***rouxii*** **Chitogels** of **BSF** were used as formaldehyde remover in a drawer.
3. Readings of concentration of **HCHO** were recorded using formaldehyde meters everyday.

Figure 2.4.3 Furniture of drawers emitting about 0.5 mg/m^3 **HCHO** (exceeding safety limit of 0.125 mg/m^3)

2.5 Biodegradability of **Chitogels** of black soldier fly **BSF**

1. Dry samples of **Chitogels** and ***rouxii*** **Chitogels** (hydrogels) of **BSF** were weighed.
2. Samples were left in soil and water was added to keep the soil wet.
3. Wet samples were weighed two times every week.

Figure 2.5 Samples of **Chitogels** and ***rouxii*** **Chitogels** of BSF in soil for bio-degradation

2.6 Detection of the presence of allergens in **Chitogels** of **BSF** using 3M Clean-Trace ALLTEC60 for detection of surface protein allergens

3M Clean-Trace ALLTEC60 detects as little as 3 µg of protein on surfaces and in hard to reach areas. It is validated for a range of allergenic proteins, including egg, milk, gluten, soy, peanut, almond and buckwheat

Figure 2.6 Detection of the presence of allergens in **Chitogels** and ***rouxii*** **Chitogels** of **BSF** using 3M Clean-Trace ALLTEC60 for detection of surface protein allergens

3. Results

3.1 Investigation of the percentage by mass of chitosan in black soldier fly **BSF** and the optimum ratio of volume of vinegar to mass of chitosan of black soldier fly **BSF** to in obtaining hydrogel of **BSF**

3.1.1 Investigation of the percentage by mass of chitosan in black soldier fly **BSF**

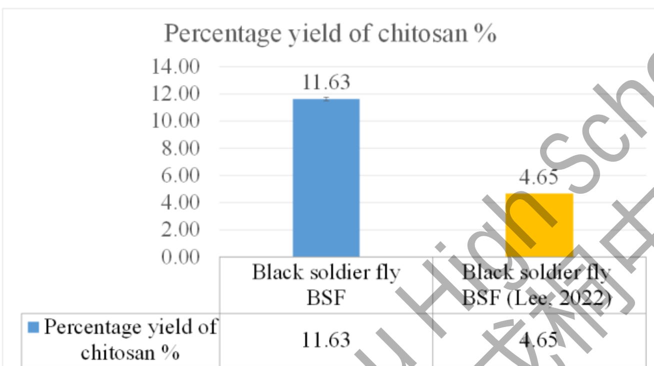


Figure 3.1.1 Graph of percentage of chitosan in black solder flyer **BSF**

Conclusion: The percentage by mass of chitosan obtained from *Hermatia illucens*, black soldier fly **BSF** was 11.63% which is comparable to the literature of 4.65% (Lee, 2022).

3.1.2 Investigation of the optimum ratio of volume of vinegar to mass of chitosan of black soldier fly **BSF** to in obtaining hydrogel of **BSF**

Table 3.1.2.1 Different ratio of volume of vinegar to mass of **BSF** for obtaining hydrogel

volume of vinegar/ mass of **BSF** (cc/g)	mass of dried hydrogel (g)
2	0.1959
4	0.2442
6	0.2765
8	0.2619
10	0.2587

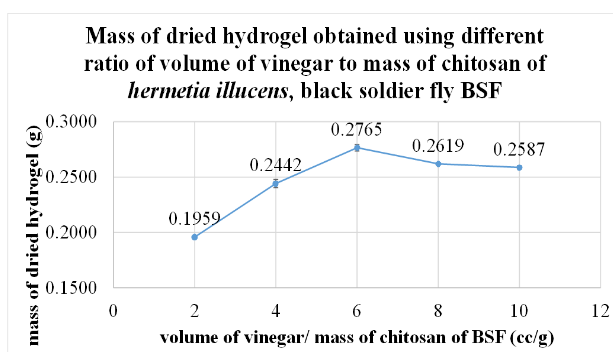


Figure 3.1.2.2 Graph of mass of hydrogel obtained using different ratio of volume of vinegar to mass of chitosan of **BSF**

Conclusion: The maximum mass of hydrogel chitosan of black soldier fly **BSF** would be obtained when 4 cm^3 to 5 cm^3 vinegar were added to 1g of chitosan of **BSF**.

3.1.3 Investigation of the effect of temperature on the percentage yield of ***rouxii* Chitogels** from chitin **BSF** in **GVFM**

Table 3.1.3.1 Percentage yield of ***rouxii* Chitogels** at different fermentation temperatures

***rouxii* Chitogel (vinegar +*Mucor rouxii*) obtained at different temperature/ degrees Celsius**	**Percentage yield of *rouxii* chitogel/ %**
30	49.1
40	50.5
50	71.0
60	53.2
vinegar only at 50	54.6

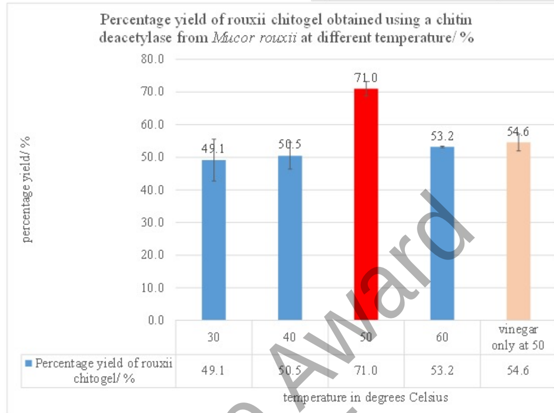


Figure 3.1.3.2 Percentage yield of ***rouxii* Chitogels** at different fermentation temperatures

Conclusion:

Fermentation of chitin BSF to ***rouxii* chitogels** by a chitin deacetylase from *Mucor rouxii* took place at 50ºC (optimum temperature which agreed with KAFETZOPOULOS, 1992) as the percentage yield was increased significantly to 71.0 % (cf. 54.6% with vinegar only)

3.2 Investigation of the structural changes using FTIR of hydrogel of chitosan of black soldier fly **BSF** before and after the absorption via condensation with formaldehyde **HCHO**.

3.2.1 Investigation of structural changes using FTIR Spectrum II

3.2.1.1 Investigation of the degree of deacetylation DD% of chitosan of black soldier fly **BSF**

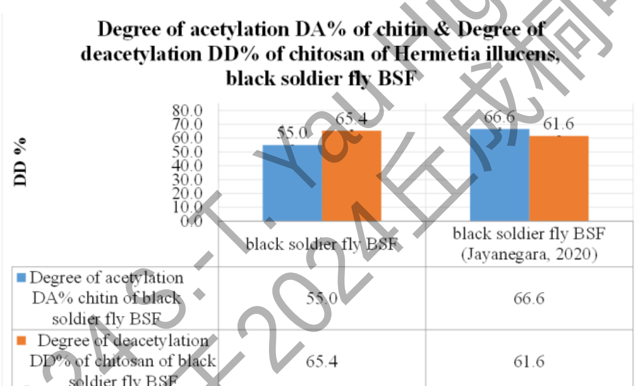
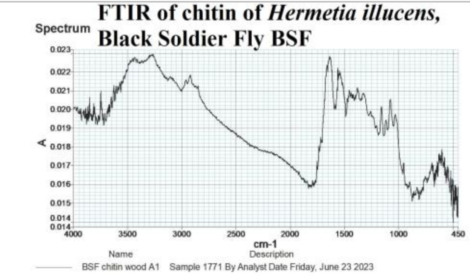


Figure 3.2.1.1 Degree of acetylation DA% of chitin and degree of deacetylation DD% of chitosan of black soldier fly **BSF**

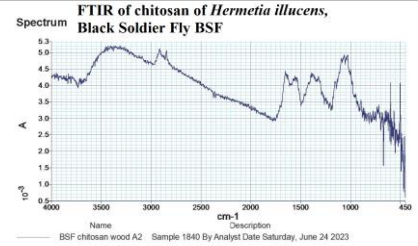
Conclusion: The degree of acetylation DA% of chitin and degree of deacetylation DD% of chitosan of black soldier fly was found to be 55.0% and 65.4% which were consistent with the literature value of 66.6% and 61.6% respectively. (Jayanegara, 2020)

3.2.1.2 Investigation of the degree of deacetylation DD% of **Chitogels** and ***rouxii* Chitogels** of black soldier fly **BSF** before and after condensation with **HCHO**



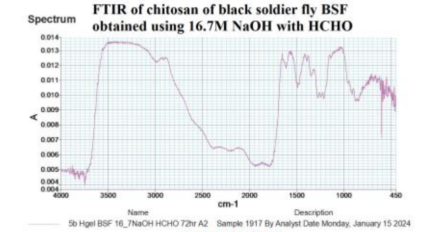
DA% 55.0%

FTIR of chitin of *Hermetia illucens,* Black Soldier Fly **BSF**



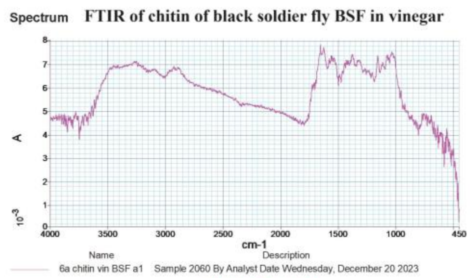
DD% 65.8%

FTIR of chitosan of *Hermetia illucens,* Black Soldier Fly **BSF**



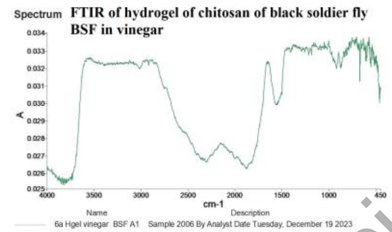
DD% 58.2%

FTIR of chitosan of black soldier fly **BSF** obtained using 16.7M **NaOH** with **HCHO (72 hrs)**



