Lampiran 1. Bagan Alir Prosedur Kerja

Pengumpulan limbah kulit udang windu *(penaeus monodon)* dan kulit udang vanname *(Litopenaeus vannamei)*

Kulit udang windu dan vaname

disortasi basah

dicuci dengan air mengalir

ditiriskan

ditimbang

Berat Kulit udang basah

Kulit udang windu 10 kg

Kulit udang vanname 10 kg

Dikeringkan

Berat kering masing-masing sampel 400 g

Dihaluskan

Serbuk masing-masing kulit udang 200 gram

Ditimbang

Disimpan dalam wadah yang tertutup rapat

Diisolasi kitosan

Lampiran 2. Bagan Alir Isolasi Kitosan Kulit Udang Windu dan Udang Vaname

Serbuk Sampel

Proses Demineralisasi

Proses Deproteinasi

Proses Depigmentasi

Nilai LC50

Proses Deasetilasi

Kitin menjadi kitosan

Kitosan

* Randemen
* Kadar air
* Kadar abu
* Kelarutan kitosan
* Derajat Deasetilasi
* Pengujian FTIR

Uji Toksisitas dengan metode BSLT

Karakterisasi Kitosan

Lampiran 3. Bagan Alir Uji Toksisitas Kitosan Windu dan Vanname

0,1g Kitosan Udang Windu dan Udang Vanname

Blanko

/Kontrol (-)

Dibuat variasi konsentrasi dari larutan induk I

Larutan Induk Baku (LIB I)

Dilarutkan dalam labu tentukur 100 ml

100 µg/ml

250 µg/ml

500 µg/ml

750 µg/ml

1000 µg/ml

* Masing-masing konsentrasi dibuat 3 kali perlakuan
* Dimasukkan dalam vial 10 ml
* Dimasukkan kedalam masing-masing konsentrasi 10 ekos larva udang
* Ditambahkan 1 tetes suspense ragi
* Vial diletakkan ditempat yang terang dan hangat
* Mortalitas dihitung setelah 24 jam

0 %

3,3 %

6,7 %

10,0 %

16,7 %

26,7 %

0 %

3,3 %

10,0 %

13,3%

16,7 %

23,3 %

Hasil Uji Toksisitas Kitosan Udang Vanname

Hasil Uji Toksisitas Kitosan Udang Windu

Lampiran 4. Sampel Yang Digunakan Pada Penelitian

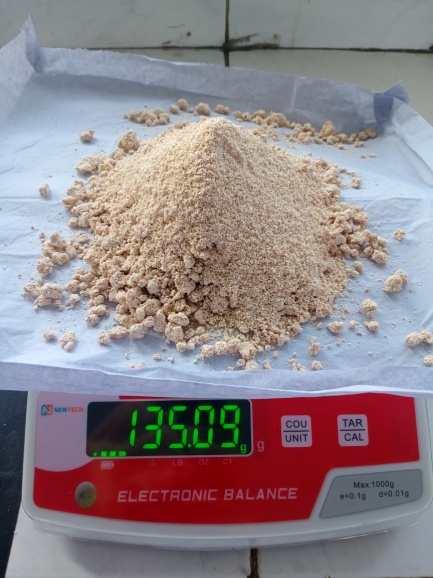
Kulit Udang Windu Kulit Udang Vaname



Kulit Udang Windu Kering Kulit Udang Vanme Kering



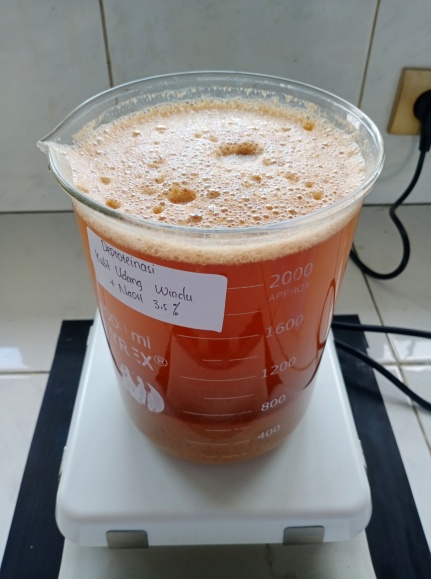
Serbuk Kulit Udang Windu Serbuk Kulit Udang Vaname



