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USE OF OKARA WASTE FOR ALGAE NUTRITION

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ABSTRACT

Chlorella vulgaris belonging to *Chlorophyta* and *Nannochloropsis oculata* belonging to *Chrythophyta* were used in the current study to evaluate whether their successive indoor growth using industrial food waste (okara) as a growth medium enriched with organic carbon and nitrogen The basic nutrient solutions were BG-II for *Chlorella*, while F2 was used for *Nannochloropsis* growth. Okara was used in four concentrations (25 , 50 , 75 and 100%) verses to control and based on its initial nitrogen content. Original waste was diluted by 4 fold of tape water prior inoculation. The investigated parameters were dry weigh ($g.l^{-1}$); total chlorophyll ($mg.l^{-1}$) and total carotenoids ($mg.l^{-1}$). Maximum dry weight of *Chlorella* was obtained with 25% of okara waste. As for *Nannochloropsis*, a slight increase was observed with all okara concentrations used. Lower okara concentration (25%) enhanced chlorophyll accumulation by *Chlorella vulgaris*, while higher concentration (100%) reached the maximum with *Nannochloropsis oculata*. Completely opposite pattern was observed with total carotene.

INTRODUCTION

The wasted human consumption yearly is accounted by about one third of the food produced **(Gustavsson et al 2013)**. The think of food waste application as feedstock in microorganisms cultivation allows the recycling of waste matters consisting of carbon, nitrogen and phosphorous compounds **(Luque and Clark, 2013).**

Algae are a group of aquatic photosynthetic organisms wide spread in nature affected with different environmental factors such as light, temperature, pH, Salinity and availability of nutrients **(Blinova et al 2015)**. Algae also defined as lower plants without any structure such as leaves, stems and roots that can use the CO_2 to produce a huge biomass **(Chu, 2012)**.

The same study estimated the minerals analysis (%) and found as (C) 28.32 %, (O) 43.80, (Na) 2.04, (Mg) 13.16, (Al) 0.92, (Si) 1.60, (Cl) 1.97 and (Ca) 8.20 **(Sudjito et al 2014)**. On dry weight basis, *Nannochloropsis oculata* contains 3.99% moisture, 24.47 % ash, 8.08 % fixed carbon and 11.44 % lipid content. The estimated global production volumes of Chlorella are 2000 tons of dry matter/year and the production values about 40 million dollars/year **(Spolaore et al 2006 and Milledge, 2012).**

Nanochloropsis is a source of different kinds of pigment such as chlorophyll , Zeaxanthin and canthazanthin and also it is considered as a source of eicosapentaenoic acid (EPA) C20:5 ω–3 **(Jorge et al 2003)**.

Growth conditions can affect the growth rate of algae according to its level in algal growth media. These conditions including CO_2 levels, pH, temperature, nutrients availability (nitrogen, phosphorus, hydrogen, potassium, zinc, boron and magnesium) and presence or absence of other organisms **(Juneja et al 2013)**. For example, presence of phosphate buffer in the growth medium of *Nanochloropsis* clouded the culture medium and makes difficulty for the light to get into the medium **(Jorge et al 2003)**.

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Algae nutrition in concern carbon represented the maximum production figure and the proper challenge is to reduce cost. In this connection, several organic and non-organic wastes were used to meet the minimizing of nutrition costs. Of these, citrate wastes were used by **(El-Sayed., 2010)** to increase induction growth and carotenoids accumulation. Furthermore, vegetative growth was markedly increased in the absence of induction stress **(El-Sayed et al 2012)**. On the other series, corn steam liquor wasted from starch industry seems to be the most proper organic carbon when used in *Chlorella vulgaris* growth **(Battah et al 2013 and El-Sayed et al 2015).**

Okara is a by-product from the soymilk industry. Raw okara, also called soy pulp, is a white or yellowish insoluble material from soybean seeds, which remains in the filter sack when pure soybeans are filtered for the production of soymilk. **(Redondo et al 2008).**

Dried and blended bread crusts, okara powder and brewing grain in *thraustochytrid Schizochytrium* mangrovei KF6 growth medium led accumulate the high-value polyunsaturated fatty acid docosahexaenoic acid **(Fan et al 2000).**

The aim of the current work is to use industrial food waste as a novel algal growth medium which in turn reduces the production costs via the utilization of initial organic carbon as well as other organic components.

