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Full Length Article

Cultivation of Algae in Vegetable and Fruit Canning Industrial Wastewater Treatment Effluent for Tilapia (*Oreochromis niloticus*) Feed Supplement

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Abstract

This work was conducted to study the possibility of cultivating algae for use as fish feed using vegetable and fruit canning industry wastewater treatment effluent. The results showed that *Chlorella vulgaris* showed successful growth in 10% initial stock solution, 20 cm water depth and for 12 days of cultivation. The algae attained a cell density of 13.72×10^5 cells/mL, biomass dry weight of 255 mg/L and protein content of 45.6%. Dried algae were used as a feed additive for tilapia (*Oreochromis niloticus* L.) cultivated in a vegetable and fruit canning industry oxidation pond. After 3 months of cultivation, fish fed 3% dried algae had a higher (P<0.05) fish survival rate and percentage weight gain than the fish fed only the commercial feed.

Keywords: *Chlorella vulgaris*; Wastewater; Feed supplement; Tilapia

Introduction

Vegetable and fruit processing can be considered an important industry in agricultural countries, but considerable amounts of wastewater are generated. Generally, wastewater is treated by anaerobic digestion followed by an aerobic wastewater treatment processes; however, after treatment, inorganic substances remain. This treatment effluent contains a high amount of macronutrients such as nitrogen and phosphorus, which can cause eutrophication when they come into contact with natural water resources. On the other hand, these substances can be developed as important food sources for the cultivation of algae with high economic significance. At present, treated wastewater is used to cultivate many species of algae such as *Spirulina* sp., *Chlorella* sp. and *Scenedesmus* sp. (Gantar *et al*., 1991; Cheunbarn and Peerapornpisal, 2010; Hongyang *et al*., 2011; Arbib *et al*., 2012; Wang *et al*., 2013). These algae can be developed into animal feed, especially as a feed mixture supplement for fish to improve yield and quality (Promya and Chitmanat, 2011; Ajiboye *et al*., 2012; Sing *et al*., 2014).

In this study, a microalgal *Chlorella vulgaris* isolated from vegetable and fruit canning industrial effluent was investigated. The study focused on suitable conditions for algal cultivation and use of the algae as a fish feed additive for tilapia (*Oreochromis niloticus* Linn.). It could reduce the capital cost of fish rearing, as well as serve as a method of treating the effluent from this factory with the highest degree of efficiency.

Materials and Methods

Wastewater Effluent Sample

The effluent was collected from a vegetable and fruit (Lungan, Lychee, Rambutan, Bamboo shoot and baby corn) canning industrial effluent, which was treated by anaerobic digestion followed by an aerated lagoon. The characteristics of the effluent were: BOD 25–35 mg/L, COD 65–80 mg/L and pH 6.5–7.2. (APHA, 1998)

Algae Isolation and Purification

Water samples were filtered and transferred to the laboratory. The streak plate technique was employed with Jaworski's medium under controlled conditions. The algae cells were isolated under light microscope (Olympus, Model CH30RF200) and identified as *Chlorella vulgaris* by morphological such as colour, cell shape and size (Peerapornpisal, 2006; Peerapornpisal, 2013). The plate culture was transferred to liquid medium to prepare a stock solution.

Suitable Condition for Cultivation of Algae

The stock solution of *Chlorella vulgaris* (OD_{560} 0.8) was inoculated in 500 mL volumetric flask with 300 mL of the effluent. Four treatments with three replications of algal culture with initial concentrations of 2, 5, 10 and 20% were evaluated. The culture flasks were continuously aerated with an electric aerator for 16 days. Cell growth was determined

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by cell counting with a hemocytometer, and the optical density was measured at 560 nm (Spectronic Genesys 5 UV-visible spectrophotometer) every two days.

The suitable initial algal concentration was cultivated in 100 L effluent in a 200 L cement pond for 15 days. The three different depths (20, 30 and 40 cm) with three replications were evaluated. The cellular concentration was determined every 3 days.

For mass culture, a suitable initial concentration of algae, depth and retention time based on the previous study was prepared in a 200 L cement pond with a 100 L working volume. The algal biomass was harvested, dried and analysed for the nutrient value according to the AOAC method (AOAC, 1984).