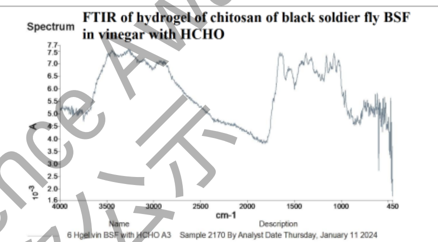
DA% 56.4%

FTIR of chitin of black soldier fly **BSF** in vinegar



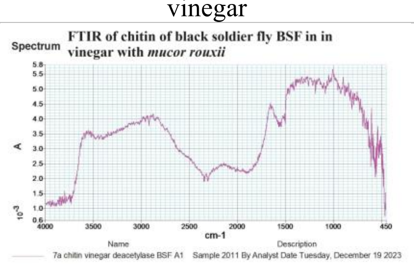
DD% 59.7%

FTIR of **Chitogels** of chitosan of black soldier fly **BSF** in vinegar



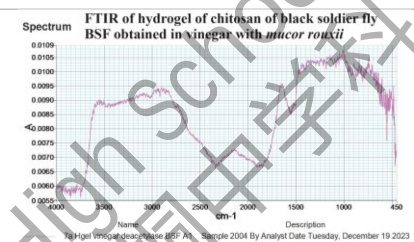
DD% 51.1%

FTIR of **Chitogels** of chitosan of black soldier fly **BSF** in vinegar with **HCHO** (72 hrs)



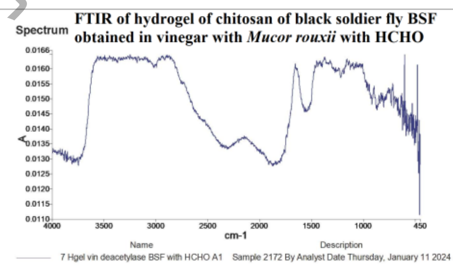
DA% 55.4%

FTIR of chitin of black soldier fly **BSF** in in vinegar with *Mucor rouxii*



DD% 61.1%

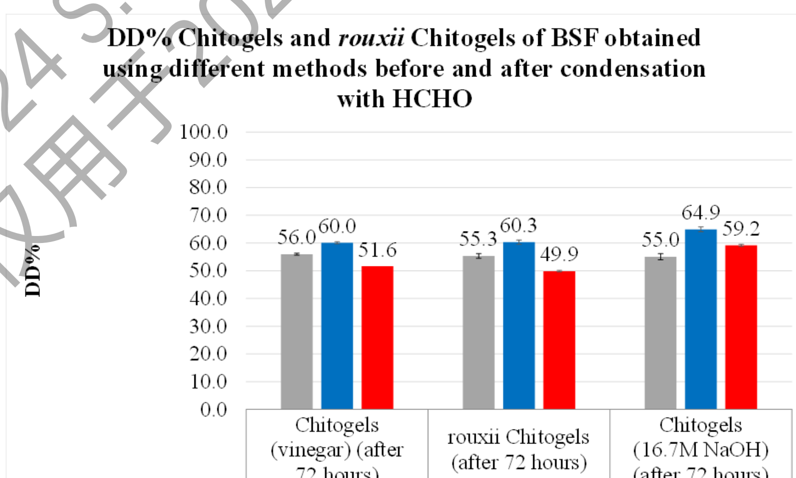
FTIR of ***rouxii*** **Chitogels** of chitosan of black soldier fly **BSF** obtained in vinegar with *Mucor rouxii*



DD% 49.9%

FTIR of ***rouxii*** **Chitogels** of chitosan of black soldier fly **BSF** obtained in vinegar with *Mucor rouxii* with **HCHO** (72 hrs)

Figure 3.2.1.2.1 FTIR of chitins , **Chitogels** and ***rouxii*** **Chitogels** of chitosans obtained using different methods with adsorbed **HCHO**



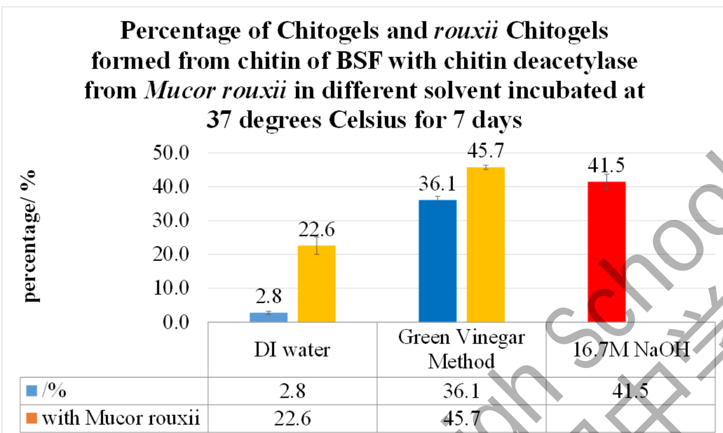
	Chitogels (vinegar) (after 72 hours)	rouxii Chitogels (after 72 hours)	Chitogels (16.7M NaOH) (after 72 hours)
DA% of chitin of BSF	56.0	55.3	55.0
DD% no HCHO	60.0	60.3	64.9
DD% with HCHO	51.6	49.9	59.2

Figure 3.2.1.2.2 DA% of chitin, DD% **Chitogels** and ***rouxii* Chitogels** of **BSF** obtained using different methods with adsorbed **HCHO** (72 hrs)

Conclusion: The DD% of ***rouxii* Chitogels** obtained using the Green Vinegar Fermentation Method (60.3%) and **Chitogels** of the Green Vinegar Method (60.0%) were consistent with that that obtained using the traditional method (16.7M NaOH) (64.9%).

There were more significant decreases in DD% of the Chitogels obtained using the Green Vinegar Fermentation Method with adsorbed **HCHO** (60.3-49.9=10.4%) and Green Vinegar Method with adsorbed **HCHO** (60.0-51.6=8.4%) than that obtained using the traditional method (16.7M NaOH) (64.9-59.2=5.7%). Thus, ***rouxii* Chitogels** obtained using the Green Vinegar Fermentation Method and **Chitogels** of Green Vinegar Method could remove **HCHO** effectively showing that structural changes took place as **-NH_2** groups in hydrogel during condensation due to formation of **-N=C between** -NH_2 in **Chitogels** and **-C=O** in formaldehyde.

3.2.2 Investigation of the percentage of **Chitogels** and ***rouxii* Chitogels** obtained from chitin of black soldier fly **BSF** using different methods

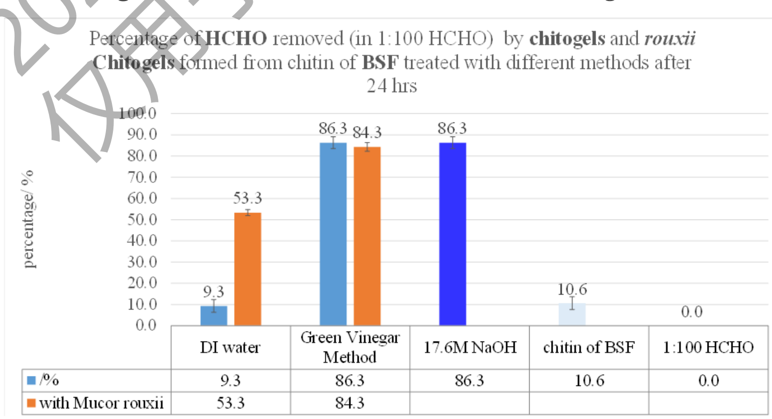


	DI water	Green Vinegar Method	16.7M NaOH
/%	2.8	36.1	41.5
with Mucor rouxii	22.6	45.7	

Figure 3.2.2 Percentage of **Chitogels** and ***rouxii* Chitogels** obtained from chitin of black soldier fly **BSF** using different methods

Conclusion: The percentage of ***rouxii* Chitogels** obtained from chitin **BSF** using the Green Vinegar Fermentation Method was 45.7% and the **Chitogels** of Green Vinegar Method was 36.1% which were 1.1 times and 87.0% of that of using the traditional method of highly corrosive 16.7M NaOH (41.5%), so the innovative Green Vinegar Fermentation and Green Vinegar Method to obtain **Chitogels** and ***rouxii* Chitogels** from chitin of **BSF** was a good alternative to eliminate the use of highly corrosive conc. NaOH.