MATERIALS AND METHODS

Algae and growth conditions

The green algae *Chlorella vulgaris* belonging to *Chlorophyta* and *Nannochloropsis oculata* belonging to *Chrythophyta* were obtained from Algal Biotechnology Unit, National Research Centre. Growth was indoor performed using two different growth media. BG-II **(Stainer et al 1971)** was used for *Chlorella vulgaris* growth. F2 growth medium **(Guillard 1975)** was used in *Nannochloropsis oculata* growth. Growth container was fully transparent Plexi Glass column with initial diameter 7.5cm x 200cm containing 2.5 l of growth medium. Continuous light was provided from one side light bank with five white cool fluorescent lamps (40waste). Free oil compressed air stream supporting aeration from the lower hold of growth container. Inoculum was laboratory prepared after centrifugation and washing two times using laboratory cooling centrifuge (RUNNE HEIDELBERG Model / RSV-200, Germany).

Industrial food waste

Okara waste was obtained from Food Technology Research Institute, soybean processing center, Agriculture Research Center, Giza, Egypt. It was freshly collected and it was freezed till used.

Chemical analysis of dried waste was done according to methods adapted by **Chapman and Pratt (1978)**. Prior inoculation, waste was diluted by 4 fold of bi-distilled water and then filtered through sequences filtered sizes. The clear obtained volume was analyzed for total nitrogen and kept in Frigidaire till it will be use. All of nutrient solutions and okara dilution were made using distill water and sterilization were performed by autoclaving at 121^{o}C for 15 min.

Experiments

The used volumes and concentrations were selected based on their initial concentration of total nitrogen in waste and original growth medium. Thus, algal growth was achieved under the concentrations of 0.0, 25, 50, 75 and 100% of wastes enriching growth media.

Growth measurement

Daily sampling for determination of dry weight, total chlorophyll and carotenes were employed. Dry weight was measurement by filtering a defined volume of the algal slurry (5-10 ml) over pre-weighted dried membrane filter (0.45 µm). Filters were dried at 105^{o}C for 30 minutes, kept over anhydrous calcium chloride till it reached to room temperature and then re-weighted. The difference between weights monitored the net dry weight of the grown alga within defined sampling time. Chlorophyll was extracted from the pre-centrifuged algal bulk by 95% DMSO **(Burnison, 1980).** Chlorophyll absorbance was measured at 666 nm and concentration was calculated (mg.g^{-1}) . To recover carotenes, saponification was performed by 5% KOH/30% MeOH and the residual was re-extracted by DMSO after the addition of 5 drops of concentrated acetic acid **(Boussiba et al 1992)**. Carotenes absorbance was measured at 468nm and concentration was calculated (mg.g^{-1}).

Growth analysis

Growth analysis; mainly growth rate (µ); doubling time (g); degree of multiplication(y) and percentage increase (inc.%) was performed using the methods adopted by **Pirt (1973)**.

RESULTS AND DISCUSSION

Chemical composition of waste

As shown in **Table (1)**; chemical composition was found to be varied in concern biochemical ingredients mainly protein. The contribution of protein fraction represented the initial content of available nitrogen that will be used in algal growth. In addition, sugars in specific to soluble forms affecting algal growth either as an organic carbon source or as chelating agents for mineral nutrition. Chemical composition of waste is one of the most important factors in concern organic carbon, nitrogen and other nutrients.

Table 1. Some chemical analysis of dried okara waste

Protein	Carbohydrate	Oil		Ash		Total carbon			
%									
38.18	24.9	25.86		7.31		45.2			
Mineral analysis									
T.Nitrogen	T.phosphorus	K	Na	Ca	Mg	Fe	Zn	Mn	Cu
%									
6.11	0.35	1.69	1.98	1.92	0.42	0.17	0.003	0.05	0.001

Okara had the ash content which in turn predicted to support algal growth medium by an adequate amount of mineral nutrition.

Otherwise, the variation of the initial ash nutrients became the most limiting factor due to the variation of natural growth habitat of both used algae *i.e.*; *Chlorella* and *Nannochloropsis*. High ash content is expected increase growth of both algae in specific Nannochloropsis due to its high salinity margin.