Feed Supplement for Tilapia

Dried algae were ground into a powder, mixed with commercial fish pellets and coated with fish oil. The samples were kept in a plastic bag, after drying in the open air. Tilapia (*Oreochromis niloticus* Linn.) were cultivated in cages (3.0×3.0 × 1.5 M) for 3 months in a vegetable and fruit canning oxidation pond. The study was divided into two treatments with three replications. Treatment 1 consisted of commercial fish pellets (CP brand product) mixed with 0% dried algae, while treatment 2 was commercial fish pellets mixed with 3% dried algae. The fish were fed twice a day (09:00–10:00 am and 3:00–4:00 pm). The quantity of feed was adjusted based on the monthly weight of the fish. The monthly weight was recorded and the feed supplied was used to compute the growth and nutrient utilisation parameters as follows:

1. Specific growth rate (%/day) = (ln W_2– ln W_1/no. of days during experiment) ×100

Where W_2 = final weight of fish, W_1= initial weight of fish.

2. Survival (%) = F1/F2×100

Where F1 = number of fish at the beginning of the experiment,

F_2 = number of fish at the end of the experiment.

3. Feed conversion ratio (unit) = feed intake (g)/fish weight gain (g)

4. Percentage weight gain (%) = $(W_2 – W_1)/W_1×100$

Where W_1 = initial weight of fish, W_2= final weight of fish.

Water Quality during Fish Cultivation

Water quality was measured every two weeks during the cultivation period. Temperature and pH were measured by multi parameter (Eutech instrument, Model PCTestr35). Nitrate, ammonia, and phosphate were measured by UV-Visible spectrophotometer (Hach Odyssey, Model DR/2500). DO, BOD, COD and TS were measured depending on procedure that has been described by APHA (1998). The four heavy metals, cadmium (Cd), copper (Cu), zinc (Zn) and mercury (Hg) were measured at the end of the experiment by ICP-MS (Agilent, Model 7500A).

Statistical Analysis

The differences of fish growth performances when use algae for fish feed supplement were analyzed. The study was divided into two treatments with three replications. The comparison and derivation were made between treatment 1 (0% algae) and treatment 2 (3% algae). Independent t-tests by compare means was performed using SPSS 16.0 for windows. $P<0.05$ was considered statistically significant.

Results

Suitable Condition for Cultivation of Algae

In this study, it was shown that algal growth was maintained in a stable manner, as evidenced by the increasing OD value and number of cells. The highest growth was found at 10% initial algae concentration. A highest cell density (16.08 × 10^5 cells/mL) was found on day 16 of the experimental period with an OD value of 0.42. This was followed by 2, 5 and 20% initial stock solution, which produced highest cell density of 13.83 × 10^5 cells/mL, 13.39 × 10^5 cells/mL and 12.43 × 10^5 cells/mL, respectively (Fig. 1 and 2). The suitable depth for algal growth was 20 cm. A highest number of cells was observed on day 15 at 18.14 × 10^5 cells/mL with an OD value of 0.546. This was followed by 30 and 40 cm depth, which produced highest cell density of 12.42 × 10^5 and 6.23 × 10^5 cells/mL, respectively (Fig. 3 and 4). The best time point for harvesting was on day 12 when the algae were completely mature.

The mass culture was done in batch system by the suitable condition with 10% initial stock solution, 20 cm depth and harvesting was done after 12 days, i.e. during the log phase. After cultivation, the algae had an OD value of 0.428, cell density of 13.72 × 10^5 cells/mL, and dry weight of 255 mg/L. The nutritional properties were protein 45.6%, fibre 8.5%, ash 17.0%, fat 15.2% and carbohydrate 10.6% of dry weight.

Fish Growth Performance on the Algae Feed Supplement

Feeding tilapia with commercial pellets and commercial pellets mixed with 3% dried algae, led to a significant difference in the fish survival rate and percentage weight gain at the 95% confidence level ($P<0.05$). There were no significant differences between Treatments 1 (0% algae) and 2 (3% algae) in the specific growth rate and feed conversion rate (Table 1).