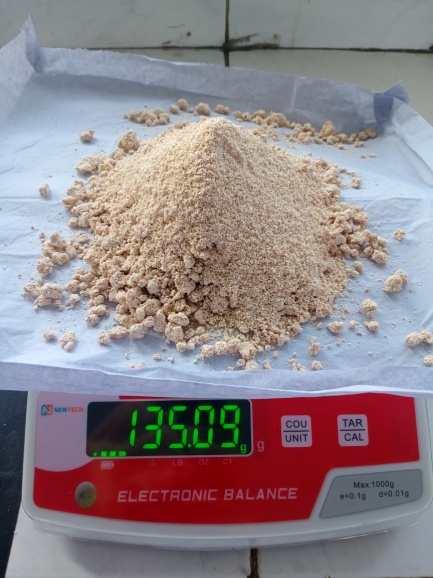
Lampiran 5. Proses Pembuatan Kitosan

1. Proses Deproteinasi

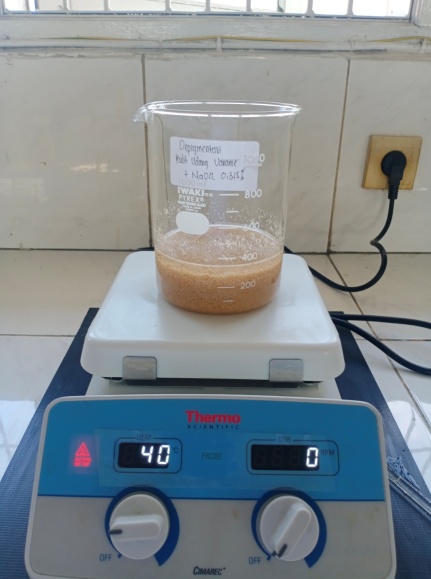
Kulit Udang Vaname Kulit Udang Windu



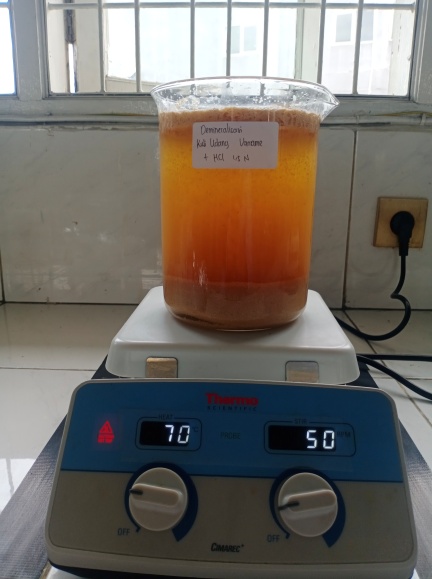
Proses pengadukan serbuk selama 4 jam dengan NaOH 3,5%