3.2.3 Investigation of the percentage of **HCHO** removed and conc. of **HCHO** remained (in 1:100 **HCHO**) by **Chitogels** and ***rouxii* Chitogels** formed from chitin of **BSF** obtained using different methods



	DI water	Green Vinegar Method	17.6M NaOH	chitin of BSF	1:100 HCHO
/%	9.3	86.3	86.3	10.6	0.0
with Mucor rouxii	53.3	84.3			

Figure 3.2.3.1 Percentage of **HCHO** removed (in 1:100 **HCHO**) by **Chitogels** and ***rouxii* Chitogels** formed from chitin of **BSF** obtained using different methods after 24 hrs

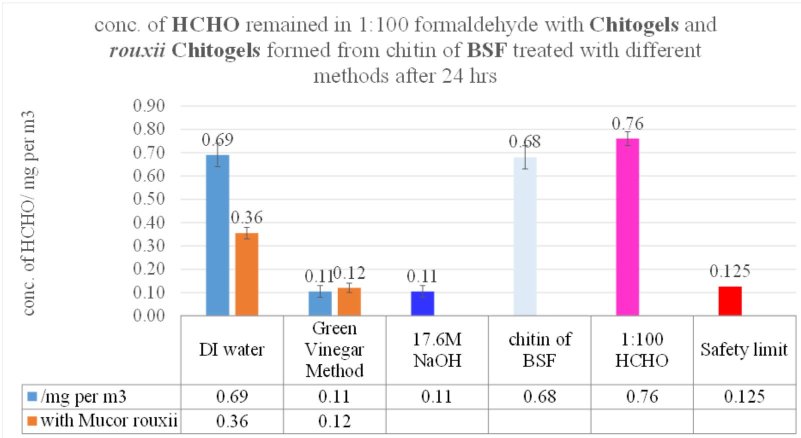


Figure 3.2.3.2 conc. of **HCHO** remained in 1:100 **HCHO** with **Chitogels** and ***rouxii* Chitogels** formed from chitin of **BSF** obtained using different methods after 24 hrs

Conclusion: Both **Chitogels** and ***rouxii* Chitogels** obtained using the Green Vinegar Method and traditional method (16.7M NaOH) could remove 86.3% **HCHO** and that of the Green Vinegar Fermentation Method could remove 86.3% **HCHO** (in 1:100 **HCHO**) in 24 hrs. The conc. of **HCHO** remained were 0.11 mg/m^3 , 0.11 mg/m^3 and 0.12 mg/m^3 which were within the safety limit (0.125mg/m^3).

3.2.4 Investigation of the efficiency of removal of formaldehyde **HCHO** by **Chitogels** of black soldier fly **BSF** with different sources of **HCHO**

3.2.4.1 Investigation of the percentage of removal of formaldehyde **HCHO** by **Chitogels** of black soldier fly **BSF** with different sources of **HCHO**

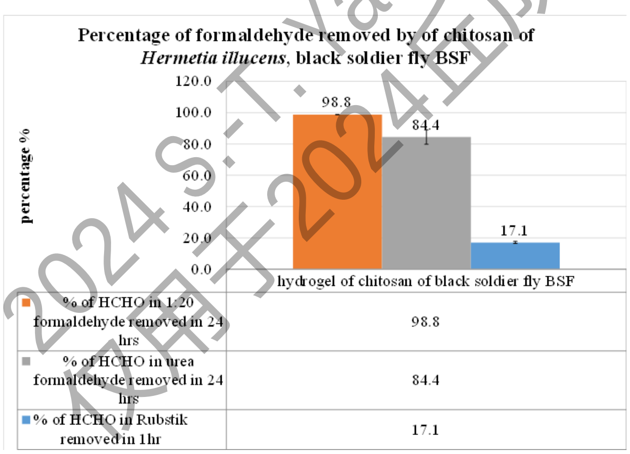


Figure 3.2.4.1 Percentage of formaldehyde removed by **Chitogels** of chitosan of **BSF**

Conclusion: **Chitogels** of black soldier fly **BSF** could remove over 98% **HCHO** in 1:20 by mass of **HCHO** in 24 hours, over 84% **HCHO** in urea formaldehyde in 24 hours and over 17% of **HCHO** in Rubstik in 1 hour (Rubstik dried in 1hr).

3.2.4.2 Investigation of the concentration of removal of formaldehyde **HCHO** by **Chitogels** of black soldier fly **BSF** with different sources of **HCHO**

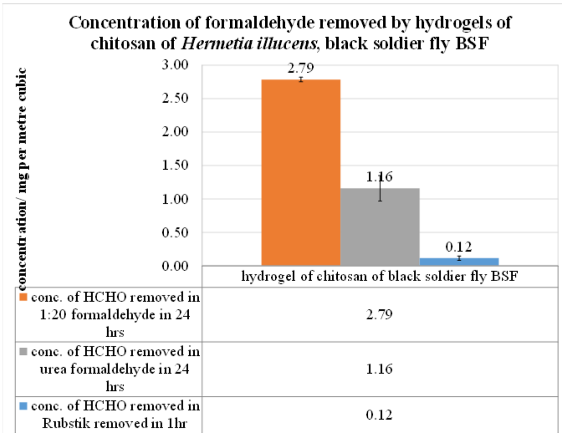


Figure 3.2.4.2 Concentration of formaldehyde removed by **Chitogels** of chitosan of **BSF**

Conclusion: **Chtiogels** of black soldier fly **BSF** could remove over 2.79 mg/m^3 **HCHO** in 1:20 by mass of **HCHO** in 24 hours, over 1.16 mg/m^3 **HCHO** in urea formaldehyde in 24 hours and over 0.12 mg/m^3 of **HCHO** in Rubstik in 1 hour.

3.2.4.3 Investigation of the concentration of formaldehyde **HCHO** remaining after removal of **HCHO** by **Chitogels** of black soldier fly **BSF** with different sources of **HCHO**

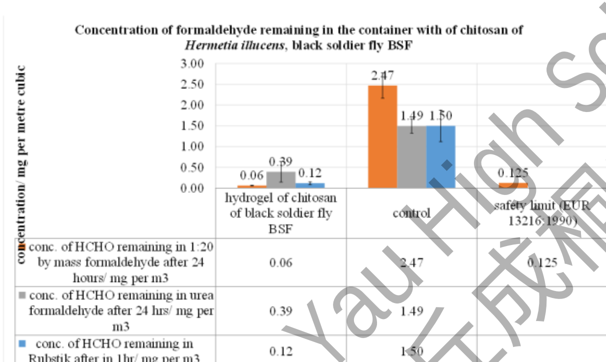


Figure 3.2.4.3 Concentration of formaldehyde remaining in the container with **Chitogels** of chitosan of **BSF**

Conclusion: **Chitogels** of black soldier fly **BSF** could remove significant amount of **HCHO** in 1:20 by mass of formaldehyde, urea formaldehyde and Rubstik (<0.5g/kg formaldehyde).

The conc. of **HCHO** in 1:20 by mass formaldehyde was (reduced from 2.47 mg/m^3 to) 0.06 mg/m^3 after 24 hrs with **Chitogels** of **BSF**; and Rubstik (reduced from 1.50 mg/m^3) to 0.12 mg/m^3 after 1 hr (dried in 1 hr) that were within safety limit of 0.125 mg/m^3 (EUR13216EN, 1990) with **Chitogels** of black soldier fly **BSF**.

3.2.5 Investigation of the efficiency of removal of formaldehyde **HCHO** by **Chitogels** and ***rouxii* Chitogels** of black soldier fly **BSF** and other commercial products

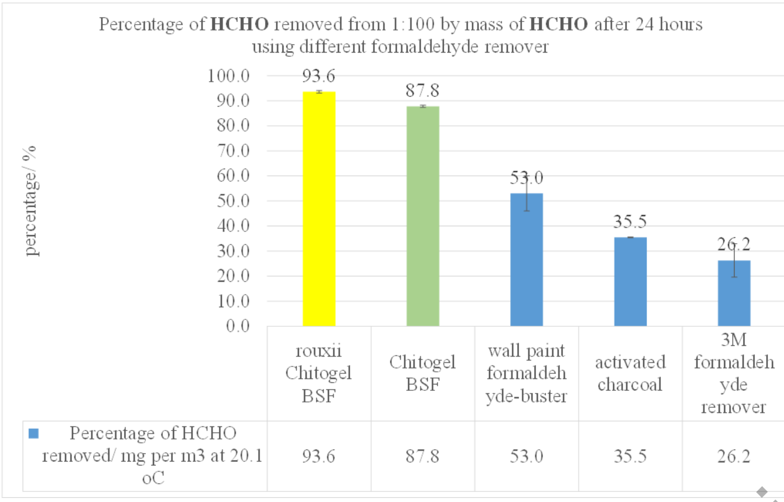


Figure 3.2.5.1 Percentage of **HCHO** removed from 1:100 by mass of **HCHO** after 24 hours using different formaldehyde remover

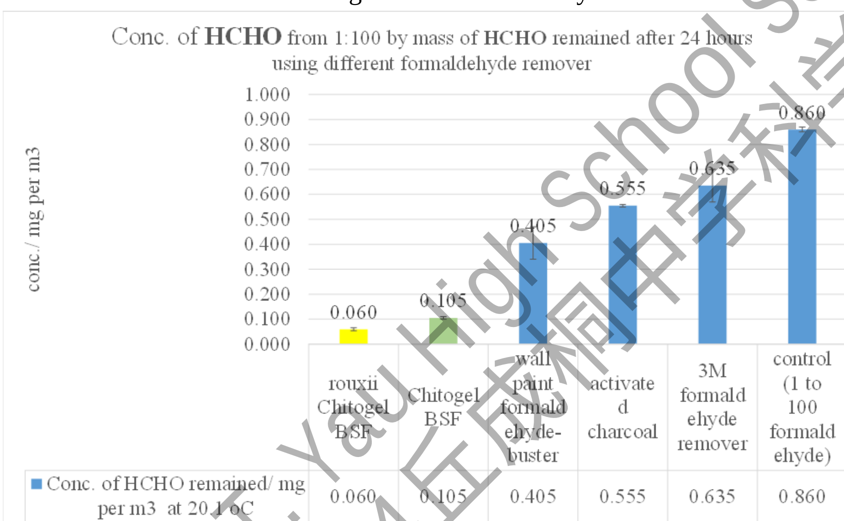


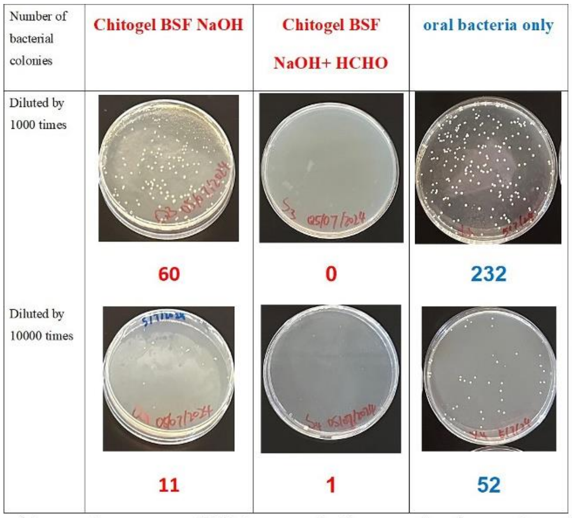
Figure 3.2.5.2 Conc. of **HCHO** from 1:100 by mass of **HCHO** remained after 24 hours using different formaldehyde remover

