

Supplementary Information

Figure 1S. Substrate preparation for feeding treatment. All the substrates were obtained from a local restaurant, a public market, and the domestic waste of Universitas Sumatera Utara.



Figure 2S. Photograph of the experimental bio-pond setup. Each bio-pond consisted of a plastic container with dimensions of $21 \times 11 \times 10$ cm and was placed on a rack to facilitate organized arrangement and handling during the experiment



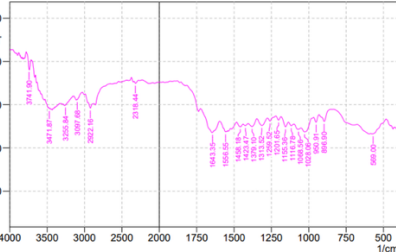
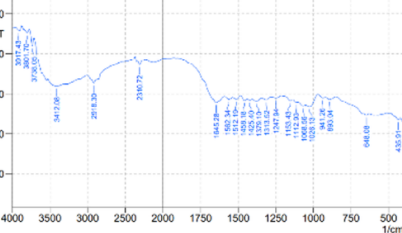
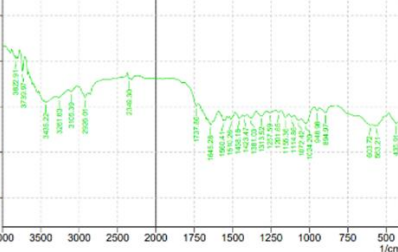
Figure 3S. Example of larval measurement using ImageJ Software, where the measurement will randomly pick 30 BSFL. The standard length will use a millimeter block of paper, which is placed next to the bio-pond.

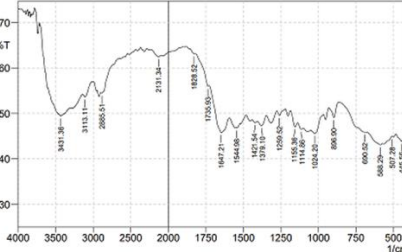
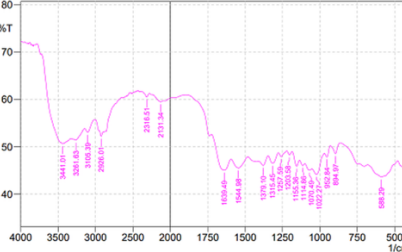


Figure 4S. Step by step larval preparation for chitin extraction and characterization

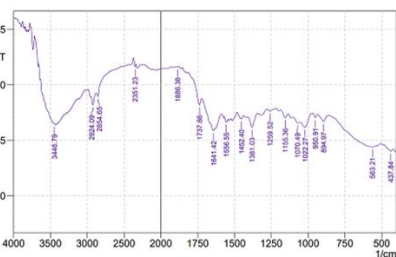


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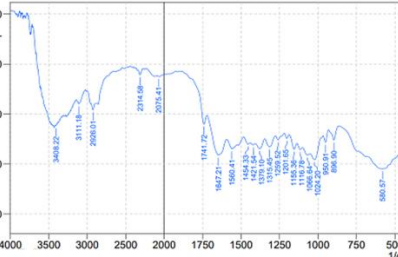
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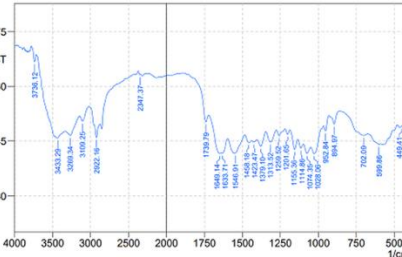
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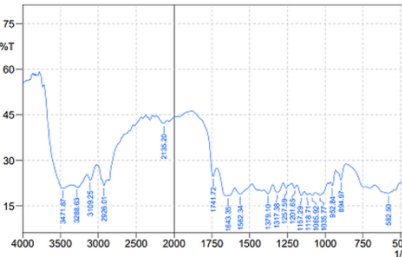
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② 二、Case study, state