Hasil akhir setelah di oven selama 24 jam



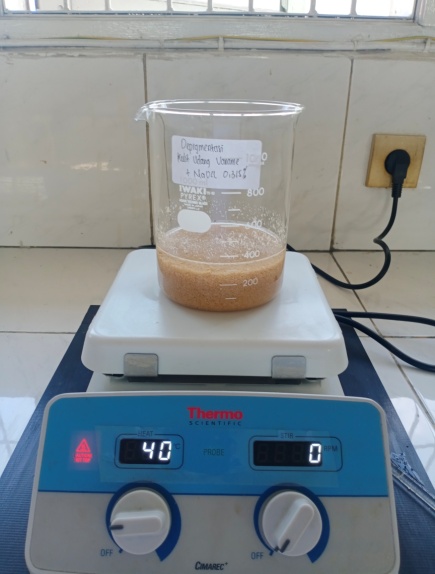
1. Proses Demineralisasi



proses demineralisasi 4 jam dengan HCl 1,5 N

Hasil Akhir Setelah Dioven 24 jam

1. Peoses Depigmentasi



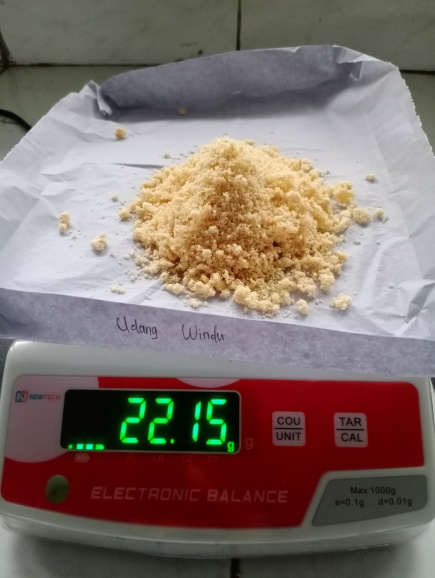
Prosesn depigmentasi dengan NaOCl 0,315% selama 1 jam