Effect of okara waste concentrations on *Chlorella vulgaris* and Nannochloropsis oculata growth

Dry weight

As shown in **Fig. (1 a and b)**, okara waste increases the dry weight accumulation of all *Chlorella vulgaris* grown cultures as compared with control cultures that received only nutrients from recommended growth medium (BG-II). The most superior okara concentration was 25% that reached the maximum growth rate. Here, the net obtained biomass was 1.12, 1.92, 1.47, 1.42 and 1.58 $g.l^{-1}$ with 0.0, 25, 50, 75 and 100% of okara enriched grown *Chlorella* grown culture, respectively. It could be concluded that 25% of okara added to algal growth medium seems to be the most proper added percent led to the sufficient growth accumulation rate.
It is predicated that 100% of okara increases the net obtained biomass within the define time of incubation due to its higher content of organic carbon and minerals, but high ash content especially sodium ion that reach 1.98% on dry weight basis and might alert growth dry weight accumulation.

The effect of sodium ions in *Chlorella vulgaris* grown culture was early studied and all of these studies claimed the growth decline of *Chlorella* and other fresh water grown algae. Under high sodium conditions, protein and chlorophyll decomposition took place with a massive accumulation of lipids and carotenoids. Such phenomenon is maximized in the presence of organic carbon. In the case of *Nannochloropsis*, lower enhancing effect on dry weight was observed due to okara waste enriching growth medium as compared with *Chlorella* culture. The dry weight increment was found with all concentrations used comparing with control culture; however, a slight variation among them was observed. Here, low growth rate could be ascribed to the natural habitat of such alga as they obligated autotrophic growth mode alga like most of marine algae species.

Organic carbon from okara waste affecting algal growth by shifting algae metabolism toward heterotrophic nutrition that minimized illumination potential.

Fungal hydrolyzed commercial food residues fed algal growth medium trace elements and vitamins. It is also contains glucose, free amino nitrogen, phosphate and most likely long chain fatty acids **(Pleissner et al 2013).**

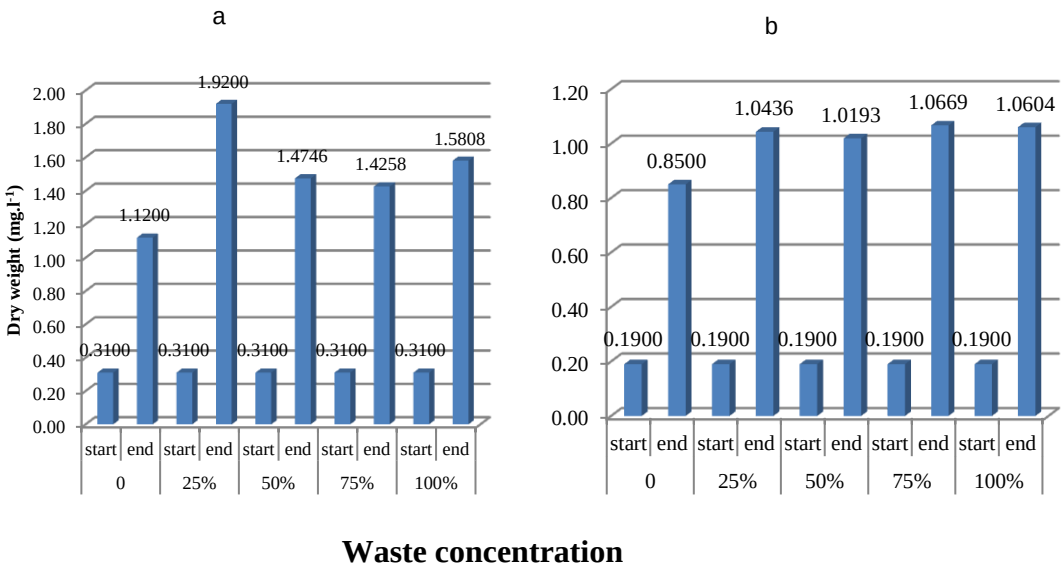

Fig. 1. Dry weight accumulation (g.l^{-1}) of *a) Chlorella vulgaris* and *b) Nannochloropsis oculata* as affected by okara waste enriching growth medium

Table 2. Dry weigh growth analysis of *Chlorella vulgaris* and *Nannochloropsis oculata* as affected by okara percentage enriching growth medium