Table 1: Growth performance of tilapia in an experimental pond using *Chlorella vulgaris* as feed supplement for three months

Parameters	Treatment 1 (commercial fish pellet+0% algae)	Treatment 2 (commercial fish pellet + 3% algae)	P-value
Specific growth rate (%/day)	2.16±0.006	2.37±0.152	ns
Survival rate (%)	61.7±9.5	76.3±5.5	*
Feed conversion rate (FCR)	0.45±0.05	0.40±0.03	ns
Percentage weight gain (%)	597.6±55.19	740±91.32	*

Mean± standard deviation. *= significant statistical differences (P<0.05), ns= no significant statistical differences

Table 2: Water quality during tilapia cultivation

Water Quality Index	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	mean ±SD
Temp (ºC)	23.6	23.4	24.5	25.6	24.2	24.6	24.4	24.3±0.7
TS (mg/L)	67	64	79	76	56	65	64	67.3±7.8
pH	7.5	7.2	6.9	7.6	7.5	6.9	7.4	7.3±0.29
DO (mg/L)	5.5	5.2	5.5	6.5	6.5	5.8	5.4	5.8±0.5
COD (mg/L)	68	80	76	65	82	75	79	75.0±6.3
BOD (mg/L)	16	18	19	15	14	19	20	17.3±2.3
NO_3^-- N (mg/L)	17	19	14	25	16	14	19	17.7±3.8
NH_3-N (mg/L)	0.14	0.16	0.23	0.15	0.30	0.24	0.23	0.2±0.06
PO_4^{3}- (mg/L)	0.11	0.32	0.16	0.22	0.30	0.23	0.18	0.22±0.07

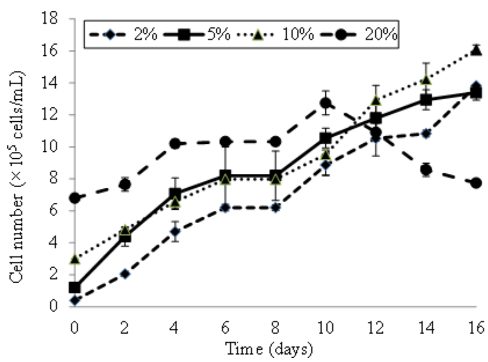

Fig. 1: Growth (cell densities) of *Chlorella vulgaris* cultivated at various initial concentrations

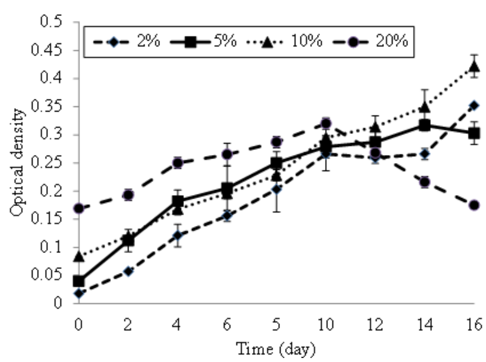

Fig. 2: Growth (OD) of *Chlorella vulgaris* cultivated at various initial concentrations

Water Quality During Fish Cultivation

The water quality results taken during the 3 months of rearing tilapia in the oxidation pond are shown in Table 2. Four heavy metals were measured. The results showed that only zinc was measured at a very low level of 0.01 mg/L, and the others were not found.

Discussion

A suitable initial concentration is considered necessary for cultivating algae. A huge number of algae are unable to survive under inappropriate conditions although they are native ones and adapted to local environment. This research found that the suitable *Chlorella vulgaris* initial stock concentration was found at 10% (OD_{560} 0.8). If the initial concentration is too low, only a few algae would continue to grow because of breaking colonies caused by photo-oxidation (Abeliovich and Shilo, 1972; Benchokroun *et al*., 2003; Baroli *et al*., 2004). This situation occurred at the 2 and 5% initially stock solution.

Also, if the initial concentration is too high, the yield would not be good because they would obstruct light from each other, and photosynthesis would be reduced (Gitelson *et al*., 1996). This situation appeared at the 20% initially stock solution, showing rapid growth only at the beginning but after 10 days of the experiment the growth slowly declined. Sedimentation also occurred, as the green colour of algal biomass was observed at the bottom of the container.

It was found that algae cultivated at 20 cm depth showed the best growth rate. During day 0–3, the algal growth was slow and increased only slightly because the algae were adapting to the new environment and had not yet started to increase in numbers. After the initial adaptation, the algae started to grow quickly during days 3–12, then grew slowly again until they reach a stationary phase (Fig. 3 and 4). However, the best time point for harvesting was on day 12 when the algae were completely mature, only a few dead cells were found together with a lower level of chlorellin. *Chlorella vulgaris* cultures usually release chlorellin (Pratt *et al*., 1945), a substance similar to a fatty acid and a hydrocarbon (Spoehr and Milner, 1949) having allelopathic activity, a property that allows it to inhibit the