Hasil Akhir setelah di oven 4 jam

1. Proses Deasetilasi

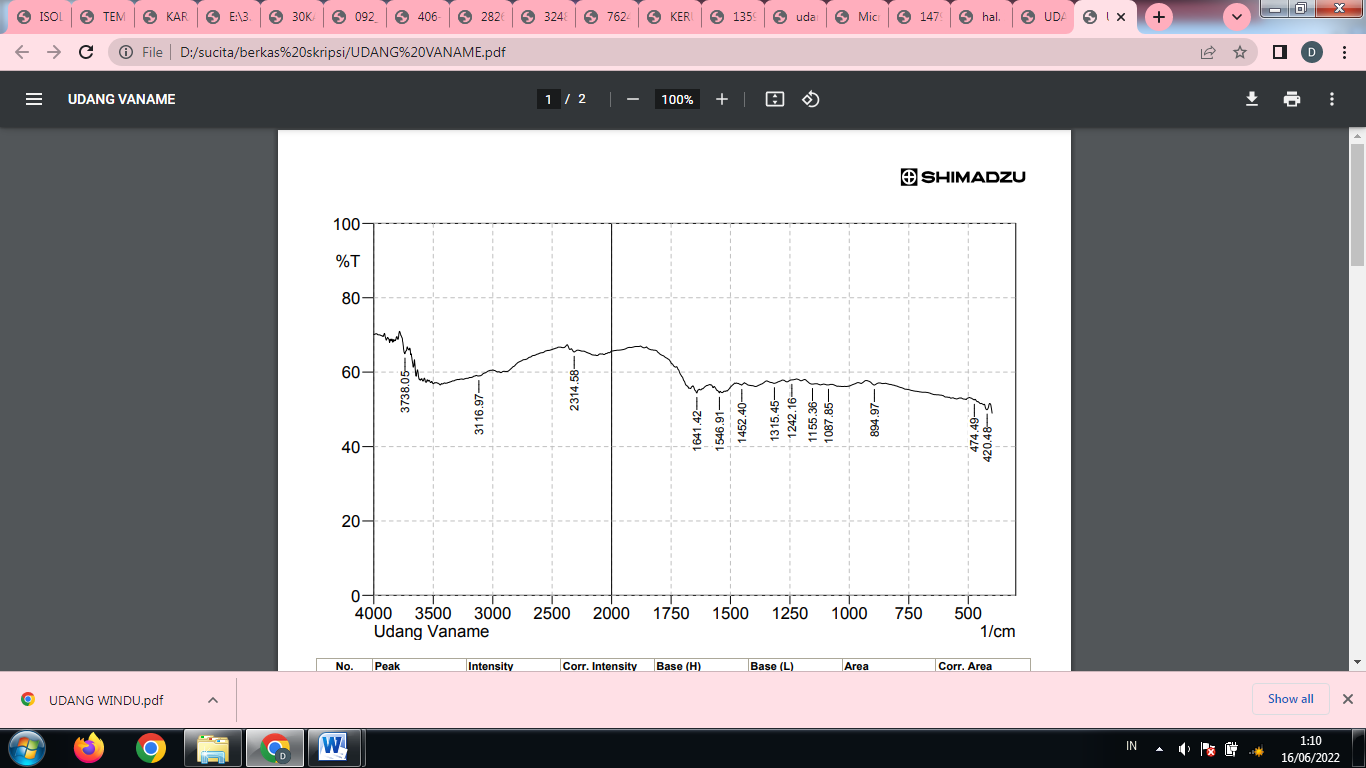


Proses tranformasi kitin menjadi kitosan dengan penambahan NaOH 60%



Hasil Akhir setelah di oven selama 24 jam

Lampiran 6. Hasil FTIR Kitosan Kulit Udang Vaname



**F**

**CA**

**E**A

**D**A

**B**A

**A**

Derajat deasetilasi pada kitosan udang vaname:

Dik: To = AC ; To = DF

T = AB ; T = DE

Ket: To = %Transmitan pada Garis datar panjang gelombang

T = %Transmitan pada puncak minimum panjang gelombang

Rumus :