Wastes %		0.0	25	50	75	100
G.R	*Chlorella*	0.092	0.130	0.111	0.109	0.116
	Nannochloropsis	0.107	0.122	0.120	0.123	0.123
D.T	*Chlorella*	7.555	5.322	6.222	6.360	5.956
	Nannochloropsis	6.477	5.697	5.777	5.624	5.644
D.M	*Chlorella*	1.852	2.629	2.249	2.200	2.349
	Nannochloropsis	2.160	2.456	2.422	2.488	2.479
P.I	*Chlorella*	72.321	83.85	78.977	78.26	80.390
	Nannochloropsis	77.647	81.79	81.360	82.19	82.082

G.R= growth rate; D.T= doubling time; D.M= degree of multiplication and P.I= percentage increase

Growth analysis confirmed such hypothesis, where the maximum growth rate was obtained with 25% of okara waste (0.130 g.l^{-1}.d^{-1}) with the lowest generation time (doubling time) was recorded as 5.32 hours as compared with other concentrations used (, 25, 50, 75 and 100%,).

During the whole incubation period (14 days) *Chlorella* biomass was folded by 1.852 to 2.63 times due to different okara concentrations used. This also resulted in 72.321 to 83.85% increase comparing with control cultures. The lowest doubling time was recorded as 5.322 hours with the proper okara concentration used (25%). On the contrary, okara seems to be inert in doubling time of N*annochloropsis*, where the neighbor values of growth rate were obtained 5.6-6.5 hours. The growth rate of C. pyrenoidosa in hydrolysate, however, was reduced by 50% as compared to growth on a defined medium **(Pleissner et al 2013)**. Here, the deduction in growth rate could be attributed to the presence of organic phosphorous of hydrlysate and/or the effect of acidic reaction media that avoided in the current study using fresh okara filtrate.

Thus, food waste may be a promising nutrients source for many algal strains. An economic evaluation of microalgae cultivation processes based on food waste is currently on-going in our laboratory, the broad range of chemicals and polymers obtainable from microalgal biomass, however, may make it economically feasible **(Foley et al 2011).**

Total chlorophyll

Data of total chlorophyll accumulation by *Chlorella vulgaris* and *Nannochloropsis oculata* **(Fig. 2 a and b)** was found in the same response of those obtained by dry weight results at 25% of okara concentration which was found to be the most effective in chlorophyll accumulation by the green alga *Chlorella vulgaris.* Other tested concentrations resulted in adverse effect on chlorophyll accumulation and led to decrease the net accumulated content. The inhibitory effect of okara concentrations more than 25% on chlorophyll content could be ascribed to the increase of initial organic carbon supported from okara waste.

In contrast, the opposite pattern was observed with *Nannochloropsis oculata* alga, where lower and hyper okara concentrations represented the maximum. Otherwise, moderate concentration represented some chlorophyll increase comparing with control cultures.

As mentioned above, organic carbon led to shift algal metabolism to heterotrophic growth mode, so chlorophyll seems to be inert. Lowest organic carbon with high nitrogen content causing high chlorophyll accumulation by algal cells as a photosynthetic machine.

With higher okara waste (100%); a slight increase was observe which might go back to the excess of nutrients on cell division and dry weight accumulation through photosynthetic processes.

As presented in **Table (3)**; growth rate was maximized with the superior concentration (25%) to reach 0.038 $g.l^{-1}.d^{-1}$ followed 100% cultures (0.006) and control cultures (0.005). Chlorophyll decline was observed with concentrations of 50 and 75% which resulted in a negative growth rate corresponding with high doubling time and lower percentage increase. The inhibitory effect of moderate okara level that observed with Chlorella was found less in *Nannochloropsis oculata*.

Total carotenoids

Total carotenoids were found to be the most responding growth parameter **(Fig. 3 a and b)**; where carotenoid of all treated cultures even control were increased. The lowest increase was observed with control cultures. Other moderate concentrations were found to have a slight enhancing effect. Hyper concentration of okara waste (100%) represented the maximum increase and markedly surpasses other concentrations used. The completely opposite pattern was observed with Nannochloropsis oculata, since the moderate concentrations represented the maximum. Hyper concentrations of okara waste might be stimulated *Chlorella* carotenoids biosynthesis due to organic carbon and high salt margin which in turn induced carotenogensis. As for Nannochloropsis alga, high salt margin from okara waste seemed to be non-effective due to the natural habitat of alga growth as a marine alga. Moderate concentration might be attributed to a sufficient organic carbon with lower nitrogen concentration comparing with the hyper okara concentration used.