THE FIVE-STAR SUBSTANCE



7-28 - 110cm

-28- FIDCI

D-28 = Carbohydrate

D-28 - Mixed Substrate

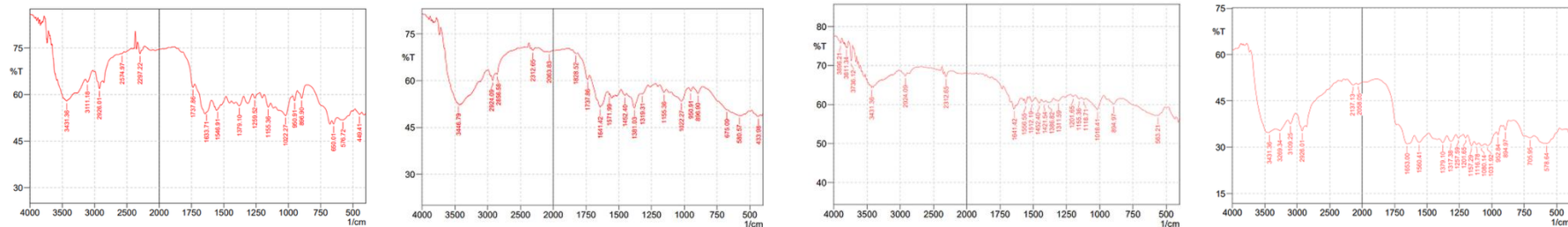


Table 1S. Raw data of the length measurement of BSFL based on four substrate profiles

No Sample	D7	D14				D21				D28			
	All	P	F	C	M	P	F	C	M	P	F	C	M
S1	4.829	14.575	4.009	11.622	13.559	13.897	11.282	13.217	19.462	15.867	10.995	18.203	13.245
S2	6.599	14.920	5.223	13.268	6.669	11.430	7.473	12.657	15.724	11.204	12.657	14.702	12.872
S3	4.805	11.074	5.620	12.414	14.748	12.840	5.869	12.766	19.868	13.031	13.066	13.845	17.227
S4	6.321	9.152	6.439	11.704	11.931	12.273	10.438	11.369	18.686	16.611	10.711	14.947	12.280
S5	4.770	6.837	4.551	13.268	7.771	13.356	6.198	9.362	20.215	14.519	12.752	14.742	22.571
S6	6.259	7.143	6.218	10.183	11.838	13.154	5.575	11.755	18.744	12.490	11.964	16.166	14.520
S7	4.214	14.147	5.669	10.290	12.163	12.986	4.604	14.491	14.017	13.483	12.935	14.617	16.121
S8	4.114	9.740	5.324	5.979	8.143	14.345	9.932	11.369	14.188	17.607	14.120	12.328	19.877
S9	6.079	9.917	5.491	11.762	15.661	11.510	11.85	11.050	18.857	11.835	11.827	11.744	15.550
S10	3.653	7.644	5.539	10.429	13.042	11.600	7.370	11.657	16.784	15.856	10.460	11.168	22.855
S11	5.903	8.163	5.169	8.829	12.303	12.173	10.423	11.940	19.437	13.596	14.854	17.116	15.560
S12	4.628	15.097	3.426	6.127	7.507	14.170	6.385	12.165	14.558	15.896	14.674	18.286	21.987
S13	4.025	10.653	4.564	11.426	11.140	16.844	5.313	13.417	20.242	15.860	12.752	15.335	19.854
S14	6.469	14.720	5.195	5.662	14.211	13.117	6.666	11.798	13.213	12.438	11.480	15.727	13.666
S15	3.769	8.531	6.519	10.586	13.743	19.437	4.713	10.011	18.111	14.349	9.884	14.312	17.545
S16	3.604	8.414	6.098	8.317	9.301	17.656	5.869	12.592	18.789	17.198	12.537	15.168	11.588