= = = 0,04

= = = 0,07

x 100 %

x 100 %

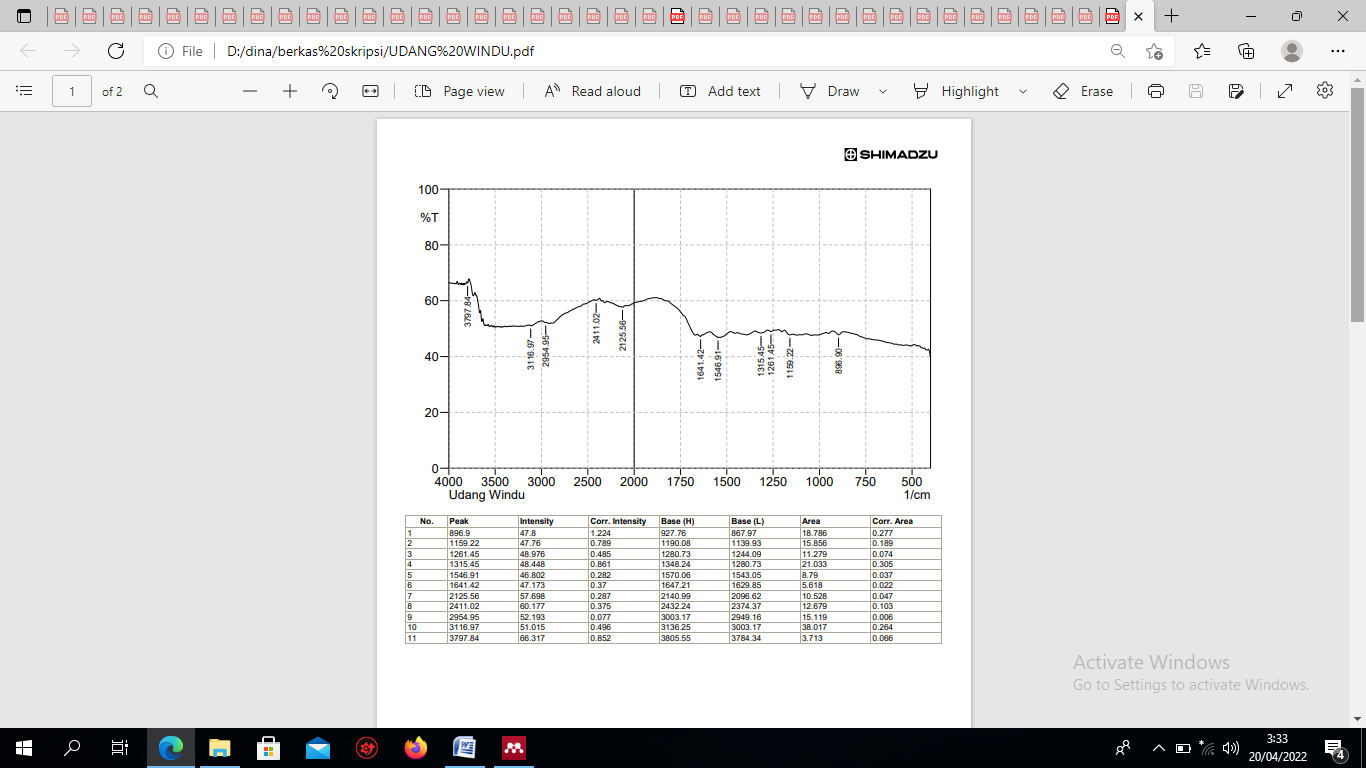
x 100 %

x 100 %

% DD = 1 – (0,42) x 100 %

% DD = 0,58 x 100 %

% DD = 58%

Lampiran 7. Hasil FTIR Kitosan Kulit Kitosan Kulit Udang Windu

**C**

**F**

E

**B**

**D**

**A**

Derajat deasetilasi pada kitosan udang windu :

Dik: To = AC ; To = DF

T = AB ; T = DE

Ket: To = %Transmitan pada Garis datar panjang gelombang

T = %Transmitan pada puncak minimum panjang gelombang

Rumus :

= = = 0,06

= = = 0,11

x 100 %

x 100 %

x 100 %

x 100 %

% DD = 1 – (0,40) x 100 %

% DD = 0,6 x 100 %

% DD = 60%

Lampiran 8. Perhitungan Kadar Air Kitosan Udang Vaname *(Litopenaeus vannamei)*

A - B

% kadar air = x 100 %

Berat sampel

Keterangan : A = Berat Cawan + Isi basah (g)

B = Berat Cawan + Isi Kering (g)

1. A = 65,7641 g

B = 65,7356 g

Berat Sampel = 0,5010 g

65,7641- 65,7356

% Kadar air = x 100% = 5,5%

0,5010

1. A = 33,5081 g

B = 33,4910 g

Berat Sampel = 0,5032 g

33,5081 - 33,4910

% Kadar air = x 100% = 3,4%

0,5032

1. A = 30,9647 g

B = 30,9479 g

Berat Sampel = 0,5002 g

30,9647 – 30,9679

% Kadar air = x 100% = 2,99%

0,5002

5,5% + 3,4% +2,99%

Kadar air rata-rata = = 5,94%

3

Lampiran 9. Perhitungan Kadar Air Kitosan Udang Windu *(Penaeus monodon)*

A - B

% kadar air = x 100 %

Berat sampel

Keterangan : A = Berat Cawan + Isi basah (g)

B = Berat Cawan + Isi Kering (g)

1. A = 64,3067 g

B = 64,2810 g

Berat Sampel = 0,5000 g

64, 3067 – 64, 2810

% Kadar air = x 100% = 5,14%

0,5000

1. A = 29,3813 g

B = 29,3509 g

Berat Sampel = 0,5068 g

29,3813 – 29,3509

% Kadar air = x 100% = 5,99%

0,5068

1. A = 35,7567 g

B = 35,7280 g

Berat Sampel = 0,5066 g

35,7567 – 35,7280

% Kadar air = x 100% = 5,66%

0,5066

5,14% + 5,99% + 5,66%

Kadar air rata-rata = = 5,59%

3

Lampiran 10. Perhitungan Penetapan Kadar Abu Kitosan Vaname *(Litopenaeus vannamei)*