Carotenoids increase is the first monitor of medium and growth conditions. Under unfavorable growth conditions cell metabolism shifted to a massive carotenoids accumulation to overcome such conditions of these conditions are nitrogen deficiency, salinity, high light intensity and organic carbon.

When data were subjected to growth analysis, 0.103 $g.l^{-1}.d^{-1}$ of total carotenoids were formed by the hyper concentration of okara waste (100 %), while the moderate concentrations (50 and 75%) resulted in about 0.04 $g.l^{-1}.d^{-1}$. Accordingly, doubling time was found to be the lowest with hyper concentrations of *Chlorella* grown cultures (6.709 h), while the moderate most effective with *Nannochloropsis* (16-19 h). Other growth parameters took the same trend.

The application of industrial food wastes in mass production was also took place and such use varied due to waste analysis and the grown algae species. Algal species that able to grow heterotrophically seems to be the most proper and promising. In carotenoids mass production, chlorophyll became function less due to low nitrogen concentration used with salinity. Here, algal metabolism shifted to perform their photosynthetic process through carotenoids using organic carbon and store such metabolites as lipids. In the absence of organic carbon and even in the presence of carbon dioxide carotenogensis that led to accumulate lipids and carotenoids is accompanied with drastic losing in dry weight.

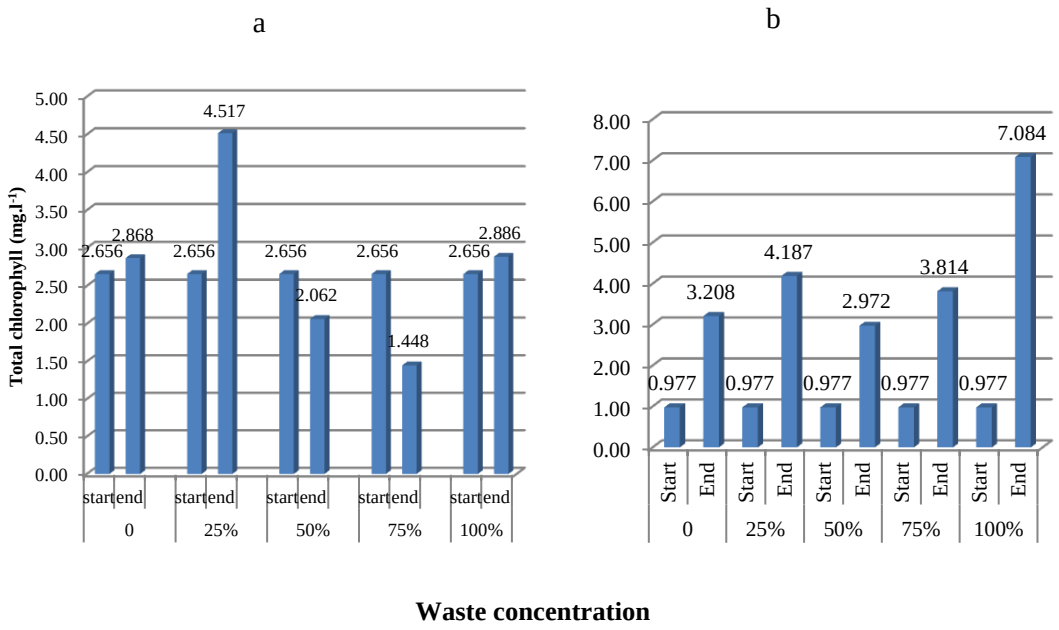

Fig. 2. Total chlorophyll accumulation (mg.l^{-1}) of *Chlorella vulgaris* and *Nannochloropsis oculata* as affected by okara waste enriching growth

Table 3. Chlorophyll growth analysis of *Chlorella vulgaris* and *Nannochloropsis oculata* growth as affected by okara percentage enriching growth medium