S17	6.365	9.290	6.382	11.402	14.745	17.824	7.405	12.870	17.441	17.787	13.217	15.367	18.044
S18	3.143	14.61	5.763	11.110	12.544	13.028	11.21	8.359	15.724	15.182	9.847	13.532	19.957
S19	6.034	7.970	6.254	13.086	11.427	15.033	11.296	12.464	16.412	16.197	13.217	12.392	14.200
S20	4.531	11.142	5.152	10.986	16.658	12.567	9.432	12.130	19.929	13.491	13.313	12.544	14.445
S21	3.885	14.92	7.104	9.104	11.937	12.377	8.596	10.107	10.558	12.172	12.903	12.268	23.999
S22	5.041	15.348	3.036	9.715	10.301	10.144	7.721	11.258	17.472	16.986	7.392	18.630	16.982
S23	3.430	8.114	6.147	6.325	9.423	13.374	11.812	12.642	18.194	17.578	12.110	15.758	19.999
S24	4.919	8.401	3.772	6.025	11.744	12.582	10.835	12.274	18.481	15.860	9.957	18.662	17.367
S25	3.541	10.147	5.123	7.402	15.085	16.010	10.569	10.338	18.350	14.135	12.757	15.977	17.556
S26	6.006	13.208	6.029	10.324	12.268	17.016	11.444	11.362	16.235	15.201	12.657	13.552	21.251
S27	5.215	15.46	5.41	8.317	12.935	11.035	9.481	13.144	15.335	14.334	12.249	12.894	14.658
S28	4.121	7.756	6.706	12.084	10.666	16.356	10.859	13.417	18.481	16.109	11.197	13.616	18.203
S29	6.365	7.012	6.013	9.436	14.545	13.470	8.139	10.395	14.395	12.329	10.512	17.780	19.294
S30	2.866	11.219	5.686	5.195	12.628	9.389	7.349	12.945	17.923	15.914	9.792	13.576	15.692
AVG	4.850	10.844	5.454	9.746	12.021	13.700	8.537	11.844	17.194	14.837	11.960	14.832	17.299
STDV	1.144	3.023	0.968	2.436	2.495	2.387	2.357	1.339	2.375	1.892	1.638	2.119	3.342

Table 2S. Raw data of the weight measurement of BSFL based on four substrate profiles

No Replication	D7	D14				D21				D28			
	All	P	F	C	M	P	F	C	M	P	F	C	M
R1	0.039	0.074	0.080	0.079	0.083	0.220	0.127	0.129	0.210	0.357	0.181	0.316	0.332
R2	0.037	0.078	0.084	0.083	0.075	0.199	0.115	0.143	0.232	0.340	0.172	0.301	0.316
R3	0.035	0.082	0.076	0.087	0.079	0.210	0.121	0.136	0.221	0.323	0.163	0.286	0.300
AVG	0.037	0.078	0.080	0.083	0.079	0.210	0.121	0.136	0.221	0.340	0.172	0.301	0.316
STDV	0.002	0.004	0.004	0.004	0.004	0.011	0.006	0.007	0.011	0.017	0.009	0.015	0.016

Table 3S. Chitin Extracted from BSFL based on four substrates at different phases of development

D7 – All Substrate					
No Replication (R)	Initial Weight (g)	Final Weight (g)	% Yield Chitin	Average Yield Chitin	Standard Deviation (%)
R1	0.6111	0.097	6.013	6.975	0.950

R2	0.63	0.09	7.000		
R3	0.6489	0.082	7.913		
D14 – BSFL with Protein based Feed Treatment (P)					
R1-P	1.985	0.195	10.179	9.775	0.415
R2-P	1.763	0.18	9.794		
R3-P	1.44	0.154	9.351		
D14 – BSFL with Fiber based Feed Treatment (F)					
R1-F	1.937	0.225	8.609	8.395	0.241
R2-F	1.756	0.208	8.442		
R3-F	1.391	0.171	8.135		
D14 – BSFL with Carbohydrate based Feed Treatment (C)					
R1-C	1.938	0.216	8.972	8.537	0.508
R2-C	1.68	0.194	8.660		
R3-C	1.093	0.137	7.978		
D14 – BSFL with Mixed Substrate based Feed Treatment (M)					
R1-M	2.108	0.195	10.810	10.306	0.488
R2-M	1.88	0.183	10.273		
R3-M	1.79	0.182	9.835		
D21 – BSFL with Protein based Feed Treatment (P)					
R1-P	2.533	0.177	14.311	14.771	0.429
R2-P	2.998	0.202	14.842		
R3-P	3.487	0.23	15.161		
D21 – BSFL with Fiber based Feed Treatment (F)					
R1-F	2.294	0.218	10.523	10.863	0.317
R2-F	2.543	0.233	10.914		
R3-F	3.022	0.271	11.151		
D21 – BSFL with Carbohydrate based Feed Treatment (C)					
R1-C	2.708	0.224	12.089	12.304	0.252
R2-C	2.644	0.216	12.241		