1. Pengulangan 1

Berat sampel = 0,5024 gram

Berat cawan kosong = 36, 8646 gram

Berat cawan + isi = 36,8750 gram

[(36,8750) – 36,8646]

% kadar = x 100% = 2,07%

0,5024 gram

1. Pengulangan 2

Berat sampel = 0,5020 gram

Berat cawan kosong = 36,4510 gram

Berat cawan + isi = 36,4614 gram

[(36,4614) – 36,4510]

% kadar = x 100% = 2,07%

0,5020 gram

1. Pengulangan 3

Berat sampel = 0,5020 gram

Berat cawan kosong = 35,1426 gram

Berat cawan + isi = 35,1524 gram

[(35,1524) – 35,1426]

% kadar = x 100% = 1,95%

0,5020 gram

2,07% + 2,07% + 1,95%

Kadar Abu rata-rata = = 1,96%

3

Lampiran 11. Perhitungan Penetapan Kadar Abu Kitosan Windu *(Penaeus monodon)*

[(Berat cawan + berat isi) – berat cawan kosong]

% kadar = x 100%

Berat sampel

1. Pengulangan 1

Berat sampel = 0,5003 gram

Berat cawan kosong = 36, 8617 gram

Berat cawan + isi = 36,8635 gram

[(36,8635) – 36,8617]

% kadar = x 100% = 0,36%

0,5003 gram

1. Pengulangan 2

Berat sampel = 0,5003 gram

Berat cawan kosong = 36,4485 gram

Berat cawan + isi = 36,4499 gram

[(36,4499) – 36,4485]

% kadar = x 100% = 0,28%

0,5003 gram

1. Pengulangan 3

Berat sampel = 0,5007 gram

Berat cawan kosong = 35,1394 gram

Berat cawan + isi = 35,1404 gram

[(35,1404) – 35,1394]

% kadar = x 100% = 0,20%

0,5007 gram

0,36% + 0,28% + 0,20%

Kadar Abu rata-rata = = 0,28%

3

Lampiran 12. Uji Toksisitas Kitosan Udang Vaname *(Litopenaeus vannamei)* dan Kitosan Udang Windu *(Penaeus monodon).*

1. Penetasan telur *Artemina salina* L.



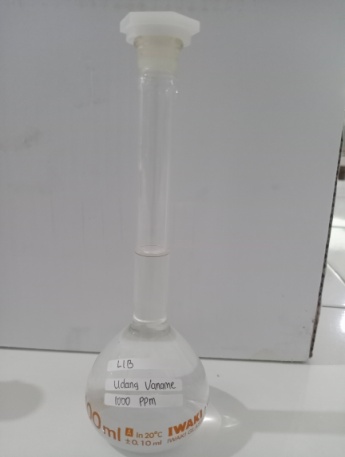


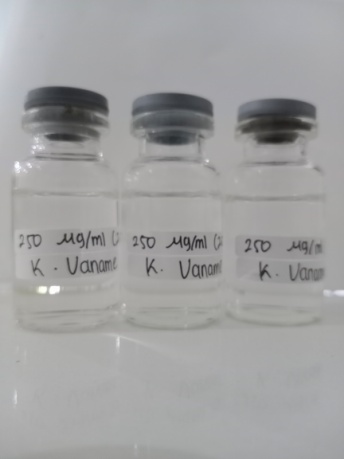
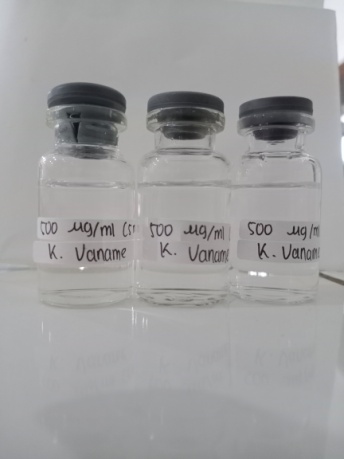
Telur *Artemia* Air laut buatan