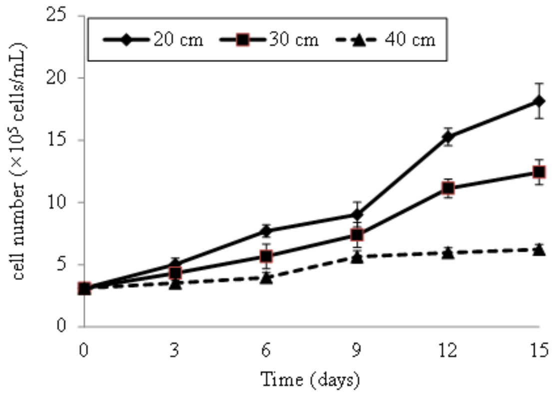

Fig. 3: Growth (cell densities) of *Chlorella vulgaris* cultivated at various depths

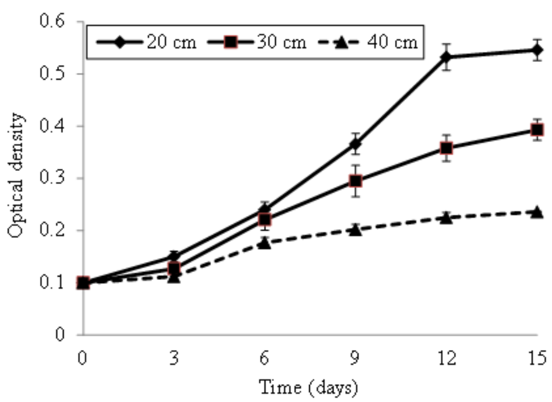

Fig. 4: Growth (OD) of *Chlorella vulgaris* cultivated at various

growth of other living organisms such as bacteria and algae (McCracken *et al*., 1980). Moreover, it has been found that chlorellin at lower concentration still allows algae to grow. However, if chlorellin is present at a concentration greater than 6.5 mg/L, it affects growth (DellaGreca *et al*., 2010). Chlorellin extracted from aged *Chlorella* sp. is still able to obstruct photosynthetic activity (Mandalam and Palsson, 1995); during this stage of harvesting, it must be collected before sedimentation.

At a depth of 30 and 40 cm, the growth of algae was less than at 20 cm. This might be due to the small size of *Chlorella vulgaris*, which enables them to spread out in the water thus increasing photosynthesis. At the high depth, light would not be able to penetrate into a water body; thus, photosynthesis will not take place and algae around the pond might not survive. This could be seen at the 40 cm depth where algae did not grow as well and the lag phase was much longer.

However, cultivation of *Chlorella vulgaris* in wastewater from the vegetable and fruit canning industry was not as efficient. This yield was not high compared with other algal cultivation methods such as cultivation in an effluent from the fertiliser production industry; in that study, the dry cell weight of the algae was 0.541 g/L after cultivation in BBM medium, the dry weight was 0.629 g/L (Toyub *et al*., 2007), and in the Trebon system, the production rate for *Chlorella* was 10 g/L (Doucha *et al*., 2005).

Normally, when algae are cultivated in wastewater, they are widely accepted as a feed supplement, especially for fish feed. However, the present study found that FCR in the both treatments were very low (Table 1), indicating that they took less feed to produce 1 kg of fish. Both fish diet formulas were not the only kind of feed for the fish, because the fish were in the oxidation pond where inorganic substrates were still high, so plankton could grow. The plankton became a natural food for the fish, so it was possible to use less feed to produce 1 kg of fish. However, the amount of algae as a protein supplement for fish depends on the type of fish. For example, *Paralichthys olivaceus* grew best when fed with 2% *Chlorella* powder (Koo *et al*., 2001), while *Sebastes schlegeli* (Hilgendorf) grew best when fed with 0.5% *Chlorella* powder (Bai *et al*., 2001).

Normally, when algae are widely accepted as a feed supplement, the supplementation would also increase the organic substances in the wastewater affecting the quality of the effluent. However, the analysis of water quality showed that the amounts of heavy metals were below the standard for inland aquaculture (MNRE, 2007) and the standard for industrial water effluents (MOSTE, 1996).

Conclusion

In conclusion, the agro-industrial effluent could be applies for microalgae cultivation, especially the strain that isolated from the effluent. With high protein and many nutrients contents, the microalgae could be applies as a feed supplement for fish to improve yield quality.

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