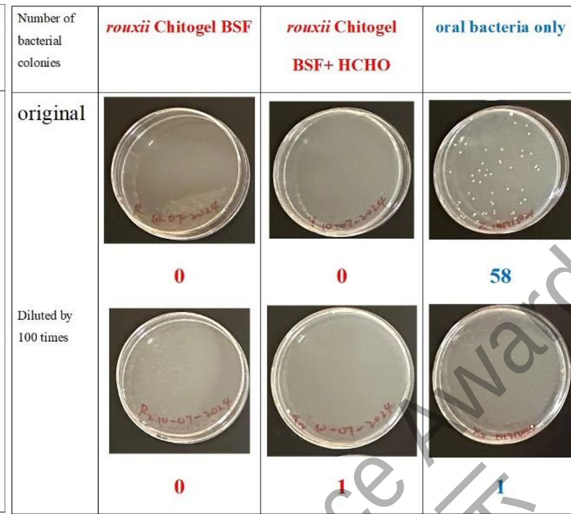
Conclusion: ***rouxii* Chitogels** (hydrogel of **BSF** obtained using chitin deacetylase from *Mucor rouxii*) and **Chitogels** showed the highest % of removal of HCHO from 1:100 **HCHO** (by mass) of 93.0% and 87.8%. Besides the concentration of **HCHO** left were 0.06mg/m^3 and 0.105mg/m^3 (within safety limits of 0.125mg/m^3). (EUR13216EN, 1990)

Thus the use of ***rouxii* Chitogels** obtained from fermentation of *Mucor rouxii* of chitin of **BSF** as formaldehyde remover was feasible and much better than commercial products such as wall paint formaldehyde-buster, activated charcoal and 3M formaldehyde remover.

3.3 Investigation of the anti-bacterial effect of **Chitogels** (hydrogels) and ***rouxii* Chitogels** of black soldier fly **BSF**

Table 3.3 Count of bacterial colonies of **Chitogels** (hydrogels) and ***rouxii* Chitogels** of black soldier fly **BSF** hydrogel in drinking water with oral bacteria before and after condensation with formaldehyde **HCHO**





(Agar plates were NOT opened afterwards; Agar plates were autoclaved before disposal)

Conclusion:

Chitogels and **rouxii Chitogels** are anti-bacterial and showed significant anti-bacterial effect towards oral bacterial. [no. of bacterial colonies of **Chitogels** (60, 11 w.r.t. dilution 10^{-3}, 10^{-4}) cf. oral bacterial (control: 232, 52) and **rouxii Chitogels** (0, 0 w.r.t. dilution 10^{0}, 10^{-2}) cf. oral bacterial (control: 58,1)] After condensation with formaldehyde, **Chitogels** of **BSF** kept showing anti-bacterial effect towards oral bacteria. [no. of bacterial colonies: **Chitogels** (0,1 w.r.t. dilution 10^{-3}, 10^{-4}) cf. oral bacterial (control: 232, 52)] and **rouxii Chitogels** (0, 1 w.r.t. dilution 10^{0}, 10^{-2}) cf. oral bacterial (control: 58, 1).

3.4 Investigation of the use of **Chitogels** in the removal of formaldehyde **HCHO** in a newly renovated Lecture Theatre, air purifier and furniture

3.4.1 Investigation of the removal of **HCHO** in a newly renovated Lecture Theatre using **Chitogels** of black Soldier fly **BSF** to remove **HCHO**

3.4.1.1 Investigation of the concentration of **HCHO** remained in a newly renovated Lecture Theatre using **Chitogels** of black Soldier fly **BSF** to remove **HCHO**

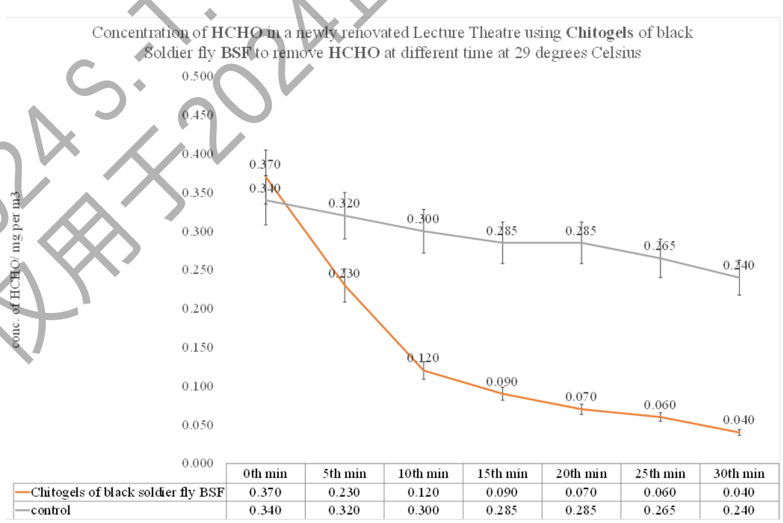


Figure 3.4.1.1 Concentration of **HCHO** in a newly renovated Lecture Theatre using **Chitogels** of black Soldier fly **BSF** to remove **HCHO** at different time at 29ºC

Conclusion: When using **Chitogels** to remove **HCHO** and 10 mins when using hydrogel of **BSF** (from **0.37** mg/m^3). Obviously, **Chitogels** removed **HCHO** effectively by condensation with **HCHO** in a newly renovated Lecture Theatre at 29ºC.

3.4.1.2 Investigation of the rate of removal of **HCHO** in a newly renovated Lecture Theatre using **Chitogels** of black Soldier fly **BSF** to remove **HCHO**

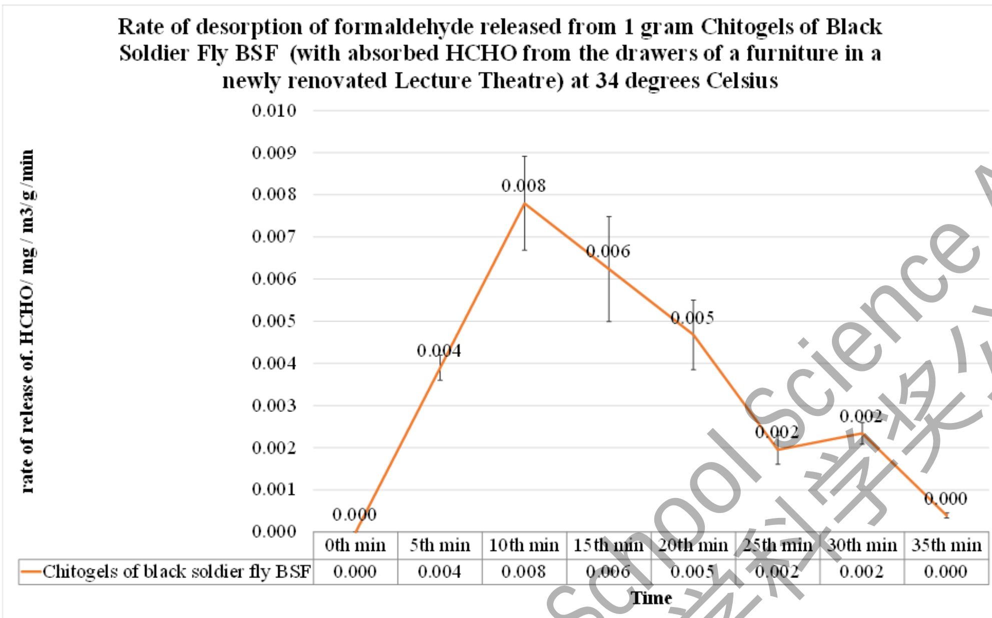


Figure 3.4.1.2 Rate of removal of **HCHO** by 1g of **Chitogels** of **BSF** in a Newly renovated Lecture Theatre at 29ºC.

Conclusion: Rate of removal of **HCHO** by **Chitogels** was 0.008 mg/m^3 /g/ min at the 10th min. Safety limit (0.125 mg/m^3) was reached at the 10th min when using **Chitogels** as **HCHO** remover. Then rate became almost zero in 25 mins. Obviously, **Chitogels** removed **HCHO** effectively by condensation with **HCHO** in a newly renovated Lecture Theatre at 29ºC.