Wastes %		0.0	25	50	75	100
G.R	Chlorella	0.005	0.038	-0.018	-0.043	0.006
	Nannochloropsis	0.085	0.104	0.079	0.097	0.141
D.T	Chlorella	126.56	18.28	-38.34	-15.99	117.145
	Nannochloropsis	8.163	6.668	8.725	7.125	4.899
D.M	Chlorella	0.111	0.765	-0.365	-0.875	0.119
	Nannochloropsis	1.714	2.098	1.604	1.964	2.856
P.I	Chlorella	7.381	41.19	-28.80	-83.46	7.950
	Nannochloropsis	69.540	76.67	67.119	74.38	86.207

G.R= growth rate; D.T= doubling time; D.M= degree of multiplication and P.I= percentage increase

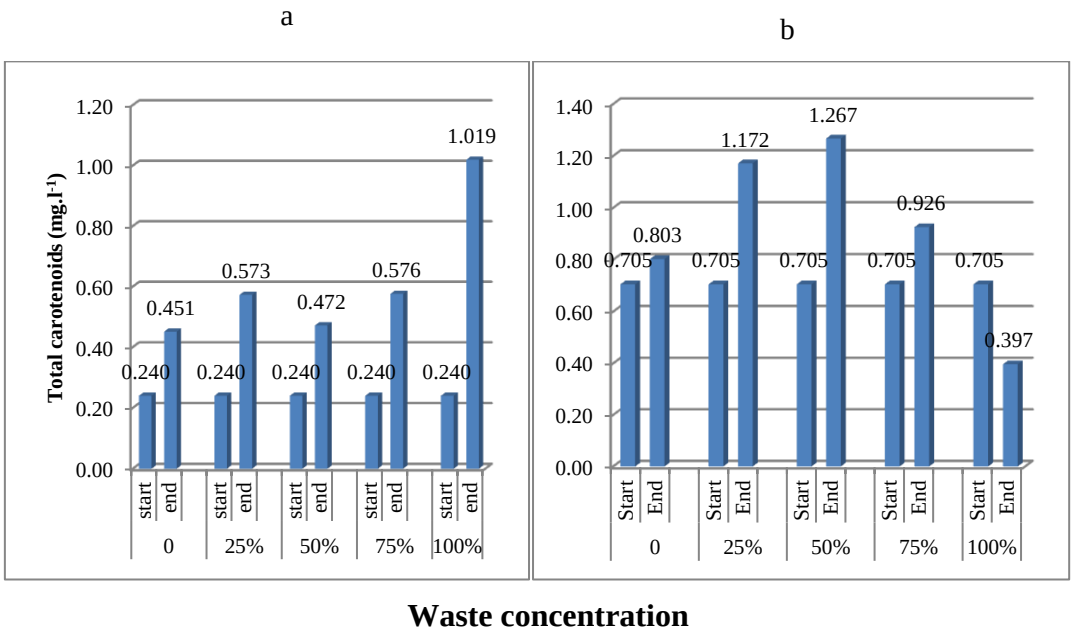

Fig. 3. Total carotenoids accumulation (mg.l^{-1}) of *Chlorella vulgaris* and *Nannochloropsis oculata* as affected by okara waste enriching growth medium

Table 4. Carotene growth analysis of *Chlorella vulgaris* and *Nannochloropsis oculata* growth as affected by okara percentage enriching growth medium

Wastes %		0.0	25	50	75	100
G.R	Chlorella	0.045	0.062	0.048	0.063	0.103
	Nannochloropsis	0.009	0.036	0.042	0.019	-0.041
D.T	Chlorella	15.378	11.155	14.361	11.082	6.709
	Nannochloropsis	75.244	19.130	16.565	35.733	-16.853
D.M	Chlorella	0.910	1.254	0.974	1.263	2.085
	Nannochloropsis	0.186	0.731	0.845	0.392	-0.830
P.I	Chlorella	46.797	58.101	49.120	58.341	76.457
	Nannochloropsis	12.100	39.787	44.334	23.782	-77.862

G.R= growth rate; D.T= doubling time; D.M= degree of multiplication and P.I= percentage increase

CONCLUSION

Okara waste as a source of both nitrogen and organic carbon source seemed to be a part of algal mass production concerning the minimizing of production costs. The response of grown alga to okara waste varied due to their natural growth habitat.

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