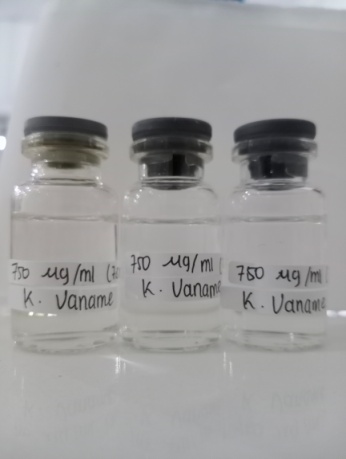
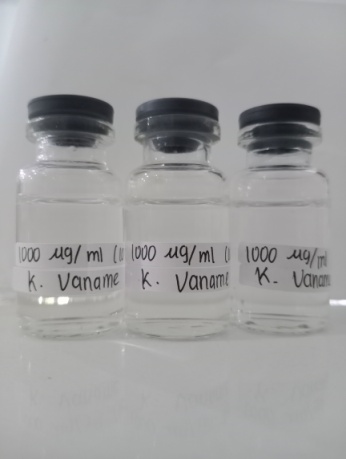
Telur dimasukkan ke tempat yang gelap Proses penetasan

1. Pengenceran Kitosan dalam Beberapa Konsentrasi Pada Uji Toksisitas

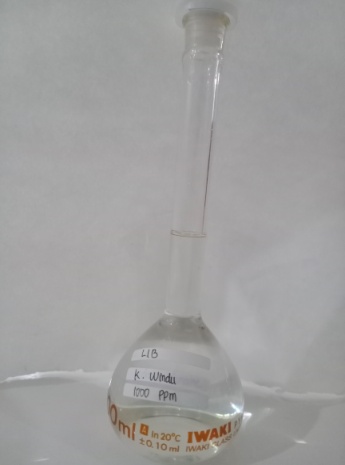
Konsentrasi Kitosan Vaname







Konsentrasi Kitosan Windu







Lampiran 13. Perhitungan Pembuatan Variasi Pengenceran Konsentrasi Larutan Uji Toksiistas

# 100 mg sampel dalam labu tentukur 100 ml

# LIB 1 => C= 100 mg ( 100.000µg / 100 ml), maka C = 1000 µg/ml

10.000 µg/ml

C1 = 1000 µg/ml x 10 ml = = 1000µg/ml

10 ml

7500 µg/ml

C2 = 1000 µg/ml x 7,5 ml = = 750µg/ml

10 ml

5000 µg/ml

C3 = 1000 µg/ml x 5 ml = = 500µg/ml

10 ml

2500 µg/ml

C4 = 1000 µg/ml x 2,5 ml = = 250µg/ml

10 ml

1000 µg/ml

C5 = 1000 µg/ml x 1 ml = = 100µg/ml

10 ml

Lampiran 14. Perhitungan LC50 Kitosan Vaname *(Litopenaeus vannamei)*

*Jumlah kematian larva uji*

% Kematian Larva = x 100%

*Jumlah larva uji*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **(C (µg/ml))** | **n**  **(jumlah larva)** | **R**  **(larva yang mati)** | **P**  **(%Mortalitas)** | **X**  **(log C)** | **Y**  **(NIlai Probit)** | **XY** | **X²** |
| 100 | 30 | 1 | 3,3 % | 2,0000 | 3,1616 | 6,3232 | 4,0000 |
| 250 | 30 | 2 | 6,7 % | 2,3979 | 3,5015 | 8,3964 | 5,7501 |
| 500 | 30 | 3 | 10,0 % | 2,6990 | 3,7184 | 10,0359 | 7,2844 |
| 750 | 30 | 5 | 16,7 % | 2,8751 | 4,0339 | 11,5977 | 8,2660 |
| 1000 | 30 | 8 | 26,7 % | 3,0000 | 4,3781 | 13,1343 | 9,0000 |
|  | | | | **ΣX =**  12,9720 | **ΣY =**  18,7935 | **ΣXY =**  49,4874 | **ΣX² =**  34,3005 |