R3-C	2.491	0.198	12.581		
D21 – BSFL with Mixed Substrate based Feed Treatment (M)					
R1-M	3.978	0.221	18.000	17.048	0.837
R2-M	3.411	0.204	16.721		
R3-M	3.055	0.186	16.425		
D28 – BSFL with Protein based Feed Treatment (P)					
R1-P	3.409	0.203	16.793	17.219	0.531
R2-P	3.7	0.217	17.051		
R3-P	4.115	0.231	17.814		
D28 – BSFL with Fiber based Feed Treatment (F)					
R1-F	3.978	0.307	12.958	12.362	0.582
R2-F	3.059	0.248	12.335		
R3-F	2.63	0.223	11.794		
D28 – BSFL with Carbohydrate based Feed Treatment (C)					
R1-C	4.064	0.29	13.996	13.101	1.126
R2-C	3.341	0.248	13.472		
R3-C	3.03	0.256	11.836		
D28 – BSFL with Mixed Substrate based Feed Treatment (M)					
R1-M	4.784	0.252	18.984	18.136	0.942
R2-M	4.136	0.226	18.301		
R3-M	3.784	0.221	17.122		

Table 4S. Water quality measurement on the catfish fish tank

Parameter	Day of Cultivation														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A															
TDS (mg/L)	74	85	99	102	100	136	149	159	159	188	213	230	234	250	256
EC (μ S/cm)	150	176	190	198	189	237	299	325	343	377	424	440	482	498	514
pH	7.93	7.93	7.93	8.02	8.07	8.12	8.12	8.15	8.22	8.25	8.24	8.30	8.31	8.31	8.28

TSS (mg/L)	<10	<10	<10	15.26	15.89	16.02	18.29	19.98	21.09	24.22	28.72	30.16	30.76	31.31	32.54
B															
TDS (mg/L)	74	90	105	103	110	129	145	158	165	178	192	220	229	246	251
EC (μ S/cm)	150	181	189	202	205	221	267	290	319	351	399	438	492	501	520
pH	7.93	7.93	7.95	7.99	8.02	8.09	8.12	8.16	8.15	8.20	8.25	8.23	8.28	8.28	8.32
TSS (mg/L)	<10	<10	<10	15.26	15.89	16.02	18.29	19.98	21.09	24.22	28.72	30.16	30.76	31.31	32.54

Table 5S. Growth and development performance of catfish (*Clarias batrachus*) reared under different dietary treatments comparing commercial feed (A) and BSFL chitin-supplemented feed (B)

No Replication (R)	Length (cm)								Weight (gram)							
	A				B				A				B			
	D0	D5	D10	D14	D0	D5	D10	D14	D0	D5	D10	D14	D0	D5	D10	D14
R1	8.7	9.7	11.5	12.4	8.5	8.9	9.3	11.1	6.7	9.3	15.9	20.3	6.2	7.2	8.3	14.3
R2	8.7	8.7	12.1	13.1	8.3	8.5	9.8	11.7	6.7	6.8	18.5	23.9	5.8	6.2	9.7	16.8
R3	8.6	9.2	10.9	13.8	8.9	9.1	10.3	12.3	6.4	8.0	13.6	27.8	7.2	7.7	11.3	19.5
R4	8.5	8.6	11.8	12.8	8.4	8.4	9.6	11.5	6.2	6.4	17.2	22.2	6.0	6.0	9.1	15.9
R5	8.8	8.9	12.3	13.4	8.2	8.7	10.0	11.9	6.9	7.2	19.6	25.6	5.6	6.7	10.3	17.7
Average	8.7	9.0	11.7	13.1	8.5	8.7	9.8	11.7	6.6	7.5	17.0	24.0	6.2	6.8	9.7	16.9
STDV	0.1	0.4	0.5	0.5	0.3	0.3	0.4	0.4	0.3	1.1	2.4	2.9	0.7	0.7	1.2	2.0