3.4.2 Investigation of the removal of **HCHO** using ***rouxii*** **Chitogels** of black Soldier fly **BSF** as filter in an air purifier

3.4.2.1 Investigation of the percentage of **HCHO** removed by ***rouxii*** **Chitogels** of black Soldier fly **BSF** as filter in an air purifier

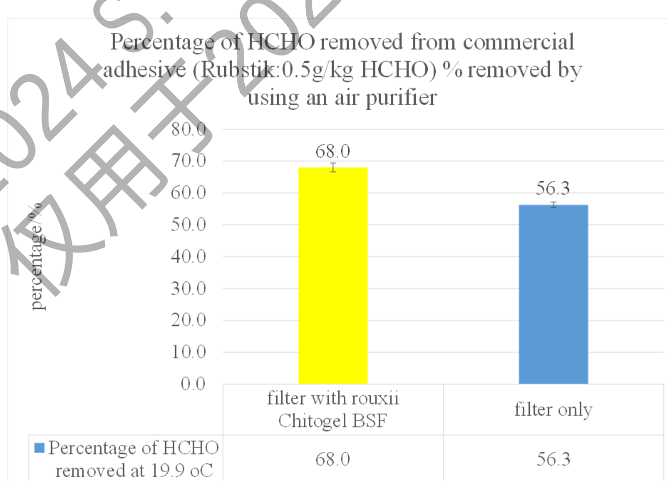


Figure 3.4.2.1 Percentage of **HCHO** removed from commercial adhesive (Rubstik:0.5g/kg **HCHO**) % removed by using an air purifier at 19.9ºC

Conclusion: The Percentage of **HCHO** removed by an air purifier with ***rouxii* Chitogels** as filter was 68.0% 19.9^{o}C which outperformed that of filter only of 56.3% by 20.8%.

3.4.2.2 Investigation of the percentage of **HCHO** removed by ***rouxii* Chitogels** of black Soldier fly **BSF** as filter in an air purifier

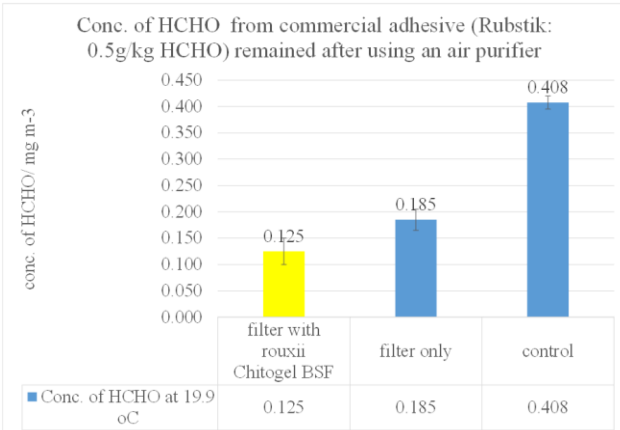


Figure 3.4.2.2 Conc. of **HCHO** from commercial adhesive (Rubstik: 0.5g/kg **HCHO**) remained after using an air purifier 19.9^{o}C

Conclusion: The conc. of **HCHO** remained was 0.125mg/m^3 when using filter with ***rouxii* Chitogels** 19.9^{o}C which was within the safety limit of HCHO of 0.125 mg/ m^3. (EUR13216EN, 1990)

3.4.3 Investigation of the removal of **HCHO** using ***rouxii* Chitogels** of black Soldier fly **BSF** in furniture

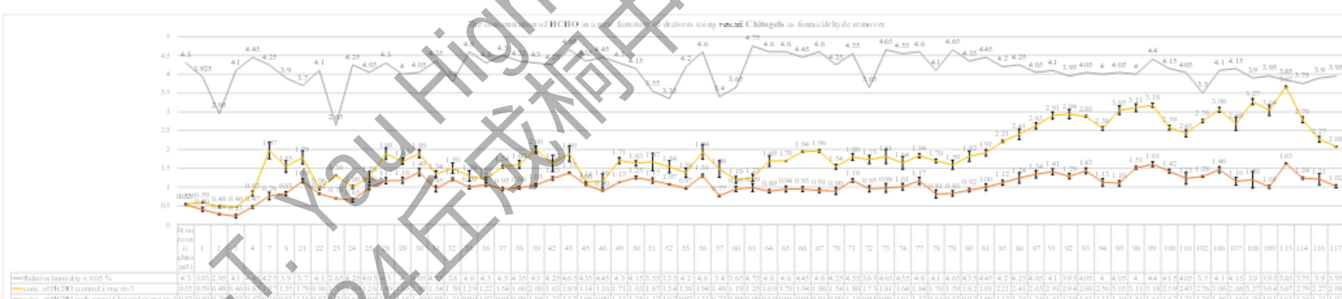


Figure 3.4.3.1 The concentration of **HCHO** in a new furniture of drawers using ***rouxii* Chitogels** as formaldehyde remover for 117 days

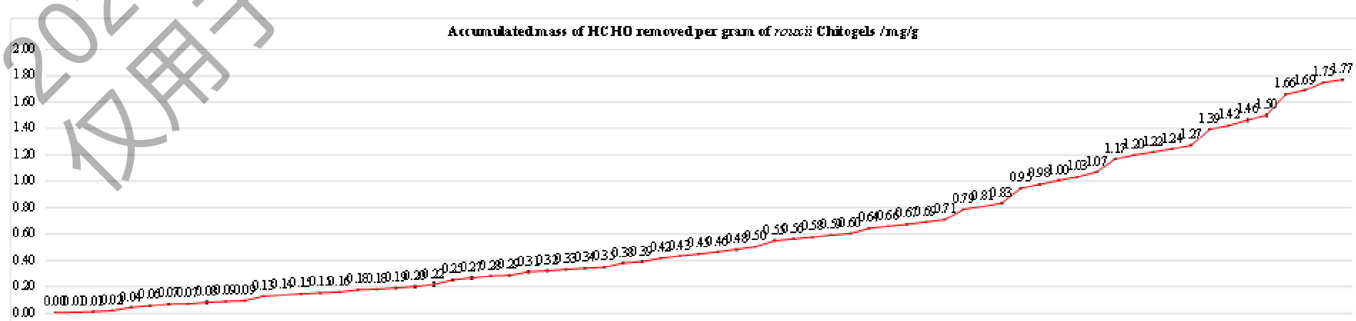


Figure 3.4.3.2 Accumulated mass of **HCHO** removed per gram of ***rouxii* Chitogels** as formaldehyde remover in a new furniture of drawers for 117 days

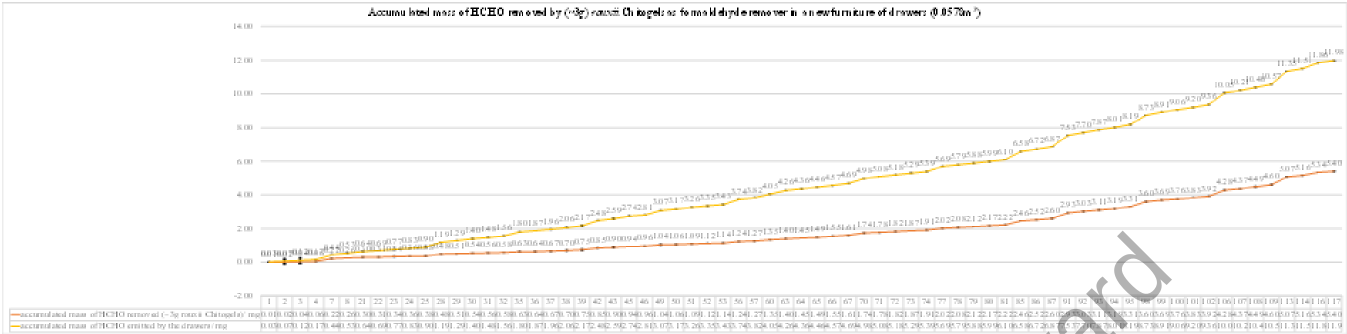


Figure 3.4.3.3 Accumulated mass of **HCHO** removed by (~3g) ***rouxii*** **Chitogels** as formaldehyde remover in a new furniture of drawers for 117 days

Conclusion: Using ***rouxii*** **Chitogels** as formaldehyde remover in new furniture such as drawers (0.0578m^3) could remove 1.77mg/g or 5.40mg/3g ***rouxii*** **Chitogels** (out of 12.0 mg control**)** **HCHO** effectively in 117 days at 31ºC or below. The concentration of **HCHO** could reach as low as 0.23 mg/m^3 in 3 days at 22ºC or below. More than (5.40÷12.0x100%=) 45.0% of **HCHO** was removed from the drawers (0.0578m^3) in 117 days.