Persamaa garis regresi linier : y = b x + a

y = konsentrasi kematian

x = log konsentrasi

n (ΣXY) – (ΣX.ΣY)

b =

n (ΣX2) – (ΣX)2

5 (49,4874) – (12,9720 x 18,7935)

b =

5 (34,3005) – (12,9720)2

(247,437) – (243,789)

b =

(171,5027) – (168,2720)

(3,6483)

b =

(3,2305)

b = 1,1293

(lanjutan)

ΣY – b ΣX

a =

*n*

18,7935 – (1,1293 x 12,9720)

a =

5

18,7935 – (14,6493)

a =

5

4,1442

a =

5

a = 0,8287

Nilai LC50 diperoleh dari antilog x, dimana x merupakan logaritma konsentrasi bahan toksik pada y = 5, yaitu nilai probit 50 % hewan uji. Sehingga diperoleh persamaan regresi

y = 1,1293 x + 0,8287

5 = 1,1293 x + 0,8287

5 – 0,8287

x =

1,1293

x = 3,6936

Maka, nilai LC50 antilog 3,6936 = 4897,79 µg/ml

Lampiran 15. Perhitungan LC50 Kitosan Windu *(Penaeus monodon)*

*Jumlah kematian larva uji*

% Kematian Larva = x 100%

*Jumlah larva uji*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **(C (µg/ml))** | **n**  **(jumlah larva)** | **R**  **(larva yang mati)** | **P**  **(%Mortalitas)** | **X**  **(log C)** | **Y**  **(NIlai Probit)** | **XY** | **X²** |
| 100 | 30 | 1 | 3,3 % | 2,0000 | 3,1616 | 6,3232 | 4,0000 |
| 250 | 30 | 3 | 10,0 % | 2,3979 | 3,7184 | 8,9165 | 5,7501 |
| 500 | 30 | 4 | 13,3 % | 2,6990 | 4,0339 | 10,8874 | 7,2844 |
| 750 | 30 | 5 | 16,7 % | 2,8751 | 4,0339 | 11,5977 | 8,2660 |
| 1000 | 30 | 7 | 23,3 % | 3,0000 | 4,2710 | 12,8130 | 9,0000 |
|  | | | | **ΣX =**  12,9720 | **ΣY =**  19,2188 | **ΣXY =**  50,5378 | **ΣX² =**  34,3005 |

Persamaa garis regresi linier : y = b x + a

y = konsentrasi kematian

x = log konsentrasi

n (ΣXY) – (ΣX.ΣY)

b =

n (ΣX2) – (ΣX)2

5 (50,5378) – (12,9720 x 19,2188)

b =

5 (34,3005) – (12,9720)2

(252,689) – (249,306)

b =

(171,5027) – (168,2720)

(3,3833)

b =

(3,2305)

b = 1,0472

(lanjutan)

ΣY – b ΣX

a =

*n*

19,2188 – (1,0472 x 12,9720)

a =

5

19,2188 – (13,5856)

a =

5

5,6332

a =

5

a = 1,1268

Nilai LC50 diperoleh dari antilog x, dimana x merupakan logaritma konsentrasi bahan toksik pada y = 5, yaitu nilai probit 50 % hewan uji. Sehingga diperoleh persamaan regresi

y = 1,0472 x + 1,1268

5 = 1,0472 x + 1,1268

5 - 1,1268

x =

1,0472

x = 3,69846

Maka, nilai LC50 antilog 3,69846 = 4994,16 µg/ml

Lampiran 16. Tabel Probit