Table 6S. Complete one-way ANOVA and Tukey HSD post-hoc results for larval length, weight, and chitin yield among the four substrate treatments (protein, fiber, carbohydrate, mixed) at each sampling point, and Welch's independent-samples t-test for catfish growth between dietary treatments. Analyses were performed in Python (SciPy and statsmodels).

(a) Larval length (mm), n = 30 per group

Sampling day	SS (between)	df (b)	MS (between)	SS (within)	df (w)	F	p-value
D-14	737.6905	3	245.8968	644.7564	116	44.240	<0.001

D-21	1176.1346	3	392.0449	542.0339	116	83.901	<0.001
D-28	428.8757	3	142.9586	635.5654	116	26.092	<0.001

(b) Larval weight (g), n = 3 per group

Sampling day	SS (between)	df (b)	MS (between)	SS (within)	df (w)	F	p-value
D-14	0.0000	3	0.0000	0.0001	8	0.875	0.4933
D-21	0.0232	3	0.0077	0.0006	8	97.577	<0.001
D-28	0.0509	3	0.0170	0.0017	8	79.816	<0.001

(c) Chitin yield (%), n = 3 per group

Sampling day	SS (between)	df (b)	MS (between)	SS (within)	df (w)	F	p-value
D-14	7.8890	3	2.6297	1.4527	8	14.481	0.0013
D-21	67.0586	3	22.3529	2.0982	8	85.228	<0.001
D-28	75.4575	3	25.1525	5.5557	8	36.219	<0.001

(d) Tukey HSD pairwise comparisons — larval length

Day	Pair	Mean diff	95% CI lower	95% CI upper	p-adj (sig)
D-14	C vs F	-4.2915	-5.8783	-2.7048	0.0 (*)
D-14	C vs M	2.2753	0.6886	3.862	0.0016 (*)
D-14	C vs P	1.0982	-0.4885	2.685	0.2766 (ns)
D-14	F vs M	6.5668	4.9801	8.1536	0.0 (*)
D-14	F vs P	5.3898	3.803	6.9765	0.0 (*)
D-14	M vs P	-1.1771	-2.7638	0.4097	0.2198 (ns)
D-21	C vs F	-3.3071	-4.762	-1.8522	0.0 (*)
D-21	C vs M	5.3501	3.8953	6.805	0.0 (*)
D-21	C vs P	1.8557	0.4009	3.3106	0.0064 (*)
D-21	F vs M	8.6572	7.2024	10.1121	0.0 (*)

D-21	F vs P	5.1628	3.708	6.6177	0.0 (*)
D-21	M vs P	-3.4944	-4.9493	-2.0395	0.0 (*)
D-28	C vs F	-2.8722	-4.4476	-1.2968	0.0 (*)
D-28	C vs M	2.467	0.8916	4.0424	0.0005 (*)
D-28	C vs P	0.0054	-1.57	1.5808	1.0 (ns)
D-28	F vs M	5.3392	3.7638	6.9146	0.0 (*)
D-28	F vs P	2.8776	1.3022	4.453	0.0 (*)
D-28	M vs P	-2.4617	-4.0371	-0.8863	0.0005 (*)