3.5 Biodegradability of **Chitogels** and ***rouxii*** **Chitogels** in soil

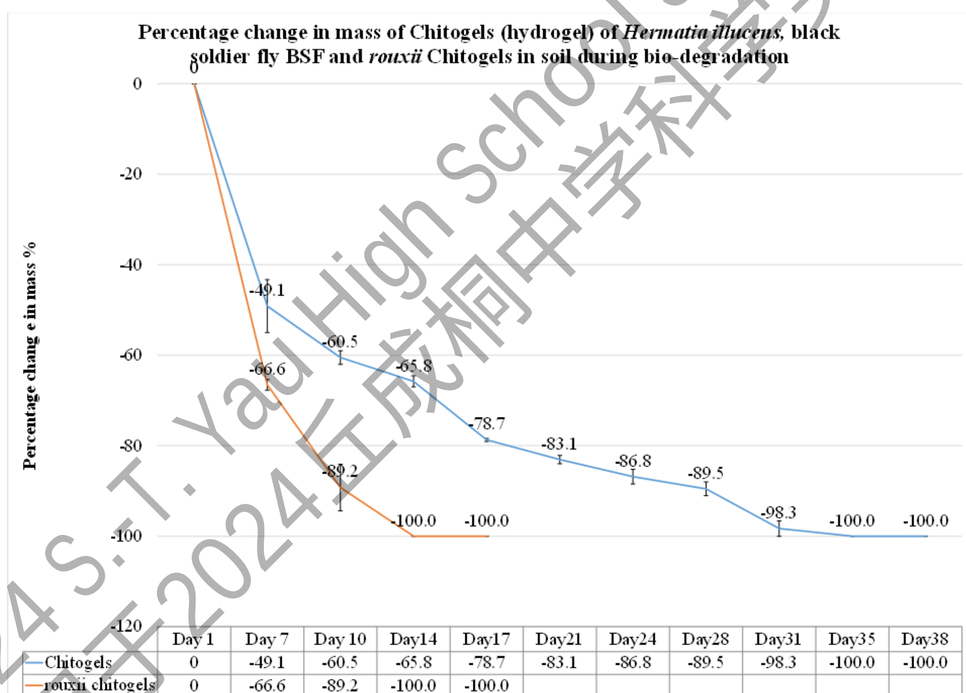


Figure 3.5 Percentage change in mass of **Chitogels** (hydrogel of *Hermatia illucens* black soldier fly **BS** remained in soil during bio-degradation

Conclusion:
Chitogels of **BSF** took 38 days and ***rouxii*** **Chitogels** took 14 days to be fully bio-degraded in soil showing that **Chitogels** and ***rouxii*** **Chitogels** were bio-degradable.

3.6 Detection of the presence of allergens in **Chitogels** of **BSF** using 3M Clean-Trace ALLTEC60 for detection of surface protein allergens

Figure 3.6 Surface protein allergens absent when **Chitogels** and of **BSF** were tested using 3M Clean-Trace ALLTEC60

Conclusion: Surface protein allergens were absent when **Chitogels** and ***rouxii* Chitogels** of **BSF** were tested using 3M Clean-Trace ALLTEC60, so **Chitogels** of BSF should be non-allergic and safe to be used in **Anti-bacterial forma-ridden Chitogels** as formaldehyde remover.

4. Findings

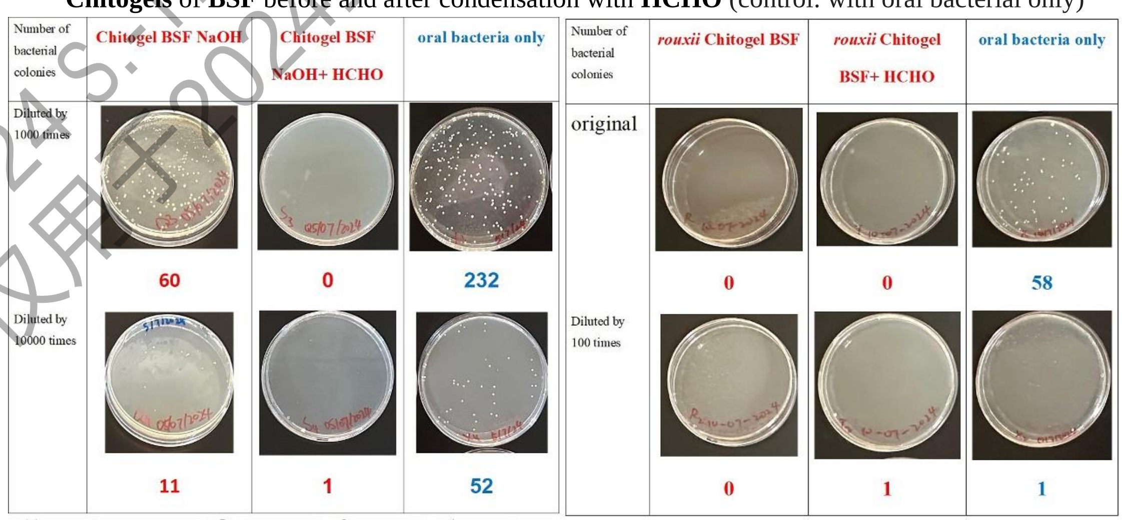
4.1 Feasibility of using hydrogel of black soldier fly (*Hermetia illucens*) obtained fermentation done by a chitin deacetylase from *Mucor rouxii* as formaldehyde remover in making **Anti-bacterial forma-ridden *rouxii* Chitogels**

The percentage yield of ***rouxii* Chitogels** (hydrogel of **BSF** obtained using chitin deacetylase from *Mucor rouxii*) from chitin of **BSF** was 71.0 at 50^{o}C. and did not involve the use of highly corrosive NaOH (cf. 41.5% from 17.6M NaOH). ***rouxii* Chitogels** and **Chitogels** showed the highest percentage of removal of HCHO from 1:100 **HCHO** (by mass) of 93.0% and 87.8% when compared with some commercial formaldehyde removers (cf. wall paint HCHO-busters: 53.0%; activated Charcoal 35.5%; 3M **HCHO** remover 26.2%). Besides the concentration of **HCHO** left were 0.06mg/m^3 and 0.105mg/m^3 (within safety limits of 0.125mg/m^3) after 24 hours at 19.9^{o}C. (EUR13216EN, 1990). Obviously, ***rouxii* Chitogels** as formaldehyde remover in making **Anti-bacterial forma-ridden *rouxii* Chitogels** is feasible.

4.2 Anti-bacterial effect of hydrogel of black soldier fly (*Hermetia illucens*) before and after condensation with **HCHO.**

Chitogels (Hydrogel**)** and ***rouxii* Chitogels** of **BSF** before and after condensation with **HCHO** all showed significant anti-bacterial effect when soaked in water with oral bacteria at different dilution factors.

Table 4.2 Number of oral bacterial colonies formed in drinking water with oral bacteria soaked with **Chitogels** and ***rouxii* Chitogels** of **BSF** before and after condensation with **HCHO** (control: with oral bacterial only)



Number of bacterial colonies	Chitogel BSF NaOH	Chitogel BSF NaOH+ HCHO	oral bacteria only
Diluted by 1000 times	60	0	232
Diluted by 10000 times	11	1	52

Number of bacterial colonies	*rouxii* Chitogel BSF	*rouxii* Chitogel BSF+ HCHO	oral bacteria only
original	0	0	58
Diluted by 100 times	0	1	1

(Agar plates were NOT opened afterwards; Agar plates were autoclaved before disposal)

Therefore, the use of **Chitogels** and ***rouxii* Chitogels** of **BSF** could make **forma-ridden Chitogels** anti-bacterial.

4.3 Efficiency of using **Chitogels** (hydrogels) of black soldier fly **BSF** (*Hermetia illucens*) as formaldehyde remover on-the-spot

4.3.1 Using **Chitogels** on-the-spot in a newly renovated Lecture Theatre as formaldehyde remover

Figure 4.3.1.1 a newly renovated Lecture Theatre (conc. HCHO of 0.30 mg/m^3, exceeding safety limit of 0.125mg/m^3)

Figure 4.3.1.2 Formaldehyde meter reading at the 10th min at 29°C

Chitogels as a formaldehyde remover was efficient as the concentration of **HCHO** reached 0.12 mg/m^3 (within safety limit) in 10 mins when using hydrogel of **BSF** (from 0.37 mg/m^3**)**. Obviously, **Chitogels** removed **HCHO** effectively by condensation with **HCHO** and absorption of **HCHO** in a newly renovated Lecture Theatre at 29°C.
Rate of removal of **HCHO** by **Chitogels** (0.008 mg/m^3 /g/ min) at the 10th min. Safety limit (0.125 mg/m^3) was reached at the 10th min. Then rate became almost zero in 25 mins cases. Obviously, **Chitogels** removed **HCHO** effectively by condensation with **HCHO** and absorption of **HCHO** in a newly renovated Lecture Theatre at 29°C.
Using hydrogel of black soldier fly **BSF** as formaldehyde remover in making **Chitogels** was an efficient way to eliminate **HCHO**.

4.3.2 Using ***rouxii*** **Chitogels** as filter in an air purifier

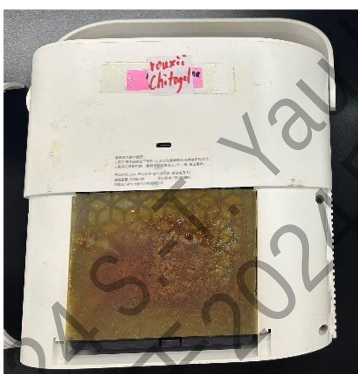


Figure 4.3.2 ***rouxii*** **Chitogels** as filter in an air purifier

The Percentage of **HCHO** removed by an air purifier with ***rouxii*** **Chitogels** as filter was 68.0% 19.9°C which outperformed that of filter only of 56.3% by 20.8%. The conc. of **HCHO** remained was 0.125mg/m^3 when using filter with ***rouxii*** **Chitogels** 19.9°C which was within the safety limit of HCHO of 0.125 mg/ m^3. (EUR13216EN, 1990) The incorporation of ***rouxii*** **Chitogels** as filter in air purifier obviously could enhance the efficiency of removal of **HCHO**.

4.3.3 Using ***rouxii*** **Chitogels** as formaldehyde remover in furniture

Using ***rouxii*** **Chitogels** (~3g) as formaldehyde remover in new furniture such as drawers could remove 5.40mg (out of 12.0mg) **HCHO** effectively in 117 days at 31°C or below. The concentration of **HCHO** could reach as low as 0.23 mg/m^3 in 3 days at 22°C or below. More than 45.0% of HCHO was removed from the drawers (0.0578m^3) in 117 days.

Figure 4.3.3 Anti-bacterial forma-ridden *rouxii* Chitogels as **HCHO** remover in new furniture of drawers emitting about 0.5 mg/m^3 **HCHO** (exceeding safety limit of 0.125 mg/m^3)

4.4 Cross-reactive allergens risks of *Hermetia illucens*, black soldier fly **BSF**

Figure 4.4 Surface protein allergens absent when **Chitogels** and hydrogel of **BSF** were tested using 3M Clean-Trace ALLTEC60

Surface protein allergens were absent when **Chitogels** and ***rouxii* Chitogels** of **BSF** were tested using 3M Clean-Trace ALLTEC60, so **Chitogels** and ***rouxii* Chitogels** of BSF should be non-allergic and safe to be used in **Anti-bacterial forma-ridden *rouxii* Chitogels** as formaldehyde remover.

5. Discussion

5.1 Using hydrogel of black soldier fly (*Hermetia illucens*) in the production of **Anti-bacterial forma-ridden *rouxii* Chitogels**

5.1.1 **Using hydrogel of black soldier fly (*Hermetia illucens*) to form Anti-bacterial forma-ridden Chitogels**

Hydrogel of **BSF** as **forma-ridden Chitogels** is environmentally friendly as hydrogel of **BSF** is bio-degradable, and the use of **BSF** is environmentally sustainable. (Hall, 2002) Chitosan in hydrogel of **BSF** could remove formaldehyde emitted from urea formaldehyde particle board via condensation (formation of **-N=C between** -NH_2 in **Chitogels** and **-C=O** in formaldehyde) and absorption. DD% of **Chitogels** decreased from 72.3% to 67.8% after condensation with formaldehyde as -**N=C** formed showing that **Chitogels** removed formaldehyde effectively via condensation of **-NH_2** in hydrogel of **BSF** and -**C=O** in formaldehyde..

Chitosan is also anti-bacterial (Ardean, 2021) making **Chitogels** eligible to be used in a variety application ranging from households to commercial including air filters, food packaging, health care, hygiene, medical, storage, ventilation and water purification systems. (Gulati, 2022)

https://photonexport.com/wp-content/uploads/2020/07/Antibacterial-Surface-768x512.jpg

Figure 5.1.1.1 Coatings of Antimicrobial Surface for Killing Coronavirus

https://d328pb8icbgz49.cloudfront.net/microban/uploads/images/built-in/_moduleZPattern/mbns20_Microban-Technology-How-It-Works-Felli.jpg

Figure 5.1.1.2 Anti-bacterial food packaging

5.1.2 **Using the Green Vinegar Fermentation Method to obtain hydrogel of chitosan of black soldier fly (*Hermetia illucens*) to obtain anti-bacterial forma-ridden *rouxii* Chitogels**

The percentage of ***rouxii* Chitogels** obtained from chitin **BSF** using the Green Vinegar Fermentation Method was 71.0 at 50°C and the **Chitogels** of Green Vinegar Method was 36.1% which were 1.1 times and 87.0% of that of using the traditional method of highly corrosive 16.7M NaOH (41.5%), so the innovative Green Vinegar Fermentation and Green Vinegar Method to obtain **Chitogels** and ***rouxii* Chitogels** from chitin of **BSF** was a good alternative to eliminate the use of highly corrosive conc. NaOH. The conc. of **HCHO** remained were both 0.11 mg/m^3 which was within the safety limit (0.125mg/m^3). Certainly, anti-bacterial **forma**-ridden *rouxii* **Chitogels** obtained using the Green Vinegar Method would no longer involve conc. NaOH in the deacetylation of chitin to chitosan.

5.2 Using **Chitogels** as a cheaper, environmentally friendly and innovative option to remove formaldehyde in real-life such as in a newly renovated Lecture Theatre

Table 5.2 Comparison of formaldehyde removal options

Methods	Technology	Concentration	Operating Temp.	Operating Cost
Recovery	Adsorption	Low, High	Ambient	Acceptable
	Membrane	High	Ambient	High cost of material
	Condensation	High	Ambient and cryogenic	High cost of energy
Destruction	Thermal Oxidation	High	~815 °C	High cost of Energy
	Catalysis	Low, High	Ambient, 200~500 °C	Acceptable
	Photo catalysis	Low, High	Ambient	Costly dopants required
	Non-thermal plasma w/wo catalyst	Low	Ambient	High cost of system assembly
	Biological/Botanical filtration	Low	-	-

https://www.researchgate.net/publication/360646327/figure/tbl1/AS:1160525400547329@1653702225104/Comparison-of-formaldehyde-removal-options.png

Traditional condensation method to remove formaldehyde is effectively but requires a high operating cost to recover formaldehyde by lowering of temperature and applying a high temperature. Condensation using hydrogel of chitosan of **BSF** is a much cheaper, environmentally friendly and innovative option as shells of BSF are leftovers during the life cycle of **BSF**. In fact, **Chitogels and *rouxii* Chitogels** removed formaldehyde effectively on-the-spot such as in a newly renovated Lecture Theatre (see **4.3.1**), as filter in air purifier (see **4.3.2**) and furniture (see **4.3.3**). **Chitogels** and ***rouxii* Chitogels** of **BSF** took 38 days and 14 days to be fully bio-degraded in soil showing that **Chitogels** and ***rouxii* Chitogels** were bio-degradable. **Anti-bacterial formma-ridden Chitogels** should be eligible for marketing and be applied to real life situation.

5.3 Daily applications of **Chitogels** and ***rouxii* Chitogels**

Chitogels and ***Rouxii* Chitogels** can be applied as formaldehyde remover in real life such as a newly renovated Lecture Theatre, air purifier and furniture.

Figure 5.3.1 a newly renovated Lecture Theatre (conc. HCHO of 0.30 mg/m^3, exceeding safety limit of 0.125mg/m^3)

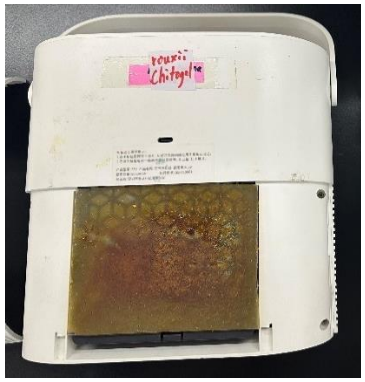


Figure 5.3.2 *rouxii* Chitogels as filter in an air purifier

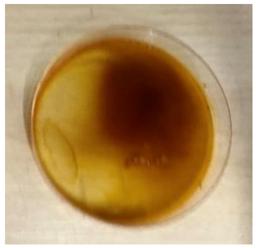
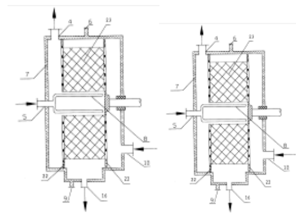
Figure 4.3.3 Anti-bacterial forma-ridden *rouxii* Chitogels as **HCHO** remover in new furniture of drawers emitting about 0.5 mg/m^3 **HCHO** (exceeding safety limit of 0.125 mg/m^3)

5.4 Cross-reactive allergens risks of *Hermetia illucens*, black soldier fly **BSF**

Surface protein allergens were absent when **Chitogels** and ***rouxii* Chitogels** of **BSF** were tested using 3M Clean-Trace ALLTEC60, so **Chitogels** of BSF should be non-allergic and safe to be used in **Anti-bacterial forma-ridden Chitogels** as formaldehyde remover.

5.5 Comparison of ***rouxii* Chitogels** and other commercial products

Table 5.5 Other methods for the reduction of formaldehyde of particleboard.

	Anti-bacterial Forma-ridden *rouxii* Chitogels 	Rotating packed bed with steam as stripping gas (CN100427522C China retrieved from https://patents.google.com/patent/CN100427522C/en) 	Reducing formaldehyde emission of urea formaldehyde-bonded particleboard by addition of amines as formaldehyde scavenge (Reducing formaldehyde emission of urea formaldehyde-bonded particleboard by addition of amines as formaldehyde scavenger - ScienceDirect) (Gulati, 2022)
Description	hydrogel of chitosan of black soldier fly **BSF** is used as formaldehyde remover, so **HCHO** could be removed irreversible by condensation with chitosan of **BSF**	(steam) stripping gas is guided into the rotating packed from a gas (steam) inlet of the rotating packed bed, wherein the free formaldehyde is diffused to the gas (steam) stripping gas to be carried away by the gas	In order to reduce the formaldehyde emission from particleboard, amines were added into the urea formaldehyde (UF) resin as formaldehyde scavenger.
Advantages	-eliminating the use of highly corrosive conc. NaOH by using the **Green Vinegar Method & Green Vinegar Fermentation Method** -removal of formaldehyde by condensation with chitosan is irreversible. **-Chitogels** are anti-bacterial, eco-friendly as they are made from chitosan of **BSF**	facilitates increased contact between the stripping gas (steam) and the free formaldehyde, leading to improved mass transfer efficiency. This allows for more effective removal of formaldehyde from the process.	-removal of formaldehyde by condensation with amines is irreversible.
Disadvantages	None	-Formaldehyde dissolved in the stripping gas (steam) would be released as the process is reversible. -Excess formaldehyde used in the condensation of urea formaldehyde will be given out during the transportation and storage of furniture.	-urea formaldehyde and amine are not bio-degradable -both urea formaldehyde and amine are toxic

-Some furniture could not fit in the rotating bed such as TV cabinet.