(e) Tukey HSD pairwise comparisons — larval weight

Day	Pair	Mean diff	95% CI lower	95% CI upper	p-adj (sig)
D-14	C vs F	-0.003	-0.0135	0.0075	0.7961 (ns)
D-14	C vs M	-0.004	-0.0145	0.0065	0.6298 (ns)
D-14	C vs P	-0.005	-0.0155	0.0055	0.4642 (ns)
D-14	F vs M	-0.001	-0.0115	0.0095	0.9893 (ns)
D-14	F vs P	-0.002	-0.0125	0.0085	0.9253 (ns)
D-14	M vs P	-0.001	-0.0115	0.0095	0.9893 (ns)
D-21	C vs F	-0.015	-0.0383	0.0083	0.2424 (ns)
D-21	C vs M	0.085	0.0617	0.1083	0.0 (*)
D-21	C vs P	0.0737	0.0504	0.0969	0.0 (*)
D-21	F vs M	0.1	0.0767	0.1233	0.0 (*)
D-21	F vs P	0.0887	0.0654	0.1119	0.0 (*)
D-21	M vs P	-0.0113	-0.0346	0.0119	0.4492 (ns)
D-28	C vs F	-0.129	-0.1671	-0.0909	0.0 (*)
D-28	C vs M	0.015	-0.0231	0.0531	0.6103 (ns)
D-28	C vs P	0.039	0.0009	0.0771	0.0452 (*)
D-28	F vs M	0.144	0.1059	0.1821	0.0 (*)
D-28	F vs P	0.168	0.1299	0.2061	0.0 (*)

D-28	M vs P	0.024	-0.0141	0.0621	0.2589 (ns)
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(f) Tukey HSD pairwise comparisons — chitin yield

Day	Pair	Mean diff	95% CI lower	95% CI upper	p-adj (sig)
D-14	C vs F	-0.1413	-1.2556	0.9729	0.9759 (ns)
D-14	C vs M	1.7693	0.6551	2.8836	0.0042 (*)
D-14	C vs P	1.238	0.1238	2.3522	0.0304 (*)
D-14	F vs M	1.9107	0.7964	3.0249	0.0026 (*)
D-14	F vs P	1.3793	0.2651	2.4936	0.0175 (*)
D-14	M vs P	-0.5313	-1.6456	0.5829	0.4662 (ns)
D-21	C vs F	-1.441	-2.7801	-0.1019	0.0355 (*)
D-21	C vs M	4.745	3.4059	6.0841	0.0 (*)
D-21	C vs P	2.4677	1.1286	3.8067	0.0016 (*)
D-21	F vs M	6.186	4.8469	7.5251	0.0 (*)
D-21	F vs P	3.9087	2.5696	5.2477	0.0001 (*)
D-21	M vs P	-2.2773	-3.6164	-0.9383	0.0027 (*)
D-28	C vs F	-0.739	-2.918	1.44	0.7071 (ns)
D-28	C vs M	5.0343	2.8554	7.2133	0.0003 (*)
D-28	C vs P	4.118	1.939	6.297	0.0014 (*)
D-28	F vs M	5.7733	3.5944	7.9523	0.0001 (*)
D-28	F vs P	4.857	2.678	7.036	0.0004 (*)
D-28	M vs P	-0.9163	-3.0953	1.2626	0.5619 (ns)

(g) Catfish growth — Welch's independent-samples t-test (control A vs treatment B, n = 5)

Parameter	Day	Mean A	Mean B	t	p-value
Length (cm)	D-0	8.66	8.46	1.525	0.1837
Length (cm)	D-5	9.02	8.72	1.270	0.2456
Length (cm)	D-10	11.72	9.80	6.421	<0.001
Length (cm)	D-14	13.10	11.70	4.472	0.0023

Weight (g)	D-0	6.58	6.16	1.377	0.2216
Weight (g)	D-5	7.54	6.76	1.296	0.2383
Weight (g)	D-10	16.96	9.74	6.209	<0.001
Weight (g)	D-14	23.96	16.84	4.545	0.0027

Significance: * = significant ($p < 0.05$); ns = not significant. Means sharing a letter in Tables 2–4 of the main text are not significantly different (Tukey HSD).