5.6 **Anti-bacterial forma-ridden *rouxii* Chitogels** meeting Sustainable Development Goals

Anti-bacterial forma-ridden *rouxii* Chitogels meet the 12th Sustainable Development Goal (SDG) of the United Nations - ensure sustainable consumption and production patterns. In this investigation, **BSF** shells were converted into chitosan which became the raw material for making **Anti-bacterial forma-ridden *rouxii* Chitogels** and hence reducing the waste production. As the life cycle of **BSF** is short (38 days), the supply of shells of **BSF** is continuous. Indeed, the production of ***rouxii* Chitogels** completes a circular economy and is an example of upcycling from less useful shells of **BSF** to more useful anti-bacterial **forma-ridden *rouxii* Chitogels**.

Anti-bacterial forma-ridden *rouxii* Chitogels also meet the 3rd SDG of the United Nations - ensure healthy lives and promote well-being for all at all ages. Formaldehyde is classified as class 1 carcinogen by the International Agency for Research on Cancer Group (WHO, 2006) which can cause many health problems such as cancer. **Anti-bacterial forma-ridden *rouxii* Chitogels** condensed with formaldehyde to give harmless substances such as water, so posing no threat to the environment. In this investigation, **Anti-bacterial forma-ridden rouxii Chitogels** could remove formaldehyde effectively in a furniture of drawers, **rouxii Chitogels** could remove 5.40mg/g **HCHO** effectively in 117 days at 31ºC or below. The concentration of **HCHO** could reach as low as 0.23 mg/m^3 in 3 days at 22ºC or below. More than 45.0% of **HCHO** was removed from the drawers (0.0578m^3) in 117 days. and in a newly renovated Lecture Theatre at 29ºC as the concentration of **HCHO** reached 0.12 mg/m^3 (within safety limit) in 10 mins when using hydrogel of **BSF** (from 0.37 mg/m^3) and hence meets the Target 3.9 of the Sustainable Development Goals - substantially reduce the number of deaths and illnesses from hazardous chemicals and air, water and soil pollution and contamination.

Figure 5.6.1 3rd SDG of UN

Figure 5.6.2 3.9 of 3rd SDG of UN

Figure 5.6.3 12th SDG of UN

Figure 5.6.4 12.5 of 12th SDG of UN

(retrieved from: https://www.un.org/sustainabledevelopment/sustainable-development-goals/)

6. Limitation

6.1 Effect of temperature on the emissions of formaldehyde

The emission rate of formaldehyde from a panel of MDF (medium density fibreboard) increased 192% between 26.1 ºC and 38.9 ºC, i.e. as temperature increases, the amount of formaldehyde emitted from a also increases. (Swankie, 2017) However it is out of our scope to control the variable of temperature on the emission rate of formaldehyde in a school laboratory.

6.2 Effect of absolute humidity on the emission of formaldehyde

The initial emittable concentration of formaldehyde from a type of medium density fiberboard in absolute humidity (AH) range of 4.6–19.6 g/m3 at 25 °C were increased by 10 times. (Huang, 2016) However it is out of our scope to control the variable of absolute humidity on the emission rate of formaldehyde in a school laboratory.

7.Further study

7.1 Effect of temperature on the emissions of formaldehyde

The emission rate of formaldehyde from a panel of MDF (medium density fibreboard) increased 192% between 26.1 ºC and 38.9 ºC, i.e. as temperature increases, the amount of formaldehyde emitted from a also increases. (Swankie, 2017) Investigation could be done inside a large incubator of air bath for further studies.

7.2 Fermentation of chitin of BSF to chitosan using chitin deacetylase

Mucor rouxii was first found to contain a chitin deacetylase which is a greener way to obtain chitosan. (Tsigos, 1999) Further studies could be done on expression of chitin deacetylase from different sources. (Yin, 2023)

Fungi	Phylum	Optimal pH/Temp. (°C)	pI	Molecular Weight (kDa)	Carbohydrate contents (%)	Refs
Mucor rouxii	Mucoromycotina	4.5, 50	3.0	75–80	30	[7]
Absidia coerulea	Mucoromycotina	5.0, 50	NA	75	NA	[8]
Rhizpus circinans	Mucoromycotina	5.5–6.0, 37	NA	75	NA	[9]
Rhizopus nigricans	Mucoromycotina	NA	NA	100	53	[10]
Mortierella sp. DY-52#	Mucoromycotina	5.5, 60	NA	50, 59	NA	[11]
Colletotrichum lindemuthianum (ATCC 56676)#	Ascomycotina	12, 60	3.7	32–33	NA	[12]
Colletotrichum lindemuthianum (DSM 63144)#	Ascomycotina	8.5, 50	3.0	150	67	[13]
Colletotrichum lindemuthianum (UPS 9) #,*	Ascomycotina	8.0, 60	NA	25	0	[14]
Aspergillus nidulans #	Ascomycotina	7.0, 50	2.8	27	28	[15]
Metarhizium anisopliae #	Ascomycotina	8.5, NA	3.6	70, 37, 26	NA	[16]
Scopulariopsis brevicaulis	Ascomycotina	7.5, 55	NA	55	NA	[17]
Saccharomyces cerevisiae Cda2p	Ascomycotina	8.0, 50	NA	43	18	[18]
Schizosaccharomyces pombe	Ascomycotina	NA	NA	NA	NA	[19]
Flammulina velutipes	Basidiomycotina	7.0, 60	NA	31	0	[20]
Cryptococcus neoformans	Basidiomycotina	NA	NA	NA	NA	[21]

#, extracellular chitin deacetylases;

*, structure available;

NA, not available.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2817921/table/t1-marinedrugs-08-00024/?report=objectonly

Figure 7.2 Sources of different chitin deacetylase (Zhao, 2010)

8. Summary

The incorporation of chitin deacetylase from *Mucor rouxii* in th**e** innovative Green Vinegar Fermentation Method could produce **Anti-bacterial Forma-ridden *rouxii* Chitogels** and eliminate the use of highly corrosive conc. NaOH in deacetylation with a yield of 71.0 at 50ºC. (cf. 41.5% from 17.6M NaOH). ***Anti-bacterial forma-ridden rouxii* Chitogels** *and* **Chitogels** showed the highest percentage of removal of HCHO from 1:100 **HCHO** (by mass) of 93.0% and 87.8% when compared with some commercial formaldehyde removers (cf. wall paint HCHO-busters: 53.0%; activated Charcoal 35.5%; 3M **HCHO** remover 26.2%). Besides the concentration of **HCHO** left were 0.06mg/m^3 and 0.105mg/m^3 (within safety limits of 0.125mg/m^3) after 24 hours at 19.9ºC. (EUR13216EN, 1990). When applied as filter in an air purifier, the Percentage of **HCHO** removed using ***rouxii* Chitogels** as filter was 68.0% at 19.9ºC which outperformed that of filter only of 56.3% by 20.8%. The conc. of **HCHO** remained was 0.125mg/m^3 when using filter with ***rouxii* Chitogels** 19.9ºC which was within the safety limit of HCHO of 0.125 mg/ m^3. When applied as formaldehyde remover in a furniture of drawers, ***rouxii* Chitogels** could remove 5.40mg/g **HCHO** effectively in 117 days at 31ºC or below. The concentration of **HCHO** could reach as low as 0.23 mg/m^3 in 3 days at 22ºC or below. More than 45.0% of **HCHO** was removed from the drawers (0.0578m^3) in 117 days. When applied in a newly renovated Lecture Theatre, **Chitogels** removed **HCHO** effectively as the concentration of **HCHO** (from 0.37 mg/m^3) reached 0.12 mg/m^3 (within safety limit 0.125 mg/m^3) in 10 mins at 29ºC. Condensation was confirmed by FTIR as DD% of **Chitogels** decreased from 59.7% to 51.1% and that of ***rouxii* Chitogels** decreased from 61.1% to 49.9% (due to formation of **-N=C between -NH_2** in **Chitogels** and **-C=O** in formaldehyde).

Chitogels and **rouxii Chitogels** are anti-bacterial and showed significant anti-bacterial effect towards oral bacterial. [no. of bacterial colonies of **Chitogels** (60, 11 w.r.t. dilution 10^{-3}, 10^{-4}) cf. oral bacterial (control: 232, 52) and **rouxii Chitogels** (0, 0 w.r.t. dilution 10^{0}, 10^{-2}) cf. oral bacterial (control: 58,1)] After condensation with formaldehyde, **Chitogels** of **BSF** kept showing anti-bacterial effect towards oral bacteria. [no. of bacterial colonies: **Chitogels** (0,1 w.r.t. dilution 10^{-3}, 10^{-4}) cf. oral bacterial (control: 232, 52)] and **rouxii Chitogels** (0, 1 w.r.t. dilution 10^{0}, 10^{-2}) cf. oral bacterial (control: 58, 1) **Chitogels** and ***rouxii* Chitogels** are bio-degradable as they took 38 days and 14 days to be fully bio-degraded in soil.

Chitogels and ***rouxii* Chitogels** are non-allergic as surface protein allergens were absent, so the marketing of **Anti-bacterial forma-ridden *rouxii* Chitogels** should be eligible for marketing.

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